Myelin Basic Protein and Human Coronavirus 229E Cross-Reactive T Cells in Multiple Sclerosis

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Multiple sclerosis (MS) is an inflammatory demyelinating neurological disease in which autoreactive T lymphocytes sensitized to myelin components of the central nervous system are postulated to contribute to pathogenesis. The possible relevance of molecular mimicry between a human coronavirus and the myelin basic protein component of myelin in the generation of this autoimmune reaction was evaluated. Myelin basic protein– and virus-reactive T-cell lines were established from 16 MS patients and 14 healthy donors and shown to be mostly CD4+. In contrast to healthy donors, several T-cell lines isolated from MS patients showed cross-reactivity between myelin and coronavirus antigens. Overall, 29% of T-cell lines from MS patients (10 donors) but only 1.3% of T-cell lines from normal control subjects (2 donors) showed an HLA-DR–restricted cross-reactive pattern of antigen activation after in vitro selection with either myelin basic protein or human coronavirus strain 229E antigens. Moreover, reciprocal reactivities were only observed in MS patients (4 donors). This establishes molecular mimicry between a common viral pathogen, such as this human coronavirus, and myelin as a possible immunopathological mechanism in MS and is consistent with the possible involvement of more than one infectious pathogen as an environmental trigger of disease.

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Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by multifocal regions of inflammation and myelin destruction [1]. Clinical disease onset is most common in young adults. Disease prevalence is unevenly distributed geographically. Available evidence suggests that the etiology of MS is multifactorial and probably involves a combination of genetic susceptibility and environmental triggering factors [2]. Genetic predisposition may involve immune response genes such as those encoded by the major histocompatibility complex and T-cell receptor (TCR) complex gene regions [3, 4]. However, the low concordance rate among monozygotic twins (25–40%) and epidemiological studies also implicate environmental factors, with microbial infections the most commonly cited triggering event associated with MS [5].

Repeated attempts have been made to identify an MS-linked pathogen. More than a dozen different viruses have been associated with MS over the years, without a clear causal relationship having yet been established [6]. Coronaviruses appear in the long list of candidates implicated in disease etiology. These enveloped positive-stranded RNA viruses are respiratory pathogens involved in up to 30% of common colds [7]. All known human isolates can be grouped into two serotypes, designated 229E and OC43. Support for their possible involvement in neurological diseases such as MS is derived from several lines of study: Related murine coronaviruses cause MS-like immune system–dependent chronic and/or recurrent CNS demyelinating diseases in rodents and primates [8]; coronavirus-like particles were observed in the brain of an MS patient [9]; murine-like coronaviruses were isolated from 2 MS patients [10]; titers of antibodies to both serotypes of human coronaviruses were elevated in the cerebrospinal fluid of MS patients compared to control subjects [11]; human coronaviruses have the capacity to replicate in cultures of neural and glial cells of human origin [12, 13]; and two groups including our own recently reported coronavirus gene expression in the brains of a significantly higher proportion of MS patients than of control subjects [14, 15].

Activated myelin-reactive cells are found in increased frequency in the peripheral circulation and the in-

trathecal compartment of MS patients compared to control subjects [16–19]. The mechanism underlying this activation of myelin-reactive T cells remains obscure. Interestingly, coronavirus infection of experimental animals leads to the activation of similar autoreactive T cells, which when transferred to naive animals, triggered experimental allergic encephalomyelitis (EAE), an autoimmune disease that is also used as a model for MS and is usually induced by the injection of myelin basic protein (MBP) or proteolipid protein (PLP) in adjuvant [20]. Although the mechanism involved in such virus-induced autoimmunity is not known, molecular mimicry was suggested as an underlying triggering event [21, 22]. Indeed, molecular mimicry has often been proposed to be involved in the pathogenesis of MS, although experimental evidence has thus far been lacking.

Presumably, the sharing of amino acid sequences between a virus and a self-antigen, such as MBP, could result in the activation by the infectious agent of T lymphocytes that recognize the shared sequences. Such T cells may be able to recognize the self-antigen, with immunopathological consequences, with or without an accompanying persistent viral infection. Such shared sequences between various viruses and MBP and PLP were reported several years ago, although biological consequences were not identified [23, 24]. We also recently found such structural mimicry between a non-structural protein of the 229E strain of human coronavirus (HCV-229E) and a region of MBP that is close to the immunodominant 84–102 site observed in MS patients [25] and is known to be encephalitogenic in experimental animals [26]. We hypothesized that molecular mimicry between this group of common respiratory pathogens and myelin constituents could underlie the initiation, persistence, and/or recurrence of the CNS-directed autoreactive immune response associated with MS. According to this hypothesis, MS would be triggered in individuals who are genetically predisposed to mount an immune response to sequences of an infecting pathogen, such as the human coronavirus, that are shared with myelin antigens. Thus, we reasoned that MS patients would preferentially express virus-myelin cross-reactive T cells.

