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The Human T Cell Response to Myelin Oligodendrocyte Glycoprotein: A Multiple Sclerosis Family-Based Study

Niklas K. U. Koehler,* Claude P. Genain,* Barbara Giesser, † and Stephen L. Hauser*

Myelin oligodendrocyte glycoprotein (MOG) is an encephalitogenic myelin protein and a likely autoantigen in human multiple sclerosis (MS). In this work, we describe the fine specificity and cytokine profile of T cell clones (TCC) directed against MOG in three nuclear families, comprised of four individuals affected with MS and their HLA-identical siblings. TCC were generated from PBMC by limiting dilution against a mixture of eleven 20-mer overlapping peptides corresponding to the encephalitogenic extracellular domain of human MOG (aa 1–120). The frequency of MOG peptide-reactive T cells was surprisingly high (range, 1:400 to 1:3,000) and, unexpectedly, cloning efficiencies were highest at low seeding densities of 10^2 or 10^3 PBMC per well. A total of 235 MOG peptide-reactive TCC were produced, all of which were CD4^+ CD8^- TCRβ^+ TCCγδ^-. All 11 MOG peptides were recognized by the TCC, and different epitopes of MOG appeared to be immunodominant in the HLA-identical siblings. The patterns of cytokine secretion by TCC from single individuals were generally similar. The healthy individuals exhibited Th2-, Th0-, and T regulatory cell 1-like cytokine profiles, whereas TCC from one sibling with MS had a striking Th1-like phenotype, producing high levels of IFN-γ and TNF-α, and low IL-4 levels. Thus, MOG-reactive T cells appear to constitute an important part of the natural T cell repertoire, a finding that could contribute to the development of autoimmunity to this protein. The Journal of Immunology, 2002, 168: 5920 –5927.

Multiple sclerosis (MS) is an autoimmune disease thought to be caused by a concerted attack of T cells, B cells, and macrophages against myelin components of the CNS (1, 2). The immune responses against the quantitatively major myelin proteins, specifically myelin basic protein (MBP) and proteolipid protein, have been well characterized, both in animal models (experimental allergic encephalomyelitis) and in human MS. MBP appears to be a critical T cell autoantigen in MS (2); however, because MBP is expressed in central and peripheral nervous system myelin, additional factors must be present to explain the specificity of MS for CNS white matter. Myelin oligodendrocyte glycoprotein (MOG) is a minor myelin protein that is exclusively expressed in the CNS and is located on the extracellular membrane of oligodendrocytes, their processes, and the outermost myelin lamellae (3–7). In several animal species, most notably non-human primates, MOG is highly encephalitogenic and induces a primary demyelinating disease that closely mimics human MS (8–14). Large concentric areas of macrophage infiltration, autoantibody deposition, and vesicular demyelination are characteristic of MOG-induced experimental allergic encephalomyelitis (15, 16). In humans, autoimmune against MOG also appears to play a causative role in the pathogenesis of MS. MOG-specific autoantibodies have been detected in situ in actively demyelinating MS lesions (15). High levels of reactivity of PBMC against MOG (17–19), high precursor frequencies of MOG-reactive T cells in serum and cerebrospinal fluid (CSF), and CSF autoantibodies (20–22) have also been associated with MS. In this study, a limiting dilution technique was used to generate MOG-reactive T cell clones (TCC) from haplolidentical siblings belonging to MS-prone families. The fine specificity and cytokine patterns of the TCC were also analyzed. Our data indicate that MOG peptide-reactive T cells occur with surprisingly high frequency in the normal T cell repertoire and raise the possibility that individual differences exist in the response to MOG that may be relevant to the pathogenesis of MS.

Materials and Methods

Subjects

Individuals from three MS families with multiple affected members were studied (Table I). Family I consisted of two unaffected parents (I-Uf and I-Um), two daughters (I-Ms1 and I-Us1) discordant for MS, and two sons (I-Ms2 and I-Us2) also discordant for MS. The affected daughter (I-Ms1) was treated with IFN-β-1b, while the affected son (I-Ms2) was untreated. Families II and III consisted of a sibling pair discordant for MS (II-Ms1 and II-Us1; III-Ms1 and III-Us2); in each, the affected individual was untreated (Table I). All affected individuals had relapsing-remitting, clinically definite MS (23). HLA typing was performed by a PCR method as previously described (23). All siblings within a given family were HLA-haplolidentical.

