For many years, the placebo-controlled randomized clinical trial has been the touchstone for clinical studies aimed at the development of effective disease treatment. Indeed, the success of this approach has led to the approval by various regulatory agencies and the subsequent widespread use of five disease-modifying agents for the treatment of multiple sclerosis (MS) in the past 12 years. Ironically, this very success now threatens the development of better therapeutic modalities.

Considerable controversy swirls over the ethical legitimacy of placebo-controlled trials. This continues despite a 2001 note of clarification by the World Medical Association about its revision in 2000 of the Declaration of Helsinki, stating that the use of placebos “may be ethically acceptable, even if proven therapy is available… where for compelling and scientifically sound methodological reasons its use is necessary to determine the efficacy or safety of a prophylactic, diagnostic, or therapeutic method” (Declaration of Helsinki). Nonetheless, virtually all ethicists agree with Temple and Ellenberg (2000) that “placebo controls are clearly inappropriate for conditions in which delay or omission of available treatments would increase mortality or irreversible morbidity in the populations to be studied.”

The concept of equipoise provides the ethical underpinning for the randomized clinical trial (RCT). This term may be used, in the construct of Miller and Weijer (2003) “as the full set of answers to the question, ‘When may a physician legitimately offer RCT enrollment to her patient?’” As originally described by Charles Fried in 1974, equipoise required that an individual researcher be genuinely uncertain about the relative merits of the treatment alternatives in a clinical trial before offering enrollment to a patient. Freedman (1987) later defined clinical equipoise as a state in which “honest, professional disagreement among expert clinicians about the preferred treatment” exists and leads to the institution of a clinical trial “with the aim of resolving this dispute.” Both authors have sought to provide a sound moral framework for the RCT. The former assured satisfaction of the physician’s “duty of care,” whereas the latter offered guidance to guarantee “overarching social approval” (Miller and Weijer 2003) of the trial. As Miller and Weijer conclude, Fried’s equipoise (FE) and Freedman’s clinical equipoise (CE) complement one another by addressing different aspects of moral concern. By satisfying the demands of the requisite fiduciary relationship between physician and patient, FE protects individual patients who are either potential or actual subjects in clinical trials. This is critically important because informed consent alone, while supporting the principle of patient autonomy as stated in the famous Belmont Report (1978), does not guarantee the protection of human subjects. Many patients readily believe that anything recommended by the physician is intended for their benefit (Levine 2003), a phenomenon termed by Appelbaum et al (1982) the “therapeutic misconception.” On the other hand, CE meets the “need of the state to protect its citizens from harm” and provides guidance for institutional review boards. Thus, successful application of these principles can allow the ethical completion of an RCT.

Given the constraints and concepts cited above, is the conduct of placebo-controlled trials ethically justifiable in MS? I believe that neither a state of clinical equipoise nor adequate protection of patient safety exists to permit the use of placebo controls for phase 3 trials in relapsing-remitting MS (RRMS). As a task force of the National Multiple Sclerosis Society concluded, a legitimate exception would allow such trials for patients who refuse currently available treatments or have failed their use (Lublin et al. 2001). However, in this circumstance ethically proper recruitment of subjects may be too difficult to allow successful completion of the trial. Whether placebo controls may be utilized in short phase 1 or 2 studies for RRMS is debatable. Clinical equipoise does exist regarding treatment of patients with clinically isolated syndromes and the use of placebo controls is arguably permissible. Similarly, their use is ethically justifiable in both primary and secondary progressive MS.
What options exist then for clinical trials in MS that will allow us to achieve the ethical social mandate to develop better treatments? Active control trials are one possibility despite the cautions about “assay sensitivity” (Ellenberg 2003). “Add-on” trials may be the best current approach for treatments whose mechanisms of action differ from those of existent therapy (Brody et al. 2003). The potential use of virtual control groups based on computer-modeling warrants consideration, but has not yet reached a state that is ready for acceptance in a major clinical trial.

The desire to achieve better treatment for MS is strong, yet the ethical requirement for the protection of human subjects remains paramount. Only with acceptance of this principal and a firm commitment among the MS community to think “outside the box” will we succeed. A workshop addressing issues in MS clinical trials, sponsored by the National Multiple Sclerosis Society to be held December 2004 in Washington, DC, should be a major step in this direction.

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The Multiple Sclerosis Lesion Project (MSLP) was designed as an international collaborative effort to study the clinical, radiologic, and pathological correlates of the MS lesion. It is based on our previous observations demonstrating profound inter-individual heterogeneity, but intra-individual homogeneity in MS lesions with respect to patterns of myelin destruction (1). Early active lesions have been classified into four subtypes: Pattern I: macrophage associated; Pattern II: antibody/complement mediated; Pattern III: distal oligodendro-gliopathy (DOD) and Pattern IV: oligodendrocyte (OG) degeneration in the periplaque white matter (PPWM). Hypoxia may contribute to tissue injury in pattern III lesions (2). Differences in chemokine receptor expression (CCR5/CCR1) between patterns II and III, suggest distinct inflammatory microenvironments (3).

To date, 286 MS cases have been classified: 15%, Pattern I; 58%, Pattern II; 26%, Pattern III; and 1%, Pattern IV. All active lesions from a given patient continue to demonstrate the identical pathological pattern, suggesting the presence of a single dominant effector mechanism of demyelination within that patient. The identification of four MS lesion patterns suggests MS may be a disease with heterogeneous pathogenic mechanisms. Furthermore, distinct patterns may require different therapeutic interventions. Clinical and paraclinical correlates are needed in order to differentiate between these pathological patterns.

Critics suggest these observations may not be generalizable to prototypic MS since they may either reflect alternative diagnoses or represent atypical or exceptionally severe MS. A study of the clinical course of 91 patients with pathologically confirmed inflammatory demyelinating disease, however, indicates that patients in this biopsy group are not unique. Eighty-two met Poser or McDonald criteria for definite/probable MS, whereas 9 patients had an isolated demyelinating syndrome (IDS) at last follow-up (4). During the follow-up period, no patients had developed symptoms, or signs revealing an alternative diagnosis. Clinical course of the 82 definite/probable MS biopsy cohort was compared with the Olmsted County MS prevalence cohort (n = 218). Although the biopsy cohort was on average older and had a higher proportion of men, the clinical course was similar to the prevalence cohort, with comparable proportions of RR and SPMS. The frequency and type of symptoms prior to biopsy differed from onset attack symptoms in the population-based cohort (motor weakness 57% vs. 22%, cerebral dysfunction 24% vs. 2%, optic neuritis 4% vs. 22% and sensory symptoms 36% vs. 56%). These less typical presentations may have in part led to brain biopsy. Although both cohorts had the same median EDSS at last follow up (3.0), the mean duration of follow up was much longer for the population based prevalence cohort. The biopsy cohort had a mean of 1.65 EDSS points higher than the population cohort after correcting for gender, age at onset, and disease duration. However, 70% of the biopsy cohort had an EDSS ≤ 4. Though a statistically significant difference was found, the disability progression of the biopsy cohort compared with the population-based cohort was clinically less significant than expected.

There was no correlation between disease duration pre-biopsy/autopsy, and the pathological pattern. However, a similar distribution of patterns I, II, and III was present in patients biopsied up to four years after disease onset, suggesting distinct patterns of myelin destruction appear to persist over a period of time (at least four years). There was no correlation between immunopathological pattern and disease course of prototypic MS, or EDSS at last follow-up. However, pathological analysis of the most severe variants of inflammatory demyelinating disease, such as neuromyelitis optica (NMO) and Balo’s concentric sclerosis, have revealed that NMO is the most severe manifestation of Ab-mediated demyelination (5), whereas all patients with Balo’s concentric lesions have a DOD, and evidence of hypoxia-mediated injury (6). A novel and highly specific serological biomarker (NMO-IgG) has been identified in NMO patients, which localizes to the perivascular region in a pattern similar to the perivascular distribution of complement activation and immunoglobulin deposition, as described in NMO lesions. This marker for the first time reliably distinguishes NMO from other inflammatory demyelinating disorders, including MS (specificity 91%; sensitivity 72%) (7).
We have also observed a striking correlation between the therapeutic response to plasma exchange (PE) in MS patients with evidence for antibody and complement activation (pattern II pathology), versus no response in pattern I (n = 3) or pattern III (n = 6) cases (p < 0.0001) (8). These observations suggest that pathologic heterogeneity has therapeutic implications. We found no correlation between anti-MOG/anti-MBP IgM/IgG antibodies and pathological pattern, disease course, PE response, or disease duration. Preliminary results suggest no association between HLA DR or DQ carrier status or HLA haplotypes, and MS patterns of demyelination, however, there is a trend towards HLA DR1 exclusively present within patterns I/II.

MRI-pathological correlates reveal that the accumulation of macrophages in a sharp border at the plaque edge characteristic of pattern I and II lesions, is highly associated with the presence of ring enhancement on Gd-MRI and hypointense T2W rims, whereas these imaging features are not seen in pattern III lesions (p < 0.0001) (9). Review of follow-up MRIs from pattern III cases does not reveal evidence for ring enhancing lesions (n = 13 cases). Cross-sectional follow-up imaging of biopsied MS patients suggest the apparent diffusion coefficient (ADC) of normal appearing white matter and cortex is higher than normal. However, the ADC of pattern III patients was statistically significantly higher than patterns I and II, for both NAWM and cortex (p < 0.03). In the serial patients (n = 6), the single pattern III patient showed marked increases in ADC values of NAWM, whereas the ADC of NAWM of pattern I and II patients remained stable. Although these ADC changes may reflect an inherent abnormality of the NAWM in pattern III cases, further studies are needed to confirm whether these ADC changes are primary, or secondary to differences in lesion load or lesion destructiveness.

We conclude that the pathological findings and resultant new insights into the underlying pathophysiology of MS based largely on biopsied patients, can be applied to prototypic MS. Furthermore, it would appear that specific immunopathological patterns, with likely differing underlying mechanisms of CNS damage, are seen across a wide clinical spectrum of MS. The clinical and radiologic observations reported to date on a large series of early MS cases continue to support pathogenic heterogeneity in MS, which persists over a period of time, rather than a single mechanism dominating the formation of all lesions. The future will lie in studying the underlying immunopathological mechanisms involved in each pattern, determining the chronicity of the patterns by identifying patients with longer disease duration prior to biopsy/autopsy, and developing pattern specific therapies and pattern specific serological, radiological, and genetic markers which reliably differentiate between these patterns.

