The Myelin-Associated Oligodendrocytic Basic Protein Region MOBP15–36 Encompasses the Immunodominant Major Encephalitogenic Epitope(s) for SJL/J Mice and Predicted Epitope(s) for Multiple Sclerosis-Associated HLA-DRB1*1501

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The Myelin-Associated Oligodendrocytic Basic Protein Region MOBP15–36 Encompasses the Immunodominant Major Encephalitogenic Epitope(s) for SJL/J Mice and Predicted Epitope(s) for Multiple Sclerosis-Associated HLA-DRB1*1501

Nicole Kerlero de Rosbo,* Joel F. Kaye,* Miriam Eisenstein, † Itzhack Mendel, 2* Romana Hoeftberger,‡ Hans Lassmann,‡ Roni Milo,§ and Avraham Ben-Nun3*

Autoimmune response to the myelin-associated oligodendrocytic basic protein (MOBP), a CNS-specific myelin constituent, was recently suggested to play a role in the pathogenesis of multiple sclerosis (MS). The pathogenic autoimmune response to MOBP and the associated pathology in the CNS have not yet been fully investigated. In this study, we have characterized the clinical manifestations, pathology, T cell epitope-specificity, and TCRs associated with experimental autoimmune encephalomyelitis (EAE) induced in SJL/J mice with recombinant mouse MOBP (long isoform, 170 aa). Analysis of encephalitogenic MOBP-reactive T cells for reactivity to overlapping MOBP peptides defined MOBP15–36 as their major immunodominant epitope. Accordingly, MOBP15–36 was demonstrated to be the major encephalitogenic MOBP epitope for SJL/J mice, inducing severe/chronic clinical EAE associated with intense perivascular and parenchymal infiltrations, widespread demyelination, axonal loss, and remarkable optic neuritis. Molecular modeling of the interaction of I-As with MOBP15–36, together with analysis of the MOBP15–36-specific T cell response to truncated peptides, suggests MOBP20–28 as the core sequence for I-A*-restricted recognition of the encephalitogenic region MOBP15–36. Although highly focused in their epitope specificity, the encephalitogenic MOBP-reactive T cells displayed a widespread usage of TCR Vβ genes. These results would therefore favor epitope-directed, rather than TCR-targeted, approaches to therapy of MOBP-associated pathogenic autoimmunity. Localization by molecular modeling of a potential HLA-DRB1*1501-associated MOBP epitope within the encephalitogenic MOBP15–36 sequence suggests the potential relevance of T cell reactivity against MOBP15–36 to MS. The reactivity to MOBP15–36 detected in MS shown here and in another study further emphasizes the potential significance of this epitope for MS. * The Journal of Immunology, 2004, 173: 1426–1435.

E xperimental autoimmune encephalomyelitis (EAE)1, a well accepted model for multiple sclerosis (MS) is a T cell-mediated autoimmune disease of the CNS induced in experimental animals by active immunization with CNS homogenate, myelin or myelin proteins and peptides thereof, or by passive transfer of activated T cells raised to these encephalitogens. Myelin proteins investigated in the context of MS can be consid-

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2 Current address: Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892-1892.
3 Address correspondence and reprint requests to Dr. Avraham Ben-Nun, Department of Immunology, Weizmann Institute of Science, Rehovot, Israel.
4 Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; MBP, myelin basic protein; PLP, proteolipid protein; MOG, myelin oligodendrocyte glycoprotein; MOBP, myelin-associated oligodendrocytic basic protein; OSP, oligodendrocyte-specific protein; mMOBP, mouse MOBP; hMOBP, human MOBP; IPTG, isopropyl-β-D-thiogalactopyranoside; Mt, Mycobacterium tuberculosis; LNC, lymph node cell; PPD, purified protein derivative; βAPP, β-amyloid precursor protein; pmMOBP, mouse MOBP peptide; phMOBP, human MOBP peptide; SI, stimulation index.
aa 35–55 (10, 11), and pMOG35–55 is also strongly encephalitogenic for H-2a mice (12) and Lewis rats (13). Other encephalitogenic epitopes of MOG have been demonstrated in other mouse strains: pMOG92–106 induces disease in SJL/J mice and pMOGs 1–22, 43–57 and 134–148 induce disease in Biozzi AB/H mice (14). In addition, Abs against MOG but not MBP or PLP, have been shown to have strong demyelinating activity. In contrast, MOBP-induced EAE has been poorly characterized.

MOBP, a CNS myelin-specific protein thought to play a role in stabilizing the myelin sheath (15–17), was recently shown to be encephalitogenic. Thus, active immunization with recombinant preparations of both the long (rOPRP1) and the short (MOBP81) MOBP isotypes could induce EAE in BALB/c (H-2b) and/or SJL/J (H-2a) mice (1). EAE characterized by an acute, albeit delayed, onset could be induced in C3H.SW (H-2a) mice with pMOBP65–85 (2), which is likely to be a cryptic epitope (our unpublished data). To further investigate the encephalitogenic potential of MOBP, Holz et al. (3) immunized SJL/J mice with pools of overlapping MOBP peptides, one of which, a pool encompassing aa 37–60 of the MOBP81 isofrom, induced EAE. In the study reported here, we determined the epitope specificity and TCR expression of encephalitogenic T cells raised to a recombinant preparation of the long isofrom of mouse MOBP (rmMOBP) in SJL/J mice, defined the major encephalitogenic epitope, MOBP15–36, documented the associated pathology in the CNS, and suggested the potential relevance to MS of autoreactivity to MOBP15–36.

