ECN2019

The European Conference on Neuroinflammation

Basic and Clinical Concepts

8-9 April 2019 | London, UK

In association with The Oxford Centre for Neuroinflammation

Oxford Centre for Neuroinflammation

Bioevents
Sharing Biomed Knowledge

Abstract Book

www.ecn2019.com

Poster Board 1

Topic: Clinical management of neuroinflammation and neurodegeneration with particular focus on multiple sclerosis

Intranasal Delivery of Methylprednisolone: A Targeted Non-Invasive Route to Control Neuroinflammation in EAE

Dunia Rassy[1], Brandon Bárcena Calderón[1], Hugo Besedovsky[2], Gabriela Meneses[1], Edda Sciutto[1]
[1]*Inmunología, Instituto de Investigaciones Biomédicas- UNAM, Mexico*
[2]*Immunphysiology, Institute of Physiology and Pathophysiology, Philipps-University Marburg, Germany*

Background: Despite disease-modifying therapies, relapses are still the hallmark of Relapsing-Remitting Multiple Sclerosis (RR-MS). The recommended treatment for these acute neuroinflammation episodes consists of high doses (500-1500mg) of intravenous (IV) methylprednisolone (MP) for 3-5 days. Nevertheless, this administration route is invasive, expensive and involves systemic exposure with multiple unwanted side effects. The current alternative delivery route, oral administration of prednisone, remains impractical due to the number of tablets required.

Objective: Explore the efficacy of intranasal (IN) MP as a non-invasive and more effective and route into the CNS to control neuroinflammation in experimental autoimmune encephalomyelitis (EAE), the most widely used model of RR-MS.

Methods: EAE was induced in 10-12-week-old female C57BL/6J mice with a 1:1 emulsion of MOG_{35-55} peptide and CFA, and 200ng of *B. pertussis* toxin. Mice with tail paralysis were randomly distributed into treatment groups: saline, IV-MP or IN-MP (200mg/Kg). Treatments were administered once a day for three consecutive days. Motor deficits and weight changes were monitored daily using the score defined by Bittner et al. Tissue and blood samples were obtained during maximum neuroinflammation to perform multiplex immunoassays and histology.

Results: IN-MP was effective at in reducing disease severity as evidenced by group means and the calculated area under the curve after 28 days. Spinal cord H&E and Luxol Fast Blue stains showed reduced infiltration and damage. IN treatment was as effective as IV treatment for reducing Th1 and Th17 cytokine levels both in the spinal cord and the periphery. No nasal epithelium damage was detected due to IN-MP administration.

Conclusion: IN administration of MP may represent a safe and non-invasive alternative for MS relapse treatment.

Poster Board 2

Topic: Clinical management of neuroinflammation and neurodegeneration with particular focus on multiple sclerosis

Exploring the Efficacy of Repeated Intranasal Administration of Low Doses of Neurotrophic Factors in Preventing Age-Related Cognitive Decline in Adult-Aged Rats

Manuel Jimenez[1], Sandra Ledesma[1], Susana Esteban[1], Floris Ilaria[2], Miquel Enseñat[3], Ana Rodriguez[3], Beatrice Lejeune[4], **David Moranta**[1]

[1]*Biology Department, University of Balearic Islands, Spain*
[2]*RD Department, Labo'Life France, France*
[3]*Production Department, Labo'Life Spain, Spain*
[4]*RD Department, Labo'Life Belgium, Belgium*

Faced with the current aging of the population, the development of therapies and/or strategies to achieve a "healthy" aging and greater autonomy in advanced ages is one of the main fronts of action of research. This work aims at evaluating the efficacy of a micro-immunotherapy formulation to prevent and/or delay cognitive deterioration associated with aging. A rat aging model (Sprague-Dawley, around 15-month-old) was used, to which a mixture of neurotrophic factors (BDNF, NGF, EPO and NT-4; 0.1 ng/ml each) in low intranasal daily doses (30 µl in each nostril), for three months was administered. During this chronic treatment, cognitive abilities were analyzed through the Barnes and Radial Maze tests. In addition, plasma levels of interleukins 6 and 10 were analyzed. Results show a clear reduction in systemic inflammatory markers in the treated group, accompanied by a slight tendency to improve cognitive abilities such as working memory, visuo-spatial and learning, analyzed by the Barnes test. The animals did not show differences in the radial maze test, which could reflect a reduction in the appetite of the animals caused by the treatment. In fact, the animals had a slight tendency to reduce weight compared to the control group, which in turn could exert beneficial effect against brain aging. Taken together, the results point micro-immunotherapy as a promising therapy to delay brain aging.

Poster Board 3

Topic: Emerging concepts for understanding and treating patients with multiple sclerosis

Effects of Induced Depression on the Animal Model of Multiple Sclerosis: An overlooked Disability

Amer Kamal[1], Ayman Mohammed[3], Ghada Al Khafaji[2], Amal Almahroos[3], Zahra Almosawi[3], Hawra Alalwan[3], Reem Abdulla[3], Fajer Alammadi[3], Ahmed Almubarak[3]
[1]*Physiology, Arabian Gulf University / College of Medicine, Bahrain*
[2]*Molecular medicine and genetics, Arabian Gulf University / College of Medicine, Bahrain*
[3]*Medicine, Ministry of Health / Salmaniya hospital, Bahrain*

Background and Objective: Depression is a common disabling symptom of multiple sclerosis (MS) with a lifetime prevalence of 50%. The aim of this study is to investigate the impact of induced depression on cuprizone mouse model of demyelination.

Methods: Mice were divided into cuprizone with no intervention (Cup-O), cuprizone undergoing induced depression (Cup-Dep) and control groups (9 to 10 per group). Depression was induced by repeated open-space forced swim and was implemented 6 days prior to the testing period. Multiple sclerosis was induced by continuously feeding six-week-old C57BL/6 male mice a 0.2% cuprizone-enriched diet. Spatial learning and memory were tested using Morris water maze while rotarod was used to assess motor function.

Results: Cognitive and motor deficits were established in cuprizone mouse model of demyelination as Cup-O had worse results than control group in Morris water maze (p0.001) and rotarod (p0.05). Induced depression was seen to exaggerate the aforementioned deficits; Cup-Dep showed a significantly declined performance in Morris water maze (p0.001) and rotarod (p0.05) in comparison to Cup-O.

Conclusion: Depression can further worsen the natural disease course of MS model. Therefore depression-ameliorating measures should be considered as a part of MS management plan based on the results of this study.

Poster Board 4

Topic: Emerging concepts for understanding and treating patients with multiple sclerosis

Analysis of Olfactory Transduction Signaling in Experimental Autoimmune Encephalomyelitis

Taekyun Shin[1], Jeongtae Kim[1], Meejung Ahn[1], Yuna Choi[1], Poornima Ekanayake[1], Chul Min Park[2], Changjong Moon[3], Kyungsook Jung[4], Akane Tanaka[5], Hiroshi Matsuda[5]
[1]*Department of Veterinary Anatomy, College of Veterinary Medicine, Jeju National University, South Korea*
[2]*Department of Obstetrics and Gynecology, School of Medicine, Jeju National University, South Korea*
[3]*Department of Veterinary Anatomy, College of Veterinary Medicine and BK21 Plus Project Team, Chonnam National University, South Korea*
[4]*Immunoregulatory Materials Research Center, Korea Research Institute of Bioscience and Biotechnology, South Korea*
[5]*Laboratory of Veterinary Molecular Pathology and Therapeutics, Graduate School, Institute of Agriculture, Tokyo University of Agriculture and Technology, Japan*

Background: Olfactory dysfunction occurs in multiple sclerosis in humans, as well as in an animal model of experimental autoimmune encephalomyelitis (EAE). The changes of olfaction-related signals remains to be further analyzed in the mouse olfactory bulbs with experimental autoimmune encephalomyelitis.

Methods: EAE was induced in C57BL/6 mice following immunization with myelin oligodendrocyte glycoprotein and adjuvant. Inflammatory lesions were identified in the olfactory bulbs as well as in the spinal cord of immunized mice. Analysis of differentially expressed genes (DEGs) was done in olfactory bulbs of EAE-affected mice by next generation sequencing, with a particular focus on changes in olfaction-related signals.

Results: Analysis of DEGs in the olfactory bulb of EAE-affected mice revealed that 44 genes were upregulated, which were primarily related to inflammatory mediators, while 519 genes were downregulated. Genes of olfactory marker protein and stomatin-like 3 were significantly downregulated (log2 [fold change] 1 and p-value 0.05), which are deeply associated with olfaction.

Conclusion: collectively, these findings suggest that olfactory dysfunction in EAE-affected mice is associated with the downregulation of some olfactory signal transduction genes, particularly olfactory marker protein and stomatin-like 3, which are involved in olfaction in an animal model of human multiple sclerosis.

*This research was supported by the National Research Foundation of Korea (Grant number: NRF-2017R1A2B4012487).

Poster Board 5

Topic: Emerging concepts for understanding and treating patients with multiple sclerosis

Dissecting functional phenotypes of microglia and macrophages in ischemia-induced neuroinflammation

Bozena Kaminska, Wenson D Rajan, Bartosz Wojtas, Bartosz Gielniewski, Anna Gieryng, Malgorzata Zawadzka
Neurobiology Center, Nencki Institute of Experimental Biology, PAS, Poland

Background. Ischemic brain injury causes inflammation, which involves activation of microglia, leukocyte and monocyte infiltration. Specific subpopulations may differentially contribute to neuroinflammation and final outcome.

Objective. Identity and involvement of immune cell subpopulations in ischemia-induced damage/repair are debatable.

Methods. Using flow cytometry we isolated microglia, bone marrow (BM)-derived macrophages and perivascular/meningeal macrophages at days 1, 3, and 7 after middle cerebral artery occlusion (MCAo) in rats. Global gene expression profiling was performed by RNA sequencing and selected findings were confirmed by qPCR and immunocytochemistry.

