Magnetization Transfer and Multicomponent T2 Relaxation Measurements With Histopathologic Correlation in an Experimental Model of MS

Paula J. Gareau, PhD,1* Brian K. Rutt, PhD,1 Stephen J. Karlik, PhD,1,2 and J. Ross Mitchell, PhD1,2

Magnetization transfer and multicomponent T2 imaging techniques were implemented to study guinea pig in vivo. A chronic-progressive model of experimental allergic encephalomyelitis (EAE) was produced, and the inflammatory component of the disease was manipulated using antibodies against integrin. The magnetization transfer ratio (MTR) and T2 relaxation properties were measured in normal-appearing white matter (NAWM) with histological comparisons. Significant reductions in both the mean MTR and the myelin water percentage were measured in NAWM of EAE guinea pig brain. However, the MTR and myelin water percentage appear to measure different aspects of pathology in NAWM in EAE. Reductions in the MTR were prevented or reversed with suppression of inflammation. However, modulation of inflammatory activity was not reflected in the measurement of the myelin water percentage. Since the amount of myelin is not expected to vary with inflammatory-related changes, these observations support our hypothesis that the MTR is sensitive to physiological changes to myelin induced by inflammation, while the short T2 component is a more specific indicator of myelin content in tissue. Pathologic features other than demyelination may be important in the determination of the MTR. J. Magn. Reson. Imaging 2000;11:586–595. © 2000 Wiley-Liss, Inc.

Index terms: magnetization transfer ratio; multicomponent T2 relaxation; normal-appearing white matter; inflammation; experimental allergic encephalomyelitis; multiple sclerosis

MAGNETIZATION TRANSFER (MT) imaging has become a focus of basic science and clinical studies because of its unique physical basis of contrast and the potential to quantify aspects of the biochemical structure and composition of tissues (1). MT contrast is obtained by applying off-resonance radiofrequency irradiation designed to saturate preferentially immobile protons of macromolecules (2,3). This magnetization energy is then transferred to mobile protons and reduces their signal intensity (SI). Careful optimization of the MT imaging sequence is required for reliable quantification. Commonly, the relative difference in SI with and without magnetization transfer, the magnetization transfer ratio (MTR), is calculated (4). Tissues with a large number of restricted protons will show a large SI decrease on application of the saturation pulse; tissues with few or no restricted protons will have a very small MT effect.

The MTR is expected to be related to the amount of myelin in tissue (5,6). Consequently, there is great interest in the application of MTR to study white matter (WM) disorders, particularly multiple sclerosis (MS). Breakdown of the macromolecular structure, such as in demyelination, is expected to cause a decrease in the MT effect and a decreased MTR. An increase in free water, as occurs in edematous lesions, is thought to cause only a slight reduction in the MTR (4). This has led to the proposal that the MTR may be useful for differentiating between types of lesions in MS.

In MS patients, reduced MTRs have been reported in WM lesions (4.7–9), and in normal-appearing white matter (NAWM) (4,8,10–12). The reductions in the MTR in NAWM tend to be small, while the range of the MTRs in individual studies of WM lesions is typically very wide (4,7). Dousset et al (4) were the first to suggest that the wide range of MTRs measured in lesions of MS patients might indicate lesions of differing degrees and grades of demyelination. Since this seminal work, several groups have measured differences in the MTR for lesions of different ages or classes (9,13–16). Others (7,17) have found no significant differences.