Materials and Methods

Mice

Female SJL/J (H-2a) were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were 2- to 3-mo-old when used in the experiments.

MOBP peptides

Mouse (pmMOBP) and human (phMOBP) MOBP peptides were synthesized in the laboratory of Prof. M. Fridkin (Department of Organic Chemistry, Weizmann Institute), using the F-moc technique with an automated peptide synthesizer (AMS422; Abimed Analys-Technik, Langenfeld, Germany). All synthesized peptides used migrated on HPLC (Spectra Series P200, RP18 Column; Merck, West Point, PA) as a major peak representing over 85% of the preparation. The peptides used in this study are shown in Table I.

Molecular modeling of I-A*

A model structure of the peptide-binding domain of I-A* was constructed on the basis of the closely related x-ray structures of I-A* (Ref. 18; Protein Data Bank [PDB] code 1iak). The y- and y-chains in the peptide binding domain of I-A* show 92% sequence identity to the corresponding chains of I-A* (alignment with FastA using the GCG Wisconsin Package; Accelrys, San Diego, CA), without any inserts or deletions. The initial model was built using the Homology module of Insight II (Accelrys). A few bumps between side chains were corrected manually by choosing different conformers and the model was energy minimized, while constraining the Ca atoms to their initial positions (module Discover in the Accelrys package).

Recombinant mMOBP (rmMOBP)

A DNA fragment coding for the long mouse MOBP corresponding to rOPRP1 in the rat (16) was amplified by PCR using murine (PL/J) spinal cord cDNA as a template and the 5′ primer, 5′-CATGGTATGGCTAGCA GTCAAAAAATGGCCCAAAGG-3′ containing an added Nhel restriction site, and the 3′ reverse primer 5′-CTCATGGATCCTACGAACT AGGAGCTCTGGT containing an added BamHI restriction site. The gel-purified PCR product was digested with Nhel/BamHI and subcloned into Nhel/BamHI sites of the pRSET expression vector (Invitrogen Life Technologies, San Diego, CA). The nucleotide sequence of the PCR product within the construct, which was obtained by direct sequencing using primers derived from pRSET (forward primer, 5′-ATGGGCGGTTTCTCTCATC-3′, and reverse primer, 5′-TAGCAGCGGATCAGGCT-3′) and an Applied Biosystems 373A DNA sequencer (Foster City, CA), confirmed an open reading frame coding for the long (aa 1–170) MOBP isofrom (GenBank accession number AF120475), preceded by (Met)-Arg-Val-Ser-(His)6-Ala-Ser.

Expression and detection of rmMOBP

Expression of rmMOBP was induced in the BL21-D3 bacterial host using isopropyl-β-D-thiogalactopyranoside (IPTG; cat. no. R0392; MBI Fermentas, Vilnius, Lithuania) and detected by Western blotting using rabbit anti-rami serum to prMOBP 88 (1). Recombinant proteins were separated on 15% SDS-PAGE and electrotransferred to a nitrocellulose membrane (Micron Separations, Westboro, MA). The filter was blocked by incubation overnight with 10% milk in PBS-Tween (0.05%), incubated with anti-prMOBP 88–103 (1:20,000), followed by HRP-conjugated goat-anti-rabbit IgG. The proteins were detected using ECL, using an ECL analysis system (cat. no. RPN 2108; Amersham Biosciences, Little Chalfont, U.K.), according to the manufacturer’s protocol.

Purification of rmMOBP

The bacterial pellets from two 12-L fermentor cultures were pooled, solubilized in lysis buffer (6 M guanidine-HCl, 0.5 M NaCl, 100 mM NaH2PO4·H2O, 10 mM Tris, pH 8.0) and sonicated (UltraSonic Processor; Heat Systems, Westboro, MA). The recombinant protein was separated on 15% SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane (Micron Separations, Westboro, MA). The filter was blocked by incubation overnight with 10% milk in PBS-Tween (0.05%), incubated with anti-prMOBP 88–103 Ab (1:20,000), followed by HRP-conjugated goat-anti-rabbit IgG. Reactive bands were detected by ECL, using an ECL analysis system (cat. no. RPN 2108; Amersham Biosciences, Little Chalfont, U.K.), according to the manufacturer’s protocol.

Induction of EAE with rmMOBP and MOBP15–36 peptide

SJL/J mice were injected s.c. at two sites in the tail base with 100 μl of emulsion composed of 80 μg of rmMOBP or 200 μg of pMOBP15–36 emulsified in CFA containing 400 μg of Mycobacterium tuberculosis (Mt) H37Ra (cat. no. 3114-25; Difco Laboratories, Detroit, MI). Mice received 300 ng of pertussis toxin (cat. no. P-9452; Sigma-Aldrich, St. Louis, MO) in 500μl of PBS in the tail vein, immediately and 48 h after the immunization. An identical booster immunization was given at two sites on the