Materials and Methods
Patients
Sixteen patients diagnosed with MS were selected at random: 7 patients showed chronic progression and 9, relapsing-remitting disease. Fourteen healthy donors were selected at random as control subjects. Donors were between 24 and 66 years old, with a mean age of 36 years. Their histocompatibility profiles were established serologically for human leukocyte antigen (HLA) class II antigens and the HLA-DR assignment was confirmed with a molecular assay (Table 1).

Antigens
Human MBP was prepared from a normal male adult human brain (Montreal Brain Bank, Douglas Hospital, Verdun, Québec, Canada) according to the procedure of Cheifetz and Moscarello [27]. Purity was monitored by sodium dodecyl sulfate–polyacrylamide gel electrophoresis: The expected single band of 21 kd was observed by Coomassie blue staining. A lyophilized stock was stored at −20°C and reconstituted to a concentration of 1 mg/ml in complete RPMI culture medium (described below) and stored at 4°C. HCV-229E, obtained from the American Type Culture Collection (ATCC; Rockville, MD), was propagated in the human embryonic lung cell line L132 [26]. Human coronavirus OC43 (HCV-OC43), also obtained from ATCC, was propagated in the human rectal tumor cell line HRT-18 [28]. Viral antigens were prepared from L132 cells infected with HCV-229E at an MOI of 0.01 at 33°C for 43 hours or HRT-18 cells infected with HCV-OC43 at an MOI of 0.004 at 33°C for 72 hours. Control antigens were prepared from parallel cultures of uninfected L132 or HRT-18 cells, respectively. The cells were lysed in the culture medium by three cycles of freezing at −70°C and thawing at 37°C, clarified at 9,600 g for 20 minutes to yield viral or control antigens, which were stored in aliquots at −70°C. Before use, infectious virus in the viral antigen preparations (10^6 tissue culture infectious doses 50% [$TCID_{50}$]/ml) was inactivated by exposure to ultraviolet light (ultraviolet illuminator, model 3-3000, Fotodyne, New Berlin, WI) for 7 minutes.

Generation of Myelin- or Virus-Reactive T-Cell Lines
T-cell lines were prepared according to a modification of the method of Pette and colleagues [29]. Briefly, peripheral blood lymphocytes were separated by Ficoll/Hypaque (Pharmacia) density gradient centrifugation and resuspended at 2 × 10^6 cells/ml in complete culture medium (Gibco) (RPMI containing 2 mM L-glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 10 mM HEPES, 50 μM 2-mercaptoethanol, 5% (vol/vol) heat-inactivated pooled AB serum [Pel-Freez, Brown Deer, WI], and 5% (vol/vol) heat-inactivated [at 56°C for 30 minutes] autologous serum). Cells were seeded at 2 × 10^5 cells per well (in 0.1 ml) into 96-well round-bottom microtiter plates (ICN/Flow), to which either MBP or viral antigen was added at a final concentration of 30 μg/ml (3 μg/well) for MBP or a final dilution of 1/25 for viral antigens.

After 3 to 4 days of incubation in a humidified atmosphere containing 5% (vol/vol) carbon dioxide, 0.1 ml of culture medium per well supplemented with 40 units of human recombinant interleukin-2 (Immunex, Seattle, WA) per milliliter was added and this was repeated every 3 to 4 days for a total of 14 days. Primary T-cell lines were screened for antigen-dependent proliferation. The T-cell lines in microtiter plates were washed twice with serum-free complete culture medium to remove free interleukin-2 (centrifugations were at 1,000g for 10 minutes), resuspended in 0.1 ml of complete culture medium per well, and split into two new 96-well round-bottom microtiter plates (50 μl/well). Autologous peripheral blood lymphocytes prepared as described

Table 1. Description of Donors Involved in the Study

Donor Group and Designation	Sex	Age (yr)	MS Diagnosis	HLA-DR	HLA-DQ
MS					
P1	M	41	RR	5 (11), 8	4, 3 (7)
P2	F	32	CP	4, 10	3, 1
P3	F	44	CP	2 (15), 6 (13)	1 (6), −
P4	F	40	RR	2 (15), 3 (17)	1 (6), 2
P5	F	33	RR	2 (15), 5 (11)	3 (7), 1 (6)
P6	F	35	RR	ND	ND
P7	M	44	RR	3 (17), 5 (11)	3 (7), 2
P8	F	45	CP	3 (17), 6 (14)	1, 2
P9	M	45	CP	4, 7	3, −
P10	F	41	CP	1, 8	1, 4
P11	M	53	CP	2 (15), 6 (13)	3, 1 (6)
P12	F	59	RR	7, 2 (16)	3, 1 (6)
P13	F	41	RR	7, 3 (17)	2, 3
P14	F	26	RR	4, 2 (15)	3, 1 (6)
P15	F	48	RR	2 (15), −	1, −
P16	M	66	CP	2 (15), 2 (17)	2, 1 (6)
Controls					
C1	F	41		2 (15), 6 (13)	1, −
C2	M	38		7	2, −
C3	M	28		4, 5 (11)	3 (7), −
C4	M	24		4, 5 (11)	3 (7), −
C5	M	32		2 (15), 7	3, 1 (6)
C6	F	24		2 (15), 4	1 (6), 3 (7)
C7	M	24		ND	ND
C8	F	26		2 (15), 4	1 (6), 3 (7)
C9	M	24		ND	ND
C10	M	26		3 (17), 6 (13)	1, 2
C11	F	24		1, 5 (11)	1, 3 (7)
C12	F	43		7, 2 (16)	2, 1 (6)
C13	M	55		6 (13), 2 (15)	1, −
C14	M	42		1, 7	1, 2

