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Correlation of Blood T Cell and Antibody Reactivity to Myelin Proteins with HLA Type and Lesion Localization in Multiple Sclerosis 1

Judith M. Greer, 2 Peter A. Csurhes, Diane M. Muller, and Michael P. Pender

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS. The numbers of autoimmune T cells and Abs specific for proteins of CNS myelin are increased in the blood in some patients with MS. The aim of this study was to investigate whether there are correlations between the specificity of the autoimmune responses in the blood, the HLA molecules carried by the patient, and the clinical features of MS, because studies on experimental autoimmune encephalomyelitis, an animal model of MS, indicate that autoimmune responses targeting particular myelin proteins and the genetic background of the animal play a role in determining the pattern of lesion distribution. We tested blood T cell immunoreactivity to myelin proteins in 100 MS patients, 70 healthy controls, and 48 patients with other neurological disorders. Forty MS patients had strongly increased T cell reactivity to one or more myelin Ags. In these 40 patients, the most robust correlation was between CD4+ T cell reactivity to myelin proteolipid protein residues 184–209 (PLP184–209) and development of lesions in the brainstem and cerebellum. Furthermore, carriage of HLA-DR4, -DR7, or -DR13 molecules by MS patients correlated with increased blood T cell immunoreactivity to PLP184–209 as well as the development of lesions in the brainstem and cerebellum. Levels of PLP190–209-specific Abs in the blood also correlated with the presence of cerebellar lesions. These findings show that circulating T cells and Abs reactive against specific myelin Ags can correlate with lesion distribution in MS and suggest that they are of pathogenic relevance.


M  

ultiple sclerosis (MS) 1 is a chronic inflammatory de-
myelinating disease of the CNS and is a common
cause of progressive disability (1, 2). Although MS is
considered to be an autoimmune disease (1, 2), the generally small percentages of individuals showing increased immunoreactivity in their blood to any one autoantigen have raised doubts that autore-
active cells in the blood are of pathogenic relevance. However, if
Ag-specific therapy for MS is to be effective, the specific Ags
being targeted in individual patients must be known. What is needed, 

therefore, is stronger evidence that autoreactive responses to myelin
Ags can correlate with some feature of MS.

Studies on experimental autoimmune encephalomyelitis (EAE), an animal model of MS, show that the distribution of lesions in the nervous system varies, depending on the Ag and immunization protocol used to induce EAE, and on the MHC and non-MHC

1 Abbreviations used in this paper: MS, multiple sclerosis; CND, patients with other CNS disorders; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalo-
myelitis; EDSS, Kurtzke Expanded Disability Status Scale; MBP, myelin basic
protein; MOBP, myelin oligodendrocyte basic protein; MOG, myelin oligodendrocyte
glycoprotein; MRI, magnetic resonance imaging; MSSS, MS severity score; OSP, oligodendrocyte-specific protein; PLP, myelin proteolipid protein; SI, stimulation
index.

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patients used in the T cell assays are shown in Table I. Blood and cerebrospinal fluid (CSF) were also collected from five patients undergoing a first attack of CNS demyelination (by MRI) brain scan. Forty-six patients (26 men; 47.7 ± 15.4 years of age at blood collection) and other CNS disorders (CND patients) were also recruited. These included 13 with cerebrovascular disease, 13 with epilepsy, 6 with Parkinson’s disease, 3 with motor neuron disease, 2 with idiopathic intracranial hypertension, 2 with cerebral aneurysm, 2 with CNS tumors, 2 with spinocerebellar degeneration, and 1 each with cerebellar hemiatrophia, sleeping disorder, and hydrocephalus. None of the CND patients had received immunosuppressant, immunomodulatory, or corticosteroid therapy in the previous 3 mo. One hundred twenty healthy controls (39 men; 36.1 ± 11.2 years of age at blood collection) were recruited from hospital and university staff. Blood from 50 of these was used only for HLA typing. The patients and controls were all of Caucasian origin, except for 1 MS patient and 1 healthy control, who were both of Asian ethnic origin.

Blood and CSF

Fifty milliliters of blood was collected by venepuncture from each subject. Five milliliters was used for HLA typing. PBMC were separated from the blood by centrifugation through Ficoll, and plasma was collected from above the PBMC layer. Up to 10 ml CSF was collected, after informed consent had been obtained, at the time of diagnostic lumbar puncture. Lymphocytes were separated from the CSF by centrifugation.