It is still not clear whether MTRs can be used effectively to judge the histopathologic state of MS lesions. MS is a diffuse and heterogeneous disease process. MS lesions are of mixed pathology, with varying amounts of demyelination, edema, inflammatory cell infiltration, gliosis, and repair (18). In NAWM, there is a combination of truly normal WM along with regions of microscopic WM disease in various states of degradation and repair (19). These mixtures of pathologic change, in NAWM and WM lesions, make the establishment of the

1John P. Robarts Research Institute, London Health Sciences Center, University Campus, London, Ontario, Canada N6A 5K8.
2Department of Diagnostic Radiology and Nuclear Medicine, London Health Sciences Center, University Campus, London, Ontario, Canada N6A 5K8.
Contract grant sponsor: Multiple Sclerosis Society of Canada.
*Address reprint requests to: P.J.G., Robarts Research Institute, 100 Perth Drive, London, Ontario, Canada N6A 5K8.
E-mail: pgareau@irus.rr.on.ca
Received October 20, 1999; Accepted February 24, 2000.

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underlying mechanism for the reduction in MTR very difficult.

Measurement of the short component of multiexponential T2 decay in WM may provide information related to myelin. For example, MacKay et al (20) reported three T2 components in human brain tissue in vivo, including a short T2 component in WM that they attributed to water bound between myelin bilayers, the so-called myelin water (21,22). They suggest that damage to myelin structure should change the “myelin water” content, and thus the short T2 component of multiexponential decay. In MS patients examined by MacKay et al (20), the average myelin water content in MS lesions was significantly reduced compared with that of WM in normal volunteers. A more recent study by this group (15) reported significantly lower mean myelin water percentages in MS lesions compared with both NAWM in MS patients and normal WM in volunteers. Does et al (23) also reported three T2 components in vivo in peripheral nerve, while our own group has reported extraction of a short T2 component from WM in guinea pig brain in vivo using an optimized imaging protocol at 4.0 T (24).

Experimental allergic encephalomyelitis (EAE) is a widely used animal model for studying MS (25,26). The acute phase of EAE (days 9–14) is characterized primarily by inflammation without signs of alterations to myelin. During the chronic progressive (CP) phase (>day 30), there can be evidence of both myelin damage and inflammation in the brain and/or spinal cord. A number of MRI techniques have been used to investigate brain WM lesions in different animal models of EAE (4.27–35), although there has never been a reference to NAWM in EAE. As in MS, the NAWM in EAE brain actually may not be normal, rather, the WM, which appears normal in standard MR images, has been shown histologically to contain regions of normal WM along with distinct regions containing large concentrations of inflammatory cells and structural changes to myelin. The extent of these abnormalities progresses with the timecourse of the disease.

Neither the MTR nor T2 have been measured in vivo during the CP phase of EAE. Previously, Stewart et al (22) measured a reduction in the myelin water percentage in demyelinated spinal cord samples. Slightly reduced MTRs have been reported in focal WM lesions examined during the acute phase of EAE; these lesions were edematous without demyelination (4). Jordan et al (36) have used MT contrast to examine chronic EAE in a marmoset model; however, the tissue MTRs were not reported.

The purpose of our study was to clarify the relationship between measured MTR and multicompontent T2 and WM pathology in an animal model of demyelinating disease. To achieve this, MT and multicompontent T2 imaging techniques were implemented to study guinea pig brain in vivo. A CP model of EAE was produced, and the inflammatory component of the disease was modulated using anti-α4-integrin treatment (37). The MTR and the T2 relaxation properties were measured in NAWM with histological comparisons. The results of these experiments support the hypothesis that pathologic features other than demyelination may be important in the determination of the MTR.

MATERIALS AND METHODS

Animal Model

EAE was induced in female Hartley (Charles River) guinea pigs (200–250 g) by an intramuscular injection of whole central nervous system (CNS) homogenate in complete Freund’s adjuvant with inactivated Mycobacterium tuberculosis. Animals were weighed and scored daily for the clinical features of EAE (Table 1). Scoring began the day after immunization.

Antibody Treatment

In this study we used a short course of antibody treatment, with anti-α4 integrin, to manipulate the infiltration of immune cells into the CNS. Treatment of either acute or chronic EAE with antibodies against integrin leads to a reversal of the clinical signs of disease and a resolution of inflammatory activity (37). Anti-α4 integrin-mediated recovery from EAE has been shown to be due to the prevention of the influx of new inflammatory cells into the CNS that are required to replace those undergoing apoptosis (38). In this study treated animals received anti-α4 integrin daily for 7 days (1 mg, subcutaneously).

