Myelin-Associated Oligodendrocytic Basic Protein: Identification of an Encephalitogenic Epitope and Association with Multiple Sclerosis

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Myelin-Associated Oligodendrocytic Basic Protein: Identification of an Encephalitogenic Epitope and Association with Multiple Sclerosis

Andreas Holz, Bibiana Bielekova, Roland Martin, and Michael B. A. Oldstone

Myelin-associated oligodendrocytic basic protein (MOBP) is an abundant myelin constituent expressed exclusively by oligodendrocytes, the myelin-forming cells of the CNS. We report that MOBP causes experimental allergic encephalomyelitis and is associated with multiple sclerosis. First, we note that purified recombinant MOBP inoculated into SJL/J mice produces CNS disease. Tests of overlapping peptides spanning the murine MOBP molecule map the encephalitogenic site to amino acids 37–60. MOBP-induced experimental allergic encephalomyelitis shows a severe clinical course and is characterized by a prominent CD4+ T lymphocyte infiltration and a lesser presence of CD8+ T cells and microglia/macrophages around vessels and in the white matter of the CNS. Second, PBL obtained from patients with relapsing/remitting multiple sclerosis mount a proliferative response to human MOBP, especially at amino acids 21–39. This response equals or exceeds the response to myelin basic protein and an influenza virus hemagglutinin peptide, both serving as internal controls. Thus, a novel myelin Ag, MOBP aa 37–60, plays a role in rodent autoimmune CNS disease, and its human MOBP counterpart is associated with the human demyelinating disease multiple sclerosis. The Journal of Immunology, 2000, 164: 1103–1109.

Lymphocytes and their products play a pivotal role in the pathogenesis of multiple sclerosis (MS), the most common demyelinating disease of humans, and in its laboratory model, experimental allergic encephalomyelitis (EAE) (reviewed in Ref. 1). A distinguishing theme for both MS and EAE is that T cells recognize and respond to specific peptides from myelin presented by selected MHC molecules. However, in MS the environmental event(s) responsible for triggering such T cell responses as well as the specific myelin Ags recognized in vivo by these T cells are largely unknown. Thus, it is important to identify the potential disease-causing Ags (peptides) in myelin and use that data to determine whether such myelin peptides mimic the sequence or confirmations of microbial peptides. Further, different myelin Ags (peptides) may be associated with different subsets of humans having MS (2, 3). Hence, the recognition of all the myelin Ags that cause disease is important and may shed light on strategies to suppress Ag-specific immunopathologic T cell responses (4, 5), give leads for identifying and testing whether a specific infectious agent is involved in the triggering of disease (6–9), and assist in identifying different subgroups of patients with disease (2). Currently, the major myelin Ags known are myelin basic protein (MBP) (10–13), proteolipid protein (PLP) (14–18), and myelin oligodendrocyte glycoprotein (19–21), although other neural proteins like MBP or PLP. The most abundant MOBP isoform was the 81 aa constituting MOBP81 protein (23, 25). Biologic analysis of MOBP indicated three major differences from MBP and PLP. First, MOBP was located exclusively in oligodendrocytes of the CNS, whereas MBP and PLP appeared both in the CNS and peripheral nervous systems (23, 24, 26). Second, in rodents, transcription of the Mobp gene occurred 2–3 days later than expression of the MBP or PLP genes (23, 27). Third, MOBP was transcriptionally active at the time of myelin compaction (23), and MOBP protein was noted at the major dense line of myelin (24), suggesting a role for this protein in myelin structure. MOBP was estimated as the third most abundant myelin constituent of the CNS (24).

Here we analyzed the role of MOBP in CNS autoimmune disease. We find that this new myelin constituent causes EAE in susceptible mice and map its encephalitogenic peptide sequence. Furthermore, we demonstrate that MOBP is involved in the pathogenesis of MS, and T cells isolated from MS patients show significantly elevated proliferative responses to human MOBP peptides.

Materials and Methods

Peptides

Peptide libraries covering the entire murine MOBP81 peptide (25) and human MOBP aa 1–70 (24) were synthesized at Chiron Technologies (San Diego, CA; see Table I and Fig. 3). The encephalitogenic PLP aa
139–151 peptide (VSLGKWLGHPDKF) (28) was used as a positive control for EAIE induction. The immunodominant human MBP aa 83–99 peptide (10, 11, 29) and influenza-hemagglutinin (Flu-HA) aa 306–318 peptide (PKYVKQNTLKLAT) (30, 31) served as control Ags for proliferation studies using human T cells.

