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Expansion of Autoreactive T Cells in Multiple Sclerosis Is Independent of Exogenous B7 Costimulation

Christian Scholz,†† Kurt T. Patton,*, David E. Anderson,*** Gordon J. Freeman,† and David A. Hafler**†††

Multiple sclerosis (MS) is an inflammatory disease of the myelinated central nervous system that is postulated to be induced by myelin-reactive CD4 T cells. T cell activation requires an antigen-specific signal through the TCR and a costimulatory signal, which can be mediated by B7-1 or B7-2 engagement of CD28. To directly examine the activation state of myelin-reactive T cells in MS, the costimulation requirements necessary to activate myelin basic protein (MBP) or tetanus toxoid (TT)-reactive CD4 T cells were compared between normal controls and MS patients. Peripheral blood T cells were stimulated with Chinese hamster ovary (CHO) cells transfected either with DRB1*1501/DRA0101 chains (t-DR2) alone, or in combination with, B7-1 or B7-2. In the absence of costimulation, T cells from normal subjects stimulated with the recall antigen TT p830–843 were induced to expand and proliferate, but stimulation with MBP p85–99 did not have this effect. In marked contrast, T cells from patients with MS stimulated with MBP p85–99 in the absence of B7-1 or B7-2 signals expanded and proliferated. Thus, MBP-reactive CD4 T cells in patients with MS are costimulation independent and have been previously activated in vivo. These experiments provide further direct evidence for a role of activated MBP-specific CD4 T cells in the pathogenesis of MS. The Journal of Immunology, 1998, 160: 1532–1538.

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4 Abbreviations used in this paper: MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; MBP, myelin basic protein; PLP, proteolipid protein; RT, room temperature; TT, tetanus toxoid.

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Here we attempted to develop techniques that may allow us to gain insight into the activation state of circulating autoreactive T cells in patients with autoimmune disease. Highly purified CD4+ T cells isolated directly ex vivo from peripheral blood were stimulated in primary cultures with self-Ag/MHC complex in the presence or absence of different costimulatory signals. The experimental readout was clonal expansion and cytokine secretion of T cells measured by ELISpot. A modified protocol was used for long-term (>7 days) cytokine secretion in the presence of T-cell stimulatory supernatant. All flow cytometry analysis was performed using a FACSort (Becton Dickinson Immunocytometry Systems, San Jose, CA). Cells were sorted twice, single-cell cloned and matched for MHC type by PCR as follows: genomic DNA was isolated by lysing 5 × 10^5 cells in 0.5 ml of 0.1% SDS and 1% NP-40 at room temperature for 1 h, followed by a FITC-conjugated goat F(ab')2 anti-mouse IgG and IgM (Tago Immunologics, Camarillo, CA), or with CTLA-4-Ig fusion protein followed by a PE-conjugated goat F(ab')2 anti-human IgG Ab (Southern Biotechnology Associates, Birmingham, AL). Flow cytometry analysis was performed on a FACSort (Becton Dickinson Immunocytometry Systems, San Jose, CA). Cells were sorted twice, single-cell cloned and matched for MHC class II and B7 expression.

Cell transfectants
cDNAs for DRB1*0101 cloned into pRSV-5 (expressing neomycin resistance) and DRB1*1501 cloned into pREP4 (expressing hygromycin resistance) were a kind gift from Dr. K. W. Wucherpfennig (Dana-Farber Cancer Institute, Boston, MA). Chinese hamster ovary (CHO) cells were either transfected with pRSV-5 and pREP4 alone (t-DR2) or together with human B7-1 cloned into pLEN (a gift of California Biotechnology, Mountain View, CA) (t-DR2/B7-1). t-DR2 was subsequently transfected with pPGK-puro (expressing puromycin resistance) (a gift from Dr. P. Laird, Whitehead Institute, Cambridge, MA) (t-DR2/B7-2). CHO cells were transfected by electroporation, grown in selective medium and stained with the following reagents: phycoerythrin-conjugated MHC class II Ab (13) (Coulter Corporation, Hialeah, FL), anti-B7-1, IgG2a (C4), anti-B7-2, IgG2a (3D1), followed by a FITC-conjugated goat F(ab')2 anti-mouse IgG and IgM (Tago Immunologics, Camarillo, CA), or with CTLA-4-Ig fusion protein followed by a PE-conjugated goat F(ab')2 anti-human IgG Ab (Southern Biotechnology Associates, Birmingham, AL). All flow cytometry analysis was performed on a FACSort (Becton Dickinson Immunocytometry Systems, San Jose, CA). Cells were sorted twice, single-cell cloned and matched for MHC class II and B7 expression.