| Table 1. Sequences* of mouse (pm) and human (ph) MOBP peptides used in the study |
|-----------------------------|-----------------------------|
| **Peptide** | **Sequence** |
| pmMOBP1–22 | SQKVAKEGPRSLSNKNQKFSEHFS |
| pmMOBP15–36 | QKFSFHEHSHBCCPFTTFLNSKR |
| pmMOBP30–50 | TFLNSKREIVDRKYSICKSGC |
| pmMOBP37–60 | EIVDRKYSICKSGCFYQKKEEDWI |
| pmMOBP55–77 | KEEDWCAACQTSRKRTSPQKP |
| pmMOBP63–89 | ACQTSRKRATSPKHPQAPASSPVRV |
| pmMOBP65–85 | QKTSRKTSPKQHPQAPASSPVRV |
| pmMOBP68–84 | SRRATSPQKHPQAPASSPVRV |
| pmMOBP73–89 | SPQKHPQAPASSPVRV |
| pmMOBP88–103 | VRAPPAKPSPPRPA |
| pmMOBP125–138 | RPERVPRPAPKAQK |
| phMOBP15–36 | QKSYEHSIIHCPTFPTFLNSK |
| phMOBP27–49 | PFTFLNSKKEIDRKYSCICKSG |
| phMOBP48–71 | SGCYQKKEIDWICCACQKTECSSR |
| phMOBP138–162 | PRPVRRPPAAKORPKPQSKQPRSS |
| phMOBP149–171 | QRPQKSKQPQPSPLRGGPAS |
| phMOBP158–181 | QPRRSSLRPGASRGGSPVKAS |

*GenBank accession numbers AF120475 and D28114 for the mouse and human sequences, respectively.

**Corresponds to the rat sequence whereby the underlined amino acids are different in the mouse sequence (LM in the mouse sequence).

* Amino acid differences in the human MOBP sequence are indicated in bold.

** Amino acids underlined in phMOBP48–71 are not present in the rat MOBP sequence.
flank 1 wk later. Following the encephalitogenic challenge, mice were observed daily and clinical manifestations of EAE were scored on a scale of 0–6; 0, no clinical signs; 1, loss of tail tonicity; 2, flaccid tail; 3, hind leg paralysis; 4, hind leg paralysis with hind body paresis; 5, hind and fore leg paralysis; 6, death.

**T cell lines and T cell proliferative responses**

SJL/J mice were injected s.c. in the foot pads with rmMOBP (100 µg) or pMOBP15–36 (150 µg) emulsified in CFA containing 200 µg of Mt. The primary proliferative response was analyzed by culturing the isolated polyclonal lymph node cells (LNC) (5 × 10^5) in microtiter wells as previously described (10), in the presence or absence of rmMOBP and overlapping MOBP peptides (0.5 and 2 µg/well). Purified protein derivative (PPD) of Mt (2.0 µg/well) was added as a positive control of proliferation. T cell lines specific for rmMOBP or pMOBP15–36 were selected in vitro as described previously (20). All T cell lines were maintained in vitro in medium containing IL-2 with alternate stimulation with the Ag, every 10–14 days as previously described (20). The analysis of the proliferative response of line T cells (1.5 × 10^6) was performed as above in the same way, in the presence of irradiated (2500 rad) syngeneic spleen cells (5 × 10^6/well) as previously described (10).

The cultures were incubated for 72 h (LNC) or 48 h (line T cells) at 37°C in humidified air containing 7.5% CO2. 

[3H]Thymidine incorporation (mean cpm of triplicate cultures) and SEM of triplicate cultures.

**Analysis of reactivity to human MOBP peptides by PBLs from MS patients and controls**

Venous heparinized blood samples were obtained from 15 healthy individuals and from 22 patients (MS Clinic; Assaf Harofeh Hospital, Zerifin, Israel) with a definite MS diagnosis who agreed upon request to be tested and were not under steroid treatment or other medication known to affect the immune system, for at least 2 mo before the time of testing. Proliferative response by PBLs separated from whole heparinized blood by Ficoll-Hypaque density gradient was assayed exactly as described previously (21, 22). Briefly, PBLs (2 × 10^7/200 µl) were cultured (37°C, 7.5% CO2) in flat-bottom 96-well plates for 6 days, in the presence or absence of phMOBP (0.5 µg and 2 µg). Cultures were pulsed with [3H]Thymidine (1 µCi/well) for the last 18 h of incubation, harvested, and counted using a Matrix 96 Direct Beta Counter (Packard Instrument, Meriden, CT). The proliferative response is measured as the [1H]thymidine incorporation expressed as mean cpm of triplicate cultures.

**FIGURE 1.** Immunogenicity of rmMOBP. A, Expression and purification of rmMOBP analyzed by SDS-PAGE and Western blotting. Lane 1, Molecular mass markers (kDa). Lanes 2 and 3, Coomassie blue-stained SDS-gel of bacterial lysate before and after IPTG induction (15 µl, equivalent to 150 µl of bacterial culture). Lanes 4 and 5, Western blot of bacterial lysate before and after IPTG induction; rabbit anti-prMOBP88–103 antiserum was used at a dilution of 1/20,000, as indicated in Materials and Methods. Lanes 6 and 7, Coomassie blue-stained SDS-gel and Western blot of purified rmMOBP (10 µg). B, Proliferative response to rmMOBP by primed LNC and short-term T cell lines. LNC isolated from SJL/J mice immunized with rmMOBP 9 days previously and derived short-term T cell lines (three cycles of stimulation in the presence of rmMOBP) were tested for their proliferative response to rmMOBP (0.5 µg, and 2.0 µg/well). PPD (2.0 µg/well) of Mt was tested as a specificity control of T cell proliferation. Each histogram represents the mean cpm × 10^3 ± SEM of triplicate cultures.

