UAlberta MS Centre Research Symposium

Brain-Gut Axis:
a focus on finding a cure for Multiple Sclerosis

May 4th 2018

Telus International Centre

University of Alberta

UAlberta MS Centre Research Symposium
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8:30AM	**Welcome & Introduction -** ***Michael Phair*** **–** Chair, Board of Governors, University of Alberta
8:45AM	***Dr. Hartmut Wekerle*** **–** Multiple Sclerosis: Brain, Gut and Microbes
9:35AM	***Dr. Kathy McCoy*** **–** The Role of the Microbiome in Educating the Developing Immune System
10:25-10:45	**Health break**
10:45AM	***Dr. Jens Walter*** **–** Impact of diet on the gut microbiome: Implications for Multiple Sclerosis
11:30 AM	***Dr. Helen Tremlett*** **–** The Gut Microbiota in (Paediatric) Multiple Sclerosis: Epidemiology
12:20PM	**Lunch**
12:45-1:45	**Poster Presentations –** See Poster Layout
1:45 PM	**Trainee Presentations**
2:30PM	***Dr. Kassandra Munger*** **–** Nutritional (and Other) Factors in MS Risk and Progression
3:20-3:35	**Health break**
3:35PM	***Dr. Tom Louie*****–** Down the hatch...eating fecal microbes opens the door to restoring diverse clinical entities along the gut-brain axis
4:25PM	**Question and Answer Panel**
5:30PM	**Closing Remarks**

~ Welcome ~

Welcome to the 3rd annual UAlberta MS Centre Research Symposium! We are very excited to host an outstanding program filled with cross disciplinary speakers who investigate a broad range of subjects that highlight the role of the brain-gut connection in MS. The brain-gut axis has emerged as a unifying concept linking the functions of the gut and brain that is operative at multiple levels including MS-associated changes in the microbial flora of the gut (the so-called microbiome), the impact of immune system on the gut's (enteric) nervous system during MS, the effects of diet on MS, the potential pathways connecting the gut and nervous system in MS as well as potential therapeutic windows for MS provided by the gut.
As always, our overarching objective is to offer people an opportunity for dialogue in which interdisciplinary discussions will enhance collaboration between our experts and their trainees in these areas, thereby accelerating progress in the field of MS research. The end of day panel discussion involves individuals from different backgrounds ensuring a diverse and informative session for all participants. The MS Centre was conceived in 2014 to foster research, education and innovative clinical care for multiple sclerosis through an interdisciplinary approach and through building a platform to service a catalyst for the exchange of ideas among scientists, clinicians and trainees at all stages of their careers.

We would like to thank everyone who has worked diligently to organize this Research Symposium, particularly Erika Johnson for her enthusiasm and dedication to its planning, and we are especially grateful for the sponsors for their assistance in supporting this event. We look forward to a meeting that encourages scientific dialogue and pleasant social interactions.

Our very best wishes,

Bradley Kerr, PhD and Christopher Power MD
Co-Directors of the UAlberta MS Centre

UAlberta MS Centre

UNIVERSITY OF ALBERTA
NEUROSCIENCE AND MENTAL HEALTH INSTITUTE

UNIVERSITY OF ALBERTA
FACULTY OF MEDICINE & DENTISTRY

~ Co-Directors of the UAlberta MS Centre ~

Dr. Bradley Kerr, PhD
Associate Professor
Anesthesiology and Pain Medicine

Dr. Bradley Kerr completed a BSc at McGill University and went on to do a PhD in the UK at King's College London. From London he moved to the California Institute of Technology for a postdoctoral fellowship and then returned to McGill for a second postdoctoral fellowship with Dr. Sam David. Dr. Kerr became a faculty member at UofA in 2007 and is an Associate Professor in the Department of Anesthesiology and Pain medicine. Dr. Kerr is a Co-Director of the UAlberta MS Centre and Assistant Chair for Research in Anesthesiology and Pain Medicine.

Dr. Christopher Power is a Professor of Neurology at the University of Alberta, holds a Canada Research Chair in Neurologic Infection and Immunity and is a Fellow of the Canadian Academy of Health Sciences. He is an clinician-scientist, focused on the causes and treatments of neuroinflammatory diseases and whose research program is supported by the MS Society of Canada, Alberta Innovates, and the Canadian Institutes of Health Research. He is also an attending consultant at the University of Alberta Hospital is Co-Director of the University of Alberta MS Centre. He is the author of over 190 peer-reviewed publications and 20 book chapters.

Dr. Christopher Power, MD, FRCPC
Professor
Canada Research Chair
Department of Medicine (Neurology)

~ Guest Speakers ~

Dr. Hartmutt Wekerle

Hartmut Wekerle was director and member of the Max Planck Institute of Neurobiology. In 2012 he was awarded a Hertie Senior Professorship, and he leads an Extended Emeritus Group. Hartmut Wekerle's scientific research focuses on the mechanisms initiating and driving multiple sclerosis and its experimental models, which imply autoimmune attack against the nervous system. Wekerle's work led to the identification of brain reactive autoimmune T lymphocytes in the immune system. Most recently, he identified the commensal bacterial gut flora as a factor triggering the pathogenic potential of immune cells. He develops and uses new imaging approaches to detail the mechanisms of autoimmune T cell migration into the brain. Wekerle has received numerous awards, including the Jung Prize, Zülch Prize, Koetser Prize, Charcot Award (MS International Federation), Grand Prix Louis D. (Institut de France), and a Koselleck Award (DFG), Jacob-Henle-Medaille (University Medical Center of Goettingen). He holds an Honorary Professorship of the University of Munich and Honorary Doctorates of the Universities of Hamburg and Wuerzburg. He is a member of the German Academy of Science (Leopoldina), Honorary Member of the Société Française de Neurologie and Honorary Member of the Cuban Neuroscience Society.