**Materials and Methods**

**Subjects**

Peripheral venous blood was obtained after informed consent from normal subjects, or patients with the relapsing-remitting form of MS either in the midst of an acute attack or within 6 mo of a relapse; none were in long-term remission. All patients were seen in the outpatient MS Clinic at the Brigham and Women’s Hospital, Boston, MA. None of the patients had received any steroids within three months of drawing blood or any immunosuppressive treatment.

**PBMCs**

PBMCs were isolated from heparinized venous blood of normal controls and patients with MS by dextran sedimentation and subsequent Ficoll-Paque density gradient centrifugation (Pharmacia Biotech, Uppsala, Sweden). Cells were resuspended at 2 × 10^6 cells/ml and incubated overnight at 37°C in complete medium (20% human AB serum, 100 U/100 μg/ml penicillin/streptomycin) (BioWhittaker, Walkersville, MD) supplemented with 10% autologous serum. DR2-positive nonadherent cells were washed twice in PBS and loaded onto a human T cell enrichment column (R&D Systems, Minneapolis, MN). Eluted cells were incubated at a density of 10^6 cells/ml for 30 min at 4°C with the following monoclonal mouse anti-human Abs: anti-CD11b, IgG2b (Mo1), anti-CD4, IgG2b (Mo2), anti-CD8, IgG2a (OKT8) (ATCC, Rockville, MD), anti CD16, IgG1 (3G8) (a gift from J.C. Unkeless, Department of Biochemistry, Mount Sinai School of Medicine, New York, NY and T. Springer, Center for Blood Research, Boston, MA), anti-CD20, IgG2a (B1) or anti-CD19, IgG1 (B4). Cells were washed twice and incubated for 30 min at 4°C with goat anti-mouse IgG-conjugated magnetic beads (Perseptive Diagnostics, Cambridge, MA). After three depletions on a magnet, the remaining cells were washed and adjusted to a density of 10^6 cells/ml in complete medium supplemented with 10% autologous serum. Cells were more than 97% CD4 positive, MHC class II positive and IL-2R low or negative, as well as CD11b, CD14, CD8, CD20, and CD16 negative.

**T cell cultures**

Ag-specific T cell lines were generated in 96-well U-bottom plates (CoStar, Cambridge, MA) as follows: as APCs, CHO cell transfectants were fixed with 0.2% paraformaldehyde for 10 min, washed twice in PBS. Fixed cells were left in complete medium for 16 h at 37°C before being pulsed for 2 h at 37°C with 100 μg/ml peptide (1% Triton X-100; Bio-Synthesis, Dallas, TX). Titration experiments with professional Ag-presenting cells (APC) from peripheral blood as well as with CHO cell transfectants have shown that peptide concentrations between 40 μg/ml (10 μm) and 400 μg/ml (100 μm) will lead to significant clonal expansion, whereas concentrations below this result in few lines even when professional APC are used. After peptide incubation unbound peptide was washed away and transfectched CHO cells were added at 5 × 10^5 cells/well to 10^5 CD4 T cells/well (final volume 200 μl). On day 7, each well was restimulated with peptide-pulsed CHO cells as used on day 0. On day 9, a final concentration of 5% v/v Technical Collaborative Biomedical Products, Bedford, MA was added to the cultures. On day 14, each original well was split into five wells: two receiving the unpulsed primary transfection CHO cell, two receiving the peptide-pulsed CHO cell and one having only complete medium supplemented with 10% human serum (BioWhittaker). Cells from the last well were reserved to single-cell clone T cell lines. When being cloned, cells from this well therefore had only received two previous in vitro stimulations (day 0 and day 7). Supernatants from cells that were stimulated during the split well assay on day 14 were collected after 40 h of culture for cytokine measurement, and each well was pulsed with 1 μCi [3H]thymidine (NEN/Dupont, Boston, MA) for the last 18 h of a 72-h incubation. Subsequently, cells were harvested (Harvester 96, Tantec, Orange, CT) and [3H]thymidine uptake was measured in a β-scintillation counter (Be-taplate 1205, Wallac, Gaithersburg, MD). Where indicated, CTLA4-Ig fusion protein (a gift from Dr. M. Collins, Genetics Institute, Cambridge, MA) at a concentration of 10 μg/ml was added throughout the T cell culture.