**Analysis of TCR Vβ gene expression by rmMOBP and phMOBP 15–36 reactive line T cells**

TCR Vβ gene expression of line T cells was analyzed by flow cytometry using available monoclonal anti-Vβ TCR Abs exactly as described previously (10). To obtain data on the potential expression of Vβ proteins for which Abs were not available to us, analysis of TCR Vβ gene usage by encephalitogenic line T cells was also conducted by PCR amplification of expressed TCR genes as described previously (11).
Results

Immunogenicity and encephalitogenic activity of rmMOBP in SJL/J mice

The expression in *Escherichia coli* of recombinant mouse MOBP (rmMOBP) using the pRSET bacterial expression vector could only be detected by Western blotting (Fig. 1A, lanes 4 and 5). Similar low levels have been reported for baculovirus expression in insect cells of the long rat rOPRP1 MOBP isoform (1), as well as for expression in *E. coli* of the short mouse MOBP81 isoform as a fusion protein with GST (3).

Coomassie blue-stained SDS-gel analysis of rmMOBP purified from the bacterial lysate showed several bands including a major band of the size expected for the His-tagged rmMOBP (Fig. 1A, lane 6). Western blotting with anti-prMOBP88–103 Ab (Fig. 1A, lane 7) confirmed that the major band corresponds to rmMOBP. The other, lower bands detected by Western blotting are likely to represent products of partial degradation of the protein within the bacterial host and/or of incomplete translation (Fig. 1A, lane 5), and/or products of degradation upon purification of rmMOBP (Fig. 1A, lanes 6 and 7). As the Ab used for detection of MOBP was raised to pMOBP88–103, it is possible that some of the bands which are seen in Coomassie blue staining and are not stained by this Ab also represent degradation products.

The immunogenicity and encephalitogenic potential of the purified rmMOBP preparation was assayed in SJL/J mice. Analysis of the recall proliferative response by draining lymph node cells isolated from rmMOBP-primed mice indicated that rmMOBP could elicit a strong T cell response in these mice (Fig. 1B).

FIGURE 2. Clinical course of rmMOBP-induced EAE. A, Active induction of EAE. SJL/J mice were injected for disease development with rmMOBP/CFA. Four of the five mice immunized developed EAE; each line represents the clinical course of disease in a single mouse. B, Passive EAE transferred by rmMOBP-specific line T cells (2 × 10^6 T cells/mouse). The clinical severity scored daily is shown for each of the two recipient mice. X indicates death.

FIGURE 3. The reactivity of rmMOBP-specific line T cells is directed primarily against a major immunodominant epitope located within MOBP15–36. rmMOBP-specific line T cells were analyzed for their proliferative response to rmMOBP (2.0 μg/well) and overlapping MOBP peptides (2.0 μg/well) spanning most of the sequence of rmMOBP. Each histogram represents the mean SI (mean cpm in the presence of Ag over mean cpm in the absence of Ag (mean background cpm ± SD = 1373 ± 87 for line I and 830 ± 54 for line II; SD ≤ 10%) of triplicate cultures. For each line, the data presented are representative of three separate experiments.
rmMOBP-reactive T cells were specific as indicated by their reactivity to synthetic MOBP peptides (see below). Active immunization of SJL/J mice with rmMOBP in CFA induced severe EAE in four of the five mice injected, starting 11 days after encephalitogenic challenge (Fig. 2A); immunization with a previous batch of rmMOBP resulted in disease in all three mice injected, albeit with a delayed onset (not shown). The clinical expression of the disease followed the classical caudo-rostral ascending paralysis typical of EAE induced in SJL/J mice with other encephalitogenic proteins.

CD4+ rmMOBP-specific line T cells are encephalitogenic in SJL/J mice

Upon the third round of in vitro selection with rmMOBP, the rmMOBP-specific T cells (Fig. 1B) were tested for their ability to passively transfer EAE in irradiated syngeneic recipients. As can be seen in Fig. 2B, the T cells were pathogenic causing in recipients severe EAE of abrupt onset (8 days after T cell transfer) from which they succumbed within 10 days after disease onset. FACS analysis of the rmMOBP-specific line T cells revealed their phenotype to be CD4<sup>+</sup>/CD8<sup>-</sup>/αβTCR<sup>+</sup> (data not shown).

Encephalitogenicity of rmMOBP in SJL/J mice is associated with an immunodominant T cell response against the encephalitogenic region MOBP15–36

The epitope specificity of the encephalitogenic rmMOBP-specific line T cells was analyzed using synthetic overlapping murine (pmMOBP) and human (phMOBP) MOBP peptides spanning most of the rmMOBP sequence (Table I). The rmMOBP-reactive T cells (line I) responded strongly to both the mouse and human peptides corresponding to aa 15–36, but not to any of the other peptides tested (Fig. 3), indicating that the reactivity of the encephalitogenic rmMOBP-specific line T cells is directed primarily to an immunodominant epitope encompassed within amino acid residues 15–36. Another short-term rmMOBP-reactive T cell line (line II) was derived from SJL/J mice immunized with another preparation of rmMOBP; the rmMOBP-reactive T cell line II also showed a single immunodominant reactivity against MOBP15–36 (Fig. 3). Accordingly, SJL/J mice were immunized for disease development with pmMOBP15–36 or phMOBP15–36. Both mouse and human peptides induced a reproducible chronic EAE in 90% of immunized mice (n = 10; Table II). Upon onset, 10–12 days after the encephalitogenic challenge (Table II), neurological impairment followed the caudo-rostral ascending paralysis typical of EAE in SJL/J mice (not shown). As both rmMOBP-reactive T cell lines responded to the same extent to pmMOBP15–36 and phMOBP15–36, which differ only by two amino acids at positions 17 and 36 (Table I), these two positions are unlikely to be of importance in recognition of the immunodominant encephalitogenic epitope of rmMOBP.

