CD8 T Cell Responses to Myelin Oligodendrocyte Glycoprotein-Derived Peptides in Humanized HLA-A*0201-Transgenic Mice

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Multiple sclerosis (MS) is a chronic inflammatory disorder of the CNS characterized by multifocal demyelination, glial scar formation, and substantial disabling axonal damage. The pathophysiology of MS is related to an inflammatory response in the CNS white matter comprising CD4+ and CD8+ T cells as well as macrophages (1). This immune reaction is thought to target myelin proteins such as myelin basic protein (MBP), proteolipid protein, myelin-associated glycoprotein, and myelin oligodendrocyte glycoprotein (MOG), a process mimicked in animal models of experimental autoimmune encephalomyelitis (EAE). Among these myelin proteins, MOG has received increasing interest as a potential target for autoimmune demyelination in MS (2–4). Myelin degeneration is in part provoked by the humoral response, as demyelinating MOG-specific Abs have been detected in MS patients (5–7). This transmembrane glycoprotein is also a target for the cellular immune response in MS because MOG-specific T cells are predominant among the myelin-reactive T cells even though MOG represents only 0.1% of total myelin proteins (8,9). MOG is highly immunogenic in a wide variety of animal models and the histopathology of MOG-induced EAE in rodents and nonhuman primates resembles that observed in MS (5,10–12). The immune reactivity toward MOG can be in part explained by its narrow cellular expression pattern. In humans and mice, MOG is almost exclusively expressed in the CNS and is barely detectable in primary and secondary lymphoid organs, restricting its ability to promote self-tolerance (13–15).

The robust association between MHC class II alleles and MS, supported by observations from EAE, has led to the initial belief that MS was a CD4-mediated disease (16,17). However, alternative effector mechanisms were suggested when the therapeutic depletion of CD4+ T cells in MS failed to reduce the relapse rate or inflammatory status of patients (18,19). CD8+ T cells could participate in the pathophysiology of MS as CD8+ T cells largely outnumber CD4+ T cells within demyelinating CNS lesions (20–22). This accumulation is likely to be Ag driven as a striking oligoclonal expansion of CD8+ T cells can be observed in the cerebrospinal fluid (23) and in lesions (24) of MS patients. Functionally, CD8+ T cells could participate in CNS tissue damage in MS. Axonal damage correlates closely with the number of CD8+ T cells and macrophages in the lesion (25) and cytotoxic CD8+ T cells closely interact with demyelinated axons in acute MS lesions (26). Moreover, human myelin-specific CD8+ T cells have been detected in the blood (27) and they induce lysis of HLA-matched oligodendrocytes in vitro (28). An active role for CD8+ T cells in autoimmune CNS demyelination is further...
strengthened by animal studies, as EAЕ can be adoptively transferred by injection of either MOG- or MBP-specific CD8+ T cells (29–31).

To further evaluate the role of myelin-specific CD8+ T cells in MS pathogenesis, it would be of importance to identify the fine specificity of the autoreactive CD8+ T cell responses. HLA-A*0201 is a common allele for which some antigenic epitopes of MBP, proteolipid protein, and myelin-associated glycoprotein have been identified in both healthy individuals and MS patients (28, 32, 33). In this study, we describe the identification of novel MOG-derived HLA-A*0201-restricted candidate epitopes and assess, in a humanized transgenic mouse model, their immunogenicity, natural processing, immunodominance, and pathogenicity in vivo.

### Materials and Methods

**Animals**

The HLA-A*0201 mice (C57BL/6 background) are transgenic for HHD and knockout for β2-microglobulin and H-2Dβ. The HHD transgene encodes for the human HLA-A*0201 H chain covalently linked to human β2-microglobulin. In addition, to optimize the interaction between the mouse CD8 co-receptor and the transgenic HLA-A*0201 molecules, the human α3 domain has been replaced by the α3 domain of H-2Dα (34, 35). These mice therefore are devoid of any murine MHC class I expression (H-2Kβ is serologically undetectable), enforcing the selection of CD8+ T cells by the transgenic HLA-A*0201 molecules. All experimental protocols were approved by the local ethic committee on animal experimentation and are in compliance with European Union guidelines.