For measurement of T cell proliferation to anti-CD3, 10^5 CD4 T cells were incubated in 96-well flat-bottom plates (CoStar) with plastic-bound anti-CD3 (2.5 μg/ml) (ATCC) and 5 × 10^5 fixed CHO cells. T-mock was transfected with pPGK-Hygro containing only the resistance gene for hygromycin and SV2-Neo-Sp65 containing only the neomycin resistance gene, t-DR2–B7–1 in pLEN (12) and t-DR2–B7–2 contains human B7–1 in pLEN (24). Transfectants were generated as described above. t-DR2–B7–1 and t-DR2–B7–2 were co-cultured with CHO cells and anti-CD3 for 72 h. [3H]Thymidine was added for the final 18 h of culture and uptake was measured as described above.

**Cytokine assays**

IL-4 and IFN-γ secretion was measured as follows: Immulon 4 microtiter plates (Dynatech, Chantilly, VA) were coated overnight at 4°C with 1 μg/ml monoclonal capture Ab (IL-4: PharMingen, San Diego, CA; IFN-γ: Endogen, Cambridge, MA) in 0.1 M sodium bicarbonate buffer (pH 9.4). Plates were blocked for 2 h at room temperature (RT) with 1% BSA (Sigma Chemical Company, St. Louis, MO) in PBS. Eighty microliters of culture supernatant or standards of recombinant cytokines (IL-4 – R&D Systems; IFN-γ – Life Technologies) were added in duplicate, incubated at 4°C overnight and washed and developed in 0.6% BSA in PBS. For detection of IL-4, plates were incubated for 45 min at RT with a biotinylated mAb (PharMingen) at 1.0 μg/ml. After washing, plates were incubated for 30 min at RT with avidin-peroxidase (Sigma) at a dilution of 1:5,000. IFN-γ was detected by incubating a polyclonal rabbit anti-human IFN-γ Ab (Endogen) at 1.0 μg/ml for 2 h at RT followed by a goat anti-rabbit IgG-horse-radish-peroxidase-labeled Ab diluted at 1:10,000 (BioSource International, Camarillo, CA). ELISA plates were developed with TM one component peroxidase substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD), samples were stopped with an equal volume of 1.0 M H_2PO_4, absorbance was measured at 450 nm using a Bio-Rad ELISA reader, and the...
cytokine concentration was calculated from a standard curve with a Bio-Rad Microplate Manager 2.1 program (Bio-Rad Laboratories, Hercules, CA).

**Single-cell T cell cloning**

Ag-reactive T cell lines were single-cell cloned at limiting dilution as follows: 0.3 cells per well were incubated at a final volume of 100 µl with 10^5 irradiated (5000 rads) allogeneic mononuclear feeder cells in complete medium with 10% human serum and 1 µg/ml phytohemagglutinin (PHA-P) (Murex, Norcross, GA). After two days of culture, 100 µl of complete medium with 10% human serum and 10% v/v T-stim were added to each well. Fresh complete medium with 10% human serum and 5% v/v T-stim was added every two to three days. Thereafter, wells were visually scored for cell growth and proliferating wells were retested for Ag specificity. Ag-specific T cell clones were expanded with PHA-P and irradiated mononuclear feeder cells, and tested for Ag-specific proliferation and secretion of IL-4 and IFN-γ as described above. Cloning efficiency ranged between 2 and 10%.