CP = chronic progressive; RR = relapsing-remitting; ND = not done.

above and irradiated with 2500 rads were added (1–2 × 10⁵ cells in 50 μl/well) as antigen-presenting cells in both microtiter plates. One of the two plates also received specific antigen, either MBP or viral antigens (same quantities as described above, in 10 μl/well), and the other plate received serum-free complete culture medium or L132 cell lysates in culture medium (10 μl/well). The cells were fed human recombinant interleukin-2 every 3 to 4 days for a total of 14 days, as described above. Antigen-specific T-cell lines were selected for expansion and proliferation assays. Cells were washed twice to remove free interleukin-2 and restimulated at a concentration of 2 × 10⁵ cells/ml in complete culture medium to which irradiated antigen-presenting cells (ratio of 5:1) and antigen were added. Proliferation assays described below were performed on an aliquot of these cells. Human recombinant interleukin-2 was added to the remaining cells every 3 to 4 days for a total of 14 days, as described above. This antigen restimulation protocol was repeated one to three times, for a total of three to five in vitro stimulations.

Antigen-Specific Proliferation Assays
Proliferation assays were performed by adding 1 × 10⁴ T cells per well into 96-well round-bottom microtiter plates to which irradiated antigen-presenting cells and various antigens were added separately in complete culture medium (duplicate or triplicate wells for each antigen, 0.1 ml/well). These specific and control antigens were MBP (4–12 μg/well; 4 μg for MBP-reactive lines and 8 μg for virus-reactive lines were experimentally established as optimal), viral antigens in infected cell lysates (final dilution 1/10 to 1/50; optimal dilution 1/25 for virus-reactive lines), tetanus toxoid (1 μg previously tested to be optimal), appropriate dilutions of uninfected cell lysates, and serum-free complete culture medium. The cells were incubated for a total of 72 hours and [³H]thymidine (1 μCi/well; Amersham) was added for the last 6 to 16 hours. Cells were harvested onto glass microfiber filters (Skatron) on a 96-well Skatron model 11050 Micro cell harvester and counted in 5 ml of Ecolite(+) scintillation fluid (ICN), using a Canberra Packard Tri-Carb 2200A scintillation counter. A stimulation index was calculated as the ratio of the radioactivity (counts per minute [cpm]) incorporated in the presence of specific antigen over its control and a value above 3.0, with at least 1,000 cpm incorporated, was considered significant. Dose-response curves were performed for each antigen and the observed proliferation was found to gradually increase up to a specific amount of anti-

gen (described above as optimal) and either remain stable or decrease if more antigen was used. MBP and viral antigens used in proliferation assays were prepared as described above.

Results

Peripheral blood lymphocytes from the 30 donors described in Table 1 were used to select, in parallel, MBP-reactive and HCV-229E–reactive T-cell lines. MBP-reactive T-cell lines could be established as frequently from MS patients as from healthy donors (an average of 4–5 lines/donor with negative results in 3 patients (P1, P7, and P16) and 3 control subjects (C3, C6, and C12)). In all but 1 MS patient (Patient 2), HCV-229E–reactive T-cell lines could also be established (an average of 5.5 lines/patient and 7.6/control). As expected from the prevalence of coronavirus infections in humans [7], all 30 donors were found to be seropositive for this virus by enzyme-linked immunosorbent assay and indirect immunofluorescence (data not shown). After three to five in vitro stimulations with either MBP or HCV-229E, the reactivities of the T-cell lines to the homologous and the heterologous antigens were tested. Reactivity was scored as positive when a stimulation index of more than 3.0 was obtained, with at least 1,000 cpm of [³H]thymidine incorporated. After three in vitro stimulations with either MBP or the viral antigens, proliferation to an unrelated control antigen, tetanus toxoid, consistently yielded background proliferation, equivalent to the reaction observed where only antigen-presenting cells were present, reflecting the selection of MBP- or virus-reactive T cells. T-cell proliferation to tetanus toxoid was observed during initial in vitro stimulations, likely re-

flecting the presence of memory cells that remained from previous tetanus vaccination of the donors. As shown in Table 2, a significantly increased proportion of T-cell lines from MS patients compared to control subjects proliferated to viral antigen after selection with MBP, or conversely with MBP after selection with viral antigen. Overall, T-cell lines from 10 of 16 MS patients and 2 of 14 control subjects showed cross-reactivity, for a proportion of such lines of 29% in MS patients (39/134 lines tested) and only 1.3% in healthy donors (2/155 lines tested). Unlike normal control subjects, 4 MS patients (P8, P9, P12, and P13) even showed reciprocal cross-reactivity profiles.

