Influencing the Encephalitogenic Potential of Autoreactive T Cells in a Humanized Murine Model of MS

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The immunological component of MS is emphasized by the identification of several specific disease-associated MHC class II alleles and the implicated involvement of myelin-specific T cells in disease pathogenesis. These observations are the basis for a humanized T cell receptor (TCR)/MHC transgenic (Tg) model to study factors important for T cell encephalitogenicity and the relevance of “molecular mimics” to disease. Tg mice were created which possess the human variable components of HLA-DR4dW4 and the TCR from a clone recognizing MBP111–129, the HLA-DR4dW4-restricted immunodominant epitope of MBP. Following active and passive EAE induction, mice developed only very mild clinical symptoms despite high levels of autoreactive T cells. Studies are currently focused on factors which influence disease development: identifying infectious mimics with increased potency (varying the antigen), and the delivery of stronger signals from the antigen-presenting cell (altering costimulation). Using positional scanning combinatorial peptide libraries and mathematical predictions to scan known microbial and human databases, we have identified several peptides derived from infectious agents that stimulate the transgenic T cells with significantly higher affinity than MBP111–129. We are now testing the ability of these high affinity peptides/pathogens to alter the initiation and severity of EAE in this model.

Additionally, studies using phenotypically diverse bone marrow-derived dendritic cells (DC) suggest that antigen presentation can significantly influence the encephalitogenicity of autoreactive T cells. Immature HLA-DR4dW4 Tg DC activate Tg T cells to a Th0/Th1 profile, but cytokine-matured DC enhance a strong Th1 shift in T cells without affecting encephalitogenicity. In contrast, immature DC in the B10.PL Tg model induce Th2 T cells incapable of causing disease, yet mature DC prime T cells to become encephalitogenic. Although striking differences exist, we are now testing whether differentially primed DC may serve as promising immune therapies. Thus, this unique humanized Tg system has been a valuable tool in our characterization of several factors contributing to T cell encephalitogenicity and the consequence of molecular mimics in autoimmunity.

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Positive Selection of a T-Independent Natural Autoreactive B Cell Clone in the Central Nervous System of Multiple Sclerosis

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The findings of our previous studies along with those of other groups have shown that intrathecal immunoglobulin (Ig)-forming B cells derived from a majority of patients with RRMS, particularly from cases in the early stage of the disease, are clonally expanded. They carry somatic mutations on their Ig heavy chain variable region (VH) genes, indicating that a T-cell dependent autoimmunity has played a role in initiating an inflammatory process in the central nervous system (CNS) of early MS and RRMS. The beneficial effects of using current immunomodulatory-therapies in these patients further confirm this hypothesis. However, little efficacy of these therapies has been observed in cases with chronic progressive MS (CPMS), secondary and primary progressive MS. This indicates that there may be an alternative T-cell independent immune pathological mechanism involved in the chronic progression of the disease. We have tested the hypothesis that a T-cell independent natural autoimmune response may contribute to the pathogenesis of CPMS. Using analysis of Ig VH genes of intrathecal B cells derived from patients with the different courses of MS, we show that a germline gene-encoded natural autoreactive B cell clone is selected to expand in the cerebrospinal fluid and in lesions of patients with CPMS. These clonally selected natural autoreactive B cells infiltrate multiple lesions and are persistently expanded during disease progression. The findings of a high frequency of this natural autoreactive B cell clone in the CSF and lesions of patients with CPMS, but not in the patients with RRMS indicates that the persistent immune inflammatory process in the CNS of MS may provoke a natural autoimmune response, which contributes to the pathogenesis of CPMS. Our studies reveal a potentially important mechanism by which natural autoreactive B cells have a pathological role in the more advanced and severe forms of autoimmune disease.