Results. We found that inflammatory microglia predominate in the ischemic brain after MCAo. At day 3 BM-macrophages accumulate in the ischemic hemispheres and display a unique transcriptomic profile indicative of the pro-regenerative, immunosuppressive phenotype. Transient depletion of peripheral macrophages with clodronate-filled liposomes reduced the number of pro-regenerative macrophages in the ischemic brain. Even if global gene expression was modified by environmental clues, respective cells expressed microglia and macrophage signature genes. At day 7, infiltrating BM-macrophages exhibited inflammatory gene expression suggesting a switch toward a pro-inflammatory phenotype. We successfully isolated and characterized transcriptomes of CD163+ perivascular/meningeal macrophages that show unique signature genes, proliferate and accumulate in ischemic parenchyma.

Conclusion. We propose that while inflammatory microglia accumulate at damaged sites, BM-macrophages recruited to the injured brain early after ischemia are pro-regenerative but they switch toward a pro-inflammatory phenotype in the ischemic parenchyma. CD163+ perivascular/meningeal macrophages proliferate, accumulate and adapt inflammatory phenotype in the ischemic parenchyma. Our results reveal cellular and functional heterogeneity of immune infiltrates after ischemia.

Supported by the NCN grant 2017/27/B/NZ3/01605.

Poster Board 6

Topic: Emerging concepts for understanding and treating patients with multiple sclerosis

Modulating Microglia via Colony Stimulating Factor-1 Receptor (CSF1R) Inhibition for Therapeutic Benefit in Multiple Sclerosis

Nellwyn Hagan, Lisa Woodworth, Amy Mahan, Matija Zelic, Dimitry Ofengeim
Rare and Neurological Diseases Therapeutic Area, Sanofi, USA

Microglia serve as the innate immune cells of the central nervous system (CNS) by providing continuous surveillance of the CNS microenvironment and initiating defense mechanisms to protect CNS tissue. Upon injury, microglia transition into an activated state altering their transcriptional profile, transforming their morphology, and producing pro-inflammatory cytokines. These activated microglia initially serve a beneficial role, but their continued activation drives neuroinflammation and neurodegeneration. Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the CNS and activated microglia and macrophages play a significant role in mediating disease pathophysiology and progression. We hypothesize that modulating microglia and infiltrating macrophages through the inhibition of colony stimulating factor-1 receptor (CSF1R) will attenuate deleterious CNS inflammation and inhibit subsequent demyelination and neurodegeneration. In human credentialing experiments, we observed an increase in CSF1R signaling components in CNS tissue derived from MS patients. This finding provided sufficient rationale to generate a novel CSF1R inhibitor for preclinical testing. *In vitro* assays utilizing primary microglia and macrophages demonstrated that our CSF1R inhibitor successfully blocked receptor phosphorylation and downstream signaling and ultimately altered cellular functions such as proliferation, survival, and cytokine production. *In vivo*, our CSF1R inhibitor decidedly improved neuroinflammation in an acute LPS model as well as neurological impairments observed in the MOG peptide-induced experimental autoimmune encephalomyelitis model of secondary progressive MS. Together, these data suggest that CSF1R inhibition warrants exploration as a strategy to modulate microglia phenotypes in the context of neuroinflammation and for therapeutic use in MS.

Poster Board 7

Topic: Genetic and immunological understandings of multiple sclerosis

Galectin-3 is necessary for postnatal subventricular zone gliogenesis

Francis Szele, Osama Al-Dahlahma, Luana Soares, James Nicholson, Swip Draijer, Victor Lu, Mayara Mundim, Eric O'Neill
Physiology, Anatomy and Genetics, University of Oxford, UK

Background
Postnatal subventricular zone neural stem cells generate forebrain glia, namely astrocytes and oligodendrocytes.

Objective
The cues necessary for this process are unclear, despite this phase of brain development being pivotal in forebrain gliogenesis. Galectin-3 regulates astrocyte proliferation in the injured adult brain, is increased in multiple brain pathologies and is pro-inflammatory.

Methods
We used postnatal brain electroporation of the subventricular zone, to overexpress, knockdown or conditionally knockout Gal-3 and the BMPR1a receptor. This was followed by immunohistochemistry, confocal microscopy and stereological quantification.

Results
Galectin-3 overexpression surprisingly did not induce inflammation in the healthy postnatal subventricular zone. This allowed investigation of inflammation-independent effects of galectin-3 on gliogenesis. Loss of galectin-3 function via knockdown or conditional removal reduced gliogenesis, whereas galectin-3 overexpression increased it. Galectin-3 overexpression also increased the percentage of striatal astrocytes generated by the subventricular zone but decreased the percentage of oligodendrocytes. These novel findings were further elaborated with multiple analyses demonstrating that galectin-3 binds to the bone morphogenetic protein receptor one alpha and functions to increase bone morphogenetic protein signaling. Conditional knockout of bone morphogenetic protein receptor one alpha abolished the effect of galectin-3 overexpression on gliogenesis.

Conclusion
Our findings thus show a novel inflammation-independent function for galectin-3; it is necessary for gliogenesis and when increased in expression can induce astrocytogenesis via bone morphogenetic protein signaling.

Poster Board 8

Topic: Genetic and immunological understandings of multiple sclerosis

B cells are recruited through the blood–CSF barrier and, dependent on their activation state, traffic more easily in multiple sclerosis

Leonardo Costa[1], Juergen Haas[1], Henriette Rudolph[2], Saskia Libicher[1], Simon Faller[1], Sven Jarius[1], Tobias Tenebaum[2], Horst Schroten[2], Brigitte Wildemann[1]
[1]*Department of Neurology, University Hospital of Heidelberg, Germany*
[2]*Department of Pediatrics, Medical Faculty Mannheim, Heidelberg University, Germany*

Background: The role of B-cells in MS immunopathogenesis is increasingly recognized. B-cells undergo compartmentalized redistribution in blood and cerebrospinal fluid (CSF) during active MS, whereby antigen-experienced memory B-cells accumulate in the CSF. While trafficking of B-cells across the blood–brain barrier has been intensively investigated, cellular diapedesis through the blood–CSF barrier (BCSFB) is incompletely understood.

Objectives: To investigate how B-cells interact with the choroid plexus to transmigrate into the CSF.
Methods: We isolated B-cells from blood samples of healthy donors (HC) and MS patients, then utilized an inverted transwell culture system of human choroid plexus papilloma (HIBCPP) cells to determine transmigration rates of distinct B-cell subsets and immunofluorescence microscopy to analyze their migration route through the epithelial barrier, cytokine assays and RT-PCR were used to determine the cytokines/chemokines mediating B-cell transmigration.

Results: Spontaneous transmigration of both HC- and MS-derived B-cells across HIBCPP cells was scant, yet increased significantly in response to B-cell specific chemokines (including CXCL-12, -13) and was further boosted upon pre-activation. Migrating cells were characterized by upregulation of several genes involved in B-cell activation and an enhanced expression of chemokine receptors CXCR4 and CXCR5, predominantly exhibited a $CD27^{+}$ memory phenotype, and chemotactic activities of this subset were more pronounced in B-cells from MS than in those from HC.

Conclusions: Our findings provide new information on how antigen-experienced B-cell phenotypes and the BCSFB act together to facilitate aberrant B-cell accumulation in the CSF of MS patients.

Poster Board 9

Topic: Genetic and immunological understandings of multiple sclerosis

The Role of Exosomes in the Pathology of High Fat Diet Induced Increase in Brain Inflammation after Ischemic Stroke

Hsing-Ni Lee, Joen-Rong Sheu, Cheng-Ying Hsieh, Chih-Hao Yang
Department of Pharmacology, School of Medicine, Taipei Medical University, TAIWAN CHINA

Objective:
Stroke as the second leading cause of death that accounts for 6.8% of deaths annually world-wide. Hyperlipidemia and Obesity have long been identified as critical risk factors for the occurrence of ischemic stroke which might be linked to the vascular remodeling or blood vessel constriction in the brain. However, exosome as mediators of intercellular communication may play critical roles in trafficking of proteins and mRNAs between cells. And recent studies found evidence showing the dysregulation of exosomal kinetics in metabolism related disorders. We aim to study the changes in exosome dynamics and the transfer of its neuro-inflammatory cargos in dictating the early onset of hypoxic susceptibility in hyperlipidemics.

Methods:
Male C57BL/6 mice were fed with high fat diet start from three weeks after birth, and their individual susceptibility to hypoxic challenge in the brain were studied at different stages (6, 9, and 12 weeks) after HFD-feeding. The transient middle cerebral artery occlusion (tMCAo) was adapted to induce regional brain damage and evaluate cytotoxic events after hypoxic challenge. Multiple indexes including morphological infarct area, neurological scores, and biochemical indicators of neuro-inflammatory events were analyzed to reflect hypoxic susceptibility after high fat diet feeding.

Results:
We found that there is a trend increase in hypoxic susceptibility (increase in infarct size, impairment of neurological coordination) which correlated to the duration of HFD-feeding. Interestingly, a significant increase in hypoxic susceptibility starts from as early as six weeks after HFD-feeding which there were no obvious changes of vascular remodeling or blood vessel constriction in the mouse brain. Besides, we provide evidence showing that the aberrant releasing of exosomal cargos in hyperlipidemic animals may be correlated to the increased hypoxic susceptible phenotypes.

Conclusion:
Our results indicate that HFD-feeding significantly increase in susceptibility to neuronal injury after hypoxic insults and targeting of exosome trafficking or its neuro-inflammatory cargos may a promising therapeutic direction for the prevention of hypoxia induced brain damage in hyperlipidemic individuals.