**Peptides, immunization, and EAE induction**

MOG55-65 (MEVGWYRSPFSRVHLYRNGK) was synthesized by Mi-
notopes. The HLA-A*0201-binding MOG peptides (Table I) and control peptides (HIV1 POL589–597: IVGAETFYV; hepatitis B virus (HBV)-A2: HBFcore18–27 FLPSDFFPSV) were synthesized by NeoMPS and dissolved in PBS or PBS-10% DMSO at a concentration of 4 mg/ml. Normal mice were immunized with 100 μg of a given HLA-A*0201-binding peptide combined with 140 μg of an I-Aβ-binding nonencephalitogenic Th peptide (HBV-IA: HBV nucleocapsid114–122 TPPATRPPNAPIL) emulsified in CFA (Difco). The emulsion (100 μl) was injected s.c. at the base of the tail. For EAE experiments, mice were immunized s.c. with either MOG55–65 and HBV-IA, MOG55–65 alone, or a combination of both MOG55–65 and MOG55–65, emulsified in CFA. Pertussis toxin (List Biologica l Laboratories) was injected i.v. at days 0 (200 ng) and 2 (400 ng) postimmunization. Clinical scores were recorded daily as previously described (14).

### Measurement of the relative affinity (RA) of peptides for HLA-A*0201

The assessment of peptide RA was performed as previously described (36). Briefly, T2 cells (TAP−, HLA-A*0201−) were incubated for 16 h with increasing concentrations of MOG peptides (from 0.1 to 100 μM) and stained with fluorescently labeled BB7.2 mAb (BD Biosciences) to quantify the surface expression of HLA-A*0201. For each peptide concentration, the HLA-A*0201-specific staining was calculated as the percentage of the staining obtained with 100 μM of the reference peptide HIVpol589. The RA was determined as the ratio between the concentrations of the tested and reference peptide that induce 20% of maximal HLA-A*0201 expression.

### Assessment of HLA-A*0201:peptide complex stability

As previously described (36), T2 cells (TAP−, HLA-A*0201−) were incubated overnight with 100 μM of each peptide at 37°C in serum-free medium. After a preincubation with 10 μg/ml brefeldin A (Sigma-Aldrich) for 1 h, cells were washed and incubated at 37°C for 0, 2, 4, or 6 h in complete medium supplemented with 0.5 μg/ml brefeldin A. To reveal HLA-A*0201 expression cells were stained with FITC-labeled BB7.2 mAb. For each time point, peptide-induced HLA-A*0201 expression was calculated as the mean fluorescence of peptide-pulsed T2 cells minus the mean fluorescence of T2 cells treated in similar conditions in the absence of peptide. Dissociation complex 50 (DT50) was defined as the time required for the loss of 50% of the HLA-A*0201:peptide complexes stabilized at t = 0.

### Generation of CTL in HLA-A*0201 mice

HLA-A*0201 mice were injected s.c. with 100 μg of peptide emulsified in IFA, in the presence of 140 μg of the I-Aβ-binding HBV-IA peptide. After 11 days, draining lymph nodes and spleen were harvested and CD8+ T cells were positively selected using magnetic beads (Miltenyi Biotec). A total of 1 × 10^6 irradiated HLA-A*0201 splenocytes pulsed with 10 μM MOG peptide in DMEM medium plus 10% FCS. CTL lines were established by weekly in vitro restimulation with irradiated spleen cells in the presence of peptide (10 μM) and 30 μM IL-2 (BioSource International).

### Generation of MOG-expressing HLA-A*0201+ cells and in vitro natural processing

An 830-bp murine MOG cDNA fragment encoding the full-length protein together with its signal sequence (37) was subcloned into a pcDNA6 vector (Invitrogen Life Technologies). A total of 3 × 10^6 HLA-A*0201 RMA cells (34) were cotransfected with 5 μg of MOG-pcDNA6 vector and 1 μg of pMaxGFP vector using Amaxa nucleofection. Cells were cultured in the presence of blastcicidin (Invigene) to select for pcDNA6 transfectants and, after 24 h, GFP+ cells were cloned using an Epics ALTRA cell sorter (Beckman Coulter). Cloned empty pcDNA6 and MOG-pcDNA6 HLA-A*0201 RMA transfectants were then grown in the presence of blastcicidine. For the analysis of natural processing, 25 × 10^6 irradiated HLA-A*0201 RMA cells expressing or not expressing MOG were cocultured with 5 × 10^6 short-term Ficol-purified CD8+ T cell lines (2 wk of in vitro restimulation). Intracellular IFN-γ was assessed by FACS after an overnight culture in the presence of blasticidin A according to the manufacturer's instructions (BD Biosciences).

### DNA vaccination

For plasmid DNA immunization, a pcDNA3.1 vector containing an 830-bp MOG sequence encoding the full-length protein (37) was prepared using Qiagen DNA purification columns (Endofree Plasmid kit, Qiagen). As previously described, cardiotoxin (latoxan) was injected in the tibialis anterior, followed 5 days later by the plasmid DNA injection in the regenerating muscles (38). Each muscle was injected with 50 μl of DNA at 1 mg/ml in PBS, such that each animal received a total of 100 μg of DNA. All i.m. injections were conducted under anesthesia (rompun/ketamine). When indicated, a second booster injection was performed 14 days after the initial DNA injection.

