15th ISNI Congress 2021

BOOK OF ABSTRACTS

International Society of Neuroimmunology

Content

NOVEMBER 9 | 2021

10:00-10:30 **Welcome Remarks**
Roland Liblau, Sonia Berrih-Aknin, Amit Bar-Or, Francisco Quintana

Plenary I

Neuroimmunology of CNS demyelinating disorders
Chairs: Samia Khoury *(Lebanon)* & David Laplaud *(France)*

10:30-11:00 **Functional involvement of the HLA-DR15 haplotype in the autoimmune pathogenesis of MS**
Roland Martin (University of Zurich, Switzerland)

11:00-11:30 **The T cell-Phagocyte Interface in Neuroinflammation**
Burkhard Becher (University of Zurich, Switzerland)

11:30-12:00 **Role of the inflammasome in MS**
Manuel Comabella (Vall d'Hebron University Hospital, Spain)

Sponsored Symposia

Sanofi Symposium - Targeting new pathways in MS pathophysiology
Chair: Gandhi Roopali

12:15-13:00 **Targeting new pathways in MS pathophysiology**
Timothy Vartanian (Weill Cornell Medicine, USA)

13:00-13:15 **Q&A**

Biogen Symposium - New research targets in neuro-inflammatory & neurodegenerative diseases
Chair: Pr Hélène Zéphir *(Neurologist Lille Hospital University - France)*

12:15-12:20 **Introduction**
Béatrice Baciotti (Medical Director Biogen France)

12:20-12:40 **NAbs as potential therapeutic targets in MS**
Pr Heinz Wiendl (Neurologist - Münster Hospital University - Germany)

12:40-13:00 **New targets of research on Alzheimer's Diseases**
Thierry Bussière Thierry Bussière *(Dementia Principal Investigator - Biogen)*

13:00-13:15 **Q&A – Conclusion & Key Message**

Plenary II

Brain-Immune system interactions in physiological conditions
Chairs: Sonia Garel *(France)* & Jon Laman *(the Netherlands)*

13:30-14:00 **Single-cell clonotype tracing of encephalitogenic T cells**
Vijay Kuchroo (Harvard Medical School and Brigham and Women's Hospital, USA)

14:00-14:30 **Anticipating pathogen invasion: Neuroimmune activation preserves gut homeostasis**
Gabrielle Belz (The University of Melbourne, Australia)

14:30-15:00 **Dissecting functions of Microglia and non-parenchymal CNS macrophages**
Steffen Jung (The Weizmann Institute of Science, Israel)

Keynote Lecture

The Newsom-Davis Lecture
Chair: Amit Bar-Or *(USA)*

15:15-16:15 **Immune mechanisms in neurologic disease**
Howard Weiner (Harvard University, Brigham and Women's Hospital, USA)

Parallel Symposia

Parallel I - Dialog between astrocytes and microglia in neuroimmunology
Chairs: Tanja Kuhlmann *(Germany)* & Ari Waisman *(Germany)*

16:30-16:55 **Microglia in early brain wiring: from circuit assembly to tissue integrity**
Sonia Garel (Université Paris, France)

16:55-17:10 **59-Astrocyte-Oligodendrocyte interaction drives central nervous system remyelination**
Irene Molina-Gonzalez (University of Edinburgh, UK)

17:10-17:35 **Regulation of CNS Macrophages in Health and Disease**
Melanie Greter (University of Zurich, Switzerland)

17:35-17:50 **249-APOE4 Impairs Microglia Response to Neurodegeneration in alzheimer's disease**
Neta Rosenzweig (Brigham and Women's Hospital, Harvard Medical School, USA)

17:50-18:15 **Astrocyte heterogeneity in Multiple Sclerosis**
Francisco Quintana (Harvard Medical School, Brigham and Women's Hospital, USA)

18:15-18:30 **308- Selective inhibition of soluble TNF promotes beneficial neuroinflammatory responses and remyelination in the cortical grey matter**
Athena Boutou (Hellenic Pasteur Institute, Greece)

Parallel II - Parasites and neuroinflammation
Chairs: Sylviane Pied *(France)* & Nicolas Blanchard *(France)*

16:30-16:55 **A conserved GABAergic system in mononuclear phagocytes - implications for infection**
Antonio Barragan (Stockholm University, Sweden)

16:55-17:10 **246-Studying the mechanisms of neuroinflammation-induced cognitive alterations associated with Toxoplasma gondii infection**
Marcy Belloy (University of Toulouse, France)

17:10-17:35 **CD4 and CD8 T cells infiltrate the brain and form brain-resident memory after blood-stage infection with P. berghei ANKA**
William Heath (The University of Melbourne, Australia)

17:35-17:50 **108-Regional cyst localisation and innate immune activation of the retina and brain in murine toxoplasmosis**
Dana Lee (Queensland University of Technology, Australia)

17:50-18:15 **Mechanisms of fatal blood barrier dysfunction during cerebral malaria**
Dorian McGavern (NINDS, USA)

Parallel III | Antibody-mediated diseases: new targets and new mechanisms
Chairs: Jérôme Honnorat *(France)* & Laurent Groc *(France)*

16:30-16:55 **The different syndromes and mechanisms of neuronal synaptic autoantibodies**
Josep Dalmau (University of Barcelona, Spain)

16:55-17:10 **191-Immunogenetics in autoimmune encephalitis and related disorders**
Sergio Muniz-Castrillo (Hospices Civils de Lyon, Hôpital Neurologique, France)

17:10-17:35 **The peculiar features of IgG4 and its role in autoimmune diseases**
Maartje Huijbers (Leiden University Medical Center, the Netherlands)

17:35-17:50 **259-The role of SWAP-70 and FCRL2 in pathophysiology of multiple sclerosis and their value as biomarker**
Elif Sanli (Istanbul University, Turkey)

17:50-18:15 **Autoimmune encephalitis and epilepsy**
Maarten Titulaer (Erasmus MC University Medical Center, the Netherlands)

18:15-18:30 **131-Clinical and laboratory features in anti-NF155 autoimmune nodopathy**
Lorena Martín-Aguilar (Hospital de la Santa Creu i Sant Pau, Spain)

Workshops

Workshop I | Innate and Adaptive Immunity in AD, Parkinson and ALS

Chairs: Bente Finsen *(Denmark)* & Stephane Hunot *(France)*

18:45-19:15 **Lymphocyte trafficking to the CNS – does provenance matter?**
Thomas Korn (Technische Universität München, Germany)

19:15-19:30 **57-CYP46A1 in the choroid plexus: an unexpected safeguard of brain function lost in Alzheimer's disease**
Afroditi Tsitsou-Kampeli (Weizmann Institute of Science, Israel)

19:30-19:45 **34-Effects of IgLON5 antibodies on neuronal cytoskeleton: A link between autoimmunity and neurodegeneration**
Jon Landa (Universitat de Barcelona, Spain)

19:45-20:00 **55-Etiology-independent disease-associated oligodendrocytes in CNS pathologies**
Mor Kenigsbuch (Weizmann Institute of Science, Israel)

20:00-20:15 **287-Disrupting the neuroimmune crosstalk in the spleen exacerbates cognitive loss in animal model of Alzheimer's disease**
Tommaso Croese (Weizmann Institute of Science, Rehovot, Israel)

20:15-20:30 **38-Repurposing the anxiolytic drug buspirone to counteract inflammation in cellular and animal models of Parkinson's disease**
Sarah Thomas Broome (University of Technology Sydney, Australia)

Workshop II | Modeling neuroinflammation using IPSC, organoids and animal models

Chairs: Sandra Amor *(the Netherlands)* & Lennart Mars *(France)*

18:45-19:15 **Th17 lymphocyte-induced neuronal cell death in Parkinson's disease**
Iryna Prots (University Hospital Erlangen, Germany)

19:15-19:30 **375-Human induced pluripotent stem cells-derived neurons to study CNS-reactive autoantibodies in COVID-19-mediated neurological syndromes**
Amandine Mathias (Lausanne University Hospital and Lausanne University, Switzerland)

19:30-19:45 **336-Mapping of radiation-induced microglia activation in whole-brain mouse histological images**
Sindi Nexhipi (National Center for Radiation Research in Oncology, Germany)

19:45-20:00 **185-Intrinsic regulation of Th17 cell pathogenicity by IL-24**
Christopher Sie (Technical University of Munich, Germany)

20:00-20:15 **90-Decline of Neural Stem Cell Resilience in multiple sclerosis**
Alexandra Nicaise (University of Cambridge, UK)

20:15-20:30 **184-Regulatory B cells required IL-2 signaling to control disease severity in EAE**
Juliette Gauthier (INSERM U1236, France)

20:30-20:45 **356-Using hiPSC-derived CNS cells as antigen presenting cells for unbiased identification of CNS-autoreactive CD8+ T cells**
Sylvain Perriot (CHUV, Switzerland)

Workshop III | Single cell analyses in neuroimmunology: essential or overrated?
Chairs: Rejeane Rua (France) & Simon Fillatreau (France)

18:45-19:15 **Oligodendroglia cell states in multiple sclerosis: insights from single-cell transcriptomics and epigenomics**
Gonçalo Castelo-Branco (Karolinska Institutet, Sweden)

19:15-19:30 **304-Single cell analysis reveals incomplete tolerance mechanisms in MOGAD patients**
Alicia Zou (University of Sydney, Australia)

19:30-19:45 **298-Diverse human astrocyte and microglial transcriptional responses to Alzheimer's pathology**
Amy Smith (University of Auckland, New Zealand)

19:45-20:00 **363-Single-cell transcriptome profiling revealed the distinct transcriptional response of microglia-like cells during herpes simplex encephalitis**
Olus Uyar (Université Laval, Canada)

20:00-20:15 **54-Exploring reported genes of microglia RNA-seq studies: uses and considerations**
Thecla van Wageningen (Amsterdam UMC, the Netherlands)

20:15-20:30 **200-CXCR4-positive T cells are increased in people with radiologically isolated syndrome**
Raphael Schneider (Keenan Research Centre for Biomedical Science, Canada)

20:30-20:45 **225-CanProCo study; Determining technical parameters for large scale single-cell RNA sequencing of MS patients**
Fiona Tea (CrCHUM, Canada)

Workshop IV | Immunomodulation and remyelination
Chairs: Abdelhadi Saoudi *(France)* & Violetta Zujovic *(France)*

18:45-19:15 **Promoting macrophage/microglia activity for remyelination**
V. Wee Yong (University of Calgary, Canada)

19:15-19:30 **187-TNFR2 regulates the response of oligodendrocyte precursor cells to neuroinflammation**
Haritha Desu (University of Miami, USA)

19:30-19:45 **17-Targeting lipophagy in macrophages improves repair in multiple sclerosis**
Melanie Loix (Hasselt University, Belgium)

19:45-20:00 **61-Exogenous fractalkine enhances oligodendrogenesis and remyelination in the cuprizone-induced demyelination mouse model**
Monique Marylin Alves de Almeida (University of Alberta, Canada)

20:00-20:15 **123-Stearoyl-CoA desaturase-1 impairs the reparative properties of macrophages and microglia in the brain**
Jeroen Bogie (Hasselt University, Belgium)

20:15-20:30 **220-Themis1/Vav1 signaling hub controls T cell pathogenicity in a mouse model of multiple sclerosis**
Rémi Marrocco (INFINITY, France)

20:30-20:45 **65-Elevated expression levels of Ephrins on Immune Cells of Patients With multiple sclerosis Affects Oligodendrocyte Differentiation**
Maya Golan (Tel Aviv Sourasky Medical Center, Israel)

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10:30-12:30 **Poster Session**

Sponsored Symposia

BMS/Celgene | S1P receptor from Central nervous system to clinical use
Chair: Roland Liblau *(France)*

12:45-13:00 **Lymphocyte dynamics in the central nervous system during health and in Multiple Sclerosis**
Pr Britta Engelhardt (Switzerland)

13:00-13:15 **Role of S1P receptors and CNS migration cells**
Pr Jack Antel (Canada)

13:15-13:30 **Targeting and involvement of S1PR modulators in Multiple Sclerosis and other pathologies**
Pr Jeffrey A Cohen (USA)

13:30-13:45 **Questions & Conclusion**

Plenary III

Plenary III | Neuroimmunology of neurodegenerative diseases and ageing
Chairs: Shohreh Issazadeh-Navikas *(Denmark)* & Guillaume Dorothée *(France)*

14:00-14:30 **Etiopathogenic relevance of CD8 T cells in Parkinson's disease: inferences from a postmortem study**
Jordi Bove (Vall d'Hebron Research Institute (VHIR), Spain)

14:30-15:00 **Systemic blockade of anti-PD-l1 can potentially overcome Trem2 polymorphism in combating Alzheimer's disease**
Michal Schwartz (Weizmann Institute of Science, Israel)

15:00-15:30 **Heterogeneity of meningeal B cells reveals a lymphopoietic niche at the CNS borders**
Marco Colonna (Washington University School of Medicine St. Louis, USA)

Parallel Symposia

Parallel IV | Barriers of the CNS: actors in neuroinflammation
Chairs: Catharina Gross *(Germany)* & Julie Ribot *(Portugal)*

15:45-16:10 **Regulation of the blood-brain barrier in health and disease**
Richard Daneman (University of California, San Diego, USA)

16:10-16:25 **282-DICAM Promotes Th17 Lymphocyte Trafficking Across the Blood-Brain Barrier during Neuroinflammation**
Marc Charabati (Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Canada)

16:25-16:50 **Modelling T-cell autoimmunity of the CNS grey and white matter**
Francesca Odoardi (University Medical Center Göttingen, Germany)

16:50-17:05 **157-Immune protection at the CNS borders: heterogeneity and maturation of meningeal macrophages**
Elisa Eme-Scolan (Immunology Center of Marseille-Luminy, France)

17:05-17:30 **Unbiased and large-scale CNS endothelium transcriptomic analyses in human and mouse**
Alexandre Prat (Université de Montréal, Canada)

17:30-17:45 **85-Oncostatin M opens the gate for T helper 17 cells during neuroinflammation**
Doryssa Hermans (Hasselt University, Belgium)

Parallel V | Autoimmune neurological disorders (other than MS)
Chairs: Anne-Katrin Pröbstel *(Switzerland)* & Patrick Vermersch *(France)*

15:45-16:10 **Neuromyelitis optica spectrum disorders: Pathobiology predicts treatment response for AQP4-IgG-associated subtype**
Brian Weinschenker (Mayo Clinic, USA)

16:10-16:25 **71-T cell-mediated neuronal destruction in GAD65-encephalitis calls for early immunosupressive treatment**
Anna Tröscher (Medical University of Vienna, Austria)

16:25-16:50 **Brain control of adaptive immunit**
Hai Qi (Tsinghua University, China)

16:50-17:05 **255-Prospective long-term tocilizumab responses in relapsing myelin oligodendrocyte glycoprotein IgG-associated disease: factors associated with post-tocilizumab relapse-freedom**
Fan Cheng (Peking Union Medical College Hospital, UK)

17:05-17:30 **New aspects of autoimmune neuromuscular junction disorders**
Angela Vincent (University of Oxford, UK)

17:30-17:45 **281-Investigating anti-inflammatory and immunomodulatory properties of brivaracetam in experimental autoimmune encephalomyelitis (EAE)**
Oumarou Ouédraogo (University of Montreal, Canada)

Parallel VI | Viruses and neuroinflammation
Chairs: Daniel Gonzalez-Dunia *(France)* & Trevor Owens *(Denmark)*

15:45-16:10 **Innate immune mechanisms of pathological forgetting**
Robyn Klein (Washington University School of Medicine – St. Louis, USA)

16:10-16:25 **127-MAVS is required to mount IFN-β- and broad innate immune responses in VSV infected microglia**
Olivia Luise Gern (TWINCORE, Germany)

16:25-16:50 **Epstein-Barr virus and multiple sclerosis: from autoimmunity to virus-induced immunopathology?**
Francesca Aloisi (Istituto Superiore di Sanità, Italy)

16:50-17:05 **156-Age-dependent meningeal macrophages protect against viral neuroinfection**
Rejane Rua (Immunology Center of Marseille-Luminy, France)

17:05-17:30 **Disentangling pathological and protective T cell responses in the CNS**
Doron Merkler (Centre Médical Universitaire Géneva, Switzerland)

17:30-17:45 **52-Human iPSC-derived astrocytes as a model to understand JCV infection in the brain**
Larise Oberholster (Lausanne University Hospital and University of Lausanne, Switzerland)

Keynote Lecture

The Immunology Lecture
Chair: Gianvito Martino *(Italy)*

18:00-19:00 **Unraveling the Complex Pathogenesis of Multiple Sclerosis Using Animal Models**
Joan Goverman (University of Washington, USA)

Workshops

Workshop V | Does our food affect our brain via the immune system?
Chairs: Caroline Pot *(Switzerland)* & Guy Gorochov *(France)*

19:15-19:45 **Diet and the gut microbiome shape the course of multiple sclerosis**
Aiden Haghikia (Ruhr-Universität Bochum, Germany)

19:45-20:00 **62-Diet-dependent regulation of TGFb impairs reparative innate immune responses after demyelination**
Mar Bosch Queralt (Technical University Munich, Germany)

20:00-20:15 **56-Sialic acid-driven untimely immune aging in obesity-Alzheimer's disease comorbidity accelerates cognitive decline**
Stefano Suzzi (Weizmann Institute of Science, Israel)

20:15-20:30 **140-Neurological alterations and intestinal dysbiosis associated with subclinical food allergy**
Kumi Nagamoto-Combs (University of North Dakota School of Medicine & Health Sciences, USA)

20:30-20:45 **171-Impact of methionine intake on neuroinflammatory processes in a spontaneous sex-biased animal model of multiple sclerosis**
Victoria Hannah Mamane (Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Canada)

20:45-21:00 **115-Dietary supplementation with conjugated linoleic acid ameliorates intestinal inflammation and CNS autoimmunity**
Ann-Katrin Fleck (University Hospital Münster, Germany)

21:00-21:15 **289-Impact of high fibre diet and its combination with intermittent fasting in CNS autoimmunity**
Eileen Liao (The University of Sydney, Australia)

Workshop VI | Brain ageing: impact of immunity and infection

Chairs: Elsa Suberbielle *(France)* & Serge Nataf *(France)*

19:15-19:45 **Uncovering immune responses driving cellular communities towards proteinopathy and cognitive decline in 1.6 million transcriptomes**
Philip De Jager (Columbia University Irving Medical Center, USA)

19:45-20:00 **110-Aging perturbs microglia function and upregulates osteopontin to accelerate oxidized phosphatidylcholine-mediated neurodegeneration**
Yifei Dong (University of Calgary, Canada)

20:00-20:15 **136-Aging blood factors promote CD8 T cell infiltration in the adult mouse brain**
Lynn van Olst (Amsterdam UMC, the Netherlands)

20:15-20:30 **342-Deranged granulopoiesis drives poor outcome of ischemic stroke in the elderly**
Giorgia Serena Gullotta (San Raffaele Scientific Institute, Italy)

20:30-20:45 **328-Aged CNS-resident cells promote a non-remitting course of experimental autoimmune encephalomyelitis**
Jeffrey Atkinson (The Ohio State University, Columbus, USA)

20:45-21:00 **267-Ageing promotes non-remitting experimental autoimmune encephalomyelitis with persistent meningeal inflammation and subpial demyelination in SJL/J mice**
Michelle Zuo (University of Toronto, Canada)

21:00-21:15 **307-Chronic demyelination-induced cell senescence is associated with motor impairment in a model of MS**
Irini Papazian (Hellenic Pasteur Institute, Greece)

Workshop VII | Neuroimmunology of the eye and the optic nerve

Chairs: Cécile Delarasse *(France)* & Sylvain Fisson *(France)*

19:15-19:45 **Microbiota and the eye – is there a gut-eye axis?**
Rachel Caspi (National Eye Institute, NIH, USA)

19:45-20:00 **202-Is it the Vaccine? Case Reports and Systematic Review of Postvaccination Neuromyelitis optica Spectrum Disorder**
Nanthaya Tisavipat (Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand)

20:00-20:15 **254-The early emergences of aquaporin 4-specific B cells characterises neuromyelitis optica spectrum disorders**
Sudarshini Ramanathan (John Radcliffe Hospital; University of Oxford, UK)

20:15-20:30 **201-Aberrant DNA methylation of Runx1 promotes Th17 induced autoimmunity in Neuromyelitis optica Spectrum Disorders**
Yuhan Qiu (West China Hospital of Sichuan University, China)

20:30-20:45 **73-ATP mediates neuropathic pain in neuromyelitis optica via microglial activation**
Teruyuki Ishikura (Osaka University, Japan)

20:45-21:00 **377-Subretinal Associated Immune Inhibition, a new antigen-specific immunosuppressive mechanism in the context of AAV gene transfer**
Gaelle Chauveau (Univ Evry, Université Paris, France)

21:00-21:15 **162-Role of P2X7 receptor in mouse experimental autoimmune uveitis (EAU)**
Cécile Delarasse (Institut de la Vision, France)

Workshop VIII | Sex chromosomes and hormones in neuroimmunology
Chairs: Nathalie Arbour (Canada) & Sophie Laffont (France)

19:15-19:45 **Sex Chromosome Effects on Autoimmunity**
Rhonda Voskuhl (UCLA, USA)

19:45-20:00 **276-In utero exposure to maternal Anti-Caspr2 antibodies alters microglial activation and development in male offspring**
Ben Spielman (The Feinstein Institutes for Medical Research, USA)

20:00-20:15 **67-Suppression of the disease-relevant brain-homing T cell in MS by nuclear receptor crosstalk**
Steven Koetzier (Erasmus MC, University Medical Center, the Netherlands)

20:15-20:30 **116-Regulatory T cells contribute to sexual dimorphism in neonatal hypoxic-ischemic brain injury**
Josephine Herz (University Hospital Essen, University Duisburg-Essen, Germany)

20:30-20:45 **264- Antidiabetic and antiaging role of Moringa oleifera on hippocampus of experimental male rats**
Pardeep Kumar (Jawaharlal Nehru University, India)

20:45-21:00 **133-CD300f immune receptor contributes to healthy aging by regulating inflammaging, metabolism and cognitive decline**
Hugo Peluffo (UDELAR, Institut Pasteur de Montevideo, Uruguay)

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Workshops

Workshop IX | Behavior and immunity
Chairs: Fabienne Brilot *(Australia)* & Asya Rolls *(Israel)*

10:30-11:00 **Neuroimmune interactions in the skin, from molecular mechanisms to therapeutic perspectives**
Sophie Ugolini (Parc Scientifique et technologique de Luminy, France)

11:00-11:15 **42-Brain-Immune Axis Regulation is Responsive to Cognitive Behavioral Therapy and Mindfulness Intervention in Crohn's Disease patients**
Ilan Karny (Ben-Gurion University of the Negev, Israel)

11:15-11:30 **92-The modulatory effect of melatonin on the behavior in rats under experimental conditions of inflammation models**
Aisance Tchang (Samara University, Democratic Republic of Congo)

11:30-11:45 **222-Role of meninges in activity-induced neurogenesis**
Matilde Marin (Immunology Center of Marseille-Luminy, France)

11:45-12:00 **277-Deleterious role of microgliosis after perinatal cerebellar injury on anxious behaviors in adult mice**
Eloi Guarnieri (Université de Montréal, Canada)

12:00-12:15 **122-Cytotoxic NK-like CD8+T cells, the reservoir of clonal cells, are related to disease activity in MS**
Emilie Dugast (CRTI-INSERM U1064, France)

Workshop X | The sensory and autonomic nervous systems: links with inflammation

Chair: Nicolas Gaudenzio *(France)*

10:30-11:00 **Neuroimmunometabolism**
Ana Domingos (University of Oxford, UK)

11:00-11:15 **63-Meningeal inflammation in multiple sclerosis induces phenotypic changes in cortical microglia that differentially associate with neurodegeneration**
Carla Rodriguez-Mogeda (Amsterdam UMC, the Netherlands)

11:15-11:30 **316-Argonaute autoantibodies are diagnostic and prognostic biomarkers for autoimmune-related sensory neuropathies**
Christian Moritz (Université de Lyon, Université Claude Bernard Lyon 1, France)

11:30-11:45 **6-Flow cytometric detection of functionally relevant ganglionic acetylcholine receptor antibodies in Autoimmune Autonomic Ganglionopathy**
Nicolás Urriola (Royal Prince Alfred Hospital, Australia)

11:45-12:00 **12-High-dimensional immune profiling in traumatic spinal cord injury patients in relation to clinical parameters**
Judith Fransen (Hasselt University, Belgium)

Workshop XI | Immunology in neuro-oncology

Chairs: Romana Höfteberger *(Austria)* & Alberto Vogrig *(Italy)*

10:30-11:00 **Glioma immunotherapy**
Gaetano Finocchiaro (Istituto Neurologico C.Besta, Italy)

11:00-11:15 **195-Cervical lymph nodes and ovarian teratomas as germinal centres in NMDA receptor-antibody encephalitis**
Adam Al-Diwani (University of Oxford, UK)

11:15-11:30 **299-Clinical spectrum and long-term outcome in n-iraes: the ciclops study**
Antonio Farina (Hospices Civils de Lyon, France)

11:30-11:45 **32-Pretreatment Neutrophil-to-Lymphocyte/Monocyte-to-Lymphocyte Ratio as Prognostic biomarkers in Glioma Patients**
Sher Ting Chim (Monash University, Australia)

11:45-12:00 **163-Identification of target antigens for glioblastoma-infiltrating CD4+ T cells**
Reza Naghavian (University of Zurich, University Hospital Zurich, Switzerland)

12:00-12:15 **212-Immune contexture of isocitrate dehydrogenase stratified human gliomas revealed by single-cell transcriptomics and accessible chromatin**
Pravesh Gupta (The University of Texas MD Anderson Cancer Center, USA)

12:15-12:30 **234-Is there a paraneoplastic neurologic syndrome associated to renal and bladder cancer?**
Macarena Villagran Garcia (Hospices Civils de Lyon, France)

Workshop XII | Myasthenia Gravis
Chairs: Sonia Berrih-Aknin *(France)* & Nils Erik Gilhus *(Norway)*

10:30-11:00 **Etiological and pathophysiological mechanism in myasthenia gravis**
Rozen Le Panse (Sorbonne Université, INSERM, AIM, France)

11:00-11:15 **294-Higher Th17 and Th1 cells in thymoma may affect development of thymoma-associated-myasthenia gravis inducing Tfh cells**
Nerve Cebi (Istanbul University, Turkey)

11:15-11:30 **36-Functional monovalency amplifies the pathogenicity of anti-MuSK IgG4 in myasthenia gravis**
Dana Vergoossen (Leiden University Medical Center, the Netherlands)

11:30-11:45 **91-Conditioned Mesenchymal Stromal Cells as tools for immunomodulation in myasthenia Gravis**
Alexandra Bayer Wildberger (Sorbonne Université, INSERM, France)

11:45-12:00 **341-Role of HIF-1 and Treg/Th17 Imbalance in the Thymus in myasthenia Gravis**
Ilayda Altinonder (Istanbul University, Turkey)

12:00-12:15 **129-Monoclonal antibody anti -IL-23 ameliorates neuromuscular defects in myasthenia Gravis mouse model**
Nadine Dragin (Sorbonne Université, France)

12:15-12:30 **247-Endogenous nucleic acids induce an organ-specific type I interferon signature in myasthenia Gravis thymus**
Cloé Payet (Sorbonne University, France)

Sponsored Symposia

Alexion | A reverse-translational story of complement in neuroinflammatory diseases
Chair: Professor Heinz Wiendl *(Germany)*

12:45-12:50 **Welcome and introductions**
Chair: Professor Heinz Wiendl (Germany)

12:50-13:05 **Complement pathway: from an ally to an enemy**
Professor Seppo Meri (Finland)

13:05-13:20 **The role of complement in the pathophysiology of infl ammatory diseases: the enemy attacks**
Professor Heinz Wiendl (Germany)

13:20-13:35 **Terminal complement inhibition in gMG and NMOSD:* an update**
Professor Sean Pittock (USA)

13:35-13:45 **Questions and close**
All

Parallel Symposia

Parallel VII | Neurons as active players in neuroinflammation
Chairs: Angela Vincent *(UK)* & Lesley Probert *(Greece)*

14:00-14:25 **Neuronal structure and function in the inflamed gray matter**
Martin Kerschensteiner (Ludwig-Maximilians University Munich, Germany)

14:25-14:40 **126-Vesicle-mediated transfer of ribosomes from glia to axons during neuroinflammation**
Christina Vogelaar (University Medical Center Mainz, Germany)

14:40-15:05 **Adaptive and innate immune mechanisms: context dependent bystanders or active players in neurodegeneration**
Hans Lassmann (Medical University of Vienna, Austria)

15:05-15:20 **81-Hypothalamic AgRP neurons control lymphoid hematopoiesis in bone marrow and Treg generation in thymus**
Tiziana Vigo (IRCCS Ospedale Policlinico San Martino, Italy)

15:20-15:45 **CD4 T cells contribute to neurodegeneration in Lewy body dementia**
David Gate (Northwestern University, USA)

15:45-16:00 **196-Cytokine-induced DNA breaks, a new player in chronic inflammation-induced behavioral impairment?**
Elsa Suberbielle (University of Toulouse III, France)

Parallel VIII | Peripheral nervous system: a central target of neuroinflammation
Chairs: Jean-Christophe Antoine *(France)* & Sharosh Irani *(UK)*

14:00-14:25 **Glial-axonal interdependency at the node of Ranvier in autoimmune injury**
Hugh Willison (South Glasgow Hospitals University, UK)

14:25-14:40 **263-In vitro anti-glycolipid antibody production by Guillain-Barré syndrome patients' derived B cells**
Ruth Huizinga (Erasmus MC, University Medical Center, the Netherlands)

14:40-15:05 **Nodo-paranodopathies: how autoantibody valency affects conduction in peripheral nerves**
Jérôme Devaux (Hospital Saint Eloi, France)

15:05-15:20 **44-Autoreactive T cells in Guillain-Barré syndrome**
Daniela Latorre (ETH Zurich, Switzerland)

15:20-15:45 **Nociceptor Neuron regulation of barrier immunity and host defense**
Isaac Chiu (Harvard Medical School, USA)

15:45-16:00 **240-Utilizing Epigenetics to Understand Mechanisms Underlying Primary Progressive Multiple Sclerosis**
Majid Pahlevan Kakhki (Karolinska Institutet, Sweden)

Parallel IX | Gut microbiota and CNS inflammation
Chairs: Jennifer Gommerman *(Canada)* & Sergio Baranzini *(USA)*

14:00-14:25 **How the microbiota regulates susceptibility to CNS autoimmunity**
Ari Waisman (University Medical Center of the Johannes Gutenberg University, Germany)

14:25-14:40 **113-Dysbiosis in the Salivary Microbiome as a Promising Biomarker for Early Detection of Multiple Sclerosis**
Daiki Takewaki (National Center of Neurology and Psychiatry, Japan)

14:40-15:05 **Myelin-specific T cell trafficking to the gut triggers neuroinflammation**
Caroline Pot (Lausanne University Hospital, Switzerland)

15:05-15:20 **135-Microbiota-derived factors modulate intestinal immune cell function via the aryl hydrocarbon receptor and influence stroke outcome**
Corinne Benakis (University Hospital Munich, Germany)

15:20-15:45 **Grey Matters – The ageing microbiome and neuroinflammation**
Jennifer Gommerman (University of Toronto, Canada)

15:45-16:00 **310-The respiratory symbionts Moraxella catarrhalis and Klebsiella pneumoniae promote pathogenicity in myelin-reactive Th17 cells**
Jenny Mannion (Trinity College Dublin, UK)

Plenary III

Plenary IV | Regeneration and neuroimmunomodulation

Chairs: Catherine Lubetzki *(France)* & Francisco Quintana *(USA)*

16:15-16:45 **Microglia roles in myelin health**
Veronique Miron (University of Edinburgh, UK)

17:15-17:45 **The central nervous system at risk – regulatory processes in health and disease**
Frauke Zipp (University Medical Center of the Johannes Gutenberg, Germany)

Keynote Lecture

The Rita Levi-Montalcini Neurobiology Lecture

Chair: Michal Schwartz *(Israel)*

18:00-19:00 **Neurovascular Interactions: Mechanisms, Imaging, Therapeutics**
Katerina Akassoglou (University of California, USA)

19:00-21:00 **Poster Session**

NOVEMBER 12 | 2021

Workshops

Workshop XIII | Immunoregulation of neuroinflammation

Chairs: Sachiko Miyake *(Japan)* & Roland Liblau *(France)*

10:30-11:00 **Myositoxicity: a dark side of The Checkpoint Immunotherapy Revolution**
Yves Allenbach (Pitié Salpetriere University Hospital, France)

11:00-11:15 **365-MCAM involvement in regulatory T cell migration across the blood brain barrier: implications for multiple sclerosis**
Stephanie Zandee (CRCHUM, UdeM, Montréa,l Canada

11:15-11:30 **96-Investigation of the opsonizing capacity of patient-derived anti-MOG antibodies**
Marie Freier (University Medical Center, Georg August University Göttingen, Germany)

11:30-11:45 **297-Flanking residues of a self-dominant peptide harnesses immunity via determining the stability of antigen-specific effector Tregs**
Youwei Lin (National Institute of Neuroscience and National Center Hospital, Japan)

11:45-12:00 **315-Impaired Treg suppressive function in MS is correlated with an age- and treatment-dependent altered Treg dynamics**
Tiziana Lorenzini (University Hospital Zürich, Switzerland)

12:00-12:15 **348-Selective deletion of IDO1+ cDC1 worsened CNS inflammation in an experimental model of multiple sclerosis**
Giulia Scalisi (University of Perugia, Italy)

12:15-12:30 **229-Regulatory B cells ameliorate chronic CNS inflammation in an interleukin-10-dependent manner**
Silke Häusser-Kinzel (University Medical Center, Göttingen, Germany)

Workshop XIV | Diversity of brain myeloid cells

Chairs: Melanie Greter *(Switzerland)* & Trevor Owens *(Denmark)*

10:30-10:45 **16-Migration and functional polarization of monocyte derived cells across the central nervous system barriers during neuroinflammation**
Daniela C. Ivan (University of Bern Switzerland)

10:45-11:00 **274-Oxysterol-production by brain endothelial cells suppresses Myeloid-Derived Suppressor Cells and promotes experimental autoimmune encephalomyelitis**
Florian Ruiz (Lausanne University Hospital and University of Lausanne, Switzerland)

11:00-11:15 **268-Neutrophil plasticity in neonatal hypoxic-ischemic brain injury**
Josephine Herz (University Hospital Essen, Germany)

11:15-11:30 **237-Microglia heterogeneity in different regions of the healthy mouse central nervous system**
Falezeh Etebar (Queensland University of Technology, Australia)

11:30-11:45 **350-IL-15 expression is distinctly modulated on myeloid cells by inflammatory factors in MS**
Negar Farzam-kia (University of Montreal, Canada)

11:45-12:00 **147-Exploring the transcriptomic diversity of live human microglia in aging, neurodegeneration, and neuroinflammation**
John Tuddenham (Columbia University Irving Medical Center, USA)

Workshop XV | Metabolism in Neuroimmunology

Chairs: Anne Dejean *(France)* & Benoit Salomon *(France)*

10:30-11:00 **Targeting the metabolism of T cells for the treatment of autoimmune disease**
Luciana Berod (University Medical Center of the Johannes Gutenberg University Mainz, Germany)

11:00-11:15 **10-iPSC-derived astrocytes from patients with Multiple Sclerosis show metabolic alterations**
Bruno Ghirotto (University of São Paulo, Brazil)

11:15-11:30 **24-Dissecting the role of IGF1R in neuroinflammation: a parallel focus on oligodendrocytes and myeloid cells**
Giuseppe Locatelli (University of Bern, Switzerland)

11:30-11:45 **182-Regulation of human oligodendrocytes process extension by the integrated stress response**
Florian Pernin (McGill University, Canada)

11:45-12:00 **233-Eomes regulates mitochondrial function and promotes survival of pathogenic CD4+ T cells during CNS autoimmunity**
Emeline Joulia (Inserm Umr1291, Cnrs Umr5051, Ut3, France)

12:00-12:15 **68-Alterations in adipokine levels in MS patient cohort project Y**
Merel Rijnsburger (Amsterdam UMC, MS Center Amsterdam, the Netherlands)

12:15-12:30 **146-Metabolic regulation of MS specific neural stem cells**
Rosana-Bristena Ionescu (University of Cambridge, UK)

Workshop XVI | Tissue-resident T cells in CNS inflammation

Chairs: Doron Merkler *(Switzerland)* & Hans Lassmann *(Austria)*

10:30-11:00 **Tissue resident memory T cells in multiple sclerosis**
Joost Smolders (University Medical Center Rotterdam, the Netherlands)

11:00-11:15 **18-CD8 T cells target enteric neurons in patients with gastrointestinal dysmotility**
Anna Brunn (University of Cologne, Germany)

11:15-11:30 **215-Tissue-resident memory CD8+ T cells drive compartmentalized and chronic autoimmune damage against CNS neurons**
David Frieser (Toulouse Institute for infectious and inflammatory diseases (Infinity), France)

11:30-11:45 **309-Regulatory T cells counteract CNS neuroinflammation via TGF-β signalling in CNS myeloid cells**
Stefan Bittner (University Medical Center of the Johannes Gutenberg University Mainz, Germany)

11:45-12:00 **319-Cytotoxic-like Eomes+ Th cells in secondary progressive multiple sclerosis**
Ben Raveney (National Institute of Neuroscience, NCNP, Japan)

12:00-12:15 **117-Activated Eomes-Tfh1 cells infiltrate the cerebrospinal fluid in early Multiple Sclerosis**
Marion Mandon (Univ Rennes, France)

12:15-12:30 **292-Contact-dependent granzyme B-mediated cytotoxicity of Th17 cells towards human oligodendrocytes**
Hélène Jamann (Université de Montréal, Montreal, QC, Canada, Canada)

Panel Discussion

Immunotherpay in MS: 8 key questions

Chairs: Giancarlo Comi *(Italy)* & Roland Liblau *(France)*

12:40-12:48 **MS one or several diseases**
Catherine Lubetzki (Sorbonne University, France)

12:48-12:56 **Is anti MOG a disease or one of the many variants of MS?**
Kazuo Fujihara (Southern TOHOKU Research Institute for Neuroscience, Japan)

12:56-13:04 **Is there any useful serum or CSF biomarker to detect and predict inflammatory activity?**
Hans Peter Hartung (Ruhr-Universität Bochum, Germany)

13:04-13:12 **Immunotherapy of MS: induction or escalation?**
Gilles Edan (University Hospital of Rennes, France)

13:12-13:20 **Is there a role for cellulary therapy?**
Frauke Zipp (University Medical Center of the Johannes Gutenberg, Germany)

13:20-13:28 **Can we fully block neuroinflammation in MS?**
Howard Weiner (Brigham and Women's Hospital, USA)

13:28-13:36 **Which combination immunotherapy can we forsee for MS?**
Patrick Vermersch (University Hospital of Lille, France)

Keynote Lecture

The Dale McFarlin Lecture

Chair: Tomas Olsson *(Sweden)*

14:00-15:00 **Translational reserach towards personalized medicine for MS and NMOSD**
Takashi Yamamura (National Institute of Neuroscience, NCNP, Japan)

Parallel Symposia

Parallel X | Drainage and immunosurveillance of the CNS

Chairs: Gabriela Constantin *(Italy)* & Alexandre Prat *(Canada)*

15:15-15:30 **153-Pericytes play a protective role during neuroinflammation**
Dila Atak (Koç University, Istanbul, Turkey)

15:30-15:55 **Breaking (Bad) Brain Barriers Beliefs**
Jonathan Kipnis (Washington University in St. Louis, School of Medicine, USA)

15:55-16:10 **169-The role of the meningeal layers in CNS autoimmunity**
Arianna Merlini (University Medical Center, Göttingen, Germany)

16:10-16:35 **Harnessing brain T cells to modulate neurodegeneration**
Adrian Liston (Babraham Institute, UK)

16:35-16:50 **322-Migration across the blood-brain barrier affects mTOR signalling in regulatory T cells**
Paulien Baeten (Hasselt University, Belgium)

16:50-17:15 **Oligodendrocyte-derived extracellular vesicles as antigen-specific therapy for CNS inflammatory demyelination**
Abdolmohamad Rostami (Thomas Jefferson University, USA)

17:15-17:30 **166-Multiple sclerosis and circadian rhythm: a systematic review**
Francisco Lima-Neto (State University of Montes Claros, Brazil)

Parallel XI | Multi-omics in Neuroimmunology
Chairs: Naomi Habib *(Israel)* & Burkhard Becher *(Switzerland)*

15:15-15:30 **82-Transcriptome guided optimization of in vitro culture conditions for adult primary microglia**
Raissa Timmerman (Biomedical Primate Research Centre, the Netherlands)

15:30-15:55 **Single Cell Analysis of T cells in the CNS**
David Hafler (Yale School of Medicine, USA)

15:55-16:10 **262-Investigating B cells and their depletion in relapsing-remitting Multiple Sclerosis using DNA methylation patterns**
Ewoud Ewing (Karolinska Institutet, Sweden)

16:10-16:35 **Integrating genome, metagenome and data science to understand MS**
Sergio Baranzini (University of California San Francisco, USA)

16:35-16:50 **326-Transcriptomic characterization of CSF reveals T and Myeloid cells as early players during MS disease onset**
Hanane Touil (Columbia University Irving Medical Center, USA)

16:50-17:15 **Applied epigenomics: insights into the pathogenesis of Multiple Sclerosis**
Maja Jagodic (Karolinska Institutet, Sweden)

17:15-17:30 **368-Large-scale phosphoproteomic mapping of RTKs reveals dynamic architecture and microglia expression in blunt TBI**
Rida Rehman (Ulm University, Germany)

Parallel XII | Immunopsychiatry: is it a nascent field?
Chairs: Roberto Furlan *(Italy)* & Luc Vallieres *(Canada)*

15:15-15:40 **Towards a clinical immunological diagnosis of psychiatric disorders**
Hemmo A. Drexhage (University Medical Center Rotterdam, the Netherlands)

15:40-15:55 **33-Encephalitis with Autoantibodies against the Glutamate Kainate Receptor (GluK2)**
Jon Landa (Universitat de Barcelona, Spain)

15:55-16:20 **Modulatory roles of the immune system in shaping animal behaviors**
Jun Huh (Evergrande Center for Immunologic Diseases, USA)

16:20-16:35 **80-Deviated B cell receptor repertoire in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome**
Wakiro Sato (National Center of Neurology and Psychiatry, Multiple Sclerosis Center, Japan)

16:35-17:00 **Remembering immunity: brain representation of peripheral immune activity**
Asya Rolls (Technion, Israel Institute of Technology, Israel)

17:00-17:15 **279-Slow disease progression and clinical heterogeneity make anti-CASPR2 encephalitis a diagnostic challenge**
Jeanne Benoit (Hospices Civils de Lyon, Hôpital Neurologique, France)

We are Neuroimmunology

We are neuroimmunology
Chair: Fabienne Brilot *(Australia)*

17:45-18:00 **Introduction**
Fabienne Brilot (University of Sydney, Australia)

18:00-18:15 **How we study neuroimmunology through autoantibodies**
Sarosh Irani (Oxford Autoimmune Neurology Group (OANG), UK)

18:15-18:30 **Neuroimmune crosstalk in neuropathic pain**
Gila Moalem-Taylor (The University of New South Wales, Australia)

18:30-19:00 **Closing Remarks**
Roland Liblau, Sonia Berrih-Aknin, Amit Bar-Or, Francisco Quintana, Fabienne Brilot, Veit Rothhammer, Luc Vallieres

ORAL PRESENTATIONS - NOVEMBER 9, 2021

Parallel I - Dialog between astrocytes and microglia in neuroimmunology

59 - Astrocyte-Oligodendrocyte interaction drives central nervous system remyelination

Irene Molina-Gonzalez[1,2,3] - Zoeb Jiwaji[3,4] - Owen Dando[3,4] - Rebecca K. Holloway[1,2,3] - James A. Febery[4] - Jeffrey A. Johnson[5,6,7,8] - Jill H. Fowler[4] - Tanja Kuhlmann[9] - Anna Williams[2,10] - Siddharthan Chandran[2,3,4] - Giles Hardingham[2,3,4] - Veronique E. Miron[1,2,3]

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Failed regeneration of myelin following central nervous system damage contributes to axon dysfunction and steady clinical decline, for which there is an unmet therapeutic demand. However, the cellular and molecular mechanisms underpinning remyelination failure are unclear. Complementing our previous work uncovering the importance of microglia in supporting remyelination, here we identify astrocytes as a key cell type regulating remyelination and reveal a novel treatment strategy targeting dysregulated astrocytic pathways to restore remyelination efficiency. Sequencing of astrocyte translatomes during successful remyelination revealed Nrf2 pathway de-activation at the time of generation of new myelin-forming oligodendrocytes, coincident with upregulation of the cholesterol biosynthesis pathway. Preventing deactivation of the Nrf2 pathway in astrocytes via transgenic overexpression was sufficient to reduce cholesterol biosynthesis pathway activation, leading to oligodendrocyte death and poor remyelination. Treating transgenic mice with a cholesterol efflux stimulator or an existing therapy which inhibits Nrf2 normalized astrocyte responses, restored oligodendrocyte survival, and

promoted remyelination. In vitro experiments revealed that cholesterol is transferred directly from astrocytes to oligodendrocytes to regulate their survival, and that this is regulated by astrocytic Nrf2 activation. Chronic human brain lesions with poor remyelination potential and increased oligodendrocyte death were enriched for an astrocyte cluster with increased Nrf2 activation and decreased cholesterol pathway activation. The timing of astrocytic Nrf2 and cholesterol pathway regulation during the course of remyelination suggests potential crosstalk with microglia to offset pro-inflammatory responses and complement their functions. In summary, we identify astrocytes as key regulators of remyelination and identify putative therapeutic strategies to restore their regenerative functions following CNS damage.

249 - APOE4 IMPAIRS MICROGLIA RESPONSE TO NEURODEGENERATION IN ALZHEIMER'S DISEASE

Neta Rosenzweig[1]* - Zhuoran Yin[1]* - Wesley Nogueira Brandao[1] - Oleg Butovsky[1,2]

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[2]Evergrande Center for Immunologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States.

** These authors equally contributed to this study.*

APOE4 is the strongest genetic risk factor for late-onset Alzheimer's disease (AD). We previously identified that APOE signaling governs the transcriptional regulation from homeostatic to neurodegenerative microglia (MGnD). However, the underlying mechanism for APOE4-mediated microglial dysregulation and its contribution to increased risk for developing AD is unknown. Here we aimed to dissect the impact of microglial APOE4 on AD pathology, using CX3CR1-CRE^{ERT2} mice crossed to APOE-KI (E3 and E4)$^{fl/fl}$:APP/PS1. We detect reduced numbers of amyloid-beta plaque associated Clec7a^{+} MGnD-microglia in APP/PS1:APOE4 KI mice and increased plaque load compared with APP/PS1:APOE3-KI mice. Moreover, APOE4-KI mice challenged with labeled apoptotic neurons, failed to respond to acute neurodegeneration, depicted by reduced numbers of phagocytic MGnD-microglia at injection site compared with APOE3-KI mice. Importantly, conditional genetic deletion of APOE4 in microglia restored MGnD-microglial response to acute neurodegeneration and in APP/PS1 mice. scRNAseq analysis showed increased proportion of MGnD-microglia in APP/PS1:APOE4 conditional KO mice, associated with reduced plaque pathology and increased astrocytic recruitment towards plaques. Furthermore, we found impaired induction of MGnD signature in AD brains of APOE4 carriers. Our findings show that APOE4 is a negative regulator of MGnD-microglia in AD, and that its genetic deletion restores the induction of MGnD signature and their crosstalk with astrocytes, associated with reduction in plaque pathology. Taken together, these findings identify a cell-intrinsic role of APOE4 in the induction of dysfunctional MGnD microglia and their impaired response to neurodegeneration, which may provide new molecular targets to modulate and restore functional microglia in AD.

308 - Selective inhibition of soluble TNF promotes beneficial neuroinflammatory responses and remyelination in the cortical grey matter

Athena Boutou[1*] - Ilias Roufagalas[1*] - Katerina Politopoulou[1] - Ray J Tesi[2] - Chris J Barnum[2] - Vasiliki Kyrargyri[1] - Lesley Probert[1]

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**These authors contributed equally.*

Neuroinflammation is important for both demyelination and remyelination of central nervous system in diseases such as multiple sclerosis. TNF is critically involved in both processes, with transmembrane TNF mediating and soluble TNF (solTNF) inhibiting remyelination. To further study the beneficial effects of solTNF inhibition in remyelination, specifically in cortical grey matter, we used a cuprizone (CPZ) demyelination model in C57BL/6 mice treated by s.c administration of XPro1595, a selective inhibitor of solTNF that crosses the blood-brain barrier. We performed immunofluorescence and high resolution 3D confocal microscopy analyses in the cortex of XPro1595-treated and control animals using sagittal slices of whole mouse brains taken at two distinct pathological time points, week 3 (microglia and astrocyte responses, start of demyelination) and week 5 (maximum demyelination), using markers for myelinating oligodendrocytes (MBP), microglia (Iba1) and reactive

astrocytes (GFAP). At week 3, XPro1595 mice had significantly increased numbers of microglia compared to controls in the deeper cortical layers, with a concomitant increase in myelin phagocytosis, as defined by colocalization of Iba1 and MBP. At the same timepoint, GFAP expression levels were also significantly increased in XPro1595 mice compared to controls, suggesting a possible correlation of microglia and astrocyte activation as an early response to CPZ demyelination in the grey matter. At week 5, XPro1595 mice had significantly higher levels of MBP in the deeper cortex compared to controls, indicating earlier remyelination. This was accompanied by higher phagocytic activity of microglia, although numbers of microglia were similar between the two groups at this timepoint. In agreement with the in vivo results, XPro1595 treatment of macrophages and microglia enhanced myelin phagocytosis in vitro. Our finding that XPro1595 promotes early microglia and astrocyte responses to demyelination in the cortical grey matter, aligns with our previous findings in EAE, where XPro1595 treatment reduces clinical disease while enhancing the expression of neuroinflammatory markers at disease onset. Taken together, our results suggest that inhibition of solTNF allows beneficial neuroinflammatory responses of microglia and astrocytes to develop, resulting in efficient phagocytosis and clearance of myelin debris and earlier remyelination in the demyelinated cortical grey matter.

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Parallel II - Parasites and neuroinflammation

246 - Studying the mechanisms of neuroinflammation-induced cognitive alterations associated with Toxoplasma gondii infection

Marcy Belloy - Charlotte Paut - Benjamin Schmitt - Emilie Bassot - Amel Aida - Anna Salvioni - Elsa Suberbielle - Nicolas Blanchard*

Toulouse Institute for Infectious and Inflammatory Diseases, Infinity, University of Toulouse, CNRS, Inserm, UPS, Toulouse, France

The intracellular parasite *Toxoplasma gondii* is a foodborne pathogen with a worldwide prevalence of around 30%. In immunocompetent individuals, initial infection is mainly asymptomatic. Yet, persisting parasites establish latent infection within cysts that are located in retinal, muscular and neuronal cells. While some individuals likely manage to clear the parasite, *T. gondii* may reactivate and cause severe encephalitis upon immunosuppression in others. *T. gondii* encephalitis is associated with uncontrolled parasite replication, leukocyte brain infiltration, and cerebral dysfunction. Moreover, increasing evidence indicate that behavioral and cognitive changes also occur during latency. In part due to the lack of standardized mouse models of the three human pathophysiological situations (clearance, latency and encephalitis), the contribution of neuroinflammatory processes in cognitive alterations, and the underlying molecular mechanisms, remain ill-defined. To address these questions, we have developed three models of type II *T. gondii* infection in C57BL/6 mice resulting in clearance, latency or encephalitis, respectively associated with resolved/low, moderate or high neuroinflammation. We have observed that mice with high neuroinflammation display major deficits in tests that evaluate risk-taking (Elevated Plus Maze and attraction to predator's urine test) and spatial memory (Barnes maze and Object Location Task). Interestingly, the ability of encephalitic mice to discriminate a new object (Novel Object Recognition) remains intact, suggesting that hippocampal dysfunction could underlie the spatial memory defect. Mice with moderate neuroinflammation show only slight spatial learning alterations and cleared mice mostly behave as uninfected control mice. Brain cytokine assessments have suggested candidate molecules that may be involved in hippocampal neuronal alterations. Studies interfering with the receptor and/or biological activities of select cytokines are underway to address their roles in *T. gondii*-associated cognitive perturbations. Our results will help dissect the neuro-immune cross-talk that regulates neuronal dysfunction during this prevalent chronic brain infection. In the absence of an effective drug eliminating cysts, these molecular pathways may suggest therapeutic targets to minimize the impact of latent brain infections on neuropsychological health.

108 - Regional cyst localisation and innate immune activation of the retina and brain in murine toxoplasmosis.

Dana Lee[1] - Paul G. McMenamin[2] - Chris Tonkin[3] - Samantha J. Dando[1,*]

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[2]*Department of Anatomy and Developmental Biology, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia*
[3]*Infectious Diseases and Immune Defence Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia*

Toxoplasma gondii, a ubiquitous neurotropic parasite, is a leading cause of posterior uveitis and encephalitis. We investigated whether *T. gondii* preferentially localised in different regions of the retina and brain; and whether microglia are activated in a regionally-dependent manner in a mouse model of acute toxoplasmosis. C57BL/6J mice were inoculated with *T. gondii* Prugniaud-tdTomato tachyzoites (low dose [$5x10^3$] n=5; high dose [$1x10^4$] n=5) or PBS control (n=5). *In vivo* retinal imaging was performed every 7 days to monitor clinical disease and initial parasite invasion. Eyes and brains were collected between days 7-28 post-infection. Retinal wholemounts and brain sections were processed for immunofluorescence staining (Tmem119 and MHC class II antibodies) and confocal microscopy. *T. gondii* cyst burden, microglia density, field area and MHC class II expression were quantified using FIJI. Clinical disease and tdTomato+ *T. gondii* parasites were observed in infected mice from day 14 using *in vivo* retinal imaging. Examination of tissues revealed that cysts were detected only in mice infected with high-dose *T. gondii.* In the retina, cysts exclusively localised in the ganglion cell layer (GCL) and inner plexiform layer (IPL); whereas cysts preferentially localised in the cortex of the brain. Despite regional specificity of *T. gondii* cysts, Tmem119+ microglia activation was widespread, evidenced by MHC class II upregulation and altered microglia phenotype in all examined CNS regions (retina: GCL, IPL, outer plexiform layer; brain: cortex, olfactory bulbs, hippocampus, cerebellum) compared to controls ($p<0.05$). *T. gondii* infection also resulted in increased Tmem119-/MHC class II+ cells in the retinal GCL, and upregulation of MHC class II on brain vasculature. These findings demonstrate regional tropism for *T. gondii* cyst formation and suggest robust microglia activation occurs during acute toxoplasmosis. Understanding the role of innate immunity in controlling *T. gondii* replication in the CNS may lead to novel immunotherapeutic targets for toxoplasmosis.

Parallel III | Antibody-mediated diseases: new targets and new mechanisms

191 - Immunogenetics in autoimmune encephalitis and related disorders

Sergio Muñiz-Castrillo, MD[1,2] - Alberto Vogrig, MD[1,2] - Anne-Laurie Pinto, MSc[1,2] - Vicente Peris-Sempere, MSc[3] - Aditya Ambati, PhD[3] - Nicolás Lundahl Ciano-Petersen, MD[1,2] - Antonio Farina, MD[1,2] - Valérie Dubois, MD[4] - Bastien Joubert, MD PhD[1,2] - Emmanuel Mignot, MD PhD[3] - Jérôme Honnorat, MD PhD[1,2]
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[2]*SynatAc Team, Institute NeuroMyoGène, INSERM U1217/CNRS UMR 5310, Université de Lyon, Université Claude Bernard Lyon 1, Lyon, France.*
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[4]*HLA Laboratory, French Blood Service, EFS Auvergne-Rhône-Alpes, Lyon, France.*

Although the discovery of neuroglial antibodies has allowed for more accurate diagnoses and better understanding of some of the pathogenic mechanisms of autoimmune encephalitis (AE) and related disorders, the exact processes leading to the immune tolerance breakdown are still unknown, but are likely the result of a complex interaction between environmental triggers and genetic traits. The human leukocyte antigen (HLA) is the main genetic factor related to autoimmunity, but only a few studies have so far been carried out in AE. Across different studies, we performed HLA genotyping in patients with AE and antibodies against glutamic acid decarboxylase 65 (GAD65, n=32), contactin-associated protein-like 2 (CASPR2, n=30), leucine-rich glioma-inactivated 1 (LGI1, n=72), adenylate kinase 5 (AK5, n=11), Yo (n=54), delta/notch-like epidermal growth factor-related receptor (DNER, n=20), and glial fibrillary acidic protein (GFAP, n=26), also taking into account their clinical phenotypes, oncological associations, and immunological characteristics. We found three different scenarios in the association between HLA and autoimmune encephalitis and related disorders. First, non-paraneoplastic limbic encephalitis with antibodies of predominantly IgG4 isotype (LGI1, CASPR2) showed strong and specific

associations with class II alleles (DRB1*07:01 and DRB1 *11:01, respectively). Second, non-paraneoplastic disorders with antibodies of predominantly IgG1 subclass or likely to be T-cell mediated were either no associated with HLA (GFAP, CASPR2-neuromyotonia) or weakly (GAD65, AK5) with the ancestral haplotype 8.1, which is related to a general predisposition to autoimmunity. Moreover, in patients with GAD65 antibodies, family history of autoimmunity was observed in 68% of 65 patients included in an interview-based study, further supporting a non-HLA dependent genetic predisposition to autoimmunity. Finally, paraneoplastic neurological syndromes (CASPR2-Morvan syndrome, Yo, DNER) showed no HLA association. Altogether, our findings reflect a variable involvement of HLA in the pathophysiology of autoimmune encephalitis and related disorders, including a likely very important role in limbic encephalitis with LGI1 or CASPR2 antibodies, the presence of a general HLA predisposition to autoimmunity along with other possible non-HLA genes in IgG1/T-cell mediated disorders, and a greater relevance of the own characteristics of the tumors in paraneoplastic neurological syndromes.

259 - The role of SWAP-70 and FCRL2 in the pathophysiology of multiple sclerosis and their value as a biomarker

Elif Sanli[1] - Emine Sekerdag Yilmaz[2] - Ece Erdag[1] - Ece Akbayir[1] - Canan Ulusoy[1] - Gizem Koral[1] - Yasemin Gursoy Ozdemir[2] - Recai Turkoglu[3] - Cem Ismail Kucukali[1] - Vuslat Yilmaz[1] - Erdem Tuzun[1]

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Objective: There are many studies on the detection of biomarkers that predict clinical prognosis in patients with multiple sclerosis (MS). In recent years, there are some evidence that B cells play an important role in the pathogenesis of MS and the disease related disability. In this study, we aimed to detect peripheral B cell-derived molecules that may play a role in MS pathogenesis and clinical progression and also to determine the value of these molecules as biomarkers.
Methods: Twelve benign MS, 18 non-benign MS and 30 healthy controls were included into the study. Microarray and bioinformatics analyzes were performed using B cell mRNA samples isolated from peripheral blood of the subjects. Expressions of molecules that differed significantly between MS groups were verified by real-time PCR. Correlations with clinical parameters were tested with the Pearson test. Selected genes were silenced with shRNA in the experimental autoimmune encephalomyelitis (EAE) model induced by proteolipid protein immunization in the C57BL/6 mouse, and the clinical parameters of the mice were evaluated.
Results: In the microarray study, FCRL2 and SWAP-70 were identified as B cell-derived molecules that showed significant expression differences in benign MS cases. Peripheral blood expression levels of these genes were found to be suppressed in MS cases compared to healthy controls. FCRL2 expression level was higher in benign MS cases than in non-benign MS cases. A negative correlation was found between SWAP-70 expression level and EDSS scores (p=0.003; R=0.422). In gene silencing studies, it was shown that suppression of the FCRL2 gene prevented the development of paralysis and weight loss in the EAE model, while suppression of the SWAP-70 gene increased the severity of the disease.
Conclusion: Our findings suggested that FCRL2 has an effect on the development of MS, and SWAP-70 may be a autoimmunity associated protective factor preventing the development of MS. Additionaly, SWAP-70 may be used as a clinical progression biomarker in MS. Differentiated B cell functions may alter disease progression in MS patients.
Keywords: multiple sclerosis, SWAP70, FCRL2, biomarker

131 - Clinical and laboratory features in anti-NF155 autoimmune nodopathy

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Objective: To study the clinical and laboratory features of anti-neurofascin-155 (NF155) positive autoimmune nodopathy (AN).
Methods: Patients with anti-NF155 antibodies detected on routine immunological testing were included. Clinical characteristics, treatment response and functional scales (mRS and I-RODS) were retrospectively collected at baseline and at follow-up. Autoantibody and neurofilament light (NfL) chain levels were analyzed at baseline and at follow-up.
Results: Forty NF155+ AN patients were included. Mean age at onset was 42.4 years. Patients presented with a progressive (75%), sensory-motor (87.5%), and symmetric distal-predominant weakness in upper (97.2%) and lower extremities (94.5%), with tremor and ataxia (75%). Patients received a median of 3 [2-4] different treatments in 46 months of median follow-up. Response to IV immunoglobulin (IVIg) (86.8%) or steroids (72.2%) was poor in most patients, while 77.3% responded to rituximab. HLA-DRB1*15 was detected in 91.3% of patients. IgG4 anti-NF155 antibodies were predominant in all patients; anti-NF155 titers correlated with mRS within the same patient (r=0.41, p=0.004). sNfL levels were higher in anti-NF155+ AN than in healthy controls (36.47pg/mL vs 7.56pg/mL, p<0.001) and correlated with anti-NF155 titers (r=0.43, p=0.001), with I-RODS at baseline (r=-0.88, p<0.001) and with maximum I-RODS achieved (r=-0.58, p=0.01). Anti-NF155 titers and sNfL levels decreased in all rituximab-treated patients.

Conclusions: Anti-NF155 AN presents a distinct clinical profile and good response to rituximab. Autoantibody titers and sNfL are useful to monitor disease status in these patients. The use of untagged-NF155 plasmids minimizes detection of false anti-NF155+ cases.

Workshop I | Innate and Adaptive Immunity in AD, Parkinson and ALS

57 - CYP46A1 in the choroid plexus: an unexpected safeguard of brain function lost in Alzheimer's disease

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Type-I interferon (IFN-I), a well-conserved anti-viral response, also protects the brain from autoimmune attacks. Yet, chronic elevation of IFN-I at the choroid plexus (CP) in aging and Alzheimer's disease (AD) debilitates brain function. Using postmortem human AD samples and a mouse model of amyloidosis (5xFAD), we demonstrate that elevation of IFN-I signaling at the CP is accompanied by reduction of CYP46A1, found here to be constitutively expressed by the CP. Overexpression of *Cyp46a1* at the CP in 5xFAD mice downregulated local IFN-I signaling and attenuated cognitive loss and amyloidosis. Furthermore, CP *Cyp46a1* could be induced, via IFNγ-SP1 regulatory axis, by a treatment mobilizing systemic immunity, previously shown to modify AD in mouse models. Transcriptomic analysis of primary mouse CP cultures exposed to the enzymatic product of CYP46A1, 24-OH, revealed suppression of IFN-I-signaling. Our results suggest CP CYP46A1 as an unexpected safeguard against chronic anti-viral-like responses that is amenable to rescue when lost.

34 - Effects of IgLON5 antibodies on neuronal cytoskeleton: A link between autoimmunity and neurodegeneration.

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Objective: To describe the IgLON5 antibody (IgLON5-ab) effects on the cytoskeleton of rat hippocampal neurons and to quantify the Neurofilament Light-Chain (NfL) levels in patients' CSF.
Methods: Primary cultures of rat hippocampal neurons were exposed during 3 weeks to purified IgG from sera of 3 patients with anti-IgLON5 disease, two with predominat IgG4 and one with predominant IgG1 IgLON5-ab subclass, or control IgG (from healthy donors or patients with other autoantibodies). The effects were visualized by immunofluorescence and confocal microscopy. Specificity was confirmed by immunoabsorption of patients' samples with HEK293 cells expressing IgLON5. Concentration of NfL in the CSF of 19 anti-IgLON5 patients was quantified by an enzyme-linked immunosorbent assay (ELISA) kit (Uman Diagnostics, Tvistevägen, Sweden).
Results: IgLON5-IgG caused, along with the expected decrease of IgLON5 clusters on the cell surface, a disorganization of the neurofilament architecture in cultures of neurons. Dystrophic neurites, axonal swellings, bulb-like structures and early termination of the dendritic processes were observed. Treatments with Patient 1 IgG resulted in a median of 307 lesions/neuron (range 81-774; 95% confidence interval [CI]:322-390), Patient 2 IgG who had predominant IgG1 IgLON5-ab, 571 lesions/neuron (range 180-908; 95% CI: 511-628) and Patient 3 IgG; 553 lesions/neuron (range 338-764; 95% CI: 510-598) vs. treatments with IgG from healthy donors that showed 63 lesions/neuron (range 7-200; 95% CI:62-84) or with other specificities, 55 lesions/neuron (range 33-

108; 95% CI:33-89) (p<0.0001). IgLON5-ab effects were not observed in patients' samples immunoabsorbed with IgLON5. Neurons cultured under hypoxic conditions resulted in a similar number of lesions when compared with the IgG of patients (305 lesions/ neuron; range: 252-434: 95% CI: 282-371). Concentration of NfL levels in the CSF of patients with anti-IgLON5 disease had a mean of 1401 pg/mL (range 249-9438pg/mL; CI: 437-2365pg/mL) compared with control CSF (mean 404 pg/mL, range 105-728 pg/mL; 95% CI:322-390) (P=0.00009)
Conclusions: patients' IgLON5 antibodies specifically disrupt the cytoskeletal organization in cultured rat hippocampal neurons resulting in dystrophic neurites and axonal swellings. The increased concentration of NfL in patients' CSF suggests a disruption of the cytoskeleton. These findings establish a link between IgLON5 autoimmunity and neurodegeneration.

55 - Etiology-independent disease-associated oligodendrocytes in CNS pathologies

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Alzheimer's disease (AD) is a complex, heterogeneous neurodegenerative disease, perturbing neuronal and non-neuronal populations. Among the later, the fate of oligodendrocytes in this disease has been the least characterized. Using single-cell transcriptomics, we identified a cellular state of oligodendrocytes associated with AD, termed disease-associated oligodendrocytes (DOLs). DOLs appear in late-stage disease, accumulate along disease progression, and could be identified both in amyloidosis and tauopathy mouse models. Moreover, we showed that amyloid-beta (Aβ) alone was not sufficient to induce expression of DOL signature *in vitro*. Apart from AD, we observed cells with DOL signature in other neurodegenerative conditions and autoimmune inflammatory conditions, suggesting response of oligodendrocytes to severe deviation from homeostasis. Immunohistochemistry revealed the presence of DOL-like cells in postmortem AD patients' brains. Using quantitative spatial analysis, we found that such cells are present in the mouse cortex in areas of Aβ plaques, suggesting an association with sites of damage. Taken together, the present study characterizes an intrinsic oligodendrocyte response program universal across central nervous system pathologies.

287 - Disrupting the neuroimmune crosstalk in the spleen exacerbates cognitive loss in animal model of Alzheimer's disease

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Introduction: The immune system and the central nervous system (CNS) are each in direct contact with the other organs of the body, though their interconnection is only starting to be fully appreciated. During the crosstalk between the CNS and the immune system in peripheral lymphoid organs, neurotransmitters present in the local tissue environments influence innate and adaptive immune responses. Interestingly, recent evidence identified hypothalamic neurons as responsible for regulating splenic innervation. In Alzheimer's disease, the hypothalamic nuclei show a substantial decrease in neuronal populations, nevertheless, to our knowledge, the impact and consequences of an impairment in the neuroimmune crosstalk in AD was never addressed so far.
Methods: To test the role of splenic innervation in animal models of AD (5xFAD) and WT mice, we adapted a surgical denervation procedure treating splenic nerve plexuses with alcohol before they enter the spleen,

selectively ablating the innervation of the spleen without affecting other organs. Subsequently, we tested the cognitive performance of all mice, and we studied their immune landscape in the spleen and blood through high-dimensional single-cell mass cytometry.
Results: Loss of the sympathetic innervation of the spleen significantly accelerated cognitive loss in 5xFAD mice with no effect on WT mice. Furthermore, loss of cognition was associated with immune rearrangements which were more pronounced in 5xFAD compare to WT mice.
Conclusions: These results show that the innervation of peripheral organs plays a crucial role in the development of a proper immune response, which in turn is needed to limit CNS pathology and support brain functioning in the context of neurodegenerative disorders.

38 - Repurposing the anxiolytic drug buspirone to counteract inflammation in cellular and animal models of Parkinson's disease

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Considerable evidence suggests that blockade of the dopamine-3-receptor (D3R) is neuroprotective and reduces inflammation in models of Parkinson's disease (PD). However, to date there are no selective D3R antagonists in the market. Recently, computational analyses have demonstrated that buspirone, an FDA-approved anxiolytic drug with serotonin 1A (Htr1a) agonist activity, also functions as a potent D3R antagonist. To test if buspirone also elicited anti-inflammatory activities via D3R blockage *in vitro*, we generated stable $Drd3^{-/-}$ and $Htr1a^{-/-}$ BV-2 microglial cell lines using CRISPR-Cas9 technology and then tested the effects of buspirone after lipopolysaccharide (LPS) challenge. We found that buspirone counteracted LPS-induced NO release ($p<0.001$), IL-1β ($p<0.01$) and TNF-α ($p<0.0001$) gene expression in WT cells, whereas it exerted limited effects in $Drd3^{-/-}$ and $Htr1a^{-/-}$ microglia. To determine if buspirone elicited neuroprotective effects *in vivo*, C57BL/6 mice were treated with the PD-mimetic rotenone (10mg/kg rotenone i.p. × 21 days) also received daily injections of either 1, 3, or 10mg/kg buspirone for 21 days. Buspirone treatment successfully mitigated rotenone-induced deficits in locomotor and exploratory behaviours in the Open Field test. Additionally, we found that rotenone caused variable degrees of toxicity across the different brain regions examined (i.e. midbrain, striatum, prefrontal cortex, amygdala, hippocampus and spinal cord) and these effects were ameliorated by buspirone co-treatment. In the midbrain, buspirone successfully restored inflammation and oxidative stress to levels comparable to healthy mice, as shown by a decrease in CD11b ($p<0.001$), IL-1β ($p<0.0001$), SOD1 ($p<0.001$) and GFAP ($p<0.01$). The drug also prevented dopaminergic cell loss in the midbrain (TH expression, $p<0.0001$) and altered the expression of endogenous neurotrophic molecules such as the neuropeptides PACAP and VIP and the neurotrophic factors BDNF and ADNP. In summary, our findings indicate that buspirone attenuates microglial polarization after LPS challenge and can mitigate rotenone-induced neurotoxicity and inflammation *in vivo*.

Workshop II | Modeling neuroinflammation using IPSC, organoids and animal models

375 – Human induced pluripotent stem cells-derived neurons to study CNS-reactive autoantibodies in COVID-19-mediated neurological syndromes

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Neurological complications associated with COVID-19 are a growing concern as the second complication after acute respiratory syndrome. Moreover, neurological symptoms can develop and/or even persist after SARS-CoV-2 acute infection raising the hypothesis of a virus-induced autoimmunity possibly mediated by central nervous system (CNS)-reactive antibodies. Here, we developed a cell-based assay (CBA) to screen for the presence of CNS-specific antibodies in serum and cerebral spinal fluid (CSF) using CNS cells derived from human-induced pluripotent stem cells (hiPSC). Human iPSC-derived neurons were incubated with serum and CSF of 40 SARS-CoV-2+ patients suffering from mild to severe neurological symptoms. As controls, we included 177 patients with inflammatory neurological diseases (IND) and 46 patients with non-IND (NIND). IgG bound to CNS cells were detected using a combination of fluorescently-labelled antibodies. IgG-associated fluorescence intensity (FI) measure was automated using a fluorescence plate reader. Serum or CSF were defined as positive using a ROUT test with a FDR at 2% on quantified FI. Each CBA well was also observed by fluorescence microscopy. We identified antibodies recognizing hiPSC-derived neurons in 36/263 (13%) study patients: 19/238 (8 %) in the serum and 24/259 (9%) in the CSF including 7 positive in both serum/CSF. Among these 36 study patients, there were 3/40 (8%) SARS-CoV-2+ patients with severe neurological symptoms, 2/46 (4%) NIND and 31/177 (17.5%) IND including 3 patients who had CNS-reactive antibodies (Hu, VGKC, NMDAR) in routine laboratory. These results were further validated by fluorescence microscopy. In conclusion, the presence of neuron-specific Abs in COVID-19 patients with neurological complications does not seem to be frequent. By contrast, IND patients exhibited twice more frequently neuron-specific antibodies, including patients known to harbor previously recognized auto-antibodies, validating the sensitivity of our assay. Finally, only 4% of patients with NIND were positive, suggesting that this assay is specific. To conclude, we believe that this hiPSC-based assay has a great potential to identify new CNS antigens in both serum and CSF samples.

336 – Mapping of radiation-induced microglia activation in whole-brain mouse histological images

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Introduction: Patients who receive cranial irradiation may show delayed complications such as cognitive decline that reduce their quality of life. As the number of brain cancer long-term survivors increases, it is of paramount importance to predict and prevent late radiation-induced side effects. Clinical observations indicate that tissue at beam edge and the periventricular zone are especially vulnerable to radiation-induced brain injury. Chronic neuroinflammation caused by persistent activation of microglia, the brain-resident immune cells, is suggested as an underlying mechanism. The aim of this study was to spatially investigate the microglia response to radiation-induced injury in whole-brain slices of proton irradiated mice. Material and methods: Immunofluorescence images stained with Iba1 (microglia) and DAPI (nuclei) were analyzed using an algorithm developed in FIJI. Area and circularity were quantified for each microglia and used to calculate the M-score, a parameter describing the activation status. The developed algorithm was applied to an open-source dataset (https://rodare.hzdr.de/record/810) of fused multimodal medical and light microscopy images from brain tissue irradiated with different proton doses, which we acquired recently. Results: Fusion of the CT-based Monte-Carlo dose simulation to the histological data of an 80 Gy irradiated mouse brain showed a clear dose-dependent increase of microglia activation. Cells with a high M-score were located in the irradiated periventricular zones and the beam edge. Conclusion and outlook: The presented automated analysis of radiation-induced microglia activation enables spatial visualization of neuroinflammation across the mouse brain. Ongoing analyses focus on

the statistical correlation of parameters like M-score, proximity to ventricles, cell migration and dose on a single cell level.

185 - Intrinsic regulation of Th17 cell pathogenicity by IL-24

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Th17 cells were initially recognized for their role in host defence against fungal and extracellular bacterial pathogens. Besides their protective role, Th17 cells are also involved in autoimmune diseases like psoriasis, rheumatoid arthritis, and multiple sclerosis. The graded amounts of immune pathogenetic effects of Th17 cells have been attributed to their microenvironment. E.g. cytokines like IL-12 and IL-27 are known to regulate Th17 cell pathogenicity through induction of IL-10. In addition to such exogenous mediators, autocrine IL-17 has also been suggested to regulate expression of Th17 effector cytokines like GM-CSF through local action of IL-24. IL-24, a member of the IL-20 cytokine superfamily, usually signals through its cell surface receptors, i.e. IL-20Rα/IL-20Rβ and IL-22Rα1/IL-20Rβ. While it has been argued that the expression of IL-24 is a feature of non-pathogenic Th17 cells, its role in pathogenic Th17 cells is ambiguous. In the present study we have shown that IL-1β also can prompt pathogenic Th17 cells to produce high amounts of IL-24. Eventually, IL-24 production in Th17 cells segregated with their capacity to produce IL-10. The genetic ablation of *Il24* (or its siRNA-mediated knockdown) in Th17 cells led to a reduction in the frequencies of IL-10^{+} cells, and reconstitution of *Il24*$^{-/-}$ Th17 cells with non-secretable IL-24 rescued IL-10 levels, suggesting that an intracellular function of IL-24 was associated with IL-10 expression in Th17 cells. In support of this idea, *Il24*$^{-/-}$ T cells were hard-wired in their failure to produce IL-10 even in the presence of exogenous sources of IL-24, and deletion of the IL-24 receptor (*Il20rb*$^{-/-}$) did not recapitulate properties of *Il24*$^{-/-}$ Th17 cells. IL-24 interacted with Grim19, a component of complex I of the electron transport chain at the inner mitochondrial membrane, and contributed in recruiting STAT3 to the mitochondrial compartment, thus modulating the nuclear availability of STAT3. An increased severity of experimental autoimmune encephalomyelitis (EAE) in *Il24*$^{-/-}$ mice was consistent with the involvement of IL-24 in IL-10-mediated regulatory circuits *in vivo*. In summary, IL-24 is associated with the production of IL-10 in Th17 cells in a strictly cell intrinsic manner and drives an autoregulatory circuit through a non-canonical (intracellular) function of IL-24. It remains to be determined to which degree this regulation is dependent on altered STAT3 dynamics in Th17 cells lacking IL-24.

90 - Decline of Neural Stem Cell Resilience in Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system, which currently lacks effective therapies that provide regeneration and stop disease progression. A suggested link is anticipated between the development of progressive MS (PMS) and ageing, as suggested by recent work identifying hallmarks of cellular senescence in numerous cell types both ex vivo, in vitro with patient cell lines, and in vivo in the post mortem MS brain. Using a new inducible system, that directly reprograms human fibroblasts into induced NSCs (iNSCs), we aimed to thoroughly characterise control and PMS patient iNSCs and progenies towards the development of a 2D and 3D in vitro model system that can be genetically manipulated using CRISPR technology. Using this model system, we aim to identify the key mechanisms driving disease progression and accumulation of

irreversible damage in PMS. We have generated stably expandable iNSC lines from patients with PMS and age-matched controls, and characterised these cells for senescence markers, phenotyped for NSC behaviours, performed bulk RNA sequencing and metabolomics, and single cell RNA and ATAC sequencing in vitro. Preliminary analysis of iNSCs and astroglial progenies have revealed a disease-associated (DA) senescent phenotype, including increased expression of cell-cycle regulators, dysfunctional cell cycling, increased DNA damage, and secretion of pro-inflammatory molecules. Sequencing data has uncovered unique clusters in the PMS iNSCs, associated with DNA damage and cell cycling. Our results highlight a novel DA cellular mechanism in PMS wherein iNSCs and their progeny become dysfunctional and lose their intrinsic cellular resilience. Further characterisation of this model system will uncover how these DA cells intrinsically become dysfunctional and how they affect their microenvironment.

184 - Regulatory B cells required IL-2 signaling to control disease severity in EAE

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B cells play important roles as effectors and suppressors in multiple sclerosis (MS), an autoimmune disease affecting the central nervous system (CNS), and in the experimental autoimmune encephalomyelitis (EAE) model of MS. Suppressive functions are mainly driven by regulatory B cells subsets (Breg) producing IL-10. However, little is known about how IL-10 production by B cell is regulated in vivo. IL-2 signaling has critical immunomodulatory effect in MS and EAE pathogenesis through the control of T cell subset homeostasis. However, the role of IL-2 on B cells is not well defined. The expression of the beta subunit of IL-2 receptor gene (Il2rb) is part of the signature of IL-10 competent B cells. We tested here the hypothesis that IL-2 signaling on B cells may be involved in Breg development and function and so in the development of MS. We have generated a new conditional mice models (cKO) invalidated for Il2rb specifically in mature B cells ($Il2rb^{fl/fl}CD19^{cre/+}$). EAE was induced by active immunization with myelin oligodendrocyte glycoprotein (MOG) peptide (p)35-55 in cKO and control mice. IL2RB deficiency in B cells enhanced disease severity. Immune cell infiltration was increased in the CNS of cKO mice while the accumulation of IL-10 secreting Breg was decreased. Functional analysis of splenic Breg revealed that B cells defective for IL-2 signaling produced significantly less IL-10 than control cells. Our results underscore that beyond T cells, IL-2 signaling contributes to the acquisition of B cell suppressive properties through at least IL-10 production and protects against EAE by a B cell intrinsic mechanism.

356 - Using hiPSC-derived CNS cells as antigen presenting cells for unbiased identification of CNS-autoreactive CD8+ T cells

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Autoreactive CD8+ T cells recognizing central nervous system (CNS) antigens (Ag) are suspected to play a central role in many neurological diseases, including multiple sclerosis (MS), narcolepsy, Rasmussen encephalitis and Susac syndrome among others. Despite numerous research efforts in the field, no CNS antigen have been convincingly associated with any of the diseases mentioned above. This situation is mostly due to the lack of a system enabling screening for autoantigens in an unbiased manner. Indeed, most research carried so far have focused on a restricted number of suspected Ag due to technical limitations.
To overcome this limitation, we are developing an *in vitro* model of human CNS based on induced pluripotent stem cells (iPSC). This system allows us to generate large amount of neurons, astrocytes and oligodendrocytes naturally expressing a vast range of CNS antigens in their natural conformation. Using these cells, we have

designed a co-culture assay to assess the activation of CD8+ T cells by Ag presented by autologous CNS cells. To detect activated T cells, those are stained with dual specificity antibodies anti-CD45 and IFNγ. Upon activation, CD8+ T cells secrete IFNγ, which is captured by the antibodies then stained with a fluorophore-labeled anti-IFNγ antibody. The frequency of activated T cells is then assessed by flow cytometry.
First, we have developed protocols to obtain enriched cultures of neurons, astrocytes and oligodendrocytes. Second, we demonstrated that these cells respond to IFNγ by increasing their Ag-presenting capacity and upregulating MHC class I molecules at the cell surface. Third, in co-culture with autologous CD8+ T cells, CNS cells pulsed with peptides are able to trigger activation of cognate Ag-specific CD8+ T cells. We are now assessing the capacity of all three CNS cell types to elicit activation of Ag-specific CD8+ T cells by presenting peptides from endogenously produced proteins in association with MHC class I molecules at membrane surface.

Overall, we now have at hand a tool allowing us to generate a complex autologous co-culture system between immune and CNS cells from any patient. Once fully validated, we will use this platform to assess the presence and frequency of CD8+ T cells targeting CNS Ag in a cohort of MS patients versus healthy donors. Ultimately, we will be in a position to identify Ag that would be specifically recognized in MS thus shading light on an important question of MS pathogenesis.

Workshop III | Single cell analyses in neuroimmunology: essential or overrated?

304 - Single cell analysis reveals incomplete tolerance mechanisms in MOGAD patients

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Objective Myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease (MOGAD) is characterised by the presence of serum autoantibodies to MOG in patients with inflammatory CNS demyelination. We interrogated the B cell repertoire of these patients with flow cytometry and single cell RNA sequencing (scRNA-seq) to elucidate the origins of MOG autoimmunity in these patients. Methods B cell immunophenotyping was performed on four seropositive MOGAD patients and four healthy controls using flow cytometry. Live CD19+ B cells were gated into naïve B cells, non-switched memory B cells, class-switched memory B cells, and plasmablasts. Three different MOGAD patients and a healthy control were sequenced on the 10X Genomics scRNA-seq platform. Naïve B cells, memory B cells, and plasmablasts were enriched from PBMCs using magnetic separation and flow cytometry, and used to construct single cell mRNA and V(D)J libraries. Commonalities between MOGAD patient repertoires were investigated. Results A significant decrease in non-switched memory B cells was observed in adult MOGAD patients compared to controls ($p=0.0238$). ScRNA-seq data revealed preferential use of IGHV4 exclusive to MOGAD patients ($p<0.0001$), as well as a strong kappa to lambda light chain preponderance (2.21 ± 0.15) that was not reported in the control (1.66). While control heavy chain CDR3 (CDR3H) sequence lengths decreased significantly from naïve to antigen experienced class-switched memory B cells and plasmablasts ($p<0.0001$), CDR3H lengths in MOGAD patients were maintained despite antigen encounter and B cell differentiation. Furthermore, MOGAD patients exhibited lower frequencies of CDR3H mutation than the control across sequenced B cell subsets. Conclusions Altered B cell proportions and shared BCR repertoire features in MOGAD indicate the presence of erroneous tolerance mechanisms both early in B cell development and post-antigen encounter. These findings support the premise that MOGAD patients are inherently predisposed to develop autoimmunity.

298 - Diverse human astrocyte and microglial transcriptional responses to Alzheimer's pathology

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To better define roles that astrocytes and microglia play in Alzheimer's disease (AD), we used single-nuclei RNA sequencing to comprehensively characterize transcriptomes in astrocyte and microglia nuclei selectively enriched during isolation *post mortem* from neuropathologically-defined AD and control brains with a range of amyloid-beta and phospho-tau (pTau) pathology. Significant differences in glial gene expression (including AD risk genes expressed in both the astrocytes [*CLU*, *MEF2C*, *IQCK*] and microglia [*APOE*, *MS4A6A*, *PILRA*]) were correlated with tissue amyloid or pTau expression. The differentially expressed genes were distinct between with the two cell-types and pathologies, although common (but cell-type specific) gene sets were enriched with both pathologies in each cell type. Astrocytes showed enrichment for proteostatic, inflammatory and metal ion homeostasis pathways. Pathways for phagocytosis, inflammation and proteostasis were enriched in microglia and perivascular macrophages. We also found distinguishable sub-sets of astrocytes and microglia characterised by transcriptional signatures related to either homeostatic functions or disease pathology. Gene co-expression analyses revealed potential functional associations of soluble biomarkers of AD in astrocytes (*CLU*) and microglia (*GPNMB*). Our work highlights responses of both astrocytes and microglia for pathological protein clearance and inflammation, as well as glial transcriptional diversity in AD.

363 - Single-cell transcriptome profiling of immune cells revealed the distinct transcriptional response of microglia-like cells during herpes simplex encephalitis

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Microglia actively participate in the control of the central nervous system (CNS) infections. An early response of these immune cells seems to be a key component for a better clinical outcome in herpes simplex encephalitis (HSE). Hence, the role of microglia during HSE has not been fully characterized. We investigated the transcriptional response of immune cells using single-cell RNA sequencing (scRNA-seq) on CD11b+ immune cells, isolated from thalamic region of three C57/BL6 mice intranasally infected with herpes simplex virus-1 (HSV-1) on day 6 p.i. Following the clustering step of immune cell populations and sub-populations, we focused on the transcriptomics of microglia, infiltrating monocytes and CNS-associated macrophages. The analysis of microglial transcriptomic signature showed higher expression of genes (Aif1, CD36, H2-Oa, H2-DMa etc.) implicated in phagocytic and antigen presentation for reactive microglia, compared to surveillant microglia. Antigen presenting microglia were also observed in electron microscopy images of HSV-1+ ventral posterolateral nucleus (VPL) of thalamus on day 6 p.i. Next, we identified cluster-specific-genes to distinguish microglia from other cells. Based on the constant levels of Tmem119 expression in microglia during HSE, we decided to perform the immunofluorescence analysis on HSV-1+ brain sections, using Tmem119 staining. We observed Tmem119+

CD68+ ramified microglia in the vicinity of highly infected spots in VPLs. On the contrary, Tmem119+ amoeboid microglia situated in the center of the infectious spots, did not express CD68. Interestingly, a gradual increase of the Iba-1+CD68+ microglia-like cells density was observed towards to the highly infected spots. In parallel, we identified a rare (7% of all cells) subset of microglia-like cells that were found only in highly infected VPLs and that expressed genes associated to neutrophils, such as Ly6G and CXCR2. This neutrophil-like microglia cells also expressed high levels of Iba-1 (Log2>5), compared to other immune cells such as surveillant microglia, infiltrating monocytes and macrophages. Our results suggest that Iba-1+CD68+ microglia-like cells found in direct contact with HSV-1+ CNS cells could correspond to neutrophillike microglia. Furthermore, increased levels of TLR-2, MyD88, IRF7 and NF-KB underlined the inflammatory state of this neutrophil-like microglia cluster. ReactomePA analysis for the neutrophil-like microglia cluster revealed NLRP3-inflammasome mediated-IL1B production and increased death receptor signaling pathways. We think that this distinct cluster of microglia participate in the exacerbated inflammatory response in HSE. Further studies for better characterizing this population will allow to alter the immune response of these cells with different immunomodulatory strategies to better control the HSE.

54 - Exploring reported genes of microglia RNA-seq studies: uses and considerations

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Recently, several studies reporting microglia RNA-seq data of Alzheimer's disease or multiple sclerosis cases versus control subjects have been published, aiming at more insight into the role of microglia in these neurological diseases. Though the raw sequencing data are often deposited in open access databases, these are not easily available for non-bioinformaticians. The most accessible source of data is what is reported in published papers. By calculating the Jaccard index between genes reported in published microglia RNA-seq studies featuring mouse models or human tissue of either multiple sclerosis or Alzheimer's disease, we found limited overlap in reported differentially expressed genes between papers of each disease. Differences in experimental setup clearly influenced the number of overlapping reported genes. Yet, even when the experimental setup was similar, the overlap in reported genes was low. We identified that papers reporting large numbers of differentially expressed genes generally showed higher overlap, indicating that limiting the restrictions on which gene is included in the paper increases the overlap with other studies. In addition, pathway analysis of reported genes (indicating possible microglia function) varied between papers, possibly affecting the conclusion on the role of microglia in disease. Though the pathology present within the tissue used for sequencing can greatly influence microglia gene expression, often the pathology present in samples used was underreported, leaving it difficult to assess the data. Whereas reanalyzing every raw dataset could reduce the variation that contributes to the observed limited overlap in reported genes, this is not feasible for labs without (access to) bioinformaticians. We propose that decreasing the restrictions on which genes are reported within papers and increasing reporting on pathology present within samples used for sequencing may increase accessibility and usefulness of published RNA-seq data.