Poster Board 10

Topic: Genetic and immunological understandings of multiple sclerosis

T-Cell Repertoire Sequencing During Pregnancy Identifies Disease Associated Clones in Multiple Sclerosis

Caren Ramien[1], Erik C. Yusko[7], Kostas Patas[1], Stefanie Gamradt[9], Jan Broder Engler[1], Nils Schweingruber[1], Anne Willing[1], Sina Cathérine Rosenkranz[1], Anke Diemert[2], Anja Harrison[1,5], Marissa Vignali[7], Catherine Sanders[7], Harlan S. Robins[7,8], Eva Tolosa[4], Christoph Heesen[1,6], Petra C. Arck[3], Alexander Scheffold[10], Kenneth Chan[7], Ryan O. Emerson[7], Manuel A. Friese[1], Stefan M. Gold[1,9]

[1]*Institute of Neuroimmunology and Multiple Sclerosis (INIMS), University Medical Center Hamburg-Eppendorf, Germany* [2]*Department of Obstetrics and Fetal Medicine, University Medical Center Hamburg-Eppendorf, Germany* [3]*Laboratory of Experimental Feto-Maternal Medicine, University Medical Center Hamburg-Eppendorf, Germany* [4]*Department of Immunology, University Medical Center Hamburg-Eppendorf, Germany* [5]*Department of Psychology, University of Central Lancashire, UK* [6]*Department of Neurology, University Medical Center Hamburg-Eppendorf, Germany*

[7]*Adaptive Biotechnologies Corp., USA* [8]*Fred Hutchinson Cancer Research Center., USA*

[9]*Department of Psychiatry Campus Benjamin Franklin, Charité Universitätsmedizin Berlin, Germany*

[10]*Department of Cellular Immunology, Clinic for Rheumatology and Clinical Immunology, Charité Universitätsmedizin Berlin, Germany*

Background: Identifying T-cell clones associated with autoimmunity has remained challenging. Intriguingly, many human autoimmune diseases including multiple sclerosis (MS) show strongly diminished disease activity during pregnancy. In MS patients the immune balance necessary to ensure successful reproduction results in a relapse reduction of up to 80% in the 3rd trimester of gestation. Pregnancy thus provides a unique research paradigm to explore dynamics of immune repertoire changes and potentially disease associated clones during active and inactive disease.

Objective: We aimed to characterize immunomodulation during pregnancy in MS Patients at the single clone level by sequencing the T-cell repertoire.

Methods: We sequenced the T-cell repertoire of carefully matched healthy women and female MS patients longitudinally over the course of pregnancy using the immunoSEQ® platform (Adaptive Biotechnologies).

Results: T-cell clonality was significantly reduced during pregnancy in MS patients, indicating that the T-cell repertoire during MS pregnancy becomes less dominated by expanded clones. Only few T-cell clones were substantially modulated during pregnancy while circulating frequency of T-cells remained unaltered. In a proof-of-concept approach, for one patient we demonstrated that clones that were highly abundant during relapse contracted during pregnancy and expanded during a postpartum relapse. In this same patient we were able to identify and track myelin- and EBNA-1-specific clones through pregnancy.

Conclusion: Our data provide evidence that profiling the T-cell repertoire during pregnancy could serve as a tool to discover and track disease-associated T-cell clones in human autoimmunity.

Poster Board 11

Topic: Genetic and immunological understandings of multiple sclerosis

Bioactive alkaloid of the bark of Cinchona exerts neuroprotective effects in a transient focal ischemia/reperfusion mice model

Kuan-Jung Lu, Cheng-Ying Hsieh
Department of Pharmacology, School of Medicine, Taipei Medical University, TAIWAN CHINA

Stroke, also known as cerebrovascular accident, is the state of ischemia that localized tissue is unable to maintain physiological function or obstruction or rupture of cerebral vessels then leads to death. Stroke is the second leading cause of mortality and morbidity in the world. In the past studies, the cinchona-alkaloids have shown the characteristics of anti-inflammation and anti-oxidation. Cinchonidine is a bioactive alkaloid which found in the bark of cinchona, and was known to have much lower toxicity and higher activity compared to other cinchona-alkaloids. However, few studies have investigated the potential clinical applications of cinchonidine. In the present study, we determined the neuroprotective effect of cinchonidine against ischemic stroke in middle cerebral artery occlusion (MCAO) mice model. The treatment of cinchonidine (5, 10 and 20 mg/kg) concentration-dependently reduced infarction volume and edema ratio in mice subjected to MCAO. The neuro-behavioral assays including neurological severity score, rotarod test, and locomotor activity were significantly improved in cinchonidine (10 mg/kg)-treated MCAO mice. Cinchonidine also reduced the expression of Iba1, a microglia biomarker, and blood-brain barrier permeability in MCAO-treated mice. In addition, the *in vivo* imaging data revealed that the treatment of cinchonidine (10 mg/kg) potently attenuates reactive oxygen species formation in mice-subjected to MCAO. Collectively, these data indicated a potent neuroprotective effect of cinchonidine in the MCAO model of mice. The antineuroinflammation and antioxidation properties of cinchonidine may contribute to its neuroprotective effect against ischemic brain injury.

Poster Board 12

Topic: Genetic and immunological understandings of multiple sclerosis

Molecular signature in brain lesions of progressive MS patients and the MS Atlas

Maria Louise Elkjaer[1,2,5], Tobias Frisch[2], Richard Reynolds[3], Tim Kacprowski[4], Mark Burton[5], Torben Kruse[5], Mads Thomassen[5], Jan Baumbach[2,4], Zsolt Illes[1,5]
[1]*Department of Neurology, Odense University Hospital, Denmark*
[2]*Department of Mathematics and Computer Science, University of Southern Denmark, Denmark*
[3]*Division of Brain Science, Imperial College London, UK*
[4]*Chair of Experimental Bioinformatics, Technical University of Munich, Germany*
[5]*Department of Clinical Research, University of Southern Denmark, Denmark*

Active lesions in the white matter (WM) of MS patients can be remyelinated or develop into inactive lesions. In the progressive phase, chronic active lesions become prominent in the WM. To investigate mechanisms behind lesion evolution, we examined the transcriptome in normal-appearing WM (NAWM), active, inactive, remyelinating, and chronic active lesions from progressive MS brains.

Next generation RNA sequencing was performed on 75 lesions and 25 controls. To investigate unique transcriptional changes in different lesion types, we examined (i) protein interactions in each lesion type by *de novo* network enrichment of differentially expressed genes (DEG); (ii) lesion-specific pathways based on DEGs only in one lesion type; (iii) signatures of significant DEGs different between at least two lesion types; (iv) expression and cellular source by immunohistochemistry (IHC), immunofluorescence (IF) and RNAscope. We created a user-friendly web interface (MS Atlas), which allows for interactively analyzing the lesion types.

Of 18722 expressed genes, 4223 were DEGs in MS compared to control. *CD26/DPP4* was among the six upregulated DEGs in NAWM expressed by microglia. We identified two clusters of 62 DEGs that separated chronic active from all other lesion types by an inverse regulation pattern. The gene of an emerging biomarker, *CHI3L1* was such unique upregulated DEG in chronic active lesions: combined IHC and RNAscope revealed expression on astrocytes in the rim. *De novo* network enrichment of DEGs with inverse regulation in chronic active *versus* remyelinating lesions identified TGFb-R2 as a central hub for remyelinating lesions: IHC and RNAscope showed TGFb-R2 expression by astrocytes.The transcriptome signature of chronic active lesions was different from all other lesion types. Microglial CD26 and astrocytic CHI3L1 may be key molecules in early and chronic active lesion evolution. TGFb-R2 expressed by astrocytes in remyelinating lesions may be important in repair. The compendium of mechanistic lesion type profiles in MS Atlas is a novel interactive tool to fuel MS research and a new basis for MS treatment hunt.

Poster Board 13

Topic: Genetic and immunological understandings of multiple sclerosis

Activin-A Directs AHR-CD73-driven Metabolic Programs to Restrain Th17 Pathogenicity and CNS Autoimmunity

Gina Papadopoulou[1], Ioannis Morianos[1], Aikaterini Trochoutsou, Aikaterini Trochoutsou[1], Maria Semitekolou[1], Aggelos Banos[1], Dimitris Konstantopoulos[2], Antigoni Manousopoulou[3], Maria Kapasa[1], Ping Wei[4], Themis Kalamatas[5], Klinta Karageorgiou[5], Federica Sallusto[6], Spiros D. Garbis[3], Fan Pan[4], Francisco J. Quintana[7], Georgina Xanthou[1]

[1]*Biomedical Research Foundation of the Academy of Athens, Cellular Immunology Laboratory, Greece*
[2]*B.S.R.C. 'Alexander Fleming', Department of Molecular Biology and Genetics, Greece*
[3]*Beckman Institute, Division of Biology and Biological Engineering, California Institute of Technology, Proteome Exploration Laboratory, USA* [4]*Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Department of Oncology and Medicine, USA*
[5]*Athens Medical Center, Department of Neurology, Greece* [6]*Institute for Microbiology, ETH, Department of Biology, Switzerland* [7]*Brigham and Women's Hospital for Neurological Diseases, Harvard Medical School, USA*

Background: Th17 cells represent key drivers of CNS autoimmune inflammation in multiple sclerosis (MS). Nevertheless, factors that control Th17 pathogenicity remain incompletely defined. Activin-A is a cytokine that exerts both pro- and anti- inflammatory functions. Our previous studies revealed that activin-A restrained Th2 allergic responses in asthmatic individuals and protects against experimental asthma.
Objective: Here, we investigated the effects of activin-A on the regulation of Th17 pathogenicity, and the molecular mechanisms involved.
Methods: The *in vivo* role of activin-A was explored following therapeutic administration in the CFA/MOG EAE model. Th17 cells were generated *in vitro* under highly-pathogenic conditions and the effects of activin-A on their transcriptional and phenotypic profile were analyzed by RNA-seq, qPCR, flow cytometry, ELISA, immunofluorescence and chromatin immunoprecipitation assays. The effects of activin-A on the regulation of Th17 pathogenicity *in vivo* was examined through transfer EAE experiments. Bioinformatics, proteomics and metabolomics analyses deciphered the molecular mechanisms underlying the effects of activin-A on Th17 pathogenicity.
Results: Administration of activin-A in vivo ameliorated disease severity and alleviated CNS immunopathology and demyelination. Activin-A stimulation in vitro repressed genes linked to Th17 pathogenicity, including *Ifng*, *Csf2*, *Il1b*, *Tbx21* and *Batf*, concomitant with an upregulation of genes associated with non-pathogenic Th17 cells, such as, *Il10*, *Maf* and *Ahr*, in a mechanism dependent on the activation of the ATP-depleting CD73 ecto-5`-nucleotidase. Aryl hydrocarbon receptor directly controlled CD73 expression in Th17 cells in response to activin-A, and was essential for endowing Th17 cells with Tr1-like phenotypic and functional characteristics. Activin-A suppressed pathogenic
Th17 signatures also through negative regulation of hypoxia-inducible factor-1α and proteins involved in aerobic glycolysis pathways.
Conclusion: Our studies uncover activin-A as a crucial negative regulator of Th17 pathogenicity and a promising therapeutic target for MS.