### ELISPOT

Peptide-specific T cells were enumerated using an ELISPOT assay as previously described (39). Ninety-six-well plates containing nitrocellulose filters (Multiscreen; Millipore) were coated overnight with sterile anti-IFN-γ mAbs at 4°C (BD Biosciences). The plates were washed three times with DMEM and saturated for 1 h with complete DMEM. Single-cell splenocytes were cultured at 1 × 10^6 cells/well and restimulated with the intended peptides at 1 or 10 μM (triplicates). Cells were cultured in parallel with an irrelevant HLA-A*0201-binding control peptide (HBV-A2) or Con A (5 μg/ml). After 40 h, plates were washed three times with PBS-Tween 20 and PBS, incubated 2 h with biotinylated anti-mouse IFN-γ (BD Biosciences), and 1.5 h with alkaline phosphatase-conjugated streptavidin.

Table I. HLA-A*0201-binding and -stabilization capacities of MOG-derived peptides

<table>
<thead>
<tr>
<th>Position</th>
<th>Amino Acid Sequence</th>
<th>RA</th>
<th>DT50 (Hours)</th>
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<tr>
<td>188–196</td>
<td>VLGPLVALI</td>
<td>0.38</td>
<td>5</td>
</tr>
<tr>
<td>181–189</td>
<td>TLFVIVFPV</td>
<td>0.58</td>
<td>4</td>
</tr>
<tr>
<td>179–188</td>
<td>KTLFVIVFPV</td>
<td>1.66</td>
<td>5</td>
</tr>
<tr>
<td>205–214</td>
<td>RLAQQFLEEL</td>
<td>4.54</td>
<td>4</td>
</tr>
<tr>
<td>114–122</td>
<td>KVTEDPPYNYV</td>
<td>6.5</td>
<td>3</td>
</tr>
<tr>
<td>166–175</td>
<td>RTFDIPHLIRV</td>
<td>4.54</td>
<td>&lt;1</td>
</tr>
<tr>
<td>172–180</td>
<td>FLVYP CKNI</td>
<td>&gt;10</td>
<td>ND</td>
</tr>
<tr>
<td>194–202</td>
<td>ALICYNWNL</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Position of the MOG peptides according to the mature MOG sequence without the signal peptide (44).*

*RA is the concentration of each peptide/concentration of the reference peptide that induce 20% of HLA-A*0201 expression obtained by 100 μM of the reference peptide.*

*Half-life of the HLA-A*0201:peptide complex.*

*ND, Not determined due to insolvency or low affinity.*
FIGURE 1. Immunogenicity of the HLA-A*0201-binding MOG peptides. Immunogenicity of the high-affinity (●), intermediate-affinity (□), and low-affinity (■) HLA-A*0201-binding MOG peptides. Groups of HLA-A*0201 mice were immunized with a given HLA-A*0201-binding MOG peptide and the HBV-IA peptide. The magnitude of the HLA-A*0201-restricted, MOG peptide-specific CD8+ T cell response was analyzed ex vivo by IFN-γ ELISPOT. To this end, splenocytes were restimulated with 10 μM of the corresponding peptides or an irrelevant HLA-A*0201-binding control peptide (HBV-A2). The data represent the frequency of Ag-specific IFN-γ-producing cells of individual mice. Four independent experiments are pooled, involving 8–10 mice for each individual peptide.

In vivo cytotoxicity

HLA-A*0201 mice were immunized s.c. with 100 μg of MOG peptide or PBS emulsified in CFA in the presence of 150 μg of the HBV-IA epitope. A booster injection of 100 μg of MOG peptide or PBS emulsified in IFA was administered s.c. 14 days later. Seventy-two hours later, PBS or MOG peptide-immunized HLA-A*0201 mice received target cells that were generated by pulsing HLA-A*0201 splenocytes for 2 h at 37°C with either 10 μM MOG-peptide or the HLA-A*0201-binding HBVc control peptide, washed, and differentially labeled with CFSE at a final concentration of 5 μM for MOG-loaded cells or 0.5 μM for HBV-A2-loaded cells. Eighteen hours after their injection, highly fluorescent (MOG loaded) or moderately fluorescent (HBV-A2 loaded) target cells were enumerated in inguinal lymph nodes and the spleen by FACS analysis (40). Ag-specific lysis was calculated as follows: 1 – ((CFSE<sub>high</sub> PBSTM/CFSE<sub>high</sub> PBS)/(CFSE<sub>low</sub> MOG/CFSE<sub>low</sub> HBV) x 100%.

