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EDITORS

David A Brown
The Westmead Institute for Medical Research. The University of Sydney

Stephen W Reddel
Brain and Mind Centre. The University of Sydney

Judith M Greer
UQ Centre for Clinical Research. The University of Queensland

Fabienne Brilot
Kids Research Institute The Children’s Hospital at Westmead. The University of Sydney

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Introduction to the Proceedings of Neuroimmunology Australia

Neuroimmunology Australia is a body that was formed in 2010, with the aim of providing a forum for discussion of immune-mediated diseases of the brain, spinal cord, peripheral and autonomic nerves, the neuromuscular junction and muscles. The members of Neuroimmunology Australia come from the fields of clinical immunology, basic immunology, neurology and neurosciences, and from hospitals, diagnostic laboratories and universities. The intention is to provide a meeting where there can be true inter-disciplinary interaction.

The first meeting was held in Brisbane in 2010, on the topic of “Antibody-mediated Neurological Disease”. The range of diseases that were discussed was wide - ranging from myasthenia gravis to schizophrenia. Subsequent meetings have also been held in Brisbane and Sydney in April 2011, August 2012, July 2014, July 2015 and now in August 2016.

The Proceedings of Neuroimmunology Australia is published to provide a permanent record of the work presented at the meetings of Neuroimmunology Australia. The proceedings have been published since the second year of the Neuroimmunology Australia annual scientific meeting.

David A Brown
President,
Neuroimmunology Australia
Trevor Owens was born in Ireland and after a BSc and MSc from McGill he obtained his PhD from the University of Ottawa in Canada in 1981. After postdoctoral training in London and Melbourne he returned to McGill in 1987 and in 1990 joined the Neuroimmunology Unit of the Montreal Neurological Institute. In 2004 he became Professor at the University of Southern Denmark, where he has been Leader of the Neurobiology Research Department since 2010. His laboratory focuses on animal models of multiple sclerosis and specifically on interactions between immune cells and glial cells in the brain and spinal cord.
PROTECTIVE ROLE FOR MICROGLIA IN NEONATAL AND ADULT CNS

Wlodarczyk, Reza Khoroooshi, Trevor Owens

Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, Odense.

Microglia are tissue macrophages of the central nervous system (CNS). Their primary functions are as part of innate CNS immunity, to clear debris and damaged cells, and to signal for immune response in the CNS. Microglia play a major role in homeostatic regulation in the CNS, including by production of Type I interferons (IFN), as well as other cytokines and chemokines. Cytokine secretion profiles and microglial phenotypes are themselves influenced by developmental and degenerative cues, including bidirectional interaction with astrocytes, and their recent phagocytic experience. They therefore interpret local needs and conditions for appropriate immune response.

Our research aims to understand how microglial regulatory profiles are triggered and how they control neuroinflammation. We apply experimental ligands to drive regulatory cytokine production in mouse models for multiple sclerosis and neuromyelitis optica and measure outcomes using transgenic reporter and gene-deficient mice to establish mechanism. Gene expression profiles of microglia from neonatal and adult mice have been compared.

Induction of Type I IFN by adult microglia protects against neuroinflammatory disease. The CD11c+ microglial subset are very effective inducers of a T cell proliferative response although ineffective at inducing effector T cells. This subset is over-represented in neonatal CNS. A neuroprotective role for neonatal microglia is shown by intrathecal transfer of these cells to alleviate EAE in adult mice. Transcriptomic analysis suggests a role in neuronal development for microglia that produce the cytokine insulin-like growth factor-1 and other factors implicated in neuronal guidance and survival.
ROLE OF MUSK IN ADAPTING PRESYNAPTIC QUANTAL RELEASE TO CHANGES IN POSTSYNAPTIC QUANTAL AMPLITUDE

Joanne Ban

Discipline of Physiology, University of Sydney

Muscle specific kinase (MuSK) is essential to develop a stable postsynaptic AChR cluster during formation of neuromuscular junction. However, it is not entirely clear how MuSK might influence neuromuscular transmission physiologically. To investigate this question, MuSK was overexpressed in muscle. Naïve mice were anesthetized and rAAV-MuSK-EGFP was injected into the right tibialis anterior (TA) muscle. The contralateral muscle of each mouse was injected with empty AAV vector. Three weeks later, the mice were again anesthetized and muscle maximum isometric contraction force was recorded from the TA muscle in vivo using a semi-isometric transducer. Tetanic activation was elicited via the nerve or by direct stimulation of the muscle in three different train lengths (400msec, 800msec, 1600msec; n=14 mice). Muscles injected with empty vector produced steady tetanic force for the first 400msec of nerve stimulation. Moderate decayed in force only observed after 800msec and 1600msec of nerve stimulation. Muscles injected with rAAV-MuSK-EGFP produced substantial decay in force during tetanic stimulation of 400msec and maintained a faster rate of force decay with the longer tetani (residual force in 400ms: 85.0% ±1.81; 800ms: 64.1% ±3.39 ; 1600ms: 45.4% ±3.61 ), compared to empty vector control muscles. In contrast, no significant decay was recorded when muscles were stimulated directly. Thus the decay in nerve-evoked force was due to failure of nerve-muscle transmission. Recovery from neuromuscular transmission failure force in rAAV-MuSK-EGFP injected muscle was assessed by comparing two successive tetani separated by different delay times (120s, 60s, 30s, 10s, 3.6s, 10.6s; n=9 mice). Delays of 0.6sec were not sufficient for full recovery of force after the first tetanus. Muscles expressing MuSK-EGFP reached 50% recovery of the initial peak force after 3.6s. These results suggest that the postsynaptic MUSK kinase system may negatively regulate neuromuscular transmission possibly via retrograde feedback control of synaptic vesicle release capacity.
INVESTIGATING POTENTIAL PATHOGENICITY OF ANTIBODIES FROM PEOPLE WITH MULTIPLE SCLEROSIS

Shannon J. Beasley¹, David V. Pow² and Judith M. Greer¹

¹The University of Queensland, UQ Centre for Clinical Research, Royal Brisbane & Women’s Hospital, Brisbane, Queensland, Australia  
²School of Medical Science, RMIT University, Bundoora, Victoria, Australia

The pathogenic role that antibodies might play in multiple sclerosis (MS) is still debated, along with the identity of molecules that could be the targets of these antibodies. In the current study, some measures which could suggest pathogenicity of antibodies from people with MS were tested.

Initially, sera from MS patients (n=58), patients with other neurological diseases (OND; n=14) and healthy individuals (n=16) were tested for their ability to opsonize human myelin and thereby increase uptake of the myelin by macrophages. All but 2 of the MS serum samples were able to induce a significant increase in myelin uptake compared to both healthy individuals and OND patients.

Next, IgG was purified from selected sera from MS patients (n=6) and healthy individuals (n=3), and tested for the ability to kill rat glial cells. The samples used were selected because they all showed elevated levels of reactivity in ELISA to myelin proteolipid protein, which has the identical sequence in humans and rats. In addition, the IgG samples were also tested in ELISA for their ability to bind to peptides of myelin oligodendrocyte glycoprotein (positive in one MS patient), several isoforms of the glutamate transporter GLAST (positive in 3 MS patients), and the cystine-glutamate antiporter c-gap (positive in 1 healthy control and 3 MS patients). Mature mixed glial cell cultures from neonatal rats were then incubated for 18 h with the purified patient or healthy control IgG, in the presence or absence of guinea pig complement, and percentages of live oligodendrocytes and astrocytes were then assessed. Oligodendrocyte cell numbers were significantly reduced following incubation with IgG from one MS patient, and in control cultures treated with the O4 antibody. Astrocyte numbers were significantly reduced following incubation with IgG from one MS patient.

This preliminary study shows that IgG from some MS patients can induce complement mediated cell cytotoxicity of glial cells. Cell cytotoxicity did not correlate strongly with the specificity of the antibodies in the ELISA assays; however, only a relatively small number of antigens were tested by ELISA. Further studies will be required to confirm these findings in a larger patient cohort.
AUTOIMMUNE AND INFLAMMATORY CONTRIBUTORS TO MENTAL DISORDERS PRESENTING ON A YOUNG ADULT PSYCHIATRIC UNIT

David A. Brown ¹, Ian Hickie ², G Galambos ³, Elizabeth Scott ²,³

¹ Neuroinflammation Research Group, The Westmead Institute for Medical Research, Sydney, Australia.
² Brain and Mind Centre, University of Sydney, Australia.
³ Young Adult Mental Health Unit, St Vincent’s Private Hospital, Sydney, Australia.

It is increasing being recognised that immune and inflammatory processes mediate a proportion of mental disorders. These range from non-specific symptom constellations arising in “conversion” disorders to defined neuropsychiatric disorders with specific antibody mediated processes targeting neurotransmitter receptors. It is clear that many specific antibody mediated diseases presenting as mental disorders are yet to be characterised.

A review of the literature regarding the changes in neuroimmunology associated with early life events and a case history will be presented demonstrating that successful immunotherapy of patients presenting with treatment resistant mental disorders where autoimmune and inflammatory processes were found to be active.
THE DISTINCTIVE ROLE OF MALT1 IN TOLERANCE AND AUTOIMMUNITY

Anne Brüstle1,2#, Dirk Brenner2,3#, Christiane B. Knobbe-Thomsen4, Maureen Cox5, Philipp A. Lang4, Karl S. Lang5, Tak W. Mak2

1 John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia
2 Campbell Family Institute for Breast Cancer Research, Toronto, ONT, Canada
3 Department of Infection and Immunity, Experimental and Molecular Immunology, Luxembourg Institute of Health, Luxembourg, Luxembourg
4 University of Düsseldorf, Düsseldorf, NRW, Germany, S. University of Essen-Duisburg, Essen, NRW, Germany
5 These authors contributed equally to this work

Effector functions of inflammatory IL-17-producing Th (Th17) cells have been linked to autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS). However, what determines Th17 cell encephalitogenicity is still unresolved. We here demonstrate that the NF-κB-regulator MALT1 is a central, cell-intrinsic factor determining the encephalitogenic potential of Th17 cells. MALT1 deficient (Malt1/-/) mice exhibit strong lymphocytic infiltration in the CNS, but do not develop any clinical signs of EAE. Despite their significantly decreased secretion of Th17 effector-cytokines, Malt1/-/ Th17 cells show normal expression of lineage-specific transcription factors indicating that MALT1 determines the encephalitogenic potential post-transcriptionally. We further show that the paracaspase activity of MALT1 is responsible for this posttranscriptional regulation. However, paracaspase-dead mutant mice (MALT1PD/PD) develop spontaneous autoimmunity in addition to their total resistance to EAE due to a diminished induction of regulatory T cells (Tregs), a T cell population negatively regulating inflammation. Our newest study analyses MALT1 as an important novel regulator of Tregs.