Poster Board 14

Topic: Genetic and immunological understandings of multiple sclerosis

In vitro Approaches to Study Inflammatory Processes

Irene Schilcher, Tina Loeffler, Stefanie Flunkert, Birgit Hutter-Paier
Neuropharmacology, QPS Austria, Austria

Background: Immune activation in the CNS and production of neurotoxic mediators are linked to various neurodegenerative diseases including Multiple Sclerosis (MS), Alzheimer's disease (AD) and Parkinson's disease (PD). Neuroinflammation, a response within the brain or spinal cord, is mediated by the production of different stimuli e.g. cytokines and interleukines. Activated glia cells are the central players for this process in the central nervous system (CNS).

Objective: Therefore, we established three different *in vitro* systems, organotypic slices, primary microglia and the BV-2 cell line for cytokine and interleukin release as neuroinflammation models.

Methods: First, to study neuroinflammation in an intact neuronal system, organotypic slices from the hippocampus or whole brain slices were prepared from early postnatal wildtype mouse pups. Second, primary micoglial cultures isolated from early postnatal wildtype mouse pups and a mouse micoglial cell line were used to investigate neuroinflammtion effects specifically on micoglia. To stimulate inflammation, all three systems were incubated with Lipopolysaccharide and cytokine release into the supernatant over time was measured by single or multiplex Mesoscale Discovery (MSD) analyses. Ibudilast or Dexamethasone served as reference items to inhibit cytokine release.

Results: Lipopolysaccharide stimulation of all three *in vitro* cultures resulted in increased cytokine release of e.g. TNF-alpha, IL-6 and IL-10 into the supernatant over time. Whereas co-incubation of Lipopolysaccharide with the reference items, Ibudilast and Dexamethasone, reversed these effects and inhibited cytokine production.

Conclusion: All three tested *in vitro* approaches serve as good models to evaluate the effect of different compounds on neuroinflammation and its related neurodegenerative diseases.

Poster Board 15

Topic: Genetic and immunological understandings of multiple sclerosis

The Role of Exosomes in the Pathology of High Fat Diet Induced Increase in Brain Inflammation after Ischemic Stroke

Shin-Wei Huang, Joen-Rong Sheu, Cheng-Ying Hsieh, Chih-Hao Yang
Department of Pharmacology, School of Medicine, Taipei Medical University, TAIWAN CHINA

Objective:
Stroke as the second leading cause of death that accounts for 6.8% of deaths annually world-wide. Hyperlipidemia and Obesity have long been identified as critical risk factors for the occurrence of ischemic stroke which might be linked to the vascular remodeling or blood vessel constriction in the brain. However, exosome as mediators of intercellular communication may play critical roles in trafficking of proteins and mRNAs between cells. And recent studies found evidence showing the dysregulation of exosomal kinetics in metabolism related disorders. We aim to study the changes in exosome dynamics and the transfer of its neuro-inflammatory cargos in dictating the early onset of hypoxic susceptibility in hyperlipidemics.

Methods:
Male C57BL/6 mice were fed with high fat diet start from three weeks after birth, and their individual susceptibility to hypoxic challenge in the brain were studied at different stages (6, 9, and 12 weeks) after HFD-feeding. The transient middle cerebral artery occlusion (tMCAo) was adapted to induce regional brain damage and evaluate cytotoxic events after hypoxic challenge. Multiple indexes including morphological infarct area, neurological scores, and biochemical indicators of neuro-inflammatory events were analyzed to reflect hypoxic susceptibility after high fat diet feeding.

Results:
We found that there is a trend increase in hypoxic susceptibility (increase in infarct size, impairment of neurological coordination) which correlated to the duration of HFD-feeding. Interestingly, a significant increase in hypoxic susceptibility starts from as early as six weeks after HFD-feeding which there were no obvious changes of vascular remodeling or blood vessel constriction in the mouse brain. Besides, we provide evidence showing that the aberrant releasing of exosomal cargos in hyperlipidemic animals may be correlated to the increased hypoxic susceptible phenotypes.

Conclusion:
Our results indicate that HFD-feeding significantly increase in susceptibility to neuronal injury after hypoxic insults and targeting of exosome trafficking or its neuro-inflammatory cargos may a promising therapeutic direction for the prevention of hypoxia induced brain damage in hyperlipidemic individuals.

Poster Board 16

Topic: Genetic and immunological understandings of multiple sclerosis

The protective Effect of Platonin against Cerebral Ischemic Stroke Induced Neuro-inflammatory Damage

Pei-yi Li, Joen-Rong Sheu, Cheng-Ying Hsieh, Chih-Hao Yang
Department of Pharmacology, School of Medicine, Taipei Medical University, TAIWAN CHINA

Objective:
Thrombosis and stroke are major causes of disability and death worldwide. However, the regular antithrombotic agents may have unsatisfactory results and side effects. Platonin, a cyanine photosensitizing dye, has been used to treat trauma, ulcers and some acute inflammation. Here, we explored the neuroprotective effects of platonin against transient middle cerebral artery occlusion (tMCAo)-induced cerebral ischemic stroke in mice.

Methods:
The middle cerebral artery occlusion was adapted to induce regional brain damage and evaluate excitotoxic events after hypoxic challenge. Indexes including morphological infarct area, neurological scores, and neuro-inflammatory indicators were analyzed to reflect hypoxic susceptibility. Platonin (200 μg/kg) were applied intraperitoneal at thirty-minute after the tMCAo surgery.

Results:
We found Platonin (200 μg/kg) substantially reduced cerebral infarct volume, brain edema, neuronal cell death and neurological deficit scores, and improved the tMCAo -reduced locomotor activity and rotarod performance. Meanwhile, Platonin (5-10 μM) potently inhibited platelet aggregation and c-Jun NH2-terminal kinase (JNK) phosphorylation in collagen-activated platelets. The antiaggregation effect did not affect bleeding time but increased occlusion time in platonin (100 and 200 μg/kg)-treated mice. Platonin (2-10 μM) was potent in diminishing collagen- and Fenton reaction-induced ·OH formation. Platonin (5-10 μM) also suppressed the expression of nitric oxide, inducible nitric oxide synthase, cyclooxygenase-2, interleukin-1β, and JNK phosphorylation in lipopolysaccharide-stimulated macrophages. tMCAo -induced expression of 3-nitrotyrosine and Iba1 was apparently attenuated in platonin (200 μg/kg)-treated mice.

Conclusion:
Platonin exhibited remarkable neuroprotective properties against tMCAo-induced ischemia in a mouse model through its antiaggregation, anti-inflammatory and antiradical properties. The observed therapeutic efficacy of platonin may consider being a novel therapeutic compound for the treatment of cerebral ischemic stroke induced neuro-inflammatory damage in clinic.

Poster Board 17

Topic: Genetic and immunological understandings of multiple sclerosis

Anti-neuroinflammatory and cell-protective effects of cinchonidine in BV2 and Neu-2A cells with oxygen-glucose deprivation

Chieh-Min Chen, Cheng-Ying Hsieh, Kuan-Jung Lu
Department of Pharmacology, School of Medicine, Taipei Medical University, TAIWAN CHINA

Stroke is a critical cerebrovascular disease, and is the second of top ten global death causes based on the report of the World Health Organization. According to the pathological mechanism, stroke can be divided into the hemorrhagic and ischemic type. Moreover, ischemic stroke, which usually caused by blood flow occlusion due to thrombosis or embolism, accounts for eighty seven percent of all stroke. The major pathophysiological mechanism mediating ischemic stroke is excitotoxicity. Too much glutamic acid excites neurons to death by inducing oxidative stress. Also, inflammatory response might further be active by recruited leukocytes, astrocytes, active microglial cells, and damaged neurons to producing pro-inflammatory cytokines in ischemic stroke. On the other hand, inflammatory response also leads to blood–brain barrier disruption causing more white blood cell infiltration and release of pro-inflammatory cytokines for amplification of inflammation following stroke. The cinchona-alkaloids are known to have characteristics of anti-oxidant and anti-inflammation, however the anti-neuroinflammatory effect of cinchonidine, a major cinchona alkaloid, has not been determined. In this study, we investigated the cell-protective and anti-neuroinflammation of cinchonidine to survey a novel therapeutic agent for treating ischemic stroke. The treatment of cinchonidine (1 μM, 5 μM and 10 μM) significantly inhibited the expression of inducible nitric oxide synthase and COX2 in oxygen-glucose deprivation (OGD)-treated BV2 cells in a concentration dependent manner. On the other hand, neuronal cell death and apoptosis in OGD-treated Neu-2A cells was reduced by the treatment of cinchonidine (1 μM, 5 μM and 10 μM). Furthermore, the cell-protective effects of cinchonidine may due to the suppression of Bax expression. These data collectively indicate the anti-neuroinflammatory and neuronal cell-protective properties of cinchonidine. Cinchonidine may be a potential therapeutic agent in treating ischemic stroke or other neuroinflammatory diseases.