Beyond these accomplishments, the MSLP has accumulated an enormous resource of interactive investigators, invaluable research experience, and a carefully organized tissue/clinical/radiographic/genetic database. This resource will uniquely allow investigators to directly define the pathogenetic role of MS susceptibility/severity genes on the development of the lesions, to evaluate the usefulness of paraclinical markers for diagnosis and subtyping of MS patients and lesion stages, and potentially allow therapeutic interventions to be more specifically tailored to lesion subtypes.

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Chemokines constitute a family of structurally related polypeptides (MacKay 2001). The chemokines and their receptors exert well-characterized roles in inflammation and physiological function of the immune system, by modulating biological responses, including migration, enzyme secretion, cellular adhesion, cytotoxicity, tumor cell growth, degranulation, and T-cell activation (MacKay 2001; Asensio and Campbell 1999; Gerard and Rollins 2001). Chemokines act through plasma membrane G-protein coupled chemokine receptors on cellular targets (Luster 1998; Rollins 1997). Chemokines and their receptors have been implicated in various pathologies of the human central nervous system (CNS) and have emerged as salient targets for therapeutic intervention (Asensio and Campbell 1999; Glabinski and Ransohoff 1999; Mennicken et al. 1999; Trebst and Ransohoff 2001).

The current study addressed the expression of CC chemokine receptors (CC-CKR) on mononuclear phagocytes in multiple sclerosis (MS). Two variables were imposed on this characterization. First, we evaluated CC-CKRs on mononuclear phagocytes in various stages of lesion evolution. Acute MS lesions (less than 1 month old) exhibit three major zones of demyelinating activity. In the inactive (IA) lesion core, myelin has been completely removed and myelin debris in macrophages is limited to neutral lipid (Lucchinetti et al. 1996). At the expanding lesion edge, in early-active (EA) zones, intracellular myelin debris includes detectable protein (including low-abundance components such as myelin oligodendroglial glycoprotein (MOG) and myelin-associated glycoprotein (MAG; Lucchinetti et al. 1996). Macrophages and monocytes in EA zones express acute-activation epitopes such as the MRPI-14 isoform of the S100 family. In the intermediate late-active (LA) zone, only the abundant myelin proteins such as myelin proteolipid protein (PLP) are found in the intracellular inclusions of macrophages, which also may be demonstrated to contain the lipid-intercalating dye luxol fast blue (LFB). Based on our prior results, we hypothesized that monocyte recruitment to MS lesions could be analyzed by focusing exclusively on the EA zones (Trebst et al. 2001). The further differentiation and activation of these cells could be monitored by evaluating the LA and IA regions of these acute lesions.

Second, we compared mononuclear phagocyte CC-CKR expression in lesions exhibiting different pathological patterns, as described by Lucchinetti, Bruck, and Lassmann (Lucchinetti et al. 2000). This categorization protocol has been reported and recently reviewed (Lassmann et al. 2001). Lesion patterns were defined by these investigators, as described in Materials and Methods. EA, LA, and IA zones of demyelination were evaluated in both lesion patterns.

We found striking differences and informative similarities in our studies of CCR1 and CCR5 expression in pattern II and pattern III lesions. In EA zones of both pattern II and pattern III lesions, infiltrating monocytes (located in perivascular aggregates and expressing MRP-14) expressed CCR1 and CCR5 in the majority of cells. This observation was consistent with our previous report, which was confined to pattern II lesions (Trebst et al. 2001). Taken in the context of data from experimental studies using CCR1 or CCR5-deficient mice, these data support a role for CCR1, and possibly CCR5, in the accumulation of monocytes in the human CNS (Rottman et al. 2000).

Regulation of CCR1 and CCR5 diverged when LA and IA zones of pattern II and pattern III lesions were compared. Specifically, percentages of CD68+ cells expressing CCR5 rose from about 30% in EA zones to approximately 80% in LA and IA regions. This observation led to the logical conclusion that CCR5 was maintained on infiltrating monocytes and upregulated on resident microglia in the lesional environment of the pattern II pathology. By contrast, CCR5 expression diminished in LA and IA zones of pattern III lesions, from about 35% in EA zones to 25% ± 30% in the older regions of these lesions. One possible interpretation of this disparity was that interferon-γ, known to upregulate CCR5 (Hariharan et al. 1999), was present at high levels in pattern II but not pattern III lesions.

Expression of CCR1 also differed. In pattern II lesions, as noted above, about 20% of infiltrating cells expressed this receptor, but CCR1-immunoreactive cells were scarce (5% ± 15%) in LA and IA regions. This finding was interpreted to indicate activation-related downregulation of CCR1 and could be recapitulated in vitro by adherence of monocytes to serum-coated tissue culture flasks (Trebst et al. 2001). By contrast, CCR1 was...
expressed on a relatively uniform proportion of CD68+ cells throughout all zones of pattern III lesions. The cumulative distinction between pattern II and pattern III lesions could be shown most clearly by calculating the ratio of CCR5+ cells/CCR1+ cells in EA, LA, and IA zones of pattern II and pattern III lesions. In EA zones, the ratio approximated 1 in both lesion types, reflecting the recent infiltration of CCR1+/CCR5+ monocytes. This ratio remained unchanged in LA and IA zones of pattern III lesions, but rose progressively in pattern II lesions, as CCR5 was upregulated and CCR1 was downregulated. In LA zones of pattern II lesions, the CCR5/CCR1 ratio was 10, and in IA zones, the ratio approached 20.

CCR8 was expressed in a similar fashion in pattern II and pattern III lesions of MS (Trebst et al. 2002). In particular, CCR8 was present on phagocytic macrophages and activated microglia in both lesion patterns. We did not detect CCR8+ lymphocytic cells in MS lesions under any circumstances (Trebst et al. 2002). Among the lesion variants of MS, patterns II and III exhibit differences defined by the mode of demyelination and the presumed inflammatory mechanisms. We undertook to determine if these differences were reflected at the level of chemokine receptor expression on mononuclear phagocytes. We found that patterns of chemokine expression were similar in some respects but dramatically different in others.

What were the similarities? First, the infiltrating monocyte population in both lesion patterns appeared to co-express CCR1 and CCR5. Further, CCR8 was expressed by phagocytic macrophages and activated microglia in both lesion patterns.

What were the differences? Most strikingly, the microglial population in pattern II lesions strongly expressed CCR5 in LA and IA zones, but not in the corresponding regions of pattern III lesions. Second, the number of cells expressing either CCR1 or CCR5 significantly changed in relation to demyelinating activity of pattern II but not pattern III lesions.

In conclusion, these studies support the hypothesis that lesional environments in patterns II and III differ, as reflected, for example, in CCR5 expression patterns, suggesting the presence of interferon-c in pattern II but not pattern III lesions. Additionally, the proposal that CCR1+/CCR5S+ monocytes accumulate in MS lesions is supported by these studies as this population was detected in both lesion types.

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MRI has been shown to be an exquisitely sensitive technique for the detection of multiple sclerosis (MS) plaques (1) and this has been confirmed in early (2) and subsequent (3) MRI-pathological studies. Early studies in the pathologic correlates of these focal MRI findings attempted to relate specific histopathologic features to these changes. However, it became apparent that these changes were not specific for pathology and that a variety of histopathologic changes, either singly or in combination, can lead to the same MRI abnormality evident as a focal lesion on the MRI scan (3). For example, the hyperintense lesions in T2-weighted imaging of MS have been attributed to demyelination (4), gliosis (5), expansion of the extracellular space (6), edema (7), vascular permeability (8), or macrophage infiltrates (9). Correlative studies of T1-weighted MRI have shown that “black holes” are markers of tissue destruction with axonal loss (10). However, it is now apparent that some of these abnormalities are reversible, at least some probably being remyelinated (11). Thus, before a given “black hole” can be considered irreversible tissue destruction, a significant period of time should elapse.

While the findings on routine MRI in MS have not proved to be directly translatable into specific histopathology, the patterns of intensity within lesions may provide important information with respect to the genesis and age of that lesion. For example, the imaging abnormalities seen at the periphery of a lesion may be indicative of the rim of macrophages at the edge of a chronic active plaque (9,12), and alternating rings of normal and abnormal signal would be indicative of Balo’s concentric sclerosis variant of MS (13).

The very earliest event in the pathogenesis of a focal lesion in MS is uncertain and this is true of its MRI correlate. The first change seen at the location of a future lesion (which will later become apparent on T2-weighted imaging) is a focal abnormality on magnetization transfer imaging (MTI) (14). This can antedate the enhancement, normally evident as an early change in routine imaging of a new lesion, by quite some time, and its pathologic basis has not been determined. Enhancement, on the other hand, has been associated with inflammatory infiltrates (9,15) and neo-vascularization (16) in the lesion, as determined by MRI-pathology correlative studies.

A number of non-routine techniques show promise for histopathologic specificity (17). The most obvious of these is magnetic resonance spectroscopy (MRS). While no formal histopathologic correlative studies have been employed with the technique, the specificity of this technique is implicit in as much as it represents an assessment of the presence, absence, increase or decrease of a specific biochemical marker which may be cell-specific. For example, N-acetyl aspartate (NAA) is a marker for axons and has been shown by MRS to be reduced in MS lesions (18), correlating with the now well-established finding of axonal loss shown in histopathologic studies (19). Similarly, the lipid peaks in MS lesions most likely indicate lipid degradation products in macrophages engaged in the demyelinating process (20). MTI shows promise of demonstrating histopathologic features at various stages of plaque formation (21,22,23). The same is true of diffusion-weighted imaging (DWI) (24), and diffusion tensor imaging elegantly displays tracts within the CNS, allowing for assessment of tractal Wallerian degeneration in MS (25). T2-weighted relaxation data can be fractionated into several components, the shortest of which, referred to as short T2 (26), correlates with the distribution of myelin (27) and may prove to be a useful tool for following demyelination and remyelination in MS patients.