Experimental Design

The following experiments were conducted in three phases, in separate groups of animals. Phase I involved the optimization, testing, and application of an MT imaging protocol and application of this protocol to study acute and chronic EAE. Phase II involved the collection of both MT and T2 measurements in the same location. Phase III involved analysis of histological preparations made from the same slices where MR imaging was performed.

In Phase I MTRs were measured in the following groups of animals. Five guinea pigs were examined longitudinally during the CP phase of EAE, as follows:

Day 35: prior to antibody treatment
Day 43: after 7 days of antibody treatment
Day 53: after 10 days off treatment

In addition, a cross-sectional study included the following groups of animals that were scanned and then sacrificed:

<table>
<thead>
<tr>
<th>Score</th>
<th>Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No abnormality</td>
</tr>
<tr>
<td>0.5</td>
<td>More than 1 day of weight loss</td>
</tr>
<tr>
<td>1.0</td>
<td>Hind limb weakness, poor righting reflex</td>
</tr>
<tr>
<td>2.0</td>
<td>Paresis, urinary incontinence, fecal impaction</td>
</tr>
<tr>
<td>3.0</td>
<td>Paralysis</td>
</tr>
<tr>
<td>4.0</td>
<td>Terminal paralysis</td>
</tr>
</tbody>
</table>
Group I \((n = 8)\): normal controls  
Group II \((n = 6)\): day 13 EAE, acute phase  
Group III \((n = 6)\): day 35 EAE, chronic phase  
Group IV \((n = 6)\): day 43, chronic phase, after antibody treatment  
Group V \((n = 5)\): day 45, chronic phase, untreated

In Phase II we applied both MT and T2 techniques in a similar cross-sectional study:

Group I \((n = 6)\): normal controls  
Group II \((n = 6)\): day 13, acute EAE  
Group III \((n = 6)\): day 33, chronic EAE  
Group IV \((n = 6)\): day 41, chronic phase after antibody treatment  
Group V \((n = 6)\): day 42, chronic phase untreated

Imaging Protocols

All imaging was performed at 4.0 T on a Varian/Siemens UNITY INOVA using a custom-built solenoid radiofrequency (RF) coil for the guinea pig head (5 cm internal diameter, 4 cm length). Anesthesia was induced with ketamine/xylazine. Animals were placed in the supine position with the head centered in the coil. A multislice T2-weighted spin-echo (SE) sequence was used to identify the axial brain image slice to be used for subsequent MT and T2 acquisitions.

Magnetization Transfer Imaging

MT imaging was performed with a proton density-weighted SE single-slice acquisition. The single-slice mode was chosen to avoid incidental MT effects. The imaging parameters were as follows: TR/TE 5000/25 msec, slice thickness 5 mm, 4 × 4 cm field of view (FOV), image matrix 256 × 128, no signal averaging. The scan time was approximately 10 minutes.

The following characteristics of the saturating pulse train were tested and optimized in a distilled water phantom and in normal guinea pig brain: average RF power \((B_1)\), frequency offset \((\Delta f)\), number of saturation pulses \((N_{sat})\) and time between pulses. A distilled water phantom was subsequently included in the FOV during application of the MT protocol, as a reference to determine the direct saturation of the MT pulse train experimentally.

T2 Relaxation Measurements

A Carr-Purcell-Meiboom-Gill (CPMG) sequence, previously developed for single-slice imaging at 1.5T (20) was implemented and optimized at 4.0 T. The pulse sequence parameters were as follows: TR/TE 5000/10 msec, echo spacing 10 msec, echo train length 32, slice thickness 5 mm, FOV 7 × 7 cm, image matrix 128 × 128, bandwidth ±32 kHz, no signal averaging. The scan time was approximately 10 minutes. A slice-selective minimum phase Shinnar-LeRoux (SLR) pulse, with a time-bandwidth product of 8 having 1% stop and 1% pass band ripple, was used for excitation (39). A 200-μsec-wide composite refocusing pulse was used, composed of three adjacent nonselective rectangular pulses, 90°-180°-90°. A set of balanced crusher gradients was applied along the slice-selective axis, surrounding each refocusing pulse. These were sign alternating and descending in amplitude as proposed by Poon and Henkelman (40).