Kathy McCoy

Dr. Kathy McCoy is a Professor in the Cumming School of Medicine and Scientific Director of the International Microbiome Centre at the University of Calgary.

Kathy is a mucosal immunologist and microbiome researcher interested in the dynamic interplay between the gut microbiota and the innate and adaptive immune systems. Using germ-free and gnotobiotic animal models together with human translational studies her research aims to understand how exposure to intestinal microbes early in life educates and regulates the developing immune system and how this impacts on susceptibility to immune-mediated diseases, such as allergy and autoimmunity, later in life.

~ Guest Speakers ~

Jens Walter

Dr. Jens Walter is an Associate Professor and Campus Alberta Innovation Program Chair for Nutrition, Microbes, and Gastrointestinal Health at the University of Alberta. After majoring in Food Technology, he became increasingly interested in probiotics and gut microbiota research. His graduate research under the supervision of Walter P. Hammes at the University of Hohenheim in Germany was on the molecular characterization of gut ecosystems and ecological studies of intestinal lactobacilli. Dr. Walter received his doctoral degree in 2003 and continued to conduct his postdoctoral research into the genetic basis of gut colonization by lactobacilli and functional metagenomics of gut ecosystems at the University of Otago in New Zealand under the supervision of Gerald Tannock. In 2006, Dr. Walter accepted a tenure track position at the University of Nebraska-Lincoln to work as a Molecular Microbial Ecologist. He received tenure in 2012 before moving to the University of Alberta, Canada, in 2014. Dr. Walter's research focuses on the investigation of ecological and evolutionary processes that shape host–microbial symbioses in the human gut, and the application of these scientific concepts to develop microbiome-targeted nutritional and therapeutic strategies to improve human health.

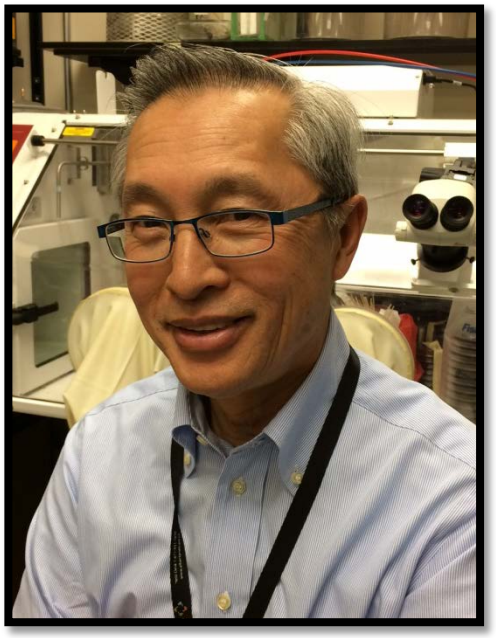
Helen Tremlett

Canada Research Chair in Neuroepidemiology and Multiple Sclerosis and Professor at the University of British Columbia, Vancouver, Canada in the Faculty of Medicine, Division of Neurology. Her research program is also funded through operating grants from the Canadian Institutes of Health Research (CIHR), the MS Society of Canada, the MS Scientific Research Foundation, the US National MS Society among others. Trained in pharmacoepidemiology and multiple sclerosis with a PhD from Cardiff University, UK. Heads the Tremlett Lab and the Epidemiology in Multiple Sclerosis research program. Research interests include: the natural history of MS; prognosis and predictors of disease progression in MS; mortality; safety and effectiveness of the immunomodulatory drugs (IMDs) for MS; pharmacogenomics; MS epidemiology; incidence and prevalence of MS; life expectancy in MS; comorbidities and MS; pregnancy and MS; impact of parental MS on childhood developmental outcomes; health administrative data; the MS prodrome; the gut microbiome and MS.

~ Guest Speakers ~

Kassandra Munger

Dr. Kassandra Munger received her BA in Biology from the University of Rochester in 1997, MS in Epidemiology from the University of Massachusetts, Amherst in 2001, and her ScD in Nutritional Epidemiology from the Harvard School of Public Health in 2009, where she is currently a research scientist in the Neuroepidemiology group in the Department of Nutrition. For over 15 years she has been working with the Nurses' Health Studies and other large cohorts to investigate the role of various infectious and dietary biomarkers, including Epstein-Barr virus and vitamin D, as well as other environmental risk factors such as obesity, and risk of multiple sclerosis. Her research interests also include whether established MS risk factors influence MS progression and whether MS patients with generally healthy lifestyles experience better outcomes.



Thomas Louie

Dr. Thomas Louie MD (Alberta '69), FRCPC is Clinical Professor, Department of Medicine, Cumming School of Medicine, University of Calgary. After infectious diseases training at UCLA and Tufts University, Dr. Louie has been a clinician investigator in Winnipeg and in Calgary since 1988. In addition to setting up infection control programs and infectious diseases services, Dr. Louie has mainly contributed to the literature on the development of newer agents in the treatment and prevention of Clostridium difficile infection. In addition he has provided fecal microbiota transplantation to patients with multiple recurrent C. difficile infection since 1996 for patients from across the Canada, and has pioneered a transition between 2010 -2013 to the use of oral fecal capsules as a low cost, highly effective and well tolerated means of repopulating the gut microbes. This approach, originally thought of as high risk, has opened the door to restoration and manipulation of the gut microbiota in medicine for the many effects the gut microbiome might have on human health. Dr. Louie will go through some of the new opportunities and challenges for microbiome restoration.