Ascertainment of lesion localization

Patient records from the time of onset of MS were reviewed, and patients were also assessed clinically at the time of venepuncture. Conventional MRI brain scans (T1, T2, and fluid attenuated inversion recovery (FLAIR)) were available for 100 of the MS patients at one or more time points throughout the disease course. The distribution of MRI lesions was determined by radiologists who were unaware of the results of the T cell assays and HLA typing. Patients were then classified according to whether they had lesions in the cerebral hemispheres, brainstem, cerebellum, optic nerve, spinal cord, and spinal roots, based on clinical and MRI findings. Clinical evidence of cerebral involvement was taken to include cognitive impairment, epileptic seizures, dysphasia, hemiparesis, hemisensory loss, and homonymous visual field defects. Clinical evidence of lesions in the brainstem and/or cerebellum included extracerebellar weakness (III, IV, or VI nerve palsy), internuclear ophthalmoplegia, facial sensory loss, trigeminal neuralgia, lower motor neuron facial palsy, nystagmus, MS-related deafness, dysarthria, dysphagia, dysphonia, limb ataxia without sensory loss, gait ataxia (without sensory loss and with negative Romberg’s sign), head titubation, or cerebellar postural tremor. Nerve root involvement was detected by the presence of radicular pain, localized muscle atrophy, and absent deep tendon reflexes.

Genomic DNA preparation and HLA typing

Genomic DNA was prepared as previously described (13) or using Nucleospin Blood XL DNA extraction kits (Clontech). Dynal low-resolution SSP kits (Dynal Biotech) were used to type for HLA-DR and HLA-DQB molecules, to a resolution equivalent to that of serotyped subgroups. For subtyping of subjects carrying DR2, DR4, and DR13, and for DQA typing, Dynal AllSet SSP high-resolution kits (Dynal Biotech) were used.

Assessment of T cell proliferation to peptides

Uptake of tritiated thymidine. T cell proliferation assays were performed using established techniques (13) on fresh PBMC or CSF cells. Human PLP and MBP were extracted from human brain tissue as previously described (13). Peptides (see Fig. 1 for sequences) were synthesized according to the human sequences by Auspep or Mimotopes. All peptides were >95% pure. The PLP and oligodendrocyte-specific protein (OSP) peptides were moderately hydrophobic and were dissolved in 0.2 M acetic acid as 5 mg/ml stock solutions. Stock solutions of the other peptides were made in water. The peptides were diluted in tissue culture medium immediately before adding to the microtiter plates at a final concentration of 5, 10, and 25 μg/ml. Con A (final concentration 2 μg/ml) and tetanus toxoid (10 Lf/ml) were used as positive controls. All assays were done in triplicate. For assay of PBMC, 1.5 × 10⁵ fresh PBMC/well were cultured in U-bottom 96-well plates (Nunc) in the presence or absence of Ag for 6 days, with [3H]thymidine being added during the final 18 h. For assay of cells from CSF, 8 × 10⁴ CSF cells plus 10⁵ irradiated (3000 rad) autologous PBMC were added to each well of the 96-well plate in the presence or absence of Ag for 6 days, with [3H]thymidine being added during the final 18 h. Cells were then harvested onto glass-fiber mats, and the cpm determined in a Betaplate counter (Beckman Coulter). To be considered a true positive response and be included as such in the study, all three wells had to show a similar increase in reactivity. Stimulation indices (SI) were determined by the formula: SI = (Mean cpm of peptide-containing wells) / (Mean cpm of control wells without peptide). The mean SI reported in Figs. 2 and 4 are the means of the maximum SI over the three peptide concentrations tested.

CFSE assays. Freshly collected PBMC were labeled with CFSE, as previously described (29), and incubated with 10 μg PLP184–199 + 10 μg PLP199–209 for 10 days in phenol red-free X-vivo 15 serum-free medium (Cambrex). Cells were then washed, stained with PerCP-labeled anti-CD4 or anti-CD8 Abs (BD Biosciences), and analyzed by flow cytometry, with gating on the lymphocyte population.