Antigens

For T cell cloning and epitope mapping studies, a series of eleven 20-mer peptides overlapping by 10 aa and corresponding to the entire sequence of the extracellular domain of human MOG (aa 1–120) were synthesized using standard F-moc chemistry and purified >95% as verified by HPLC and mass spectrometry (Research Genetics, Huntsville, AL). In addition to the peptides, 82 positively reacting TCC from family I were tested for reactivity against recombinant proteins corresponding to the extracellular domains of recombinant human (rh)MOG and recombinant rat (rr)MOG, produced in Escherichia coli and purified as described previously (24). The purity of these recombinant proteins was confirmed by SDS-PAGE and silver staining. For 26 MOG-reactive TCC from family I, fine mapping of...
epitopes was performed using a panel of multiple-mer peptides overlapping by 1 aa (Chiron Mimotopes, San Diego, CA).

**Establishment of MOG peptide-reactive TCC**

PBMC were isolated from freshly drawn heparinized blood by Ficoll density centrifugation (Pharmacia Biotech, Uppsala, Sweden). PBMC were incubated at a cell density of 4 × 10^6/ml in AIM-V medium (Life Technologies, Gaithersburg, MD) for 3 days in a bulk culture with a mixture of the 11 synthetic MOG peptides, at a final concentration of 10 μg/ml for each peptide, using a 50-ml tissue culture flask (Nunc, Roskilde, Denmark). After 3 days, the cells were harvested by resuspending, washed twice in RPMI 1640 to remove dead cells and Ag, and counted. Viable cells were seeded in AIM-V medium (Life Technologies) in the presence of rIL-2 (10 U/ml) and IL-4 (4 U/ml) (both from Life Technologies) at densities of 10^2, 10^3, 10^4, and 10^5 cells/well using 96-well round-bottom plates (Costar, Cambridge, MA). A total of 10^3 irradiated autologous PBMC per well were added to the three lower densities. Wells (120 or 96) were plated per dilution for each individual (Table I). After 3 days, 100 μl of the TCC/well was assayed by a proliferation assay. TCC were generated as described above. PBMC from three other IFN-γ-cytokine supernatants from 92 MOG peptide-reactive TCC from family I were determined by ELISA. The same pooled supernatant of each TCC was used in all cytokine assays. The following cytokines were measured: IFN-γ, TNF-α, IL-10, IL-6, IL-4 (all from BioSource International, Camarillo, CA), and TGF-β (R&D Systems, Minneapolis, MN). T cells were rested for 4–6 days, washed, and resuspended in AIM-V. A total of 10^5 T cells/well of each clone were stimulated with 5 × 10^5 Ag pulsed APC/well in 250 μl AIM-V/well, using a 96-well round-bottom plate. MOG peptide-pulsed APC were prepared as described above. Supernatants were harvested after 2 days and stored at −70°C until use. The ELISA were performed according to the manufacturer’s instructions. The measured cytokine concentrations were corrected against background cytokine secretion from the APC by subtracting cytokine concentrations produced from APC alone incubated with MOG peptide.

The detection limits of the cytokine kits were as follows: human IFN-γ, <4 pg/ml; human TNF-α, <1 pg/ml; human IL-4, <2 pg/ml; human IL-6, <2 pg/ml; human IL-10, <5 pg/ml; and TGF-β, <5 pg/ml. Cytokine levels below the detectable concentration limit were set at that limit. Cytokine ratios were calculated by dividing IFN-γ or TNF-α (both Th1 cytokines) by IL-4 (Th2 cytokine) concentrations.

**Results**

**Phenotype and epitope specificity of MOG-reactive T cells**

The subjects, their disease status and HLAA-DR haplotype, the number of seeded wells for the four tested dilutions, the total number of generated MOG peptide-reactive TCC, the immunodominant MOG peptides, the number of TCC against the dominant peptide, and the estimated frequency of MOG peptide-reactive T cells are summarized in Table I. The optimal concentration of Ag used in this study was established in preliminary experiments using a range of 2–100 μg/ml. Frequencies of MOG peptide-reactive TCC were calculated by dividing the number of reactive wells by the total number of PBMC plated at each dilution.