Of interest, there was no relationship between severity of neurological dysfunction or MS diagnosis and cross-reactivity patterns, which involved as many patients with an Expanded Disability Status Scale (EDSS) score lower than 5 as patients with an EDSS score equal to or higher than 5 (data not shown) and were observed in patients with both the relapsing-remitting and the chronic progressive disease (see Table 1).

Although there was no apparent correlation between HLA type and reactivity patterns, it is interesting to note that the presumably more pathologically relevant recognition of MBP by virus-reactive T cells observed in 8 MS patients included 5 HLA-DR2^{+} individuals (P4, P5, P12, P15, and P16), who were presumably genetically more susceptible to develop MS [3]. However, virus-myelin cross-reactivity was absent in 3 of the 8 HLA-DR2^{+} MS patients and all of the 6 HLA-DR2^{+} control subjects. Examples of proliferation profiles illustrating restricted or cross-reactive responses are shown in the Figure. All lines tested (15 from 4 MS

Table 2. Patterns of Antigen Reactivity of Myelin Basic Protein (MBP)– or Virus-Reactive T-Cell Lines[a]

Donor Group	Selecting Antigen	Antigen Reactivity[b] MBP	Virus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Proportion and Percentage of T-Cell Lines Obtained for Each Selecting Antigen[d]
MS	MBP	+	−	0	0	4	4	4	1	0	2	7	2	1	0	3	8	5	0	41/51 (80%)
		+	+	0	1	0	0	0	4	0	1	2	0	0	1	1	0	0	0	10/51 (20%)
	Virus	−	+	5	0	6	0	8	2	7	1	4	2	4	7	3	5	0	0	54/83 (65%)
		+	+	0	0	0	5	2	0	0	7	2	0	0	8	2	0	1	2	29/83 (35%)
Controls	MBP	+	−	7	5	0	2	3	0	8	3	6	4	4	0	4	1			47/48 (98%)
		+	+	0	1	0	0	0	0	0	0	0	0	0	0	0	0			1/48 (2%)
	Virus	−	+	3	11	6	12	9	5	10	7	6	17	8	10	1	1			106/107 (99%)
		+	+	0	0	0	0	0	0	0	0	0	1	0	0	0	0			1/107 (1%)

Header spanning: "No. of T-Cell Lines Obtained per Donor[c]" / "Donor Designation" over columns 1–16.

[a]MBP- or virus-reactive T-cell lines from the peripheral blood of two donor groups (16 MS patients and 14 healthy control subjects) were selected by in vitro growth in the presence of the appropriate selecting antigen as described in the methods section.

[b]Presence (+) or absence (−) of reactivity to MBP or virus after 3 or 4 in vitro stimulations with selecting antigen. Reactivity was considered positive when the stimulation index of a [³H]thymidine incorporation assay was 3.0 or more and at least 1,000 cpm was incorporated.

[c]Total number of T-cell lines obtained with each selecting antigen that exhibited the indicated antigen reactivity (donor designations from 1 to 16 refer to MS patients P1, P2, etc., and controls C1, C2, etc., as described in Table 1).