More recently, great interest has focused on the white matter not associated with MS plaques, the so-called “normal-appearing white matter” (NAWM). Techniques such as MRS (28) and DWI (29) have shown diffuse abnormalities in the white matter which appears normal by routine MRI techniques. Thin-slice MRI (30) and high-field strength magnets (31) have revealed very small focal abnormalities in the NAWM. Formal pathologic
correlative studies have not been done to determine the basis of these findings. However, pathologic studies of grossly normal-appearing white matter have shown microscopic abnormalities, particularly axonal loss (32) thought to be due to Wallerian degeneration as a result of axonal destruction in plaques (33).

On routine T2-weighted and proton density studies, there is a subtle abnormality intermediate in intensity between plaque and the remaining NAWM (34). This has been referred to as “dirty-appearing white matter” (DWM). Preliminary data from our studies would indicate that in addition to axonal loss, there is a prominent loss of myelin in these areas of DWM (35,36).

Thus, it would appear that in the future our understanding of the histopathology and pathogenesis of a given MS plaque may be determined by the superimposition of data from a variety of MRI techniques, both routine and those which are present are in the research sphere. Moreover, MRI-pathology correlations of the abnormalities in non-lesional MS white matter (NAWM and DWM) may provide important insights into diffuse and probably degenerative processes in this disease which up to recently was thought to be exclusively multifocal and inflammatory in its pathogenesis.

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Despite extensive clinical and experimental investigations on the genetics, immunology, imaging and clinical investigations of the disease, fundamental questions still remain over the etiopathogenesis of multiple sclerosis. There are many reasons for attempting pathological classifications of multiple sclerosis. First is the need to try to predict and control clinical behavior in patients by trying to determine cases that are similar or different in order to differentiate etiopathogenic comparisons one from the other. In the absence of a definitive etiology, much has to be inferred from the study of the extensive body of experimental literature which has produced similar conditions in laboratory situations. This approach has both practical and conceptual limitations, but does yield some information as to what could be happening in multiple sclerosis. Secondly, modern diagnostic imaging techniques have transformed both the clinical management of MS (9), and the potential to carry these etiopathogenic inferences to a new level. The revival of the suggestion that MS is not a single homogeneous process has led to an attempt to develop a classification of type and stage, which will be recognizable by non-invasive imaging techniques, and will enable tailoring therapy to the individual patient.

Classifications and staging systems also depend on delineating the state of progression of the disease. Classically the disease has been delineated into acute, sub-acute (acute on chronic) or chronic (10), but it is important to recognize that the definitions which govern the designation of each of these stages may vary. In this regard experimental evidence as to the progression of similar inflammatory diseases has been extremely useful (10). Recent work from the laboratory of Prineas and Barnett have raised the suggestion that the progression of the disease may not show classical inflammatory parameters early. This has led not only to questions as to etiopathogenesis, but as to the ability to stage lesions. Nonetheless, MS is a disease with common neuropathological processes including edema, inflammation, axonal damage, demyelination, remyelination and gliosis. Each of these plays a role in both the development and the resolution of the lesions, and each will have a role in determining the clinical and radiological presentation. It becomes critical to incorporate these elements in any common classification.

Although most classifications of the disease has traditionally relied on determining the temporal nature of the process, with the report by Luchinetti et al. (6,7), an attempt was made to determine the pathogenetic basis for the disease by examining the various components of lesions seen at surgical biopsy and relating them to experimental processes. While this approach has not been uniformly used, and may not be operant in all cases, an enormous amount of information has been generated (8), and has led to the delineation of various forms, with suggestions as to etiopathogenesis. One of these includes the suggestion that many of the changes are related to local changes in tissue blood flow. This idea has been picked up by Prineas and Barnett in their recent study of what they postulate is the earlier stage of inflammation where the predominant lesion appears to be ischemic rather than inflammatory (1).

Most of the current classifications and staging systems have not incorporated two very important features of the disease that are increasingly recognized to have enormous clinical and diagnostic significance. The first of these is the status of a normal appearing white matter (NAWM), which is increasingly found to play a significant role both clinically, and also potentially in the development of new lesions. The second is the realization that many of the clinical and prognostic parameters of MS depend on the degree and severity of axonal (2,4,5), as well as cortical (3) pathology. Both these features need to be incorporated into common classifications and staging systems. Reliance on older concepts that cover only demyelination, remyelination and inflammation, exclude the utility of this most important aspect of the disease.

At the moment however, pathological classification, with attendant in vivo diagnostic imaging, remains of
great importance in determining type of disease, and in
drawing some conclusions as to either etiology or pro-
gression. However, the lack of certainty as to what these
changes represent, together with incomplete pathologi-
cal and clinical data will continue to impose limitations
on their utility. A utilitarian approach in the future that
searches for a classification based on pathological, clin-
ical and radiological information will ultimately prove
to be of more value.

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STEM CELL THERAPY IN EAE: NON-INVASIVE IN VIVO TRACKING OF CELL BIODYNAMICS AND RELATION TO CLINICAL DISEASE STATUS

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Keywords: cell migration; EAE; iron oxide particles; magnetic labeling; MR imaging; stem cell therapy

Background: Stem cell therapy can attenuate the clinical disease score in EAE. Central to the future success of stem cell transplantation in MS is the ability of transplanted cells to migrate from the site of transplantation to relevant foci of disease, while exhibiting remyelinating and trophic properties. A new approach to non-invasively assess how far and at what speed cells are migrating is the use of MRI and magnetically labeled cells.

Objectives: To assess the biodynamics of cell migration of transplanted neurospheres and to correlate the distance and speed of cell migration to the clinical disease status.

Methods: Newborn mouse neural spheres were labeled with iron oxide nanoparticles and BrdU. Chronic EAE was induced in mice by immunization with MOG peptide. Clinical scores were assigned daily. (Undifferentiated) stem cell transplantation was performed on day 6 following EAE induction in 8 mice, all of which underwent MRI. Serial MRIs, were performed in vivo using a 4.7 Tesla scanner. High resolution ex vivo imaging was performed at 9.4 Tesla. The distance of migration was calculated from the 3D MRI dataset, and validated by histology of BrdU- and Prussian blue (iron) positive cells.

Results: Magnetic labeling did not affect differentiation of neurospheres into the three neural lineages in vitro. Astrocytes comprised 41 ± 5%, oligodendrocytes 27 ± 7%, and neurons 1.0 ± 1%. In vivo MRI showed that at days 1–2 after transplantation, cells migrated to the white matter tracts, with further continuation at days 13–14 (peak first relapse) and 30 (chronic phase). Most migration occurred early during the acute phase of disease. The clinical score demonstrated a good correlation between disease severity and migration. The histological distribution of labeled cells were found to match the MRI findings, with differentiation of cells occurring in vivo.

Conclusions: The observation that the greatest degree of migration occurred very early in the course of disease highlights the narrow time window in which transplantation of remyelinating cells may be effective for obtaining clinical results. These results show that inflammatory signals associated with the clinical score modulate migration in a positive manner.

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WHITE MATTER ATROPHY CORRELATES WITH CLINICAL DISABILITY IN PATIENTS WITH PELIZAEUS-MERBACHER DISEASE

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Keywords: clinical disability; demyelinating disease; leukodystrophy; neuroimaging; Pelizaeus-Merbacher disease; white matter

Background: Axonal degeneration contributes to clinical disability in the acquired demyelinating disease multiple sclerosis (MS). Axonal degeneration occurs during acute attacks, associated with inflammation, and during the chronic progressive phase of the disease in which inflammation is not prominent. To explore the role of interactions between oligodendrocytes and their axons in CNS demyelinating disease, we analyzed the brains of rodents and humans with a null mutation in the gene encoding the major CNS myelin protein, proteolipid protein (PLP1). These studies demonstrated a length-dependent axonal degeneration without significant demyelination in the CNS of PLP1 null mice as well as in the CNS of patients with a PLP1 null mutation, suggesting that disruption of PLP1-mediated axonal-glial interactions was the likely cause.

Objectives: To extend these studies to patients with other PLP1 mutations, we analyzed the brains of an additional 30 PMD patients by MRI. Twenty-five of these patients had one of a variety of PLP point mutations and 5 had a duplication of the region of the PLP gene.

Methods: For each patient we measured total brain volume (TBV), white matter volume (WMV), mid-corpus callosal area, and intercaudate distance (ICD). In addition, we calculated a functional disability score (FDS) for each patient which was developed specifically for this purpose.
Results: Comparison of the MRI and the functional data demonstrates that white matter volume decreases linearly with functional disability, suggesting that disability is the direct result white matter atrophy. Since intercaudate distance also correlates with the FDS, this simple linear measurement must also be proportional to white matter volume, and can thus be used as a substitute measurement when volumetric data are not available. Corpus callosal area also correlates with functional disability, but not as well as WMV or ICD.

Conclusions: Taken together, these data demonstrate that white matter atrophy is the likely cause of neurological disability in patients with PMD, regardless of mutation type. In addition, they support the notion that oligodendrocyte dysfunction is an important cause of neurological disability in patients with MS.

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ANTI-MYELIN ANTIBODIES: FREQUENCY, STABILITY AND CLINICOPATHOLOGIC ASSOCIATIONS IN A BIOPSY MS COHORT

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Keywords: anti-myelin antibodies; biopsy; clinical course; immunopathology; marker stability; MOG antibodies

Background: Antibodies against myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) are present early in MS and may be an early predictor of conversion to definite MS (MOG) or marker for overall brain damage due to MS (MBP).

Objectives: To report the frequencies of MBP and MOG antibodies in patients with biopsy proven demyelinating disease, and investigate their association with HLA DR genotype and immunopathological patterns and to ascertain their reproducibility over time.

Methods: The biopsy cohort consisted of 71 patients with pathologically confirmed inflammatory demyelinating disease with adequate follow up, stored sera and DNA available for testing. Follow up and EDSS scores were ascertained by interview and examination, telephone interview and chart review.