While absent in young Malt1/-/ mice, Treg numbers increase in older Malt1/-/ mice as well as in Malt1-/- mice subjected to experimentally induced inflammation. Natural (n) Tregs are totally absent in Malt1 deficient mice but inducible (i)Tregs isolated from WT and Malt1-/- mice are indistinguishable with respect to their suppression abilities. Malt1-/- iTregs also express higher levels of Toll-like receptor (TLR) 2. Treatment of WT and Malt1-/- Th cells in vitro with the TLR2 ligand Pam3Cys strongly enhances the induction and proliferation of Malt1-/- iTregs. Our data suggest that MALT1 supports nTreg development in the thymus but suppresses iTreg induction in the periphery during inflammation. MALT1 is therefore not only a key molecule for Th17 cell function, but plays a critical role in Tregs, contributing to immune tolerance in the steady-state while facilitating immune reactivity under stress conditions.
DEVELOPMENT OF AN INTERNATIONAL DATABASE FOR RESEARCH INTO NEUROIMMUNOLOGICAL DISEASES (eNID)

Katherine A Buzzard, Stephen W. Reddel, Sean Riminton, and Helmut Butzkueven

The nervous system is vulnerable to inflammatory damage caused by the immune system leading to variety of neuroimmunological diseases. These diseases are often chronic, requiring long-term management. They are generally treatable, however they are often associated with periods of significant disability. Although individually rare, collectively neuroimmunological diseases affect a significant number of individuals worldwide and account for a disproportionately heavy burden on health care systems. Like so many rare diseases, a lack of evidence-based information poses significant challenges for clinicians. Disease registries can be powerful and cost-effective instruments for expanding our knowledge of diseases and treatments. They are particularly useful in rare diseases where they can provide the opportunity to pool ‘real world’ data to reach a sufficient sample size for epidemiological and clinical research.

We are developing an international registry for patients with neuroimmunological diseases, known as eNID (electronic NeuroImmunology Database). eNID is being built as an extension to MSBase, a highly successful international registry for patients with Multiple Sclerosis. Work has begun on developing the data templates for 4 diseases to be initially included in eNID: myasthenia gravis, CIDP, neurosarcoidosis and autoimmune encephalitis. Disease specific interest groups are being established to coordinate the design of the data templates. We will be providing an update on the current status of eNID.
Recruitment of cells to the CNS is a fundamental component of neuroinflammation and is driven by an array of chemoattractants and adhesion molecules. However, the precise molecular signals that drive immune cells into the CNS in distinct forms of neuroinflammation are incompletely understood. In addition, resolution of neuroinflammation and repair of the damaged CNS is also driven by recruitment to the CNS of cells that drive these processes. We have identified that pathogenic Th17 cells utilise two distinct chemokine receptors for entry to the CNS at distinct phases of neuroinflammation in the EAE model. CCR6 brings an early wave of Th17 cells into the CNS in EAE, which is followed by a CCR2-dependent influx of highly pathogenic Th17 cells at later stages of neuroinflammation. Indeed, we show that CCR2+CCR6-Th17 cells define a subpopulation of highly pathogenic Th17 cells dependent on IL-23 that produce the pro-inflammatory cytokine GM-CSF. Thus, while CCR6 continues to be required for Treg recruitment to the CNS as inflammation proceeds, Th17 cells increasingly depend on CCR2 for entry. To take advantage of this knowledge of the changing requirements for chemokine receptors as inflammation progresses, we have modified mesenchymal stem cells (MSCs), a cell-based immunomodulatory and reparatory therapy of potential utility in MS, to express CCR6 or CCR2. We show that these modified MSCs have enhanced recruitment to the CNS at distinct phases of disease and are more inhibitory to CNS inflammation than non-CNS tropic control MSCs. Together, these findings enhance our knowledge of cell recruitment to the CNS during neuroinflammation, provide impetus to understand in more detail the dynamic requirements for leukocyte entry to the inflamed CNS, and provide strong rationale to improve homing of cell based therapeutics for neuroinflammatory diseases to target them more readily to the inflamed CNS.
EVIDENCE OF BRAIN WHITE MATTER INTEGRITY REPAIR IN CHRONIC HIV INFECTION: A DIFFUSION TENSOR IMAGING STUDY

Lucette A. Cysique, James R. Soares, John Geng, Maia Scarpetta, Kirsten Moffat, Michael Green, Bruce J. Brew, Roland Henry, Caroline Rae

1Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia
2Neuroscience Research Australia, Randwick, NSW, Australia
3St. Vincent’s Hospital Sydney, Darlinghurst, NSW Australia
4Peter Duncan Neuroscience Unit, St. Vincent’s Hospital Centre for Applied Medical Research, Darlinghurst, NSW Australia
5Department of Neurology, University of California San Francisco, California, USA

Rationale: Diffusion Tensor Imaging (DTI) is a method that assesses the brain white matter (WM) integrity. WM injury represents one of the hallmarks of HIV-related brain involvement. Abnormality of DTI measures such as Fractional Anisotropy (FA) and Mean Diffusivity (MD) can reflect axon loss, gliosis, and inflammation. However, once HIV-positive persons are stable on antiretroviral treatment, it is unclear what the degree of WM injury is.

Objective: Quantify the degree of WM integrity in chronic and virally suppressed HIV-positive (HIV+) persons and determine predictors of WM integrity.

Methods: DTI was conducted 40 HIV-negative and 82 HIV+ men with comparable demographics (mean age 55 ± 6) and life style factors. DTI was acquired across 32 gradient directions; FA and MD maps were quantified using Itrack IDL. Using the ENIGMA DTI protocol FA and MD values were extracted for each participant and in 11 skeleton regions of interest (SROI) from standard labels in the JHU ICBM-81 atlas covering major striato-frontal and parietal tracks, which are typically altered by HIV.

Results: Between-groups ANOVAs revealed no statistically significant differences in FA and MD across the 11 SROI except for trends of lower FA and higher MD in the HIV+ group relative to the HIV- group in the Fornix (p=.06). A higher brain antiretroviral penetration was independently associated with higher WM integrity in projection and commissural fibers (both FA and MD, p<.05-p<.006). A higher CD4-T cell count and greater immune recovery were independently associated with higher WM integrity in the projection and association fibers (both FA and MD, p<.04-p<.009).

Conclusions: We are the first to demonstrate extensive WM repair in chronic HIV infection and linked it immune improvement as well as specific antiretroviral treatments. DTI could potentially be used as a method for quantifying brain repair in chronic HIV+ persons.
PROTECTION AGAINST DEVELOPMENT OF A MOUSE MODEL OF MULTIPLE SCLEROSIS BY A PARASITE-DERIVED 68-MER PEPTIDE

Aakanksha Dixit¹, Sheila Donnelly², Maria A. Lund², John P. Dalton³, Judith M. Greer¹

¹The University of Queensland, UQ Centre for Clinical Research, Brisbane, Australia,
²University of Technology Sydney, The School of Life Sciences, Sydney, Australia,
³Queen's University Belfast, School of Biological Sciences, Belfast, United Kingdom

Helminths (parasitic worms) can exert protective effects on autoimmune diseases by modulating the type of immune response, and deliberate infection is being explored as a potential therapeutic strategy for autoimmunity. However, the use of live helminths as therapeutic agents for autoimmune disease has a number of drawbacks, and it would be preferable to identify and use the immunomodulatory components of the helminths. Previously it has been shown that the immunomodulatory activity of the liver fluke Fasciola hepatica resides in its excretory-secretory products (FhES), and further analysis of FhES has identified 3 major components: an 68 amino acid alpha helical cathelicidin-like peptide (FhHDM1), a cathepsin L-cysteine protease (FhCL1), and peroxiredoxin (FhPrx).

In the current study, the ability of these three components to modify the course of a relapsing-remitting experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS) was tested. FhHDM1 was the most effective, significantly (p<0.0001) reducing the overall severity of the disease and the number of relapses compared to mice treated with vehicle alone or mice treated with FhPrx. The effects were long-lasting, with mice continuing to show benefits for up to 70 days following a single course of FhHDM1 treatment. Preliminary investigation of the mechanism of action of FhHDM1 suggests that it is not affecting the adaptive arm of the immune response, but is exerting significant effects by modulation of innate pro-inflammatory immune responses. The data suggest that this parasite-derived peptide has potential as a novel treatment for patients with MS.
Optic neuritis (ON) is an inflammatory disease of the optic nerve characterised by pain and reduced visual acuity. ON has been associated with multiple sclerosis and neuromyelitis optica spectrum disorders (NMOSD). A distinct clinical subset of ON, presenting with recurring symptoms, is defined as either recurrent optic neuritis (rON) or chronic relapsing inflammatory optic neuritis (CRION). CRION is characterised by multiple episodes of ON involving one or both optic nerves, which occur within weeks or months of each other. CRION is particularly corticosteroid responsive, if not corticosteroid dependent. Recent evidence suggests that a subset of individuals with rON/CRION, particularly those with bilateral optic neuritis, is shown to be associated with the presence of anti–myelin oligodendrocyte glycoprotein (MOG) antibodies. Anti-MOG antibodies are expressed exclusively in the central nervous system. The clinical spectrum of the anti-MOG antibody associated disease is still being defined. However, the antibody has been shown to be associated with severe and often bilateral CRION, spinal cord inflammatory lesions affecting mainly the conus medullaris, and aquaporin-4 antibody negative NMOSD. We report the case of a 37 year old post-partum Indonesian female, who presented to a tertiary hospital in Sydney with relapsing severe bilateral optic neuropathy which was associated with MRI evidence of optic nerve inflammation and anti-MOG antibodies within both the serum and cerebrospinal fluid.
Introduction: Dysregulation of the complement system is a key factor in the pathogenesis of all forms of age-related macular degeneration (AMD). In geographic atrophy, complement activation has been linked to the progressive expansion of the macular lesion via genome-wide association studies, although how this process occurs is unclear. Using a rat model of photo-oxidative stress and inflammation, we explore the role of microglia/macrophages in propagating complement activation within expanding lesions over a 2-month period, as chronicled via Optical Coherence Tomography (OCT).