ELISA

Plasma was collected from each patient and stored at −70° C until required. The total IgG concentration in each sample was determined using Bindarid radial immunodiffusion (RID) kits (The Binding Site). For use, each plasma sample was adjusted to 100 μg/ml total IgG. PLP184–199 and PLP199–209 in 0.2 M bicarbonate buffer (0.5 μg/ml) were coated onto Nunc PolySorb ELISA plates, plates were blocked with 2% skimmed milk in PBS-Tween 20, and 100 μl of each diluted plasma sample was added in duplicate to the plates and incubated overnight at room temperature in a humidified chamber. Each plate also contained four dilutions of a high-titer-positive control reference serum. Anti-human IgG-alkaline phosphatase (Sigma-Aldrich) was used as secondary Ab and was detected with

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* RR-MS indicates relapsing-remitting MS; SP-MS, secondary progressive MS; PP-MS, primary progressive MS; EDSS, Kurtzke Expanded Disability Status Scale.

a MSSS scores were determined as previously described (28). MSSS scores were not available for those patients for whom an EDSS score was not available.
FIGURE 1. Proliferative responses to myelin Ags. The percentages of MS patients and CND patients responding to each myelin Ag with a stimulation index greater than or equal to the mean ± 2 SD of the healthy control group are shown. * p = 0.02 or ** p = 0.006 compared with healthy control group by χ² analysis with Yates’ correction, as required. The mean background cpm ± SD for these assays was 1913 ± 968, with a range of 603-4321 cpm for MS patients, 372-3671 cpm for healthy controls, and 412-4142 cpm for CND patients. The mean SI ± SD for healthy controls in response to each Ag was: h-PLP, 1.44 ± 0.76; PLP184–199, 1.42 ± 0.65; PLP190–209, 1.36 ± 0.58; PLP262–276, 1.44 ± 0.71; h-MBP, 2.25 ± 2.01; MBP12–100, 1.89 ± 1.76; MBP151–171, 1.41 ± 0.67; MOG10–44, 1.83 ± 0.72; MOG41–55, 1.52 ± 1.12; MOG82–86, 1.25 ± 0.52; MOG121–135, 1.32 ± 0.64; MBP28–46, 1.53 ± 0.65; MOBP17–55, 1.33 ± 0.36; OSP42–52, 1.30 ± 0.47; OSP62–71, 1.21 ± 0.48; OSP86–93, 1.20 ± 0.58; OSP102–121, 1.42 ± 0.71; OSP148–196, 1.22 ± 0.48; OSP202–218, 1.40 ± 0.50.

Assessment of effect of PLP190–209-specific T cells and Abs in C3H/HeJ mice

All animal experiments were approved by the Animal Ethics Committee of University of Queensland. PLP190–209-specific CD4⁺ T cell lines were generated by removing the draining lymph nodes from female 8–10-wk-old C3H/HeJ mice (Animal Resources Centre, Canning Vale, WA, Australia) immunized 10 days earlier with 50 μg PLP190–209 in CFA, and culturing peptide-reactive lymph node cells. The lines were stimulated in vitro every 10–14 days with peptide and irradiated splenocytes as APCs for five rounds. The resultant lines were >99% CD4⁺ T cells, as determined by flow cytometry. PLP190–209-specific Abs were obtained by immunoadsorption purification of Ab from the sera of the C3H/HeJ mice immunized with PLP190–209. The immunoabsorbent was prepared by coupling PLP190–209 to cyanogen bromide-activated Sepharose 4B (Amersham Biosciences), as per the manufacturer’s instructions. For induction of EAE with T cell lines, activated T cell blasts were enriched by centrifugation through Ficoll 3 days after activation with PLP190–209 and irradiated splenocytes and washed; 5 × 10⁶ T cell blasts were injected i.v. into naive mice. Five of the mice also received 500 μg affinity-purified PLP190–209-specific Ab i.v. 1 day after the T cells and then again 7 days later. Mice were assessed for development of EAE, and they were perfused with modified Karnovsky’s fixative (7) within 2 days of onset of neurological signs. Brains were embedded in HistoResin (Leica Instruments) and 1-μm sections were cut every 10 μm through the brainstem and cerebellum. These were stained with cresyl violet to assess inflammation (7). Whole cross-sections were photographed on a Zeiss Axiohot microscope. The percentage area of each cerebellum covered by lesions was determined using NIH Image software.

