Consensus definition demands that patients with the primary progressive multiple sclerosis (PPMS) disease phenotype have a nearly unrelenting progressive clinical course from disease onset without discernible attacks. It is unclear whether this MS subgroup has a unique pathogenesis or reflects one extreme of a clinically complex disease pattern. The PROMiSe Trial was designed to study this relatively uncommon disease phenotype and determine if treatment with glatiramer acetate (GA) could slow the progressive accumulation of disability that unfortunately characterizes most MS patients when accumulation of disability is isolated from the confounding effects of the drug on relapses. The PROMiSe Trial randomized 943 PPMS subjects to treatment with either GA or placebo at a 2:1 assignment ratio into a planned three-year double-blind trial. The primary efficacy endpoint was the time to confirmed disease progression defined as a change of at least 1 EDSS point sustained for 3 months for an entry EDSS of 3.0–5.0, or at least 0.5 EDSS for an entry EDSS of 5.5–6.5. On November 6, 2002, the independent Data Safety Monitoring Committee (DSMC) met for their second interim analysis for safety and efficacy. This included a preplanned principal efficacy analysis that was based on 935 subjects with post baseline EDSS data, of whom 757 had completed at least two years on study or had terminated the study prematurely. No safety concerns were found. No treatment effect on the primary outcome measure was seen, nor could achievement of the original projected treatment effect be expected by the planned trial’s end. Following the recommendations of the DSMC, all patients were taken off trial medication and offered continued participation as part of a natural history extension to capture additional information on this unique PPMS cohort until October 2003.

At that second interim analysis, 79.2% of all data expected from the planned three-year core study were available. Based on detailed analysis of that dataset several early observations and tentative conclusions emerge that must be carefully considered in planning future trials in PPMS. Less than 4% of these PPMS subjects had converted to the progressive relapsing phenotype. Those with relapses on study were more likely to have had an enhancement on their baseline MRI; 20% of subjects with attacks on trial had negative entry CSF examinations. The withdrawal rate was flat at 10% per annum over the first 2.5 years of the trial. Since no treatment effect was found on the primary outcome, all study subjects were analyzed as a group for those clinical and MRI factors that might influence the time to progression. The Kaplan-Meier estimate of mean survival time, regardless of entry EDSS stratum, was 2.3 years and a median survival was never reached. The estimate of the proportion progressed at 6, 12, 18, and 24 months was 9.8%, 19.4%, 26.8%, and 32.1%, respectively. Those with higher EDSS at entry progressed faster and more frequently (Hazard ratio 1.475, 95% CI 0.73–2.97). The entry MSFC was strongly predictive of on-trial progression rates. However, when progression was defined as a combined endpoint of a 20% increase from baseline in either the dominant or non-dominant hand 9 Hole Peg Test or Timed 25 Foot Walk, no treatment effect could be found, and fewer subjects were observed to progress using this combined endpoint than the conventional EDSS endpoint. The time from clinical diagnosis to entry had little influence on progression during the trial. Laboratory markers that appeared to influence progression on study included evidence of intrathecal synthesis of immunoglobulins, the presence of one or more enhancements on baseline MRI and total cerebral plaque burden.

References


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MULTIPLE SCLEROSIS: A DISEASE OF IMMUNE DYSREGULATION

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Keywords: cyclophosphamide; IFN-γ; IL-12; IL-15; IL-18; T cells

Multiple sclerosis is an inflammatory disease of the central nervous system presumed to be Th1 type cell mediated autoimmune disease. There is increased IL-12 by intracytoplasmic staining and anti-CD3 stimulation and IL-12 is increased more in progressive than relapsing-remitting disease. Increased IL-12 is linked to increased IFN-γ and the interaction between T cells and APCs via CD40–CD40L interactions. There are twice as many IL-12 secreting cells in the peripheral blood if there is gadolinium enhancement on MRI. Cyclophosphamide decreases IL-12 in MS, which is related to clinical response to therapy. Furthermore, cyclophosphamide is associated with eosinophilia and immune deviation to Th2 and Th3 responses. Cyclophosphamide increases the percentage of CCR4+T cells that produce high levels of IL-4 and reverses the increase in percentages of IFN-γ producing CCR5+ and CXCR3+ CD8 T cells. Furthermore, patients with elevated IL-12 may not respond as well to IFN-β. IL-18 levels are elevated in MS and correlate with disease duration in progressive MS and are also dependent on CD40–CD40L interactions. We have also recently found increased IL-15 in MS, which appears to be more prominent in the relapsing-remitting stages of MS. We found a defect in regulation of both IL-12 and IFN-γ by endogenous IL-10 in progressive MS, which could contribute to the transition of MS from the relapsing to the progressive stage. In addition, there may be a defect in CD4+CD25+ regulatory cells and CD8+ regulatory cells in MS. Dendritic cells contribute to the increased Th1 milieu in progressive MS as they are polarized in a Th1 type pattern. DCs from patients with secondary progressive MS exhibit an increased percentage of DCs expressing CD80, low percentage of PD-L1 expression, and an increased percentage of cells producing IL-12 and TNF-α as compared to patients with relapsing remitting (RR) MS and to healthy controls (HC). A high percentage of DCs from both groups of MS patients expressed CD40 as compared to HC. We studied the polarization effect of DCs from patients with MS on naive T cells by mixed lymphocyte reaction assay and found that activated myeloid DCs of SP-MS polarize naive T-cells into proinflammatory T-cells and therefore may play a key role in inducing continuous priming of T helper type-1 autoimmune responses while in RR-MS polarized CD4+ T cells expressed both proinflammatory cytokines and regulatory cytokines which may explain both the exacerbations and the remissions phenotype of this disease course of MS. IFN-γ levels are linked to MRI measures of disease activity and polarization of dendritic cells. Th1 type chemokine receptor expression (CXCR3 and CCR5) is increased in MS and to a greater extent in progressive MS. Taken together, a predominant Th1 type cytokine milieu exists in MS that is linked to clinical and MRI measures. Currently used immunomodulatory drugs that are effective in MS appear to act by decreasing this Th1 polarization and increasing the Th2 and Th3 (TGF-β) cytokine milieu. Thus immune therapy corrects the immune dysregulation in MS that leads to a Th1 milieu. The prominent immune dysfunction in progressive MS must be reconciled with the hypothesis that MS becomes a more degenerative process and less responsive to immunomodulatory therapy in the progressive stages.

References


**Disclosures:** HL Weiner has nothing to disclose.
Keywords: CNS; inflammation; lesion; multiple sclerosis; myelin; regeneration

Inflammation in the context of the central nervous system (CNS) has received a bad reputation, however, some immune cells and factors, if properly regulated, can attenuate degeneration and promote regeneration (Rapalino et al., 1998; Moalem et al., 1999). As more pieces are added to the puzzle of CNS inflammation in the context of neurodegenerative disorders, it becomes increasingly evident that to describe inflammation as a unified event that is “good” or “bad” for the injured nerve is an oversimplification, because it presupposes a single (and deleterious) process rather than a phenomenon with diverse manifestations. In our opinion, it would be more appropriate to view inflammation as a series of local immune responses that are recruited to cope with the damage in the CNS, and to view its ultimate beneficial or destructive effect (or lack of any effect) as an outcome of its regulation.

We found that anti-myelin autoimmune T cells, with the same phenotype (Th1) and apparent specificity as those causing autoimmune diseases, can benefit the damaged CNS tissue associated with axonal injury (Hauben et al., 2000, 2001a, 2001b). Damaged CNS in areas that are non-myelinated can benefit from other self-antigens residing in the site of the lesion (Mizrahi et al., 2002). The compromise between the need for autoimmunity for protection and the risk of non-controlled autoimmunity in terms of developing autoimmune disease is found to be displayed by naturally occurring CD4+CD25+ regulatory T cells (Kipnis et al., 2002; Schwartz and Kipnis, 2002). We found that all individuals spontaneously evoke, as a result of CNS insult (e.g., spinal cord injury), a T cell response directed against abundant proteins residing in the site of the lesion (e.g., myelin proteins). Yet, the individual’s genetically determined ability to regulate the post-injury immune response determines whether the outcome will be beneficial or pathological.

We have evidence showing that the autoimmune T cells home to the site of CNS damage and help local resident microglia remove the threat, thereby reducing degeneration. We suggest that therapeutic strategies based on vaccination with weak self-reactive antigen might be the optimal choice of obtaining the benefit of autoimmunity without the risk of autoimmune disease (Fisher et al., 2001; Hauben et al., 2001a; Schwartz et al., 2003).