~ Panel Members ~

Dr. Garry Wheeler

Dr. Garry Wheeler is an Exercise Physiologist and Registered Psychologist in the Province of Alberta. He is currently President of the Alberta and Northwest Territories Division of the Multiple Society of Canada. He has published more than 47 articles in peer reviewed journals during his academic career and has over 80 conference abstracts and presentations in scientific conferences to his name. Garry specialized in two areas of publications: physiology and psychology. His main topics of study were the effects of chronic exercise stress on endocrine profiles and males and retirement in athletes with disability respectively. In addition he has also co-authored and edited a number of text books and chapters of books in the Adapted Physical Activity domain. For his work in the area of disability sports he was awarded the 2007 International Paralympic Committee Scientific Award in Seoul Korea for outstanding research and services to athletes with disability. Other career roles have included: member of the International Paralympic Sports Science Committee; publishing and writing in the health fitness and lifestyle area and Coaching Association of Canada. In the latter area he has been responsible for creating ethics modules for coaches of children in Canada.

Margaret Prociuk – RN

Margaret graduated from Vancouver Community College as a registered nurse in 1986. She went on to obtain her Bachelor of Science in Nursing from the University of Alberta in 1993. During her working career, she focused mainly in the area of intensive care and cardiac surgery and returned to University in 2006 to pursue her Master's. In 2009, she graduated with a Master of Nursing from Athabasca University and worked in primary care prior to joining the Multiple Sclerosis clinic in 2014. The Nurse Practitioner role in the MS clinic is to facilitate patient care and access and support physician work flows as well as research initiatives. Margaret has become an MS Certified Nurse since joining the clinic and was the recipient of the MS Society Professional Care award in 2015.

~ Panel Members ~

Debra Vollrath

Deb Vollrath graduated from the University of Alberta with a Bachelor of Science in Laboratory Medicine in 1996. Her keen interest in research aided in the discovery of the Bordetella Pertussis gene that is responsible for turning this organism's virulence factors on and off. This discovery has created an opportunity to develop a more potent vaccine for whooping cough. Deb worked as a Laboratory Technologist for a few years prior to pursuing a rewarding career as a Pharmaceutical Sales Representative with both Pfizer and Janssen-Ortho. Deb was working as a Hospital Specialty Representative for Janseen-Ortho when she experienced her first debilitating MS relapse in 2009. Eventually, Deb had to stop working due to her MS symptoms. It was very hard for her to accept this "forced retirement" at such an early stage of a promising career. The silver lining was that by making her health her "full time job", she was able to spend more time with her husband and two active children. Deb continues to battle MS on a daily basis. Her interest in research and development has her keenly interested in new and advancing drugs/treatments for MS.

Dr. Kassandra Munger

Dr. Jens Walter

~ Poster Session Layout ~

P01 – Carlos Camara-Lemarroy, University of Calgary

Patients with early MS and an altered intestinal barrier have a distinct immunologic phenotype
Carlos R. Camara-Lemarroy, Jennifer Hahn, Claudia Silva, Luanne Metz, V. Wee Yong

Background and purpose: Patients with Multiple Sclerosis (MS) have increased intestinal permeability. An altered intestinal barrier could lead to the translocation of gut commensals and associated molecules into the circulation that alter immunologic responses. Our aim was to measure the serum levels of Intestinal Fatty-Acid Binding Protein (IFABP), a marker of intestinal barrier integrity, in patients with early MS/Clinically Isolated Syndrome (CIS). Inflammatory mediators including multiple cytokines, chemokines, growth factors and matrix metalloproteases were also quantified.
Materials and methods: We included Calgary site patients from the Minocycline-CIS trial (Metz et al., New Engl J Med 376:2122, 2017), who experienced a first clinical demyelinating event (29 patients with a mean age of 32.2 ± 9.1 years, where 62% were female). We used 10 age and sex matched healthy individuals as controls and 5 patients with Inflammatory Bowel Disease (IBD) as positive controls (for IFABP levels only). Pre-treatment blood-samples were used to measure IFABP by ELISA and cytokine levels by Luminex® Assay. Clinical (EDSS), demographic and outcome measures (conversion to Definite MS) were analyzed.
Results: Patients with early MS/CIS have higher concentrations of IFABP than controls, indicating an altered intestinal barrier. IFABP levels were not associated with age or sex, but they were significantly correlated with EDSS. When comparing patients with an altered intestinal barrier to those with an intact barrier, we found differences in the concentrations of several immune mediators. Notably, patients with an altered intestinal barrier had higher concentrations of TNF-alpha, MMP-1, and MMP-1/TIMP-1 ratio, while patients with an intact barrier had higher concentrations of GCSF, GMCSF, IP10, MCP-3 and IL-18.
Conclusions
Some patients with early MS/CIS have an altered intestinal barrier. This subgroup also has a distinct immunologic phenotype. The clinical relevance of these differences remains to be elucidated.