Results: 61 patients had MS and 10 patients had an isolated demyelinating syndrome (IDS). The mean duration of disease was 6.3 ± 5.7 years with median EDSS of 3.0 (range 0.0–8.5). For MS patients, 23% had MBP IgG, 39% had MBP IgM, 34% had MOG IgG and 61% had MOG IgM. For 10 IDS patients with mean follow up of 5.8 ± 4.9 years since attack, 1 had MBP IgG, 2 had MBP IgM or MOG IgG and 5 had MOG IgM in sera drawn at last follow up. Clinical course and disease severity were similar for both seropositive and negative patients. Serial blood draws were collected in 4 patients, at 3 monthly intervals, 9–22 months after disease onset; 3 were positive for MOG IgM at some point in their course and subsequently became negative on at least 3 serial testings. Similar changes were seen for the other postulated markers. Immunopathological classification was available on 51 of 71 patients; 11 pattern I, 29 pattern II and 11 pattern III. MBP and MOG antibody seropositivity did not correlate with immunopathological patterns. There was no association between seropositive status of any of the antibodies tested and HLA DR hetero or homozygosity.

Conclusions: Frequencies of myelin antibodies in biopsy proven MS patients are similar to rates previously reported for prototypic MS. There was no association between these antibodies and HLA DR status or immunopathology. Although fluctuations in MOG IgM antibody status in patients with definite MS may reflect disease activity, further longitudinal studies are needed to determine whether this is a stable biomarker.

Disclosures: SJ Pittock has nothing to disclose.

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CXCL13 IN AUTOIMMUNE DEMYELINATING DISEASE

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Keywords: animal models; chemokines; dendritic cells; experimental autoimmune encephalomyelitis; helper T cells; neuroinflammation

Background: Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are chronic, relapsing demyelinating diseases of central nervous system (CNS) white matter. Histologically
and EAE are characterized by perivascular white matter infiltrates, composed of T cells, B cells and myeloid cells, within demyelinating plaques. We speculated that the formation of organized CNS infiltrates during autoimmune demyelination might be governed by chemokines, such as CXCL13, that are normally involved in the development of lymph node and spleen during embryogenesis. CXCL13 is a lymphoid chemokine that attracts both B cells and a subset of T cells bearing the CXCR5 receptor. Here we test our hypothesis that CXCL13 is ectopically expressed in the CNS during EAE and drives chronic inflammation within the brain and spinal cord.

**Objectives:** To investigate the role of CXCL13 in experimental autoimmune encephalomyelitis.

**Methods:** EAE was induced in susceptible strains of mice by active immunization with myelin antigens or by the adoptive transfer of myelin-reactive lymph node cells. Representative animals were sacrificed at various points during the clinical course and CNS CXCL13 levels were measured by RT-PCR, RNAse protection assays and/or Western blot analysis. In parallel studies, spinal cord mononuclear cells were analyzed by flow cytometric analysis to detect CXCR5+ leukocytes. The clinical course of EAE was compared in CXCL13-deficient and wildtype mice as well as in wildtype mice treated with neutralizing anti-CXCL13 or isotype matched control antibodies.

**Results:** We readily detected CXCL13 on both the mRNA and protein level in spinal cords from mice afflicted with EAE but not from healthy controls. CXCL13 expression rose steadily throughout the course of relapsing-remitting disease. CXCR5+ CD4+ T cells, bearing a memory cell surface phenotype, accumulated in spinal cords beginning with the first exacerbation. Blockade of CXCL13 attenuated adoptively transferred disease. Consistent with these results, CXCL13 deficient mice experienced a relatively mild disease course characterized by an absence of relapses.

**Conclusions:** CXCL13 is ectopically expressed in the CNS during EAE and plays a pathogenic, non-redundant role during the effector phase of the disease. Most strikingly, ablation of CXCL13 abrogates relapses. Agents that block CXCL13 interactions might be ameliorative in the treatment of multiple sclerosis or other organ-specific autoimmune disorders.

**Disclosures:** BM Segal has nothing to disclose.

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induces CNS inflammation and motor dysfunction. Upon adoptive transfer to the naïve syngeneic recipients, the \textit{in vivo} pre-activated T10/T22 specific gamma delta T-cells alone are capable of inducing the pathology and clinical symptoms.

**Conclusions:** The recognition of activation/infection induced self-antigens such as T10/T22 by gamma delta T-cells may contribute to the initiation of autoimmune diseases. Thus, an important ‘trigger’ for autoimmune diseases such as multiple sclerosis may occur through the activation of peripheral gamma delta T-cells during the course of an infection.

**Disclosures:** JH Yeh has nothing to disclose.

**Funding:** JHY is a post-doctoral fellow of the National MS Society (FG 1535-A-1).

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**PHASE TWO TRIAL OF FAMPRIDINE-SR IN MULTIPLE SCLEROSIS**

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**Keywords:** functional outcome measures; gait disturbance; motor dysfunction; potassium channel blocker; timed 25 foot walk; weakness

**Background:** Previous studies of the potassium channel blocker, fampridine (4-aminopyridine), in multiple sclerosis (MS) have reported improvement in various aspects of neurological function including measures of mobility and muscle strength.

**Objectives:** This was a phase two, double-blind, placebo-controlled, parallel-group trial to evaluate the safety and efficacy of fampridine-SR in subjects with MS.

**Methods:** In this multi-center study, 206 subjects were randomized equally to one of four cohorts: fampridine-SR 10, 15, 20 mg bid or placebo. An initial placebo run-in period (2 wk) was followed by periods of dose escalation (2 wk), stable treatment (12 wk), down-titration (1 wk), and follow-up evaluation (2 wk off drug). Eligible subjects were ages 18–70 yr with MS (as defined by McDonald) who had adequate cognitive function and could perform required study procedures, including a timed 25-foot walk twice (required range: 8–60 sec). The primary efficacy variable was percent change from baseline in average walking speed over 25 ft. Secondary variables were Lower Extremity Manual Muscle Test (LEMMT), Ashworth Score, clinician’s global impression of change, subject global impression, 9 Hole Peg Test, Paced Auditory Serial Addition Test 3\(^{rd}\), the Multiple Sclerosis Quality of Life Inventory, and the 12-Item MS Walking Scale. Safety was assessed by monitoring adverse events, changes in vital signs, clinical laboratory test results, ECG, and physical examination.

**Results:** In total, 195 subjects from all 4 groups completed the trial: fampridine-SR bid 10 mg (n = 50), 15 mg (49), 20 mg (51), and placebo (45). The Timed 25-Foot Walk demonstrated a significant effect for all dose groups compared to placebo at the first visit after up-titration. There was a strong trend toward increased walking speed during the stable dose period. LEMMT demonstrated statistically significant improvement across doses at up-titration and during stable treatment (p = 0.007). Additional analyses of efficacy are ongoing. The adverse event profile was consistent with previous experience and included dizziness, insomnia, paresthesiae, and nausea that were mostly mild to moderate in severity. Two subjects receiving 20 mg bid experienced seizures during the trial, one from an accidental overdose.

**Conclusions:** These preliminary results demonstrating a strong trend on the Timed 25-Foot Walk, a statistically significant increase in leg strength, and a favorable safety profile support the continued investigation of fampridine-SR in MS.

**Disclosures:** A Goodman, J Cohen, T Vollmer, and M Johnson are consultants for Acorda Therapeutics. M Katz, A Blight, and R Cohen are employees of and hold equity interest in Acorda Therapeutics.

**Funding:** Supported by Acorda Therapeutics.
USE OF CVB-BASED VACCINES TO MODIFY AN IMMUNE RESPONSE TO SELF

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Keywords: autoimmunity; CVB; interleukin-4; TMEV; vaccine; viral vectors

Background: Studies have demonstrated that epitope spreading is responsible for some of the pathology observed in the Theiler's murine encephalomyelitis virus (TMEV) model of multiple sclerosis (MS). Mice develop self-reactivity to epitopes of proteolipid protein (PLP) as well as myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP).

Objectives: In these studies, our goal was to determine if we could utilize a viral vector strategy to attenuate the cellular immune response to a PLP epitope that in the TMEV model of MS.

Methods: In these studies, we used the TMEV model of demyelination to test the hypothesis that vaccination with a coxsackievirus B (CVB)-based vaccine containing a PLP epitope and/or the coding sequence for interleukin-4 (IL-4) could redirect the immune response from a harmful Th1-dominated response toward a benign Th2-dominated response. Mice were i.p. vaccinated with 2 doses of $2 \times 10^5$ TCID50 of the CVB vaccine either before or after TMEV infection.

Results: Survival studies demonstrated that survival was not impaired by vaccination in the presence of TMEV infection. Mice receiving the chimera containing PLP178–191 and IL-4 experienced a depressed delayed type hypersensitivity (DTH) response compared with mice receiving the vaccine containing only PLP178–191 (0.07 ± 0.02 vs. 0 + 0.03 × 10^-3 mm change in footpad swelling), supporting the hypothesis that the IL-4 containing vaccine could reduce the cellular immune response in animals receiving this vaccine. Mice receiving the PLP178–191/IL-4 chimera also had higher levels of PLP-specific IgG1 as determined by ELISA compared to mice receiving the vaccine containing PLP alone. IgG2a levels were similar in both groups of mice.

Conclusions: Together, these data demonstrate that this immunization approach can be used successfully to modulate the self-reactive immune response in TMEV-infected mice. We are undertaking studies to assess the impact to these vaccines on demyelination.

Disclosures: KM Drescher has nothing to disclose.

Funding: No funding reported.

COMBINATION OF PACLITAXEL AND VITAMIN B12CN ATTENUATES DEMYELINATION MORE EFFECTIVELY THAN PACLITAXEL ALONE IN AN ANIMAL MODEL OF MS

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Keywords: citrulline; interferon; paclitaxel; peptidyl arginine deiminase; remyelination; vitamin B12

Background: Previously we described the effects of low dose paclitaxel (taxol) in attenuating disease in a primary demyelinating transgenic mouse (ND4) (Moscarello et al., 2002, Mult Scler 8:130) and in EAE models (Cao et al., 2000, J Neuroimmunol 108:103). These studies revealed a significant and modest suppression of clinical signs.

Objectives: Determine whether combination of paclitaxel and vitamin B12 is more effective in attenuating demyelination than paclitaxel alone.