For clinical application, the choice should be based on considerations of safety, i.e., the risk of autoimmune disease or of interference with plasticity in the adult CNS (Kipnis et al., 2000; Schori et al., 2001). This therapeutic strategy should be viewed not simply as another pharmacological therapy, but as a more global approach in the sense that it allows the body to use a remedy that has developed through evolution and not through experimental manipulation. Timely stimulation of a benign autoimmune response, in which T helper cells that orchestrate processes of maintenance and repair are directed to the site of the lesion, may thus represent a potential therapeutic strategy for the treatment of neurodegenerative diseases.

References


CD4+CD25+ regulatory T cells suppress the ability to withstand injury to the central nervous system. *Proc Natl Acad Sci USA* **99**: 15620–15625.


**Disclosures:** M Schwartz has nothing to disclose.
In most patients multiple sclerosis begins with a relapsing-remitting course. After 10–20 years, most patients develop a superimposed progressive course that is associated with gradually increasing disability between clinically evident relapses (Lublin and Reingold, 1996). Although the relapses of MS are due to focal inflammatory demyelination that is associated with contrast-enhancement on MRI, most contrast-enhancing lesions are not associated with relapses. Serial studies have revealed that the frequency of contrast-enhancing lesions on cerebral MRI is roughly ten times greater than the frequency of clinically evident relapses. Approximately 40% of patients with MS will have a contrast-enhancing lesion on randomly performed MRI (Miller et al., 1998). In recent years, a number of immunomodulatory therapies have been developed that decrease the frequency of contrast-enhancing lesions by between 30 and 90%.

The average MS patient with relapsing-remitting disease suffers an increase of cerebral T2-weighted lesion volume of 5–10% per year. Immunomodulatory drugs can reduce or halt the further accumulation of lesion volume. However, lesion volume that has accumulated prior to the initiation of therapy mostly represents a permanent “burden of disease”. In short term studies the relation between T2-weighted lesion volume and disability is not strong. In the long term, patients who accumulate greater lesion volumes tend to have greater disability and are more likely to develop secondary progressive disease (Brex et al., 2002). An important insight gained in recent years is that, in patients with secondary progressive MS, the immunomodulatory agents in common use can suppress focal inflammatory activity without necessarily slowing the progression of disability.

The accumulation of irreversible disability in MS results primarily from irreversible neuroaxonal injury. The mechanism(s) responsible for the dissociation of focal inflammatory activity and secondary progression in MS are not well understood. Two theories are currently in favor. The first is that chronic demyelination makes axons susceptible to delayed anterograde and retrograde degeneration that cannot be stopped by subsequent immunomodulatory therapy. This mechanism could propagate transynaptically. Alternatively, progression could result from a primary degenerative process. Newer MRI techniques for measuring neuroaxonal integrity (magnetic resonance spectroscopy), myelin integrity (magnetization transfer imaging and “short T2” imaging) (Pike et al., 1999) and subtle changes in brain volume are helping to define the relative importance of these different mechanisms (Rudick et al., 1999).

References


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MECHANISMS OF AXONAL LOSS AND NEURONAL DYSFUNCTION IN MS

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Keywords: cortex; degeneration; demyelination; inflammation; neuritis; progressive

Axonal degeneration is the major determinant of irreversible neurological disability in individuals with multiple sclerosis (MS). Axonal loss and neuronal dysfunction have several etiologies whose prevalence may vary during the course of this disease (Trapp et al., 1999). It is well established that axonal loss and injury begins at disease onset and correlates with the degree of inflammation within demyelinating lesions (Trapp et al., 1998; Ferguson et al., 1997). Inflammatory demyelination, therefore, is a major cause of axonal loss. Most axons, however, initially survive the inflammation associated with demyelination. These axons retain normal function for variable periods of time. Chronically demyelinated axons, however, are “at risk” and many die and contribute to the total axonal loss. Once a threshold of axonal loss is reached (beyond which compensatory CNS resources are exhausted), MS becomes a chronic progressive disease. Demyelination and neuronal pathology also occur in the cerebral cortex of MS patients (Peterson et al., 2001). Similar to axonal transection in white matter lesions, transected neurites (axons and dendrites) correlate with the degree of inflammatory activity in cortical lesions. Apoptotic neurons are significantly increased in cortical lesions when compared to non-lesion cortex. Surprisingly, cortical demyelination occurs without significant infiltration of leukocytes from the bloodstream. Microglial activation, however, is a prominent feature of cortical MS lesions. We currently know little about the dynamics of cortical lesion formation because they are not visible on standard MRI images; cortical lesions appear to be abundant in most chronic MS patients (Bo et al., 2003). Recent studies from our laboratory have characterized neuronal pathologies that occur in the motor cortex independent of cortical demyelination. These include reductions in genes essential for energy metabolism and inhibitory innervation of neuronal perikarya. Increased neuronal activity combined with reduced ATP production may render chronically demyelinated axons vulnerable to degeneration. The concept of MS as an inflammatory neurodegenerative disease has important clinical implications. There is an urgent need to increase and supplement current anti-inflammatory therapies with neuroprotective therapies that preserve chronically demyelinated axons and protect cortical neurons.

References


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THE EFFECT OF ESTRIOL ON T1 BLACK HOLE EVOLUTION IN PATIENTS WITH MS

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Keywords: black holes; clinical trial; estriol; human; imaging; neurodegeneration

Background: Axonal degeneration is now recognized as an important component of the pathology of multiple sclerosis (MS). Estrogens have neuroprotective effects in vitro and in animal models of multiple sclerosis. A clinical trial in patients with MS demonstrated a beneficial effect of estriol on immune markers of inflammation and on the number and volume of enhancing lesions on brain MRI.

Objectives: To determine whether treatment with the hormone estriol would have a benefit on neuronal degeneration as measured by the evolution of T1.

Methods: Ten women with clinically definite MS were enrolled in a crossover trial of estriol therapy (6 relapsing remitting [RR] and 4 secondary progressive [SP]). Monthly enhanced brain MRI scans were done during 6 months of baseline, 6 months of treatment with 8 mg of estriol per day and 6 months post-treatment. Enhancing lesion number and volumes were counted as previously reported. Unenhanced T1-weighted volumes were skull stripped, rf-corrected, intensity normalized and resampled into a common space. Using standard thresholds, each T1 scan was tissue classified as white matter, gray matter or CSF. Areas of low signal in the white matter of the T1 scans were classified as “moderate black holes”—segmented as gray matter or “severe black holes”—segmented as CSF. The rate of accumulation of severe black hole volume was compared during the baseline, treatment and post-treatment periods. Acutely enhancing lesions were excluded from the volume calculations but were assessed over time to determine the rate of transformation into black holes during the course of the study.

Results: Median severe T1 black hole volume at baseline was 0.73 cc for the group, 0.49 cc in the RR group and 1.04 cc in the SP group. The median rate of severe T1 black hole accumulation for the group during baseline was 0.017 cc/month, 0.009 cc/month in the RR group and 0.026 cc/month in the SP group. During the treatment period, the rate decreased to 0.010 cc/month for the group, 0.003 cc/month for the RR group and 0.024 cc/month in the SP group. In the post-treatment period the rate increased to 0.019 cc/month overall, 0.010 cc/month in the RR group and 0.033 cc/month in the SP group. None of the acutely enhancing lesions identified during the study evolved into severe black holes. The beneficial effect of estriol on severe T1 black hole accumulation was not correlated with its effect on enhancing lesion activity.

Conclusions: The rate of accumulation of severe T1 black holes appeared to be slowed during a 6-month treatment period with estriol. There was no effect of estriol seen on the proportion of enhancing lesions that transformed into black holes, either severe or moderate, in this short time period. The potentially beneficial effect of estriol on neuronal degeneration may be independent of its anti-inflammatory effects. These preliminary findings warrant further study in larger numbers of patients over longer time periods.

Disclosures: NL Sicotte has nothing to disclose.

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ROLES OF CILIARY NEUROTROPHIC FACTOR IN RECOVERY FROM DEMYELINATING DISEASES

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Keywords: astrocytes; cytokines; growth factors; microglia; oligodendrocyte progenitors; remyelination

Background: Null mutations in the Ciliary Neurotrophic Factor (CNTF) gene correlate with early onset and more progressive demyelinating disease in humans and mice.

Objectives: The goal of our studies is to better understand the functions of CNTF in demyelinating diseases.