Image Analysis

The MTR was calculated as:

\[
MTR = \left(1 - \frac{M_s}{M_0}\right) \times 100\%
\]

where \(M_s\) is the mean SI of pixels from an image without saturation and \(M_0\) is the mean SI of the same pixels from an image with saturation. The mean SI was determined from 16 to 20 pixels (each 0.16 × 0.3 mm) positioned in the NAWM of the corpus callosum (Fig. 1). For T2 analysis, WM regions of interest (ROIs) were also specified in the NAWM of the corpus callosum. Decay curves were fitted by both monoexponential and multiexponential functions. The non-negative least squares (NNLS) algorithm, modified by Whittall and MacKay (41), was applied to each curve for multiexponential fitting. An F test was used, with a significance level of 1%, to determine whether the fit created by the multiexponential function was significantly better than that created by the monoexponential function. We compared monoexponential and multiexponential fits to the same data using the F test function (42):

\[
F = \frac{RSS_{mono}/v_{mono}}{RSS_{multi}/v_{multi}}
\]

where \(RSS_{mono}\) is the sum of the squares of the residuals from the monoexponential fit, \(RSS_{multi}\) is the sum of the squares of the residuals from the multiexponential fit, \(v\) is the degrees of freedom and equals \(n - k\), where \(n\) is the number of data points and \(k\) is the number of parameters in the fitting function. In our study \(v_{mono} = 30\) (32 − 2) and \(v_{multi} = 26\) (32 − 6). Where a multiexponential analysis provided the best fit for the NAWM T2 signal decay, one T2 peak was measured in the “short” T2 bin (5–40 msec), and one T2 peak was measured in the “medium” T2 bin (40–120 msec). The amplitude in each T2 bin corresponds to the proton signal intensity for that T2 pool interpolated to TE = 0 msec. The area of the short T2 component of the multiexponential decay corresponds to the myelin water percentage. The image signal-to-noise ratio (SNR) was defined as the mean SI in the sample divided by the standard deviation of background noise in air. White matter lesions identified in the images, and confirmed by light microscopy, were excluded from this stage of the analysis.

Phase III: Correlative Histopathology

Image-guided dissection of the brain was performed to obtain a brain tissue slice corresponding to the MR image slice from which the MTR and T2 measurements were obtained. Contiguous sections (5 μm) were stained with hemotoxylin & eosin to assess cellular infiltration and solochrome-R-cyanin to demonstrate myelin (Fig. 1). Sections were examined and scored...
blindly for pathologic changes in two categories, encephalitis and myelin pallor (Table 2).

Statistics
Statistical analysis consisted of one-way ANOVA with a Tukey test for multiple comparisons. The relationship between the MTR and the pathologic scores was tested using the Spearman’s rank correlation coefficient. Statistical significance was defined as $P < 0.05$.

RESULTS
Phase I
MT Imaging Sequence Optimization

The MTRs in the distilled water phantom and WM were examined at three different average $B_1$ values (2.5, 4.2, and 5.9 $\mu$T), for six different offset frequencies ranging from 600 to 3000 Hz with $N_{sat} = 20$. As expected, the MTR was reduced as $\Delta v$ increased for all power levels, and there was a positive relationship between the average RF power and the MTR. The WM MTR, measured at the lowest offset frequency, was 88% for the highest power level, 67% for the intermediate power, and 29% for the low power condition. The effects of direct saturation were assessed by examination of the MTR of the distilled water phantom. At the highest power ($\mu$T) the apparent MTR for distilled water ranged from 18% at 600 Hz offset to 10% at 3000 Hz offset, indicating a considerable amount of direct saturation. With less power the MTR was reduced to below 10% when $\Delta v$ was greater than 1000 Hz.