[d]Proportion and percentage of T-cell lines (from 16 MS or 14 control donors) that were selected with either MBP or virus and yielded the indicated antigen reactivity profiles. Overall, 29% (10 + 29, or 39, out of 51 + 83, or 134) of T-cell lines from MS patients (10/16 donors) and 1.3% (1 + 1, or 2, out of 48 + 107, or 155) of T-cell lines from healthy donors (2/14 donors) were cross-reactive to MBP and virus (4 patients and no controls showed reciprocal cross-reactivity).
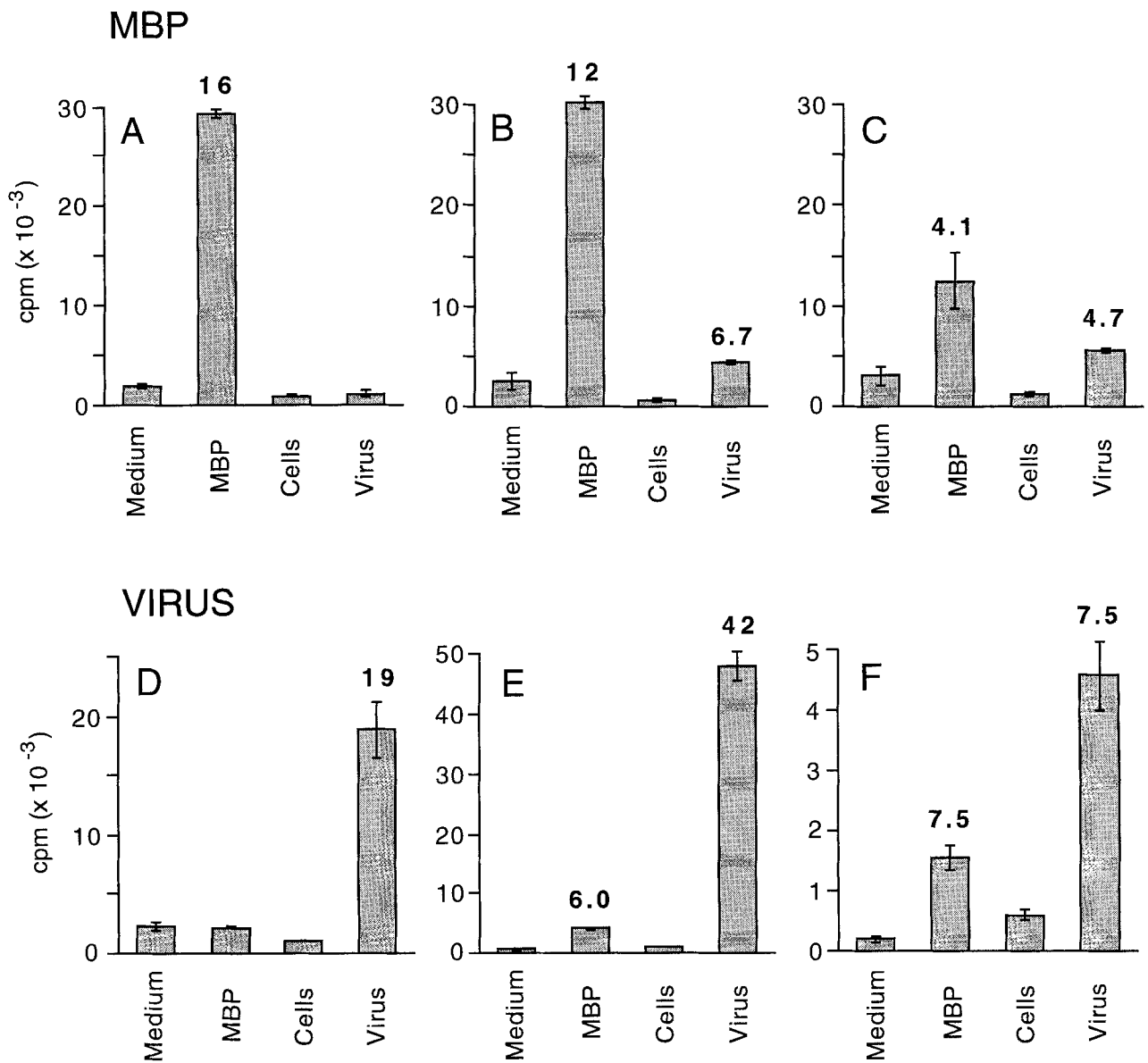
Typical proliferation profiles of T-cell lines established from MS patients by selection with either myelin basic protein (MBP) or human coronavirus. With human coronavirus 229E or MBP as the selecting antigen, T-cell lines were derived from MS patients and healthy donors and their reactivity to both viral and MBP antigens was evaluated in proliferation assays. The proliferation profiles of representative T-cell lines from MS patients are shown: MBP was the selecting antigen for T-cell lines used in (A, B, C) and HCV-229E for those in (D, E, F). (A) MBP reactivity only (Patient P9). (B, C) Cross-reactive T-cell lines from Patient P6. (D) Virus reactivity only (Patient P9). (E, F) Cross-reactive T-cell lines from Patient P8. The stimulation index is indicated on top of each significant reactivity (>3.0; >1,000 cpm), as well as the standard deviation for each count-per-minute result. "Medium" represents a proliferation assay in the presence of antigen-presenting cells only (used as control for MBP) and "Cells" represents a lysate from uninfected L132 cells that serves as a control to ascertain the virus specificity of the reactions. Tetanus toxoid consistently yielded background proliferation levels similar to "medium."

patients and 5 from 2 healthy donors), except 1, were shown to be CD4$^+$ by cytofluorometry, the exception being a CD8$^+$ line from 1 MS patient.

Another 11 T-cell lines obtained from 3 MS patients (3 from Patient P1, 4 from Patient P2, and 4 from Patient P10) by repeated in vitro stimulation with MBP lost their reactivity to MBP, even though they did react with virus (data not shown). This reactivity was not antigen independent since it was not observed with antigen-presenting cells only or with the control antigen (tetanus toxoid). Loss of reactivity to antigen was not observed with T-cell lines selected in the presence of virus. Two (P1 and P10) of the 3 MS patients from whom this unusual T-cell reactivity profile was observed did not yield T-cell lines with cross-reactivity patterns involving both MBP and virus, as 10 other MS patients did. Loss of reactivity to MBP used as an in vitro selecting antigen has been previously observed and was accompanied by the acquisition of reactivity to measles virus [30]. It remains possible that these cells are indeed virus-MBP cross-reactive but have become nonresponsive to MBP, possibly because of suppressor epitopes found on this molecule and not on the virus. Indeed, T-cell lines and clones were recently reported to be generated at a higher frequency and proliferate better with a synthetic peptide homologous to an immunodominant epitope of MBP rather than the whole molecule [31].