Methods: Each mouse received 4 injections of paclitaxel intraperitoneally once/week for four weeks. Controls received phosphate-buffered-saline (PBS). Vitamin B12 was given once/week intraperitoneally on a continuing basis.

Results: Consistent with the reduction of clinical signs, we found a reduction in astrocytosis, increased myelin basic protein (MBP) in white matter tracts, ultrastructural evidence of remyelination a reduction of MBP citrullination. Decreased citrullination was due to a reduction of peptidyl arginine deiminase (PAD) mRNA and protein in brain. In addition to stabilizing microtubules paclitaxel induces an increase in microtubules in differentiated oligodendrocytes in culture. The enhanced effect of the paclitaxel and B12CN resulted in activation of endogenous interferon-beta in the brain of ND4 mice. Interferon-gamma expression was reduced in the combination treated mice. Part of this mechanism involved the stabilization of STAT-1, an interferon responsive transcription factor, by methylation. The increase in activated STAT-1 in the brains of combination treated mice following interferon-beta binding is
indicative of the functional nature of this treatment (Mastronardi et al., 2004, J Immunol 172:6418).

**Conclusions:** Combination of taxol treatments with vitamin B12-CN (B12-CN) resulted in enhancement of suppression of cumulative clinical signs in the ND4 mouse. A possible therapeutic mechanism of action of paclitaxel involves Toll-like receptor signaling and the activation of interferon-beta.

**Disclosures:** FG Mastronardi is supported by Transition Therapeutics Inc. MA Moscarello is a consultant for Transition Therapeutics Inc. 

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**Keywords:** binding antibodies; bioavailability; interferon-beta; MxA protein; neutralizing antibodies; observational

**Background:** MS patients develop neutralizing antibodies (NABs) mainly in the first 1–2 years of treatment with interferon-beta (IFNβ). Binding antibodies (BABs) are commonly used to screen for NABs; BABs usually precede NAB development. With continuous treatment, >60% of neutralizing antibody (NAB) positive patients on IFNβ-1b lose their NABs (Rice et al., 1999). MxA induction, a marker for IFNβ bioavailability, is consistently inhibited in the presence of persistent high titres of NABs (Bertolotto et al., 2003). It is unknown if MxA bioavailability re-appears once NABs disappear. We explored MxA responses in patients treated with IFNβ ≥ 4 years, and compared past BAB, NAB status to the present.

**Objectives:** To explore MxA response to IFNβ in MS patients who have lost their NABs over time.

**Methods:** We measured MxA induction in lymphocytes of MS patients who fulfilled the following criteria: continuous treatment with a single IFNβ preparation for ≥ 4 years with past BAB and NAB measurement. Control groups included either MS patients receiving their first injection of IFNβ or in the first 6 months of treatment with an IFNβ. Patients had blood drawn for BABs (ELISA), NABs (positive NABs ≥ 100 Kawade units), pre- and post-injection MxA levels (MxA normalization ratio) on 2 visits to UBC MS Clinic.

**Results:** 32 patients participated in the study, 3 withdrew; 23 were female. Three patients with previously high NAB titres were now negative, with mean (SD) ‘present’ MxA induction of 19.33 (6.36). This was much higher than long-term patients remaining NAB positive (n = 6), 0.93 (1.64) and similar to the MxA induction of one long-term group (n = 12) never found to be NAB positive, MxA 24.10 (13.19). Control groups were patients receiving their first injection of IFNβ and patients in their first 6 months of therapy (n = 8), their mean MxA induction was 24.81 (18.92) that was similar to those patients NAB negative at present, regardless of previous NAB status.

**Conclusions:** Some MS patients on IFNβ lost their NABs with treatment continuation and regained MxA induction to IFNβ injection. Conversely, those with continued high NAB titres continued to have no MxA induction to IFNβ injection. Long-term treated patients, NAB-negative, continued to show good MxA induction. Further investigation is needed to confirm these findings, and to ascertain clinical outcomes.

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K+ CHANNEL KV3.1 AND MYELIN PROTEIN OSP/CLAUDIN-11 MODULATE OLIGODENDROCYTE PROGENITOR FUNCTION

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Keywords: ion channels; membrane potential; migration; myelin; oligodendrocyte; proliferation

Background: Development of oligodendrocyte progenitor cells (OPC) is regulated by the timely expression of distinct ion channels and modulation by growth factors. The activity of different ion channels and transport systems is dependent on changes in the cell resting membrane potential, which in turn has been implicated in controlling protein synthesis, cell proliferation and migration. Alteration of OPC membrane potential may occur due to a changing ionic environment during development and during some disease states, such as multiple sclerosis, spinal cord injuries or leucodystrophies. Regulating the resting membrane potential might provide a direct control of the behavior of the OPC. We have identified a potassium (K+) channel, Kv3.1, as an associated protein of oligodendrocyte specific protein (OSP)/claudin-11 via yeast two-hybrid assay. K+ channel block and membrane depolarization adversely affects OPC proliferation, migration and axon myelination, specifying the importance of K+ channels in OPC function. OSP/claudin-11 is essential for forming CNS myelin and testicular tight junctions. It is involved in proliferation and migration of oligodendrocyte progenitor cells (OPC) and is a candidate autoantigen in multiple sclerosis.

Objectives: Our objective in the present study was to establish the presence of Kv3.1 potassium channel, its relative contribution to K+ conductance, its association with OSP/claudin-11 and its role in proliferation and migration of OPC.

Methods: A combination of immunohistochemical, electrophysiological and biochemical methods were used.

Results: Blocking Kv3.1 specific currents or knocking out Kv3.1 gene decreased the sustained K+ current component of OPC by 50–75%. OPC from OSP/claudin-11 knock out mice had reduced K+ currents indicating that the interaction with OSP/claudin-11 is important for K+ channel function. Blocking Kv3.1 specific currents or knocking out Kv3.1 gene significantly decreased proliferation and migration of OPC.

Conclusions: These results suggest that Kv3.1 currents represent a significant component of outward K+ conductance in OPC and Kv3.1 protein in association with OSP/claudin-11 is important in regulating OPC proliferation and migration, important processes for myelin formation, maintenance, and repair.

Disclosures: SK Tiwari-Woodruff has nothing to disclose.

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A DOUBLE-BLIND, RANDOMIZED, CONTROLLED STUDY OF ORAL PIRFENIDONE FOR TREATMENT OF SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS

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Keywords: cytokines; double-blind; pirfenidone; secondary progressive; TNF-alpha; treatment

Background: Currently, there are no proven treatments for secondary progressive multiple sclerosis (MS) in stabilizing or reversing the neurological disabilities associated with this disease. Pirfenidone, an orally effective small molecule, was found to be therapeutically beneficial in improving and stabilizing the disabilities in two independent open-label studies in secondary progressive MS.

Objectives: We studied the effects of pirfenidone in a phase II double-blind, randomized, and controlled, clinical trial in secondary progressive MS for 12 months.

Methods: Eleven (11/18) patients (61%) on oral placebo and sixteen (16/25) patients (64%) on oral pirfenidone remained in the study for 12 months. Seven (7/18) patients (39%) in the placebo and nine (9/25) patients (36%) in the pirfenidone groups dropped out from the study.

Results: The oral pirfenidone significantly (p < 0.001) improved the Scripps Neurological Rating Scale (SNRS) scores as early as 3 months and the scores remained improved for 6 and 12 months as compared to the baseline SNRS scores of this group. In contrast, the baseline SNRS scores of patients on oral placebo did not significantly improve at 3, 6 or 12 months of the study. In addition, the patients on oral pirfenidone had significantly (p < 0.05) higher rank in improved SNRS scores from their individual baseline scores than the patients on oral placebo at 3, 6 and 12 months. Oral pirfenidone was also associated with a marked improvement in bladder dysfunction in eight (8/16) patients (50%) as com-
pared to three (3/11) patients (27%) on placebo. In addition, oral pirfenidone reduced the incidence of relapses from five (5/11) patients (45.5%) on placebo compared to two (2/16) patients (12.5%) on pirfenidone (p < 0.027). Other indices of MS such as EDSS scores, and magnetic resonance imaging failed to reveal any statistically significant differences between the oral placebo and the oral pirfenidone.

Conclusions: In view of the findings of the present clinical trial that oral pirfenidone significantly improved the clinical conditions (SNRS) of the secondary progressive MS patients, a larger, multicenter, double-blind, randomized, controlled trial is recommended.

Disclosures: JE Walker has nothing to disclose.
Funding: No funding reported.

EFFECT OF INTERFERON BETA-1A ON GREY MATTER ATROPHY PROGRESSION IN RELAPSING-REMITTING MULTIPLE SCLEROSIS

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Keywords: brain atrophy; grey matter atrophy; interferon beta-1a; longitudinal; MRI; trial

Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder of the central nervous system (CNS) that involves most prominently the white matter (WM) of the brain. It has been demonstrated that substantial gray matter (GM) damage in the brain occurs from the earliest stages of the disease. Two recently concluded clinical trials showed that Avonex® (IFN beta-1a; Biogen, Inc., Cambridge, MA) slows down the rate of brain atrophy progression in the second and third year of the trial. However, more detailed information on the effect of this disease-modifying treatment (DMT) on the topography of CNS atrophy has not been provided yet.

Objectives: To determine whether Avonex® slows down progression of brain atrophy by reducing predominantly GM or WM damage, or both.

Methods: Thirty-one patients with MS have been included in the study and followed for 3 years. The inclusion criteria were: relapsing-remitting (RR) MS, Expanded Disability Status Scale (EDSS) 0–5.5 and one exacerbation in the 12 months prior to study entry. At study entry, the therapy with Avonex® has been offered to all patients. Fourteen patients started treatment with Avonex®, whereas 17 decided to delay the start of DMT and served as study controls. Both groups had quantitative cranial MRI scans at study entry and after three years, and standardized clinical assessments every 6 months. Brain parenchymal fraction (BPF), grey matter fraction (GMF) and white matter fraction (WMF) percent changes were calculated using SPM99 software program.