Methods: Adult SD rats were exposed to 1000 lux bright light for 24 hours (LD). Following light exposure, OCT images were captured at 0, 3, 7, 14 and 56 days following LD (n=4), to map histological changes of the outer nuclear layer (ONL) and retinal integrity. For other animals, eyes and retinas were collected at each of the aforementioned time points, and assayed for expression and localisation of complement genes (qPCR and in situ hybridisation), and deposition of the complement activation product C3d (immunohistochemistry).

Results: Following LD, OCT revealed a significant thinning of the ONL which culminated in a substantial lesion in the superior retina by 7 days. This lesion slowly expanded over the ensuing post-exposure period, and reached maximal thinning at 56 days (P<0.05). In correlation, the expression of all complement genes assessed (C1s, C2, C3, C4a, CFB, CFD, SERPING1, CFH and CFI) was persistently up-regulated throughout the LD time course. Some, including C1s, C3 and C4a, remained up-regulated even after 56 days post-exposure (P<0.05). Using in situ hybridization, C1s and C3 mRNA were found to co-localise with subretinal microglia/macrophages, which were present at the edges of the expanding lesion up to 56 days after LD and were in close proximity to C3d deposits at the lesion edges.

Conclusion: Our findings suggest that complement activation at the edges of atrophic lesions is promoted by the recruitment of subretinal microglia/macrophages, and that this may facilitate its chronic expansion over time. Consequently, the modulation of microglial activation and recruitment may be a useful therapeutic avenue to curtail deleterious complement activation and slow the progression of atrophic AMD.
INNATE IMMUNE RESPONSE IN TRAUMATIC SPINAL CORD INJURY (SCI), KILLING OR HEALING?

Masoud Hassanpour Golakani¹, Mohammad Ghaleb Mohammad², Hui Li¹, Manvendra Saxena¹, Samuel Breit¹, Marc Ruitenberg³, Gill Webster⁴, and David Brown¹,⁵

¹Laboratory of Neuroinflammation, AMR & UNSW, Sydney, NSW, Australia
²Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Sharjah, Sharjah, UAE
³Queensland Brain Institute, UQ, Brisbane, Queensland, Australia.
⁴Innate Immunotherapeutics, Auckland, New Zealand
⁵Westmead Institute for Medical Research, ICPMR and Westmead Hospital NSW 2145, Australia.

Background: Inflammatory responses post spinal cord injury (SCI) may be detrimental or beneficial. However the dynamics of these inflammatory responses are largely unknown. In this study we aimed to characterize the various immune cells in and around the injury site and their relationship with locomotor functional recovery at various time points post SCI in order to identify cell populations that might be targeted therapeutically. We also investigated the role of MIS416, a newly discovered drug that modulates the myeloid-targeted immune response in EAE and is currently in phase 2b clinical trial for the treatment of secondary progressive multiple sclerosis (SPMS).

Methods: Severe contusive SCI (70 Kdyne) was induced in mice and locomotor function was assessed on day 1,7,14, 21 and 28 post SCI using the Basso Mouse Scale (BMS). A subset of animals had MIS416 administered intravenously 24 hr following SCI and every 7 days thereafter .The entire spinal cord was removed and immune cells were isolated, characterized, and examined using FACS, immunohistochemistry (IHC) and immunofluorescence (IF). Immune cell subsets were correlated with behavioral assessment at various time points post injury.

Results: In untreated mice, peripherally derived immune cells progressively increased over the course of recovery from SCI, most of which were CD8+ and CD4+ (predominantly IFNg+) T-cells and myeloid DCs (mDCs). IHC and IF revealed infiltrating CD11c+ and CD3+ cells in the injury core, GFAP+ astrocytes surrounding the core, and Iba-1+ microglia dispersed throughout the entire cord. The number of infiltrating macrophages and CD11c+B220+ cells were correlated with functional recovery, which showed both beneficial and detrimental effects depending on the time post SCI. MIS416 treated mice showed significantly augmented functional recovery compared to saline treated control mice (p<0.0001; 2way ANOVA) from day 7 onwards. This work is ongoing to investigate the cellular mechanism of MIS416 in this respect.

Conclusions: These findings highlight that the dynamics of the innate immune responses post SCI might be manipulated to augment functional recovery.
CSF CYTOKINE/CHEMOKINE PROFILE IN POST-HERPES SIMPLEX AUTOIMMUNE ENCEPHALITIS

Kavitha Kothur1, Deepak Gill2, Melanie Wong3, Shekeeb S Mohammad1, Sushil Bandodkar4, Susan Arbunkle5, Louise Wienholt6, Russell C. Dale1

1Neuroimmunology group, Institute for Neuroscience and Muscle Research, The Children’s Hospital at Westmead, University of Sydney, Sydney, NSW, Australia;
2Department of Neurology and Neurosurgery, The Children’s Hospital at Westmead, Sydney, NSW, Australia;
3Department of Clinical Immunology, The Children’s Hospital at Westmead, Sydney, NSW, Australia;
4Department of Biochemistry, The Children’s Hospital at Westmead, Sydney, NSW, Australia;
5Department of Pathology, The Children’s Hospital at Westmead, University of Sydney;
6Department of Clinical Immunology, Royal Prince Alfred Hospital, Sydney, NSW, Australia

Background: Herpes simplex viral encephalitis (HSE) can induce relapses related to autoimmune encephalitis. The immunological studies describing pathophysiology during this transition phase and post-HSE neurological syndromes are limited. There are no longitudinal cytokine/chemokine studies of the transition from HSE to autoimmune anti-NMDAR encephalitis.

Aim: To examine the longitudinal CSF cytokine/chemokine profile of HSV-induced NMDAR autoimmunity and the latent CSF cytokine/chemokine profile in post-HSE chronic/relapsing neurological syndromes.

Patients and Methods: We measured serial CSF cytokine/chemokines (n=34) in one patient during acute HSE and subsequent anti-NMDAR encephalitis using multiplex bead assay and ELISA, and compared the findings with two patients with anti-NMDAR encephalitis without pre-existing viral infection. We also compared cytokines/chemokines in latent CSF samples of three children with previous HSE who had ongoing chronic or relapsing neurological symptoms (2.5yr-17 yrs after HSE) with noninflammatory neurological controls (n=20).

Results: Acute HSE showed elevation of a broad range of all Th subset related cytokine/chemokines, whereas the post-HSE anti-NMDAR encephalitis phase showed persistent elevation of 2 of 5 Th1 (CXCL9, CXCL10), 3 of 5 B cell (CXCL13, CCL19, APRIL) mediated molecules and IFN-α, which was more pronounced than anti-NMDAR encephalitis. All three chronic post-HSE cases showed persistent elevation of CXCL9, CXCL10 and IFN-α, and there was histopathological evidence of chronic lymphocytic inflammation in one biopsied case. Two of three chronic cases showed a modest response to immune therapy.

Conclusion: This is the first study to examine longitudinal CSF cytokine/chemokines during the evolution of viral induced autoimmune encephalitis. There is persistent CSF upregulation of cytokines/chemokines in post-HSE chronic or relapsing neurological symptoms, which may be modifiable with treatment.
INVESTIGATING THE ROLE OF KYNURENINE PATHWAY OF TRYP TOPHAN METABOLISM IN MONOCYTES IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS

Michael D. Lovelace\textsuperscript{1,2}, Bianca Varney\textsuperscript{1}, and Bruce J. Brew\textsuperscript{1,2,3}

\textsuperscript{1}Applied Neurosciences Program, Peter Duncan Neurosciences Research Unit, St Vincent’s Centre for Applied Medical Research, Sydney, NSW, Australia
\textsuperscript{2}Faculty of Medicine, St Vincent’s Clinical School, University of New South Wales, Sydney, NSW, Australia
\textsuperscript{3}Department of Neurology, St Vincent’s Hospital, Sydney, NSW, Australia

Progressive forms of multiple sclerosis (MS) lack effective therapies and almost universally lead to a long period of severe disability prior to death. Key features of MS involve sustained autoimmunity, recurrent episodes of overt and subclinical demyelination, impaired remyelination from oligodendrocyte precursor cells (OPCs), and neurodegeneration. The Kynurenine pathway (KP), the major route of tryptophan (TRP) catabolism, impacts all of these processes. The KP generates several downstream neuroactive metabolites including neurotoxic quinolinic acid (QUIN), 3-hydroxykynurenine (3-HK) and neuroprotective metabolites Kynurenic acid (KYNA) and Picolinic acid (PIC). Chronic KP activation (via upregulation of KP enzymes IDO-1 and KMO) leads to the production of neurotoxic metabolites and impaired innate repair mechanisms preventing remyelination.