Poster Board 18

Topic: Genetic and immunological understandings of multiple sclerosis

Activation of the necroptosis signaling in cortical neurons in MS

Carmen Picon, Rachel James, Mazarakis Nicholas, Richard Reynolds
Medicine, Imperial College, UK

Background: Cortical pathology in secondary progressive MS (SPMS) is associated with a more severe clinical course and the presence of subpial grey matter (GM) lesions with significant neuronal loss and inflammatory infiltrates in the subarachnoid space. Our previous work suggests that TNF could be a key molecule driving cortical pathology in MS.

Objective: We investigated the hypothesis that TNF produced in the meninges leads to pathological changes in the underlying cortical neurons.

Methods: We studied changes in the balance in TNF signaling pathways in the GM from 30 SPMS cases and 10 controls. Lentiviral vectors carrying the TNF and interferon-gamma genes were injected into the subarachnoid space of DA rats to test the hypothesis.

Results: TNFR1 was significantly up-regulated in SPMS compared to controls, while no differences were found in the expression levels of TNFR2. A downregulation of CYLD and BCL2 was found in SPMS compared to controls, key proteins involved in the regulation of TNF pathways leading to apoptosis. MS cases showed a down-regulation of cleaved active caspase 8 whereas no differences were found in the number of cleaved caspase 3 cells between groups. In contrast, MS cases showed a significant increase in the key proteins of the necroptotic pathway, phospho-RIPK3 and phospho-MLKL (p-MLKL). The density of neurons expressing p-MLKL and p-RIP3 was significantly increased in MS cases compared to controls. We found MLKL oligomers only in MS, a sign of activated necroptosis. Finally, persistent cytokine production over 1 month in DA rats produced chronic meningeal inflammation and increased levels of the necroptosis markers p-MLKL and p-RIPK3 in the underlying cortical neurons, similar to the MS cortex.

Conclusions: Our data show there is a shift in the balance of TNF dependent signaling pathways towards TNFR1-mediated necroptosis in cortical neurons, which could be responsible for the neurodegeneration observed in the GM of MS patients.

Poster Board 19

Topic: Genetic and immunological understandings of multiple sclerosis

CD83 Expressed on Dendritic Cells and Microglia Controls Inflammatory Autoimmune Responses in the Periphery and the Central Nervous System

Andreas Wild[1], Lena Krzyzak[1], Silvia Seubert[2], Jochen Mattner[3], Alexander Steinkasserer[1]
[1]*Department of Immune Modulation, University Hospital Erlangen, Germany*
[2]*Department of Neurology, University Hospital Erlangen, Germany*
[3]*Institute of Microbiology, University Hospital Erlangen, Germany*

Introduction: CD83, a well-known surface marker for mature dendritic cells (DCs), is also expressed on activated B and T cells as well as on regulatory T cells and plays a crucial role for T cell development during thymic selection. In addition, also antigen-presenting cells (APCs) of the CNS, like microglia acquire CD83 expression under defined conditions. However, the precise role of membrane-bound (mCD83) expressed on DCs and especially microglia was largely unknown when the present investigations were initiated.

Objectives: To unravel the biological function of CD83 expression on DCs and microglia both under homeostatic and pathologic conditions using conditional knock-out (cKO) mice.

Methods: We used DC- and microglia-specific cKO mice to investigate the function of CD83 in these cells under steady state and patho-physiological conditions. In particular, we assessed the outcome of autoimmune neuroinflammation in these mice using the experimental autoimmune encephalomyelitis (EAE) model.

Results: CD83-deficient DCs are characterized by an over-activated phenotype leading, on the one hand to faster clearance of acute infections, but on the other hand to an impaired resolution of autoimmune inflammation by subverting Treg suppressive capacities. Interestingly, further data showed that CD83 expression by microglial cells is differentially regulated by acute and chronic inflammatory stimuli. Additionally, CD83 cKO microglia provide less trophic support during neuroinflammation, thus exacerbating the course of EAE.

Conclusion: Here we show for the first time that CD83 expression on DCs and microglia is essential for the resolution of neuroinflammatory autoimmune responses within the CNS.

Poster Board 20

Topic: Genetic and immunological understandings of multiple sclerosis

LXR-mediated Lipid Networks Modulate T-cell Function and are Dysregulated in People with Multiple Sclerosis

Kirsty Waddington[1,2], Marsilio Adriani[1], Eden Chrifi-Alaoui[1], Dylan Owen[3], Iveta Ivanova[3], Petra Nytrova[4], Eva Kubala Havrdova[4], Rachel Farrell[5], Elizabeth Jury[1], Inés Pineda-Torra[2]

[1]*Centre for Rheumatology, University College London, UK*

[2]*Centre for Cardiometabolic Medicine, University College London, UK*

[3]*Department of Physics and Randall Division of Cell and Molecular Biophysics, King's College London, UK*

[4]*Department of Neurology and Centre of Clinical Neuroscience, Charles University in Prague, Czech Republic*

[5]*Institute of Neurology and National Hospital of Neurology and Neurosurgery, University College London, UK*

Background:
The pathogenesis of multiple sclerosis (MS) is associated with autoreactive $CD4^+$ T-cells and dysfunctional regulatory T-cells. Lipid metabolism is altered in MS, including signalling via the liver-X-receptor (LXR), a transcriptional regulator of lipid metabolism with immunomodulatory actions.

Objective:
We investigated whether altered LXR signalling contributes to T-cell dysfunction in people with MS via the regulation of cholesterol and glycosphingolipid-enriched plasma membrane (PM) microdomains, essential for T-cell activation.

Methods:
RNA-Sequencing was performed on $CD4^+$T-cells from people with relapsing MS (pwRMS) and healthy controls (HCs, n=10/group). PM-lipids were quantified by flow cytometry using filipin (cholesterol), cholera-toxin B (glycosphingolipids) and di-4-ANEPPDHQ (fluidity). Serum lipids were measured in pwRMS (n=47), progressive MS (pwPMS) (n=39), and HCs (n=30) by NMR spectroscopy.

Results:
In healthy T-cells, LXR activation increased PM fluidity by upregulating cholesterol transporters ABCA1/G1 and glycosphingolipid biosynthesis enzyme UGCG. In the context of T-cell receptor stimulation this altered the distribution and phosphorylation of signalling components at the immune synapse, increased cytokine production, and reduced proliferation.

Transcriptomic analysis revealed increased expression of LXRβ in $CD4^+$T-cells from pwRMS. Moreover, a subset of LXR target genes was differentially expressed, including reduced expression of UGCG and increased levels of SREBP1c, a key regulator of fatty acid biosynthesis. Additionally, PM fluidity was increased in responder ($CD4^+CD25^-CD127^+$) and regulatory ($CD4^+CD25^+CD127^-$) T-cell subsets from pwRMS.

Circulating lipids affect both T-cell function and LXR activation. Serum levels of high-density lipoprotein cholesterol and Apo-A1 were significantly reduced in pwRMS and pwPMS. The lipid composition of lipoprotein particles was also significantly altered compared to HCs, and between patient groups. Thus, changes in lipid metabolism may be associated with progression.

Conclusion:
These data support dysregulation of LXR-mediated T-cell lipid metabolism in pwMS. Work is ongoing to assess whether differences in PM-lipids in pwMS are regulated by LXR, and whether they correlate with clinical outcomes.

Poster Board 21

Topic: Genetic and immunological understandings of multiple sclerosis

A molecular characterization of meningeal inflammatory infiltrates in the progressive multiple sclerosis brain

Laura Fuentes Font[1], Colin Glover[2], Richard Reynolds[1]
[1]*Department of Medicine, Imperial College London, UK*
[2]*RIA, MedImmune, UK*

The presence of lymphoid-like immune cell aggregates in the leptomeninges is suggested to promote damage to the cerebral cortex and play a role in accumulating disability in multiple sclerosis. To explore the molecular mechanisms that drive their formation, cryosections were cut from five cortical blocks per case from 55 SPMS and 14 control brains. Meningeal tissue was dissected and RNA extracted. Affymetrix HTA 2.0 GeneChips were used to obtain the meningeal transcriptome and gene expression determined using R package Limma. Differentially expressed genes with FC2 and FDR0.05 were used to perform gene set enrichment analysis using WebGestalt and gene networks constructed using R package WGCNA. When comparing controls with highly inflamed MS cases, alterations were mainly found in expression of homing chemokines and receptors and in cytokines that enhance B cell survival, proliferation and antibody and IFNγ production, such as IL10, IL18, PPBP, CXCR4, HSPA7, XBP1, CS1 and CD27. Considering these are molecules with chemotactic and co-stimulatory properties, changes on their expression could be underscoring alterations on how B and T cells are interacting on the meninges; furthermore, modifications in genes involved in the development of lymphatic vessels (LYVE1) and cell motility, survival and antigen presentation (HLA-B) were prominent. Gene network analysis revealed 5 network modules whose eigengenes were highly correlated with disease status and lymphocytic infiltration. Functional enrichment yielded a list of functions, including cell adhesion, protein folding and pro-inflammatory processes. Subsequent to microarray data analysis a panel of 55 inflammatory genes was chosen for validation by TaqMan OpenArrays. Highly significant, strong and moderately strong, correlations were found between the TaqMan and Affymetrix data for most individual genes, providing robustness to our meningeal transcriptomics data. We have identified molecular cues that are likely to mediate meningeal inflammation in MS that suggest a dysregulation of pathways that are critical for B-cell trafficking and recruitment into the CNS.