P02 – Andrew Caprariello, University of Calgary

Biochemically-altered myelin triggers autoimmune demyelination
Andrew V. Caprariello, James A. Rogers, Megan L. Morgan, Vahid Hoghooghi, Jason R. Plemel, Adam Koebel, Shigeki Tsutsui, Jeffrey F. Dunn, Lakshmi P. Kotra, Shalina S. Ousman, V. Wee Yong, Peter K. Stys

The etiology of inflammatory demyelination in multiple sclerosis (MS) remains unresolved. Conventional thinking holds that lesions are caused by unchecked immune attacks on CNS myelin, a concept recapitulated in an animal model called experimental autoimmune encephalitis (EAE). Unlike EAE, however, the identity and target of pathological self-antigens in MS remain elusive. Peripheral targets have been the field's primary focus, but we sought to test whether destabilized CNS myelin can initiate its autoimmune demise. To test this theory, mice were fed 0.2% cuprizone for two weeks, an exposure brief enough to destabilize but not destroy myelin. Abbreviated cuprizone treatment was followed by immune adjuvant (identical to standard EAE but *without* myelin peptides), after which a two-week incubation period on normal chow allowed time to mount potential immune responses. Excitingly, cuprizone-altered myelin was sufficient to trigger robust inflammatory demyelination particularly of the corpus callosum. Brains were gadolinium-enhancing on T1 MRI, which correlated with infiltrating, myelin-reactive T cells and peripheral macrophages/monocytes. Shorter or longer cuprizone feeding reduced the inflammatory infiltrate, demonstrating that myelin immunogenicity depended not only on a stimulated immune system but also on the extent and timing of biochemical myelin modification. We next tested whether protein-arginine deiminases (PAD), enzymes known to destabilize myelin and to correlate to MS lesion severity, contributed to myelin-induced inflammation. A small molecule PAD inhibitor delivered during only the cuprizone induction phase prevented the inflammatory reaction to myelin destabilization. Our Cuprizone Autoimmune Encephalitis (CAE) model therefore provides proof-of-concept as well as a molecular pathway through which subtle damage to brain myelin triggers MS-like inflammatory pathology.

P03 – Ana Catuneanu, University of Alberta

Sex differences in central nervous system plasticity and pain in a mouse model of multiple sclerosis
A. Catuneanu, J.W. Paylor, I. Winship and B.J. Kerr

Aim of Investigation: Multiple Sclerosis (MS) is a neurodegenerative autoimmune disease with many known structural and functional changes in the central nervous system. A well-recognized, but poorly understood, complication of MS is chronic pain. Furthermore, little is known regarding the influence of sex on the development and maintenance of MS-related pain. This is important to consider, as MS is a predominantly female disease. The current project uses the experimental autoimmune encephalomyelitis (EAE) mouse model of MS to examine key pain processing regions in the spinal cord of male and female mice.
Methods: Following EAE induction, pain behaviours were assessed using von Frey monofilaments and the acetone test. At disease onset (indicated by partial tail paralysis), spinal cord tissue was extracted and prepared for Golgi-Cox and immunohistochemical staining to visualize features of wide dynamic range (WDR) neurons within the dorsal horn.
Results: Both male and female EAE mice demonstrated increased frequency and duration of nociceptive behaviours, however this response was often lateralized in females. Sholl analysis of dendritic arborization revealed WDR neurons from male EAE mice showing behavioural hypersensitivities are significantly less complex, whereas the opposite effect was observed in EAE males lacking pain behaviours. Exploration of potential mechanisms for this finding revealed sex differences in axonal injury and demyelination, microglial activation and extracellular matrix integrity.
Conclusions: This study reveals morphological and inflammatory differences in the spinal cord associated with pain development between female and male mice with EAE, supporting the idea of differentially regulated pain pathways between sexes. Results from this study may indicate future sex-specific targets that are worth investigating their functional role in pain circuitry.

P04 – Matthew Doan, University of Alberta

Brain peroxisome injury is associated with neuroinflammation in multiple sclerosis
M. Doan, M. Mamik, L. Saito, P. Chen, W. Branton, R. Rachubinski, T. Hobman, C. Power

Multiple sclerosis (MS) is a progressive neuroinflammatory disease associated with demyelination in the central nervous system. Peroxisomes are a major organelle involved in the detoxification of reactive oxygen species, β-oxidation of fatty acids and the production of plasmalogens. Peroxisomal dysfunction in the brain potentially plays a key role in demyelination, as oligodendrocytes contain an abundance of peroxisomes. Our current working hypothesis is that neuroinflammation negatively impairs peroxisome function at the transcript and protein levels, exacerbating disease, leading to further demyelination. To explore this hypothesis *in vitro*, we used differentiated THP-1 (macrophage-like) and MO3.13 (oligodendrocyte-like) cell lines exposed to inflammatory molecules (LPS and TNFα) to determine changes in transcript levels of select peroxin (PEX) and metabolic genes using sq-RT-PCR analysis. To verify our findings, we analyzed autopsied brain samples from MS and non-MS control patients at both the transcript and protein levels. In cell lines, a decrease in transcript levels with inflammatory stimuli was found for a majority of peroxisome genes including *PEX3*, *PEX5*, *PEX11β*, *PEX16*, *PEX19* and *catalase* in a cell-type dependent manner. Similar altered expression levels were observed in MS brain tissue, including decreases in several PEX gene transcript levels along with a reduction in *PMP70* and *catalase* when compared to non-MS controls at the protein level. Furthermore, we are currently investigating therapeutic options to rescue peroxisome function at the protein levels. We have identified fenofibrate and 4-phenylbutyrate as possible agents to help alleviate peroxisome perturbations. Our studies are investigating both *in-vitro* and *in-vivo* effects of these drugs at the protein level of key peroxisomal proteins that appear to be affected by inflammatory conditions. Together, our data suggest neuroinflammation negatively affects peroxisome biogenesis and metabolic processes both in cell cultures and MS brain tissue. Further research is being done to identify the specific pathway(s) associated with peroxisomal injury in MS patients and how therapeutics can potentially halt demyelination and/or facilitate remyelination.