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Neuroimmunology from the CNS’s Point of View: Immuno-Competence of Parenchymal CNS Cells Controls the Pathogenesis of EAE

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Multiple sclerosis is an inflammatory, T lymphocyte-mediated disease of the central nervous system (CNS). Within the CNS, microglial cells have been proposed to play a potential role during priming, expansion, and polarization of auto-aggressive T cells under inflammatory conditions. To this day however, there is no in vivo evidence that microglia/T cell interactions actually impact on inflammation within the CNS. We systematically evaluated the functional contribution of CNS-parenchymal cells in Ag-presentation, co-stimulation and cytokine production/polarization during MOG induced EAE. By combining transgenicity with bone-marrow chimerism, we have generated mice in which the genotype and functional properties of the CNS is distinct from that of the systemic immune compartment. After engraftment of donor bone-marrow cells in the systemic compartment and perivascular space, EAE was induced by active immunization as well as adoptive transfer of MOG-reactive T cells. Our data show that MHC-II expression within the CNS parenchyma is absolutely critical for the development of inflammation and clinical EAE, showing that parenchymal cells are needed to present Ag to infiltrating auto-aggressive T cells to manifest disease. Furthermore, CD40/CD154 interactions within the CNS control clinical disease and leukocyte infiltration into the parenchyma. As with CD40, mice in which IL-12 cannot be produced by CNS-resident cells, do not develop clinical EAE. However, the lack of IL-12 does not inhibit leukocyte infiltration. Preliminary data suggest that T cell encephalitogenicity is inhibited upon their entering a CNS environment from which IL-12 is absent. Taken together, our data demonstrate that parenchymal cells are critical players during T cell activation, migration and differentiation in vivo during EAE.

N-Acetyl Aspartate is a Specific Marker of Mature White Matter Axons in Vivo

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Axonal degeneration is a major cause of permanent neurological disability in MS (see Bjartmar and Trapp, Curr Opin Neurol 14: 271–278, 2001, for review). Axonal transection begins at disease onset, but remains clinically silent due to compensatory brain mechanisms. Non-invasive monitoring of axons is therefore essential for the study of disease progression and evaluation of ongoing therapy in MS patients. The neuronal compound N-acetyl aspartate (NAA), as measured by magnetic resonance spectroscopy (MRS), is a promising monitor of axonal pathology. It has been reported, however, that NAA is expressed also by oligodendroglial lineage cells in vitro. These observations raise concerns whether NAA alterations in vivo reflect neuronal/axonal pathology only. In order to investigate NAA specificity for white matter axons, transected rat optic nerves undergoing Wallerian degeneration were analyzed by HPLC and immunohistochemistry. In transected adult nerves, loss of NAA correlated with axonal degeneration. Non-proliferating oligodendrocyte progenitors, oligodendrocytes, and myelin were abundant in these axon-free nerves. In non-transected contralateral nerves, NAA increased significantly (42%) over time. After transection at postnatal day 4 (P4), total NAA decreased by 80% (P14) and 94% (P20). In these developing axon-free nerves containing high densities of oligodendrocyte progenitors, 25–33% of the progenitors were proliferating as determined by BrdU-labeling. These data validate MRS measurements of NAA as a specific in vivo monitor of axonal pathology and disease progression in MS patients. In addition, the results indicate that neuronal activity can modulate NAA levels, and that some NAA in developing white matter may occur in proliferating immature oligodendrocytes.

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Short-Term Effect of Intravenous Methylprednisolone on Whole Brain Atrophy in Multiple Sclerosis

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Background: Brain parenchymal fraction (BPF) is a highly reproducible measure of whole brain atrophy that correlates with disability in MS patients. The magnitude of short-term fluctuations and effects of corticosteroids are unknown.

Aim: To examine short-term stability of BPF and other volumetric MRI measurements in MS and determine the effects of intravenous methylprednisolone (IVMP).

Methods: MRI scans were obtained from 10 MS patients immediately before and weekly for 8 weeks after IVMP (1 g daily for 3 days) followed by oral prednisone (12 days). Comparison was made to 5 untreated MS patients matched for degree of brain atrophy and disease course. BPF, whole brain magnetization transfer ratio (MTR), T2 lesion volume, T1 hypointense lesion volume, and gadolinium enhancing lesion volume were calculated using a reproducible, fully automated image analysis method.

Results: There were substantial within-subject fluctuations in BPF over the first four weeks following IVMP, which then stabilized over the second four weeks (p = 0.03). Secondary-progressive MS patients demonstrated a persistent decrease in BPF after IVMP. In contrast, relapsing-remitting MS patients did not demonstrate this decrease. The effects of steroids on other quantitative MRI parameters are currently being analyzed.