Induction of EAIE and clinical scoring
EAIE induction was performed according to established protocols (18, 32). Sixty micrograms MOBP fusion protein, 100 μg MOBP peptides, or 150 μg PLP aa 139–151 peptide were used as inducing agents. These Ags were emulsified in CFA containing 5 mg/ml Mycobacterium tuberculosis H37RA (Difco Laboratories, Detroit, MI) and injected s.c. at the base of the tail in female SJL/J mice (The Jackson Laboratory, Bar Harbor, ME). Simultaneously, 150 ng pertussis toxin (List Biological Laboratories, Campbell, CA) was given i.v. and 48 h later. Animals were scored and weighed daily. Clinical scoring was according to the following scale: 0, normal animal, no clinical signs of EA; 1, completely limp tail; 2, impaired righting reflex with completely limp tail; 3, partial hind limb paralysis; 4, complete hind limb paralysis; 5, hind and fore limb paralysis, moribund.

Generation of MOBP-GST fusion protein
The open reading frame encoding rat MOBP81 protein (23) was PCR amplified using the primers 5’-CGGAATTCTGATGAGTCAAAAAGTG-GCC and 5’-CATCATCAGAGTGTTCCAC. The PCR product was EcoRI/XhoI digested and ligated unidirectionally into the EcoRI/XhoI sites of plasmid pGEX-KG (33) (kindly provided by Dr. John Patterson, The Scripps Research Institute, La Jolla, CA). DNA sequencing confirmed the integrity of the resulting GST-MOBP plasmid expressing rat MOBP aa 1–81 in frame with the GST protein. Recombinant fusion protein was made in Escherichia coli strain BL21 after induction with 0.5 mM isopropyl β-D-thiogalactoside (IPTG) and affinity-purified with glutathione beads as described (33). In brief, exponentially growing bacterial cultures were IPTG induced, and after a 2-h incubation period cells were collected by centrifugation. Bacteria were lysed with lysozyme (10 μg/ml) in 25 mM sodium-HEPES buffer, pH 7.5, containing 15 mM isopropyl β-D-thiogalactoside (IPTG) and affinity-purified with glutathione beads as described (33). To characterize MOBP functionally, we engineered a cDNA expressing a fusion protein of the major rodent MOBP (MOBP81) isoform and the GST protein. The fusion gene was expressed in E. coli, and the soluble product was purified by affinity chromatography (Fig. 1). Subsequently, several MOBP-specific bands were observed by using anti-MOBP Ab (Fig. 1A). This result likely indicates an altered migration behavior of the highly basic fusion protein or its partial degradation. However, a unique protease cleavage site within the fusion protein (33) allowed the enzymatic cleavage and removal of MOBP from GST after incubation with thrombin protease (Fig. 1B); the resulting recombinant 12-kDa MOBP was consistent with the full-length MOBP molecule (23), and no MOBP-specific degradation products were detected.

Inoculation of GST-MOBP (60 μg/mouse) induced EAIE in female SJL/J 6- to 8-wk-old mice (Fig. 1C; five of five mice inoculated). Tissues obtained 14 days after immunization contained mononuclear cell infiltrates within the CNS, around blood vessels, and at both subventricular and white matter areas of the parenchyma. Immunohistochemical study revealed that these infiltrates were primarily CD4+ T lymphocytes with a minority of CD8+ T cells. Control animals (four mice per group) inoculated with PBS/CFA or GST/CFA lacked any such mononuclear cell infiltration into the CNS.

MOBP peptides spanning aa 37–60 induce severe clinical EAIE in SJL/J mice
We next sought the peptide sequence(s) of the MOBP responsible for the encephalitogenic activity. Overlapping peptides, each containing 15 aa, were synthesized that covered the entire rodent MOBP molecule (25). These peptides were combined into six separate pools (Table I); individual pools were emulsified in CFA and the separate emulsions injected into SJL/J mice (seven to eight mice per group; two separate experiments). Of these six pools, only pool 4, consisting of MOBP aa 37–60, induced acute EAIE (Table I). The pool 4 inoculum produced characteristic clinical signs (Table I) of weight loss, limp tail, paresis, ataxia, and paraplegia. The mean disease onset was 10 days after the initial inoculation, and the mean maximum severity of disease was 3.5 on a scale of 5 (Table I). The incidence rate of EAIE induced by the MOBP peptides was 75% (six of eight mice).
Histologic and immunohistochemical evaluation of MOBP aa 37–60 induced EAE