Results: Three patients in the control arm have been excluded from the study, because they started DMT before the study conclusion. Baseline demographic, clinical, and MRI measures were well matched between the two study arms. None of the patients on Avonex® developed neutralizing antibodies during the study. Mean percent change in BPF (–1.2% vs. –2.2%, p = 0.003) and GMF (+3.6% vs. –4.2%, p < 0.0001) favored the Avonex® treatment arm. Both treatment groups developed similar amounts of WM atrophy over 3 years (p = NS).

Conclusions: The results of this preliminary study indicate that Avonex® may slow down brain atrophy progression preventing predominantly the development of GM atrophy.

Disclosures: R Zivadinov has nothing to disclose.
Funding: No funding reported.

CLINICAL CHARACTERISTICS OF HISPANIC PATIENTS WITH RELAPSING-REMITTING MS

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Keywords: Caucasian; Hispanic; MRI; prevalence; relapse rate; relapsing-remitting

Background: MS is uncommon in Hispanics with a prevalence of 5–8/100,000 in Latin America. There is scant information regarding the clinical features of MS in Hispanic individuals.

Objectives: To study the clinical and radiographic characteristics of relapsing-remitting (RR) MS in Hispanics.

Methods: We reviewed medical records of all Hispanic and Caucasian patients seen for the first time at an academic MS center in New York City during a 1-year period. The diagnosis of MS was made according to the McDonald criteria. Relapses were determined by history or diagnosed prospectively while patients were followed. Fisher’s exact test, unpaired t-test, or the Wilcoxon
rank-sum test were used to assess differences between the Hispanic and Caucasian patients.

**Results:** Of the 79 patients diagnosed with RR MS, 9 (11%) were Hispanic and 64 (81%) were Caucasian. The 2 groups had similar age at onset, gender, type of initial demyelinating event, and length of follow-up. Although the mean number of relapses between the 2 groups did not differ, the Hispanic patients had a significantly shorter mean disease duration (4.2 ± 3.4 vs. 9.9 ± 8.1 years, p = 0.024) and a higher average annualized relapse rate (ARR) (2.1 ± 2.3 vs. 0.8 ± 0.9 exacerbations per year, p = 0.004) than the Caucasians. More Hispanics (8/8, 100%) than Caucasians (38/62, 62%) met the McDonald criteria for MRI dissemination in space (p = 0.04). 67% and 73% of the Hispanics and Caucasians, respectively, were treated with a disease modifying agent (DMA) and the average duration of therapy was similar between the groups (22.2 ± 20.5 vs. 23.0 ± 20.3 months, p = 0.92). After beginning therapy, the Caucasians had a 50% reduction in the ARR (0.8 ± 1.9 vs. 1.6 ± 3.0; p = 0.14). The average ARR in the Hispanic group was nearly identical before and after (1.3 ± 0.9 vs. 1.3 ± 1.1, p = 0.95) therapy was initiated.

**Conclusions:** In this study, Hispanics had a significantly higher ARR than Caucasians. Furthermore, a significantly greater proportion of Hispanics fulfilled the McDonald criteria for MRI dissemination in space. After beginning treatment with a DMA, there was a 50% reduction in the ARR for Caucasians, while no change was seen in the Hispanic patients. As the Hispanic population in the US is expected to rise nearly 40% over the next 15 years, there is a need for a large prospective study of Hispanic patients with MS, including their response to DMAs.

**Disclosures:** J Derwenskus has nothing to disclose.

**Funding:** No funding reported.

**HERPESVIRUSES AS POSSIBLE ETIOLOGY OF MS**

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**Keywords:** antibodies; first episode; herpesviruses; relapse; remission; viral DNA

**Background:** The hypothesis of viral infection in the etiology of MS has been investigated for several years. However, until today no single virus has been isolated from tissues of MS patients.

**Objectives:** We are studying the herpesvirus family (HSV1, HSV2, VZV, CMV, EBV, HHV6, HHV7 and HHV8) as a possible etiology of MS. These viruses are able to remain in latency in lymphocytes and have common epitopes among them and probably with cellular proteins (i.e. myelin). These characteristics could be a key in the initiation of an autoimmune mechanism, reappearing every time a virus is reactivated. We are investigating samples of 100 MS patients for the presence of viral DNA in serum, lymphocytes and CSF and viral antibodies in serum samples. Serum, lymphocyte and CSF samples are collected during the first MS attack and serum and lymphocyte samples during remission and relapse periods. Comparison of viral presence will be performed between three groups of samples.

**Methods:** MS patients’ samples as well as 20 CSF and serum samples from patients with other neurological disorders (OND) are under investigation. To detect IgG and IgM of herpesviruses ELISA kits are used with exception of HHV7 and HHV8 where ‘in-house’ protocols will be used. PCR reactions were standardized using 10, 100 and 1000 copies of purified viral DNA. PCR is followed by Southern blots for optimization of detection.

**Results:** Herpesvirus DNA was detected in 36 out of 50 CSF first attack MS samples analyzed so far. The most frequent viral DNA is HSV1 DNA found in 13 samples followed by EBV DNA in 9 samples. All herpesviruses, with the exception of HHV8, are present in the 50 MS CSF samples. The viral DNA presence in the serum is lower than in CSF. HSV1 DNA is present in 8 patients (CSF and serum) but 5 more patients have HSV1 DNA only in CSF. In the control group only 2 serum and no CSF samples are positive for viral DNA. IgG antibody detection of 5 viruses in 100 first attack MS samples and 20 OND controls do not show significant difference. The prevalence of IgM is 7% in the control group and 16% in MS patients.

**Conclusions:** Although these results are only preliminary and the number of patients is small, we can observe a significant difference of viral presence between MS and control samples. This difference could correspond to an acute infection coexisting with the symptoms of the first MS attack.

**Disclosures:** D Koptides has nothing to disclose.

**Funding:** National Multiple Sclerosis Society RG3321A1/2, and The Cyprus Institute of Neurology and Genetics.
THE VALINE66 METHIONINE POLYMORPHISM OF BRAIN DERIVED NEUROTROPHIC FACTOR IN RECURRENT NEUROMYELITIS OPTICA AND MULTIPLE SCLEROSIS

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Keywords: Devic’s neuromyelitis optica; disability progression; genetics; MRI; neurotrophins; optocarpal MS

Background: Recurrent neuromyelitis optica (R-NMO) is a demyelinating disease involving the optic nerves and the spinal cord that usually spares the brain parenchyma. Brain derived neurotrophic factor (BDNF), a neurotrophin produced by neurons and immune cells, contributes to neuronal survival and repair. The Valine66Methionine (V66M) polymorphism of BDNF is functional and affects intracellular trafficking and activity dependent secretion.

Objectives: To investigate the associations between the BDNF V66M polymorphism and clinical characteristics in R-NMO and relapsing remitting multiple sclerosis (RR-MS).

Methods: Six R-NMO patients and 71 RR-MS patients enrolled in 3 separate studies were evaluated using the EDSS and MRI. The V66M genotype was characterized using allele discrimination.

Results: Five of the 6 R-NMO patients (83.3%) had the methionine allele: 1 patient (16.7%) was V66V, 4 patients were V66M (66.7%) and the 1 remaining patient (16.7%) was M66M. In the RR-MS population: 49 (69%) patients were V66V, 18 (25.4%) were V66M, and 4 (5.6%) patients were M66M, which is similar to the distribution in a control population. Despite the small sample size, the frequencies of the V66V, V66M and M66M genotypes in R-NMO were significantly different from those in MS ($\chi^2 = 6.48$, p = 0.039). Further assessments of possible associations between optico-spinal MS presentation and the V66M polymorphism are underway. Long term quantitative MRI data, available for a subset of RR-MS patients, is being analyzed to determine the associations between genotype and MRI parameters.

Conclusions: Our findings suggest that V66M polymorphism is associated with R-NMO but not with susceptibility to RR-MS. In RR-MS, this polymorphism may be associated with disease course. These findings require confirmation in a larger cohort but the BDNF genotype shows promise in distinguishing between the different types of demyelinating diseases.

Disclosures: M Ramanathan has nothing to disclose.

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ACUTE PARTIAL TRANSVERSE MYELITIS WITH NORMAL CEREBRAL MRI: TRANSITION RATE TO CLINICALLY DEFINITE MS (CDMS)

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Keywords: classification; clinically isolated syndromes; diagnosis; magnetic resonance imaging; prognosis; transverse myelitis

Background: To date, there have been only a few long-term follow-up studies that have addressed the issue of conversion from APTM to CDMS in patients with initially normal cerebral MRIs.

Objectives: To determine the long-term risk of developing clinically definite multiple sclerosis (CDMS) in patients with acute partial transverse myelitis (APTM).

Methods: We studied 30 consecutive patients with clinical evidence of APTM. Patients with symmetric severe acute transverse myelitis were considered to have complete transverse myelitis and were excluded. All patients underwent spinal and cerebral MRIs, 13 underwent cerebrospinal fluid (CSF) analysis, and 11 patients underwent evoked potential studies. Various other studies were performed to assess for connective tissue disease and causes of acute partial transverse myelitis other than demyelinating disease.

Results: After an average follow-up of 61.1 months, all laboratory and clinical evidence, including relapse history, indicated that 3 patients developed lesions on cerebral MRI and could be classified as CDMS by either Poser criteria (2 patients) or MacDonald criteria (1 patient). Relapses limited to the spinal cord clinically were seen in 14/30 (46.6%) patients. Oligoclonal bands were seen in 8/13 (62%) patients; 1 patient transitioned...
to CDMS. Unifocal lesions of the cord were seen in 19/30 (63%) patients, multifocal lesions were seen in 8/30 (27%), and 3/30 (10%) had negative MRIs. The 3 patients who converted to CDMS did so within 5 years of the onset of myelitis.

Conclusions: APTM with normal cerebral MRI had a low rate of conversion to CDMS in this long-term study. The presence of oligoclonal bands in several patients who have yet to develop evidence of cerebral demyelination may indicate a limited utility for this test in patients with APTM.

Disclosures: TF Scott has nothing to disclose.

Funding: No funding reported.