Conclusions: IVMP treatment consistently produced short-term fluctuations in BPF lasting approximately 4 weeks. The direction of change differed between subjects and appeared to differ between relapsing and progressive subjects. Studies employing quantitative MRI parameters as endpoints need to postpone imaging studies at least 4 weeks after steroid treatment. Final data analysis regarding short-term fluctuations of BPF and other volumetric lesion measurements with and without IVMP will be presented. This data will facilitate design of future MS studies that use quantitative MRI parameters as endpoints, and may clarify the effects of corticosteroids on MS pathology.

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Central Nervous System Myelin Protein Gene Products Are Robustly Expressed, and Modulate Apoptosis in Non-Neural Tissues

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The proteins of myelin are perceived as a unique group of proteins that have evolved to form the myelin sheath in the nervous system. We show that different isoforms of myelin basic protein (MBP) and proteolipid protein (PLP) genes are expressed in different tissues including blastocyst, testes, and thyroid/parathyroid. In the blastocyst, classical DM20 transcripts are more abundant than classical PLP and the recently described srPLP/srDM20 transcripts. Both GOLLI and MBP transcripts are found in the blastocyst, with GOLLI being the predominant isoform. In testes, PLP, DM20, srPLP, and srDM20 transcripts are abundantly detected with RT-PCR. PCR products have been sequenced in these tissues and match the published sequences for MBP and PLP genes. Blastocysts, human trophoblasts, and testes each show different MBP and PLP isoforms on Westerns, suggesting each isoform has specific functions in these tissues. Using in situ hybridization and immunocytochemistry, we find PLP message and/or protein in trophoblast layer of blastocysts, immortalized human trophoblasts, and spermatogonia. Combined in situ hybridization/immunocytochemistry and TUNEL shows abundant PLP products in TUNEL+ cells. In jimpy mouse, a PLP mutant characterized by abundant oligodendrocyte death and in PLP overexpressors, PARP and activated caspase-3 are significantly elevated in testes, suggesting abnormal expression of PLP functions in non-CNS tissues. The finding of CNS myelin proteins in non-neural tissue raises the intriguing question as to whether they play a role in the induction of tolerance during development, and, if they are abnormally expressed in these non-CNS tissues, in induction of autoimmune diseases including multiple sclerosis.

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Contrast Letter Acuity as a Candidate Visual Outcome Measure for the MS Functional Composite

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Visual loss is one of the most common and disabling aspects of neurologic impairment in multiple sclerosis (MS). The MS Functional Composite (MSFC) includes a timed 25-foot walk, 9-hole peg test, and paced auditory serial addition test (PASAT-3), but does not include a measure of visual function. Adding a visual dimension to the MSFC will likely increase its applicability in MS populations. The purpose of these investigations was to examine construct validity and feasibility for contrast letter acuity as a candidate visual outcome measure for the MSFC. Binocular low-contrast Sloan letter chart testing (LCSLC) was administered to a subgroup of participants in the International Multiple Sclerosis Secondary Progressive Avonex Controlled Trial (IMPACT). Data from an ongoing study of visual outcome measures at the University of Pennsylvania (Penn Study-relapsing-remitting MS) were also analyzed. LCSLC testing was performed by examining technicians. Rank-correlations of LCSLC scores (# letters correct at 5%, 2.5%, 1.25% contrast levels) with MSFC and EDSS scores were modest to moderate, supporting construct validity for LCSLC:

| LCSLC vs. MSFC Z-Score | LCSLC vs. EDSS Score |
|------------------------|----------------------|
| IMPACT (N=65)           |                     |
| $r_s=0.34$ to $0.63$,   | $r_s=-0.23$ to $-0.48$, |
| $p=0.008-0.0001$       | $p=0.08-0.0002$     |
| Penn Study (N=24)       |                     |
| $r_s=0.58$ to $0.68$,   | $r_s=-0.35$ to $-0.52$, |
| $p=0.004-0.0004$       | $p=0.09-0.009$      |

Among 5 visual outcome measures in the Penn Study, LCSLC also best distinguished MS patients from age-matched controls on the basis of visual function ($p=0.03-0.008$, logistic regression analysis). Contrast letter acuity (LCSLC) testing demonstrates construct validity and feasibility for administration to patients with relapsing-remitting and secondary-progressive MS, and has excellent potential as a candidate visual component for the MSFC.