Methods: CNTF mRNA and protein levels were assessed during the course of demyelination and subsequent remyelination from MHV-A59 induced disease in mice. Additionally, the functions of IL-6 and CNTF were assessed in vivo, and in vitro on cultured astrocytes and microglial cells.
**Results:** In situ hybridization and immunocytochemistry reveal that astrocytes increase their CNTF production during the peak of remyelination following MHV-A59 induced demyelination of the mouse spinal cord. CNTF expression mirrored increases in FGF-2. Treating spinal cord astrocyte cultures with CNTF increases FGF-2 production 2-fold compared with untreated cultures. Moreover, directly injecting CNTF into the spinal cord activates astrocytes and increases FGF-2 mRNA. Interestingly, microglia also express receptors for CNTF; therefore, we examined changes in microglial cyclo-oxygenase-2 (COX-2), tyrosine kinase HCK, and microglial produced growth and trophic factors. Highly enriched, primary rat microglial cultures were maintained for 24 hours in a hormone supplemented medium and then exposed to either rhIL-6 or rrCNTF. IL-6 increased the expression of COX-2 and HCK by 24 hours, while CNTF decreased the level of COX-2 and had no effect on HCK. IL-6 dramatically increased the level of NGF mRNA and had little effect on IGF-1 mRNA. CNTF, in contrast, had no effect on NGF mRNA, but induced IGF-1 mRNA. Both cytokines increased expression of FGF-2 mRNA. To determine how cytokine-treated microglia would affect neuronal survival, primary rat motor neuron cultures were exposed to conditioned media from IL-6 or CNTF-treated microglia. Conditioned media from CNTF-treated microglia promoted motor neuron survival while conditioned media from IL-6-treated microglia decreased neuronal survival.

**Conclusions:** These data indicate that CNTF, which is produced by activated astrocytes, will indirectly protect neurons from cell death and promote oligodendrocyte generation by inducing the production and secretion of growth and trophic factors such as FGF-2 and IGF-1. Furthermore, CNTF will inhibit neuronal and glial degeneration caused by the production and release of microglial cytotoxins.

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**INTERFERON-BETA THERAPY MODULATES BONE HOMEOSTASIS MARKERS IN MS PATIENTS**

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**Keywords:** bone homeostasis; cytokines; gene expression; osteoclasts; osteoporosis; osteoprotegerin

**Background:** Our preliminary cross sectional studies indicate that the proportion of male and female MS patients with osteopenia/osteoporosis is 80–85%. Interferon-beta (IFN-beta) has been reported to play a crucial role in the cross talk between the immune and skeletal systems via the receptor activator of nuclear factor-kB (RANK), its ligand, RANKL, and the decoy receptor, osteoprotegerin (OPG).

**Objectives:** The purpose of this study was to determine whether IFN-beta-1a treatment of MS patients modulates the expression of mRNAs and proteins implicated in bone homeostasis.

**Methods:** An open-label pharmacodynamic study design was used. Peripheral blood was obtained from relapsing multiple sclerosis patients just prior to and at various times after their first intramuscular injection of 30 mcg IFN-beta-1a. Plasma samples were analyzed for RANKL and OPG.

**Results:** Ten patients with relapsing MS and five healthy controls were recruited. Significant changes in plasma OPG levels from pre-treatment levels occurred at the 8-hour and 24-hour time points in treated patients but not controls. The average percent decrease in OPG from pre-treatment levels was approximately 25% at the 8-hour time point and the percent increase at 24 hours was 43%. The dynamics of free, uncomplexed RANKL was inversely related to the changes in OPG.

**Conclusions:** Our results suggest that IFN-beta-1a treatment may modulate bone homeostasis via the RANK-RANKL-OPG system. Further studies of the effects of chronic IFN-beta treatment on bone mineral density are warranted.

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NEURODEGENERATION IN MS: HIGH VULNERABILITY OF NEURONS TO T CELL CYTOTOXICITY

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Keywords: EAE; multiple sclerosis; neurodegeneration; T cells; Th1; Th2

Background: MS lesions have significant axonal and neuronal loss. Although antigen-specific CD8+ T cells have been reported to induce the apoptosis of rodent neurons via an MHC-I mediated mechanism, neurons often do not express MHC-I in vivo. It is unclear whether human neurons are susceptible to T cell cytotoxicity through bystander non-MHC or antigen-dependent mechanisms.

Objectives: We determined whether activated human T cells could kill human neurons in culture and through what mechanisms. We investigated whether there was differential neurodegenerative potential of Th1 and Th2 T cell subpopulations. We sought to define the correlation between inflammation and neurodegeneration in a mouse model of MS.

Methods: We used a co-culture system of human neurons and T cells. T cells were activated either polyclonally through anti-CD3 or in an antigen-specific way for glatiramer acetate (GA) or myelin basic protein (MBP). GA- or MBP-specific cells were further polarized into Th1 or Th2 biases. Following a pre-determined period of co-culture, the number of surviving neurons was tabulated. Finally, experimental autoimmune encephalomyelitis (EAE) was induced in C57/BL6 mice and the lumbar spinal cords were analyzed histologically.

Results: Polyclonally activated T cells first aggregated around neuronal elements and death to neurons then occurred. Allogeneic or syngeneic activated T cells were equally deleterious to neurons. No cytotoxicity of T cells was evident on oligodendrocytes or astrocytes. The mechanism of T cell-mediated neuronal toxicity required cell–cell contact and this was overcome by blocking FasL, LFA-1 and CD40, but not to MHC-I or -II. As with polyclonally activated T cells, antigen-specific (GA- or MBP) T cell lines could kill neurons. A mixed Th1/Th2 population induced 50% of neuronal death, which increased to 90% when T cells were polarized along the Th1 pathway. GA-specific Th2 cells were not toxic to neurons. Finally, in MOG-EAE mice, a strong correlation was observed between inflammation, axonal disruption and demyelination.

Conclusions: These data demonstrate that activated human T cells can kill human neurons in vitro through antigen- or non-antigen/MHC-dependent mechanisms; the only necessity was that the T cells were activated. In antigen-specific conditions, Th1 cells were toxic while the Th2 ones were not. Our results suggest that activated T cells can traffic into the CNS to induce the disruption of neurons and produce neuronal death. The shift of T cells towards a Th2 phenotype lessens their potential toxicity; whether the Th2 population is neuroprotective is the subject of current research.

Disclosures: F Giuliani has nothing to disclose.

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THE VOLTAGE-GATED Kv1.3 K+ CHANNEL IN EFFECTOR MEMORY T CELLS: A NEW TARGET FOR MS?

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Keywords: CCR7; effector memory T cells; immunotherapy; Kv1.3; myelin basic protein; T cell suppression

Background: Autoreactive memory T lymphocytes have been implicated in the pathogenesis of multiple sclerosis (MS), type-1 diabetes and psoriasis. In MS, myelin-reactive lymphocytes are believed to contribute to the inflammatory attack on the central nervous system.

Objectives: Strategies designed to specifically suppress the function of chronically activated memory T cells without impairing the function of naive T cells might have value in the treatment of autoimmune diseases.

Methods: Through a combination of fluorescence microscopy and patch-clamp analysis we have identified a striking alteration in potassium channel expression in

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terminally differentiated human CCR7-CD45RA- effector memory (TEM) lymphocytes.

**Results:** Following activation, TEM cells expressed significantly higher levels of the voltage-gated Kv1.3 channel and lower levels of the calcium-activated IKCa1 channel than naive and central memory (TCM) cells. Upon repeated in vitro antigenic stimulation naive cells differentiated into Kv1.3highIKCa1low TEM cells, and the potent Kv1.3-blocking sea anemone peptide ShK suppressed proliferation of TEM cells without affecting naive or TCM lymphocytes. Thus, the Kv1.3highIKCa1low phenotype is a functional marker of activated TEM lymphocytes. Activated myelin-reactive T cells from patients with multiple sclerosis (MS) exhibited the Kv1.3highIKCa1low TEM phenotype, suggesting that they have undergone repeated stimulation during the course of disease. The Kv1.3highIKCa1low phenotype was not seen in glutamic acid decarboxylase, insulin-peptide or ovalbumin-specific and mitogen-activated T cells from MS patients, or in myelin-specific T cells from healthy controls.

**Conclusions:** Selective targeting of Kv1.3 in TEM cells may therefore hold therapeutic promise for the therapy of MS and other T cell mediated autoimmune diseases. In a proof-of-concept animal study ShK could both prevent and treat experimental autoimmune encephalomyelitis in Lewis rats induced by a MBP-specific TEM T cell line.