Histopathology of MOBP-associated EAE

Fig. 4 documents pathological manifestations typical of EAE induced in mice by MOPB15–36, encompassing the major encephalitogenic epitope of MOBP for SJL/J mice. Histopathological examination of brain and spinal cord from mice, 33 days after encephalitogenic challenge, revealed intense inflammation (Fig. 4A). The infiltrate consisted of a high number of macrophages (Fig. 4E), T lymphocytes (Fig. 4F), and some eosinophilic granulocytes (Fig. 4Aa). Confluent perivascular and subpial demyelination was found mainly in spinal cord (Fig. 4B), whereas in the brain, demyelination was more prominent at the central portions of the trigeminal roots (Fig. 4, H and Hh). The demyelination in the optic nerves (Fig. 4I) was most conspicuous. No complement deposition was found in sections stained for complement C9 (Fig. 4D), suggesting a minimal pathogenic role for Abs in pmMOBP15–36-induced EAE. In areas of inflammation and demyelination, βAPP-positive axonal spheroids were abundant (Fig. 4, C, Cc, and G),

Table II. Active induction<sup>a</sup> of EAE with pmMOBP15–36 in SJL/J mice

<table>
<thead>
<tr>
<th>Encephalitogen</th>
<th>Disease Incidence</th>
<th>Mean Day of Onset</th>
<th>Mean Maximal Clinical Score</th>
<th>Histology&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmMOBP15–36</td>
<td>4/5</td>
<td>12.7 ± 0.3</td>
<td>2.10 ± 1.07</td>
<td>+++</td>
</tr>
<tr>
<td>pmMOBP15–36</td>
<td>5/5</td>
<td>12.0 ± 2.2</td>
<td>3.10 ± 0.97</td>
<td>+++</td>
</tr>
<tr>
<td>pmMOBP357–60f</td>
<td>0/10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> As described in Materials and Methods.

<sup>b</sup> Edema and perivascular inflammation.

<sup>c</sup> Spans the pool of peptides reported to induce EAE in SJL/J mice by Holz et al. (3).
indicating that MOBP-related pathogenic autoimmunity can be associated with substantial axonal injury and axonal loss.

**Modeling of MOBP/I-A' interactions: prediction of MOBP20–28 as the preferred core binding sequence of MOBP15–36**

The data reported by Holz et al. (3) on MOBP-induced EAE in SJL/J mice contrast markedly with our data. Encephalitogenic activity was reported to be associated with a pool of overlapping peptides spanning aa 37–60, while their pool of peptides spanning aa 13–36 which encompasses MOBP15–36 did not cause disease. In view of this discrepancy, we have studied the potential modes of interaction of MOBP with I-A' by molecular modeling.

As the x-ray structure of I-A' is not available, a model structure of the peptide-binding domain of I-A' was constructed on the basis of the closely related x-ray structures of I-Ak (18). The binding preferences of MHC class II I-A molecules have been described previously (23). The most notable feature of I-A molecules is the minor role of pocket 1 as compared with HLA-DR/I-E molecules and the relatively restrictive nature of pocket 9. In the case of I-A', pocket 9 shows strong preference toward proline residue. This is in concert with the substitution of the highly conserved asparagine in position α69 by threonine. Thus, in most MHC structures, asparagine α69 forms two hydrogen bonds with the peptide backbone: it acts as a hydrogen donor to the backbone carbonyl of P8 and as an acceptor of the NH hydrogen of P9. The replacement of asparagine α69 by threonine is in accord with the placement in pocket 9 of a proline residue, which does not have NH hydrogen.

The model structure of I-A' (Fig. 5A) shows that pocket 1 is deep and lined with aromatic side chains, and is slightly negative. Aliphatic-hydrophobic side chains line pocket 4, making it mostly neutral and slightly negative near the surface. This pocket is likely to accommodate a variety of non-negative side chains. Pocket 6 is polar and neutral, and of average size, and pocket 9 is neutral and mixed polar and hydrophobic. Based on these observations, we constructed a 20 × 4 scoring table, in which each entry is an estimate of the likelihood of a given side chain to be accommodated in one of the four binding pockets of I-A'. We then wrote a computer program that uses a nine-residue moving window to scan the whole rmMOBP sequence for segments that are likely to bind to all four pockets. Several nine-residue segments were predicted as candidate I-A' binding sequences. Those with a proline in position P2 were not considered because they cannot form the conserved hydrogen bonds with asparagine β82 of the I-A'. Other nonameric sequences with predicted potential binding capacity, such as MOBP69–77, MOBP96–104, and MOBP105–113, which have a proline in pocket 6 that prevents formation of the two conserved hydrogen bonds with asparagine α62 of I-A', were also considered as less probable binding sequences. The sequences predicted to be with a preferred binding mode to I-A' were therefore MOBP20–28, MOBP66–74, MOBP77–85, MOBP83–91, and MOBP158–166. Nonameric sequences with predicted lower binding affinity potential, such as MOBP6–14 and MOBP41–49, may also constitute epitopes stimulatory for T cells. However, we only tested the encephalitogenic potential of peptides encompassing the preferred binding sequences. Thus, while immunization with prMOBP15–36 could induce EAE in SJL/J mice (Table II), SJL/J mice immunized with prMOBP65–85 or prMOBP158–181, encompassing three of the other four preferred I-A' binding sequences, did not develop EAE (data not shown). Modeling the potential mode of interaction of the encephalitogenic region, MOBP15–36, with I-A' (Fig. 5A) predicted MOBP20–28 as the only preferred core binding sequence present within MOBP15–36.