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Attitudes Regarding Assisted Suicide in Patients with Multiple Sclerosis

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Purpose: To assess attitudes about assisted suicide in patients with multiple sclerosis (MS)

Methods: Surveys were sent to 576 MS patients registered with a county MS association.

Findings: Of respondents (N=405), 79% were female, median age was 50 (range 21–78); 61% lived with a partner, and 69% were educated beyond high school. The median EDSS score was 6 (range 0–9). Imagining a severe worsening of their MS without recovery, 53% would want a medication to end their life, whereas 61% would consider it “under some circumstances.” Most (61%) would like these available to patients, but only 3% would have taken one during the past year. Most (63%) thought they should be available only through a physician. A minority (22%) thought that these medications should not be available. Factors rated very important in forming these opinions included having control (64%), pain (63%), loss of purpose/enjoyment (58%), loss of cognition (56%), dependency (50%), religious beliefs (37%), timing (36%), financial (31%), what others would think (20%), having a long life (20%), and legality (19%). Those who would consider assisted suicide under some circumstances were younger ($p=0.02$), had lower EDSS scores ($p=0.04$), scored higher on a hopelessness scale ($p=0.05$), and scored lower on a spirituality scale ($p<0.0001$). They did not have excess depression ($p=0.44$).

Conclusions: Most MS patients want medications available to end their life under some circumstances, but only through a physician. Factors important in forming these opinions are control, pain, loss of purpose/enjoyment, loss of cognition, dependency, hopelessness, spirituality, and disability.
Co-Stimulatory Molecule and Cytokine Expression by Monocytes Derived from MS Patients with Secondary Progressive Course of Disease

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Co-stimulatory molecule expression or inflammatory and regulatory cytokines produced by monocytes may be used to predict prognosis or response to treatment with INF-β in MS patients. We focused our studies on monocytes due to their purported important role in initiating and perpetuating the immune response in MS. Cross-sectional and longitudinal studies were performed on freshly isolated peripheral blood monocytes derived from MS patients with RR or SP forms of disease, before and during IFN-β treatment.

Co-stimulatory molecule expression (CD80/86 on monocytes, or CD28/40/154 on T cells) was measured by flow cytometry. We used three different techniques (RNase protection assay, ELISA assay and intracellular cytokine staining) to assess the levels of monokines.

Only CD86 expression was 2-fold higher in SPMS patients compared to HC. Treatment of RRMS with interferon-β led to reduced CD86 expression on monocytes compared to pretreatment levels. We observed an increase of IL1, IL6 and TNF-α but not IFN-γ production by all three methods in untreated RRMS and SPMS patients compared to HC and IFN-β treated RRMS patients. Increased levels of IL-10 and IL-12 mRNA as well as IL-12 protein production were observed in SPMS patients. IFN-β treated RRMS patients had lower levels of monokines compared to untreated RRMS or SPMS patients. MO derived cytokines may prove to be key indicators of transition of the disease to a more advanced stage.

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Human Mesenchymal Stromal Cells Form Oligodendrocytes After Transplantation into a Rat Model of Neurodegenerative Disease

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During the course of multiple sclerosis, myelin is destroyed and oligodendrocytes are depleted. The persistence of neurological symptoms can be attributed to lack of repair of the myelin sheath and axonal injury. One approach to therapy would be to use exogenous cell in a transplant paradigm to replace the damaged oligodendrocytes and regenerate myelin. We have transplanted human bone marrow mesenchymal cells into a rat model of demyelination and observed the presence of newly formed oligodendrocytes. Bone marrow stromal cells have previously been shown to be a source of multi-potential cells that can differentiate into adipocytes, osteoblasts, myoblasts and cells which express the astrocyte marker GFAP. Our studies have utilized the myelin deficient (MD) rat. These animals have an X-linked point mutation in the gene for proteolipid protein (PLP) that results in a failure to form mature oligodendrocytes and myelin, and die at 21 days postnatal. Human MSCs were injected into the lateral ventricle of one day postnatal male rat pups. At 20 days postnatal, the animals were sacrificed and frozen sections were prepared. The injected MD rats demonstrated robust expression of proteolipid protein near the injection site and increased expression of myelin markers; Rip and myelin basic protein. Uninjected MD rats express virtually no PLP and very small amounts of MBP and RIP. We also noted that the injected brains also contained GFAP+ astrocytes and NCAM+ neurons which could be labeled with antibodies to human antigens indicating they originated from the transplanted cells. Our data suggest that mesenchymal stromal cells can serve as an autologous source of cells to facilitate transplantation following dysmyelinating or demyelinating disease.