Analysis of brains and spinal cords revealed widespread infiltration of mononuclear cells throughout the CNS (Fig. 2), but no infiltration in the peripheral nervous system or other peripheral tissues. In the CNS, mononuclear cells were noted in perivenular, periventricular, and subpial areas (Fig. 2, A and B), as well as in the CNS parenchyma, primarily in white matter. CNS inflammation was observed in all animals inoculated with the peptide pool spanning MOBP aa 37–60. The amount of infiltration observed in animals correlated with the severity of clinical disease. A myelin-specific stain (Luxol fast blue) revealed moderate myelin breakdown (data not shown). The degree of demyelination in MOBP-inoculated animals was similar to that observed in mice in which EAE was induced with PLP aa 139–151.

Immunohistochemical analysis showed that the infiltrates contained mostly CD4⁺ T lymphocytes (Fig. 2, C and F) accompanied by F480⁺ macrophages/microglia (Fig. 2D), fewer CD8⁺ T lymphocytes (Fig. 2E), and very few B lymphocytes (not shown). The infiltration profile of MOBP-induced EAE was indistinguishable from that produced by inoculation with a PLP encephalitogenic peptide used as a control. None of the other five peptide pools caused EAE when similarly inoculated into SJL/J female mice, nor did PBS/CFA produce disease (seven to eight mice per group; experiment confirmed on two occasions).

Four overlapping peptides of the encephalitogenic site were individually administered to SJL/J mice (five mice per group). All four peptides initiated infiltration of mononuclear cells, primarily CD4⁺, in the CNS. A myelin-specific stain (Luxol fast blue) revealed moderate myelin breakdown (data not shown). The degree of demyelination in MOBP-inoculated animals was similar to that observed in mice in which EAE was induced with PLP aa 139–151.

T cells from MS patients respond to human MOBP peptides by specific proliferation

Lastly, we turned our attention to whether MOBP is a potential target Ag for lymphocytes from MS patients. Human MOBP (24)
is 97% homologous with the common amino-terminal part of murine MOBP. Sequence analysis identified the presence of putative peptide-binding motifs for the MS-associated HLA-DR alleles DRB5*0101 (DR2a), DRB1*0401, and also a partial motif for DRB1*1501 (also referred to as DR2b) in human MOBP aa 21–39 (data not shown) that mapped near the murine MOBP encephalitogenic region. This observation of a shared encephalitogenic region in SJL/J mice and DR2-positive MS patients had also been found earlier (36–38). Accordingly, we detected proliferation against a peptide from human MOBP aa 37-60 in the healthy donors and patients (Table I).