Stereotactic injections, histology, and immunocytochemistry

For stereotactic injections, adult male HLA-A*0201 mice were anesthetized using rompun/ketamine and placed on a stereotactic frame. A total of 5000 U of mouse IFN-γ (5 μl volume at 5 μl/min, BioSource International), or sterile PBS (vehicle) were injected through a finely drawn capillary into the lateral ventricle of the brain. The coordinates used for the injections from bregma were for the left ventricle, caudal −0.13, lateral +1, ventral −2.5. For histology, deeply anesthetized mice were transcardially perfused with 4% paraformaldehyde. Tissues were embedded in paraffin, and tissue sections were stained with H&E or Luxol fast blue. Immunocytochemistry was performed using Mac-1, HLA-A (HCA2), human β3-microglobulin, and CD3-specific mAbs on paraffin sections and Ab binding was revealed using a biotin-avidin peroxidase system (Sigma-Aldrich). Labeling was visualized with 3,3′-diaminobenzidine-tetra-hydrochloride (Sigma-Aldrich) (41). Human postmortem brain tissue samples were obtained from a 41-year-old female without brain pathology and a chronic active lesion from a 41-year-old male with secondary progressive MS. Brain biopsies were obtained from the archives of the Center of Brain Research (Vienna, Austria).

Enumeration of CNS-infiltrating CD8 T cells

Cryostat sections were fixed in ice-cold acetone for 10 min, dried, and incubated in TBS with 0.5% BSA before application of the primary Abs. For confocal microscopy analysis, fluorescent double labeling with primary Abs from different species (rabbit-anti-CD3 and rat-anti-CD8), Abs were applied simultaneously at room temperature. After washing with TBS, secondary Abs consisting of goat-anti-rat Cy3 and goat-anti-rabbit Cy2 (both Jackson ImmunoResearch Laboratories, 1:200) were applied simultaneously for 1 h at room temperature. Fluorescent preparations were examined using a confocal laser scan microscope (Zeiss) equipped with an argon laser (488 nm excitation) and an HeNe laser (543 nm excitation). Scanning with the 488- and 543-nm lasers was performed sequentially. In all cases, a pinhole size leading to an optical section of 0.5 μm was chosen. The recordings were stored in two different color channels (green: Cy2; red: Cy3) and overlaid to produce a two-colored picture.

Statistical analysis

EAE day of onset was compared using Mann-Whitney test, whereas disease occurrence and mortality were compared using the log-rank test, and cumulative severity, maximal severity, and the histopathological quantification using the unpaired Student t test.
Results

Identification of HLA-A*0201-binding MOG peptides

Potential HLA-A*0201-binding human MOG peptides were identified using computer algorithms (BIMAS, SYFPEITHI) able to predict their ability to stabilize HLA-A*0201 by scoring the independent binding properties of individual residues (42, 43). Table I summarizes the eight unique MOG peptides conserved between mice and humans that were identified and synthesized. These sequences are contained within the extracellular domain (MOG114), the membrane-associated domain (MOG166, MOG172, MOG179, MOG181, MOG188, MOG194), and the intracellular domain (MOG205) (44). MOG194, a highly hydrophobic peptide, was synthesized but could not be analyzed due to its insolubility. We evaluated in vitro the capacity of these peptides to bind (RA) and stabilize (DT50) the HLA-A*0201 molecule (Table I), using human HLA-A*0201+ TAP- T2 cells. According to these criteria, the peptides were classified as high affinity (MOG179, MOG181, MOG188), intermediate affinity (MOG114, MOG205), and low affinity (MOG166, MOG172). All the high- and intermediate-affinity peptides, except for MOG166, formed stable complexes with HLA-A*0201 (DT50 > 3 h). The low affinity of MOG172 prohibited the accurate assessment of complex stability.

FIGURE 3. Natural processing and immunodominance of the HLA-A*0201-binding MOG peptides. Frequencies of IFN-γ-secreting CD8+ T cells specific for the high-affinity (■), intermediate-affinity (□), and low-affinity (▲) MOG peptides are presented after one (A) or two (B) MOG DNA immunization. Splenocytes from MOG-immunized mice were collected 14 days after the last MOG DNA immunization. Single-cell suspensions were restimulated with 10 μM of the corresponding peptides or a control HBV-A2 peptide for 40 h in an IFN-γ ELISPOT assay. The figure presents the pooled data of six independent experiments enumerating the epitope-specific CD8+ T cells per million CD8+ T cells in the spleen of five (single vaccination) or nine (double vaccination) individual mice. The total CD8+ frequency was assessed by FACS analysis.