Hence, the results presented above together with the molecular modeling analysis of MOBP/I-A' interactions strongly suggest that the region of MOBP encompassing amino acids 15–36 contains

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**FIGURE 5.** Modes of preferred interaction of I-A' and HLA-DRB1*1501 with MOBP15–36. The crystal structure of HLA-DRB1*1501 was used for modeling (PDB code 1bx2) with the MOBP16–27 peptide mapped onto the bound peptide in 1bx2. The structure of I-A' was modeled as described in the text. The solvent-accessible surface of I-A' and HLA-DRB1*1501 is colored according to the electrostatic potential: red for negative, blue for positive, and yellow for neutral. The peptide is shown in green. A, Top and side views of mouse MOBP18–30 bound to I-A', where the core sequence MOBP20–28 (HFSIHCCPPP) is bound in pockets 1–9 of I-A'. B, Top and side views of human MOBP16–27 bound to HLA-DRB1*1501, where the core sequence MOBP18–26 (SEHFSIHCC) is bound to pockets 1–9 of HLA-DRB1*1501. In the side views, part of the surface of I-A' or HLA-DRB1*1501 is omitted to expose the peptide-binding pockets and the internal part of the molecule appears as a dark area.
FIGURE 6. MOBP15–36-reactive T cells recognize the molecular modeling predicted core sequence MOBP20–28, but are more efficiently stimulated by longer peptides. A, Proliferative response to phMOBP15–36 and rmMOBP by phMOBP15–36-primed LNC and derived short-term T cell lines. LNC isolated from SJL/J mice 9 days previously, and derived short-term T cells (three cycles of stimulation in the presence of phMOBP15–36), were tested for their proliferative response to phMOBP15–36 and rmMOBP (0.5 μg, and 2.0 μg/well). Each histogram represents the mean cpm × 10⁻³ ± SEM of triplicate cultures. PPD (2.0 μg/well) was tested as a specificity control of T cell proliferation. B, Proliferative response of phMOBP15–36-specific line T cells to truncated peptides spanning MOBP15–36. After four cycles of stimulation with phMOBP15–36, the highly specific line T cells were tested for their reactivity to truncated peptides spanning MOBP15–36 (0.2 and 1.0 μg/well). Each histogram represents the mean SI (SD ≤ 10%) of triplicate cultures (mean background cpm ± SD = 1607 ± 110). The data presented are representative of three separate experiments.

Encephalitogenic pMOBP15–36-specific line T cells recognize the predicted nonameric core epitope MOBP20–28, but are optimally stimulated by longer peptides

Fig. 6A shows the recall proliferative response to phMOBP15–36 by lymph node cells isolated from SJL/J mice 9 days after immunization. As can be seen, phMOBP15–36 is immunogenic, and the elicited T cells recognize both phMOBP15–36 and rmMOBP. Following two further rounds of in vitro selection with phMOBP15–36, the line T cells were highly specific (Fig. 6A). Analysis of their reactivity against truncated peptides spanning MOBP15–36 indicated recognition of the predicted core epitope MOBP20–28 by pMOBP15–36-reactive line T cells (SI = 6.5; Fig. 6B); accordingly, the line T cells did not react to pmMOBP15–24 or pmMOBP23–35. However, stronger reactivity was observed to peptides longer than the core epitope, with pmMOBP18–31 being the minimal epitope which could optimally stimulate the cells (SI = 30; Fig. 6B). Transfer of activated pMOBP15–36-reactive T line cells into syngeneic recipients induced clinical EAE (Fig. 7; mean day of onset, 12.75 ± 0.43; mean maximal clinical score ± SEM, 2.5 ± 0.25) associated with perivascular inflammation in the brain and spinal cord and with demyelination mainly in the spinal cord (data not shown), similar to that seen in active immunization with pMOBP15–36 (Fig. 4).

Encephalitogenic MOBP-reactive line T cells display TCR diversity despite focused reactivity against MOBP15–36

The encephalitogenic line T cells selected in vitro for reactivity to rmMOBP were analyzed for TCR Vβ gene expression (Table III). After five rounds of selection in vitro, rmMOBP-specific line T cells were found by FACS to express TCR Vβ2 (11%), Vβ4 (20%), Vβ6 (12%), Vβ7 (6%), Vβ14 (6%), and Vβ17 (24%). PCR analysis also revealed the gene usage of TCR Vβ1, Vβ3, Vβ10, and Vβ16 (Table III). A similar diverse TCR Vβ gene expression by the line T cells raised to the major immunodominant epitope recognized by the rmMOBP-specific line cells was observed. FACS analysis of the phMOBP15–36–specific line T cells revealed expression of Vβ2 (9.5%), Vβ4 (19%), Vβ6 (7.9%), Vβ7 (25%), Vβ14 (11.3%), and Vβ17 (11.7%), and PCR analysis revealed the additional expression of Vβ1, Vβ10, Vβ15, and Vβ16 (Table III). Taken together, these results indicate that, despite their recognition
of a major immunodominant epitope located within aa 18–35. MOBP-reactive T cells elicited in SJL/J mice are highly heterogeneous as indicated by their diverse TCR Vβ gene expression. The use and similar distribution of the same Vβ gene families by encephalitogenic rmMOBP-selected and pMOBP15–36-selected line T cells further suggest that the reactivity to rmMOBP is primarily focused toward reactivity against the immunodominant epitope(s) within MOBP18–35.