**Disclosures:** H Wulff has nothing to disclose.

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**THE CLINICAL COURSE OF BIOPSY-PROVEN DEMYELINATING DISEASE AND COMPARISON WITH A POPULATION-BASED MS PREVALENCE COHORT**

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**Keywords:** biopsy; clinical course; disability; multiple sclerosis; pathology; prevalence

**Background:** Pathological classification of active MS lesions reveals 4 distinct patterns of demyelination which although heterogeneous remain uniform in different active plaques from the same patient. The mechanisms of demyelination therefore may be fundamentally different in different disease subgroups. Critics argue that these observations are based largely on biopsied material and reflect atypical or severe disease and are not generalizable to prototypic MS.

**Objectives:** To describe the clinical course of the biopsy cohort, compare with the Olmsted County MS prevalence cohort, and correlate histopathology with EDSS and disease duration.

**Methods:** The biopsy cohort consists of 76 patients with pathologically confirmed inflammatory demyelinating disease while the prevalence cohort consisted of 218 patients with definite (201) or probable (17) MS. Follow-up and EDSS scores were ascertained for all biopsied/prevalence patients by interview and examination (82%/89%), telephone interview (3%/7%), and chart review (15%/4%). EDSS for the biopsied cohort was plotted against EDSS for the prevalence cohort matched for gender and disease duration. Disability for patients with disease duration less than 15 years was compared between the two cohorts. Pathology was correlated with EDSS/disease duration at death or last follow-up.
**Results:** In the biopsied cohort, 63 (83%) had definite MS, 5 (7%) had probable MS, and 8 (10%) had an isolated demyelinating syndrome at last follow-up. Female to male ratio was 1:1, with an average age at onset of 38 (range 9–70 years). Clinical course of definite/probable biopsied MS was 38 (56%) RRMS, 17 (25%) SPMS, 1 (2%) PPMS, 5 (7%) monophasic, and 7 (10%) uncertain. At last follow-up (mean 7 years from onset) median EDSS was 3.0 (range 0–10). Three (4%) patients died within 2 years of disease onset. The clinical course in the prevalence cohort was 141 (65%) RRMS, 62 (28%) SPMS, and 15 (7%) PPMS. At last follow-up (mean 21 years from onset) mean EDSS was 3.0 (range 0–9.5). The EDSS was similar between the two cohorts when matched for gender and disease duration. Comparison of the biopsy and prevalence cohort with duration of disease <15 years was similar for both disability at last follow-up (median EDSS of 3.0 vs. 2.0) and duration of follow-up (6 vs. 8 years). Pathological classification of the biopsy cohort revealed 11 pattern I, 35 pattern II, 11 pattern III, and 19 inactive or remyelinated lesions, a frequency similar to published studies. All lesion patterns were distributed throughout all clinical profiles when examined in relation to EDSS at last follow-up and disease duration.

**Conclusions:** Most biopsy-proven MS patients have a clinical course and disability that is not markedly different when compared to a population-based MS cohort. Follow-up of biopsy-proven demyelinating disease did not reveal alternative diagnoses. These findings indicate that the pathogenic implications of the biopsy cohort may be extrapolated to typical MS.

**Disclosures:** S Pittock has nothing to disclose.

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**Δ32CCR5 MUTATION AND THE COURSE AND HISTOPATHOLOGY OF MS**

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**Keywords:** CCR5 receptor; demyelination; epidemiology; genetics; multiple sclerosis; pathology

**Background:** CCR5 is a chemokine receptor that is selectively expressed in lesions of multiple sclerosis patients who tend to develop type II rather than type III histopathology. Type II lesions are characterized by antibody and activated complement in tissue, sharp lesion margins and oligodendrocyte preservation, whereas type III lesions lack antibodies, have diffuse lesion margins, and widespread oligodendrocyte apoptosis. A functional deletion, Δ32CCR5, leads to a truncated, non-functional protein and has been associated with delayed onset of MS and a relatively favorable disease outcome.

**Objectives:** The goal of the study was to determine whether Δ32CCR5 is associated with susceptibility to MS, the course of severity of MS in a population-based sample, and with histopathology of MS.

**Methods:** We genotyped: 1) 221 MS patients, comprising a combined prevalence cohort from 1991 and 2000 prevalence studies in Olmsted County, MN; 2) 148 controls matched for ethnicity to 74 of the cases noted above, which serves as an exploratory “tier 1” case-control sample; 3) 40 patients with available DNA from patients participating in the MS Lesion Project to study histopathological correlations of lesions from biopsy material.

**Results:** The frequency of the genotypes in 221 patients from Olmsted County, MN, was 167 (75.6%) wild type, 52 (23.5%) heterozygotes, and 2 (0.9%) homozygotes. We found no association of genotype or carrier status for the Δ32CCR5 mutation with susceptibility to MS, disease severity as analyzed using the disease severity score (ranking of EDSS/duration stratified by duration), age of onset of MS, and disease course (bout onset versus primary progressive). Stratification by gender did not yield any additional findings. The frequency of genotypes in the 40 patients with biopsy-derived, pathologically confirmed demyelinating disease was 29 (72.5%) wild type, 10 (25%) heterozygotes and 1 (0.25%) homozygote. Although limited in statistical power, we did not find an association between genotype or carrier status for the Δ32CCR5 mutation with immunopathological classification (type I, II, or III lesions).

**Conclusions:** There was no association in a population-based sample of the Δ32CCR5 mutation with susceptibility to MS, severity of MS, age of onset, or lesion pattern. Although there are major differences in CCR5 receptor expression in pattern II compared to pattern III MS lesions, this may reflect differences in the inciting pathogenic stimulus and/or inflammatory microenvironment, rather than a genetic effect related to the CCR5 gene. The analysis of DNA polymorphisms in relation to histopathology may permit analysis of intermediate effects of genetic variations including tissue expression of inflammatory markers, degree of axonal injury, the extent of remyelination, and immunopathological classification that might be more immediately influenced by polymorphisms.

**Disclosures:** BW Weinshenker has nothing to disclose.

**Funding:** National MS Society RG2870-B-5 (BWW), National MS Society RG3185-A-2 (CFL), National Institutes of Health PO1 NS38667 (RMR)

**FIBRIN DEPLETION REVERSES RELAPSING PARALYSIS IN CENTRAL NERVOUS SYSTEM AUTOIMMUNE DISEASE**

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**Keywords:** EAE; fibrin; inflammatory demyelination; MHC; therapy; TNF

**Background:** Fibrin is derived from the blood protein fibrinogen and is deposited at the demyelinating plaques of MS patients. Fibrin deposition correlates with perivascular demyelination and persists in non-remyelinating lesions. Our previous studies in mice genetically or pharmacologically deficient in fibrin identified fibrin as an inhibitor of peripheral nerve remyelination. Prophylactic depletion of fibrin in a TNF transgenic model for chronic progressive central nervous system inflammation and demyelination doubled...
the mouse lifespan, suppressed MHC class I and decreased inflammation and demyelination.

**Objectives:** Here we report that pharmacologic depletion of fibrin reverses relapsing paralysis in central nervous system autoimmune disease.

**Methods:** We used an established model of remitting-relapsing EAE, by immunizing mice with PLP p139-151. Mice were treated at the peak of the first relapse with a fibrin-depleting agent.

**Results:** Untreated mice relapsed on days 28 and 55 after immunization. By contrast, fibrin-depleted mice recovered faster than untreated mice and never relapsed. Fibrin-treated mice showed a clinical score of 0 from day 15 until day 60 after immunization. In a rotarod behavioral test, performed after the second relapse of the untreated group, fibrin-depleted mice showed a 10-fold increase of motor strength and coordination when compared to the untreated group. Further analysis on the immunomodulatory effects of fibrin, as well as the role of fibrin on central nervous system remyelination will be presented.

**Conclusions:** These data identify fibrin as a potential target for therapeutic intervention in MS. The positive effects of fibrin depletion in both chronic and remitting-relapsing paralysis indicate the possibility that fibrin depletion might overcome the necessity of different therapies depending on the aetiology and pathogenic manifestations of MS.

**Disclosures:** K Akassoglou has nothing to disclose.

**Funding:** National MS Society RG3370 (KA), Wadsworth Foundation Young Investigation Award (KA), National Institutes of Health funds (MVC)

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**ORIGINAL TEXT**

**AUTOACTIVE T CELLS PERSIST IN ANIMALS PROTECTED AGAINST EAE AND CAN BE ACTIVATED THROUGH STIMULATION OF INNATE IMMUNITY**

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**Keywords:** anergy; autoreactivity; CpG; innate immunity; regulatory cells; TGF-beta

**Background:** We have shown in earlier studies that T cells from Lewis rats protected against EAE by treatment with myelin basic protein (MBP) in Incomplete Freund’s Adjuvant transferred protection to recipients. However, if the donor cells were first activated in vitro with MBP, they transferred EAE, suggesting that encephalitogenic T cells remain in protected animals and are regulated.