200 - CXCR4-positive T cells are increased in people with radiologically isolated syndrome

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In-vitro and animal model studies of multiple sclerosis (MS) suggest that aberrantly activated immune cells migrate across the blood-brain barrier into the central nervous system, where they cause or contribute to the formation of MS lesions. Neurological symptoms emerge when such lesions appear in critical areas of the brain or spinal cord. The radiologically isolated syndrome (RIS) describes a situation where a person without typical MS symptoms has magnetic resonance imaging (MRI) of the brain, revealing the characteristic lesions seen in people with MS (pwMS). Early initiation of disease-modifying therapy (DMT) can improve the mid- and long-term

prognosis of pwMS, but currently, there are no clear evidence-based guidelines as to how to manage pwRIS. While people with RIS (pwRIS) are at high risk of developing MS, the mechanisms underlying the conversion from RIS to MS are incompletely understood. Identifying pwRIS who are at increased risk of conversion would allow patients and clinicians to be proactive and take steps to prevent the accumulation of disability and optimize health outcomes. We hypothesize that studying the immune mechanisms underlying RIS will provide a better understanding of the disease processes leading to MS and help identify novel prognostic biomarkers for pwRIS. We measured the immune cell activation profiles of pwRIS, pwMS and healthy controls using mass cytometry (cytometry by time of flight, or CyTOF). We used a 37-marker panel that included markers of T cell activation. In our cohort of 11 healthy controls, 10 pwRIS, and 6 pwMS, we observed a significantly higher number of CXCR4-positive CD4 and CD8 T cells in the blood of pwRIS compared to healthy controls. CXCR4 is a chemokine receptor that has recently been shown to define subsets of activated T cells that are increased in the blood of pwMS and decrease after initiation of DMT. Our data suggest that CXCR4-expression on T cells may identify those pwRIS who show evidence of immune cell activation and may benefit from initiation of DMT even before the onset of MS symptoms. Prospective clinical validation is underway to confirm this hypothesis.

225 - CanProCo study; Determining technical parameters for large scale single-cell RNA sequencing of MS patients

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The heterogenic clinical presentation and unpredictable disease trajectory of multiple sclerosis (MS) remains a prevalent challenge for patient care. Predictive and prognostic biomarkers are lacking, but much needed. The Canadian Prospective Cohort to Understand Progression in MS (CanProCo) study involves collection of biological, radiological, and clinical data from 1000 MS patients early in the disease course, with blood collected annually for five years. Single-cell RNA sequencing will be performed on 200 MS patient's peripheral blood mononuclear cells (PBMCs) prior to treatment initiation. Single cell libraries were generated, and deep sequencing (70k reads/cell) was performed on one relapsing-remitting MS patient (18,627 cells), one sex-matched healthy control (14,148 cells) on BD Rhapsody, and the same MS patient's PBMCs sequenced on the 10X Genomics platform (8,968 cells). Transcriptomic profiles were integrated and compared across both platforms, and the number of sequenced cells and depth of sequencing (reads/cell) were analyzed to determine ideal sequencing parameters for the 200 CanProCo PBMCs. Unbiased and biased clustering identified 12 putative cell clusters. The proportion of each cell type and top ten differentially expressed genes in each cell cluster was similar across both sequencing platforms. By downsampling the number of cells, the proportion of each cell cluster remained similar, even at 500 cells. T cells were extracted and unbiasedly reclustered to identify nine unique T cell clusters. Rare T cell populations, like gamma-delta T cells, were still detectable at 8,968 cells. Despite the variation in sequencing depth (12k vs 70k reads/cell), the number of unique genes and the transcriptomic profile of putative cell types were similar. Comparison of the number of transcripts and unique mapped genes showed that the level of sequencing saturation varied across cell types, which highlighted an important consideration when determining optimal sequencing depths for specific cells of interest. These preliminary data have guided sequencing plans for CanProCo patient PBMCs. Single cell transcriptomic profiles of MS patients will be eventually analyzed alongside extensive clinical, imaging, and demographic data. Understanding the transcriptomic profile at a single-cell resolution will elucidate gene signatures which may dictate disease trajectory and treatment response and reveal novel prognostic biomarkers to improve patient care and outcomes.

Workshop IV | Immunomodulation and remyelination

187 - TNFR2 regulates the response of oligodendrocyte precursor cells to neuroinflammation

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MS is a chronic autoimmune disease characterized by inflammation, demyelination and degeneration in the CNS. Tumor Necrosis Factor (TNF) is a pleiotropic cytokine that has been implicated in the pathophysiology of various neurological disorders, including multiple sclerosis (MS). Our lab has significantly contributed to elucidating the complex role of TNF in CNS autoimmunity. By ablating TNFR2 from all oligodendrocyte lineage cells in CNP-cre:TNFR2$^{fl/fl}$ mice we showed that TNFR2 promotes remyelination following EAE (Madsen et al., 2016). Furthermore, CNP-cre:TNFR2$^{fl/fl}$ mice showed exacerbation of the acute phase of EAE, which is mainly driven by immune cell infiltration, suggesting that oligodendroglial TNFR2 might play a role in regulating the immune-mediated neuroinflammatory response as well (Madsen et al., 2020). To investigate this function and dissect the role of TNFR2 specifically in oligodendrocyte precursor cells (OPCs), we assessed the transcriptional profile of WT and TNFR2$^{-/-}$ OPCs cultured in vitro and exposed to inflammatory cytokines. Upon stimulation, OPCs shift away from their canonical phenotype and towards an immunomodulatory phenotype. This shift is exacerbated in TNFR2$^{-/-}$ OPCs, suggesting that TNFR2 suppresses the immunomodulatory role of OPCs. To validate these transcriptional changes in vivo, we generated PdgfrαCre^{ERT2}:TNFR2$^{fl/fl}$:EYFP mice to conditionally ablate TNFR2 in OPCs and induce concomitant expression of EYFP. In the MOG$_{35-55}$ experimental autoimmune encephalomyelitis (EAE) model of MS, ablation of TNFR2 in OPCs resulted in earlier onset and peak disease, preceded by accelerated infiltration of peripheral immune cells and increased microglia number in the spinal cord. In addition, microglial cells sorted from PdgfrαCre^{ERT2}:TNFR2$^{fl/fl}$:EYFP mice at pre-symptomatic EAE showed increased inflammatory gene expression, suggesting that OPC-microglia interactions are modulated by TNFR2 and play a role in the onset of EAE. Overall, our data point at TNFR2 signaling in OPCs as an important pathway in the modulation of neuroinflammation in CNS disease, supporting the idea that TNFR2 could be a promising new target for MS therapy.

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17 - Targeting lipophagy in macrophages improves repair in multiple sclerosis

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Foamy macrophages containing abundant intracellular myelin remnants are an important pathological hallmark of multiple sclerosis. Reducing the intracellular lipid burden in foamy macrophages is considered a promising therapeutic strategy to induce a phagocyte phenotype that reduces neuroinflammation and promotes central nervous system repair. Recent research from our group showed that sustained intracellular accumulation of myelin-derived lipids skews these phagocytes towards a disease-promoting and more inflammatory phenotype.
Our data now demonstrates that disturbed lipophagy, a selective form of autophagy that helps with the degradation of lipid droplets, contributes to the induction of this phenotype. Stimulating autophagy using the natural disaccharide trehalose reduced the lipid load and inflammatory phenotype of myelin-laden macrophages. Importantly, trehalose was able to boost remyelination in the ex vivo brain slice model and the in vivo cuprizone-induced demyelination model.
In summary, our results provide a molecular rationale for impaired metabolism of myelin-derived lipids in macrophages, and identify lipophagy induction as a promising treatment strategy to promote remyelination.

61 - Exogenous fractalkine enhances oligodendrogenesis and remyelination in the cuprizone-induced demyelination mouse model

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Multiple Sclerosis (MS) is an autoimmune and neurodegenerative disorder that leads to the damage or loss of myelin, an insulating layer that coats and protects nerve axons. Current disease-modifying therapies are ineffective for progressive MS, which is characterized by worsening of the disease with no improvements. Treatments for progressive MS could be achieved by stimulating the production of new oligodendrocytes from resident oligodendrocyte precursor cells (OPCs). Our lab has shown that fractalkine (FKN), an immunological chemokine, stimulates oligodendrogenesis in the normal adult brain (Watson, de Almeida et al. 2021 Stem Cell Rep). Here, we asked whether fractalkine enhances oligodendrogenesis in a demyelinated brain.
To answer this question, we used OPC lineage-tracing mice (PDGFRαCreERT2;RosaYFPSTOP/+ and PDGFRαCreERT2;RosaTdTomato/mGFP) that underwent cuprizone demyelination. We show intracerebroventricular infusion of FKN into demyelinated OPC lineage-tracing mice increases de novo oligodendrocyte genesis in the cortical grey and white matter. Interestingly, FKN increases OPC proliferation only in the cortical grey matter. We also show FKN decreases engulfment of newborn oligodendroglial cells by microglia and/or macrophages. Finally, we demonstrate FKN infusion leads to increased remyelination in the cortical grey matter. These results suggest FKN modulates microglia function and that OPCs in the cortical white and grey matter respond differently to exogenous FKN. We have previously shown that in addition to microglia, FKN receptor (CX3CR1) is also expressed in OPCs, albeit at lower level (Watson, de Almeida et al. 2021 Stem Cell Rep). Thus, both cell types are poised to respond to FKN. Using microglia-OPC co-cultures, we are currently investigating the mechanism of FKN-mediated oligodendrogenesis. In summary, our results show FKN is a novel pro-regenerative molecule in a cuprizone mouse model of demyelination.

123 - Stearoyl-CoA desaturase-1 impairs the reparative properties of macrophages and microglia in the brain

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Failure of remyelination underlies the progressive nature of demyelinating diseases such as multiple sclerosis. Macrophages and microglia are crucially involved in the formation and repair of demyelinated lesions. Here we show that myelin uptake temporarily skewed these phagocytes towards a disease-resolving phenotype while sustained intracellular accumulation of myelin induced a lesion-promoting phenotype. This phenotypic shift was controlled by stearoyl-CoA desaturase-1 (SCD1), an enzyme responsible for the desaturation of saturated fatty acids. Monounsaturated fatty acids generated by SCD1 reduced the surface abundance of the cholesterol efflux

transporter ABCA1, which in turn promoted lipid accumulation and induced an inflammatory phagocyte phenotype. Pharmacological inhibition or phagocyte-specific deficiency of SCD1 accelerated remyelination *ex vivo* and *in vivo*. These findings identify SCD1 as a novel therapeutic target to promote remyelination.

220 - Themis1/Vav1 signaling hub controls T cell pathogenicity in a mouse model of Multiple Sclerosis

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Although Multiple Sclerosis (MS) is the leading cause of disability among young adults in the world, the mechanisms underlying its development remain poorly understood. In 2007, the first Genome Wide Association Study (GWAS) revealed the implication of many genetic factors, including immune-related genes. Even though deeper GWAS have later been conducted on larger cohort and confirmed the predominance of immune genes, genetic susceptibility factors only account for less than 50% of MS etiology. This could be explained by the implication of environmental factors, but also by the epistasis (gene-gene interaction), a phenomenon that is not addressed in GWAS studies. This prompted us to investigate the epistasis between two MS susceptibility genes, Themis1 and Vav1, in the susceptibility to develop EAE (Experimental Autoimmune Encephalomyelitis), an animal model of MS. Themis1 and Vav1 are two TCR signaling molecules which interact following TCR engagement. In this study, we show that the Themis1-Vav1 signaling hub controls the encephalitogenicity of conventional T cells. We used a natural variant of Vav1 that was shown to reduce susceptibility to central nervous system (CNS) inflammation in mice, together with a T cell-conditional deletion of Themis1. When taken separately, both mutations triggered a mild reduction of EAE severity, whereas this reduction was much stronger when both genes were mutated simultaneously. Functional studies revealed that this finding was independent on Tregs functions and unrelated to the impact of Themis1 on thymic selection. Rather, it resulted from decreased production of pro-inflammatory cytokines (IFN-γ, IL-17, and GM-CSF), together with reduced T cell infiltration in the CNS. Our current work aims at determining the underlying mechanisms, by analyzing the pathways impacted by both mutations. Altogether, our study reveals an epistatic interaction between Vav1 and Themis1, which form a signaling hub controlling Tconv encephalitogenicity. Our work also provides a rationale for examining gene complexes, rather than separated genes, to deeper understand MS etiology.

65 - Elevated expression levels of Ephrins on Immune Cells of Patients With Multiple Sclerosis Affects Oligodendrocyte Differentiation

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Background: Remyelination in multiple sclerosis (MS) lesions often remains incomplete despite the presence of oligodendrocyte progenitor cells (OPCs), leaving axons permanently demyelinated and vulnerable to degeneration. Ephrins are membrane-bound ligands activating tyrosine kinase signaling proteins that are known to have an inhibitory effect on oligodendrocyte regeneration. Moreover, ephrins A1, A2, A3, & B3 were identified in MS lesions. Objectives: Since these ephrins are expressed on immune cells membrane, we hypothesized that their expression levels might be changed on immune cells of MS patients and can affect OPCs differentiation in the brain. Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from 43 untreated MS patient and 27 aged matched healthy controls, and were sujected to flow cytometry analysis for the expression of ephrin-A1, A2, A3 & B3 on PBMCs, and T cell subpopulations. PBMCs were co-cultured with 293T-cells and immunostained for ephrins signaling, or with OPCs to study the effect on their differentiation in-vitro. Results: We found an increased expression of ephrinA2, A3 & B3, especially on T cell subpopulations. We have also showed that the expression of ephrins on immune cells of patients with RR-MS but not of HC, increases the forward

signaling pathway through their receptors and has an inhibitory effect on the differentiation of oligodendrocyte precursor cell (OPCs) in vitro. Conclusions: Our study findings support the concept that the immune activity of T cells in patients with RR-MS has an inhibitory effect on the differentiation capacity of OPCs through the expression and forward signaling of ephrins.

ORAL PRESENTATIONS - NOVEMBER 10, 2021

Parallel IV | Barriers of the CNS: actors in neuroinflammation

282 - DICAM Promotes T_H17 Lymphocyte Trafficking Across the Blood-Brain Barrier during Neuroinflammation

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The migration of circulating leukocytes across the blood-brain barrier (BBB) into the central nervous system (CNS) is a key driver of multiple sclerosis (MS) pathogenesis. The monoclonal antibody natalizumab proved that pharmaceutically obstructing this process is an effective therapeutic approach for treating relapsing-remitting MS (RRMS). Unfortunately, the clinical efficacy of natalizumab is somewhat offset by its incapacity to control the progressive forms of MS (PMS), and by life-threatening side effects in RRMS rising from the ubiquitous expression of its molecular target VLA4 and the consequent impairment of CNS immunosurveillance. In this study, we identify Dual Immunoglobulin domain containing Cell Adhesion Molecule (DICAM) as a novel cell trafficking molecule preferentially expressed by RORγt-driven IL17-secreting $CD4^+$ T helper (T_H17) lymphocytes. In MS, we found that the frequency of circulating $DICAM^+$ memory $CD4^+$ T cells is increased in patients with clinically and radiologically active RRMS and PMS disease courses, and that the expression of its ligands is upregulated on BBB endothelium in brain lesions. Using multiple *in vitro* and *in vivo* approaches, we show that neutralizing DICAM reduces murine and human T_H17 cell trafficking into the CNS. Lastly, we demonstrate that blocking DICAM with a monoclonal antibody alleviates disease severity in four distinct experimental autoimmune encephalomyelitis (EAE) models, including relapsing-remitting and progressive disease models. Collectively, our data showcase DICAM as a

therapeutic target to impede the migration of disease-inducing leukocytes into the CNS in both RRMS and PMS, and establish its blocking with a monoclonal antibody as an effective therapy with promising potential.

157 - Immune protection at the CNS borders: heterogeneity and maturation of meningeal macrophages

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Immune responses in the central nervous system (CNS) are increasingly shown to influence cognition and neuropathology. The CNS interfaces are protected by the blood-brain barrier, but also the meninges, a set of three membranes (pia, arachnoid and dura mater). The meninges are highly vascularized and contain a dense network of resident immune sentinels, such as meningeal macrophages (MM) in the dura-mater. In this study, we investigated their spatial heterogeneity and the mechanisms underlying their evolution over time. Using immunohistochemistry, flow cytometry, and single-cell RNA sequencing, we observed that populations of MM (MHC-II+ and MHC-II-) had different transcriptomic profiles and coexisted along vasculature and nerves. While MHC-II- MM were abundant in neonate mice, MHC-II+ MM appeared progressively over time. Using CXCR4CreER and CXCR3CreER lineage-tracing mice pulsed at 2.5 weeks and chased at 3 months old, we confirmed that adult MHC-II- MM were long-lived. In contrast, MHC-II+ MM were derived from infiltrating monocytes as well as differentiation of MHC-II- MM. We then investigated the mechanisms underlying this MHC-II+ MM maturation, which was previously shown to be partially linked with the microbiota. First, we observed higher number of MHC-II+ MM clusters in the meninges of MAVS-/- mice compared to controls. This could be due to faint leakage of microbial products at steady state, creating a low-grade inflammation and maturation of MHC-II+ MM. Second, we observed a decreased population of MHC-II+ MM in adult IFNγ-/- mice and adult mice with a T cell deficiency (TCRα-/-). Therefore, activated T cells producing IFNγ could promote MHC-II+ MM differentiation. Finally, we discovered that food intake affects the proportion of MHC-II+ MM. Indeed, under caloric restriction, both adult and young mice have a lower MHC-II+ MM proportion compared to controls. This suggests that food intake influences the implementation and/or the conservation of MHC-II+ MM. Overall, we show that the meninges are populated by two populations of MM with specific developmental trajectories and localizations, and that their maturation relies on the influence of a complex balance between immune and environmental factors.

85 - Oncostatin M opens the gate for T helper 17 cells during neuroinflammation

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Blood-brain barrier (BBB) dysfunction is an intrinsic feature of neurodegenerative and -inflammatory diseases, including multiple sclerosis (MS). Oncostatin M (OSM) cytokine levels are elevated in the blood and brain of MS patients. We previously demonstrated that OSM exerts neuroprotective and remyelination-promoting functions after central nervous system (CNS) damage, warranting its potential therapeutic use. However, OSM's role in neuroinflammation and BBB function is poorly understood. To investigate the role of OSM signalling in a neuroinflammatory setting *in vivo*, we induced experimental autoimmune encephalomyelitis (EAE) in wild-type and OSM receptor (OSMRβ) deficient mice. CNS immune cell infiltration and BBB leakage were analysed at different timepoints during disease. Surprisingly, OSMRβ deficient mice exhibited milder EAE symptoms and the lack of a disease peak, which was associated with diminished T helper 17 (Th17) cell infiltration into the CNS and

reduced BBB leakage. Effects of OSM on inflamed BBB-endothelial cells (ECs) were further investigated *in vitro* using primary mouse brain microvascular ECs and the human cerebral microvascular EC line (hCMEC/D3). *In vitro*, OSM promotes secretion of the Th17-attracting chemokine CCL20 by inflamed BBB-ECs, whereas it downregulates intercellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expression. Using flow cytometric fluorescence resonance energy transfer (FRET) measurements, we found that OSM-induced endothelial CCL20 promotes activation of the ICAM-1 ligand, lymphocyte function-associated antigen 1 (LFA-1), characterized by a conformational change. This effect was abrogated when CCL20 was neutralized in the EC conditioned medium, or when CCR6 was blocked on Th17 cells. Finally, we found that OSM reduced the transendothelial electrical resistance of BBB-ECs in control and inflammatory conditions by downregulating cell-cell junction expression of Claudin-5 and VE-cadherin. Together, these data show that OSM contributes significantly to BBB dysfunction in neuroinflammation by inducing permeability while recruiting Th17 cells via enhanced endothelial CCL20 secretion.

Parallel V | Autoimmune neurological disorders (other than MS)

71 - T cell-mediated neuronal destruction in GAD65-Encephalitis calls for early immunosupressive treatment

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Autoimmune encephalitis has gained increasing recognition in recent years and can be categorized into T cell-mediated encephalitis, and antibody-mediated encephalitis, where antibodies target extracellular epitopes. In GAD65-encephalitis the underlying pathomechanism is still not fully understood. As GAD65 is an intracellular protein, antibodies are unlikely to have a direct pathogenic effect. However, a direct pathogenic effect of T cells in these patients remains to be proven. To elucidate the underlying pathomechanism of GAD65-encephalitis patients, we collected FFPE brain tissue of 17 patients with disease duration ranging from 0.3 to 59.6 years and compared them to a non-neurological disease cohort (n=7) by performing histopathological and whole-genome transcriptomics. Our histopathological examinations revealed that especially in the very early stages of the disease cytotoxic T cells (CTLs) are present in the parenchyma in significantly elevated numbers. Part of these CTLs contained Granzyme B+ granules and were found in apposition to neurons. T cell numbers however rapidly decrease with longer disease duration. Whole-genome transcriptomics underlined this finding as many pathways linked to T cell immunity are upregulated in cases with a shorter disease duration. Moreover, we found high numbers of plasma cells in the perivascular space of bloodvessels as well as in the parenchyma, also indicated by an overrepresentation of Fc-receptor signalling pathways and B cell homeostasis clusters in the transcriptomics data. Furthermore, we evaluated recent neuronal destruction, indicated by APP+ axonal bulbs, which was predominantly seen in cases with short disease duration and high T cell counts. Our data suggests early neuronal destruction by cytotoxic T cells, followed by plasma cell influx. MRI scans underline this disease progression as initial hippocampal swelling is followed by atrophy. With this unprecedented evaluation of high numbers of brain resections of GAD65-encephalitis patients, we provide evidence for the hypothesis of GAD65-encephalitis as being T cell mediated. The early, irreversible neuronal destruction is a valid explanation for the incomplete response to immunosuppressive therapy of most GAD65-encephalitis patients. Taken together, our results call for early immunomodulatory treatment to ameliorate the disease outcome.

255 - Prospective long-term tocilizumab responses in relapsing myelin oligodendrocyte glycoprotein IgG-associated disease: factors associated with post-tocilizumab relapse-freedom

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Introduction: Tocilizumab (TCZ), an anti-interleukin 6 receptor antibody, shows promise in treating myelin oligodendrocyte glycoprotein IgG-associated neurological disease (MOGAD). However, large prospective studies are lacking while determinants for post-biologic disease response remain unclear. We prospectively evaluated long-term TCZ responses in relapsing MOGAD and associated factors for post-TCZ relapse-freedom. **Methods:** 195 MOG-positive and anti-aquaporin-4 negative adult MOGAD patients with relapses despite ≥3 immunosuppressants from 5 highly-specialised centres received intravenous TCZ (8mg/kg 4-weekly) for 4 years (2017–2021). We characterised pre- and post-TCZ annualised relapse rates (ARR) and Expanded Disability Status Scale (EDSS) scores, radiological progression, adverse events (AE), features of those with and without post-TCZ relapses, and elucidated factors for relapse-freedom and odds ratios (OR) by logistic regression. **Results:** Mean patient age, MOGAD onset age and disease duration were 47.9, 42.1 and 5.8 years. There were 566 pre-TCZ clinical demyelinating episodes (mean 2.9/patient, mean pre-TCZ immunosuppressants 5.2/patient). 63.1 % previously used rituximab. Post-TCZ initiation, 21.0% relapsed over 4 years (75.5weeks to first relapse). TCZ reduced mean cohort ARR (1.81 to 0.11,p<0.0001) and ARR 18 months pre and post-TCZ initiation (2.06 to 0.08,p<0.0001), with longer duration to first post-TCZ relapse in those with 1 previous relapse than ≥2 relapses (107.3 vs 71.0weeks,p=0.0012) and similar ARR reduction (-92% vs -95%). Previous rituximab-users had lower ARR during TCZ treatment than rituximab (0.11 vs 0.53,p<0.0001). TCZ improved EDSS score (3.36 to 2.87,p=0.0007). 84.6% and 61.0% showed no post-TCZ EDSS or radiological progression. Post-TCZ 4-year survival was 90.8% (18 deaths, none treatment-related). 81.0% developed AEs. 43.1% developed infections (5.1% severe). Post-TCZ relapse-freedom independently associated with 1 pre-TCZ relapse (OR4.38,p=0.003), pre-TCZ MOGAD duration<2years (OR2.19,p<0.001), onset age<40 (OR1.83,p=0.001) and absence of autoimmune comorbidities (OR3.52,p=0.002). **Conclusion:** TCZ reduces MOGAD relapse and disability over 4 years. Superior responses associated with 1 pre-TCZ relapse, pre-TCZ MOGAD duration<2years, onset age<40 and no autoimmune comorbidities, identifying those likely to show greatest TCZ response. Efficacy of earlier TCZ initiation after first MOGAD relapse requires further investigation.

281 - Investigating anti-inflammatory and immunomodulatory properties of brivaracetam in experimental autoimmune encephalomyelitis (EAE)

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Epilepsy is a chronic central nervous system condition affecting 1 in 100 individuals around the world. One third of patients suffer from drug-resistant epilepsy (DRE), characterized by recurrent seizures despite appropriate trials of anti-epileptic drugs (AEDs). Identification of several autoimmune epileptic encephalitis suggest a potential contribution of the immune system to refractory seizures. Accumulating data support a biological link between neuroinflammation and chronic epilepsy. Our team has previously reported an increased proportion of CD4 T cells in the peripheral blood compartment of adults DRE, with a higher frequency of proinflammatory Th17/Th1 cells. Furthermore, we reported significantly higher levels of the marker of neuronal injury sNfL in aging subjects with DRE compared to controls. Numerous observations suggest that certain AEDs such as brivaracetam and levetiracetam display immunomodulatory and neuroprotective properties but their impact on the immune cell profile and capacity to mount a robust response is unknown. Multiple sclerosis, the prototypical inflammatory disease of the CNS, is associated with a significantly increased risk of seizures. We hypothesized that novel generation AED brivaracetam, which is associated with lower lymphocyte counts and a mild increase of infections, could show benefits in the animal model of multiple sclerosis (experimental autoimmune encephalomyelitis (EAE)) through anti-inflammatory and/or neuroprotective properties. We compared the impact of exposure to

brivaracetam and lacosamide, another novel generation AED with a different mechanism of action, on activation of human peripheral blood mononuclear cells and on development of EAE. Our data show that brivaracetam and lacosamide do not impair proliferation or activation of immune cells in vitro and in vivo, while exposure to brivaracetam is associated with a mild reduction of GM-CSF+ human T cells in vitro and a lower frequency of CNS-infiltrating CD8 T cells at day 7 after MOG immunization in vivo. Prophylactic administration of brivaracetam or lacosamide do not delay EAE onset but is associated with a significantly less severe clinical course of active EAE in female C57BL/6 mice compared to controls. These data suggest that novel generation AEDs show a class effect alleviating the course of EAE through mostly neuroprotective mechanisms without significantly impairing the immune response.

127 - MAVS is required to mount IFN-β- and broad innate immune responses in VSV infected microglia

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Several RNA viruses can infect the central nervous system (CNS), which typically is associated with viral encephalitis. This inflammatory condition can result in long-term neurological sequelae and even lethal disease. Upon infection of the CNS by neurotropic viruses, tissue-resident cells sense the virus through different pattern recognition receptors (PRRs) and mount innate immune responses. Important PRRs in the control of viral infections are members of the diverse group of the Toll-like receptors (TLRs), the RIG-I-like receptors (RLRs), and the cGAS/STING axis. In most previous studies, transgenic mice or cell lines with complete deletion of single sensing components were used. Hence, the question remained unsolved how individual CNS-resident cell types respond to virus infection. We addressed the role of molecular sensing pathways in various different CNS-resident cell types that are relevant for the induction of innate immune responses upon exposure to vesicular stomatitis virus (VSV). We isolated neurons from murine embryos and generated primary astrocytes and microglia from newborn mice. Such experiments were performed with wild type (WT) and MAVS deficient mice. The resulting WT and MAVS KO primary cells were *in vitro* infected with VSV at MOI 0.5 and interferon (IFN)-β responses were monitored. Microglial responses to VSV infection were further analyzed by RNA-sequencing. Upon infection with VSV, cultured neurons did not mount IFN-β responses. Infection with the VSV variant M2, which inhibits the induction of type I IFN responses less efficiently than VSV, induced a significant secretion of IFN-β in WT neurons. However, neurons lacking MAVS did not respond by IFN-β production neither to VSV nor VSV M2. WT astrocytes mounted notable IFN-β responses to VSV and VSV M2, whereas no IFN-β could be detected in astrocytes deficient of MAVS. Similarly, microglia devoid of MAVS showed clearly diminished IFN-β responses upon VSV M2 infection when compared with WT controls, whereas VSV did not induce IFN-β. Transcriptomic analyses of VSV infected microglia indicated that the induction of a wide variety of innate immune signature genes was dependent on MAVS. Our results indicate that upon VSV infection of neurons, astrocytes and microglia MAVS triggering is needed to induce antiviral IFN-β responses. In microglia, MAVS signaling was needed for the orientation towards an immunoactive state.

156 - Age-dependent meningeal macrophages protect against viral neuroinfection

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Due to the vital importance of the Central Nervous System (CNS), its potential infection and inflammation have to be tightly controlled. The surface of the CNS is connected to the periphery by a rich and complex tissue, the meninges. They contain a vast network of macrophages subdivided in at least two subpopulations endowed with elusive functions: a neonatal, MHC-II negative macrophage population, and an age-dependent population expressing MHC-II. Using in situ-histocytometry, flow cytometry, and single-cell RNA sequencing approaches, we show that those populations have opposite dynamic behaviors in response to in vivo peripheral challenges such as LPS, SARS-CoV2 and lymphocytic choriomeningitis virus (LCMV), with an apparent contraction of the MHC-II+ population. Focusing on LCMV infection in experimental mouse models and using innovative pharmacological and genetic depletion strategies, we show that MM represent an early line of protection against this neuroinvasive pathogen. In their absence, specific areas in the meninges become highly infected, leading to fatal brain disease. While their intrinsic sensing of viral replication through the Mitochondrial antiviral-signaling protein (MAVS) is dispensable, sensing of IFNs through the STAT1 pathway plays an important role in controlling viral spread. Unexpectedly, the age-dependent MHC-II+ macrophage population has a major role in controlling neuroinfection, and this is independent of the MHC-II molecule itself. This work helps understand the spatial organization of the brain defense system and the cellular and molecular mechanisms involved in CNS protection.

52 - Human iPSC-derived astrocytes as a model to understand JCV infection in the brain

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JC virus (JCV) is the causative agent of progressive multifocal leukoencephalopathy (PML), a devastating disease of the central nervous system (CNS) that results in the widespread formation of lesions across the brain parenchyma. Because of the human selective nature and cell specificity of the virus, there has been a lack of proper culture systems and animal models to study JCV infection. For this reason, even half a century after its discovery, many questions regarding JCV infection in the brain remains unresolved. JCV is able to establish a lytic infection of oligodendrocytes, which results in rapid demyelination, the pathological hallmark of the disease. While the infection of astrocytes is well documented in vivo, the exact role of these cells in the JCV pathophysiology remains unknown. Here, using astrocytes derived from human induced pluripotent stem cells (hiPSC), we are able to address some of these uncertainties combining microscopy, molecular and proteomic analysis. First, we show that hiPSC-derived astrocytes are susceptible to JCV with a significant increase in viral titer over time as assessed by fluorescence microscopy and confirmed by qPCR. More precisely, JCV particles and filamentous structures, formed by viral core proteins, localize to the cell nucleus as verified by immunofluorescence and transmission electron microscopy. We further demonstrate, by proteomic analysis of JCV-infected astrocytes, an increase of proteins associated with the cell cycle and the DNA damage response,

reflecting what is observed for other polyomaviruses. We also show that extracellular vesicles (EVs) derived from JCV-infected astrocytes are associated with JCV viral capsid protein and genomic DNA, suggesting a potential role of EVs in JCV propagation within the CNS. Taken together, these results show that human iPSC-derived astrocytes provide an excellent model to study JCV biology in glial cells and closely reflect what is observed in vivo. This unique human-based in vitro model will help to broaden our understanding of JCV infection in the brain and pave the way to the development of effective therapeutic strategies against PML.

Workshop V | Does our food affect our brain via the immune system?

62 - Diet-dependent regulation of TGFb impairs reparative innate immune responses after demyelination

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Pro-regenerative responses are required for the restoration of nervous system functionality in demyelinating diseases such as multiple sclerosis (MS). Yet, the limiting factors responsible for poor CNS repair are only partially understood. Here, we test the impact of Western diet (WD) on phagocyte function in a mouse model of demyelinating injury that requires microglial innate immune function for a regenerative response to occur. We find that WD feeding triggers an ageing-related, dysfunctional metabolic response that is associated with impaired myelin debris clearance in microglia, thereby impairing lesion recovery after demyelination. Mechanistically, we detect enhanced transforming growth factor beta (TGFb) signalling, which suppresses the activation of the liver X receptor (LXR)-regulated genes involved in cholesterol efflux, thereby inhibiting phagocytic clearance of myelin and cholesterol. Blocking TGFb or promoting triggering receptor expressed on myeloid cells 2 (TREM2) activity restores microglia responsiveness and myelin debris clearance after demyelinating injury. Thus, we have identified a druggable microglial immune checkpoint mechanism regulating the microglial response to injury that promotes remyelination.

56 - Sialic acid-driven untimely immune aging in obesity-Alzheimer's disease comorbidity accelerates cognitive decline

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Obesity is a strong risk factor for Alzheimer's disease (AD), but the underlying mechanisms remained elusive. Using an AD mouse model (5xFAD) under obesogenic diet, we found accelerated cognitive loss and altered brain cellular landscape accompanied by untimely systemic immune aging. Non-targeted plasma metabolomics revealed that *N*-acetylneuraminic acid (NANA), the predominant sialic acid, was specifically elevated in obese-AD comorbid mice, and was inversely correlated with cognitive performance and directly correlated with splenic frequencies of immune-suppressive cells. Single-nucleus RNA-sequencing of the visceral fat identified macrophages as a potential source of sialic acid. Short-term treatment with NANA of middle-aged wild-type mice recapitulated the immune deviations observed in comorbid mice, and human T cells exposed to NANA displayed reduced proliferation and increased exhaustion. Our study identified sialic acid-driven immune dysfunction as the missing link between obesity and AD, and potentially other AD-predisposing conditions.

140 - Neurological alterations and intestinal dysbiosis associated with subclinical food allergy

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In some individuals, certain types of food allergies, such as cow's milk and hen's egg allergies, produce only mild symptoms. These subclinical food allergies have been implicated in behavioral and mood disorders from observations that allergen elimination diets improve symptoms. However, clinical studies attempting to validate the link between food allergies and neuropsychiatric disorders have reported inconsistent results, likely due to cohort variability. Thus, without sufficient pathological evidence and defined mechanism, the contributory role of food allergies in brain dysfunction has been debated. To test the causal effect of a food allergy on behavior under controlled conditions in subjects with the same allergy type, diet, environment, and genetic background, we developed a mouse model of non-anaphylactic cow's milk allergy by sensitizing male and female C57BL/6J mice to a bovine whey allergen, beta-lactoglobulin (BLG: Bos d 5). Hypothesizing that behavioral changes are neurological manifestations of non-anaphylactic responses triggered by allergen-induced intestinal impairment in sensitized individuals, we evaluated affective and cognitive behavior in sham and BLG-sensitized mice after allergen exposure. Associated changes in the intestines, brain and immunologic factors in the blood were also examined. As expected, BLG-sensitized mice had significantly higher levels of allergen-specific IgE than the sham mice, even though they did not exhibit anaphylactic reactions upon allergen exposure. Furthermore, the fecal microbiome profiles of sensitized mice were distinct from the sex-matched sham mice, indicating intestinal dysbiosis. Behavioral manifestations were sex-dependent, and anxiety and depression-like behavior were observed only in BLG-sensitized male mice but not in female mice. In sensitized male mice, decreased immunoreactivity for intestinal tight junction protein, occludin, and elevated plasma cytokines and chemokines were found, suggesting that intestinal barrier impairment triggered systemic immune responses. In addition, perivascular astrogliosis and demyelination were evident in the brain, providing potential neural substrates for the observed behavioral changes. These results substantiated the contribution of food allergy to intestinal and brain pathologies that may explain behavioral symptoms. Additional investigations are in progress to elucidate the role of altered microbiota and the immune cells on the nervous system.

171 - Impact of methionine intake on neuroinflammatory processes in a spontaneous sex-biased animal model of multiple sclerosis

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Introduction: Multiple Sclerosis (MS) is a chronic neuroinflammatory disorder more common in women than men, and characterized pathologically by multifocal areas of demyelination, and neuronal degeneration in the context of central nervous system (CNS) infiltration by inflammatory immune cells. Western diet, obesity, and related comorbidities promote a shift towards an inflammatory immune cell phenotype and are associated with a higher

risk of MS onset as well as less favorable disease course. Dietary methionine restriction (MR) without caloric restriction prolongs lifespan, decreases markers of inflammation in obese mice, and is protective in a model of inflammatory bowel disorder. Moreover, dietary MR improves metabolic health via sexually dimorphic mechanisms. We found that methionine pathway is induced upon T lymphocyte activation in vitro and methionine restriction affects the effector function and proliferation of TH17 lymphocytes, considered pathogenic in MS and its animal model, experimental autoimmune encephalomyelitis (EAE). Our objective is therefore to study the manipulation of T lymphocyte methionine metabolism as a new therapeutic avenue for controlling neuroinflammatory diseases such as MS in both sexes. **Methods:** Spontaneous EAE in transgenic male and female TCR1640/SJL mice (T lymphocyte receptor specific for MOG) exposed to low methionine (MR) vs. control or methionine supplemented (M+) diet. Clinical evaluation and flow cytometry studies are used to assess disease incidence and characterize immune cell distribution and activation. RNA- and 16S rRNA- sequencing are used to evaluate the impact of methionine intake on T lymphocytes transcriptomic profile and composition of gut microbiota. **Results:** Dietary MR is associated with a lower weight gain and a significantly delayed onset of neurological deficits in both males and females with a near complete abrogation in males. This is paralleled with a lower number of immune cells as well as pro-inflammatory T lymphocytes in the spleens and CNS at presymptomatic and chronic stages of spontaneous EAE. Finally, T lymphocytes transcriptomic profile and gut microbiota composition reveal differences according to sex. **Conclusion:** Our results demonstrate the beneficial impact of restriction of methionine intake on clinical course and neuroinflammatory processes in a spontaneous EAE model mimicking sex difference in MS, establishing a novel link between methionine metabolism and neuroinflammation.

115 - Dietary supplementation with conjugated linoleic acid ameliorates intestinal inflammation and CNS autoimmunity

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Since first-line disease-modifying treatments of multiple sclerosis (MS) can been associated with various side effects and have limited impact on the processes related to disease progression, complementary alternative therapeutic options have come into focus in recent decades. In particular, the modulation of processes along the gut-central nervous system (CNS) -axis by dietary factors have been intensively investigated. Our experiments in a spontaneous mouse model of MS demonstrated that dietary supplementation with the polyunsaturated fatty acid conjugated linoleic acid (CLA) improved CNS autoimmunity, which is accompanied by an attenuation of gut barrier dysfunction and inflammation as well as an expansion of suppressive intestinal mononuclear phagocytes. These beneficial effects of dietary supplementation with CLA were not abrogated upon antibiotic eradication of the gut microbiota, highlighting their dispensable role in mediating CLA-effects. Instead, we observed several direct anti-inflammatory effects of CLA on murine mononuclear phagocytes, such as an increased IL-10 production, a balanced ROS/glutathione response and the ability to suppress T cell proliferation. Furthermore, in a human pilot study involving 15 relapse-remitting MS patients under first-line disease-modifying therapy, we confirmed that dietary supplementation with CLA for 6 months significantly improved the anti-inflammatory features of circulating mononuclear phagocytes compared to baseline. In conclusion, our results implicate that CLA is a potent modulator of the gut-CNS axis, targeting intestinal mononuclear phagocytes, which in turn regulate encephalitogenic T cell responses. However, further evaluation should be conducted in larger clinical trials to evidence the complementary therapeutic potential of CLA in MS.

289 - Impact of high fibre diet and its combination with intermittent fasting in CNS autoimmunity

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Dietary fibre is known to benefit health through promoting healthy gut microbiota and boosting the production of bacteria-derived metabolites, particularly short chain fatty acids. High fibre intake confers protection against various preclinical models of immune-mediated disorders including allergy, lupus and colitis, but its impact in multiple sclerosis (MS) remains elusive. Intermittent fasting (IF) attenuates disease in animal models of MS, including experimental autoimmune encephalomyelitis (EAE). Thus, the effects of high-fibre (HF) diet and its combination with IF in MS is of great therapeutic interest. C57BL/6 mice were fed on either a HF or zero-fibre (ZF) content diet and were either given ad libitum (AL) food access or subjected to 24-hour fasting-feeding cycles. Immune profiles were analysed by flow cytometry – (1) before EAE induction after 5 weeks of feeding and (2) on day 40 post EAE induction. Mice were culled both after feeding and fasting to assess the temporal effects of IF. Splenic Th17 cells and blood Ly6Chi monocytes were increased in the HFAL group compared to the ZFAL group under basal conditions before EAE induction. Acute fasting decreased Th17 cells in HFIF mice and decreased Ly6Chi monocytes in both HFIF and ZFIF groups compared to corresponding AL groups, whilst refeeding restored Th17 and monocyte levels to that of AL groups. Consistent with these results, the HFAL group had the highest EAE incidence, whilst IF reduced disease incidence, delayed disease onset and reduced clinical severity compared to AL feeding of the same diet, with the HFIF intervention being the most protective against EAE. In accordance, ex vivo antigen-specific Th17 response was greatest in the HFAL group and was greatly dampened in the HFIF group. Altogether, HF feeding was not protective against EAE development, whilst IF in combination with HF feeding rescued clinical disease parameters, indicating complex interactions between the two interventions.

Workshop VI | Brain ageing: impact of immunity and infection

110 - Aging perturbs microglia function and upregulates osteopontin to accelerate oxidized phosphatidylcholine-mediated neurodegeneration

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Oxidized phosphatidylcholine (OxPC) species found in multiple sclerosis (MS) lesions are harmful molecules that promote neurodegeneration and which require microglia for clearance (Dong et al., Nature Neurosci 2021). Here, we aimed to determine how aging affects microglia responding to OxPC-mediated neurodegeneration by comparing OxPC-induced spinal cord white matter lesions from young (6-week-old) and aging (52-week-old) mice. Microscopy analysis showed OxPC-induced lesions from aging mice had greater total lesion volume and axonal loss, as well as reduced oligodendroglial counts compared to lesions from young mice. Single cell RNAseq of live cells sorted from homogenized spinal cords identified multiple distinct microglia/macrophage clusters and aging associated signatures. The latter include the upregulation of interferon signaling and antigen presentation genes in the healthy aging spinal cord compared to young spinal cord. Moreover, microglia/macrophages in the aging OxPC-induced spinal cord lesions significantly elevated genes associated with neuroinflammation such as *Spp1*, *Ifitm3*, *Cd74*, and *Lgals3* compared to young spinal cords after OxPC injection. Spinal cord sections were then examined by spatial RNAseq which showed that aging associated changes identified by single cell RNAseq were anatomically enriched within the OxPC lesions. Notably, both single cell and spatial RNAseq revealed the upregulation of liver x receptor (LXR) activated genes by microglia in response to OxPCs, and LXR activation by GW3965 attenuated OxPC mediated neurodegeneration in both young and aging mice. Conversely, the aging-upregulated gene *Spp1* (osteopontin) significantly increased the severity of OxPC mediated neurodegeneration, at least in part by altering microglia/macrophage function. Thus, aging is associated with prominent transcriptomic changes by microglia/macrophages in the spinal cord, which are enhanced during OxPC induced

neurodegeneration. These findings also suggest that pathways associated with LXR activation and osteopontin signaling may regulate aging mediated neurodegeneration and MS progression.

136 - Aging blood factors promote CD8 T cell infiltration in the adult mouse brain

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Aging is the greatest cause of neurodegenerative disease worldwide and is characterized by altered immunological processes and cognitive decline. Advanced age presents a plasma proteome with increased levels of cytokines and chemokines that have detrimental effects on the brain. Here, we postulate that aged plasma harbors pro-aging factors that drive immunological changes within the periphery and the brain. We first characterized the immune landscape using cytometry by time-of-flight (CyTOF) upon aging in 20 month old mice and observed that memory T cells expanded in the circulation and that specifically effector CD8 T cells expressing programmed cell death protein 1 (PD-1) and tissue-resident memory CD8 T cells accumulated in the aged brain. Injections of aged plasma derived from old mice decreased the frequency of circulating naïve T cells and increased regulatory T cells and patrolling Ly6C^{lo} PDL1^{+} monocytes in the blood of adult receiving mice. In addition, aged plasma led to higher numbers of splenic memory CD8 T cells and Ly6C^{lo} PDL1^{+} monocytes. Finally, we found that CD8 T cells accumulated within the brain parenchyma of adult mice treated with aged plasma, which correlated to higher levels of vascular cell adhesion molecule 1 (VCAM-1) expression on the brain vasculature. Taken together, our data highlights the role of CD8 T cells in the aging brain and suggests involvement of age-associated proteins in the circulation that cause CD8 T cell migration and expansion in the brain parenchyma.

342 - Deranged granulopoiesis drives poor outcome of ischemic stroke in the elderly

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Ischemic stroke is one of the leading causes of disability and mortality worldwide, recognizing aging as a prominent risk factor and determinant of dismal outcome. Aging is known to lead to overall frailty due to multifactorial changes, but pathogenic mechanisms underlying poor outcome remain unclear. Here we show that deterioration in elderly stroke is preceded by neutrophil accumulation in the brain.
With high-dimensional single-cell profiling of the blood we could delineate after stroke diverse granulocyte clusters whose quantitative release is deranged in the old. Functionally, adoptive transfer of neutrophils from old mice into young stroke mice leads to worse outcome. Conversely, rejuvenation of the hematopoietic niche by

transplantation of total bone marrow from young into aged mice rebalanced the number of granulocyte-monocyte progenitors (GMPs) in the marrow and the circulating neutrophil subsets, and led to ameliorated brain injury and stroke outcome.
Our results demonstrate how age-related alterations of granulocytes have a relevant pathogenic role in one of the main cerebrovascular disorders affecting the world population.

328 - Aged CNS-resident cells promote a non-remitting course of experimental autoimmune encephalomyelitis

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Chronological age is a risk factor for the development of progressive multiple sclerosis (pMS), both the presentation of primary pMS, and the conversion from relapsing-remitting to secondary pMS. Accumulation of disability during pMS correlates weakly with the frequency of contrast enhancing radiological lesions, and is relatively unresponsive to disease modifying therapies that target peripheral immune cells. Conversely, slowly expanding lesions with a rim of activated microglia, and widespread microglial activation, are more characteristic of pMS. Collectively, these observations have led to the hypothesis that CNS injury in progressive disease is mediated to a greater extent by CNS-resident cells than CNS-infiltrating leukocytes. To assess the effect of biological ageing on autoimmune neuroinflammation, we compared Th17 adoptive transfer experimental autoimmune encephalomyelitis (EAE) in young versus middle-aged mice. Middle-aged transfer recipients exhibited an exacerbated clinical disease course with high peak disability scores and mortality rates, and a non-remitting course. In contrast, young recipients displayed a milder course with no mortalities, and the majority underwent remission. While the total number of $CD45^{hi}$ immune cells in the CNS did not differ significantly between the 2 groups, infiltrates in in middle-aged mice had a relative preponderance of donor $CD4^{+}$ T cells and neutrophils, and a dearth of B cells. CNS GM-CSF levels were elevated in middle-aged versus young recipients. During the early phase of disease, neutralization of GM-CSF reduced disease severity in middle-aged recipients to a level comparable to young recipients. However, during later stages of EAE, anti-GM-CSF treatment did not curtail clinical EAE severity or mortality rates in middle-aged recipients. Experiments with reciprocal bone marrow chimeras demonstrated that the age of the host mice, as opposed to the bone marrow donors, determines the clinical phenotype. Microglia in the inflamed aged CNS exhibited a distinctive pro-inflammatory phenotype and transcriptome. These results suggest that the aged CNS micro-environment, which includes highly activated microglia, supports a robust local encephalitogenic T cell response and drives a non-remitting clinical course. The exacerbated clinical course in aged mice appears to be GM-CSF driven at presentation. Alternative mechanisms contribute to disability progression as the disease evolves.

267 - Ageing promotes non-remitting experimental autoimmune encephalomyelitis with persistent meningeal inflammation and subpial demyelination in SJL/J mice

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Current therapies for multiple sclerosis (MS) reduce the frequency of relapses by modulating adaptive immune responses, but fail to stop progressive disability. Subpial demyelination, meningeal inflammation and neurodegeneration are key hallmarks of progressive MS, however mimicking these features in a single animal model has been challenging. Adoptive transfer of proteolipid protein-primed Th17 cells into young (~8 weeks old) SJL/J recipient mice induces experimental autoimmune encephalomyelitis (EAE) characterized by subpial demyelination associated with microglial/macrophage activation, disruption of the glial limitans, and evidence of an oxidative stress response that is topologically associated with foci of immune cells in the meninges. However, after peak disease young adoptive transfer SJL/J nevertheless remit. Since age is a risk factor for developing progressive MS, we hypothesized that adoptive transfer of proteolipid protein-primed Th17 cells into aged (~8-

12 months old) SJL/J recipient mice would promote non-remitting disease. Indeed, transfer of cells in aged recipient mice resulted in a non-remitting clinical course compared to young recipients. Although both young and old recipient mice harboured similar numbers and types of CNS-resident T cells, compared to young mice old EAE mice showed persistent meningeal inflammation, and enhanced cortical pathology including increased subpial demyelination, microglia/macrophage activation, glial limitans disruption, axonal injury and synapse loss compared to young EAE mice which by day 25 post-adoptive transfer had largely recovered. Furthermore, compared to young mice, old EAE mice had higher levels of neurofilament light chain in their serum and exhibited more pronounced brain volume loss by MRI. Single-cell RNA sequencing analysis of the meninges and the cortex at the peak of disease, when disease severity is similar between aged and young mice, revealed an altered abundance of B cells and neutrophils in the brains of old mice that were confirmed using flow cytometry. In conclusion, adoptive transfer of proteolipid protein-primed Th17 cells into young and aged recipient mice provides opportunities to assess how aging impacts meningeal inflammation and brain pathology, and provides a valuable tool for assessing the effect of disease modifying therapies on brain-intrinsic phenotypes that are often observed in progressive MS.

307 - Chronic demyelination-induced cell senescence is associated with motor impairment in a model of MS

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Advancing age is the strongest predictor for the transition from the early relapsing phase of MS that is considered primarily inflammatory, to PMS that is thought to be mainly neurodegenerative. Cell senescence (CS) is a biological process associated with aging that might be responsible for progressive worsening in MS. To test the hypothesis that CS contributes to axonal damage and motor impairment in chronic demyelination. we used a novel model of CNS demyelination induced by prolonged dietary cuprizone (CPZ) in C57BL/6 mice. Established markers were used to study the DNA damage response (DDR) and CS in different cell types at characteristic pathological time points of disease. Motor performance was assessed with the rotarod and grip strength tests. We found that senescent cells accumulate in the corpus callosum during demyelination in young mice. Increased expression of senescence-associated β-galactosidase (SA-β-gal) activity starting from the early phase of demyelination was observed in CPZ-treated mice compared to naïve controls. We also found increased immunostaining for the phosphorylated histone H2AX (γH2AX form), an early sign of DNA damage, and increased autofluorescence due to lipofuscin accumulation in chronic lesions compared to naïve controls. Chronic demyelination was associated with loss of motor performance as measured by rotarod and grip strength tests. Interestingly, low-dose rapamycin treatment, a potent inhibitor of mTOR which inhibits CS, prevented this loss of motor performance and also showed a trend towards reduced SA-β-gal staining in the medial corpus callosum in the chronic lesions. We next tested the effect of natural aging on the development of demyelinating lesions, motor function and the development of CS using this model. Demyelination, neuroinflammation (microglia) and axonal damage were delayed in aged (12- and 24-month-old) compared to young mice. Despite this delay in pathology, CS was increased in the corpus callosum of aged compared to young mice, seen by SA-β-gal staining and autofluorescence elements. Aged mice also showed accelerated loss of motor function by the rotarod test. Together, these results indicate that chronic demyelination induces premature CS of non-neuronal cells in brain white matter, which is associated with motor impairment. Also, natural aging exacerbates the development of CS and motor dysfunction induced by chronic demyelination.

Workshop VII | Neuroimmunology of the eye and the optic nerve

202 - Is it the Vaccine? Case Reports and Systematic Review of Postvaccination Neuromyelitis Optica Spectrum Disorder

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Background: The triggers of neuromyelitis optica spectrum disorder (NMOSD) are obscure. Vaccination have been observed to precede certain cases of NMOSD. Amidst the Coronavirus disease 2019 (COVID-19) pandemic, mass vaccination has unveiled two cases of newly diagnosed NMOSD following COVID-19 vaccination so far.

Objectives: To present two cases of post-COVID-19-vaccination NMOSD and systematically review previous reports.

Methods: Searching of Ovid MEDLINE and EMBASE databases was done independently by two investigators using predefined search terms related to NMOSD and vaccination. Newly diagnosed NMOSD cases fulfilling the 2015 International Panel for NMO Diagnosis criteria with symptoms presenting between 2-30 days after vaccination were included together with 2 cases from this report. Using a standardized table, data on age, sex, comorbidity, vaccine name, type, and dose number, duration from vaccination to symptom onset, clinical phenotypes, MRI findings, CSF profiles, severity of attack, initial and maintenance treatment, number of relapses after vaccination, and clinical outcomes were extracted and compared.

Results: Ten cases of postvaccination NMOSD, aging between 15-46 years, were identified. Nine patients (90%) presented with transverse myelitis and 3 (30%) with optic neuritis. The median duration from vaccination to onset was 9 (range 2.5-14) days. Five patients (50%) tested positive for aquaporin 4 (AQP4) antibody and one had a family history of NMOSD. Three-fourths of AQP4-IgG seropositive patients with myelopathy had short-segment transverse myelitis. The reported vaccines included CoronaVac, ChAdOx1 nCoV-19, yellow fever, quadrivalent influenza, H1N1 influenza, quadrivalent human papilloma virus, Japanese encephalitis, rabies, and recombinant hepatitis B virus together with tetanus-diphtheria-pertussis vaccines. All patients received high-dose steroids for initial treatment and 2 received additional therapeutic plasma exchange. Maintenance therapy was given in 4 patients. Five patients (50%) had no subsequent relapse within the follow-up period ranging between 3-34 months. Almost all patients returned to baseline functional status.

Conclusions: Postvaccination NMOSD is a rare condition that has been observed with various types of vaccines. The short temporal relationship between vaccination and onset of NMOSD and the family history of NMOSD in one patient indicate that vaccine might be a trigger for genetically predisposed individuals.

254 - The early emergence of aquaporin 4-specific B cells characterises neuromyelitis optica spectrum disorders

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Background Immunoglobulin G (IgG) antibodies to aquaporin-4 (AQP4) are pathogenic in patients with neuromyelitis optica spectrum disorder (NMOSD). We sought to identify the developmental stages of AQP4-specific B cells in NMOSD, and characterise the evolution and pathogenicity of their B cell receptors (BCRs).
Methods Peripheral blood mononuclear cells (PBMCs) were isolated from 4 NMOSD patients and 2 healthy controls. PBMCs were single cell sorted from new emigrant, naïve and memory B cells, and differentiated into antibody secreting cells *in vitro*. Supernatants were tested for AQP4 and HEK-cell reactivity and these monoclonal antibodies (mAbs) were recombined and characterised.
Results 2/7680 (0.026%) BCRs from healthy controls showed AQP4 or HEK-reactivity. In contrast, across 4 NMOSD patients, 36/14304 B cells were AQP4-specific (0.25%,p<0.0001). The AQP4-specific B cells showed increasing mutational rates and reducing frequencies across stages of B cell development comprising 1:148 (0.67%) new emigrant but only 1:936(0.1%) mature naïve and 1:710(0.14%) memory B cells (p<0.0001). This result was mirrored by the frequency of HEK-reactive B cells (n=30). There was no preferential Ig variable heavy or light chain family usage in AQP4-specific BCRs, but 18/18(100%) HEK-reactive B cells were of the polyreactive IGHV4-34 family. Both AQP4-specific and HEK-reactive sequences had a more positive CDR3 charge compared to non-reactive sequences (p=0.0046,p<0.0001). The AQP4-specific new emigrant B cells showed the longest CDR3 lengths compared to HEK-reactive and non-reactive sequences (p=0.0038,p<0.0001). Binding strengths revealed increasing avidities in memory vs naïve vs new emigrant derived mAbs. 3 AQP4-specific memory IgGs that had acquired somatic mutations were reverted back to their germline precursor (unmutated common ancestor UCA), and retained AQP4-specific binding. These UCAs demonstrated similar complement mediated cytotoxicity to new emigrant, naïve, and memory mAbs *in vitro*; and *in vivo,* had the potential to cause astrocyte loss.
Conclusion In NMOSD patients, we observe both AQP4-specific central and peripheral checkpoint perturbations with frequent AQP4-specific reactivities in the earliest new emigrant B cells. Their characterisation across a developmental lineage revealed differences in sequences, mutation rates, CDR3 characteristics and binding strengths with the identification of UCAs that retain high binding strength and pathogenic potential.

201 - Aberrant DNA methylation of Runx1 promotes Th17 induced autoimmunity in Neuromyelitis Optica Spectrum Disorders

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73 - ATP mediates neuropathic pain in neuromyelitis optica via microglial activation

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Intractable neuropathic pain is a common symptom of neuromyelitis optica spectrum disorder (NMOSD). Current therapeutics for NMOSD pain, including antiepileptic agents, anti-spasticity medications, and opioids, provide insufficient relief of the symptoms. However, the underlying mechanism of NMOSD pain remains to be elucidated. The aim of this study was to establish a novel animal model of NMOSD pain and to investigate its pathogenic mechanism. We established a NMOSD pain model by injecting anti-AQP4 recombinant autoantibodies (AQP4-Ab) from NMOSD patient plasmablasts into rat spinal cords at the tenth thoracic vertebral level. Development of mechanical allodynia was confirmed in the NMOSD pain model. In this study, we sought to clarify whether ATP is involved in the pathogenesis of NMOSD pain. Damage-associated molecular patterns (DAMPs), including ATP, are known to accelerate innate immune responses. Moreover, the pivotal roles of ATP and purinergic receptors have been demonstrated in a peripheral neuropathic pain model. Transcriptome analysis of NMOSD rat spinal cord

revealed upregulation of some purinergic receptor-related genes. AQP4-Ab mediated extracellular ATP release from rat astrocytes *in vitro.* Pharmacological inhibition of ATP receptor reversed mechanical allodynia in the NMOSD pain model. Furthermore, transcriptome analysis revealed elevated level of IL-1β in the NMOSD spinal cord. Inhibition of microglial activation and neutralization of IL-1β also attenuated neuropathic pain in the NMOSD rat model. In patients with NMOSD, CSF ATP concentration was significantly higher in the acute and remission phases than those of multiple sclerosis or other neurological disorders. ATP is previously shown to activate microglia and further promote secretion of IL-1β, which result in the activation of neuronal cells in a peripheral neuropathic pain model. However, there has been no previous report investigating the involvement of ATP in NMOSD. In this study NMOSD pain model was established and ATP, microglial activation, and IL-1β secretion was shown to orchestrate the pathogenesis of NMOSD-related neuropathic pain.

377 - Subretinal Associated Immune Inhibition, a new antigen-specific immunosuppressive mechanism in the context of AAV gene transfer

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While subretinal injection of adeno-associated virus (AAV) gene therapy vectors can successfully treat several inherited retinal diseases, some patients display inflammatory events. The eye is known as an immune-privileged site but anti-capsid and anti-transgene immune responses have been reported in some patients, possibly contributing to the loss of transduced cells. We previously reported that a subretinal injection of AAV8 triggers a systemic anti-transgene T-cell response in a dose-dependent manner, and that an antigen introduced into the subretinal space can provide a systemic antigen-specific immunosuppression referred to as subretinal-associated immune inhibition (SRAII). Here, we hypothesize that peptides from the transgene product, have utility as SRAII inducers when introduced simultaneously with the AAV. A single subretinal injection of AAV8 with peptides from the transgene product was performed. The transgene cassette encoding GFP and HY male antigen, containing MHC class I- and MHC class II-restricted T cell epitopes (UTY and DBY peptides immunodominant in H-2b female mice), was packaged into AAV8 under the ubiquitous PGK promoter and injected subretinally with or without HY peptides at day 0 in female wild type C57BL/6 and pathophysiological rd10 mice. ELISpot and multiplex assays were done 3 to 14 days post injection for systemic anti-transgene specific primary T-cell response evaluation, or at day 21 (following a subcutaneous challenge at day 14 with HY peptides adjuvanted in CFA) for memory T-cell response analysis. We found in both models that: (i) subretinal injection of 2.10e9 or 5.10e10 vg of AAV8-PGK-GFP-HY triggered a dose-dependent systemic primary and memory anti-transgene Th1/Tc1 responses, (ii) the simultaneous co-injection of AAV8 and of HY peptides inhibited both CD8+ and CD4+ T-cell specific primary and memory responses against HY even at high dose (5.10e10 vg) of AAV. SRAII phenomenon seems to be a powerful systemic immunosuppressive mechanism specific to a transgene expressed in an eye. Since we have confirmed these results in the rd10 pathophysiological context, co-injection of the transgene product and the therapeutic vector may be considered as a new immunomodulatory strategy to control inflammatory reactions in the context of ocular gene therapy.

The authors have to declare that a patent has been filed based on these results

162 - Role of P2X7 receptor in mouse experimental autoimmune uveitis (EAU)

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Uveitis are inflammatory eye diseases of various origins which can be very debilitating or even blinding. Damaged cells release high level of extracellular ATP which constitutes a potent danger signal. The purinergic receptor P2X7 is the main sensor of high amounts of ATP and its activation triggers an inflammatory response and/or induces cell death. Thus, P2X7 may contribute to the development of retinal damages via its expression on immune cells. Our data showed that P2X7 is expressed in the different immune cell subpopulations in the retina during EAU. Given the multiple functions of P2X7, its activation could play distinct roles in the development of the disease

depending on the cell type that expresses it. We investigate the function of P2X7 in EAU using new conditional P2X7 knockout mouse line. While we did not find any significant effect of total P2X7 invalidation, the severity of EAU was significantly reduced when P2X7 was specifically depleted, in particular in retinal microglia/mononuclear phagocytes. Thus, P2X7 conditional knock-out mice allow us to define the role of P2X7 in EAU.

Workshop VIII | Sex chromosomes and hormones in neuroimmunology

276 - In utero exposure to maternal Anti-Caspr2 antibodies alters microglial activation and development in male offspring

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Exposure in utero to maternal anti-brain antibodies (IgG) has been associated with an increased risk of having a child with Autism Spectrum Disorder (ASD). Caspr2 protein (Contactin-associated protein-like 2, encoded by the ASD risk gene, CNTNAP2) is one of the most common targets of these maternal antibodies. We have shown that male, but not female mice born to dams immunized with the extracellular portion of Caspr2 show an ASD-like phenotype. Here, we examine the role of microglia in mediating the ASD-like phenotype in mice exposed in utero to anti-Caspr2 ("Anti-Caspr2") IgG or to adjuvants alone ("Control"). We observed increased microglial activation in E18.5 and adult Anti-Caspr2 male mice, corresponding to decreased dendritic arborization and reduced spines in the CA1 region of the hippocampus. Single nucleus (sn) RNA-seq of adult hippocampus from Anti-Caspr2 and Control male mice demonstrates that microglia may be halted in an immature, pruning stage in these mice. In adulthood, depletion and subsequent reconstitution of microglia using PLX3397, an inhibitor of colony stimulating factor 1 receptor, does not restore normal dendritic arborization nor does it change the activated phenotype of microglia. This suggests that in utero exposure to maternal anti-Caspr2 IgG affects microglial programming in males but not females, with male microglia arrested in an immature/pre-microglia stage, leading to sustained pruning. We hypothesized that early suppression of microglial activation using captopril, an FDA approved drug for hypertension and heart failure that has previously been shown to suppress microglial activation, will prevent the decreased dendritic arborization and reduced spines in the hippocampus that have been noted in Anti-Caspr2 male mice. Preliminary data from this study shows that captopril suppresses microglial activation in anti-Caspr2 male mice compared to vehicle-treated anti-Caspr2 male mice. If successful, this study will make important advances that integrate an understanding of environmental risk factors and microglial function to explore novel therapeutic strategies.

67 - Suppression of the disease-relevant brain-homing T cell in MS by nuclear receptor crosstalk

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In multiple sclerosis (MS), pro-inflammatory $CD4^+$ T cells invade the central nervous system, but it remains elusive which subset contributes to disease activity. In this study, we aimed to uncover pro-inflammatory T-cell subsets driving disease activity to better predict and treat the MS course. We identified with FACS distinct pro-inflammatory $CD4^+$ T helper (Th) cell subsets based on receptor expression profiles: Th1 ($CCR6^-CCR4^-CXCR3^+$; IFN-

y^{dim}GM-CSF^{low}IL-17^{neg}), Th17 (CCR6^{+}CCR4^{+}CXCR3^{-}; IFN-y^{neg}GM-CSF^{dim}IL-17^{high}), Th17 DP (CCR6^{+}CCR4^{+}CXCR3^{+}; IFN-y^{low}GM-CSF^{dim}IL-17^{dim}) and Th17.1 cells (CCR6^{+}CCR4^{-}CXCR3^{+}; IFN-y^{high}GM-CSF^{high}IL-17^{low}). Cells from several distinct MS cohorts (pregnant, treatment-naive early-stage, late-stage disease) and compartments (blood, cerebrospinal fluid, postmortem brain tissue) were assessed. Furthermore, cytokine production (Luminex), transmigration and glucocorticoid sensitivity (proliferation, activation) were determined *in vitro*. Multidrug resistance 1 (MDR1) and glucocorticoid (GR) receptor expression were measured with qPCR and FACS. We demonstrated that circulating Th17.1 cells are reduced in treatment-naive patients with rapid disease onset and are elevated in patients responsive to natalizumab (anti-VLA-4 mAb). Memory CD4^{+} T cells from pregnant MS patients that experienced a relapse postpartum, produced high levels of Th17.1-related IFN-γ and GM-CSF as compared to pregnant non-relapsing and control groups (n=19). Relapsing patients had decreased frequencies of circulating Th17.1 cells after delivery, suggesting migration into the central nervous system. Accordingly, Th17.1 cells predominated the cerebrospinal fluid from 15 early MS in contrast to 8 neurological control patients *ex vivo* and preferentially crossed human brain endothelial layers *in vitro*. This corresponded to a glucocorticoid resistance profile (MDR1^{high}GR^{low}), which characterized Th17.1 cells present in MS brain tissue (*in situ* and *ex vivo*; n=14). Their glucocorticoid insensitivity was reversed *in vitro* by female hormones and vitamin D_3. These results indicate that glucocorticoid-resistant Th17.1 cells are promising candidate markers for predicting MS disease activity and that the interplay between steroid hormones may be used to suppress this brain-homing T cell.

116 - Regulatory T cells contribute to sexual dimorphism in neonatal hypoxic-ischemic brain injury

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Neonatal encephalopathy caused by hypoxia-ischemia (HI) is a major cause of death and disability in newborns. Clinical and experimental studies suggest a sexual dimorphism in HI induced brain injury and therapy responses. A major hallmark of HI pathophysiology is the infiltration of peripheral immune cells into the injured brain. However, the specific role of regulatory T cells (Tregs) is still unknown. Nine-day-old mice were exposed to HI by ligation of the right common carotid artery followed by 1 h hypoxia (10% oxygen). Using immunohistochemistry, flow cytometry and microarray analyses, Tregs were investigated in the brain, spleen and blood 24 h post HI. The functional role of Tregs was evaluated by acute Treg depletion in DEREG mice. Brain injury, neuroinflammatory responses and vascular injury were analyzed via immunohistochemistry and western blot 48 h and 7 days after HI. Females revealed an increased cerebral Treg infiltration, coinciding with elevated chemokine receptor expression. Treg depletion in females aggravated HI-induced brain tissue injury, associated with enhanced microglia and endothelial activation and leukocyte infiltration. Treg depletion in males resulted in neuroprotection, associated with reduced astrogliosis and vascular injury. *Ex vivo* isolated female Tregs displayed an increased immunosuppressive activity associated with an altered transcriptional profile compared to male Tregs. The present findings demonstrate that Tregs from female mice provide endogenous neuroprotection, whereas Tregs from male mice enhance secondary neurodegeneration. As potential mechanisms, we identified intrinsic transcriptional differences associated with enhanced anti-inflammatory activity of female Tregs and non-immunological detrimental effects of male Tregs, related to vascular damage. Our study emphasizes the urgent need for sex-stratified clinical and pre-clinical analyses.

264 - Antidiabetic and antiaging role of Moringa oleifera on hippocampus of experimental male rats

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Objective: The objective of this study was to investigate protective effects of methanolic extract of Moringa oleifera leaves on membrane function, mitochondrial and antioxidant enzymes, oxidative stress biomarker, and expression of glucose transporters in hippocampus of diabetic aging male rats.

Methods: Young (3 months) adult (12 months) and aged (24 months) rats will be diabetic by using alloxan monohydrate. Methanolic extract of Moringa oleifera leaves (MOL) was administered i.p. at a dose of 200 mg/kg/day for 30 days to both control and diabetic aging rats. The effect of extract on serum glucose, glycated hemoglobin, plasma insulin and the levels of thiobarbituric acid reactive substances (TBARS), hydroperoxides (HP), conjugated dienes (D), activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-reductase (GRD) and reduced glutathione content (GSH) were estiated. Metformin and atorvastatin were used as standard drugs. A detailed study was carried on membrane fluidity, lipofuscin, antioxidant enzymes and DNA degradation to identify the antidiabetic and antiaging role of MOL using biochemical ,molecular and histochemical study.

Results: Present study shows that there was a similar pattern of increased TBARS, lipofuscin, DNA degradation and glucose transporters and a decrease in membrane fluidity, glutamate dehydrogenase , Na^+ K^+ ATPse, antixodant enzymes activities in both aging and diabetes. MOL treatment helped to reverse the age related changes studied, to normal levels, elucidating an anti-aging, antidiabetic and neuroprotective action. MOL effectively countered the diabetes-induced structural abnormalities of hippocampus of aging rats.

Conclusions: MOL was found to be an effective treatment in stabilizing and normalizing the neuronal functions; therefore this therapy can be considered an alternative to be explored further as a means of diabetic and aged related disorders control. The results of this study will be useful for pharmacological modification of the aging process and applying new strategies for control of age related disorders including metabolic syndrome.

133 - CD300f immune receptor contributes to healthy aging by regulating inflammaging, metabolism and cognitive decline

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Emerging evidences suggest that immune receptors participate in many aging related processes such as metabolism, inflammation, and cognitive decline. CD300f is a lipid sensing immune receptor that shares many properties with TREM2. CD300f has been shown to be important to limit the severity of inflammatory conditions by negatively regulating the innate immune system. The absence of SiglecE inhibitory immune receptor shortened mice lifespan, and aging-induced microglial and macrophage metabolism alterations can influence cognitive decline. This puts forward the question whether immune receptors expressed on myeloid cells, and in particular CD300f, can regulate systemic aging-related processes such as metabolism, inflammation and ultimately aging and healthy lifespan. We aged CD300f-/- and WT mice and followed them closely for 30 months. Strikingly, three different cohorts of male and female CD300f-/- mice showed an important reduction in lifespan. Moreover, it was

observed under both specific pathogen free (SPF) conditions and in a closer to real-life housing/immunologic environment. No single evident cause of death in CD300f-/- mice could be determined as expected for aging driven multi-cause deaths. Aging enhanced the progressive liver accumulation of immune infiltrates in CD300f-/- mice from six months of age. The brain also showed increased inflammaging as observed by RNAseq. Interestingly, CD300f deficiency drove cognitive decline in 18-24 months old mice, as observed by alterations in Novel Object Recognition and Barnes Maze learning and memory tests. Brain hypometabolism observed by ^{18}FDG PET scans was observed in aged 18 months old CD300f-/- female mice. These brain function alterations were correlated with increased lipid droplet containing microglia, increased expression of Disease Associated Microglia genes, alterations in some AD associated genes and in frailty markers. In support of the increased frailty of aged CD300-/- mice, they also display motor coordination deficits at 25 months. While adult male and female CD300f-/- mice showed no alteration in glucose buffering capacity, aged female CD300f-/- mice showed alterations in glucose buffering and reduced hepatic gluconeogenesis capacity. Taken together, we present novel data supporting the hypothesis that the study of the biology of immune receptors in the context of aging contributes to the elucidation of novel predictors of both health and lifespan, and may identify therapeutic targets for attenuating aging and abrogating age-related diseases.

ORAL PRESENTATIONS - NOVEMBER 11, 2021

Workshop IX | Behavior and immunity

42 - Brain-Immune Axis Regulation is Responsive to Cognitive Behavioral Therapy and Mindfulness Intervention: Observations from a Randomized Controlled Trial in Patients with Crohn's Disease

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Nemirovsky A and Ilan K contributed equally.

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**The institutional ethics committees of Soroka Medical Center and Rabin Medical Center approved the trial. All participants were given a detailed written and oral description of the research project and provided their written informed consent.*

Background Crohn's disease (CD) is a chronic inflammatory bowel disease associated with psychological stress that is regulated primarily by the pituitary-hypophysis-adrenal (HPA) axis. Here, we determined whether the psychological characteristics of CD patients associate with their inflammatory, and microbial state, and whether a 3-month trial of cognitive-behavioral and mindfulness-based stress reduction (COBMINDEX) impacts their inflammatory process.

Methods Circulating inflammatory markers, a wide range of psychological parameters related to stress and well-being, and microbial stool samples were measured before and after COBMINDEX.
Results CD patients exhibited increased peripheral low-grade inflammation compared with HCs, demonstrated by interconnected inflammatory modules, and significantly higher anti-commensal antibodies levels. Notably, higher IL-18 levels correlated with higher score of stress and a lower score of wellbeing in CD patients. COBMINDEX induced significant microbial changes in both beta and alpha diversity and was accompanied by changes in inflammatory markers that coincided with changes in cortisol: changes in serum levels of cortisol correlated positively with those of IL-10 and IFN and negatively with those of MCP-1. Finally, inflammatory markers of CD patients at baseline predicted COBMINDEX efficacy and correlated with possible microbial targets; higher levels of distinct cytokines and cortisol at baseline, correlated negatively with changes in disease activity, distress scores, and specific bacterial phyla.
Conclusions CD patients have a characteristic immunological and microbial profile that correlates with well-being, psychological stress, and the disease process. We suggest that COBMINDEX induces stress resilience in CD patients, which not only impacts their well-being, but also their disease-associated inflammatory and microbial process.

92 - The modulatory effect of melatonin on the behavior in rats under experimental conditions of inflammation models

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Inflammation and the action of proinflammatory cytokines on brain structures is one of the factors of cognitive dysfunction, adaptive behaviour and neurodegenerative diseases (Alzheimer's disease, multiple sclerosis, Parkinson's disease). We investigated an ability of the epiphyseae hormone melatonin to modulate behavioral responses in adult male Wistar rats under semi-chronic exposure to Salmonella typhi lipopolysaccharide (LPS). The LPS administration was used as an adequate model for the development of peripheral and central inflammation. Rats in Group A (n=15) received the LPS injections (i.p., 50 μg/kg) for 10 days. Rats in Group B (n=15) received LPS (50 μg/kg) with melatonin (orally, 0.5 mg/kg) for 10 days. Rats in the control group (n=15), received 1 ml of physiological solution. The behavior of the rats in all experimental groups was tested in the following experimental conditions: an open field, an elevated plus maze, the Barne's maze, an extrapolation escape task. The rats' behavior was observed for two hours after the experimental exposures on days 1, 3, 5 and 10. One day after the LPS administration, groups A and B demonstrated the decrease in horizontal motor activity in the open field and the increase in anxiety in the elevated plus maze. The most pronounced behavioural effects were seen on the 10th day after the LPS administration. Rats in Group A, showed the higher level of motor activity, and entered more often into the central sector in the open field. In the elevated plus maze, the melatonin increased the time the rats in group A spent in the open arms in comparison to rats in group B in the same experimental conditions. In the Barne's maze, the rats in group A spent less time to find the true shelter. In the extrapolative escape setting, the rats which received melatonin performed the escaping task faster in stressful situation. Based on the results of this study, it could be concluded that melatonin maintains the higher level of motor activity in rats and increases their cognitive function under conditions of peripheral and central inflammation. It is likely that the effects of melatonin could be related to its immunomodulatory and neurotropic attributes.

222 - Role of meninges in activity-induced neurogenesis

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In line with their protective role on the central nervous system, meninges provide trophic factors that promote neurogenesis in the developing and adult brain. Meninges contain a large population of resident immune cells,

including macrophages, at the interface between the brain and the periphery. As adult neurogenesis is promoted by environmental factors such as physical activity, we investigated whether meningeal macrophages could influence adult neurogenesis upon voluntary exercise. Using innovative transcranial delivery of the CSF-1R inhibitor, PLX3397, we depleted meningeal macrophages in mice undergoing exercise for 2 weeks. Using immunohistochemistry, we observed that neurogenesis was increased in the subventricular zone of placebo-injected running mice. Importantly, meningeal macrophages depletion abolished this effect. To better understand the underlying mechanisms, we performed single-cell RNA sequencing of meningeal cells in mice with or without exercise. Even at steady state, meningeal macrophages expressed Insulin Growth Factor 1 (IGF1), a peptide hormone playing a role in neurogenesis. However, LysM-Cre : Igf1fl/fl mice did not present a defect in neurogenesis, suggesting that other neurogenic factors might be involved. We are now exploring candidate genes based on our single-cell RNA datasets. We hope that this work will open up new perspectives on the modulation of neurogenesis.

277 - Deleterious role of microgliosis after perinatal cerebellar injury on anxious behaviors in adult mice

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Up to 19% of very preterm infants have been diagnosed with perinatal cerebellar injuries (CBI) during their neonatal intensive care unit stay. Perinatal cerebellar hemorrhage (CBH) and postnatal infection are known as two major risk factors for neurodevelopmental impairments in preterm infants. Using a newly designed translational mouse model of CBI, we described a massive CBI-induced microgliosis prior to long-term neurobehavioral deficits. To study if microglial cell contributes to the pathogenesis of CBI, we experimentally induced right-sided CBH and/or early inflammation state (EIS) in 2 days-old transgenic mice (B6.129P2(Cg)-*Cx3Cr1*$^{CreERT2\text{-}EYFP/iDTR}$) with or without transient right-sided cerebellar microglial cell depletion. Sensorimotor and neurobehavioral testings were blindly performed at P4-P14 (Grasping reflex acquisition) and at P60-P85 (Marbles burying, Open field, Y maze, Elevated Plus Maze, Social recognition test, Morris water maze, Rotarod and Footprint analysis). Our preliminary results suggested that without microglial cell depletion, males exposed to EIS spent significantly (*P=.014*) more time in the opened arms of the elevated plus maze (20.7±4.2 %, *n*=5) compared to controls (5.3±1.3 %, *n*=6). Similarly, in the CBH+EIS group without microglial cell depletion, males tended to spend more time in the opened arms of the elevated plus maze (12.9±3.0 %, *n*=10) and more time in the central area of the open field (8.2±1.3 %, *n*=10) compared to controls (5.3±1.3 % and 6.2±1.2 % respectively; *n*=6). Microglial cell depletion prior to insult seemed to attenuate these altered anxious behaviors (time in opened arms of the elevated plus maze: EIS 9.0 ± 1.8 %, *n*=11; time in the central area of the open field: CBH+EIS 7.4 ± 0.9 %, *n*=7). Although a larger number of animals is required to validate these results and perform sex difference analysis, our model underlined alterations of anxious behaviors in adult mice exposed to CBI. By decreasing the numbers of microglial cells prior to insult, we may protect the developing cerebellum from injuries and improve long-term functional outcomes.

122 - Cytotoxic NK-like CD8+T cells, the reservoir of clonal cells, are related to disease activity in MS

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Keywords: Multiple sclerosis, NK-like CD8+T cells, Cytotoxicity

Memory CD8+ T cells are key players in multiple sclerosis (MS) as they predominate at the lesion sites of the central nervous system (CNS), with an oligoclonal repartition both in the periphery and the lesions, but yet, the culprit CD8+T cells driving autoimmune inflammation have not been identified. We hypothesized that the cells able to provoke damages in the CNS, may have a specific phenotypic and functional pattern. To identify a cell subtype involved in MS, we used a single-cell high dimensional profiling of peripheral memory CD8+ T cells combined with TCRβ sequencing on a cohort of MS patients, healthy controls (HC) and patients with other inflammatory diseases of the CNS (OID). The involvement of a cells subtype in MS was then confirmed by flow cytometry analysis. We then deepened the knowledge of the identified cells with RNA sequencing analysis. Finally, we analyzed the function of these cells with functional assay and determined their infiltration in the CNS by immunofluorescence. The single-cell analysis allows us to identify an effector memory CD8+ T cells subtype increased in MS patients compared to HC and OID. These cells expressed numerous markers usually associated to NK cells (among other the CD94 marker) and cytotoxicity (Perforin, Granzyme, Granulysin). These cells, that we thus called NK-like CD8+ T cells, are preferentially found during relapse episodes and the high throughput sequencing of the TCR repertoire in the samples and comparison of single cell TCR shows that these cells belong to a reservoir of peripheral oligoclonal cells. The increase of these cells subtype in MS patients is also confirmed at the protein level by the flow cytometry analysis on blood samples. Interestingly, we also found the CD8+CD94+ T cells in MS lesions and particularly in the chronic active lesions. Finally, the functional assay shows that these NK-like CD8+ T cells exert cytotoxicity function against the K562 cell line. The K562 cells being devoid of MHC molecules, this result suggests that the cell cytotoxicity that we observed is absolutely independent of the TCR involvement. Taken together, our data are the first to describe a memory CD8+ T cells with NK-like properties that are specific of MS patients, belong to an oligoclonal reservoir of peripheral T cells and that are mobilized in the periphery during inflammation to enrich lesions of the CNS. These NK-like CD8+ T cells exert cytotoxicity against target cells independently to any TCR involvement.

Workshop X | The sensory and autonomic nervous systems: links with inflammation

63 - Meningeal inflammation in multiple sclerosis induces phenotypic changes in cortical microglia that differentially associate with neurodegeneration

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Meningeal inflammation strongly associates with demyelination and neuronal loss in the underlying cortex of progressive MS patients, thereby contributing significantly to clinical disability. However, the pathological mechanisms of meningeal inflammation-induced cortical pathology are still largely elusive. By extensive analysis of cortical microglia in post-mortem progressive MS tissue, we identified cortical areas with two MS-specific microglial populations, termed MS1 and MS2 cortex. The microglial population in MS1 cortex was characterized by a higher density and increased expression of the activation markers HLA class II and CD68, whereas microglia in MS2 cortex showed increased morphological complexity and loss of P2Y12 and TMEM119 expression. Interestingly, both populations associated with inflammation of the overlying meninges and were time-dependently replicated in an *in vivo* rat model for progressive MS-like chronic meningeal inflammation. In this recently developed animal model, cortical microglia at 1 month post-induction of experimental meningeal inflammation resembled microglia in MS1 cortex, and microglia at 2 months post-induction acquired a MS2-like phenotype. Furthermore, we observed that MS1 microglia in both MS cortex and the animal model were found closely apposing neuronal cell bodies and to mediate pre-synaptic displacement and phagocytosis, which coincided with a relative sparing of neurons. In contrast, microglia in MS2 cortex were not involved in these synaptic alterations, but instead associated with substantial neuronal loss. Taken together, our results show that in response to meningeal inflammation, microglia acquire two distinct phenotypes that differentially associate with neurodegeneration in the progressive MS cortex. Furthermore, our *in vivo* data suggests that

microglia initially protect neurons from meningeal inflammation-induced cell death by removing pre-synapses from the neuronal soma, but eventually lose these protective properties contributing to neuronal loss.

316 - Argonaute autoantibodies are diagnostic and prognostic biomarkers for autoimmune-related sensory neuropathies

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The identification of autoantibody (Ab) biomarkers in neurological disorders can assist clinicians to establish a diagnosis and choose an appropriate treatment. However, many idiopathic patients lack clear biomarkers, despite hints towards a dysimmune context. Recently, we described Abs against the family of Argonaute (AGO) proteins (mainly AGO1 and AGO2) as a serum biomarker for a dysimmune context in neurological diseases. Here, we aimed at describing the frequency and distribution of AGO Abs among peripheral neuropathies and to retrospectively characterize the antibody regarding its clinical relevance.

Via a newly established conformation-specific enzyme-linked immunosorbent assay (ELISA), we screened the sera of 823 subjects for AGO1 Abs: 433 patients with peripheral neuropathies, 274 with systemic autoimmune diseases (AID) already known to be associated with AGO Abs (formerly anti-Su) as a positive control cohort, 116 healthy controls (HC) as a negative control. Cell-based assays (CBA) were used as a validation method. IgG subclass and titer were determined via ELISA and CBA.
Among peripheral neuropathy patients, 28/433 (6.5%) had AGO1 Abs. In sensory neuronopathy (SNN) patients (17/132 [12.9%]), the frequency was significantly higher than in non-SNN neuropathies (11/301 [3.7%]; p=0.001), AID (16/274 [5.8%]; p=0.02), and HC (0/116 [0%]; p<0.0001). Among all SNN patients without any known dysimmune context, AGO1 Abs were detected in 5/59 (8.5%). We then compared the clinical pattern of the SNN in 17 patients with and 115 without AGO1 Abs, there was no difference in term of age and sex, but patients with AGO1 Abs had a higher SNN score (median 12,2 [25-75%-int: 11-12,7] vs. 11.0 [8,2-11], p= 0.004), the face was more frequently affected (8/17 [47%] vs. 14/75 [19%], p=0.01), and global areflexia was more frequent (13/17 [76%] vs. 29/75 [39%], p=0.01). Interestingly, the m-RANKIN had significantly decreased upon treatment in AGO1 Abs-positive SNN patients (p=0.02) but not in AGO1 Abs-negative patients (p=0.76). Multivariate analysis confirmed that this difference in the disease course of the two SNN groups upon treatment was significant (p=0.01) and linked to the presence of AGO1-Abs (p=0.004). AGO1-Abs titers ranged from 100-100,000 in the neuropathy cohorts and from 100-10,000 in the autoimmune disease cohorts. IgG subclass was mainly IgG1 (>80% of patients), but also IgG3 and 4 was found in 20-30%.
Our findings demonstrate a potential diagnostic and prognostic benefit of AGO1 Abs as a novel and early biomarker for autoimmune-related SNN.

6 - Flow cytometric detection of functionally relevant ganglionic acetylcholine receptor antibodies in Autoimmune Autonomic Ganglionopathy

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Autoimmune Autonomic Ganglionopathy is a rare disorder of the autonomic nervous system. Less than half of new diagnoses are associated with antibodies to the ganglionic acetylcholine receptor (gnACHR). Antibodies to the gnACHR have traditionally been detected by radioimmunoprecipitation assays (RIA) or by Luciferase

Immunoprecipitation system (LIPS). These assays are highly sensitive but are unable to give information on whether the antibodies are functionally relevant or not. Furthermore, they require expensive radioligands and sacrificial animals or specialized transfected cell lines and as such, have not been widely implemented across diagnostic laboratories. Additionally, difficulty with establishing adequate surface staining on gnACHR transfected cell lines has made this latter approach unsuitable. Patch-clamp and immunofluorescence studies with pathologically relevant antibodies to the gnACHR have established receptor internalization (modulation) as the mechanism of pathology. We describe a flow cytometric assay that demonstrates the effect of functional gnACHR autoantibodies (through receptor modulation) that can be implemented in a high throughput, diagnostic service. The assay gives congruent results with the established RIA, but is safe, and requires no specialized transfected cell lines or animals, and has the potential to reduce false positive results.

12 - High-dimensional immune profiling in traumatic spinal cord injury patients in relation to clinical parameters

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Spinal cord injury (SCI) causes irreversible damage to the nerve tissue of the spinal cord, which results in the loss of motor and sensory functions below the injury level. An inflammatory immune reaction is triggered following a SCI, leading to advanced secondary tissue damage during a secondary injury phase. This study aimed to extensively analyse the circulating immune cell composition in traumatic SCI patients, including the (sub)acute ((s)aSCI) and chronic (cSCI) disease phase, in relation to clinical parameters. High-dimensional flow cytometry was performed on peripheral blood mononuclear cells of traumatic SCI patients and healthy controls (n=18 each). SCI blood samples were collected at multiple time points in the (sub)acute (0 days to 3 weeks post-SCI) and chronic (6 to >18 weeks post-SCI) disease phase up to a total of 46 SCI samples. Total and $CD4^+$ T cell frequencies were increased in cSCI patients. $CD4^+$ T cells and B cells were shifted towards memory phenotypes in (s)aSCI and cSCI patients, respectively. Most profound changes were observed in the B cell compartment. Interestingly, decreased immunoglobulin (Ig)G^+ and increased IgM^+ B cell frequencies reflected disease severity, as these correlated with American Spinal Injury Association (ASIA) impairment scale (AIS) scores. Post-SCI B cell responses consisted of an increased frequency of B cells and B cell subsets expressing the survival receptor CD74. Expression of CD74 was also elevated on B cell subsets of cSCI but not (s)aSCI patients. In conclusion, post-SCI inflammation is driven by memory immune cell subsets. The elevated CD74 expression on B cells of SCI patients suggests the potential involvement of CD74-related pathways in post-SCI B cell responses. Monitoring of circulating IgM^+ and IgG^+ B cell levels could aid in the clinical evaluation and prognosis of SCI patients.