We previously showed that monocytes (not lymphocytes) significantly upregulate KP enzymes after inflammatory stimuli like IFN-γ. Monocytes enter the CNS parenchyma during normal immune surveillance and in MS, playing a role in BBB breakdown. The role of activated monocytes in MS has been relatively unappreciated until recently, where several lines of evidence have shown direct roles in key stages of MS: 1) monocytes are directly associated with Oligodendrocytes, e.g. at Nodes of Ranvier and importantly, they initiated demyelination; 2) blood CD16+ monocytes are reduced in MS patients and in the EAE mouse MS model, where they traffic to the brain and predict disease severity; 3) monocytes are enriched in CSF and lesions, suggesting monocyte entry as key events in MS; 4) Preventing monocytes from CNS entry prevents EAE progression. In this study, we are exploring how monocytes participate in MS pathogenesis, using both in vitro cultured monocytes treated with a candidate MS drug (Laquinimod). Our preliminary data shows the first evidence that Laquinimod reverts the inflammatory phenotype of IFN-γ stimulated human monocytes, reducing significantly IDO-1 expression and secreted QUIN while increasing neuroprotective KYNA levels. Further experiments will progress this study in both in vitro and in vivo models. Peripheral monocytes thus are a rational therapeutic target for KP modulation for MS treatment.
LG1-1 LIMBIC ENCEPHALITIS PRESENTING AS ALTERED BEHAVIOUR, COGNITIVE IMPAIRMENT, AND VISUAL HALLUCINATIONS

Abul KM Mamun, Julia Thompson, L Allport

Autoimmune limbic encephalitis is an increasingly recognised treatable cause of subacute seizures, confusion and psychiatric symptoms. Limbic encephalitis associated with serum and CSF antibodies to the LGI1 component of the voltage-gated potassium channel complex in the brain has been associated with faciobrachial dystonic seizures (FBDS), psychiatric manifestations, confusion and subclinical seizures, and cognitive and memory decline. The condition is most commonly autoimmune, although paraneoplastic associations have been reported. In addition patients have been noted to have an increased incidence of hyponatraemia and MR hyperintensity within the medial temporal lobe (MTL) structures, although CSF cell count and biochemistry is frequently normal. Manifestations of LGI1-antibody-associated limbic encephalitis have been documented to respond well to immunotherapy, although generally poorly to antiepileptic medications. Emerging research has also further highlighted that delays in diagnosis and starting treatment often mean that patients do not improve to their baseline, and may have ongoing functional limitation from their memory deficits, highlighting the importance of early treatment and recognition. We report a case of a 55 year old Asian gentleman presenting to a tertiary Sydney Hospital with strikingly altered psychological behaviour, confusion, visual hallucinations and headache for 1 week. He was admitted following a nocturnal seizure, with initial investigations unremarkable despite mild hyponatraemia. He became progressively more confused with bilateral asymmetrical temporal epileptiform changes noted on EEG and bilateral medial temporal lobe hyperintensity on MRI and positive serum and CSF LGI1 antibodies, and was managed with immunotherapy and antiepileptic medications. This case further reinforced the clinical spectrum of this increasingly recognised cause of autoimmune encephalitis and the need to early diagnosis.
ZIKA VIRUS (ZIKV) CO-INFECTION IN A PATIENT WITH VARICELLA ZOSTER VIRUS (VZV) MENINGITIS

Dev Nathani, Walid Matar, Tomas Jensen

Mr OM is a 37 year old gentleman who became unwell the day after returning to Australia (Day 1) after spending a week each in Uruguay and Brazil. He had fevers, malaise, dry cough, vomiting, headache, facial sores, arthralgia and watery diarrhoea. On Day 5, a fine, erythematous non-pruritic maculopapular, blanching rash appeared on his limbs and torso, prompting presentation to an Emergency Department (ED).

He has hyperlipidaemia and had acute pancreatitis in 2015. He has no regular medications and denies a history of allergies or adverse drug reactions. He was unsure of his immunisation status. OM migrated from Uruguay around 2000 and lives with his partner and children. He works for the water company, replacing water meters. He has never smoked and drinks alcohol occasionally.

He denied sick contacts, animal exposure, new sexual partners, travel to rural areas or swimming in open water. He had no pre-travel vaccinations and did not recall tick or mosquito bites. Blood tests showed mildly elevated gamma-glutamyl transferase of 78 and mild leucopaenia of 3.2X10^9/L (Neutrophils 1.8; Lymphocytes 0.8). Acute ZIKV serology was sent. He improved with supportive care and was discharged. The rash soon resolved.

OM re-presented thrice to ED between Days 16-18 with similar symptoms; the last visit resulted in admission under Neurology. Examination revealed only dry, raised painless non-herpetiform skin lesions over the left temporal and right occipital regions. Alanine transferase was mildly elevated at 56 and C-Reactive Protein was <1. Non-contrast CT Brain, CT Venogram, MRI Brain and plain chest radiograph were all normal. Cerebrospinal fluid (CSF) Protein was high at 2.47g/L and CSF Glucose was 2.8mmol/L. Cell count (in 10^6/L) showed 6 red cells, 8 polymorphs and 378 mononuclears. Acyclovir and Ceftriaxone were commenced and later ceased when Polymerase Chain Reaction (PCR) of CSF VZV DNA returned positive. OM improved and was discharged on Day 22.

Convalescent ZIKV IgG titre (Day 22) rose from <10 to 320 and IgM changed from negative to positive indicating a recent ZIKV infection. ZIKV PCR in CSF (Day 18), blood (Day 20), urine and saliva (Day 47) was negative. OM remained well on follow up (Day 47).

ZIKV appears to be neurotropic with associations with congenital microcephaly, Guillain-Barré syndrome, myelitis, meningoencephalitis and encephalitis. OM is a probable case of ZIKV infection according to WHO definition. ZIKV PCR was negative but timing of test was well after ZIKV PCR is known to be positive. Duration of ZIKV PCR positivity in CSF is unknown. Thus OM either has PCR-negative ZIKV meningitis with concurrent VZV infection or has herpes sine zoster manifesting as VZV meningitis possibly triggered by acute ZIKV infection. The latter may have occurred via an as yet undetermined mechanism. Further study of this possible interaction could be useful.
LEUKOCYTE MICROPARTICLES FROM MULTIPLE SCLEROSIS PATIENTS PROMOTE INFLAMMATORY PHENOTYPES OF HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

Angelica Panopoulo, Anna Zinger, Simon Hawke, Georges E. Grau

Vascular Immunology Unit, Sydney Medical School, The University of Sydney, NSW, Australia.

Multiple Sclerosis (MS) is a complex inflammatory disorder leading to central nervous system (CNS) demyelination. Blood brain barrier (BBB) disruption is a key hallmark of acute MS pathology, a complex process permitting the transmigration of autoaggressive immune cells into the CNS with aspects yet to be elucidated. Microparticles (MPs) are small vesicles shed from cell membranes. Their production through cell stimulation or apoptosis is thought to influence their composition and function in both biological and pathological processes. We studied the inflammatory effects of MPs generated during peripheral blood mononuclear cell (PBMC) stimulation with cytokines, TNF and IFN-γ, on human brain microvascular endothelial cells (HBMVECs). PBMCs from healthy controls, treated and untreated MS patients were cultured and used to generate MPs. MPs were then purified, washed and cocultured with HBMVECs overnight. We discovered that MPs from cytokine-stimulated PBMCs were capable of inducing an activated phenotype in HBMVECs. Flow cytometric analysis of co-cultures showed enhanced expression of adhesion molecules, ICAM-1 and VCAM-1, on HBMVECs, similar to that seen upon TNF stimulation, in the presence of MPs from activated but not resting PBMCs. Furthermore, we used Electric Cell Substrate Impedance Sensing to determine the effects of MPs on endothelial barrier integrity. We found that cytokine-stimulated MPs from MS patients were capable of disrupting the integrity of tight junctions that connect endothelial monolayers to cause a decrease in resistance. These findings suggest that stimulated MPs from MS patients possess a stronger capacity to disrupt endothelial monolayers than stimulated MPs from healthy donors and that MPs from cytokine-stimulated immune cells are biologically different from constitutively generated MPs during resting states. In the context of MS, it is plausible that MPs from activated immune cells are responsible for initiating inflammatory lesions and events leading to transmigration of immune cells into the CNS. Overall, these findings describe an alternative model of leukocyte-endothelium cross-talk that is relevant in the early stages of neuroinflammation.
ANTI-GABA B RECEPTOR ANTIBODY MEDIATED AUTOIMMUNE ENCEPHALITIS

Kevin DJ O’Connor¹,², Srimathy Vijayan¹, Christine Bundell³,⁴, David Nolan², Mina John², Catherine Franconi¹, Wai K Leong¹

¹Department of Neurology, Royal Perth Hospital, Perth, WA 6000
²Department of Clinical Immunology, Royal Perth Hospital, Perth 6000
³Clinical Immunology PathWest Laboratory Medicine QE II Medical Centre, Nedlands, WA 6009
⁴Pathology and Laboratory Medicine University of Western Australia, Nedlands, WA 6009

Here we report the first case of anti-GABA B antibody receptor encephalitis in Western Australia, of a male who presents with limbic encephalitis (LE), with a prodrome of worsening neurological function and seizures. After identification of the antibody, strongly positive both in serum and CSF by indirect immunofluorescence using the transfected cell line Euroimmun neurology mosaic substrate, he was promptly treated with glucocorticoids, plasmapheresis and rituximab. A work up for occult malignancy was undertaken; full body CT and PET scan showed avid mediastinal lymphadenopathy. Endobronchial ultrasound was performed, and endobrochial brushings were initially negative for malignancy. Subsequently repeat testing confirmed small cell lung cancer 9 months after presentation, and he is currently receiving chemotherapy. Nine months later he continues to make a significant clinical improvement from a neurological perspective.

Conclusion: Our case of anti-GABA B receptor antibody encephalitis is consistent with what is reported in the literature. The most important association is with small cell lung cancer, with up to 50% of patients having this tumour type, and are known to have poorer outcomes in this context. Treatment with glucocorticoids, plasmapheresis and rituximab has had ongoing sustained benefit in our patient independent of treatment for the underlying small cell lung cancer.
MULTIFOCAL MOTOR NEUROPATHY: A SINGLE CENTRE EXPERIENCE

Kevin DJ O’Connor¹,², Srimathy Vijayan¹, Christine Bundell³,⁴, David Nolan², Mina John², Catherine Franconi¹, Wai K Leong¹

¹Department of Neurology, Royal Perth Hospital, Perth, WA 6000
²Department of Clinical Immunology, Royal Perth Hospital, Perth 6000
³Clinical Immunology PathWest Laboratory Medicine QE II Medical Centre, Nedlands, WA 6009
⁴Pathology and Laboratory Medicine University of Western Australia, Nedlands, WA 6009

OBJECTIVE: To characterize a single centre cohort of patients with multifocal motor neuropathy (MMN) with extended follow-up.

BACKGROUND: MMN is an immune-mediated demyelinating neuropathy with prevalence of 1-2/100,000. A consistent feature is high intravenous immunoglobulin (IVIG) requirement, and latter development of treatment refractoriness. Other immunomodulatory therapies have not shown consistent benefit, but reports of off-label rituximab use have suggested promise. Our experience is stabilization of disease progression may occur with concurrent rituximab therapy.

DESIGN/METHODS: A single-center cohort study was conducted involving the state quaternary neuropathy service in Western Australia, a population of just over 2.5 million. Patients with MMN, all of whom received IVIG therapy during follow up, were enrolled. All patients had clinical assessment by one neurologist.