**Results**

**Tetanus toxoid-specific T cells can be clonally expanded in the absence of B7 costimulation**

To assess the costimulation requirements of peripheral blood lymphocytes in an Ag-specific system, we transfected CHO cells with either the MHC class II (DRA*0101, DRB1*1501) (t-DR2) alone or in combination with either B7–1 (t-DR2/B7–1) or B7–2 (t-DR2/B7–2). Stable transfectants were obtained after sorting and limiting dilution cloning (Fig. 1). CD4 T cells from peripheral blood, enriched by negative depletion to a purity of more than 97%, were cultured with CHO cells that were pulsed with and without the recall Ag tetanus toxoid p830–843 peptide, or the immunodominant MBP p85–99 peptide. A total of over 5000 short term T cell lines were generated (Table I), and a summary of all the data is shown in Figure 2. Specifically, in five of seven normal individuals, costimulation-independent T cell lines were detected in response to TT p830–843 pulsed t-DR2 while no detectable [3H]thymidine incorporation was observed to MBP p85–99 pulsed t-DR2 (Fig. 2A). The lack of MBP-reactive T cells in normal individuals was not due to their absence, as clonal expansion and proliferation was observed with CHO APCs co-expressing DR2 and B7–1 (Fig. 2B) or DR2 and B7–2 (Fig. 2C). These data indicate that in humans, proliferation and clonal expansion of T cells to recall Ags can be elicited independently of B7 costimulation. In contrast, proliferative responses and clonal expansion to naive Ags require B7 costimulation. Moreover, these experiments established a novel method to examine differential costimulation requirements and thus the activation state of autoreactive CD4 T cells immediately ex vivo.

**Autoreactive MBP-specific T cells are B7 costimulation independent in MS**

We then examined whether MBP-reactive T cells from patients with MS were dependent upon costimulation with B7–1 or B7–2 for clonal expansion as assessed by [3H]thymidine incorporation. In contrast to normal individuals, costimulation-independent Ag-specific [3H]thymidine uptake was observed when T cells were stimulated by MBP p85–99 pulsed t-DR2. As with controls, representative cpm values are shown in Table I, and a summary of the data is shown in Figure 2. Remarkably, seven out of twelve patients tested showed between 2 and 8% MBP p85–99 specific lines. As expected, reactivity to TT p830–843 pulsed t-DR2 was comparable to healthy controls (Fig. 2D). These data provide direct evidence for a difference in activation requirements of MBP-specific T cells in patients with MS compared with normal individuals.

**Costimulation requirements of peripheral blood T cells in MS are Ag-specific**

To determine whether the B7 costimulation independence of MBP-reactive T cells in patients with MS was Ag-specific, we examined the total CD4 T cell population without antigenic bias.
Purified CD4 T cells were incubated with plastic bound anti-CD3 and CHO cells without (t-mock) or with costimulatory molecules (t-B7–1, t-B7–2), and [3H]thymidine uptake was measured. T cells from both normal individuals and patients with MS required costimulation for proliferation (Fig. 2G). These data indicate that the costimulation independence of MBP-reactive T cells from patients with MS is related to a subpopulation of Ag-reactive T cells as opposed to all TCR-expressing lymphocytes.

Clonal expansion in the absence of B7 costimulation cannot be blocked by addition of CTLA4 Ig

As B7.1 and B7.2 can be expressed on activated T cells, we next wished to determine whether T cell expansion in the presence of t-DR2 plus peptide was due to costimulation by T cells. To examine this we compared the frequency of MBP-reactive T cells after stimulation of purified CD4 T cells from MS patients with CHO APCs transfected with DR2 after adding CTLA4-Ig fusion protein (10 μg/ml) to block B7.1 and B7.2 costimulation to all steps of the T lymphocyte culture. As can be seen in Table II, there was no difference in the frequency of Ag-specific T cells after culture of CD4 T cells in the presence or absence of CTLA4-Ig. In contrast, the CTLA4-Ig fusion protein could block B7-dependent costimulation of resting T cells (data not shown).