The effects of increasing the duty cycle of the saturating pulse train was tested by varying $N_{sat}$ (from 10 to 60) and the time between the saturation pulses. The
MTR in WM increased with \( N_{\text{sat}} \), approaching a maximum value with \( N_{\text{sat}} \) equal to 50. The MTR measured in distilled water with \( N_{\text{sat}} \) equal to 50, however, was considerable. With \( N_{\text{sat}} \) equal to 30, we varied the time between the saturation pulses from 3.75 to 15 msec, resulting in duty cycles of 80.5%–60.8%. Within this range of values the time between the pulses had only a mild effect on the MTR for WM. The trend was a reduction in the MTR with increasing time between the pulses (decreasing duty cycle) from 55.4% at 3.75 msec to 48% at 15 msec.

The parameters of our optimized sequence were chosen to minimize the amount of direct saturation while maximizing the MT saturation and the GM-WM MTR. The final optimized parameters were as follows: MT saturation produced by a train of 30 15-msec-wide Gaussian RF pulses with average \( B_1 \) of 4.2 \( \mu \)T, applied 1.5 kHz off resonance. The time between pulses was 3.75 msec, resulting in a duty cycle of 80% for the saturating RF pulse train. The mean square saturating power (\( P_{\text{sat}} \)) and effective flip angle (\( \theta_{\text{sat}} \)), calculated as described in Berry et al (43), were approximately 20 \( \mu \)T\(^2\) and 961°, respectively. The amount of direct saturation, estimated from SI measurements in the distilled water phantom, was found to be less than 7% for the optimized MT sequence.

**Longitudinal Study**

Figure 2 shows the results of the longitudinal study. At day 35 of the CP phase of EAE, before treatment, the mean MTR measured in the NAWM was significantly reduced from the normal average value of 54.5% ± 2.35% (cross-sectional study; group I). Treatment with anti-\( \alpha \)-4 integrin produced a dramatic recovery of clinical signs of EAE. Concurrently, the average MTR returned to near the normal baseline value. When treatment was removed, the clinical signs rapidly reversed. At day 53, 10 days after treatment was stopped, the average MTR was again significantly reduced from normal and was not significantly different from the value measured prior to treatment.

**Cross-Sectional Study: Groups I–V**

The mean MTRs (A) and pathologic scores (B) for groups I–V are shown in Fig. 3. The mean MTR in NAWM of group II animals was not significantly different from the normal mean value of 54.5% ± 2.35%, measured for group I. Histologic evaluation of these brains showed evidence of only minor inflammation. A significant reduction in the MTR was measured for groups III and V, where correlative histology showed evidence of both inflammation and myelin pallor in NAWM. For group IV, the antibody-treated animals, the average MTR was not significantly different from normal. The pathologic scores for both encephalitis and myelin pallor were decreased for sections examined from group IV animals.

The relationship between the MTR results and the pathologic scores was examined by plotting the individual MTRs against the pathologic scores for all animals. A significant correlation was found between the MTR and both encephalitis (\( r^2 = 0.65; P < 0.001 \)) and myelin pallor (\( r^2 = 0.45; P < 0.001 \)). Moreover, the two pathologic scores are themselves strongly correlated with \( r^2 = 0.89 \).