Statistical analyses

Statistical analyses were done using GraphPad Prism version 4.00 (GraphPad Software). T cell and Ab data from the patients and controls were initially tested to determine whether they were normally distributed within each group. This was not found to be the case, and thus the Kruskal-Wallis test for non-parametric data was used to compare all groups together. If this gave a p of <0.05, then Dunn’s multiple comparison test was used to compare pairs of groups. The frequencies of HLA phenotypes and genotypes in patients and controls were compared using χ² analysis with 2 × 2 contingency tables. Yates’ correction was applied when the number of positive samples in any test was <5. p values were corrected for multiple comparisons using the method of Bonferroni according to the formula Pc = 1 – (1 – Pf)², where P is the uncorrected p value, Pc is the corrected p value, and n is the number of comparisons.

Results

T cell proliferation to myelin Ags

Initially, T cell proliferation to 20 myelin Ags (human PLP and MBP and 18 peptides derived from the human sequences of PLP, MBP, MOG, myelin oligodendrocyte basic protein (MOBP), and OSP; see Fig. 1 for details) and the positive control Ags, tetanus toxoid and Con A, was assessed in fresh PBMC from 100 MS patients, 46 CND patients, and 70 healthy controls. For MOBP, MOG, and OSP peptides, only 18 CND patients were tested. None of the MS or CND patients had received immunosuppressants or immunomodulatory agents for at least 3 mo before blood collection. There were no significant differences among the three groups for mean responses to tetanus toxoid or Con A. The percentages of individuals with SI greater than or equal to the mean ± 2 SD in the healthy control group are shown for each myelin Ag in Fig. 1. The only significant differences between the percentages of MS patients and healthy controls responding to the Ags were for PLP184–199 (p = 0.02) and PLP190–209 (p = 0.006).

To determine the phenotype of the cells proliferating in response to PLP184–199 and/or PLP190–209, CFSE assays were used to analyze T cell proliferation in 10 MS patients who were selected on the basis of having responded to the PLP peptides. In 8 of the 10 MS patients, the cells responding to PLP184–199 and PLP190–209...
were predominantly CD4^+ T cells (Fig. 2). In the other two pa-
tients, proliferating cells comprised both CD4^+ T cells and CD8^+ T cells.

In total, 40 individuals with MS had SI at least 2 SD above the
mean of the healthy subjects for one or more myelin proteins. The
clinical notes and MRI data at the time of venepuncture of these 40
MS patients were reviewed to determine the distribution of lesions
at that time point. Reactivity to each myelin protein was analyzed
according to whether the patients had lesions in the cerebral hemi-
spheres, brainstem, cerebellum, optic nerve, spinal cord, and nerve
roots (Table II). The most striking observation was that patients
who showed increased reactivity to PLP_{184–199} and/or PLP_{190–209}
were more likely to have lesions in the cerebellum or brainstem
\((p = 0.02)\), and to carry DR4, DR7, or DR13, but not DR3 \((p =
0.01)\), than were patients who did not show increased T cell re-
 sponses to these PLP peptides. An increased number of patients
responding to the PLP peptides also had lesions in the optic nerve
compared with patients not showing increased responses to PLP,
but this did not reach statistical significance.

**Immunoreactivity to PLP and correlation with lesion
localization**

We sought to confirm these associations in the larger group of 100
MS patients. The MS patient group was subdivided on the basis of
clinical or MRI evidence of lesions in the brainstem and/or cere-
bellum before or at the time of venepuncture. The mean SI for all
peptides were determined and compared between MS patients with
lesions in these areas, MS patients without lesions in these areas,
healthy controls, and CND patients. There was a significantly in-
creased proliferative response to PLP_{184–199} and PLP_{190–209} in the
group of MS patients with lesions in these areas \((n = 51)\), com-
pared with MS patients without lesions \((n = 49)\), CND patients,
and healthy controls (Fig. 3), but not for any of the other 18 myelin
Ags, Con A, or tetanus toxoid (not shown).