Reactivity to phMOBP by PBLs from MS patients and healthy individuals

PBLs isolated from MS patients and healthy individuals were tested for their proliferative responses to overlapping synthetic peptides spanning the human MOBP sequence with the proline/arginine-rich repeat boxes (aa 96–135) excluded (2). As can be seen in Table IV, each of the peptides stimulated PBLs from at least one patient and/or healthy individual of the 22 untreated MS patients and 15 control individuals tested. The analysis with these cohorts of MS patients showed no significant difference in reactivity between MS patients and controls, and no highly significant preferential reactivity to a particular MOBP peptide. However, PBLs from both MS patients and control individuals showed a tendency for reactivity against MOBP1–23, MOBP15–36, and MOBP65–87 with no significant difference between MS and controls. Obviously, larger cohorts of HLA-typed MS patients need to be investigated for their reactivity to MOBP peptides. Nevertheless, these results, when taken together with the data on MS patients reported by Holz et al. (3) (see Discussion), suggest that phMOBP15–36, which encompasses the major encephalitogenic epitope for SJL/J mice, also contains an epitope for human T cells.

### Table III. TCR Vβ gene expression by MOBP-reactive line T cells elicited in SJL/J (H-2b) mice

<table>
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<th>phMOBP15–36-Specific T Cells</th>
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* a, ++, +++ indicate relative intensity of the signal detected on the autoradiogram.
* b Expressed as the percentage of TCR Vβ/CD4+ T cells.
* c, mAb not available.
* d NT, not tested; NA, not applicable to this mouse strain.

### Table IV. Incidence* of response to phMOBP in MS patients and healthy controls

|--------|------|------|------|------|------|------|------|------|--------|--------|--------|

* Number of responders over total tested.

Discussion

MOBP has only recently been identified as a potential target Ag in MS (1–3), and very little is known with regard to the immunopathophysiology of EAE associated with an autoreactivity against this CNS-specific myelin component. This study defines the autoimmune T cells involved in MOBP-related disease, and details the associated pathology in the CNS. As shown above, immunization of SJL/J mice with rmMOBP elicit CD4+ T cells directed primarily against a single immunodominant encephalitogenic region, MOBP15–36, which induce severe/chronic EAE associated with pathology in the CNS characterized by intense perivascular and parenchymal inflammatory infiltrates, widespread demyelination, axonal loss and optic neuritis. Molecular modeling analysis predicted MOBP20–28 as a potential core sequence with preferred binding mode to I-Ab harbored within MOBP15–36; this prediction was substantiated by experimental data demonstrating that MOBP15–36-specific T cells recognize MOBP20–28. Nevertheless, as also observed for MOG35–55-specific T cells (11), MOBP15–36-reactive T cells required peptides longer than the core epitope for optimal stimulation. In contrast to their highly focused epitope specificity, the encephalitogenic MOBP-reactive T cells were diverse in their expressed TCR Vβ genes. Such heterogeneity in TCR V gene usage by T cells focused with respect to their epitope specificity is also a characteristic of MOG-induced T cells encephalitogenic for H-2b mice, which recognize the major immunodominant encephalitogenic epitope, MOG37–52, with MOG40–48 required as a core sequence (11). Our recent studies at the clonal level indicate that the extensive TCR diversity of MOG37–52-reactive T cells may be related to recognition of multiple overlapping epitopes within MOG37–52, all of which include the nonameric core sequence MOG40–48 (L. Mendel, N. Kerlero de Rosbo, J. Kaye, M. Eisenstein, and A. Ben-Nun, manuscript in preparation). Although MOBP20–28 is highly likely to be the core sequence of the immunodominant epitope MOBP15–36, as indicated by the analysis of specific T cells to the truncated peptides spanning MOBP15–36 supported by the molecular modeling prediction, the possibility should also be investigated that the extensive TCR diversity of MOBP15–36-specific T cells may be related to their different specificities for overlapping stimulatory epitopes but with distinct core epitopes within aa 15–36, such as reported for reactivity to PLP139–151 (24) and more recently for MBP89–101 in SJL/J mice (25).

Our results are in conflict with the study of Holz et al. (3) with regard to the identification of the major encephalitogenic epitope of MOB for SJL/J mice. Using pools of overlapping peptides spanning MOBP81 (a rat short MOBP isofrom of which the first 68 aa common to all MOBP isoforms have 100% homology with the long mouse MOBP isofrom), they show that one of these pools, comprising four overlapping peptides spanning MOBP aa 37–60, induced clinical EAE in SJL/J mice (3). In our study, a different
region, MOBP15–36, was found to comprise the immunodominant and major encephalitogenic epitope, and MOBP37–60, which encompasses the sequence spanned by the peptides comprising pool 2 reported as encephalitogenic by Holz et al. (3), did not stimulate the encephalitogenic rmMOBP-specific line T cells (Fig. 3), nor did the MOBP37–60 peptide induce EAE in our SJL/J colony (Table II). Furthermore, while we defined MOBP15–36 as the major encephalitogenic region for MOBP-induced EAE in SJL/J mice, the pool of four overlapping peptides spanning aa 13–36, which encompasses MOBP15–36, was reported as nonencephalitogenic in the study of Holz et al. (3) with their SJL/J mice.