P05 – Timo Friedman, University of Alberta

MicroRNA-RNA interactions within a pain-centered EAE mouse model: an unbiased predictive computational approach utilizing Next Generation Sequencing

T.Friedman , M. Saad Yousuf , A. Cantuneanu , B. Kerr

MicroRNA (miR) are short non-coding RNA regulatory molecules characterized by their ability to interfere with the translation process of RNA. Experimental autoimmune encephalomyelitis (EAE) is an inducible disease state with widespread pathway dysregulation at the protein and RNA level, including immune and inflammatory pathways. EAE is used for its similarities to Multiple Sclerosis (MS), as it mimics many aspects of motor disturbance in the disease, but also the heterogeneous pain syndromes across individuals. Although miRs have been studied in EAE, their role in tissues of the PNS is poorly understood. As the pain experienced by both MS patients and EAE mice may arise in the periphery, we examined the pattern of miR expression in the dorsal root ganglion (DRG) of mice with EAE. We collected DRG samples from male and female mice with EAE that exhibited signs of pain hypersensitivity or not, as well non-disease controls. The samples were purified for both miR and RNA and sequenced by Next Generation Sequencing. RNA and miR statistically differing between groups were queried through online pathway databases for potential biological functions or theoretical RNA targets respectively. We have begun to validate miR-30a/d as it was strongly downregulated in our female cohort, but not male mice with pain. We have confirmed the upregulation of predicted miR-30 targets such as Stau1 and Magt1, providing evidence of our microarray's validity. In female mice, miR-30a/d may have a role in the induction of pain, although its specific function is still being investigated.

P06 – Joanna Jung, University of Alberta

Calnexin role in T cell Transmigration into the central nervous system

J. Jung, T. Paskevicius, P. Eggleton, C. Power, M. Michalak

Multiple sclerosis (MS) is a chronic, progressive disease characterized by the destruction of central nervous system (CNS) myelin and often the underlying axons. Leukocyte infiltration into the CNS is an underlying cause of its pathological conditions. Leukocyte crossing requires cascade of physiological changes in brain blood barrier (BBB) endothelial cells and cells of immune system. Activation of brain endothelia cells involves changes in expression and activity of adhesion molecules that provide the adhesiveness necessary for extravasation. Cell adhesion molecules of the Ig superfamily play key roles in leukocyte transmigration. They play critical roles in the immune response and are upregulated endothelial cells in response to inflammatory stimuli and contribute to the adhesiveness of transmigrating cells. Among the CAMs that play important roles in the trafficking of leukocytes through the BBB are Intercellular Adhesion Molecule 1 (ICAM-1), Vascular Cell Adhesion Molecule 1 (VCAM-1), and Platelet Endothelial Cell Adhesion Molecule 1 (PECAM-1). In the animal models of MS - experimental autoimmune encephalomyelitis (EAE), immunization with myelin proteins results in the symptoms of demyelinating inflammation of CNS resembling MS.

Calnexin (Canx) is a type I integral membrane protein and molecular chaperone that resides in the lumen of endoplasmic reticulum and is involved in the quality control and trafficking of glycoproteins. We discovered that MS patients have an unusually high abundance of CANX in BBB endothelial cells, while deficiency of Canx in mice confers resistance to experimental autoimmune encephalomyelitis (EAE), the animal model recapitulating the key pathological features of MS. We show that T cells specific conditional knockout of Canx did not result in the EAE resistance. Instead, the absence of Canx did prevent T-cells transmigration across BBB brain endothelial cells[4], suggesting essential role for Canx in the function of BBB in T cells transmigration. We have also identified fatty acid binding protein 5 (FABP5), a member of a family of highly conserved, cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands, as a novel binding partner of Canx that might contribute to its role in pathology of EAE. Deficiency in FABP5 also protects against EAE suggesting that potentiation of the Canx/FABP5 complex may be a key to the induction of MS, and that long-lived formation of the Canx/FABP5 complex increases susceptibility to MS.

P07 – Kyla Coates, University of Calgary

The mechanisms of fatigability from whole-body exercise and its relationship to chronic fatigue in people with multiple sclerosis: Preliminary results.

K. Coates, S.J. Aboodarda, S. Jarvis, E. Giroux, L. Metz, G.Y. Millet

The present study investigated whether chronic perceived fatigue in people with Multiple Sclerosis (PwMS) was associated with greater neuromuscular fatigability during cycling.
Eleven PwMS with high levels of perceived fatigue (HF), ten PwMS with low levels of perceived fatigue (LF), and eleven healthy controls (CON) completed an incremental cycling task to volitional exhaustion. By employing an innovative cycle ergometer, neuromuscular assessments were performed at baseline, every 3 minutes during cycling, and immediately after exhaustion. Maximal voluntary contractions (MVC) and electrical stimulation of the femoral nerve (PNS) were used to quantify voluntary activation (VA) and muscle contractile ability (PT). The EMG responses to PNS (Mmax) and transcranial magnetic stimulation (MEP) during MVC were used to quantify corticospinal excitability (MEP/Mmax).
MVC and VA dropped over time to a similar extent in all groups ($P<0.003$). A greater decrease in PT relative to baseline was observed in HF compared to CON at the last commonly completed stage (stage 3)($P=0.03$), and exhaustion ($P=0.019$). From the first neuromuscular evaluation onward, HF displayed a lower PT compared to LF ($P=0.041$), but both groups declined over time ($P < 0.001$). MEP/Mmax was significantly lower in HF compared to CON ($P= 0.041$) at all time points.
This study demonstrates that both peripheral and central mechanisms contribute to exercise-induced neuromuscular fatigability in PwMS with higher levels of chronic perceived fatigue.