*Does the increased reactivity to PLP_{184–209} correlate with the
severity of MS?*

Because lesions in the brainstem and cerebellum may correlate
with the burden of disease and duration of MS, we looked at T cell
responses to PLP_{184–209} as a function of the MSSS (28), which is
determined from the Kurtzke Expanded Disability Status Scale
score and the duration of MS. Overall, there were no differences
with respect to the MSSS between MS patients with brainstem
and/or cerebellar lesions and MS patients without brainstem or
cerebellar lesions (Table I). Whereas there was increased reactivity
to PLP_{184–209} in patients who had an MSSS of \(\geq 2.00\) and who had
lesions in the brainstem or cerebellum, there was no similar in-
crease in PLP reactivity in patients who did not have lesions in
these areas, even in those with a high MSSS (Fig. 4). This finding,
together with the lack of increased responses to other myelin Ags
in the group with brainstem/cerebellar lesions compared with the
group without lesions in these areas (Table II), indicates that the ob-
erved differences in responsiveness to PLP_{184–209} are not due to the
former group having a greater overall burden of disease.

We also looked for increased immunoreactivity to PLP_{184–209}
and development of lesions in the brainstem/cerebellum in the
early stages of MS. Both PBMC and CSF cells were available
within 1 day of a MRI brain scan for five patients undergoing the
first attack of CNS demyelination. One of these patients presented
with a brainstem lesion and had only one other lesion on MRI
scans of the brain and spinal cord. Cells from both the blood
and the CSF of this patient showed strongly increased T cell reactiv-
ity against both PLP_{184–199} and PLP_{190–209} (Fig. 5). None of the other
four patients showed any evidence of brainstem or cerebellar le-
isons or increased reactivity to PLP_{184–199} or PLP_{190–209}. None of
the five patients had increased blood or CSF T cell reactivity to
MBP_{82–100} or MOG_{41–55} (not shown).

**Carriage of HLA-DR4, -DR7, -DR13, or -DQ3 correlates with
an increased risk of developing lesions in the brainstem and/or
cerebellum**

Most Caucasian MS patients carry DRB1*1501 (DR15) (Table
III); however, we have previously found that the strongest T cell
reactivity against PLP_{184–209} occurs in patients carrying DR4 or
DR7 (13). PLP_{180–199} and PLP_{190–209} bind with moderately high
affinity to HLA-DRB1*1501 molecules, and PLP_{180–199} has also
been shown to bind with high affinity to DR4, but only poorly to
DR3 (30). Analysis of HLA type in MS patients (including an
additional 12 patients on immunomodulatory therapies and not in-
cluded in the T cell assays) and 120 healthy Caucasian controls
showed increases in the percentages of individuals carrying DR4,
DR7, and DR13 in the subgroup of MS patients who developed
brainstem/cerebellar lesions, compared with those who did not de-
velop lesions in these areas (Table III). In contrast, the percentage
of patients carrying DR3 was significantly increased in patients
who did not develop brainstem or cerebellar lesions compared with
those who did develop lesions in these areas \((P = 0.004)\).

The HLA types can be subgrouped based on the sequence of the
HLA molecule. Several positions within the HLA sequences are
important in determining whether a particular Ag can bind to that
HLA molecule. Typing to the molecular level indicated that the
frequency of carriage of those HLA-DR4, -DR7, and -DR13 al-
leles encoding HLA-DR molecules with either arginine, alanine,
lysine, or glutamine, but not a glutamic acid, at positions 71 or 74
of the \(\beta\)-chain (which form the P4 pocket of the peptide-binding
cleft of the HLA molecule) \((i.e., DRB1^\*0401, DRB1^\*0404,
DRB1^\*1303, DRB1^\*1312, and all DRB1^\*07) was significantly
increased in MS patients with brainstem and/or cerebellar lesions
compared with MS patients without lesions in these areas (17.1%) ($P_{c}/H110050.0000002$).

There was also a correlation between carriage of HLA-DQ3, which is in linkage dysequilibrium with HLA-DR4 and which can also occur with HLA-DR7, -DR11, and -DR13, and development of lesions in the brainstem and cerebellum (Table III).

