

GBS-CIDP Foundation International Research Report 2013

Dear All,

For many years, the GBS-CIDP Foundation International has supported research. While the grants are small by NIH standards, we fill an important need in the GBS and CIDP and related diseases research arena by offering seed money that leads to NIH or MRC funded projects.

This year we took a comprehensive look at what has been done with your donations. We hope you will agree the results are impressive.

We started our grant program in 2004. Below is a brief report from each awardee. Almost all have been highly successful resulting in a publication, a completed project, or a larger grant. In some cases all of these resulted from the award.

In addition to this report, we have attached as individual pdf files, the research publications from some of these.

The Foundation looks forward to continuing and with your support expanding our grant program.

Sincerely yours,

Ken and Phil for the GBS CIDP Board

2004

Prof K.A. Sheikh, MD

Johns Hopkins, Baltimore, MD

Determination of anti-ganglioside antibody affinity in patients with GBS

Antibody affinity and nerve injury in GBS is a complex issue. Our work points out at least 3 issues:

1) Anti-ganglioside antibody affinity/avidity is likely not related to selective targeting, i.e., sensory vs motor nerve fiber recognition. Fine specificity of the antibodies is more relevant to this aspect. We had to clone a new anti-GD1a antibody with an order of magnitude higher affinity to make this point and also tested AMAN sera in this context. This was published in Brain 2008. See attached articles. GBS/CIDP foundation support acknowledged.

2) Antibody affinity/avidity seems to be a factor in nerve fiber injury. This was shown with different GM1 ligands with low to extremely high affinities. In this study a monoclonal antibody with low affinity did not cause nerve injury, GBS patient anti-GM1 antibodies with moderate affinities produced nerve injury, and cholera toxin subunit B (ligand with highest affinity for GM1) produced more severe injury. This was published in J Neuroscience 2010. See attached articles. GBS/CIDP foundation support acknowledged.

3) We also have unpublished data, which show that GBS patients have a range of affinities but the majority are in the moderate range. The antibody affinities are not as high as for example in M G. The caveat with the data are that these are cross-sectional data on antibodies in circulation. We have reason to believe that higher affinity antibodies may have shorter circulating half-lives. In order to produce injury moderate affinity antibodies must have robust Fc effector functions. We have preliminary data that indicate that Fc functions of anti-ganglioside antibodies are critical in producing nerve inflammation and injury.

Prof B. Arnason, MD

University of Chicago, Chicago, IL

A New Approach to Treatment of the Guillain-Barre Syndrome

The basic idea was that we could use a construct we had developed with interesting immunomodulatory properties to treat EAN. It did not work: we still don't quite understand why it didn't. It didn't work in EAE either even while it did work in an animal model for ITP. The construct is comprised of multiple copies of the CH2 region of IgG arranged linearly with the cysteines replaced by lysines to assure an open configuration so that the construct cross links multiple copies of all 3 classes of Fc gamma receptors, both activating and inhibiting, expressed on macrophages and thereby induce an off signal for cytokine secretion. Although this particular experiment failed other attempts, in addition to the ITP study, did not. When we put an antigenic peptide on the construct, the peptide was preferentially taken up by macrophages and dendritic cells with 10-100 times the antibody production seen when antigen alone was given. We now have US, Japanese, European and Australian patents for our technology, a start-up company called Iterative Therapeutics, a wet lab in an industrial complex across town, and 2 Small Business grants (SBIRs) from the NIH. One is to use our construct to augment the efficacy of Herceptin, since our construct really activates NK cells, perhaps because they express only activating receptors, and the other is to develop a vaccine to Botulinum toxin, a vaccine of potential interest to militaries concerned about bioterrorism. Another company has licensed our technology for an anthrax vaccine that they are developing. The funded projects, should they work out, will provide proof of principle, or so we hope, since if it works in one cancer it might well work in others, and ditto for vaccines. Thus, though the EAN project failed, the concept is still alive

Prof. Hugh Willison

Institute of Neurological Sciences, Glasgow, Scotland

Synthetic disialylgalactose immunoabsorbents deplete anti-GQ1b antibodies from autoimmune neuropathy sera

Acute and chronic autoimmune neuropathies, including Guillain-Barré syndromes (GBS) are often characterized by the presence of autoantibodies that react with neural gangliosides. Evidence from human and animal studies indicates that anti-ganglioside antibodies play a primary neuropathogenic role, and their rapid elimination from the circulation through specific immunoabsorption therapy thus has the potential to ameliorate the course of the disease. Here we have tested this therapeutic principle in the Miller Fisher variant of GBS that is associated serologically with acute phase anti-GQ1b ganglioside immunoglobulin G (IgG) antibodies, and in chronic ataxic neuropathies associated with persistently elevated immunoglobulin M (IgM) antibodies that react with GQ1b, GD3 and other disialylated gangliosides. Human and mouse anti-GQ1b IgG and IgM antibodies may also react with GD3, suggesting the shared terminal disialoside epitope could be involved in antibody binding. We thus synthesized the terminal trisaccharide, NeuAc(alpha2-8)NeuAc(alpha2-3)Gal common to GQ1b and GD3, and conjugated it to bovine serum albumin (BSA). This disialylgalactose glycoconjugate (DSG-BSA) binds anti-GQ1b antibodies in 32/58 (55%) human sera containing IgG or IgM anti-GQ1b antibodies at titres up to 1/130 000; it also binds a wide range of mouse monoclonal anti-GQ1b and -GD3 antibodies. When conjugated to Sepharose as mock therapeutic immunoaffinity columns, the immobilized trisaccharide (DSG-Sepharose) eliminates anti-GQ1b antibodies from positive sera in proportion to their level of binding to DSG-BSA. Oligosaccharide-specific immunoabsorption therapy thus provides a new therapeutic approach to anti-GQ1b antibody-associated syndromes that could be applied to clinical practice. Furthermore, modification of the immobilized oligosaccharide epitopes to incorporate other glycan structures may allow this approach to be adapted to other forms of autoimmune neuropathy associated with uniform anti-glycolipid antibody profiles.