Workshop XI | Immunology in neuro-oncology

195 - Cervical lymph nodes and ovarian teratomas as germinal centres in NMDA receptor-antibody encephalitis

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Autoantibodies against the extracellular domain the N-methyl-D-aspartate receptor (NMDAR) NR1 subunit cause a severe and common form of encephalitis. To better understand their generation, here, we aimed to identify human germinal centres actively participating in the NMDAR-specific autoimmunization by sampling patient blood, cerebrospinal fluid, ovarian teratoma tissue and, directly from the putative site of human CNS lymphatic drainage, cervical lymph nodes. From 285 sera (108 patients, 28 with ovarian teratoma), positive teratoma status was associated with a higher frequency of NR1-IgA positivity (OR=3.11; P<0.0001) and lower frequency of NR1-IgM (OR=0.48; P=0.012), particularly early in disease (receiver operating characteristic curve area under = 0.86 at day 10). Consistent with this immunoglobulin class switching, teratoma samples showed intratumoral production of both NR1-IgG and NR1-IgA and single cell RNA sequencing of teratomas showed expanded, highly-mutated IgA clones with an OT-restricted B cell population. Multiplex histology identified germinal centre structures containing dense T and B cell foci, follicular dendritic cells, lymphatics and high endothelial vasculature: these closely resembled lymph node and tonsil architectures, suggestive of tertiary lymphoid structures. Cultured teratoma explants and dissociated intratumoral B cells secreted NR1-IgGs in culture, consistent with functional NR1-specific germinal centre activity. Moreover, from patients with NR1-antibody encephalitis, but not control participants, B cells cultured from cervical lymph nodes produced NR1-IgG in 3/7 cultures, most notably from samples with the highest serum NR1-IgG (P<0.05) and cervical lymph node C-X-C motif ligand 13 levels. Our multimodal evaluations provide convergent anatomical and functional evidence of NMDAR-autoantibody production from active germinal centres within both intratumoral tertiary lymphoid structures and cervical lymph nodes. Furthermore, we provide methods to directly inform systemic immunological contributions across diseases of the central nervous system.

299 - CLINICAL SPECTRUM AND LONG-TERM OUTCOME IN N-IRAES: THE CICLOPS STUDY

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Neurological Immune Related Adverse Events (n-irAEs) of immune checkpoint inhibitors (ICIs) constitute an emerging group of neurological disorders with heterogeneous pathogenic mechanisms and clinical phenotypes. Although rare (1-3% of patients treated with ICIs), n-irAEs are often highly disabling, and can be fatal. However, the long-term outcomes of n-irAEs remain largely unknown. In this nationwide, observational retrospective study, we studied the clinical presentations and outcomes of a large real-world cohort of n-irAEs patients. Cases were selected after interrogation of the databases of the French Reference Center for paraneoplastic neurological syndromes (PNS) and the OncoNeuroTox network. Only patients with a reasonable exclusion of alternative diagnoses were included. We identified a total of 147 cases (59% male, mean age 63.69 years) whose clinical phenotypes were: irEncephalitis (32,5%), irMeningitis (5,5%), irDemyelinating syndromes (6%), irNeuropathy (26%), and irMyopathies/Neuromuscular Junction disorders (NMJ) (36%). In 6% of cases overlapping irEncephalitis and irNeuropathy were observed. Severe (CTCAE grade≥3) toxicity was registered in 94%. According to the updated 2021 PNS diagnostic criteria, 73.5% of cases were non-PNS, 12% definite PNS, 11% probable PNS, 3.5% possible PNS. First-line immune therapy (≥1 among steroids, intravenous immunoglobulins, plasma exchange) was administered in 91% of cases, and 80% of them improved after treatment (change in mRS score≥1). After a median follow-up of 12 months (range 0-50), the rate of patients with a poor outcome (residual mRS at last visit ≥3) varied according to the clinical phenotype: 69% for irEncephalitis, 25% for irMeningitis, 55.5% for irDemyelinating syndromes, 76% for irNeuropathy, 46% for irMyopathies/irNMJ disorders. 32.5% patients died. The most common cause of death was cancer progression (35.5%), followed by neurological toxicity (27%). Neurological relapses were observed in 8% of the cases. Cancer progression was observed for 51% of patients, with a median time to cancer progression of 11.9 months. ICI rechallenge was performed in 12% of patients, of which 12% experienced a neurological relapse. Our findings illustrate the clinical heterogeneity of n-irAEs. Outcomes varied greatly depending on the initial clinical presentation. In addition, our data suggest that ICI withdrawal in n-irAE patients may favor cancer progression, while ICI rechallenge appears safe in selected cases.

32 - Pretreatment Neutrophil-to-Lymphocyte/Monocyte-to-Lymphocyte Ratio as Prognostic Biomarkers in Glioma Patients

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Background: In the glioma microenvironment, elevations in immune cell ratios are posited to reflect the host's systemic response to malignancy. Interactions between immune cells and neoplastic cells can contribute to tumour pathogenesis and progression, in addition to anti-cancer cell-specific targeting. Given the dearth in clinically significant molecular markers to predict prognosis, there is potential for peripheral immune cell ratios to serve as low-cost and readily available prognostic markers. **Objectives:** This study evaluated the ability for pretreatment peripheral immune cell ratios (Neutrophil-to-Lymphocyte Ratio, NLR, and Monocyte-to-

Lymphocyte Ratio, MLR) to predict overall survival (OS) and modified Rankin Scale (mRS) at admission, 6 months and 12 months post-diagnosis. It also explored relationships between peripheral immune cell counts and clinicopathological parameters (tumour location, tumour size, tumour grade, IDH-1 mutation and MGMT promoter methylation status). **Methods:** Pretreatment NLR and MLR were analysed retrospectively in 64 glioma patients from Royal Melbourne Hospital. OS was evaluated with the Kaplan-Meier method and log rank test. Prognostic factors for OS and mRS were evaluated with univariate and multivariable regression analyses. **Results:** We found that, compared to lower pretreatment NLR (≤4.7), higher pretreatment NLR (>4.7) predicted higher mean admission mRS (mean 3.31 vs 2.40, p<0.001) and 6-month mRS (mean 3.60 vs 2.44, p=0.019). Higher pretreatment NLR was associated with poor functional outcome (mRS 3-6) at admission (p<0.001) and 6 months (p=0.001). Higher pretreatment MLR (>0.35) predicted poorer OS in glioma patients (median 57.0 ± 6.6 weeks, 95% CI 43.4-70.6, p=0.024). Higher NLR was associated with larger tumour size (≥5cm) (p=0.02). **Conclusion:** To our knowledge, this was the first study to evaluate the association between immune cell ratios and mRS, and the most comprehensive study to date in evaluating the associations between immune cell ratios and the clinicopathological features of glioma patients. This study demonstrated that NLR and MLR can serve as prognostic markers to predict functional outcomes and OS in glioma patients. These findings allow us to identify high-risk patients in need of further treatment and advance understanding of the role of immune cells in glioma pathogenesis, which allows for the development of therapeutic targets.

163 - Identification of target antigens for glioblastoma-infiltrating CD4+ T cells

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Glioblastoma (GBM) is considered an immunologically "cold" tumor, characterized by a low mutational load and relative scarcity of $CD4^+$ and $CD8^+$ T cells in the tumor. Recent tumor vaccination approaches have provided promising results particularly with respect to stimulating tumor infiltrating lymphocytes that are CD4+. Therefore, finding highly immunogenic antigen(s) that are able to stimulate CD4+ T cells against the tumor is crucial. The aim of this project is to isolate disease-relevant $CD4^+$ T cell clones (TCCs) in a case of GBM, in whom we applied a highly individualized tumor vaccination using peptides, and to identify their target antigens using an unbiased epitope discovery approach. We search for targets among glioblastoma-related antigens, all human proteins, viruses and bacteria including gut microbiota. Vaccine peptides were used to stimulate bulk tumor-infiltrating lymphocytes (TILs) in vitro, and some were identified as strong stimulators of bulk TILs including peptide-126 from the Sin3A protein. $CD4^+$ TCCs were isolated and expanded using peptide-126 and were tested for their proliferative capacity as well as function. Positional scanning peptide libraries (ps-SCL) combined with a dedicated bioinformatics approach were used as unbiased antigen discovery approach. TCC88 was tested with ps-SCL and the response was used to create a scoring matrix. This scoring matrix was used to search in several large scale databases for the above targets among tumor antigens, self-peptides and antigens from infectious agents. So far, we could demonstrate that TCC88 recognizes multiple glioblastoma-derived target antigens, but also a large number of targets from bacteria and particularly gut microbiota. The potential importance of these observations for future tumor vaccination approaches will be discussed.

212 - Immune contexture of isocitrate dehydrogenase stratified human gliomas revealed by single-cell transcriptomics and accessible chromatin

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The immune cell composition of isocitrate dehydrogenase wild type (IDH-wt) glioma patients significantly differs compared to IDH-mutant (IDH-mut) yet a detailed and unbiased understanding of their transcriptomic and epigenetic landscapes remains elusive. To this end, we performed single-cell RNA-sequencing (scRNA-seq) and single cell- Assay for Transposase-Accessible Chromatin using sequencing (sc-ATAC-seq) on ~100,000 tumor-associated immune cells from seventeen IDH mutation classified primary and recurrent human gliomas and non-glioma brains (NGBs). Our analyses revealed sixty-two transcriptionally distinct myeloid and lymphoid cell states within and across glioma subtypes and we noted microglial attrition with increasing disease severity concomitant with invading monocyte-derived cells (MDCs) and lymphocytes. Specifically, certain microglial and monocyte-derived subpopulations were associated with antigen presentation gene modules, akin to cross-presenting dendritic cells. As tissue macrophages exhibit multifaceted polarization in response to microenvironmental cues, we clarify the existence of microglia/macrophage functional states beyond M1/M2 paradigms exemplified by the presence of palmitic-, oleic- acid, and glucocorticoid responsive polarized states. We identified cytotoxic T cells with poly-functional cytolytic states mostly in recurrent IDH-wt gliomas. Furthermore, ligand-receptor interactome analyses showed a preponderance of antigen presentation/phagocytosis over the checkpoint axis in IDH-wt compared to IDH-mut gliomas.
Additionally, our sc-ATAC-seq analyses revealed differences in regulatory networks in NGBs, IDH-mut and IDH-wt glioma associated immune cells. In particular, we noted reduced microglial usage of an iron recycling SPIC transcription factor and Interferon Regulatory Factors (IRFs); IRF1 and IRF2 in IDH-wt relative to IDH-mut and NGBs. Unique features such as amplification of 11- Zinc Finger Protein accessibility were restricted to MDCs. Finally, sc-ATAC-seq profiles of $CD8^+$ exhausted T cells from IDH-wt showed strong enhancer accessibility on CTLA-4, Layilin and TIM-3 but no enrichment on PD1 was seen. In summary, our study provides unprecedented granular detail of transcriptionally defined glioma- specific immune contexture that can be exploited for immunotherapy applications.

234 - Is there a paraneoplastic neurologic syndrome associated to renal and bladder cancer?

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Bladder and renal cancers rank 5th and 9th in frequency among all types of malignancies and follow prostate cancer among urological neoplasms. Paraneoplastic neurologic syndromes (PNS) are immune-mediated remote effects of cancer. Urological cancers are not frequently associated with PNS and heterogenous clinical and paraclinical data have been reported in this regard. We performed a retrospective nationwide study and a systematic review of the literature of all patients with renal or bladder cancer and suspected PNS. We excluded patients with a remote cancer diagnosis from clinical onset (>5 years), presence of a more recent or commonly

associated cancer, confirmed alternative diagnosis or insufficient neurological data. We identified 10 renal and 6 bladder cancer patients with a potential PNS in our cohort; additionally, we identified 17 and 14 cases from the literature, respectively. Overall, median age at onset of neurological symptoms was 66 years (renal) and 68 (bladder), with a male predominance (59% and 65%). Pathological examination showed that 92% of renal were clear cell carcinoma, while among bladder cancers, 75% were urothelial and 20% neuroendocrine or small cell. The interval between neurological symptoms and cancer diagnosis was <2 years for 88% (renal) and 75% (bladder) of patients. There was a predominance of cerebellar symptoms (37% renal, 50% bladder) followed by encephalitides-syndromes in renal (33.3%) and sensory neuronopathy/encephalomyelitis disease spectrum in bladder (35%). Neural autoantibodies (Ab) were found positive in 48% (renal) and 90% (bladder), of which 30% (renal) and 94% (bladder) were high or intermediate risk Ab. Interestingly, neural antigens were detected in 3 cases of urothelial bladder cancer with PNS displaying cerebellar symptoms (2 Ri, 1 Yo). According to the updated PNS Criteria, 59% (renal) and 85% (bladder) scored for possible PNS or higher. Given its low frequency, a better characterisation of renal and bladder cancer related PNS is needed to guide the clinician in the setting of neurological symptoms that could herald or relate to these tumours. This PNS may have a predilection for cerebellar involvement considering the clinical pictures observed. There seems to be a stronger association between bladder malignancies and PNS, which is supported by the previously reported expression of neural antigens by urothelial cancers. Another occult cancer most frequently associated with PNS should always be excluded.

Workshop XII | Myasthenia Gravis

294 - Higher Th17 and Th1 cells in thymoma may affect development of thymoma-associated-myasthenia gravis inducing Tfh cells

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Myasthenia gravis (MG) is an autoimmune disease mediated by autoantibodies mainly against the acetylcholine receptor (AChR). Thymic pathologies associated with MG differ and a small subgroup of MG is associated with thymoma. On the other hand, thymoma is not always accompanied with MG and contribution of immune activity of thymomas to MG development is not well understood. Based on the findings in MG without thymoma, thymoma associated immune activity has been assessed in thymoma associated MG (TAMG) in comparison to non-MG patients with thymoma (TOMA). Clinically diagnosed MG patients with AChR antibodies and pathologically proven thymoma (n: 22) were included. TAMG patients, 13 with and 9 without immunosuppressive treatment, were compared with TOMA (n: 9) and healthy controls (HC, n: 38). Intracellular IL-21, IL-4, IL-17A, IL-10, IFN-□□were measured in $CD4^+$ T cells by flow cytometry after stimulation of peripheral blood mononuclear cells (PBMC) with PMA/ionomycin for 4 hours. T helper (Th) subsets according to the expression of chemokine receptors ($CXCR3^+CCR6^-$: Th1) and ($CXCR3^-CCR6^+$: Th17), as well as CXCR5, ICOS or PD-1 expression on $CD4^+$ T cells were analyzed. Non-parametric tests were used for statistical analysis. In TAMG patients, IL-21 and IL-4 productions of $CD4^+$ T cells were increased compared both to HC ($p < 0.001$, $p = 0.002$) and TOMA patients ($p < 0.001$, $p = 0.022$). $PD\text{-}1^+$ and $CXCR5^+PD\text{-}1^+$ (circulating T follicular cells, cTfh) T cells were also higher in $CD4^+$ T cells in TAMG compared to HC (both $p < 0.001$) and TOMA ($p = 0.017$, $p = 0.021$). IL-17A production of $CD4^+$ T cells ($p < 0.001$, $p = 0.024$), Th17 phenotype ($p < 0.001$, $p = 0.035$) and ICOS expression of $CD4^+$ T cells were increased both in TAMG and TOMA patients, irrespective of CXCR5 (all $p < 0.001$), compared to HC. On the other hand, IFN-□ production of $CD4^+$ T cells and Th1 phenotype were lower in TAMG patients than HC ($p = 0.006$, $p = 0.005$), whereas Th1 cells were increased in TOMA ($p = 0.034$) and IFN-□ production of $CD4^+$ T cells was also relatively higher than in TAMG. To the contrary, IL-10 production was also increased both in TAMG and TOMA patients in comparison to HC ($p < 0.001$, $p = 0.013$). Higher IL-21, IL-4 and cTfh in TAMG implicate the thymic activity

involvement in autoantibody production similar to MG without thymoma which was also associated with lower IFN-g production. Higher Il-17 and IFN-g in thymoma may have induced the change to autoantibody producing state in TAMG.

36 - Functional monovalency amplifies the pathogenicity of anti-MuSK IgG4 in myasthenia gravis

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An expanding group of autoimmune diseases is now recognized to be hallmarked by pathogenic IgG4 autoantibodies. A substantial part of the autoantigens in these diseases have a function in the nervous system. It is poorly understood how IgG4 autoantibodies cause these severe autoimmune disorders, because IgG4 was classically viewed as anti-inflammatory and benign. IgG4 is the only human antibody able to undergo Fab-arm exchange; where IgG4 half-molecules exchange with unrelated IgG4s in the circulation in a stochastic and continuous manner. This renders the great majority of IgG4 functionally monovalent for its antigen. We investigated whether functional monovalency of IgG4 contributes to the pathophysiology of muscle-specific kinase (MuSK) myasthenia gravis (MG), one of the best characterized IgG4 autoimmune diseases. MuSK a key molecule in the organization and maintenance of the neuromuscular junction (NMJ). In mice, MuSK MG patient-derived monoclonal monovalent anti-MuSK IgG4s rapidly caused progressive and severe myasthenic muscle weakness, whereas the bivalent equivalents did not cause overt muscle weakness or were less potent. Therefore, the functional monovalency of anti-MuSK IgG4 amplified the pathogenicity *in vivo*. This may be explained by the opposing effects of bivalent and monovalent MuSK antibodies on MuSK signaling (i.e. activating vs inhibiting) in the NMJ, as demonstrated in cultured myotubes. These findings suggest that isotype switching to IgG4 autoantibodies is a critical step in the development of MuSK MG and establishes functional monovalency as a novel pathogenic determinant in IgG4-mediated autoimmunity.

91 - Conditioned Mesenchymal Stromal Cells as tools for immunomodulation in Myasthenia Gravis

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Myasthenia gravis (MG) is an autoimmune neuromuscular disorder due to autoantibodies directed against the neuromuscular junction. Conventional treatments are limited by hefty adverse effects mandating the set-up of novel therapies. Mesenchymal Stromal Cells (MSC) are multipotent progenitors that modulate immune and inflammatory responses. Research grade (RG-) MSC conditioned by a co-culture step with peripheral blood mononucleated cells (PBMC) improved the clinical outcomes in a humanized MG mouse model (NSG-MG) (Sudres *et al*., JCI Insight, 2017). To shift towards a clinical perspective, we replaced RG-MSC by clinical grade (CG-) MSC. Here, we aim to characterize the phenotypic and gene expression profiles of resting and conditioned CG-MSC, as well as to validate their functional capacities *in vitro* and *in vivo*. Therefore, CG-MSC derived from adipose tissue were conditioned by PBMC (cMSC), left untreated (rMSC), or activated by IFN-γ (γ-MSC) as a control. Flow cytometry phenotyping (65 antibodies) showed that despite close global profiles, cMSC most remarkable phenotypic traits include increased CD54, CD273 and CD49a expression and reduced HLA-DR expression. The intensity of modulation was culture dependent. At variance, major changes in MSC phenotype were induced by IFNγ activation in agreement with litterature (increased CD54, HLA-ABC, HLA-DR, CD47, reduced CD59). Single

cell clustering by mass cytometry (CyTOF, Fluidigm, 31 antibodies) confirmed the proximity between cMSC and rMSC and underlined the phenotypic alterations induced by IFNγ. Gene expression profiles were analyzed by RNA-seq (Illumina). Comparing rMSC with cMSC, we observed the differential expression of 244 genes. Meanwhile, IFN-γ increased the number of deregulated genes. Indeed, 2089 and 3614 genes were differentially expressed upon comparison of γ-MSC with rMSC and cMSC respectively. *In vitro* immunomodulating capacities were evaluated by PBMC proliferation inhibition assays and showed that the cMSC supernatant was the only one able to reduce proliferation by at least 50%. Finally, the *in vivo* efficacy of CG cMSC was challenged in our MG-NSG model. cMSC-treated mice presented MG scores lowered by 50% compared to untreated mice from as early as 2 weeks post-injection. To sum-up, this work unveiled treatment-dependent phenotypic markers of MSC and demonstrated that immunomodulation capacities *in vitro* and *in vivo* are enhanced by cellular conditioning.

341 - Role of HIF-1 and Treg/Th17 Imbalance in the Thymus in Myasthenia Gravis

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Myasthenia gravis (MG) is an autoimmune disease, in which T cell-dependent autoantibodies cause weakness in skeletal muscles. Thymic pathologies such as lymphoid hyperplasia and thymoma can be observed in patients. Some studies indicate an imbalance between T helper 17 cells (Th17) and regulatory T cells (Treg) in the thymus of MG patients. On the other hand, hypoxia-inducible factor 1 (HIF-1) is shown as a critical regulator of Th17/Treg balance. HIF-1, which is activated in a STAT3-dependent manner, works as a direct transcriptional activator of the major Th17 transcription factor RORγt. Furthermore, HIF-1 forms a complex with RORγt by recruiting p300 to activate Th17 signature genes. HIF-1 also inhibits Treg differentiation by targeting FOXP3 for proteasomal degradation. We sought an explanation for the Th17/Treg imbalance by expression analysis in thymic tissues and thymocytes. RNA was extracted from thymic tissues of 12 thymoma-associated myasthenia gravis (TAMG), 12 thymoma without MG (THY), and 13 MG with hyperplasia (HPMG) patients and from thymocytes of 4 HPMG, 3 TAMG, and 4 THY patients. All MG patients had acetylcholine receptor autoantibodies and had not received immunosuppressive treatment by the time of thymectomy. Relative expressions of *TDT, CTLA4, FOXP3, GITR, HIF1A, IL21, RORC, CCR6, IL6, STAT3, CD25* and *IL2* were analyzed using qPCR. *18S* and *HPRT* were used as housekeeping genes. In tissue samples, *HIF1A* was significantly higher in TAMG in comparison to HPMG (0.031 vs. 0.0045, p = 0.049). *RORC* also showed a significantly high expression in TAMG compared to the HPMG group (0.053 vs 0.025, p= 0.039) which was supported by higher expression of *CCR6* in TAMG compared to HPMG. *IL2* was significantly lower in TAMG compared to HPMG (1.08 vs. 3.24, p = 0.007) and lower than in THY. *FOXP3* also indicated a tendency of lower expression in TAMG compared to HPMG. Supporting our findings in tissue samples, we found a tendency for higher expression of *HIF1A* and *RORC* and a lower expression of *FOXP3* in TAMG compared to HPMG in thymocytes. The results demonstrated differences probably related to metabolic changes in thymic pathologies. Increased RORγt in TAMG implicates Th17 lineage involvement which is promoted by HIF1A. HIF1A may contribute to Treg/Th17 imbalance also by FOXP3 degradation. Lower IL2 expression supports the impairment of Treg maturation in TAMG. In conclusion, HIF-1 may be the pathological driver of Treg/Th17 imbalance in TAMG.

129 - Monoclonal antibody anti -IL-23 ameliorates neuromuscular defects in Myasthenia Gravis mouse model

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Autoimmune myasthenia gravis (MG) is a rare neuromuscular disease due to auto-antibodies directed against acetylcholine receptors (AChRs) located at the neuromuscular junction. The induced neuromuscular junction (NMJ) dysfunctions lead to muscle weakness and fatigue. Previous data showed that pathogenic Th-17 cells are increased in sera of $AChR^+$ MG patients.

Therefore, we aimed to study the effect of a monoclonal anti-IL-23 antibody that targets pathogenic Th17 cells, in the classical experimental MG mouse model (EAMG). EAMG is based on active immunization of C57BL6 mice with torpedo fish-AChR. This model allows us to determine the impact of anti-IL-23 treatment on EAMG clinical score and the production of anti- AChR antibodies.
We observed that the anti-IL-23 treatment significantly ameliorated muscular EAMG clinical score. In addition, analysis by electromyography of muscle single fibers, in the tibialis anterior, showed that the treatment improved the transduction signal at the neuromuscular junction. Hence, myasthenic mice receiving the anti-IL-23 treatment did not displayed the typical MG decrement following repetitive fiber stimulations illustrating an improvement in the neuromuscular transmission. At a molecular level, the anti-IL-23 antibody stimulated gene expression of factors involved in muscle regeneration, and reduced the IL-17 related inflammation in the tibialis anterior. Finally, we observed a significant decreased production of the IgG2b antibody induced by the treatment.
Together, these data suggest that targeted IL-23 therapy may induce significant clinical amelioration in EAMG due to potential concomitant beneficial effects on the autoantibody production and on the skeletal muscle functional defects.

247 - Endogenous nucleic acids induce an organ-specific type I interferon signature in Myasthenia Gravis thymus

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Myasthenia gravis (MG) is a neuromuscular disease due to autoantibodies against the acetylcholine receptor (AChR). Thymic abnormalities are associated with MG such as ectopic germinal centers with B cells producing anti-AChR antibodies. The MG thymus is also characterized by the overexpression of interferon (IFN)-β and IFN-stimulated genes. IFN-β play a central role in MG as it can induce the overexpression of α-AChR by thymic epithelial cells (TECs) and favor the auto-immune reaction against α-AChR.

A chronic IFN-I signature is observed in periphery in Type I interferonopathy diseases and other autoimmune diseases such as SLE or dermatomyositis. We studied if an IFN-I signature was also present in the serum and PBMC of MG patients but no IFN-I signature was observed. We concluded that MG might be an organ-specific interferonopathy. IFN-I are produces upon pathogen infections but can also be associated with sterile inflammation due to defects in nucleic acid metabolism or a poor resolution of inflammation. We previously demonstrated that Poly(I:C), a dsRNA molecule considered as mimicking viral infection, induces α-AChR expression by TECs via IFN-β release but so far no specific viral signature has been found. However, we recently demonstrated that molecules mimicking endogenous nucleic acids (dsDNA or dsRNA) and that can activate innate immunity signaling pathways induced the overexpression of IFN-β and the expression of α-AChR in human TECs or in the thymus of treated mice. Where those nucleic acids could come from? It is known that the thymus is the site of huge apoptotic processes for thymic selection. Therefore, we hypothesize that endogenous nucleic acids could be released by necrotic thymocytes. Upon contact with endogenous nucleic acids, TECs would overproduce IFN-β, which induces α-AChR that could induce self-sensitization and thymic changes leading to MG.

Parallel VII | Neurons as active players in neuroinflammation

126 - Vesicle-mediated transfer of ribosomes from glia to axons during neuroinflammation

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Neurons are highly specialized and polarized cells exhibiting long processes, among them axons in which mRNA transport is crucial and local protein synthesis contributes to regenerative processes. We recently discovered transfer of ribosomes from Schwann cells to peripheral axons upon injury. In experimental neuroinflammation of the central nervous system (CNS) we now observed an increase of axonal ribosomes in the corticospinal tract (CST) indicating a similar and novel upregulation of protein synthesis machinery within CNS neurons. Notably, ribosomes were hardly detectible in healthy CST axons. Using conditional RiboTracker mice, we here found that these axonal ribosomes originate from oligodendrocytes. Furthermore, axons from wild type cortical explants displayed tagged ribosomes when incubated with conditioned medium from RiboTracker oligodendroglia. Differential ultracentrifugation revealed the presence of ribosomes in microvesicles and exosomes, and incubation of cortical axons with these vesicle fractions indeed resulted in ribosome transfer to the axons. Our work suggests that injured CNS axons receive ribosomes from oligodendroglia during inflammatory pathology, and that ribosomes are transferred via extracellular vesicles. These findings strengthen the concept of axons functioning partially independent of the cell body.

81 - Hypothalamic AgRP neurons control lymphoid hematopoiesis in bone marrow and Treg generation in thymus.

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Hypothalamic Agouti-related expressing (AgRP) neurons are involved in the generation of adrenergic signals that control the differentiation of mesenchymal stromal cells (MSC) in bone marrow (BM) and the generation of regulatory T lymphocytes in thymus. In BM, the interaction of MSC expressing beta 3 adrenergic receptors (B3AR) with hematopoietic precursors provides distinctive functional units to regulate hematopoiesis. The thymus, where discrete stromal areas provide the microenvironment for the maturation of T lymphocytes, is innervated by adrenergic fibers and contains MSC. Whether or not thymic MSC express B3AR is as yet unknown, as is the possible contingent effect of adrenergic activation of MSC on T-cell maturation.

We have postulated that AgRP neurons may control adrenergic activation of B3AR-expressing stromal cells, impacting on the commitment of hematopoietic stem cells (HSC) in BM and of T-cell precursors in thymus. In a model of T-cell-mediated central nervous system (CNS) inflammation, experimental autoimmune encephalomyelitis (EAE), we have demonstrated that adrenergic signals activating B3AR+ stromal cells in BM promote lymphoid hematopoiesis and mobilization of BM precursors to the thymus, where B3AR+ stromal cells promote the generation of Treg. We showed that AgRP neurons signal to B3AR+ cells both in BM and thymus, inducing HSC differentiation and Treg generation. Finally, we have discovered that AgRP neurons are activated in EAE and in multiple sclerosis, as shown by an increase in serum AgRP peptide levels, suggesting AgRP neuron activation as a possible marker of neuroinflammation.

Overall, our study strongly suggests the existence of a neural connection that involves AgRP neurons and B3AR-expressing stromal cells in BM and thymus and controls the differentiation of HSC and T-lymphocytes.

196 - Cytokine-induced DNA breaks, a new player in chronic inflammation-induced behavioral impairment?

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Neuroinflammation accompanies many neuropsychiatric and neurodegenerative diseases. Proinflammatory cytokines, such as interleukin-1 beta (IL-1b), are detected at elevated levels in the blood and the brain in these chronic diseases, yet the contribution of this cytokine to the pathological outcome remains largely unstudied. In acute inflammation, increased IL-1b levels disrupt behavior and cognition notably by impairing glutamate balance in the brain. However, the mechanisms by which the cytokine may specifically and durably cause behavioral abnormalities in chronic inflammation are unknown. Since epigenetic processes are critical player in neuronal plasticity and function, we hypothesized that durable effects of IL-1b in the brain may rely on epigenetic mechanisms. DNA double-strand break (DSB) response has emerged as major processes in the control of epigenetic mechanisms underlying cognition. The balance between DSB production and repair is tightly regulated in neurons in response to neuronal activity and DSB may regulate activity-dependent gene expression. Here, we explored whether chronic exposure to IL-1b caused behavioral deficits by altering the DNA DSB response in neurons. Using chronic exposure of adult mice to IL-1b, thanks to subcutaneously implanted osmotic minipumps, we investigated the impact of chronic IL-1b on innate and cognitive behaviors, on the distribution of brain cells populations, on DNA DSB response markers, and on IL-1b related signaling in neuronal cells. We showed that chronic exposure to IL-1b impaired consolidation of spatial memory, without any overt changes in glial populations nor neurogenesis. Despite a peripheral infusion of the cytokine, by disrupting IL-1b signaling through its receptor in neurons, we showed that chronic IL-1b-induced cognitive impairment were due to a direct effect of the cytokine on the neuron. Finally we found that chronic exposure to IL-1b increases DSB levels in neurons and that DSB response signaling in neurons was critical to IL-1b-induced behavioral deficits. Our results shed light on novel pathological mechanisms in inflammation control of gene expression in neurons that could apply to a wide array of neuro-immune diseases.

Parallel VIII | Peripheral nervous system: a central target of neuroinflammation

263 - In vitro anti-glycolipid antibody production by Guillain-Barré syndrome patients' derived B cells

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The Guillain-Barré syndrome is an acute immune-mediated neuropathy characterized by rapidly progressive muscle weakness. Antibodies crossreactive with glycolipids present in myelin and/or axonal membranes are found in approximately half of the patients and are presumed to initiate the nerve damage. Antibody levels vary considerably between patients both at disease onset and during follow-up but the reason for this is unknown. We hypothesize that patients with high titer antibodies have circulating B cells which have the potential to produce anti-glycolipid antibodies in vitro. B cells from six GBS patients were sorted into plasmablasts and CD27 positive and negative B cells. Cells were cultured in vitro with a cocktail of IL-2/6/21 with or without CpG to stimulate antibody production. Anti-GM1 and anti-GQ1b antibodies were present in the serum of five and one patient respectively as determined by ELISA. Anti-GM1 IgG antibodies were detected in the culture supernatant of peripheral blood plasmablasts from half of the patients. These cells did not require stimulation with CpG. Anti-GM1 IgG antibodies were also detected in the supernatant of CD27+ B cells from these same patients but only after CpG stimulation. CD27- B cells did not produce anti-glycolipid antibodies in vitro. Follow up samples were investigated from two patients who demonstrated in vitro anti-GM1 antibody production. In one patient anti-GM1 antibodies were still produced by plasmablasts and CD27+ cells isolated one week after the start of treatment. Serum anti-GM1 IgG antibody titers only decreased by one (2-fold) dilution step or remained the same

after one week in patients who demonstrated in vitro antibody production. These data indicate that GM1-specific B cells circulate in the peripheral blood of GBS patients at the time of hospital admission and in some patients also after one week. The presence of GM1-specific cells within the CD27+ population of B cells may suggest ongoing B cell activation and differentiation. The data provide a rationale for B-cell targeted therapy in case standard treatment is not effective in reducing pathogenic antibody levels.

44 - Autoreactive T cells in Guillain-Barré syndrome

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Guillain-Barré syndrome (GBS) is considered an autoimmune disorder of the peripheral nervous system (PNS) in which the contribution of pathogenic autoreactive T lymphocytes targeting PNS antigens has been strongly supported by *in vivo* studies. However, the underlying immune-mediated mechanisms in humans are far from clear. The overall aim of this study is to gain insights into this issue by investigating the existence and providing an in-depth characterization of the autoreactive T cell response in GBS patients during the acute and recovery phases of the disease. Flow cytometry analysis of *ex-vivo* PBMCs revealed altered frequencies in $CD4^+$ and $CD8^+$ T cell subsets in GBS patients, thus pointing to an involvement of T cells in the disease. Notably, by using a recently established sensitive workflow based on *ex vivo* T cell screenings, generation of single T cell clones and TCR sequencing, here we reveal the existence of self-reactive T cells in GBS patients. Memory $CD4^+$ T cells targeting self-antigens of the PNS were detected in all GBS patients analyzed so far, whereas they resulted almost absent in healthy controls. Moreover, by analyzing more than 400 autoreactive single T cell clones, we found that these cells show a polyclonal TCR repertoire, target multiple epitopes of the self-antigens with some immunodominant regions and are mostly HLA-DR restricted. Collectively, our data provide the first solid description of self-reactive T cells directed against PNS myelin proteins in GBS patients, thus further supporting the notion of GBS as an autoimmune disease and opening new perspective for biomedical application.

240 - Utilizing Epigenetics to Understand Mechanisms Underlying Primary Progressive Multiple Sclerosis

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Primary-progressive multiple sclerosis (PPMS) is characterized by accumulation of clinical disability from the onset, affecting 5-15% of all MS patients. The mechanisms underlying MS progression are still largely unknown and there are very limited treatment options for progressive MS. DNA methylation plays a role in MS etiology and provides a molecular interface that mediates the impact of genetic and environmental factors on the risk of developing disease. We aimed to exploit the stability and reversibility of DNA methylation marks to identify epigenetic changes that associate with progressive MS states and to characterize the functional consequences of identified epigenetic changes. Genome-wide analysis of methylation in blood demonstrated a significant differentially methylated region (DMR) in neuroblastoma break point family (NBPF) locus on chromosome 1 specifically in PPMS compared to RRMS and secondary progressive (SPMS) patients (n=140, $p=5x10^{-6}$). We confirmed significant difference in the methylation levels at several CpGs in the DMR in an independent cohort of PPMS (n=36) and RRMS (n=48) patients. Moreover, we found that two SNPs are strongly associated with the

methylation levels at multiple CpGs in the DMR in both analyzed cohorts (p-val. range 10^{-21}-10^{-13}) and the variants showed some evidence of association with the risk of developing PPMS (n_{PPMS}=477, $n_{RRMS+SPMS+HC}$=13.254, p<0.03, OR=1.2). The same variants associated with reduced expression of several genes in the locus in the brain in public data. Moreover, our functional analysis revealed that the genomic sequence of the DMR can play a role as promoter and enhancer and that this function depends on methylation level, suggesting DMR may control expression of neighboring genes. Correlation network analysis using publicly available RNA-sequencing data from *postmortem* brain tissue further implicated the *NBPF* locus genes in brain pathology of PPMS. In order to functionally test the impact of PPMS-associated DMR, we have recently developed and optimized targeted epigenome-editing systems to induce stable locus-specific changes of DNA methylation *in-vitro*. By utilizing these tools, we found that reducing the DNA methylation level at the PPMS-associated DMR led to transcriptional alteration of nearby genes in neuroblastoma cell line. Our data suggest that the genetic variation in the *NBPF* locus may predispose for PPMS possibly via control of DNA methylation and expression of genes in the locus.

Parallel IX | Gut microbiota and CNS inflammation

113 - Dysbiosis in the Salivary Microbiome as a Promising Biomarker for Early Detection of Multiple Sclerosis

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Background: Multiple sclerosis (MS) generally starts as relapsing-remitting form (RRMS) and often shifts into secondary progressive MS (SPMS). Although early diagnosis and sufficient treatment certainly leads to the prevention of later disability, it is sometimes difficult owing to the lack of decisive biomarker. While alterations of the gut microbiome in patients with MS are proven repeatedly all over the world, the characteristics of salivary microbiome remain unclear at present.
Objectives: The aim of this study was to reveal the characteristics of salivary microbiome in patients with various clinical subtypes of MS and explore its potential as a diagnostic biomarker.
Methods: We comparatively analyzed salivary microbiome of 59 RRMS patients, 12 SPMS patients, 20 atypical MS patients, 20 neuromyelitis optica spectrum disorder patients, and 60 healthy controls (HC) by 16S rRNA gene sequencing data from saliva samples.
Results: There were large differences in the overall composition of salivary microbiome between each MS subtype and the HC groups. The abundances of two major phyla, namely Proteobacteria and Actinobacteria, were reciprocally changed in all MS groups compared with the HC group. Each patient group had a lot of genera and species having significant changes in abundance in comparison with HC. Changes in some microbial data had significant association with disease activity of the patients. Then we explored the potentials of diagnostic biomarker between the salivary and fecal microbiome in the exactly matched RRMS and HC cohorts by combining the species or genera selected by the random forest algorithm in machine learning, followed by confirmation with 10-fold cross-validation. We ascertained that the area under the curve (AUC) value distinguishing RRMS from HC based on the saliva data was 0.94 in a discovery cohort and 0.83 in a validation cohort, which markedly surpassed the corresponding AUC values based on the feces data. Even in the comparison between HC and RRMS (disease duration ≦5 years) and between HC and RRMS (EDSS score ≦1), the AUC value based on the saliva data remained 0.84 and 0.88, respectively.
Conclusion: We revealed the dysbiosis of salivary microbiome in various clinical subtypes of MS, which might support early detection of this disease.

135 - Microbiota-derived factors modulate intestinal immune cell function via the aryl hydrocarbon receptor and influence stroke outcome

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The gut microbiota is a key modulator of immune cells in brain diseases including stroke. Microbial metabolites sourced from the dietary tryptophan are ligands of the aryl hydrocarbon receptor (AHR) and orchestrate the intestinal mononuclear phagocytes (MNP) – first sensors of microbial products. Here, we seek to determine whether microbial ligands of AHR regulate intestinal MNPs and influence stroke outcome.

Using untargeted metabolomics of the intestinal fecal content from mice subjected to stroke, we identified the tryptophan metabolism as one of the top pathways significantly regulated after stroke in comparison to control mice. This was associated with an increase after stroke of AHR activity in the blood and the upregulation of the AHR prototypic gene member of the cytochrome P45 family *Cyp1a1* in MNPs (CD11c+CD11b+) isolated from the small intestine. Fecal metabolite extract enriched for AHR ligands was isolated from stroke or sham mice and co-cultured with MNPs for gene expression. Whereas *Ahr* expression remained unchanged, the downstream-AHR gene *Cyp1a1* was up-regulated in MNPs challenged with the stroke-associated metabolite extract.

To address the functional role of AHR signalling pathway in MNPs, we subjected CD11c-AHR ($Itgax^{Cre}Ahr^{flx}$) deficient mice and littermate controls to stroke. We identified that the macrophage-like dendritic cells (defined as CD11c+MHCII+CD11b+CD64+ cells) are increased in the brain after stroke with a concomitant decrease in the small intestine. This effect was abolished in the gut of mice not expressing AHR in CD11c cells. In addition, CD11c-AHR deficient mice have a smaller infarct size in comparison to the littermate controls. This phenotype was confirmed using the AHR antagonist CH223191 supplemented in the diet of mice for two weeks prior inducing stroke. Importantly, we found that velocity properties of CD11c+ isolated from mice subjected to stroke was increased in an *in vitro* migration assay and was abolished in cells not expressing AHR. Here we showed that stroke regulates microbial ligands of the AHR affecting intestinal CD11c cell migration and influencing the development of the brain ischemic lesion.

These results revealed a new gut-brain axis via the microbiota-derived AHR ligands as immunomodulators of mononuclear phagocyte trafficking and influencing stroke outcome. These findings may allow for the identification of microbial metabolites-based therapies to reduce the neuroinflammatory response to stroke.

310 - The respiratory symbionts *Moraxella catarrhalis* and *Klebsiella pneumoniae* promote pathogenicity in myelin-reactive Th17 cells

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The respiratory tract is home to a diverse microbial community whose influence on local and systemic immune responses is only beginning to be appreciated. Increasing reports have linked the airways with the trafficking of myelin-specific T cells in the pre-clinical stages of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Myelin-reactive Th17 cells are important pathogenic effectors in MS and EAE but are innocuous immediately following differentiation. Conversion to an ex-Th17 cell phenotype appears to be a critical step in their acquisition of pathogenic potential, but little is known about the mechanisms that mediate this process. We hypothesize that the airways are a critical site in the immunopathogenesis of EAE, where respiratory tract bacteria express crucial factors that promotes encephalogenicity in Th17 cells. We transferred myelin-specific Th17 cells to congenic recipient mice exposed to a range of human respiratory symbionts and monitored disease severity and T cell trafficking. Disease was exacerbated in mice exposed to the Proteobacteria species *Moraxella catarrhalis* and *Klebsiella pneumoniae,* but not the Firmicute species *Veillonella parvula* (commonly associated with healthy human lungs), compared to PBS administered controls. Disease susceptibility

was reduced in germ-free mice compared to conventionally housed mice but was partially restored in germ-free mice colonised with *K. pneumoniae*. In the pre-clinical stages of disease, we found a significant increase in the frequency of GM-CSF$^+$ and GM-CSF$^+$IFNγ$^+$ donor CD4 T cells in the lungs of mice exposed to *M. catarrhalis* or *K. pneumoniae*, compared to *V. parvula*-exposed mice or PBS controls. We also found elevated expression by donor Th17 cells of key trafficking molecules including CCR6 and CXCR6 in the lungs of these mice. *In vitro*, dendritic cells exposed to these respiratory bacteria secrete high concentrations of the critical pathogenic cytokine IL-23 and Th17-polarised cells co-cultured with these bacteria-stimulated dendritic cells also displayed a significant increase in GM-CSF and IFNγ expression. Our data indicates that exposure to the respiratory symbionts *Moraxella catarrhalis* and *Klebsiella pneumoniae* promotes expression of key pathogenic molecules in myelin-specific Th17 cells and supports the concept that perturbations in the respiratory microbiota may contribute to the pathophysiology of CNS autoimmune disease.

ORAL PRESENTATIONS - NOVEMBER 12, 2021

Workshop XIII | Immunoregulation of neuroinflammation

365 - MCAM involvement in regulatory T cell migration across the blood brain barrier: implications for multiple sclerosis

Stephanie Zandee[1,*] - Jennifer Sebali[1] - Carla Rodriguez Mogeda[1] - Antoine Fournier[1] - Camille Grasmuck[1] - Fiona Tea[1] - Olivier Tastet[1] - Evelyn Peelen[1] - Tessa Dhaeze[1] - Xavier Ayrignac[1] - Lyne Bourbonnière[2] - Marc Charabati[1] - Boaz Lahav[1] - Alain Bouthillier[1] - Robert Moumdjian[1] - Catherine Larochelle[1] - Alexandre Prat[1]

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In multiple sclerosis (MS), effector T cells enter the central nervous system (CNS) using cellular adhesion molecules (CAMs), such as Melanoma cell adhesion molecule (MCAM). Immune cell entry into the CNS leads to blood-brain barrier (BBB) and blood-meningeal barrier (BMB) breakdown and lesion formation. Regulatory T cells (Treg) are a key component of immune tolerance, protecting against autoimmune disease. Treg are present in a subset of MS patients and can produce anti-inflammatory IL-10. Little is known about Treg entry and function within the CNS. Here, we sought to understand whether Treg migrate across the BBB using MCAM and are functional within the inflamed CNS.

Flow cytometry was used to study MCAM expression on peripheral blood (PB) and cerebrospinal fluid (CSF) derived Treg from MS patients and healthy controls (HC) as well as on BBB endothelial cells. Confocal microscopy confirmed MCAM presence on the BBB in MS lesions. Various co-inhibitory molecules, chemokine receptors (CCR), functional markers and cytokines were assessed on *in vitro* generated MCAM$^+$ vs MCAM$^-$ Treg (iTreg) using flow cytometry and qPCR. Boyden migration assays were used to study migratory potential of HC vs untreated RRMS iTreg. Presence of MCAM$^+$ Treg in the CNS of experimental autoimmune encephalomyelitis (EAE) affected mice was assessed by confocal microscopy. Moreover, lesions dissected from fresh MS brain tissue, derived from autopsy, contained MCAM$^+$CD4$^+$CD25^{hi}CD127^{lo} cells, indicating these cells are capable of entering the CNS.

MCAM was increased on PB derived Treg of untreated RRMS patients compared to HC. MCAM$^+$ Treg were superior in anti-inflammatory cytokine production (IL-10 Granzyme B) and show higher expression of co-inhibitory molecule CTLA-4 and chemokine receptors CCR5 and CCR6. The frequency of MCAM$^+$ Treg was increased in the CSF compared to PB and migration assays showed MCAM$^+$ iTreg migrate faster than their MCAM$^-$ counterparts. Treg preferentially migrated over the BBB compared to the BMB. Furthermore, MCAM$^+$ Treg accumulated in the CNS of EAE affected mice as well as in MS lesions as determined by flow cytometry.

MCAM is involved in Treg homing to the inflamed CNS. MCAM$^+$ Treg seem better equipped to migrate and are superior in anti-inflammatory cytokine production to potentially dampen down CNS inflammation.

96 - Investigation of the opsonizing capacity of patient-derived anti-MOG antibodies

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Over the last years, evidence condensed that B-cell derived antibodies play an important role in the development of central nervous system (CNS) demyelinating disorders such as neuromyelitis optica and myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease. Especially for the latter group of patients, which show antibodies against MOG in the blood, the precise function of these CNS-reactive antibodies is still unknown. We hypothesize that in these patients, MOG-specific antibodies opsonize traces of MOG in peripheral lymphatic organs, subsequently triggering CNS inflammation and demyelination. To prove whether antibodies isolated from patients with MOG antibody-associated disease are indeed capable of opsonizing their target antigen, we investigated *in vitro* their capacity to promote internalization of membrane-bound MOG by human antigen-presenting cells. For the generation of dendritic- and macrophage-like cells, $CD14^+$ myeloid cells were isolated from human peripheral blood of healthy donors and cultured in the presence of distinct cytokines. Flow cytometry analysis of the generated cells revealed that macrophage-like cells highly expressed Fc receptors I, II and III. By contrast, dendritic-like cells only expressed Fc receptor II. In further experiments, differentiated phagocytes were incubated with fluorescently labeled MOG alone and in the presence of anti-MOG antibody positive serum, and antigen uptake was assessed by flow cytometry. Both dendritic- and macrophage-like cells were capable of internalizing human MOG protein expressed on the surface of human embryonic kidney 293A cells. Six of eight patient-derived anti-MOG antibody positive serum samples fostered the uptake of membrane-bound MOG by myeloid antigen-presenting cells. In this regard, the phagocytosis rate of macrophage-like cells was stronger enhanced compared to dendritic-like cells, which may be due to the different expression of Fc receptors. These findings indicate that anti-MOG antibodies isolated from patient blood opsonize membrane-bound MOG, resulting in an increased phagocytic activity of human antigen-presenting cells. We therefore conclude that opsonization of CNS antigen by peripheral MOG-directed antibodies can play a central role in triggering inflammatory CNS demyelination and may be a new target for therapeutic approaches.

297 - Flanking residues of a self-dominant peptide harnesses immunity via determining the stability of antigen-specific effector Tregs

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The immune system elicits selective responses against non-self antigens while avoiding harmful responses towards self antigens, which can cause autoimmunity and induce tumor development. However, the precise mechanism underlying such abnormal immune responses and antigen-dependent variability of their manifestations are unclear. Thus, sufficient antigen-specific therapy that restores balanced immunity is not yet viable.

We investigated how different encephalitogenic peptides produce distinct clinical characteristics of experimental autoimmune encephalomyelitis (EAE), irrespective of their genetic background. The peptide PLP139-151 caused relapsing EAE in SJL/J mice and progressive EAE following repetitive immunization. Contrastingly, PLP136-150 induced monophasic EAE without progressive capacity. This was due to the efficient expansion and maintenance of a $CD69^+CD103^+$(DP) subset of $CD4^+CD25^+$ regulatory T-cells (Tregs), preferentially in the brain tissue. The DP-subset of Tregs (DP-Tregs) included the most effector Tregs that co-expressed other transcriptional factors of

pathogenic effector T-cells besides Foxp3 such as RORt, Tbet, or Eomes. The effector Tregs were sequentially induced days after the induction of the corresponding effector T-cells, and demonstrated high antigen specificity facilitated by cognate antigens and was correlated with the disease course. Notably, altered peptide studies revealed that the MHC binding and T-cell receptor (TCR) contact sites of PLP136-150 and PLP139-151 were identical. However, without the additional MHC binding site in PLP136-150 that stabilizes the peptide, it would fluctuate in the MHC peptide-binding pockets. Furthermore, truncated peptide studies revealed that the flanking residues influenced the binding capacity and were closely correlated with the kinetics of DP-Tregs, which was achieved by the availability and duration of TCR signaling.
To sum up, the flanking residues of encephalitogenic peptides influenced the stability of the MHC-peptide complex, TCR signaling, and stability of antigen-specific effector Tregs, determining the balance with effector T-cells. These results augment our understanding of immunity, which may help in the development of alternative therapies that consider the mutual balance of autoimmunity and tumor immunity, such as inverse vaccination, and the design of efficient peptide-based anti-tumor therapy through the manipulation of the stability of disease antigen-specific effector Tregs.

315 - Impaired Treg suppressive function in MS is correlated with an age- and treatment-dependent altered Treg dynamics

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Multiple sclerosis (MS) is considered a prototypic autoimmune disease. It affects the central nervous system of young adults, and T- and B cells play important roles in its pathogenesis. To elucidate the functional role of regulatory T cells (Tregs) in peripheral immune tolerance in MS, we assessed their frequency, phenotype, and function at the bulk and clonal level. By flow cytometry, we analyzed 16 untreated patients with relapsing-remitting MS (RR-MS), 12 Natalizumab-treated RR-MS patients, and 15 healthy donors (HD). We found that younger (<37 year-old) treated RR-MS patients showed a significant reduction of Foxp3+CD127lo T cells compared to HD (p=0.033), resulting from a decreased Foxp3 expression (p=0.002) and an increased CD127 expression (p<0.0001). By contrast, younger untreated and older treated RR-MS patients had an increased CD127 expression (p=0.015 and 0.019, respectively), without a significantly reduced Foxp3 expression. In addition, younger treated RR-MS patients displayed a significant decrease of GPA33+CD45RA+ Tregs compared to HD (p=0.03). These Tregs are naïve thymus-derived, and tend to diminish during aging. Overall, MS patients do not show an accelerated lowering of GPA33+CD45RA+ Tregs during aging. Younger treated RR-MS patients showed decreased CD39+ cells in CD4+CD25+CD127loFoxp3+ compared to HD (p=0.001). Nevertheless, older treated RR-MS patients exhibited signs of altered migratory capacity, i.e. an increased CD103 expression on Tregs (p=0.028). Then, we used a [3H] thymidine assay to measure Treg-mediated suppression of polyclonal T cell proliferation. We analyzed 7 RR-MS patients compared to 4 HD, and we confirmed that overall RR-MS patients-derived Tregs have an impaired function. However, 4/7 patients maintained a residual suppressive capacity (>50% of the mean HD function). A preliminary analysis suggests that an increased CD39 expression is positively correlated with a partially conserved Treg function in RR-MS patients (p=0.028). Finally, we generated clones from thymus- and peripheral immune system- derived Tregs, from two MS patients. From both populations (GPA33+ and GPA33-, respectively) we could sort and clone cells responding to stimulation with myelin peptides, as shown by up-regulation of CD137 and HLA-DR. We found that the function of 6 Foxp3+ TCCs recapitulate the suppressive capacity observed at the bulk level. We aim to identify functional Treg-derived TCCs, which could be used for adoptive Treg therapy.

348 - Selective deletion of IDO1+ cDC1 worsened CNS inflammation in an experimental model of multiple sclerosis

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Dendritic cells (DCs) are specialized antigen presenting cells, highly adapted to sense pathogens and induce adaptive immune responses activation. They form a complex network of phenotypically and functionally distinct subsets: cDC1 and cDC2 conventional DCs and plasmacytoid DCs (pDCs). cDC1 and cDC2 function both in initiating immune responses against pathogens and in maintaining self-tolerance. It is known that tolerogenic cDCs, expressing the tryptophan metabolic enzyme indoleamine 2,3-dioxygenase 1 (IDO1), control inflammation trough regulatory T cell induction in an experimental autoimmune encephalomyelitis (EAE). However, the specific regulatory cDC subset lowering CNS inflammation is poorly defined.
Here, we first analyzed the IDO1 expression in cDC subsets and found that it is selectively expressed only in mature CCR7$^+$ cDC1. Interestingly, IRF8 and BATF3, cDC1 specific transcription factors, control *Ido1* expression, through a specific AP-1-IRF composite element in *Ido1* promoter and its CAS9-mediated silencing abrogate *Ido1* expression. Moreover, IDO1 confers to CCR7$^+$ cDC1 a tolerogenic signature, characterized by increased PDL1 expression and inhibition of T cells proliferation. Then we elucidated the role of tolerogenic IDO1$^+$ cDC1 in controlling CNS inflammation. Surprisingly, the selective loss of IDO1 in cDC1 worsened EAE symptoms, promoting CNS immune cell infiltration, likewise in mice lacking cDC1. Such mice showed severe EAE symptoms, a greater CNS immune cell infiltration and increased pathogenic cytokines compared to control. We also observed a significant decrease in PDL1 expression and a reduction of TGF-β and regulatory T cells frequency, relative to control. Finally, preclinic data were confirmed in single cell RNA-seq analysis of multiple sclerosis (MS) patients. Accordingly, we found that IDO1 was the only tryptophan metabolic enzyme expressed by cDC1 in cerebrospinal fluid (CSF) in relapsing remitting (RR) MS patients compared to healthy donors, but a slight IDO1 cDC1$^+$ decrease was observed in RR blood.
These data highlight how IDO1$^+$ cDC1 contribute to control EAE pathogenesis and might be considered a potential biomarker in neuroinflammatory disease. A decrement of circulating IDO1$^+$ cDC1 may correspond to a disease exacerbation in RR patients. Indeed, the cDC1-mediated tolerogenic response may represent a strategy to establish a long-term tolerance to block CNS inflammation in early MS patients and promote myelin reconstitution.

229 - Regulatory B cells ameliorate chronic CNS inflammation in an interleukin-10-dependent manner

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Background/Objectives: In multiple sclerosis (MS), B cells foster central nervous system (CNS) inflammation via antigen presentation and cytokine secretion. Hence, inflammatory responses within the CNS involve B and T cells, but also myeloid cells and microglia. In the current view, an inflammatory circuit between these CNS-established and -resident cells attributes to chronic progression in MS. Thus, it is crucial to identify factors, which inhibit these processes. Evidence accumulates that besides their role as T cell activators, B cells have relevant anti-inflammatory properties. They regulate immune cells e.g. via secretion of interleukin (IL)-10. Hence, we investigated whether B cell-derived IL-10 modulates infiltrating myeloid cells as well as CNS-resident microglia, which both are considered being crucial for full activation of encephalitogenic T cells. **Methods**: Murine bone-marrow derived macrophages (BMDM) and primary microglia were cultured with B cells or their supernatant *in vitro*; IL-10 was removed by genetic ablation or neutralizing antibodies. We assessed functional changes of BMDM/microglia by checking their ability to activate T cells. To investigate B cell regulation *in vivo*, we depleted C57BL/6 mice of B cells using anti-CD20 antibodies. Further, we reconstituted B cell-depleted mice with IL-10-deficient B cells. After induction of experimental encephalomyelitis (EAE) via MOG peptide, we examined activation of infiltrating myeloid cells and microglial by ELISA and flow cytometry. **Results:** After removal of B cell-derived IL-10 *in vitro*, BMDM and microglia showed an increased secretion of pro-inflammatory cytokines, an upregulation of co-stimulatory molecules and an enhanced capacity to activate T cells. *In vivo*, depletion of B cells or reconstitution with IL-10-deficient B cells worsened EAE and increased the number of CNS-infiltrating cells. Exacerbation was associated with an enhanced expression of molecules involved in antigen-presentation by infiltrating myeloid cells and microglia. **Conclusion**: We showed that B cells are capable of shaping immune cells

in an anti-inflammatory manner, diminishing inflammatory responses of CNS-infiltrating as well as -resident cells. These findings can be of importance, as they imply that the sole presence of B cells within the CNS is not by definition pathogenic. B cells can also be immunoregulatory and potentially relevant for controlling CNS intrinsic inflammation associated with clinical progression.

Workshop XIV | Diversity of brain myeloid cells

16 - Migration and functional polarization of monocyte derived cells across the central nervous system barriers during neuroinflammation

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The central nervous system (CNS) parenchyma is enclosed by meninges, the blood-brain barrier (BBB), the choroid plexuses (ChP) and the glia limitans, which collectively maintain CNS homeostasis. Trafficking through these barriers, monocyte-derived cells (MoCs) gain access to the CNS in several inflammatory diseases including multiple sclerosis (MS), exerting detrimental or beneficial functions in a context-dependent manner. How and where these macrophages acquire their different functional commitments during CNS invasion remains however unclear. Studying the *in vitro* interaction of MoCs with endothelial (BBB) and epithelial (ChP) barriers of the CNS, we showed that pro- or antiinflammatory functional specification drastically reduced MoC interaction with CNS endothelial cells but not with ChP epithelium. ChP analysis during autoimmune CNS inflammation in mice revealed increased CCR2+ MoC presence and functional polarization in the stroma, thus indicating the ChP as a potential CNS entry site for MoCs. Notably, depending on their activation status, endothelial and epithelial CNS barrier cells differentially regulate transcription of the signature pro and anti-inflammatory enzymes iNOS and arginase-1 in MoCs. Through *in vitro* and *in vivo* observations, we could describe the existence of an IL1β/GM-CSF axis between MoCs and endothelial BBB cells leading to the upregulation of arginase-1 in MoCs. Our data indicates the ChP as a MoC entry gateway during neuroinflammation and highlights how the interaction with the CNS barriers can significantly affect the functional status of inflammatory cells during autoimmune inflammation.

274 - Oxysterol-production by brain endothelial cells suppresses Myeloid-Derived Suppressor Cells and promotes experimental autoimmune encephalomyelitis

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Cholesterol-25-hydroxylase (Ch25h) and its downstream oxidized cholesterol metabolite, 25-hydroxycholesterol (25-OHC), promote experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis (MS). However, the cellular source of Ch25h during neuroinflammation and the underlying molecular mechanisms remains unknown. We generated a floxed eGFP-reporter-ch25h knock-in mouse and report increased Ch25h expression in CNS endothelial cells during EAE. Furthermore endothelial-specific Ch25h deletion dampened EAE. Mechanistically, RNA sequencing and lipidomic analysis of brain endothelial cells revealed that Ch25h deletion affect lipid biosynthetic transcriptional program and induces a remodeling of secreted lipids, notably Prostaglandin E2 (PGE2) known to drive polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) expansion. PMN-MDSCs have been shown to promote EAE recovery. In-vitro 25-OHC suppressed PMN-MDSC expansion while and PGE2 increased it. Accordingly, PMN-MDSC infiltration was increased in the CNS of Ch25h endothelial cell-deleted mice during EAE and proliferation of CD4 T cells was reduced. Strikingly, enhancing

immature neutrophils circulation resulted in an almost complete protection of EAE in absence of Ch25h and further increased CNS infiltrating PMN-MDSC. Our findings reveal a unique role for Ch25h in blood-brain barrier endothelial cells, trough secretion of 25-OHC and PGE2 that antagonistically acts on PMN-MDSC expansion during neuroinflammation.

268 - Neutrophil plasticity in neonatal hypoxic-ischemic brain injury

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Neonatal encephalopathy caused by hypoxia-ischemia (HI) is a major cause of death and disability in children. A major hallmark of HI-induced brain injury is the infiltration of neutrophils into the injured brain. While we have previously proven the detrimental role of these cells in the early phase after HI, their impact on endogenous delayed HI-induced regenerative processes is unknown. By the use of light sheet microscopy and flow cytometry, we observed a biphasic infiltration pattern of neutrophils peaking 1 and 7 days after HI. Analysis of neutrophil activation demonstrated a significant decrease in ROS production and in the frequency of hyperactivated/aged neutrophils at day 7 compared to day 1 after HI. Furthermore, we detected a significantly reduced expression of Ly6B and Ly6G on $CD14^{+}CD101^{-}$ neutrophils and a decreased frequency of $VEGFR1^{+}CD49^{+}$ neutrophils, both recently associated with neuroregenerative and angiogenic neutrophils. According to that, neutrophils, isolated from HI-injured brains 7 days after HI induced an increase in the number and lengths of vessel sprouts in organoid vascularization assays. Importantly, in contrast to neuroprotective effects of early neutrophil depletion, depletion of neutrophils at the second infiltration peak increased HI-induced neuronal loss and vascular injury. With regard to neurodevelopmental processes, we observed a reduction of mature myelinating oligodendrocytes, accompanied by an increased amount of immature oligodendrocytes. Together, these data indicate that early infiltrated neutrophils differ from late neutrophils in phenotype and function, with neutrophils in the secondary disease phase likely to contribute to secondary neuroprotection and endogenous regeneration, involving angiogenesis and vascular remodeling.

237 - Microglia heterogeneity in different regions of the healthy mouse central nervous system

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The CNS comprises highly specialised and functionally distinct tissue regions; however, little is known about how microglia are adapted to different regions of the neural parenchyma. This study aimed to investigate heterogeneity of microglia within different neuroanatomical regions of the healthy adult mouse CNS. Microglia were isolated from the olfactory bulbs, cortex, hippocampus, cerebellum and retina from 8-week-old female C57Bl/6J mice using FACS (n=4 biological replicates, each consisting of cells pooled from n=5 mice) for bulk RNA-sequencing. Differential gene expression analysis revealed three distinct microglia clusters (i) cortical and hippocampal microglia; (ii) olfactory bulb and cerebellar microglia; (iii) retinal microglia. Regulation of immune responses, inflammatory responses, cell signalling and activation were the most overrepresented biological processes as determined by Gene Ontology analysis. Further analysis demonstrated that genes involved in antigen processing and presentation, chemokine production, phagocytosis and antimicrobial activity were differentially expressed between microglia populations. Having shown that microglia isolated from different regions of the healthy CNS were transcriptionally heterogeneous, we next examined the phenotype and function of these microglia populations using morphological analysis and phagocytosis assays respectively. Sholl analysis of

microglia in Cx3cr1-gfp/+ mice (n=6) demonstrated that cerebellar and retinal microglia had fewer intersections and longer processes compared to other microglia types, and olfactory bulb microglia displayed the highest ramification index ($p<0.05$). Olfactory bulb microglia also demonstrated the greatest capacity to phagocytose *E. coli* bioparticles *in vitro*. Taken together, these results demonstrate that microglia in different regions of the healthy mouse CNS are phenotypically, transcriptionally and functionally heterogeneous, suggesting that these resident macrophages are highly adapted to their microenvironment.

350 - IL-15 expression is distinctly modulated on myeloid cells by inflammatory factors in MS

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Although the salience of immunopathology in multiple sclerosis (MS) has been established, the mechanisms involved are incompletely characterized. We posit that IL-15 contributes to MS neuropathology, given its role in the proliferation, differentiation, and survival of immune effector cells, such as CD8 T lymphocytes. Additionally, a greater proportion of myeloid cells in the brain of MS patients (macrophages and microglia) express IL-15 versus non-MS controls. Thus, we aim to identify inflammatory factors contributing to elevated IL-15 levels, investigate the properties of IL-15 expressing cells, and finally characterize the signaling pathways mediating IL-15 expression in myeloid cells. GM-CSF, which can be produced by encephalitogenic T cells, is being increasingly recognized as integral to the pathobiology of MS and EAE, the animal model commonly used to study MS. We found that GM-CSF significantly increased the proportion of IL-15$^+$ M0 and M1 macrophages from both healthy donors and MS patients. While GM-CSF increased the proportion of IL-15$^+$ M2 macrophages from healthy donors, it did not have this effect on M2 from MS donors. Interestingly, GM-CSF also increased IL-15 expression on primary adult human microglia. Additionally, we found that GM-CSF increased ICAM-1 expression on macrophages and microglia, as well as the percentage of macrophages expressing CD80 compared to unstimulated counterparts. In depth analysis of flow cytometry data showed that IL-15 expressing populations of macrophages and microglia had greater expression of MHC class I and II molecules, as well as ICAM-1, compared to IL-15 negative counterparts. Lastly, we utilized pharmacological inhibitors of MAPK (U0126) and JAK2 (AG490) phosphorylation to identify the signaling pathways mediating the effect of GM-CSF on myeloid cell IL-15 expression. These blockers reduced basal IL-15 expression and/or reduced the effect of GM-CSF on myeloid cells. Due to off-target effects of these inhibitors, however, their impact on IL-15 expression may be mediated by more than one signaling pathway. Our data demonstrate that GM-CSF mediates IL-15 expression on human myeloid cells and also increases the expression of co-stimulatory factors involved in antigen presentation and T cell activation. Importantly, we have shown that IL-15+ myeloid cells may have enhanced antigen-presenting capacities in the context of T cell activation.

147 - Exploring the transcriptomic diversity of live human microglia in aging, neurodegeneration, and neuroinflammation

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The microglial field still lacks comprehensive interrogation of human microglial heterogeneity at the single-cell level across the central nervous system (CNS) and neurodegenerative diseases. We hypothesized that single-cell sequencing of human microglia from a broad array of neurological diseases and CNS regions would reveal diverse microglial subtypes and functional states with specialized roles in specific regions or diseases. Here, we present an updated microglial population structure, demonstrate the use of an optimized protocol for joint IHC-RNAscope staining to confirm our findings in situ, and leverage this dataset to annotate microglial subpopulations in glioblastoma (GBM), a disease where microglia are known to play a pivotal role. Using

the 10x Genomics Chromium platform and our pipeline for extracting live human microglia, we profiled microglia from an array of neurological diseases including Amyotrophic Lateral Sclerosis (ALS), Alzheimer's (AD), Multiple Sclerosis, Parkinson's, Temporal Lobe Epilepsy and deceased individuals with no or mild cognitive impairment. We sampled diverse CNS regions including neocortex (BA4, BA9, BA20/21), substantia nigra, hippocampus, and spinal cord. With over 212,000 cells after stringent pre-processing, we identified 12 microglial subsets. We identify an intriguing central divide between oxidative and heterocyclic metabolism and subsets associated with antigen presentation, motility, and proliferation. We also demonstrate enrichment of gene sets associated with AD and other dementias, ALS, and glioma in a small microglial subset with strong homology to disease-associated (DAM) and lipid-associated microglia previously reported in mouse models of AD. To confirm our findings, we developed an optimized pipeline for dual-detection IHC and RNAscope that allows us to precisely localize transcripts within microglia in situ. We use this pipeline to discriminate multiple microglial subsets in the same field of view across CNS regions and diseases.

Finally, as the identity of GBM-associated microglial subtypes remains unclear, we mapped 15,000 myeloid cells from twenty subjects with single-cell GBM data onto our reference using a pairwise deep learning approach. We saw enrichment of our DAM-like, stress-inflammation, and proliferation-associated clusters and depletion of clusters associated with antigen-presentation and homeostasis. We are evaluating the correlation of these signatures with clinical outcome in GBM.

Workshop XV | Metabolism in Neuroimmunology

10 - iPSC-derived astrocytes from patients with Multiple Sclerosis show metabolic alterations

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Multiple sclerosis (MS) is a multifactorial demyelinating disease that affects more than two million people worldwide and although many progress has been made in the field, it still has no cure. Several studies have shown that astrocytes, which compose the major cell population in the human brain, play a key role in MS pathology, however most of the literature focuses on the Experimental Autoimmune Encephalomyelitis model due to the impossibilities on obtaining brain biopsies of MS patients. Since there are several differences between human and mice astrocytes at the functional and transcriptional levels, there is an urgent need to develop strategies to assess patients' cells in a non-invasive way. In this sense, induced pluripotent stem cells (iPSC) appear as a powerful tool to study disease-related molecular mechanisms of complex diseases like MS, as they can be differentiated into any resident cell population of the human brain. Here we successfully obtained and characterized iPSC-derived astrocytes from three MS patients and three age and sex-matched controls and performed functional assays in these cells including electron microscopy, flow cytometry, cytokine measurement, gene expression, in-situ respiration and metabolomics. We observed several differences between the groups, with an enrichment of genes associated with mitophagy, mitochondrial ion transport and neurodegenerative processes in patients' cells. Next, using electron microscopy, we observed increased mitochondrial fission in the MS group, which was followed by a decreased mitochondrial to nuclear DNA ratio, indicating decreased mitochondrial content in these cells. Then, we observed in this same group an increase in superoxide and MS-related proinflammatory chemokines production. Additionally, using Seahorse assays, we observed an increased electron transport capacity and proton leak in patients' astrocytes, which is in line with the increased oxidative stress observed in these cells. Finally, our metabolomics analysis indicated a distinct metabolic profile between control and MS astrocytes, with a deficiency in aminoacid catabolism and increased sphingolipid metabolism in patient's cells,

which have already been linked to MS. To our knowledge, this is the first study thoroughly describing the metabolic profile of iPSC-derived astrocytes from patients with MS, validating this model as a very powerful tool to study disease mechanisms and to perform drug targeting assays *in vitro*.

24 - Dissecting the role of IGF1R in neuroinflammation: a parallel focus on oligodendrocytes and myeloid cells

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Insulin-like Growth Factor 1 (IGF-1) signalling is critically involved in metabolic control and survival of several cell types. More specifically in the central nervous system (CNS), IGF-1 has been principally studies as a regulator of differentiation and myelination of oligodendrocyte (ODC) precursor cells and as a neuroprotective and immunomodulatory molecule. Interestingly, IGF-1 and its receptor (IGF1R) are increased at demyelinating lesions in the CNS of multiple sclerosis patients and of the animal model experimental autoimmune encephalomyelitis (EAE). However, the potential therapeutic importance of IGF-1 is still crucially hindered by the unclear functions of IGF-1 in several cell types, and more specifically on mature ODCs and resident myeloid cells, which together regulate CNS homeostasis. To better explore the role of IGF1R in these cells during autoimmune demyelination, we therefore utilized novel mouse models carrying a deletion of IGF1R specifically in mature ODCs or, in a separate line of experiments, in CNS-resident microglia/macrophages. Notably, while the absence of IGF1R from ODCs led to significant protection against clinical development of neuroinflammation in the EAE model, the absence of the receptor from resident myeloid cells did not significantly affect disease course. Taken together, our data show that cell-specific IGF-1 signaling differentially impact inflammation in the CNS playing complex role which goes beyond their mere cell survival.

182 - Regulation of human oligodendrocytes process extension by the integrated stress response.

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Early multiple sclerosis lesions feature relative preservation of oligodendrocytes (OLs) but with withdrawal ("dying back") of their myelin processes. With disease progression, OLs are lost. Contributors to this pathology include metabolic stress (ischemia), as well as proinflammatory molecules (interferon γ, IFNγ and tumor necrosis factor α, TNFα) and excitotoxins (glutamate). Previous findings have shown that metabolic stress reduced process outgrowth in dissociated cell culture and glutamate reduced ensheathment of synthetic nanofibers. We have now shown that TNFα and IFNγ inhibit ensheathment of synthetic nanofibers without cell cytotoxicity as measured in dissociated cell culture. We have used bulk RNA sequencing to identify mechanisms underlying process retraction by human OLs subjected for 48 hours to these disease relevant mediators. Our transcriptomic analyses indicate that the metabolic insult down-regulates the expression of a broad array of gene pathways while inducing the integrated stress response (ISR) pathway. The ISR is a major regulator of protein production and can preserve cell energy by attenuating protein translation. Reduced protein synthesis could impact directly OL process retraction in MS. Constituents of the ISR can be detected in active MS lesions. Our data show that enhancing ISR activation *in vitro* with Sephin1 reduces process outgrowth under basal and metabolic stress (low glucose, LG) conditions. Inhibiting the ISR with ISRIB enhances process outgrowth under LG conditions. We found that the pro-inflammatory mediators IFNγ and TNFα are associated with the induction of a wide range of molecular pathways. Almost 50% of upregulated genes induced by TNFα were also upregulated in the IFNγ condition. In contrast with previous studies examining rodent oligodendrocyte progenitor cells, ISR induction was not detected following IFNγ stimulation of human OLs. Pathways activated by IFNγ and TNFα include STAT1/IRF-1 signaling, which impairs

dendritic outgrowth in neurons. Glutamate induced only a limited impact on gene transcription implicating a direct role on cell process retraction. Our comparative studies indicate the need to consider both the specific injury mediators and the distinct cellular mechanisms of responses engaged by human OLs as one seeks effective neuroprotective therapies for multiple sclerosis.

233 - Eomes regulates mitochondrial function and promotes survival of pathogenic CD4+ T cells during CNS autoimmunity

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Eomes is a transcription factor which role is mostly known in Natural Killer cells and in CD8 T cells as a key regulator of IFN-g production and cytotoxicity. Studies in Humans have shown that Eomes belongs to a susceptibility locus associated with Multiple Sclerosis (MS). Moreover, an increase of Eomes$^+$ CD4 T cells was reported in cerebrospinal fluid and PBMC from patients with secondary progressive MS. It has also been reported that Eomes$^+$ CD4$^+$ T cells accumulate in inflamed tissues of patients with other chronic inflammatory disorders as rheumatoid arthritis, psoriaris, inflammatory bowel disease or cancer. However, we still don't know neither the precise role of Eomes in CD4 T cell compartment nor the contribution of Eomes$^+$ cells in the pathophysiology of these inflammatory diseases.

Using a mouse model of MS, the experimental autoimmune encephalomyelitis, we showed that Eomes deletion in antigen-specific CD4 T cells leads to a strong reduced disease severity. Eomes deficiency decreases CD4 T cell ability to accumulate into the central nervous system during the course of the disease. We also showed that Eomes-deficient CD4 T cells exhibit a decreased overall survival. In order to decipher the molecular mechanisms involved, RNA-sequencing was performed comparing Eomes-competent *vs* Eomes-deficient CD4 T cells. Our analyses revealed that the deletion of Eomes impacts mostly pathways related to mitochondrial metabolism. Indeed, Eomes-deficient cells exhibited reduced oxidative phosphorylation linked to altered mitochondrial respiration. This impaired mitochondrial function is associated with a downregulation of Romo1, a key player in metabolism through its role in maintaining the mitochondrial cristae structure. Accordingly, electron microscopy analyses revealed aberrant cristae structure in Eomes-deficient cells contrary to Eomes$^+$ cells which exhibit well-organized mitochondrial network. We also show that altered mitochondrial architecture in Eomes-deficient cells is associated with reactive oxygen species-dependent cell death.

Altogether, we identify a key role of Eomes as a coordinator of mitochondrial function and antioxidant responses, thereby promoting long-term survival of pathogenic CD4 T cells in inflamed tissues.

Deciphering the link between Eomes and Romo1 will lead to a better understanding of the role of Eomes in the pathophysiology of MS and other inflammatory diseases which could open new therapeutic strategies targeting mitochondrial actors.

68 - Alterations in adipokine levels in MS patient cohort project Y

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Current immunomodulatory and anti-inflammatory therapies for the treatment of multiple sclerosis (MS) prove to be relatively effective, however fail to concomitantly stop progressive neurodegeneration and do not reverse acquired disability, creating a high and unmet clinical need. The proportion to which genetic and environmental factors contribute to the etiology of MS is still incompletely understood, however an interesting association between MS etiology and obesity has recently been shown, with obesity greatly increasing the risk to develop MS. Altered balance of adipokines, white adipose tissue hormones, plays an important role in the low-grade chronic inflammation in play during obesity by their pervasive modification of local and systemic inflammation. V*ice versa*, inflammatory factors secreted by immune cells affect adipokine functional pathways. We hypothesize that in MS, there is a detrimental feedforward loop between pro-inflammatory adipokines and pro-inflammatory factors, which significantly contributes to chronic inflammation and neurodegeneration. We measured the plasma levels of the three highest circulating adipokines (adiponectin as anti-inflammatory and leptin and resistin as pro-inflammatory) in patient cohort Project Y. This is the first population based cohort worldwide in which MS patients (n=288) and controls (n=124) of the same year of birth of an entire country are included, and includes patients in relapse-remitting state and both primary (PPMS) and secondary progressive MS (SPMS). We used ELISA to measure adipokines and correlated outcomes to extended disability status scale (EDSS), disease duration, number of relapses, treatment status and neurofilament light (NfL), a biomarker for neurodegeneration. We observed significant higher adiponectin levels in male MS patients which was most apparent in PPMS males. In this group, high adiponectin levels associated with lower number of relapses, however in patients with high NfL levels, indicating increased progression, adiponectin levels were also increased. There were no differences in leptin or resistin levels between control and MS, however resistin levels decreased in patients that used disease modifying drugs. These data indicate that adipokines might be used as biomarkers for progression or treatment response, and more in-depth analyses will be performed to pinpoint their exact roles.

146 - Metabolic regulation of MS specific neural stem cells

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Progressive multiple sclerosis (PMS) is a chronic demyelinating and neurodegenerative disease of the central nervous system currently lacking effective treatment. Premature cellular ageing in the stem cell compartment of the PMS brain, with an increased accumulation of cell stress signatures, has been incriminated as a potential driver of remyelination failure. Cellular metabolism has been shown to regulate cell stress pathways and undergo reprogramming in aged cells, making it a promising target for therapeutic intervention. To uncover metabolic signatures of the stem cell compartment of the PMS brain with potential involvement in the pathobiology of the disease we employed a human directly reprogrammed brain stem cell (iNSC) system, where patient iNSCs retain the phenotypic and transcriptomic ageing markers of the brain. iNSC lines derived from patients with PMS were profiled and compared to age-matched control iNSC lines in terms of (1) transcriptional landscape through mRNA sequencing; (2) bioenergetic profile through extracellular flux analysis, (3) intracellular and extracellular metabolic landscape through untargeted metabolomics, and (4) mitochondrial morphology and fitness traits at single-cell resolution through a combination of flowcytometry and immunocytochemistry techniques. Our work identifies gene expression alterations in several signalling pathways involved in metabolic regulation in PMS iNSC compared to controls. PMS iNSCs display an altered bioenergetic profile, with increased mitochondrial and glycolytic

functions compared to age-matched control iNSCs. Several metabolic pathways are altered in PMS iNSCs, including glycolysis, pentose phosphate pathway, glutamine metabolism, and several amino acid metabolisms. Lastly, PMS iNSCs exhibit alterations of mitochondrial morphology and fitness traits. Our preliminary results suggest that PMS iNSCs retain a distinctive metabolic signature, making them a promising tool for investigating the metabolic control of brain stem cell dysfunction in the context of PMS. Further interrogating PMS iNSC metabolism and its involvement in regulation of cell stress may provide patient specific molecular insights and aid in therapeutic testing, enhancing precision medicine approaches.

Workshop XVI | Tissue-resident T cells in CNS inflammation

18 - CD8 T cells target enteric neurons in patients with gastrointestinal dysmotility.

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Full-thickness colonic specimens of 30 patients with GI dysmotility were investigated. Clinically, abdominal tumors and acute inflammation due to ulcerative colitis, Crohn's disease, and diverticulitis were excluded by thoroughly inquired past medical history, including defaecation diary, abdominal ultrasound, Wexner's, and Longo's scores. Furthermore, colonoscopy, colon contrast medium clyster, abdominal CT, MR defaecography, Hinton test were performed; pudendal nerve conduction velocity was determined.

A two-stage disease affecting the enteric nervous system was identified in these patients. CD8 T cell-dominated inflammation was active in patients with a clinical history of less than six years. CD8 T cells were closely attached to vital neurons of the submucosal and myenteric plexus which had upregulated MHC class I antigen. In contrast, only single CD4 T cells and CD68 macrophages were scattered throughout the intestinal wall but were not preferentially associated with enteric neurons. Beyond six years, there was no evidence for further inflammation; however, 74.8% of enteric neurons of the submucosal and myenteric plexus had vanished. The majority of remaining neurons was apoptotic.

Our further observation of CD8 T cell-mediated inflammation with CD8 T cells attached to esophageal neurons in sporadic cases of achalasia support the hypothesis that clinically relevant CD8 T cell-mediated immune reactions are not confined to the colon, but may also affect the upper GI tract.

Thus, a CD8 T cell-mediated immune reaction against enteric neurons may cause dysmotility of the upper and lower GI tract. It will be important to identify potential candidate neuronal autoantigens recognized by CD8 T cells and to diagnose patients in the phase of active inflammation prior to irreversible neuronal loss.

215 - Tissue-resident memory CD8+ T cells drive compartmentalized and chronic autoimmune damage against CNS neurons

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One Sentence Summary: In mouse models of CNS autoimmunity, sustained tissuedamage depends on autoreactive CD8+ T cells that adopt a tissue-resident memory phenotype.

The mechanisms underlying the chronicity of autoimmune diseases of the central nervous system (CNS) are largely unknown. In particular, it is unclear whether tissueresident memory T cells (Trm) contribute to lesion pathogenesis during chronic CNS autoimmunity. Herein, we observed that a high frequency of brain-infiltrating CD8+ T cells exhibit a Trm-like phenotype in human autoimmune encephalitis. Using mouse models of neuronal autoimmunity and a combination of T single-cell transcriptomics, high-dimensional flow cytometry and histopathology, we show that pathogenic CD8+ T cells behind the blood-brain barrier adopt a characteristic Trm differentiation program, and we reveal their phenotypic and functional heterogeneity. In the CNS, autoreactive CD8+ Trm sustain focal neuroinflammation and progressive loss of neurons, independently of recirculating CD8+ T cells. Consistently, a large fraction of autoreactive CD8+ Trm exhibits proliferative potential as well as pro-inflammatory and cytotoxic properties. Persistence of Trm in the CNS and their functional output, but not their initial differentiation, was crucially dependent on CD4+ T cells. Collectively, our results point to CD8+ Trm as essential drivers of chronic CNS autoimmunity and suggest that therapies targeting this compartmentalized autoreactive T cell subset would fill an unmet medical need.

309 - Regulatory T cells counteract CNS neuroinflammation via TGF-β signalling in CNS myeloid cells

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Despite success in anti-inflammatory therapies in multiple sclerosis (MS), persistent inflammatory processes within the central nervous system (CNS) driving neurodegeneration are still poorly understood and are not addressed by current treatment approaches. In order to assess novel therapeutic schemes with clinical potential, we investigated the local CNS-specific effects of adoptive regulatory T cell (Treg) transfer in ongoing CNS neuroinflammation. We found that Tregs migrating into the CNS are even able to suppress experimental neuroinflammation when given after symptoms have emerged. Within the inflamed CNS, T effector cells locally screen and interact with potential target cells, whereas Tregs show a place-bound behavior. Strikingly, Treg-T helper 17 (Th17) cell contacts happen frequently but transiently and very shortly, we exclude direct cell-to-cell suppression as major mode of action. The suppressive effect of Tregs is strictly antigen-specific and Tregs preferentially form stable and long-lasting contacts with CNS myeloid cells. RNA-sequencing of CNS myeloid cells as well as detailed mechanistic analyses revealed that blocking TGF-β eventually resulted in decreased effector function of Th17 cells with a crucial mediator role of CNS myeloid cells. Single cell sequencing from human cerebrospinal fluid samples confirmed the role of the discovered experimental pathways in MS patients. Thus, we provide evidence that antigen-specific Tregs display their suppressive effect in the CNS by modulation of CNS myeloid cells. Our findings support that revisiting the concept of Treg cell-based treatment in MS is an attractive option in light of current progress in clinical cell-based therapeutic approaches.

319 - Cytotoxic-like Eomes+ Th cells in secondary progressive multiple sclerosis

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Multiple sclerosis (MS) often presents with a relapsing/remitting course (RRMS) that can transition into a secondary progressive form (SPMS). SPMS exhibits worsening disability that accumulates over time and was previously thought to result from neurodegenerative processes occurring at a constant rate. Recent advances in SPMS research have now implicated active immune processes in pathogenesis and the rate of disability increase is known to be variable over time and between individuals, with stationary and progressive disease states. The factors leading to SPMS transition and disease worsening are unknown. We recently described that a circulating population of cytotoxic-like Eomes$^+$ Th cells were significantly increased in peripheral blood of a group of SPMS patients, and the level of these cells corresponded to patients with active worsening disability (Raveney *et al.* PNAS, 2021).

We observed through genotyping analysis that particular Eomes-related SNPs are associated with SPMS and may be related to a more rapid transition from RRMS and thus, we have investigated the functional consequences of these SPMS associated haplotypes.

Eomes$^+$ Th cells were found infiltrating into SPMS brain tissue in autopsy samples and these cells had a restricted TcR repertoire and expressed the cytotoxic effector molecule granzyme B. As we have linked Eomes$^+$ Th cells with increased brain atrophy in SPMS, these data suggest the expansion of a CNS epitope-specific population invading the brain and generating damage via a cytotoxic mechanism.

SPMS was associated with increased granzyme B production by peripheral Th cells and flow cytometry identified Eomes$^+$ Th cell subsets expressing granzyme B that can be distinguished by their cell surface phenotype. Such Th cells expressing the chemokine receptor CX3CR1, are linked to worsening symptoms in SPMS and could also be found in SPMS brain autopsy samples and in a novel mouse model of SPMS.

This study expands the understanding of Eomes$^+$ Th cell pathogenic mechanisms in chronic neuroinflammation, revealing potential biomarkers for SPMS progression and new therapeutic targets for SPMS treatment.

117 - Activated Eomes-Tfh1 cells infiltrate the cerebrospinal fluid in early Multiple Sclerosis

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Tertiary lymphoid structures (TLS) are reported in the meninges of patients with multiple sclerosis (MS), especially at the progressive stage, and are strongly associated to cortical lesions and disability. Besides B cells, these structures comprise follicular helper T (Tfh) cells that are crucial to support B cell differentiation. Tfh cells play a pivotal role in amplifying autoreactive B cells and promoting autoantibody production in several autoimmune diseases however very few is known about their role in MS. To this purpose, we analyzed the phenotype of Tfh cells in the blood of 39 healthy controls (HC), 41 untreated relapsing-remitting MS (RRMS) patients and in the cerebrospinal fluid (CSF) and paired blood of 10 untreated RRMS patients, all sampled at the onset of the disease. Using an *in vitro* model of blood-brain barrier, we assessed the trans-endothelial migratory abilities of the different Tfh cell subsets. We completed our MS Tfh description through bulk RNA sequencing of CSF-Tfh cells and blood-Tfh cells in 8 clinically isolated syndrome (CIS) patients. Although no significant alterations of circulating Tfh cells were observed by cytometry, we showed in the CSF of early RRMS patients an important infiltration of Tfh1 cells, with a high proportion of activated PD1-expressing cells. In parallel, transmigration study demonstrated that Tfh1 cells present an increased ability *in vitro* to cross a model

of BBB. Finally, RNAseq analysis showed that CSF-Tfh cells from CIS patients display a pro-inflammatory Tfh1 signature and a high expression of Eomes which was found associated with several genes implicated in cytotoxicity. Altogether, transmigration analysis suggests that Tfh that have crossed the BBB preferentially harbor a Tfh1 phenotype explaining their enrichment in the CSF. Then, the presence of activated pro-inflammatory Tfh1 cells inside the CSF of early MS patients may contribute to the reactivation of autoreactive memory B cells directly inside the CNS and participate to the formation of TLS fueling the disease. In addition, high expression of Eomes and cytotoxicity genes by these enriched Tfh1 points toward characteristics of tissue resident memory cells as it was demonstrated in other autoimmune diseases. This work was funded by grants from GMSI (Grant for MS Innovation) by Merck KGaA (CrossRef Funder ID: 10.13039/100009945), from ARSEP Foundation, from LabEx IGO and from INCR (Institut des Neurosciences Cliniques de Rennes).