The HLA type was predictive of where lesions would occur, with 59 of 68 MS patients (86.8%) who carried DR4, DR7, or DR13 developing brainstem or cerebellar lesions; in contrast, only 14 of 44 individuals (31.8%) who did not carry DR4, DR7, or DR13 developed brainstem or cerebellar lesions ($p_{c}/H110052.5/109; OR = 14.0 (5.5–36.2))

Thus, carriage of DR4, DR7, or DR13 gives a relative risk of 2.7 for development of brainstem or cerebellar lesions. Forty of 51 individuals (78.4%) who carried DQ3 developed brainstem or cerebellar lesions; in contrast, only 32 of 60 (53.9%) of those who did not carry DQ3 developed brainstem or cerebellar lesions ($p_{c}/H110050.005; OR = 3.2 (1.4–7.4))

Carriage of HLA-DR4, -DR7, or -DR13 correlates with increased T cell reactivity to PLP184–209

When the T cell reactivity to PLP184–209 was analyzed on the basis of the HLA-DR type, the strongest responses occurred in MS
FIGURE 3. T cell responses to PLP$_{184-199}$ and PLP$_{190-209}$ are increased in patients who had lesions in the brainstem and/or cerebellum. Blood samples from MS patients who had lesions in the brainstem and/or cerebellum before or at the time of blood collection (BS/Cb lesions), MS patients without lesions in these areas before or at the time of blood collection (no BS/Cb lesions), healthy controls (HC), and CND patients were tested for T cell reactivity to PLP$_{184-199}$ and PLP$_{190-209}$ in proliferation assays. The mean SI ± SEM against the PLP peptides were significantly increased in the MS patients with BS/Cb lesions compared with the other groups (*, *p < 0.01; **, *p < 0.001, as determined by the Kruskal-Wallis test followed by Dunn’s multiple comparison test).

FIGURE 4. Increased levels of reactivity to PLP$_{184-209}$ in five patients presenting with a first attack of CNS demyelination. PBMC and CSF cells were collected within 1 day of a MRI brain scan and were tested for proliferation in response to PLP$_{184-199}$, PLP$_{190-209}$, MBP$_{82-100}$, and MOG$_{41-55}$. Patient no. 3 was the only one of the five to have lesions in the brainstem and/or cerebellum (lesion in the pons) and also the only patient with increased T cell reactivity to the PLP peptides in either the blood or CSF; this patient had increased T cell reactivity to both PLP$_{184-199}$ and PLP$_{190-209}$ in the blood (SI >6) and the CSF (SI >4), compared with background proliferation in the absence of Ag (SI = 1). There was no increased reactivity to MBP$_{82-100}$ or MOG$_{41-55}$ in any of the blood or CSF samples (not shown).

FIGURE 5. T cell responses to PLP$_{184-209}$ in five patients presenting with a first attack of CNS demyelination. PBMC and CSF cells were collected within 1 day of a MRI brain scan and were tested for proliferation in response to PLP$_{184-199}$, PLP$_{190-209}$, MBP$_{82-100}$, and MOG$_{41-55}$. Patient no. 3 was the only one of the five to have lesions in the brainstem and/or cerebellum (lesion in the pons) and also the only patient with increased T cell reactivity to the PLP peptides in either the blood or CSF; this patient had increased T cell reactivity to both PLP$_{184-199}$ and PLP$_{190-209}$ in the blood (SI >6) and the CSF (SI >4), compared with background proliferation in the absence of Ag (SI = 1). There was no increased reactivity to MBP$_{82-100}$ or MOG$_{41-55}$ in any of the blood or CSF samples (not shown).

Table III. HLA-DR and HLA-DQ typing in MS patients and healthy controls

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*p < 0.004 for comparison of MS patients with brainstem/cerebellar lesions and MS patients without brainstem or cerebellar lesions (p = 0.004). **p < 0.05 for comparison of MS patients with brainstem or cerebellar lesions and MS patients without brainstem or cerebellar lesions before correction for multiple comparisons; the corrected *p values are not significant.

Significantly different compared to both MS patients with brainstem or cerebellar lesions and healthy controls before correction for multiple comparisons; after correction, significantly different from healthy controls (p < 0.05).
For HLA-DQ, the mean SI for reactivity to PLP<sub>184–199</sub> and PLP<sub>190–209</sub> was not significantly higher in DQ3<sup>+</sup> MS patients than in DQ3<sup>+</sup> MS patients (SI = 2.7 ± 0.6 vs 2.2 ± 0.2) (Fig. 6B), suggesting that the marked increase in the number of MS patients with brainstem or cerebellar lesions who carry DQ3 and show increased reactivity to PLP<sub>184–209</sub> may be due primarily to the strong linkage disequilibrium of DQ3 with all DR4 and some DR13 alleles (and to a lesser extent with DR7). Only 5 of the 100 MS patients in the study carried DQ3 in the absence of either DR4, DR7, or DR13, and the response to PLP<sub>184–209</sub> was only marginally increased in those patients. Interestingly, the association of increased T cell reactivity to PLP<sub>184–209</sub> with carriage of DR4, DR7, DR13, or DQ3 was not observed in healthy subjects, indicating its clear relationship to MS (Fig. 6B).