2005

Dr. M.N. Rasband

University of Connecticut Health Center, Farmington, CT

The Role of Schwann Cell Microvilli at Nodes of Ranvier and in Guillain-Barre Syndrome

Autoantibodies to gangliosides such as GM1 have been proposed to disrupt nodes of Ranvier in peripheral motor nerve fibers and lead to Guillain-Barré syndrome, an autoimmune neuropathy characterized by acute limb weakness. At the nodes in myelinated axons, voltage-gated Na⁺ channels are highly concentrated and facilitate rapid action potential conduction. Using a disease model caused by immunization with gangliosides, we showed that Na⁺ channel clusters were disrupted or disappeared at abnormally lengthened nodes concomitant with deposition of IgG and complement products at the acute phase with progressing limb weakness (Susuki et al. J Neurosci 2007). Paranodal axoglial junctions, the nodal axonal cytoskeleton, and Schwann cell microvilli, all of which stabilize Na⁺ channel clusters, were also disrupted. During recovery, complement deposition at nodes decreased, and Na⁺ channels redistributed on both sides of affected nodes. These results suggest that Na⁺ channel alterations occur as a consequence of complement-mediated disruption of interactions between axons and Schwann cells. Our findings support the idea that the axonal form of Guillain-Barré syndrome is a disease that specifically disrupts the nodes of Ranvier. We also showed that gangliosides themselves are highly accumulated at and near nodes, and contribute to the formation and maintenance of the nodal Na⁺ channel clusters. In the mutant mice lacking gangliosides including GM1, ion channel clusters and structures at and near nodes were remarkably disrupted (Susuki et al. Glia 2007). Abnormal molecular organization at paranodes became more prominent with age. Furthermore, the defects were more

prevalent in ventral than dorsal roots, and less frequent in mutant mice lacking the b-series gangliosides but with excess GM1. These results indicate that gangliosides contribute to stability and maintenance of nodes of Ranvier, and further support the critical roles of anti-ganglioside antibodies in Guillain-Barré syndrome.

Publications supported by this grant

Susuki K, Rasband MN, Tohyama K, Koibuchi K, Okamoto S, Funakoshi K, Hirata K, Baba H, Yuki N. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. *J Neurosci* 2007;27:3956-67

Susuki K, Baba H, Tohyama K, Kanai K, Kuwabara S, Hirata K, Furukawa K, Furukawa K, Rasband MN, Yuki N. Gangliosides contribute to stability of paranodal junctions and ion channel clusters in myelinated nerve fibers. *Glia* 2007;55:746-57

2005 – 2007

Prof R.A.C. Hughes

Dept. of Clinical Neuroscience - Kings College London, England

A Pilot Randomized controlled trial of Methotrexate for Chronic Inflammatory Demyelinating Polyradiculoneuropathy RMC Trial

The GBS/CIDP Foundation grant funded the largest trial of an immunosuppressive agent for CIDP yet performed. The results were published in the prestigious international journal *Lancet Neurology* [RMC Trial Group 2009]. The trial was a high quality randomized study undertaken in 25 centers in 5 European countries. It compared methotrexate with identical appearing dummy or placebo tablets in people with CIDP already taking intravenous immunoglobulin or corticosteroids. After about 16 weeks, the dose of corticosteroids or intravenous immunoglobulin was decreased by 20% every 4 weeks provided that the participants did not worsen. The primary outcome was achieving a greater than 20% reduction in the dose of corticosteroids or intravenous immunoglobulin in the last 4 weeks of the trial compared with the first 4 weeks. Fifty-nine people completed the trial. Fourteen (52%) of the 27 taking methotrexate were able to reduce their corticosteroid or intravenous immunoglobulin dose but, unexpectedly, so were 14 (44%) of the 32 taking the dummy tablets. Thus the trial did not show any benefit from taking methotrexate but it did show, against expectations, that 44% were taking more corticosteroids or intravenous immunoglobulin than they needed. This has affected the design of subsequent CIDP treatment trials and had a major effect on the management of CIDP worldwide. It has led to the recommendation of drug holidays to check the need for continued treatment.

The grant paid for the employment of Dr. Mohamed Mahdi-Rogers, a neurologist originally from Sierra Leone, and allowed him to complete his training in London, UK, and secure a position as a Neuromuscular Disease Fellow. Thus the grant added to the small army of doctors qualified to look after people with CIDP. During his fellowship Dr. Mahdi-Rogers also completed an epidemiological study and a cost study of CIDP in south-east England [Mahdi-Rogers and Hughes 2013] and a Cochrane review of immunosuppressive treatment for CIDP [Mahdi-Rogers et al. 2013]. Support from the GBS/CIDP Foundation International has been acknowledged in all these publications.

Mahdi-Rogers M, Hughes RAC. Epidemiology of chronic inflammatory neuropathies in south east England. *European Journal of Neurology* 2013; [Epub ahead of print] PubMed PMID: 23679015.

Mahdi-Rogers M, van Doorn PA, Hughes RAC. Immunomodulatory treatment other than corticosteroids, immunoglobulin and plasma exchange for chronic inflammatory demyelinating polyradiculoneuropathy. Cochrane Database of Systematic Reviews 2013; CD003280.

RMC Trial Group. Randomized controlled trial of methotrexate for chronic inflammatory demyelinating polyradiculoneuropathy (RMC trial): a pilot, multicentre study. *Lancet Neurol* 2009; 8: 158-164.

2006

Prof K.A. Sheikh

Johns Hopkins, Baltimore, MD

Reversal of anti-ganglioside antibody mediated inhibition of nerve regeneration with erythropoietin

Guillain-Barre' syndrome (GBS) is a monophasic immune neuropathic disorder in which a significant proportion of patients have incomplete recovery. The patients with incomplete recovery almost always have some degree of failure of axon regeneration and target reinnervation. Anti-ganglioside antibodies (Abs) are the most commonly recognized autoimmune markers in all forms of GBS and specific Abs are associated with the slow/poor recovery. We recently demonstrated that specific anti-ganglioside Abs inhibit axonal regeneration and nerve repair in preclinical models by activation of small GTPase RhoA and its downstream effectors. The objective of this study was to determine whether erythropoietin (EPO), a pleiotropic cytokine with neuroprotective and neurotrophic properties, enhances nerve regeneration in preclinical cell culture and animal models of autoimmune neuropathy/nerve repair generated with monoclonal and patient derived Abs. Primary neuronal cultures and a standardized sciatic crush nerve model were used to assess the efficacy of EPO in reversing inhibitory effects of anti-ganglioside Abs on nerve repair. We found that EPO completely reversed the inhibitory effects of anti-ganglioside Abs on axon regeneration in cell culture models and significantly improved nerve regeneration/repair in an animal model. Moreover, EPO-induced proregenerative effects in nerve cells are through EPO receptors and Janus kinase 2. Signal transducer and activator of transcription 5 pathway and not via early direct modulation of small GTPase RhoA. These preclinical studies indicate that EPO is a viable candidate drug to develop further for neuroprotection and enhancing nerve repair in patients with GBS.

Prof U. Dianzani

University of Eastern Piedmont, Novara, Italy

Search for biological markers of inflammatory demyelinating polyneuropathies development and progression: analysis of patients displaying defective function of the Fas apoptotic pathway

Is Defective Fas-Mediated T-Cell Apoptosis a Pathogenetic Mechanism in CIDP?