RESULTS: 19 patients (12 males, 8 females) were identified. Age at symptom onset ranged from 17 to 60 years. The duration of follow up ranged from 23 to 155 months (mean 94 months). Every individual had upper limb involvement at diagnosis with 3/19 developing lower limb involvement. Sensory symptoms were reported in 8/19. Anti-IgM GM1 antibodies were positive in 1/19 (5%) cases (tested interstate), however 18/19 cases were negative when tested using the Euroimmun anti-ganglisoide antibody immunoblot commercial assay through PathWest. Repeat testing on 14 of the cases that had available serum, using the Generic anti-ganglisoide antibody immunoblot commercial assay, revealed 6/14 (43%) positive for anti-IgM GM1 antibodies, and 2 patients had concurrent positives with anti-IgM GD1b and anti-IgM GM4, all of which previously tested negative. IVIG greater than 1g/kg/month was required to maintain clinical stability in most treated patients. Rituximab was used in 4 cases with disease progression despite IVIG 2g/kg/month, with improved disease control, but no reduction in IVIG requirements. A single patient with clinical response to concurrent quinidine therapy was observed.
DEVELOPING GUIDELINES FOR OUTCOME MEASURES IN PRECLINICAL MYASTHENIA STUDIES

Bill Phillips

Potential therapeutics that appeared promising in animal studies of neuromuscular diseases have too often proved expensive failures in subsequent clinical trials. This points to the need for more consistency and better documentation of preclinical animal studies (1, 2). In September 2014, two-dozen researchers met in Bethesda MA, USA to thrash out guidelines for animal models of myasthenia gravis. The discussion and resultant guidelines covered active immunization and passive transfer models of anti-acetylcholine receptor and anti-MuSK myasthenia gravis, the influence of animal holding conditions, the need for blind assessment and statistics reporting among other things (3). This was really just a beginning. One important unsettled issue concerns outcome measures for assessing disease severity in myasthenic mice and rats. Many different grading schemes have been published in different studies by different groups. Even widely reported weakness-grades differ, from lab to lab, in how they are interpreted and how animals are scored. Improved assessment and reporting of preclinical studies will require ongoing discussion within the myasthenia research community before broad consensus emerges. Consistent grading and reporting is needed if the work of preclinical researchers is to be translated into better treatment options for patients. Meetings like ours can help by discussing what we think might be the most robust and relevant primary outcome measure/s for rodent studies of myasthenia.

INVESTIGATING THE AUTOIMMUNE T-CELL RESPONSE TO THE DOPAMINE-2 RECEPTOR IN MOVEMENT AND PSYCHIATRIC DISORDERS

Deepti Pilli, Nese Sinmaz, Sudarshini Ramanathan, Fiona Tea, Vera Merheb, Tina Nyguen, Alicia Zou, Russell Dale, Fabienne Brilot

Brain Autoimmunity Group, Institute for Neuroscience and Muscle Research, Kids Research Institute, The Children’s Hospital at Westmead
Discipline of Child and Adolescent Health, Sydney Medical School, University of Sydney, Sydney, Australia

Background: An autoimmune aetiology in movement and psychiatric disorders has been proposed upon discovery of autoantibodies targeting various neuronal receptors and synaptic proteins. One of these targets is the dopamine-2 receptor (D2R). Anti-D2R antibodies are IgG, a subclass which is dependent on the involvement of active CD4+ T cells for its generation. Alternatively, in patients who are anti-D2R antibody-seronegative, the immune response against D2R may predominantly be mediated by active CD8+ T cells. However, the role of these T cells is yet to be elucidated.

Objective: To identify and characterise the T cell response to D2R in paediatric patients with movement and psychiatric disorder

Methods: Using whole blood from patients and controls, activation of CD4+ and CD8+ T cells by overlapping 15-meric human D2R peptides was determined via flow cytometry. The release of IFN-, an inflammatory cytokine, upon stimulation with human D2R peptide library was evaluated by ELISpot.

Results: Compared to controls, CD4+ T cells from patients demonstrated greater activation towards multiple peptides spanning the D2R protein. In particular, most patients responded to aa51-75, aa156-195, aa206-265, aa356-380, and aa381-443 whereas aa81-115, aa121-155, and aa256-315 elicited activation in some patients. In comparison, CD8+ T cell activation was observed less and stimulated namely by aa156-195, aa256-315, and aa356-380. Preliminary data suggests that IFN- secretion was generated by aa206-265, aa316-365, and aa356-380.

Conclusion: Movement and psychiatric disorder patients exhibit an enhanced T cell activation by D2R, which is more evident in the CD4+ subset than in the CD8+, and appear to have an increased IFN- secretion. Investigating the D2R-specific T cell response will provide a holistic understanding of the immune function in movement and psychiatric disorders.
IN VIVO EFFECTS OF IgG1 AND IgG4 AUTOANTIBODIES SPECIFIC FOR MYELIN PROTEOLIPID PROTEIN ON REMYELINATION

Hannah Savage, Shannon Beasley, Aakanksha Dixit, Judith Greer

University of Queensland, University of Queensland Centre for Clinical Research, Brisbane, QLD

Multiple Sclerosis (MS) is a demyelinating disease of the central nervous system that affects approximately 2.5 million people worldwide with females affected twice as often as males. In patients with relapsing remitting MS, remyelination occurs at sites of demyelination, repairing the damage to a certain extent. Eventually, this remyelination process fails and as such patients are then diagnosed with secondary progressive MS. The cause of the failure in the remyelination process is unknown and of great interest to us. Antibodies are known to play an important role in many autoimmune diseases and it is hypothesised that autoantibodies direct against components of myelin may play a key role in MS: they may be pathogenic or may be potential biomarkers of different stages of MS. Studies from our lab and others have previously shown that MS patients have elevated levels of auto-antibodies in their serum. Specifically it was determined that some of these antibodies are against the most abundant protein in myelin, myelin proteolipid protein (PLP180-230), however the isotype of the anti-PLP antibodies differs from patient to patient. The current study aims to assess whether anti-PLP180-230 antibodies of IgG1 and IgG4 isotypes, purified from MS patients, can inhibit or slow remyelination within the central nervous system of mice with cuprizone-induced demyelination. Multiple techniques are being used to assess the effects, including diffusor tensor imaging, histology and immunohistochemistry. It is anticipated that the different isotypes may have different effects in vivo.
ANALYSIS OF THE BINDING SPECIFICITY OF ANTIBODY TO DOPAMINE-2 RECEPTOR IN PAEDIATRIC AUTOIMMUNE MOVEMENT AND PSYCHIATRIC DISORDERS

Nese Sinmaz, Deepti Pilli, Alicia Zou, Vera Merheb, Russell Dale, Fabienne Brilot

Brain Autoimmunity Group, Institute for Neuroscience and Muscle Research, The Kids Research Institute, Children’s Hospital at Westmead, Discipline of Child and Adolescent Health, University of Sydney, Sydney, Australia.

Objective: To investigate the target epitope of autoantibodies to dopamine-2 receptor (D2R) in paediatric autoimmune movement and psychiatric disorders. Defining D2R antigenic region is important to elucidate functional effects of autoantibodies, and offer novel treatment opportunities.

Method: We performed mutational analysis of the extracellular domains of human D2R by serial and single point mutations. Expression of mutant D2R at the cell surface on human embryonic kidney cells was evaluated by confocal microscopy and flow cytometry, and the effects on binding of anti-D2R antibodies was assessed by flow cytometry live cell-based assay.

Results: 35 anti-D2R antibody-positive patient sera did not recognize NTermD1RcD2R chimeric receptor, a construct consisting of extracellular N-terminal domain of dopamine-1 receptor (D1R) on the backbone of WT D2R. These results suggest that antibodies do not recognise the extracellular loops of D2R but they recognise D2R extracellular N-terminus. To define anti-D2R antigenic region with N-terminus, lucy D2-22 D2R was constructed, the addition of the lucy tag was necessary to aid in cell surface expression. We found that 25/35 (71%) patients recognised an epitope within amino acids 23-37. Next, we assessed the influence of N-linked glycosylation within this region via N23Q mutant. From these 25 patients positive to aa23-37, only 22/25 (88%) recognised N23Q mutant, suggesting that N-glycosylation in this region does not influence antibody binding. Furthermore, we explored the influence of mouse D2R (D26E/A29P) on human anti-D2R autoantibody binding. Interestingly, we found that 10/25 (40%) of patients do not recognise glycosylated mouse D2R. To assess the influence of mouse N-glycosylation in human anti-D2R antibody binding we created the N23Q/D26E/A29P. We found that the lack of glycosylation at position 23 reinstated human anti-D2R antibody binding (24/25; 96%). To abolish epitope binding to human D2R we subcloned lucy R20K/P21G/F22W/N23Q/D26E/A29P mutant, where 11/25 (44%) did not recognise this construct.

Conclusion: Extracellular amino acid substitution at positions 23-29 of human D2R N-terminus abolish binding of human anti-D2R antibodies. Human anti-D2R antibody binding to mouse D2R is dependent on glycosylation. These results indicate the global importance of N-terminus conformation in anti-D2R antibody binding.
**SERINE 727 PHOSPHORYLATION OF STAT1 PROTECTS FROM SEVERE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS**

Pattama Songkhunawej and Markus Hofer

School of Life and Environmental Sciences, The University of Sydney, Australia

Interferon-beta (IFN-β) is a commonly used treatment for multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS). However, its beneficial use is often limited by neurological side effects and insufficient immune responses, resulting in exacerbations and disease progression. The signal transducers and activators of transcription 1 (STAT1) is a key transcription factor that mediates IFN-β signalling. STAT1 activity is regulated by phosphorylation of tyrosine 701 (pY-STAT1) which is essential for STAT1 activation, and serine 727 (pS-STAT1) whose function is poorly understood. While pS-STAT1 has been shown to regulate STAT1 transcriptional activity in vitro, the biological relevance of pS-STAT1 in vivo remains unclear. Reduced pS-STAT1 levels have recently been linked to MS pathogenesis. The present study investigated the role of pS-STAT1 in the CNS in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. Transgenic mice that express serine727-alanine mutant STAT1 (STAT1 KI mice), on average, developed more severe disease compared to wild type mice. Moreover, the clinical disease was comparable in STAT1 KI and STAT1 knock-out mice, indicating that the severe EAE previously reported in STAT1-deficient mice may be a consequence of deficient pS-STAT1. While histopathological changes were comparable between all genotypes, differences were observed in the expression of various proinflammatory cytokines that have been implicated in EAE pathogenesis. This supports a regulatory role of pS-STAT1 in the IFN response in the CNS. Furthermore, the protective effects of pS-STAT1 during neuroinflammation suggest that it may be a promising target in understanding MS pathogenesis.
TARGETING KYNURENINE PATHWAY FOR THE TREATMENT OF MULTIPLE SCLEROSIS

Gayathri Sundaram¹,², Gilles J Guillemin¹,⁴ and Bruce J Brew¹,²,³

¹Peter Duncan Neurosciences Research Unit, St Vincent’s Centre for Applied Medical Research, oralAustralia
²St Vincent’s Clinical School, University of New South Wales, Australia
³Neurology, St Vincent’s Hospital, Australia
⁴Neuroinflammation group, Faculty of Medicine and Health Sciences, Macquarie University, Australia

Background: Kynurenine pathway (KP) is a tryptophan catabolic pathway, implicated in multiple sclerosis (MS) pathogenesis. Current therapies for MS only appear to slow down the progression of the disease but it is unclear whether they are directly affecting disease pathogenesis. Hence, we hypothesize that the dissecting and modulating this pathway may provide significant understanding and amelioration of MS pathogenesis.