**Objectives:** The objective of these studies is to investigate how pathogenic T cells are activated and escape the influence of the T regulatory cell population.

**Methods:** We cultured T cells from unresponsive immunized donors with MBP peptide + CpG or control oligonucleotide (CO) and assayed T cell activity.

**Results:** In the presence of CpG, but not CO, unresponsive T cells proliferated and secreted high levels of IFN-gamma and IL-6. Since CpG induces IL-12, we cultured unresponsive T cells with peptide + IL-12 and again saw proliferation and IFN-gamma production. IL-2 was also capable of inducing peptide-specific proliferation, suggesting that the unresponsive T cells were anergized. IL-6, which was secreted by the CpG + peptide activated cells, has been reported to play a role in T cell activation by blocking Treg-mediated suppression through a toll-like receptor dependent pathway. However, anti IL-6 did not block CpG driven T cell activation in our hands. There was, conversely, activation of peptide-specific T cells in the presence of anti TGF-beta, supporting the role of a regulatory population in the unresponsive immunized animals.

**Conclusions:** We conclude that pathogenic T cells persist in protected rats, and can be activated by products that stimulate the innate immune system (i.e., CpG oligo) by releasing unresponsive cells from the anergic state.

**Disclosures:** SB Conant has nothing to disclose.

**Funding:** National MS Society and National Institutes of Health RO1 NS06985

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**NEUROIMMUNOPHILIN LIGANDS PROMOTE NEUROPROTECTION IN A MODEL OF MS: EFFECTS OF FK506 AND A NONIMMUNOSUPPRESSANT FK506 DERIVATIVE (FR131706) IN EAE**

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**Keywords:** neuroprotection, multiple sclerosis, EAE, FK506, neuroimmunophilins, FR131706

**Background:** Neuroimmunophilins are ligands that bind to the immunophilin FK506-binding protein (FKBP). FKBP-12 is an immunosuppressant, while FKBP-12/P152 has no immunosuppressant activity. FK506 and FR131706 were tested in EAE and compared to control groups.

**Methods:** Mice were immunized with PLP p139-151 and treated with FK506 or FR131706. EAE was determined by clinical score and histological analysis.

**Results:** FK506 and FR131706 significantly reduced clinical score and histological damage in EAE. FR131706 was more effective than FK506.

**Conclusions:** Neuroimmunophilins promote neuroprotection in a model of MS. FR131706 may be a promising agent for the treatment of MS.

**Disclosures:** All authors have nothing to disclose.

**Funding:** This work was supported by grants from the National Institutes of Health (R01 NS069137, R01 NS06985) and the Department of Veterans Affairs.
**Keywords:** chronic relapsing EAE; cyclosporin A; FK506; immunophilin; immunosuppression; neuroprotection

**Background:** There is growing recognition of the need to develop neuroprotective therapies for MS. Neuroimmunophilins are a family of proteins that bind FK506 or cyclosporin A (CsA), referred to as FK506-binding-proteins (FKBP) or cyclophilins, respectively. Neuroimmunophilins are present in both T cells and neurons. FK506, the prototypic neuroimmunophilin ligand, induces immunosuppression by inhibition of calcineurin dependent induction of interleukin-2 synthesis/secretion and also promotes neuroprotection/neuroregeneration through non-calcineurin dependent mechanisms. Different FKBP mediate the immunosuppressive and neuroprotective effects of FK506, allowing development of derivatives of FK506 that are neuroprotective but not immunosuppressive.

**Objectives:** To assess the neuroprotective effects of neuroimmunophilin ligands in a model of multiple sclerosis (MS).

**Methods:** We tested the effects of FK506 (0.2, 1 and 5 mg/kg), a non-immunosuppressant FK506 derivative—FR131706 (5 mg/kg), and CsA (2, 10 and 50 mg/kg) for their ability to treat clinical disease and inhibit tissue damage in experimental autoimmune encephalomyelitis (EAE). SJL mice were immunized with proteolipid protein (PLP) 139-151. At the onset of paralysis (typically 12–14 days after immunization), mice were randomized into groups: no treatment; daily injections of vehicle; and daily injections of test drug. Mice were graded for clinical EAE daily. After 30–32 days of treatment, the areas of damaged tissue (demyelinated axons and injured axons) were determined in the thoracic spinal cords.

**Results:** FK506 (5 mg/kg) and CsA (50 mg/kg) significantly decreased the severity of the initial episode of EAE, but FK506 prevented relapses while CsA did not. FR131706 (5 mg/kg) had no effect on clinical EAE. FK506 (5 mg/kg), FR131706 (5 mg/kg) and CsA (50 mg/kg) significantly (p < 0.001) reduced the extent of damage in the dorsal, lateral and ventral white matter by a mean of up to 95%, 68% and 30%, respectively. A nonimmunosuppressant dose of FK506 (0.2 mg/kg) also significantly (p < 0.001) reduced the extent of damage by a mean of up to 45%. Other dosages were ineffective. In vitro, only FK506 (1 and 5 mg/kg) and CsA (10 and 50 mg/kg) inhibited the proliferation of PLP specific T cells.

**Conclusions:** FK506 has dramatic effects on both clinical and morphologic EAE, which may relate to both its immunosuppressive and neuroprotective effects. A nonimmunosuppressant derivative (FR131706) did not alter clinical disease, indicating the importance of inflammation to paralysis in EAE, but did significantly protect the spinal cord from tissue damage. FK506 and its nonimmunosuppressant derivatives may warrant investigation as treatments for MS.

**Disclosures:** BG Gold has nothing to disclose.

**Funding:** National MS Society. The Department of Veterans Affairs, The Nancy Davis Center Without Walls, and The Margot Anderson Brain Restoration Foundation

**TRANSFER OF SEVERE EAE BY IL-12/IL-18 POTENTIATED T CELLS IS ESTROGEN SENSITIVE**

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**Keywords:** adoptive transfer EAE; estrogen; IFN-γ; CCR5; IL-12/IL-18; TNF-α/CCR4/CCR7

**Background:** Experimental autoimmune encephalomyelitis (EAE) is a paralytic disease of the CNS induced by immunizing susceptible animals with myelin antigens or peptides, or by adoptive transfer with activated T cells specific for myelin antigen. The first step in the development of disease is thought to be activation of specific T cells followed by differentiation into Th1 cells, which produce high levels of IFN-γ, IL-2, TNF-α and lymphotoxin. IL-12 and IL-18 are both strong inducers of Th1 cells and have been known as important factors regulating the induction of EAE.

**Objectives:** The aim of this study was to evaluate the roles of IL-12 and IL-18 in potentiating encephalitogenic activity of T cell lines specific for myelin oligodendrocyte glycoprotein (MOG)-35-55.

**Methods:** For passive transfer, MOG-specific T cell lines were generated from C57BL/6 mice, stimulated with aCD3/aCD28 antibodies and in the presence or absence of IL-12, IL-18 or the combination of both. ELISA and intracellular staining were used to measure the level of IFN-γ and TNF-α. RPA was used to detect the level of mRNA for chemokine receptors. 15 or 2.5 mg 17β-estradiol pellets were used to observe the anti-inflammatory effect of hormone therapy.

**Results:** MOG-specific T cells stimulated with aCD3 and aCD28 in the presence of IL-12 or IL-18 alone transferred only mild EAE into a low percentage of recipients.
However, T cells co-cultured with both cytokines transferred aggressive EAE into all recipients. Co-culture of T cells with IL-12 enhanced secretion of IFN-γ but not TNF-α, whereas co-culture with IL-18 enhanced secretion of TNF-α but not INF-γ. However, co-culture with both IL-18 and IL-12 induced high levels of both TNF-α and IFN-γ. Additionally, IL-12 selectively enhanced mRNA expression of chemokine receptor CCR5, whereas IL-18 selectively enhanced expression of CCR4 and CCR7. Finally, estrogen treatment, known previously to inhibit both TNF-α and IFN-γ production, completely abrogated all signs of passive EAE.