Starting from the concept that the immune response in the peripheral nervous system is shut down through T-cell apoptosis, we aimed to assess whether defective T-cell apoptosis had a role in chronic inflammatory demyelinating polyneuropathy (CIDP) as shown for other autoimmune diseases, such as MS (Comi et al., 2000). We studied the Fas apoptotic pathway in patients with CIDP, Guillain-Barré syndrome (GBS), and in healthy controls, finding that CIDP patients displayed significantly higher T-cell survival to Fas-mediated apoptosis than both controls and GBS patients, whereas GBS patients and controls showed no difference. Moreover, the percentage of Fas-resistant (Fas-r) subjects, defined by the survival of more than 82% of their T cells to Fas-mediated apoptosis, was 52% in CIDP patients, 2% in controls, and 0% in GBS patients (Comi et al., 2006). The reason why we compared CIDP and GBS patients lies in the self-limiting nature of GBS as opposed to the persistent immune system activation of CIDP. Our hypothesis was that T-cell apoptosis might be a factor influencing, when effective, the self-limitation of the inflammatory process that we observed in GBS, or when defective, the disease

persistence typical of CIDP. This concept was later reinforced by the finding that patients with acute-onset CIDP (a-CIDP) can be distinguished very early in the disease from patients with GBS by showing defective T-cell apoptosis. To show that, we used a twofold approach: initially, we compared Fas function in retrospective patients analyzed after diagnosis of GBS or a-CIDP and subsequently performed a prospective study analyzing Fas function at the onset of an acute inflammatory polyneuropathy and following up patients for the development of GBS or a-CIDP for at least 6 months. In both cases, we found that a-CIDP patients displayed significantly higher T-cell survival to Fas mediated apoptosis than controls and GBS patients. Moreover, single-patient analysis showed that all a-CIDP patients were Fas-r compared with one control and no GBS patients (Comi et al., 2009). One important question was whether Fas function may vary during the disease course. To clarify that, we tested the stability of Fas-mediated apoptosis with a second test at a time frame of about 1 year and found that variability was always <5%. We next tested whether the Fas pathway impairment had an inherited component, as was previously found in MS (Comi et al., 2000). We evaluated Fas-mediated T-cell death in families of Fas-r CIDP patients and found that in each family there was at least one Fas-r parent (Comi et al., 2006). In previous studies, we showed that Fas-r patients with ALPS or MS produce molecules exerting a dominant-negative effect on Fas function (Comi et al., 2000; Ramenghi et al., 2000; Dianzani et al., 2003). To evaluate whether these molecules were also detectable in CIDP, we fused activated CD4+ T cells derived from Fas-r patients with the Fas-s HUT78 T-cell lines and cultured hybrid cells under the selective pressure of anti-Fas monoclonal antibodies (MAbs). Fusions from the Fas-r patients gave rise to Fas-r hybrid cell lines, whereas fusion from Fas-s control subjects did not (Comi et al., 2006). Studying Fas-mediated cell death in CIDP patients with different clinical courses, we found that patients with a chronic progressive (CP) course had a significantly higher cell survival than patients with an RR course. Indeed, 70% of CP patients were Fas-r compared to only 20% of RR patients. After dividing CIDP patients according to neurophysiologic features at diagnosis, we found that patients with secondary axonal pattern had a significantly higher cell survival than those with a pure demyelinating form. All patients from the former group were Fas-r compared with only 24% of those from the latter (Comi et al., 2006). Finally, to better characterize the apoptosis defect, we evaluated the function of the extrinsic and intrinsic pathways of apoptosis by assessing the activation of caspase-8 induced by Fas triggering, and caspase-9 induced by etoposide, selectively acting on mitochondria in T-cell lines from Fas-r CIDP patients and Fas-s controls. Results showed that caspase-8 activity was significantly lower in CIDP patients than in controls, whereas no difference was found in caspase-9 activity. To assess whether defective caspase-8 activation was ascribable to increased expression of the FLIP inhibitor, we evaluated FLIP expression by Western blotting in the T-cell lines obtained from the same group of patients. Results showed no difference between patients and controls (Comi et al., 2006).

Our studies on T-cell apoptosis show that defective Fas function is a predisposing factor for CIDP development. Moreover, we provide evidence that T-cell Fas function may be a marker for identifying patients with an aggressive course and, in the case of acute onset, for discriminating between patients with a self-limiting disease and patients prone to develop a chronic course.

2007

Prof B. Soliven

The University of Chicago, Chicago, IL

Spontaneous Autoimmune Polyneuropathy in the NOD Mouse

The antigen/s and the triggering mechanisms causing chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) remain elusive. B7-2 is one of the molecules expressed by antigen presenting cells and is required for optimal activation of T lymphocytes. When B7-2 is eliminated in non-

obese diabetic (NOD) mice, animals do not develop diabetes, but instead they develop a spontaneous autoimmune polyneuropathy (SAP), which mimics CIDP in many ways.

Funding support from the GBS/CIDP Foundation International has facilitated our efforts to identify potential target antigens in B7-2 knockout NOD mice using a combination of immunologic methods that include proliferation assay, cytokine secretion, adoptive transfer and intravenous tolerance studies. We found the myelin P0 is one of the auto-antigens involved in the development of autoimmune neuropathy in this model, and that this protein is also expressed by Schwann cells in the pancreas. These findings suggest that a subset of patients with CIDP, particularly those with increased susceptibility to develop type 1 diabetes, may be mediated by autoimmunity against myelin P0. Data from this pilot grant were included in a manuscript published in the Journal of Immunology (Kim et al., 2008), and formed the basis of a successful NIH grant application.

Prof K.A. Sheikh

Johns Hopkins, Baltimore, MD

Neuroprotection and Enhancement of Nerve Repair with Erythropoietin in EAN

Erythropoietin (EPO) is a pleiotropic cytokine originally identified for its role in erythropoiesis. In addition, in various preclinical models EPO exhibited protective activity against tissue injury. There is an urgent need for potent treatments of autoimmune driven disorders of the peripheral nervous system (PNS), such as the Guillain-Barre' syndrome (GBS), a disabling autoimmune disease associated with relevant morbidity and mortality. To test the therapeutic potential of EPO in experimental autoimmune neuritis (EAN) - an animal model of human GBS - immunological and clinical effects were investigated in a preventive and a therapeutic paradigm. Treatment with EPO reduced clinical disease severity and if given therapeutically also shortened the recovery phase of EAN. Clinical findings were mirrored by decreased inflammation within the peripheral nerve, and myelin was well maintained in treated animals. In contrast, EPO increased the number of macrophages especially in later stages of the experimental disease phase. Furthermore, the anti-inflammatory cytokine transforming growth factor (TGF)-beta was upregulated in the treated cohorts. In vitro experiments revealed less proliferation of T cells in the presence of EPO and TGF-beta was moderately induced, while the secretion of other cytokines was almost not altered by EPO. Our data suggest that EPO revealed its beneficial properties by the induction of beneficial macrophages and the modulation of the immune system towards anti-inflammatory responses in the PNS. Further studies are warranted to elaborate the clinical usefulness of EPO for treating immune-mediated neuropathies in affected patients.