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Vitamin D as an Inhibitor of MS: Mechanism for 1,25-Dihydroxyvitamin D3 Prevention of Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is a demyelinating disease involving genetic and environmental risk factors. Inheriting genetic risk factors is not sufficient to cause MS; exposure to environmental risk factors is also required. MS may be preventable if these environmental risk factors can be avoided. Much evidence suggests that sunlight is protective, whereas a lack of sunlight is a risk factor for MS. The vitamin D endocrine system is exquisitely responsive to sunlight, suggesting that sunlight may exert its protective effects through vitamin D. We have investigated this hypothesis using the hormone, 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3], as an inhibitor of experimental autoimmune encephalomyelitis (EAE), a model for MS. Cells from mice with a transgenic T cell receptor (TCR-tg) specific for myelin basic protein (MBP) peptide were transferred into normal mice, and the recipients were treated with 1,25-(OH)2D3 or vehicle and immunized with MBP peptide. The mock-treated mice developed EAE, but the 1,25-(OH)2D3-treated recipients remained largely disease-free. Immunofluorescence, proliferation studies, and cytokine analysis showed that the hormone-treated and mock-treated mice both had TCR-tg cells that proliferated to MBP peptide and produced IFN-γ, but not IL-4, in the lymph nodes. The mock-treated mice also had these activated TCR-tg cells in the CNS, but the 1,25-(OH)2D3-treated mice had only naïve TCR-tg cells in the CNS. Thus, 1,25-(OH)2D3 did not promote autoreactive T cell deletion, or T helper type 2 development, or inhibit T helper type 1 development in the periphery. Instead, the 1,25-(OH)2D3 inhibited autoreactive T cell activation in the CNS.

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Cytokine Transduced T Cells in the Treatment of Allergic Encephalomyelitis

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An imbalance between auto-aggressive and suppressor T cells is perhaps of major importance in the pathogenesis of certain autoimmune diseases. Regulatory T cells may protect against chronic inflammation by secreting immune-suppressive cytokines such as TGF-β and IL-10. Thus, switching auto-aggressive into regulatory T cells through gene therapy might protect against myelin damage in MS. Earlier we showed a protective effect of TGF-β in allergic encephalomyelitis (EAE) in mice. Since active TGF-β is fibrogenic, we have used a gene therapy strategy in which MBP-specific auto-aggressive CD4 cloned TH1 cells are transduced with the cDNA for latent TGF-β and injected at the onset of EAE. Although the untransduced TH1 cells aggravated the PLP-induced EAE, the TGF-β/MBP cells caused a marked amelioration. The protective effect of these cells was abolished by anti-TGF-β, showing that active TGF-β produced in vivo was responsible. Similarly transduced T cells of a specificity unrelated to myelin proteins had no effect. Spinal cords taken 12-50 days after TGF-β/MBP cells contained TGF-β cDNA, but not thereafter. This finding was in agreement with the observation that TGF-β/MBP T cell recipients were resistant to the induction of EAE relapses by bacterial superantigen or endotoxin within 2 weeks but not >6 weeks after the T cell transfer. In previously untreated mice, endotoxin-induced EAE relapses could be prevented by simultaneously injecting TGF-β/MBP T cells. Similar studies with IL-10 transduced TH1 cells were much less successful and IL10/MBP T cells appeared to persist for a shorter time in the spinal cord. Currently, we are using this gene therapy approach also in trying to prevent the development of spontaneous EAE in MBP specific TCR transgenic mice.