Histopathology was scored under low magnification (2.5–40×). Representative examples of solochrome-R cyanin-stained brain sections for groups I and III are shown in Fig. 4A and B, respectively. In the normal WM of the corpus callosum the myelin stains a dark bluish purple color and appears compact (Fig. 4A). In NAWM examined from sections obtained on day 35, during the CP phase of EAE, prominent regions of myelin pallor can be identified within the WM (Fig. 4B; arrows). These regions of myelin pallor are intimately associated with the presence of inflammatory cells throughout the CP phase of EAE. An area of myelin pallor is shown at higher magnification in Fig. 4C. This representative section was obtained from an untreated animal of
group V (day 45). With antibody treatment the inflammatory exudate in the WM is diminished. Without the presence of inflammatory cells the degree of myelin pallor in these areas is minor and the WM appears nearly normal. This is illustrated in an example from a group IV animal (Fig. 4D).

**Phase II**

Figure 5 shows average MTRs (A) and myelin water percentages (B) for all groups in phase II. The normal WM MTR for group I animals in phase II had an average value of 57.25% with a standard deviation of 3.3%. This differs by less than 3% from the average MTR measured in normal WM of group I in phase I animals, demonstrating the high reproducibility of the MTR determined by this technique. For groups III and V, the untreated, chronic EAE animals, the mean MTR for NAWM was significantly reduced from the normal mean value. The mean MTR for groups II and IV, the acute EAE animals and the antibody-treated animals, respectively, were not significantly different from normal. These observations are equivalent to those measured in the matched animal groups in the longitudinal study of Phase I.

With the optimized guinea pig head solenoid RF coil used in this study, we achieved a substantial gain in SNR, and two T2 components were extracted in all normal WM analyzed. The mean image SNR, calculated in the WM in the first echo image, was approximately 700, with no signal averaging. In our previous studies (24), the highest average SNR achieved was 270, using a quadrature birdcage RF coil and the same CPMG imaging sequence with four signal averages. Under these conditions we extracted the short T2 component with a probability of 88% in normal guinea pig brain WM (24).

The mean myelin water percentage for normal guinea pig WM was 28% (±6.1 SD). To obtain an estimate of the variability in extracting the short T2 component in WM, we performed repeated measures in one animal. A guinea pig was scanned two times in succession (scans a and b) and then repositioned and scanned a third time (scan c). The myelin water percentages were (a) 21%, (b) 22%, and (c) 27%; the mean value was 23%, with a standard deviation of 3.46%.

For all EAE groups in phase II, the mean myelin water percentage in NAWM was significantly less than the normal WM mean value. Unlike the MTR, this measurement was not influenced by modulations in inflammatory activity due to antibody treatment. There was no significant difference between any two groups of EAE animals. Two peaks were extracted in NAWM in 20 of the 22 images analyzed. In the other two cases, a mono-exponential analysis provided the best fit for the NAWM T2 signal decays, implying a negligible level of myelin water in these two cases.

The relationship between the individual MTR and myelin water percentage measurements is illustrated in Fig. 6. There was no significant correlation between these two quantities.

**DISCUSSION**

In this study, significant reductions in both the mean MTR and the myelin water percentage were measured in NAWM of EAE guinea pig brain in vivo. These measurements detected abnormalities in NAWM that could
not be visualized with standard imaging techniques but that were confirmed by light microscopy. However, the MTR and myelin water percentage appear to measure different aspects of pathology of NAWM in EAE. This is in agreement with Vavasour et al (15), who clearly demonstrate the disparities in MTR and T2 maps of normal and MS brain.

In our cross-sectional study, changes in the MTR of NAWM occurred in parallel with changes in the clinical signs of disease. The recovery of clinical signs with anti-α4 integrin treatment, and the rapid reversal when treatment was stopped, are probably related to the resolution of inflammation, which is known to occur with this antibody treatment. This observation suggests that the changes in the MTRs measured in the NAWM, with treatment, were related to inflammatory activity.