Prof R.P. Lisak

Wayne State University, Detroit, MI

Identifying Targets of Tumor Necrosis Factor Alpha (TNF- α) Signaling that Inhibit Schwann Cell (SC) Differentiation

We previously showed that some inflammatory cytokines, primarily interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α), which are found in the peripheral nerve of patients with GBS and CIDP, as well as in several animal models of GBS, are able to inhibit Schwann cells from expressing glycolipids associated with myelination in vitro. As part of our ongoing investigation into the relationships between inflammatory cytokines and Schwann cell biology, we have looked at the effects of both of these cytokines on Schwann cells. We are currently analyzing a very large set of data using gene array technology to determine if TNF- α alters the pattern of gene expression induced by cAMP, which simulates many of the effects of axolemma on Schwann cells in vitro. The gene array studies might provide a gene discovery approach to identify potential targets for decreasing or reversing

inflammation---induced nerve demyelination. Although many laboratories, including our own, have shown that Schwann cells can upregulate major histocompatibility antigens (MHC) in vitro in response to inflammatory cytokines, in clinical and experimental inflammatory neuropathies, as well as in other diseases of the PNS, there is controversy over whether Schwann cells can upregulate major histocompatibility antigens or whether MHC expression is limited to infiltrating inflammatory cells. We postulated that Schwann cells in culture that are expressing myelin associated molecules, as many of them would be in a mature myelinated nerve, might be resistant to upregulation of MHC class II molecules as well as the adhesion molecule, intercellular adhesion molecule ---1 (ICAM---1), also upregulated by cytokines. When we incubated undifferentiated Schwann cells with IFN-g, the most potent stimulator of MHC Class II, both MHC Class II and ICAM---1 were up-regulated in many of the cells. However, when Schwann cells were treated with cAMP in the presence or absence of IFN-g, we found that when Schwann cells were stimulated by cAMP to differentiate and express myelin associated molecules, they did not express MHC Class II or ICAM---1. This indicates that the state of differentiation and perhaps myelination influences the response of SC to pro-stimulatory cytokines.

Dr. E.E. Ubogu

Baylor College of Medicine, Houston, TX

CXCR3 Blockade and Inflammatory Demyelinating Polyradiculoneuropathies (IDP)

Funds from this award, in addition to funds received from the Baylor College of Medicine New Investigator Start-Up program supported the isolation, purification and characterization of primary human endoneurial endothelial cells from sciatic nerves (1). These cells are responsible for forming the restrictive blood-nerve barrier. This work has led to the development of an in vitro blood-nerve barrier system to study molecular determinants and signaling pathways potentially relevant to pathologic leukocyte trafficking in GBS and CIDP. Using this model, we demonstrated the effect of physiological pro-inflammatory cytokine stimulus at the blood-nerve barrier and showed the importance of alpha M integrin and intercellular adhesion molecule-1 [ICAM-1] (2), providing a framework to better understand pathologic leukocyte trafficking potentially relevant to targeted therapies for AIDP. Recent preliminary data using a reliable mouse model of AIDP called severe murine experimental autoimmune neuritis (sm-EAN) shows that CXCR3 blockade using a proprietary drug called AMG487 improves the clinical manifestations of sm-EAN when giving after disease onset (3), suggesting that drugs that block specific chemokine receptors may be useful future therapy for peripheral nerve inflammatory disorders such as AIDP.

2008

Dr. I.S.J. Merkies

Universiteit Maastricht, The Netherlands

Peripheral Neuropathy Outcome Measures Standardization (PeriNom) Study

Different methods have been used to study patients with inflammatory polyneuropathies. The use of proper outcome measures is very important to facilitate adequate follow-up and evaluate treatment effects. The PeriNomS study aimed to expand the clinimetric knowledge on outcome measures at selected levels of outcome (mainly at the impairment, activity & participation, and quality of life levels) in patients with Guillain-Barré Syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP), multifocal motor neuropathy (MMN) and monoclonal gammopathy of undetermined significance related polyneuropathy (MGUSP).

The PeriNomS study consisted of two parts:

The cross-sectional part focusing on comparative validity and reliability of the outcome measures, in which 122 clinically stable patients participated.

The longitudinal part was performed to obtain responsiveness data that may help to differentiate between comparable valid and reliable measures. The longitudinal part was an international, multi-centre collaborative approach of 26 neuromuscular centres. Newly diagnosed patients and patients with a relapse of their polyneuropathy (n=163) have been included and were examined 3 times (T0, T3, T12 months) in MMN (n=26) and MGUSP (n=23) or 5 times (T0, T1, T3, T6, and T12 months) in GBS (n=55) and CIDP (n=59). The inclusion period ended on January 1st, 2013, the database was closed and the main data at the impairment and activity and participation were presented at the 196th workshop, held in Naarden, the Netherlands under the auspice of the European NeuroMuscular Centre (ENMC). The ultimate goal was to get international consensus on a specific core set of outcome measures to be used in future clinical trials and follow-up studies in patients with inflammatory polyneuropathy, particularly focusing on GBS, CIDP, MGUSP, and MMN. At the 196th ENMC workshop, consensus was met for most illnesses at the various levels of assessing outcome. A paper on this will be published soon and at the PNS 2013 a presentation will be given on this topic.

Timeframe: the project was completed and all targets of inclusion were met, for both cross-sectional and longitudinal recruitments.