Materials and Methods: Experimental autoimmune encephalomyelitis (EAE) mice were either treated with modulators acting at different stages of KP such as 1-methyl tryptophan (1-MT; upstream inhibitor) and Ro 61-8048 (mid-stream inhibitor) for 7 days or untreated. Clinical severity was measured based on the approved clinical scale (CS; 0-5). KP metabolites (tryptophan (TRP), kynurenine (KYN), kynurenate (KYNA), picolinate (PIC) and quinolinate (QUIN)) in plasma and CNS tissues of mice with EAE (treated and untreated) and wildtype were measured by HPLC and GCMS. KP gene expressions were measured by qRT-PCR. Nicotinamide adenine dinucleotide (NAD+) was measured using commercially available kit. Statistical analysis was performed using ANOVA with Bonferroni post hoc test.

Results: In EAE untreated mice, KP activation was steadily up-regulated and directly proportion to its disease severity (n=7; p<0.01) compared to wildtype. Additionally neuroprotective compounds KYNA and PIC were significantly down regulated (p<0.05), whereas the neurotoxic compound QUIN was significantly up regulated (p<0.05) in both the periphery and CNS tissues of EAE mice (untreated) compared to wildtype. However synthesis of NAD+ from QUIN was significantly downregulated (p<0.01) in untreated EAE induced mice indicating chronic accumulation of QUIN during disease pathogenesis. EAE induced mice treated with KP modulators significantly reduced the clinical severity (1-MT (CS 2.5 to 1.5; p<0.05) and Ro 61-8048 (CS 2.5 to 0.25; p<0.05) compared to untreated mice. The decreased severity in Ro 61-8048 group was associated with increased KYNA and PIC (p<0.05) and decreased QUIN (p<0.05) compared to untreated controls. Decreased QUIN was associated with decreased QPRT enzyme synthesis (p<0.05) in Ro 61-8048 group compared to untreated control.

Discussion: We have shown that the KP is activated in EAE mice and treating with modulators had significant clinical benefit by altering the production of neuroactive compounds, thereby providing important insights into new therapeutic strategies for limiting MS.
SURVIVAL OF LCMV-INFECTED IRF9-DEFICIENT MICE REQUIRES PD1/PD-L1 MEDIATED FUNCTIONAL EXHAUSTION

Tamara Suprunenko, Martina Fink and Markus Hofer

School of Life and Environmental Sciences, The Charles Perkins Centre and the Bosch Institute, Maze Crescent G08, The University of Sydney, NSW, Australia

The interferon regulatory factor 9 (IRF9) is a key component of type I interferon (IFN-I) signalling and critical for the host anti-viral response. In contrast to wild-type mice that develop lethal lymphocytic choriomeningitis following intracranial infection with lymphocytic choriomeningitis virus (LCMV), IRF9-deficient mice develop a transient disease. However, absence of IRF9 results in viral persistence and functional exhaustion of LCMV-specific CD8+ T-cells. This state of T-cell dysfunction is characterised by poor effector function and sustained expression of inhibitory receptors, in particular programmed death-1 (PD-1), and its ligand PD-L1. Here we investigated the contribution of PD-1/PD-L1-dependent functional exhaustion on the survival of LCMV-infected IRF9-deficient mice. For this, we crossed IRF9-deficient mice with mice lacking PD-L1. Interestingly, the absence of PD-L1 did not revert the phenotype of IRF9-deficient mice infected with LCMV. Instead, IRF9 x PD-L1 double-deficient mice developed a lethal wasting disease, suggesting that PD-L1 dependent functional exhaustion is crucial for the survival of LCMV-infected IRF9-deficient mice.

We next investigated if PD-L1-dependent functional exhaustion is due to the absence of IFR9 specifically, or the result of disturbed IFN-I signaling by comparing IRF9-deficient mice with mice lacking the IFN-I receptor (IFNAR). Similar to IRF9-deficient mice, IFNAR-deficient mice survived intracranial infection with LCMV. However, the clinical course in IFNAR-deficient mice was significantly milder. Despite this, viral RNA levels remained similar compared to IRF9 KO mice and there were only minor differences in expression of key pro-inflammatory cytokines in the peripheral organs.
PHENOTYPIC CONSEQUENCES OF THE CONDITIONAL DELETION OF STRAWBERRY NOTCH HOMOLOG 2 FROM ASTROCYTES IN THE BRAIN OF MICE

Taylor E. Syme¹, Magdalena Grill¹,², and Iain L. Campbell¹

¹School of Life and Environmental Sciences and the Bosch Institute, University of Sydney, NSW, Australia
²Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Austria

Introduction: Strawberry notch homolog 2 (Sbno2) is a putative transcription factor the expression of which we have identified previously to be both constitutive and strongly upregulated by various cytokines (including IL-6) and by lipopolysaccharide (LPS) in astrocytes in the central nervous system (CNS). Although these observations suggest Sbno2 may have roles in astrocyte homeostasis and in the regulation of the inflammatory response little is known concerning its function in the healthy or inflamed CNS. Therefore, the aim of this study was to further delineate the function of Sbno2 by generating a mouse model for the cell-specific deletion of Sbno2 in astrocytes.

Methods: Mice (Sbno2fl/fl) were generated in which the introns flanking exons 8-10 of the murine Sbno2 gene were floxed. The Sbno2fl/fl mice were then crossed with GFAP-Cre mice to conditionally disrupt the Sbno2 gene in astrocytes (Sbno2-AstKO mice). Primary cultures of astrocytes were obtained from Sbno2-AstKO and control mice and the physical and molecular phenotypes of these cells were compared. Mice were given dual, staggered intra-peritoneal injections of purified E. coli LPS and various markers for neuroinflammation were analysed.

Results: Sbno2-AstKO offspring developed normally and displayed no outward phenotype up to at least 12 months of age. In cultured astrocytes from Sbno2-AstKO mice neither un-recombined Sbno2 mRNA nor SBNO2 protein were detectable, confirming the Cre-mediated disruption of the Sbno2 gene. The morphology of astrocytes from Sbno2-AstKO mice was indistinguishable from control mice despite having increased levels of STAT3 protein and the astrocyte markers glial fibrillary acidic protein (GFAP) and aldehyde dehydrogenase 1 family, member L1 (ALDH1L1). The level of GFAP mRNA was higher in the cerebrum of adult Sbno2-AstKO mice compared with age-matched control littermates. Following LPS injection, the expression of various inflammatory and glial markers was found to be similarly affected between Sbno2-AstKO and control mice.

Conclusions: A new model allowing the conditional deletion of the Sbno2 gene in specific cell types was successfully generated. Our initial findings reveal that loss of Sbno2 in astrocytes, (1) does not compromise the development or viability of these cells and is associated with only subtle phenotypic changes, and (2) does not overtly alter the neuroinflammatory response following systemic LPS challenge. It is expected that further insight to the function of Sbno2 in astrocytes will be gained from ongoing studies profiling the transcriptome of Sbno2-AstKO cells.
MOG AUTOANTIBODY EPITOPE RECOGNITION: SHEDDING LIGHT ON EPITOPE STABILITY AND PROTEIN CONFORMATION

Fiona Tea, Tina K Nguyen, Vera Merheb, Sudarshini Ramanathan, Nese Sinmaz, Deepti Pilli, Alicia Zou, Russell Dale, Fabienne Brilot

Brain Autoimmunity Group, Institute for Neuroscience and Muscle Research, Kids Research Institute, oralThe Children’s Hospital at Westmead, Discipline of Child and Adolescent Health, Sydney Medical School, University of Sydney, Sydney, Australia

Background: Due to autoimmune attack against the myelin sheath in central nervous system (CNS) demyelinating diseases, brain myelin proteins have been extensively studied as autoantigenic targets. Autoantibodies targeting myelin oligodendrocyte glycoprotein (MOG autoantibodies) have been recently associated with subsets of human CNS demyelinating diseases, such as bilateral optic neuritis. By site-directed mutagenesis, we aim to determine whether the epitope recognised by MOG autoantibodies from adult CNS demyelinating patients would be similar to those described in children.

Methods: Human MOG mutant P42S was subcloned and stably expressed by lentiviral transduction in HEK293 cells. After confirming cell surface mutant MOG expression by flow cytometry and confocal microscopy, we assessed sera MOG autoantibody binding using a flow cytometry live cell-based assay. Furthermore, to investigate the implication of antibody binding to an altered MOG protein conformation, we also analysed MOG autoantibody binding to paraformaldehyde-fixed MOG-expressing HEK293 cells.

Results: In comparison to antibody binding to MOG wild-type, 80% (20/25) of MOG autoantibody-positive adult patients had reduced binding to the P42S MOG mutant. Longitudinal analysis indicated stable epitope recognition overtime. Only 4 out of 28 (14%) adult MOG autoantibody-positive patients bound to paraformaldehyde-fixed MOG-expressing HEK293 cells.