292 - Contact-dependent granzyme B-mediated cytotoxicity of Th17 cells towards human oligodendrocytes

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Multiple sclerosis (MS) is characterized by the loss of myelin and of myelin-producing oligodendrocytes in the central nervous system (CNS). Pro-inflammatory CD4 Th17 cells are considered pathogenic in MS. We recently showed that Th17 cells establish prolonged contact with oligodendrocytes and induce process damage and cell death. Although we uncovered that CD29-triggered glutamate release by Th17 cells contributes to this process damage, mechanisms driving oligodendrocyte cell death remained unknown. Using fluorescent and brightfield in vitro live imaging, we found that compared to Th2-polarized cells, Th17-polarized cells showed greater interactions with human oligodendrocytic cell line MO3.13 and primary human oligodendrocytes, displaying longer duration of contact, lower mean speed and higher rate of vesicle formation at the sites of contact. To shed light on the mechanisms involved in the detrimental Th17-oligodendrocyte encounter, we investigated the properties of Th17 cells upon close interaction with such glial cells. Using single cell RNA sequencing analysis, we observed that Th17 cells upon direct contact with oligodendrocytes upregulated the mRNA expression of 71 genes, among them pro-inflammatory cytokines and chemokines such as IL-17A, IFN-γ and granzyme B. We confirmed by ELISA that secretion of CXCL10, IFN-γ, TNF, IL-17A and granzyme B were induced upon direct contact in cocultures of human Th17 cells with human oligodendrocytes. In addition, we confirmed by flow cytometry and immunofluorescence that granzyme B expression was upregulated in Th17 compared to Th2 cells. This lytic enzyme was more abundant in Th17 cells in direct contact with oligodendrocytes or MO3.13 cells compared to Th17 cells separated by an insert. Moreover, granzyme B was found in oligodendrocytes and MO3.13 cells following direct contact with Th17, suggesting that granzyme B released by Th17 cells entered in target cells: oligodendrocytes. To confirm granzyme B–mediated cytotoxicity, we showed that recombinant human granzyme B induced MO3.13 cells and oligodendrocyte death. Furthermore, pre-treatment of Th17 cells with an irreversible granzyme B blocker (Ac-IEPD-CHO) or a natural granzyme B blocker (serpina3N) improved survival of MO3.13 cells after coculture with Th17 cells. In conclusion, we demonstrated that human Th17 cells form biologically significant contacts with human oligodendrocytes and exert direct toxicity by releasing granzyme B.

Parallel X | Drainage and immunosurveillance of the CNS

153 - Pericytes play a protective role during neuroinflammation

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Background: Pericytes play a key role in blood-brain-barrier (BBB) maturation and maintenance of immune homeostasis in the central nervous system (CNS). Ablation of pericytes in developing mice causes vascular abnormalities, BBB leakage and, increased infiltration of leukocytes to the CNS in later life both in steady-state and pathological conditions. However, the effect of pericyte ablation during neuroinflammation in the presence of a fully mature BBB is unknown. **Aim:** In this study, we first aimed to generate a tamoxifen inducible pericyte ablation model in adult mice. Then, we investigated the effect of pericyte loss on neuroinflammation in an experimental allergic encephalomyelitis (EAE) model. **Methods:** PDGFRβ-PA2-CreER$^{+/-}$ mice (JAX Mice, 030201) were crossed with Rosa26-DTA176$^{+/+}$ mice (JAX Mice, 010527). After genotyping, 9-10 weeks old PDGFRβ-Cre^{+}/DTA176^{+} (Cre+) mice were injected with 0.1mg/g tamoxifen (TAM) for two, three, or five consecutive days to induce the expression of diphtheria toxin in PDGFRβ+ pericytes. PDGFRβ-Cre^{-}/DTA176^{+} (Cre-) mice were used as controls. After 15 days, animals were sacrificed to assess pericyte coverage and numbers, and *PDGFRβ* gene expression in the cortex and spinal cord. After analysis, 2X TAM injection model was selected for EAE studies. MOG35-55 (Hooke Labs, EK2110) was used to induce EAE 15 days after TAM injection. Cre- and wild-type C57BL/6 mice were used as controls. Weight change and clinical scores were monitored for 28 days. Analysis was done by repeated measures 2-way ANOVA multiple comparison test. **Results:** There was a 75% (+-SEM=0.9192) decrease in pericyte coverage in the cortex ($p<0.01$) and 40% (+-SEM=2.385) decrease in the spinal cord ($p<0.01$) after 2X TAM injection (n=7). Similarly, Pdgfrβ gene expression was decreased to 55% (+-SEM=2.230) in the cortex ($p=0.017$) and 59% (+-SEM=4.511) in the spinal cord ($p=0.048$). During EAE, pericyte-ablated mice (n=7) had a more severe clinical score after Day 20 (p=0.0086, 0.0036, 0.0054, 0.07,0.041). Pericyte ablated mice lost more weight than the wild type mice (p=0.0020, 0.0101, 0.0039, 0.0064, 0.0181, 0.0091, 0.0006 from Day16 to 28). In addition to the spinal cord lesions, inflammatory foci were also detected in the periventricular region in the brain of the pericyte ablated mice but not in control mice. **Comment:** We have generated and characterized a TAM inducible pericyte ablation model in adult mice that does not require injection of a toxin. By using this model, pericytes were shown to play an important role during the resolution of inflammation.

169 - The role of the meningeal layers in CNS autoimmunity

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The meninges, composed of the leptomeninges (pia and arachnoid layers) and the pachymeninx (dura layer), play an important role in the initiation and progression of central nervous system (CNS) autoimmunity. However, at present the contribution of each meningeal layer to the autoimmune process remains unclear. We detected a strongly contrasting involvement of the leptomeninges and the dura in experimental autoimmune encephalomyelitis (EAE). While the leptomeninges were highly inflamed during the autoimmune process, the dura mater was only marginally affected. We identified several properties of the dural and leptomeningeal milieu that could explain their distinct participation. First, dural vessels expressed lower levels of adhesion molecules that are necessary for the binding of the pathogenic effector T cells to the endothelium. Accordingly, 2-photon intravital microscopy showed that effector T cells had fewer interactions with the dural compared to the leptomeningeal endothelium. Further, activation of T cells in the dura during EAE was lower when compared to

the leptomeninges and prevented the formation of a local inflammatory process. This was not due to intrinsic defects in the T cells, nor to incompetence of the dura antigen presenting cells (APCs) in activating the T cells. Instead, the low activation of CNS-reactive T cells in the dura was due to the low availability of the cognate antigen: unlike the leptomeningeal APCs, dural APCs failed to spontaneously present myelin or neuronal antigens to autoreactive T cells. Indeed, dural APCs were able to do so only when the antigen was exogenously added. Finally, trafficking of CNS-reactive T cells through the lymphatic vessels of the dura and deep cervical lymph nodes was very limited during EAE. In these structures, we did not detect either antigen drainage or autoreactive T cell activation. Consequently, depletion of the dural lymphatic system did not have any effect on the EAE clinical course or immune cell infiltration in the CNS.

In conclusion, due to its intrinsic vascular properties and reduced access to CNS autoantigens, the dura is largely shielded from the neuroinflammatory process. The permissive nature of the leptomeningeal vessels to effector T cell trafficking and the availability of CNS antigens to local APCs place the leptomeninges as the crucial site for initiation of CNS inflammation.

322 - Migration across the blood-brain barrier affects mTOR signalling in regulatory T cells

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Multiple sclerosis (MS) is characterized by dysfunctional regulatory T cells (Tregs), both in the periphery and in the central nervous system (CNS). It was previously shown that in an inflammatory environment, Tregs can acquire Th1 and Th17 characteristics. We hypothesized that migration across an inflamed blood-brain barrier (BBB) induces a functional and phenotypical loss in Tregs. Using FOXP3 reporter mice, we found that exFOXP3 Tregs accumulate in the CNS of EAE animals over time, suggesting that migration into the CNS destabilizes Tregs. To elucidate the mechanism, a human *in vitro* model of the BBB, using the hCMEC/d3 endothelial cell (EC) line seeded in Thincerts, was employed. Tregs of healthy donors (HD) and MS patients were allowed to migrate for 24h, and RNAseq was performed on the non-migrated and migrated fractions. We identified that pro-inflammatory pathways (i.e. Th17 signalling) are increased and suppressive molecules(i.e. STAT5, LEF1, BACH2) decreased. These results were validated on protein level by flow cytometry: FOXP3, Helios and CD25 are decreased while IL6R, RORγt and MCAM are increased after migration across BBB-ECs. To identify changes on the functional level, a suppression assay was performed after migration. Preliminary data indicates that the suppressive capacity of Tregs is lower in the migrated vs non-migrated fraction. One of the most prominently affected suppression-related pathways was the mTOR pathway. Indeed, treatment of migrated Tregs with the mTORC1 inhibitor rapamycin restores and even augments their suppressive capacity. Finally, we sought evidence for this phenotype switch in MS patients. It is known that CD49d is expressed on inflammatory and non-suppressive Tregs. Indeed, we confirmed the presence of 2 Treg subpopulations in the peripheral blood of HD and MS patients, based on CD49d expression. Analysis of paired blood and CSF samples of MS patients at diagnosis, revealed that $CD49d^+$ Tregs are highly enriched in the CSF.
In conclusion, Tregs undergo a pathogenic phenotypic switch and loss of suppressive function after passage over BBB-ECs. Using the clinically relevant compound rapamycin, the functional stability of migrated Tregs can be restored.

166 - Multiple sclerosis and circadian rhythm: a systematic review

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, characterized by axonal and neuronal demyelination resulting from an autoimmune inflammatory response. Studies suggest that desynchronization of circadian rhythms is associated with neurodegenerative and metabolic disorders, such as MS. Objective: The aim of this study was to investigate the relationship between circadian rhythm and MS through a systematic review. Methods: A comprehensive and systematic literature review was performed using online databases, namely Cochrane, LILACS, PubMed and SciELO, with no date limit until April 17, 2021, to identify all clinical trials reported in English, Portuguese or Spanish. The strategy used in the search was based on the combination of the following descriptors: (multiple sclerosis) AND (circadian rhythms), (multiple sclerosis) AND (biological clock), (multiple sclerosis) AND (melatonin), (multiple sclerosis) AND (biological rhythms), (multiple sclerosis) AND (circadian cycle). After the initial search, articles in duplicate, triplicate or quadruplicate were excluded. The list of articles was then evaluated by two independent reviewers. The evaluation steps were: title, abstract and full text. Disagreements between reviewers were resolved by a third reviewer. Results: 65 papers were found in the initial search. Of these, 42 were disregarded after title analysis and 15 after reading the abstract, resulting in 8 articles. After reading the full text, 3 articles were considered eligible and used as the basis for conducting this review. Better tolerability was observed in nighttime corticosteroid treatment, with progressive improvement of symptoms and reduction of adverse events. There was no disturbance in the circadian rhythm during the treatment. In addition, circadian cortisol release was more pronounced in patients with relapsing-remitting multiple sclerosis compared to healthy control subjects. Conclusions: There is a difference in the tolerability of corticosteroid treatment of MS patients depending on the time of drug administration. The analysis presented may contribute to a better elucidation of the relationship between circadian rhythm and its perturbations in neurodegenerative conditions such as MS.

Parallel XI | Multi-omics in Neuroimmunology

82 - Transcriptome guided optimization of in vitro culture conditions for adult primary microglia

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Microglia, the resident tissue macrophages of the central nervous system (CNS), are key players during brain homeostasis as well as during neurodevelopmental and neurodegenerative disorders. Detailed knowledge of their cell biology is therefore of pivotal importance, and primary cell cultures provide an excellent means to obtain such knowledge. However, recent studies have demonstrated that the morphology and transcriptome of classical primary microglia culture models only partially recapitulate that of mature homeostatic *in vivo* microglia. We have therefore exposed primary microglia from adult rhesus macaques to a variety of culture conditions including exposure to different soluble factors, and several serum replacement approaches, and compared their morphologies and transcriptomes to those of mature, homeostatic *ex vivo* microglia. This enabled us to develop a new, partially serum-free, monoculture protocol, that yields high numbers of ramified cells. However, the transcriptomes of *in vitro* and *ex vivo* microglia remained substantially different. Analysis of differentially expressed genes inspired us to perform coculture experiments in ultra-low attachment plates leading to the formation of spheres. In such spheres, microglia signature genes were strongly induced, and the transcriptome better resembled that of *ex vivo* homeostatic microglia than adherent monocultured microglia did. These data provide new anchor points for the optimization of homeostatic primary microglia *in vitro* cultures, which contribute to better *in vitro* models to study microglia in health and disease.

262 - Investigating B cells and their depletion in relapsing-remitting Multiple Sclerosis using DNA methylation patterns

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Background: Multiple Sclerosis (MS) is a chronic inflammatory disease characterized by autoimmune destruction of myelin sheaths and neuroaxonal degeneration in the central nervous system (CNS). Previous reports have indicated widespread differences in DNA methylation in B cells from MS cases compared to healthy controls, and B cells have come under renewed interest due to the effectiveness of treatments that deplete B cells, such as rituximab. DNA methylation is an epigenetic mechanism used by cells to control gene expression, that is stable but reversible that could provide insight in understanding complex disease mechanisms as well as be a target for treating disease.
Objectives: To characterize epigenetic changes and their functional consequences in $CD19^+$ B cells from MS patients, and to investigate the treatment related changes in $CD4^+$ T cells and $CD14^+$ monocytes following B cell depletion with rituximab.
Methodology: e measured DNA methylation in CD19+ B cells sorted from peripheral blood of a cohort of 115 individuals comprising treatment-naïve MS patients, treated MS patients and various types of controls, which we compared and integrated with previously analyzed cohort (RRMS, n = 12; HC, n = 10). We also quantified DNA methylation in $CD4^+$ T cells and $CD14^+$ monocytes sorted from peripheral blood of RRMS patients (n = 17) at baseline and 6 months after rituximab treatment. DNA methylation was measured using Illumina Infinium HumanMethylationEPIC arrays.
Results: Meta-analysis of the two B cell cohorts revealed 3 003 differentially methylated positions (DMPs) between RRMS and HC (adjusted p-value < 0.05). Pathway analyses of the genes associated with differential methylation implicated dysregulation of genes involved in B cell development, survival, metabolism, motility and activation. Rituximab treatment resulted in 388 DMPs in CD4+ cells and 2478 DMPs in CD14+ cells (adjusted p-value < 0.05). Pathway analyses of candidate differentially methylated CpGs associated with changes in T helper differentiation and monocyte motility and activation being affected by B cell depletion.
Conclusion: Our data establish that B cells from MS patients acquire a distinct epigenetic profile connected to changes in multiple pathways of importance for B cell functions. Furthermore, we demonstrate changes in other cell types following B cell depletion as a therapeutic modality.
Keywords: MS, Multiple Sclerosis, Disease Modifying Treatment, rituximab, Mabthera, B cells, Epigenetics, DNA Methylation, EWAS

326 - Transcriptomic characterization of CSF reveals T and Myeloid cells as early players during MS disease onset

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The characterization of cerebrospinal fluid (CSF) lymphocytes is crucial to better understand important contributors to MS disease activity. Previously, their fragile nature and low cell counts often restricted investigations to utilizing fresh samples, and thus key lymphocyte signatures triggering and propagating CNS-compartmentalized inflammation remain unclear. To address this gap, we established a reliable CSF-cryopreservation protocol adapted for single cell RNA sequencing (sc-RNA-Seq). Next, using sc-RNA-Seq, we characterized the profile of CSF cells from untreated RRMS n=23, Ocrelizumab-treated progressive MS n=6, and patients with other neuroinflammatory diseases ONID n=6. After removing doublets, RBCs, and low-quality cells, we analyzed a total of 175,529 CSF cells. Top level clustering revealed 86% T-cells, 10% myeloid cells, 2% NK-cells,

1% B-cells, 0.5% plasma cells/plasmablasts and 0.5% plasmacytoid-DCs. Overall, we report 30 distinct CSF-cell clusters arranged into: T cells (NK T cells, dg-T, CD4/CD8 central memory T TCM, effector memory T TEM), ab-T cell and subclusters, myeloid cells (microglia-like, monocytic, myeloid DC, granulocytes), NK, plasmacytoid DC, Bcells and plasmablasts. We noted higher T-cell proportions in RRMS, at least in part driven by CD40L+ CD4 TCM, while the myeloid cell cluster proportions were lower, particularly the monocyte-like cells. This is in line with other flow cytometry-based data, and highlight the pivotal role of T and myeloid cells during MS disease onset. We also report significantly higher proportions of plasma cells in the MS groups compared to the ONID group; these cells are nearly absent in the latter. To investigate the origin of the microglia-like cells, we examined cells from a ONID female patient who had a bone-morrow transplantation from a male donor. The identification of microglia-like cells with a male phenotype post-transplantation in the CSF of this female patient indicates that these cells potentially acquire a microglia-like profile after CNS infiltration from the bone-marrow. Finally, the major cell clusters and gene expression were conserved in the cryopreserved samples, reflecting a successful cryopreservation process. Overall, our findings offer a higher resolution level to characterize CSF cells, which is useful to identify novel molecular and cellular mechanisms triggering MS disease onset, and those propagating CNScompartmentalized inflammation leading to disease progression.

368 - Large-scale phosphoproteomic mapping of RTKs reveal dynamic architecture and microglia expression in blunt TBI

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Traumatic Brain Injury (TBI) is a highly complicated pathology involving multiple events occurring simultaneously including, but not limited to, neuroinflammation, blood brain barrier disruption, apoptosis and alteration in neuronal signalling. Microglia plays a critical role in injury-related neuroinflammation and acts as a double-edged sword in brain trauma. To understand the temporal dynamics of neuroinflammatory process in traumatic Brain Injury (TBI), we performed an advanced unbiased approach using large-scale array phosphoproteomics to map 14 different families of receptor tyrosine kinase mediated signalling in a murine mild blunt TBI model and developed an R software-based analysis pipeline for analysing protein array data, available on open-access GitHub repository PROTEAS (PROTein array Expression AnalysiS; github.com/Rida-Rehman/PROTEAS. The initial investigation of temporal activation revealed that most signalling events initiate as early as 3 hours post TBI, thereby limiting our time window for acute intervention. After establishing the temporal significance, we performed a large-scale in-depth analysis of 223 tyrosine and serine/theronine kinase proteins and identified distinct signalling modules at 3 hours after trauma. Our results revealed that, among other significant targets, phosphorylated levels of B-cell specific Bruton's tyrosine kinase (Btk) and wound healing associated cMet (HGFR) were upregulated as soon as 3h post injury in microglial cells. Small molecule inhibition of Btk failed to produce an effect in signalling pattern or behavioural assessment whereas cMet emerged as a selective modifier of the early microglial response. cMet blockade prevented the induction of microglial inflammatory mediators, reactive microglia morphology, and TBI-associated responses in neurons, vessels, and brain extracellular matrix. Acute or prolonged cMet inhibition altered microglial morphology to ramified (resting state), compared to amebiod (activated) in sham controls, ameliorated neuronal survival and significantly improved motor performance. Our findings identify cMet as a modulator of early neuroinflammation in TBI with translational potential and indicate

several receptor tyrosine kinase (RTK) families as possible additional targets for TBI treatment. We conclude that acute microglia inactivation by cMet (expressed in microglia) inhibition and promotion of resting microglial state mitigates pathological response as early as 1-day post TBI.

Parallel XII | Immunopsychiatry: is it a nascent field?

33 - Encephalitis with Autoantibodies against the Glutamate Kainate Receptor (GluK2)

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Objective: To report the identification of antibodies against the glutamate kainate receptor subunit 2 (GluK2-abs) in patients with autoimmune encephalitis and describe the clinical-immunological features and antibody effects.

Methods: Rat neuronal cultures were used to precipitate the antigen of two sera from 8 patients with similar rat brain immunohistochemistry staining. Samples from 596 patients with different neurological disorders and 23 healthy controls were screened by cell-based assay (CBA) with GluK2-expressing HEK293 cells. GluK2-ab effects were determined by confocal microscopy in cultured neurons and electrophysiology in GluK2-expressing HEK293 cells.

Results: Patients' antibodies precipitated GluK2. GluK2 antibody-specificity was confirmed by: CBA, immunoprecipitation, GluK2-immunoabsorption, and GluK2 knockout brain immunohistochemistry. Two of the 8 samples showed reactivity with GluK2 epitopes shared with GluK1 or GluK3 subunits that GluK2 immuno-absorption abrogated completely. Six of 8 patients developed acute encephalitis and clinical or MRI features of predominant cerebellar involvement (4 presenting as cerebellitis, which in 2 patients caused obstructive hydrocephalus), and 2 patients had other syndromes (1 with cerebellar symptoms). One of the 8 samples showed mild reactivity with non-kainate glutamate receptors (AMPAR and NMDAR) leading to identify 6 additional patients with GluK2-abs among patients with anti-AMPAR (5/71) or anti-NMDAR encephalitis (1/73). Patients' antibodies internalized GluK2-containing receptors in HEK293 cells and neurons; these effects were reversible in neurons. In addition, a significant reduction of GluK2-mediated currents was observed in cells treated with patients' GluK2 serum; these functional effects were not mediated by receptor blocking but by antibody-mediated receptor internalization.

Interpretation: GluK2-abs associate with an encephalitis with prominent clinicoradiological cerebellar involvement. The antibody effects are predominantly mediated by internalization of GluK2-containing receptors.

80 - Deviated B cell receptor repertoire in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

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Background: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a chronic, intractable disorder, characterized by chronic fatigue, post-exertional malaise, sleep disorders, cognitive impairment, autonomic dysfunctions, etc. So far, there is no objective diagnostic marker, which is an obstacle in diagnosis and development of treatments for ME/CFS. Recent findings indicate the involvement of neuroinflammation with B cell abnormalities in ME/CFS, including various B cell abnormalities: skewed functional B cell subsets; impaired B cell differentiation and survival; and presence of autoantibodies against b-adrenergic or muscarinic cholinergic receptors. Objectives: Development of blood biomarker using deviated B cell receptor repertoire in ME/CFS
Methods: ME/CFS patients met Canadian criteria are enrolled. Age and sex-matched healthy subjects were recruited as control. The peripheral blood mononuclear cells (PBMCs) were purified and B cell receptor (BCR) repertoire were analyzed by next-generation sequencing and bioinformatics tool. Using the same samples, B cell subset analyses were performed by flowcytometry and anti-autonomic nerve receptor antibodies (anti-b1/b2/M3/M4) were measured (CellTrend GmbH.). Receiver operating characteristic (ROC) analysis were performed. New patient group were recruited to evaluate reproducibility. Results: There was a biased usage of several IGHV/IGHD/IGHJ genes in peripheral blood B cells from ME/CFS patients. ROC analysis indicated a possibility of distinguishing patients from healthy controls. A new patient group data suggested reproducibility of the BCR repertoire analysis. B cell clones using IGHV3-30 and IGHV3-30-3 genes were more frequent in patients with an obvious infection-related episode at onset, negatively correlated with disease duration and correlated to expression levels of interferon response genes in plasmablasts. Analysis of CDR3 length distribution of IGHV3-30/3-30-3 revealed that significantly higher frequencies at specific lengths from patients. Measurement of antibodies demonstrated a significantly increased frequency of anti-b1/2 adrenergic receptor antibody-positive ME/CFS patients. The frequency of IGHV3-30 and IGHV3-30-3 genes are more frequent in these antibody-positive patients. Conclusions: BCR repertoire analysis could be developed as a valuable tool to help diagnose ME/CFS.

279 - Slow disease progression and clinical heterogeneity make anti-CASPR2 encephalitis a diagnostic challenge

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Introduction: Autoimmune encephalitis with antibodies against contactin-associated proteinlike 2 (CASPR2 encephalitis) encompasses a broad range of both limbic and non-limbic symptoms. Previous studies suggested it may progress insidiously, possibly delaying diagnosis and treatment. We aimed to characterize the onset mode of CASPR2 encephalitis and assess how it influences the outcome of the disease.
Methods: We analysed retrospectively the medical records of a French cohort of 48 CASPR2 encephalitis patients. Telephone interviews with 35 of them and their relatives were conducted to obtain more complete data and analyze long-term outcomes. Cross-sectional assessment of autonomy and quality of life were performed using the Functional Activity Questionnaire and SF-36 scales, respectively.

Results: Forty-seven patients (98%) were males. Median age was 64 years. Median times to diagnosis and to disease peak were 10 and 16 months, respectively. At onset, prominent symptoms were altered general state, seizures, mood and sleep disorders, behavioral disorders, and neuropathic pain. 83% of patients presented with symptoms from only one of the three following categories: limbic-, cerebellar- or peripheral nervous system (PNS)- related symptoms. Because of slow disease progression and initially incomplete presentations, the current diagnostic criteria were not fulfilled in any patient at the early stage of the disease. At last visit, persisting symptoms were frequent but minor in most patients, 69% of them having a modified Rankin scale score ≤2. Cross-sectional analysis revealed preserved autonomy and good quality of life in most patients.
Conclusions: CASPR2 encephalitis initial presentations are highly variable, and the current diagnostic criteria are insufficiently sensitive at the early stage. However, awareness of the three categories of symptoms we highlighted (limbic, cerebellar, PNS) associated with male sex, age over 50, asthenia, altered general state, dysautonomia, or insomnia, should allow to identify the patients sooner in the course of their disease.

Poster Presentations

Antibody-mediated diseases: new targets and new mechanisms

223 - Skewing of SAG mediated therapy for a predominant Th1 during Visceral Leishmaniasis on triggering CD2 epitope

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Background Visceral leishmaniasis is a macrophage associated disorder which is linked with a profound decrease in the immunotherapeutic potential of the infected subjects leading to a marked reduction in the CD4 linked Th1 protective immune response. It greatly affects the liver leading to abnormal levels of SGPT and SGOT. Also the patients suffering from VL have been reported to be coinfected with Hepatitis C during some circumstances. Simultaneously the patients in Bihar are showing unresponsiveness towards SAG which is still a first line of drug in many countries around the world against Visceral Leishmaniasis. We have previously reported down regulation of CD2 co receptor on the surface of CD4 cells in patients suffering from Visceral Leishmaniasis. Stimulation of CD2 epitope with antiCD2 antibody has led to a remarkable increase in the Protein kinase C alpha mediated phosphorylation on CD2 co receptor on CD4 T cells, induction of IFN-γ led Th1 dominated immune response, a substantial increase in the lymphoblast population and this response remained Th1 dominated even in the presence of Th2 predominant conditions signified with rIL4. Studies in the 1980s showed that biological immunomodulators such as interferon (IFN)-γ can provide a missing signal and enhance the activity of antimonials in the treatment of VL and CL.

Methodology/Principal Findings In the present part of the study we have tried to evaluate the use of CD2 antibody as an immunotherapeutic agent along with SAG in ensuring treatment of BALB/c mice induced with experimental Visceral leishmaniasis. It has been found in the present set of studies that stimulation of CD2 co receptor along with along with therapeutic dose of SAG has led to the enhancement in the release of IFN-gamma which leads to the release of TNF-alpha and activates the macrophages. An increase in the NO mediated killing further observed by the activated macrophages leading to the reduction in the parasitic load.
Conclusions/Significance The results indicate that enhancing the immune potential of a VL patient will help in the better response of Sodium Antimony Gluconate which is the first line of drug against VL in many countries. These results further proven by insilico studies also emphasized the above results that SAG is the best available alternative for combating drug resistance.
Key words: Visceral Leishmaniasis (VL), rIL4 (Recombinant IL4), T helper prototype 1

230 - Immunopathogenesis and proposed clinical score for identifying Kelch-like Protein-11 encephalitis

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In this study we report the clinical features of Kelch-like protein 11 antibody (KLHL11-Ab)-associated paraneoplastic neurological syndrome (PNS), design and validate a clinical score to facilitate the identification of patients that should be tested for KLHL11-Abs, and examine in detail the nature of the immune response in both the brain and the tumour samples for a better characterization of the immunopathogenesis of this condition. The presence of KLHL11-Abs was retrospectively assessed in patients referred to the French Reference Center for PNS and Autoimmune Encephalitis with (i) Ab-negative PNS (limbic encephalitis [n=105], cerebellar degeneration [n=33]) and (ii) Ab-positive PNS (Ma2-Ab encephalitis [n=34], NMDAR-Ab encephalitis with teratoma [n=49]). Additionally, since January 1, 2020, patients were prospectively screened for KLHL11-Abs as new usual clinical practice. Overall, KLHL11-Abs were detected in 11 patients (11/11, 100% were male; their median [range] age was 44 [35-79] years), 9 of them from the Ab-negative PNS cohort, 1 from the Ab-positive (Ma2-Ab) cohort, and 1 additional prospectively detected patient. All patients manifested a cerebellar syndrome, either isolated (4/11, 36%) or part of a multi-system neurological disorder (7/11, 64%). Additional core syndromes were limbic encephalitis (5/11, 45%) and myelitis (2/11, 18%). Severe weight loss (7/11, 64%) and hearing loss/tinnitus (5/11, 45%) were common. Rarer neurologic manifestations included hypersomnia and seizures (2/11, 18%). An associated cancer was found in 9/11 (82%) patients, it was most commonly (7/9, 78%) a spontaneously regressed ("burned-out") testicular germ cell tumour. A newly designed clinical score (MATCH score: male, ataxia, testicular cancer, hearing alterations) with a cut-off ≥4 successfully identified patients with KLHL11-Abs (sensitivity 78%, specificity 99%). Pathological findings showed the presence of a T-cell inflammation with resulting anti-tumour immunity in the testis and one chronic, exhausted immune response – demonstrated by immune checkpoint expression - in the metastases and the brain. In conclusion, these findings suggest that KLHL11-Ab PNS is a homogeneous clinical syndrome and its detection can be facilitated using the MATCH score. The pathogenesis is probably T-cell mediated, but the stages of inflammation are different in the testis, metastases, and the brain.

258 - Can OSMS be differentiated from NMOSD and MS by peripheral blood immunophenotyping?

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The differential diagnosis of multiple sclerosis (MS) includes Opticospinal MS (OSMS), and Neuromyelitis Optica Spectrum diseases (NMOSD), which are clinically similar to MS. There is no biomarker for the diagnosis of OSMS, which can be misdiagnosed as NMOSD or MS. In this study, we aimed to develop B lymphocyte-based diagnostic biomarkers for distinction of OSMS from other demyelinating autoimmune disorders.
Method: Twenty-three relapsing remitting MS (RRMS), 15 relapsing optic neuritis (RON), 17 OSMS and 21 age-sex matched healthy donors were recruited for the study. Peripheral blood immunophenotyping was done by flow cytometry and CD19+ B cells were sorted by magnetic-activated cell sorting (MACS) from peripheral blood mononuclear cells (PBMCs). Agilent Human 8X60 K Oligo Microarray was used for gene level expression identification of isolated B lymphocytes. Following normalization, differentially expressed genes (DEGs) were obtained using R software. Functional enrichment analysis (gene ontology and pathway analysis) was performed for DEGs using DAVID database. Expression levels of selected DEGs were quantified by real time quantitative polymerase chain reaction (RT-PCR) for validation.
Results: No difference was found between study groups in terms of total T and B cell populations. OSMS patients showed significantly increased peripheral blood subsets of NK cells, regulatory B cells ($CD19+CD38^{high}CD24^{high}$) and plasmablasts ($CD19^{+}CD38^{++}CD138^{-}$) than other groups. IL10 producing $CD3^{+}CD4^{+}CD25^{+}CD127^{-}CD49^{+}$ Treg cells were elevated in the NMOSD and OSMS group. In microarray analysis and PCR studies, OSMS patients showed increased expression of CSF3R, CCL4L2, CCL20 and IL-18R genes compared to other disease groups.
Conclusion: OSMS patients show a distinct peripheral blood phenotype pattern that is different from conventional MS patients. NK cells, plasmablasts and chemokines attracting these cell types are increased in the peripheral blood of OSMS patients. Whether these factors may influence preferential involvement of the spinal cord and optic nerves need to be determined by future clinical and animal model studies.

Keywords: Multiple sclerosis, opticospinal MS (OSMS), neuromyelitis optica spectrum diseases (NMOSD), B cells, microarray

317 - The antibody repertoire of inflammatory sensory neuronopathies targets pathways of the innate and adaptative immune system.

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Dysimmune sensory neuronopathies (SNN) are heterogeneous disorders affecting neurons in dorsal root ganglia. Some are paraneoplastic, others occur with systemic autoimmune diseases (Sjöegren syndrome) or remain isolated. Antibodies (AB) occur in paraneoplastic (anti-Hu AB) and non-paraneoplastic SNN (anti-FGFR3 AB) but the pathophysiology of dysimmune SNN is still wholly unknown. Recent evidences indicate that AB targeting proteins of the immune system are produced during autoimmune diseases giving hints at their underlying mechanisms. Thus systemic approaches addressing the entire antibody repertoire of targeted antigens promise a more comprehensive understanding of immune disease mechanisms. Material/methods. We systematically analyzed the dysimmune SNN AB repertoire against proteins of the immune system pathways with two protein arrays. ProtoArray covering 7,634 human proteins was used with 38 SNN (16 with non-paraneoplastic dysimmune SNN comprising 7 with and 9 without anti-FGFR3 AB; 8 with paraneoplastic SNN and anti-Hu AB; 14 controls comprising 7 other neuropathies and 7 healthy controls). HuProt array covering 15,797 human proteins was used to test 43 subjects (12 with non-paraneoplastic dysimmune SNN and no AB; 31 controls comprising 22 other neuropathies and 9 healthy controls). We annotated the immune-system-related proteins among the repertoires and performed overrepresentation analyses via the Reactome database of PantherDB software. Serum INF alpha-2, IFN-gamma, IL 1-beta, IL6, IL17, and TNF alpha were measured by Bio-Plex Pro™ Reagent Kit III. **Results:** Dysimmune SNN sera interacted with a significantly higher number of proteins of immune system pathways than other study groups. Likewise, more pathways of the innate immune system, adaptative immune system, and cytokine signaling system were significantly overrepresented in dysimmune SNN than in healthy controls, anti-

FGFR3-neagtive SNN, or paraneoplastic SNN. Anti-FGFR3-positive SNN were more reactive with immune system proteins than anti-FGFR3-negative patients, both regarding the number of targeted proteins and the number of overrepresented pathways. Cytokine levels were higher in anti-FGFR3-negative patients than in anti-FGFR3-positive ones for IFN alpha-2 and TNF alpha. **Conclusion:** The antibody repertoire of non-paraneoplastic SNN may be an imprint of disease-relevant immunological pathways. The identified repertoires of targeted antigens involve cytokine signaling pathways that may also participate to the pathogenesis of Sjögren Syndrome.

164 - GABAAR autoantibodies have differential effect on GABAAR and contribute differently to GABAAR encephalitis

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Autoantibodies against central nervous system proteins are increasingly being recognized in association with psychiatric, neurological and neuro-degenerative disorders. Although a growing number against a variety of proteins have been identified, a causal link between specific antibodies and disease etiology is anecdotal at best, as most studies to date are on mixtures of antibodies present in the CSF and not with single molecularly identifiable antibodies. It is possible that each individual antibody in the CSF operates in a unique fashion and/or combination of mechanisms, a so-called 'fingerprint', to alter receptor function and thus contribute to disease progression. To resolve this issue in the present study, we have utilized patient derived monoclonal antibodies against specific $GABA_AR$ subunits; α1- and α1γ2-subunits. Intriguingly our studies could show that the α1 and α1γ2 subunit specific antibodies have different function altering fingerprints that could contribute to disease progression. Specifically, we found that the α1 antibody directly affects $GABA_AR$ function on a very short time scale and diminishes GABA currents. This decrease in GABA currents subsequently leads to increased network excitability. Interestingly, binding of the α1γ2 antibody to receptors appears to have no direct effect on receptor function and/or internalization. We are therefore currently investigating the role of the benzodiazepine site, which is also located at the interface of the α1γ2 subunits, as a possible mediator of effector mechanisms in more depth. Taken together, these results can influence treatment strategies and help make treatment more specific.

265 - Antibodies targeting both Caspr1 and CNTN1 in patients with autoimmune nodopathies

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Background: Antibodies anti-Caspr1 or anti-CNTN1 are present in a subset of patients with aggressive-onset autoimmune nodopathies (EAN/PNS 2021) with early axonal involvement and poor response to IVIg. Recently, anti-paranodal antibodies targeting both Caspr1 and CNTN1 were reported in two patients with acute-onset autoimmune neuropathies. Methods: We tested anti-Caspr1 antibodies in sera from a cohort of 18 CIDP patients with anti-CNTN1 antibodies. Anti-Caspr1 and anti-CNTN1 IgG were tested by CBA and ELISA, and also IgG subclasses and pre- and post-treatment Ab titers were investigated by ELISA. Clinical data were retrospectively collected. Results: We identified two patients with antibodies reacting against both CNTN1 and Caspr1 separately. Patient 1 was a 54 y/o man with a subacute-onset axonal neuropathy with thoracic and lumbar back pain, asymmetric tetraparesis, distal sensory disturbances, ataxia and dysphonia. Concomitantly he presented with a nephrotic syndrome with leg edema, ascites, hypoproteinemia and proteinuria. Renal biopsy demonstrated a membranous glomerulonephritis. At onset, anti-Caspr1 and anti-CNTN1 antibodies were IgG4 predominant but also anti-Caspr1 and anti-CNTN1 IgG3 were detected. After treatment, Ab titers decreased and only anti-Caspr1

IgG4 were detected. Both, nephrotic syndrome and neuropathy improved with treatment with steroids and rituximab. Patient 2 was a 74 y/o man with an acute-onset demyelinating neuropathy with a relapsing-remitting course with oculomotor palsy, ptosis, decreased reflexes, sensory ataxia, tremor, tetraparesis and respiratory involvement that required ventilation. He responded temporarily to plasma exchange. Anti-Caspr1 and anti-CNTN1 antibodies were IgG4 predominant and also anti-CNTN1 IgG3 were detected. Conclusion: Our observations suggest that anti-paranodal antibodies targeting both Caspr1 and CNTN1 epitopes are present in a small number of patients with aggressive-onset autoimmune nodopathies. Their detection is crucial to improve the clinical management of these patients.

372 - Anti-GM1 flow cytometry cell based assay is more specific for the diagnosis of AMAN than ELISA methods.

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Guillain-Barré Syndrome (GBS) has several variant forms, one of which is termed acute motor axonal neuropathy (AMAN). AMAN has been linked to a preceding Campylobacter jejuni infection and formation of antibodies to GM1. Anti-GM1 bind to the nodes of Ranvier and activate complement and/or activate macrophages. The detection of anti-GM1 antibodies, especially of IgG isotype, is useful for the diagnosis. However, there are different methods to detect the presence of these autoantibodies. Our laboratory used for many years a home-made ELISA technique. Our objective was to compare our method with the Incat ELISA method, the commercial Bülhmann ELISA and a cell-based assay using flow cytometry method (FCM) with incorporation of GM1 in the membrane of living cells for the diagnosis of AMAN. The later technique, contrary to ELISA methods, allows the presentation of carbohydrate epitopes similar to the presentation at the level of the nodes of Ranvier. Polyneuron Pharmaceuticals AG have developed a glycopolymer (PN1018) presenting multiple mimics of this natural GM1 carbohydrate epitope that is able to compete in vitro and in vivo with the fixation of anti-GM1 antibodies.
We performed a monocentric retrospective study with selection of 24 sera from AMAN patients, 18 anti-GM1 IgG positive sera from non-AMAN patients coming from the same neurological department suffering from different pathologies (ALS (1), CIDP (1), GBS (3), NMM (4), NORB (1), HNPP (1) Psychosis (1), Facial Paralysis (1), mononeuropathy (1), medullar compression (1), Sensitive Neuropathy (1), Proximal Deficit (1), Radiculalgia (1)) and sera from healthy controls. We first compared the anti-GM1 reactivity among the four tests and then analyzed the effect of adding the competitor PN1018 in the reactions. FCM is the more sensitive method for AMAN diagnosis (75.0%). Among all anti-GM1 positive sera from non-AMAN, 78.8% are negative using FCM. PN1018 fully inhibited the binding of anti-GM1 using FCM of positive sera from AMAN while the polymer only partially inhibited the signal in ELISA. Moreover, the polymer did not inhibit the signal in ELISA with sera from non-AMAN patients suggesting that these anti-GM1 antibodies recognize other epitopes non-exposed in living cells and probably not involved in AMAN.

26 - A Long Acting B Cell Depleting BiTE (Bi-Specific T Cell Engager) Significantly Ameliorated EAE Symptoms without Potential CRS Risk

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Key words: BiTE, B cell depletion, EAE, CRS

B cells play critical roles in the pathophysiological progression in autoimmune diseases including multiple sclerosis (MS). B cell depletion by monoclonal antibodies (such as anti-CD20) has been proved to bring clinical benefits for autoimmune disease patients. In this study, we developed a novel pegylated anti-CD3 x anti-CD19 compound which significantly ameliorated symptoms on the animals of Experimental Autoimmune Encephalomyelitis (EAE, the animal model of MS) without the potential risk of developing cytokine release syndromes (CRS). Compared to the non-pegylated parent compound blinatumomab (approved for treating B cell lineage leukemia or lymphoma), the pegylated compound binds to CD3ε on T cells and CD19 on target cells with much reduced affinity than blinatumomab and selectively depletes B cells with high level CD19 expression in PBMC, which has been reported to be antibody secreting plasma cells. By measuring the released cytokines of the healthy donor derived whole blood incubated for 2hrs, 6hrs and 24hrs with different concentrations of the compound, we found that the compound barely induced cytokines, while blinatumomab induced significantly high-level cytokines. At the high doses of 30 and 100mg/kg administered to the humanized transgenic animals (hCD3ehCD19), the compound did not induce any observable toxicity. For studying the potential therapeutic efficacy to MS, we treated the MOG1-125 induced EAE animals (using hCD3ehCD19 transgenic mice) with clinical scores of 3 post model construction by this compound along with the control drug MEDI-551 (an anti-CD19 monoclonal antibody approved for treating neuromyelitis optica spectrum disorder (NMOSD) in adults), we found: although MEDI-551 eliminated all B cells in the spleen, the clinical symptoms of the EAE animals were not ameliorated comparing with untreated EAE group; on the contrary, the compound depleted only 50% of B cells in the spleen, yet the clinical score of the compound treatment group was significantly reduced than the untreated EAE group. Furthermore, pharmacokinetics study demonstrated that in vivo elimination half-life of the compound in wild type C57BL/6 mice was equivalent to one week in humans for the single dose of 1mg/kg. More studies on this compound are still ongoing.

50 - Transcriptome analysis of ependymal cells exposed to autoantibodies from Neuromyelitis Optica patients

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Background: At the interface between cerebrospinal fluid (CSF) and brain parenchyma lies ependyma. This epithelium is composed of glial cells called ependymocytes presenting crucial functions. Through the expression of specialized transporters, it contributes to the bidirectional exchanges of water, ions, metabolites and to the clearance of toxic wastes between CSF and interstitial fluids. Ependymocytes also possess tufts of multiple motile cilia regulating CSF circulation and is are a niche for subventricular neural stem cells. Finally, ependyma plays a role in immune processes since it controls leukocyte diapedese, it expresses numerous cytokine/chemokine or complement receptors and produce cytokines and chemokines in response to microbial infections. Neuromyelitis Optica (NMO) is a severe neuroinflammatory disease associated with autoantibodies (NMO-IgG), directed against aquaporin 4 (AQP4) mainly expressed at astrocytic foot processes. NMO-IgG are present in patient's CSF during attacks, and are known to trigger astrocyte dysfunction leading to neuroinflammation with immune cell infiltration, demyelination and axonal loss. Interestingly, ependymal cells also express AQP4 and evidences of ependymal alteration are reported in NMO. **Aim:** Evaluate the transcriptome profile of ependymal cells after exposure to NMO-IgG autoantibodies. **Methods:** NMO-IgG were purified from AQP4 antibodies positive patients' plasma. IgG from healthy donors (CTRL-IgG) and non-treated (NT) conditions were used as controls. Primary ependymal cell cultures were treated during 24h with NMO-IgG or control conditions (n=3) and bulk RNA sequencing (RNAseq) was performed following flow cytometry cell sorting. **Results:** NMO-IgG increased 295 and decreased 70 transcripts expression compared to NT and CTRL-IgG differentially increased 118 transcripts and decreased 80 transcripts compared to NT condition. Functional analysis performed with Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA) showed a strong increase of gene sets related to immune and cell proliferation pathways in NMO-IgG condition and some pathways related to immune activity in CTRL-IgG

condition. Analysis of cytokine and chemokine expression in the RNAseq dataset and by qPCR showed a downregulation of Il6 and an increase of CXCL13, CCL12, CCL6, CXCL9 and TNF-α compared to NT and CTRL-IgG condition. **Conclusions:** These results suggest that NMO-IgG modulate the transcriptome profile of ependymal cells, and highlight the potential role of ependymal cells in neuroinflammation.

101 - Antiphospholipid antibodies are elevated in clinically isolated syndrome relative to other clinical subtypes of multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disorder targeting the central nervous system (CNS). Lipids make up more than 80% of myelin, the protective sheath surrounding axons and potential target of MS pathology. Elevated antiphospholipid antibodies (aPLs) have been implicated in MS but are poorly characterized in different MS clinical subtypes. We conducted an exploratory cross sectional analysis of aPLs in a diverse MS cohort compared to those with clinically isolated syndrome (CIS), a CNS demyelinating event often followed by transition to MS. Serum IgM levels of β2-Glycoprotein I (BGP), Cardiolipin (CL), Phosphatidyl-choline (PC), -ethanolamine (PE), -inositol (PI), -serine (PS), and Sphingomyelin (SM) aPLs were measured by enzyme-linked immunosorbent assay in healthy controls (HC, n=35), CIS (n=20) and different clinically confirmed MS subtypes (n=83) including relapse remitting (RRMS, n=33), secondary progressive (SPMS, n=30), and primary progressive (PPMS, n=20) MS. CIS participants had higher levels of aPLs against CL, PS, PC, PE, SM, and PI than HC (ANOVA with Tukey multiple comparisons, $p<0.05$). Only aPL levels to CL and PS were elevated in MS participants versus HC ($p<0.05$). No differences in aPL to BGP was detected between groups. Independent of age and sex, elevated aPL levels to CL (β (95% CI)=0.231 (0.08-0.38)), PC (β=0.180 (0.07-0.29)), PE (β=0.217 (0.10-0.34)), and SM (β=0.173 (0.07-0.28)) were associated with a higher likelihood of being CIS relative to HC ($p<0.005$). Elevated aPL to PS (CIS: β=0.223 (0.10-0.35); MS: β=0.101 (0.01-0.19)) and PI (CIS: β=0.209 (0.10-0.33); MS: β=0.088 (0.003-0.17)) were associated with a higher likelihood of being CIS or confirmed MS relative to HC ($p<0.05$). Setting a positive response cut off at HC levels +1.5SD, responses to these 6 aPL were detected in 3-11% of HC, 25-40% of CIS, and 12-24% of pooled MS participants. Low response rates to each aPL in RRMS (3-18%) and SPMS (0-13%), compared to higher rates in PPMS (25-50%), meant aPL to CL, PE, and PS distinguished between the different confirmed MS group types (Fisher Exact, $p_{adj}<0.008$). IgM antibodies have been linked to complement-mediated pathology associated with certain types of MS. Prevalence of similar aPL in PPMS and CIS groups is suggestive of similar pathological processes in these groups, and highlights the need to further characterize the relevance of aPL in the pathophysiology of different MS clinical subtypes.

102 - The functional significance of hnRNP A1 in oligodendrocyte biology in experimental models of multiple sclerosis

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Multiple sclerosis (MS) pathology is characterized by oligodendrocyte death and damage, but the underlying mechanisms remain unknown. RNA binding proteins (RBP) dysfunction, including nuclear depletion, nucleocytoplasmic mislocalization of RBPs from the nucleus to the cytoplasm, and the formation of cytoplasmic stress granules (SGs), are characteristic of several neurologic diseases, including amyotrophic lateral sclerosis and frontotemporal dementia. Recent data indicate that dysfunctional RBPs may contribute to the pathogenesis of MS. Previous work from our lab demonstrated increased incidence of nucleocytoplasmic mislocalization of the RBP heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) in neurons from MS patients as compared to controls. Interestingly, other studies have indicated that hnRNP A1 regulates the expression of myelin related genes, which are critical for oligodendrocyte myelination. Therefore, the hypothesis in this study was that dysfunctional hnRNP A1 and SG formation might be contribute to the biology of oligodendrocyte in MS. For this purpose, C57BL/6 female mice were used to induce experimental autoimmune encephalomyelitis (EAE) using MOG_{35-55}. Spinal cord tissues were removed at the peak of EAE and examined for detection of the hnRNP A1 dysfunction and stress granule assembly factor 1 (G3BP), a marker of SG formation, in oligodendrocytes. Mouse primary oligodendrocytes were cultured and treated with the following cytokines (2.5μg/mL, 24h): IFN-γ, TNF-α , and IFN-γ/ TNF-α, to assess hnRNP A1 localization and SG formation. siRNA against hnRNP A1 in oligodendrocyte CG4 cells was used to determine its effects on oligodendrocyte health. Our in vivo findings showed that hnRNP A1 dysfunction, including hnRNP A1 nucleocytoplasmic mislocalization and SG formation in oligodendrocytes is prevalent in the spinal cord white matter of EAE animals. Quantification also showed a significant increase in the percent of oligodendrocytes with hnRNP A1 mislocalization and SG formation following IFN-γ/TNF-α treatment as compared to control cells (in vitro). Furthermore, siRNA knockdown of hnRNP A1 in CG4 cell line decreased oligodendrocyte complexity, increased cytotoxicity, and induced activation of necroptosis and apoptosis pathways. Taken together, this suggests that RBP dysfunction plays a crucial role in oligodendrocyte biology and might be an underlying mechanism that contributes to MS pathogenesis.

Keywords: Multiple Sclerosis, Experimental autoimmune encephalomyelitis, Oligodendrocyte, Heterogenous nuclear ribonucleoprotein A1, Stress granule.

103 - Autoimmunity to an RNA binding protein drives neurodegeneration in an *in vitro* model of multiple sclerosis

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Neurodegeneration, the progressive loss of structure and function of neurons underlies permanent disability in multiple sclerosis (MS), yet its mechanisms remain incompletely understood. MS patients make antibodies to the RNA binding protein (RBP) heterogeneous nuclear ribonucleoprotein A1 (A1), which we have identified to contribute to A1 dysfunction and neurodegeneration in MS models. Dysfunctional RBPs (including A1) have become a pathogenic hallmark of many neurodegenerative diseases including MS and results in (i) nucleocytoplasmic mislocalization of the RBP, (ii) increased formation of stress granules and (iii) altered RNA metabolism. Although dysfunctional A1 is a feature of MS, it is unknown whether this dysfunction triggers or occurs as a result of neurodegeneration. Therefore, we hypothesize that the addition of A1 antibodies to neuronal cultures will result in an exacerbation of A1 dysfunction preceding neurodegeneration.

In the present study we treated embryonic mouse primary cortical neurons with A1 or control IgG antibodies (20 ug/mL) for 6, 12 or 24 hours. Neurons were analyzed quantitatively for antibody uptake, A1 mislocalization, stress granule formation, and neurodegeneration with markers of necroptosis and neurite length. Additionally, RNAseq was performed to detect changes in RNA metabolism.

Compared to controls, A1 antibodies were preferentially taken up by neurons at 6, 12 and 24 hours ($p \leq 0.01$) resulting in (i) increased A1 mislocalization at 6 and 12 hours ($p \leq 0.001$), (ii) increased stress granule formation at

12 and 24 hours ($p \leq 0.05$), (iii) increased markers of necroptosis at 12 hours ($p \leq 0.001$), and (iv) a reduction in neurite length at 24 hours ($p \leq 0.05$). RNAseq analysis identified 468 differentially expressed genes, with several pathways of interest enriched in the data set.

Neurons treated with A1 antibodies showed that A1 dysfunction, including mislocalization, stress granule formation, and altered RNA metabolism, occurred prior to increased markers of necroptosis and reduced neurite length. This is indicative of A1 dysfunction triggering rather than occurring as a result of neurodegeneration. These data reveal a novel mechanism of neurodegeneration involving autoimmunity to an intraneuronal target, which may be targeted to inhibit permanent disability in MS.

124 - Immunization with the multiple sclerosis candidate autoantigen of KIR4.1 promotes murine autoimmune encephalomyelitis

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Multiple sclerosis (MS) involves immune cells including B and T cells but the actual targets of the immune response are still undefined. The ATP-sensitive inward rectifying potassium channel KIR4.1 expressed by glial cells is suspected as an autoantigen since high seric levels of IgG against the extracellular sequence (e1) were reported in some MS patients, but whether this domain can be encephalitogenic remains elusive. Here, using ELISA with the e1 sequence (amino acids 83-120) as coating peptide, we indeed obtained high seric levels of anti-e1 IgG in a subset of MS/CIS patients from Nantes and Louvain, with 6-8% frequency. To investigate the encephalitogenicity of the e1 peptide, C57Bl6 mice were immunized with e1 peptide and the development of anti-e1 antibodies and of clinical/neuropathological signs of autoimmune encephalomyelitis was followed after immunization challenge. Detection of high seric anti-e1 IgGs by ELISA at one month and two months after immunization indicates that tolerance to e1 could be broken. Strikingly, after the last challenge, one third of the mice developed signs of typical EAE with later signs of atypical EAE. Accordingly, neuroinflammation and demyelination in sick mice occurred widely and heterogeneously in the CNS from forebrain, cerebellum to the spinal cord with immune cell infiltration mainly T cells and macrophages localized in white matter tracts. Another observation of e1-immunized sick mice was the presence of B cells in brain periventricular and white matter perivascular regions as well as in spinal cord meninges. Our data confirm higher levels of antibodies directed against the e1 extracellular loop of KIR4.1 in a subset of MS patients. Moreover, the development of demyelinating neuroinflammation in e1-immunized mice supports KIR4.1 as a valid candidate autoantigen in MS. Our results favor the heterogeneity in the autoimmune aspect of the disease, in which specific subgroups with discrete autoreactivites for CNS antigens may be further delineated in the MS spectrum. Supported by Inserm, Region Pays de la Loire and Antares association.

150 - T cells in NMOSD patients – insights from the peripheral blood and the CSF

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Neuromyelitis optica-spectrum disorder (NMOSD), a rare autoimmune disease of the central nervous system (CNS), preferentially affects the optic nerve and spinal cord. Most patients exhibit pathogenic aquaporin 4 (AQP4) antibody, which targets the water channel on astrocytes. However, the role of T cells in NMOSD pathogenesis is still not understood. Therefore, we performed an in-depth characterization of T cell subsets in the periphery and the cerebrospinal fluid (CSF) of NMOSD patients (n=30) by multi-colour flow cytometry and T cell receptor (TCR) sequencing. For the evaluation of the flow cytometry data our cohort of treatment naïve NMOSD patients was strictly age- and sex matched with healthy donors (HD) and treatment-naïve relapsing remitting MS (RRMS) patients. Data analysis revealed that naïve NMOSD patients displayed more activated T cells in the CSF and more terminally differentiated T cells with features of senescence in the peripheral blood compared to RRMS patients as well as HDs.
Also in the TCR sequencing, treatment naïve NMOSD patients show a higher clonality in the CD4+ and CD8+ compartment compared to HD and RRMS implying a clonal expansion of T cells. Initial TCR data analysis of a larger NMOSD cohort (n=180) supports the hypothesis of an antigen-driven T cell response. Taken together, our data confirm a role for T cells in NMOSD disease pathogenesis.

154 - Acquisition of viral and self antigens by B cells in virus infected brain

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Multiple sclerosis (MS) is an inflammatory demyelinating disease which affects the central nervous system. The recent success of anti-CD20 treatment suggests that B cells are involved in pathogenesis. Our study was based on the hypothesis that viral infections provoke B cell infiltration into tissues, and we aimed to elucidate the parameters governing B cell migration into the brain, lung and skin. We established a model system using genetically modified virus injection into the striata of transgenic mice with B cell receptors specific for viral antigens or for the myelin oligodendrocyte glycoprotein (MOG). B cells infiltrate the brain from day 2 post injection. Immunofluorescent microscopy of infected tissue – lung and brain – and intravital two-photon imaging of infected brain and skin showed intimate contact between the antigen-specific B cells and antigen-expressing cells in the infected area. ELIspot analyses demonstrated the ability of brain-infiltrated, antigen-specific B cells to present antigen to T cells ex vivo. Antibody-secreting B cells could be detected in the brain parenchyma for more than 28 days after injection. These results suggest that B cells are able to acquire both viral and self antigens directly in infected tissue, but require another signal, possibly T cell derived, in order to survive and secrete antibody. Better understanding of B cell biology in brain is likely to improve the development of better therapeutics for MS.

173 - Effects of patient-derived anti-NMDA receptor antibodies on synaptic function and cortical network activity

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Anti-NMDAR encephalitis is a severe neuropsychiatric disorder associated with autoantibodies against NMDA receptors, which give rise to a broad variety of symptoms. Initially, patients present with prominent psychiatric manifestations with psychosis, hallucinations and behavioral changes, which then progress to severe memory loss, seizures and autonomic instability, often requiring prolonged treatment in intensive care unit. Previous studies aiming to elucidate underlying mechanisms focused mainly on hippocampal effects of these autoantibodies, helping to explain mechanistic causes for cognitive impairment. However, antibodies' effects on higher cortical network function, where they could contribute to psychosis and/or seizures, have not been explored in detail until now. We therefore generated patient-derived, monoclonal antibody against the NR1 subunit of NMDAR and tested its effects on *in vitro* cortical neuronal cultures using imaging and electrophysiological techniques. Remarkably, we observed that hNR1 antibody gradually increases the overall activity of cortical networks to a hyper-excitable state by increasing neuronal firing rates. This hyper-excitability seems to be a result of reduced inhibition due to a specific impairment of inhibitory neuron output. Together, these findings provide a novel, cortex-specific mechanism of antibody-induced impairment of inhibition, highlighting regional specificity underlying the pathology of autoimmune encephalitis.

204 - IgA production decrease is associated with B cell differentiation and proliferation defects in multiple sclerosis

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The efficacy of B-cell depleting therapies in multiple sclerosis (MS) has proven the B cell contribution to pathogenesis. However, the failure of TACI-Ig, a treatment that blocks B cell differentiation into plasma cells has pointed out a potential protective function of plasma cells in MS. The aim was to explore IgA production and plasma cell differentiation in MS patients. IgA levels were measured by ELISA in MS patients (n=33) and healthy controls (n=15). B cell differentiation was assessed in vitro. IgA-memory B cells were sorted by flow cytometry. Libraries preparation and sequencing were performed by Next Generation Sequencing Platform of Curie Institute. We report a lower concentration of IgA in MS patient serum compared to controls. A lower proliferation and differentiation of B cells from MS patients was observed and associated with reduced IgA secretion. RNA sequencing analysis of IgA memory B cells showed a specific transcriptomic signature in MS. Two blockers of proliferation were found upregulated and 1 gene of cell survival was downregulated. Our results suggest that IgA decreased production is linked to a defect of proliferation and B cell differentiation in MS.

Keywords: IgA, plasma cells, multiple sclerosis

218 - LONG-TERM PROGNOSTIC VALUE OF INTRATHECAL ANTI-NMDAR ANTIBODIES PERSISTENCE

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Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is defined by the presence in the cerebrospinal fluid (CSF) of antibodies targeting the NMDAR. Most patients significantly improve with immunotherapy, but 20% remain with cognitive, behavioral, and motor disabilities, and 12-20% present relapses. Several biomarkers have been proposed, but the prognostic value of persistent CSF antibodies after the acute phase remains unknown. We conducted a retrospective observational study to describe the clinical features of patients with relapses and poor outcomes, and to assess the value of persistent CSF NMDAR antibodies as prognostic biomarkers.A total of 78 patients with available CSF and detailed clinical evaluations on subsequent follow-ups were included. At 12 months follow-up, 38/61 (62%) patients tested had persistent detectable CSF NMDAR antibodies; at 24 months, 20/32 (62%); at 36 months, 17/27 (62%); at 60 months, 7/9 (77%); and at any point later than 60 months, 5/6 (83%). At last follow-up (median 1288 days; range 135-2081), 60/78 (76%) patients had good outcomes (mRS <3), and those with poor outcomes (mRS ≥3) tested at 12 months had more frequently persistent NMDAR antibodies (13/35, 37%, vs 2/21, 9%; p=0.03). Patients with relapses (18/78, 23%) had less commonly an underlying tumor (0/18, 0%, vs 14/60, 23%; p = 0.03) and had longer delay of first-line treatment (median 34 days, range 8-393, vs 15 days, range 1-1402; p=0.03). Twelve (12/18, 66%) relapsed after 24 months, and all those tested at this point and prior to the relapse had persistent CSF NMDAR antibodies (6/6, 100%, vs 9/20, 45%; p=0.02). During relapses, 1 patient suddenly died, 1/17 (88%) had persistent CSF antibodies, 2/17 (11%) had new MRI lesions, 6/17 (35%) had inflammatory CSF. All patients with relapses were treated with immunotherapy, and 11/17 (64%) with second-line or alternative drugs. The chronic maintenance of azathioprine or mycophenolate mofetil was not associated with outcomes or relapses. The persistence of CSF NMDAR antibodies at 12 and 24 months might be respectively associated with worse outcomes and more frequent relapses. Further prospective studies should confirm our findings and investigate novel therapies with an impact on CSF antibodies.

241 - Paraneoplastic Cerebellar Degeneration: The Importance of Including CDR2L as a Diagnostic Marker

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Paraneoplastic neurological syndromes (PNS) are rare immune-mediated diseases triggered by cancer, and characterized by circulating onconeural antibodies directed against antigens expressed by neurons and tumor cells. One of the most common forms of PNS is paraneoplastic cerebellar degeneration (PCD). In patients with PCD and ovarian or breast cancer, the dominant onconeural antibody is anti-Yo, detected in serum and cerebrospinal fluid (CSF). Anti-Yo targets two intracellular antigens, cerebellar degeneration-related protein 2 (CDR2) and CDR2-like (CDR2L), expressed in Purkinje neurons in the cerebellum. The interaction between anti-Yo and CDR proteins is thought to mediate Purkinje neuron dysfunction and death, leaving the patients in a severely disabled state. Onconeural antibodies identified in the sera or CSF of patients are key diagnostic biomarkers for PCD. Commercial line assays are available, but the diagnostic value of these tests has been questioned due to low test specificity. The most established commercial tests for anti-Yo detection, line immunoassay and cell based assay (CBA), use CDR2 as the Yo antibody target. After establishing that CDR2L, not CDR2, is the major Yo antigen target in PCD, we assessed the value of including CDR2L as a diagnostic marker to increase the specificity for Yo antibody detection. We developed two in-house techniques, CBA for CDR2L and western blot analysis with recombinant CDR2 and CDR2L proteins. CBA for CDR2L identified PCD patients with 100% accuracy, whereas the western blot analysis was less accurate, most probably related to differently expressed epitopes of CDR2L detected in each of the assays. However, the western blot analysis detected fewer false positive samples compared to the line immunoassays. Based on our findings, we conclude that the accuracy of anti-Yo PCD diagnosis is greatly improved when including CDR2L as a diagnostic marker.

273 - Antibodies against the flot1/2 complex in patients with multiple sclerosis

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Introduction. Multiple sclerosis (MS) is an autoimmune disease of the central nervous system in which the target antigen(s) of the immune response remains elusive. Recently, the presence of antibodies against the flotillin-1/2 (FLOT–1/2) complex has been described in a MS patients cohort, representing the 1-2% of the patients. Flotillin-1 and flotillin-2 are homologous proteins that are found in lipid raft microdomains at the plasma membrane. They are involved in axon regeneration and neuronal differentiation, endocytosis, T-lymphocyte activation and membrane protein recruitment. Our study aims to analyse the presence of antibodies against the FLOT-1/2 complex in our MS patients' cohort. **Methods.** Serum and CSF samples from 210 patients fulfilling the McDonald diagnostic criteria for MS were included in the study (n serum = 170, n CSF = 60). The control group was composed of 200 serum samples (53 healthy donors and 147 with other neuroinflammatory disorders). Anti-FLOT1/2 antibodies were tested by cell-based assays using HEK293 cells co-transfected with mammalian-expression vectors encoding human FLOT1 and FLOT2. First, all the samples were analysed for IgG reactivity over commercial slides containing biochips with cells co-expressing FLOT1 and FLOT2 proteins and then positivity was confirmed by immunocytochemistry over in house co-transfected HEK293 cells. **Results.** From 370 serum samples, we identified 5 MS patients with antibodies against the FLOT-1/2 complex (5/170, 2.9%), whereas none of the control sera tested showed reactivity in cell-based assays (0/200, 0%), being differences observed statistically significant (p = 0.015). Moreover, 3 of the anti-FLOT1/2 positive patients showed this positivity in other serum samples extracted at different moments of their disease. Anti-FLOT1/2 antibodies were detected in 1 CSF sample of MS patients (1/60, 0.02%). Positivity of the 5 serum and the 1 CSF sample was confirmed by immunocytochemistry over *in house* co-transfected HEK293 cells. IgG subclasses of anti-FLOT-1/2 antibodies were predominantly IgG1 and IgG3. **Conclusion**. Flotillin-1/2 complex may be a target antigen of autoantibodies in a subgroup of patients with MS. In our cohort, they were found on 2.9% of patients with MS, which is in concordance with another study recently published. Further studies are needed to understand the pathological relevance of anti-FLOT-1/2 autoantibodies in this disease.

338 - Does Fc-glycosylation influence the pathogenicity of myelin-specific antibodies ?

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Antibodies (Ab) mediate their effector function tanks to the crystallisable fragment called: Fc-domain. This fragment is able to bind to the Fc receptors and also to activate the complement. The affinity of these interactions can be influenced by the glycosylation of the Fc-domain and more precisely of the highly-conserved asparagine 297 (N297). This modulation greatly expands the functional potential of IgG antibodies beyond their subclass. The scientific aim is to assess whether Fc-glycosylation detected on autoantibodies in demyelinating diseases influences inflammation and disease severity. To this end the project is particularly interested in Myelin Oligodendrocyte Glycoprotein (MOG) specific antibodies which are implicated in a new group of emerging demyelinating inflammatory disease called: MOGAD for MOG-IgG-associated disorders. To study the functional potential of Fc- glycosylation we study the pathogenicity of murin MOG-IgG1 specific Fc-glycovariants in our animal models of MOGAD with the ambition to better understand the molecular and cellular immune mechanisms which are implicated. To this end we cloned MOG specific IgG1 mAb 8-18C5 and produced different Fc-

glycovariants under polarising glycosylation conditions. All glycovariants had an identical affinity for the target antigen MOG. The N174 glycan moieties on recombinant 8-18C5 produced by HEK cells, are fucosylated (G0F), while in YB2 cells it remained hypofucosylated (G0). The pathogenicity of Fc-variants was assessed in the mouse model of experimental autoimmune encephalomyelitis (EAE). A single 50 □g injection of the G0F variants aggravated MOG35-55 induced EAE in C57BL/6 mice. Strikingly, hypofucosylated variants caused a more aggressive EAE associated with increased mortality. To assess the underlying cellular mechanisms we analyzed tissue damage in the central nervous system. Increased inflammation and demyelination was demonstrated by immunohistochemistry for the G0F variant relative to the G0 8-18C5 recipients. This was correlated with a CD8 T cell infiltrate for all 8-18C5 treated mice and a significant increase in the magnitude of the CD4 T cell response for the G0 variant relative to the G0F 8-18C5. This study provides novel insight into the functional impact of Fc-glycosylation in antibody-mediated demyelinating diseases. Our results demonstrate the increased pathogenicity of hypofucosylated MOG antibodies which is of particular interest for MOGAD.

357 - The role for parvalbumin interneurons and male vulnerability in maternal anti-Caspr2 antibody induced ASD

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Autism Spectrum Disorder (ASD) is a heterogenous group of neurodevelopmental disorders that is characterized by impairments in social interactions, communication and the presence of stereotypic behaviors. ASD affects 1 in every 58 children in the United States and is four times more prevalent in boys than in girls. Both genetic and environmental factors converge on deficits in the GABAergic system, suggesting that inhibitory interneurons might be particularly susceptible and contribute to ASD pathophysiology. Several studies, including our own, have demonstrated that 10-20 percent of mothers of a child with ASD harbor brain-reactive antibodies (IgG). One target of these antibodies is Caspr2, a protein involved in neural development and synaptic transmission, and present in up to 40% of mothers with anti-brain antibodies and an ASD child. We have developed a model in which female mice are immunized with Caspr2 and harbor endogenous polyclonal anti-Caspr2 IgG throughout gestation. Male offspring, but not female offspring, display ASD-like behaviors and exhibit cortical abnormalities including a reduction in the GABAergic parvalbumin interneurons (PV) in the cortex and hippocampus. We did not observe changes in the total GABA neuron population suggesting that exposure in utero to anti-Caspr2 IgG affect PV interneuron selectively. Perineuronal nets (PNN) are specialized extracellular matrix components that specifically surround PV interneurons and are implicated in the regulation of their function. The association of PNN with PV cells were visualized using immunohistochemistry and staining with the marker Wisteria Floribunda Agglutinin. While mice exposed in utero to Control IgG showed positive correlation in the hippocampus between PV and PNN expression as measured by intensity, such correlation was lost in mice exposed in utero to anti-Caspr2 IgG. Since alterations to PNNs have been shown to influence PV interneuron activity, and dysregulation of these cells is a proposed mechanism underlying ASD; ongoing studies are focused on the trajectory of PV interneuron development and the effect of exposure in utero to anti-Caspr2 IgG on the intrinsic physiology of PV interneurons. If successful, this study will offer significant insight into the contribution of sex to the cell autonomous function of PV interneurons in ASD risk.

291 - Serum IgG of NMDAR-antibody positive encephalitis patients delays seizure onset and prolongs seizure duration in rats

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Although N-methyl D aspartate receptor (NMDAR) encephalitis often presents with seizures, exact role of NMDAR antibodies in seizure pathogenesis pends to be characterized. Our aim was to investigate the role of serum antibodies in generation of seizures in NMDAR-antibody positive patients.
Immunoglobulin G (IgG) purified from peripheral blood of NMDAR encephalitis patients (n=10), IgG from healthy controls (HC) and saline were administered intracerebroventricularly into non-epileptic Wistar rats. Electroencephalography (EEG) recordings and behavioral tests were done before and after IgG administration. To detect possible changes in seizure threshold, subconvulsive dose (35 mg/kg) of pentylenetetrazole (PTZ) was intraperitonealy administered. Acutely induced spike-wave discharges (SWDs) were compared between groups.
Time from injection to emergence of SWDs and seizure duration were significantly increased in the NMDAR encephalitis group compared to HC and saline groups. Also, horizontal and vertical activity in open field maze as well as latency, maximum speed and total distance in rotarod were significantly decreased in the NMDAR encephalitis group ($p<0.05$).
These findings suggest that NMDAR antibodies may have more of an anti-convulsive impact rather than pro-convulsive in the early disease phase of antibody administration. Serum antibodies of NMDAR encephalitis patients might be alleviating the excitatory effect of thalamocortical projections through down-regulation of NMDARs. Future work will investigate the complicated role of NMDAR antibodies in the immunopathogenesis of seizures.
Key words: autoimmune encephalitis, epilepsy, seizures, NMDAR, antibodies

Autoimmune neurological disorders (other than MS)

300 - A Quarter-century Report on Siriraj Neuromyelitis Optica Spectrum Disorder Registry

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Background: Neuromyelitis optica spectrum disorder (NMOSD) is a rare autoimmune demyelinating disorder of the CNS with a racial predilection towards Asian and African descents. The relapsing nature of the disease leads to high accumulative disability.
Objectives: To demonstrate the characteristics, morbidity, and mortality of NMOSD patients at Siriraj Hospital, a university hospital in Thailand.
Methods and materials: Medical record of patients attending the *Multiple Sclerosis and Related Disorders Clinic* at Siriraj Hospital between January 1994 and July 2021 were reviewed retrospectively. Patients fulfilling the 2015 International Panel for NMO Diagnosis criteria were included. Descriptive statistical analysis was performed on the demographics, treatment, visual outcome, motor outcome, Expanded Disability Status Scale (EDSS), and mortality. Data were compared between aquaporin 4 (AQP4)-IgG seropositive and seronegative patients.
Results: From the 165 NMOSD patients included, 145 were AQP4-IgG seropositive and 20 were seronegative. The overall mean age at onset was 37.48 +/-14.29 years with female-to-male ratio of 14:1. The initial presentation of AQP4-IgG seropositive and seronegative NMOSD were similar with transverse myelitis as the most common phenotype (44.8% vs 50%), followed by optic neuritis (41.1% vs 30%). Area postrema syndrome presented more commonly in the seronegative group (6.2% vs 30%, p=0.004). Ninety percent of patients had relapsing disease with 73.1% of seropositive patients experiencing a relapse within 2 years whereas only 45% of the seronegative do (p=0.01). The median number of attacks was 4 (1-40) and 3 (1-15), respectively (p=0.067). AQP4-IgG seropositive patients suffered from more frequent optic neuritis attacks (1 (0-16) vs 0.5 (0-5), p=0.004) and unilateral blindness (42.8% vs 5%, p=0.001). Majority of patients (46.2% and 75%) remained ambulatory with EDSS less than 4. Eleven mortality cases were identified (6.9% and 5%) with infection being the leading cause of death.

Conclusions: NMOSD in Thai patients most commonly presents with transverse myelitis or optic neuritis. AQP4-IgG seropositive patients had higher risks of unilateral blindness.

40 - Latest trick about H1N1 influenza vaccine induced autoimmunity and its association with HLA-DQB1*0602 genotype

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Background: H1N1 pandemrix vaccine induced narcolepsy is a rare disabling disorder, characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations and sleep paralysis. Several studies demonstrated the association of narcolepsy with H1N1 pandemrix vaccine and HLA-DQB1*0602 genotype in various ethnic groups. Thus, the goal of this study was to determine the prevalence of HLA-DQB1*0602 allele in narcoleptics patients vaccinated with H1N1 pandemrix vaccine, and the use of HLA-DQB1*0602 allele in diagnosing narcolepsy. In addition, narcolepsy attributed car accidents and job problems were assessed.

Methods: We studied 44 narcoleptic patients, 30 patients with other types of excessive daytime sleepiness (EDS) and 50 healthy age and sex matched individuals from January 2015 to February 2016 in this case-control study. Patients and controls filled out a questionnaire including items about car accidents due to sleepiness and job problems. International classification of sleep disorders-2 criteria was used as the gold standard for diagnosis of narcolepsy. The DNAs isolated from whole blood samples were collected from the patients and controls to assess the presence of HLA-DQB1*0602.

Results: The results showed that HLA DQB1*0602 was present in 4 (8%) individual of controls and 20 (45.5%) patients with higher prevalence in patients with cataplexy (78.9%) than patients without cataplexy (p<0.001). The sensitivities of the DQB1*0602 for diagnosing narcolepsy with cataplexy and narcolepsy without cataplexy were 78.9 and 20; specificities were 88 and 72.4, respectively. 18.2% of patients had car accidents due to sleepiness and 68.2% suffered from job problems.

Conclusion: Our study showed DQB1*0602 genotype frequency was high in narcoleptic patients especially in narcolepsy with cataplexy. HLA-DQB1*0602 genotype can't be used for diagnosing narcolepsy without cataplexy. However, further study with large sample size is needed to verify the association of H1N1 pandemrix vaccine induced narcolepsy with HLA-DQB1*0602 genotype, and the use of HLA-DQB1*0602 genotype in diagnosing narcolepsy.

Keywords: Cataplexy; HLA-DQB1*0602; Narcolepsy; Sleep disorder

41 - Immunoreactivity to *Campylobacter jejuni* in Guillain-Barré syndrome and correlation with ganglioside autoantibody profile: case-control study

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Guillain-Barré syndrome (GBS) is the commonest postinfectious autoimmune peripheral neuropathy of undiscerned aetiology. *Campylobacter jejuni* (*C. jejuni*) is the most widely reported antecedent infection in GBS is. In this case-control investigation, we assessed the contribution of *C. jejuni* in GBS and ganglioside autoantibody response in a tertiary care hospital from India. This case-control study was performed on 150 treatment-naive patients with GBS and 150 age and sex matched controls from the same community. IgM immunoreactivity for *C. jejuni* was detected by enzyme-linked immunosorbent assay (ELISA) in sera of patients and control subjects. Autoantibody response was evaluated for single ganglioside targets GM1, GM2, GD1a, GD1b, GT1b, GQ1b as well as all possible heterodimeric complexes in patients' sera. The immunoreactivity against *C. jejuni* was compared between demyelinating and axonal subtypes of GBS. *C. jejuni* infection was found in 48/150 (32%) of GBS patients compared to 4/150 (2.7%) of controls. This garners evidence of significantly higher immunoreactivity against *C. jejuni* (p<0.001, OR=17.170 CI=6.005-49.128) in the patient group compared to controls. However, the anti-*C.*

jejuni immunoreactivity was not found to significantly differ between demyelinating and axonal subtypes of GBS (P=0.585, OR=0.79, CI=0.342-1.832). Further, C. *jejuni*-preceded patients demonstrated no association with any specific single ganglioside or ganglioside complex (GSC) autoantibodies. In this large case-control study, *C. jejuni* was observed to be an antecedent trigger of GBS and ascertaining a significantly higher prevalence among patients than controls.

Keywords: Post-infection, AIDP, AMAN, Polyneuropathy, GB syndrome.

105 - Rituximab treatment in opsoclonus-myoclonus-ataxia syndrome: prognostic factors and outcome, a single centre experience

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Objectives: Opsoclonus-myoclonus-ataxia syndrome (OMAS) is a rare neuroinflammatory disorder associated with neuroblastoma (NB) in 40-50% of the cases, defined by the presence of opsoclonus, myoclonus or ataxia and behavioural disturbances. Aim of the study was to describe clinical characteristics and long-term outcome of OMAS paediatric patients treated with Rituximab (RTX).
Methods: we collected data including age at onset, presence and characteristics of NB, treatment approach, number of relapses of 12 patients who received treatment with RTX. To assess the outcome, OMS rating scores recorded before RTX treatment and after 6 and 12 months, along with clinical and neurodevelopmental evaluations, were reviewed.
Results: 50% of the patients presented OMAS associated with NB. Mean OMS score at onset was 8.67 (range 5-13). All patients presented chronic-relapsing course and poor response to corticosteroids first-line treatment; 5/12 presented multiple relapses before RTX. No major adverse event was observed. At last follow-up (mean 7.12 years, range 0.97-16.79), all patients showed OMS score reduction (mean 3.42, range 0-10), except one. Fifty % of the patients presented developmental delay or cognitive deficiencies.

Conclusion: RTX treatment resulted to be safe and effective on neurological symptoms. Cognitive and psychomotor improvement were evident, but further studies with larger cohort are needed.

106 - Soluble CD27 is a sensitive biomarker of inflammation in autoimmune encephalitis

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Background: Autoimmune encephalitis (AE) comprises a group of rare, severe neuroinflammatory conditions. Current routine biomarkers of neuroinflammation are often normal in AE which therefor can be difficult to diagnose in patients with seizures, cognitive or neuropsychiatric symptoms. Soluble CD27 (sCD27) and soluble B-cell maturation antigen (sBCMA) in cerebrospinal fluid (CSF) are sensitive biomarkers of neuroinflammation, but they have not yet been investigated in AE. Methods: Concentrations of sCD27 and sBCMA were measured in CSF from 37 symptomatic controls (SCs) without overt neurological disease and 20 untreated AE patients (12 with anti-N-methyl-D-aspartate receptor (NMDAR) antibodies, 8 with anti-leucine-rich glioma-inactivated 1 (LGI1)

antibodies). Results: CSF concentrations of sCD27 were significantly increased in untreated NMDAR (median 1571 pg/ml; p<0.001) and untreated LGI1 (median 551 pg/ml; p<0.05) AE patients compared to SCs (median 250 pg/ml). CSF sBCMA was significantly increased in untreated NMDAR AE patients (median 832 pg/ml) compared to SCs (median 429 pg/ml). Receiver operating characteristic curve analysis of untreated AE patients versus SCs showed an area under the curve of 0.97 for sCD27 and 0.76 for sBCMA. For sCD27 a cut-off value of 304 pg/mL resulted in 100% sensitivity, 73% specificity and a negative predictive value of 100% in discriminating untreated AE from SCs. Conclusion: CSF sCD27 is a sensitive biomarker of neuroinflammation in AE with an excellent ability to discriminate AE from SCs and can potentially be used to rule out AE early on during the diagnostic process.

121 - IgG4-mediated autoimmune diseases have a normal (IgG4) B cell compartment

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The collaboration project Target to b! is financed by the PPP Allowance made available by Top Sector Life Sciences & Health to Samenwerkende Gezondheidsfondsen (SGF) to stimulate public-private partnerships and co-financing by health foundations that are part of the SFG.

Recently a new niche of autoimmune diseases hallmarked by dominant pathogenic IgG4 autoantibodies was identified (IgG4-AID). These include several neuropathies, skin blistering disorders, nephropathies and a haemolytic disorder. It is unknown why IgG4 predominates these disorders. We hypothesized that dysregulated B cell maturation or aberrant isotype switching results in overrepresentation of IgG4 B cells and plasma cells. We examined the B-cell landscape of 11 MuSK myasthenia gravis and 10 pemphigus vulgaris patients (two archetypical IgG4-AIDs) using flow cytometry with validated EuroFlow B cell markers and compared to age-matched healthy donors. B cell subset counts at all maturation stages were largely normal in two IgG4-AIDs, except for a reduction in immature and naïve CD5+ cells likely related azathioprine treatment. IgG4 B cell and plasma cell counts were normal in IgG4-AID patients. However, we found a relative increase of $CD20^{-}CD138^{-}$ (3-fold) and $CD20^{-}CD138^{+}$ (10-fold) plasma cells in both patient groups versus age-matched controls. These cells precede long-lived plasma cells residing in the bone marrow and are linked to other (non-IgG4 mediated) autoimmune diseases. Additionally, we found a non-isotype specific increase in CD21- memory B cells in pemphigus patients (2.5-fold). In conclusion, in patients with IgG4-AID we did not find indications of impaired B cell maturation or increased levels of IgG4+ memory B cells or plasma cells. These results argue against aberrant B cell biology in these patients. Instead, the IgG4 predominance in autoantibodies may be antigen-driven, or the result of IgG4-inducing T cell help. The increase in mature plasma cell counts is striking and warrants further research.

149 - TREK1 as critical regulator of the myovascular unit.

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$K_{2P}2.1$ (TREK1), a two-pore domain potassium channel, has been implicated as important regulator of the leukocyte transmigration into the central nervous system. Of note, immune-cell infiltration into the muscle is also a pathogenic hallmark of idiopathic-inflammatory myopathies (IIM); however, the underlying mechanisms remain to be elucidated. In this study, we investigated the role of $K_{2P}2.1$ in the peripheral autoimmune response of IIMs.

We were able to detect $K_{2P}2.1$ expression in primary skeletal muscle and endothelial cells of murine and human origin. We demonstrate an increased pro-inflammatory cell response, adhesion and transmigration by pharmacological blockade or genetic deletion of $K_{2P}2.1$ *in vitro* and *in vivo*. Conversely, these features are reduced by activation of this potassium channel. In summary, $K_{2P}2.1$ is critically involved in the regulation of immunological processes in the skeletal muscle, especially immune cell trafficking. These regulatory mechanisms might be impaired in IIMs and therefore represent targets for new therapeutic strategies.

155 - Anti-NMDA-Receptor Encephalitis in Filipino Adults: A Case series in a tertiary government hospital in the Philippines

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194 - Complement activation by MOG and AQP4 antibodies

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Autoantibody associated demyelinating diseases of the central nervous system (CNS) are rare diseases with often severe clinical manifestations. Autoantibodies against myelin oligodendrocyte glycoprotein (MOG), which is expressed on the outermost layer of myelin sheaths and on oligodendrocytes, are found in MOG-antibody associated disease (MOGAD). Autoantibodies targeting aquaporin 4 (AQP4), a water channel expressed on the endfeet of astrocytes, are found in the majority of patients with neuromyelitis optica spectrum disorder (NMOSD). Both, MOG and AQP4 antibodies can induce complement dependent cellular cytotoxicity (CDC), leading to the formation of the terminal complement complex (TCC) and consecutive cell damage. Whereas CDC is a main pathological hallmark in NMOSD, the role of complement activation in MOGAD is less clear. Furthermore, we have recently found major differences in antibody binding to the six major MOG isoforms. In this study, we aimed to compare complement activation of rodent and human antibodies to different MOG and AQP4 isoforms. Therefore, we used different approaches. First, we investigated the lactate dehydrogenase release of HEK293 cells transfected with different MOG isoforms or AQP4 M23 to compare the amount of complement induced cell damage caused by autoantibodies. To visualize complement activation, we also stained the TCC complex using a C9 neoantigen specific antibody. Additionally, the concentration of complement factors C3a and C5a in the supernatant of patient sera and complement treated cells was investigated. AQP4 antibodies showed stronger complement activation compared to MOG antibodies, but in both cases, complement activation was dependent on the antibody titers. Furthermore, anti-C9 neoantigen antibody showed TCC deposition on the cell surface after incubation with patient sera together with active complement. Moreover, with our analysis of C3a and C5a complement factors and the comparison of different MOG isoforms we aim to further elucidate the contribution of CDC on the pathogenicity of MOG and AQP4 antibodies. This study is supported by the Austrian Science Funds (FWF, project P32699)

197 - Ablation of interleukin-19 improves motor function in a mouse model of amyotrophic lateral sclerosis

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Objective: Neuroinflammation by activated microglia and astrocytes plays a critical role in the disease progression of amyotrophic lateral sclerosis (ALS). We previously identified interleukin-19 (IL-19) as a negative-feedback regulator to limit pro-inflammatory response of microglia in autocrine/paracrine manners, and we also revealed that treatment with IL-19 effectively suppressed experimental autoimmune encephalomyelitis. However, it remains uncertain how IL-19 contributes to ALS pathogenesis. Here, we investigated the role of IL-19 in ALS using transgenic mice carrying human superoxide dismutase 1 with G93A mutation ($SOD1^{G93A}$ Tg mice).

Method: We generated IL-19deficient SOD1^{G93A} Tg (IL-19$^{-/-}$/SOD1^{G93A} Tg) mice by crossing SOD1^{G93A} Tg mice with IL-19$^{-/-}$ mice and evaluated disease progression, motor function, and survival rate as well as pathological and biochemical alternations. In addition, the effect of IL-19 on glial cells were assessed using the primary microglia and astrocyte cultures from the embryonic brains of SOD1^{G93A} Tg mice and IL-19$^{-/-}$/SOD1^{G93A} Tg mice.
Result: Expression level of IL-19 was upregulated in the primary microglia and the lumbar spinal cords of SOD1^{G93A} Tg mice compared to those of wild type mice. Unexpectedly, IL-19$^{-/-}$/SOD1^{G93A} Tg mice showed a partial improvement of motor function. Ablation of IL-19 in SOD1^{G93A} Tg mice increased expression levels of both neurotoxic and neuroprotective factors such as tumor necrosis factor-α, IL-1β, glial cell line–derived neurotrophic factor (GDNF), and transforming growth factor-β1 in the lumbar spinal cords. The primary microglia and astrocytes from IL-19$^{-/-}$/SOD1^{G93A} Tg mice showed TNF-α upregulation, which induced GDNF release from astrocytes.
Conclusion: Our findings suggest that inhibition of IL-19 signaling may alleviate ALS symptoms by astroglial GDNF production due to microglial TNF-α release.

203 - Prolonged T cell expansion in neurological manifestations associated with COVID-19

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Neurological manifestations associated with COVID-19 (Neuro-COVID) have emerged as a significant cause of morbidity in the ongoing coronavirus pandemic. Our previous data indicates an altered immune landscape in the cerebral spinal fluid (CSF) of Neuro-COVID patients despite SARS-CoV-2 not being detectable in the CSF compartment. There might be a higher risk of neurological autoimmunity if certain immunological alterations persist after viral clearance.
Hence, we recruited Neuro-COVID patients with prior history of systemic, but not neurological manifestations of SARS-CoV-2 infection, who subsequently developed Guillain-Barré syndrome (GBS) after viral clearance (post-COVID GBS). We collected CSF and blood cells, and compared them to those derived from Neuro-COVID patients during acute SARS-CoV-2 infection and to non-infected control donors. Using single-cell transcriptomics, we identified expansion of CD4+ and CD8+ memory T (Tm) cells in the CSF of all Neuro-COVID patients, more significantly in patients with post-COVID GBS. Interestingly, these cells showed signs of exhaustion only during acute infection. By sequencing their antigen receptors, we were able to confirm the clonal expansion of these Neuro-COVID-associated T cells in both Neuro-COVID and in post-COVID GBS patients. Our results provide an in depth understanding of how SARS-CoV-2 immune response in the CNS might trigger secondary autoimmunity against peripheral nerves.

216 - Cerebrospinal fluid proteomics in recent-onset Narcolepsy type 1 reveals ongoing inflammatory response

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Narcolepsy type 1 (NT1) is a rare, chronic and disabling neurological disease characterized by excessive daytime sleepiness and by cataplexy (sudden loss of muscle tone). NT1 is characterized pathologically by a selective and almost complete loss of neurons producing the orexin neuropeptides in the lateral hypothalamus. Genetic and environmental factors strongly suggest the involvement of the immune system in the loss of orexin neurons. Cerebrospinal fluid (CSF) secreted locally and surrounding the CNS is considered an accessible window into CNS pathological processes. We hypothesized that in NT1 patients close to disease onset, biomarkers of inflammation

and neurodegeneration could be detected in the CSF. Therefore, we performed a proteomics analysis of the CSF from 21 recent-onset NT1 patients compared to two groups of controls: (i) patients with somatoform disorders, and (ii) patients with sleep disorders other than NT1, to control for any potential effect of sleep disturbances on CSF composition. To achieve a high sensitivity analysis, the most abundant proteins were depleted, and after pre-fractioning, samples were analyzed by Nano-flow liquid chromatography tandem mass spectrometry (nano-flow LC–MS/MS) using the latest generation of hybrid Orbitrap mass spectrometer. Our study allowed the identification and quantification of 1943 proteins, providing a remarkably deep analysis of the CSF proteome. The differential analysis of CSF proteome identified 71 and 76 proteins differentially regulated between NT1 patients compared to controls (i) and (ii), respectively. Interestingly, proteins involved in the regulation of immune responses were differentially regulated between NT1 patients and the control groups. Of particular note, the TGFBR2 protein was down-regulated in NT1 patients. Given the role of TGF signaling in immune tolerance, our data suggest a disruption of immunological tolerance that could promote aberrant (auto)immune responses. To further analyze the disturbed biological processes, we took advantage of the ingenuity pathway analysis tool that revealed enrichment in pathways related to acute inflammatory responses signaling, infiltration and activation of leucocytes as well as complement activation. These pathways were significantly up-regulated in NT1 patients compared to both groups of controls. Our data therefore reveals an altered composition of the CSF proteome in NT1 patients close to disease onset, which points to an ongoing inflammatory process.