In a preliminary verification of clonality, we used a semiquantitative reverse transcriptase–polymerase chain reaction procedure [32] to identify the Vβ molecule on the TCR of 2 MBP-reactive T-cell lines obtained from 1 MS patient (P9). The T-cell line that recognized only MBP exclusively expressed the Vβ4 chain and the T-cell line that reacted to both MBP and HCV-229E only expressed the Vβ13 molecule (data not shown).

The proliferative responses to MBP and viral antigens were blocked by monoclonal antibodies to HLA class II antigens (for example, stimulation indices of 118 to MBP and 7.2 to virus of an MBP-reactive line were reduced to 0.73 and 1.1, respectively, while an isotype control antibody had no effect), and were not observed with HLA-mismatched antigen-presenting cells, which is consistent with an HLA-restricted response (data not shown). HLA-DR was identified as the restriction element, as previous studies also showed with MBP-reactive T cells [29].

Finally, it was of interest to evaluate the virus specificity of the T-cell lines that reacted to both MBP and HCV-229E antigens. For that purpose, MBP- or HCV-229E–selected, cross-reactive T-cell lines obtained from 6 patients (P11–P16) (see Table 2) were also tested for reactivity to HCV-OC43 antigens. We observed no cross-reactivity to this other human coronavirus serotype, despite the fact that donors were sero-

positive for this virus and HCV-OC43–reactive T-cell lines could be generated from the MS patient for whom it was tried (P13). This observation strengthens the possibility that the observed cross-reactivity patterns are the result of molecular mimicry between HCV-229E antigens (or possibly virus-induced antigens) and MBP.

Discussion

The observation of an HLA-restricted proliferation to both myelin and viral antigens of T-cell lines is consistent with dual specificities of the cross-reactive T cells for MBP and virus. Since less than 10% of the culture wells contained antigen-reactive T cells, the Poisson distribution suggests that an antigen-specific T-cell line derived from an individual microculture well most likely represented a monoclonal response to the myelin or viral antigen, as reported by others [16]. The detection of a unique Vβ molecule on the TCR of a T-cell line that recognized both MBP and viral antigens is also consistent with clonality, although this will need to be confirmed by sequencing of the variable regions of the TCR from several cross-reactive T-cell lines. The possibility that some of the observed cross-reactive patterns resulted from the selection of independent virus- and MBP-reactive T cells present in the overall population by either virus or MBP antigens cannot be formally excluded but would in itself also be an interesting observation since its preferential occurrence in MS patients is consistent with a possible pathological relevance. The often lower stimulation indices observed with the heterologous antigen compared to the homologous selecting antigen (see Fig, data not shown) suggest a less efficient recognition of the heterologous antigen. This could be explained if peptides processed from the heterologous antigens fit only imperfectly and with lower affinity within the complex between the HLA antigen and the TCR of the T cells selected with the homologous antigen.

The frequent MBP reactivity of MS patient–derived T cells selected with in vitro virus exposure is consistent with the possibility that the activation of at least a portion of the MBP-reactive T cells observed in both the periphery and the CNS of MS patients [16] could have been triggered in some of these patients by an otherwise innocuous respiratory coronavirus infection. There was no difference in frequency of T-cell cross-reactivity between patients with the relapsing form of disease compared to those with progressive disease, and no correlation with severity of neurological dysfunction. This suggests that development of cross-reactivity is not a direct consequence of CNS tissue injury.

Of interest, T-cell lines from 8 MS patients showed MBP reactivity after selection with virus. Five of these patients bear the HLA-DR2 haplotype presumably as-

sociated with genetic susceptibility to disease, although 3 other MS patients and 6 control subjects were also HLA-DR2$^+$ and showed no virus-myelin T-cell cross-reactivity. This suggests that cross-reactivity is not a direct consequence of the presence of this HLA haplotype. It is also noteworthy that reciprocal cross-reactivities (MBP reactivity of virus-reactive T-cell lines and virus reactivity of MBP-reactive T-cell lines) were observed in 4 MS patients but none of the normal control subjects. The presumed CNS migration of activated T cells sensitized to encephalitogenic epitopes of MBP could contribute to the initiation or propagation of the disease in these MS patients, either by direct cytotoxicity or indirectly through the secretion of soluble factors. Even though it was recently suggested that HCV-229E may be neurotropic [13, 14] (A. Bonavia et al, unpublished data, 1996), a persistent CNS infection would not be necessary for such autoimmune events to occur.