**Phenotype of cells**

CD4, CD8, CD45, TCRαβ, and TCRγδ expression were determined with a FACSscan (BD Biosciences, Mountain View, CA), using the mAbs CD4 FITC, CD8 PE, CD45 PE-Cy5, pan-TCRαβ FITC, and pan-TCRγδ PE (all from Immunotech, Marseilles, France), according to the manufacturer’s instructions. A PE-labeled isotype control was used to exclude nonspecific binding.

<table>
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<tr>
<th>Table I. <strong>Summary of TCC reactive to MOG</strong></th>
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* I, II, and III, Family designation; M, affected with MS; U, unaffected; f, father; m, mother; s, sibling; 1, female; 2, male.
not shown). All TCC were CD4⁺CD8⁻CD45⁺TCRαβ⁺TCRγδ⁻ by FACS analysis.

The TCC were tested in proliferation assays for their reactivity against the individual MOG peptides, rhMOG and rrMOG. All 11 MOG peptides were recognized by the TCC. Three MOG-reactive wells from I-Uf, as well as one well each from I-Ms2 and I-Us2 seeded at 10² or 10³ PBMC/well, reproducibly recognized four or more MOG peptides. Despite the identical DR/DQ haplotype of the siblings in each of the three families, the TCC from each individual revealed a distinct repertoire of reactivity against MOG peptides (Table I and Fig. 2). Dominant patterns for the haploidentical siblings can be summarized as follows: in family I, 23 of 45 (51%) TCC from I-Ms2 recognized aa 11–30, 36 of 72 (50%) TCC from I-Us2 recognized aa 21–40, and 5 of 19 (26%) TCC from I-Us1 recognized aa 31–50; in family II, 8 of 26 (31%) TCC from II-Ms1 recognized aa 71–90 and 7 of 17 TCC from II-Us1 (41%) recognized aa 1–20; and in family III, 10 of 22 (46%) TCC from III-Ms1 recognized aa 41–60 and 6 of 13 (46%) TCC from III-Us2 recognized aa 101–120. Thus, it is evident that no single linear peptide of MOG was immunodominant in peripheral blood T cells derived from HLA-identical individuals.

The pattern of reactivity of TCC against MOG peptides was also highly sensitive to the seeding density (Fig. 3). For individual I-Us2, at 10² PBMC/well the TCC equally recognized aa 11–30 and 21–40; however, TCC specific to aa 11–30 were fewer if seeding of PBMC occurred at 10³ cells/well, and diminished further at 10⁴ PBMC/well. At these higher seeding densities the TCC predominantly recognized aa 21–40; however, at higher seeding densities the TCC predominantly recognized aa 21–40; in contrast, TCC from the affected brother I-Ms2 tended to react against aa 21–40 at all densities.

The epitope specificities of 26 TCC from the three haploidentical siblings in family I, reactive to MOG peptides aa 11–30, 31–40, and 31–50, were more finely mapped in proliferation assays using individual 12-mer MOG peptides overlapping by 1 aa each (Fig. 4). Sixteen TCC from I-Ms2 and I-Us2, specific to aa 11–30, exhibited identical peptide reactivity, delineating a fine specificity to aa 13–24. Seven TCC from I-Us2 and two TCC from I-Us1, reactive to aa 21–40, had a fine specificity to aa 30–40 and aa 23–32, respectively. One TCC from I-Us1, reactive to aa 31–50, had a fine specificity to aa 36–46. Thus, immunodominant responses to some MOG epitopes with identical fine specificity could be identified both within individuals and across some, but not all, HLA-identical subjects.
Not all MOG peptide-reactive TCC were also reactive against the extracellular domain of whole MOG. A total of 82 TCC from family I were tested for their reactivity to rhMOG and rrMOG. Of these, 37 TCC (45%) showed a positive proliferative response to rhMOG and 43 TCC (52%) showed a positive proliferative response to rrMOG. The frequency of peptide-reactive clones that also recognized rhMOG was variable: 12 of 20 TCC (60%) from I-Ms2 and I-Us2, specific to MOG aa 11–30, and 31–50, using synthetic 12-mer MOG peptides overlapping by 1 aa. TCC of the DR/DQ haploidentical siblings from family I, I-Ms2, I-Us1, and I-Us2, were analyzed. Reactive MOG peptides are shaded, and suitable target epitopes are framed. TCC specific to aa 11–30 from I-Ms2 and I-Us2, and TCC specific to aa 21–40 from I-Us2, delineated identical fine specificities to aa 13–24 and 30–40, respectively. Numbers at the left identify individual overlapping peptides.