FIGURE 4. HLA-A*0201 expression in the CNS of HLA-A*0201 mice. CNS sections of noninjected mice (A and C) or 40 h after IFN-γ injection (B and D) in the left brain ventricle were stained with either human β2-microglobulin-specific (A and B) or HLA-A-specific (HCA2) Abs (C and D). No basal HLA-A*0201 expression was observed (A and C), while IFN-γ injection induces marginal HLA-A*0201 expression as detected by the covalently linked human β2-microglobulin (B). Data in A–D show representative images of two mice per group. HLA-A*0201 expression was assessed in the CNS of four HLA-A*0201 mice 21 days after immunization with 5 μg of MOG35–55 (G–J). Inflammatory cells and meningeal cells, but also occasional glia cells, are positive for the HHD fusion protein as detected by human β2-microglobulin mAbs (G). Shown in more detail are microglia cells (H) and astrocytes (I). Two astrocytes (arrows) next to a vessel with HLA-A*0201-positive endothelium (arrowhead) are shown in J. To illustrate HLA-A expression, HCA2-stained human CNS sections without CNS pathology (E) and a chronic active MS lesion (F) are shown. HLA-A*0201 expression was detected using mAbs recognizing the HLA-A H chain (HCA2) (C–F) or human β2-microglobulin (A, B, and G–J). The staining was visualized by peroxidase conversion of 3,3’-diaminobenzidine-tetrahydrochloride, with a hematoxylin counterstain. Bars indicate the scale in micrometers.
Immunogenicity of the HLA-A*0201-binding MOG peptides

To assess the capacity of the MOG peptides to elicit an HLA-A*0201-restricted CD8$^+$ T cell response in vivo, we used HLA-A*0201-transgenic mice that express a chimeric HLA-A*0201 monochain with an H-2D$^b$-derived α3 domain covalently linked to human β2-microglobulin (34). These mice have proven to be a valuable tool in the preclinical evaluation of antitumor and antiviral strategies, as well as for the identification of cancer Ags (45–49). In these mice, the peripheral CD8$^+$ T cell pool is reduced in size (∼6% of splenic T cells), but not in the diversity of its TCR repertoire (50). The HLA-A*0201 mice were immunized with each of the HLA-A*0201-binding MOG peptides in the presence of an immunogenic I-A$^b$-binding viral peptide to elicit CD4$^+$ T cell help (HBV-IA). As shown in Fig. 1, the evaluation of the peptide-specific CD8$^+$ response by IFN-γ ELISPOT revealed a very limited immunogenicity for the two MOG peptides with low affinity or short DT$_{50}$ (MOG$_{166}$ and MOG$_{172}$), while all five MOG peptides (MOG$_{188}$, MOG$_{181}$, MOG$_{179}$, MOG$_{205}$, MOG$_{114}$) that form stable HLA-A*0201 complexes were highly immunogenic, inducing a sizeable CD8$^+$ T cell response in the majority of mice.

Natural processing of the HLA-A*0201-binding MOG peptides

To assess whether the selected MOG peptides are naturally processed from native MOG protein and presented in the context of HLA-A*0201, we created HLA-A*0201$^+$ RMA clones transfected with full-length murine MOG cDNA. These MOG-expressing HLA-A*0201$^+$ RMA cells (Fig. 2A) were cocultured with short term CD8$^+$ T cell lines specific for each of the identified MOG peptides and intracellular IFN-γ production was used to assess CD8$^+$ T cell activation. RMA cells transfected with an empty vector pulsed or not with exogenous MOG peptides served as positive and negative controls, respectively. Fig. 2B shows representative FACS plots of the various CD8$^+$ T cell lines. With the exception of the MOG$_{185}$-specific line, all CD8$^+$ T cell lines were stimulated by the MOG-transfected cells, increasing the level of IFN-γ expression and augmenting the frequency of cytokine-producing cells. These data indicate that apart from MOG$_{188}$, all MOG peptides are naturally processed (Fig. 2, B and C).

To explore the natural processing and immunodominance of the MOG epitopes further, we vaccinated HLA-A*0201 mice i.m. with a DNA construct encoding full-length murine MOG. The magnitude of the CD8$^+$ response was assessed by IFN-γ ELISPOT following ex vivo restimulation with the identified MOG peptides. A single MOG DNA immunization induced a detectable CD8$^+$ response only to MOG$_{181}$ (Fig. 3A) that was further enhanced by boosting with a second MOG DNA vaccination (Fig. 3B). The booster vaccination also revealed a sizeable CD8$^+$ T cell response against MOG$_{179}$, but only occasional responses to MOG$_{188}$, MOG$_{205}$, and MOG$_{114}$. Therefore, the peptides can be classified as follows: two of three high-affinity peptides represent immunodominant epitopes (MOG$_{181}$, MOG$_{179}$), while subdominant responses were induced against MOG$_{188}$, MOG$_{205}$, and MOG$_{114}$: the latter two peptides having an intermediate affinity for HLA-A*0201. The two unstable MOG peptides, MOG$_{166}$ and...
MOG\textsubscript{172}, failed to induce a detectable CD8\textsuperscript{+} T cell response. Taken together, these data indicate that both MOG\textsubscript{181} and MOG\textsubscript{179} are immunogenic, naturally processed, and immunodominant, and as such represent novel candidate epitopes for myelin-specific CD8\textsuperscript{+} T cell responses in HLA-A*0201 humans.