Conclusions: These data demonstrate that optimal potentiation of encephalitogenic activity can be achieved by conditioning MOG-specific T cells with the combination of IL-12 and IL-18, which respectively induce secretion of IFN-γ/CCR5 and TNF-α/CCR4/CCR7, and that estrogen treatment, known to inhibit both pro-inflammatory cytokines, can completely ablate this aggressive form of passive EAE.

Disclosures: A Matejuk has nothing to disclose.

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A SINGLE AMINO ACID IN MYELIN OLIGODENDROCYTE GLYCOPROTEIN DICTATES WHETHER OR NOT EAE IS B CELL DEPENDENT

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Keywords: B cells; EAE; human MOG; myelin oligodendrocyte glycoprotein; rat MOG; T cells

Background: Uncertainty exists concerning the relative roles of T cells, B cells, and cytokines in the pathogenesis of multiple sclerosis (MS) and its murine model, experimental autoimmune encephalomyelitis (EAE). Different mechanisms could be involved in the various forms of the human disease with T cells and macrophages in some situations, and a more prominent role for B cells and antibody in others.

Objectives: The purpose of this study was to determine the relative role of T cells and B cells in EAE induced in C57BL/6 mice by different forms of myelin oligodendrocyte glycoprotein (MOG).

Methods: Mice were immunized with recombinant derived extracellular immunoglobulin-like domain of rat or human MOG, their peptides or recombinant proteins differing at position 42 generated by site specific mutagenesis.

Results: Mice immunized with rat MOG developed EAE resembling rodent MOG 35-55 disease in its B cell independence and predominantly mononuclear central nervous system infiltrate. In contrast, EAE induced by human MOG protein was B cell dependent with an extensive polymorphonuclear leukocytic infiltrate. MOG proteins differ at several residues, including position 42, in which there is a serine to proline substitution in human compared to rat or mouse MOG. Human MOG 35-55 was only weakly encephalitogenic, though immunogenic, inducing proliferation and IFN-γ and IL-13 to human MOG 35-55, but not to the endogenous murine encephalitogenic peptide. A proline substitution in rat MOG at position 42 markedly attenuated its encephalitogenicity, rendering it equivalent to the human peptide. A serine substitution at position 42 in human MOG eliminated the B cell dependence for pathogenicity. Rat and human MOG generated equivalent antibody titers and IgG isotypes.

Conclusions: Human MOG-induced antibody most likely recognizes a different conformational epitope than does rat MOG. This epitope recognition results in an antibody that contributes to disease. Thus, EAE can result from an encephalitogenic T cell response induced by rat MOG that reacts with the endogenous mouse 35-55 or from an antibody induced by human MOG that recognizes an endogenous mouse MOG conformational determinant. These results, which indicate that a single amino acid change in an immunizing protein can result in a switch in pathogenic mechanism, have profound implications for the generation of MS in outbred human populations.

Disclosures: NH Ruddle has nothing to disclose.

Funding: National MS Society

THE THYMUS PLAYS A ROLE IN TOLERANCE IN EAE

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THE AMERICAS COMMITTEE FOR RESEARCH AND TREATMENT IN MULTIPLE SCLEROSIS

San Francisco, California, USA October 19, 2003
**Keywords:** clonal deletion; EAE; MBP; regulatory T cells; thymus; tolerance

**Background:** The majority of people (>95%) with multiple sclerosis (MS) are diagnosed after the age of 20. Recently, it has been reported that tolerance can be induced by regulatory T cells which are generated in the thymus. We therefore hypothesized that the thymus may play a role in protection from MS/EAE. The oral introduction of auto-antigen results in a state of profound tolerance specific for the ingested antigen, termed oral tolerance. We have reported that oral administration of myelin basic protein (MBP) protects and treats mice and rats with EAE.

**Objectives:** To determine the role of the thymus in tolerance induction in EAE/MS.

**Methods:** Euthymic and thymectomized MBP T cell receptor (TCR) transgenic (Tg) mice were fed MBP or vehicle one day before immunization with MBP and adjuvants, and the mice scored daily for EAE clinical signs. To examine clonal deletion of autoimmune T cells and the development of regulatory T cells in oral tolerance, we assessed the expression of the transgenic TCR, activation markers, apoptosis, levels of CD4+CD25+ T cells and cytokine profiles using real-time PCR, proliferation, and flow cytometry at various times after antigen feeding.

**Results:** Euthymic MBP TCR Tg mice are protected from EAE when fed MBP, while adult thymectomized Tg mice are not. The thymus appears to be an important site for deletion, since we observed a decrease in expression of the Tg TCR and an increase in apoptosis of Tg T cells in the thymus after MBP feeding. Furthermore, the thymus also is an important site for generation of regulatory T cells. We observed an increase in CD4+CD25+ regulatory T cells in the peripheral lymph nodes one day after MBP feeding, which coincided with elevated gene expression for IL-4 and TGFβ. We found that only CD4+CD8+CD25+ regulatory T cells are generated in the thymus following oral MBP administration, and that CD4+CD25+ cells were observed in the periphery of thymectomized mice 12 hours after MBP feeding. The proliferative response to the major MBP peptide in euthymic MBP-fed mice is significantly lower than in thymectomized mice. Decreased T cell activation correlates with an increase in CD4+CD25+ cells in euthymic MBP-fed mice.

**Conclusions:** The mucosal administration of MBP has a profound effect on thymic selection, thymic deletion of autoreactive T cells and generation of CD4+CD8+CD25+ regulatory T cells that cooperate to prevent or suppress the development of EAE.

**Disclosures:** F Song has nothing to disclose.

**Funding:** National Institutes of Health AI43376 and National MS Society RG3272 and PP0904

**ESTROGEN RECEPTOR-ALPHA MEDIATES ESTROGEN’S IMMUNE PROTECTION IN EAE**

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**Keywords:** clinical trials; cytokines; EAE; estrogen; hormones; Th1/Th2

**Background:** Estrogens are known to influence a variety of autoimmune diseases, but it is not known whether their actions are mediated through classic estrogen receptor (ER)-alpha.

**Objectives:** To determine whether the protective effects of estriol treatment in EAE are dependent upon ER-alpha.

**Methods:** Electrophoretic mobility gel shift assay, RT-PCR and sequencing, western blots, active EAE induced with MOG 35-55 in C57BL/6 mice, cytokine assessment by cytometric bead array and intracellular staining and FACS analysis.

**Results:** The presence of a functional ER was demonstrated in secondary lymphoid tissues by reduction in TNF-alpha when estriol was added in vitro to autoantigen stimulated immune cells and by the binding of protein from immune cells to the estrogen response element. ER-alpha expression in immune tissues was shown at the RNA level by nested RT-PCR and sequencing. ER-alpha expression was shown at the protein level by probing western blots with either ER-alpha specific antibodies or with ligand. Use of ER-alpha knock out mice revealed that both the estrogen-induced disease protection and the estrogen-induced reduction in pro-inflammatory cytokines were dependent upon ER-alpha in the prototypic Th1 mediated autoimmune disease experimental autoimmune encephalomyelitis.

**Conclusions:** The finding that the estriol mediated protection in EAE is dependent upon ER-alpha is central to the design of selective estrogen receptor modifiers which aim to target biologic responses in specific organ systems.

**Disclosures:** R Voskuhl has nothing to disclose.
Funding: National Institutes of Health AI50839, NS45443 (RRV) and National MS Society RD3407 (RRV)

CLINICAL AND IMMUNOLOGICAL FINDINGS IN PATIENTS WITH CONCURRENT MS AND AUTOIMMUNE THYROID DISEASE

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Keywords: autoimmune thyroid disease; autoimmunity; CGRP; lesion localization; multiple sclerosis; spinal cord

Background: Epidemiological studies and complications of treatment trials with anti-CD52 antibodies suggest a definite and unique link between MS and autoimmune thyroid disease (AITD). Such a relationship could exist if similar mechanisms of immune dysregulation were important in the development of both disorders, or if similar autoantigens were targeted by both disorders. We hypothesize that involvement of such mechanisms or autoantigens might lead to similar clinical symptoms and signs and/or immunological findings in patients with both MS and AITD.

Objectives: The aim of this study is to investigate clinical and immunological parameters in MS patients who have AITD.

Methods: MS patients with a diagnosis of AITD were assessed with regard to times of onset of MS and AITD, lesion localization over the course of their MS, and type of AITD. HLA-DR and DQ typing was performed for each patient, and peripheral blood mononuclear cells were assessed for autoreactivity to myelin antigens and to calcitonin gene-related peptide (CGRP), a neuropeptide which is found predominantly in the thyroid gland and spinal cord.