P08 – Chieh-Hsin Lee, University of Alberta

Characterization of immune cell populations of clinically isolated syndrome patient conversion to multiple sclerosis

C. Lee, M. Nakhaei-Nejad, D. Barilla, F. Giuliani

Clinically isolated syndrome (CIS) is the prodromal phase of multiple sclerosis (MS) disease course, with patients having experienced only one neurological episode. Given that up to 70% of CIS patients develop subsequent MS, it is important to differentiate those who will and will not convert to RRMS in the future. This will not only allow for earlier treatment but also help improve our understanding of the MS disease course. This study aimed to: (1) dissect the prevalence of various PBMC subsets in CIS and RRMS patients who are not being treated with any disease modifying therapy; (2) identify "biomarkers" that predict progression from CIS to RRMS; and (3) examine whether there is a difference in the PBMC subpopulations between early and late phases of the disease in RRMS.
Multi-colour flow cytometry panels were designed to identify up to 50 peripheral blood lymphocyte subpopulations. We compared patients with CIS who do (CIS-C) or do not (CIS-N) covert to RRMS at a later point with relapsing remitting multiple sclerosis (RRMS) patients, which are further divided into early RRMS (RRMS-E) and late RRMS (RRMS-L).
In the B cell compartment, naïve/transitional cells are significantly higher in RRMS-L than RRMS-E, which is similar to CIS-C. In females, RRMS-L have a higher amount than both RRMS-E and CIS-C. Pre-switch memory (CD27+IgD+) cells are significantly higher in RRMS-E than CIS-N. In the T cell compartment, the CD4-to-CD8 ratio is higher in the RRMS group than CIS-N. With both T helpers, TfH and Th22, numbers being lower in RRMS than the two CIS groups. CD8+CD127+CD45RA-, a memory subpopulation, is higher in RRMS than CIS-N, while CD8+CD62L-, an effector subpopulation, is lower in RRMS than CIS-N.
These results show that CIS patients who do or do not convert to RRMS are different in some immune subpopulations, with those who convert being similar to those already diagnosed with RRMS in some lymphocyte subsets. They also show that in many subpopulations the levels of converter CIS patients are more similar to early than late RRMS patients. These results partially support the view that CIS patients who convert are functionally the same as RRMS and suggest that peripheral blood markers may be used to predict future conversion of CIS patients to RRMS and allow for earlier treatment of the disease.

P09 – Olga Lekontseva, University of Alberta

Delivering education to patients with MS – a survey of Canadian Neurologists

O. Lekontseva, P. Smyth, C.S. Hodgson

Objectives: Meeting education needs of people diagnosed with multiple sclerosis (MS) is increasingly challenging given the complexity of the disease, treatment options, and shared decision--making. If people with MS are not satisfied with the education received from their neurologist, they turn to independent sources of information such as internet. Our research questions were: (1) at the time of diagnosis, what resources, if any, are used by Canadian neurologists to educate their patients with MS and (2) is there a relationship between years since graduation from medical school or gender and types of education provided?

Methods: We developed a database of Canadian (excluding Quebec) neurologists (N=838) from online, searchable, provincial databases of the Colleges of Physicians and Surgeons of Canada. A postcard survey was mailed to a random sample (33%) of neurologists. Data were analysed using SPSS.

Results: Of 277 neurologists mailed the survey, 68 (24%) replied. Mean time from medical school graduation was 24 years (range: 7--43 years); 31% were female. The sample was representative of the overall Canadian neurologist population.

Results indicate that 68% provide MS education while 31% refer. Most (81%) use multiple strategies: information on reputable websites (76%); printed handouts (MS society 52%, drug company 57%, made by their practice 28%); and/or education sessions (28%). Physicians who graduated from medical school less than 20 years ago were more likely to recommend websites (90%) compared to those graduating more than 20 years ago (65%); the opposite was true for use of MS society handouts (29**%** and 70%, respectively). Women were more likely to provide websites and use drug company handouts than men.

Conclusions: Our study demonstrates that the majority of neurologists deliver MS education to their patients; however, educational methods were utilized differently by generational cohorts and gender. A limitation to the study was the small sample size.

P10 – Brienne McKenzie, University of Alberta

Inflammasome activation and pyroptosis in oligodendrocytes and microglia during multiple sclerosis and experimental autoimmune encephalomyelitis

B.A. McKenzie, M.K. Mamik, L. B. Saito, R. Boghozian, M.C. Monaco, E.O. Major, J-Q. Lu, W.G. Branton, C. Power

Inflammasome-associated caspases mediate the maturation and release of the proinflammatory cytokines IL-1b and IL-18, and activate the pore-forming protein gasdermin D (GSDMD). Recently, GSDMD was shown to be the primary executioner of pyroptosis, a form of programmed proinflammatory cell death downstream of inflammasome activation. Excessive GSDMD pore formation causes local osmotic swelling and the formation of pyroptotic bodies, which eventually burst leading to cell lysis. Although evidence has emerged for inflammasome activation and pyroptosis in neurological diseases, the role of GSDMD in neuroinflammation remains uncharacterized. Multiple sclerosis (MS) is the prototypic inflammatory demyelinating disease of the central nervous system (CNS). Here, we report molecular evidence for GSDMD-mediated inflammasome activation and pyroptosis in both macrophages/microglia and, unexpectedly, in myelin-forming oligodendrocytes (ODCs) in the CNS of patients with MS and in the associated animal model, experimental autoimmune encephalomyelitis (EAE). We observe inflammasome activation, GSDMD expression, and pyroptosis in human microglia and ODCs *in vitro* following exposure to MS-relevant inflammatory stimuli (including ATP and TNFa), which is rescued by inhibiting caspase-1 with the small molecule inhibitor VX-765. Further, we demonstrate that direct GSDMD inhibition using an siRNA-based approach suppress pyroptosis in human microglia. VX-765 treatment of EAE animals reduced expression of inflammasome- and pyroptosis-associated proteins in the CNS, reduced neuroinflammation, prevented axonal injury, and improved neurobehavioral performance. Thus, GSDMD-mediated pyroptosis in multiple glial populations represents a previously unrecognized mechanism of inflammatory demyelination and presents a unique therapeutic opportunity for mitigating neuroinflammation.