253 - Microglial Uptake and Degradation of Amyloid β is Affected by Inhibition of the Lysosomal Protease Cathepsin Z

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Introduction: Microglia, the innate immune cell of the brain parenchyma, are involved in clearance of extracellular Amyloid β (Aβ) deposits, which are a hallmark of Alzheimer's disease (AD). The lysosomal proteins cathepsin Z (CtsZ) and cathepsin D (CtsD) that possess exopeptidase and endopeptidase activity respectively, are involved in a number of diseases. We have previously shown CtsZ to be expressed in microglial lysosomes in AD, and in microglia from a mouse model of AD. CtsD, which is already known to be able to cleave Aβ, has also been shown to be upregulated in AD, and in microglia from mouse models of AD. The aim of the present study was to investigate the role of CtsZ in microglial endocytic uptake and lysosomal degradation of Aβ. Methods: Primary murine microglia were stimulated with Aβ labeled with the lysotracker pHrodo (Aβ-pHrodo) or unlabeled Aβ, and further treated with the CtsZ inhibitor, AMS36, or vehicle, and analyzed with flow cytometry, quantitative PCR, or Western blotting. Results: AMS36 treatment both affected the microglial uptake and degradation of Aβ. Thus, AMS36 treated cells, simultaneously exposed to Aβ, for 24-72hrs contained less Aβ at all timepoints, with a maximum uptake at 48hrs. Microglia exposed to Aβ for 24hrs followed by AMS36 treatment, after depletion of Aβ, up to 120hrs showed a greater capacity to degrade Aβ up to 96hrs compared to vehicle-treated microglia. Microglial Aβ exposure for 24hrs, led to a marginal increase in CtsZ mRNA expression after 72hrs, and to a significant increase in the mRNA expression of CtsD between 24-72hrs. No effect of treatment with AMS36 was found on the mRNA expression of CtsZ and CtsD. The CtsZ protein expression appeared not to be affected by Aβ exposure. Conclusion: These results suggest a role of CtsZ both in the uptake and in the degradation of Aβ. The uptake of Aβ decreased with treatment with the CtsZ inhibitor, AMS36, and the degradation of Aβ occurred more rapidly compared to vehicle-treated microglia. The accelerated degradation of Aβ after AMS36 treatment, indicates an anti-inflammatory role for AMS36 making it a drug candidate for further investigations, or indicates that the activity of other lysosomal proteases, such as CtsD, is increased by the treatment.

285 - Immunologic landscape of patients with chronic inflammatory demyelinating polyneuropathy

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Background: Chronic inflammatory demyelinating polyneuropathy (CIDP) is an autoimmune disease of the peripheral nervous system. Various players of the immune system may be implicated in the pathogenesis of CIDP, however each player's nature and function remain largely unknown. We aimed to explore the immunologic landscape of CIDP and identify important immunologic culprits.
Methods: We performed single-cell RNA sequencing using peripheral blood mononuclear cells (PBMCs) obtained from patients with CIDP and healthy controls (HCs). Flow cytometry and enzyme-linked immunosorbent assay (ELISA) were used to validate the study results.
Results: In the analysis with single-cell RNA sequencing, PBMCs from patients with CIDP exhibited hyperinflammatory responses across all type of cells, as compared to those from HCs. Sub-clustering analysis of B cells revealed hyperinflammatory signals in naïve and memory B cells, but not in transitional B cells. Notably, a subcluster analysis of transitional B cells showed down-regulation of IL-2 production pathway in CIDP patients (vs. HCs). In the analysis using flow cytometry, the number of regulatory T cell (CD3+CD4+CD25+FoxP3+) was similar between patients with CIDP and HCs, whereas that of regulatory B cell (CD19+24+38+) was lower in patients with CIDP. In the analysis using ELISA, the levels of pro-inflammatory cytokines (TNF-α, IL1-β, IL2, IL5, IL6, and IL-15) were higher in patients with CIDP than HCs, while those of anti-inflammatory cytokines were comparable.
Conclusions: Immunologic landscape of PBMCs in CIDP patients may be distinct from that in HCs, as the former demonstrates hyperinflammatory signatures. Among the various immunological players, those in the B-cell lineage may be importantly implicated in the pathogenesis of CIDP, which warrants future studies.

312 - Incidence of AQP4-IgG+ NMOSD Appears Stable Over Time: Population-Based Study Over 9-Years at Penang Island, Malaysia

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Background: AQP4-IgG+ NMOSD is now known to be more prevalent among Blacks and East Asians as compared to Whites and Austronesians, suggesting certain degree of genetic predisposition. There is also no latitude gradient observed (at least among East Asians). While globally the incidence of multiple sclerosis is increasing over time, the temporal trend of NMOSD incidence has not been extensively investigated.
Objective: A population-based study was conducted to investigate the temporal trend of NMOSD incidence over 9 years (2012-2020) at Penang Island, Malaysia.
Methods: Neurologists (adult and paediatric) at 6 hospitals with neurology service at Penang Island participated in this study. Patients fulfilling the 2015 IPND criteria and were AQP4-IgG+ were included. Date of 1st attack / initial symptom was used as "incidence date" to determine the "real incidence", rather than using date of diagnosis which might lag behind the actual onset date.

Results: Of the 53 patients identified, 27 were residents of Penang Island [22 Chinese (East Asians), 5 Malays (Austronesians)], while the remaining (residents of neighbouring areas and 2 non-citizens) were excluded. Among the 22 Chinese patients, 12 were incident cases from 2012-2020, giving an annual incidence of 3.36/million. When the 9-year duration was divided into three 3-year periods (2012-2014, 2015-2017, 2018-2020), there were 4 incident cases in each period, corresponding to annual incidences of 3.42/million, 3.36/million, and 3.32/million, respectively, which were relatively stable. Four of the 5 Malay patients were incident cases (annual incidence: 1.76/million), with 2 cases, 1 case, and 1 case, respectively, in the three 3-year periods. On prevalence day (31st December 2020), there were 19 living Chinese patients and 4 living Malay patients, giving a prevalence of 4.73/100,000 among Chinese and 1.51/100,000 among Malays.
Conclusion: Our study shows that, when calculated according to racial groups, the incidence (new cases) of AQP4-IgG+ NMOSD appears relatively stable over time, suggesting that genetic predisposition, rather than potential environmental risk factors, may play a more important role in the disease pathogenesis. Larger studies over a longer duration in different geographical locations and racial groups will be useful to confirm this.

314 - Properties of Novel CD20 Expressing Natural Killer Cell Subtype and Its Relationship with Multiple Sclerosis

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Introduction: CD20 expression is not restricted to B cells**.** Previously, CD3+CD20dim T cells were found to be associated with prognosis in people with MS (pwMS), have a more activated phenotype and were shown to be depleted after rituximab. Recently, we have observed that a fraction of NK cells also expresses low levels of CD20.
Objectives: To identify the characteristics of CD20 expressing NK cells and investigate their relationship with MS.
Methods: First, peripheral blood mononuclear cells (PBMC) from 10 healthy controls (HCs),12 untreated and 12 rituximab treated pwMS were stimulated with PMA/Ionomycin to identify the features of CD3-CD19-CD56+CD20dim cells. Next, magnetically sorted CD56+ cells obtained from HCs were stimulated with PMA/Ionomycin or IL-2+IL-15 w/wo K562 cells. Expression of CD107a, NKp46, IFN-γ, GM-CSF and TNF-α were measured. Then, cytotoxicity assay was performed by incubation of K562 cells together either with CD20+ or CD20- NK cells. Finally, ratio of CD3-CD19-CD56+CD20+ cells were measured in the PBMC and CSF samples of patients and controls [31 newly diagnosed pwMS, eight inflammatory demyelinating disorders (IDD), 12 other inflammatory neurological disorders (OIND), 15 non-inflammatory neurological syndrome (NINS) and 14 HCs].
Results: The percentage of CD56+CD20+ cells in patients with demyelinating diseases (MS: 9.052, n=31; IDD: 8.273, n=8) was higher than in patients with non-demyelinating disorders (OIND: 5.621, n=12; NINS: 5.805, n=15) and HCs (3.036, n=14) ($p < 0.001$) and these cells were more abundant in the CSF compared to blood. CD56+CD20+ cells were enriched in the CD56bright population. Stimulation studies showed that CD107a, NKp46, IFN-γ, GM-CSF and TNF-α expressions are significantly higher in CD56+CD20+ cells compared to CD20- NK cells. There was an increased apoptosis in K562 cells cocultured with CD20+ NK cells. Finally, CD20+ NK cells were depleted after rituximab therapy.
Conclusions: We have identified a new subset of NK cells expressing CD20. These cells have increased degranulation capacity, express cytokines more abundantly and are more cytotoxic than CD20 negative NK cells. Clinically, CD56+CD20+ cells are enriched in pwMS and IDD compared to controls. Our findings suggest that CD20+ NK cells may be prominent mediators of inflammation in MS and represent a novel target for CD20 targeting therapies.

340 - A CASE OF RADIOLOGICALLY ISOLATED SYNDROME DEVELOPING BALO'S CONCENTRIC SCLEROSIS LESIONS ON LONG-TERM FOLLOW-UP

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Radiologically isolated syndrome (RIS) depicts a clinical scenario with white matter lesions satisfying multiple sclerosis (MS) magnetic resonance imaging (MRI) criteria in individuals without clinical features of an index or previous clinical demyelinating bout. Balo's concentric sclerosis (BCS) on the other hand, is an inflammatory demyelinating disease often seen as a monophasic variant of MS, characterized by concentrically layered demyelinating lesions in the cortical white matter. This is the first reported case to our knowledge, whereby a RIS patient developed characteristic BCS lesions on follow-up. A 49-year-old male with an incidental left frontal lobe lesion on MRI underwent stereotactic biopsy procedure to rule out malignant etiologies. The lesion was pathologically diagnosed as a demyelinating plaque. He was followed-up both clinically and radiologically, as a biopsy verified RIS patient. He remained asymptomatic with normal neurological examination throughout the three-year post-biopsy follow-up period. The last follow-up MRI in August 2021 revealed multiple demyelinating lesions, some showing concentrically arranged layers, with additional progression of the index lesion to BCS. Our case highlights the heterogeneity of RIS as a clinico-radiological entity, requiring further sub-classification of its spectrum for improved management.

361 - IgG antibodies against HNK1 epitope in anti-MAG neuropathies

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In peripheral neuropathies with antibodies against Myelin Associated Glycoprotein (MAG), an IgM monoclonal gammopathy recognizes a specific epitope called Human Natural Killer 1 (HNK1) that is shared by NK lymphocytes and several components of the peripheral nerve myelin including the MAG. An ELISA method, using a synthetic glycan which mimics the natural HNK-1 carbohydrate epitope, is an alternative of the anti-MAG ELISA. Using this ELISA, we detected reactivity of an IgG isotype in some patient sera. The objective was to determine the significance of positive anti-HNK1 IgG antibodies in anti-MAG neuropathies and control patients. We tested 32 anti-MAG patients and 80 controls (20 chronic inflammatory demyelinating polyradiculoneuropathies (CIDP), 11 Lewis Sumner syndromes (SLS), 24 multifocal motor neuropathies (MMN), 16 multiple sclerosis (MS) and 9 inherited neuropathies (Charcot Marie Tooth neuropathies, CMT). Sera were considered positive if the titre of IgG anti-HNK1 was above the 95th percentiles of the dosages of 22 healthy blood donors. IgG anti-HNK1 antibodies were positive in 59% of anti-MAG neuropathies (19/32), but with differences between clinical phenotypes: 2/8 (25%) with paraesthesia and hypoesthesia, 11/14 (79%) with sensory ataxia, 3/5 (60%) with motor deficiency ($p = 0.04$). Median titre of the antibodies was 72 (interquartile 55-98). IgG anti-HNK1 titre was correlated with sensory deficiency evaluated with the INCAT sensory sum score ($r = -0.4$, $p = 0.04$), but not with age, disease duration, motor deficiency, disability, motor and sensory nerves amplitudes, motor unit number index (MUNIX), IgM anti-MAG titres or IgM anti HNK1 titres. Frequency and titres of IgG anti-HNK1 antibodies were higher in anti-MAG neuropathies than in CIDP (10% positive, median 25), MMN (17% positive, median 29), MS (13% positive, median 17) and CMT (no positive, median 17), but without statistically significant difference with SLS (36% positive, median 62). Conclusion: IgG anti-HNK1 antibodies are more frequently detected in anti-MAG neuropathy

than in other neurological pathologies. Interestingly, IgG anti-HNK1 are related to the clinical phenotype and the sensory deficiency. Their presence could reflect a spreading of the autoimmune reaction in some anti-MAG neuropathies and could explain part of the phenotypic variability of the disease.

364 - Multiparametric analysis highlight soluble CD146, membrane CD146 and GRP56 expression for CIDP stratification

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Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is the most frequent immune-mediated polyneuropathy belong to the spectrum of causally treatable neuropathies. The pathology includes typical CIDP and atypical variants according to clinical presentation, severity, evolution profile, electrophysiological parameters and finally, according to the response to different treatments. The first-line treatment may be either IVIG (inefficient in 30% of CIDP cases) or corticotherapy (inefficient in asymmetric Lewis and Sumner syndrome (LSS)). Immunosuppressants or immunomodulators such as rituximab are second-line treatment. Our aim was to identify biomarkers for the diagnosis, the prediction of severity or the stratification of CIDP. We performed multiplex assays of 14 biomarkers (cytokines, chemokines and vascular markers including CD146s) and immune cell profiling using multicolor flow cytometry including CD146 and GPR56. CD146 is expressed by IL-17 producing cells and is potentially involved in lymphocyte recruitment. GRP56, expressed by cytotoxic T lymphocytes and NK cells, inhibits immune cell recruitment. We analyzed samples from 25 CIDP, 41 ALS patients and 24 healthy controls. We then correlate the biomarkers with the severity of the disease using clinical scores ONLS and RODS and electrophysiological score MUNIX.
Elevated inflammatory biomarkers are associated with neuroinflammatory state as Illustrated by markers variation in both ALS and CIDP compared to healthy patients. However, none of the analyzed soluble biomarker variation were specific of CIDP patients. CIDP is associated with the specific increase of CD146 positive Effector Memory CD4+ T cells and CD146 positive TEMRA CD4+ T Cells. Concerning the severity score, CD146s correlates with both severity scores (RODS (rp=0.284; p=0.048) and MUNIX (rp=0.303; p=0.038)) whereas no correlation is observed with any of the other soluble marker. Frequency of $GPR56^{+}$ CD8 lymphocytes is inversely correlated with the MUNIX scores and the increase of GPR56+ NK cells is associated with asymmetric presentation. Finally, Ward method's hierarchical clustering using 3 biological variables (CD146+ CD4 T cells, CD146s and GRP56+ CD8 T cells) discriminates CIDP patients in 2 clusters. Interestingly, patients suffering from typical CIDP are exclusively in the first cluster while the second cluster is mainly composed of Lewis and Sumner cortico-resistant forms of CIDP and patient with higher MUNIX score.

Barriers of the CNS: actors in neuroinflammation

29 - Role of the angiogenic factor Ang-2 in myeloid recruitment into the inflamed CNS

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In multiple sclerosis (MS) resident and infiltrating myeloid cells are the predominant inflammatory cells within lesions of the CNS. Studies have shown a detrimental role of infiltrating monocytes aggravating disease progression and outcome in a mouse model of MS. To study the mechanisms involved in migration of myeloid cells into the CNS we here focus on the growth factor Angiopoietin-2 (Ang-2). Ang-2 is part of the Ang/Tie system critical for the regulation of vascular development, maturation and stability during embryogenesis as well as for vessel remodelling during pathology. We established a transgenic mouse model with inducible endothelial cell specific overexpression of Ang-2 (EC-Ang2). These animals slowly develop a chronic disease characterized by

progressive blood vessel enlargement, increased vascular leakage and accumulation of myeloid cells in numerous peripheral tissues. We crossbred EC-Ang2 mice with the myeloid cell knock-in reporter mice CX3CR1$^{+/GFP}$/CCR2$^{+/RFP}$ generating EC-Ang2/CX3CR1$^{+/GFP}$/CCR$^{+/RFP}$ mice allowing to distinguish CNS-resident GFP+ and inflammatory RFP+ myeloid subsets. Quantitative analysis of immune cell infiltrates using flow cytometry revealed a strong increase of CCR2+ infiltrating macrophages as well as lymphocytes in the brain and spinal cord of EC-Ang2 mice during steady state. Interestingly, male EC-Ang-2 mice displayed a higher number of infiltrating immune cells in comparison to age-matched female transgenic mice. Analysis of precise cell localization in brain sections by immunofluorescence microscopy suggests that Ang-2 recruits myeloid cells mostly to the CNS interfaces, since CCR2+ and CCR2/CX3CR1+ double positive cells accumulated mainly in the leptomeninges and within blood vessels. The CNS parenchyma on the other hand was devoid of those cells. Subjecting female EC-Ang2 mice to active EAE, a common mouse model for MS, revealed however that moderate Ang-2 overexpression does not lead to significantly increased CNS immune cell infiltrates and consequently does not aggravate EAE disease course. Nevertheless, the role of Ang-2 in neuroinflammation could be masked by the strong inflammatory environment created by our adjuvant-based EAE model and furthermore dependent on sex and Ang-2 concentration gradually increasing over time. In summary, endothelial Ang-2 overexpression results in immune cell accumulation in the CNS during steady state, while its role during neuroinflammation remains to be investigated.

130 - Exploring versican as a primary inhibitor of remyelination in models of multiple sclerosis

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The demyelination that characterizes multiple sclerosis (MS) lesions can be repaired to a degree in some individuals, though in majority of patients, this repair is inadequate. Remyelination has the potential to restore function in the central nervous system (CNS). In order to promote remyelination in MS lesions, the inhibition of oligodendrocyte precursor cell (OPC) recruitment and differentiation must be overcome. Targeting the deposited extracellular matrix (ECM) factors in the lesion to create a more permissive environment for OPCs is a promising therapeutic strategy. Versican, and likely its V1 isoform, has been identified as a prominent inhibitory chondroitin sulfate proteoglycan (CSPG) that is upregulated in the MS lesion. In this thesis, I sought out to determine the outcome of the interaction between newly available purified V1 and OPCs in culture. I observed a robust inhibitory effect of V1 as a substrate and as a soluble treatment on OPCs *in vitro*. Using the lysolecithin (LPC) murine model of demyelination and remyelination, where versican upregulation in CNS lesions appears to be in macrophages/microglia from previous studies, I conditionally deleted versican from CCR2+ monocytes to evaluate the outcome on OPC presence in the lesion. The conditional versican knockout was dependent on the administration of tamoxifen and resulted in reduced V1 in the lesion compared to controls. With less V1 in the lesion, there was an increase in oligodendrocyte lineage cells during the documented period of remyelination in the LPC lesion. These results are promising in proposing a target for future therapeutic strategies to improve remyelination in MS lesions.

143 - Determining the distribution of proinflammatory astrocytes and their interactions with T cells in Multiple Sclerosis Progression

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Multiple Sclerosis (MS) is a progressive, autoimmune disorder of unknown etiology that is characterized by demyelination, neurodegeneration, and immune infiltration into the central nervous system (CNS). Understanding the complex interplay between infiltrating immune cells and CNS resident cells, as well as the molecular mechanisms involved in this interaction, is imperative to understanding the etiology of the disease. Inflammatory astrocytes, one of the first described subtypes of reactive astrocytes, have been shown in the center and expanding edge of active demyelinating lesions, as well as in chronic lesions. However, both their abundance throughout the course of disease and their role(s) in the disease process have yet to be determined.

Using a murine model of MS, experimental autoimmune encephalomyelitis (EAE), we aimed to determine the frequency of inflammatory astrocytes, identified by C3 and GFAP co-staining, throughout disease progression, as well as their cell-to-cell interactions. We show that inflammatory astrocytes are increasingly present in EAE lesions, starting from the presymptomatic stage through chronic time points. In addition, using spectral flow cytometry, we show the unique presence of inflammatory astrocytes at chronic stages of disease. Moreover, we observe that inflammatory astrocytes extend their processes and envelop T cells, infiltrating immune cells known to play a role in EAE and MS. We also show astrocytes, when polarized to an inflammatory profile *in vitro*, increase their production of hyaluronan synthases- key proteins required to produce hyaluronan (HA). HA is a known ECM molecule involved in polarizing T cells to an encephalitogenic profile and promoting a pro-inflammatory environment. Our results suggest that inflammatory astrocytes could play a pathogenic role in EAE through their interactions with T cells, via production of HA. This provides a novel mechanism by which inflammatory astrocytes contribute to the pathophysiology of EAE and MS.

160 - A specific perforin inhibitor inhibits CD8 T cell mediated neuroinflammation

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In the development of neuroinflammatory diseases, disruption of the blood-brain barrier (BBB) and subsequent infiltration of immune cells into the brain represent key events. Susac's syndrome (SuS) is a rare neurological disease, patients present with encephalopathy, visual disturbances, and hearing deficits. Its pathogenesis remains enigmatic, but an autoimmune process leading to an endotheliopathy has been postulated. We recently showed based on human data and a newly-developed transgenic mouse model experiments that SuS is a CD8 T cell-mediated endotheliopathy resulting from perforin-dependent CD8 T cell-mediated killing of endothelial cells (ECs). Perforin is a non-redundant pore-forming toxin of CD8 T cells, whose function is to deliver granzyme proteases into the target cell cytosol, in turn triggering target cell apoptosis (Voskoboinik et al. Nat Rev Immunol 2015). We used a transgenic mouse model that allows expression of the *Influenza* virus hemagglutinin (HA) as a neo-antigen in the BBB-ECs as well as the choroid plexus (Gross et al. Nat Comm 2019). CD8 T cell-mediated endotheliopathy is then induced by an adoptive transfer of activated cytotoxic CD8 T cells (CTLs) specific for HA, which results in weight loss and motor dysfunction. Three days after the induction of the disease, mice were treated with the perforin inhibitor SN34960 for 4 days. CNS infiltrating CTLs were evaluated. In parallel a co-culture model using primary cultures of CTLs and BBB-ECs was conducted in order to further characterize the interactions between these two cell types. We found *in vitro* that antigen-specific apoptosis of EC-HA increases with time, which may explain the microhemorrhages and BBB disruption observed. We also found that treatment with a specific perforin inhibitor decreases EC apoptosis and, *in vivo,* allows full recovery of mouse motor function. Our results suggest that a perforin-dependent antigen-specific destruction of BBB-ECs, by CTLs, induces microhemorrhages thus allowing T cells to infiltrate the CNS and is responsible for the clinical effects observed in SuS. Clinical and histological effects can be reversed by using a perforin inhibitor, with EC survival, preservation of the BBB, and allowing a quick and complete recovery of motor performance. Perforin's non-redundant role in the cytotoxic interaction of ECs and CTLs makes it a potential target for therapeutic intervention in SuS patients in the future.

178 - Induction of hypoxia related programs in astrocytes exacerbates Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) a chronic inflammatory disease of the central nervous system (CNS) which is characterized by demyelination, neuro-axonal degeneration and astrogliosis resulting in transient but also accumulating

disability. The mode of neuro-axonal degeneration has been extensively studied but remains unconfirmed up to date. The hypothesis of a focally distributed energetic crisis, called "virtual hypoxia" is one frequently discussed paradigm. Consequently, hyperoxigenated and chronic mild hypoxia treatment studies were conducted in the animal model of MS, experimental autoimmune encephalomyelitis (EAE). Paradoxically, both showed beneficial effects. In our presenting study we analyzed if an astrocyte-specific activation of hypoxia related pathways after the onset of EAE would improve the clinical outcome. Therefore, we made use of a mouse strain with a tamoxifen inducible astrocyte-specific knock out (ko) of the cellular oxygen sensors and hypoxia negative regulators prolyl hydroxylase domain-containing protein (PHD) 2 and 3 (namely Aldh1l1-cre^{ERT2}; *Phd2*$^{fl/fl}$; *Phd3*$^{fl/fl}$). Here, a ko induction prevents the post-translational hydroxylation and finally ubiquitination of the hypoxia-inducible factor alpha subunits (HIF1a and HIF2a), resulting in their nuclei translocation and an increased hypoxia related gene expression. In a therapeutic approach, we induced the *Phd2/Phd3* ko (after the onset of first EAE symptoms. Surprisingly, during the 30 days post disease onset *Phd2/Phd3* ko animals suffered from disease progression shown by worsened EAE scores, weight losses and reduced neurological fitness (measured by Rotarod). Flow cytometric analysis of spinal cord isolated single cells showed a significantly increased number of infiltrated immune cells, in particular of inflammatory active CD4+ cells. By using the translating ribosome affinity purification (TRAP) method we performed a bulk RNA-seq analysis of astrocyte specific mRNAs. Astrocytes of both EAE groups (wt and ko) highly expressed pan reactive, A1 and A2 specific genes in contrast to controls. A deeper analysis of wt and ko animals highlighted 60 significantly differentially expressed genes (DEGs) enriched in distinct biological processes. We conclude that under EAE conditions the strong induction of hypoxia related gene pathways in astrocytes exacerbates clinical EAE by inducing an inflammatory permissive astrocyte phenotype.

179 - Immune Reconstitution after Autologous Hematopoietic Stem Cell Transplantation in Multiple Sclerosis

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system, in which autoreactive CD4+ T cells and memory B cells play important roles. Autologous hematopoietic stem cell transplantation (aHSCT) is a procedure, in which patients undergo chemotherapy and thereafter receive back their own hematopoietic stem cells. This procedure blocks MS disease activity efficiently and in the majority of patients permanently, most likely by renewal of the adaptive immune system, that is T- and B cells. We aim to understand the dynamics and extent of immune reconstitution of adaptive immune cells after aHSCT and find immunological correlates explaining the excellent efficacy of aHSCT in MS. Since the approval of aHSCT for MS in Switzerland in 2018, more than 30 MS patients were treated in Zurich and donated biomaterials. In the first 27 patients, we studied immune reconstitution using explorative, multidimensional flow cytometry, T cell receptor (TCR) sequencing and telomere length profiling. 3-6 months after the transplantation, naïve T cells were present at very low numbers, while a certain memory cell population, the effector memory (EM) T cells quickly reconstituted to pre-aHSCT levels. Using a variety of approaches, we show that early EM T cells partially consist of carry-over T cells, show a more senescent and exhausted phenotype, while the renewal of T cells increases greatly one year after aHSCT. These observations add to our current understanding of immune-renewal by aHSCT in MS. Our data supports the renewal of a majority of the T cell compartment late(r) after the transplantation, while carry-over T cells remain in the early phase.

190 - LFA-1 integrin controls microglia-neutrophil interaction in the central nervous system during experimental autoimmune encephalomyelitis

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Microglia, the resident immune competent cells of the central nervous system (CNS), have a pivotal pathological role during brain inflammatory disorders. We have previously reported that intrathecal injection of anti-LFA-1

integrin blocking antibody significantly reduces microglia activation in experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis (MS). Since LFA-1 is a leukocyte adhesion molecule promoting cell-cell contacts, we investigated the role of this integrin in the interactions between microglial cells and neutrophils, which are considered key contributors to the CNS inflammation during EAE. Two-photon laser scanning microscopy (TPLSM) *in vivo* experiments performed during the acute EAE phase showed that a large proportion of infiltrated neutrophils was moving slowly in the proximity of pial vessels in the sub-arachnoid space (SAS) of the spinal cord, suggesting their potential engagement in cell-cell interactions with CNS resident cells. To verify this hypothesis, we took advantage of CX3CR1-GFP mice, which display green fluorescent microglial cells. Our results demonstrated that infiltrating neutrophils interact with microglia in the spinal SAS in TPLSM experiments. Moreover, *in situ* LFA-1 blockade induced a substantial loss of neutrophil compartmentalization near blood vessels together with a strong reduction of neutrophil-microglia interactions. To further understand the functional involvement of LFA-1 in neutrophil-microglia contacts, we set up *in vitro* co-cultures of microglia isolated from EAE mice with neutrophils from healthy mice. Notably, our live microscopy data confirmed that control neutrophils tend to establish stable engagements with microglia whereas LFA-1 blockade significantly impaired long-lasting cell-cell contacts. Moreover, our in vitro live-imaging results also suggested that interfering with LFA-1 dependent cell-cell interactions, reduces the activated, pro-inflammatory microglia phenotype during EAE. Collectively, our data suggest that LFA-1 has a role in neutrophil-microglia interactions in the inflamed CNS and that targeting these cell-cell contacts may represent a promising therapeutic approach in CNS inflammatory diseases.

210 - Encephalitogenic T cells show elevated mannose receptor C-type 2 levels in multiple sclerosis

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Multiple sclerosis (MS) is an idiopathic autoimmune disease of the central nervous system (CNS). It is characterized by a disturbance of the blood-brain barrier (BBB), and infiltration of immune cells attacking the host's own CNS tissue. Previously, encephalitogenic MCAM$^+$Th17 cells were linked to an increased ability to infiltrate the CNS tissue in MS. An in-depth analysis based on proteomics data showed elevated protein levels of the mannose receptor C-type (MRC) 2 in these cells. Here, we present first results addressing the relevance of MRC2 in MS using the following methods: 1) Flow cytometry to determine the abundance of MRC2 on peripheral blood mononuclear cells (PBMCs) in untreated relapsing remitting (RRMS) patients in comparison to healthy donors, 2) immunohistochemistry of pre-active / active MS lesions on post-mortem brain tissue, 3) analysis of MRC2 expression on human *in vitro* polarized CD4$^+$ Th1, Th2, Th17 cells and CD8$^+$ Tc1, Tc2, Tc17 cells by flow cytometry, RT-qPCR and / or immunofluorescence microscopy, and 4) immunohistochemistry on CNS tissue derived from MOG35-55 induced experimental autoimmune encephalomyelitis (EAE) C57BL/6 mice. Preliminary flow cytometry analysis of PBMCs from 5 untreated RRMS patients in remission showed elevated proportion of MRC2$^+$ CD4$^+$ and CD8$^+$ T cell subpopulations when compared to healthy donors. Indeed, MRC2 expression was significantly co-localized with perivascular accumulating CD8$^+$ cytotoxic T cells entering pre-active and active CNS lesions on post-mortem brain tissue from 5 MS patients. *In vitro* polarized pro-inflammatory T helper (Th1) and T cytotoxic subpopulations (Tc17) showed increased MRC2 expression. Similar to human MRC2 expression, preliminary data showed Mrc2 co-localization with infiltrating leukocytes at the peak of EAE development. Our first results indicate a role for MRC2 in pro-inflammatory activated peripheral immune cells, particularly CD8$^+$ cytotoxic T cells / CD4$^+$ helper T cells localized in MS / EAE lesions, respectively. Future analysis including *in vitro* migration assays using human primary endothelial cells and *in vivo* studies using the EAE mouse model will elucidate whether MRC2 indeed represents a novel therapeutic target to interfere with MS disease development and progression.

232 - Astrocyte TrkB may regulate copper transport and foster demyelination in multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system (CNS) characterized by demyelination, inflammation and neuronal damage. Astrocytes are the largest population of glial cells in the CNS and participate to both repair and inflammatory reactions occurring during neuroinflammation. In fact, the activation of specific intracellular signalling pathways may drive glial response from beneficial to detrimental, depending on the stimuli offered by the local inflamed milieu.
Here we investigated the contribution of the neurotrophin receptor TrkB in astrocytes to demyelination. Histological studies in MS lesions showed that astrocyte TrkB finely demarcated chronic demyelinated areas and was paralleled by neurotrophin loss, suggesting that a role for astrocyte TrkB in demyelination in response to stimuli other than neurotrophins. In vitro approaches highlighted that TrkB supported glia migration and proliferation even in absence of neurotrophins, indicating transactivation of TrkB signalling in response to inflammatory or toxic mediators. In vivo modeling of MS showed that mice with astrocyte-specific TrkB deletion were resistant to demyelination induced by autoimmune or toxic insults. Neuropathological investigations in MS and model lesions revealed upregulation of copper transporters in glia cells, and evidenced TrkB-dependent expression of the copper transporter CTR1 on glia cells during neuroinflammation. In vitro experiments confirmed that astrocyte TrkB supported expression of CTR1 via modulation of glial calcium flux in response to stimuli distinct from neurotrophins, thus leading to copper uptake and release which in turn caused oligodendrocyte and myelin loss.
Collectively, these data demonstrate a novel demyelination mechanism supported by astrocyte copper and dependent on astrocyte TrkB, and open to the possibility of restoring proper copper homeostasis as therapeutic target in MS.

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244 - Considering the role of brain neutrophils in psoriasis – relationship between peripheral autoinflammation and central nervous system

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Neutrophils are the most abundant leukocytes in the circulation. Despite their short half-life, they are extremely potent immune cells. They provide the primary defense against bacterial and fungal infections and are principal components of tissue infiltrates during autoinflammatory disorders. Cutaneous recruitment of neutrophils serves as a typical histopathologic hallmark of psoriasis – a mixed autoimmune and autoinflammatory skin disease.
On the other hand, neutrophils play an important role in a wide range of diseases with a neuro-immune background, such as multiple sclerosis, neurodegenerative disorders, pain, stroke and rheumatological arthritis. Clinical observations and scanty experimental data evidence that disturbances in the neuro-immune interactions are closely related to the development of psoriasis. Nevertheless, the involvement of neutrophils mobilized to the brain as a potentially significant element of the puzzling pathogenesis of that skin disease has not been studied so far.

In the present study we assessed the number of neutrophils in brains of mice immunized by imiquimod (an immune response modifier, a TLR7/8 agonist). Topical application of Aldara cream, in which imiquimod is the active component, is a well-described animal model of psoriasis.
The main observation of our study was that skin treatment with imiquimod-containing cream led to neutrophil infiltration to the brain. Surprisingly, while the number of neutrophils in the skin and blood gradually increased already in the first three days of imiquimod administration, the recruitment of neutrophils to the brain was seen only on the 6th day after the induction of the disease and was negatively correlated with the macroscopic psoriatic-like changes.
For a long time, presence of neutrophils in the brain was considered as an inflammatory, harmful condition. However, recently more attention is focused on the impact of these cells in maintaining the brain homeostasis. Our data indicate that neutrophils may represent a hitherto unexplored route of immune communication to the brain in the course of chronic skin autoinflammation.

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252 - Anti-inflammatory effects of siponimod on astrocyte

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Objectives: Siponimod is the first oral disease-modifying drug recently approved for active secondary progressive multiple sclerosis. It acts as a functional antagonist of sphingosine-1-phosphate (S1P) receptor 1 ($S1P_1$) via $S1P_1$ internalization and an agonist of S1P receptor 5 ($S1P_5$). Although $S1P_1$-expressing lymphocyte is the primary target of siponimod in terms of prevention of lymphocytic infiltration into the central nervous system, it is still uncertain whether siponimod directly affects the functions of astrocytes which express S1P receptors. Here, we investigated the effects of siponimod on astrocytes using mouse primary culture.
Methods: Mouse primary astrocyte-enriched cultures were isolated from mixed glial cell cultures prepared from newborn C57BL/6 mouse brains. Astrocytes were activated by stimulation with 1 μg/ml lipopolysaccharide (LPS) or 20 μM hydrogen peroxide for 24 h following pretreatment with 0–1,000 nM siponimod 1 h prior. To block the binding of siponimod to $S1P_1$ or $S1P_5$, astrocytes were pretreated with 1 μM W146 ($S1P_1$ antagonist) or 10 μM suramin ($S1P_5$ antagonist) before siponimod administration. mRNA expression levels of cytokines, neurotrophic factors, and antioxidants were examined by quantitative PCR. Protein production levels of cytokines were evaluated by ELISA. Activation of nuclear factor-kappa B (NF-κB) was assessed by nuclear translocation of NF-κB using immunostaining.
Results: Siponimod significantly suppressed NF-κB activation, and mRNA and protein levels of pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) in activated astrocytes. By contrast, siponimod did not affect the expression levels of neurotrophic factors and antioxidants in activated astrocytes. W146 interfered with the suppressive effects of siponimod on astroglial production of IL-6 and TNF-α, but not IL-1β. In addition, treatment with W146 *per se* reduced activated astroglial production of IL-1β and IL-6, but not TNF-α. Moreover, siponimod more effectively suppressed astroglial IL-6 production than W146. By contrast, treatment with suramin did not alter astroglial production of these pro-inflammatory cytokines. These data suggested that siponimod might exert anti-inflammatory effects on astrocytes by mainly $S1P_1$ antagonization and partially other pathway(s) independent of $S1P_1$ signaling.
Conclusions: Our findings indicated that siponimod shows broader anti-inflammatory effects on astrocytes compared to $S1P_1$ antagonist. Siponimod might suppress disease progression of multiple sclerosis in part by direct inhibition of astroglial neuroinflammation.

302 - In vivo imaging of the glia limitans with a new aquaporin 4 - mRuby3 reporter mouse

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Immune surveillance of the immune privileged central nervous system (CNS) is provided by the CNS barriers which establish compartments that differ with respect to their accessibility to immune cells and immune mediators. One of these barriers is the *glia limitans* which is formed by astrocyte end-foot processes and the parenchymal basement membrane. The *glia limitans* is located at the surface of the brain and the spinal cord parenchyma and separates the CNS border compartments that are accessible to peripheral immune cells from the CNS parenchyma. The *glia limitans* provides a barrier to immune cells as penetration of immune cells across the *glia limitans* correlates with clinical disease onset in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. Two-photon intravital microscopy (IVM) has advanced our understanding of CNS immunity. However, a limitation of current IVM technologies is the lack of simultaneous visualization of the brain barriers and immune cells, which hampers correct allocation of immune cells to CNS border compartments or CNS parenchyma. To overcome this drawback, we have established an aquaporin-4 (AQP4) mRuby3 reporter mouse that allows to visualize the polarized expression of AQP4-mRuby3 on astrocyte end-foot processes as a maker for the *glia limitans.* Two-photon imaging of the brain and spinal cord of AQP4-mRuby3 mice verified *in vivo* visualization of the *glia limitans* at the surface of the CNS parenchyma, and along perivascular spaces. Simultaneously imaging of immune cell entry into the CNS allowed their allocation to subarachnoid, perivascular and parenchymal locations. The AQP4-mRuby3 reporter mouse will thus be suitable to study the role of the *glia limitans* in controlling CNS immunity.

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306 - Barriers of the CNS: actors in neuroinflammation - PosterAssociation of choroid plexus enlargement with disease severity and cortical atrophy in early multiple sclerosis

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Neuroinflammation is a pathophysiological hallmark of multiple sclerosis. The migration of peripheral inflammatory cells through the brain barriers towards the central nervous system (CNS) has a close mechanistic link to neuroinflammation and neurodegeneration. Robust translational models to reliably quantify and track neuroinflammation and the peripheral to CNS-cross-talk in both mice and humans are currently lacking. The choroid plexus (ChP) plays a pivotal role in regulating the trafficking of immune cells from the brain parenchyma into the cerebrospinal fluid (CSF) and is a key structure in the initiation of inflammatory brain responses. In a translational framework, we looked into the integrity and multidimensional characteristics of the ChP under inflammatory conditions and hypothesized whether ChP volumes could act as an interspecies marker of neuroinflammation that closely interrelates with functional impairment. Therefore, we explore ChP characteristics in neuroinflammation in patients with multiple sclerosis and in two well established experimental mouse models, cuprizone diet-related demyelination and experimental autoimmune encephalomyelitis. We demonstrate that ChP enlargement-reconstructed from MRI-is highly correlated with neuroinflammatory activity and disease progression, both in the studied mouse models and in humans. In addition, pharmacological modulation of the blood-CSF barrier with natalizumab prevents an increase of the ChP volume. ChP enlargement was strongly linked to emerging functional impairment as depicted in the mouse models and in multiple sclerosis patients. We were able to show that ChP characteristics are robust and translatable hallmarks of acute and ongoing neuroinflammatory activity in mice and humans that could serve as a promising interspecies marker for

translational and reverse-translational approaches. Furthermore, choroid plexus can assist to identify patients at high risk for progression who may benefit from early treatment.

311 - B cells and their progenitors reside in non-diseased meninges

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The CNS is ensheathed by the meninges and cerebrospinal fluid, and recent findings suggest that these CNS-associated border tissues have complex immunological functions. Unlike myeloid lineage cells, lymphocytes in border compartments have yet to be thoroughly characterized. Based on single-cell transcriptomics, we here identified a highly location-specific composition and expression profile of tissue-resident leukocytes in CNS parenchyma, pia-enriched subdural meninges, dura mater, choroid plexus and cerebrospinal fluid. The dura layer of the meninges contained a large population of B cells under homeostatic conditions in mice and rats. Murine dura B cells exhibited slow turnover and long-term tissue residency, and they matured in experimental neuroinflammation. The dura also contained B lineage progenitors at the pro-B cell stage typically not found outside of bone marrow, without direct influx from the periphery or the skull bone marrow. This identified the dura as an unexpected site of B cell residence and potentially of development in both homeostasis and neuroinflammation.

324 - Neutrophils functions and homeostasis in epileptic patient, impact on BBB disruption ?

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Epilepsy is characterized by the presence of a leaky blood brain barrier (BBB) and permanent neuro-inflammation that contribute to neuronal hyperexcitability. Neutrophils (PMNs), key cells of the innate immune system, are also important mediators of inflammation-induced vascular and tissue injury, and their improper activation may lead to oxidative stress and exaggerated inflammatory reactions. Despite some evidence of their impact on neuropathology and BBB disruption, little attention has been focused in epilepsy. We attempt to clarify PMNs homeostasis in patients suffering of intractable temporal lobe epilepsy with hippocampal sclerosis.
Human brain Tissues and blood were obtained from surgery and pre-surgical evaluation. Using flow cytometry on whole blood samples, we evaluated i) PMNs subpopulations as senescent PMNs (PS) ($CXCR4^{high}$ and $CD62L^{low}$) and angiogenic PMNs (PA) ($VEGFR1^{+}$, $CD49d^{+}$); ii) PMNs activation and degranulation by CD11b and CD62L expression; iii) oxidative stress via Diydroethidine (DHE) oxidation. Cytokines level in sera were determined by Simoa technology and brain infiltration of PMNs by immunohistochemistry. All these informations will be correlated with clinical markers of disease severity.
In blood of epileptic patients, a recent seizure induces an increase of PMN/ lymphocyte ratio (NLR) as well as an upregulation of CD11b and downregulation of CD62L reflecting PMNs activation. PS subpopulation, a harmful

subset producing high levels of ROS, are significantly elevated compared to control and correlated with IL-6 levels in sera. In parallel, ROS species increase in blood of epileptic patient. PA subpopulation, characterized by angiogenic properties and able to migrate to hypoxic tissue, decrease in epileptic patient and are inversely correlated with the frequency of seizures. In the same line, significant PMNs infiltration is observed in epileptic tissue and higher in patients suffering of a recent seizure.
Improper activation of PMNs after a seizure may lead to oxidative stress and exaggerated inflammatory reactions. Release of Oxidative species as ROS or enzymes as elastase or MMP9 by activated PMNs could induce to Blood Brain Barrier permeability known to participate to epileptogenesis. This project will be the first to evaluate PMNs activation in epileptic patient and could open new perspectives in the development of innovative immunotherapy strategies.

331 - The NKG2D ligand ULBP4, an upregulated stress signal in MS that shapes CD8 T cells behaviour

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Extensive evidence points to pathogenic roles for immune mediators in the pathobiology of multiple sclerosis (MS); nevertheless, the contribution of specific immune mediators remains incompletely resolved. We have identified the NKG2D pathway as a relevant player in MS pathology. Our goal is to uncover the contribution of NKG2D and its ligands (NKG2DL) to the typical tissue injury observed in the brain of MS patients. Using post-mortem human samples, we evaluated the presence of NKG2DL in the brain of MS patients and controls. Although we detected mRNA for the eight identified human NKG2DLs in brain samples, only ULBP4 protein expression was detectable by western blot. Moreover, ULBP4 levels were greater in MS lesions and normal appearing white matter from MS patients compared to grey matter from the same patients as well as brain tissue from controls. Using immunohistofluorescence, we identified astrocytes as the main population expressing this ligand. Interestingly, ULBP4-expressing astrocytes were often localized around small blood vessels suggesting that astrocytes' end feet participating in the blood brain barrier can interact with infiltrating immune cells expressing NKG2D. To identify possible triggers of ULBP4, we tested various cellular stressors observed in MS lesions on primary cultures of human astrocytes. Such stressors, including oxidative stress, reticulum endoplasmic stress as well as the presence of pro-inflammatory cytokines, induced an increase of ULBP4-expressing astrocytes. Finally, we observed the presence of soluble ULBP4 in CSF of MS patients and controls, with a smaller shed/soluble form of 25kDa that was significantly elevated in CSF from female MS patients compared to controls and male MS patients. To demonstrate the functional impact of soluble ULBP4, we evaluated the effect of this ligand on various functions of CD8+ T lymphocytes. We showed that ULBP4 enhanced the production of the pro-inflammatory cytokines GM-CSF and IFNγ. In addition, it increased CD8+ T lymphocytes motility and favored a kinapse-like behavior when these cells were cultured in the presence of human astrocytes. CD8+ T lymphocytes from MS patients were especially altered by the presence of soluble ULBP4 compared to healthy controls. In conclusion, our study provides new evidence for the involvement of NKG2D and its ligand ULBP4 in MS pathology and points to this ligand as a viable target to specifically block the NKG2D pathway.

332 - IL-27-exposed human astrocytes exhibit altered immune properties and modify the profile of encountered human T lymphocytes

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by immune cell infiltration, loss of myelin, and neuronal cell death. The cytokine interleukin-27 (IL-27) triggers both pro- and anti-inflammatory responses upon binding its receptor (IL-27R). IL-27 reduces EAE severity but its role in MS patients is unresolved. We showed that IL-27 is elevated in MS brains and that human astrocytes in both MS lesions and *in-vitro* express IL-27R. *We posit that IL-27 alters astrocytes' immune properties that can modify the immune profile of encountered T cells.* Transcriptomic analysis showed that IL-27-exposed human astrocytes upregulated multiple immune genes. We validated that IL-27-treated astrocytes secreted elevated amount of chemokines (CXCL9, 10 and 11), as well as IL-18BP, an inhibitor of the pro-inflammatory cytokine IL-18. By flow cytometry, we detected greater levels of molecules potentially acting on T cells such as PD-L1 and HLA-E, as well as ICAM-1, an adhesion molecule involved in T cell infiltration and immunological synapse formation. Thus, we investigated whether the astrocyte response to IL-27 shapes the immune profile of CD4 and CD8 T cells with which they come in contact. Indeed, upon contact with IL-27-exposed astrocytes, CD4 and CD8 T cells increased T-bet and Eomes expression, suggesting changes in T cell polarization. Moreover, IL-27-treated astrocytes increased surface expression of immune mediators such Fas and PD-L1, which can respectively trigger apoptosis and inhibitory signal in T cells, and reduced the chemokine receptor CXCR3. Using our recently optimized live imaging assay to capture T cell movements in co-culture with human neural cells, we showed that CD8 T cells exhibited a higher motility when encountering IL-27-treated astrocytes compared to untreated astrocytes, suggesting a preponderance of kinapse-like interactions instead of synapse-like ones. Finally, we observed that CD8 T cells from MS patients present a higher motility in contact with IL-27- treated astrocytes compared to cells from healthy donors. Our results support the notion that IL-27 distinctly alters functions of human astrocytes and consequently shapes T cell immune profile and motility, especially in CD8 T cells from MS patients.

333 - NKG2D and its ligand ULBP4 shape human CD8 Tcell-astrocytes interactions in the context of MS

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Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by multifocal areas of myelin/oligodendrocyte loss, axonal damage, glial cell activation and immune cell infiltration. Although the key role of the immune system in MS is well established, the contribution of specific immune molecules remains incompletely unresolved. We identified NKG2D, an activating immune (co)receptor, as a key factor shaping the interactions between CNS and immune cells in the context of MS. The NKG2D pathway is involved in T cell cytokine production, cytotoxicity and migration across endothelial cells, especially in inflammatory and autoimmune diseases. We have previously demonstrated that NKG2D contributes to disease progression in passive experimental autoimmune encephalomyelitis (EAE). We showed that one NKG2D ligand called MULT1 is upregulated in the CNS and cerebrospinal fluid of EAE mice especially at disease peak. We detected the full and shed forms of MULT1 in EAE samples. We investigated whether the NKG2D pathway contributes to T cell-CNS cell interactions in the context of MS using human primary cultures. We found that a proportion of primary human astrocytes and neurons expressed the NKG2D ligand called ULBP4. Using our recently optimized live-spinning disk microscopy assay, we characterized the interactions between human astrocytes or neurons and activated human CD8 T lymphocytes (expressing NKG2D). We showed that blocking NKG2D decreased stable interactions between astrocytes and CD8 T lymphocytes, increased CD8 T cell motility and promoted kinapse-like behavior by CD8 T cells. This impact was not observed when CD8 T lymphocytes were cultured with human neurons suggesting that the NKG2D pathway is more involved in astrocyte-CD8 T cell interactions than in neuron-CD8 T cell contacts. Notably, we detected a soluble form of ULBP4 (sULBP4) in the CSF of MS patients. We observed that the addition

of sULBP4 to our co-cultures decreased CD8 T cell-astrocyte stable interactions, increased motility and modified CD8 T cell behaviour. Furthermore, we observed that those effects were more prominent for CD8 T cells isolated from MS patients compared to healthy donors. Our results suggest that human NKG2D+ CD8 T lymphocytes infiltrating the brain parenchyma could interact with ULBP4-expressing astrocytes and that such interactions shape CD8 T cell motility behavior especially in MS patients.

349 - Elucidating mechanisms of EAE resistance in BAFF-tg mice

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Background: The extent of inflammation in the central nervous system (CNS) in Multiple Sclerosis (MS), is dictated by a balance of anti- and pro-inflammatory mechanisms. In recent years, we and others have shown that B cells regulate the mouse model of MS, experimental autoimmune encephalomyelitis (EAE). Our lab has previously shown that antibody-secreting IgA^+ plasma cells (PCs) can attenuate EAE, and BAFF-transgenic (BAFF-tg) mice which have excessive numbers of IgA^+ PCs are highly resistant to both active and passive EAE. My hypothesis is that protection against the development of EAE occurs at the T cell effector stage of EAE in BAFF-tg mice.
Methods: Adoptive transfer (A/T) EAE was performed by harvesting lymph nodes and spleens from wildtype (WT) B6 donor mice immunized against MOG_{35-55} on day 9 post-immunization. Cells were cultured with MOG_{35-55} peptide and IL-23 for 72hrs, then transferred into recipient mice. Donor cells retrieved from recipients were identified by a congenic marker (CD45.1) or via CFSE labelling prior to transfer. Donor cells were then analyzed by flow cytometry at different time points post-transfer.
Results: I previously showed that BAFF-tg recipient mice are protected from A/T EAE and have less $GM\text{-}CSF^+$ $CD4^+$ T cells in the CNS compared to WT mice, suggesting that the mechanism of BAFF-dependent EAE suppression does not need to be present during priming, however it is possible that this mechanism may still operate in the periphery. To test this, I tracked transferred T cells in the spleen, lungs, gut, and blood of WT vs. BAFF-tg mice at timepoints before EAE onset. At 24hrs and 48hrs post-transfer, $CD45.1^+$ donor cells can be detected in the spleen, but few are present in the lung and gut. By 96hrs post-transfer, many $CD45.1^+$ cells are present in the spleen, lungs, and gut. Notably, $CD45.1^+CD4^+CD44^+CD62L^-$ cells are expanded at 96hrs, suggesting that donor effector T cells have been reactivated in the recipients and have proliferated. Using CFSE to label transferred cells before injection, I demonstrated similar kinetics.
Conclusions: Adoptively transferred encephalitogenic T cells exhibit altered abundance in the periphery of BAFF-tg mice. Future experiments will investigate regulatory mechanisms that can account for this finding.

353 - CLMP promotes immune cells trafficking across the blood-brain barrier during neuroinflammation.

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The integrity of the blood-brain-barrier (BBB) is compromised in Multiple Sclerosis (MS). This alteration allows recruitment and migration of peripheral immune cells into the CNS through the expression of various cell adhesion molecules. Using bulk-RNA sequencing of human BBB endothelial cells, we have identified CAR-Like Membrane Protein (CLMP) as an adhesion molecule overexpressed in inflammatory conditions. Although CLMP is involved in adipocyte maturation and in the development of obesity, its implication in CNS physiology and in the development of MS has not been studied yet. Our hypothesis is that CLMP is implicated in immune cells migration across the BBB.
To assess CLMP expression, we have used qPCR, western blot and flow cytometry on peripheral blood mononuclear cells (PBMCs), immunohistochemistry on human and mouse CNS tissues and in vivo molecular magnetic resonance imaging (mMRI) on C57Bl/6 mice affected by experimental autoimmune encephalomyelitis (EAE). Finally, in vitro adhesion and migration assays with human PBMCs were used to determine the implication of CLMP in immune cell migration across human BBB endothelial cells (BBB-ECs).

We demonstrate CLMP is upregulated during inflammation of primary cultures of human BBB-ECs. Using flow cytometry of human PBMCs, we show that CLMP is overexpressed on B lymphocytes and monocytes in MS patients compared to healthy control subjects. In accordance with this, in autopsy brain tissue from MS patients, we show that CLMP expression on vessels is associated with the infiltration of inflammatory cells invading the perivascular space and the parenchyma. This overexpression of endothelial CLMP was present only in active MS lesions, and not in healthy control brain tissue. We also found CLMP+ immune cell infiltrates in the perivascular area of MS plaques. In addition, using immunohistochemistry and molecular MRI, we detected CLMP+ vessels in the CNS of MOG35-55 – immunized C57BL/6 EAE mice, but not in sham animals. In infiltrated spinal cord areas of EAE mice, we also detected CLMP+ immune cells in the parenchyma. Moreover, using a static adhesion assay we show that leukocytes strongly adhere to CLMP. Finally, the use of a blocking antibody against CLMP reduced the migration of immune cells across human BBB endothelial cells.
We propose that CLMP is an adhesion molecule used by immune cells to access the CNS. CLMP could represent a biomarker of MS activity and a target for a new treatment of neuroinflammatory conditions.

373 - DICAM: A new cell adhesion molecule involved in myeloid cells infiltration to the CNS.

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Disruption of the blood-brain barrier (BBB) and migration of leukocytes from the periphery to the central nervous system (CNS) are early events in lesion formation in multiple sclerosis (MS). Among CNS-infiltrated leukocytes, macrophages are important contributors to inflammation and tissue damage. Monocytes readily cross the BBB to infiltrate the CNS, differentiate into macrophages and express pro-inflammatory cytokines. Using proteomic and RNA sequencing techniques, we have identified Dual Ig domain containing Cell Adhesion Molecule (DICAM) as a new adhesion molecule expressed by human TH17 lymphocytes and BBB endothelial cells (ECs). The expression and function of DICAM in MS pathogenesis remain unexplored. The current study aims to evaluate DICAM's role in myeloid cell migration to the CNS during neuroinflammation. Firstly, to investigate the expression of DICAM by myeloid cells, we performed flow cytometric analyses of peripheral blood mononuclear cells (PBMCs) from patients with RRMS, SPMS and PPMS, age and sex-matched with healthy control (HC) samples. Using FlowSOM clustering, we demonstrated that DICAM is more expressed by classical monocytes in PBMCs compared to other peripheral myeloid populations. Comparing HC and MS patients, we observed that both intermediate/non-classical and classical monocytes from RRMS and SPMS patients overexpress DICAM compared with matched HC. To further explore the DICAM expressing cell profile, we are currently analysing cytokine expression by DICAMpos vs DICAMneg PBMCs by flow cytometry. Preliminary results indicate that higher frequency of DICAM+ classical monocytes express IL6 compared with DICAMneg cells, suggesting a more pro-inflammatory role. Finally, the role of DICAM in monocyte adhesion to the BBB-ECs was assessed in vitro by blocking DICAM in a flow adhesion assay on a monolayer of human BBB-ECs. Different experimental autoimmune encephalomyelitis models (EAE, animal model of MS) were used to explore DICAM function in vivo. From those experiments, we showed that the blockade of DICAM restricts the adhesion of monocytes on human BBB-ECs and decreases disease severity in several EAE models. This study aims to characterize the role of DICAM in harmful myeloid cell migration across the BBB. Our preliminary data shows that DICAM is preferentially expressed in pathogenic myeloid cells. Therefore, this adhesion molecule might be involved in MS pathogenesis and could arise as a new therapeutic target for MS.

376 - Comparison of markers of inflammation and neurodegeneration in cerebrospinal fluid as predictors of survival in patients with amyotrophic lateral sclerosis

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Objective: To compare markers of neurodegeneration [neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH)] and markers of inflammation [chitotriosidase-1 (CHIT1), chitinase-3-like protein 1 (YKL-40) and monocyte chemoattractant protein-1 (MCP-1)] as biomarkers of survival in cerebrospinal fluid (CSF) of patients with amyotrophic lateral sclerosis (ALS).

Methods: Protein biomarkers were determined in CSF of 94 patients with ALS using enzyme-linked immunoassays. Univariate survival analyses were performed using the Kaplan-Meier analysis, and the log-rank test was conducted to determine differences between the survival curves (n = 94, censored: 14%). A Cox regression survival model was calculated including 8 established clinical predictors of survival in ALS (n = 84, censored: 13%). For this purpose patients were stratified as low and high biomarker level based upon the median concentration of the biomarker in the total ALS cohort.

Results: Univariate survival analyses revealed a significantly shorter survival in patients with ALS having high levels of pNfH ($\chi^2 = 12.69$, $p < 0.001$), NfL ($\chi^2 = 12.34$, $p < 0.001$), CHIT1 ($\chi^2 = 7.62$, $p < 0.01$), YKL-40 ($\chi^2 = 14.05$, $p < 0.001$) and MCP-1 ($\chi^2 = 8.45$, $p < 0.01$). In a multivariate Cox regression model, high levels of pNfH (HR: 3.36, 95% CI: 1.87 – 6.04, $p < 0.001$), NfL (HR: 2.06, 95% CI: 1.23 – 3.46, $p < 0.01$), CHIT1 (HR: 2.13, 95% CI: 1.22 – 3.73, $p < 0.01$), YKL-40 (HR: 2.17, 95% CI: 1.21 – 3.90, $p = 0.01$) and MCP-1 (HR: 2.49, 95% CI: 1.47 – 4.21, $p = 0.001$) were independently associated with a shorter survival. Patients with both high NfL and YKL-40 levels harbored a significantly shorter survival (median: 15.3 months, range: 1.43 – 49.0 months) compared to those patients with both low NfL and YKL-40 levels (median: 45.5 months, range: 2.03 – 74.5 months; $p < 0.0001$), but did not when compared to those patients with low NfL yet high YKL-40 levels (median: 20.2 months, range: 0.47 – 77.3 months; $p = 0.74$).

Conclusion: This study highlights the importance of CSF biomarkers to predict survival in patients with ALS. Furthermore, it demonstrates that patients with ALS without pronounced neurodegeneration have a short survival in the presence of neuroinflammation, e.g. reflected by low NfL levels and high YKL-40 levels. These findings may have implications for future stratification of patients in clinical trials.

Behavior and immunity

141 - IL-6 affects neurogenesis and gliogenesis of SVZ progenitors producing behavioral phenotypes reminiscent of neurodevelopmental disorders

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Epidemiologic studies have demonstrated that perinatal infections and several other maternal immune challenges during pregnancy increase the risk of offspring developing neurodevelopmental disorders that include autism (ASD), schizophrenia and depression. However, the mechanisms linking inflammation to perinatal brain development are not fully understood. Animal models that aim to reproduce maternal immune activation (MIA) using bacterial and viral mimetics during gestation have shown long lasting behavioral changes in the offspring reminiscent of human neurodevelopmental disorders, including communication, cognitive and social deficits. These studies also highlighted the importance of inflammatory cytokines in inducing these behavioral changes. Notably, interleukin-6 (IL-6) injected mid-gestation can cross the placenta and the fetal blood-brain barrier, reproducing behavioral deficits of MIA models. IL-6 can directedly affect neural stem cells (NSCs) and neural progenitors (NPs) developing postnatally in the SVZ. These progenitors produce neurons, astrocytes and oligodendrocytes that populate late developing structures relevant to the behavioral deficits. Here, we have increased IL-6 from postnatal days 3-6, when the SVZ is rapidly expanding. Using Nestin-CreERT2 fate mapping

we show the IL-6 decreased neurogenesis in the dentate gyrus of the dorsal hippocampus, astrogliogenesis in the amygdala and oligodendrogenesis in the corpus callosum, all by ~50%. Moreover, the IL-6 treatment elicited behavioral changes classically associated with neurodevelopmental disorders. IL-6 injected male mice lost social preference in the social approach test, spent ~30% less time socially engaging with sexually receptive females and produced ~50% fewer ultrasonic vocalizations during mating. They also engaged ~50% more in self-grooming and had an increase in inhibitory avoidance. Altogether, these data provide new insights into the biological mechanisms linking perinatal immune activation to complex neurodevelopmental brain disorders. Supported by grants from the Governor's Council for Medical Research and Treatment of Autism awarded to SWL and FJV.

217 - Neural precursor cells tune striatal connectivity via secretion of IGFBPL-1.

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The adult brain retains endogenous neural precursors cells (eNPCs) in two major neurogenic niches, the subgranular zone (SGZ) in the hippocampus and the subventricular zone (SVZ). While in humans and rodents the SGZ contributes to memory functions by generating new neurons that integrate into hippocampal circuitry, the role of eNPCs of the SVZ is less clear. SVZ-eNPCs contribute to the generation of new neurons fated to the olfactory bulb (OB) in rodents but not in humans where they are thought to contribute to striatal neurogenesis as a result of tissue damage.

Here, we show in mice and humans a novel non-neurogenic physiological role of adult SVZ-eNPCs in supporting cognitive functions by regulating striatal neuronal activity. We first provide evidence that GABAergic transmission between parvalbumin-expressing fast-spiking interneurons (FSIs) and medium spiny neurons (MSNs) is tuned by SVZ-eNPCs via secretion of Insulin-Like Growth Factor Binding Protein Like 1 (IGFBPL-1) that, in turn, regulates the Insulin-Like Growth Factor (IGF-1) signalling cascade. Consistently, selective ablation of SVZ-eNPCs and in vivo disruption of the IGF-signalling determine the impairment of intrastriatal coherence. A finding associated with a higher failure rate of GABAergic transmission mediated by FSIs and with striatum-related behavioural dysfunctions impairing decision making. Human validation studies revealed IGFBPL-1 expression in the SVZ and in foetal and induced-pluripotent stem cell-derived NPCs as well as a strong correlation in neurological patients between SVZ pathological damage, reduction of striatum volume and impairment of information processing speed.

All in all, our results highlight a novel non-neurogenic homeostatic role exerted by SVZ-eNPCs on striatal GABAergic neurons that might contribute to cognitive processes involving decisions-making tasks.

219 - Sleep increases the migration of T-cells towards CCL19 in humans via growth hormone and prolactin signaling

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Sleep promotes the formation of adaptive immunity, as shown in experiments demonstrating increased antigen-specific responses if participants sleep following vaccination. The consistently observed reduction of T-cell numbers in blood during sleep compared with nocturnal wakefulness might reflect an enhanced migration of T-cells to secondary lymphatic organs (SLOs), which could support local T-cell responses and thus provide a mechanism underlying the immunoenhancing effects of sleep. However, direct evidence for this notion is lacking. To determine whether sleep indeed promotes the migratory potential of T-cells towards SLOs, healthy participants were examined in the sleep laboratory during a normal sleep-wake cycle and during 24 hours of continuous wakefulness. We collected whole blood every 4 hours for the determination of T-cell migration using Transwell® chemotaxis assays in the presence or absence of the chemokine CCL19, which is crucial for T-cell trafficking to SLOs. We show that sleep increases the spontaneous as well as CCL19-directed migration of total CD3, CD4, CD8 T-cells, and naïve T-cell subsets. To show that the effects of sleep were mediated by soluble molecules present in the plasma, we incubated T-cells from healthy donors with plasma that had been collected at night during the sleep and wake conditions of the *in vivo* experiment. These experiments revealed a higher CCL19-directed migration when T-cells had been incubated with plasma collected from sleeping compared to awake participants. This effect was suppressed after addition of growth hormone (GH) and prolactin (PRL) antagonists to the sleep plasma, suggesting that these two hormones, which were increased during sleep in the *in vivo* experiments, mediated the sleep effects. This finding is in line with additional *in vitro* experiments demonstrating increases in T-cell migration towards CCL19 following incubation with GH and PRL. Together, our series of experiments show that sleep selectively promotes the migration of T-cells towards CCL19 by enhancing GH and PRL signaling, and thus reveal a potential underlying mechanism of the supporting effect of sleep on adaptive immunity.

228 - Generation and characterization of TRE-TNFR2 mice, a novel gain-of-function transgenic for cell-specific overexpression of TNFR2

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Tumor necrosis factor (TNF) has been implicated in the pathophysiology of a variety of neurological disorders, including multiple sclerosis (MS). TNF exists in two forms: transmembrane TNF (tmTNF), which functions via cell-to-cell contact by activating TNF receptor 2 (TNFR2), and soluble TNF (solTNF), which preferentially binds and activates TNF receptor 1 (TNFR1). Studies in MS and its animal models have shown that solTNF-TNFR1 signaling is detrimental due to activation of neurotoxic pathways, and tmTNF-TNFR2 signaling is protective due to activation of reparative processes. Our laboratory has been at the forefront in the investigations of tmTNF-TNFR2 signaling in MS using loss-of-function genetic approaches, demonstrating that TNFR2 in oligodendrocyte lineage cells and microglia has reparative potential. Nevertheless, to fully understand the protective mechanisms initiated downstream of TNFR2 activation and identify targetable molecules for the development of MS therapeutics, a gain-of-function approach is warranted. For this purpose, we generated TRE-TNFR2 transgenic mice to induce timed overexpression of TNFR2 in the cell of interest based on administration of doxycycline (DOX) in tet-on systems, or removal of DOX in tet-off systems. We designed our construct so that TNFR2 and an eGFP reporter are simultaneously driven by a bidirectional tet responsive element (TRE) and two minimal cytomegalovirus (CMV)

promoters in opposite orientation to visualize cells with TNFR2 overexpression by eGFP fluorescence. TRE-TNFR2 mice were characterized upon crossing with various tTA and rtTA transgenics, demonstrating efficient and specific overexpression in neurons, oligodendrocyte lineage cells, myelinating oligodendrocytes and astrocytes. Furthermore, overexpressed TNFR2 was found to be functional, as TNFR2-dependent activation of intracellular signaling, specifically PI3K/AKT, was observed both *in vivo* and *in vitro*.To directly address the reparative functions of TNFR2 in the CNS during demyelinating disease, we subjected mice with TNFR2 overexpression in myelinating oligodendrocytes (PLPtTA:TRE-TNFR2) to MOG_{35-55} experimental autoimmune encephalomyelitis (EAE), a model of MS, and found a marked improvement of the clinical outcome. While studies to identify the specific mechanisms underlying this effect are still ongoing, these data validate TNFR2 as a promising target for therapy in MS and potentially other neurological disorders associated with neuroinflammation.

359 - The role of astrocytic PERK in learning and memory

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Misfolded protein accumulation in the brain and memory impairment are common underlying features in various neurodegenerative disorders. Misfolded protein accumulation in the endoplasmic reticulum (ER) lumen leads to ER stress and, in response, cells initiate the unfolded protein response (UPR). The UPR is partly mediated by the activation of ER transmembrane protein PKR-like ER kinase (PERK). Activated PERK phosphorylates eukaryotic initiation factor 2 alpha (eIF2α), which apart from mediating global translation attenuation, can also affect learning and memory. Astrocytes can regulate various biological processes in the CNS, which are instrumental for memory formation. Neuronal PERK has previously been suggested to affect spatial working memory and cognitive flexibility but the role of astrocytic PERK in memory and learning has not been investigated. We hypothesized that astrocyte specific PERK deletion *in vivo* will affect memory and learning. We conducted open field, Y maze and Morris water maze behavioral tests in two age cohorts (12 and 18 months) of control and astrocyte specific (GFAP-Cre) PERK knockout mice ($PERK^{Astro\text{-}KO}$) to assess general locomotor activity, short term spatial working memory, long term reference memory and cognitive flexibility respectively. We found that there is no significant difference between control and $PERK^{Astro\text{-}KO}$ mice in open field, Y maze and Morris Water Maze tests for both age group. Moreover, there is no significant difference in the ER stress marker and proinflammatory cytokine gene expression between control and $PERK^{Astro\text{-}KO}$ mice in the hippocampus and cortex region. Additionally, we detected no change in the expression of proteins involved in PERK-eIF2α signaling between $PERK^{Astro\text{-}KO}$ and control mice in cortex and hippocampus. Overall, our results indicated that PERK deletion from GFAP-expressing astrocytes *in vivo* does not affect general locomotion or learning and memory.

370 - Innate vs. Adaptive Immunity, Brain Structure and Behavior in Active Pediatric Crohn's Disease

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Objective: Crohn's disease (CD) is an inflammatory disease of the intestine associated with a range of extra-intestinal manifestations including those affecting the brain. Gray and white matter changes and decreased cognitive performance have been found in adults with CD, though findings have been inconsistent, without immune-mechanistic evaluation. We have shown similar effects for pediatric CD. We here study if the peripheral immune response during active intestinal disease is associated with brain structures and behavior. **Methods:** Sixty children 10-15 years (19 CDActive, 23 CDRemission, 18 Healthy) underwent structural MRI, diffusion weighted imaging, neuropsychological assessment, disease severity rating and phlebotomy for immune gene expression. Cortical thickness, surface area and volume were analyzed with Freesurfer. White matter axial diffusivity, fractional anisotropy, isotropic free water were obtained via diffusion compartment model. Immune gene expression was assayed via multiplex Nanostring®. Analyses included group comparison, regression, gene set

enrichment and variance analyses via GeneOntology -and were adjusted for demographics, steroid and anti-TNF therapy. **Results:** Compared to disease and healthy controls, CDActive patients demonstrate widespread reduced cortical thickness, cortical volume, surface area, subcortical volume, and poorer behavioral function (memory, mood, fatigue). Consistent structural differences were found for insula, cuneus, thalamus and posterior regions. Several white matter tracts showed increased isotropic free water (neuroinflammation) and decreased axial diffusivity (axonal integrity). We found upregulation of innate (myeloid leukocyte mediated immunity) and downregulation of adaptive immunity (T cell costimulation) in active CD and this was associated with smaller brain volumes and thickness, increased free water and fatigue. Memory and mood was predicted by a network of single genes (i.e. complement). **Conclusions:** Gray and white matter is reduced in active pediatric CD, particularly in brain regions critical for cognition, emotion and pain processing, and autonomic control/immune regulation, and this reduction is not explained by medical therapies. Importantly, we found inflammatory gene transcriptional signatures that correlate with reduced brain structures, neuroinflammation, and poorer behavioral function during active disease. This study provides first insights in immune mechanisms underlying brain manifestations in pediatric CD with implications for development.

Brain ageing: impact of immunity and infection

39 - Age-related changes in the central nervous system and peripheral immune system in experimental autoimmune encephalomyelitis

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Background: The debut of multiple sclerosis (MS) at an older age is associated with increased risk of presenting a primary progressive form, earlier conversion to the secondary progressive form and greater disability accumulation. These facts could be due to the impact of immunosenescence in elderly MS patients. Objectives: To study the impact of ageing on clinical course, on the central nervous system (CNS) and on the peripheral immune system in experimental autoimmune encephalomyelitis (EAE).

Methods: 8-week-old (young) and 40-week-old (aged) C57BL/6J RccHsd mice were immunised with myelin oligodendrocyte glycoprotein (MOG)35-55 and EAE clinical follow-up was performed daily. Spinal cord and spleen from non-immunized mice as well as EAE mice at inflammatory phase (14 days post-immunization; dpi) and chronic phase (28 dpi) were collected. In spinal cord, immunofluorescence stainings were performed to evaluate histopathology and real-time PCR was performed to determine gene expression. In splenocytes, immune cell subsets and intracellular cytokines were analysed by flow cytometry, polyclonal and MOG-specific proliferative capacity were assessed by thymidine incorporation assay and cytokines from MOG-stimulated splenocyte supernatants were measured by Luminex assay. Statistical analysis was performed to compare young and aged mice at each time point and their evolution pattern along the disease. Results: Aged mice showed a more severe EAE clinical course compared to young mice. An increase in inflammatory infiltration, axonal damage and demyelination was observed in the CNS of aged mice at 28 dpi, as well as higher gene expression of IL-1b, reactive astroglia, reactive and M2 microglia/macrophage markers and lower expression of neuronal recovery marker. Regarding the peripheral immune response, we found age-related changes in adaptive immunity, such as in the stages of differentiation and exhaustion of T cells, in regulatory T cells and in cytokine-producing T cells, while few changes were observed in B cell subsets. In innate immunity, we found age-related changes in the stages of NK maturation. Furthermore, splenocytes from aged mice showed a decreased polyclonal proliferative capacity at 28 dpi and an increased production of cytokines in supernatants when stimulated with MOG at 14 dpi.

Conclusions: Altogether, our data indicate that ageing has an impact on the CNS and the peripheral immune system, suggesting that age influences EAE immunopathogenesis.

Disclosures: MD, HE, LC-B, MC and CE declare no competing financial interests. LMV has received speaking honoraria or participated in advisory boards from Biogen, Celgene, Sanofi-Genzyme, Merck, Novartis, and Roche. XM received speaking honoraria and travel expenses for scientific meetings, has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past 3 years with Actelion, Alexion, Bayer, Biogen, Celgene, EMD Serono, Genzyme, Immunic, Medday, Merck, Mylan, Nervgen, Novartis, Roche, Sanofi-Genzyme, Teva Pharmaceutical, TG Therapeutics, Excemed, MSIF and NMSS.
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43 - Neuroimmunological Consequences of COVID-19 in Older African Americans and Their Risk for Alzheimer's Disease.

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Alzheimer's Disease (AD), like COVID-19, involves disruption to the immune system and has a disproportionate impact on African Americans. Both diseases are also known to damage the hippocampus, a key structure within the brain responsible for encoding and storing new information. Noteworthily, AD and COVID-19 share several risk factors and comorbidities, such as age, gender, hypertension, diabetes, and APOE ε4 expression. Such evidence may explain the mutualistic relationship between AD and COVID-19. In this study, 30 older African Americans (ages 60 and above), including 15 who are COVID-19 survivors, and 15 that were not infected with SARS-CoV-2, underwent immunological, cognitive, and neural assessments to examine if infection by SARS-CoV-2, the causative agent for COVID-19, further accelerates CD8+ T-cell senescence, the aging of the immune system, thereby increasing risk for and progression of AD. A deeper understanding of the linkages between COVID-19 and AD may result in both better treatments for the long-term neurological consequences of COVID-19 as well as advances in the field of AD and related dementias.

Keywords: Alzheimer’s Disease, COVID-19, SARS-CoV-2, neuroinflammation, African American, Neurodegeneration, T-cells, cellular senescence, aging, Neuroimmunology, high resolution fMRI, medial temporal lobe.

107 - Klotho Expression in Aging & Disease

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Klotho is a regulatory anti-aging protein that is significantly expressed in the choroid plexus, plays an important role in regulating the immune response in the CNS, and diminishes with age. Klotho deficiency is characterized by premature inflammaging phenotypes, including neurocognitive deficits. The choroid plexus is a reservoir for HIV, and as HIV infection can cause HIV-associated neurocognitive disorders—an inflammaging phenotype, we hypothesized that klotho may play a role in the development of this neurocognitive deficit. In this project, we sought to characterize patterns of age-related klotho decline in primates as well as to determine if there was a correlation between klotho expression in the choroid plexus and HIV infection. Using formalin-fixed, paraffin-embedded sections of *Rhesus macaque* choroid plexus with and without SIV infection, we examined klotho expression using immunohistochemistry. Although klotho expression was varied in the uninfected *Rhesus macaques*, there was an overall decreasing trend with the advancement of age. In SIV-infected *Rhesus macaques*, the choroid plexus had significantly less klotho expression than the uninfected control group regardless of age (p=0.01). This significant decrease of klotho in SIV infected individuals could help to explain the development of HIV-associated neurocognitive disorders. However, the exact mechanism in which SIV diminishes klotho expression is unknown and additional studies are warranted to examine this phenomenon.

118 - Patterns of premature immunosenescence in multiple sclerosis patients

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It has been shown that aging significantly impairs development and progression of autoimmune diseases and neurodegenerative disorders. Immunosenescence has also been linked to multiple sclerosis (MS) as elderly patients show a decrease in disease activity and reduced efficacy of disease-modifying therapies.
Using multi-parameter flow cytometry, we evaluated age-dependent changes the peripheral blood and cerebrospinal fluid (CSF). Therefore, we performed a comprehensive immune phenotyping in age- and sex-matched relapsing-remitting MS (RRMS) patients and primary progressive MS (PPMS) patients in comparison to respective controls.
Our data show significant age-dependent alterations in the peripheral CD8 T cell compartment in healthy donors (HDs) and MS patients. Whereas HDs exhibited a severe age-related decline in the expression of the immunoregulatory molecules KLRG1, LAG3, and CTLA-4 on memory CD8 T cells, this age-related decline was completely abrogated in MS patients. Moreover, the expression of the costimulatory molecule CD226 (DNAM-1) on CD8 memory T cells from HDs was elevated with age. This age-related increase in CD226 expression was not present in MS patients. Of note, we identified a potential connection between premature immunosenescence and disease activity, as the CD226 expression on memory CD8 T cells correlated positively with disability scores in young MS patients (≤ 50 years). In addition, several senescence-associated T cell phenotypes correlated positively with serum neurofilament light chain levels in young MS patients, suggesting a possible link to clinical disease manifestation. In an independent cohort, we investigated immunological changes in the CSF. We observed an age-related decline of several immune cell subsets specifically in PPMS patients, indicating a differential age-dependent regulation of inflammation in the CSF.
Overall, our data suggest that aging in MS is associated with an imbalance between costimulatory and immunoregulatory signaling by CD8 memory T cells favoring a pattern of premature immunosenescence in young MS patients, potentially affecting disease activity.

125 - Modulation of neuroendocrinal and peripheral immunogical biomarkers by rehabilitation in sarcopenic subjects

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Sarcopenia is an aged-related condition characterized by loss of muscle mass and function, whose risk factors include, among others, inflammation and a complex imbalance of the neuroendocrine system. No pharmacological agents have been FDA approved for the treatment of sarcopenia, and the management of this disease is primarily focused on physical therapy for muscle strengthening and gait training. Because the crosstalk between the neuroendocrine and immune system is modulated by rehabilitation, the aim of this study was to verify the efficacy of the rehabilitation in reducing inflammation in sarcopenic patients.
Sixty sarcopenic patients undergoing a specifically-designed rehabilitation program, were enrolled in the study. At the time of recruitment (T0), and at the end of the rehabilitation program (30-days; T1) patients underwent a

comprehensive geriatric multidimensional evaluation that included lower extremity function evaluation with the Short Physical Performance Battery (SPPB), fall risk assessment evaluation (Tinetti score), and the evaluation of performance in activities of daily living (Barthel index), as well as the analysis of the plasmatic concentration of pro-inflammatory (IL-1β, TNFα, IL-6, IL-18) and anti-inflammatory cytokines (IL-10) and the quantification in serum of neurotransmitters noradrenalin, adrenalin, dopamine, and serotonin.
Rehabilitation resulted in a significant improvement of physical and cognitive conditions. This was accompanied by significantly increased concentrations of IL-10 and noradrenalin (p=0.02 and p= 0.016, respectively) that were positively correlated with the improvement in the scores of the Tinetti (p=0.02) and of the (SPPB) tests (p=0.004). IL-18 concentration was significantly reduced as well at T1 (p = 0.008), and this was negatively correlated with Barthel index (p= 0.0085) and SPPB (p= 0.05) test scores. Results herein show a correlation between the rehabilitation efficacy and the reduction of the inflammation, and identify the peripheral immunological and neuroendocrine biomarkers which are modulated by rehabilitation.

161 - P2X7 receptor, a promising therapeutic target in Alzheimer's disease

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The purinergic receptor P2X7 is activated by high amount of ATP. Damaged/dying cells and activated glial cells release ATP that acts as a danger signal. Our works showed that P2X7 is involved in pathological processes of various diseases of the central nervous system. The most studied function of P2X7 is activation of the NLRP3 inflammasome leading to the release of the proinflammatory cytokine IL-1β. In a mouse model of age-related macular degeneration, P2X7 was up-regulated, leading to increased production of IL-1β. Interestingly, activated P2X7-dependent pathways are different according to the pathological environment. We showed that in amyloid model of Alzheimer's disease, P2X7 play a critical role in chemokines release associated with altered neuronal functions. In addition, we showed that P2X7 also contributes to the cognitive deficits linked to the development of Tauopathy indicating that P2X7 antagonists might be ideal candidate drugs to treat Alzheimer's disease and Tauopathies.
In summary, P2X7 was involved in different physiopathological processes, highlighting its potential as a therapeutic target in pathologies of the central nervous system.

248 - Towards manipulating microglial subsets in humans: an approach to polarize microglia in a targeted fashion

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The dimension of microglial heterogeneity in human physiology and pathophysiology remains poorly explored. We recently generated a single-cell RNA-sequencing dataset consisting of 212.000 microglial transcriptomes of purified human microglia from a broad array of neurological diseases including Alzheimer's, Amyotrophic Lateral Sclerosis, Parkinson's disease, Multiple Sclerosis, Temporal Lobe Epilepsy and individuals with no or mild cognitive impairment, sampled from multiple brain regions. Hierarchical clustering identified 12 microglial clusters with specific subsets associated with antigen presentation, motility, and proliferation; we further report a central divide between microglial subsets based on metabolic identities (oxidative vs. heterocyclic), likely reflecting metabolic changes between homeostatic and immunologically activated microglial subsets. Using the Connectivity Map (CMap) resource, a comprehensive catalog of cellular signatures representing systematic genetic and pharmacologic perturbation, we identified transcription factors (TFs), RNA-binding proteins (RBPs) and compounds associated with cluster-specific gene signatures. Following validation using internal single-nucleus RNA sequencing data along with published bulk RNA-sequencing and proteomic datasets from human microglia, we are using overexpression and CRISPR/Cas9 genome editing to genetically perturb different *in vitro*

models of microglia in order to validate the identified regulators and to build models for studying our identified microglial subsets *in vitro*.
Comparison of cluster-specific gene signatures with the CMap resource also identified pharmacologic compounds associated with specific microglial subsets. The molecular signatures of our identified homeostatic-active clusters were associated with BRD-K39187410, an anti-amyloidogenic agent and the mTOR inhibitor Torin 2, while immunologically active clusters were associated with Camptothecin, a topoisomerase inhibitor. Preliminary gene expression data generated from a microglial cell line (HMC3 cells) treated with the identified compounds suggest the potential of these compounds to polarize microglia towards cluster-specific expression profiles. This will enable the functional characterization of human microglial subtypes.

286 - Neuroprotective effects of Ibudilast, a Phosphodiesterase IV inhibitor in mouse model of Parkinson's disease

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Background: Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting about 2% of the population over 65 years of age. Ibudilast (IBD), a inhibitor of the phosphodiesterase IV (PDE IV), has recently been shown to exert neuroprotective effects in an stroke and Alzheimer transgenic mouse model and in hypoxic-ischemic damage in the rat brain. It activates the cAMP-dependent protein kinase (PKA)/cAMP regulatory element-binding protein (CREB) signaling pathway and it inhibits inflammation.

Objectives: In the present study, we examined neuroprotective effects, if any, of IBD drug, a inhibitor of the phosphodiesterase IV in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD.

Methods: Experimental animal is muscular weighing 25–35 g of 3–4-month-old. The drug was given four times at 12 h intervals by gavation (10–50 mg/kg) in animals made parkinsonian following two doses of MPTP (30 mg/kg, i.p.) injection for 10 consecutive days. Control mice were injected with the same volume of pure DMSO. Brain was used for biochemical and histopathological study for glial cell-derived neurotrophic factor (GDNF). Evaluation concerned dopamine content in the striatum, tyrosine hydroxylase (TH) protein and α-synuclein, TNF-α, IL-6, and IL-1β expression measured in both control and treatment group. High-pressure liquid chromatography, Western blot analysis, and real time RT-PCR methods were applied.

Results: MPTP-induced striatal dopamine depletion was significantly attenuated by higher dose of IBD. MPTP-induced catalepsy and akinesia, as well as loss in swim ability, were blocked dose-dependently by IBD. These results indicate that the observed neuroprotective effects of IBD from its significant antioxidant and anti-neuro inflammatory action. Our study demonstrated that chronic administration of IBD attenuated astroglial reactivity and increased GDNF production in the striatum. Moreover, IBD reduced TNF-α, IL-6, and IL-1β expression. It also prevented TH protein decrease and increased α-synuclein level in treated rats.

Conclusions: Present data show a neuroprotective effect of the PDE IV specific inhibitor IBD against dopaminergic neuron degeneration, suggesting that PDE IV inhibitors might be a potential treatment for Parkinson's disease. Diminished inflammation and an increased level of GDNF may provide a better outcome in the later stages of neurodegeneration.

295 - Invivo and invitro effects of resveratrol in experimental models of Parkinson's disease

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BACKGROUND AND PURPOSE:

The MPTP-induced PD model is characterized by chronic inflammation, oxidative stress and loss of the dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). Resveratrol (RT) improves cognition and limits neuroinflammation in the brain. However, the beneficial effects of RT in ameliorating Parkinson's

disease (PD) remain unknown. Therefore, we aimed to elucidate the pharmacological Invivo and invitro effects and mechanisms of action of RT in experimental models of PD.

EXPERIMENTAL APPROACH:

We utilized SH-SY5Y cells exposed to RT (20μM) against 1-methyl-4-phenylpyridinium iodide (MPP+) as an in vitro PD model. Cell viability and apoptosis were analyzed via the MTT assay and flow cytometry. Mitochondrial morphology, apoptotic markers such as B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated X protein (Bax), mitochondrial respiratory capacity and ROS were measured by a mitochondrial tracker, a Seahorse analyzer and a MitoSOX-Red dye.

For in vivo PD model, behavioral tests, Nissl staining and immunohistochemistry were used to evaluate the protection of RT (50 mg/kg body weigh). The levels of tyrosine hydroxylase (TH), cAMP response element-binding protein (CREB) and cytokines levels (IL-1β, IL-6 and TNF-α) were analyzed by by Western blotting, RT-PCR and quantitative PCR analysis.

RESULTS:

RT decreased MPP+ -induced apoptosis in SH-SY5Y cells and human dopaminergic neurons. RT also increased mitochondrial respiratory capacity, decreased ROS production and restored mitochondrial morphology. RT reversed the MPP+ -induced reductions of phosphorylated CREB, PGC-1α, and TH, while the protective effects were blocked by the PKA inhibitor H-89 and via PGC-1α siRNA. In mice treated with MPTP, RT significantly improved motor functions. Importantly, RT prevented both dopaminergic neuronal loss and the reduction of phosphorylated CREB and glial activation, decreasing the levels of IL-1β, IL-6 and TNF-α, as well as their respective receptors in the SNpc of MPTP-treated mice.

CONCLUSION :

RT may be a promising neuroprotective agent for the treatment of neurodegenerative disorders such as Parkinson's disease. Results suggest that RT may play a neuroprotective role via modulating the mitochondrial-mediated signaling and CREB pathway in MPTP/MPP+-induced PD.

339 - Autophagy and progressive multiple sclerosis

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90 000 Canadians live with multiple sclerosis (MS): a neurodegenerative autoimmune disease characterized by demyelinating lesions in the central nervous system (CNS). “Relapsing-remitting” (RR-) is the most common form of MS and is aptly defined by symptom relapse followed by periods of remission. Within 10 years of disease onset, approximately half of RRMS patients transition to a secondary progressive (SP) phenotype in which symptoms no longer remit and disability accrues perpetually. Disease modifying therapies - which aim to prolong remissions - are available for RRMS patients; however, these treatments are ineffective in SPMS. SPMS patients are often left with limited treatment options, in part because the mechanisms behind the RR- to SPMS transition remain poorly understood. Currently, age is the only consistent predictor of progression. We speculate that processes underlying aging of the immune system and CNS may contribute to the development of progressive MS. One such process is autophagy: a molecular recycling pathway that degrades and clears exhausted organelles and debris. Autophagy dysfunction is thought to underlie normal aging. Intriguingly, autophagy also plays numerous roles in both innate and adaptive immunity. **We aimed to explore the role of autophagy in the development of SPMS.** To accomplish this, peripheral immune cells (PBMCs) from RR- and SPMS patients have been analyzed for autophagy markers via both western blot and flow cytometry – the level of which could reveal crucial differences in disease phenotypes. To date, we have found differential expression in several crucial autophagy markers, including ATG5 and LC3-II/I. To complement the human results, we have also used flow cytometry examine autophagy markers expressed by peripheral immune cells in a murine model of MS known as “1C6” experimental autoimmune encephalomyelitis (EAE). 1C6 EAE mirrors human MS: the mice display a relapsing-remitting disease course that gradually transitions to a progressive phase. Thus far, we have identified several specific cell types (e.g., CD4+ T cells, CD8+ T cells, CD11b+ macrophages, CD11c+ dendritic cells, B220+ B cells) with significantly altered autophagy activity in the progressive phase of 1C6 EAE (compared to naïve and relapse phases). Elucidating the

mechanism behind progression can provide the foundation for novel therapeutics and thus, prevent the immense personal, social, and economic burden that accompanies SPMS.