In the attempt to understand the discrepancy between our findings and that of Holz et al. (3), the interaction of rmMOBP with I-A\(^{\text{a}}\) was modeled to predict I-A\(^{\text{a}}\)-binding sequences of rmMOBP. The computer modeling predicted several nonameric sequences likely to bind well to I-A\(^{\text{a}}\), specifically MOBP20–28, MOBP66–74, MOBP77–85, MOBP83–91, and MOBP158–166, all of which include a preferred proline binding to pocket 9. Our model did not predict any high affinity binding to I-A\(^{\text{a}}\) by the peptides comprised in the peptide pool spanning MOBP37–60 reported to be encephalitogenic by Holz et al. (3), none of which contain a proline residue; however, low affinity binding to I-A\(^{\text{a}}\) could be predicted for the sequence RKYSICKSG, which is present within their peptides A3-2 (MOBP37–51) and A4-2 (MOBP40–54) (3). Together with the lack of reactivity by encephalitogenic rmMOBP-reactive T cells to the peptide pmMOBP37–60 (Fig. 3), these observations suggest that MOBP37–60 may contain a minor or cryptic encephalitogenic epitope. Our studies in C3H.SW mice suggest that the epitope which we had demonstrated to be encephalitogenic in mice immunized with the rat MOBP peptide prMOBP65–85 (2) may also be cryptic; indeed, rmMOBP-specific line T cells raised from rmMOBP-immunized C3H.SW mice did not respond to prMOBP65–85 or neighboring overlapping peptides (data not shown). A cryptic encephalitogenic epitope within PLP104–117, has also been described for PLP-induced EAE in SJL/J mice (26).

The lack of encephalitogenicity reported by Holz et al. (3) for their pool 2 which spans MOBP15–36, the major encephalitogenic demonstrated in this study, could be attributed to truncation of crucial residues flanking the core MOBP20–28 at the N-terminal (peptide A9-1) or the C-terminal (peptide A8-1) ends. Another possibility is that the encephalitogenicity of their peptides A8-1 and/or A9-1 was hampered by reactivity to the other peptides in pool 2. It is also possible that our contrasting data result from differences in immunization protocols.

Further studies are required to determine whether MOBP-induced EAE in SJL/J mice is associated with two major encephalitogenic epitopes, rather than a major encephalitogenic epitope and a cryptic encephalitogenic epitope, and how the pathogenic mechanisms differ with different protocols used in different studies to result in failure to detect encephalitogenic epitope(s).

The potential contribution to the pathogenesis of MS by auto-reactivity to MOBP was investigated by us (2) and by Holz et al. (3). In our previous report, analysis of the reactivity to overlapping human MOBP peptides by PBLs from cohorts of 10 MS patients and 10 healthy control individuals revealed a preferential reactivity both in MS patients and control individuals to pHMOBP65–87, which contains the encephalitogenic cryptic epitope of MOBP for H-2\(^{b}\) mice (2). In the present analysis of other cohorts of 22 MS patients and 15 healthy individuals (Table IV), reactivity to MOBP1–23, MOBP15–36, and MOBP65–87 was observed in some MS patients and controls, but incidence was not significantly different between the two groups. These results together with the study of Holz et al. (3) which showed significantly different MOBP-specific reactivity between MS patients and healthy individuals, particularly for stimulation by MOBP21–39 (corresponding to MOBP20–38 in the present study), in which they identified a putative HLA-DR motif, support the possibility that autoreactivity against MOBP15–36 may contain an MS-relevant epitope(s).

Molecular modeling of the interaction of MOBP15–36 with one of the HLA molecules predominantly associated with MS (27–30), HLA-DRA*0101, HLA-DRB1*1501 (thereafter referred to as HLA-DRB1*1501), predicted several core sequences, MOBP17–25, MOBP18–26, MOBP25–33, and MOBP29–36, with potential binding capacity to HLA-DRB1*1501. The energy minimization, however, preferred the binding mode to HLA-DRB1*1501 of MOBP18–26, suggesting that it is a likely core sequence for HLA-DRB1*1501-associated epitopes within MOBP15–36. MOBP17–25 as a potential core sequence binding to HLA-DRB1*1501 places a tyrosine in pocket 1, which according to our model may be too big to be accommodated in pocket 1 without enforcing some conformational changes in the HLA-DRB1*1501 molecule. The possibility of a conformational change in MHC molecules enforced by peptide binding has been previously reported (31–34). MOBP18–26 is the nonameric sequence predicted by our modeling to bind with a lower energy (higher affinity) to HLA-DRB1*1501 (lower than for MOBP25–33). Fig. 5B shows top and side views of MOBP16–27 bound to HLA-DRB1*1501 with MOBP18–26 as the core sequence accommodated in the cleft. Although the serine and cysteine residues of MOBP18–26 at P1 and P9, respectively, do not seem to fill their respective pockets completely, the P4 aromatic/hydrophobic phenylalanine and the P6 hydrophobic isoleucine fit well in pockets 4 and 6, respectively, and are likely to provide sufficient anchorage. In addition, there is structural evidence that high affinity binding of peptide to MHC class II molecule can be achieved without large anchor residues (35). In this study, 9 of the 22 MS patients studied for reactivity against MOBP peptides were HLA-typed (not shown); of these, three were HLA-DRB1*1501-positive. It is worth noting that two of these three HLA-DRB1*1501-positive patients reacted against MOBP15–36, supporting the molecular modeling prediction that this region also encompasses an HLA-DRB1*1501-associated epitope. It is of significance that these predicted HLA-DRB1*1501-binding sequences map within the major immunodominant encephalitogenic region for SJL/J mice. It is interesting to note that for other myelin proteins such as MBP and MOG, major encephalitogenic epitopes have been shown to be highly relevant to MS, in particular MBP89–101 (36, 37) and MOG35–55 (10, 22) encephalitogenic for H-2\(^{a}\) and H-2\(^{b}\) mice, respectively. However, further analysis with larger cohorts of HLA-typed MS patients and controls should be conducted to evaluate the relative contribution of the autoreactivity to MOBP in MS pathogenesis and to determine the preferential epitopes involved.

References
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