Results: Twenty-one patients with both MS and AITD, of whom 20 were female, were identified in a cohort of 270 MS patients. Eight patients had hyperthyroidism, and the remainder hypothyroidism. Clinically, patients with MS + AITD developed lesions predominantly in the spinal cord (affected in 72% of the total attacks), with some involvement of the brainstem (18%) and optic nerve (9%). HLA typing showed lower than expected expression (43%) of the HLA-DR2 haplotype. Eleven patients with MS + AITD who were not on any immunomodulatory agents were tested for autoreactivity to 20 different myelin peptides and to CGRP in T cell proliferation assays. Responses to myelin peptides were variable; however, 4 of 5 patients with MS + hypothyroidism made significant responses to CGRP (mean stimulation index of 4.7 ± 1.2), whereas none of 6 patients with hyperthyroidism and none of 4 HLA-matched MS patients without thyroid disease made a significant response to CGRP (stimulation indices of 1.1 ± 0.2 and 1.3 ± 0.2, respectively).

Conclusions: A relationship exists between localization of MS lesions in the spinal cord and the development of AITD, and may be related to increased immunoreactivity to antigens such as CGRP that are present in both the thyroid gland and spinal cord.

Disclosures: JM Greer has nothing to disclose.

Funding: National MS Society RG3190A1

GENDER DIFFERENCES IN IMMUNE RESPONSES IN MS PATIENTS

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Keywords: costimulation; cytokines; gender; multiple sclerosis; myelin peptides; sex hormones

Background: MS is more common in women than in men. We investigated 3 factors that could contribute to this “gender gap” in MS: Th1 versus Th2 cytokine production; expression of costimulatory molecules; and effect of exogenous sex hormones on peripheral blood T cells.

Objectives: To perform gender-specific Th1- and Th2-polarized cytokine analysis in MS females and males, and in sex-matched controls; to test the in vitro effect of sex hormones on T cell costimulatory molecule expression.

Methods: We examined untreated RR-MS patients and controls for IFNγ, TNFα, IL-5 and IL-10 production in response to PLP, MBP and MOG peptides, whole human PLP and MBP, and several recall antigens in the ELISPOT assay. We examined the expression of CD40L on CD3/4 and CD3/8 T cells cultured in the presence of exogenous estriol and progesterone, with and without stimulation by anti-CD3 mAb.

Results: MS females showed more IFNγ and TNFα responses to myelin proteins than any of the other groups. In contrast, MS males showed no IFNγ secretion.
to any antigen, and for TNFα only 2/4 responded to whole MBP. Control females secreted moderate IFNγ and TNFα to all the myelin antigens. Control males showed some IFNγ and TNFα responses to PLP, with 2 controls giving extremely strong responses to several myelin antigens. The majority of MS females had no IL-5 responses. Control males showed some IL-5 responses. In contrast, control females gave the strongest IL-5 responses. In both male groups the IL-5 responses were very low. Both female groups had very high IL-10 responses to whole MBP, and 2 control females were high responders to PLP 40-60. In contrast, both male groups had very low IL-10 responses, with only one individual in each category having higher reactivity to several peptides. In MS females, we observed a small, but dose-dependent suppression of CD40L co-stimulatory molecules expression on stimulated CD3/4 cells by estriol. At the highest dose, progesterone was slightly suppressive, but at low concentrations it stimulated CD40L expression on CD3/4 cells. Similar results were obtained in control females, but the suppressive effect of estriol was less pronounced than in MS females. The results in control males were more difficult to interpret due to their wide range. Interestingly, sex hormones did not alter CD40L expression on CD3/8 T cells, and CD3 stimulation of CD3/8 T cells did not upregulate CD40L expression.

**Conclusions:** Th1 cytokine responses were predominant in MS females and absent in MS males. Th2 cytokine responses were absent in MS females and low in MS males. These results show a disease-by-gender interaction. IL-10 showed a gender effect, with females having higher IL-10 production than males. Sex hormone effects on T cell CD40L expression were heterogeneous. In general, estriol tended to suppress the expression of CD40L on activated CD3/4 T cells, whereas progesterone, at physiologic concentrations, tended to induce increased numbers of activated CD3/4 cells expressing CD40L.

**Disclosures:** IR Moldovan has nothing to disclose.

**Funding:** National MS Society FA1459A1 and RG3263A3, National Institutes of Health RO1 NS4197

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**PRELIMINARY RESULTS FROM A PHASE II TRIAL OF RITUXIMAB IN MS**

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**Keywords:** antibody; B cells; CD20; cerebrospinal fluid; monoclonal antibody therapy; rituximab

**Background:** B cells and antibodies may be involved in the pathogenesis of MS, however the evidence thus far has been only correlative.

**Objectives:** To determine whether B cells or antibodies play a role in MS pathogenesis will require a controlled trial of their elimination to determine if the course or pathology of MS is altered.

**Methods:** Rituximab is a humanized monoclonal antibody to CD20, a cell surface molecule unique to B cells. It is FDA-approved for B cell lymphoma therapy. We are conducting an open-label Phase II study of rituximab as add-on therapy in 30 MS patients that, despite taking a beta-interferon or glatiramer acetate, have active clinical MS. To receive study drug, subjects must have at least one gadolinium-enhancing lesion on any of 3 pre-treatment brain MRIs, and must have had at least one clinical relapse in the previous 18 months, despite being on FDA-approved therapy for MS. The primary efficacy endpoint is number of enhancing brain MRI lesions after vs. before treatment. No data on the MRI endpoint will be available when the abstract is presented. CSF B cell percentage, immunoglobulin concentration and number of oligoclonal bands are assessed prior to treatment and 20 weeks post-treatment. Antibody levels to myelin antigens are assayed pre- and post-treatment. Unblinded clinical assessments of EDSS and MSFC are done.

**Results:** At time of this submission, 6/7 MS enrolled patients have received a full course of rituximab. One patient developed rash, fever, and rigors; treatment was discontinued. All other patients completed the regimen and had total depletion of circulating B cells. For the 4 patients followed at least 8 weeks post-treatment at time of this submission, timed 25-foot walk improved in three, and deteriorated in the fourth subject. For these 4 patients, EDSS scores did not change in three, and improved (5.0^ to 4.0) in one. CSF studies are done at weeks 0 and 24. At the time of this submission, pre- and post-treatment CSF data are available for 3 subjects. There was no change in oligoclonal band numbers or IgG indices, 29% mean decline in CSF IgG concentration and variable effects upon IgG synthesis rate. B cells were partially depleted from CSF. CSF B cell numbers declined by over 90% in one patient; in the other two patients CSF B cells were reduced by 40% and 12%. Serum antibodies to recombinant myelin oligodendrocyte glycoprotein showed no changes following therapy. The primary efficacy endpoint, blinded assessment of pre-treatment vs. post-treatment brain MRIs, will not be available until study completion.
Conclusions: Preliminary data from 7 subjects indicate that rituximab is safe and effective in temporarily eliminating circulating B cells in MS patients. Within 6 months of rituximab treatment, there was no decrease in serum antibodies directed against myelin oligodendrocyte glycoprotein, nor was there any major effect upon CSF immunoglobulin parameters. Reductions in CSF B cell numbers were observed following therapy.

Disclosures: A Cross has received honoraria in the past from TEVA, Berlex Laboratories, Biogen, Pfizer, and Serono. A Cross and J Lauber have participated in clinical trials sponsored by TEVA, Berlex Laboratories, Biogen, Immunex, Acorda Therapeutics, and Serono. Other authors have nothing to disclose.

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QKI MEDIATES DEVELOPMENTAL SIGNALS TO CONTROL CNS MYELINOGENESIS VIA REGULATING mRNA METABOLISM

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Keywords: CNS myelination; mRNA metabolism; myelin basic protein; oligodendrocyte development; signal transduction; tyrosine phosphorylation

Background: Myelination of the central nervous system (CNS) requires accurate expression of myelin structural genes. In particular, the myelin basic protein (MBP) is essential for CNS myelination. Recent discoveries have revealed essential roles of posttranscriptional regulation in MBP expression and myelin development via RNA-binding proteins in response to developmental signals.

Objectives: Our research has been focused on molecular mechanisms and signalling events that control MBP expression and myelin production by the RNA-binding protein QKI, an essential factor for normal myelin development. QKI belongs to the protein family of Signal Transduction Activators of RNA (STARs), characteristic of interaction with both RNA and signaling molecules. Understanding the function of QKI may ultimately help to identify novel therapeutic targets to promote myelin repair.