To understand more fully how the pathology of EAE was influencing the MTRs, we performed cross-sectional studies in which separate groups of animals were scanned and then sacrificed at various stages of EAE. A statistically significant reduction in the MTR in NAWM was measured for all untreated chronic EAE animals. The NAWM in these animals contained large quantities of inflammatory cells invading the neural parenchyma and produced confluent areas of myelin pallor. In this study we found that the areas of myelin pallor, in sections obtained during the CP phase of EAE, were closely associated with inflammatory cell infiltration. Antibody treatment prevented new inflammatory cells from entering the CNS; after 7 days of treatment the MTRs were close to the mean MTR for normal controls, the presence of inflammatory cells in the NAWM was diminished, and at the same time the degree of myelin pallor was dramatically reduced.

No statistically significant reductions in the MTR were measured in NAWM examined during the acute phase of EAE, and histopathology of the NAWM in these animals revealed only a minor degree of inflammation. Similarly, Doussset et al (4) reported slight reductions in the MTR in inflammatory WM lesions of acute EAE (days 12–14) guinea pigs.

Our mean MTR for all normal WM (55.8%), obtained

![Figure 4](image-url)
Phase II. Mean MTRs (A) and myelin water percentages (B) for animals of groups I–V. Error bars represent the standard error of the mean. * = group means that are significantly different from the normal value.

The pathologic score for myelin pallor is not a quantitative measure of myelin loss, rather, it is a semiquantitative measure of the amount of myelin that is abnormal, inferred from the absence of myelin staining. The myelin pallor score may reflect demyelination and/or the displacement of myelinated axons accompanying the inflammatory process. The rapid reversal of clinical signs with antibody treatment is unlikely to be due to remyelination; instead this may be related to the resolution of inflammatory activity and the return to normal myelin organization. Thus the recovery of MTR with antibody treatment may also reflect a resolution of inflammatory activity, and not remyelination.

Large reductions in MTR have been reported in enhancing or active MS lesions; the MTR may afterward remain stable, recover, or worsen (44–46). Filippi et al (47) showed that substantial changes in MTRs in MS lesions were correlated with changes in blood-brain barrier permeability; MTRs were substantially reduced in gadolinium-enhancing lesions but recovered after enhancement ceased. The authors of that paper propose that this may be a reflection of periods of demyelination followed by remyelination. Our results may suggest an alternative explanation: that substantial reductions in the MTRs occur due to inflammatory-related changes to the structure of WM and that MTR recovery may be due to antibody-mediated reduction in the blood-brain barrier permeability to inflammatory immune components.

There is good evidence that the short T2 component in WM is related to myelination (15,20–23). The amount of water between myelin bilayers is expected to be proportional to the amount of myelin. MacKay and colleagues (15,20) have been the pioneering group utilizing multicomponent T2 determinations in MS patients. The myelin water percentage has been shown to vary for different MS lesions (20) and in EAE (22). To the best of our knowledge, this is the first time multicomponent T2 measurements have been applied to study EAE in vivo. We have measured a reduction in the short T2 component in all EAE.
NAWM compared with normal WM, which suggests that there is a significant loss of myelin in NAWM that does not change over the course of the disease. Perhaps most interestingly, modulation of inflammatory activity by the anti-α4 integrin treatment was not reflected in the measurement of the myelin water percentage as it was in the measurement of MTR. Since the amount of myelin is not expected to vary with inflammatory-related changes, this observation supports our hypothesis that the MTR is sensitive to physiological changes in NAWM induced by inflammation, while the short T2 component is a more “pure” or specific measure of myelin content in tissue.

Pathologic tissue characterization is of critical importance in the understanding of MS. As first proposed in 1992 by Dousset et al (4), the possibility exists for using MTRs to discriminate between highly demyelinated MS lesions and lesions with no demyelination but minor inflammation. However, the challenge continues to be in the characterization of those lesions that do not fall at the limits of the spectrum of pathologic change. Our results indicate that a dramatically lower MTR in NAWM compared with normal WM, does not necessarily imply a loss of myelin; it may, alternatively, indicate either changes in myelin content or structural changes to myelinated tissue that occur in response to inflammation. Additional studies are required to clarify further the underlying physiological mechanisms that relate myelin status in EAE to both measures of short T2 fraction and magnetization transfer.

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