The coronavirus-myelin T-cell cross-reactivity patterns observed with much increased frequency in MS patients compared to control subjects, and which were specific for one of the two human coronavirus serotypes, are consistent with the molecular mimicry hypothesis of MS pathogenesis. Our finding that such cross-reactivity can occasionally be found in some control donors raises the issue of what prevents many individuals with autoreactive T cells from developing autoimmune disease [33].

Although the identity of the cross-reactive epitopes remains to be determined, it has now been reported that memory T lymphocytes that are cross-reactive to distantly related viruses may not require striking structural homologies, and that apparently unrelated peptides can stimulate the same T lymphocytes, albeit to different degrees [34]. Moreover, only four native MBP residues were shown to be capable of stimulating MBP-reactive T cells [35]. Such studies recently culminated in the elegant identification of viral peptides that could activate MBP-reactive T-cell clones generated from MS patients [36]. The cross-reactive sequences were predicted from the experimentally determined structural motifs required for binding of a peptide homologous to the immunodominant domain of MBP to HLA-DR2 molecules [37], which have been associated with genetic susceptibility for the development of MS. Peptides that could activate T-cell clones specific for the immunodominant epitope of MBP were from diverse pathogens, some of which have been associated with MS: herpes simplex virus, Epstein-Barr virus, adenovirus type 12, influenza virus type A, and reovirus type 3, as well as *Pseudomonas aeruginosa*. Interestingly, a peptide from the human coronavirus was predicted as bearing the necessary structural requirements, although it could not activatate the T-cell clones studied. This may be a reflection of the experimental bias toward the immunodominant epitope on MBP, the limited

number of T-cell clones studied, or the difficulty in predicting all relevant viral epitopes. Indeed, the identification of the biologically relevant cross-reactive epitopes between human coronavirus and MBP may in part require the use of purified proteins and peptides. Such studies are now in progress. Moreover, it will be important to look for the presence of such virus-myelin cross-reactive T cells within the CNS of MS patients.

Our current findings regarding T-cell cross-reactivity between the human coronavirus and the myelin antigen MBP in some MS patients could be extended to numerous pathogens [21, 22, 36] and other endogenous CNS antigens, such as PLP, myelin-associated glycoprotein, myelin oligodendrocyte glycoprotein, cyclic-nucleotide phosphodiesterase [38], and αB-crystallin [39], and this may account for the lack of coronavirus-MBP cross-reactive patterns in some MS patients. Virus-induced, T cell–mediated autoimmunity by molecular mimicry provides a unifying hypothesis that could explain both the suspected genetic involvement of immune response genes and the difficulty in associating one specific pathogen with MS. It is also consistent with the reported associations between viral infections, including those of the upper respiratory tract, and exacerbations of MS [40], as well as with the beneficial effects on MS patients of interferon beta [41–43], which has among its many other actions an antiviral property.

References
1. Ffrenchconstant C. Pathogenesis of multiple sclerosis. Lancet 1994;343:271–275
2. Sadovnick AD, Ebers GC. Epidemiology of multiple sclerosis—a critical overview. Can J Neurol Sci 1993;20:17–29
3. Hillert J. Human leukocyte antigen studies in multiple sclerosis. Ann Neurol 1994;36:S15–S17

4. Steinman L, Oksenberg JR, Bernard CCA. Association of susceptibility to multiple sclerosis with TCR genes. Immunol Today 1992;13:49–51

5. Kurtzke JF. Epidemiologic evidence for multiple sclerosis as an infection. Clin Microbiol Rev 1993;6:382–427

6. Talbot PJ. Implication of viruses in multiple sclerosis. Médecine/Sciences 1995;11:837–843

7. Myint SH. Human coronaviruses—a brief review. Rev Med Virol 1994;4:35–46

8. Murray RS, Cai GY, Hoek K, et al. Coronavirus infects and causes demyelination in primate central nervous system. Virology 1992;188:274–284

9. Tanaka R, Iwasaki Y, Koprowski HJ. Intracisternal virus-like particles in the brain of a multiple sclerosis patient. J Neurosci Res 1976;28:121–126

10. Burks JS, DeVald BL, Jankovsky LD, Gerdes JC. Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. Science 1980;209:933–934

11. Salmi A, Ziola B, Hovi T, Reunanen M. Antibodies to coronaviruses OC43 and 229E in multiple sclerosis patients. Neurology 1982;32:292–295

12. Pearson J, Mims CA. Differential susceptibility of cultured neural cells to the human coronavirus OC43. J Virol 1985;53:1016–1019

13. Talbot PJ, Ékandé S, Cashman NR, et al. Neurotropism of human coronavirus 229E. Adv Exp Med Biol 1994;32:339–346

14. Stewart JN, Mounir S, Talbot PJ. Human coronavirus gene expression in the brains of multiple sclerosis patients. Virology 1992;191:502–505