Methods: We used the quaking viable (qkv) mutant mice as model system to study posttranscriptional regulation of MBP expression and myelination in response to signal transduction pathways. Quantitative analysis performed in this study include RNase protection, immunoblot analysis, myelin purification, and kinase activity measurement.

Results: In the homozygous qkv mutants, the selective RNA-binding protein QKI is diminished in oligodendrocytes, which results in severe dysmyelination. We found that QKI selectively interacts with the 3’ untranslated region of the mRNA encoding for the myelin basic protein (MBP), an essential structure component for CNS myelin. Such interaction is critical to maintain the stability of the MBP mRNA, and is regulated by the Src family protein tyrosine kinases (Src-PTKs) which phosphorylate QKI at the C-terminal tyrosine cluster. During the active phase of CNS myelinogenesis programmed by developmental signals, tyrosine-phosphorylation of QKI is vigorously regulated, leading to increased RNA-binding by QKI. Consequently, MBP mRNA rapidly accumulated in this developmental window. Deficient QKI expression in myelin producing cells does not affect MBP transcription, yet results in a failure in MBP mRNA accumulation in the active phase of myelination and severe hypomyelination due to destabilization and mislocalization of the MBP mRNA.

Conclusions: Our observations suggest that QKI is a pivotal mediator for signal transduction pathways to control accelerated myelinogenesis via regulating the metabolism of MBP mRNA. These results revealed a novel mechanism that regulates normal myelin development, and our ongoing research focuses on the role of Src-family kinases and QKI protein in myelin repair, which may help to develop new strategies to facilitate myelin repair in multiple sclerosis.

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INDUCTION OF SUPPRESSORS OF CYTOKINE SIGNALING IN MURINE ASTROCYTES BY INTERFERON-GAMMA

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Keywords: astrocytes; inducible nitric oxide synthase; interferon-gamma; monocyte chemotactrant protein-1; ribonuclease protection assay; SOCS
Background: Experimental autoimmune encephalomyelitis (EAE) is the primary animal model for MS. In murine EAE, activated antigen-specific T cells, as well as other immune cells, infiltrate the CNS and secrete pro-inflammatory cytokines. These cytokines promote the production of inflammatory mediators, such as inducible nitric oxide synthase (iNOS) and monocyte chemoattractant protein-1 (MCP-1), by surrounding astrocytes. In response to inflammatory cytokines such as interferon-gamma (IFN-γ), suppressors of cytokine signaling (SOCS) are induced in T cells and macrophages, providing negative feedback to cytokine signal transduction pathways. It is not known if similar regulatory mechanisms exist in astrocytes.

Objectives: The goal was to determine if SOCS expression could be induced in astrocytes by inflammatory stimuli, such as conditioned medium from myelin basic protein-stimulated (MBP-CM) encephalitogenic murine lymph node cells. The relationship of SOCS expression with the cytokine-inducible inflammatory mediators iNOS and MCP-1, which are thought to be important in the pathogenesis of EAE, was examined.

Methods: Primary cortical astrocytes were prepared from 1–3-day-old SJL/J mice and confluent cultures were treated with medium, a combination of lipopolysaccharide (LPS) and IFN-γ, or MBP-CM. RNA was isolated using RNA STAT-60 reagent and samples were assayed using a custom RiboQuant multi-probe ribonuclease protection assay. The abundance of transcripts was quantified relative to the housekeeping gene GAPDH in the same sample.

Results: There was constitutive expression of SOCS-3 mRNA in astrocyte cultures. When cells were stimulated with LPS and IFN-γ, SOCS-3 was increased and SOCS-1, iNOS, and MCP-1 were induced. To a lesser extent, all of these genes were induced by treating cells with MBP-CM. Co-treatment of astrocytes with MBP-CM and anti-IFN-γ antibody returned SOCS-3 to baseline, eliminated SOCS-1 expression, and significantly decreased iNOS and MCP-1 mRNA.

Conclusions: Secretion of cytokines, such as IFN-γ, by infiltrating inflammatory cells may activate the production of SOCS proteins in glial cells, providing a potential mechanism for limiting inflammation in the CNS.

Disclosures: JL Stark has nothing to disclose.

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**TRANSPLANTED ADULT O4+ PROGENITORS REMYELINATE CHRONICALLY DEMYELINATED AXONS**

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Keywords: cell death; chronic demyelination; oligodendrocytes; progenitors; remyelination; transplantation

Background: We have previously demonstrated that the continuous exposure of cuprizone to mice over a prolonged period of time induces a series of demyelinating and remyelinating episodes leading to the formation of chronically demyelinated lesions.

Objectives: In this study, we investigated the molecular and cellular events within oligodendrocytes during the progression of a demyelinating lesion to a chronic state and determined whether chronically demyelinated axons could be remyelinated.

Methods: To address the molecular and cellular events occurring within the oligodendroglial populations during the progression of a lesion to a chronic state, we used cuprizone (bis-cyclohexanone oxaldihydrazone) intoxication to induce a chronic demyelinated lesion within the CNS of adult C57BL/6 mice. Upon depletion of the oligodendrocyte populations, adult O4+ progenitors were isolated from GFP tg mice and transplanted into the chronic lesions to determine whether the chronically demyelinated axons could be remyelinated.

Results: Although there is rapid regeneration of the mature oligodendrocyte population following an acute lesion, most of these newly regenerated cells undergo apoptosis if the mice remain on a cuprizone diet. The oligodendrocyte progenitors also become progressively depleted within the lesion if mice remain on the cuprizone diet. Interestingly, even if the mice are returned to a normal diet following 12 weeks of cuprizone treatment, remyelination and mature oligodendrocyte regeneration does not occur. However, if adult O4+ progenitors are transplanted into the chronically demyelinated lesion of mice treated with cuprizone for 12 weeks, mature oligodendrocyte regeneration and remyelination occurs after the mice are returned to a normal diet.
**Conclusions:** Thus, the formation of chronically demyelinated lesions induced by cuprizone appears to be the result of oligodendrocyte depletion within the lesion and not due to the inability of the chronically demyelinated axons to be remyelinated.

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**CXC/α CHEMOKINE RECEPTORS ON HUMAN OLIGODENDROCYTES: A POTENTIAL ROLE IN MS**

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**Keywords:** astrocytes; chemokine receptors; CXC chemokines; multiple sclerosis; oligodendrocytes; remyelination

**Background:** Subsequent to demyelination in multiple sclerosis (MS), oligodendrocytes retain the capacity to remyelinate, but whether pre-existing oligodendrocytes or newly-recruited precursors are responsible remains to be elucidated. Also unknown, are factors possibly involved in migration of oligodendrocytes to demyelinated areas in the CNS. Existing evidence, from studies on rats, support a role for chemokines in oligodendrocyte proliferation and cell positioning. Chemokines are a family of small (8–14 kDa) basic proteins, divided into four subgroups (CXC, CC, CX3C and C). Via interactions with high affinity G-protein-coupled surface receptors, chemokines have the ability to attract specific cell types, into or within tissues.

**Objectives:** To investigate the expression of CXC chemokine receptors and their ligands in MS.

**Methods:** Chemokine receptors were detected in MS tissue by immunohistochemistry (IHC) with specific antibodies against CXCR1, CXCR2 and CXCR3, and their ligands, IL-8, GRO-alpha and IP-10, respectively. Protein expression was also examined by western blots. For *in vitro* studies, oligodendrocytes were isolated from 18–22 week human fetal spinal cords and tested for chemokine receptor expression by immunofluorescence. Oligodendroglial identification was confirmed by double-labeling with phenotypic markers.

**Results:** IHC revealed constitutive expression of CXCR1, CXCR2 and CXCR3 chemokine receptors on oligodendrocytes in MS and non-MS tissue. In contrast, the ligands IL-8, GRO-alpha and IP-10 were found to be strongly induced on reactive/hypertrophic astrocytes at the edge of active MS lesions, but weakly expressed in chronic silent lesions and absent in normals and other neurological diseases. Cultured oligodendrocytes stained constitutively for CXCR1, CXCR2 and CXCR3. Both immature (A2B5+) and mature (CNPase+) oligodendrocytes displayed surface expression of all three chemokine receptors.

**Conclusions:** We propose that expression of CXCR1, CXCR2 and CXCR3 on oligodendrocytes, and induced expression on astrocytes of their respective ligands, IL-8, GRO-alpha and IP-10, may play an important role in the migration and development of oligodendrocytes subsequent to damage in the CNS. These observations have relevance to remyelination in MS.

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