Role of PLP<sub>184–209</sub>-specific Abs in development of brainstem and cerebellar lesions

Abs against myelin components have been suggested to play a role in lesion development in MS. PLP is a integral membrane protein on the surface of oligodendrocytes and is present throughout the myelin lamellae, including on the outmost loop of myelin (31), and the PLP<sub>184–209</sub> is located on the extracellular face of PLP (32).

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Because PLP<sub>190–209</sub>-specific Abs correlated with the presence of cerebellar lesions, we asked whether T cells and Abs were both required for the development of cerebellar lesions. To address this, we used C3H/HeJ mice, which develop predominantly brainstem and cerebellar lesions when immunized with PLP<sub>184–199</sub> (5, 7). The encephalitogenic T cells are CD4<sup>+</sup> and I-A<sup>+</sup> restricted. I-A<sup>+</sup> is the mouse ortholog of HLA-DQ, and the residues making up the Ag-binding pocket of the I-A<sup>+</sup> molecule most closely align to HLA-DQ3. Mice were injected either with PLP<sub>184–199</sub> in CFA subcutaneously, or with 5 × 10<sup>6</sup> PLP<sub>190–209</sub>-specific T cells i.v. One and 3 days later, five of the mice injected with the PLP<sub>190–209</sub>-specific T cells were injected i.v. with 500 µg immunofluorescence-purified PLP<sub>190–209</sub>-specific Ab. On the first or second day of neurological signs, mice were perfused and the CNS was removed for histological analysis. All mice developed lesions in the brainstem; however, none of 30 mice injected with T cells alone developed cerebellar lesions (Table IV). In contrast, all 10 mice immunized with PLP<sub>190–209</sub> in adjuvant and 3 of 5 mice injected with PLP<sub>190–209</sub>-specific T cells and Abs developed cerebellar lesions (Table IV).
Discussion

This study demonstrates that autoimmune reactivity detected in the peripheral blood can correlate with the clinical and MRI features of MS, and it provides evidence for a pathogenic role for autoimmunity in MS. We found a highly significant correlation between increased T cell and Ab reactivity to the 184–209 region of PLP, carriage of HLA-DR4, -DR7, or -DR13, and development of brainstem and/or cerebellar lesions. This is striking because, in the C3H/HeJ mouse, PLP190–209-specific autoreactivity causes lesions predominantly targeting the brainstem and cerebellum (5, 7). In the present cross-sectional study, we found that 56% of MS patients with brainstem or cerebellar lesions had increased T cell reactivity to PLP184–209. This is likely to be an underestimate because blood with brainstem or cerebellar lesions had increased T cell reactivity during an attack, and because there are major fluctuations in the frequency of circulating T cells reactive to PLP184–209, with surges of increased reactivity sometimes preceding relapses (16). Our findings do not mean that patients not carrying DR4, DR7, or DR13 and/or not showing increased reactivity to PLP184–209 cannot develop brainstem or cerebellar lesions, but the likelihood that lesions will occur in these areas is substantially increased if they do.

Three lines of evidence from human studies support a causal role of autoreactivity to PLP in the targeting of the brainstem and cerebellum in MS. First, we have shown herein that 21 of 22 individuals with strongly increased T cell reactivity to PLP184–209 in the blood had brainstem and/or cerebellar lesions at the time of testing and that elevated levels of T cell reactivity against PLP184–209 were present in both the blood and CSF and correlated with a brainstem lesion during the first attack of CNS demyelination, when only one other lesion could be detected by MRI. Second, in a previous longitudinal study, we investigated the frequency of myelin-specific T cells in the blood during a 12–18-mo period in five patients, and correlated surges in the frequency of these cells with the presence of gadolinium-enhancing MRI brain lesions (16). Two of these patients, who were also included in the present study, had relapses involving the brainstem (patient MS35) or the cerebellum (patient MS48), and in both cases there was a surge in the frequency of T cells reactive to PLP184–199 and/or PLP190–209 just before the onset of these attacks (16). Third, in the study of Tuohy et al. (24), in which lesion localization and PLP reactivity were assessed longitudinally in 10 patients with monocentric monophasic syndromes suggestive of MS, 2 of 3 patients with anterior brainstem syndrome showed increased reactivity to the PLP180–210 region of PLP and 2 of these patients additionally carried HLA-DRB1*04. In the present study we also found that MS patients with cerebellar lesions had higher circulating levels of Abs against PLP190–209 than did healthy controls and MS patients without cerebellar lesions.