15. Murray RS, Brown B, Brian D, Cabirac GF. Detection of coronavirus RNA and antigen in multiple sclerosis brain. Ann Neurol 1992;31:525–533

16. Zhang JW, Markovicplese S, Lacet B, et al. Increased frequency of interleukin 2-responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. J Exp Med 1994;179:973–984

17. Sun JB, Link H, Olsson T, et al. T-cell and B-cell responses to myelin-oligodendrocyte glycoprotein in multiple sclerosis. J Immunol 1991;146:1490–1495

18. Zhang Y, Burger D, Saruhan G, et al. The T-lymphocyte response against myelin-associated glycoprotein and myelin basic protein in patients with multiple sclerosis. Neurology 1993;43:403–407

19. Derosbo NK, Milo R, Lees MB, et al. Reactivity to myelin antigens in multiple sclerosis—peripheral blood lymphocytes respond predominantly to myelin oligodendrocyte glycoprotein. J Clin Invest 1993;92:2602–2608

20. Watanabe R, Wege H, ter Meulen V. Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. Nature 1983;305:150–153

21. Oldstone MBA. Molecular mimicry and autoimmune disease. Cell 1987;50:819–820

22. Barnett LA, Fujinami RS. Molecular mimicry: a mechanism for autoimmune injury. FASEB J 1992;6:840–844

23. Jahnke U, Fischer EH, Alvord EC. Sequence homology between certain viral proteins and proteins related to encephalomyelitis and neuritis. Science 1985;229:282–284

24. Shaw SY, Laursen RA, Lees MB. Analogous amino acid sequences in myelin proteolipid and viral proteins. FEBS Lett 1986;207:266–270

25. Ota K, Matsui M, Milford EL, et al. T-cell recognition of

an immunodominant myelin basic protein epitope in multiple sclerosis. Nature 1990;346:183–187

26. Jouvenne P, Mounir S, Stewart JN, et al. Sequence analysis of human coronavirus 229E messenger RNAs 4 and 5—evidence for polymorphism and homology with myelin basic protein. Virus Res 1992;22:125–141

27. Cheifetz S, Moscarello MA. Effect of bovine basic protein charge microheterogeneity on protein-induced aggregation of unilamellar vesicles containing a mixture of acidic and neutral phospholipids. Biochemistry 1985;24:1909–1914

28. Mounir S, Talbot PJ. Sequence analysis of the membrane protein gene of human coronavirus OC43 and evidence for O-glycosylation. J Gen Virol 1992;73:2731–2736

29. Pette M, Fujita K, Kitze MD, et al. Myelin basic protein-specific T lymphocyte lines from MS patients and healthy individuals. Neurology 1990;40:1770–1776

30. Richert JR, Reuben-Burnside CA, Deibler GE, Kies MW. Peptide specificities of myelin basic protein-reactive human T-cell clones. Neurology 1988;38:739–742

31. Inobe J-i, Yamamura T, Kunishita T, Tabira T. T lymphocyte lines and clones selected against synthetic myelin basic protein 82-102 peptide from Japanese multiple sclerosis patients. J Neuroimmunol 1993;46:83–90

32. Labrecque N, McGrath H, Subramanyam M, et al. Human T cells respond to mouse mammary tumor virus encoded superantigen—V-beta restriction and conserved evolutionary features. J Exp Med 1993;177:1735–1743

33. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994;12:991–1045

34. Selin LK, Nahill SR, Welsh RM. Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses. J Exp Med 1994;179:1933–1943

35. Gautam AM, Lock CB, Smilek DE, et al. Minimum structural requirements for peptide presentation by major histocompatibility complex class II molecules—implications in induction of autoimmunity. Proc Natl Acad Sci USA 1994;91:767–771

36. Wucherpfennig KW, Strominger JL. Molecular mimicry in T-cell mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell 1995;80:695–705

37. Wucherpfennig KW, Sette A, Southwood S, et al. Structural requirements for binding of an immunodominant myelin basic protein peptide to DR2 isotypes and for its recognition by human T cell clones. J Exp Med 1994;179:279–290

38. Riethmuller A, Kalbus M, Dubois E, et al. T-cell-reactivity against CNPase (a minor myelin component) in multiple sclerosis patients and normals. J Neuroimmunol 1994;54:191

39. van Noort JM, van Sechel AC, Bajramovic JJ, et al. The small heat-shock protein αB-crystallin as candidate autoantigen in multiple sclerosis. Nature 1995;375:798–801

40. Panitch HS. Influence of infection on exacerbations of multiple sclerosis. Ann Neurol 1994;36:S25–S28

41. Duquette P, Girard M, Despault L, et al. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 1993;43:655–661

42. Paty DW, Li DKB, Duquette P, et al. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 1993;43:662–667

43. Jacobs L, Cookfair D, Rudick R, et al. Results of a phase III trial of IM recombinant beta interferon as treatment for MS. J Neuroimmunol 1994;54:170