Poster Board 22

Topic: Genetic and immunological understandings of multiple sclerosis

Role of TWIK2 channels on the immune system

Li-Ming Lee[1], Juncal Fernandez-Orth[1], Tobias Ruck[1], Stefan Bittner[2], Nicole Bobak[3], Florian Lesage[3], Sven Meuth[1]

[1]*Institute for Translational Neurology, Department of Neurology, University Hospital Münster, Germany*

[2]*Department of Neurology, University Medical Center of the Johannes Gutenberg-University Mainz, Germany*

[3]*LabEx ICST, Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, and Université de Nice Sophia-Antipolis, France*

Background:
Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system (CNS) characterized by demyelination and neuroaxonal degeneration. In previous studies, K_{2P} channels have been shown to play a significant role in the disease pathogenesis of MS and in its animal model, experimental autoimmune encephalomyelitis (EAE). Here, we are interested in TWIK2, a silent K_{2P} channel not producing any currents at the plasma membrane, but being active in intracellular lysosomes where it affects the functioning of the organelle. In addition, TWIK2 expression is pronounced in spleen and peripheral blood lymphocytes suggesting a physiological role in immune cells, which are essential in MS pathogenesis.

Objective:
To unravel a potential functional role of TWIK2 in the regulation of immune cell functions under physiological and pathophysiological conditions.

Methods:
To address our aims, we will subject TWIK2 knock out ($TWIK2^{-/-}$) animals to EAE, and perform subsequent in-depth *ex vivo* immunological and histological analysis as well as mechanistic studies *in vitro*.

Results:
Based on our preliminary results, we could observe that $TWIK2^{-/-}$ animals were less susceptible to EAE, with delayed disease onset and a significantly ameliorated disease course. Additionally, inflammatory infiltrates and demyelination in the CNS were less prevalent and less IL-17A secretion in the periphery was found in $TWIK2^{-/-}$ animals compared to WT.

Conclusion:
Therefore, we hypothesize that TWIK2 channels can modulate immune cell functions under pathophysiological conditions and might provide a potential therapeutic target for MS and other autoimmune disorders

Poster Board 23

Topic: Genetic and immunological understandings of multiple sclerosis

Neuroinflammation causes changes to the nodes of Ranvier in multiple sclerosis normal appearing white matter

Patricia Gallego Delgado[1], Joanna Meng[1], Rachel James[1], Eleanor Browne[1], Aldo Faisal[2], Richard Reynolds[1]
[1]*Brain Sciences, Imperial College London, UK*
[2]*Bioengineering, Imperial College London, UK*

Background: In addition to the focal demyelinating lesions in multiple sclerosis (MS), both imaging and neuropathological analyses have demonstrated the presence of a more diffuse pathology in both the white and grey matter, including changes to the structure of nodes of Ranvier in the normal appearing white matter (NAWM). The presence of the axo-glial junctions of the myelin end loops and the proper clustering of Nav channels at the node and Kv1.2 channels at the juxtaparanode are crucial for fast action potential conduction.

Objective: Study the structural and functional consequences of nodal pathology and the role of inflammation in them.

Methods: We have examined the expression of Caspr1, Nav, Kv1.2 and SMI32 in NAWM areas from post-mortem progressive MS brains compared to controls. This axo-geometrical data was then integrated into a computational model of an axon developed with NEURON. To test our hypothesis, rats were injected into the cerebral subarachnoid space with lentiviral vectors for lymphotoxin-α and interferon-γ, and nodal changes were examined 3 months later. Furthermore, a cerebellar tissue culture model was used to induce nodal pathology by the activation of microglia with interferon-g and glutamate.

Results: The paranodal domain in MS NAWM tissue was 21.7% longer on average than in the control, and associated with stressed/damaged axons and activation of microglia. Moreover, we found a higher proportion of axons with Kv1.2 channels dislocated towards the paranode. When these changes were inserted into the computational model, we observed an exponential decrease in velocity as the paranodal peri-axonal space increases reaching conduction failure when the axons were less than 1µm of diameter. The same changes in paranodal length and Kv1.2 channel dislocation were observed in the corpus callosum of our rat model, and were associated to microglia/astrocyte activation.

Conclusion: Microglial activity could trigger nodal pathology in MS NAWM contributing to axonal degeneration and subsequent conduction deficits.

Poster Board 24

Topic: Genetic and immunological understandings of multiple sclerosis

The Diverse Roles of Matrix Metalloproteinase (MMP)-2 and MMP-9 in the Induction and Progression of Murine Experimental Autoimmune Encephalomyelitis (EAE)

Miriam Burmeister[1], Hanna Gerwien[1], Anna Chashchina[1], Jian Song[1], Ghislain Opdenakker[2], Lydia Sorokin[1]
[1]*Institute of Physiological Chemistry and Pathobiochemistry, University of Muenster, Germany*
[2]*REGA Institute, University of Leuven, Belgium*

Background
Gelatinases, MMP-2 and MMP-9, are critical for induction of EAE, a murine model disease for multiple sclerosis, and in their absence there is no CNS leukocyte infiltration or disease symptoms[1]. In WT mice, gelatinase activity correlates with sites of initial leukocyte penetration of the CNS parenchyma and is required for leukocyte chemotaxis across the blood-brain barrier (BBB)[2]. Chimeric mice, carrying *Mmp2*$^{-/-}$ */9*$^{-/-}$ double knockout immune cells, show significantly reduced CNS leukocyte infiltration, suggesting a role for immune cell-derived MMP-2/-9 in the peripheral immune response[2].

Objective
To define how MMP-2 and MMP-9 affect EAE induction and progression.

Methods
Confocal microscopy, Flow cytometry, qPCR, proliferation assays

Results
Chimeras lacking immune cell-derived gelatinases and *Mmp9*$^{-/-}$ mice show delayed onset of EAE, implicating MMP-9 in EAE induction. However, both *Mmp2*$^{-/-}$ and *Mmp9*$^{-/-}$ mice show increased EAE severity and absence of a recovery phase, with long-term persistence of inflammatory cuffs. Flow cytometry reveals no differences between *Mmp2*$^{-/-}$, *Mmp9*$^{-/-}$ and WT mice in proportions of effector (T_{eff}) and regulatory T cells (T_{regs}) in the periphery and CNS at peak EAE but preliminary data suggest altered T_{reg} function and reduced Th17 pathogenicity in the absence of the gelatinases.

Conclusion
MMP-9 is critical for penetration of BBB, while both gelatinases are required for the recovery phase and act independently of each other at later EAE stages. Absence of immune cell-derived gelatinases results in cell-intrinsic defects in T_{reg} function and Th17 pathogenicity, which are independent of CD25/IL-2 and TGF-β signalling. MMP-2/-9 substrates on T_{regs} and T_{effs} are under investigation.

[1]Agrawal, S., Anderson, P., Durbeej, M., van Rooijen, N., Ivars, F., Opdenakker, G. and Sorokin, L. M. (2006). J. Exp. Med. 203, 1007-1019; [2]Song, J., Wu, C., Agrawal, S., Korpos, E., Wang, Y., Faber, C., Schäfers, M., Körner, H., Opdenakker, G., Hallmann, R. and Sorokin, L.(2015). Cell Reports 10, 1040-1054.

Poster Board 25

Topic: Genetic and immunological understandings of multiple sclerosis

Antigen Specific T Regulatory Cells Activated by Antigen and Type-2 Cytokines Inhibit Induction of EAE

Bruce Hall[1,3], Giang Tran[1], Suzanne Hodgkinson[1,2], Paul Wilcox[1], Nirupama Verma[1], Catherine Robinson[1]
[1]*Immune Tolerance Laboratory, Ingham Institute, Liverpool Hospital, UNSW Australia, Australia*
[2]*Liverpool Hospital, Multiple sclerosis and neuroimmunology, Australia*
[3]*Liverpool Hospital, Renal Medicine, Australia*

Background. $CD4^+CD25^+Foxp3^+$T regulatory cells(Treg) can be activated by specific antigen and Type-II cytokines interleukin-4 (IL-4) and IL-5 to control autoimmunity.

Objective. To examine if naïve Treg cultured with specific antigen MBP and rIL-4 or rIL-5 can inhibit EAE.

Methods. Lewis rats were immunized with MBP in FCA. Treg from naïve rats were cultured with MBP and rIL-4 for 4 days. To further activate rIL-4 and MBP activated Treg, they were cultured for 3 days with MBP and rIL-5. Cultured cells were assayed by FACS and RT-PCR to determine their phenotype and were given ivi to rats 9 days after immunization.

Results. Treg cultured with rIL-4 and MBP, retained a $CD4^+CD25^+Foxp3^+$T phenotype, and had mRNA for *il-5ra* but no *il-5* consistent with a Ts2 phenotype. $5x10^6$, but not $5x10^5$, of these Ts2 cells arrested progression of EAE at day 14 all rats recovering in 2 days, whereas controls had more severe disease and recovered 4-5 days later. $5x10^6$ naïve Treg had no effect on EAE.
Ts2 cells re-cultured with rIL-5 and MBP, also retained a $CD4^+CD25^+Foxp3^+$T phenotype, and had mRNA for *il-5ra, il-5, il-4* but no Th1 or Th17 cytokines. They also expressed Th2 associated transcription factors *gata-3* and *irf4,* giving them a Th2-like Treg phenotype. $5x10^5$ Th2-like Treg delayed onset of EAE by 2-3 days, reduced peak disease and accelerated recovery.

Conclusion. Naïve Treg activated by specific autoantigen and Type II cytokines produce Treg that can markedly reduce the severity of EAE. Such cells, responsive to IL-5 have therapeutic potential.

Poster Board 26

Topic: Genetic and immunological understandings of multiple sclerosis

Studies on Subsets of CD4^{+}CD25^{+}CD127^{lo}T Regulatory Cells in Blood of Patients with Multiple Sclerosis after Treatment with Alemtuzumab

Suzanne Hodgkinson[1,2], Andrew Lam[1], Chris Chiu[1], Nirupama Verma[1], Meena Sharma[2], Sue Baker[2], Giang Tran[1], Bhumika Limbu[2], Catherine Robinson[2], Bruce Hall[1]
[1]*Ingham Research Institute, Immune Tolerance Group, UNSW Sydney, Australia*
[2]*Neurology Liverpool Hospital, Multiple Sclerosis and Neuroimmunology, Australia*

Objective. To examine changes in the three Treg populations in MS patients treated with alemtuzumab.