Conclusion: Taken together, the proline at position 42 of human MOG is important in adult MOG autoantibody recognition, and this binding remains stable overtime. Importantly, correct protein conformation is crucial for proper autoantibody detection. Greater characterisation of MOG antibodies will drive the development of novel antibody therapies to improve patient management and treatment.
LGI1-RECEPTOR ANTIBODY LIMBIC ENCEPHALITIS: A CASE SERIES REVIEW OF FBDS, COGNITIVE IMPAIRMENT, AND THE INFLUENCE OF TREATMENT ON LONG TERM OUTCOME

Thompson Julia, Murchison Andrew, Irani Sarosh

Leucine-rich glioma-inactivated 1 (LGI1)-antibody encephalitis is a treatable disease within the ever-expanding group of autoimmune encephalitides. The illness is typically characterized by the subacute onset of amnesia, confusion, and seizures in middle age, with approximately 60% of patients showing medial temporal lobe T2 hyperintensities. It has been previously identified to affect men more than women. The most characteristic seizure syndrome associated with the LGI1 antibody is the recently termed entity of faciobrachial dystonic seizures (FBDS). Previous smaller retrospective and prospective observations have shown that immunotherapies produce a more marked reduction in FBDS than antiepileptic drugs, with AEDs leading to a number of adverse events. Furthermore, there appears to be an emerging temporal trend, with onset of FBDS often being followed by the development of cognitive impairment (CI) in about 60% of cases.

We present data from our multi-centre multi-national case review of over 100 patients with LGI1 encephalitis and FBDS ahead of publication. This large case series reviews the clinical course and associated features of the condition, investigations including imaging and EEG findings, in addition to treatment responses and outcomes, and is the largest case series reviewed to date. Particular attention was paid to predictors of cognitive impairment and timing and effects of treatments to gain further clinico-pathological insight into this illness.

From this case series we have gleaned further understanding of this condition, both clinically and pathologically. Importantly this study also highlights the inks between FBDS and cognitive impairment, and further demonstrated that prevention of the cognitive impairment is crucial, with earlier diagnosis, FBDS cessation, and more rapid initiation of immunotherapy leading to better long term clinical outcomes.
THE MICROGLIA PHENOTYPE IS ALTERED IN AN IL-6-DRIVEN CYTOKINOPATHY OF THE CENTRAL NERVOUS SYSTEM

Phillip K. West¹, Oleg Butovsky² & Iain L. Campbell¹

¹School of Life and Environmental Sciences & Bosch Institute, The University of Sydney, NSW, Australia
²Center for Neurologic Diseases, Department of Neurology, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, USA

Introduction: The cytokine interleukin-6 (IL-6) is known to be a causal factor in neuroinflammatory disease in humans. However, an understanding of the mechanisms via which IL-6 mediates central nervous system (CNS) inflammation and injury is unclear. A transgenic mouse (termed GFAP-IL6 mice) model with CNS-restricted production of IL-6 replicates many of the key neuropathological and behavioural changes found in human neuroinflammatory disease. Microglia, the tissue-resident macrophages of the CNS, may be a major target of IL-6 and effector cells in IL-6-mediated neuroinflammation. Therefore the objective of this study was to gain an understanding of the nature of the microglial response to IL-6 in the CNS.

Methods: WT and GFAP-IL6 mice with EGFP-labelled macrophages (MacGreen – MG) were generated. Combinations of in situ (immunohistochemistry and confocal microscopy) and ex vivo (flow cytometry) approaches were employed to determine the morphology, distribution, number and molecular signature of microglia in the brain of age-matched MG/WT compared with MG/GFAP-IL6 mice.

Results: Compared with MG/WT, MG/GFAP-IL6 mice displayed significantly increased numbers of EGFP+ cells throughout the brain, which were negative for the peripheral monocyte-specific marker 4C12. This increase in microglia number was due to significantly increased proliferation, particularly in younger GFAP-IL6 mice, as shown by BrdU incorporation. Three-dimensional reconstructions and morphometric analysis revealed MG/WT microglia exhibited a typical highly ramified morphology with long, fine primary and secondary processes. In contrast, MG/GFAP-IL6 microglia exhibited a dramatically altered morphology, with stunted, thickened primary processes and reduced secondary branching. Finally, compared with MG/WT, microglia in MG/GFAP-IL6 mice had an altered molecular signature with significantly decreased P2RY12 levels, while FCRLS levels were increased and 4D4 levels were unchanged.

Conclusions: These studies indicate that microglia are a major responder cell to IL-6 in the CNS that undergo dramatic morphological transformation and increased proliferation. In addition, exposure to IL-6 in the CNS can mediate a marked alteration in the unique molecular signature that defines these tissue-resident myeloid cells.
PATIENTS WITH NFKBIA MUTATIONS ARE LESS RESPONSIVE TO TREATMENT WITH MIS416 FOR PROGRESSIVE MULTIPLE SCLEROSIS

Jun Yan¹, Gill Webster², Judith Greer¹

¹UQCCR, the University of Queensland, Brisbane, Australia
²Innate Immunotherapeutics, Auckland, NZ

MIS416 is a new treatment for people with progressive multiple sclerosis (MS), and is now in Phase 2B trials. The proposed mechanism of action of MIS416 is via modulation of a major NF-kB related pro-inflammatory pathway. Importantly, however, we have recently found that ~15-20% of patients with progressive MS carry novel mutations in the gene NFKBIA, which encodes a key regulator of this pathway, IκB-α. In comparison, <1% of healthy individuals carry the same mutations. In vitro analyses suggest that the NFKBIA mutations, when carried as homozygous or compound heterozygous mutations, significantly alter the activation of NF-kB. We therefore hypothesized that a proportion of MS patients in the MIS416 trial would carry homozygous or compound heterozygous NFKBIA mutations, and that they would be less likely to respond in a positive manner to MIS416, as NF-kB activity would be regulated differently in those patients.

To investigate this, we have sequenced genomic DNA extracted from patients enrolled in the initial Phase 1B/2A trial of MIS416 (n=12) to identify mutations in the promoter region of NFKBIA, and also examined some of the patients for the effect of these mutations on immune responses, and correlated the mutations with clinical outcomes in the treatment of MIS416. Three of the 12 patients carried NFKBIA mutations. Notably, these 3 patients were the lowest responders to MIS416, in terms of immune parameters and clinical outcome measures (EDSS, SF-36 PCS, SF-36 MCS, 25ft Walk, 9-hole peg test, PASAT and FSS).

We are currently sequencing NFKBIA in patients enrolled in the phase 2B MIS416 trial. Of 59 patients sequenced so far (out of 90 patients enrolled in the Phase 2B trial), 46% of patients have been found to carry a variety of mutations/polymorphisms. In a preliminary analysis based on immune markers, it appears that patients with NFKBIA mutations are over-represented in the group of non-responders to MIS416.

This study suggests that the presence of NFKBIA mutations may influence the response of patients to treatment with MIS416, and knowledge of these mutations may be important in enabling appropriate selection of patients who are most likely to benefit from MIS416 in the future.
TRANSENDOTHELIAL MIGRATION EFFICIENCY OF LEUCOCYTES ACROSS IN VITRO BLOOD BRAIN BARRIER DECREASED BY FINGOLIMOD TREATMENT

Anna Zinger¹, Simon Hawke¹,²,³ and Georges E. Grau¹

¹Vascular Immunology Unit, Dept of Pathology, Sydney Medical School, The University of Sydney, Medical Foundation Bldg, 92 Parramatta Rd, Camperdown NSW 2050 Australia
²Brain and Mind Centre, The University of Sydney, 94 Mallett St, Camperdown NSW 2050 Australia,
³Central West Neurology and Neurosurgery, 93 Byng St, Orange NSW 2800 Australia.

Background: While multiple sclerosis (MS) is a disease of the brain, there is clear evidence of immune activation outside the central nervous system (CNS). For many years it has been considered an autoimmune disorder where auto-aggressive T cells target the CNS, causing inflammation. Trans-endothelial migration (TEM) of leucocytes across BBB is one of the earliest CNS changes in MS leading to brain parenchymal lesions. Most acute lesions in MS are angiocentric and it is known that perivenular lymphocytic infiltration occurs in the absence of parenchymal abnormalities in the normal appearing white matter. Even when myelin and oligodendrocytes are not present, like in the retina, blood vessel abnormalities have been found. Subsequent MRI studies showed that the earliest MRI abnormality in lesion formation is the breakdown of BBB, altogether leading to the view that MS might primarily be a disorder of blood vessels.

Goal: We aim to examine the effect of the disease state and treatment on the TEM of leucocytes across the BBB.

Methods and results: We collected peripheral blood mononuclear cells (PBMCs) from treated MS patients and migrated them through an in vitro BBB model (Neuroprobe, USA). Leucocyte numbers, subsets and phenotypes were assessed by flow cytometry. We demonstrated that CD4+ and CD8+ T cells migrated more efficiently across BBB than T cells isolated from healthy subjects. Furthermore, fingolimod treatment significantly reduced migration and adherence of the cells to the membrane, consistent with known effects of fingolimod on rolling, adhesion or transmigration. We also found that there is a higher proportion of Breg cells in migrating B cells population from healthy and fingolimod treated subjects than in non-treated MS patients.