**Ag-specific T cells can secrete IL-4 in the absence of [3H]thymidine incorporation**

As no [3H]thymidine uptake was observed after primary stimulation of T cells from control subjects with MBP p85–99 in the absence of B7 costimulation, we examined whether any signal was induced in these naive T cells. This was of particular interest as cytokine secretion of MBP-reactive T cells in many cases does not correlate with [3H]thymidine uptake (25). We examined approximately 1500 CD4 T cell lines for secretion of IL-4 or IFN-γ after different primary APC stimuli. Ag-specific IL-4 secretion was detected when MBP p85–99 pulsed t-DR2 was used to generate T cell lines in normal individuals; a similar frequency of nonproliferating, IL-4-secreting T cells had occurred during the initial and the second stimulation. We then determined whether the IL-4-secreting T cells could be induced to differentiate further after primary TCR stimulation in the absence of costimulation. Wells containing IL-4-secreting T cells generated during 14 days of culture and two stimulations with t-DR2/peptide were cloned at 0.3 cells/well with PHA-P and secreted IFN-γ (442 pg/ml with peptide compared with 6 pg/ml without peptide) secreted IFN-γ by either normal individuals or patients with MS. The frequency of nonproliferating, IL-4-secreting T cells was similar to the frequency of the proliferating lines, which were detected when t-DR2/B7–1 or t-DR2/B7–2 were used as APCs. The amounts of IL-4 secreted ranged between 39 and 154 pg/ml, and a small sample of the representative T cell lines are shown in Figure 3B. Stimulation of T cells with TT p830–843 and t-DR2 in the absence of costimulation also generated nonproliferating, IL-4-secreting T cell lines in one out of five healthy individuals (15% and 17% IL-4-secreting lines in two separate experiments) and were not detected in any of six MS patients examined (data not shown). Thus the presence of IL-4-secreting nonproliferating T cells is not specific for autoreactive T cells.

To examine whether clonal T cell expansion was required for the measurement of IL-4 secretion, we examined culture supernatants 48 h after initial stimulation of CD4 T cells with t-DR2 CHO cells pulsed with MBP p85–99 and were unable to detect IL-4 (data not shown). Thus, the secretion of IL-4 in the absence of [3H]thymidine incorporation after 14 days of culture indicated that some degree of clonal expansion and perhaps differentiation of IL-4-secreting T cells had occurred during the initial and the second stimulation. We then determined whether the IL-4-secreting T cells could be induced to differentiate further after primary TCR stimulation in the absence of costimulation. Wells containing IL-4-secreting T cells generated during 14 days of culture and two stimulations with t-DR2/peptide were cloned at 0.3 cells/well with PHA-P and allogeneic whole mononuclear cells, which provide a costimulatory signal. Four clones from two different IL-4-secreting lines were examined. These PHA-P-stimulated T cell clones now proliferated in response to Ag and secreted IFN-γ but not IL-4. For example, T cell line JE 1 that did not incorporate [3H]thymidine in response to stimulation with TT p830–843 pulsed t-DR2 (427 cpm with peptide compared with 476 cpm without peptide) secreted IL-4 (442 pg/ml with peptide compared with 39,368 cpm with peptide) secreted IFN-γ (79,734 cpm with peptide compared with 39,368 cpm without peptide) secreted IFN-γ.

**Table II. Representative proliferative responses ([3H]thymidine incorporation) of peripheral blood T cells stimulated with t-DR2 plus peptide**

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*CD4+ T cells from subjects with MS or normal controls were stimulated with CHO cells transfected with DR2 pulsed with MBP p85-99 or TT p830-843. Representative values of [3H]thymidine incorporation with and without Ag stimulation of T cell lines generated after culture (see Materials and Methods) are shown. Patients with MS generated T cell lines that proliferated to MBP in the absence of costimulation.
270 pg/ml without peptide) but did not secrete IL-4. Five T cell clones derived from four proliferating lines that secreted IFN-γ but no IL-4 were also generated, and as expected they did not change the cytokines secreted (data not shown).