Methods. Blood was collected from MS patients(n=9) before treatment with alemtuzumab and three months later. Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll and subjected to flow FACS. CD4^{+}CD25^{+}CD127^{lo}Foxp3^{+}T regulatory cells(Treg) into naïve Treg that express CD45RA (Population I). CD45Ra is lost on activation and the most activated Treg increase expression of Foxp3 and CD25 (Population II) whereas less activated Treg do not increase CD25 and Foxp3 expression(Population III). Activated Treg migrate to sites of inflammation by expression of chemokine receptors similar to effector T cell subtypes; Th1-like(CXCR3), Th17-like(CCR6).

Results. Alemtuzumab increased the proportion of Treg compared to effector CD4^{+}T cells, with an increase in CCR6, but not CXCR3, expressing activated Treg.

Conclusion. In a small cohort, alemtuzumab led to a greater proportion of Treg, with a reduction in naïve Treg and an increase in activated Treg expressing CCR6 the Th17 associated chemokine receptor.

Poster Board 27

Topic: Genetic and immunological understandings of multiple sclerosis

Studies on Subsets of CD4^{+}CD25^{+}CD127^{lo}T Regulatory Cells in Blood of Patients with Multiple Sclerosis

Suzanne Hodgkinson[1,2], Nirupama Verma[2], Andrew Lam[2], Chris Chiu[2], Meena Sharma[1], Sue Baker[1], Bhumika Limbu[1], Catherine Robinson[2], Giang Tran[2], Bruce Hall[2]
[1]*Liverpool Hospital ool, Multiple Sclerosis and Neuroimmunolgy, Australia*
[2]*Immune Tolerance Laboratory, Ingham Institute and UNSW Sydney, Australia*

Background. CD4^{+}CD25^{+}CD127^{lo}Foxp3^{+}T regulatory cells (Treg) can be divided into subtypes by FACS analysis. Naïve Treg express CD45RA (Population I) and this marker is lost on activation. The most activated Treg increase expression of Foxp3 and CD25 (Population II) whereas less activated Treg do not express CD45RA but have normal levels of expression of CD25 and Foxp3 (Population III). Further naïve Treg circulate from blood to lymph and express CCR7, whereas activated Treg migrate to sites of inflammation express chemokine receptors similar to effector T cell subtypes.

Objective. To examine if MS had differences in the three Treg populations.

Methods. Blood was collected from MS patients(n=36) and age/sex matched healthy donors(HD)(n=20). Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll and subjected to flow FACS to identify naïve (CD4^{+}CD25^{+}CD127^{lo}FoxP3^{+}CD45RA^{+}) and activated (CD4^{+}CD25^{+}CD127^{lo}Foxp3^{+}CD45RA^{-})Treg. Chemokine receptor staining identified Th1-like(CXCR3), Th17-like(CCR6) and circulating Treg(CCR7).

Results. MS patients as a group had less naïve Treg, but this overlapped with low normal. As a group MS patients had higher Population II than HD, in particular those with no clinical MS in the last 3 months. Population III was similar.
CCR5 was mainly in Population I, and CCR6 and CXCR3 in population II and III. A minority of MS patients had CCR6 expression lower than HD in population II, but there was no difference in CXCR3 expression.

Conclusion. We identified differences in some MS patients, including more highly activated Treg and less Th17-like CCR6^{+}Treg. Interpreting these observations requires more patients and clinical correlation.

Poster Board 28

Topic: Genetic and immunological understandings of multiple sclerosis

Dissecting the Role of Pattern Recognition in Initiation of Anti-myelin Autoimmune Responses

Filipa Marques Ferreira, Thorsten Buch
Institute of Laboratory Animal Science, University of Zurich, Switzerland

As the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE) can be actively induced in C57BL/6 mice by co-administration of myelin oligodendrocyte glycoprotein peptide and complete Freund adjuvant, together with pertussis toxin. The requirement of adjuvant components for successful EAE induction suggests that there may be a role for pattern recognition receptors (PRR) in the onset of this disease. PRR recognise pathogen- or damage- associated molecular patterns, engaging the innate and adaptive immune systems. Even though there is abundant literature implicating PRR and their downstream signalling molecules in the development of EAE, no molecule or receptor has yet been identified as a major player in this model.
It is known that MyD88 knockout mice are resistant to EAE. Surprisingly, studies in our lab show that this effect is not due to TLR signalling, the main upstream signalling pathway of MyD88, as in our hands Tlr23479 knockout mice develop EAE to the same extent as control mice. Thus, we hypothesise that there are alternative pathways involved in EAE development. In order to study these pathways, we have generated knockout mice for key molecules of other PRR pathways: Mavs (RLR pathway), Tram (TLR pathway), Card9 (CLR, NLR and TLR pathways) and Sting (CDS pathway). These knockout mouse lines develop EAE, with variable levels of delay in onset and severity than their littermate controls.

Poster Board 29

Topic: Genetic and immunological understandings of multiple sclerosis

Transcriptomic analysis of reactive human iPSC-derived astrocytes induced by neuroinflammatory cytokines

Sylvain Perriot[1], Guillaume Perriard[1], Mathieu Canales[1], Amandine Mathias[1], Renaud Du Pasquier[1,2]
[1]*Neuroscience Research Centre, CHUV, Switzerland*
[2]*Service of Neurology, CHUV, Switzerland*

Astrocytes occupy a central place in neuroinflammatory diseases, such as multiple sclerosis (MS). Recent studies in mice have identified two clear states of astrocyte reactivity, A1 and A2, respectively induced by neuroinflammation and transient ischemia. However, due to the difficulty in obtaining human astrocytes, validity of these data in a human context remains to be established.
Here, we aimed at better characterizing human astrocyte reactivity in different neuroinflammatory conditions. To address this issue, we took advantage of our recently published serum-free technique to obtain resting astrocytes from human induced pluripotent stem cells (hiPSCs). We generated hiPSC-derived astrocytes from healthy donors and MS patients and stimulated them with major neuroinflammatory cytokines (IL-6, IL-1β and/or TNFα) to assess their transcriptomic profile in response to these stimuli.

Transcriptomic analysis of reactive astrocytes showed first that each of these three cytokines lead to the modulation of a specific set of genes, triggering a unique activation profile of astrocytes. Second, gene ontology analysis revealed that IL-6 triggered the upregulation of genes mainly involved in cell adhesion, CNS development and ion transport while IL-1β and TNFα led to the upregulation of genes mainly involved in the inflammatory response, interferon signaling and defense against viruses.

In conclusion, our study reveals specific activation states of astrocytes in response to neuroinflammatory cues, suggesting distinct functionalities in different inflammatory contexts. As each neuroinflammatory disease is associated to a different inflammatory CNS milieu, our data call for a more precise characterization of reactive astrocytes in a given disease to decipher their role in a such condition. Better understanding of these reactive states would lead to a better understanding of astrocyte roles in neuroinflammatory diseases and may allow identifying new therapeutic targets.

Poster Board 30

Topic: Genetic and immunological understandings of multiple sclerosis

A20 critically controls microglia activation and inhibits inflammasome-dependent neuroinflammation

Sofie Voet[1,2], Conor Mc Guire[1,2], Nora Hagemeyer[3], Arne Martens[1,2], Anna Schroeder[4], Peter Wieghofer[3,10], Carmen Daems[5], Ori Staszewski[3], Lieselotte Vande Walle[1], Marta Joana Costa Jordao[3], Mozes Sze[1,2], Hanna Vikkula[1,2], Delphine Demeestere[1,2], Griet Van Imschoot[1,2], Charlotte L. Scott[1,2], Esther Hoste[1,2], Amanda Gonçalves[1,2,6], Martin Guilliams[1,2], Saskia Lippens[1,2,6], Claude Libert[1,2], Roos Vandenbroucke[1,2], Ki-Wook Kim[7], Steffen Jung[7], Zsuzsanna Callaerts-Vegh[8], Patrick Callaerts[5], Joris de Wit[4], Mohamed Lamkanfi[1], Marco Prinz[3,9], Geert van Loo[1,2]

[1]*Center for Inflammation Research, VIB, Belgium*
[2]*Department of Biomedical Molecular Biology, Ghent University, Belgium*
[3]*Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Germany*
[4]*Center for Brain & Disease Research, VIB, Belgium*
[5]*Department of Human Genetics, KU Leuven, Belgium*
[6]*Bio-Imaging Core, VIB, Belgium*
[7]*Department of Immunology, Weizmann Institute of Science, Israel*
[8]*Laboratory of Biological Psychology, KU Leuven, Belgium*
[9]*BIOSS Centre for Biological Signalling Studies, University of Freibur, Germany*
[10]*Institute of Anatomy, University of Leipzig, Germany*

Microglia, the mononuclear phagocytes of the central nervous system (CNS), are important for the maintenance of CNS homeostasis, but also critically contribute to CNS pathology. Here we demonstrate that the nuclear factor kappa B (NF-κB) regulatory protein A20 is crucial in regulating microglia activation during CNS homeostasis and pathology. In mice, deletion of A20 in microglia increases microglial cell number and affects microglial regulation of neuronal synaptic function. Administration of a sublethal dose of lipopolysaccharide induces massive microglia activation, neuroinflammation, and lethality in mice with microglia-confined A20 deficiency. Microglia A20 deficiency also exacerbates multiple sclerosis (MS)-like disease, due to hyperactivation of the Nlrp3 inflammasome leading to enhanced interleukin-1β secretion and CNS inflammation. Finally, we confirm a Nlrp3 inflammasome signature and IL-1β expression in brain and cerebrospinal fluid from MS patients. Collectively, these data reveal a critical role for A20 in the control of microglia activation and neuroinflammation.