Prof P.A. van Doorn

Erasmus MC, The Netherlands

Autonomic Failure and pain in Guillain-Barre Syndrome: A skin biopsy study

We performed a prospective study in 32 patients with Guillain-Barré syndrome (GBS) or its variants to correlate intraepidermal nerve fiber density (IENFD) at the distal leg and lumbar region with pain, autonomic dysfunction, and outcome. In the acute phase, IENFD was reduced in 60% and 61.9% of patients at the distal leg and lumbar region, respectively. In the acute phase, 43.7% of patients complained of neuropathic pain. Their IENFD at the distal leg was significantly lower than in patients without pain ($P < .001$) and correlated with pain intensity ($r_s = [1]0.51$; $P = .003$). Intriguingly, also patients with the pure motor variant of GBS and pain had low IENFD. At 6-month follow-up, only 3 patients complained of persisting neuropathic pain, whereas 3 patients reported late-onset pain symptoms. IENFD in the acute phase did not predict presence or intensity of pain at 6-month follow-up. IENFD in the acute phase did not correlate with clinical dysautonomia or GBS severity at nadir. However, it correlated with poorer GBS disability score at 6 months ($P = .04$), GBS score at nadir ($P = .03$), and clinically probable dysautonomia ($P = .004$). At 6-month follow-up, median IENFD remained significantly low both at the distal leg ($P = .024$) and lumbar region ($P = .005$). Double and triple staining confocal microscope studies showed diffuse damage of myelinated dermal nerves along with axonal degeneration, and mononuclear cell infiltration. Unmyelinated and myelinated skin nerves are diffusely affected in GBS and its variants, including the pure motor form. IENFD declines early, remains low over time, correlates with pain severity in the acute phase, and may predict long-term disability.

Prof R.A. Lewis

Wayne State University. Detroit, MI

Database of Multifocal Motor Neuropathy and Other Inflammatory Neuropathies

The project was to create a registry/database of patients with Multifocal Motor Neuropathy and if successful to be utilized in the future for other inflammatory neuropathies. The funds were utilized to contract with the University of Maryland Bioinformatics Department to assist in the creation of the database, and the implementation of a web-based data entry system. A committee of neurologists

active in the Inflammatory Neuropathy Consortium met at the INC meetings and dialogued by email to work on the information that would be put into the database.

A number of issues were identified that required attention before a product could be implemented. These included:

1. HIPPA compliance and patient anonymity,
2. Developing a method to make sure that patients actually had MMN,
3. Keeping the amount of information that was required to a minimum but having enough information to be useful, and
4. Getting physicians to use the registry/database.

It was decided that the initial product would be a registry that would include demographics, whether the patient met either AANEM or EFNS criteria, whether conduction block was identified, whether there were any sensory signs or symptoms and whether GM1 antibodies were present. In addition, information on treatments used and their efficacy was included.

The project took over 2 years, but the registry was developed and was initiated. However, full implementation stalled and is currently not active. The major problem that occurred was the dismantling of the University of Maryland Bioinformatics Department. The registry is still available and there has been interest (Baxter remains interested) in working with Dr. Leonard van den Berg and others to reinvigorate the project. One approach that is very appealing is to utilize the mechanism created for IGOS to do a very similar project on MMN.

In summary, the project on creating an MMN registry and database was partially successful in that a product was created that can be utilized in the future. The difficulties in implementation were frustrating but the overall goal of developing a network of centers that can do future treatment trials in MMN is moving forward.

2009

Prof K.A. Sheikh

University of Texas, Houston, TX

Engineering chimeric protein(s) to enhance nerve repair in antibody-mediated preclinical models of autoimmune neuropathy

We had funding for one year but spent at least 2 years on this project. Progress has been slow on this one and include generation of six different constructs of human and mouse EPO conjugated with 3 different mouse IgG Fc isotypes. We have tried to express mouse EPO constructs in a mammalian cell line and had only limited success with one construct. The level of protein expression is extremely low. Despite our best efforts, we have been able to generate sufficient chimeric protein to carry out pharmacokinetic and efficacy studies in animals. The project is currently dormant due to funding. But once funding is available we plan to have some of these chimeric proteins expressed in sf9 insect cells. I have talked with protein expression experts and they do not have any additional suggestions except trying the insect cells. Apparently, expressing chimeric proteins in mammalian systems is tedious and unpredictable hit or miss. If insect expression provides us sufficient chimeric protein then will test this in animal studies. I have asked the lab to prepare a power point containing key data showing protein generation, which is attached. All EPO-Fc constructs and transfected CHO cell lines are available as a resource for sharing.

Dr. B.C. Jacobs

Erasmus MC, The Netherlands

Prognostic models for Guillain-Barré syndrome.

Guillain-Barré syndrome (GBS) has a highly variable clinical course and outcome. Despite the fact that various studies described prognostic factors in GBS, these are difficult to apply in clinical practice. To support patients, family and neurologists we have developed various validated prognostic models which are based on clinical characteristics which can be easily obtained at the bedside of the patient. These models were developed to predict in individual patients with GBS the chance of respiratory failure (Walgaard et al. Brain 2010) and severe disability at one to six months (Walgaard et al. Neurology 2011). These models have found their way to the clinic and are for example used in the Dutch National Guideline for the treatment of GBS. The next step was to further improve and validate these prognostic models in a large cohort of GBS patients. Such numbers of patients can only be reached by large scale international collaboration. With support from the GBS-CIDP Foundation International we have initiated the International GBS Outcome Study (IGOS). IGOS is conducted by the members of the Inflammatory Neuropathy Consortium (INC). The aim of the IGOS is to identify the clinical and biological predictors of the clinical course and outcome of GBS. Supported by this grant a website and web based data entry system and database was developed by a professional web designer. Data are collected at 7 standard time points during a follow-up of one year, but there is an opportunity to expand the follow-up to 2 and 3 years. Using this system detailed and standardized data are obtained regarding the demography, preceding events, neurological deficits, diagnostic investigations, treatment, complications and outcome. Several validated outcome scores are used to describe in detail the most important consequences to the patients, including disability, pain and fatigue. After the prototype website was developed various extensive test rounds were conducted in participating centers from various countries to further improve the website and database. Data are collected in a coded form without reference to personal information and stored in an environment which is highly secured and protected. The website and data base is up and running and currently used by more than 100 research centers from North- and South-America, Europe, Asia and Australia participating in IGOS. The website is maintained daily and can be visited at www.gbsstudies.org. At this website a GBS tool is presented to predict the clinical course in individual patients.

Dr. H. C. Lehmann

Heinrich-Heine University, Germany

Schwann cell replacement therapy for chronically denervated nerves

Our project is based on the well-recognized clinical dilemma that patients with Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyneuropathy (CIDP) often show an incomplete recovery and poor functional outcome after a prolonged, severe disease course.

Previous experimental studies suggested that incomplete recovery in peripheral nerve disorders is partially attributable to morphological changes in the distal segments of chronically denervated nerves. There is evidence that Schwann cells, which provide trophic support under normal conditions, become atrophic and lose their ability to support the regeneration of axons after prolonged denervation.