P11 – Katherine Mifflin, University of Alberta

Voluntary wheel running has sex specific effects on modulation of nociceptive behaviour in the murine model a multiple sclerosis

K.A. Mifflin, M.S. Yousuf, K.C. Thorburn, M.E. Peréz-Muñoz, J. Huang, G. Tenorio, and B.J. Kerr

Aim of Investigation: Multiple Sclerosis (MS) is an inflammatory neurodegenerative autoimmune disease whose main symptoms include sensory and motor dysfunction. Yet, ~50% of patients experience chronic neuropathic pain during their disease course. The development of this pain is not yet understood and few effective treatments are available. Previous work from our laboratory explored voluntary exercise as a possible treatment for chronic pain in female mice. We interested to see if this effect would also be seen in males given the known sex differences in both chronic pain and MS. Thus, our current project explores sex differences in the modulation of nociceptive behaviour via daily voluntary wheel running.

Methods: MS is modeled in our laboratory through the murine model: experimental autoimmune encephalomyelitis (EAE). Mice of both sexes were given access to running wheels for 1 hour a day until disease onset. Mechanical allodynia was tested using von Frey hairs in male and female mice at disease onset (measured by start of tail paralysis). Differences in immune cells and soluble immune factors, known to be different in male and female mice, were explored by various methods (ELISAs cytokine analysis, western blots, proliferation assays). Sex differences in blood brain barrier integrity were also explored (Western blot analysis). We also explored the role of dorsal root ganglia hyperexcitability in culture neurons using calcium imaging.

Results: Overall, significant sex differences in the effects of running on nociceptive behaviour and immune system of mice with EAE were found. We were surprised to find that daily voluntary wheel running only effectively reduced nociceptive behaviour in female mice but not male mice. Exploration of potential biological mechanisms for this effect revealed differences in peripheral cytokine profiles, blood brain barrier integrity and dorsal root ganglia hyperexcitability. Overall, males were found to have an increased inflammatory peripheral cytokine profile, reduced blood brain barrier integrity and increased dorsal root ganglia hyperexcitability. Female on the other had showed a reduction in inflammatory peripheral cytokines and decreased dorsal root ganglia hyperexcitability.

Conclusions: Taken together our results indicate that the inflammatory response generated in EAE is distinct between the sexes and its modulation by daily exercise can have sex specific effects on disease related outcomes. This also suggests that sex specific exercise regimes may be needed to see a reduction in nociceptive behaviours in both sexes.

P12 – Leina Saito, University of Alberta

Generation of innate immune molecules by Oligodendrocytes depends on maturation state

L.B.Saito, M.C. Monaco, W. Branton, E.A. Cohen, E.O. Major, C. Power

Introduction: Oligodendrocytes are myelin-forming cells of the central nervous system, where the maturation status of the cell dictates myelination competence. In inflammatory demyelinating diseases such as multiple sclerosis (MS), oligodendrocyte stress and cell death is often observed in environments with pathogenic neuroinflammation and neurodegeneration. It is currently unknown whether oligodendroyctes are capable in functions other than myelination, such as mounting an immune response. We investigated whether human oligodendrocytes could generate innate immune responses upon exposure to MS relevant inflammatory stimuli. These results were compared to immune responses observed in clinical samples

Methods: Differentiated and undifferentiated human progenitor-derived oligodendrocytes (PDOs) were exposed to TNFα for 24 hours. Oligodendrocyte cell markers and innate immune gene expression were analysed by qRT-PCR. ELISA and Type I IFN bioassay were performed on supernatants of TNFα exposed cells. Immunofluorescence (IF) analysis on caspase-1 (CASP1) and gasdermin D (GSDMD), as well as total process length by actin staining was assessed and quantified on TNFα exposed PDOs. Deep sequencing was performed on total RNA extracted from normal appearing brain white matter of MS and non-MS patients. Nanostring analysis was also performed on brain sections from MS and non-MS patients.

Results: TNFα exposure led to suppression of oligodendroycte cell markers *OLIG1* and *PLP*. TNFα stimulation of PDOs induced expression of Type I IFN-associated (*IFNB*, *IRF3*, and *IRF7*) and inflammasome-associated (*NLRP3, CASP1)* genes; with responses by differentiated PDOs being >2-fold greater than undifferentiated PDOs. *MX1* and *BST2* genes were also highly expressed in PDOs after TNFα exposure, however *IFNA* and *IFNL* were not detected. CASP1 and GSDMD proteins were detected in TNFα exposed PDOs by IF. Average processes length and number on PDOs were also greatly reduced in cells exposed to TNFα. IL-1β and IL-18 release into supernatants could not be detected from TNFα stimulated PDOs, but Type I IFN activity could be detected. Analyses of human brains indicated induction of Type I IFN- and inflammasome-associated genes in MS compared to non-MS brains.