Cytokine production

None of the MOG peptide-reactive TCC secreted TGF-β above background levels produced by the APC. Besides TGF-β, only IL-6 was produced in low amounts by the APC, and in all analyses IL-6 production of the TCC was corrected against background levels of secretion by APC alone. Cytokine concentrations (measured by ELISA) for TNF-α, IFN-γ, IL-4, IL-6, and IL-10 to T regulatory cell 1 (Tr1) T cells in picograms per milliliter) for TNF-α, IL-4, IL-6, and low levels of IFN-γ and TNF-α. TCC from I-Us1 exhibited a Th0-like pattern, secreting high levels of IFN-γ, IL-4, and IL-10, but low levels of TNF-α and IL-6. TCC from I-Uf had a Th1-like cytokine profile, producing high levels of IL-10, but otherwise low amounts of all other cytokines. Thus, the cytokine production of MOG-specific TCC differed between individuals, and TCC generated from a single individual tended to display a homogeneous cytokine profile. To determine whether the cytokine profiles of MOG peptide-reactive TCC were stable in vitro, the levels of IFN-γ and IL-4 were measured sequentially from 16 TCC from I-Uf and I-Us2. For each TCC, four measurements were obtained over a 10-wk period, beginning with the second and ending with the fifth restimulation. No shift in cytokine profile was observed for any of the clones (data not shown).

Discussion

These studies were undertaken because of emerging data implicating MOG, a CNS-specific myelin protein, as an autoantigen in human MS (15). We found that the frequency of TCC against MOG peptides was remarkably high, up to 1:444 in the healthy sibling I-Uf. Notably, the best cloning efficiencies were observed at the lowest seeding densities, e.g., 10² or 10³ PBMC/well. These data are similar to our analysis of the characteristics of the circulating T cell repertoire to MOG in naive, unimmunized marmosets (46). Taken together, these findings clearly demonstrate that MOG-reactive T cells are highly prevalent in the peripheral T cell compartment. Although no individual in the current study demonstrated reactivity to rMOG in short-term proliferation assays of freshly isolated PBMC, previous studies have demonstrated a high level of proliferation against MOG in some MS patients (17, 18) and a high precursor T cell frequency in peripheral blood and CSF (1:7299 and 1:450, respectively) enumerated by measuring IFN-γ levels.
in response to stimulation with MOG (20, 22). However, in our experience, proliferative responses of whole PBMC to rhMOG or rrMOG are equally prevalent in MS and control individuals (19).

In contrast to MOG, relatively low frequencies of MBP-reactive T cells, generally in the range of $10^{-5}$–$10^{-6}$, have been described by traditional limiting dilution methods in MS patients (31, 32, 68). A similar low frequency has been reported against another quantitatively major myelin protein, proteolipid protein (32). Using different approaches, such as those based upon detection of a known MBP-specific TCR chain or by cloning in the presence of exogenous IL-2, much higher estimates of MBP-reactive T cells, in the range of $10^{-3}$–$10^{-2}$, have been reported (33, 34). The use of synthetic peptides, in contrast to recombinant or native protein, is known to further increase both the number and diversity of generated MBP-reactive TCC (35–37).

In the normal repertoire, the finding of a high frequency of T cells reactive against some myelin Ags suggests that homeostatic mechanisms must be active to prevent autoimmune demyelination in the absence of provoking triggers. It is well established that MBP- and MOG-reactive T cells present in naive animals are potentially encephalitogenic following activation in vitro and adoptive transfer (38–40). The regulatory mechanisms responsible for containment of autoreactive T cells are not fully defined; however, possibilities include anti-idiotypic suppressor cells (41–44) and self-MHC-reactive T cells (28–30). Anti-idiotypic suppressor cells were found to contribute to prevention of spontaneous autoimmunity in double-transgenic mice engineered to express encephalitogenic TCR molecules (44). Self-MHC-reactive T cells proliferated together with Ag-specific T cells following stimulation with tetanus toxin in vitro to display a regulatory (Tr1) cytokine phenotype producing IL-10 and to suppress T cell proliferation and Ig production (28–30). These cells appear to form an important part of the T cell repertoire and have been found at frequencies $1:50$ by single T cell cloning in a healthy human subject (45). Lower estimates of MOG-reactive T cells at higher seeding densities might be explained by the presence of MHC-reactive cells that suppressed the growth of MOG-reactive T cells when cultured at high cell concentrations. The presence of such regulatory T cell populations in the peripheral blood of healthy individuals could provide an effective means to control expansion of MOG-reactive T cells.