Discussion

We directly examined the costimulation requirements to activate MBP as compared with tetanus toxoid-reactive CD4 T cells from normal controls and MS patients. In the absence of costimulation, T cells from normal subjects stimulated with the recall Ag tetanus toxoid p830–843, but not with the naïve Ag MBP p85–99, were induced to expand and proliferate. In contrast, T cells from patients with MS stimulated with MBP p85–99 in the absence of B7–1 or B7–2 signals expanded and proliferated. A high frequency of non-proliferating T cell lines stimulated in primary culture with MBP p85–99 either in the presence or absence of CTLA 4-Ig fusion protein. The relative proportion of MBP p85–99 reactive T cells (Δcpm > 500, SI > 2) was the same in the presence of (5/60) or absence of (5/60) CTLA 4-Ig fusion protein.

Over the past decade, the structural basis for recognition of myelin Ags has been investigated and both MHC and TCR contact residues of immunodominant peptides of both MBP and PLP have been defined (24, 26, 27). It has been observed that the immunodominant myelin peptides and the TCR used to recognize the Ags are similar between patients with MS and normal individuals. Perhaps this is not surprising considering that in rodents prone to develop EAE, the MHC/peptide/TCR complexes involved in the disease are the same before and after disease induction. Instead, investigation of the EAE model has indicated that the minimal requirement for inducing experimental inflammatory CNS disease that at times can appear almost identical to MS are the presence of activated, myelin-reactive T cells. This leads to the postulate that understanding the activation state of myelin-reactive T cells in patients with MS is critical in understanding the disease’s pathogenesis.

Previous investigations have suggested that circulating MBP and/or PLP-reactive T cells in the blood of patients with MS are in a different state of activation as compared with normal individuals. Using an hprt+ mutant assay, eleven of 258 mutant T cell clones cultured by mitogen from the peripheral blood of five of six MS patients showed strong reactivity to MBP, as compared with none of 114 clones grown from blood of normal subjects (28). More recently, we investigated a total of 72 subjects with MS as to whether myelin-reactive T cells exist in a different state of activation as compared with myelin-reactive T cells cloned from the blood of normal individuals. While there were no differences in the frequencies of MBP and PLP-reactive T cells after primary antigen stimulation, the frequency of MBP or PLP but not TT-reactive T cells generated after primary rIL-2 stimulation were significantly higher in MS patients as compared with control individuals (22). Primary rIL-2-stimulated MBP-reactive T cell lines were CD4 positive and recognized MBP epitopes 84–102 and 143–168 similarly to MBP-reactive T cell lines generated with primary MBP stimulation. Based on more recent data, we postulate that the higher frequency of T cells observed with primary IL-2 as opposed to antigen culture resulted from the induction of apoptosis by antigen of MBP-reactive T cells activated in vivo, which was partially prevented by primary culture with IL-2 (27).

As the CHO cells could not process whole Ag, we examined the peptide MBP p85–99. This peptide exhibits high affinity binding to DRB1*1501 (29), and is processed and presented to a high proportion of MBP-reactive T cells in patients with MS and normal
individuals with the DR2 haplotype (24, 26, 27). It is important to note that this does not necessarily imply that MBP is the initiating antigen in MS. It has been demonstrated that in EAE, regardless of the initiating event, there is activation and recruitment of autoreactive lymphocytes specific for epitopes distinct from the disease-inducing epitope from chronic tissue damage (13, 23). Thus, it is likely that any number of different myelin antigen peptides could be used to examine the state of activation of the immune system in the recognition of CNS Ags.