Conclusions: While the disease-modifying effects of fingolimod could be explained by its profound effect on reducing the numbers of circulating potentially auto-aggressive lymphocyte populations, we postulate that fingolimod may also have a direct immunomodulatory effect on the residual circulating lymphocyte populations or even on brain endothelial function, thereby inhibiting TEM.
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## PARTICIPANTS

<table>
<thead>
<tr>
<th>Name</th>
<th>Email address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joanne Ban</td>
<td><a href="mailto:joanne1021b@yahoo.com.my">joanne1021b@yahoo.com.my</a></td>
</tr>
<tr>
<td>Shannon Beasley</td>
<td><a href="mailto:shannon.beasley@uq.edu.au">shannon.beasley@uq.edu.au</a></td>
</tr>
<tr>
<td>Stefan Blum</td>
<td><a href="mailto:neurotwink@gmail.com">neurotwink@gmail.com</a></td>
</tr>
<tr>
<td>Bruce Brew</td>
<td><a href="mailto:B.Brew@UNSW.edu.au">B.Brew@UNSW.edu.au</a></td>
</tr>
<tr>
<td>David Brown</td>
<td><a href="mailto:D.Brown@amr.org.au">D.Brown@amr.org.au</a></td>
</tr>
<tr>
<td>Anne Bruestle</td>
<td><a href="mailto:Anne.Bruestle@anu.edu.au">Anne.Bruestle@anu.edu.au</a></td>
</tr>
<tr>
<td>Sophie Bouffler</td>
<td><a href="mailto:u5185436@anu.edu.au">u5185436@anu.edu.au</a></td>
</tr>
<tr>
<td>Christine Bundell</td>
<td><a href="mailto:Chris.Bundell@health.wa.gov.au">Chris.Bundell@health.wa.gov.au</a></td>
</tr>
<tr>
<td>Katherine Buzzard</td>
<td><a href="mailto:kabuzzard@gmail.com">kabuzzard@gmail.com</a></td>
</tr>
<tr>
<td>Iain Campbell</td>
<td><a href="mailto:iain.campbell@sydney.edu.au">iain.campbell@sydney.edu.au</a></td>
</tr>
<tr>
<td>Ian Comerford</td>
<td><a href="mailto:iain.comerford@adelaide.edu.au">iain.comerford@adelaide.edu.au</a></td>
</tr>
<tr>
<td>Alastair Corbett</td>
<td><a href="mailto:alastair.corbett@sydney.edu.au">alastair.corbett@sydney.edu.au</a></td>
</tr>
<tr>
<td>Lucette Cysique</td>
<td><a href="mailto:lcysique@unsw.edu.au">lcysique@unsw.edu.au</a></td>
</tr>
<tr>
<td>Aakanksha Dixit</td>
<td><a href="mailto:uqadixit@uq.edu.au">uqadixit@uq.edu.au</a></td>
</tr>
<tr>
<td>Stephen Duma</td>
<td><a href="mailto:sduma@cym.org">sduma@cym.org</a></td>
</tr>
<tr>
<td>Nilisha Fernando</td>
<td><a href="mailto:nilisha.fernando@anu.edu.au">nilisha.fernando@anu.edu.au</a></td>
</tr>
<tr>
<td>Masoud Hassanpour</td>
<td><a href="mailto:m.hassanpour@amr.org.au">m.hassanpour@amr.org.au</a></td>
</tr>
<tr>
<td>Sumana Gopinath</td>
<td><a href="mailto:sumanagopinath@gmail.com">sumanagopinath@gmail.com</a></td>
</tr>
<tr>
<td>Judith Greer</td>
<td><a href="mailto:j.greer@uq.edu.au">j.greer@uq.edu.au</a></td>
</tr>
<tr>
<td>Markus Hofer</td>
<td><a href="mailto:Markushofer@sydney.edu.au">Markushofer@sydney.edu.au</a></td>
</tr>
<tr>
<td>Stacey Jankelowitz</td>
<td><a href="mailto:stacey.jankelowitz@sydney.edu.au">stacey.jankelowitz@sydney.edu.au</a></td>
</tr>
<tr>
<td>Hayley Inglis</td>
<td><a href="mailto:h.inglis@uq.edu.au">h.inglis@uq.edu.au</a></td>
</tr>
<tr>
<td>Michael Jackson</td>
<td><a href="mailto:michael.jackson@flinders.edu.au">michael.jackson@flinders.edu.au</a></td>
</tr>
<tr>
<td>Kavitha Kothur</td>
<td><a href="mailto:kavitha.kothur@health.nsw.gov.au">kavitha.kothur@health.nsw.gov.au</a></td>
</tr>
<tr>
<td>Amy Kunchok</td>
<td><a href="mailto:amykunchok@gmail.com">amykunchok@gmail.com</a></td>
</tr>
<tr>
<td>Nyoman Kurniawan</td>
<td><a href="mailto:n.kurniawan@uq.edu.au">n.kurniawan@uq.edu.au</a></td>
</tr>
<tr>
<td>Michael Lovelace</td>
<td><a href="mailto:m.lovelace@amr.org.au">m.lovelace@amr.org.au</a></td>
</tr>
<tr>
<td>Abul Mamun</td>
<td><a href="mailto:mamunk51@gmail.com">mamunk51@gmail.com</a></td>
</tr>
<tr>
<td>Jennifer Massey</td>
<td><a href="mailto:jennifer.massey@hotmail.com">jennifer.massey@hotmail.com</a></td>
</tr>
<tr>
<td>Emily Mathey</td>
<td><a href="mailto:emily.mathey@sydney.edu.au">emily.mathey@sydney.edu.au</a></td>
</tr>
<tr>
<td>Pam McCombe</td>
<td><a href="mailto:pamela.mccombe@uq.edu.au">pamela.mccombe@uq.edu.au</a></td>
</tr>
<tr>
<td>Mastura Monif</td>
<td><a href="mailto:masturamonif@gmail.com">masturamonif@gmail.com</a></td>
</tr>
<tr>
<td>Dev Nathani</td>
<td><a href="mailto:devnathani@gmail.com">devnathani@gmail.com</a></td>
</tr>
<tr>
<td>Manisha Narasimhan</td>
<td><a href="mailto:manisha.narasimhan@health.nsw.gov.au">manisha.narasimhan@health.nsw.gov.au</a></td>
</tr>
<tr>
<td>Benjamin Nham</td>
<td><a href="mailto:benjaminsb.nham@gmail.com">benjaminsb.nham@gmail.com</a></td>
</tr>
<tr>
<td>Tina Nguyen</td>
<td><a href="mailto:tina-nguyen@live.com">tina-nguyen@live.com</a></td>
</tr>
<tr>
<td>Kevin O'Connor</td>
<td><a href="mailto:kevindoconnor@gmail.com">kevindoconnor@gmail.com</a></td>
</tr>
<tr>
<td>Trevor Owens</td>
<td><a href="mailto:towens@health.sdu.dk">towens@health.sdu.dk</a></td>
</tr>
<tr>
<td>Angelica Panopoulos</td>
<td><a href="mailto:apan3955@uni.sydney.edu.au">apan3955@uni.sydney.edu.au</a></td>
</tr>
<tr>
<td>Bill Phillips</td>
<td><a href="mailto:william.phillips@sydney.edu.au">william.phillips@sydney.edu.au</a></td>
</tr>
<tr>
<td>Sekhar Pillai</td>
<td><a href="mailto:norwestpaedneurology@gmail.com">norwestpaedneurology@gmail.com</a></td>
</tr>
<tr>
<td>Name</td>
<td>Email</td>
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<td>--------------------------------------------</td>
</tr>
<tr>
<td>Deepti Pilli</td>
<td><a href="mailto:dpil5654@uni.sydney.edu.au">dpil5654@uni.sydney.edu.au</a></td>
</tr>
<tr>
<td>John Prineas</td>
<td><a href="mailto:john.prineas@sydney.edu.au">john.prineas@sydney.edu.au</a></td>
</tr>
<tr>
<td>Sudarshini Ramanathan</td>
<td><a href="mailto:darshi_r@hotmail.com">darshi_r@hotmail.com</a></td>
</tr>
<tr>
<td>Stephen Reddel</td>
<td><a href="mailto:swreddel@sydneyneurology.com.au">swreddel@sydneyneurology.com.au</a></td>
</tr>
<tr>
<td>Alasdair Robertson</td>
<td><a href="mailto:alasdair.robertson@doctors.org.uk">alasdair.robertson@doctors.org.uk</a></td>
</tr>
<tr>
<td>Marc Ruitenber</td>
<td><a href="mailto:m.ruitenber@uq.edu.au">m.ruitenber@uq.edu.au</a></td>
</tr>
<tr>
<td>Hannah Savage</td>
<td><a href="mailto:hannah.savage@uq.net.au">hannah.savage@uq.net.au</a></td>
</tr>
<tr>
<td>Manvendra Saxena</td>
<td><a href="mailto:m.saxena@amr.org.au">m.saxena@amr.org.au</a></td>
</tr>
<tr>
<td>Elisabeth Shiner</td>
<td><a href="mailto:elizabeth@sydneyneurology.com.au">elizabeth@sydneyneurology.com.au</a></td>
</tr>
<tr>
<td>Nese Sinmaz</td>
<td><a href="mailto:nsin4816@uni.sydney.edu.au">nsin4816@uni.sydney.edu.au</a></td>
</tr>
<tr>
<td>Pattama Songkhunawej</td>
<td><a href="mailto:pson6851@uni.sydney.edu.au">pson6851@uni.sydney.edu.au</a></td>
</tr>
<tr>
<td>Gayathri Sundaram</td>
<td><a href="mailto:g.sundaram@amr.org.au">g.sundaram@amr.org.au</a></td>
</tr>
<tr>
<td>Tamara Suprunenko</td>
<td><a href="mailto:tsup5163@uni.sydney.edu.au">tsup5163@uni.sydney.edu.au</a></td>
</tr>
<tr>
<td>Taylor Syme</td>
<td><a href="mailto:tsym3155@uni.sydney.edu.au">tsym3155@uni.sydney.edu.au</a></td>
</tr>
<tr>
<td>Fiona Tea</td>
<td><a href="mailto:ftea7693@sydney.university.edu.au">ftea7693@sydney.university.edu.au</a></td>
</tr>
<tr>
<td>Hooi Ling Teoh</td>
<td><a href="mailto:sakesushi@yahoo.com">sakesushi@yahoo.com</a></td>
</tr>
<tr>
<td>Julia Thompson</td>
<td><a href="mailto:Jtho4262@med.usyd.edu.au">Jtho4262@med.usyd.edu.au</a></td>
</tr>
<tr>
<td>Padmaja Tummala</td>
<td><a href="mailto:padmaja.tummala@anu.edu.au">padmaja.tummala@anu.edu.au</a></td>
</tr>
<tr>
<td>Philip West</td>
<td><a href="mailto:pwes8497@uni.sydney.edu.au">pwes8497@uni.sydney.edu.au</a></td>
</tr>
<tr>
<td>Alicia Wilson</td>
<td><a href="mailto:alicia.wilson@anu.edu.au">alicia.wilson@anu.edu.au</a></td>
</tr>
<tr>
<td>Jun Yan</td>
<td><a href="mailto:j.yan@uq.edu.au">j.yan@uq.edu.au</a></td>
</tr>
<tr>
<td>Anna Zinger</td>
<td><a href="mailto:annazinger@gmail.com">annazinger@gmail.com</a></td>
</tr>
<tr>
<td>Alicia Zou</td>
<td><a href="mailto:azou2297@uni.sydney.edu.au">azou2297@uni.sydney.edu.au</a></td>
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