Thus we wanted to explore the underlying cellular mechanisms of this chronic denervation condition in GBS and CIDP. Moreover we pursue the idea that axonal regeneration within those chronic denervated nerve segments in patients with GBS and CIDP could be enhanced by transplantation of freshly extracted, healthy Schwann cells. To address this clinically important issue we adapted and modified an animal model, which mimics the situation of chronic denervation, comparable to those of GBS/CIDP patients after a prolonged disease course with secondary axonal loss. The project was started in 2010 and is still ongoing. The initial phase of this project was used to explore different experimental paradigms to address basic practical issues in this animal model such as timing, fate and application

route of endoneurial Schwann cell transplantation. Our preliminary experiments suggest that the transplantation of Schwann cells into chronically denervated nerve stumps can enhance regeneration, provided that narrow temporal-spatial conditions are carefully taken into account. We also observed that grafted Schwann cells do not survive over prolonged time periods, which has implications for the primary mode of action by which those grafts enhance regeneration of injured axons (transient growth factor expression versus remyelination).

In ongoing experiments we are currently investigating if Schwann cells that are exposed to an inflammatory environment like in GBS and CIDP preserve their regeneration promoting phenotype and their utility to serve as cellular graft for this model. We anticipate that the preclinical data which are generated by our proof-of-principle study will provide important information how future cell-replacement studies have to be designed to achieve highest benefit for patients with GBS and CIDP.

2010

Dr. I Nachamkin

University of Pennsylvania, Philadelphia, PA

Genome Wide Association Study of Guillain-Barre Syndrome

No work was completed on this project. Money has been returned.

Prof J. Devaux

Research Center of Neurobiology-Neurophysiology of Marseille,
Marseille, France

The role of antibodies against proteins of the node of Ranvier in the pathogenesis of Guillain-Barre syndrome: finding novel immune targets.

Background Chronic inflammatory demyelinating polyneuropathy (CIDP) is a peripheral nervous system (PNS) disease with progressive or relapsing-remitting (RR) course¹. An autoimmune attack against the PNS myelin, with the involvement of both the cellular and humoral components of the immune response is the main pathogenic event². A central role is played by T helper (TH) cells secreting cytokines supporting macrophage and B cell activation². Recent research has shift the interest from a traditional view of an imbalance between pro-inflammatory TH1 and anti-inflammatory TH2 cells as the main mechanism of autoimmunity, to a more complex scenario where TH17 cells control the pro-inflammatory component, modulating also the activity of TH1 cells³. Most data on the TH17 role have been obtained in common autoimmune diseases, including multiple sclerosis (MS), but their role has also been suggested in CIDP since TH17 cells and IL-17 levels have been found to be increased in active CIDP⁴ in the peripheral blood.

Osteopontin (OPN) is a cytokine that may influence development of autoimmune diseases through its capacity to enhance both TH1 and TH17 cell responses^{5,6}. High OPN levels have been detected in several autoimmune diseases, including EAE and MS, especially during relapses⁵. Moreover, single nucleotide polymorphisms (SNPs) of the OPN gene have been associated with several autoimmune diseases⁷⁻⁹, and a link has been suggested with the increased protein levels¹². In particular, we detected four SNPs (+282T>C in exon VI; +750C>T in exon VII; +1083A>G and +1239A>C in 3' UTR) of the OPN gene which form three haplotype combinations (haplotype-A: 282T-750C-1083A-1239A; haplotype-B: 282C-750T-1083A-1239C; haplotype-C: 282C-750T-1083G-1239C) displaying associations with several autoimmune diseases; in fact, subjects carrying haplotype-B or -C displayed 1.5 higher risk of MS, type 1 diabetes, and systemic lupus erythematosus, and 8 fold higher risk of autoimmune lymphoproliferative syndrome (ALPS), than haplotype-A homozygotes. Several data suggested that these haplotypes influence OPN levels and, in particular, haplotype-B and -C are associated with higher levels of OPN possibly because their mRNAs was more stable than the haplotype-A mRNA⁹. The OPN role in MS is supported by the finding that OPN-deficient mice are resistant to progressive EAE and have frequent

remissions¹⁰; in these mice, myelin-reactive T cells produce more IL-10 and less IFN γ than in wild type mice. A further point is that Steinman et al.¹¹ showed that low levels of anti-OPN auto-antibodies (auto-Ab) were produced in EAE mice, and boosting their production by DNA vaccinations with a plasmid encoding OPN substantially ameliorated the chronic course of EAE. These findings are in line with reports suggesting that production of auto-Ab against pro-inflammatory cytokines plays a physiologic role in down modulation of the inflammatory response¹².

Several studies have explored the role of OPN in autoimmune diseases, but little is known about its role in inflammatory polyneuropathies. A role is suggested by the finding that OPN is constitutively expressed in the PNS and up-regulated in rat experimental autoimmune neuritis¹³. It is noteworthy that OPN can inhibit T cell apoptosis¹⁴ that we previously showed to be defective in CIDP¹⁵.

In this context, this study may bridge the knowledge gap on the role of other apoptotic pathways which may be involved in controlling T cell survival in parallel to the direct pathway mediated by Fas.

Given these premises, the main objectives of this project were: a) to explore the role of OPN and anti-OPN auto-antibodies in CIDP; b) to further characterize apoptotic pathways involved in T cell survival in CIDP. Specific aims were the following: 1) To evaluate the levels of OPN and anti-OPN auto-antibodies in CIDP and GBS and assess their correlations with disease status (relapse, remission, chronic phase). 2) To type the OPN gene haplotypes and analyze other genes involved in T cell apoptosis in CIDP and GBS patients.

Patients and methods. We enrolled 62 patients with CIDP¹⁶, 30 with Guillain-Barré syndrome (GBS) and 50 age matched healthy controls. We assessed OPN plasma levels with a commercially available ELISA kit (IBL diagnostics). The presence of anti-OPN antibodies was demonstrated by western blot, whereas anti-OPN Ab levels were assessed with a custom made ELISA. Thereafter, we compared OPN plasma levels in patients CIDP with different clinical outcomes (progressive vs. relapsing course, axonal vs. demyelinating damage). Finally, we analyzed OPN gene haplotypes (+1239A>C), known to influence protein plasma levels, and variants of Perforin (PRF) gene that is involved in apoptosis and play a role several autoimmune diseases¹⁷.

Results. CIDP patients displayed significantly higher OPN plasma levels compared to both GBS patients and healthy controls (median value in ng/ml: 250 vs 165 and 160 respectively, $p=0.015$).

OPN plasma levels were higher in patients with progressive vs. relapsing course and secondary axonal damage vs. demyelination (median value in ng/ml: 270 vs. 165 and 240 vs. 170 respectively, $p=0.023$ and $p=0.035$).

Moreover, we found that CIDP patients showed higher anti-OPN antibody plasma levels compared to controls (median titer 0.36 vs. 0.14, $p=0.02$).