PREDICTING FACTORS OF INITIAL DEMYELINATING EVENT (IDE) SEVERITY AND RECOVERY

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Keywords: initial demyelinating event; monosymptomatic; polysymptomatic; predictors; recovery; severity

Background: IDE presentations include acute myelitis, optic neuritis (ON), brainstem and/or cerebellar dysfunction. Baseline demographic, clinical and MRI characteristics may predict severity and recovery from IDE, and time to subsequent MS onset. Such predictors could guide patient management.

Objectives: To 1) characterize a cohort of patients followed prospectively from IDE onset, 2) determine which baseline demographic, clinical and MRI characteristics predict IDE severity and recovery.

Methods: Data on all UCSF MS Clinic patients seen within 6 months of IDE onset was entered prospectively into an ACCESS database. Scores on EDSS, Functional Systems (FSS) and visual acuity (VA) were used to define IDE severity shortly after onset and recovery over 6 months. Severity of IDE was defined as mild = FSS 0–1 or VA \geq 20/40, moderate = FSS 2 or VA 20/50–20/190, severe = FSS 3 and more, or VA < 20/200. Recovery 6 months afterwards was defined as complete = FSS 0 or VA 20/20, fair = FSS 1 or VA better or equal to 20/40, poor = FSS 2 or more, or VA of 20/50 or worse.

Results: We identified 119 such patients (84 women) with an average onset age of 34 ± 10 years, 58 of whom received IV methylprednisolone at the time of onset. IDE was monosymptomatic in 105 patients (51 spinal cord, 31 brainstem, 4 cerebellar, 19 ON), and polysymptomatic in 14. Onset severity was mild in 46%, moderate in 47%, and severe in 7%. Recovery was complete in 46%, fair in 49%, and poor in 4% of patients. Average age, sex ratio and ethnicity were similar across groups of various severity and recovery. Patients with mild IDE were more likely to fully recover than patients with moderate or severe IDE (Fisher’s exact test, p < 0.005). Patients with polysymptomatic presentations were more likely to have a worse recovery regardless of onset severity (Fisher’s exact test, p < 0.005).

Conclusions: Explanatory variables including MRI that are potentially predictive of the severity and recovery of IDE, and time to second episode will be tested in univariate and multivariate models. This preliminary work has resulted in an ongoing international effort that will merge data on patients seen prospectively since IDE onset to expand our findings. DNA will be collected from these patients to determine if specific genetic polymorphisms predict IDE severity, recovery and time to subsequent exacerbation.

Disclosures: T West has nothing to disclose.

Funding: No funding reported.

THERAPY OF RELAPSING EAE BY NEUTRALIZATION OF LYMPHOCYTE CHEMOTACTANT CYTOKINE IL-16

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Keywords: demyelination; EAD; IL-16; immunotherapy; paralysis; relapse

Background: Infiltration of the CNS by CD4+ Th1 cells precedes onset and relapses of experimental autoimmune encephalomyelitis (EAE). We reported that (B6 × SJL) F1 (H-2b/s) mice, with severe relapsing-remitting disease, had extensive infiltration by CD4+ T cells compared to C57BL/6 (B6) (H-2b) mice, which developed mild low-relapsing disease in response to myelin oligodendrocyte peptide 35–55 (MOG35–55).

Objectives: This observation led us to search for mechanisms that specifically regulate trafficking of...
CD4+ T cells in relapsing H-2b/s mice. We found that the CD4+ cell chemoattractant cytokine IL-16 has an important role in regulation of relapsing EAE.

**Methods:** We analyzed levels of IL-16 and identified sources of IL-16 immunoreactivity in CNS throughout relapsing-remitting disease. Finally, effects of treatment with neutralizing IL-16 antibody on clinical and pathohistology of relapsing disease were evaluated.

**Results:** We found that production of IL-16 within the CNS of mice with EAE correlated well with the extent of CD4+ T cell and B cell infiltration during acute and relapsing disease. In the CNS IL-16 was produced by infiltrating CD4+ T cells, CD8+ T cells and B cells. Treatment with neutralizing anti-IL-16 antibody successfully reversed paralysis and ameliorated relapsing disease. In treated mice, diminished infiltration by CD4+ T cells, lesser demyelination and more sparing of axons were observed.

**Conclusions:** We show an important role of IL-16 in regulation of relapsing EAE and describe a novel therapeutic approach to specifically impede CD4+ T cell chemoattraction in EAE, by neutralization of IL-16. Our findings have high relevance for the development of new therapies for relapsing EAE and potentially MS.

**Disclosures:** DS Skundric has nothing to disclose.

**Funding:** No funding reported.

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**ANTI-GLATIRAMER ACETATE ANTIBODY LEVELS IN TREATED MULTIPLE SCLEROSIS PATIENTS**

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**Keywords:** enzyme linked immunosorbert assay, ELISA; glatiramer acetate (GA); immunoglobulin G, IgG; optical density, OD; peripheral blood mononuclear cells, PBMC; pokeweed mitogen, PWM

**Background:** Previously, GA has been shown to induce antibodies in up to 100% of treated patients. These antibodies have also been shown to peak at month 3, but gradually decrease, and over time return to pretreatment levels.

**Objectives:** To study the kinetics of anti-GA antibodies in treated MS patients.

**Methods:** In a randomized, double-blinded placebo controlled study, we analysed the kinetics of anti-GA antibodies, and also both in vitro and in vivo total IgG levels in 16 GA treated and 9 placebo patients. Serum samples and PBMCs were collected pretreatment and every 3 months thereafter, for up to 36 months. Anti-GA antibodies were measured using a direct ELISA method. Serum IgG were measured using nephelometry. PBMCs were cultured for 7 days with and without pokeweed mitogen (PWM), and secreted IgG measured by ELISA.

**Results:** All placebo patients (n = 9) tested negative for anti-GA antibodies. In the treated group, all patients (n = 16) were negative having a mean value of 0.19 ± 0.03 OD for anti-GA antibodies at pretreatment, but became positive at month 1, peaking at month 3 (1.64 ± 0.37 OD), and remained so for the duration of the study (36 months). The difference between pretreatment and during treatment was significant (p < 0.005) throughout the study. Serum IgG levels did not differ significantly between treated and placebo patients. Spontaneous (without PWM) IgG secretion levels was not different between placebo and treated patients. However, within the treated patients there was a significant increase in spontaneous IgG secretion (p < 0.05). PWM-induced IgG secretion was slightly increased following treatment.

**Conclusions:** Glatiramer acetate, like other immunomodulatory drugs, is highly immunogenic. Antibodies to GA peaked at month 3, as shown in other studies and remained elevated for at least 3 years. The clinical significance of these antibodies needs to be explored. The fact that spontaneous IgG secretion increases in treated patients probably indicates a switch from a TH1 to a TH2 response.

**Disclosures:** Dr Oger has received grant support from Berlex, Schering AG, Biogen and Serono. He has served as a consultant and received honoraria and travel grants from Berlex, Biogen, Serono and Teva Neuroscience.

**Funding:** No funding reported.

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**TH1 SKEWING OCCURS IN THE CNS, NOT THE PERIPHERY, DURING EAE**

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**Keywords:** animal model; chemotaxis; CNS migration; cytokines; experimental autoimmune encephalomyelitis; Th1/Th2

**Background:** Experimental autoimmune encephalomyelitis (EAE) is an autoimmune demyelinating disease of the central nervous system (CNS) that is mediated by
CD4+ T cells specific for myelin peptides. It is widely depicted as a prototype of Th1-mediated organ specific autoimmunity. In this study, we analyzed the cytokine profile of myelin-specific T cells from lymph node, spleen and CNS during EAE on the single cell level using a highly sensitive Elispot assay.

**Objectives:** To analyze the cytokine repertoire of myelin-reactive T cells in the CNS and periphery during EAE.

**Methods:** Elispot assays for IFN-gamma, IL-2, IL-4, IL-10 and IL-5 were performed on lymph node cells, splenocytes and spinal cord mononuclear cells harvested from mice with EAE. These studies were complemented by 2-color fluorispot assays and flow cytometric cytokine secretion assays (CSA) to define the nature of myelin-specific cytokine producing cells in detail.

**Results:** Our studies demonstrate that the myelin-specific T cell repertoire is more diverse than previously portrayed, with comparable numbers of IL-4, IL-5 and IFN-gamma producers detected in lymph nodes and spleen. Two color fluorispot analysis revealed that IL-4/IL-5, and IFN-gamma were secreted by distinct sub-populations. By contrast, cells isolated from the inflamed CNS represent a uniform population, exclusively secreting IFN-gamma. Cytokine secretion analysis (CSA) of splenocytes from symptomatic mice confirmed the presence of CD4+ PLP-specific cells secreting either IL-4 or IFN-gamma. Chemotaxis assays demonstrated that PLP-specific Th1, but not Th2, cells preferentially migrate towards chemokines found in the CNS of mice with EAE such as MIP1alpha, SLC and RANTES.

**Conclusions:** Our studies suggest that EAE should be classified as a Th1-mediated disease based on the biological functions of the effector cell population within the target organ as opposed to in the periphery. Th1 skewing occurs within the CNS at least in part due to preferential recruitment/retention of IFN-gamma producers in response to locally produced chemokines.

**Disclosures:** BM Segal has nothing to disclose.

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**PROSPECTIVE MEMORY IN RELAPSING-REMITTING AND SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS**

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**Keywords:** cognition; explicit memory; memory; prospective memory; relapsing-remitting; secondary progressive

**Background:** Prospective memory (PM) is the ability to remember to complete tasks that have been planned for the future—for example, remembering to take medication at the appropriate time.

**Objectives:** The primary objectives of the present study were to examine PM in MS patients and evaluate whether an “implementation intentions” mnemonic that had been effective in healthy elderly would improve PM in MS patients.

**Methods:** Participants with MS and without MS (controls) played a board game, mimicking a week in everyday life, which included 59 PM tasks. Participants were tested on noticing when actions were to be completed and on remembering the particular actions. Half the participants formed implementation intentions (e.g., stating “I intend to pick up my niece at 3 pm” and picturing themselves picking up the niece) and half rote-rehearsed.