### Table I. MOBP aa 37-60 induces EAE in SJL/J mice

<table>
<thead>
<tr>
<th>Pool Peptide</th>
<th>Mouse MOBP Position</th>
<th>No. Mice with EAE/Total Inoculated</th>
<th>Maximum Score</th>
<th>Infiltration Composition of Infiltrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 A3-1 MSQKMAKEGPRLSK</td>
<td>1–15</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>1 A4-1 KMAKEGPRLSKNNQKF</td>
<td>4–18</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>1 A5-1 KEGPRLSKNNQKFSEH</td>
<td>7–21</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>1 A6-1 PRLSKNNQKFSEHPSI</td>
<td>10–24</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>2 A7-1 SKNQKFSEHFSIHCC</td>
<td>13–27</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>2 A8-1 QKFSFHSIHCCPPF</td>
<td>16–30</td>
<td>0/8</td>
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<td>No</td>
</tr>
<tr>
<td>2 A9-1 SEHFSIHCCPPFTFL</td>
<td>19–33</td>
<td>0/8</td>
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<td>No</td>
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<tr>
<td>2 A10-1 FSIHCCPPFTFLNSK</td>
<td>22–36</td>
<td>0/8</td>
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</tr>
<tr>
<td>3 A11-1 HCCPPFTFLNSKREI</td>
<td>25–39</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>3 A12-1 PPTFLNSKREIVDR</td>
<td>28–42</td>
<td>0/8</td>
<td>0</td>
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<tr>
<td>3 A12-2 TFLNSKREIVDRKY</td>
<td>31–45</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>3 A12-2 NSKREIVDRKY</td>
<td>34–48</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>4 A3-2 REIVDRKYSICKSGC</td>
<td>37–51</td>
<td>6/8</td>
<td>3.5</td>
<td>++++ CD4⁺, CD8⁺ T cells, B cells, macrophages/activated microglia</td>
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<tr>
<td>4 A4-2 VDRKYSICKSGCFYQ</td>
<td>40–54</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>4 A5-2 KYICKSGCFYQKKE</td>
<td>43–57</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>4 A6-2 ICKSGCFYQKKEEDW</td>
<td>46–60</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>5 A7-2 SGCFYQKKEEDWICC</td>
<td>49–63</td>
<td>0/8</td>
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<td>No</td>
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<tr>
<td>5 A8-2 FYQKKEEDWICCACQ</td>
<td>52–66</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>5 A9-2 KKEEDWICCACQKTR</td>
<td>55–69</td>
<td>0/8</td>
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<td>No</td>
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<tr>
<td>5 A10-2 EDWICCACQKTRRLR</td>
<td>58–72</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
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<tr>
<td>6 A11-2 ICCACQRTLRRRSRR</td>
<td>61–75</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>6 A12-2 ACQRTLRRRSRRSRT</td>
<td>64–78</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>6 A13-1 KTRLRRRSRRSTPRKK</td>
<td>67–81</td>
<td>0/8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>PLP139–151</td>
<td>8/8</td>
<td>4.5</td>
<td>++++ CD4⁺, CD8⁺ T cells, B cells, macrophages/active microglia</td>
<td></td>
</tr>
<tr>
<td>PBS/CFA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>No</td>
</tr>
</tbody>
</table>

* A panel of overlapping peptides from mouse MOBP81 protein was synthesized and combined into six pools as indicated. Animals were inoculated with 100 μg of each peptide per pool. Only peptides in pool 4 induced clinical and histological EAE. The PBS/CFA control and the remaining MOBP peptide-injected mice failed to develop either clinical or histological evidence of disease. The encephalitogenic peptide PLP aa 139–151, used as a positive control, induced EAE. Similar results were obtained in two other experiments with four mice per group.

**Discussion**

The main findings of this report are, first, the mapping of a MOBP peptide sequence encephalitogenic in SJL/J mice, and, second, the association of human MOBP with MS. The link of MOBP with CNS autoimmune disease by these two lines of evidence might lead to further the understanding of the multifactorial disease MS, the cause of which is unknown.

The knowledge of association of MOBP (or any other autoantigen) with CNS autoimmune disease may result in uncovering MS triggering factors/agents because self-Ags may act as molecular mimics for a specific and critical pathogen involved in disease initiation (7). Studies of monozygotic twins, of case clusters, and of MHC types document that individuals who develop MS must have an appropriate immunogenetic background influenced by several MHC genes likely involved in presenting a limited number of “self” myelin Ags (39–44). However, the discordance among monozygotic twins (39, 45), the epidemiologic studies of susceptibility or resistance to MS in individuals migrating to areas with high or low incidence of disease before their 15th year of age (46), the association of MS with viral infections (47, 48), as well as outbreaks of MS in the Faroe Islands (49) indicate that environmental factors, presumably infections, are also important for disease pathogenesis. Hence, it is important to know about all molecules involved in MS. Our data links MOBP with MS. Therefore,
it will be of interest to identify sequences from microbial agents that have linear or conformational fits (6–9) with human MOBP and testing their abilities to elicit cross-reactive immune responses.