Conclusions: Activated PDOs express innate immune responses in a differentiation state-dependent manner, with evidence of inflammasome activation without IL-1β release. The observed immune responses also recapitulate the innate immune responses in MS brains. These processes could affect cellular survival and the potential for remyelination.

P15 – Sahar Shahidi, University of Calgary

Designing a novel 3-D in-vitro scaffold to define mechanisms underlying neuronal myelination
S. Shahidi, M. Janmaleki, S. Riaz, A. Sanati nezhad, N. Syed,

All nervous system functions in animals require neuronal assembly during development and the ensuring communications between large networks of neurons, which are often difficult to monitor in the intact brain. Assessing cellular and molecular mechanisms of neuronal myelination is critical to demonstrate how myelination and demyelination processes occur in vertebrate models to understand developmental and neurodegenerative diseases such as multiple sclerosis.
Objectives: One of the main objectives of my research is to design/refine a substrate that acts as a scaffold enabling neuronal growth in a 3-D environment and Assess the real-time myelination at the level of single axons and their corresponding glia.
Methods: 1- using Glass bottom Petri dishes were coated with PDL and Laminin, collagen dishes and GelMA dishes wich develop a 3-D environment for neuronal growth.
Results and discussion: When DRGs were myelinated in PDL and laminin coated dishes, myelination was more difficult since axons stick strongly to the substrate and as such do not permit SC encapsulation. The reason why collagen model was successful in facilitating SC in accessing axons and wrapping around to myelinate was because of the three-dimensional matrix of collagen and GelMA which allowed axons to be freely suspended thus permitting the wrapping around.

P16 – Ahmed Elkady – University of Alberta

Discriminative Analysis of Regional Evolution of 5-year Deep Gray Matter Changes Reveals a Linear Relationship of Iron Decrease with Disease Duration and Severity in Relapsing-Remitting Multiple Sclerosis
Ahmed M. Elkady, Dana Cobzas, Peter Seres, Gregg Blevins, Alan H. Wilman

Introduction: Quantitative Magnetic Resonance Imaging (MRI) using combined R2* and Quantitative Susceptibility (QS) mapping has been recently proposed for Discriminative Analysis of Regional Evolution (DARE) of iron and myelin longitudinal changes in Multiple Sclerosis (MS) Deep Gray Matter (DGM) [1]. We apply DARE to analyze 5-year DGM changes in Relapsing-Remitting MS (RRMS).
Methods: A 10-minute 4.7T 10-echo gradient-echo acquisition was used to compute R2* and QS [2-4] in 22 RRMS and 22 age/sex-matched (P=0.45; P=0.3) control subjects for baseline and 5-year follow-up. Automatic segmentation [5] of the Caudate Nucleus (CN), PUtamen (PU), THalamus (TH), Globus Pallidus (GP), Red Nucleus (RN), Substantia Nigra (SN), and Dentate Nucleus (DN) were used to quantify mean R2*/QS and normalized DGM regional volumes. Parametric and non-parametric mixed factorial analysis (α=0.05) of bulk DGM structures was implemented to investigate longitudinal, group and interaction effects, while DARE employed non-parametric analysis of RRMS compared to control group effects. Linear regression was performed between all significant bulk interaction and DARE results with disease duration and MS Severity Scale (MSSS).
Results: Significant interaction was only found for TH volume (Q = 4E-5; η^2 = 0.33). Specific paired t-tests indicated that there were no longitudinal effects for the control group, while 50% of the variance in the data was explained by longitudinal effects of the MS group for TH volume (Q = 3E-4; η^2 = 0.50). Significant iron decrease was demonstrated in several DGM regions, with the highest effect sizes reported for TH mean QS (Q = 7.0E-3; η^2 = 0.27) and GP mean R2* (Q = 1.1E-2; η^2 = 0.20). Regression analysis revealed a linear regression of iron decrease in GP mean R2* with disease duration (r = 0.5; P = 1.8E-2), and iron decrease in CN mean R2* with MSSS (r = 0.51; P = 1.8E-2).
Conclusion: Longitudinal decrease in DGM iron levels and in thalamic volumes are a pathologic feature in RRMS over a period of 5 years. DGM Iron decrease correlates with disease duration and severity, possibly due to depletion of iron from oligodendrocytes that are normally abundant in the DGM.

P17 – Samira Samtleben, University of Alberta

Role of oxidoreductase Ero1a in astrocytes of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE)

S. Samtleben, F. Giuliani, B. Kerr, C. Power, T. Simmen

All nervous system functions in animals require neuronal assembly during development and the ensuring communications between large networks of neurons, which are often difficult to monitor in the intact brain. Assessing cellular and molecular mechanisms of neuronal myelination is critical to demonstrate how myelination and demyelination processes occur in vertebrate models to understand developmental and neurodegenerative diseases such as multiple sclerosis.
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P18 – Jaqueline Rowley, University of Alberta

Sit Less with MS: A multi-component sedentary behaviour intervention for patients with multiple sclerosis

J. Rowley, S. Aminian, G. Mehrabani, R.W. Motl, P.J. Manns

People with multiple sclerosis are less physically active and more sedentary than people without MS. In Canada, there are published physical activity guidelines specifically for people with MS, yet levels of activity remain low. Traditional approaches to increase moderate-vigorous physical activity, such as structured exercise programs, can be challenging for many people with MS, especially those with walking disability. Focusing on smaller changes such as decreasing prolonged sitting, and increasing light-intensity activities throughout the day, may be a more feasible and sustainable approach to activity behavior change in those with MS. In this paper we describe a multi-component web-based sedentary behaviour intervention designed to help people with MS to sit less and move more.

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