It is unlikely that increased PLP184–209-specific immunoreactivity is merely a marker for brainstem/cerebellar lesions, because this same region of PLP induces lesions in the same region of the CNS in C3H/HeJ mice. We do not know why T cells specific for the PLP184–209 region have a predilection for the brainstem and cerebellum in MS patients and in C3H/HeJ mice. There is no leakage of the blood-brain barrier (33) or up-regulation of MHC class II, B7-1 (CD80), B7-2 (CD86), or a variety of chemokine receptors in these regions of the CNS in normal mice (D. M. Muller, M. P. Pender, and J. M. Greer, unpublished), indicating that these factors are not responsible. The most likely explanation is that the form or distribution of PLP in the brainstem and cerebellum is different from that in other regions of the CNS, or that other molecules found solely in these areas share some sequence homology with PLP. In support of the former idea, several studies have shown that regional differences in PLP concentrations do occur (34, 35); in support of the latter, a neuronal homolog of PLP known as M6b is expressed strongly in the molecular layer of the cerebellum (36).

The current study shows that Abs against PLP184–209 can target lesions to the cerebellum in mice and suggests that it may also do so in patients with MS.

Several common HLA-DR types, including DR4, DR7, and DR15, share largely overlapping peptide-binding repertoires, and these differ considerably from the peptide-binding repertoires of DR3 and DR12 (37). This is in keeping with our findings on the HLA-DR types of individuals responding to PLP184–209. Additionally, carriage of HLA-DR4, -DR7, and -DR13 types that do not have a glutamic acid residue at position 71 or 74 of the β-chain also correlated strongly with development of brainstem and/or cerebellar lesions and T cell reactivity to PLP184–209. We have previously shown that the primary progressive form of MS (PP-MS) with HLA-DR types that do have a glutamic acid residue at position 71 or 74 of the β-chain (38), and that patients with PP-MS have less T cell reactivity to PLP184–209 than do patients with other forms of MS (13). These observations are in keeping with the current findings and suggest that PLP184–209 is not a major target Ag for T cells in most patients with PP-MS.

It is interesting that of the proteins that are inserted only into the CNS myelin membrane, and not into peripheral nervous system myelin (PLP, OSP, MOBP, and MOG), the frequency of increased blood T cell reactivity to each protein among MS patients increased with the abundance of the protein in CNS myelin. Thus, increased T cell reactivity occurred most frequently against PLP (constituting 50% of CNS myelin protein), then against OSP and MOBP (5–10% of CNS myelin protein), and least against MOG (0.05% of CNS myelin protein) (Fig. 1). In contrast, increased T cell reactivity to MBP, which makes up ~30% of the protein of both CNS and peripheral nervous system myelin, was uncommonly observed in our MS patients, as we also found in an earlier study (13).

Although we did not see statistically significant correlations between increased reactivity to other myelin Ags and lesion distribution, it is possible that such correlations do exist. For example, we observed clinical evidence of spinal nerve root involvement in three patients, each of whom showed increased reactivity to MBP. This is notable because the spinal nerve roots in the peripheral nervous system are a major site of demyelination and nerve conduction abnormalities in EAE induced by immunization with MBP (3, 4) or by injection of MBP-specific T cells (39). Additionally, 8 of 11 patients showing increased reactivity against MOG had clinical evidence of cerebral involvement. This is interesting because immunization with MOG has been shown to induce cerebral cortical demyelination in marmosets (40) and rats of certain MHC types (10). Further investigations with larger numbers of patients who exhibit these particular clinical manifestations are needed to determine whether these are robust associations. Additionally,
References