The sensory and autonomic nervous systems: links with inflammation

5 - Amla Attenuates Ischemic Injury Through Inhibition of Inflammatory Response by Modulating JAK2/ STAT3/SIRT1 in Rats

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Introduction: Excessive neuroinflammation plays detrimental role in brain injuries and is an important contributor to the pathogenesis of ischemic stroke. Amla has been reported to exhibit many pharmacological effects including alleviating brain injury.
Objective: This study was designed to evaluates the protective effect of amla fruit extract against ischemic stroke induced by cerebral ischemia-reperfusion (I/R) injury and explore its underlying mechanisms on ischemic stroke in rats.
Method: Cerebral I/R injury was induced by middle cerebral artery occlusion (MCAO) for 2 hours followed by 24 hours of reperfusion. Male SD rats were treated with amla fruit extract (orally) after 3 hrs of middle cerebral artery occlusion. Inflammatory cytokines (interleukin-5 and interleukin--6) in the serum were detected by ELISA. Moreover, terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay was done for determining the neuronal apoptosis and Western blot assay also performed for estimating the expression of several proteins.
Results: Amla significantly lowered serum levels of interleukin-5 and interleukin-6. The number of TUNEL positive cells was found to be lower in the amla treated group than in the MCAO group of rats. Furthermore, the present study also showed that amla attenuates altered expression of Janus kinase 2 (JAK-2), sirtuin-1 (SIRT-1) and signal transducer and activator of transcription 3 (STAT3) protein levels in comparison to MCAO group.
Conclusion: The study demonstrated amla's neuroprotective actions on cerebral ischemia by reducing inflammation mediated by STAT3/JAK2/SIRT1, and warrant further evaluation.

318 - A mannan-conjugated myelin antigen inhibits the induction of CNS autoimmunity by $CD11b^{+}Ly6C^{hi}$ monocytes

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Autoimmune demyelinating diseases of the central nervous system (CNS), such as multiple sclerosis (MS) are characterized by infiltration of T and B lymphocyte and myeloid cells, which cooperate to cause inflammation, demyelination and irreversible neuron damage in the white and grey matter of the brain and spinal cord. Recently attention has been given to the involvement of immature $CD11b^{+}$Ly6Chi myeloid progenitors in the development of experimental autoimmune encephalomyelitis (EAE), although their relevance for MS is not yet understood. We previously showed that MOG35-55 (MOG) conjugated to an oxidized form of the mannan polysaccharide (OM-MOG) prevents and treats EAE by inducing antigen-specific T cell anergy and a peripheral type 2 myeloid response. Here, we wished to investigate the possibility that $CD11b^{+}Ly6C^{high}Ly6G^{-}$ ($Ly6C^{hi}$) and $CD11b^{+}Ly6G^{+}Ly6C^{low}$ ($Ly6G^{+}$) myeloid precursors are involved in immune tolerance induced by OM-MOG. Using the chemotherapeutic drug gemcitabine, we depleted $Ly6C^{hi}$ and $Ly6G^{+}$ cells prior to or after the onset of EAE and examined the effects upon disease course and peripheral myeloid cell populations in OM-MOG-treated mice. We confirm previous studies that $Ly6C^{hi}$ myeloid cells are critical for the onset of EAE, and that immature $Ly6C^{hi}MHCII^{-}$ cells are transiently depleted from the periphery during the effector phase of disease. Contrary to expectations, we show that OM-MOG immune tolerance in EAE is not dependent upon gemcitabine-sensitive $Ly6C^{hi}$ and $Ly6G^{+}$ myeloid cells, but instead prevents and treats disease by selectively retaining $Ly6C^{hi}$ cells in the peripheral lymphoid organs and blood and by up-regulating their production of PD-L1, which in turn is a critical mediator of OM-MOG tolerance.

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Dialog between astrocytes and microglia in neuroimmunology

78 - FAILURE OF ALZHEIMER'S MOUSE BRAIN NEURAL PRECURSORS TO SUPPORT MICROGLIAL-MEDIATED AMYLOID BETA CLEARANCE

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Background: Microglia remove Amyloid beta (Aβ), but fail to reduce Aβ burden in Alzheimer's disease (AD). We previously demonstrated failure in immune-modulatory properties of resident AD brain neural precursor cells (NPC). We hypothesized that AD-brain NPC fail in supporting microglial-mediated Aβ clearance, enabling Aβ accumulation.

Methods: CD11b+ microglia were extracted from 7 months old 5xFAD mice and activated with LPS to mimic AD brain inflammatory environment. Microglial Aβ content was measured by image analysis. NPC were isolated from newborn wild type (*wt*) brain, and from transgenic mice expressing GFP under the Nestin promoter, crossed with 5xFAD and *wt* littermates.

Results: Double-staining for Iba1+ microglia and Aβ in 7-months old 5xFAD brain sections, and feeding freshly isolated CD11b+ brain cells with fluorescent-labeled Aβ, demonstrated a microglial subpopulation exhibiting high Aβ-phagocytic activity. LPS activation increased latex beads uptake but reduced the fraction of high Aβ-phagocytic microglia. Time-lapse microscopy showed that co-culturing with newborn NPC did not affect the total amount of Aβ uptake by microglia. Rather, NPC significantly increased the fraction of microglia with high Aβ-phagocytic activity. NPC-enriched Subventricular zone extracts, and freshly isolated NPC from *wt* 7-months old mice, induced significant increase in the fraction of microglia with high Aβ-phagocytic activity. SVZ extracts and freshly-isolated NPC from 5xFAD mice failed to increase high Aβ-phagocytic microglial fraction. Finally, *wt* NPC induced a mildly stronger effect than 5xFAD NPC on the degradation of phagocytosed-Aβ in microglia.

Conclusions: Resident AD brain NPC fail to support Aβ clearance by microglia, leading to accelerated disease pathogenesis.

99 - Antioxidant enzyme expression in the mouse spinal cord in multiple sclerosis models

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Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) defined by immune cell infiltration, demyelination and axonal injury, resulting in neurological impairments. MS pathophysiology has been studied for years, and oxidative stress has been found to have a crucial role in MS neurodegeneration. Studies using the experimental autoimmune encephalomyelitis (EAE) animal model of MS and neuroinflammation show activated immune cells can promote neurodegeneration via the release of free radicals such as reactive nitrogen/oxygen species. Moreover, we recently reported that oxidized phosphatidylcholines (OxPC) found in MS brain lesions kill human neurons and oligodendrocytes in vitro and promote neurodegeneration when injected into the spinal cords of mice. Together, these results suggest that oxidative stress is an essential mediator of neurodegeneration and that there is a need to understand how to protect the CNS from oxidative stress to develop new MS therapeutics. Given that microglia in the CNS has protective functions during injury and neurodegeneration, we hypothesize that the initial response of microglia following demyelination is a protective one and involves the upregulation of key endogenous antioxidant enzymes. To understand the mechanisms underlying the antioxidant response in the CNS, we aimed to compare the expression of Superoxidize dismutase-1 (SOD1), Heme Oxygenase-1 (HO-1), Peroxiredoxin-5 (PRDX5), and glutathione peroxidase-4 (GPX4) between multiple MS animal models, including spinal cord injections of OxPC and lysophosphatidylcholine (LPC) as well as

MOG induced EAE. Our results demonstrate that all four tested antioxidant enzymes were highly upregulated by CD68+ microglia/macrophages in the OxPC model. For the LPC model, SOD1, PRDX5 and GPX4 enzymes were upregulated by CD68+ microglia/macrophages. For the EAE model, the expression of SOD1, HO-1, and PRDX5 by CD68+ cells significantly increased inside the lesion. Together, these observations suggest that the upregulation of HO-1 and GPX4 by CD68+ microglia/macrophages may be a response against a specific stress stimulus, while SOD1 and PRDX5 seem to be the result of a general antioxidative response of microglia upon inflammation. Our work sheds light on the protective role of macrophages and microglia during oxidative injury and helps us better understand how the antioxidative response of microglia changes based on different danger signals.

138 - Exploring the role of the Astrocytic Succinate Receptor in Neuroinflammation

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Compelling evidence exists that metabolites can act as signalling molecules in both health and disease. Succinate, an intermediate metabolite of the tricarboxylic acid cycle, has been found to play a key signalling role in conditions of persistent neuroinflammation. Succinate accumulates in the cerebrospinal fluid (CSF) of mice with chronic experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS), and in secondary progressive MS (SPMS) patients. Extracellular succinate subsequently signals via autocrine and paracrine signalling through its cognate succinate receptor 1 (SUCNR1) to modulate inflammatory responses in the central nervous system (CNS).
However, while the role of SUCNR1 in myeloid cells (e.g., microglia) is currently under active investigation, how the succinate-SUCNR1 signalling axis functions in astrocytes, and to what extent it contributes to neuroinflammation, is still unknown.
We have first used neural stem cells (NSCs) obtained from SUCNR1 knock-out ($SUCNR1^{KO}$) and wild type ($SUCNR1^{WT}$) mice to obtain $SUCNR1^{KO}$ and $SUCNR1^{WT}$astrocytes. We found that $SUCNR1^{KO}$ astrocytes had a dysfunctional phenotype characterised by the decreased expression of canonical astrocyte markers, decreased metabolic capacity, and a decreased response to pro-inflammatory stimuli.
Next, we used a new inducible system that directly reprograms human fibroblasts into induced neural stem cells (iNSCs) to obtain patient specific human (h)iNSCs from SPMS patients and age-matched controls. These lines have been transduced with an inducible CRISPR-Cas9 construct to prevent the expression of *SUCNR1* ($SUCNR1^{iKO}$) under specific conditions *in vitro*. The $SUCNR1^{iKO}$ hiNSCs lines were then differentiated into astrocytes to obtain a 2D *in vitro* model system. Preliminary data show successful integration of the construct in $SUCNR1^{iKO}$ hiNSCs, which will be then used to thoroughly characterise the role of the succinate-SUCNR1 signalling axis in human astrocytes
Our results describe for the first time a novel role for SUCNR1 in mouse astrocytes, which lose their ability to respond to pro-inflammatory conditions *in vitro* and show decreased metabolism after knockout. Further, we provide the basis for a new *in vitro* model that can be used to uncover how human astrocytes lacking SUCNR1 may function and how this can affect their responses to inflammatory conditions.

251 - Targeting microglial miR-155 enhances microglia response to neurodegeneration and improves cognitive functions in Alzheimer's disease model

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Microglia are the resident immune cells in the CNS that regulate brain development, maintenance of the neuronal network, and neurodegenerative disease progression. Our group identified homeostatic (M0) and neurodegenerative microglia (MGnD), also referred to as disease-associated microglia (DAM), that are regulated by the reciprocal suppression of TGFb and induction of APOE signaling in multiple neurodegenerative disease models. Understanding whether the MGnD phenotype is beneficial or detrimental in Alzheimer's disease (AD) progression is currently one of the major questions for therapeutic approaches in AD. miR-155 is a pro-inflammatory miRNA that modulates the inflammatory responses in innate immunity. However, its role in AD pathogenesis remains unknown. We found that conditional ablation of microglial miR-155 at 1.5 months of age in APP/PS1 mice significantly increased the expression of MGnD genes, including *Apoe, Clec7a*, and *Spp1*, enhanced interferon signaling, and suppressed inflammatory signaling, including TNF-a and NF-kB in 4-month-old APP/PS1 mice. Moreover, using immunohistochemistry and proteomics, we found the induction of the MGnD microglial phenotype was correlated with increased amyloid plaque compaction, reduced neuritic dystrophy, and enhanced microglial phagocytosis and synaptogenesis. Conditional ablation of microglial miR-155 in APP/PS1 mice at 1.5 months of age improved behavioral cognitive performance based on Y maze and fear conditioning at 8 months of age. These findings support the beneficial role of the MGnD microglia phenotype in chronic neurodegeneration and serve as the basis for the therapeutic strategy to induce MGnD-microglia for the treatment of AD.

343 - Knocking out IKKβ selectively from CNS microglia results in earlier onset of EAE

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The Nuclear Factor-κB (NF-κB) is a transcription factor ubiquitously expressed by all cells and with various functions in health and disease. It has pivotal roles in immunity and has been shown to be involved in the pathology of many autoimmune diseases, including Multiple Sclerosis (MS). Its functions can be either beneficial or detrimental, depending on the cell type and the disease context. For example, in Experimental Autoimmune Encephalomyelitis (EAE), a model of the autoimmune neuroinflammatory part of MS in mice, a conditional depletion of IKKβ kinase, a key component of NF-κB activation, from total myeloid cells results in disease amelioration. However, the specific function of IKKβ in different subpopulations of myeloid cells, and particularly in CNS resident myeloid cells microglia, in neuroinflammation and demyelination remains unknown and is the aim of our study. We used a genetic approach to specifically deplete IKKβ in microglia in an inducible manner, under the control of the myeloid Cx3cr1 promoter. IKKβF/F were crossed with mice expressing the tamoxifen-inducible Cx3cr1-Cre recombinase to produce iCx3cr1- Cre_IKKβF/F (KO) mice. FACS analysis revealed that the gene recombination was maintained 28 days after tamoxifen in the self-renewing microglia of these mice only and not in peritoneal macrophages, which were rapidly replaced by non-recombined bone marrow progenitors. Consequently, to examine the role of microglial IKKβ in neuroinflammation, we induced EAE at 28 days after tamoxifen in KO and control mice by immunizing them with MOG peptide 35-55. We found that knocking out microglial IKKβ resulted in significant earlier onset of EAE, paralleled by earlier demyelination and faster immune infiltration in the spinal cord. Immune analysis revealed no differences between the two groups in T cell activation in the periphery at the onset. Spinal cord microglia in the KO group expressed higher levels of MHC Class II at a preclinical stage (dpi8), possibly reflecting a different level of activation. To further examine the role of microglial IKKβ in demyelination we used the cuprizone model and found no differences between the two groups at any experimental time point tested. Collectively, our data show that microglial IKKβ has an important regulatory role in autoimmnune neuroinflammation, the mechanism of which is currently under investigation, but not in toxin induced demyelination or in remyelination. Supported by the Greek General Secretariat for Research and Technology (GSRT) and the Hellenic Foundation for Research & Innovation (H.F.R.I) grant (Act 1156).

Diversity of brain myeloid cells

109 - Convergence of Alzheimer's Disease Risk Variants on TREM1 Surface Expression

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Late-onset Alzheimer's disease (LOAD) is an age-related neurodegenerative disease characterized by cognitive decline and accumulation of amyloid pathology. Several LOAD risk factors have been identified that are highly expressed in innate immune cells, including CD33, TREM2, TREM1, and SPI1. Our group and others have found that the CD33, TREM1 and SPI1 risk alleles are associated with cis or trans effects leading to alterations of TREM1 and TREM2 expression, such that decreased TREM1 expression and a decrease in the TREM1 to TREM2 ratio are associated with LOAD risk. To clarify the effect of these risk alleles on TREM1 expression in the context of pathology, monocytes isolated from individuals with risk alleles for SPI1 (rs1057233), CD33 (rs3865444), or TREM1(rs6910730) were exposed to amyloid beta (AB). In individuals with the $CD33^{AA}$ protective allele, AB stress induced TREM1 expression while no change was observed in those with the $CD33^{CC}$ risk allele. $CD33^{CC}$ monocytes also demonstrated impaired AB uptake. However, when $CD33^{CC}$ monocytes were treated with a TREM1 agonistic antibody following AB stress, AB uptake was rescued and apoptosis was decreased. Similarly, TREM1 knockdown in $CD33^{AA}$ microglia-like cells resulted in increased proapoptotic BAX expression. These results demonstrate the protective effects of TREM1 in the context of AB stress. SPI1 encodes the transcription factor PU.1, a negative regulator of TREM1. While the SPI1 risk allele is known to increase PU.1 expression, the SPI1 protective allele increased TREM1 expression in response to AB stress, as did the TREM1 protective allele. These findings demonstrate that the genetic outcomes of the CD33, SPI1, and TREM1 risk alleles converge to reduce TREM1 protein expression. While these results clarify the protective effect of TREM1 in LOAD, its mechanism in preventing pathogenesis remains unknown. Ongoing work aims to understand how the role of TREM1 as an amplifier of inflammatory signals may be protective. Stimulation of innate immune receptors by viral or bacterial antigens has been shown to induce TREM1 expression in macrophages, and TREM1 in turn amplifies the inflammatory response. This synergistic activity may mediate improved phagocytosis of AB and prime microglia to respond to their environment in LOAD.

151 - Dynamic response and role of perivascular macrophages in Parkinson's disease pathogenesis

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Parkinson's disease (PD) is a movement disorder characterized by the loss of dopaminergic neurons (DN) in the substantia nigra (SN) and the presence of intraneuronal inclusions enriched in alpha-synuclein (alpha-syn), a protein believed to have prion-like propagation/aggregation properties. While mechanisms underlying the progression of neurodegeneration remain poorly understood, accumulating evidence suggests that pathological neuro-immune interactions could play a critical role in PD pathogenesis. In particular, microglial activation and T-cells are found in the SN of PD patients. The presence of circulating cells within the diseased brain may indicate alterations of the blood-brain barrier (BBB) whose structural and functional properties are tightly controlled by perivascular elements such as pericytes and perivascular macrophages (MPVs). The importance of MPVs in CNS pathomechanisms is a recent concept that has notably found echo in proteinopathies such as Alzheimer's disease. However, their response and pathogenic role in PD have never been investigated so far. Using a mouse model of degenerative synucleinopathy we showed that the number of PVMs dynamically increases within the SN during the course of neurodegeneration. We further demonstrated that local cell proliferation accounted for this PVM recruitment with no involvement of peripheral immune cell engraftment. Importantly, similar increase in PVM density was observed in the *postmortem* brain of PD patients. The specific ablation of PVMs aggravates the demise of DN in our animal model, revealing their neuroprotective potential. This feature is very likely associated to their

immunomodulatory properties and their ability to clear toxic molecules from the perivascular space. Thus, in the absence of PVMs, enhanced neurodegeneration correlates with an increased extravasation of T-cells that prove to be deleterious as shown in T-cell deficient mice. Furthermore, we found that compared to microglial cells, PVMs are much more efficient in phagocytosing α-syn aggregates and that *in vivo* PVM depletion dramatically increases synucleinopathy spreading in mice injected with toxic α-syn assemblies. Overall, our results highlight the importance of PVMs in regulating the toxicity and spreading of synucleinopathy and suggest that immunotherapeutic approaches in PD could be oriented selectively to maintain or even boost the beneficial functions of immune cells like PVMs.

352 - Xenon gas treatment to restore microglial functions in Alzheimer's disease

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder affecting more than 30 million people in worldwide. Protein aggregation plays a key role in brain dysfunction in AD. This aggregation occurs because of changes in the innate immune system that occur with aging. Emerging evidence shows that dysregulation of microglia plays a significant role in the onset and progression of AD. Our group recently identified a microglia transition between homeostatic (**M0**) to neurodegenerative (**MGnD**) microglia signature, also referred to as disease associated microglia (DAM), that are present in acute and chronic neurodegeneration diseases, including multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and AD. However, the contribution of MGnD phenotype to disease progression and how to restore the homeostatic microglia functions to treat AD still an open question. Recent studies showed that Xenon (**Xe**) gas treatment has a neuroprotective role. Xe is currently used in human patients as an anesthetic and as a neuroprotectant in treatment of brain injuries. Xe penetrates blood brain barrier, which can make it effective therapeutic. Thus, we hypothesize that Xe-gas treatment has a protective immunomodulatory role to restore microglia functional phenotype in neurodegenerative diseases and can serve as a novel treatment for AD. We discovered that Xe-treatment (70%) via inhalation modulates microglia phenotype switch from MGnD-neurodegenerative to M0-homeostatic associated with reduction in Ab-plaque pathology and neuroinflammation in APP-PS1 mice. Interesting, Xe-gas treatment directly also affect the peripheral immune response with an increase in "wound healing" signature in monocytes and Neutrophils from spleen. Moreover, Multiple treatments with Xenon suppress circulating neurodegenerative neutrophils from APOE e4 mice and decrease proinflammatory response from neutrophils in the brain of APP/PS1 mice after 2 months of treatment. Together, scRNA sequence analyses show that microglia remain in intermediate state during disease progression with a decrease in chemokine response. In conclusion, these data demonstrated that Xe-gas treatment modulates microglial and neutrophils phenotype which pushes the balance towards repair. These provides evidence that Xe-gas treatment directly induces microglia protective functions and reduces Aβ load to treat AD.

Gut microbiota and CNS inflammation

60 - Assessing the Ability of Bacterial Cell Wall Fragments Derived from Gut Inducing a Brain Immune Response

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It is recognized that the gut microbiota influences the development and function of the brain through molecules such as short chain fatty acids that can directly cross the blood-brain barrier; however, it is unknown if bacterial cell wall fragments possess this similar ability to access the brain and influence its function. We seek to investigate if such a mechanism exists and contributes to altered brain health. After injecting Sprague Dawley rats via an intraperitoneal injection with alkyne MDP, a synthetic bacterial cell wall fragment with a "clickable" handle at different concentrations (5 mg/kg or 49 mg/kg) of MDP and time points (2, 4, or 24 hours), tissues relevant in

assessing an activated immune response were collected. The RNA from these tissues were then extracted, converted into cDNA, and then submitted to real-time PCR to quantify the levels of gene expression under these different conditions. Furthermore, tissue sections from these rats were prepared using a cryostat and subsequently subjected to immunohistochemistry and, an azide-alkyne click reaction to determine if the synthetic fragment was present in the brain. The results show that MDP can upregulate pro-inflammatory cytokines such as IL6 and IL1B gene expression in the brain. This response is also seen in other regions of the body such as in the spleen, although the induction of these genes of interest occurred at a lower fold. In addition, innate immune receptors NOD2 and TLR4 experience levels of fluctuation across the time point and concentration treatments. Altogether this suggests that the cell wall fragment bacterial peptidoglycan can elicit an immune-activated response.

77 - SYSTEMIC MICROBIAL AGENTS INDUCE NEURODEGENERATION IN ALZHEIMER`S DISEASE MICE

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Objectives: Neurodegeneration is considered the consequence of misfolded proteins' deposition. Little is known about external environmental effects on neuro-degeneration. Infectious agents -derived Pathogen-associated molecular patterns activate microglia, key players in neurodegenerative diseases. We hypothesized that systemic microbial pathogens may accelerate neurodegeneration in Alzheimer's disease through microglial activation.
Methods: We examined the effects of infectious environment and microbial Toll-like receptor agonists on cortical neuronal loss and on microglial phenotype in wild type versus 5xFAD-transgenic mice.
Results: We first examined the effect of a naturally bred environment on the neurodegenerative process. Accelerated cortical neuron loss occurred in 5xFAD mice grown in a natural ("dirty") environment in comparison to SPF environment. Environmental exposure had no effect on cortical neuron density in wt mice. To model the neurodegenerative process caused by the infectious environment, we injected systemically the bacterial endotoxin lipopolysaccharide. LPS caused cortical neuronal death in 5xFAD, but not wt mice. We then used the selective retinoic acid receptor alpha agonist, Am580, to regulate microglial activation. In primary microglia isolated from 5xFAD mice, Am580 markedly attenuated iNOS expression, without canceling their basic immune response. Intracerebroventricular delivery of Am580 in 5xFAD mice reduced significantly the fraction of (neurotoxic) iNOS+ microglia and increased the fraction of (neuroprotective) TREM2+ microglia. Furthermore, intracerebroventricular delivery of Am580 prevented neurodegeneration induced by microbial TLR-agonists.
Conclusion: Exposure to systemic infections causes neurodegeneration in brains displaying amyloid pathology. AD brains exhibit increased susceptibility to microbial TLR neurotoxicity, which accelerates neuronal death. Microglial modulation protects the brain from microbial TLR-induced neurodegeneration.

134 - Dynamics and functional changes of colonic intraepithelial lymphocytes and innate lymphoid cells upon CNS autoimmunity

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Multiple Sclerosis (MS) is a $CD4^+$ T-cell-mediated autoimmune disorder of the central nervous system (CNS). Although an association between MS and inflammatory bowel diseases has been observed, the relationship between gut inflammation and neuroinflammation is not fully understood. Recent evidence demonstrated that encephalitogenic Th17 cells infiltrate colonic lamina propria prior motor impairment in experimental autoimmune encephalomyelitis (EAE), the murine model of MS. Here we aimed to study the dynamics of gut mucosa lymphocytes during EAE development.
Our data shows that colonic intraepithelial lymphocytes (IEL) composition changes during the time-course of EAE development. Specifically, $TCR\alpha\beta^+CD4^+$ and $TCR\alpha\beta^+CD8\alpha\beta^+$ frequencies are increased, and IFNγ production is augmented in EAE mice compared to healthy control. Moreover, natural $TCR\alpha\beta^+CD8\alpha\alpha^+$ IELs, a population with

suppressive potential, is reduced upon EAE development. These changes were accompanied by a decreased expression of the short-chain fatty acid receptor GPR43 in colonic TCRαβ^{+}CD8αα^{+} IELs and a reduced content of propionate (C3) in EAE mice compared to controls, suggesting an important role of gut microbiota composition in IELs function. Accordingly, i*n vitro* experiments shows that GPR43 stimulation in this population is necessary for IL-10 production and PD-1 expression. The recruitment of these pro-inflammatory IELs into the gut mucosa and the posterior migration to the CNS appears to be mediated by the kinetics expression the chemokine receptor CXCR3.

Together with the accumulation of the pro-inflammatory IELs in the colonic mucosa at maximum disease severity, there are increased numbers of innate lymphoid cells (ILCs). ILCs are primarily tissue resident cells and are particularly abundant at the mucosal surfaces. However, our results indicates that ILCs not only are present in the CNS of healthy mice at low numbers but are increased upon EAE development and produced higher amounts of IL-17.

Thus, our data shows that both innate and adaptive lymphocytes distribution are altered in colonic gut mucosa during autoimmune neuroinflammation and suggests that changes in the gut environment are relevant in the development of CNS autoimmunity. Currently, we are investigating how an autoimmune attack inside the CNS may lead to IELs and ILCs alteration in the intestine and the implication of this findings in neuroinflammation.

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172 - Sex-specific impact of methionine cycle manipulation on disease progression, T cell and gut microbiota in EAE

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Introduction: Multiple sclerosis (MS) is an inflammatory and demyelinating disease of the central nervous system (CNS) with a sex bias towards women. Proinflammatory TH1 and TH17 cells are considered pathogenic in MS and its animal model, experimental autoimmune encephalomyelitis (EAE). Obesity, western diet and gut dysbiosis increase inflammation whereas dietary methionine restriction (MR) is associated with lower weight gain and reduced inflammation. T cells upregulates components of methionine metabolism upon activation and MR reduces the expansion of TH17 cells in vitro. Therefore, we hypothesize that limiting the activity of the methionine cycle will improve EAE by modulating TH17 cells through sex-specific epigenetic mechanisms and modification of the gut microbiota. Methods: Active EAE is induced by immunization with MOG35-55 in male and female C57BL/6 mice exposed to MR or control diet. Clinical scores, flow cytometry and 16S rRNA sequencing are used to characterize the properties of immune cells and the gut microbiota. Immunofluorescence and confocal microscopy are used to characterize the blood-brain barrier, demyelination, neural damage and glial activation in the CNS. Results: Our preliminary results show that dietary MR is associated with a significantly delayed onset of neurological symptoms, with clinical differences in disease evolution between male and female. This is associated with a reduced number of immune cells and pathogenic T cells in the spleen and CNS respectively at presymptomatic, and at onset and peak stages. MR diet is associated with modification of the gut microbiota suggesting a shift towards an anti-inflammatory profile. Conclusions: MR ameliorates the clinical course and neuroinflammatory processes in EAE in a sex-dependent manner, and could represent a new therapeutic avenue to improve MS.

236 - Gut microbiota regulates CNS inflammation in experimental autoimmune encephalomyelitis through microRNA-21

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Background

Involvement of microbiota has been correlated in the pathogenesis of several auto immune disorder including multiple sclerosis (MS). Metabolites derived from gut microbiome are known to influence the intestinal immune environment. Even though, there is no clear mechanistic reason for the initiation of inflammatory reactions by gut residing microbiome, leading to CNS inflammation. Here, we explored that in experimental autoimmune encephalomyelitis (EAE) gut residing gram negative bacteria can directly initiate the inflammatory response through microRNAs. These results showed the relationship between circulating inflammatory miRNA and gut microbiota in EAE pathogenesis.

Objectives

- To elucidate the candidate miRNA and microbiome involved in the pathogenesis of MS, using EAE model.
- To know the role of gut microbiome in generation of circulating exosomes and miRNA.

Methods

We generated gut microbiome dysbiosis model mice by oral gavage of non-absorbing antibiotics cocktail. The model mice were subjected to experimental autoimmune encephalomyelitis (EAE) by injecting MOG_{35-55} peptide in CFA. MOG $tetramer_{35-55}$ reactive $CD4^+$ T cells (%) were evaluated from the lymphocytes isolated from the SI, spleen and CNS. Cell-free and exosomal miRNA from Plasma/Serum of MS patients and EAE mice were isolated and expression analysis were performed. Microbial abundances were analysed with 16S V3–V4 amplicon sequencing. Epithelial and endothelial cells were used for miRNA transfection analysis.

Results

Dysbiosis of gut microbiome is shown to ameliorate signs of EAE, along with a notable reduction in the expansion of MOG $tetramer_{35-55}$ reactive $CD4^+$ T cells frequency in the SI leading to the decreased number of T cells migration to the CNS. We also revealed substantial increase in the circulating and exosomal expression of miR-21a-5p both in EAE and MS patients. But miR-21a-5p was significantly low in dysbiosis EAE mice and healthy samples. Fecal microbiome analysis showed increased gram-negative strains in EAE mice. Further *in vitro* experiments with gut epithelial cells showed that LPS stimulation is enough to induce the secretion of miR-21a-5p. Transfection with miR-21a-5p mimics to the naïve T cells express increased inflammatory cytokines IL17 and GM-CSF. Notably, these miRNAs has a direct role in expands MOG+ pathogenic T cells in EAE.

Conclusion

The results indicate that gut microbiome would significantly influence the T cell pathogenicity through miRNA-21-5p in EAE.

245 - Oral microbiome characterization in multiple sclerosis patients

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351 - Effect of the aging gut microbiome on CNS inflammation in EAE

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Multiple sclerosis (MS) is an autoinflammatory disorder of the central nervous system (CNS), wherein aberrant immune activation results in stripping of myelin and axonal damage. The risk of developing MS and the trajectory of disease progression are closely linked with environmental factors and age, respectively. The gut microbiome of MS patients differs from that of healthy individuals, and gut microbiota from MS patients has been shown to promote disease when transplanted into mouse models. Moreover, the gut microbiome has been shown to play an important role in shaping the phenotype of microglial cells which are themselves dysregulated in MS. I

hypothesize that an aged gut microbiome in MS patients may induce microglia activation, thereby promoting disease progression.
The fecal microbiota transplant (FMT) model allows the introduction of human fecal bacterial populations into antibiotic-treated or germ-free mice. We recruited pairs of sex-matched, same-household subjects with an age difference of 30+ years for stool sample donation. Demyelination is subsequently induced by adoptive transfer of encephalitogenic T cells from proteolipid protein (139-151)-immunized donor mice. Pathology in the CNS is then examined using flow cytometry, histology, and immunofluorescence.
Depending on the donor pair, FMT from healthy young or aged individuals into young mice can alter EAE with aged FMT from some donors inducing more severe EAE. Immunological readouts reflect disease severity, where FMT recipient mice with worse disease have pronounced $Iba1^+$ cell accumulation in the CNS, near sites of meningeal lymphoid aggregation.
Young and aged healthy controls were recruited for this pilot study. Although some pairs exhibited a difference in the clinical and pathological presentation of EAE comparison between pairs showed variable effects, This variability is expected given that we are not pre-selecting subjects based on environmental parameters such as diet or disease. Our next steps will be to investigate how MS patient-derived fecal microbiota impact EAE, using samples obtained by the International MS Microbiome Study. Samples from age-matched relapsing-remitting (progression-resistant) and secondary progressive MS patients, as well as their respective household controls will be compared to determine how gut microbiota influences MS progression. This work may provide a novel avenue for developing a therapeutic approach for targeting disease progression.

367 - Exploring neuromotor tests and microbiome biomarkers to enhance developmental screening in preterm infants: Preliminary analysis.

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Background: The early developmental window of the gut microbiome occurs in parallel with the nervous system development, which may be influenced by early life exposure including variation in gut microbiota colonization. Emerging evidence are now highlighting how gut microbiota signatures are distinct between term and preterm infants. Therefore, this study aims to explore if gut microbiota colonization, as an early biomarker, is associated with neurodevelopmental delay at term-corrected age in infants born prematurely.
Methods: Fecal samples are collected at birth (<7 days) and at term-equivalent corrected age from a longitudinal observational cohort of preterm infants (recruitment at 29 to 36 weeks of gestation). Genomic DNA was extracted from each sample followed by amplification and sequencing of V3-V4 region from 16S RNA. Taxonomy abundance tables were then generated from the FASTQ data obtained, using QIIME2 (v2020.2) for further analysis. At term-corrected age, two neuromotor examination were performed to evaluate neurodevelopmental outcomes: The Amiel-Tison Test (ENTAT) and the Global Motor Assessment (GMA).
Results: PCOA cluster analysis allowed to delineate abnormal neurological assessment at term-corrected age using ENTAT examination alone or in combination with GMA. Differential abundance analyses showed a significant higher prevalence of opportunistic pathogens at birth and at term-corrected age in the abnormal neurological exam group. At birth, microbiota showed a higher prevalence of *Enterococcus sp*. in the abnormal neurological examination group, with a log fold of 2,31 *(*P*=0.02). Interestingly, few preterm infants with an abnormal neurological exam showed a severely high proportion of *Escherichia Shigella* within their microbiota, which was not observed in infants with a normal neurological examination.
Summary: Opportunistic pathogen levels are higher at term-corrected age in infants presenting an abnormal neurological exam, while their commensal bacteria decrease. The microbiota diversity at term corrected age is also diminished in infants presenting an abnormal neurological examination compared to those without neurodevelopmental delays.

Neuroimmunology of the eye and the optic nerve

250 - Intraocular administration of adeno-associated virus results in systemic and local immune response in non-human primates

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The clinical success of Adeno Associated Virus (AAV)-mediated gene therapy for a retinal degenerative disorder (Leber's Congenital Amarousis) has paved the way for development and testing of a new stream of therapies in this domain. One of the reasons for this success is attributed to a combination of the 'low immunogenicity' of AAVs and the 'immune privilege' of the eye. However, several studies and results from clinical trials have brought both these assumptions into question. As of today, there has not been any evidence correlating ocular immune responses or inflammation to antibodies in serum. So, we aimed to systematically evaluate anti-AAV antibodies in serums and examine clinical signs of ocular inflammation in 41 NHPs that had received intraocular injections with the different AAV serotypes. The total antibodies present in the serum that can bind specifically to the AAV capsid are known as Binding Antibodies (BABs), and a subset of these called Neutralizing Antibodies (NABs) can render the AAV inactive. We tested the levels of BABs and NABs against commonly used AAV serotypes (AAV2, AAV5, AAV8 and AAV9) in the serums collected from NHPs. We observed significantly higher pre-existing serum BABs against AAV8 and AAV9 compared to other serotypes. Analysis of both BABs and NABs in the serums collected post-injection revealed a dose-dependent increase, irrespective of the serotype or the mode of injection. Lastly, we were able to demonstrate a co-relation between the serum BAB levels with clinical grading of inflammation and levels of transgene expression. Our results indicate that testing serum antibodies can serve as an early and relatively non-invasive method for detecting ocular inflammation post gene therapy.

Immunology in neuro-oncology

2 - Target discovery in Glioblastoma multiforme: Immunomodulation of the Hypoxic Tumor Microenvironment

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Target discovery in Glioblastoma multiforme: immunomodulation of the Hypoxic Tumor Microenvironment
Glioblastoma multiforme (GBM) is a tumor of the central nervous system, to this date it is the most aggressive primary brain tumor in adults and it remains mainly incurable. Currently, there are several treatments available including surgery and adjuvant chemotherapy, however these invasive therapies, in addition to the aggressiveness of the tumor, determine a low survival rate in patients. Incidence increases with age and males are more often affected. Beyond rare instances of genetic predisposition and irradiation exposure, there are no known glioblastoma risk factors. The World Health Organization classifies Glioblastoma as primary and secondary GBM. Primary glioblastoma develops *de novo* while the term "secondary glioblastomas" refers to forms of glioblastomas that evolve from recurrent forms of grade II or grade III astrocytomas.
As other form of solid tumors Glioblastoma goes undetected from the immune system due to the establishment of an immunosuppressive environment. The induced immunosuppression caused mainly by GBM stem-like cells (GSCs), or tumor-initiating cells that are thought to be responsible for tumor maintenance, progression, recurrence, and resistance to therapy causing a major dilemma for immunotherapy and new drugs development. GSCs cells reside in multiple sites located inside the tumor mass characterized by hypoxia, acidic stress, and/or glucose restriction. Hypoxia has been shown to promote a stem-like state in tumor cells by activating pro-migratory and pro-invasive factors. Therefore, modulating its mechanisms could contribute to develop therapeutic agents capable of targeting GSCs stemness.

With this work we intend to find new therapeutic target on the tumor cells by also modulating the immunosuppression state of the tumor microenvironment caused by the hypoxic response. The possibility to find new therapeutic antibodies targeted against specific tumoral antigens would allow the immune system to be awakened and activated against the tumoral cells to produce a tumoral regression or a slower tumoral progression.

278 - In Vivo Two-Photon Imaging Reveals Infiltrating Glioma Cells Local Effects on Neuronal Responses to Sensory Stimulation

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Glioblastoma, the most common and aggressive form of primary brain tumor, grows by diffusely infiltrating the surrounding brain tissue. In recent years there has been a growing interest in the functional interactions between glioma cells and neurons in the brain tumor microenvironment. Nearly 80% of brain tumor patients suffer from seizures, and in mouse models of glioma, neuronal activity has been recently implicated in increased glioma growth and proliferation. Our lab has previously reported a mouse glioma model in Thy1-GCaMP6f mice that shows spontaneous seizure activity at the infiltrative margins of the tumor and that such neuronal hyperexcitability progresses in tandem with glioma growth. These findings necessitate a better understanding of glioma induced neuronal excitability and the underlying mechanisms. Here, we implanted cranial windows into Thy1-GCaMP6f mice with or without cortically injecting mCherry labeled glioma cells. We employed 2-photon imaging in order to characterize neuronal activity at the single cell level both spontaneously and during whisker stimulation. We observed an intermingling of tumor cells and excitatory neurons in our imaging fields at the infiltrative tumor margin and sought to investigate changes in synchronous activity among these neurons. To do so, we first derived the $\Delta F/F$ time series for each neuronal cell body and identified peaks in the signal by thresholding above the time series baseline. We then applied a gaussian convolution function to these identified peaks in order to more accurately and probabilistically determine correlation in synchronized calcium events. From here, we determined an average Pearson correlation value for each neuron based on correlations of each neuron's peaks with the peaks from all other neurons in the same imaging field. For each neuron we generated an average correlation value and a tumor burden score, based on the neuron's proximity to the mCherry+ tumor cells in the imaging field. We observed that neurons with higher tumor burden had greater average synchrony scores compared to neurons with low tumor burden and neurons from the control (no tumor) brains. Whisker stimulation caused a significant increase in synchrony scores specifically in the high tumor burdened neurons. These results show that infiltrative tumor cells are locally affecting neuronal firing patterns and provide new insights into how glioma cells may affect neuronal responses to physiologic sensory stimulation.

Immunomodulation and remyelination

4 - Cannabinoid Treatment Benefits in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis, A Review of the Literature

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ABSTRACT-PURPOSE: Cannabinoid treatment benefits in multiple sclerosis (MS), and its animal model experimental autoimmune encephalomyelitis (EAE), include clinicopathologic manifestations of immunomodulatory, neuroprotective, and analgesic effects. Experience with the use of cannabinoid medications for symptomatic relief of neurologic manifestations of inflammation is rapidly expanding. This paper reviews the dynamic neuropathologic deterioration of the central nervous system (CNS) in human MS, examines the neurologic outcomes following cannabinoid use in MS and EAE, and discusses implications for attenuating disease progression. METHODS: A systematic review by literature search through PubMed, ProQuest and EBSCO electronic databases was conducted for relevant studies reported since 2006 on Cannabidiol (CBD)-Tetrahydrocannabinol (THC) use in MS and EAE. Study selection, quality assessment and data extraction were

performed by 3 reviewers, and 20 studies were included. **RESULTS**: Treatment with cannabinoids, including Nabilone and nabiximols (Sativex®) oromucosal mixed CBD:THC spray formulations, resulted in decreased numeric rating scales (NRS) scores for spasticity, pain intensity, and sleep quality, reduced bladder overactivity, CNS mononuclear infiltrates, animal hindlimb stiffness, and expression levels of pro-inflammatory cytokines and transcription factors. **CONCLUSION**: Cannabinoid add-on therapy favorably impacts MS patient satisfaction by reducing neurologic impairment and improving quality of life. Taken together with its neuroimmunologic and neuropathologic effects in experimental models, it is conceivable that long-term pharmacotherapy with cannabinoids could improve the overall prognosis of patients with progressive forms of MS by slowing the accumulation of demyelinative lesions, decreasing the burdens of neuroinflammation and neuronal loss, thus allowing for longer periods of remyelination and ultimately interrupting the accrual of disability.

9 - Microglia and monocyte-derived macrophage contribution in myelin debris clearance during remyelination

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Multiple sclerosis is a demyelinating disease. Despite the successes in reducing disability, regenerative therapies are lacking. Remyelination is a regenerative process, associated with lower disability. Remyelination necessitates the clearance of inhibitory myelin debris, which is impaired in MS. Myelin debris is mainly phagocytosed by CNS resident microglia and monocyte-derived macrophages (MDMs). However, it is unknown to what extent microglia and MDMs phagocytose myelin debris. I hypothesize therefore that microglia and MDMs phagocytose myelin debris to differing extents in an experimental model of MS. I induced focal demyelination by intraspinal injection of LPC transgenic mice ($Micro^{TdT}$). $Micro^{TdT}$ flurorescently tag microglia, which allows differentiation from MDMs. The experimental endpoints were peak (3 days) and end (7 days) of phagocytosis. To compare microglia and MDMs phagocytic capacities, I measured microglial and MDM densities as well as volumes of the engulfed myelin debris. I found that microglia and MDMs have similar densities at 3 days, but microglia expanded to monopolize the LPC lesion by 7 days. Still, microglia and MDMs phagocytose myelin debris equally at 3 and 7 days. To understand how myelin debris clearance proceeds in the absence of microglia, I ablated microglia by genetically inserting the diphtheria toxin (DT) receptor into microglia and treating these mice and controls with DT. I found that MDMs compensate for microglial loss by phagocytosing more myelin debris, with no slowing of myelin debris clearance. Microglia and MDMs jointly phagocytose myelin debris. Future work will characterize the transcriptomes of phagocytosing microglia and MDMs. Understanding phagocytic mechanisms will provide targets to ultimately boost remyelination.

13 - Inhibiting the interaction of CSPGs with their receptors to promote remyelination in CNS injuries

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Glial scar formation is a common mechanism employed in the CNS to tackle the flood of inflammation that occurs following an injury, as witnessed in animal models of spinal cord injury. This presents as a protective barrier around the site of insult, but the end result is that spontaneous remyelination is also prevented due to the prohibition of growth inhibition of processes. Chondroitin sulfate proteoglycans (CSPGs) are one of the key components of the glial scar, produced in excess by reactive astroglia. Under abnormal conditions, CSPGs suppress regeneration by interacting with their inhibitory receptors from two families, namely, NogoR family (NgR1, 3) and protein tyrosine phosphate receptor family (PTPRS, F). Recently a natural ligand for CSPGs, a proliferation inducing ligand (APRIL, TNFSF13), had been identified in our lab. In our study, we have found that APRIL curtails the interaction of different CS types (glycosaminoglycan side chains, GAGs) with their receptors PTPRS and NgR1 in ELISA experiments. *In vitro* experiments were set up using mouse embryonic primary neurons, in which the different CS-GAGs suppress the growth of neurites and the addition of APRIL to these cultures rescued the average neurite length, implying that APRIL might possess certain regeneration potential. To further explore this possibility, *ex vivo* experiments were set up and chemical demyelination was induced by treating organotypic mouse cerebellar slices with lysolecithin, also marked by a proportional increase in the amount of CSPGs. Treating these tissues with APRIL brought about a greater amount of remyelinated axons compared to the control slices. Taken together, APRIL appears to be a prospective candidate in inducing regeneration as an antagonist of CSPGs.

21 - Pepducin P2pal-18S: attenuates neuroinflammation in a murine experimental autoimmune encephalomyelitis (EAE)

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Background: Proteinase-activated receptor 2 (PAR2) is a proteinase-activated G-protein-coupled receptors that is elevated in the central nervous system (CNS) in human multiple sclerosis and in its murine autoimmune encephalomyelitis (EAE) *in vivo* model, for which EAE is markedly reduced in PAR2-null mice. We therefore hypothesized that a receptor-selective PAR2 antagonist, Pepducin P2pal-18S, would attenuate the progression of EAE in the murine MS/EAE disease model in part by affecting immune cell function that is regulated by PAR2. **Methods**: To evaluate the impact of PAR2 blockade on EAE progression, P2pal-18S (10mg/kg) was administered on day 0 and day 10 in the murine EAE MS protocol and motor disability was monitored. Spinal cords were isolated and assessed by immunohistochemistry for axon myelination, T cell- and macrophage- specific markers at the peak of disease expression (day 15). Serum was isolated from EAE-affected mice to survey the circulating inflammatory and anti-inflammatory cytokines (Luminex assay). To analyze the effect of P2pal-18S on T cell and macrophage function 1. its reversal of PAR2-stimulated calcium signaling was measured, 2. antiCD3/CD28-activated splenocytes were treated with Pepducin in vitro and proliferation was monitored and 3. the pepducin effect on bone marrow-derived cytokine-mediated macrophage differentiation in vitro was assessed. The impact of P2pal-18S on macrophage differentiation was evaluated by monitoring cytokine-induced M1/M2 phenotype differentiation (semi quantitative PCR). Supernatants were collected from treated splenocytes and bone marrow-derived macrophages to analyze cytokine production (Luminex). **Results**: EAE mice treated with P2pal-18S exhibited markedly diminished paralysis and clinical scores compared to controls; and CNS T cell and macrophage infiltration and demyelination were decreased. Further, P2pal-18S decreased anti CD3/CD28-triggered lymphocyte proliferation. In addition, P2pal-18S prevented cytokine-induced macrophage M1/M2 differentiation. The decrease of serum GM-CSF in P2pal-18S–treated EAE mice paralleled the decreased production of GM-CSF by pepducin-treated CD3/CD28-activated splenocytes. **Conclusions**: We conclude that PAR2 plays a key role in EAE/antigen-induced MS-related CNS neuroinflammation and that this GPCR may represent a novel therapeutic target for treating MS and other neuroinflammatory diseases.

27 - Microglia preserve myelin integrity in the central nervous system

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Effective central nervous system (CNS) function requires myelin to be of good structural integrity. Poor cognitive function with ageing and neurodegenerative disease is associated with loss of myelin integrity, such that myelin is less compact, thicker, unravelling, forming outfoldings, and degenerating. However, the fundamental mechanisms instructing myelin formation, maintenance and integrity are unclear. Although central nervous system macrophages have been implicated, it is unknown which macrophage populations are involved and which aspects of myelin health they influence. To address this, we sought to investigate the specific roles of microglia in myelin health using a recently developed transgenic model in which deletion of the FIRE super-enhancer of the Csf1r gene ($FIRE^{\Delta/\Delta}$) leads to an absence of microglia, while retaining perivascular macrophages. We found that in the absence of microglia in $FIRE^{\Delta/\Delta}$ mice, myelin was still formed in the white matter. However, $FIRE^{\Delta/\Delta}$ mice showed a loss of integrity of myelin, with myelin which was less compacted, thicker, unravelling and forming outfoldings, culminating in myelin degeneration. $FIRE^{\Delta/\Delta}$ mice showed deficits in cognitive function concomitant with loss of myelin integrity and preceding myelin degeneration. Similar loss of myelin integrity was observed in a human condition (ALSP) whereby heterozygous mutations in CSF1R result in reduced white matter microglia and dementia. In summary, we demonstrate that whereas microglia are dispensable for developmental myelin formation, they are required for the preservation of myelin integrity and associated cognitive function, and the maintenance of myelin with ageing. Our findings highlight microglia as promising therapeutic targets for conditions where myelin integrity and preservation are compromised, such as in ageing and neurodegenerative disease.

30 - Modulation of P2X7 receptor activity in experimental autoimmune encephalomyelitis models.

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Multiple sclerosis (MS) is a chronic autoimmune and demyelinating disease of the central nervous system (CNS), characterized by axonal loss and neuronal degeneration. MS is one of the most common inflammatory diseases of the CNS and affects over 2.5 million people worldwide with a higher incidence in women. Despite its complex pathogenesis, evidence support a Th1/Th17 autoimmune component of the disease driving: (1) chronic inflammatory processes in the spinal cord and brain and (2) loss of cortical neurons and axonal degeneration in the central nervous system. These events are under the control of microglial cells, the resident macrophages. Current treatments remain ineffective during its progressive phase. Therefore, there is a great need to discover

new therapeutical targets. P2X7 receptor (P2RX7) is a cell surface ion channel that senses ATP released from cells as endogenous danger signals during inflammation. The receptor is largely implicated during inflammatory events such as inflammasome, pro-inflammatory cytokine production, differentiation and survival of lymphocytes, … The objective here will be to better understand the role of P2RX7 in the pathophysiology of experimental autoimmune encephalomyelitis models (EAE, mouse models of MS). To this end, we will use the AAVnano technology based on AAV vectors coding for single-chain antibodies named nanobodies (Nbs) specific of P2RX7. We demonstrated that the latter can efficiently target and modulate the activity of P2RX7 in the periphery as well as in the CNS (particularly microglia and T cells) during the course of EAE. Preliminary data suggested that AAVnano coding for P2RX7 Nb antagonists led to an exacerbation of the EAE clinical signs. The effect of the anti-P2RX7 Nb agonists is ongoing. This translational approach will evaluate the therapeutical potential of anti-P2RX7 Nbs during MS and potentially other inflammatory, autoimmune and/or neurodegenerative diseases.

Keywords: P2RX7, neuroinflammation, EAE, microglia, MS

31 - Optimization of antibody linked to immunoregulatory cytokines: in vitro and in vivo evaluation

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Cytokines are potent modulators of the activity of the immune system and provide a significant therapeutical potential in many pathologies. However, their application is narrow because of their off-target actions leading to important side effects (systemic toxicity). To circumvent this matter, immunocytokines, resulting from the fusion of antibody and cytokine, allow a targeted delivery of the biomolecules and are being considered in the treatment of auto-immune disease. In this context, we aim to develop new biomolecules named Nb-cytokines by combining nanobodies (Nbs, single-chain antibodies) to a cytokine. Given its roles during inflammation acting on the inflammasome, T cells and myeloid cells, we chose P2X7 receptor as a working prototype using 7E2, an anti-P2RX7 Nb developed in the laboratory. We then conjugated 7E2-Nb to the immunoregulatory IFNβ with different linker types and lengths to generate several 7E2-IFNβ immunocytokines. Once immunocytokines are produced, the specificity and functionality of each Nb-cytokine candidate will be analyzed *in vitro* using cellular models expressing P2X7 receptors and/or IFNβ receptors. The selection is made according to: (i) their ability to activate the IFNβ signaling pathway through flow cytometry and ii) their capability to inhibit cell proliferation. The selected 7E2-IFNβ immunocytokines will be further evaluated *in vivo* in Experimental Autoimmune Encephalomyelitis (EAE), a murine model of multiple sclerosis. This work will provide a proof-of-concept study demonstrating that the optimization of such biomolecules will provide new therapeutical perspectives for a large spectrum of inflammatory and autoimmune pathologies.

35 - Myeloid derived suppressor cells are biomarkers for Fingolimod treatment efficacy in multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. In recent years, there has been a remarkable increase in the number of available treatments for the relapsing-remitting MS. Therefore, neurologists need tools (e.g. biomarkers) to help them determining the most effective treatment for each patient, avoiding side effects and reducing costs to the Health System. Fingolimod (FTY720), an oral disease-modifying drug approved for RR-MS, acts as a sphingosine-1-phosphate receptor modulator (S1PR) and can bind to S1PR1-5. FTY720 prevents lymphocyte egress from lymphoid tissues via S1PR1. However, it has been described some other biological actions over other immune cells. Myeloid-Derived Suppressor Cells (MDSCs) are a heterogeneous population of immature myeloid cells whose immunosuppressive activity was enhanced by FTY720 via S1PR1,3,5 in different autoimmune models. Previous work of our group in the MS model experimental autoimmune encephalomyelitis (EAE) showed that a high MDSC abundance in blood at the onset of the clinical course is a good biomarker of a less severe clinical course. In the present work, we interrogate whether the abundance of MDSCs at the onset of EAE can be related to a high efficacy to FTY720 treatment. For this purpose,

EAE mice were orally administered Vehicle or 3mg/Kg FTY720 since disease onset, during 2 weeks. Before starting treatment, immune cells were analyzed in the blood of each animal. Our data indicated that MDSC content in the peripheral blood was associated with a milder EAE disease course in both analyzed groups. Interestingly, most of the FTY720-treated animals presented a lower maximum clinical score, and a higher/faster recovery, ending the follow-up with lower residual scores. However, a small proportion of the FTY720 EAE mice showed a clinical course similar to vehicles (non responders), which was invariably associated to a very low abundance of MDSCs at EAE onset. Cox regression analysis showed that the risk to reach a clinical score ≥3 (median value of the vehicle group) was lower when EAE animals presented a high level of peripheral MDSC abundance at disease onset, regardless of T lymphocyte abundance, or day/score at onset. In sum, our data indicate that MDSCs are good biomarkers for FTY720 treatment efficacy, especially to distinguish non responder mice, before starting the treatment, which may have important therapeutic consequences for the future MS management.

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37 - Myeloid-derived suppressor cell function is a key factor behind the clinical course severity in multiple sclerosis

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature myeloid cells with a regulatory role in multiple sclerosis (MS). In the context of the animal model of MS, MDSCs are involved in T cell suppression within the CNS. It has been established that the proportion of monocytic MDSCs is related to the previous severity of the EAE clinical course and tissue damage extent. Moreover, our group has observed the presence of MDSCs in the CNS of MS patients, being mainly circumscribed to areas with spontaneous capacity of remyelination. Interestingly, our preclinical data pointed to MDSCs as bioindicators of the future clinical course severity since the abundance of MDSCs in the peripheral blood at the onset is inversely correlated with the clinical and histopathological severity of the disease course.

In the present work, we perform *in vivo* and *in vitro* approaches using both human MS and EAE samples to investigate whether functional differences of MDSCs are behind the high variability of the MS clinical course. The correlation between MDSCs and the viability of T cell was analyzed in the CNS from MS patients with different disease severity. Our data showed that the higher abundance of MDSCs in active lesions was clearly related to a lower density of their target cells, i.e. T lymphocytes. In parallel, MS patients with milder clinical courses showed a higher density of apoptotic T cells. Thus, we considered studying whether functional differences of MDSCs would be related to the severity of EAE clinical course as well. Firstly, the clustering analysis of the clinical and immunological variables measured at EAE onset identified two well-defined animals groups with different clinical course severities. As a next step, we corroborated that the differences in the abundance of MDSCs in both groups of EAE mice were not only numerical, but also functional showing different immunosuppressive activity. Finally, transcriptomic profile analysis by RNAseq corresponding to MDSCs from mild EAE mice showed the downregulation of signaling pathways related to the activation of the pro-inflammatory immune response, pointing to a notable immunosuppressive phenotype. In sum, our results seem to indicate that clinical course severity would be related not only to the abundance of MDSCs but also to a more immunoregulatory function, suggesting that the improvement of MDSC functions should be a promising strategy to efficiently modulate the MS severity.

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47 - Circulating microRNAs in RRMS patients treated with dimethyl fumarate in the phase 4 TREMEND trial

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Introduction:
Aberrant microRNAs (miRNAs) expression is associated with a variety of diseases.
Objectives:
We examined miRNA signatures in the peripheral blood of treatment-naïve MS patients at different time points during dimethyl fumarate (DMF) treatment and related them to neurofilament light (NFL) levels in the CSF and blood.
Aims:
To identify miRNAs that are associated with DMF efficacy.
Methods:
In the phase 4 TREMEND trial, 210 blood samples were collected from 52 treatment-naïve patients at baseline (BL) and after 3, 6, 12 and 24-months of DMF treatment, and from 22 healthy controls. By microarray, 1,085 out of 2,549 miRNAs were two-folds above the background levels, and were included in the statistical analyses (cut off FDR≤0.05). NFL was measured by Simoa.
Results:
By self-organizing map plot, most miRNA changes were observed after 6-months of DMF treatment. With time course analysis, 18 miRNAs had an altered expression pattern, some with continuous decline (e.g. miR-4999-3p) or increase (e.g. miR-146-5p) over 24 months of treatment. Using limma, 41 miRNAs were altered between at least 2 time points among samples with high and low NFL; 21 of these were increased after 12 months in low-NFL samples compared to high-NFL samples at 6 months. Particularly, miR-16-5p and miR-4306 were downregulated in high-NFL samples after 6 months compared to baseline. This pattern persisted, as these two miRNAs were also downregulated at 12 months in high-NFL compared to low-NFL samples at 3 months. These two miRNAs followed an inverse pattern if NFL decreased: they were upregulated in low-NFL samples at 12 months compared to high-NFL samples at 6 months. Two other miRNAs, miR-940 and miR-4665-3p had an inverse pattern compared to miR-16-5p and miR-4306: they were upregulated if NFL was increased at 6 months. This pattern was also supported by their upregulation at 12 months in NFL-high compared to NFL-low samples at 3 months.
Conclusion:
We found altered dynamics in the miRNA signature during the early and late DMF treatment periods. Altered expression peaked after 6 months. This may either reflect mechanisms of DMF or change in MS disease-related pathways. The majority of miRNA changes comparing samples with high and low NFL occurred between 6 months and 12 months: particularly, miR-16-5p and miR- 4306 increased in low-NFL samples that could reflect treatment response while miR-940 and miR- 4665-3p increased in high-NFL samples suggesting ongoing or new disease activity.

48 - Investigating the therapeutic outcomes of niacin in the EAE model of multiple sclerosis

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Multiple sclerosis (MS) is an inflammatory and progressive disease of the central nervous system (CNS), characterized by immune cell infiltration, demyelination, and neurodegeneration. During neuroinflammation, the

immune system can promote both injury and recovery; thus, strategies to enhance remyelination and prevent degeneration may include harnessing the beneficial aspects of neuroinflammation without incurring the detriments. Our previous research has found niacin (vitamin B3) to promote remyelination in the lysolecithin demyelination model through increased phagocytosis of inhibitory myelin debris by macrophages and microglia. However, the therapeutic effect of niacin in experimental autoimmune encephalomyelitis (EAE), an MS model with involvement of both the innate and adaptive immune systems, remains unknown. Thus, we tested the hypothesis that administration of niacin would ameliorate EAE clinical score of disability and reduce neuropathological parameters through modulation of immune activity. To investigate this hypothesis, niacin was administered to EAE animals daily via oral gavage, starting on the day of immunization. We found that niacin ameliorated the severity of disability scores of EAE. By analyzing tissues at peak disease severity using immunohistochemistry, we determined that niacin decreased the expression of microglial/macrophage markers CD206 and CD68, and restored myelination to control levels. Through PCR evaluations, we found that niacin-administered EAE had an increase in BDNF expression in the spinal cord, as well as a trend towards elevated NT-3 and decreased Jagged-1. Our data suggests that niacin reduces disease phenotype in the EAE model of MS through modulation of immune activity and release of neurotrophic factors. Understanding the impact of niacin on inflammation and remyelination in the CNS is a critical step towards its translation into MS clinical trials, potentially improving treatment options for individuals living with MS.

51 - T cells from MS patients in higher disability status insensitive to an immune-suppressive effect of sulfatide

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS), characterized by relapse-remitting disease course in an early phase followed by disability progression in a later stage. Whereas chronic inflammation accompanied with degeneration are the key pathological features, the pathogenesis of MS, particularly progressive MS, remains unknown. Sulfatide is a major glycolipid component of myelin, and previous studies in experimental autoimmune encephalomyelitis mouse models showed its immune-protective functions. On the other hand, the concentration of sulfatide increases in serum and cerebrospinal fluid of patients with MS, particularly those in a progressive disease course. Here we show that a myelin-glycolipid sulfatide displays an ability to suppress the proliferation of polyclonally activated human T cells. Whereas sulfatide is known to activate NKT cells, our results show that sulfatide directly suppresses T cell proliferation, independent of NKT cells. We next examined the effects of sulfatide on T cell activation obtained from patients with MS (19 patients with MS and 10 HS). We showed that sulfatide inhibited the proliferation of T cells obtained from patients with MS bearing mild disability. Since sulfatide is a major component of myelin, sulfatide is inevitably released during myelin turnover or at myelin injury or demyelination sites. Our results suggest that sulfatide released via this process would suppress T cell activation and prevents an excessive reaction against myelin proteins. In the context of MS, where relapsing and remitting inflammation in the CNS is a crucial feature, sulfatide would serve beneficial functions in milder cases by promoting remission through its suppressive effect on T cells. Importantly, the suppressive effect of sulfatide was impaired in T cells obtained from patients with MS in higher disability status. Our study suggests that an escape of T cells from immunosuppression by sulfatide is associated with disease progression in an advanced stage.

58 - The anti-inflammatory corticosteroid medrysone drives oligodendrogenesis, beneficial reactive astrogliosis and remyelination in a cuprizone-mediated chronic demyelination model

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Demyelinating diseases such as multiple sclerosis result from inflammatory insults, lead to oligodendrocyte death and myelin loss and are, among other reactions, accompanied by neural stem cells (NSCs) proliferation. Bordering the corpus callosum (CC), the dorsal wall of the subventricular zone (d-SVZ) harbors NSCs which can potentially generate new oligodendroglial cells, however, only little is known about the actual contribution of these d-SVZ to remyelination. Therefore, taking advantage of a cuprizone-dependent chronic demyelination model, we investigated the consequences of CC damage and subsequent d-SVZ reactions in aged mice. In addition, medrysone, an FDA approved anti-inflammatory corticosteroid predicted by our previous studies to promote oligodendroglial cell fate decision, was applied. Results generated ex vivo revealed an increase in oligodendrocyte (OL) numbers and improved axonal myelination in postnatal cerebellar organotypic slice cultures after medrysone application. Moreover, medrysone treatment of dSVZ derived cells led to increased numbers of NG2- and GFAP-positive cells in culture. In addition, a non-neurotoxic astroglial profile was rescued by medrysone, highlighting the impact of this drug on astroglial cells. In vivo, medrysone application to chronically demyelinated hGFAP-GFP reporter mice regulated astroglial heterogeneity-related genes at dSVZ, followed by increased numbers of NSCs (Hopx+) and activated Type-B-NSCs (GFP+, EGFR+) in the first week of recovery. Moreover, medrysone induced early mature OLs (GSTπ+), nodes of Ranvier/paranodes (Caspr+) and mature myelinating oligodendrocytes (APC-CC1+) as well as a recovery of MBP expression in the CC. Interestingly, two subtypes of hybrid A1-A2 reactive astrocytes were found during the recovery phase in response to medrysone: (i) A1-A2 astrocytes (GFP+, C3d+, S100a10+) and (ii) remyelination-related astrocytes (GFP+, C3d+, STAT3+) expressing molecules related to neuroprotection (STAT3/S100a10). The sustained C3d expression suggests that in order to foster myelination, this hybrid phenotype is required. Based on these findings we conclude that medrysone can successfully foster myelin restoration and beneficial reactive astrogliosis from depicted NSC subpopulations and thus serves as a potential drug for future myelin repair approaches.

64 - Myeloid Derived Suppressor Cells are biomarkers of relapse recovery in multiple sclerosis patients

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Multiple sclerosis (MS) is a chronic, inflammatory and demyelinating disease of the central nervous system. One of the main characteristics of MS is the great variability of symptoms and the unpredictability of its disease course. Relapsing Remitting MS (RRMS) patients suffer periods of neurological disability (relapses) followed by partial or full recoveries (remissions). Due to the mentioned MS variability, it is very important to find biomarkers that help the clinicians make decisions about relapse management from early stages of the disease. On the other hand, the existence of the spontaneous remissions points to the existence of endogenous regulatory factors that control the immune response. Myeloid-Derived Suppressor Cells (MDSCs) are a heterogeneous group of immature regulatory cells which has been shown to be crucial for the control of disease severity in the murine model of MS, experimental autoimmune encephalomyelitis (EAE). Data from our group indicated that a high level of MDSCs on

peripheral blood at disease onset foreseen a milder disease severity and a lower tissue damage extent. Importantly, the level of MDSCs at the peak of EAE are a good biomarker of a greater clinical recovery. In view of these results, we designed a translational study on untreated RRMS patients who had not received corticosteroids in the last 6 months and who experienced their first relapse up to one year before blood sampling. Blood was collected at baseline and one year later, in order to check the correlation between MDSCs and different demographic and clinical data, e.g. RMN, EDSS, age, time elapsed from relapse to sampling. Our data indicated that MS patients presented a higher abundance of MDSCs than healthy controls (HC), exclusively in those patients whose samples at baseline were collected close to relapse. In those patients, the level of MDSCs at baseline was inversely correlated with the EDSS at that moment, but also at one year later. Interestingly, MS patients who fully recovered from relapse showed a higher abundance of MDSCs than those who recovered only partially. Based on these results, we can point to peripheral blood MDSCs content as a putative future bioindicator of relapse recovery in RRMS patients.

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72 - The effects of the Bruton's tyrosine kinase inhibitor evobrutinib in bone marrow-derived macrophages

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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system, in which lymphocytes play a central role in driving pathogenesis. Although innate myeloid cells like dendritic cells, monocytes, macrophages and microglia have prominent roles in MS pathogenesis such as antigen-presentation and the production of inflammatory cytokines, they are less commonly linked to the disease. Most treatments target cells of the adaptive immune system. A promising new target to suppress the strongly activated phenotype of B cells – with potentially less long-term side effects than CD20 depletion – may be the inhibition of Bruton's tyrosine kinase (BTK). BTK is not only crucial for B cell activation and differentiation but also implicated in Fc receptor signaling, leading to myeloid cells being most likely affected by BTK inhibition. Here, we investigated the effects of evobrutinib as an BTK inhibitor on bone marrow-derived macrophages (BMDM) *in vitro.* BMDMs were generated from naïve C57Bl/6 mice and differentially and functionally assessed following BTKi. To investigate the impairment of Fc receptor-dependent activity, we performed phagocytosis assays using fluorescently labeled Ovalbumin in combination with anti-Ovalbumin antibody. Our data showed a consistent increase of phagocytic activity after BTK inhibition independent of the polarization of macrophages or presence of antigen-specific antibodies. Next, we examined the effect of BTK inhibition on the differentiation of macrophages and used the expression of inducible nitric oxide synthase (iNOS) and Arginase 1 (Arg1) to distinguish between M1 and M2 polarization. According to our data, the M2 polarization was significantly and dose-dependently inhibited by the BTK inhibitor while the LPS-inducedM1 polarization seemed to be BTK-independent. Currently, we are investigating Fc-dependent activation strategies to examine the phosphorylation of key signaling proteins such as Syk, BTK, PLCγ2 and ERK1/2, as well as the FcR internalization, expression of activation markers and cytokine production following BTKi. Preliminary results show an inhibition of phosphorylation as well as downregulation of MHC II and proinflammatory cytokines like IL6, IFNγ and TNF. Taken together, this proposes the idea that in macrophages,

evobrutinib enhances the debris removal capacity while inhibiting the production of proinflammatory cytokines and suppressing antigen-presentation leading to a potential restraint of MS pathology.

79 - Fibrosis of PDGFRβ+ cells following spinal cord demyelination is exacerbated with age

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Remyelination is the endogenous response to demyelination like that seen in multiple sclerosis. In people with MS remyelination is heterogeneous and often insufficient for functional recovery. Age and the inhibitory lesion environment have both been implicated in impaired remyelination. Recently fibrosis of the CNS has been shown to occur following immune mediated injury. However, it remains unclear how this process effects remyelination, and if age has any influence on it. We hypothesize that PDGFRβ+ cells respond following experimental demyelination and form a fibrotic scar that is inhibitory to oligodendrocyte lineage cells and is exacerbated with age. To test this hypothesis, we used the lysolecithin model to demyelinate ventral white matter of the spinal cord in young (2-3 months) and middle-aged (12 months) mice. We see that PDGFRβ+ cells begin to form a scar area by 7 days post induction (dpi); the scar area persists throughout the lesion life span until 21 dpi. Cells of the oligodendrocyte lineage are scarce in areas occupied by these PDGFRβ+ cells at all time points. In middle-aged animals the scar area is more significant than young animals and corresponds with delayed oligodendrocyte response. We finally use electron microscopy and toluidine blue stains to analyze the influence of age on remyelination and the fibrotic scar area at the ultrastructural level. We find that age is a significant factor in determining the response of PDGFRβ+ fibrotic cells following demyelination and is not hospitable to oligodendrocyte lineage cells. It remains to be seen if this response is directly related to remyelination or if it can be targeted for therapeutic intervention.

86 - MS patient lymphocytes induce chronic active demyelinating lesion in mouse spinal cord.

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Multiple sclerosis (MS) is an inflammatory, autoimmune and demyelinating disease of the central nervous system (CNS). Classically, experimental autoimmune encephalomyelitis (EAE) is used to study inflammation and immune system activation, whereas focal demyelination models, such as intra CNS lysophosphatidylcholine (LPC) injection, are used to study demyelination/remyelination process. However, those models do not allow the study of demyelination/remyelination in an inflammatory context. Therefore, we established a new humanized model by grafting human lymphocytes (LY) in LPC induced lesion. We showed that some MS patients LY prevent remyelination in Nude mice 3 weeks after injection.
We decided to follow up the lesion evolution up to three months after the grafting. We grafted the LY of 4 healthy donors (HD) and 10 MS patients (10 mice per individual) in demyelinated lesion of Nude mouse spinal cord. We followed 1 week, 1, 2 and 3 months after the lesion /graft the behavioural changes using the rotarod, the notched beam and the grip test. At 3 months, we also measured the somatosensory evoked potential (SSEP) to assess the physiological changes due to the persistence (or not) of the lesion. Next, we evaluated the lesion volume and the extent of inflammation by electron microscopy and immunohistochemistry.
We provided evidence that MS patient LY graft elicited 3 months after their grafting the installation of a chronic active lesion with scaring tissue along with myelin decompaction, microglial cell engulfment of myelinated axons suggesting an ongoing demyelination. Furthermore, while non-grafted mice and mice grafted with HD LY improved over time, probably due to remyelination, mice grafted with MS patient LY continued to make errors on the notched beam and had a weakened forelimb grip, probably due to the persistence of inflammation and demyelination. We also observed an increase of sensory evoked potential latency in mice grafted with MS patients LY.

Proposing such a novel humanized mouse model of focal chronic demyelination and inflammation is a step forward to elaborate new therapeutic intervention to prevent the development of these so-called "chronic active" lesions which are more frequently observed in patients with a severe evolution of MS, at risk of developing higher disability.

112 - Upregulation of fibulin-2 in multiple sclerosis lesions inhibits oligodendrogenesis

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Remyelination is a spontaneous repair response that occurs in multiple sclerosis (MS), but it eventually fails with disease progression. A cause of remyelination failure is the altered extracellular matrix (ECM) in lesions. Using bioinformatic analysis of published proteomic and RNA databases for MS and its experimental autoimmune encephalomyelitis (EAE) model, we have identified fibulin-2 (FBLN2) as a highly upregulated ECM member in lesions. Here, we examined the expression of FBLN2 in lesions of MS and EAE, and addressed whether this ECM molecule could affect remyelination.

By immunofluorescence confocal microscopy and Imaris 3D rendering, we found increased levels of FBLN2 in EAE and MS lesions, localized to $GFAP^+$ astrocytes; this is in accordance with our in vitro data of elevation of FBLN2 in activated astrocytes but not macrophages and microglia. FBLN2 did not affect adhesion in tissue culture, but was strongly inhibitory for maturation of human or mouse oligodendrocyte precursor cells (OPCs) to oligodendrocytes, correspondent with cell death in real time experiments. To assess FBLN2-mediated mechanisms in OPCs, various signaling inhibitors were used. Blocking Notch signaling pathway overcame the FBLN2 inhibition. Next, we reduced FBLN2 in EAE lesions by using adeno-associated viruses (AAV) guided to infect reactive astrocytes and delete their FBLN2 through CRISPR/Cas9. FBLN2 deficiency resulted in a better clinical recovery during EAE which was correspondent with more $olig2^+$ oligodendrocyte lineage cells in lesions compared to AAV-control injected mice.

Overall, these results suggest FBLN2 as a new extracellular matrix inhibitor of oligodendrocytes and myelin repair through affecting NOTCH signaling pathway. Overcoming FBLN2 has the therapeutic potential to improve remyelination and prognosis in MS.

128 - Enhanced but retained pathogenicity of Th17 cells during natalizumab treatment-implications for disease rebound after treatment cessation?

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After natalizumab (NAT; Tysabri®) treatment cessation, some multiple sclerosis (MS) patients may experience a severe disease rebound, exceeding pretreatment levels of disease activity. NAT cessation rebound pathophysiology is still unclear; however, it has been linked to interleukin-17-producing T-helper (Th17) cells. We demonstrate that during NAT treatment, melanoma cell adhesion molecule (MCAM+) and C-C chemokine receptor 6 (CCR6+) Th17 cells (MCAM+CCR6+Th17 cells) gradually acquire a pathogenic profile, as long-term, but not short-term treated NAT patients displayed increased proinflammatory cytokine production. Investigation of effects on key step of MS pathophysiology revealed that Th17 cells derived from long-term treated NAT patients displayed an increased potential for brain endothelial barrier impairment and enhanced capacity to affect human stem cell-derived oligodendrocytes. This was accompanied by an increase in Th17 cell frequencies in the cerebrospinal fluid of NAT patients, as compared to treatment-naïve MS patients. Long-term NAT treatment led to profound gene regulation in MCAM+CCR6+Th17 cells as compared to treatment-naïve MS patients, including

significant enrichment of genes associated with Th17 pathogenicity and altered metabolic functions. Notably, transcriptional signatures of Th17 cells derived from NAT patients, who later developed a disease rebound upon treatment cessation, additionally exhibited enhanced IFNγ-signaling and migratory properties. Enhanced brain infiltration of NAT cessation rebound patient-derived MCAM+CCR6 Th17 cells was illustrated *in vivo* in a humanized mouse model and in brain biopsy histology from a rebound patient. In conclusion, our data indicates that peripheral blood accumulated MCAM+CCR6+Th17 cells might be implicated in disease rebound pathophysiology, thus monitoring changes in Th17 cell pathogenicity in patients before/during NAT treatment cessation might enable rebound risk prediction in the future.

148 - Iguratimod improves clinical course and pathological changes in a mouse model of secondary progressive multiple sclerosis

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Objectives: Experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), usually shows a chronically stable course after acute phase. We reported a novel model of secondary progressive MS (SPMS) induced by immunization with myelin oligodendrocyte glycoprotein peptide 35-55 (MOG_{35-55}) in oligodendroglia-specific *Cx47* inducible conditional knockout (*Cx47* icKO) mice, which showed a relapsing progressive course at the chronic phase (Zhao et al., *PNAS*, 2020). As we also reported efficacy of iguratimod (IGU), an anti-rheumatic drug, in acute EAE, we aimed to evaluate effects of IGU in this SPMS model. **Methods**: *Cx47* icKO (*Plp-CreERT; Cx47*fl/fl) mice were immunized with MOG_{35-55} to induce EAE. Following the peak of acute EAE, IGU (50 mg/kg, twice a day) or methylcellulose (control) was orally administered from 17 days postimmunization (dpi) to 50 dpi. **Results**: Clinical signs of EAE and demyelinated areas were decreased in IGU-treated mice than control mice at the chronic phase ($p<0.0001$ and $p=0.011$, respectively). Areas of $CD3^+$ T cells, $F4/80^+$ macrophages, $NOS2^+Iba1^+$ microglia, and $C3^+GFAP^+$ astroglia in the lumbar spinal cord lesions were significantly smaller in IGU-treated mice than methylcellulose-treated mice ($p<0.05$ for all). Furthermore, microglia circularity was also decreased in IGU-treated mice than methylcellulose-treated mice ($p=0.01$). **Conclusions**: Therapeutic administration of IGU was clinically and pathologically effective for the newly established SPMS model. IGU could be a novel therapeutic candidate for SPMS.

175 - Establishing the role of miR-150 in Multiple Sclerosis and experimental autoimmune encephalomyelitis

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MicroRNAs (miRNAs) are small noncoding RNA molecules that have an important role in the fine tuning of all biological processes and are often found to be dysregulated in diseases, such as multiple sclerosis (MS). MS primarily affects young adults resulting in progressive disability which is caused by chronic inflammation, demyelination and axonal loss in the central nervous system. We have previously shown that microRNA-150 (miR-150) levels are elevated in the cerebrospinal fluid of MS patients compared to controls. We aimed to further investigate the role of miR-150 in vivo by generating miR-150 knock-out (KO) and knock-in (KI) mice using CRISPR/Cas9. To gain more insight into the pathophysiological role of miR-150, we induced experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. After induction of EAE, miR-150 KO mice displayed

ameliorated disease compared to wild type littermate controls, while miR-150 KI mice presented with more severe disease. Further analysis demonstrated an increased number of CD4+ Foxp3+ cells in the lymph nodes at the priming stage of the disease, whereas reduced infiltration of CD4+ cells was observed at the peak of the disease in the miR-150 KO mice compared to wild type counterparts. Additionally, miR-150 KO CD4 cells had greater tendency to differentiate in vitro towards regulatory T cells (Tregs). These results are further supported by RNA sequencing data from CD4 cells at the priming stage, which demonstrated upregulation of genes that correlate with the Treg phenotype in miR-150 KO animals. Our ultimate goal is to further explore the role of miR-150 in Tregs, which will be achieved by performing RNA sequencing and methylation analysis of Tregs from miR-150 transgenic animals.

180 - Soluble IFN-beta receptor in multiple sclerosis patients. Association with the clinical response to IFN-beta treatment

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Interferon beta receptor 2 subunit (IFNAR2) can be produced as a transmembrane protein but also as a soluble form (sIFNAR2) that is generated by alternative splicing or proteolytic cleavage, which has both agonist and antagonist activities for IFN-β. However, its role regarding the IFN-β therapy used for relapsing-remitting multiple sclerosis (RRMS), is unknown. We evaluate the short and long-term effects of IFN-β therapy on sIFNAR2 production and their association with the clinical response in MS patients.

Eighty-eight RRMS patients were included and evaluated at baseline, 6 and 12 months from treatment onset. A subset of the sample was classified as responders and non-responders to IFN-β therapy. sIFNAR2 serum levels were measured by ELISA. mRNA expression for IFNAR1, IFNAR2 splice variants, MxA and proteases were assessed by RT-PCR. The short-term effect was evaluated in PBMC from RRMS patients after IFN-β stimulation in vitro.

Protein and mRNA levels of sIFNAR2 increased after IFN-β treatment. According to the clinical response, only non-responders increased sIFNAR2 significantly at both protein and mRNA levels. sIFNAR2 gene expression correlated with the transmembrane isoform expression and was 2.3-fold higher. IFNAR1 and IFNAR2 slightly increased after treatment while MxA expression increased significantly. After short-term IFN-β in vitro induction of PBMC, 6/7 patients increased the sIFNAR2 expression.

IFN-β administration induces the production of sIFNAR2 in RRMS and higher levels might be associated to the reduction of therapeutic response. Thus, levels of sIFNAR2 could be monitored to optimize an effective response to IFN-β therapy.

181 - Astroglial connexin 43 is a novel therapeutic target for a chronic multiple sclerosis model

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Objectives: Connexin (Cx) 43 gap junction channel proteins are overexpressed in chronic lesions of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalitis (EAE), at chronic phase, reflecting astrogliosis. We aimed to elucidate the role of overexpressed Cx43 in MS by therapeutic administration of a novel CNS-permeable pan-Cx blocker, INI-0602, in chronic EAE. **Methods**: EAE was induced by immunizing myelin oligodendrocyte glycoprotein peptide$_{35-55}$ in 35 C57BL6 mice. Following the peak of acute EAE, INI-0602 (40 mg/kg) or saline was intraperitoneally administered every other day from day postimmunization (dpi) 17 to dpi 50. **Results**: The clinical signs of EAE were significantly attenuated at chronic phase and demyelinated areas were reduced in INI-0602-treated mice compared with saline-treated mice. Infiltration of $CD3^+$ T cells, $Iba1^+$ microglia, $F4/80^+$ macrophages and $C3^+GFAP^+$ A1 astroglia in the lumbar spinal cord lesions significantly decreased in INI-0602-treated mice compared with saline-treated mice. Flow cytometry analyses of $CD4^+$ T cells isolated from the central nervous system tissues revealed significant decrease in the proportions of Th17 and Th17/Th1 cells at dpi 24 and Th1 cells at dpi 50. Furthermore, INI-0602 treatment of astroglia in vitro suppressed calcium-orientated communication in a dose-dependent manner and significantly decreased the Cx43 expression density. **Conclusion**: These results suggest that the overexpressed astroglial Cx43 in chronic EAE and MS lesions exacerbate neuroinflammation. Thus, astroglial Cx43 is a novel promising therapeutic target for chronic progressive MS.

192 - A broad effect of ocrelizumab on the peripheral immune component in early relapsing-remitting multiple sclerosis

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Introduction: Ocrelizumab is a recombinant monoclonal humanized anti-human CD20 antibody that selectively targets and depletes CD20-expressing B cells, which are believed to play a critical role in Multiple Sclerosis (MS).
Objectives: The purpose of this study is to analyse B and T cells subsets during and after ocrelizumab-induced B cell depletion.
Aims: This project will allow us to better understand ocrelizumab mechanism of action.
Methods: Using spectral flow cytometry, we analyzed the phenotype of T cells, B cells and monocytes before Ocrelizumab administration and after 24 and 48 weeks of therapy in the peripheral blood of 33 treatment-naive Relapsing-Remitting-MS patients from the ENSEMBLE study.
Results: We confirmed a profound decrease in the B cell subset with a notable increase in CD49d (VLA-4) and CD11a (LFA-1) expression, two markers of homing and migration cell, on remaining circulating B cells. The percentage of CD4 and CD8 cells did not change over time, but within T cell subsets, we observed a reduction in the percentage of circulating effector memory $CD8^+$ T cells ($CD45RA^-CCR7^-$), particularly those expressing CCR5 associated with CD161 at an intermediate level. The frequency of effector memory CD4+ T cells was also decreased while a compensatory increase of naive CD4 T cells ($CD45RA^+CCR7^+$) was observed. In parallel, the percentage of terminally differentiated effector memory ($CD45RA^+CCR7^-$) and central memory in $CD4^+$ and $CD8^+$ T cells ($CD45RA^-CCR7^+$) are not modified. Percentage of $CD8^+CD20^+$ T cells, a small subset of T cells containing a higher frequency of central memory cells than the $CD8^+CD20^-$ subset, was also reduced in Ocrelizumab treated patients. The frequencies of $CD4^+$ and $CD8^+$ Treg subsets and $CD8^+Perforin^+GranzymeB^+$ T cells were not modified. Additionally, we observed a significant increase in circulating $CD14^+$ monocytes. Given the pleiotropic functions of monocytes, in depth profiling of this population in currently under investigation.

Conclusion: Treatment with Ocrelizumab was associated with profound depletion of B cells. In addition, changes in the distribution of other immune subsets were observed shortly after treatment. Our findings suggest that treatment with Ocrelizumab may also directly or indirectly affect other immune cell subsets in the periphery. Further investigations are ongoing to understand this effect and the biological implications of these findings.

221 - The CD226 risk variant Gly307Ser associated with autoimmune diseases increases IFN-γ production by CD8 T cells

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The Gly307Ser (rs763361) single-nucleotide polymorphism (SNP) on CD226 has been identified as a risk factor for several inflammatory diseases, including Multiple Sclerosis (MS). To date, the cellular and molecular mechanisms whereby this variant affects the immune response remain to be addressed. The aim of our study was to gain further insight on the role of CD226 in CD8 T cells and on the impact of the amino acid change Gly307Ser on CD226 functions.
Peripheral blood mononuclear cell (PBMCs) from 32 age- and sex-matched donors were genotyped for rs763361 polymorphism and were analyzed by flow cytometry and mass cytometry for the distribution of different CD8 T cell compartments (naive, Temra, effector memory, central memory). In addition, the expression of T cell-related activation markers was investigated using high dimensional analysis to reveal differentially expressed clusters. CD8 T cells were purified and stimulated in vitro with anti-CD3 and anti-CD28 or anti-CD226 agonist mAbs and assessed by flow cytometry for survival, proliferation and cytokine production. Finally, IFNγ-related pathways (IFNGR, IL12R, Tbet and Eomes) were analyzed and the phosphorylation of STAT1 and STAT4 was quantified using phosphoflow.
Flow and mass cytometry analyses uncovered no major differences in the phenotype of PBMC between the two variants, except for the immune dysregulation of two clusters: $CD8^{+}CD45RA^{+}CD226^{+}CD27^{low}TIGIT^{low}EOMES^{low}$ and $CD8^{+}PD1^{+}CD226^{high}CD27^{high}TIGIT^{low}$. CD8 isolated from donors carrying the risk allele showed a selective increase of IFNγ production upon TCR or CD226 engagement, while the production of other cytokines (IL-17, IL-4, IL-2, TNF, Perforin, Granzyme B, GranzymeA) was similar to CD8 T cells carrying the protective allele. Moreover, CD8 T cells from donors carrying the risk allele presented an increased phosphorylation of STAT4, consistent with their increased production of IFNγ. The link between CD226 and STAT4 activation is currently under investigation. Understanding the impact of this MS-associated genetic variant may provide essential pathophysiological knowledge for driving selection of novel therapies.

226 - Retained efficacy with extended rituximab dosing intervals in relapsing-remitting multiple sclerosis: a Swedish single-centre observational study.

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B cell depletion with rituximab (RTX) is associated with high treatment efficacy in relapsing-remitting multiple sclerosis (RRMS), with low risk of rebound phenomena with treatment interruptions, but also increased risk of mainly bacterial infections. In order to reduce infection risks, further prompted by COVID19, RTX standard dosing intervals were extended up to 24 months in a single centre setting.
Aim of the study is to determine risk of disease flares in RRMS patients with extended RTX dosing intervals.
We retrospectively analysed all RRMS patients (n=719) treated with RTX included in two ongoing observational drug trials, COMBAT-MS (EudraCT 2016-003587-39) and MultipleMS (2017-002634-24). Data were extracted

from the Swedish MS registry for demographics, diagnosis, treatment history, clinical relapses and magnetic resonance imaging (MRI). Relapses and/or new MRI T2 lesions/contrast enhancing lesions, were recorded at first sign of clinical or MRI disease activity, or the lack thereof, at most recent visit/MRI for patients without signs of disease activity. The maximum interval (MI) between RTX doses before a registered outcome was used as a proxy for the efficacy of different treatment protocols.
Out of 697 patients with valid clinical data, 35 (5%) suffered a relapse, 19 of which occurred after the first RTX dose. Of the remainder, 13 patients had a relapse with a MI of 6±3 months (n=181, 7.2%), 3 with MI extended to 12±3 (n=313, 1%), and no relapse was found with MI of 18±3 or >21 months (n=140 and n=63, respectively). MRI activity was detected in 54/572 subjects (including 6 after first dose), of which 24/152 (15.8%) with MI 6±3 month, 16/244 (6.5%) with MI of 12±3 months, 9/116 (7.8%) with MI of 18±3 months and 5/60 (8.3%) with MI of >21 months. No major differences were found among the four groups concerning demographics, type of prior treatment and disability, except for a lower number of RTX doses in the 6±3 month MI group (mean number of RTX doses 3.3, 5.8, 6.8 and 6.1, respectively; ANOVA $p<0.001$). Flow cytometric B lymphocyte profiles and additional follow up data regarding treatment duration will be included in the final presentation.
Extending the RTX dosing intervals was not associated with signs of rebound disease activity in our study. Further studies are needed to establish if dose interval extension of B cell depleting treatments can improve the benefit-risk ratio regarding disease activity versus infections.

227 - MHC class I and MHC class II reporter mice enable the characterization of immune oligodendroglia

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Emerging evidence from mouse models and human MS brain tissue indicate that there are subsets of oligodendroglia that express antigen presentation transcripts in the setting of inflammation. Whether these immune oligodendroglia (iOPCs/iOLs) express MHC protein, present antigen, survive, differentiate and remyelinate is currently unknown. To facilitate the investigation of iOPCs/iOLs we generated two new reporter lines by targeting MHC class I beta chain, beta-2-microglobulin (B2m), and MHC class II-associated invariant chain (CD74). B2m-TdTomato and CD74-TdTomato reporter mice lines were generated by Crispr/Cas9 mediated replacement of the stop codon with a P2A-TdTomato-WPRE-pA transgenic sequence. B2m and CD74 reporter expression were characterized in MOG_{35-55} immunized EAE mouse brain and spinal cord. The percentage of oligodendroglia expressing MHC class I and MHC class II (Olig2 immunoreactive, TdTomato expressing cells), was significantly increased in MOG_{35-55} immunized mice with EAE score > 0 compared to pre-clinical score 0 mice in both brain and spinal cord of B2m-TdTomato and CD74-TdTomato animals. The severity of the EAE clinical score was significantly correlated with an increased percentage of TdTomato+ oligodendroglia in MOG_{35-55} EAE spinal cord in both B2m-TdTomato and CD74-TdTomato mice. On an individual animal basis, the level of inflammatory activity determined by the mean TdTomato gray value also significantly correlated with the percentage of TdTomato+ oligodendroglia. When comparing lesion to non-lesion spinal cord regions in individual animals, there was no significant difference in TdTomato+ oligodendroglia inside and outside of lesions. The majority of TdTomato+ oligodendroglia in MOG_{35-55} EAE spinal cord were not oligodendrocyte progenitors, as they were not immunoreactive to PDGFRa. B2m-TdTomato and CD74-TdTomato reporter animals are valuable new tools that enable the characterization of iOPCs/iOLs and other cells expressing MHC class I and MHC class II molecules. We found that oligodendroglial expression of MHC class I and MHC class II was increased with increasing levels of inflammatory activity and unexpectedly, we did not see an enrichment of iOPC/iOLs near spinal cord lesions, suggesting that CNS inflammation is widespread in this model. As TdTomato+ cells can be visualized in live animals, longitudinal *in vivo* imaging studies may help reveal the microenvironmental niche and fate of these subsets of immune oligodendroglia.