On the genetic side, OPN gene variants did not seem to be differently distributed among tested subjects (data not shown). On the contrary, PRF gene variations which are known to influence protein function, were differently distributed in patients compared to controls. A91V variations were present in 19% of patients versus 9% of controls ($p>0.05$). Moreover, we found two CIDP patients carrying new PRF mutations which were found to display a biologic activity.

Conclusion. This is the first report highlighting the role of OPN in CIDP. Increase of OPN plasma levels is associated to a chronic disease evolution, being absent in a self-limiting disease like GBS and more frequent in CIDP sub-forms with aggressive evolution. Moreover, patients with CIDP show higher anti-OPN Ab levels. A further step would imply the characterization of outcome in relation to both OPN and anti-OPN Ab levels. Neither protein nor antibody levels seem to be influenced by the genetic background since we found no difference in OPN SNPs between patients and controls. PRF gene variations/mutations may represent a predisposing factor for CIDP development.

CCL2-CCR2 blockade as targeted immune- modulatory therapy for inflammatory demyelinating polyradiculoneuropathies.

Funds from this award, in addition to funds received from the National Institutes of Health supported our work on the effects of functional inhibition of CCR2 signaling in immune-mediated demyelinating neuritis using the sm-EAN model. Preliminary data obtained following sm-EAN induction using a CCR2 knockout mouse showed that these mice were resistant to developing clinical manifestations of the disease, suggesting that CCR2 may be essential in the pathogenesis of acute demyelinating neuritis (3). Unpublished work (in preparation for submission) shows that a commercially available CCR2 inhibitor RS 102895 effectively treated mice with sm-EAN days after disease onset, with evidence of improved electrophysiologic parameters, and reduced inflammation and demyelination compared to untreated controls. Data from this study further suggests that targeted inhibition of specific chemokine receptors may be useful future therapies for AIDP and related disorders. Funds from the Foundation also supported the publication of a review article on chemokines in peripheral nervous system inflammation published in 2011(4).

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Chiang S, Ubogu EE. The role of chemokines in Guillain-Barré syndrome. *Muscle and Nerve* 2013 (In press. On-line version: DOI: 10.1002/mus.23829, published February 28th, 2013).

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2011

Dr. C. Como

University of Eastern Piedmont Amedeo Avogadro, Italy

The role of osteopontin and anti-osteopontin antibodies in CIDP

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a peripheral nervous system (PNS) disease with progressive or relapsing-remitting (RR) course¹. An autoimmune attack against the PNS myelin, with the involvement of both the cellular and humoral components of the immune response is the main pathogenic event². A central role is played by T helper (TH) cells secreting cytokines supporting macrophage and B cell activation². Recent research has shift the interest from a traditional view of an imbalance between pro-inflammatory TH1 and anti-inflammatory TH2 cells as the main mechanism of autoimmunity, to a more complex scenario where TH17 cells control the pro-inflammatory component, modulating also the activity of TH1 cells³. Most data on the TH17 role have been obtained in common autoimmune diseases, including multiple sclerosis (MS), but their role has also been suggested in CIDP since TH17 cells and IL-17 levels have been found to be increased in active CIDP⁴ in the peripheral blood.

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detected four SNPs (+282T>C in exon VI; +750C>T in exon VII; +1083A>G and +1239A>C in 3' UTR) of the OPN gene which form three haplotype combinations (haplotype-A: 282T-750C-1083A-1239A; haplotype-B: 282C-750T-1083A-1239C; haplotype-C: 282C-750T-1083G-1239C) displaying associations with several autoimmune diseases; in fact, subjects carrying haplotype-B or -C displayed 1.5 higher risk of MS, type 1 diabetes, and systemic lupus erythematosus, and 8 fold higher risk of autoimmune lymphoproliferative syndrome (ALPS), than haplotype-A homozygotes. Several data suggested that these haplotypes influence OPN levels and, in particular, haplotype-B and -C are associated with higher levels of OPN possibly because their mRNAs were more stable than the haplotype-A mRNA⁹. The OPN role in MS is supported by the finding that OPN-deficient mice are resistant to progressive EAE and have frequent remissions¹⁰; in these mice, myelin-reactive T cells produce more IL-10 and less IFN γ than in wild type mice. A further point is that Steinman et al.¹¹ showed that low levels of anti-OPN auto-antibodies (auto-Ab) were produced in EAE mice, and boosting their production by DNA vaccinations with a plasmid encoding OPN substantially ameliorated the chronic course of EAE. These findings are in line with reports suggesting that production of auto-Ab against pro-inflammatory cytokines plays a physiologic role in down modulation of the inflammatory response¹².

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Conclusion. This is the first report highlighting the role of OPN in CIDP. Increase of OPN plasma levels is associated to a chronic disease evolution, being absent in a self-limiting disease like GBS and more frequent in CIDP sub-forms with aggressive evolution. Moreover, patients with CIDP show higher anti-OPN Ab levels. A further step would imply the characterization of outcome in relation to both OPN and anti-OPN Ab levels. Neither protein nor antibody levels seem to be influenced by the genetic background since we found no difference in OPN SNPs between patients and controls. PFR gene variations/mutations may represent a predisposing factor for CIDP development.

Dr G. Zhang

The University of Texas Medical School of Houston, TX

Examining antagonistic/synergistic effects of IVIg and erythropoietin (EPO) in a model of autoimmune neuropathy

OBJECTIVE: To examine the potential antagonistic/synergistic effects of IVIg and EPO in preclinical models of anti-ganglioside antibody (Ab)-mediated neuropathy and inhibition of axon regeneration. **BACKGROUND:** Anti-ganglioside antibodies (Abs) are commonly present in patients with Guillain-Barré syndrome (GBS). We and others have shown that intravenous immunoglobulins (IVIg) can prevent anti-ganglioside Abs mediated complement dependent nerve cell/fiber injury. Our recent studies suggest that erythropoietin (EPO) enhances nerve repair in preclinical models of GBS. IVIg is now the standard treatment for GBS and a new trial to examine the efficacy of EPO in GBS would have to be done as add on therapy with IVIg. It is necessary to demonstrate that EPO and IVIg do not have antagonistic effects. It would also be important to know whether these two medications have synergistic effects to design an effective administration schedule in patients with GBS. **DESIGN/METHODS:** Previously published preclinical models of autoimmune neuropathy mediated by anti-ganglioside Ab were used to examine the potential antagonistic/synergistic effects of IVIg and EPO. **RESULTS:** Our results show that individual treatments with either IVIg or EPO provide significant protection, i.e., postpone the anti-ganglioside Ab- and complement-mediated neural injury to the neurites and neuronal cell body, in the neurocytotoxicity assays. Notably, no antagonistic effects were seen with the combined use of IVIg and EPO in these assays. Our data suggest that EPO and IVIg have synergistic effects in protecting neuronal cells from this anti-ganglioside Ab-mediated complement-dependent injury. **CONCLUSIONS:** Our results support the conclusion that EPO can be useful add-on therapy to the current standard IVIg treatment for GBS. This novel combination therapy can be potentially beneficial for protecting nerve from injury during the acute phase and enhancing nerve repair in the recovery period in GBS patients. **Acknowledgements:** These studies were supported by GBS/CIDP Foundation and NIH/NINDS (NS42888 and NS54962).