The disease patterns of MS are variable, and the pathology of multiple sclerosis is highly diverse (50). Hence, several immunopathogenetic mechanisms may contribute to disease onset and its progression (2). The fact that autoreactivity against MBP, PLP, myelin oligodendrocyte glycoprotein, and, as demonstrated here, MOBP, is observed during the course of MS indicates by itself the complexity of immunopathological mechanisms involved in CNS...
FIGURE 3. T lymphocytes from several MS patients show a vigorous and specific proliferative response to MOBP. MOBP-, MBP-, and Flu-HA- peptide-specific T cell proliferation was analyzed in MS patients (n = 8) and healthy donors (n = 7) using IL-7-modified primary proliferation. This assay expands Ag-specific memory and in vivo-activated T cells (34). Briefly, a total of 60 × 10⁵ PBMC from each individual was tested per Ag (1 μM). PBMC were stimulated for 7 days in the presence of peptide and IL-7 (10 ng/ml). After this period, cells were pulsed with [³H]thymidine and cpm were measured by scintillation counting. A. Raw data for a representative MS patient. The lines for the cut-off and the background are indicated. The cut-off line represents two stringent criteria: first, a stimulation index larger than 2 and, second, a cpm (cpm) larger than background level plus 3 SD. Note that the cut-off for Ag-specific proliferation was high above background values. Between zero and five positive wells were detected for each Ag, which is consistent with precursor frequency estimates for these autoreactive cells in the range of one to five per million. These data are characteristic of those observed with the other six positively responding MS patients studied. B. Proliferation data for lymphocytes obtained from MS patients and healthy donors and normalized for proliferation to Flu-HA (internal control). The differences of MOBP-specific proliferation between MS patients and healthy donors were statistically significant (χ² calculated based on the number of positive wells per each Ag, p = 0.0008). Furthermore, although the proliferative response to MOBP peptides was comparable to or higher than those to Flu-HA in the MS patients, the responses to Flu-HA were greater in the control population (p < 0.01; Mann-Whitney rank sum test).

autoimmune disease. To fully understand them is ultimately required for development of effective treatments. Therefore, it will be of great interest to evaluate and compare the immune responses to MOBP and other MS-associated autoantigens in the heterogeneous disease phenotypes in both the animal model and MS (2, 22, 50) and the time when T lymphocytes recognize these molecules during the course of MS. Current studies evaluate such responses prospectively in MS patients.

We report that EAE can be induced with recombinant rodent MOBP81 protein and, by systematically assaying overlapping peptides spanning the entire murine MOBP81 protein, we mapped a MOBP encephalitogenic epitope to aa 37–60. The clinical, histological, and immunohistochemical evaluation of MOBP-induced EAE revealed no significant differences when compared with a PLP encephalitogenic peptide: clinical signs were acute, and disease included severe hind leg paralysis, CD4⁺ T lymphocytes and macrophages dominated the mononuclear cell infiltrate, and a focal myelin breakdown was observed. Recently, it was shown that another isoform of rat MOBP is able to cause clinical and histological signs of EAE in SJL/J mice (51). Rat MOBP170 (e.g., rOPRP1; Ref. 24) was expressed and purified in a baculovirus expression system, and, similar to our findings (Table I and Fig. 2), EAE was observed when recombinant MOBP170 was administered to SJL/J mice (51). We used recombinant rat MOBP81 protein for EAE induction in SJL/J mice (Fig. 1). Because the rodent MOBP81 and MOBP170 protein isoforms are identical in their first amino-terminal 69 aa (23–25), it can be concluded that at least one encephalitogenic domain must be contained in MOBP aa 1–69. In agreement with this consideration, we find that an encephalitogenic domain is located in the MOBP peptide sequence aa 37–60 (Table I and Fig. 2). In our and in Maata et al. (51) reports, recombinant rat protein was used for EAE induction in mouse. Rat and mouse MOBP81 are highly conserved and show only a single conserved amino acid change at position 5 (Val → Met) (25). This amino acid substitution is located outside the identified encephalitogenic site in SJL/J mice (MOBP aa 37–60). Accordingly, the synthesized mouse peptides at positions aa 37–60 and the bacterially expressed recombinant rat protein both caused EAE in SJL/J mice.

MS is an autoimmune disease affecting the CNS. Therefore, potential autoantigens associated with MS must reside within brain and spinal cord. It is important to note that MOBP is specifically expressed by oligodendrocytes (23). Expression of MOBP outside the CNS has not been observed (23, 24). In contrast, the MS-associated autoantigens MBP and PLP both are expressed by oligodendrocytes, but also in the peripheral nervous system (52–54) as well as by cells in the thymus (55). In agreement with the CNS-specific expression of MOBP, we do not detect mononuclear cell infiltration in peripheral organs or the peripheral nervous system after inoculation of SJL/J mice with the encephalitogenic MOBP peptide pool. The strict target-organ-specific expression of MOBP makes this protein an attractive autoantigen for MS, in particular after the demonstration of an association of MOBP with CNS autoimmune disease.

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References


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