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Myelin-associated Oligodendrocytic Basic Protein in CNS Autoimmune Disease

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Myelin-associated oligodendrocytic basic protein (MOBP) is an abundant myelin component synthesized exclusively by oligodendrocytes in the central nervous system (CNS). Recently, we have reported that MOBP is able to induce experimental allergic encephalomyelitis (EAE) in mice and is associated with multiple sclerosis (MS) (Holz et al, *Journal of Immunology* 2000 164:1103). We have extended our initial work and show now that two murine MOBP epitopes, amino acids (aa) 20–38 and aa 41–59, are capable to induce clinical as well as histological EAE in SJL/J mice. We demonstrate that T-cell lines specific for these MOBP epitopes are mainly of the CD4+ T helper 1 cell subtype. They proliferate in response to MOBP peptides with significant stimulation indexes. As shown by flow-cytometric analysis, MOBP-specific T-cell lines in response to the corresponding peptides produce IFN-γ, but not IL-4. Such T-cell lines adoptively transferred CNS disease. Recipient mice developed signs of EAE including hind leg paralysis 5–7 days after transfer of MOBP-specific T-cell lines. Most important, we have extended these studies to the association of MOBP with human MS. We demonstrate by flow-cytometric analysis that CD4+ T-lymphocytes isolated from the blood of MS patients during clinical exacerbations respond to MOBP peptides predominantly by the production of IFN-γ and TNF-α, but not IL-4. Our results show that the abundant and CNS-specific myelin constituent MOBP is likely an important target antigen in CNS autoimmune disease.

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Comparison of Brain 4 T T1 Histograms between Multiple Sclerosis and Control Subjects

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\( T_1 \) mapping offers a precise way to characterize brain and has potential for objective evaluation of macroscopic and microscopic disease burden in MS. Water proton \( T_1 \) values differ between gray matter (GM), white matter (WM) and CSF, and these differences have been used to generate segmented brain images. In MS, brain \( T_1 \) values are often increased, markedly in lesions, and subtly in normal appearing white matter (NAWM). In this study we compare \( T_1 \) data collected from 13 MS patients and 9 controls using an imaging variant of the Look-Locker technique implemented on a 4 T MRI instrument. A non-selective adiabatic pulse was used for inversion, and the recovery was sampled at six times post-inversion. Thirty-three 3-mm thick axial slices were collected with in-plane resolution of 1.5 mm x 1.0 mm. \( T_1 \) maps were calculated using a two-parameter function. \( T_1 \) histograms were generated and means, modes, and widths were determined for WM and GM distributions by parametric fitting. Repeated measure precision was excellent. Compared to controls, average MS WM \( T_1 \) distribution means were increased 4% (\( P < 0.005 \)) and the MS GM \( T_1 \) distribution means were increased 2.4% (\( P < 0.005 \)). There were no significant differences in distribution widths, skewness’, or volume fractions between groups. The WM distributions were symmetric and \( T_1 \) values determined from histograms were in excellent agreement with those determined by region-of-interest analysis of NAWM. Fast \( T_1 \) mapping techniques combined with histogram analyses provides an efficient way to quantify disease burden in MS.

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Space-Local Spectral Texture Map Based on MR Images of MS Patients

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The exquisite contrast sensitivity of MRI makes it an excellent tool to monitor disease activity in relapsing-remitting and secondary progressive MS. Total lesion volume has become the most frequently used MR-based quantitative measure of disease status in clinical trials. However, measurement of lesion volume alone may not reflect the complete underlying pathophysiology of the disease process. In particular, there is recent evidence which suggests that there may be widespread demyelination and axonal damage in normal appearing white matter (NAWM). Thus utilization of new techniques which are more sensitive to white matter damage may provide additional valuable information about the pathological progress of MS. We are using the Stockwell transform (ST), originally developed for analyzing geophysics data, to derive texture in MR exams of MS patients. The ST is a modification of the Fourier transform and provides both spatial and frequency information in an image. It allows detection of subtle intensity changes over space and time. Thus, by applying the ST to a MR image, we can generate a new detailed high resolution map of texture which is not visible in the conventional MR images. We use these texture maps to enhance multi-spectral analyses of patient images. This new information may provide additional insight into underlying pathology, demyelination and axonal loss and aid radiologists in detecting subtle abnormalities in the NAWM.

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