**Focal HLA-A*0201 expression in the CNS of HLA-A*0201 mice during inflammatory conditions**

One limiting factor for tissue damage by CD8\textsuperscript{+} T cells is the low level of MHC class I expression on the cell surface of CNS glial cells (51, 52). We, therefore, assessed HLA-A*0201 expression by immunohistochemistry in the CNS of HLA-A*0201 mice. As shown in Fig. 4, no constitutive expression of HLA-A*0201 was detected in untreated HLA-A*0201 mice (Fig. 4, A and C), contrasting with both expression of class I on microglial cells in wild-type rodents (51, 52), and the basal HLA-A expression of blood vessel lining endothelial cells in sections of an unaffected human brain (Fig. 4E). To assess whether inflammatory mediators could improve MHC class I expression in the CNS, we injected IFN-\(\gamma\) (5000 U) via stereotaxis in the left ventricle of the brain using an established protocol (51, 52). This procedure only marginally improved HLA-A*0201 expression in the CNS of HLA-A*0201 mice (Fig. 4, B and D). CNS inflammation enhances HLA-A expression on endothelial cells, lymphocytes, macrophages, and activated microglial cells in MS (Fig. 4F). We, therefore, wondered whether the expression of HLA-A*0201 could be further induced by generating a CNS inflammatory response. To this end, we immunized HLA-A*0201 mice with the encephalitogenic I-A\(^d\)-binding MOG epitope MOG\textsubscript{35-55}. Immunized mice developed inflammatory infiltrates in the spinal cord provoking focal demyelination (data not shown). In this inflammatory context, HLA-A*0201 expression was observed on some meningeal cells (Fig. 4G), infiltrating inflammatory cells (Fig. 4, G and H), and occasional glial cells within the lesion (Fig. 4, I and J). Thus, despite an undetectable basal level, HLA-A*0201 expression can be induced on CNS glial cells during inflammatory conditions.

As a final selection criterion, we assessed the in vivo cytotoxicity of the MOG-specific CD8\textsuperscript{+} T cell response induced against the three high-affinity peptides (MOG\textsubscript{188}, MOG\textsubscript{181}, and MOG\textsubscript{179}). As shown in Fig. 5, immunization with MOG\textsubscript{188} and MOG\textsubscript{181} elicited cytotoxic responses in vivo eliminating 29 and 45% of Ag-specific target cells, respectively. Unexpectedly, MOG\textsubscript{179} induced an IFN-\(\gamma\)-producing but noncytotoxic CD8 T cell response, representing an unusual but not unique observation (53). Consequently, with MOG\textsubscript{188} representing a subdominant epitope and MOG\textsubscript{179} failing to induce a cytotoxic CD8\textsuperscript{+} T cell response, MOG\textsubscript{181} was selected to pursue the active induction of CD8\textsuperscript{+} T cell responses in HLA-A*0201 mice. Immunization with MOG\textsubscript{181} did not induce overt clinical symptoms in the HLA-A*0201 mice (\(n = 4\), data not shown). Therefore, the encephalitogenicity of the MOG\textsubscript{181}-specific CD8\textsuperscript{+} T cell response was assessed in combination with a suboptimal dose of MOG\textsubscript{35-55} that augments HLA-A*0201 expression in the CNS (Fig. 4, G–J) but generates only very mild clinical symptoms (Fig. 6A). Clinical and histopathological analysis revealed that this coimmunization led to an accelerated and aggravated EAE severity compared with HLA-A*0201 mice immunized with the suboptimal dose of MOG\textsubscript{35-55} alone or supplemented with an irrelevant HBV-A2 peptide (Fig. 6, B–D, Table II). In the mild EAE induced by MOG\textsubscript{35-55}, 62.5% of mice developed some clinical signs (maximum severity = 1.6), on average, 16 days after immunization. At day 21, some CNS inflammation and demyelination was observed which largely recovered by day 45 (Table II, data not shown). Adding a CD8\textsuperscript{+} T cell response with irrelevant specificity (HBV-IA plus MOG\textsubscript{35-55}) failed to affect EAE incidence, onset, and severity (Table III) and generated only few CNS-infiltrating CD8\textsuperscript{+} T cells (Table II). However, inducing a MOG\textsubscript{181}-specific CD8\textsuperscript{+} T cell response (MOG\textsubscript{181} + MOG\textsubscript{35-55}) accelerated disease onset (day 12), aggravated disease incidence (100%), and severity (maximum severity = 3.1) and proved lethal in some cases.