Poster Board 31

Topic: Genetic and immunological understandings of multiple sclerosis

Single Nucleus RNA Sequencing of Post-Mortem Multiple Sclerosis Cortical Grey Matter

Amy Smith[1,2], Prashant Srivastava[1], Paul Matthews[1,2], David Owen[1], Richard Reynolds[1]
[1]*Division of Brain Sciences, Imperial College London*
[2]*UK Dementia Research Institute, Imperial College London*

Background:
Multiple sclerosis (MS) is an inflammatory neurodegenerative disease associated with molecular pathological changes across all cell types in the brain. Defining how individual cell types are affected would better characterise inflammatory processes in the disease, identifying cells most susceptible to inflammation-mediated injury.

Objective:
Previous bulk tissue RNASeq and microarray studies have identified gene expression pathways disrupted in cortical grey matter (GM), but questions remain about the specific cell-types associated with these changes. Our aim has been to identify cell-specific transcriptomic changes in MS cortical GM to characterise pathogenic changes in gene expression.

Methods:
We investigated single nucleus transcriptomic changes in a cohort of MS cases with high (n=10) and low (n=10) levels of inflammation, as well as in non-neurological disease controls (n=8). All samples were taken from the cingulate gyrus, an area previously identified as showing increased meningeal inflammation and GM pathology. GM dissected from snap frozen tissue blocks was dounce-homogenized and nuclei isolated using sucrose gradient centrifugation. Single nuclei were lysed and RNA barcoded using the 10X Genomics Chromium dropseq machine. Transcriptomic data were analysed using 10X Cell Ranger software and single cell packages in R. Bulk tissue RNASeq was performed concurrently on the same tissue blocks.

Results:
All the major cell types of the brain could be identified based on their nuclear transcriptomes. We identified distinct sub-clusters of astrocytes and oligodendrocytes distinguishing the non-diseased controls and the MS patients. Cluster-based differential gene expression analyses will be described, with ongoing work to determine spatial expression patterns using RNAScope in situ hybridisation.

Conclusion:
This transcriptomic data at the single nucleus level has allowed us to identify cell-specific changes associated with different degrees of inflammation in MS GM. These data can be used to define disease and inflammation-associated pathways that promise the potential to identify new therapeutic targets for neuroprotection.

Poster Board 32

Topic: Genetic and immunological understandings of multiple sclerosis

Selective inhibition of soluble TNF promotes macrophage phagocytosis of myelin debris and remyelination of demyelinated lesions

Athena Boutou[1], Maria Karamita[1], Vasiliki Kyrargyri[1], David Szymkowski[3], Christopher Barnum[2], RJ Tesi[2], **Lesley Probert**[1]
[1]*Laboratory of Molecular Genetics, Hellenic Pasteur Institute, Greece*
[2]*INmune Bio, INmune Bio Inc*
[3]*Xencor, Xencor, USA*

Remyelination is a spontaneous regenerative process of the adult CNS that, for unknown reasons, largely fails in multiple sclerosis (MS). The transmembrane form of TNF (tmTNF) and TNFR2 are known to be critical for remyelination in experimental models, but an opposing inhibitory effect of soluble TNF (solTNF) on remyelination has only recently been discovered. Using the cuprizone demyelination remyelination model, s.c. administration of a selective inhibitor of solTNF named XPro1595 that crosses the intact blood-brain barrier in pharmacological concentrations, did not prevent toxin-induced oligodendrocyte loss and demyelination, but permitted profound early remyelination and prevented disease-associated neurodegeneration and decline in motor performance. Remyelination in the XPro1595-treated mice was associated with higher proportions of large foamy macrophages containing PLP-immunoreactive myelin degradation products and improved clearance of myelin debris in lesions compared to vehicle controls, suggesting that Xpro1595 enhances remyelination by increasing phagocytosis of myelin debris by CNS macrophages. To further investigate this effect here we used an *in vitro* myelin phagocytosis assay. Peritoneal macrophages were isolated from C57BL/6 mice and cultured in medium containing myelin in the absence or presence of XPro1595 (100 ng/ml). XPro1595 significantly increased the amount of macrophage myelin phagocytosis, as measured at 16 and 20 h of culture by increased numbers of MBP-immunoreactive cells. These results confirm that solTNF inhibits the pro-repair potential of macrophages to phagocytose myelin debris. The *in vitro* myelin phagocytosis assay represents a useful tool for investigating the molecular mechanisms of functional phenotype switching in macrophages, for identifying additional therapeutic targets for increasing remyelination, and for testing the differential contributions of microglia and peripheral macrophages in lesions. Together, our results suggest that selective inhibition of solTNF by brain-penetrating agents such as XPro1595 promotes remyelination by increasing the presence of pro-repair over pro-inflammatory macrophages, and represents a promising approach for achieving neuroprotection in progressive MS.

Poster Board 33

Topic: Genetic and immunological understandings of multiple sclerosis

Genetic Variability in Response to Abeta Deposition Influences Alzheimer's Risk

Dervis Salih[1], Sevinc Bayram[3], Sebastian Guelfi[2], Regina Reynolds[2], Maryam Shoai[2], Mina Ryten[2], Jonathan Brenton[1], David Zhang[2], Mar Matarin[2], Juan Botia[2,4], Runil Shah[2], Keeley Brookes[5], Tamar Guetta-Baranes[5], Kevin Morgan[5], Eftychia Bellou[6], Damian Cummings[1], Valentina Escott-Price[6], Frances Edwards[1], John Hardy[2]

[1]*Neuroscience, Physiology and Pharmacology, University College London, UK*
[2]*Institute of Neurology, University College London, UK*
[3]*Hitachi Rail Europe Ltd, Hitachi, UK*
[4]*Department of Information and Communications Engineering, Universidad de Murcia, Spain*
[5]*Human Genetics, School of Life Sciences, University of Nottingham, UK*
[6]*Dementia Research Institute, Cardiff University, UK*

Note: I work on the genetics of Neuroinflammation in Alzheimer`s disease

Background
Genetic analysis of late-onset Alzheimer's disease (AD) risk has previously identified a network of primarily microglial genes that form a transcriptional network. In transgenic mouse models of amyloid deposition we have previously shown that the expression of many of the mouse orthologues of these genes are coordinately up-regulated by amyloid deposition.

Objective
To use statistical comparison of an improved RNA-seq-generated amyloid-responsive mouse network with previous human AD genome-wide association studies to predict new genetic risk loci for the disease.

Methods
We generated a high-resolution transcriptional network that responds strongly to amyloid deposition using transgenic mouse models of AD for RNA-seq and weighted gene co-expression network analyses. We used a gene-based statistical approach to identify human genes that show significant association with AD using SNP data from the International Genomics of Alzheimer's Project.

Results
This statistical comparison of the mouse amyloid-responsive network with AD genome-wide association studies identifies five new genetic risk loci for the disease (*OAS1*, *CXCL10*, *LAPTM5*, *ITGAM* and *LILRB4*), in addition to confirming the importance of established risk genes such as *TREM2* and *ABI3*. These new and established AD risk genes form a transcriptional network that is conserved in human transcriptome datasets.

Conclusion
This work suggests that genetic variability in the microglial response to amyloid deposition is a major determinant for Alzheimer's risk, and discovery of these genes may help to predict the risk of developing AD. These findings also provide insights into the mechanisms underlying AD for potential drug discovery.

Poster Board 34

Topic: Genetic and immunological understandings of multiple sclerosis

Investigating CCN3 in Multiple Sclerosis

Michelle Naughton[1], Jill Moffat[1], Kristen Hawkins[2], Nira de la Vega Gallardo[1], Andrew Young[1], John Falconer[1], Andrew Hogan[3], Paul Moynagh[1,3], Neil Robertson[4], Rachael Kee[6], Ben Pearson[2], Bruno Gran[5], Stella Hughes[6], Gavin McDonnell[6], Owain Howell[2], Denise C. Fitzgerald[1]
[1]*Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, UK*
[2]*Institute of Life Sciences, Swansea University, UK*
[3]*Institute of Immunology, National University of Ireland Maynooth, Ireland*
[4]*Welsh Neuroscience Research Tissue Bank, University Hospital of Wales, UK*
[5]*Clinical Neurology, Division of Clinical Neuroscience, University of Nottingham School of Medicine, UK*
[6]*Belfast Health and Social Care Trust, Belfast City Hospital, UK*

Multiple sclerosis (MS) is an immune-mediated disease that attacks myelin in the central nervous system (CNS). Unfortunately, no therapies exist to promote myelin repair. We recently demonstrated the first evidence of CCN3 production by immune cells and its pro-myelinating properties. This study aims to address whether CCN3 expression is altered in MS by quantifying CCN3 in the periphery (plasma, immune cells) and CNS (CSF and brain tissue) using ELISA, western blot and immunohistochemistry.

Plasma CCN3 levels were comparable between MS and control samples, but were significantly higher in progressive versus relapsing-remitting MS. Patients on interferon-b displayed elevated CCN3 levels compared to natalizumab. An association between increasing BMI and CCN3 levels was observed in controls as reported previously, but this effect was absent in the MS cohort. To investigate whether CCN3 levels in plasma and cerebrospinal fluid (CSF) were linked, paired samples were analysed from MS patients who underwent lumbar puncture at diagnosis. Samples from patients who underwent CSF shunting due to idiopathic intracranial hypertension (IIH) served as controls. No correlation in CCN3 levels occurred between plasma and CSF in IIH but a significant correlation was seen in MS. Qualitatively, distinct CCN3 isoforms were detected in plasma that were absent in CSF.

CCN3 was detected in neurons, astrocytes and blood vessels in the CNS by immunohistochemistry. Although overall CCN3 levels were comparable between non-affected, demyelinated and remyelinated tissue, the profile of expression varied dramatically. This investigation provides the first comprehensive profile of CCN3 expression in MS and provides rationale to determine if CCN3 contributes to the regenerative capacity of the CNS.