**Results:** Preliminary results are based on data from 24 adults with relapsing-remitting or secondary progressive MS (11 females, 13 males; age 25–58; mean education 15.0 yr) and 15 without MS (controls; 7 females, 8 males; age 25–57; mean education 15.8 yr). Adults with MS were significantly less successful on the PM tasks than the controls, both in terms of noticing when actions were to be completed and remembering the particular actions. Half the participants formed implementation intentions (e.g., stating “I intend to pick up my niece at 3 pm” and picturing themselves picking up the niece) and half rote-rehearsed.

**Results:** Preliminary results are based on data from 24 adults with relapsing-remitting or secondary progressive MS (11 females, 13 males; age 25–58; mean education 15.0 yr) and 15 without MS (controls; 7 females, 8 males; age 25–57; mean education 15.8 yr). Adults with MS were significantly less successful on the PM tasks than the controls, both in terms of noticing when actions were to be completed and remembering the particular actions. Among participants with MS, implementation intentions did not improve PM performance on the game as a whole, although it slightly improved performance on the final round of the game. People who were older, had higher EDSS scores, or had lived with MS longer did more poorly on the PM tasks. Participants
had relatively good awareness; those who rated themselves better on PM in everyday life tended to demonstrate better performance.

**Conclusions:** People with MS had more difficulty performing PM tasks than did controls. Clinicians can expect that MS patients who are older, have higher EDSS scores, or have had MS longer will experience greater difficulties with PM tasks, and patients are likely to be aware of their deficits. It is possible that the implementation intentions mnemonic may be helpful to some people with MS, with extended practice.

**Disclosures:** DM Clawson has nothing to disclose.

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**COGNITIVE STATUS AFTER 10 YEARS OF PROSPECTIVE EVALUATION IN PATIENTS WITH RELAPSING MULTIPLE SCLEROSIS**

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**Keywords:** cognition; glatiramer acetate; longitudinal; memory; neuropsychological testing; responders

**Background:** In the pivotal study of glatiramer acetate (GA), improvements occurred in neuropsychological test scores during two years of treatment regardless of whether patients received GA or placebo, likely due to practice effects. Because most of the patients originally enrolled in this study are still being followed in an open-label study 10 years after baseline testing, we had an opportunity to reassess neuropsychological function after a much longer period, when it is more likely to have changed.

**Objectives:** The primary objectives were to assess long-term changes in neuropsychological status following 10 years of prospective evaluation and to determine whether baseline characteristics and changes in neuropsychological function after 2 years predict long-term changes.

**Methods:** Participants in the pivotal trial of GA repeated the Brief Neuropsychological Battery of Neuropsychological Tests an average of 10.6 ± 0.4 years after their initial baseline evaluation.

**Results:** Demographics and baseline cognitive test scores were similar for the subset of patients who were re-evaluated (n = 153) compared to those who were not evaluated (n = 98). On average, tests of memory and semantic retrieval showed stable function over 10 years of follow-up, but tests of attention showed declines for the group as a whole. Using a threshold of a 0.5 SD decline to define significant worsening, individual tests showed declines in 27–49% of participants and a composite score showed worsening in 19%. After 10 years of follow-up, there were minimal cognitive differences between those who were originally randomized to begin GA immediately or to delay treatment for 2–3 years. Controlling for age, gender, and education level, cognitive tests tended to worsen more in participants with lower baseline cognitive test scores and higher EDSS scores. The change in cognitive test scores during the first 2 years of observation was predictive of 10-year changes.

**Conclusions:** Most patients with relapsing MS had stable cognitive performance during 10 years of prospective evaluation. Patients with impairment at baseline and interim evaluation were the ones most likely to worsen during extended follow-up. The overall group stability may reflect benefits of immunotherapy, but conclusions are limited by an incomplete understanding of the natural history of cognitive impairment in MS.

**Disclosures:** The authors have received honoraria from Teva Neuroscience.

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**RANDOMIZED CLINICAL TRIAL OF AN ENERGY CONSERVATION COURSE FOR PERSONS WITH MS**

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**Keywords:** energy conservation strategies; fatigue; quality of life; randomized clinical trial; self-efficacy; work simplification

**Background:** Fatigue is a common and very disabling symptom for persons with MS. The MS Council for Clinical Practice Guidelines, 1998, on Fatigue and
MS reported that teaching energy effectiveness (conservation) strategies was a well-established practice but there was no scientific evidence to support its efficacy.

**Objectives:** To assess the short-term efficacy and effectiveness of six-week energy conservation (EC) course by Packer et al., 1995, on fatigue impact, quality of life, and self-efficacy for persons with MS.

**Methods:** In this randomized controlled trial, 169 persons with MS were randomly assigned to an immediate intervention group or a delayed control group using a crossover design. The outcome measures: Fatigue Impact Scale, SF-36 Health Survey, and Self-Efficacy for Performing EC Strategies were measured before and after EC course and no intervention periods. Data were analyzed from intent-to-treat and compliers methods.

**Results:** A mixed effects analysis of variance model determined that when individual subscales were analyzed separately, seven out of nine Fatigue Impact Scale analyses were decreased significantly (p < 0.05/3) and six out of nine SF-36 analyses expected to change were significantly increased (p < 0.05/8). The secondary outcome, Self-Efficacy for Performing EC Strategies Assessment increased significantly (p < 0.05) post-course compared to pre-course.

**Conclusions:** Results support the efficacy and effectiveness of the EC course to decrease fatigue impact, and increase self-efficacy and some aspects of quality of life. This EC course taught by occupational therapist is a legitimate non-pharmacological approach for managing fatigue for persons with MS.

**Disclosures:** VG Mathiowetz has nothing to disclose.

**Funding:** Supported by the National Multiple Sclerosis Society.

**ASPARTOACYLASE IS EXPRESSED PREDOMINANTLY IN OLIGODENDROCYTES AND IS DECREASED IN MS PLAQUES**

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**Keywords:** aspartoacylase; axonal-glial interactions; demyelination; MR spectroscopy; NAA; oligodendrocytes

**Background:** N-Acetylaspartate (NAA) is present in high concentrations in neurons in the central nervous system. Because of its abundance and relative cell specificity, measurement of NAA levels by magnetic resonance spectroscopy (MRS) has become a common tool for the analysis of neuronal pathology in patients with a variety of neurological diseases, including multiple sclerosis (MS). The steady-state level of NAA reflects the balance between NAA synthesis and NAA hydrolysis. Interestingly, lack of expression of the enzyme that hydrolyzes NAA, aspartoacylase (ASPA) causes the leukodystrophy, Canavan’s disease, suggesting that NAA might also be involved in myelination. Consistent with this notion, previous experiments have demonstrated that NAA can donate acetyl groups to myelin lipid.

**Objectives:** To investigate further the regulation of NAA synthesis and breakdown, we produced a rabbit polyclonal antibody to a region of the aspartoacylase protein, and used it to analyze ASPA expression in both mouse and human CNS.

**Methods:** Aspartoacylase expression was evaluated by immunohistochemistry of brain sections and oligodendrocytes in culture as well as by Western blotting of brain and cell protein extracts.

**Results:** We find ASPA expression mainly in differentiated oligodendrocytes co-expressing one of the myelin proteins or adenomatosis polyposis coli (APC) protein, both in intact brain and in primary cultures of mouse oligodendrocytes. This observation supports the possibility that neuronally produced NAA can serve as a substrate for myelin lipid and protein synthesis. In addition, analysis of tissue from the brains of several patients with MS demonstrated decreased ASPA expression within the plaque area, probably the result of reduced numbers of oligodendrocytes.

**Conclusions:** Therefore, reduced white matter NAA levels in MS patients, measured by MR spectroscopy are probably due, at least in part, to a reduction in NAA synthesis rather than an increase in NAA hydrolysis. The transient reductions reported in patients with MS likely are the consequence of more subtle disturbance in axoglial interactions.

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CEREBROSPINAL FLUID METABOLIC PROFILES DIFFERENTIATE MS TYPES

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Keywords: cerebrospinal fluid; glucose; lactate; metabolism; polyol; progression

Background: Relapse and progression are the two clinical phenomena used to type multiple sclerosis (MS). While relapse is clearly a function of inflammation, the mechanism of progression is less clear. This study investigated the role of glucose metabolism in progression. On the basis of findings linking abnormal polyol metabolism to white matter disease and to MS, we hypothesized that progression is in part mediated by an increase in glucose metabolism by the astroglial polyol pathway.

Objectives: To differentiate relapsing-remitting (RR) from secondary progressive (SP) types on the basis of cerebrospinal fluid (CSF) glucose metabolites, particularly polyol pathway metabolites.

Methods: We measured concentrations of sorbitol and fructose—the polyol pathway metabolites of glucose—as well as myoinositol, glucose, and lactate in premortem CSF and plasma samples from 100 patients and 12 healthy controls. 25 samples each came from patients designated as RR in remission; RR in relapse; SP; and SP with relapse. We used gas chromatography-mass spectrometry and a glucose/lactate analyzer to measure glucose and metabolites. Comparisons were made with nonparametric statistics with alpha adjusted to \(p < 0.005\) for multiple tests.

Results: SP patients had significantly elevated CSF glucose and lactate levels compared to RR patients, irrespective of current clinical state (medians = 64.2 vs. 57.2 mg/dl and 1.74 vs. 1.52 mmol/l, respectively). CSF glucose and lactate levels did not correlate significantly with IgG index, CSF albumin, or CSF cell counts. In comparison to healthy controls, both types had significantly elevated CSF sorbitol and plasma lactate. Contrary to our hypothesis, types did not differ in terms of CSF polyol pathway metabolites.

Conclusions: Our results suggest that CSF metabolic profiles are abnormal in MS and differentiate RR and SP types. Elevated CSF sorbitol indicates increased metabolism of glucose by the polyol pathway—an anaerobic alternative to glycolysis—in both types. CSF accumulation of lactate—the neuronal substrate for aerobic glucose metabolism—in SP patients is consistent with impaired mitochondrial glucose metabolism—an already postulated mechanism of MS progression. These CSF findings and the elevated plasma lactate in both types warrant further study to determine their significance for energy metabolism and potentially for fatigue in MS.

Disclosures: WT Regenold has nothing to disclose.

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