235 - STEMS: neural stem cells transplantation in progressive multiple sclerosis. A phase I study

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The pathophysiological mechanism underlying the progressive course of multiple sclerosis (PMS) is sustained by a low burning central nervous system (CNS)-confined chronic inflammatory process weakening self-repair properties and leading to neurodegeneration and irreversible disability. The lack of effective treatments for PMS represents an unmet need; innovative pro-regenerative treatment strategies should combine neuroprotection and immunomodulation. It has been variably shown that neural precursor cells (NPCs), once in vivo transplanted within the CNS in animal models of MS, could promote neuroprotection and remyelination by releasing molecules capable of immunomodulation, trophic support and neural plasticity. Here we present the result of the STEMS study, a prospective, not randomized, open label, single dose escalation phase I clinical trial, aimed at evaluating the feasibility, safety, and tolerability of intrathecally transplanted human fetal NPCs (hfNPCs) in 12 patients with PMS. Patients were divided in four treatment cohorts and received a single escalating dose of cells. Enrolled patients were under immunosuppressive treatment to prevent hfNPCs rejection and received adequate antibiotic and antiviral prophylaxis. To reduce hypersensitivity reactions, premedication with steroid was given immediately before the slow intrathecal infusion of hNPCs performed through a lumbar puncture. Follow up was conducted for 96 weeks after administration with clinical monitoring for survival, safety, tolerability and overall changes in the neurological status, assessed through neurological, neuroradiological and neurophysiological examinations. At the end of follow-up, no severe adverse reactions were reported. Further, cerebrospinal fluid (CSF) analysis of proteins, metabolites, chemokines, and cytokines - performed before and three-months after transplantation - showed significant changes. A shift towards a more anti-inflammatory profile was recorded for cytokines and chemokine while protein pathways' analysis showed a clear involvement of extracellular matrix and plasma membrane receptors suggesting a possible crosstalk between hfNPCs and blood-brain barrier. Although reserving judgment on the therapeutic effects, our study clearly showed that hfNSC therapy in PMS is feasible, safe and tolerable.

238 - The role of MHC-II in efficient OPC differentiation and remyelination

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Multiple sclerosis (MS) is an immune-mediated inflammatory disease of the central nervous system (CNS), characterised by demyelination, oligodendroglial loss and axonal injury. While there are no treatments targeting myelin repair, our lab has shown that regulatory T cells (Treg) can drive oligodendrocyte progenitor cell (OPC) differentiation and remyelination. The immune response mediated by these Treg is known to be mostly dependent on MHC-II, which plays a key role in antigen presentation and T cell activation. As MHC-II is also upregulated by microglia, astrocytes, and oligodendrocytes, and with the importance of microglia and astrocytes in myelin regeneration, we hypothesised that MHC-II is required for efficient OPC differentiation and remyelination. To test this hypothesis, we used *in vitro* pure OPC cultures and an *in vivo* model of lysolecithin-induced demyelination in WT and MHC-II-deficient mice. Surprisingly, we found that Treg cells significantly drive OPC differentiation independent of MHC-II *in vitro*–an effect that was comparable to that of MHC-II-expressing WT mice. Immunofluorescence staining of spinal cord sections containing demyelinated lesions revealed the absence of MHC-II does not significantly affect the number of oligodendrocyte lineage cells, proliferating OPCs and differentiated oligodendrocytes, but did impair the density of proliferating microglia and astrocytes. No

difference was observed between WT and MHC-II KO mice in the healthy spinal cord, nor was there any effect on myelin debris clearance prior to remyelination. There was also no effect on lesion size, indicating MHC-II deficiency does not increase the burden of demyelination. Together, these findings suggest a novel MHC-II-independent mechanism of Treg in driving efficient OPC differentiation, as well as a possible requirement for MHC-II in glial proliferation. Ongoing work is investigating whether MHC-II is required for remyelination *in vivo* and the mechanism(s) by which Treg function beyond what is classically known in regeneration.

Keywords: MHC-II; glia; remyelination.

239 - Modulation of Tr1 differentiation by a SNP associated to MS

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Multiple sclerosis (MS) is an auto-immune chronic inflammatory disease of the central nervous system. MS results from immune disruption due to a complex interaction between environmental factors and genetic predisposition. There is a central role for T cells with reduced T regulatory cell function. Costimulation of the complement regulator CD46 and T cell receptor promotes T cell activation and converts inflammatory Th1 cells into Type I regulatory T cells (Tr1), reducing interferon γ secretion and increasing interleukin (IL)-10 production. This Th1/Tr1 switch is defective in MS patients characterized by impaired production of IL-10 and increased production of IL-17. Furthermore, genome-wide analysis studies have highlighted a non-synonymous SNP in another costimulatory molecule CD226/ DNAM1 (rs763361), associated with risk for MS. This SNP has been shown to alter Foxp3 Treg suppressive activity, and CD4+ T cells carrying the risk allele produce significantly more IL-17 upon CD226 costimulation. However, CD226 is also a marker of Tr1 cells when co-expressed with LAG3 and CD49b, and its expression is increased on CD46-induced Tr1.

Our study aims to unravel the effect of coligation of CD46 and CD226 on T cell activation and to decipher whether expression of the risk allele of CD226 modulates the regulatory CD46 pathway. Our data suggest a crosstalk between CD46 and CD226 that regulates cytokine production and that T cells from healthy donors homozygous for the risk allele of CD226 have an altered Tr1 pathway characterized by reduced IL-10 secretion. These data highlight how a SNP associated to MS may participate to the inflammatory conditions observed in these patients.

257 - Inflammatory demyelinating milieu tailors transplanted neural precursor cell differentiation to oligodendrocyte fate after lumbar intrathecal delivery

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Background: Transplantation of neural precursor cells (NPCs) has emerged as a potential therapeutic approach for neurodegenerative diseases like multiple sclerosis (MS). In this context, the epicenter of many studies conducted on the experimental autoimmune encephalomyelitis (EAE), has been the determination of the optimal conditions and routes for the NPCs delivery. Objective: This study is the first to underline the potential for migration and differentiation of NPCs toward an oligodendrocyte lineage, following minimally invasive lumbar intrathecal NPCs administration in chronic EAE. Methods: MOG_{35-55} EAE was induced in 3 experimental groups of C57BL/6 mice. The first group (n=13) received 10^6 GFP^+ NPCs-aggregated neurospheres in phosphate buffer (PBS), the second (n=10) was injected with PBS and the last one consisted of the sham-operated control animals (n=5). In every case, a bolus of NPCs was administered through the cauda equina-occupying lumbar cistern at the pre-clinical phase which was 8 days post induction (dpi) of EAE. Routine histopathology stains and

immunohistochemistry were conducted to evaluate the disease progression and triple immunofluorescence to detect spatial migratory and differentiation patterns of the cells. Results: Significant improvement of the clinical course (50 dpi score <1, p = 0.004) and lower disease burden (area under the curve (AUC); 56.33±5.01) were observed in the NPC-treated mice, after an acute clinical relapse. Decrease in demyelination ($p < 0.01$), axonal loss ($p < 0.05$) and immune cell populations were additionally recorded. GFP^+ NPCs migrated within the subarachnoid space of the spinal cord and brain (mean distance 39.68±2.93mm; max 91.13mm). Two groups of NPCs were detected; the less differentiated subarachnoid NPCs and the noticeably differentiated parenchymal, that were predominant within the lower lumbosacral white matter fascicles. Both NPCs groups expressed mature oligodendrocyte markers such as MBP, Olig2, Nogo-A and BCAS1 (15-25%) and very low percentages of neuronal antigens, thus favoring the oligodendrocyte lineage in situ. Conclusion: Our research suggests that lumbar transplantation of NPCs contributed to EAE amelioration by promoting oligodendrocyte differentiation in the otherwise demyelinated loci, suggesting that intrathecal lumbar administration may be a plausible therapeutic intervention in EAE.

261 - ApoA-I mimetic peptide 5A boosts remyelination by promoting myelin debris clearance

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Failure of remyelination underlies the progressive nature of demyelinating diseases. Recently, we and others demonstrated that impaired remyelination ensues in part due to a dysfunctional innate immune response in the central nervous system (CNS). Sustained accumulation of myelin-derived lipids and formation of lipid droplets, combined with an inability to process and export these lipids, was found to induce a disease-promoting phagocyte phenotype. Here, we find that the apoA-I mimetic peptide 5A, a molecule well-known to promote the stabilization and activity of the lipid efflux transporter ABCA1, markedly enhances remyelination in the cerebellar brain slice and cuprizone models. Guided by immunohistochemical and lipidomics analysis, the pro-regenerative impact of peptide 5A was attributed to increased uptake of remyelination-inhibiting myelin debris through the fatty acid translocase CD36. On a transcriptional level, peptide 5A controlled CD36 expression through the ABCA1-JAK2-STAT3 signalling pathway. Collectively, our findings indicate that peptide 5A promotes the induction of a repair-permissive environment by stimulating the clearance of inhibitory myelin debris, potentially having broad implications for therapeutic strategies aimed at promoting remyelination.

284 - Characterization of BTK expression in lesions of multiple sclerosis and its animal models

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The depletion of B-cells by anti-CD20 monoclonal antibodies has strong efficacy in multiple sclerosis (MS). Recent B-cell targeting clinical trials have focused on the inhibition of B-cell signalling through Bruton's tyrosine kinase (BTK), a non-receptor tyrosine kinase that mediates B-cell activation and maturation. The interest in BTK inhibitors in MS has intensified by the recognition that BTK is expressed by and may mediate the activity of mononuclear phagocytes including microglia. However, the spatial localization and extent of BTK and activated BTK (pBTK) expression in lesions of MS and its models are not well characterized despite the interest in the field. Thus, we hypothesized that BTK and pBTK would be persistently elevated in microglia/macrophages in CNS lesions of MS and its models. Using immunohistochemistry, we determined that there is a widespread expression of BTK and pBTK in active lesions of MS, corresponding predominantly to Iba1-expressing microglia/macrophages by Imaris 3D-rendering of co-localization. In spinal cord lesions inflicted by the local injection of oxidized phosphatidylcholine (OxPC) or lysolecithin, BTK and pBTK are localized to lesions with a high density of CD68-

positive microglia/macrophages and not in the normal-appearing white matter (NAWM). In the experimental autoimmune encephalomyelitis (EAE) model in mice during peak clinical severity, BTK and pBTK signals are widespread in the spinal cord but are restricted to lesions compared to NAWM. BTK and pBTK do not appear to associate extensively with astrocytes in the mouse specimens. Our results show that BTK and pBTK are not detected in NAWM, but upon injury, microglia and macrophages recruited to the insult upregulate and maintain their expression in lesions.

313 - Challenges in the Context of Inducing Immune Tolerance in Multiple Sclerosis

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Multiple sclerosis (MS) is considered a prototypic organ-specific autoimmune disease that affects the central nervous system of young adults and particularly women. MS has a complex etiology involving genetic and environmental risk factors. The pathomechanisms of MS, although not completely understood yet, are likewise intricate, with autoreactive CD4+ T cells, proinflammatory B cell and other immune cell types playing a role, and current evidence supports the concept that breakdown of self-tolerance is a key event in MS pathogenesis. Existing therapies for MS are mainly immunomodulatory and/or immunosuppressive and are therefore not devoid of side effects. Thus, the induction of antigen-specific immunological tolerance represents an important therapeutic goal in MS. However, tolerance induction still faces several difficulties. A sound knowledge of the target antigens, the underlying pathomechanisms of the disease, and the presumed mechanism(s) of action of the tolerance-inducing approach is essential for a successful translation of such strategies. Equally challenging is to demonstrate mechanistic proof-of-concept of tolerance-inducing interventions, as the optimal assays to unequivocally assess the induction of immune tolerance and that should be performed along clinical trials are still required. In particular, the optimization of sensitive, robust methods allowing: 1) a thorough phenotypic characterization of the immune compartment; 2) the assessment of low frequency autoreactive T cells and the long-term reduction or change of their responses; 3) the detection of regulatory cell populations and their immune mediators, and 4) the validation of specific biomarkers indicating reduction of neuroinflammation and damage, is a key aspect that need to be mastered to develop tolerance-inducting approaches that can be safe and successfully delivered to patients.

330 - No evidence of rebound MS-disease activity with presence of CD20+T cells at B-cell reconstitution after rituximab

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In the last decade, the increased use of anti-CD20 treatment in patients with multiple sclerosis (pwMS) has been highly effective in reducing relapse rates and new inflammatory disease activity. A growing amount of evidence has implicated CD20+ Tcells as the disease pathogenic cells and possible targets of anti-CD20 agents.

We characterised CD20+ Tcells using flow cytometry in a cohort of pwMS with blood sampling 437±214 days after anti-CD20 treatment (Rituximab, n=47; Ocrelizumab, n=4). We neither found a correlation between time since last dose and levels of CD20+ T cells, nor differences in CD20+ T-cell percentages in anti-CD20 treated pwMS as compared to other disease modifying treatments (DMT, n=18) and healthy donors (HD, n=15). In addition, independently of the treatment, approximately 40% of CD20+ Tcells displayed a CD4+ phenotype enriched for memory cells. No evidence of clinical rebound disease activity was observed in the cohort despite presence of CD20+ T cells.

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NR, KAH, CSC, MK, FAN have nothing to disclose.

334 - TGFβ-induced upregulation of PD-1 in Tregs is impaired in multiple sclerosis

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Programmed death protein-1 (PD-1) is expressed on B and T cells and regulates the function of these immune cells. Upon interaction with one of its ligand PD-L1 or PD-L2, PD-1 inhibits TCR/CD3 signalling. Furthermore, PD-1/PD-L1 engagement induces metabolic changes that result in the formation of peripherally induced regulatory Tregs (iTregs) rather than effector T cells. PD-L1 can induce Tregs in vitro also enhancing their immune-suppressive function. The generation of iTregs is further dependent on TGFb. Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) that is mediated by a dysregulated immune system. It can lead to severe neurological issues and is one of the major causes for disability in young adults. Tregs play an important role in controlling autoimmune disease and have been shown to be defective in MS patients. To understand the role of PD-1 on Treg development in MS, PBMCs of MS patients and healthy controls were activated by CD3/CD28 stimulation and differentiated into Tregs (induced Tregs/iTregs) by the addition of TGFb, IL-2 and all-trans retinoic acid. We could show that TGFb upregulates PD-1 on in vitro generated iTregs from both MS-patient as well as healthy control PBMCs in a dose-dependent manner. MS-patient iTregs were much less able to upregulate PD-1 upon stimulation with Treg-inducing cytokines compared to healthy control PBMCs indicating a potentially impaired TGFb-signalling in MS-Tregs. Indeed, in previous studies our group found that a number of miRNAs targeting the TGFb-pathway is upregulated in naïve CD4+ T cells of MS-patients compared to healthy controls leading to a dysregulation of TGFb-signalling. Inhibition of a combination of some of the upregulated miRNAs prior to iTreg induction of PBMC of SPMS and PPMS patients led to an increased expression of PD-1 implying a role of the miRNAs in the impaired upregulation of PD-1 in iTregs of MS patients. Through the impairment of the PD-1/PD-L1 axis, a dysregulation in TGFb signalling could further impair iTreg development and function in MS contributing to the pathology of the disease.

346 - IgD$^-$CD27$^-$ double negative B cells of multiple sclerosis patients have a pro-inflammatory migratory phenotype and function

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INTRODUCTION: Pro-inflammatory age-associated immunoglobulin (Ig)D$^-$CD27$^-$ double negative (DN) B cells are abnormally elevated in the peripheral blood and cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients. Recently, we reported that DN B cells resemble mature class-switched memory (CSM) B cells. This study aimed to investigate the migratory phenotype and function of DN B cells in MS. **METHODS:** Expression of pro-inflammatory chemokine receptors C-X-C chemokine receptor (CXCR)3 and CXCR5, cell adhesion molecules lymphocyte function-associated antigen 1 (LFA-1), very late antigen 4 (VLA-4) and activated leukocyte cell adhesion molecule (ALCAM) and the activation marker CD80 was measured on DN, IgD$^-$CD27$^+$ CSM and IgD$^+$CD27$^-$ naive B cells in both CSF of newly diagnosed MS patients (n=3) and peripheral blood of healthy controls (HC, n=25) and MS patients (n=53) by flow cytometry. Using an *in vitro* chemotaxis assay, migration of MS (n=7) B cell subsets towards the chemokines CXCL10 or CXCL13 was studied. Expression of the T-box transcription factor T-bet, previously described in another pathological age-associated B cell subset, was measured on B cell subsets of HC (n=25) and MS patients (n=49). **RESULTS:** Peripheral blood DN B cells showed the highest T-bet expression compared to naive and CSM B cells. Furthermore, DN B cells showed similar expression of CXCR3 and CXCR5 compared to naive and CSM B cells, respectively. Expression levels of LFA-1, VLA-4 and ALCAM on circulating DN B cells were intermediate

between those of naive and CSM B cells. The majority of DN B cells in peripheral blood and CSF expressed LFA-1, VLA-4 and ALCAM. Preliminary results showed increased frequencies of $CXCR3^+$ and $CD80^+$ DN B cells in CSF ([22-82%] and [22-75%], respectively) compared to paired peripheral blood ([2-19%] and [3-12%], respectively), whereas the frequency of $CXCR5^+$ DN B cells was decreased in CSF ([38-64%] and [80-88%], respectively). In addition, MS DN B cells showed a high migration capacity towards CXCL10 (CXCR3 ligand) and CXCL13 (CXCR5 ligand) that was similar to CSM B cells and higher than that of naive B cells. **CONCLUSION:** DN B cells presented with a migratory phenotype in favour of migration towards the central nervous system in MS. The high migration capacity of DN B cells towards pro-inflammatory chemokines *in vitro* and the activated phenotype of CSF DN B cells further underlie the potential importance of DN B cells in MS pathology.

347 - Characterisation of the innate and adaptive immune system after ocrelizumab treatment in multiple sclerosis

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BACKGROUND AND AIM: B cell depletion by the anti-CD20 antibody ocrelizumab (OCR) is a highly effective treatment for relapsing-remitting (RR) and primary progressive (PP) MS. This study aimed to investigate the phenotypic changes of the immune system after OCR treatment in both RRMS and PPMS patients over time. **METHODS:** Peripheral blood mononuclear cells (PBMC) were collected of 18 RRMS and 22 PPMS patients before, 6 months (m) and 12m after OCR initiation. For 2 out of 18 RRMS patients, additional PBMC were collected before the next OCR infusion that was delayed with 3m due to the COVID-19 pandemic. Clinical characteristics at baseline were for RRMS and PPMS patients on average, respectively: age – 42 and 48 years, sex – 61 and 41% female, EDSS score – 3 and 5 and disease duration – 9.6 and 5.2 years. In-depth immunological phenotyping was done by flow cytometry using a sophisticated panel of 29 markers. Subsets of B cells, T cells, monocytes, dendritic cells (DC) and natural killer (NK) cells were analysed. **RESULTS:** For all patients, the frequency of $CD20^+$ B cells was strongly reduced after 6 (0.8%) and 12m (0.6%) of OCR compared to baseline (12.0%). The frequency of naive (IgD^+CD27^-) B cells was reduced, whereas transitional ($CD24^{++}CD38^{++}$), double negative (IgD^-CD27^-) and class-switched memory (IgD^-CD27^+) B cells were increased after 6 and 12m of OCR. Delaying the 18 mo dose with 3 mo increased the frequency of B cells in both RRMS patients (from 0.01% to 2.4% and 0.04% to 3.9%) with an enhanced repopulation of naive B cells. For both RRMS and PPMS patients, $CD3^+CD20^+$ T cells completely disappeared after 6 and 12m of OCR treatment, whereas the frequency of total $CD4^+$, $CD8^+$ and regulatory T cells was not affected. No differences were observed in the frequency of inflammatory monocytes ($CD14^+CD16^+$), plasmacytoid DC ($CD123^+CD11c^-$) and myeloid DC ($CD123^-CD11c^+$/$CD123^-CD11c^-$) after 6 and 12m of OCR. Interestingly, the number of $CD20^+$ NK cells ($CD56^{dim}CD16^+$/$CD56^{high}CD16^-$) was reduced after 6 and 12m of OCR. For all immune cell subsets, no significant difference between 6 and 12m was observed. **CONCLUSION:** Besides depletion of $CD20^+$ B, T and NK cells, OCR treatment induced changes in the distribution of B cell subsets in both RRMS and PPMS patients. Understanding the effect of OCR on the distribution of innate and adaptive immune cell subsets in MS will contribute to unravelling their role in MS pathology.

Immunopsychiatry: is it a nascent field?

8 -Association study between the *TLR1, TLR2* and *TLR6* genes and schizophrenia in Mexican patients.

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Introduction: Schizophrenia is considered a complex and chronic psychiatric disorder characterized for positive, negative, and cognitive symptoms. Alterations in the expression of toll-like receptors (TLRs) and immunological markers have been reported in patients with schizophrenia. TLRs are responsible for initiating the innate immune response (Chen et al., 2019). It has been proposed that polymorphic variants of the *TLR1, TLR2* and *TLR6* genes could be associated with alterations in expression levels in patients with schizophrenia (Kozlowska et al., 2018). Objective: Analyze the presence of association between the *TLR1, TLR2* and *TLR6* gene polymorphisms and schizophrenia compared with a control group. Methods: The study included 300 patients diagnosed with schizophrenia according to DSMIV-R and DSM-5 criteria and 300 healthy participants. Genotyping was performed using allelic discrimination with TaqMan probes by real-time PCR for *TLR1* (rs4833095, rs5743596, rs4833093), *TLR2* (rs3804099, rs7656411, rs5743709) and *TLR6* (rs5743810, rs3775073, rs5743827) gene polymorphisms. Statistical analysis of genotypes and alleles was performed by chi-square. Linkage disequilibrium (LD) was obtained using the Haploview program and haplotype analysis using the THESIAS program. Gene-gene interaction analysis (GXG) was carried out using the MDR program. Results: We found association between rs4833093/TLR1 (χ2=12.3, 2 gl, p=0.0021), rs5743709/TLR2 (χ2=23.8, 2 gl, p=0.0001) and rs3775073/TLR6 (χ2=12.6, 2 gl, p=0.0018) polymorphisms and schizophrenia. Haplotype analysis showed a high frequency of AT of TLR2 gene in patients with schizophrenia compared with control group. GXG analysis found an epistatic effect between *TLR1* and *TLR2* genes, increasing 2.72 times the risk of developing schizophrenia. Conclusion: Our findings support the evidence suggesting that TLR1, TLR2 and TLR6 genes are involved in the developing of schizophrenia.

83 - Autoantibodies, routine and novel markers of neuroinflammation in people with atypical psychiatric disease

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Background

Increasingly, the immune system is recognized as participating in the pathophysiology of psychiatric disease. There is renewed interest in biomarkers identifying immune activation.

Methods

Patients with atypical psychiatric (AP) disease (both psychotic and mood-related) were referred to a research immunology clinic by their psychiatrist. In these patients, we measured serum and cerebral spinal fluid (CSF) autoantibodies with other routine and novel markers of neuroinflammation, including CSF cytokine estimation and compared with cohorts of non-inflammatory (NI) controls, viral infectious controls and patients with autoimmune encephalitis.

Results

Thirty-five AP patients were enrolled (29 females; 6 males), alongside 17 NI controls. Six AP patients had first episode psychosis, 7 had depression, 3 had schizophrenia. Others had a mix of both psychotic and mood disorder features, making their disease difficult to classify. Ten patients had a history of another autoimmune condition and 11 had a family history of autoimmunity.

Low-mid titre (1:80-1:640) anti-nuclear antibodies (ANAs) without associated positive extractable nuclear antigen antibodies (ENA) were noted in 20 patients. The predominant pattern was speckled (13/20, 65%); other patterns were mitotic spindle apparatus (2) or homogenous and speckled (2). One patient had a high titre 1:2560 speckled ANA associated anti-SSa/Ro60 antibodies and also high thyroperoxidase antibodies (900IU/mL; normal < 35IU/mL). Six patients had raised anti-thyroglobulin antibodies and 6 patients had raised thyroperoxidase antibodies. Two patients had an elevated thyroid stimulating hormone (TSH).

No onconeuronal or limbic encephalitis antibodies were identified on serum or CSF. Two patients had CSF pleocytosis (>5 monocytes), 4 had raised CSF protein (>0.45g/L), 3 had CSF restricted oligoclonal bands and 11 had raised CSF neopterin (>20nm/L). CSF cytokines: Granulocyte-macrophage colony-stimulating factor, and interferon gamma were associated with psychiatric disease when compared to NI controls. Eleven patients had at least one CSF cytokine at a level beyond 4 standard deviations of the NI cohort.

Conclusion

In this cohort, there is a subset of patients with serum and/or CSF findings suggestive of immune activation. This data supports more extensive investigation of patients presenting with psychiatric disease across a broad diagnostic spectrum. Further study in a larger cohort is needed to validate these results.

Metabolism in Neuroimmunology

14 - Oxygen levels affect astrocyte homeostasis and immune functions

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There is increasing evidence that astrocytes play an essential role in the pathogenesis of multiple sclerosis (MS) and other neuroinflammatory conditions. MS is characterized by inflammation in the CNS, which results in demyelination and injury of axons. Astrocytes have the potential to contribute to MS disease progression by producing factors that activate or attract immune cells or are toxic to neighbouring cells, and by inhibiting remyelination and axonal regeneration. Astrocytes may also have a beneficial role, as they may protect and support oligodendrocytes and neurons. One method by which the function of astrocytes is studied, is by the use of cell cultures. When using cell cultures, it is vital that the conditions in which cells are grown mimic the environment from which they were extracted. However, in conventional cell culture the amount of oxygen that cells encounter is almost four times higher (21%) than what they encounter in the brain (3-6%). Previous studies have indicated that astrocytes are sensitive to changes in oxygen levels. Therefore, using a variable oxygen control incubator, in which oxygen concentrations can be controlled, we investigated how astrocytes behave when cultured in oxygen conditions similar to what they encounter in the brain, compared to atmospheric oxygen levels. We found that culturing astrocytes at 3% oxygen results in changes in astrocyte morphology and proliferation rate. In addition, astrocytes cultured at 3% oxygen level are less reactive and repopulate scratch insults less efficiently than those cultured at 21% oxygen. Moreover, at 3% oxygen, astrocytes display lower expression of immunoregulatory molecule CD40 and produce less IL-6, both under resting conditions and when stimulated by either LPS or IL1α/TNFα/C1q. Our results indicate that oxygen levels impact some of the functional readouts of in vitro astrogliosis and that astrocytes display a generally less reactive phenotype when cultured at physiological oxygen levels, compared to atmospheric oxygen levels. This highlights the importance of taking oxygen concentrations into account when studying reactive astrocyte responses. Moreover, considering that hypoxia is associated with MS disease progression, these findings set the stage for further studies into the effect of hypoxia on astrocyte functions in neurological disorders such as MS.

25 - Systemic metabolic activation by cold exposure modulates circulating monocytes and attenuates neuroinflammation

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Obesity is linked to development of metabolic and inflammatory diseases. However, effects of a negative energy balance and a metabolically active phenotype on the immune system and immune-mediated diseases are poorly understood. Here we use cold exposure as an inducer of energy expenditure, which mainly acts by activating the UCP1-mediated brown adipose tissue thermogenesis. We show that cold exposure modulates monocytes and consequently T cell priming, resulting in decreased disease severity in a mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Specifically, we found that cold exposure reduces monocytes in the bone marrow and changes their immunologic and metabolic phenotype in the circulation. Exposure to cold temperatures decreases the EAE severity independent of UCP1-mediated thermogenesis. Cold exposure reduces pathogenic T cell cytokine expression and MHCII expression of monocytes during EAE. Depleting the monocytes via genetic or pharmacological CCR2 blockade abolished T cell cytokine expression at EAE onset, implying that cold exposure may affect T cell priming via modulation of monocytes. Accordingly, EAE is unchanged when cold exposure is applied only during the effector phase of the disease. Our work provides systematic overview on the immune changes during exposure to cold and could have implications in prevention and treatment of immune-mediated diseases.

87 - Bcl6 controls meningeal Th17 cell-B cell interaction in murine neuroinflammation

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B cell-rich ectopic lymphoid tissue (eLT) can develop in the meninges of chronic multiple sclerosis (MS) patients and correlates with disability in chronic MS. In eLT, T cells promote B cell differentiation and class-switching. We hypothesized that the transcription factor Bcl6 in CD4+ T cells would be a promising candidate to control meningeal interactions between Th17 cells and B cells because it enables T cells to induce B cell differentiation and class-switching and exacerbates two variants of experimental autoimmune encephalomyelitis (EAE). To further characterize such Th17 cell-B cell interactions in the central nervous system, we utilized the adoptive transfer EAE (AT-EAE) model. We transferred myelin-specific Th17 cells from CD4*Cre*Bcl6*fl/fl*2D2*tg* mice (Bcl6-deficient) and Bcl6-competent (Bcl6+) 2D2*tg* littermates into recipient mice. We observed that Bcl6-deficient Th17 cells induced significantly less severe AT-EAE than Bcl6-competent Th17 cells. More abundant B cell-rich eLT developed in the meninges after transfer of Bcl6-expressing Th17 cells. Thus, our data suggest that Bcl6 expression in Th17 cells promotes the accumulation of meningeal B cells. We additionally performed a transcriptional characterization of meningeal versus parenchymal leukocytes at single-cell level in the spinal cord of AT-EAE recipient mice of Th17 cells from both genotypes. In this characterization of leukocytes in the inflamed spinal cord, we found B cell infiltrations exclusively in the meninges and a CD4+ T cell-dominated inflammation in

the parenchyma. Furthermore, we identified class-switching to the isotypes IgG1, IgG2b and IgG3 and an increased proliferation of meningeal B cells both controlled by Th17 cells in a Bcl6-dependent manner. We thus demonstrate that meningeal B cell accumulation, differentiation and class switch depended on Bcl6 expression in Th17 cells.

119 - Dimethyl fumarate restricts antioxidative capacity of T cells and thereby controls T cell-mediated autoimmunity

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Dimethyl fumarate (DMF), an approved treatment for multiple sclerosis (MS), effects central nervous system (CNS) resident cells and immune cells. In order to investigate the molecular mechanism of DMF in different T cell subsets, we examined the metabolic effects in T cells and their potential link to cellular stress-response leading to apoptosis. Here, we show that DMF treatment of human T cells causes a strong alteration of the metabolic profile and restrains their antioxidative capacities by decreasing intracellular levels of the reactive oxygen species (ROS) scavenger glutathione. This leads to an increase in mitochondrial ROS levels accompanied by an elevated mitochondrial stress response. These enhanced ROS levels in T cells not only resulted in increased apoptosis, but were crucial for proliferation and proinflammatory cytokine production. These changes were responsible for the well-known DMF-mediated amelioration of CNS inflammation in a mouse model of MS. Indeed, DMF immune-modulatory effects on T cells were abrogated by pharmacological interference of ROS production. By analyzing samples from MS patients before and during DMF treatment, we could prove that DMF restricts the metabolism of activated T cells thereby facilitating apoptosis and limiting proinflammatory responses. Together, these data indicate that DMF serves as an immune-metabolic drug targeting the antioxidative capacities of T cells thereby modulating their immune function. In addition, these results provides novel insight into the role of oxidative stress in modulating cellular immune responses and T cell-mediated autoimmunity.

137 - Craniotomy surgical damage elicits inflammatory changes and alters leukocyte metabolism to promote Staphylococcus aureus biofilm infection

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Craniotomy is a neurosurgical procedure involving removal and replacement of a skull fragment (bone flap) to access the brain. Despite precautions, 1-3% of craniotomies are complicated by infection, about half due to *Staphylococcus aureus* forming a biofilm on the bone flap. Infections carry substantial morbidity, often requiring the bone flap to be discarded concomitant with long-term antibiotic therapy. This study aimed to understand how the release of damage-associated molecular patterns (DAMPs) following a craniotomy contributes to biofilm formation and infection persistence. A mouse craniotomy model was used to compare spatial and temporal

attributes after sterile sham surgery vs. *S. aureus* infection. Flow cytometry revealed differential leukocyte influx into the brain, subcutaneous galea, and bone flap. Monocytes were the major population recruited to the brain, whereas granulocytic myeloid-derived suppressor cells (G-MDSCs) and neutrophils (PMNs) were most numerous in the galea and bone flap. These patterns were similar for sham and infected mice at day 3 post-surgery, even in the presence of significant bacterial burden (10^5-10^6 CFU) suggesting that surgical damage alone is a major driver of the acute immune response. However, divergence was seen at later time points (days 7-28), notably in the galea, where monocytes were enriched in sham animals but G-MDSCs remained elevated in the infected group. Profiling single-cell metabolism *ex vivo* with SCENITH, G-MDSCs and PMNs in the brain and galea displayed a high glucose dependence in both sham and infected mice, whereas glycolytic capacity was increased only during infection. Although pro-inflammatory cytokine production (IL-1β, TNF-α, and IL-6) was significantly increased in tissues of infected vs. sham animals, this was not sufficient to prevent biofilm formation. Furthermore, *in vitro* metabolic analyses of microglia co-cultured with live *S. aureus* biofilm showed a preferential upregulation of oxidative phosphorylation compared to glycolysis, suggesting that biofilm elicits an anti-inflammatory phenotype that promotes infection persistence. Collectively, these results suggest that DAMPs released from damaged tissue following craniotomy mask infection to induce a maladaptive inflammatory response promoting biofilm formation. The biofilm can then metabolically reprogram microglia leading to its persistence. Supported by the NIH National Institute of Neurological Disorders and Stroke (R01NS107369).

209 - BETA 2 ADRENERGIC RECEPTOR MODULATES METABOLIC REPROGRAMMING IN MACROPHAGES

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Macrophages are a very heterogeneous cell population that resides within tissues and are responsible for the maintenance of tissue homeostasis. Upon tissue infection, macrophages are promptly activated and start to orchestrate the innate immune response, mediated by the production of chemokines and cytokines that will attract and activate other immune cells. Metabolic reprogramming, which provides fast ATP production and metabolic mediators that possess the capacity to drive inflammation, is an essential process for macrophages to acquire a pro-inflammatory phenotype. Neuro-immune interactions occur in the majority of the tissues and can modulate immune responses by different mechanisms. Catecholamine produced by sympathetic nervous fiber binds to adrenergic receptors expressed by immune cells and induces different outcomes. Although there is an increase in the studies regarding adrenergic modulation on immune effector functions, little is known about how adrenergic receptors can modulate the metabolic reprogramming of macrophages. By treating bone marrow-derived macrophages with fenoterol, a beta 2 adrenergic receptor (B2AR) agonist, we showed that B2AR engagement induces downregulation in the gene expression of Nox1 and Inos, essential enzymes for the production of reactive oxygen species (ROS). Accordingly, upon B2AR activation total ROS, and nitric oxide production also were diminished. The formation of mitochondrial ROS and lactate (a by-product of glycolysis) was also reduced, indicating that B2AR activation limits glycolysis. Mechanistically, B2AR sustains oxidative phosphorylation (OXPHOS) by reducing ROS formation, which compromises mitochondria function and favors glycolysis. Interesting, this process is independent of fatty acid oxidation. These data can explain why animals lacking B2AR exclusively in monocytes present worsen diseases progress during the mouse model of multiple sclerosis (EAE). Importantly, the influence of B2AR in TNFa production is independent of metabolic reprogramming.

213 - Alterations of blood cholesterol homeostasis does not affect inflammation and progression of experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is a chronic inflammatory and autoimmune disease of the central nervous system (CNS) leading to neuronal damage and invalidating neurological deficits. The etiology of MS is multifactorial there is evidences that environmental factors play a major role in disease causation. In line with this concept, obesity and elevated peripheral cholesterol levels has been associated with adverse MS outcomes. Studies show that lipid-lowering strategies such as statins exhibit benefits during MS and its experimental autoimmune encephalomyelitis (EAE) model. However, the outcomes of studies using statins in MS patients are contradictory, and it was uncertain that the cause of this benefit were actually dependent of lower serum cholesterol due to their pleiotropic immunomodulatory and neurotrophic effects. Thus, the role of cholesterol metabolism during MS remain unclear and largely debated and more research are needed to clarify the causal role of circulating cholesterol during the pathogenesis of MS. Using EAE model, we assessed the importance of circulating cholesterol by two different strategies: the genetic deletion of LDLr, which causes a significant increase of plasma cholesterol and the use of human PCSK9 inhibitors alirocumab that reduces specifically PCSK9-mediated LDLr degradation and consequently lowers total blood cholesterol concentrations. We show that changes in circulating cholesterol levels does not affect development of EAE disease nor modulate the adaptive immune responses during the development of CNS autoimmunity. Our results suggest the non-deleterious role of cholesterol in neuro-inflammatory diseases and point to the largely debated non-lipid related protective effects of statins in patients with MS.

270 - COL-3 and its parent compound doxycycline reduce microglial inflammation by limiting glucose-mediated oxidative stress.

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A number of studies, including ours, have shown that the response of brain microglial cells to inflammatory stimuli is partially fueled by glucose. Here, we wished to determine whether the anti-inflammatory effects of the non-antibiotic tetracycline COL-3 and its parent compound doxycycline (DOX) towards brain microglia result from an effect on glucose metabolism. For that, we used post-natal mouse microglial cells in culture activated by LPS or α-Synuclein amyloid aggregates (αSa) to model neuroinflammatory processes as they may occur in Parkinson disease (PD). Under LPS or αSa stimulation, COL-3 (10, 20 μM) and DOX (50 μM) efficiently repressed the expression of the microglial activation marker protein Iba-1 and the stimulated-release of TNF-α, a prototypic pro-inflammatory cytokine. The inhibitory effects of COL-3 and DOX on TNF-α were reproduced by dexamethasone and apocynin (APO), an inhibitor of the superoxide-producing enzyme NADPH oxidase. This last finding suggested that COL-3 and DOX might operate themselves by restraining oxidative stress-mediated signaling events. Quantification of intracellular reactive oxygen species (ROS) revealed that COL-3 and DOX were indeed as effective as APO in reducing oxidative stress and TNF-□ release in activated microglia. Interestingly, ROS inhibition with COL-3 or DOX occurred together with a strong reduction of microglial glucose uptake and NADPH synthesis. This indicated that COL-3 and DOX might reduce microglial oxidative burst activity by limiting glucose-dependent synthesis of NADPH, the requisite substrate for NADPH oxidase. Coherent with this possibility, the glycolysis inhibitor 2-deoxy-D-glucose restrained NADPH synthesis and reproduced the suppressive action of COL-3 and DOX in activated microglia. Thus, we propose that COL-3 and its parent compound DOX may exert anti-

inflammatory effects on microglial cells by inhibiting glucose-mediated ROS production. Overall, COL-3 and DOX may be of potential therapeutic interest for chronic CNS pathological conditions such as PD, typically characterized by inflammatory processes and oxidative stress-mediated reactions. Supported by France Parkinson [DOXYPARK, GAO 2018] and Capes-Cofecub [project #Me928/19].

280 - PINK1 modulates onset and severity of experimental autoimmune encephalomyelitis

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Background: Reactive oxygen species and oxidative damage are observed in normal aging brain but are exacerbated in many neurological pathologies such as Parkinson's disease and multiple sclerosis (MS). In MS, immune cell infiltration and glial cell activation cause persistent inflammation and intensify ROS production. Susceptible targets of ROS are the mitochondria of both neurons and oligodendrocytes. Oxidative damage causes, among other, mitochondrial mutations, protein and lipid oxidation and metabolic hindrance, ultimately leading to cellular death.
Objectives: We evaluated the impact of interfering with mitochondrial homeostasis through PINK1 deletion in the experimental autoimmune encephalomyelitis (EAE), a murine model for MS.
Methods: Active EAE was induced in mice deficient for PINK1, a major protein for mitochondrial homeostasis, and wild-type C57BL/6 mice. Clinical score was used to follow the severity of the disease. Spleen, lymph nodes and central nervous system of mice were analysed by flow cytometry to assess cellular infiltration and microglial activation. Demyelination and neuronal injury were assessed by immunofluorescence and serum neurofilament (sNfL) was quantified by SIMOA.
Results: Our result show that deletion of PINK1 plays a dual role in EAE, where it affects both the onset of neurological symptoms and their severity in the chronic phase. We found that in PINK1 KO mice, the onset of EAE is mildly delayed. In contrast, the severity of the clinical neurological deficits is higher during the chronic phase of the disease compared to wild-type controls. In the chronic but not the acute phase of the disease this was paralleled by an elevated level of biomarker of neuronal injury neurofilament light chain.
Conclusions: Our data suggest that PINK1 plays a dual role in the development of EAE in both male and females. Further studies will show how PINK1 deficiency influence demyelination and neuronal injury.

323 - Deoxycytidine kinase phosphorylation status and activity in activated T cells are affected by exposure to cladribine

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Activation of cladribine (2CdA), a drug approved for multiple sclerosis, is driven by a high ratio of deoxycytidine kinase (dCK)/5'nucleotidase. In view of their high dCK content, lymphocytes are preferential target for 2CdA. We demonstrated that the 2CdA-induced apoptosis in stimulated T cells is correlated with enhanced dCK expression and activity. Up to 16 dCK phosphorylation sites have been described to date but little is known about how they affect dCK activity. Our objective was to assess the differential composition of post-translational dCK isoforms in healthy donor T cells activated or not with anti-CD3/CD28 antibodies in presence/or absence of 2CdA. We used Phos-tag™ electrophoresis, which traps phosphorylated proteins thereby reducing their migration according to their phosphorylation status. Cell lysates treated with alkaline phosphatase were used to define the control band corresponding to de-phosphorylated dCK and this latter was much reduced in unstimulated cells. Lysates from activated T cells showed five separate areas of phosphorylated dCK isoforms. Areas were fewer in lysates from activated T cells exposed to 2CdA, with a profile that appeared specific to the treatment. As areas 4 and 5 were consistently observed in all samples tested and could be reliably measured, we focused our analysis on these two areas. Our data suggest that exposure to 2CdA results in a shifted composition of phosphorylated dCK isoforms, which might be related to the activity of the enzyme and thereby influence the susceptibility of activated T cells

to the drug. Further analysis of dCK phosphorylation status and activity in lymphocytes from 2CdA-treated multiple sclerosis patients will help understand the impact of 2CdA on pathological immune responses related to central nervous system autoimmunity.

344 - Ablation of TMEM97, a regulator of cholesterol homeostasis, improves functional recovery in the EAE model of multiple sclerosis

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Multiple Sclerosis (MS) is a CNS disease that affects more than 2.5 million people worldwide. It is an inflammatory demyelinating disorder in which failure of remyelination contributes to disability. For this reason, myelin repair has emerged as a key mechanism to be targeted for therapeutic purposes in MS. A major component of CNS myelin is cholesterol. Indeed, approximately 70% of the cholesterol within the CNS is found in the myelin sheaths produced by oligodendrocytes (OLs), highlighting the high demand of cholesterol in myelin biogenesis and maintenance. Although emerging evidence suggests that cholesterol synthesis and regulation pathways are affected in individuals with MS and in animal models of the disease, the implications of cholesterol dysfunction in MS are underappreciated. To shed light on how cholesterol homeostasis is disrupted in MS, here we investigate the role of the recently identified sterol sensor transmembrane protein 97 (TMEM97)/sigma-2 receptor. TMEM97 is an ER-resident protein that controls intracellular cholesterol trafficking from the lysosomes to various cellular compartments. It is transcriptionally regulated in low-sterol conditions, thus indicating its importance in maintaining cholesterol homeostasis. TMEM97 has been implicated in the pathophysiology of neurodegenerative disorders such as traumatic brain injury, Alzheimer's, Parkinson's and Huntington's disease, but its role in MS has never been addressed. In this study, we induced TMEM97 germline knockout mice with MOG_{35-55} experimental autoimmune encephalomyelitis (EAE), a model of MS, and observed a significant improvement of the clinical outcome compared to wild-type control mice. Consistent with these data, *TMEM97*$^{-/-}$ mice showed fewer spinal cord demyelinating lesions, less gliosis, and significantly more surviving OLs than control mice. These data suggest that TMEM97, by modulating cholesterol function, may play a detrimental role in EAE, warranting further analyses. Cholesterol is essential for multiple functions, including synaptogenesis, axonal guidance, dendrite formation, myelin biogenesis, and synaptic plasticity. As a result, cholesterol dysfunction can exacerbate CNS disease. Understanding the role of cholesterol regulation in disease conditions is critical and could pave the way for novel therapeutic strategies for MS patients.

362 - Decreased cortical pro-inflammatory cytokines after oral administration of silymarin in obese mice

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Obesity is a chronic low-grade systemic inflammatory state that is the sixth leading cause of death worldwide. Obesity gradually activates the immune system, inducing a constant release of pro-inflammatory cytokines in low amounts produced in part by the visceral adipose system, leading to the activation of inflammation pathways and contributing to severe complications such as insulin resistance. Obesity-associated systemic inflammation causes increased blood-brain barrier permeability, glial activation, and secondary neuroinflammation, risk factors for developing other brain pathologies. Among the brain structures affected with neuroinflammation associated with

obesity are the cortex, hippocampus, and brain stem. Several neuroprotective drugs have been tested to reduce neuroinflammation secondary to obesity. One of them with promising results is silymarin, a complex of flavonolignans from Silybum marianum, which has anti-inflammatory effects. Male mice received a high-fat diet (HFD) for 12 weeks to evaluate how oral silymarin administration modulates obesity-induced neuroinflammation. Bodyweight was monitored weekly, fasting glucose and intraperitoneal glucose tolerance test were determined after 12 weeks of diet and after 14 days of oral administration of 100 mg/kg of silymarin.
Brain TNFα, MCP1, and CX3CL1 levels before and after silymarin treatment were determined by ELISA.
The HFD group gained significantly more weight from the third week of the diet than the control group. Fasting glucose and the glucose tolerance test were significantly higher in HFD mice. After silymarin treatment, the mice lost significant weight compared to the beginning of the treatment.
The HFD group showed significantly higher cortical MCP1 values, which decreased after silymarin treatment. Although the cortical TNFα values were not substantially different between the untreated groups; however, silymarin administration reduced TNFα levels in both treated groups. Also, silymarin administration reduced fractalkine in the treated animals.
Overall our results show a relevant reduction of the cortical pro-inflammatory cytokines, also improving glucose metabolism of obese mice.
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Modeling neuroinflammation using IPSC, organoids and animal models

49 - Epigenetic regulation of microglial activation during Staphylococcus aureus craniotomy infection

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A craniotomy is a neurosurgical procedure involving the excision of a section of the skull (bone flap), providing access to the intracranial compartment for the treatment of conditions including brain tumors, epilepsy, or cranial bleeds. Despite prophylaxis, infectious complications after craniotomy range from 1-3%, triggering the need for at least one additional surgery and an extensive antibiotic regimen for treatment. Half of craniotomy infections are caused by Staphylococcus aureus (S. aureus), which forms a biofilm on the bone flap that evades immune-mediated clearance. During biofilm infection, immune cells enter a phenotypically distinct activation state typified by unique metabolic and transcriptional signatures. Previous studies have identified epigenetic mechanisms to be partially responsible for this activation phenotype. Epigenetic changes allow for rapid responses to external stimuli, and we hypothesized that S. aureus elicits epigenetic modifications in CNS immune cells to establish a counter-productive anti-inflammatory response that promotes bacterial survival during craniotomy infection. We designed a high-throughput in vitro screen of an epigenetic compound library using primary mouse microglia, which identified Bromodomain and Extraterminal domain (BET) family members and various histone deacetylases (HDACs) as critical for regulating microglial cytokine production in response to S. aureus. Additionally, in vivo experiments revealed a critical role for HDACs in modulating the host response during S. aureus craniotomy infection as revealed by increased bacterial burden in animals treated with the pan-HDAC inhibitor panobinostat. Collectively, these findings implicate acetylation mark homeostasis as an epigenetic mechanism for regulating craniotomy infection chronicity and outcome.

69 - Devising a growth medium for the recapitulation of a homeostatic profile in cultured human microglia

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Efforts to understand microglial function in health and diseases have long been hindered by the lack of culture models that recapitulate their *in situ* properties. Microglia isolated from the brain undergo transcriptional alterations, with downregulation of homeostatic markers. Previously, murine studies have evidenced the drastic

effect of serum in growth medium on microglia functionality. A chemically-defined serum-free medium that promotes the survival of murine microglia has been formulated. Yet, no such alternative for the maintenance of human microglia has been established. We aimed to characterize the impact of culture media on human microglia function and transcriptome in the hope of devising a formulation that can better recapitulate the homeostatic microglia signature. Human microglia were isolated from non-pathological, surgically resected brain tissues of epileptic patients by magnetic activated bead sorting of CD11b+ cells and cultured in a variety of growth media for RNA sequencing and functional assays. Viability assessment revealed that human microglia can be cultured in serum-free condition in the absence of growth-promoting factors such as M-CSF, provided that adequate supplements are present in the culture. The choice of growth media, and the presence of serum had a significant influence on the capacity of the cells to phagocytose myelin debris and to secrete cytokines following lipopolysaccharide treatment. A culture medium that promotes a highly phagocytic cellular phenotype was identified. The expression of key disease relevant classes of genes was heavily influenced in human microglia cultured under various media conditions relative to freshly isolated *ex vivo* microglia isolated from the same donors. Our study highlights the significant influence of culture conditions on the transcriptional and functional state of primary human microglia.

94 - Orally administered silybin improves most of the biochemical and behavioral outcomes in MPTP-induced parkinsonism murine model

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Parkinson's Disease (PD) is the second most frequent neurodegenerative disease with motor dysfunction secondary from lost dopaminergic neurons in the nigrostriatal axis in old patients. Actual choice therapy consists of levodopa; however, its long-term use promotes treatment resistance and secondary effects. Hence, it is necessary to find new therapeutic alternatives, such as neuroprotective agents. Among these alternatives is silymarin, due to its neuroprotective role by exerting its antioxidant, anti-inflammatory, anti-apoptotic properties, and its dopamine (DA) preserving effect in MPTP-treated mice. To elucidate the role of silybin (Sb), the primary bioactive compound in silymarin, in the neuroprotection of silymarin in the PD context, Sb was administered orally to determine dopamine levels, biochemical markers and behavior. Mice received 30 mg/kg of MPTP intraperitoneally for 5 consecutive days to induce the PD model. The same days, Sb was co-administered orally to evaluate its dose-dependent conservation of striatal DA at day 7 post-treatment. The best DA conservative dose of Sb was used to evaluate the Sb effect on biochemical context: BDNF, TNFα, IL10, lipid peroxidation, and mitochondrial reduction capacity. Sb's effect on bradykinesia, gross and fine motor skills, equilibrium, and muscle strength was evaluated using pole, traction, beam, and nest building tests. Results showed that oral Sb at 100 mg/kg dose conserved DA levels about 60%, higher Sb doses did not modify DA content, so 100 mg/kg was elected as the best dose to compare biochemical and behavioral tests. Sb preserved BDNF content, diminished TNFα to basal levels and reduced lipid peroxidation in the striatum and substantia nigra in the MPTP mice. Sb preserved mitochondrial function in substantia nigra of the MPTP group but had no effect in the striatum. Behaviorally, Sb-treated MPTP group improved turning down and landing behavior in the pole test, demonstrating better gross motor skills and reduced bradykinesia in mice. Fine motor skills improved on Sb-treated MPTP mice, demonstrated in the beam test, where mice reduced their relative error-index and higher escape ratio in beam and traction tests. Also, Sb improved equilibrium and muscle strength in MPTP mice. 100 mg/kg of Sb showed to be a potential alternative in PD treatment by exerting anti-inflammatory, antioxidant effects, BDNF and DA preservatory effects, and improving motor behavior in MPTP treated mice.

104 - Heterogeneity of cytokine responsive astrocytes during the course of experimental autoimmune encephalomyelitis

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Neurological diseases are often characterized by aberrant inflammation. Although there are benefits of inflammation in the central nervous system (CNS), uncontrolled neuroinflammation can produce negative effects. It is known that glial cells contribute to the onset and progression of neuroinflammation in neurological diseases. Astrocytes and microglia are involved in the inflammatory process by producing a number of immunological factors such as cytokines and chemokines. It has been observed that long term exposure to these factors can result in local tissue damage. However, it is unknown if all astrocytes are capable of responding to the same cytokines. To examine this, we used flow cytometry to quantify cell surface cytokine receptor expression on astrocytes from several regions of the CNS and during the course of experimental autoimmune encephalomyelitis (EAE). Our data show the sparse but dynamic expression of pro- and anti-inflammatory cytokine receptors such as IL-10R, TGFβRII, IFNγR, and IL-17R. Our data also indicates the IL-10 and TGFβ receptors are co-expressed by a distinct population of astrocytes. We hypothesize that there are subpopulations of astrocytes that mediate specific cytokine responses and these cells may play an important role in regulating neuroinflammation with distinct functions.

120 - A Novel 3D Model of the Blood Brain Barrier

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Rare neurological autoimmune diseases like aquaporin 4 (AQP4) seropositive neuromyelitis optica spectrum disorder (NMOSD) or myelin oligodendrocyte glycoprotein (MOG) antibody associated disease (MOGAD) are associated with autoantibodies targeting neuronal or glial antigens. Under physiological conditions, the highly selective blood brain barrier (BBB) prevents those antibodies from gaining access into the central nervous system (CNS). However, after immune-mediated activation, the BBB becomes permeable, thus facilitating the penetration of autoantibodies into the CNS. The underlying mechanisms are still not fully understood and human tissue culture models are urgently needed. Here, we report a novel 3D printed model of the BBB to investigate the migration of autoantibodies through the BBB and their pathogenic effects on glial cells. Specifically, a cell-laden hydrogel is 3D inkjet printed onto a laser cutter-fabricated chip containing either human astrocytoma cells (U373) expressing AQP4 or human oligodendrocytoma cells (MO3.13) expressing myelin oligodendrocyte glycoprotein (MOG). To resemble the BBB, microchannels coated with Geltrex as a basement membrane substituent and a layer of human umbilical vein endothelial cells (HUVEC), were introduced. Hydrogel composition and antigen expression of cells were optimized to reach good cell viability up to 8-12 days. Tightness was tested by dextran- and antibody-diffusion assays and observed under a fluorescence microscope. Dextran-diffusion assays showed tight HUVEC layer formation as most of the dextran remained inside the channels. Diffusion assays with fluorescent-labeled antibodies showed spreading into the hydrogel and specific binding of the target cells after insertion into uncoated channels. This promising model of the BBB could help elucidating pathological mechanisms and BBB function in neurological autoimmune diseases such as NMOSD and MOGAD. Further optimizations and experiments will include cell and extra cellular matrix component refinements, implementation of a perfusion system, introduction of other antibodies and cytokines and investigating the pathogenicity of autoantibodies to astroglial, oligodendroglial and neuronal antigens. This study is supported by the Austrian Science Funds (FWF, project P32699).

158 - Multimodal Imaging of Radiation-Induced Brain Injury – an Open Data Approach

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Radiation-induced brain injury following radiotherapy can be a severe side effect in neuro-oncology. It is defined by various pathological changes, like blood-brain barrier damage, neuroinflammation, and reduced neurogenesis. This, in turn, can cause long-term side effects such as cognitive decline, which impacts the patient's quality of life. Proton therapy is a novel radiation modality that reduces the dose delivered to the normal tissue. Nevertheless, there have been reports of brain lesions after proton therapy, indicating the need for a more profound understanding of the underlying cellular changes. To investigate the biological mechanisms behind radiation-induced brain injury, we followed the reverse translation approach and established a mouse model for proton irradiation of brain subvolumes. Subsequently, a dose-finding study was performed to evoke clinically relevant side effects, using a dose range of 40 Gy – 85 Gy. Every animal received a computed tomography for treatment planning, upon which a Monte Carlo dose simulation was calculated. Mice were observed for up to six months by longitudinal magnetic resonance imaging with final histopathological analysis. The histology included an H&E staining, as well as immunofluorescence imaging of the main brain cell types (neurons, astrocytes, microglia), myelin, the Nestin protein, and the proliferation marker Ki67. As next step, the dose simulation, all imaging data, and the DSURQE brain atlas were registered into a common coordinate system, allowing the fusion of all modalities with each other. This extensive data set enables a profound analysis of radiation injury on a cellular level and a correlation of these changes to clinically relevant medical imaging. To exploit the full potential of these experiments, we made all imaging data publicly available to the scientific community under CC BY 4.0 ("Slice2Volume: Fusion of multimodal medical imaging and light microscopy data of irradiation-injured brain tissue in 3D."; https://rodare.hzdr.de/record/915). Our Open Data approach can support answering current and future research questions. For example, a present study investigates the role of neuroinflammation in radiation-induced brain injury by analyzing microglia activation.

176 - Human in vitro model to study the role of glial cells in multiple sclerosis

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Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system (CNS), where the pathological hallmarks include demyelination, axonal loss, astrogliosis and inflammation. Finding an effective treatment option for progressive disease form has been largely unsuccessful. Recent data suggest that innate immune responses in the CNS are promoting disease progression. Among others, the CNS-resident astrocytes and microglia are mediating these events, but their role in the pathogenesis needs further clarification. Bidirectional communication of glial cells may lead to enhanced immune responses, disturbed metabolism, loss of trophic support and axonal injury.

Here we utilized technologies of human induced pluripotent stem cells (hiPSCs) and microfluidics to reveal the role of astrocytes and microglia in the pathogenic processes of MS. HiPSC lines from healthy controls and patients suffering from MS were used. We induced a reactive astrocytes and microglia phenotype with inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β, lipopolysaccharide (LPS) and interferon (IFN)-γ and characterized their inflammatory phenotype. Bidirectional communication of human astrocyte and microglia were studied with a novel microfluidic chip.

Cytokine-stimulated hiPSC-astrocytes (iAstro) experienced a typical change in morphology that is known hallmark of astrogliosis. In addition, iAstro showed increased gene and protein expression of inflammatory mediators. Moreover, hiPSC-microglia (iMG) express cell type specific markers, and perform typical functions, like phagocytosis and intracellular calcium transients. Stimulation of iMG activated inflammatory signaling pathways and induced secretion of inflammatory mediators. Lastly, we engineered a novel microfluidic device for studying the bidirectional communication of iAstro and iMG in controlled, compartmentalized culture environment. Co-cultures of iAstro and iMG were successful and microglial chemotaxis through the microtunnels was demonstrated. In conclusion, our human in vitro MS model is promising and this model enables to study the role of astrocyte and microglia in the inflammatory environment and their bidirectional communications.

189 - Cannabigerol and Neuroinflammation : in vitro and in vivo studies

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Multiple sclerosis (MS) is a widespread chronic neuroinflammatory and neurodegenerative disease. Microglia play key role in the pathogenesis of an animal model of MS (experimental autoimmune encephalomyelitis) (EAE) via releasing cytokines and reactive oxygen species as nitric oxide (NO). The effect of cannabigerol (CBG) itself on microglial inflammation (low and high inflammation grades) and EAE induced splenocyte inflammatory response has been hardly investigated before. In this study, we aimed to investigate the effect of CBG on microglial inflammation, neurological scoring and inflammatory cells' response in EAE mice. In the present study, CBG attenuated microglial production of NO, inducible nitric oxide synthase (iNOS) and tumor necrosis factor- α (TNF-α) stimulated by the inflammatory inducer, lipopolysaccaride (LPS) at different concentrations. CBG significantly reduced the MOG-induced astrocytosis in lumbar sections of spinal cords. It also significantly decreased neuronal loss shown to be induced by MOG peptide in these sections. All MOG-treated mice developed a severe disease that peaked by day 15 post immunization. In contrast, the clinical manifestations of EAE were attenuated in mice receiving four injections of CBG at days 12 to 15 upon immunization. In a therapeutic prospective, our results suggest that CBG may represent a therapeutic opportunity in MS, based on its multi-target properties.

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205 - Establishment and characterization of induced pluripotent stem cell lines from multiple sclerosis patients

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Multiple sclerosis (MS) is a complex demyelinating, autoimmune disease of the central nervous system (CNS) affecting approximately 2.5 million people globally. Treatment options for this uncurable disease are limited, especially for the progressive disease types, and the pathogenesis is poorly understood. Currently, the most used models for MS research are animal models such as an experimental autoimmune encephalomyelitis (EAE). Investigating of human diseases with animal models is challenging due to species-specific differences. Therefore, using human cells, such as patient-specific human induced pluripotent stem cells (hiPSCs) is beneficial for investigating disease mechanisms and for discovering new drug targets. They provide an unlimited cell source for differentiation of CNS cell types. Currently, MS patient-specific cell models are not commonly used but highly needed. Here, we generated and characterized MS patient-specific hiPSC lines from six patient. MS-hiPSC lines were produced with genome integration-free Sendai virus transduction from peripheral blood mononuclear cells (PBMCs) of MS patients. Cell lines were characterized for pluripotency markers using immunocytochemistry (ICC) and flow cytometry. *In vitro* differentiation capacity was determined through embryoid body formation and ICC analysis of germ layer markers. Moreover, karyotype and mycoplasma analysis were performed, and the removal of viral vectors and transgenes was confirmed with qPCR. MS-iPSC lines expressed typical pluripotency markers (NANOG, OCT4, SOX2, SSEA-4, TRA1-81) and differentiated into three germ layers mesoderm (SMA), endoderm (AFP) and ectoderm (OTX2). In addition, MS-iPSCs were free of mycoplasma and used viral vectors and transgenes, and they had normal karyotype. All in all, produced MS-specific hiPSC lines are highly useful tools for MS disease modelling, and drug screening and discovery.

256 - Regulation of microglial TNF production by noncoding RNAs in multiple sclerosis lesions

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Multiple sclerosis (MS) is characterized by the formation of inflammatory lesions with infiltration of autoreactive T cells leading to demyelination and axonal degeneration, but also to microglial activation and upregulation of the levels of the pro-inflammatory, and potential, toxic cytokine TNF. By combining adoptive transfer of myelin-specific T cells with an axonal lesion in mice, we have previously observed that IFN-ɣ-expressing, myelin-specific T cells exacerbate microglial activation and induce the expression of TNF protein in microglia. Thus, we hypothesized that the production of TNF in innate microglia in T cell-inflamed MS-like lesions in the mouse and in MS lesions, is regulated by IFN-γ through specific regulatory, noncoding RNAs (ncRNAs) such as microRNAs, long-noncoding RNAs, and circular RNAs being involved in post-transcriptional gene regulation.
To test the hypothesis, we first investigated the transcriptomes of IFN-γ- and vehicle-stimulated primary, murine microglia, which by using ELISA could be termed TNF^{high} and TNF^{low} microglia, respectively. IFN-γ had major impact on the entire microglial transcriptome, including TNF mRNA. Among the regulated ncRNAs, six TNF-related ncRNAs, including two TNF mRNA-targeting microRNAs, were selected as our candidates. Quantitative analysis of MS-like lesions in mice has shown that the expression of the ncRNA candidates coincides with the expression of TNF mRNA and protein, CD11b immunoreactivity, and infiltration of IFN-γ-$mRNA^+$ T cells. Preliminary *in situ* hybridization results show that the ncRNA candidates are expressed in T cell-infiltrated MS lesions. The function of the ncRNA candidates is currently being investigated in IFN-ɣ- and vehicle-stimulated primary, murine microglia using lipidoid nanoparticles containing identical or complementary sequences of the ncRNA candidates.
The perspective is to identify new therapeutic targets for MS patients, in which microglial TNF production can be modulated in a way where high toxic levels of TNF in T cell-inflamed lesions are prevented, but where the protective functions of baseline levels of TNF are maintained.

272 - Pathways for protection in cortical brain lesions in multiple sclerosis

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Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system (CNS) in young adults. MS is believed to have an autoimmune etiology. Subpial cortical lesions and grey matter pathology are major hallmarks of secondary progressive MS (SPMS). Cortical subpial slowly expanding lesion, which consists of a demyelinated core with a rim of activated microglia and macrophages, are characteristic of SPMS. To date there are very few approved therapies for SPMS, and most of them are most effective at the disease stage associated with inflammation. An intact blood brain barrier (BBB) makes it difficult to access the lesions, making therapeutics for relapsing remitting MS (RR-MS) further ineffective in SPMS. The most commonly-used animal model for MS is experimental autoimmune encephalomyelitis (EAE), which involves significant BBB breakdown with infiltration of inflammatory Th1/Th17 CD4 cells. On the contrary, the SPMS pathology is not associated with overt inflammation. We wish to establish animal models which would enable therapeutic developments. We aim to create subpial cortical lesions which mimic SPMS pathology by creating meningeal inflammation, cortical inflammation without BBB breakdown with activated microglia/macrophages. We induced cortical infiltration and demyelination and meningeal inflammation via subarachnoid delivery of TNFalpha and IFN gamma to mice immunized for EAE. We induced cortical inflammation by feeding cuprizone to mice without EAE immunization. Preliminary data showed focal aggregates of activated microglia in cortical grey matter in these mice. These aggregates were intensified by subarachnoid injection of cytokines, and by intrathecal injections of lipopolysaccharide or neurodegeneration-primed microglia. We will further optimize and establish this animal model that mimics the hallmark pathology of SPMS and investigate the underlying pathological mechanism and kinetics. We will apply candidate therapeutics for amelioration of SPMS like pathology induced in the animal model. This will improve our understanding of SPMS and lead to better treatment of SPMS.

275 - Loss of miRNA-132/-212 function does not influence the clinical course of E. coli meningitis in mice

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Objectives: Bacterial meningitis is still associated with high mortality and long-term neurological sequela. In older adults, *Escherichia coli* (*E. coli*) is a relevant cause of meningitis. Micro RNAs (miR) are small non-coding RNAs that regulate gene expression by binding to mRNAs and thereby either inhibit their translation or cause their degradation. Expression of miR-132/-212 is increased upon activation of Toll-like receptor 2 and 4 by bacterial components. MiR-132/-212 contribute to the suppression of inflammation by negative regulation of the MyD88-dependent pathway and down-regulation of acetylcholine esterase levels. In the central nervous system (CNS), miR-132/-212 influence neuronal morphogenesis, tau phosphorylation, and blood-brain-barrier integrity. Thus, miR-132/-212 might play a role in the pathophysiology of bacterial meningitis.
Methods: In 15 miR-132$^{-/-}$/-212$^{-/-}$ and 15 miR-132$^{+/+}$/-212$^{+/+}$ mice, meningitis was induced by intracerebral (i.c.) injection of 4 x 10^4 CFU *E. coli* K1. Mice were monitored for 14 days post infection (p.i.) including weighing and assessment of motor performance in the tight rope test. Bacterial concentrations in cerebellum and spleen homogenates were determined by quantitative plating.
Results: 8 of 15 miR-132$^{+/+}$/-212$^{+/+}$ mice (53.3%) and 8 of 15 miR-132$^{-/-}$/-212$^{-/-}$ mice (53.3%) died from the i.c. *E. coli* infection between 60 and 180 hours p.i.. Survival curves did not differ significantly (log-rank test: chi square=0.01; p=0.92). Weight loss and decline of motor performance did not differ significantly between miR-132$^{+/+}$/-212$^{+/+}$ and miR-132$^{-/-}$/-212$^{-/-}$ mice. Bacterial concentrations in cerebellum and spleen did neither differ

between miR-132$^{+/+}$/-212$^{+/+}$ and miR-132$^{-/-}$/-212$^{-/-}$ mice that died from the infection (cerebellum: p=0.96; spleen: p=0.06) nor between those that survived the infection after 14 days (cerebellum: p=0.80; spleen: p=0.44; Mann-Whitney U-test).

Conclusion: Loss of miR-132/-212 function did not influence clinical outcome in our mouse model of *E. coli* meningitis leading to death in approximately 50% of the animals within 1 week p.i.. Nonetheless, modulation of miR-132/-212 appears to be a promising strategy to improve the therapy of bacterial CNS infections. Infection models with a different infection route (e.g., intranasal) or additional antibiotic treatment, or experiments using overexpression of miRNA-132/-212 might reveal an impact of miR-132/-212 in bacterial meningitis.

335 - Perinatal cerebellar injury induces persistence of an aberrant M1/M2 polarization profile of activated microglial cells

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Background: Extreme preterm infants are exposed to multiple inflammatory stressors over their neonatal period including perinatal cerebellar hemorrhage (CBH) and postnatal infection, known as two major risk factors for neurodevelopmental impairments. Given the dual involvement of microglia in immune and non-immune functions across the central nervous system, they may play a central role in the pathogenesis of cerebellar injury in developing brains by perpetuating inflammation and disrupting neuronal signaling refinement.

Methods: Conditional transgenic mice dependent on diphtheria toxin intracerebellar injection to deplete *CX3CR1*-positive cells (mononuclear phagocytes including microglial cells) were bred and exposed to CBH at P2 combined or not with early inflammation (LPS). Using this transgenic mouse model allows microglial cells depletion prior to cerebellar insult. Microglia phenotypic changes across time will be analyzed by flow cytometry. Neuronal structural alterations will be assessed using immunostainings on Purkinje cells, microglia and GABAergic synapses.

Results: Our preliminary data showed that prior to insult at postnatal day 2, the predominant phenotype of activated microglial cells is M2 pro-repair (48,74% *n*=3-6) compare to M1 pro-inflammatory phenotypes (0,88%; *n*=3-6) analyzed by flow cytometry from whole brain tissue (*n*=8-9). Two weeks after being exposed to a combination of perinatal insults (postnatal day 15), mouse pups showed a significant change of their M1/M2 ratio compared to controls. We observed a persisting increase of M1 phenotype (7,51%; *n*=4-7) after LPS exposure as a single insult compared to controls (1,66%; *n*=4-6), along with a decrease in activated microglial cells expressing M2 phenotype in all groups: CBH alone (26,30%, *n*=2), LPS (8,90%; *n*=4-7*;**P=0.*0025) and CBH-LPS (12,53%, n=4-7,**P=0.0025) compared to controls (35,04%, *n*=4-6). Qualitative assessment of P15 immunostainings showed a decrease of GAD67 expression after exposure to the double insult compared to control group.

Conclusions: Perinatal insults exposure lead to lasting changes in M1/M2 proportion from activated microglia, which may translate alterations of further immune responses to stressors and a certain vulnerability in the recovery phase of cerebellar injury.

366 - Generation and characterisation of multiple sclerosis patient-specific iPSC-derived neurons and 3D neuro-spheroids.

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Background Multiple sclerosis (MS), an inflammatory autoimmune disease characterized by myelin and axonal damage, is the major cause of progressive neurological disability in young adults. Majority of MS patients have a continuous accumulation of disability throughout the lifespan that leads to progressive MS (PMS), incurable at the moment. The possibility of generating patient-specific neurons from induced pluripotent stem cells (iPSCs) will help to study *in vitro* pathogenic mechanisms and possible therapeutic intervention. The project aims at: i) the characterization of several iPSC lines derived from twin pairs discordant for disease (MS patients and their healthy siblings); ii) differentiating neural precursors (NPCs) into functional neurons to evaluate their molecular

phenotype at baseline and after challenge with stressors to mimic the pathogenic process of the CNS; iii) to perform functional toxicity and neuroprotective assays on hiPSC-derived neuro-spheroids. **Methods** iPSC lines (generated in the Neuroimmunology lab, OSR) were differentiated into neurons and characterized via qRT-PCR and immunofluorescence. Functional assay on iPSC-derived neurons evaluating oxidative stress response (CellROX) was performed by FACS. The electrophysiology profiles have been evaluated by voltage and current clamp recordings. iPSC lines were differentiated into 3D neuro-spheroids comprised of mature neurons and glial cells (astrocytes and oligodendrocytes) for 8-12 weeks before cytotoxic exposure, followed by functional neurite outgrowth assay. **Results** iPSC lines from three twin pairs discordant for disease were differentiated towards NPCs and glutamatergic neuronal lineage and were detailly characterized. qRT-PCR analysis has identified significantly higher expression for VGLUT1 (SLC17A7) in three twin patients and one control line. Patch-clump technique identified lower Na+ and K+ currents in neurons derived from MS-affected patients compared to neurons from healthy siblings. CellROX assay identified a higher oxidative stress response in neurons derived from MS patients. 3D neuro-spheroids were differentiated from one twin pair, and the preliminary setup of functional neurite outgrowth assay is currently ongoing. **Conclusions** The iPSC-based disease-in-a-dish offers a promising system not only to find novel therapeutic approaches but also to undercover molecular and cellular pathogenic mechanisms that dictate the susceptibility of patients' cells to MS disease.

Multi-omics in Neuroimmunology

45 - CSF proteome in multiple sclerosis subtypes related to brain lesion transcriptomes

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Multiple sclerosis (MS) is an inflammatory neurodegenerative disease. Identification of specific molecular markers that reflect the pathology and disease course is difficult because of the dynamic complex pathogenesis.
We used a two-step proteomic approach to identify markers in the CSF of multiple sclerosis (MS) subtypes: (i) Discovery proteomics compared 169 pooled CSF from MS subtypes and inflammatory/degenerative CNS diseases (NMO spectrum and Alzheimer disease) and healthy controls. (ii) Next, 299 proteins selected by comprehensive statistics were quantified in 170 individual CSF samples. (iii) Genes of the identified proteins were also screened among transcripts in 73 MS brain lesions compared to 25 control brains (www.msatlas.dk).
F-test based feature selection resulted in 8 proteins differentiating the MS subtypes, and secondary progressive (SP)MS was the most different also from controls. Genes of 7 out these 8 proteins were present in MS brain lesions: *GOLM* was significantly differentially expressed in active, chronic active, inactive and remyelinating lesions, *FRZB* in active and chronic active lesions, and *SELENBP1* in inactive lesions. Volcano maps of normalized proteins in the different disease groups also indicated the highest amount of altered proteins in SPMS. Apolipoprotein C-I, apolipoprotein A-II, augurin, receptor-type tyrosine-protein phosphatase gamma, and trypsin-1 were upregulated in the CSF of MS subtypes compared to controls.
This CSF profile and associated brain lesion spectrum highlight non-inflammatory mechanisms in differentiating CNS diseases and MS subtypes and the uniqueness of SPMS. Using multi-omics of the human CNS compartment, can be a novel approach to identify disease-specific markers.

199 - Baseline and dynamic changes in serum neurofilament light to predict impending MS relapses

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Background and Objectives: Serum concentrations of neurofilament light chain (sNfL) have shown promise as a clinically useful biomarker of present and future disease activity in patients with multiple sclerosis (MS). Other fluid biomarkers such as Glial Fibrillary Acidic Protein (sGFAP) may provide complementary information. Our objective was to assess the predictive abilities of baseline sNfL and GFAP and longitudinal change in sNfL in a cohort of patients with active MS.

Methods: As part of an international clinical trial testing mesenchymal stem cells, participants with active MS were closely monitored for one year. Visits every three months or less included clinical assessments, MRI scans and serum draws. sNfL and sGFAP concentrations were quantified with Single Molecule Array immunoassay. We used Anderson-Gill Cox regression models with and without adjustment for age, sex, disease subtype, disease duration and expanded disability status score (EDSS) to estimate the rate of relapse predicted by baseline and longitudinal changes in sNfL levels.

Results: 58 Canadian and Italian participants with MS were enrolled in this study. Over the follow-up, 34 relapses were recorded in 19 patients. Patients with relapses had higher baseline sNfL compared to patients without relapses (20.9 pg/mL vs. 11.4 pg/mL; p = 0.0062). Conversely, baseline sGFAP did not differ between relapsing and non-relapsing patients. Cross-sectional analyses of baseline sNfL revealed that a two-fold difference in baseline sNfL, e.g. from 10 to 20 pg/mL, was associated with a 2.3-fold increased risk of relapse during follow-up (95% confidence interval 1.65–3.17). Longitudinally, a two-fold increase in sNfL level from the first measurement was associated with an additional 1.46 times increased risk of relapse (1.07–2.00). The impact of longitudinal increases in sNfL on the risk of relapse were most pronounced for patients with lower baseline values of sNfL (<10 pg/mL: HR = 1.54, 1.06–2.24). These associations remained significant after adjustment for potential confounders.

Discussion: Patients with high baseline sNfL but not sGFAP concentrations, and those with low-to-moderate baseline sNfL values who had longitudinal increases were more likely to experience relapses. These patients may benefit from earlier treatment optimisation.

214 - Molecular signature of bronchoalveolar cells suggests a lung-brain axis in Multiple Sclerosis patients in relation to smoking

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Despite compelling evidence of the contribution of smoking, and lung inflammation in general, in Multiple Sclerosis (MS) susceptibility and progression, the precise mechanisms underpinning such effect remain elusive. This is likely due to the limited access to the primary cells mediating the impact of smoke exposure in the lungs, bronchoalveaolar (BAL) cells. In this study, we aimed to examine the molecular changes occurring in BAL cells from MS patients in relation to smoking and in comparison to healthy controls (HC). We profiled genome-wide DNA methylation and hydroxymethylation in BAL cells from female MS (n=17) and HC (n=22) individuals, using Illumina Infinium EPIC, and performed RNA-sequencing in non-smokers. The most prominent changes were found in relation to smoking, with 1376 CpG sites (adjusted $P < 0.05$) differing between MS smokers and non-smokers. Approximately 30% of the affected genes overlapped with smoking-associated changes in HC, leading to a strong common smoking signature in both MS and HC after gene ontology analysis. Smoking in MS patients resulted in additional discrete changes related to neuronal processes. Methylome and transcriptome analyses in non-smokers suggest that BAL cells from MS patients display subtle (not reaching adjusted $P < 0.05$) but concordant changes in genes connected to reduced transcriptional/translational processes and enhanced cellular motility

compared to HC. This molecular signature was consistent with findings from animal studies of MS-like disease presenting the lungs as a potential site of immune cell priming prior to CNS infiltration. Our study provides molecular insights into the impact of smoking on lung inflammation and immunopathogenesis of MS and supports the hypothesis of a relationship between the lungs and the central nervous system in the context of MS.

260 - Genome-wide DNA methylation of cerebrospinal fluid cells in Multiple Sclerosis

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Objective

We aimed to establish and optimize a genome-wide DNA methylation approach to investigate methylation changes in immune cells from cerebrospinal fluid (CSF) cells of multiple sclerosis (MS) patients with the prospect of better understanding disease pathogenesis.

Methods

Cells from CSF of relapsing-remitting MS patients (RRMS, n=5) and age- and sex-matched non-inflammatory neurological disease controls (NINDC, n=5) were extracted by lumbar puncture. Whole-genome bisulfite sequencing (WGBS) libraries were subsequently generated using the post-bisulfite adaptor tagging (PBAT) protocol, which was originally designed for single-cells and here adapted to small cell numbers. Statistical methods based on limma, methylkit and RADmeth were applied for detection of differentially methylated positions (DMPs) and regions (DMRs), which were subsequently validated in a larger cohort (RRMS, n=18 and non-MS controls, n=12). Furthermore, DMP and DMR levels were correlated with RNAseq-based transcriptome data from overlapping samples (n=17), as well as array-based public data (n=26) [1]. We then performed Gene Ontology analysis on genes that associate with DMPs/DMRs to infer affected pathways and functions.

Results

We first explored the global landscape of CpG methylation and found that transcriptional start sites (TSSs) and promoter regions had low methylation levels, while features such as internal introns had high methylation levels. Statistical analysis based on three distinct methods identified 2710 DMPs and 4330 DMRs between RRMS and NINDCs, which were significantly enriched in immune cell migration and cell adhesion, among other pathways. In total, 75% (89/119) and 73% (350/480) of testable DMPs and DMRs, respectively, validated in an independent cohort with the criteria of minimal methylation difference of 5%. Comparison of promoter-associated DMPs and DMRs with RNAseq data from overlapping individuals as well as Affymatrix-based data from an independent MS cohort [1] showed inverse correlation, confirming deregulation of those genes. Noticeably, ARRB2 and HCP5, known to participate in cell migration, were found to be hyper- and hypo- methylated and consistently lower and higher expressed in RRMS patients.

Conclusions

Differential methylation of gene promoters correlates with differential expression of genes involved in regulation of immune cell migration and cell adhesion pathways in CSF cells of RRMS patients.

Brynedal B, Khademi M, Wallstrom E, Hillert J, Olsson T, Duvefelt K: **Gene expression profiling in multiple sclerosis: a disease of the central nervous system, but with relapses triggered in the periphery?** *Neurobiol Dis* 2010, **37:**613-621.

360 - Integrative analysis of TCR sequencing and transcriptomic profiling in Multiple Sclerosis at the single cell level

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Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system (CNS). The key initiating process of MS is the infiltration of immune cells through the blood-brain barrier, and the consequent development of axonal damages and demyelinating lesions as a consequence to the inflammatory response within the CNS. T cells are defined by their T cell receptor (TCR) sequences, which help T cells achieve highly specific TCR-dependent antigen recognition and triggers downstream signaling of T cells, a crucial biological process in normal and diseases conditions.
TCR plays a critical role in T-cell-mediated immune responses in MS and profiling the TCR repertoire in different immune subsets has attracted a growing interests. However, much effort is needed to understand the functional relevance of TCR profiling to help an unbiased interpretation of the biology of T cells and to estimate the effect of TCR repertoire on their phenotypes in the context of MS.
Recent High throughput single-cell technologies are now used to profile simultaneously clonotypes defined by T and B cells receptor and phenotype defined by gene expression. We performed single-cell RNA sequencing in PBMC samples of 3 MS patients and 3 healthy donors through the simultaneous profiling of the TCR repertoire and the transcriptome of T and B cells immune subsets. We observed different degrees of clonal expansion among the identified and annotated T cell subsets. In particular, Natural Killer T (NKT) and Mucosal-associated Invariant T (MAIT) cell subsets show the highest proportions of clonal cells. The subsets of high clonally expanded NKT and MAIT cells from MS patients exhibit distinct functional signatures compared to healthy controls and were enriched in inflammatory and metabolic pathways. These preliminary analyses imply that functional differences between clonally expanded T cells subset from MS and healthy controls could be an important tool to explore the functional landscape of TCR repertoire in MS disease development.

371 - A TraIN for cell communication molecules: Translating from Immunology to Neuroscience

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Dynamic communication between cells is essential for a healthy functioning of the human body. In the brain, cell-to-cell communication shapes connectivity during development and plasticity through adulthood, sometimes resulting in psychiatric disorders associated with connectomics such as autism, schizophrenia or vulnerability to addiction among others. Further, available treatments for these and other psychiatric disorders are often insufficient, ineffective and have broad or ill-defined molecular targets, while current understanding of molecular mechanisms for cell communication is substantially more advanced in the immune system than in the brain (partly due to the cellular and tissue intrinsic characteristics of the two). Here, we hypothesize that leveraging knowledge produced in immunology will accelerate hypothesis generation of gene function in the brain. For this, we are developing an open source bioinformatics platform that will help researchers to mine long lists of genes from omics experiments and prioritize candidate genes for follow up functional studies. Briefly, publicly available RNAseq datasets of purified human immune cells (1500 samples from 5 immune cell types) and of postmortem human brain (356 samples from 10 brain regions) were filtered by surface molecules and combined with Protein-Protein Interaction data to define potential pairs of interacting surface molecules and generate a database of Pairs of Cell-to-cell Communication Molecules (PCCMs). This resulted in over 500 PCCMs with expression in both brain and immune system, and 224 cell surface genes with gene function knowledge (Gene Ontology terms,

"Biological Process" category) associated with the immune system but not the brain, and therefore considered to have potential for translation. In the future, researchers will be able to input lists of genes in the online TraIN platform and output a list of ranked PCCMs with linked information on relation to disease and "researchability" (i.e. druggable targets, intracellular signaling, availability of animal models and molecular tools, etc). TraIN is meant to join the toolkit for secondary analysis of transcriptomics data, particularly focusing on surface molecules for cell communication.

Myasthenia Gravis

66 - Association of micro RNA expression with circulating follicular helper T cells in Myasthenia gravis

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Myasthenia gravis (MG) is an antibody mediated autoimmune disease targeting the neuromuscular junction. Accumulating evidence has shown that follicular T cells (Tfh), C-X-Chemokine Receptor 5 (CXCR5) expressing CD4 T cells present in second lymph organs, play vital role in B cells mature and antibody production. CXCR5-positive CD4 T cells exist in the peripheral blood and were defied as circulating Tfh (cTfh). Inducible T-cell co-stimulator (ICOS), expressed on the Tfh, is crucial for T-B interaction. We revealed that ICOS highly expressing cTfh ($ICOS^{high}$cTfh) elevated in MG compared with healthy subjects (HS) and the frequency of $ICOS^{high}$cTfh was correlated with disease severity in MG. However, it is unknown what cause the skew of Tfh in MG. In this study, we focused on the micro RNA (miRNA) which is a small single-stranded non-coding RNA molecule and regulates gene expression. Among miRNA, mir146a and mir155 were reported to be associated with Tfh. We analyzed the expression of mir146a and mir155 in peripheral blood mononuclear cells (PBMC) from 24 anti- acetylcholine receptor antibody positive MG and 12 HS by using real time PCR. The expression of mir146a and mir155 in PBMC were higher in MG than in HS with significance. Besides, the expression level of mir155 showed the significant correlation with the frequency of $ICOS^{high}$cTfh in MG. Several previous reports showed that mir155 promotes the accumulation of Tfh. Our result indicates that mir155 expression may influence on the Tfh skew in MG.

266 - PROSPECT OF WEARABLE TECHNOLOGY IN REHABILITATION AND MANAGEMENT OF MYASTHENIA GRAVIS

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Objectives: Myasthenia gravis (MG) is a chronic autoimmune disease of the nervous system, which is still incurable. In recent years, with the progress of immunosuppressive and supportive treatment, the therapeutic effect of MG in the acute stage is satisfactory, and the mortality rate has been greatly reduced.
To study role of wearable device (MI Band) to monitor daily life routine activities on movement and rehabilitation data in MG patients.

Methods: Total of 14 MG patients were taken as subject and wearable monitoring device (MI band) were put on the wrist of MG patients for 30 days and a questionnaire was filled out by each patient. In all subjects, blood pressure, blood glucose was measured on daily basis with day to day data of their monitoring of step count, calorie burnt, motion time, sleep monitoring, calorie consumption, monitoring heart rate to know daily routines and recording them for health purpose. Wearable bands, automatically provides a cueing sound with sensing alert when MG patients move out of the geo-fenced area and which stays until the subject resumes walking in virtual boundary.

Results: Present results shown that wearable device reading showed there was a normal heart rate, more calorie burnt with better control of sugar control and average good sleep count in more physically workout, include walking in MG patients compared to less physically workout MG patients, identified by professional

physiotherapists. MI band reading showed that after changing lifestyle routine among less physically active MG patients, their neuromusclar loss and wandering events normalize with less requirement of drug dose. Mi band is an increasing option for caregivers and families trying to reduce wandering and better care giving to MG patients.

Conclusions: By using, these wearable devices ensured their health awareness with more concerned towards exercising and demonstrate the benefit of such a context-aware system and motivate further studies. Wearable devices and technology have introduced a new way for caregivers and families to prevent the dangers of wandering in senior loved ones with MG.

374 - Novel biomarkers associated with autoimmune Myasthenia Gravis: a pilot study using two different proteomic approaches

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Autoimmune Myasthenia Gravis is a disease characterized by antibodies to molecules of the neuromuscular junction, and more generally, acetylcholine receptor (AChR). These antibodies are crucial for the diagnosis. Biological markers could be important for clinical follow-up and prediction of severe cases. Here we used Somascan and Olink technologies that both target specific proteins in biological fluids. The SomaScan assay uses modified aptamers, while Olink technology is based on the proximity extension assay using match pair of antibodies.

In the current study, we analyzed one pilot cohort including 15 steroid-untreated MG patients and nine controls, and we focused on the 172 molecules common to both proteomic approaches. Analysis of differentially expressed proteins revealed 54 significant proteins with SomaScan and 31 with Olink, 20 proteins being common. Most of the deregulated proteins were upregulated in MG patients (18 upregulated, two downregulated). These results were identical in the two techniques. In addition, the comparison of the p-values between both methods reveals a very good correlation ($p< 0.0001$), suggesting that the changes observed in this study were repeatable.

Among the deregulated proteins, there were two metalloproteases (MMP9, MMP10) and 2 chemokines (CCL8 and CXCL16). Interestingly, MMP10 and S100A12 that were previously shown to be the most increased in MG patients in another Olink study (Molin et al., 2017), were confirmed in our study. MMP9 was previously shown to be increased by ELISA (Helgeland et al., 2011). In addition, for several proteins, their levels were correlated with the clinical score, such as CXCL16 and IL17RA. Finally, several of the deregulated proteins have not been previously associated with MG disease.

In conclusion, this work shows that proteomic analyses can reveal new insights in the field of MG disease. Correlation between the level of the proteins and clinical data indicated that these biomarkers could have physiological relevance. Finally, the very good correlation between the two technologies and the fact that among the deregulated molecules, some of them were already described in the literature support the robustness of these proteomic approaches. However, the size of the cohort was small, and validation in a higher number of patients would be required.