Little is known of the fine specificity of the T cell response to MOG in humans or in patients with MS. In this study, all 11 MOG peptides were recognized by the TCC, and different peptides were found to be immunodominant in HLA-identical siblings. Observed
epitope dominance was also critically influenced by the initial seeding density of PBMC. As the MHC and exogenous events are generally considered to be the principal factors that shape the T cell repertoire, the current in vitro observations highlight the additional importance of the immunological microenvironment as a determinant of the repertoire.

Some of the predominant epitopes of MOG detected in this study contain peptides that experimentally have been shown to be encephalitogenic (46–50, 69, 70). These findings underscore the potential importance of TCC specific to aa 11–30 (aa 13–24), which also exhibited a strong Th1-like cytokine profile in the patient I-Ms2.

Notably, a diverse repertoire of epitopes within the extracellular domain of MOG were recognized by TCC, both within individuals and between HLA-identical siblings. In rodents with acute autoimmune disease, the repertoire of pathogenic autoreactive T cells may recognize an extremely limited array of protein epitopes to the immunizing Ag or tissue, and determinant spreading to a wider range of epitopes may develop over time (51–53). In humans, as in other outbred species (46), the response to large protein Ags appears to be far more diverse than in rodents, although dominant epitopes may be identified in association with some HLA haplotypes (54). Even in monozygotic twins, the allospecific T cell repertoire was found to differ between individuals (55, 56). Whereas heterozygosity at MHC loci is one likely contributor to diversity of epitope recognition in outbred individuals, these findings strongly suggest that epigenetic factors represent the predominant influence on the circulating T cell repertoire.

Even if the frequency and epitope specificity of autoreactive T cells are similar between individuals with MS and controls, it is possible that their patterns of cytokine secretion differ. In rodents, the production of TNF-α and lymphotixin correlated with encephalogenicity of MBP-specific TCC (57). High levels of TNF-α have been identified in MS brain lesions (58), and some reports indicate that levels of Th1 cytokines produced by PBMC (59–62), by CFSE (63), or by myelin-reactive TCC or lines (35, 64) have also been associated with disease activity in MS. One analysis of cytokine secretion of 30 MBP-specific TCC revealed elevated IFN-γ:IL-4 ratios in MS patients (65). By contrast, other studies failed to demonstrate clear differences in cytokine profiles of MBP-reactive TCC or T cell lines between patients and controls (66, 71).

We found that many MOG peptide-reactive TCC exhibited cytokine profiles with different admixtures of Th1- and Th2-type cytokines, supporting the concept that a strict separation of T cells in the Th1 and Th2 phenotype may not be applicable to humans (65, 72). The cytokine production of TCC differed between the individuals, but TCC derived from one individual exhibited a rather homogenous cytokine profile. We observed Th2-, Th0-, and Th1-like cytokine patterns in the healthy individuals and a striking Th1-like cytokine profile in the affected sibling I-Ms2. Longitudinal analysis of IFN-γ:IL-4 ratios of individual MOG peptide-reactive TCC during the culture period indicated that the cytokine profiles of the TCC in vitro remained stable. Finally, no correlation between the cytokine patterns and epitope specificities of MOG-reactive TCC could be identified, analogous to previous observations of MBP-specific TCC.

In summary, we provide evidence that MOG peptide-reactive T cells occur with extraordinary high frequency, both in MS patients and in their healthy siblings. Therapy with IFN-β appeared to reduce the number of MOG peptide-reactive T cells that could be generated. We also show that the dominant epitopes recognized by MOG peptide-reactive TCC differ in HLA-identical siblings, suggesting that epigenetic factors, or non-HLA genes, play a major role in shaping the T cell repertoire to this Ag. Although these results are consistent with a possible role for T cell responses directed against MOG in the pathogenesis of MS, no clear disease-specific changes in the T cell frequency or repertoire could be identified. This conclusion is similar to that reached by Lindert et al. (67) in a study of five unrelated MS patients and controls who were not HLA-matched. However, a striking Th1-like cytokine profile was present in one of our MS patients, leaving open the possibility that an enhanced proinflammatory T cell response, directed against MOG, may be present in a subset of MS patients and could correlate with the presence of MOG-mediated tissue damage in situ.

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References