These experiments do not address the issue as to how MBP p85–99-reactive T cells become costimulation independent for proliferation. One possibility is that the MBP-reactive T cells are chronically stimulated in the CNS in the presence of B7 costimulation. In this regard, Miller and colleagues demonstrated the involvement of both intermolecular epitope spreading to the PLP p139–151 peptide in relapsing-EAE induced with the MBP p84–102 epitope and intramolecular epitope spreading to the PLP p139–151 peptide in RRMS (13). Of particular interest is that the expression of co-p178 –191 epitope in R-EAE induced with the PLP p139 –151 102 epitope and intramolecular epitope spreading to the PLP p139 –151 peptide in relapsing-EAE induced with the MBP p84 –99 epitope appears to have important consequences for epitope spreading. Thus, the expression of B7–1 costimulation molecules in an inflammatory disease site may be of importance in the induction of Th1 cells and chronic inflammation and disease. In brain tissue of patients with MS, we similarly observed increased expression of B7–1 in acute MS plaques from early disease cases but not in the inflammatory lesions associated with stroke (15). Thus, chronic stimulation of T cells in the CNS by self-antigen may lead to their activated state as observed in these studies. However, it is also possible that viruses cross-reactive with myelin peptides that induce the expression of B7 co-stimulatory molecules on systemic APCs induce activated myelin-reactive T cells (30). In this regard, experiments using a PCR-based approach to measure the frequency of MBP-reactive T cells are more consistent with the hypothesis of a cross-reactive Ag (31).

These experiments demonstrate that expansion of autoreactive MBP-specific T cells in MS are independent of exogenous B7 costimulation. This could result from either a different state of T cell activation or alternatively to the expression of B7 on the T cell surface. This question was addressed by a number of experiments. First, using FACS analysis, we examined peripheral blood CD4 T cells from MS patients and healthy controls and found no difference in B7–1 or B7–2 expression (data not shown). Secondly, in other experiments, we could demonstrate that B7–1 or B7–2 expressed on human T cells does not provide a costimulatory signal sufficient for T cell activation (32). Third, we showed that an anti-CD3 signal in the absence of B7–1 or B7–2 could not stimulate the population of resting CD4 T cells. Finally, the addition of CTLA4-Ig fusion protein, which blocks B7.1 and B7.2 costimulation, did not decrease the frequency of MBP p84–102-reactive T cells after CHO-DR2 stimulation of MBP p84–102-reactive T cells. Together, these observations make it unlikely that costimulation through B7/CD28 is provided by other activated T lymphocytes in the population. As activated T cells can be independent of a costimulatory signal because of intracellular events, we favor the hypothesis that a small number of MBP and other myelin-reactive T cells have been recently activated in the CNS in the presence of B7 costimulation and thus do not require a second signal for clonal expansion and proliferation in vitro. Nevertheless, whatever mechanisms allow MBP-reactive T cells in MS patients to be independent of an exogenous costimulatory signal, these studies provide further direct evidence demonstrating that MBP-specific CD4 T cells in patients with MS have been previously activated in vivo.

While circulating myelin-reactive T cells are present in normal subjects, pathologic autoimmune responses do not occur without other events that induce the activation of autoreactive T cells. There is accumulating evidence that B7 molecules are important in regulating the response to self-Ags (13–15, 32). Thus it was of interest to observe low level secretion of IL-4 in response to MBP p85–99/DRB1*1501 in the absence of costimulatory molecules. It is possible that in vivo stimulation of naïve autoreactive T cells in the absence of costimulation may induce IL-4 secretion.
which down-regulates the response to autoantigens. This is of particular interest as IL-4 secretion by MBP-reactive T cells has been shown to ameliorate EAE induced by activated MBP-reactive T cells (33). It should be noted that the cytokine response of differentiated autoreactive human T cells activated in the absence of costimulation, which results in anergy, may be different from naive T cells as observed in these experiments. In this regard, we observed that IL-4-secreting CD4 T cell lines induced in the absence of costimulation can differentiate into IFN-γ-secreting cells in the presence of costimulation, and thus are functionally distinct.

In summary, our results indicate that autoreactive T cells can be clonally expanded with Ag presentation in the absence of B7 costimulatory signal and are not clonally deleted but instead are induced to secret IL-4. These experiments provide further direct evidence for a role of activated MBP-specific CD4 T cells in the pathogenesis of MS.

Note Added in Proof. Since submitting this manuscript, we have become aware of a similar observation entitled “Decreased dependence of myelin basic protein reactive T cells on CD28-mediated costimulation in multiple sclerosis patients: a marker of activated/memory T cells” by A. E. Lovett-Racke et al., in press in the Journal of Clinical Investigation.

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