1) Zhang, G., Bogdanova, N., Song, J., Sheikh, K. Synergistic effects of IVIg and erythropoietin (EPO) in preclinical models of autoimmune neuropathy. *Neurology* 2013; 80: P01.154.

Dr B.C. Jacobs

Erasmus MC, The Netherlands

Implementation of the International Guillain-Barre Syndrome Outcome Study (IGOS)

The International GBS Outcome Study (IGOS) is a worldwide prospective cohort study conducted by the International Neuropathy Consortium (INC) that aims to identify the clinical and biological determinants of disease progression and recovery in GBS. A website and database for inclusion of patients and data in this study was recently developed with support from the GBS-CIDP Foundation. We aim to include at least 1000 patients with GBS in IGOS. The grant was used to implement the study and we performed the following activities: (1) Neurologists from all over the world were approached to participate. At present (September 2013) more than 250 neurologists from 181 research centers from 18 countries are participating or preparing to participate. (2) The IGOS secretary supported these participants to obtain

approval from the local Institutional Review Boards (IRB). The current state is that 106 centers have IRB approval. (3) Information sheet for patients were translated in 8 foreign languages by the participant and official translation agencies. (4) We facilitated the data entry and conducted regularly data quality controls. (5) Maintenance and updates of the IGOS website and database (see www.gbsstudies.org). (6) Support with registration, transport and storage of the collected biomaterials. (7) Two meetings for the IGOS consortium were organized, which were held during the INC congress in Rotterdam, The Netherlands in July 2012, and during the PNS congress in Saint-Malo, France in June 2013. IGOS officially started in July 2012. At present 208 GBS patients are included from 14 countries including the UK (53 patients), USA (41 patients), Italy (25 patients), Denmark (25 patients), Netherlands (24 patients), Germany (13 patients), Spain (12 patients), Japan (6 patients), France (3 patients), Argentina (3 patients), Canada (3 patients), Belgium (1 patient), Australia (1 patient), and Malaysia (1 patient). Researchers from Bangladesh, Brazil, India and Taiwan also have shown interest to participate and are in the process of acquiring IRB approval. IGOS will result in the world's largest prospectively collected clinical database and biobank which will be used to understand the processes that determine disease cause, progression and recovery. These insights will form the basis to accurately predict the clinical course of individual patients with GBS and to develop new strategies to personalize therapy.

Dr. I.S.J. Merkies

Universiteit Maastricht, The Netherlands

Peripheral Neuropathy Outcome Measures Standardization (PeriNoms) Study

See above and attached report

2012

Dr C.A. Massaad

The University of Texas Health Science Center, Houston, TX

Comparing sialylated IgG and Fc fragments with IVIG in an anti-ganglioside antibody induced neuropathy model of GBS

High doses of Intravenous immunoglobulin (IVIG), requiring infusion over several days, are widely used for the treatment of Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). Recent studies indicate that autoantibody- and Fcγ receptor-mediated inflammatory cascade, is abrogated by a sialic acid-enriched minor populations present in commercial IVIG preparations. We have recently established that Fcγ receptor activation is critically involved in neural injury in a mouse model of GBS. In this study, we investigated the effects of sialic acid-enriched IVIG (seIVIG) and compared them with whole/native IVIG (nIVIG) and sialic acid-deficient IVIG (sdIVIG) in an established model of nerve repair in which anti-ganglioside antibodies significantly inhibit axon regeneration. We used lectin affinity-chromatography to generate various IVIG fractions and used them in a passive transfer model of anti-ganglioside antibody-mediated inhibition of axon regeneration. Behavioral, electrophysiological, and morphological studies showed beneficial effects of nIVIG and seIVIG on abrogating the adverse effects of anti-ganglioside antibodies on nerve repair. Notably, seIVIG was equally efficacious at a dose tenfold lower than the nIVIG. sdIVIG was ineffective in modulating anti-glycan antibody-mediated inhibition of nerve repair. Further, our lectin chromatographic studies indicate that various commercial IVIG preparations have up to 2-fold variation in seIVIG content. Our findings have translational implications as smaller amounts of IVIG and shorter infusion times are preferable because common side effects of IVIG relate with rate of infusion and amount administered. Shorter infusion times are likely to increase patient acceptance of repeated IVIG infusions necessary for the treatment of chronic immune neuropathies such as CIDP.

Dr E.P. Simpson

Methodist Neurological Institute, Houston, TX

CD4+CD25+ regulatory T cells as Potential Biomarkers of Pathogenesis and Response in Therapy in CIDP Patients

No work was completed on this project and the monies are being refunded.

Dr. L. Cousens

EpiVax, Inc., Providence, RI

Tregitopes: A Novel Immunomodulatory Therapy for CIDP

Regulatory T cells (Treg) suppress inflammation and therefore are critically important for resolution of autoimmune chronic inflammatory demyelinating polyneuropathy (CIDP). CIDP treatment focuses on inhibiting inflammation, and thus demyelination and secondary axonal loss. IVIG, an approved CIDP therapy, induces expansion of Treg in a number of human diseases and animal models. The efficacy of IVIG has been demonstrated in clinical trials, although the precise mechanism of action by which IVIG exerts its immunomodulatory effects is not clearly understood. Alternative therapies are needed, as IVIG cost and availability remain major barriers to meeting the medical needs of CIDP patients. In recent work, EpiVax has identified one trigger for Treg expansion, natural T regulatory cell epitopes (Tregitopes) contained in immunoglobulin G, and therefore in IVIG. Tregitope peptides have now been shown to replicate the effects of IVIG in a number of in vitro and in vivo models. The IVIG-like effects of Tregitopes have been validated by independent laboratories in mouse models of Type-1 Diabetes, multiple sclerosis, transplant rejection, allergy and asthma, thus confirming our primary observations. We propose Tregitopes as a potential substitute for IVIG in the treatment of certain autoimmune diseases. We reason that CIDP will also respond to Tregitope immune-modulating therapy, and that this may prove to be a more specific and accessible therapy relative to IVIG. We are studying the therapeutic application of Tregitopes to ameliorate disease in the mouse model of CIDP disease. These studies will provide critical insight into basic mechanisms by which Tregitopes engage Treg to induce tolerance and will define key parameters for Tregitope therapy in CIDP.