### Table II. Histopathological analysis*

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<th>Day</th>
<th>Immunogen</th>
<th>n</th>
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<td></td>
<td></td>
<td>Inflammatory index</td>
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<td>12</td>
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<td>0.5 (±0.5)</td>
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<td>57 (±27)*</td>
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<td></td>
<td>MOG\textsubscript{35-55} Plus HBV-A2</td>
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<td>0.3 (±0.3)</td>
<td>1 (±1)</td>
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<tr>
<td>21</td>
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<td>1.3 (±0.4)</td>
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</tbody>
</table>

*The histopathology was assessed 12 and 21 days after immunization. The inflammatory index represents the average number of perivascular inflammatory infiltrates per H&E-stained section. Ten to 15 cross-sections were quantified in the spinal cord and 2 in the cerebellum. T cell density was quantified as the average number of CD3\textsuperscript{+} or CD8\textsuperscript{+} cells/mm\textsuperscript{3} in three lumbar spinal cord cross-sections. Data represent mean values ± SEM. Statistical significance was tested using MOG\textsubscript{35-55} plus HBV-A2 as control group. *\(p < 0.05\); **\(p < 0.005\).

### Table III. Clinical severity of EAE*

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>n</th>
<th>Mean Day of onset (SEM)</th>
<th>Mean Maximum Severity (SEM)</th>
<th>Mean Cumulative Severity (SEM)</th>
<th>Disease Occurrence (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOG\textsubscript{35-55}</td>
<td>8</td>
<td>16.2 (±1.5)</td>
<td>1.6 (±0.5)</td>
<td>31 (±11)</td>
<td>5/8 (62.5)</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>MOG\textsubscript{35-55} Plus MOG\textsubscript{181}</td>
<td>9</td>
<td>12.4 (±0.7)*</td>
<td>3.1 (±0.4)*</td>
<td>81 (±18)*</td>
<td>9/9 (100)***</td>
<td>2/9 (22)</td>
</tr>
<tr>
<td>MOG\textsubscript{35-55} Plus HBV-A2</td>
<td>8</td>
<td>18.6 (±1.8)</td>
<td>1.5 (±0.5)</td>
<td>25 (±10)</td>
<td>5/8 (62.5)</td>
<td>0/8 (0)</td>
</tr>
</tbody>
</table>

*The pooled data from two independent experiments are presented. Statistical significance was tested using MOG\textsubscript{35-55} plus HBV-A2 as control group. *\(p < 0.05\); **\(p < 0.005\).

* Diseased animals only.
to 22% of HLA-A*0201 mice (Fig. 6B, Table III). This aggravation was characterized by a strong CNS inflammatory response at day 12, which persisted at day 21, and even at day 45. Parenchymal lesions contained CD3 and Mac-3-positive cells (Table II, data not shown). Moreover, during the initiation of disease at day 12, a dominant CD8+ T cell infiltration was observed in spinal cord lesions (Fig. 6D, Table II). These data illustrate that MOG181-specific CD8+ T cell responses can complement the encephalitogenic CD4 response in humanized HLA-A*0201 mice by accelerating the encephalitogenic process and worsening disease evolution.

Discussion

Using humanized transgenic mice that harbor an HLA-A*0201-restricted CD8+ T cell population, we have characterized HLA-A*0201-binding MOG epitopes of potential clinical interest. As self-Ags shape the peripheral T cell repertoire, we have selected peptides conserved between mice and humans to account for a potential tolerogenic effect of the studied MOG self-peptides. This in vivo approach permitted us to identify five human MOG-derived peptides that 1) form stable complexes with HLA-A*0201, 2) are immunogenic, and 3) with the exception of MOG188 are naturally processed. In vivo immunization with native MOG elicited immunodominant IFN-γ-producing CD8+ T cell responses against MOG179 and MOG181. Among the two immunodominant MOG peptides, only MOG181 elicited a cytotoxic CD8+ T cell response able to exacerbate suboptimal EAE, and as such represents a strong candidate epitope for the myelin-specific CD8+ T cell response in HLA-A*0201 MS patients. These results illustrate the flexibility provided by humanized mice as an intermediate for human epitope discovery in the context of autoimmunity. It is known that the correlation between candidate epitopes identified in humanized mouse models and those observed in humans is not absolute. Differences in the T cell repertoire, and in the Ag-processing machinery, may distinguish the immunogenic Ag landscape between species. Nevertheless, empirical evidence demonstrates a large overlap between virus- and tumor-specific CD8+ T cell responses in HLA-A*0201 mice and humans, validating these mice for the identification of CD8+ T cell epitopes relevant for human diseases (38, 48, 54, 55).