The sensory and autonomic nervous systems: links with inflammation

211 - The Potential of Systemic Contribution in Neuropathic Pain

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Although several clinical studies report the presence of a persistent, low-grade systemic chronic inflammation (SCI) in various chronic pain conditions, its contribution to neuropathic pain remains poorly understood. We aim to understand the potential contribution of SCI to chronic pain by using a well-established partial sciatic nerve ligation (PSNL) mouse model of neuropathic pain. To target a myriad of inflammatory mediators at once, we tested bone marrow cell extracts (BMCE), a substance rich in anti-inflammatory cytokines and growth factors, as a potential treatment modality. Our results revealed that serum from mice having PSNL surgery contained different protein profiles compared to those of sham animals. While serum from sham animals transferred intravenously to naive mice did not change their pain sensitivity, the serum from PSNL mice triggered mechanical and cold hypersensitivity in both the paws and the orofacial area of the naive mice after the intravenous administration. Interestingly, it appears that mechanical and cold allodynia triggered by day 30 PSNL serum lasts longer than that of day 1 PSNL serum. Furthermore, we have found that a regimen of systemic BMCE administration significantly ameliorated PSNL-induced mechanical and cold allodynia. The serum from these BMCE-treated mice again had a separate protein profile from that of vehicle-treated mice and did not induce mechanical and cold allodynia when transferred to naïve mice. These findings not only demonstrate that SCI is present in some form after the induction of nerve injury and may be implicated in the development and maintenance of neuropathic pain, but also shows a therapeutic potential of systemic BMCE administration in providing analgesia. Further elucidating these connections will provide a better understanding of the mechanisms behind the pathogenesis of neuropathic pain and pave the way for new therapeutic strategies for chronic pain.

293 -Neuroprotective Effect of Enalapril Against Neuropathic Pain Induced By Chronic Constriction Injury of The Sciatic Nerve In Mice

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Introduction: Enalapril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor drug class that works on the renin-angiotensin-aldosterone system, which is responsible for the regulation of blood pressure and fluid and electrolyte homeostasis. The present study was designed to investigate the effect of enalapril (angiotensin-converting enzyme inhibitor) on the chronic constriction injury of sciatic nerve induced neuropathic pain in mice model.

Methods: The neuropathic pain was induced by four loose ligations of the right sciatic nerve in mice. Enalapril (100, 200, and 300 mg/kg) and pregabalin (40 mg/kg) were intragastric administered for 8 consecutive days from the 7th day post-surgery.The battery of behavioral tests, i.e. plantar, pin prick, tail flick, tail pinch, rota rod tests, were performed to assess the degree of thermal and mechanical hyperalgesia in ipsilateral paw and tail, and motor in-coordination activity respectively. In addition, the biochemical tests, i.e. total protein, thiobarbituric acid reactive substances and reduced glutathione, were also performed in sciatic nerve tissue samples. Afterward, immunofluorescence and Western blot were utilized to examine the activation of glial cells and the expression of inflammatory cytokines, respectively.

Results: The administration of enalapril (100, 200 and 400 mg/kg, p.o.) significantly attenuated chronic constriction injury-induced rise in peripheral as well as central pain sensitivity (thermal and mechanical) along with impairment of motor in-coordination activity. Further, it also produces ameliorative effects on chronic constriction injury-induced rise in thiobarbituric acid reactive substances and decrease in glutathione levels when compared with a normal control group.

Conclusion: It may be concluded that angiotensin-converting enzyme inhibitor may be a potential new target for the management of neuropathic pain. These assays first indicated that enalapril exerted an antinociceptive effect on chronic constriction injury -induced neuropathic pain, and might be attributed to the anti-inflammatory and neuroprotective activities of enalapril.

Neurons as active players in neuroinflammation

98 - Optogenetics defines RNA binding protein dysfunction in a model of neurodegeneration in multiple sclerosis (MS).

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Evidence indicates that neurodegeneration (NDG) is a prominent feature and the primary driver of disability in MS. Yet, knowledge of the molecular mechanisms of NDG in MS, as well as treatment options for NDG are lacking. Data from our lab indicates that dysfunction of the RNA binding protein (RBP) heterogeneous ribonucleoprotein A1 (A1) may contribute to MS pathogenesis. In this study, we established an *in vitro* optogenetic paradigm of A1 dysfunction and assessed how somatic MS-associated genetic mutations in A1 cause its molecular dysregulation in the pathogenesis of NDG in a cellular model of MS. To accomplish this, we analyzed how mutations affect A1 cellular localization, cluster kinetics and stress granule (SG) formation – a marker of NDG. Real-time, *in vitro* optogenetics were performed using reversible, blue light (BL) stimulated, optogenetic A1 protein expression plasmids, containing wild-type (WT) and mutant A1 tagged with Cryptochrome 2 (Cry2) and mCherry, expressed in HEK293T. Using BL stimulation followed by a period of recovery (imitating an MS relapse), revealed that MS-associated A1 mutations, p.P275S and p.F281L, caused significant A1 cytoplasmic mislocalization compared to WT (cytoplasmic/nuclear localization ratio: p.P275S=1.14; p.F281L=0.85; WT=0.59). The kinetics of cytoplasmic cluster formation [half-maximal formation time ($KA_{1/2}$) (minutes): p.P275S=40; p.F281L=42; WT=55] and dissociation of A1 [half-maximal dissociation time ($KD_{1/2}$) (minutes): p.P275S=11; p.F281L=21; WT=18] were significantly altered with A1 mutations. A1 mutations altered the quantity (clusters/cell: p.P275S=3.2; p.F281L=2.1; WT=3.4) and size [average cluster size (μm^2): p.P275S=0.49; p.F281L=0.37; WT=0.24] of A1 clusters. A1 mutations also caused SG formation to occur more quickly [$KA_{1/2}$ (minutes): p.P275S=55; p.F281L=51; WT=73] and frequently (fold change of cells with SG: p.P275S=1.6; p.F281L=2.3; WT=1.1). These results: (1) provide evidence that mutations in A1 promote A1 mislocalization, self-association clustering, altered RBP function, and cell stress leading to SG formation, and (2) indicate a potential link between A1 protein dysfunction and NDG in MS pathogenesis, which may allow for therapies that attenuate NDG and inhibit disability in MS.

100 - Altered RNA Binding Protein Expression Contributes to the Pathogenesis of Neurodegeneration in a Multiple Sclerosis Model

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Dysfunctional RNA binding proteins (RBPs) have been shown to contribute to the pathogenesis of neurodegeneration (NDG) in neurologic diseases, however in Multiple Sclerosis (MS) the mechanism of NDG is incompletely understood. Our lab has shown that neurons from MS brains as compared to controls, exhibit a pathogenic feature of the RBP, heterogeneous nuclear ribonucleoprotein A1 (A1). This feature includes decreased nuclear staining of A1, suggesting a loss-of-function phenotype. The exact mechanism of how loss-of-function of A1 may affect neuronal health is largely unknown. In this study, we modelled loss-of-function of A1 in vitro using differentiated Neuro-2a cells (dN2a; a neuronal cell line) and siRNA to A1 (siA1). RNA-sequencing (RNAseq) followed by Gene ontology (GO) analysis was employed to examine differentially expressed (DE) transcripts and biological process affected by loss-of-function of A1. Molecular consequences of loss-of-function of A1 was assessed through examining dN2a cells for neurite outgrowth, cytotoxicity, and stress granule (SG) formation. SiA1 entered N2a cells and significantly decreased nuclear fluorescence ($p<0.001$) and A1 protein expression compared to siControl ($p=0.003$). RNAseq and GO analysis revealed 1521 DE transcripts, which were involved in multiple biological processes including RNA metabolism, cell death, neuronal function, RBP complex and neurite outgrowth. SiA1 treated dN2a cells also showed a significant reduction in neurite branching and neurite length compared to siControl ($p<0.0001$), as well as an increase in cell cytotoxicity ($p=0.04$). Additionally, loss-of-function

of A1 negatively impacted SG formation, by reducing both number of SGs as well as the size of the ones that formed ($p<0.0001$). Loss-of-function of A1 modelled in dN2a cells showed that A1 plays an important role in neuronal health and viability as revealed through its detrimental affects on neurite outgrowth, cytotoxicity, and SG formation. These findings reveal a mechanism by which A1 dysfunction, as modeled by decreased nuclear expression of A1, may contribute to the pathogenesis of NDG in MS.

168 - Macrophages Facilitate Repair of Damaged Ribbon Synapses After Noise-Induced Hearing Loss

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Noise-induced hearing loss is a major health problem, affecting nearly 26 million Americans between the ages of 20-69 (NIDCD 2010). Exposure to prolonged noise can cause rapid loss of cochlear ribbon synapses between presynaptic sensory inner hair cell (IHC) receptor and postsynaptic peripheral axons of spiral ganglion neurons (SGNs). Such synaptic loss leads to gradual axon degeneration and ultimately death of SGNs and contributes to auditory perceptual dysfunctions leading to difficulty in speech recognition and listening in noisy environments and may be key to the generation of tinnitus or hyperacusis. Moreover, absence of functional SGNs limits the performance of hearing aids and cochlear implants or any future hair cell regeneration strategies. We have recently demonstrated that macrophages migrate immediately into the damaged IHC-synaptic region and directly contact the damaged synaptic connections following noise trauma (Kaur et al., 2019). However, the functional consequences of these contacts remain elusive. To examine the role of macrophages in noise-induced cochlear synaptopathy, cochlear macrophages were eliminated using colony stimulating factor 1 receptor (CSF1R) oral inhibitor, PLX5622 which led to a robust reduction (~98%) in macrophage numbers without any changes in the density of peripheral leukocytes or adverse effects on normal cochlear function, structure, or inflammation. PLX5622-mediated macrophage elimination did not affect the degree of loss of ribbon synapses (~40-50%) at 1-day after noise trauma when compared to damaged cochlea with intact macrophages. By 14-days after noise trauma, damaged synapses underwent spontaneous repair in the presence of macrophages however, in the absence of macrophages such spontaneous repair of synapses was significantly impaired. Repopulation of macrophages into the cochlea lacking macrophages partially repaired the noise-damaged synapses and increased the density of the postsynaptic AMPA receptors on axon nerve terminals per IHC which positively correlated with partial recovery of hearing function assessed by auditory brainstem response thresholds and wave 1 amplitudes at suprathreshold sound levels. Together, these results imply that macrophages do not prevent or worsen synaptic loss but are necessary and sufficient to facilitate the repair of damaged ribbon synapses after noise trauma. Future studies will examine the mechanisms by which macrophages facilitate synaptic repair in damaged cochlea.

283 - Investigating the Role of NPC Perturbations as a Contributor to Neurodegeneration in Models of Multiple Sclerosis

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Background: Our lab has discovered that dysfunction of the RNA binding protein (RBP) heterogeneous nuclear ribonucleoprotein A1 (A1) is a feature of neurons in multiple sclerosis (MS) cortex. RBP dysfunction has been shown to cause neurodegeneration in other neurologic diseases suggesting that a similar mechanism may be involved in the pathogenesis of neurodegeneration in MS. A feature of RBP dysfunction includes mislocalization from the nucleus to the cytoplasm resulting in loss of RBP function in the nucleus. Recent data suggests that RBP dysfunction is linked to nuclear pore complex (NPC) defects resulting in impaired nucleocytoplasmic transport and downstream cell damage.
Objectives: We hypothesize that A1 dysfunction will alter the structure of the NPC, leading to defects in nucleocytoplasmic transport and negatively impacting neuronal health.

Methods: To model A1 dysfunction, we used siRNA targeting A1 (siA1) as a loss of function paradigm. Neuro-2a cells were treated with control siRNA (siNEG) and siA1. 16 hours post-transfection, cells were differentiated into non-dividing neuron-like cells. 72 hours post-transfection, cells were fixed and immunostained for A1 and markers of the NPC (Pom-121, Nup98, RanGAP1, Lamin B, RanBP2). Corrected total cellular fluorescence (CTCF) was measured using ImageJ. NPC marker morphology was categorized as normal or abnormal based on the scientific literature.
Results: Cells treated with siA1 had significantly less A1 fluorescence as compared to cells treated with siNEG ($p=0.008$), demonstrating the efficacy of the siA1 treatment. Furthermore, siA1 treated cells exhibited a significantly higher percentage of cells with abnormal staining patterns of NPC markers, including POM121 ($p=0.005$), Nup98 ($p=0.004$), RanGAP1 ($p=0.004$), and Lamin B ($p=0.02$), as compared to siNEG cells. The morphological staining pattern of RanBP2 was not significantly affected by siA1 treatment ($p=0.068$).
Conclusion: The increased prevalence of abnormal nuclear and NPC staining in cells with knockdown of A1 suggests a link between A1 dysfunction and the NPC structure. Alterations in the structure of the NPC as a consequence of A1 dysfunction could result in abnormal nucleocytoplasmic transport and negatively impact cellular health.

288 - Study of aqueous extract of *Terminalia chebula* and Allophylus serratus on aluminium-induced neurotoxicity: Relevance to sporadic amyotrophic lateral sclerosis

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Background: Aluminium (Al) is potential to cause neurological disorders in human and animals, it's accumulation in the brain has been linked to various neurodegenerative diseases. Al exposure causes an alteration in the several ions in the body and causes toxicity. These multiple mechanisms lead to the several neurodegenerative diseases, including sporadic amyotrophic lateral sclerosis (sALS).

Objective: The goal of the current work is to study the possible neuroprotective effect of *Terminalia chebula* (TC) and Allophylus serratus (AS) leaves extract on ions homeostasis, memory functions, motor learning ability, cytokine production and the oxidative status in different brain regions of rats in aluminium chloride ($AlCl_3$)-induced neurodegeneration rat model.

Methods: Male Wistar rats were divided into five groups, Group I was received normal saline; Group II was administered orally with $AlCl_3$ (150 mg/kg b. wt.); Group III was received aqueous extract of *TC* (200 & 500 mg/kg b. wt.) , Group IV was received aqueous extract of *AS* (200 & 500 mg/kg b. wt.) and Group V was received combined treatment with ($AlCl_3$+ TC + AS). All the groups were treated for 30 days. Various behavioural, biochemical and histopathological parameters were estimated in aluminium exposed animals.

Results: The treatment with $AlCl_3$ caused a significant elevation in the concentration of aluminium and calcium ions, malondialdehyde, acetylcholinesterase actiivty and nitrite/nitrate levels, while a significant reduction in the motor incoordination, memory deficits and level of magnisium and sodium ions, glutathione peroxidase activity was observed in the brain. Meanwhile, the combined treatment with ($AlCl_3$ +TC +AS) was found to restore the investigated parameters to be near the normal values. The study suggests that the treatment with TC and AS could protect the motor neurons from the toxicity that caused by Al via improving the antioxidant status, and downregulating inflammatory cytokines, BDNF, and by preventing glutamate excitotoxicity. histopathological results shown TC and AS prevent the neurofibrillary tangle formation and neuronal damage.

Conclusion: It may concluded that the TC and AS were dose-dependently effective in managing the $AlCl_3$-induced neurodegeneration and thereby to unlock a platform to find a cure for amyotrophic lateral sclerosis.

301 - Control of Neuroinflammation by alpha and beta blockers

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Parkinson's disease can cause neuropsychiatric disturbances which can range from mild to severe. This includes disorders of speech, cognition, mood, behaviour, and thought. In addition to cognitive and motor symptoms, PD can impair other body functions. Sleep problems are a feature of the disease and can be worsened by medications.Sleep and wakefulness are behavioral and physiological activities. It is a modified form of the basic rest activity cycle. Humans usually fall asleep by entering in non Rapid Eye movement sleep, a phase accompanied by characteristic changes in the Encephalogram (EEG). The person next moves to REM sleep, which is characterized not only by rapid eye movements but also by inhibition of skeletal muscle tone. These two states alternate with each other during sleep cycle. It has been found that REM sleep is generated as a result of excitation of Cholinergic PS on neurons and inhibition of monaminergic PS off neurons. Moreover the REM sleep deprivation induced increase in Na-K ATPase activity which is partially mediated by NE. Serotonin has beenfound to increase during REM sleep. These facts implicate that both serotonin and norepinepherine are involved during REM sleep. The present study has been initiated to find out the effect of Norepinepherine and Serotonin and the blockers Prazosin and Propranolol in different permutations and combinations on Na-K ATPase activity.Brain from the male wistar rats was extracted and subjected to homogenization, synaptosome was prepared and Na-K ATPase activity was estimated under the influence of NE, 5HT, Prazosin and Propranolol in different combinations. Both NE and 5HT increase the Na-K ATPase activity individually and also synergistically when used in combination but in presence of receptor antagonists a decrease is observed. Moreover Prazosin and Propranolol also decrease the basal values of Na-K ATPase activity. Bioinformatics work carried out supported that the studies might prove beneficial in exploring the therapeutic possibilities for some of the Parkinson’s and other movement disorders.

345 - Alterations of brain neurotransmitters and metabolites in a rat model of Huntington’s disease

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Background: Huntington's disease (HD) is a neurodegenerative disorder that results from the destruction of neurons in the basal ganglia, and oxidative stress has been implicated in its pathogenesis. Alterations in dopamine (DA) function and neurotransmission have a significant role in the motor and cognitive symptoms of HD since it is well-known that glutamate receptor function is modulated by activation of DA receptors.
Objectives: Present study aimed to investigate the changes in various neurotransmitters and their metabolites in 3-nitropropionic acid (3-NP)-induced oxidative stress in a rat model of HD and explored the mechanisms of action.
Methods: 48 animals of 3-NP induced HD rat model were studied. Determination of various classical neurotransmitters (dopamine, norepinephrine, acetylcholine, glutamate, serotonin, gamma-aminobutyric acid (GABA), and adenosine and neuropeptides (cholecystokinin, dynorphin, neurotensin, substance P) in was carried out using high performance liquid chromatography HPLC) (1100 series, Agilent Technologies Inc., Santa Clara, CA, USA) with green fluorescence detection was utilized to quantify metabolite concentrations. Standards were also run after every fourth sample as controls. Concentrations were corrected for potential metabolite loss during extraction using α-ABA as an internal standard.
Results: The mean values of various neurotranmitter, norepinephrine, dopamine, GABA and serotonin levels in striatum and cerebral cortex of 3-NP rat HD models were significantly decreased compared to control group, which consequently, may changes motor and non-motor symptoms in HD rat models. There was a significant increased in levels of glutamate and acetylcholine in striatum and cerebral cortex of treatment group. There was a significant alternations in adenosine, cannabinoids and neuropeptides, metabolites values in treatment group compared to control.
Conclusion: Brain neurotransmitters play a vital role in brain functioning and also have important function in HD status. It remains to be examined the clinical efficacy of such neurotransmitters and to investigate in-depth the neural networks suggested in the extrapyramidal system. Thus, it can be concluded that restoring the neurotransmitters balance in the brain may prevent or delay the symptoms of movement disorders.

355 - Development of White Matter Circuits Underlying Context Fear Conditioning: An Ex-vivo Diffusion Tensor Imaging Study

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Background. Early-life immune activation is linked to increased risk for developmental disorders associated with deficits in hippocampal learning. Lipopolysaccharide (LPS) on postnatal day (P)21 produces learning deficits in context fear conditioning from P24-P26. We believe that P21-P24 is a critical window for the formation of circuits underlying spatial learning, involving medial prefrontal cortex (mPFC), dorsal hippocampus (dHP), and ventral hippocampus (vHP). **Methods.** Ex-vivo diffusion tensor imaging (DTI; 60 directions; 2um) was used to examine changes in white matter across P16, P20, P24, P30, and P60. To examine effects of immune activation on white matter development, brains collected at P24, P30, and P60 were treated with LPS (100ug/mL/kg; i.p.) or Saline on P21. DTI metrics were calculated within mPFC, dHP, vHP, and white matter tracts that run through these regions. **Results across P16-P60 in untreated and saline-treated rats.** In dHP, fractional anisotropy (*FA*) increased from P16 to P20-P60. In vHP, *FA* increased from P16 to P24-P60 and P20 to P60. Axial diffusivity (*AD*) in both dHP and vHP increased from P16 to P24-P60. Furthermore, in tracts that run through dHP, *FA* increased from P16 to P24 and P60, and P20 to P60. In tracts that run through vHP, *FA* increased from P16 to and from P20 to P24-P60. In tracts that run through both dHP and vHP, *FA* increased from P16 to P24-P60 and from P20 to P60. In tracts that run through mPFC, *FA* increased from both P16 to and from P20 to P24 and P60. Overall, these results suggest that there may be an increase in axon density or myelination of white matter in these brain regions and tracts from early development (P16/P20) to later development (P24-P60). **Results across P24-P60 in rats treated with LPS or Saline on P21.** Within dHP, *FA* increased from P24 to P60 and P30 to P60, regardless of P21 treatment. In vHP, *FA* also increased from P24 to P60 regardless of P21 treatment. There was no significant interaction or main effect of P21 treatment. These results suggest there may also be increased axon density or myelination across later development (P24/P30 to P60), and that a single hit of LPS at P21 is insufficient to cause detectable changes in white matter development. **Conclusion.** This research provides insight into white matter growth during the juvenile period, as it may be associated with behavioral and cognitive development during this time.

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Parasites and neuroinflammation

165 - An investigation of microglial cell activation in neurocysticercosis

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Neurocysticercosis (NCC) a parasitic infection of the central nervous system (CNS) caused by the larvae of the cestode *Taenia solium,* is the leading cause of adult acquired epilepsy in the developing world. A suprising clinical manifestation of NCC is that viable larvae can exist in the brain for extended periods with no symptomatology but when they die clinical symptoms develop. The hallmark for symptomatic NCC is neuroinflammation, however, the neuroinflammatory mechanisms underlying this disease are grossly understudied. Particularly unknown is the role that microglial cells, (the resident immune cells of the brain) play during the neuroimmune response to *T. solium* infection. To investigate the neuroinflammatory effects of the parasite, we stimulated cultured mouse organotypic brain slices (OBSs) with taenia larvae homogenate for 24 hours. These were compared with untreated control slices, and slices stimulated with lipopolysaccharide (LPS), an established neuroimmune activator. The potential immunosuppressive effects of the Taenia larvae on microglial activation was assessed by concurrently treating OBSs with LPS and Taenia larvae homogenate. Inflammatory activation of microglial cells was measured by immunostaining for the inflammatory transcription factor NFIL6, a robust marker for cell activation. Computational analyses were carried out to quantify microglial activation on images obtained using confocal microscopy. Confirmation of neuroinflammation in the treatment groups was done by measuring the release of inflammatory cytokines in culture media. We found that the co-application of LPS and Taenia larvae homogenate

suppresses the microglial activation and pro-inflammatory cytokines release that we observed in the LPS only treatment group, this constitutes an anti-inflammatory effect that could explain how Taenia larvae are able to suppress an inflammatory response whilst still viable in the brain. Microglial activation was not observed in untreated slices or in slices treated with Taenia larvae homogenate only, a cytokine response was also not observed. This data makes valuable contributions towards understanding the neuroinflammatory mechanisms underlying this debilitating disease. It also gives valuable insights that parallel the clinical manifestations of NCC and has implications for potential treatments for NCC.

303 - A non-canonical autophagy is involved in the transfer of Plasmodium-microvesicles to astrocytes

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Cerebral malaria is a neuroinflammatory disease induced by *P. falciparum* infection. In animal models, the neuro-pathophysiology of cerebral malaria results from the sequestration of infected red blood cells (iRBCs) in microvesicles that promotes the activation of glial cells in the brain. This activation provokes an exacerbated inflammatory response characterized by secretion of pro-inflammatory cytokines and chemokines, leading to brain infiltration by pathogenic $CD8^+$ T lymphocytes. Astrocytes are a major subtype of brain glial cells that play an important role in maintaining the homeostasis of the central nervous system, the integrity of the brain-blood barrier and in mounting local innate immune responses. We have previously shown that parasitic microvesicles (*Pb*A-MVs) are transferred from iRBCs to astrocytes. The present study shows that an unconventional LC3-mediated autophagy pathway independent of ULK1 is involved in the transfer and degradation of *Pb*A-MVs inside the astrocytes. We further demonstrate that inhibition of the autophagy process by treatment with 3-methyladenine blocks the transfer of *Pb*A-MVs, which remain localized at the astrocytic cell membrane and are not internalized. Moreover, bafilomycin A_1, another drug against autophagy promotes the accumulation of *Pb*A-MVs inside the astrocytes by inhibiting the fusion with lysosomes, and prevents ECM in mice infected with *Pb*A. Finally, we establish that RUBCN or ATG5 silencing impede astrocyte production of CCL2 and CXCL10 chemokines induced by *Pb*A stimulation. Altogether, our data suggest that a non-canonical autophagy-lysosomal pathway may play a key role in cerebral malaria through regulation of brain neuro-inflammation by astrocytes.

305 - An indispensable role of CD2 in initiating T cell proliferation during Cerebral Visceral leishmaniasis

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Visceral Leishmaniasis is a macrophage associated disorder in which the annual incidence is 500,000 people with 90% cases in India, Sudan, Bangladesh and Nepal and annual death rate is 59,000. It can also lead to cerebral leishmaniasis. While analysing the sequence of signalling events we have shown that *Leishmania donovani* parasites, somehow manage to make gross impairment in the protein kinase C transduced antigen signals from exterior to within the T cells. In this context there was strong indication that such a defect often could be linked to inability of these T cells to mount an effective response against *Leishmania donovani*. This was seen very strongly as patients demonstrated a very low production of many cytokines, that form the basis forgetting rid of the parasite through an immunological intervention. This reflects that pathogenesis of progressive symptomatic VL is due to a net Th2 cell state of CD4 T cells. Therefore in the present study, T cells of VL patients were examined for their marked immune response dysfunction through assessing their ability to proliferate in response to *Leishmania donovani* antigen in vitro. The key features looked were cell cycle pattern, expression of CD25+cells on T cells, percentage of lymphocytes converted into lymphoblasts, percentage of activated T lymphocytes and IL-2 production. These parameters were evaluated in T cells both before and after stimulation of their CD2

antigen. Stimulation with antiCD2antibody made an impact in the cell cycle by inducing an increase in the percentage of CD4 T cells transforming from G0 to G2/M stage. This was well supported with an increase in the number of CD69 positive CD4 T lymphocytes by several folds. Also a drastic increase in lymphoblast cells was noticed on CD2 stimulation. Interestingly, an evidence for an immunological switch over from Th2 to Th1 became distinct due to CD2 mediated alteration in T cells of VL patients. Thus we further assessed whether an activation of CD2 antigen on T cells in VL individuals resulted in restricting the ability of Th2 phenotype of CD4 cells to produce IL4.

Key words: Visceral Leishmaniasis (VL), Interferon Gamma (IFN-γ), Interleukin-2 (IL-2),

337 - Role of senescence induced by Plasmodium in astrocytes in the pathogenesis of Cerebral Malaria

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Cerebral malaria (CM) is a fatal neurological disease due to *Plasmodium* (*P.*) *falciparum,* infection, which kills 405,000 people annually. A central event involved in the pathogenesis of CM is the sequestration of infected red blood cells in the cerebral capillaries and the underlying activation of glial cells secreting pro-inflammatory cytokines and chemokines that contribute to the inflammatory process of CM. However, molecular mechanisms involved in the activation of the pro-inflammatory response of glial cells during CM remains to be determined. In this study, we examine using the experimental model of CM induced by *P. berghei* ANKA (*Pb*A) if a process of senescence induced during infection participates in the inflammatory response of glial cells. Gene expression profiling of selected senescence biomarkers was done in brains of two mouse models used to differentiate protective from pathological mechanisms during *Pb*A-induced malaria: C57BL/6 CM susceptible (CM^S) versus C57BL/6.WLA-Berr2 resistant (CM^R) mice. Of note, C57BL/6 mice infected with 10^6 *Pb*A infected red boos cells develop CM at day 7 post-infection. Data showed an increase of DNA damage markers P-ATM, cell cycle inhibitory markers p38MAPK, GADD45γ, pp53 and $p21^{WAF1}$ but not $p16^{INK4}$ transcripts, exclusively in the brain of CM^S versus CM^R mice. These data were confirmed by immunoblotting on brain protein extracts. Fluorescence microscopy analysis of brain sections after staining showed an over expression of the enzyme senescence-associated β-galactosidase during CM and confirmed the increased expression by astrocytes only of p21, pp53, Bclx and P-ATM in CM^S infected mice. In addition, levels of cytokines and chemokines involved in the senescence-associated secretory phenotype (SASP) such as IL-6, TNF-α, IFN-γ, MCP-1 and IP-10 were also more produced in infected CM^s than CM^R mice. Finally, inhibition of senescence process by treatment with senolytic drugs, Quercetin and Dasatinib protects CM^S mice against PbA-induced neuropathology and reduced the expression of senescence markers such as $p21^{WAF1}$ and IL-6 in the brain. Taken together, our data provide evidence a key role for parasite-induced senescence in astrocytes in CM, in which p21 is a major player.

Peripheral nervous system: a central target of neuroinflammation

28 - Pharmacological and Epigenetic Regulators of the NLRP3-Inflammasome Activation in Alzheimer's Disease

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Activation of the NLRP3 inflammasome-complex results in the production of IL-18, Caspase-1 and IL-1. Although these cytokines have a beneficial role in promoting inflammation, an excessive activation of the inflammasome and the consequent constitutive inflammatory status plays a role in a number of human pathologies including Alzheimer's Disease (AD). MicroRNAs (miR-) target the 3'UTR region of NLRP3, preventing the activation of the inflammasome, thus inhibiting IL-18, Caspase-1 and IL-1 _production. Because Stavudine (D4T), an antiretroviral drug, was recently shown to inhibit inflammasome activation, we verified whether its effect is mediated by the modulation of miR-7, miR-22, miR-30 and miR-223, miRNAs that bind the same *NLRP3*- mRNA-UTR region and interfere with protein translation, reducing NLRP 3activation. PBMC of twenty AD patients and ten sex-matched healthy controls (HC) were stimulated with Lypopolisaccaride (LPS)+Amyloid-beta (A42) in the absence/presence of D4T. Expression of genes within the inflammasome complex and of miR- was evaluated by RT-PCR; cytokines and caspase-1 production was measured by ELISA. NLRP3, ASC, IL-1and IL-18 expression, as well as IL-18, Il-1 _and caspase-1 production, were significantly augmented (*p< 0.05*) in LPS+A42-stimulated PBMC of AD patients. D4T reduced the expression of inflammasome genes and cytokines production (*p< 0.005)*. miR-7, miR-22, miR-30 and miR-223 expression was significantly increased in LPS+A42-stimulated PBMC of AD patients and was not modulated by D4T. These results suggest that the inhibitory effect of mRNAs on NLRP3 inflammasome is lost in AD patients and indicate that the ability of D4T to reduce the activation of the NLRP3 inflammasome is not mediated by mRNAs modulation.

152 - Silencing pulmonary sensory neurons impacts influenza pathogenesis

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BACKGROUND: During influenza A virus (IAV) infection, the driver of disease severity is often an aberrant inflammatory response with various cell types contributing to the pathogenesis. The lungs are densely innervated by pulmonary sensory neurons, critical for detecting changes within the respiratory tract. However, to date, the contribution of these to IAV disease severity is yet to be investigated. We recently showed that during IAV respiratory viral infection these pulmonary sensory neurons undergo significant changes and take on a neuroinflammatory phenotype, particularly in response to the aberrant inflammation in the lungs. Here, we sought to provide the first insight of the role pulmonary sensory neurons play in IAV pathogenesis and disease severity. METHOD: Using a murine model (C57B6/J mice, 8-10 weeks age) of IAV respiratory infection (Auckland/1/09 H1N1, $6x10^3$ PFU), pulmonary sensory neuron activity (specifically, TRPV1+ neurons expressing the $NaV^{1.8+}$ channel) was silenced by the drug QX-314 (100µM) inhaled twice daily from 3 days post-infection. Disease severity was measured using whole body plethysmography, clinical scoring, gene expression and cytokine levels. RESULTS: Silencing the activity of pulmonary sensory neurons with QX-314 in IAV-infected mice resulted in a more severe weight loss and increased clinical symptoms compared to vehicle treated IAV-infected mice. QX-314 treatment in IAV-infected mice also resulted in increased pulmonary concentration of IL-6 compared to vehicle. However, no differences in breathing parameters such as tidal volume were observed between treatments in IAV-infected mice. The vagal sensory ganglia, containing pulmonary sensory neurons, showed a significant increase in the expression of interferon stimulated genes *Irf9* and *Ifit2* in QX-314 treated IAV-infected mice compared to vehicle. In addition, the neuropeptide genes *Calca* and *Tac1* were significantly decreased indicating a potential change in inflammatory modulating neuropeptide release in the pulmonary system. Finally, the ganglia showed a significant increase in *P2x3* expression, a key receptor via which the epithelial ATP activates vagal sensory neurons. CONCLUSION: This data combines raises the possibility that the vagal sensory ganglia and pulmonary sensory neurons play an active role in regulating IAV pathogenesis. Modulation of their activity may therefore be a promising novel therapeutic approach in reducing the severity of influenza virus infection.

183 - Melatonin Provides Cellular Protection in a Cell-Specific Manner

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Melatonin, a neurohormone produced by the pineal gland, has been reported to have neuroprotective and immune-modulatory properties. The hypothesis tested is that melatonin provides protection against neuroinflammation associated with neurodegeneration. Human macrophages derived from (THP-1) cells were used to represent a peripheral immune cell model while primary human astrocytes (HA) were used to represent an immune competent cell derived from the brain. Cells were exposed to LPS at 0.1 µg/mL to induce an inflammatory response and dosed with increasing concentrations of melatonin for 24 hours. Cell viability (ATP content), proliferation (cell number), and the levels of the proinflammatory cytokine interleukin-6 (IL-6) quantitatively assessed the protective and immune-modulating properties of melatonin. In macrophages, administration of melatonin increased ATP content in a dose dependent manner, with a significant increase detected at 5 and 10 µMol doses. LPS decreased ATP content but addition of melatonin increased the ATP levels significantly at 5 and 10 µMol. HA responded similarly at low doses of melatonin, but high doses caused a decrease resulting in a bell-shaped curve. The HA exposed to LPS and melatonin showed the same trend. Both cell types showed no significant difference in cell numbers upon the administration of LPS, melatonin, or both. This indicates that melatonin may modulate metabolic processes, leading to increase in ATP generation, rather than stimulating cell growth. Cytokine IL-6 was used as a biomarker for inflammation, which was not detected in the supernatant of macrophages. However, basal levels were detected in HA, which may play a normal functional role. As expected, administration of LPS significantly increased IL-6 production compared to control in both macrophages and astrocytes. No significant change was observed after treatment with melatonin in either cell type. Based on these observations, melatonin appears to modify ATP levels in both macrophages and astrocytes in a dose dependent manner. The changes in ATP levels are not reflective of cell number which could suggest that melatonin plays a role in modifying mitochondrial function. LPS increased production of IL-6, but melatonin did not alter this response in either cell type. Therefore, the general protective effects of melatonin may be related to its improvement of cellular energetics. More experiments are needed to better understand this role of the neurohormone.

188 - Assessing Macrophage Heterogeneity in Chronic Peripheral Nerve Injury

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The extent of cellular heterogeneity within the peripheral nerve following injury has only recently been described. It includes schwann cells that provide trophic support and remyelination of regrowing axons, as well as nerve-associated fibroblasts, vascular endothelial cells, and leukocytes for regulating the microenvironment. Importantly, monocyte-derived macrophage populations that enter the nerve also appear to be a primary cell population driving nerve repair, at least acutely, as evidenced by the secretion of repair promoting factors, such as VEGF, Gas6 and MCP1, which promote angiogenesis, remyelination, and axonal regrowth, respectively. Despite our knowledge of the spatiotemporal characteristics and critical roles of macrophage populations in acute injury, they also persist at chronic stages of injury, yet their role at this stage is unknown. As such, our first goal was to provide a detailed characterization of this population in chronic injury using single cell transcriptomics to inform a hypothesis for their chronic function. We collected nerve samples from uninjured, acute (5 days) and chronic (65 days) injured mice, then generated single cell suspensions and performed sequencing. Importantly, our single cell suspension preparation did not include FACS enrichment of macrophages which would exclude rare populations of macrophages that may not express classic markers used for enrichment. Unsupervised clustering and differential gene expression uncovered five distinct clusters of macrophages across timepoints. While two of these macrophage clusters were well-represented across timepoints, one cluster ($Gpc3^+$, $Smoc2^+$) was unique to the acute timepoint, and two clusters (Dcn^+, $Egr1^+$; $Ifitm3^+$, $Plac8^+$) were unique to the chronic timepoint. By comparing our dataset to other publicly available single cell datasets, we found similar populations, but also discovered two novel clusters ($Gpc3^+$, $Smoc2^+$; Dcn^+, $Egr1^+$). These populations were uniquely enriched for genes associated with regeneration, and response to interleukin 1, respectively. We further compared our unique chronic macrophage population exclusively to our acute injury macrophages and revealed a substantial increase

in genes associated with interferon-beta and gliogenesis, suggesting chronic macrophages remain activated in a pro-inflammatory state beyond what is critical for nerve repair. What this means functionally is yet to be determined and provides a foundation for our future studies.

207 - Autoantibody screening in Guillain-Barré Syndrome

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Introduction: Guillain-Barré Syndrome (GBS) is an acute inflammatory neuropathy with a heterogeneous presentation. Although some evidences support the role of autoantibodies in its pathogenesis, the target antigens remain unknown in a substantial proportion of GBS patients. The objective of this study is to screen for autoantibodies targeting peripheral nerve components in Guillain-Barré Syndrome.

Methods: Autoantibody screening was performed in serum samples from all GBS patients included in the International GBS Outcome study by 11 different Spanish centers. The screening included testing for anti-ganglioside antibodies, anti-nodo/paranodal antibodies, immunocytochemistry on neuroblastoma-derived human motor neurons and murine dorsal-root ganglia (DRG) neurons, and immunohistochemistry on monkey peripheral nerve sections. We analyzed the staining patterns of patients and controls. The prognostic value of anti-ganglioside antibodies was also analyzed.

Results: None of the GBS patients (n=100) reacted against the nodo/paranodal proteins tested, and 61 (61%) were positive for, at least, one anti-ganglioside antibody. GBS sera reacted strongly against DRG neurons more frequently than controls both with IgG (6% vs 0%; p=0.03) and IgM (11% vs 2.2%; p=0.02) immunodetection. No differences were observed in the proportion of patients reacting against neuroblastoma-derived human motor neurons. Reactivity against monkey nerve tissue was frequently detected both in patients and controls, but specific patterns were only detected in GBS patients: IgG from 13 (13%) patients reacted strongly against Schwann cells. Finally, we confirmed that IgG anti-GM1 antibodies are associated with poorer outcomes independently of other known prognostic factors.

Conclusion: Our study confirms that (1) GBS patients display a heterogeneous repertoire of autoantibodies targeting nerve cells and structures, (2) gangliosides are the most frequent antigens in GBS patients and have a prognostic value, (3) further antigen-discovery experiments may elucidate other potential antigens in GBS.

Sex, chromosomes, and hormones in neuroimmunology

74 - Involvement of genes from DLK1-DIO3 locus in multiple sclerosis: autonomous coordinated regulatory role of miRNA genes

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Our recent global miRNome analysis identified an increase in expression of a large number of miRNA genes from the *DLK1-DIO3* imprinted locus in peripheral blood mononuclear cells (PBMC) of relapsing-remitting multiple sclerosis (RRMS) patients compared to healthy individuals; these changes were observed in men, but not in women. Besides 54 miRNA genes this locus contains genes, encoding proteins (*DLK1*, *RTL1*, *DIO3*), long non-coding RNA (*MEG3*, *MEG9*, *MEG8*), small nucleolar RNAs (*SNORD112*, *SNORD113*, *SNORD114*). Genes localized at imprinted loci are usually characterized by coordinated regulation of expression and are prone to act together. The present study aimed to validate our data on association of miRNAs from the *DLK1-DIO3* locus with RRMS and to investigate the expression of other genes from this locus in MS. RT-qPCR expression analysis was performed in PBMC of 36 RRMS patients (16 men and 20 women) and 20 age-matched individuals without neurological disorders (10 men and 10 women). The RT-qPCR of 17 miRNAs (miR-431, -127-3p, -379, -376c, -381, -410, -656, -337-3p, -370, -655, -494, -323b-3p, -654-3p, -539, -668, -300 and -380) from *DLK1-DIO3* locus followed by ANOVA with Tukey post-hoc test showed the increased levels of all miRNAs in RRMS men compared to healthy men ($0.94<Log_2FC>4.06$, $p_{adj}<0.05$). In women, miRNAs levels in two groups did not differ. Spearman correlation analysis showed 23% and 55% significantly correlated pairs in healthy men and women, respectively, while in RRMS the number of correlated pairs reaches 80% and 90% in men and women, respectively ($r>0.52$, $p<0.05$). The expression analysis of all other genes from the *DLK1-DIO3* locus (*DLK1*, *RTL1*, *DIO3*, *MEG3*, *MEG9*, *MEG8*, *SNORD112*, *SNORD113* and *SNORD114*) showed no differences in their levels in RRMS patients compared to healthy controls in both men and women. Our study indicates the massive sex-specific coordinated dysregulation of at least 17 miRNA genes, but not other genes from *DLK1-DIO3* locus, in RRMS development. Further investigation of observed miRNA dysregulation in PBMC subpopulations as well as the impact of imprinting on this phenomenon will add more insight to the missing heritability problem and sexual dimorphism in MS. This study was supported by the Grant No. 20-75-00046 from Russian Science Foundation.

139 - Effects of limited bedding and nesting on anhedonia and neuroimmune function: a model for postpartum depression

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Approximately 60% of new mothers experience postpartum mood disturbances known as the “baby blues.” Most mothers recover but 15% of those mothers go on to develop postpartum depression, yet only 3% of preclinical research even mentions postpartum depression (Wisner et al, 2013). The present studied aimed to examine limited bedding and nesting (LBN) conditions as a rodent model for postpartum depression in new mothers by examining alterations to anhedonia and neuro-immune function. First time mothers underwent a series of sucrose preference tests (prior to breeding, during gestation and postpartum) to examine depressive-like behavior. On embryonic day 19, animals were placed into one of two groups: limited bedding and nesting or control living conditions. Results of this study replicated previous work in our lab showing that first time mothers experience anhedonia or depressive-like behaviors immediately postpartum (Poscillico and Schwarz, 2016) but the present study observed no effect of the LBN condition. Interestingly, regardless of condition, sucrose preference data revealed approximately 40% of new mothers display anhedonia postpartum while the others show no change, suggesting some mothers are more susceptible to depressive-like symptoms postpartum. Brain

tissue was collected from these animals at either postnatal day 2 or 9 and assessed for neuro-immune function. Results indicate increased IL-6 postpartum compared to non-pregnant animals in the dorsal hippocampus and medial prefrontal cortex as well as increased IL-1B in the ventral hippocampus. These finding suggest, although short term LBN may not be an ideal model for postpartum depression, there are some inherent differences in anhedonia susceptibility in individual animals and further work is continuing to better understand and predict which animals are vulnerable and which are resilient to postpartum anhedonia.

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142 - Effect of peripartum stress on postpartum maternal behavior and associated endocrine substrates

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The purpose of this study is to measure the impact of prenatal stress on postpartum maternal behavior in addition to the levels of estrogen receptor alpha (ERα), specifically in the medial preoptic area (mPOA), as well as measuring the effects of stress on anhedonia by sucrose preference test. A limited bedding and nesting (LBN) condition was applied to half of the cohort. They received 250 mL of bedding compared to the control group which received 4000 mL of bedding. The purpose of this condition is to simulate a stressful environment which simulates stressors a new mother might experience when lacking adequate resources to care for her infant. Maternal behaviors of LBN and control animals were recorded twice daily for 30 minute time periods, once in the morning and once in the evening. The footage was then analyzed and hand-scored on a minute-by-minute basis, choosing from a list of 17 behaviors. A mixed model analysis was used to analyze the data. A sucrose preference test was performed to measure for anhedonia using a 1% sucrose solution for 48 hours at 4 timepoints throughout the study (prior to breeding, E20-22, P0-2, P7-9). Additionally, tissue samples were collected from a total of 60 animals (same animals that were observed with maternal behavior) and were sliced at 30 µm. Slices were then stained, mounted and analyzed via microscope to examine cell count of ERα in the MPOA. The analysis of maternal behavior, although ongoing, is anticipated to exhibit a decrease in positive maternal behaviors within the LBN group, characterized by postures such as arch back nursing, pup licking, and retrieving pups. In regards to the sucrose preference test, we found the most significant decrease in preference among both LBN and control groups during the P0-P2 and P7-P9 time points when compared to baseline measurements. Specifically, in the P7-P9 time point, there are distinct groups of dams with high and low preference, (suggesting that stressors do not have a uniform effect on the entire cohort, regardless of group condition.) In regards to the stained tissue, we anticipate a larger proportion of ERα found in the mPOA to be correlated with more positive maternal behavior. This analysis is ongoing. With a lack of research which specifically focuses on postpartum depression, the use of an animal model proposes a new approach in observation. The findings of this study should prove to be beneficial in elucidating biomarkers of PPD to aid in the proper and accurate diagnosing of cases.

Single cell analyses in neuroimmunology: essential or overrated?

23 - Optimized protocols for the isolation of astrocytes for single cell analysis

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Astrocytes have a variety of functions in development and homeostasis. In addition, astrocytes are one of the first responders to insults to the CNS, including neuroinflammatory conditions such as multiple sclerosis (MS). Paralleling their diverse role in homeostasis, both the phenotype of resting astrocytes, as well as that of the

reactive astrocyte response is heterogeneous. Several studies by others and us have underscored that the roles of astrocytes in neuroinflammation vary depending on the stage and pathological processes involved. With the advent of genetic tools to study specific cell types, we will be able to dissect the precise role of astrocytes in pathogenic processes such as neuroinflammation, demyelination, as well as in myelin repair. However, phenotyping astrocyte subsets in the context of their cellular environment, as well as isolating these subsets for further analysis is paramount to understanding which avenues to explore and monitoring astrocyte responses in and ex vivo. Here, we present optimized protocols for the isolation of astrocytes, as well as infiltrating immune cells and other CNS resident cells, for downstream single-cell based applications such as spectral flow cytometry and single cell RNA sequencing.

53 - Novel cell-based analysis reveals region-dependent changes in microglial dynamics in grey matter in a cuprizone model of demyelination

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Microglia are key players in Multiple Sclerosis (MS), expressing many susceptibility genes for this disease. They constantly survey the brain microenvironment, but the precise relationship between microglial dynamics and pathological processes in MS remains unknown. We used a model of chronic MS, induced in mice by dietary cuprizone, a copper chelator that causes oxidative stress, mitochondrial dysfunction, and selective loss of oligodendrocytes, resulting in extensive demyelination of both white and grey matter in the brain. As in MS, cuprizone-treated mice show early and extensive microglial activation in both white and grey matter. However, histopathology used for identifying microglial modifications has had limited spatial and temporal resolutions and was mainly focused on pathology in white matter areas. Using high-resolution confocal and 2-photon imaging and a newly developed approach for analysing individual microglial cell dynamics, MicroApp, we found that heterogeneity in microglial morphology and function is disease and region-dependent and is associated with differences in demyelination and remyelination. In particular, we found that in cortical layer 5 and hippocampal CA1 microglia became activated very early in response to cuprizone, before detectable demyelination and showed region-specific characteristics. In cortical layer 5 microglia formed nodules with increased phagocytic activity while in CA1 they changed morphology becoming less ramified and more hypertrophic. Demyelination had similar region-specificities, starting earlier in cortical layer 5 but being more complete in hippocampal CA1. In contrast, in cortical layer 2/3, microglia were not significantly involved in phagocytosis and demyelination, but instead showed changes during remyelination, becoming hyper-ramified with slower process movement, thereby maintaining similar local tissue surveillance properties. Thus, profiling of microglial activation using specific morphological, functional and motility parameters may be useful as a sensitive biomarker for disease progression in the grey matter in MS.

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70 - Single-cell transcriptomics of oligodendrocyte and microglia lineages at different stages in experimental autoimmune encephalomyelitis

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Multiple sclerosis is an inflammatory demyelinating disease in the central nervous system (CNS). Experimental autoimmune encephalomyelitis (EAE) has similar pathology and pathogenesis as MS, and is wildly used as an animal model for MS. Oligodendrocytes (OLs) are myelin sheath forming cells in the CNS, which ensure the rapid saltatory conduction in CNS. Microglia, as one of the immune cells in CNS, plays an important role in immune monitoring, antigen presentation, and debris removal. In previous study, we found that MHC-I/II genes were

significantly increased in oligodendrocyte lineage cells in EAE at peak stage. Herein, we established relapsing-remitting EAE model in mouse, collected and dissociated spinal cord tissue at both remission and relapse stages. We used FACS sorting to enrich OLs and microglia, then performed single cell RNA-sequencing. As previously observed, OLs from EAE expressed higher levels of MHC-I/II genes, such asH2-d1, H2-k1, H2-q7, Cd74, and B2m. Interestingly, these genes still had high expression levels even at remission stage. Homeostatic microglia and active microglia expressed lower levels of inflammatory response genes at relapse stage compare to remission stage, such as Nfkbid, Nlrp3, Rel, Ccl3, Ccrl2, Cxcl13, and Il1a. We hypothesize that microglia may have weaker proinflammatory and phagocytic function at relapse stage than remission stage. Ongoing and future experimentation involves functional validation and mechanisms examination.

206 - Investigation of Novel Blood Biomarkers in Multiple Sclerosis Using scRNA-Seq and 21-color Flow Cytometry

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Background: In multiple sclerosis (MS), the course of the disease and the response to treatments are highly variable and unpredictable. The identification of novel readily measurable biomarkers reflecting pathogenic processes underlying clinical presentation is crucial to guide rational treatment decisions and render MS patient care more efficient. With the advances in high-parameter technologies, it is now possible to uncover changes at the single-cell level. Combined with the use of easily accessible biobanked samples, these technologies can facilitate the biomarker discovery process. **Objectives**: Our goal is to identify novel blood biomarkers in MS using single cell RNA-Sequencing (scRNA-Seq) and evaluate their potential using a 21-color FACS panel applied to biobanked peripheral blood mononuclear cells (PBMCs). First, we will verify whether samples are stable through cryopreservation. Second, we will evaluate the potential of novel MS biomarkers for prediction of disease activity, disease progression and treatment response. **Methods**: We used scRNA-Seq to detect differences in PBMC gene expression between untreated relapsing-remitting MS patients (n=3) and healthy controls (n=3). We conducted a differential gene expression analysis to identify novel biomarkers. Next, we designed a 21-color FACS panel to simultaneously study the seven biomarker candidates on biobanked PBMCs. We ensured the stability of cell subsets following cryopreservation by comparing the levels of each marker on fresh PBMCs, PBMCs cryopreserved for one week, and cryopreserved for two months (n=7). **Results**: Notably, scRNA-Seq analysis showed that antigen presenting cells (APCs) and natural killer (NK)/NKT cells showed the most transcriptional changes. We identified seven biomarker candidates in these cell types: lysozyme, S100A9, DAP12, IL7R, beta 2-microglobulin, integrin beta 2 and CD69. Our 21-color FACS panel allowed us to delineate more than 10 cell populations by unbiased clustering. In addition, we found that samples remained highly correlated through freeze-thaw cycles and with cryopreservation duration, indicating that the use of biobanked samples for a large biomarker study is reliable. **Conclusion**: Our approach of combined scRNA-Seq and 21-color flow cytometry allowed us to identify seven candidate biomarkers in MS that will be investigated simultaneously in APCs and NK/NKT cells. Our next step will be to correlate flow cytometry findings will clinical and radiological data.

Innate and Adaptive Immunity in AD, Parkinson and ALS

93 - Investigating a dysregulated gut-immune-brain axis underlying neurodegeneration in Parkinson's disease

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Recently, mounting evidence links disrupted immunity to Parkinson's disease (PD) and notably the participation of intestinal inflammation. Using a mouse model, we made a seminal discovery that disruption of a PD-related

gene, Pink1 (PTEN-induced kinase 1) induces the presentation of mitochondrial antigens by dopaminergic neurons following C. *rodentium* infection via gavage, triggering a targeted neuronal attack by antigen-specific T cells and PD symptoms. In this follow-up study, our aim is to provide a comprehensive spatial-temporal characterization of the immune responses along the gut-immune-brain axis in Pink1 KO and littermate controls at 1-, 2-, 4- and 24-weeks post-infection (w.p.i.). Using immunohistochemical staining and multiparametric flow cytometry, we interrogated infiltrating immune cell dynamics in nervous tissues, colon as well as cervical and mesenteric lymph nodes to determine at which timepoint and tissues pronounced immune responses are detected. We found higher density of immune cells in the brain and spinal cord in both infected Pink1 KO and wild type at 2-w.p.i. Furthermore, we ascertained the colon as the major site of immune activation where conventional dendritic cells (cDCs) expanded at 1-w.p.i. while more T cells were detected at 2-w.p.i. Alterations in immune cell populations and activation were less evident in the lymph nodes. To dissect the molecular changes in cDCs and T cells contributing to autoimmunity, we performed single cell transcriptomics and found several differentially expressed genes in the colon of infected Pink1 KO compared to control groups. At 1-w.p.i., upregulated genes in cDC1 and CD8 T cells were involved in NF-kappaB signaling and cytotoxicity. In infected Pink1 KO cDC2 and CD4 T cells, there was higher expression of genes important in cell adhesion and differentiation. At 2-w.p.i., genes associated with antigen processing and presentation via MHC class I and type I interferon signaling pathways were highly expressed in both cDCs of infected Pink1 KO. Accordingly, infected Pink1 KO CD4 and CD8 T cells expanded, and upregulated genes related to apoptotic and pro-inflammatory processes. In summary, we posit that antigen-specific T cells instigating neuronal attack are primed in the colon due to dysregulated innate and adaptive responses during the early time course of PD progression.

329 - Regulatory T cells decrease A1-like C3-positive reactive astrocytes in Alzheimer-like pathology

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Increasing evidence support a key role of peripheral immune processes in the pathophysiology of Alzheimer's disease (AD), highlighting an intricate interplay between resident glial cells in the central nervous system and both innate and adaptive peripheral immune effectors. We previously showed that regulatory T cells (Tregs) have a beneficial impact on disease progression in Alzheimer-like pathology, notably by modulating the microglial response associated with Abeta deposits in a mouse model of AD-like amyloid pathology. Besides microglia, it now appears evident that astrocytes also play a critical role in neuroinflammatory processes associated with AD. Different phenotypes of reactive astrocytes have recently been characterized, including A1 neurotoxic and A2 neuroprotective subtypes. Here, we further analyzed the impact of Tregs immunomodulation on astrocyte reactivity in response to amyloid pathology. Modulation of Tregs did not significantly impact the magnitude of global cerebral astrocytosis, nor altered pan astrocyte reactivity in the close vicinity of cortical amyloid deposits. Further morphological analyses by 3D imaging did not evidence any variation in the number, morphology, or branching complexity of astrocytes upon immunomodulation of Tregs. However, early transient depletion of Tregs modulated the balance of reactive astrocyte subtypes, resulting in increased A1-like C3-positive astrocytes associated with amyloid deposits. Conversely, early depletion of Tregs decreased A2-like phenotypes of reactive astrocytes associated with larger amyloid deposits. Intriguingly, modulation of Tregs also impacted the cerebral expression of several markers of A1-like subsets in healthy mice, as well as C1q and TNFa, factors known to be involved in the functional polarization of A1 reactive astrocytes. This study suggests that Tregs contribute to modulate and fine tune the balance of reactive astrocyte subtypes in AD-like amyloid

pathology, by dampening C3-positive astrocytes in favor of A2-like neuroprotective phenotypes. This effect of Tregs may partly relate to their capacity at modulating steady state astrocytes reactivity and homeostasis. Our data further highlight the need for refined tools, strategy of analysis, and more precisely defined markers of activation phenotypes for better deciphering the complexity of astrocyte reactivity in neurodegeneration and other neuroinflammatory conditions.

Tissue-resident T cells in CNS inflammation

95 - The two-pore-domain potassium channel $K_{2P}18.1$ mediates translation of T cell receptor signals during thymic T_{reg} development

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A distinct T cell receptor (TCR) signal is essential for the development of thymus-derived regulatory T cells (tT_{reg}). However, the mechanism how TCR signal strength is translated into the different T cell fates is still largely unclear. We identified that the two-pore-domain potassium channel $K_{2P}18.1$ is an essential player in the translation of TCR signals towards tT_{reg} development.
Mice with a functional knock-out of $K_{2P}18.1$ showed reduced T_{reg} numbers with thymic origin due to an impaired tT_{reg} development; whereas genetic activation of $K_{2P}18.1$ led to elevated tT_{reg} numbers by enhanced tT_{reg} development. Mechanistically, we showed that NF-□B signaling following TCR activation increases $K_{2P}18.1$ expression in tT_{reg} precursor cells. A high expression level of $K_{2P}18.1$ provides the driving force for sustained Ca^{2+} influx that is crucial for successful NF-□B- and NFAT- dependent FoxP3 expression and thus the stable generation of tT_{reg}. Analysis of human thymus biopsy samples and recent thymic emigrants from peripheral blood revealed expression of $K_{2P}18.1$ also on human tT_{reg}. A dominant-negative missense variant of $K_{2P}18.1$ in multiple sclerosis (MS) patients was associated with fewer T_{reg} and a poor clinical outcome of MS. These results could be confirmed in the context of experimental autoimmune encephalomyelitis (EAE), an animal model for MS. Inhibition of $K_{2P}18.1$ led to a worse EAE disease course with reduced numbers of tT_{reg}, compared to control mice. However, a pharmacological activation of $K_{2P}18.1$ ameliorated the disease course by elevation of tT_{reg} numbers.
Overall, our results demonstrate that $K_{2P}18.1$ translates the TCR signal into tT_{reg} development in mice and humans.

114 - Critical role for $CD4^+$ T cells during *Staphylococcus aureus* craniotomy infection

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Craniotomy is a neurosurgical procedure required to gain access to the brain for tumor resection or epilepsy treatment. Infectious complications occur at a frequency of 1-3%, with approximately half caused by *Staphylococcus aureus* (*S. aureus*) that forms a biofilm on the bone flap. Our recent scRNA-seq study revealed the transcriptional heterogeneity of infiltrating leukocyte populations during *S. aureus* craniotomy infection, which included the presence of T cells and NKT cells in the brain. In the current study, intracellular cytokine staining revealed an equivalent number of Th1 and Th17 infiltrates in the brain at days 7 and 14 post-infection, suggesting that T cells may influence inflammatory responses during *S. aureus* craniotomy infection. Bacterial burden in the brain, subcutaneous galea, and bone flap was significantly higher in RAG1 knockout (KO) compared to wild type (WT) mice at days 3, 7, and 14 post-infection, suggesting a critical role for T cells in infection containment. This was supported by findings in IFN-γR KO mice, where *S. aureus* titers

were significantly elevated out to day 28 post-infection in the galea. The similar phenotypes of IFN-γR and RAG1 KO mice suggested that T cells are critical for regulating the antibacterial properties of other leukocyte and microglial subsets. This was supported by the adoptive transfer of *in vitro* skewed Th1 or Th17 cells, which reduced the exaggerated bacterial burden in RAG1 KO mice to levels observed in WT animals. These findings demonstrated an essential cross regulatory role of Th1 and Th17 responses for infection containment. Interestingly, adoptively transferred $CD4^+$ Th17 cells that migrated to the infected brain favored the production of Th1-associated cytokines such as IFN-γ and TNF-α over IL-17A. Single cell RNA-seq (scRNA-seq) identified a significant hypoxia- and glycolytic signature in the brain of RAG1 KO mice, particularly in granulocytes and microglia, with a concomitant decrease in Type I and II Interferon responsive genes (ISG, IRF, IFIT, GBP etc.). These results are suggestive of T cell crosstalk with other immune cell populations in the brain by altering their activation and metabolic states. Collectively, these findings reveal the importance of adaptive immunity in dictating the outcome of craniotomy infection, likely by regulating the activity of infiltrating granulocytes and resident microglia. Supported by the NIH National Institute of Neurological Disorders and Stroke (R01NS107369).

132 - Synthetic delivery of IL2 expands the brain regulatory T cell population and effectively prevents pathological neuroinflammation

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An adaptive immunological component to neuroinflammatory pathology is increasingly being recognized. The recent identification and characterization of a small population of regulatory T cells (Tregs) resident in the brain allows for their potential therapeutic exploitation. As the most potent known anti-inflammatory mediators, Tregs have the capacity to reduce inflammation and promote tissue repair, with a key limitation being the small number present in brain tissue. Here we demonstrate that brain-specific IL2 delivery allows the efficient expansion of the brain-resident Treg population. This approach, bringing brain IL2 concentrations up to the normal physiological range of serum, allows a 10-fold accumulation of Treg numbers within the brain, without any increase in the Treg numbers in circulation or peripheral tissues. Mice with brain-specific IL2 delivery were protected from experimental traumatic brain injury, stroke and multiple sclerosis, with substantial reductions in inflammation and histological damage, and improvements in clinical measurements. These results validate synthetic IL2 delivery as an effective protection against pathological neuroinflammation, with potential translation to the patient context.

208 - Investigating the role of Tissue-resident memory T cells in chronic CNS autoimmune disease

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Multiple sclerosis (MS) is a chronic inflammatory disease characterized by demyelinating lesions of the central nervous system (CNS) likely due to uncontrolled activation of auto-reactive T lymphocytes against CNS self-antigens. New therapeutic options against MS include the use of molecules preventing T cell migration to the CNS. However, despite these molecules are showing very significant clinical effect, a large proportion of patients still experience disease relapse and, for a proportion of them, a secondary progressive disease course. One possible explanation could be that a fraction of autoreactive T cells has acquired the property to stably reside within the CNS and promote inflammation locally, independently of *de novo* recruitment of recirculating T cells. Supporting this, recent reports showed the presence of T cells with a so-called “Tissue-resident memory” (Trm) phenotype within the demyelinating lesions of MS patients. Therefore, to better investigate whether Trm cells actively play a role in sustaining the chronicity of MS, it is essential to characterize this population over time, phenotypically, molecularly and functionally.
Using a model of experimental autoimmune encephalomyelitis (EAE), in which the disease is triggered by auto-reactive CD4+ T lymphocytes following immunization with myelin-derived antigens, our data showed that (1*) bona fide* Trm cells effectively develop during the chronic phase of EAE, (2) are insensitive to Fingolimod treatment,

and (3) express inflammatory cytokines. These results support our hypothesis that Trm cells could be involved specifically in the chronic phase of the disease and could sustain inflammation in the CNS due to their pro-inflammatory potential, even in the absence of *de novo* recruitment of auto-reactive T-cells from the periphery. Further investigations about Trm functions in MS could uncover previously unappreciated mechanisms sustaining chronic inflammation and could open the door to new therapeutics against this chronic CNS disease.

Viruses and neuroinflammation

3 - A case of COVID-19 encephalitis with anti-GFAP antibody-positive meningoencephalomyelitis

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Introduction: COVID-19, caused by SARS-CoV-2, is increasingly recognised to produce neurological manifestations. Anti-glial fibrillary acidic protein (GFAP) antibody (Ab)-mediated astrocytopathy frequently have paraneoplastic or parainfectious aetiologies, although culpable infective agents are rarely identified. **Methods:** We describe a case of COVID-19 and adenovirus encephalitis preceding the development of anti-GFAP meningoencephalomyelitis. **Results:** A 49 year-old woman was admitted with headache and fever with positive SARS-CoV-2 nasal PCR. Over the next 72 hours, she developed drowsiness, agitation and confusion. CT head was normal. Cerebrospinal fluid (CSF) analysis showed leukocyte 28/mm^3, red cells 5/mm^3, protein 0.8g/dL and glucose 2.2mmol/L. She started empirical intravenous aciclovir and cefotaxime. Agitation improved, allowing MRI brain to be performed. This showed right anteromedial temporal lobe T2 hyperintensities with restricted diffusion. CSF returned positive SARS-CoV2 and adenovirus PCR results on Day 10 (negative for HSV, VZV, enterovirus PCR and microbial culture). COVID-19 and adenovirus encephalitis was diagnosed. 5 days dexamethasone and 14 days total aciclovir were given with clinical improvement. However, on Day 20, she developed new quadriparesis, myoclonus and fluctuating consciousness. Repeat MRI showed new right temporal and parietal T2 hyperintensities, patchy enhancing cord lesions, cauda equina and conus medullaris meningeal enhancement. Repeat CSF showed leukocyte 185/mm^3 (90% lymphocytes), red cells 4/mm^3, protein 1.9g/dL and glucose 1.3mmol/L, with negative SARS-CoV-2 and adenovirus PCR. Serum and CSF anti-GFAP Ab were positive (1:128 and 1:64, respectively). Other autoimmune encephalitis, paraneoplastic and connective tissue antibodies, and infective and malignancy screens were negative. After 3 days of high-dose methylprednisolone with prednisolone taper, power, speech and cognition began improving over the subsequent 2 weeks. After 1 month, meningeal enhancement resolved on repeat MRI and CSF parameters normalised. She started mycophenolate, and further improved at 3 months follow-up. **Conclusion:** We report the first case of COVID-19 encephalitis preceding anti-GFAP meningoencephalomyelitis, a diagnosis supported by characteristic imaging findings and steroid-responsiveness. We postulate COVID-19 and adenoviral infections immunologically precipitated anti-GFAP astrocytopathy.

46 - Genome-wide human endogenous retrovirus (HERV) transcripts in MS brain lesions

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HERV expression in MS brain lesions may contribute to chronic inflammation, but expression of genome-wide HERVs in different MS lesions is unknown. We examined the HERV expression landscape in different MS lesions compared to control brains. Transcripts from 71 MS brain samples (NAWM, active, chronic active, inactive, repairing/remyelinating lesions) and 25 control WM were obtained by next-generation RNA sequencing and mapped against HERV transcripts across the human genome. Differential expression of mapped HERV-W and HERV-H reads between MS lesion types and controls was analyzed. Out of 6.38 billion high-quality paired end reads, 174 million reads (2.73%) mapped to HERV transcripts. There was no difference in HERVs expression level

between MS and control brains, but HERV-W transcripts were significantly reduced in chronic active and remyelinating lesions. Of the four HERV-W transcripts exclusively present in MS, ERV3633503 located on chromosome 7q21.13 close to the MS genetic risk locus had the highest number of reads. In the HERV-H family, 75% of transcripts located to nearby 7q21-22 were overrepresented in MS, and ERV3643914 was expressed more than 16-times in MS compared to control brains. In conclusion, transcripts of different HERV families were much less abundant than DNA in the genome suggesting their transcription is mostly repressed or silenced in the brain. Novel HERV-W and HERV-H transcripts located to chromosome 7 regions were uniquely expressed in MS lesions, indicating their potential role in brain lesion evolution.

76 - TLR2 activation protects against neurotropic bunyavirus infection

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Rift Valley fever virus (RVFV) is a mosquito-borne, encephalitic bunyavirus that can invade the central nervous system in severe cases, infecting mature neurons. Initial innate immune responses are activated by pathogen associated molecular patterns (PAMPs) that bind host-encoded pattern recognition receptors (PRRs). Activation of these receptors leads to the induction of multiple antimicrobial pathways including the transcription of interferons. Type I interferon can block diverse viral infections, including RVFV, in many cell types. However, we found that Type I interferon did not attenuate RVFV infection in mature primary neurons. Therefore, we reasoned that neurons may be responsive to PRRs that induce additional defense mechanisms to control infection. To identify PRR ligands that were antiviral against RVFV in primary neurons, we screened a diverse set of 75 PAMPs for their ability to block infection. We found that Toll Like Receptor 2 (TLR2) agonists, including the classical TLR2/1 ligand PAM_3CSK_4, were antiviral against RVFV in neurons. Moreover, we found that this TLR2 agonist showed antiviral activity against the distantly related bunyavirus, La Crosse virus, suggesting that TLR2 signaling may broadly protect neurons from bunyavirus infections. In neurons, PAM_3CSK_4 stimulation did not induce interferons or interferon-stimulated genes; in contrast, we observed early and transient induction of NF-kB-dependent genes including CXCL10. Importantly, in a mouse model of RVFV-induced encephalitis, we found that intracranial delivery of PAM_3CSK_4 suppressed RVFV replication by more than 6 logs in the brain leading to increased survival from infection. This work may guide the development of new therapies for encephalitic bunyavirus infection.

84 - Fas/FasL contributes to HSV-1 brain infection and neuroinflammation

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The Fas/FasL pathway plays a key role in immune homeostasis and immune surveillance. In the central nervous system (CNS) Fas/FasL is involved in axonal outgrowth and adult neurogenesis. However, little is known about the role of the Fas/FasL pathway in herpes encephalitis. In this study, we used a neuropathogenic clinical strain of herpes simplex virus type 1 (HSV-1) to explore infection-induced inflammation and immune responses in the mouse brain and the role of Fas/FasL in antiviral CNS immunity. HSV-1 infection induced infiltration of Fas- FasL-bearing monocytes and T cells in the brain and an up-regulation of Fas and FasL expression on resident astrocytes and microglia within the infected sites. Upon infection, Fas- and FasL-deficient mice (lpr and gld) were partially protected from encephalitis with a decreased morbidity and mortality compared to wild type mice. Fas receptor stimulation abrogated HSV-1 induced activation and inflammatory reactions in microglia from WT mice, while lack of Fas or FasL led to a more pronounced activation of monocytes and microglia and also to an enhanced differentiation of these cells into a pro-inflammatory M1 phenotype. Furthermore, the specific immune response was more efficient in Fas- and FasL-deficient mice with significantly higher numbers of infiltrating HSV-1-specific cytotoxic T cells in the brain. Our data indicate that the Fas/FasL pathway leads to excessive neuroinflammation during HSV-1 infection, which is associated with a diminished anti-viral response and an excessive neuroinflammation.

89 - Brain-infiltrating B cells in MS: from peripheral induction to local effector function

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During the pathogenesis of MS, B cells escape from peripheral tolerance, enter the CNS, associate with lesions and produce antibodies. The precise B-cell subsets and mechanisms involved in these events are underexplored. In this study, we aimed to explore the triggering and development of brain-infiltrating B cells to better understand the cause of MS. We found that T-box transcription factor T-bet and its surrogate marker CXCR3 define brain-infiltrating B cells in MS. Naive B cells of MS patients were prone to develop into $CXCR3^+$ class-switched populations under IFNGR- and TLR9-inducing, follicular T cell-like conditions *in vitro*. The presence of risk variant IFNGR2 augmented the sensitivity of human B-cell lines and blood B cells of MS patients to IFN-γ. EBV infection of class-switched B cells corresponded to increased CXCR3 surface expression. $CXCR3^+$ class-switched B cells preferentially crossed brain endothelial monolayers *in vitro* and accumulated in the blood of clinical responders to natalizumab. *Ex vivo* CXCR3-expressing $CD19^+$ cells were enriched in post-mortem CSF, meninges and brain tissues compared to blood samples of n=7 MS donors. The ratios of antibody-secreting cells (ASC; $CD3^-CD38^{high}CD27^{high}$) and B cells ($CD3^-CD19^+CD38^{dim/-}$) were elevated in meningeal and brain tissues of MS donors (n=22), in contrast to non-demented controls (n=10). The presence of $CD4^+$ memory T cells positively correlated with ASC/B-cell ratios in 11 white matter lesions, which was reversed in 15 normal-appearing white matter samples. CD45 levels on brain-derived ASC were reduced, suggesting long-term persistence. Accordingly, $CXCR3^+$ class-switched B cells of natalizumab-treated MS patients were highly capable of differentiating into ASC, which corresponded to high EBV copy numbers in memory B cells. These findings implicate that $CXCR3^+$ class-switched B cells are induced by both IFN-γ and infectious triggers, thereby promoting their recruitment and maturation into ASC in the MS brain. The known association of such B cells with age possibly contributes to their local persistence.

97 - Interactome-based analysis of GWAS data delineates the mechanisms involved in multiple sclerosis-Epstein Barr virus association.

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We performed an interactome-based analysis of GWAS data to assess the association of candidate non-heritable factors with autoimmune diseases. We selected 20 gene modules whose products directly interact with environmental exposures potentially involved in autoimmunity ("candidate interactomes"). Using ALIGATOR program, we tested whether interactomes were enriched with genes containing - within 20 kb upstream/downstream of their transcribed region - nominally significant associated SNPs identified in GWA studies for 9 autoimmune and non-autoimmune diseases. Epstein-Barr virus and other Herpesviruses interactomes resulted enriched with Multiple Sclerosis (MS)-associated genes, differently from other autoimmune diseases. We also found that MS-associated SNPs were likely located in the regulatory regions of interactomes genes, potentially affecting their expression level. To assess the functional implications of in silico results, we tested whether MS-associated interactomes genes were differently expressed in PBMCs and brain cortex transcriptomes from MS patients with different disease course: MS-associated EBV interactome genes resulted enriched in PBMCs of Primary Progressive and in normal appearing grey matter and in grey matter lesions of Secondary Progressive MS patients. Taken together these findings could suggest a role of EBV in the progressive features of MS besides the inflammatory ones. We performed a pathway analysis to identify biological processes potentially involving MS-associated interactomes genes: CD40 signaling emerged as the most implicated pathway. To understand whether CD40 dysregulation in MS patients could be driven by specific virus-host interactions, we evaluated CD40 expression level in endogenous EBV-infected lymphoblastoid lines and in brain lesions: both showed a significantly dysregulated CD40 expression in MS patients compared to controls. Finally, to translate these results into a therapeutical perspective, we used a recently developed tool (Priority Index) enabling to prioritized drug targets on a genetic basis. We found that the top-ranked pharmaceutical targets for MS were enriched with MS-associated EBV interactome genes. This study strongly supports the involvement of Herpesviruses in MS pathophysiology, with potential implications for prevention and therapeutic strategies.

144 - Neurocognitive Heterogeneity in HIV: Cognitive Phenotypes and Immune Characteristics

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Introduction: HIV-1-associated neurocognitive disorder (HAND) persists despite the success of combination antiretroviral therapy. Chronic inflammation and immune dysfunction may contribute to this persistence. HAND is often represented as a single variable reflecting the severity of deficits. However, research has shown HAND to be heterogeneous, with variability in cognitive profiles. Studies have not investigated the immunological characteristics of HAND subtypes; therefore, this study examined the immune/inflammatory profiles of empirically identified subtypes of HAND. Methods: HIV+ adults (193 in number; ages 26-73; 86% virally suppressed) in the Temple/Drexel Medicine Comprehensive NeuroHIV Center (CNHC) Cohort completed comprehensive neuropsychological (NP) assessments and blood specimen collection. Based on a previously described latent class analysis (LCA) of NP assessments, patients were assigned to one of four classes that differed in level and type of cognitive impairment: cognitively intact (37%), mild motor impairment (18%), mild memory impairment (29%), and moderate mixed impairment (16%). Serum markers of immune activation/inflammation were analyzed using ELISA (sCD14, sCD163) and Luminex (IL-6, IL-8, IL-16, IL-18, IL-1β, IFN-γ, IP-10, MIP-1β, MCP-1, MMP-3, MMP-10, TNF-α, s100β, NSE, VEGF) and compared across NP subtypes using analysis of variance. Results: NP classes differed significantly in IP-10, MMP-10, NSE, and TNF-α. Specifically, the memory impairment group demonstrated higher IP-10 (p=.021) and higher MMP-10 (p=.049) than the moderate mixed impairment group. It also demonstrated higher NSE than the motor impairment group (p=.040) and higher TNF-α (p=.039) than the intact group. Analyte values were typically highest in the two mildly impaired groups and lowest in the intact and moderate mixed impairment groups. Conclusion: Findings support the cognitive and immune heterogeneity of HAND, namely, three subtypes of HAND that differ in both severity and pattern of impairment and have distinct immune profiles. Moreover, results demonstrate an inverted U-shaped relationship between inflammation and severity of impairment. Future research will determine whether these immune characteristics reflect causal mechanisms and whether these subtypes differ in CSF immune markers and longitudinal outcomes. Results may ultimately lead to the development of adjuvant therapies that target specific immuno-related cognitive phenotypes associated with HAND.

145 - GENETIC AND IMMUNOLOGICAL CONTRIBUTORS TO VIRUS-INDUCED PARALYSIS

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Neurological diseases such as Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis (MS), Parkinson's disease (PD), and epilepsy can, in some cases, stem from antecedent viral infections. Predisposing genetic risk factors for neurological dysfunction and immunological responses vary among genetically diverse individuals, resulting in a myriad of potential neurological outcomes to viral infection. We use Theiler's Murine Encephalomyelitis Virus

(TMEV) to model heterogeneity in virally induced neurological phenotypes and immune responses in Collaborative Cross (CC) mice. The CC resource consists of genetically distinct and reproducible mouse lines, thus providing a population model with genetic heterogeneity similar to humans. We compared TMEV-induced immune responses in different CC strains by measuring levels of 23 cytokines and chemokines at different stages of infection. Levels of cytokines and chemokines varied by strain across multiple phases of infection, confirming the host genetic background as a source for heterogenous viral response. We therefore haplotyped each CC strain for genomic regions previously linked with TMEV pathogenesis and viral clearance or persistence, individual cytokine levels, and TMEV-relevant gene expression. For loci previously associated with TMEV-induced immune responses, we identified several haplotypes co-segregating with the most severe TMEV-induced neurological outcome, limb paralysis, observed at 90 days post-infection (90dpi). We hypothesized that cytokine and chemokine levels measured at 90dpi were associated with limb paralysis at 90dpi. Using stepwise regression methods, we demonstrated a significant relationship between IL-1A, RANTES, and limb paralysis at 90dpi. To better understand these interactions, we are working to evaluate how genetic background influences post-infection immune responses and neurological outcomes throughout the course of infection by identifying clusters of strains with shared immunological and phenotypic profiles. Overall, these findings provide insight into the complex roles of immune response in the pathogenesis of virus-associated neurological diseases influenced by host genetic background.

177 - CNS-endogenous TLR7 and TLR9 induce different immune responses and effects on EAE

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Innate receptors, including Toll like receptors (TLRs), are implicated in pathogenesis of CNS inflammatory diseases such as multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). TLR response to pathogens or endogenous signals includes production of immunoregulatory mediators. One of these, interferon (IFN)β, a Type I IFN, plays a protective role in MS and EAE. We have previously shown that intrathecal administration of selected TLR ligands induced IFNβ and infiltration of blood-derived myeloid cells into the CNS, and suppressed EAE in mice. We have now extended these studies to evaluate a potential therapeutic role for CNS-endogenous TLR7 and TLR9. Intrathecal application of Imiquimod (TLR7 ligand) or CpG oligonucleotide (TLR9 ligand) into CNS induced IFNβ expression, with greater magnitude in response to CpG. CNS extraparenchymal CD45+ cells were identified as source of IFNβ. Intrathecal CpG induced infiltration of monocytes, neutrophils, CD4+ T cells and NK cells, whereas Imiquimod did not recruit blood-derived CD45+ cells. CpG, but not Imiquimod, had a beneficial effect on EAE, when given at time of disease onset. This therapeutic effect of CpG on EAE was not seen in mice lacking the Type I IFN receptor. Our findings show that TLR7 and TLR9 signaling induce distinct inflammatory responses in the CNS with different outcome in EAE and point to recruitment of blood-derived cells and IFNβ induction as possible mechanistic links between TLR9 stimulation and amelioration of EAE. The protective role of TLR9 signaling in the CNS may have application in treatment of diseases such as MS.

193 - Immuno-thrombotic mechanisms in stroke patients with COVID-19

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Coronavirus disease 2019 (COVID-19) is associated with increased thrombotic risk. Cerebrovascular events in patients with SARS-CoV-2 infection display a higher severity and mortality rate. However, whether thrombo-inflammatory mechanisms leading to stroke in patients affected by SARS-CoV-2 present peculiar characteristics and whether the virus can directly infect thrombus components remains uncertain. We performed a systematic pathological analysis on cerebral thrombi retrieved from patients with large vessel occlusion (LVO) stroke affected by COVID-19 (n=7 patients) and COVID-19 negative controls (n=23) matched for stroke aetiology and acute treatment. We searched for the presence of the SARS-CoV-2 receptor within the thrombotic material, analyzed the presence of SARS-CoV-2 particles, and analyzed the retrieved thrombi's structural composition and immune signature. Stroke patients with COVID did not differ from control patients in terms of blood leukocytes and neutrophils count but had, as expected, higher inflammatory markers (C-reactive protein). We found rare not organized endothelial cells and scant angiotensin-converting enzyme (ACE2) positive cells in thrombi of both groups without significant differences. Electron microscopy (EM) failed to identify virus particles. No differences were found between thrombi of COVID-19^{+} and control patients in the structural components (red blood cells, fibrin, von Willebrand Factor [vWF], platelets), complement complex C5b-9, monocytes/macrophages and lymphocytes. Interestingly thrombi of COVID-19 patients differed from controls in terms of granulocyte density.
In conclusion, cerebral thrombi of COVID-19 stroke patients are characterized by a peculiar immune signature. Tailored anti-inflammatory strategies might be envisaged in counteracting cerebral thrombosis in COVID-19 infection.

224 - IFN-β expression and IFNAR signalling of all CNS-resident cells is needed to control herpes simplex encephalitis

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Herpes Simplex Virus 1 (HSV-1) is a neurotropic virus of the *Herpesviridae* family that affects the majority of the global population. In most individuals, HSV-1 establishes latency in the trigeminal ganglia. After reactivation, in rare cases the virus can enter into the central nervous system (CNS) and then cause severe encephalitis, which is typically associated with neurological sequelae. Upon HSV-1 infection, type I interferon signalling is of key relevance to control viral replication. However, it is not clear which CNS cells contribute in the type I IFN mediated antiviral defence. To determine the source of protective IFN-β within the CNS during herpes encephalitis under *in vivo* conditions, we analysed the survival of mice with ablation of the IFN-β gene in selected CNS cell subsets including neurons, astrocytes and long-lived CNS-resident myeloid cells following intracerebral inoculation of HSV-1. Wildtype mice mainly survived the infection with a body weight loss that normally restored within 21 days, whereas conventional IFN-β$^{-/-}$ mice succumbed to infection. Mice with a selective ablation of IFN-β in neurons, in astrocytes and in long-lived CNS-resident myeloid cells showed an enhanced susceptibility to infection when compared with wildtype mice suggesting that protective IFN-β is produced by all major CNS-resident cells during HSE. Moreover, to address which cell types have to be triggered by IFN-β in order to promote survival, we infected mice that lack type I interferon receptor (IFNAR) expression in cells of the neuroectodermal lineage, in neurons, in astrocytes and in long-lived CNS-resident myeloid cells such as microglia. Wildtype mice mainly survived,

whereas conventional IFNAR$^{-/-}$ mice succumbed to the infection. Mice with a selective ablation of IFNAR in cells of the neuroectodermal lineage, in neurons, in astrocytes and in long-lived CNS myeloid cells showed enhanced susceptibility to infection compared to wildtype mice. Finally, RNA sequencing analysis revealed distinct transcriptomic signatures upon selective cell subset IFNAR triggering during HSV-1 infection highlighting the importance of cell type specific antiviral programs within the infected CNS. These data indicate that IFNAR signaling of several CNS-resident cells plays an essential role to restrict HSV-1 replication and spread within the CNS and thus to promote survival.

321 - Establishment of latent HSV1 genomes by promyelocytic leukaemia protein scaffolding and role in reactivation

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During a lifetime, almost every human will be infected with neurovirulent Herpes Simplex Virus type 1 (HSV1), which establishes a lifelong latent infection. Primary infection likely occurs in muco-cutaneous tissue of the mouth and nose, where lytic infection produces virus that spreads via axons of sensitive neurons of the face, to infect trigeminal ganglia (TG). These depots house the neurons somata, and become the site of latent genomic reservoirs. Herpetic keratitis (HK) is the consequence of reservoir reactivation, which produces virions that infect the cornea via anterograde transport, to elicit lytic inflammatory disease. The unilateral presentation of HK in almost all patients is testament to a natural state of antiviral protection that limits reactivation from neurons after primary infection. Studies in our animal model demonstrated the cause to be host-virus infection kinetics, where the ipsilateral (i)TG becomes infected before protection establishes, and the contralateral is infected afterwards. Thus, reactivations are ipsilateral to the primary infection site. Latent HSV1 genomes are encapsulated in nuclear body (NB) aggregates, enshrouded in a polymerised promyelocytic leukaemia (PML) protein shell. PML isoforms are expressed during acute infection of the iTG, with interferons (IFNs) implicated. While PML-NB facilitate epigenetic repression of HSV1 transcription by chromatinisation of genomes, the mode of action and specific functional outcomes during establishment of latency, and when reactivation is triggered, are not well understood. Different PML-NB morphological patterns develop during infection, which give clues about HSV1-PML-NB interactions during these events, but they need to be studied further. Using viral reactivation assays, PML knock-out mice, immuno-histological and molecular techniques, this study aims to uncover the role of PML-scaffolded structures and the interaction with HSV1 to establish and maintain latent reservoirs. Furthermore, the immune responses that drive PML-NB formation and maintenance will be studied in detail. This will provide a deeper understanding of how epigenetic silencing of HSV1 by PML-NB can be exploited to elicit a permanent latent state. Defining these pathways of epigenetic control could also enable their exploitation for treating other diseases.

354 - A Magnetic Resonance Imaging Study of Prenatal Zika Virus Infection Using a Novel Rat Adapted Virus

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Zika Virus (ZIKV) is a flavivirus that, in cases of in utero fetal infection, is known to cause microcephaly and other neurological and developmental disorders. Like humans, rats exhibit immunosuppression during pregnancy which allows the Zika Virus to infect fetuses in utero. Building on our previously developed model of prenatal ZIKV infection, we are currently using a novel rat-adapted virus that has been passaged on rat cochlear microglial cells (Mocha). This virus is expected to produce a stronger infection in rats than a human-derived virus. Pregnant rats

were infected at gestational day 18 as previously established by our model. They were inoculated either with the virus, a diluent control, a UV-inactivated virus (iZIKV), or a non-neutralizing antibody combined with the virus. This antibody was included to increase infection in vivo through antibody-dependent enhancement (ADE). This process can drive entry of the virus into cells that express an Fc receptor, a receptor located on immune cells and involved in antibody recognition. Nonpregnant female rats were also inoculated with diluent, iZIKV, ZIKV, or antibody+ZIKV. Febrile response was measured for seven days post-injection. This was to replicate prior results in which non pregnant rats exhibited an increase in temperature following ZIKV inoculation that pregnant females did not. These results will also allow us to identify the timeline of infection in the adult animals. Pups were born to pregnant females at five days post-injection. At postnatal day 2, one male and one female were selected from each litter for ex-vivo structural magnetic resonance imaging (MRI). Regions associated with cognitive and motor deficits common in prenatal ZIKV infection were chosen for assessment. Cortex, hippocampus, and cerebellum were segmented and volume relative to overall brain size compared. Animals exposed to either ZIKV or antibody+ZIKV were expected to have overall decreased brain volume as well as possible decreased relative volume of these regions. In a second experiment, we collected tissue from fetuses 24 hours, 48 hours, and 72 hours following maternal infection. Peripheral and brain tissue were collected to assess immune response, apoptosis, and response to viral infection in utero to determine timeline of fetal infection. These findings will validate our model as etiologically relevant for ZIKV induced microcephaly and will allow us to identify which brain areas are more affected by the virus.

379 - COVID-19 AND NEUROLOGICAL COPMLICATIONS

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On March 11th, 2020, Coronavirus Disease 2019 (COVID-19) was affirmed by the World Health Organization as pandemic. The cause of the severe acute respiratory syndrome that became known as COVID-19 was a novel coronavirus, SARS-CoV-2. Evidence showed these viruses can affect different human systems. Neurological manifestations are the second most common symptom after respiratory symptoms. COVID-19 can develop neurological complications either by the direct effect on the nervous system during the acute phase or indirectly by immune-mediated infection, which may appear even after months following the acute phase.

SARS-COV-2 has a large enveloped with spike proteins on its surface. By these surface proteins, SARS-COV-2 binds to the human angiotensin-converting-enzyme receptor 2 (ACE2) on human cells. ACE2 is expressed in many tissues like the lung parenchymal, kidney, pancreas, CNS, including neurons and glial cells. After binding to ACE2, the enzyme TMPRSS2 help to virion entry and virion releases its RNA. RNA is translated to proteins which are necessary for virion and finally the RNA are assembled into a new virion and exits the cell.

SARS-CoV-2 infects olfactory epithelium and reaches the CNS via the olfactory neurons. The high expression of ACE2 and TMPRSS2 on the olfactory epithelium has a significant role in transferring the virus into CNS through olfactory neurons. The second rout occur when SARS-CoV-2 infects peripheral nerves and the virus uses the axonal transport machinery (retrograde transport) to access the CNS. The third route is about the role of leukocytes. Coronavirus can spread to the CNS via infected immune cells, such as monocytes, neutrophils and T cells. Some evidence showed immune cells can express the binding receptors of coronaviruses. So they serve as the reservoirs for virus particle. About the fourth rout is assumed, Coronaviruses can attack to ACE2 receptors on the endothelial cells of brain vessels caused disruption in BBB and virus spread into CNS.

There are some more important injuries in COVID-19 such as, hypoxia, immune injury and cognition impairment. Infections can lead to brain hypoxia. Hypoxia in brain subsequently causes edema in brain cells, cerebral vasodilation, cerebral blood flow impediment and headache. If hypoxia continues, brain function will be reduced and drowsiness, bulbar conjunctival edema and more complex problems like coma can be observed. As, the patients with COVID-19 often suffer from severe hypoxia this may lead to subsequent nervous system damage.

COVID-19 can lead to brain damage and chronic inflammation via the activation of glial cells. Glial cells by producing high level of inflammatory factors enhance the brain damage. Cytokine storms cause to disruption the integrity of BBB, which provokes the neuro inflammatory process. Neuro inflammation has been implied as an

important factor in neurodegenerative disorders and psychiatric pathologies, including acute psychosis, schizophrenia, and autism spectrum disorder.

Clinical findings in COVID-19 patients showed neuronal complications such as epilepsy, intracranial infections, encephalitis, meningitis, dizziness, depression, Parkinsonism, confusion, headache, insomnia, stroke, myelitis, etc. Although these documents are at preliminary stages, and we need more information about the virus, but special attention must be given to patient with

COVID-19 for preventing neurological damage in the future until a specific treatment is available.