Of the eight human MOG peptides selected based on their putative HLA-A*0201-binding motif, two peptides (MOG166 and MOG172) revealed only a limited stability with HLA-A*0201 and fell below the threshold for efficient T cell priming, as suggested previously (56). Five other MOG peptides (MOG114, MOG179, MOG181, MOG188, MOG203) possessed a medium to high affinity for HLA-A*0201, formed stable HLA-A*0201:MOG peptide complexes, and all induced sizeable HLA-A*0201-restricted CD8+ T cell responses in vivo. Given that these MOG peptides are self-Ag derived, such a tight correlation between immunogenicity and MHC:self-peptide stability is uncommon, as T cells bearing a TCR complementary to stable complexes of high affinity are usually purged from the repertoire either in the thymus or periphery (57). As such, our observations are in support of the idea that, in primary and secondary lymphoid organs, MOG is expressed at levels insufficient to tolerate the peripheral CD8+ T repertoire (14, 15). A large interindividual variation was observed in the intensity of the CD8+ T cell response elicited against a given epitope. Although the HLA-A*0201 mice harbor a diverse CD8+ T cell repertoire, its reduced size might limit the abundance of a given clone in the naive repertoire. As the clonal frequency varies between mice, this could occasionally result in frequencies insufficient to meet the threshold ensuring the productive encounter between specific T cell and cognate Ag (58, 59).

To assess which epitopes are naturally processed and can thus be generated as targets of a MOG-specific CD8+ T cell response, we assessed the Ag-presentation capacity of MOG-expressing HLA-A*0201+ RMA cells in combination with short-term peptide-specific CD8+ T cell lines. Predicting epitopes on the sole basis of their binding motif has a success rate of approximately one of three to reveal naturally processed peptides (60). It was, therefore, unexpected to find that four of five immunogenic MOG peptides were processed and presented (MOG114, MOG179, MOG181, MOG203). Only MOG188-specific CD8+ T cell lines failed to be restimulated in vitro by MOG-expressing HLA-A*0201+ RMA cells. In addition, MOG DNA immunization in vivo elicited a low frequency of MOG181-specific CD8+ T cell responses in a minority (two of nine) of HLA-A*0201-transgenic mice. Together, these data indicate that the MOG188 peptide is inefficiently processed making it subdominant (61). Among the four remaining naturally processed epitopes, dominant CD8+ T cell responses were observed after DNA immunization for the high-affinity peptides MOG181 and MOG179. The intermediate-affinity peptides revealed subdominant CD8+ T cell responses, suggesting a direct role for peptide binding to HLA-A*0201 in the immunodominance hierarchy of the MOG peptides.

To be pathogenic, invading myelin-specific T cells require local restimulation by CNS self-Ags (62–64). The low basal HLA-A*0201 expression in the CNS of HLA-A*0201 mice might therefore not be favorable to the local restimulation of invading myelin-specific CD8+ T cells and might well render CNS target cells refractory to direct cytotoxicity. Indeed, inducing myelin-specific CD8+ T cells by immunization with MOG181 was efficient in generating CNS-infiltrating T cell responses, but failed to generate clinical or histological evidence of CNS tissue damage (data not shown). However, eliciting a MOG181-specific CD8+ T cell response concomitant with CD4-mediated EAE in HLA-A*0201 mice, aggravated the clinical and histopathological severity of disease. As inducing an irrelevant CD8+ T cell response had no such effect, this suggested that the MOG181-specific CD8+ T cell response that dominates the inflammatory infiltrate at disease onset contributes directly to the effector stage of EAE in the CNS. In addition, it underlines that a concomitant myelin-specific CD4+ T cell response can 1) improve CD8 recruitment to the CNS; 2) heighten local HLA-A*0201 expression on glial cell populations, which enhances direct CD8+-mediated cytotoxicity; and/or 3) enhances CD8 T cell release of detrimental cytokines and proteases.

This study, beyond characterizing new MOG-derived epitopes, provides additional evidence for the deleterious role of autoreactive CD8+ T cells in CNS inflammatory diseases. In addition to MS, detrimental CD8+ T cell responses are associated with neurodegenerative disorders such as Rasmussen’s disease and paraneoplastic cerebellar degeneration, where activated cytotoxic CD8+ T cells can be detected juxtaposed to degenerating neurons (26, 65). Our model reveals that such detrimental CD8+ T cell responses indeed exacerbate the clinical evolution of a demyelinating CNS disease when targeting human MHC class I:myelin-peptide complexes. Consequently, therapeutic inhibition of the deleterious autoreactive CD8+ T cell response might provide a clinical benefit for several human CNS inflammatory diseases including MS.

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Disclosures

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References


