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Myelin Antigen-Specific CD8+ T Cells Are Encephalitogenic and Produce Severe Disease in C57BL/6 Mice

Deming Sun,2* John N. Whitaker,* Zhigang Huang, Di Liu, † Christopher Coleclough,‡ Hartmut Wekerle,§ and Cedric S. Raine¶

Encephalitogenic T cells that mediate experimental autoimmune encephalomyelitis (EAE) are commonly assumed to be exclusively CD4+1 T cells, but formal proof is still lacking. In this study, we report that synthetic peptides 35–55 from myelin oligodendrocyte glycoprotein (pMOG35–55) consistently activate a high proportion of CD8+ αβ T cells that are encephalitogenic in C57BL/6 (B6) mice. The encephalitogenic potential of CD8+ MOG-specific T cells was established by adoptive transfer of CD8-enriched MOG-specific T cells. These cells induced a much more severe and permanent disease than disease actively induced by immunization with pMOG35–55. CNS lesions in pMOG35–55 CD8+ T cell-induced EAE were progressive and more destructive. The CD8+ T cells were strongly pathogenic in syngeneic B6 and RAG-1−/− mice, but not in isogenic β2-microglobulin-deficient mice. MOG-specific CD8+ T cells could be repeatedly reisolated for up to 287 days from recipient B6 or RAG-1−/− mice in which disease was induced adoptively with ≤1 × 106 T cells sensitized to pMOG35–55. It is postulated that MOG induces a relapsing and/or progressive pattern of EAE by eliciting a T cell response dominated by CD8+ autoreactive T cells. Such cells appear to have an enhanced tissue-damaging effect and persist in the animal for long periods.


The availability of encephalitogenic T cells from animals with experimental autoimmune encephalomyelitis (EAE) has opened fruitful approaches in the definition of pathogenetic pathways and immunotherapeutic investigations in the demyelinating diseases (1–3). Using this technology, the analysis of TCR usage (3, 4), cytokine production (5, 6), and adhesion molecule profiles of autoreactive T cells entering the CNS (7, 8), as well as subsequent interactions of these cells with resident glia (9), have revealed a profile of T cell properties important for disease production.

EAE has been induced in rodents by sensitization with a number of myelin proteins, including myelin basic protein (MBP) (10, 11), proteolipid protein (PLP) (12, 13), myelin oligodendrocyte glycoprotein (MOG) (14–16), myelin-associated glycoprotein (17), and myelin oligodendrocyte basic protein (18). Previous studies of the functional characteristics of encephalitogenic effector cells during EAE have focused on MBP- and PLP-reactive T cells. These studies have led to the general consensus that, unlike autoimmune diabetes in which both CD4+ and CD8+ T cells participate (19, 20), effector T cells in EAE are exclusively CD4+ and αβ TCR+ (21). Recently, MOG-induced EAE has attracted increasing attention, particularly because MOG-reactive T cells are readily found in the circulation in multiple sclerosis (MS) patients (22, 23), and MOG-derived synthetic peptides reproducibly induce EAE in a variety of mouse strains. In particular, MOG induces forms of chronic and relapsing demyelinating disease in mice (24–26) and primates (27, 28), the latter having much in common with human MS. Using similar experimental procedures, we have now shown that C57BL/6 (B6) mice immunized with a synthetic peptide representing residues 35–55 of MOG (pMOG35–55) consistently generated CD8+ αβ TCR+ encephalitogenic T cells. We have also demonstrated that adoptive transfer of enriched CD8+ MOG-specific T cells from B6 mice induces a more severe clinical and pathologic disease after adoptive transfer than disease induced by active immunization with pMOG35–55.

Materials and Methods

Animals

B6, SJL/J, RAG-1−/−, MHC class II−/−, and β2m-microglobulin (β2m)−/− mice were obtained from The Jackson Laboratory (Bar Harbor, ME), and housed in a National Institutes of Health-approved and American Association for the Accreditation of Laboratory Animal Care-accredited facility.

Cells and reagents

All T cells were cultured in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% selected FCS (Life Technologies), 5 × 10−5 M 2-ME, and penicillin/streptomycin (100 μg/ml). pMOG35–55 (MEVGWYRSPFSRVRHYLRNGK), pMOG40–54 (YRSPFSRVRHYLRNG), and synthetic peptide representing residues 178–191 of PLP (pPLP178–191) (NTWTTCQSIAFPSK) synthetic peptides were produced by the Molecular Resource Center at St. Jude’s Children’s Research Hospital (Memphis, TN), mAbs GK1.5 (specific for mouse CD4) and 2.43 (specific for mouse CD8) were obtained from the American Type Culture Collection (Manassas, VA), Abs specific for mouse H-2Kb/H-2Db (28-8-6), and penicillin/streptomycin (100 μg/ml). pMOG35–55 (MEVGWYRSPFSRVRHYLRNGK), pMOG40–54 (YRSPFSRVRHYLRNG), and synthetic peptide representing residues 178–191 of PLP (pPLP178–191) (NTWTTCQSIAFPSK) synthetic peptides were produced by the Molecular Resource Center at St. Jude’s Children’s Research Hospital (Memphis, TN), mAbs GK1.5 (specific for mouse CD4) and 2.43 (specific for mouse CD8) were obtained from the American Type Culture Collection (Manassas, VA), Abs specific for mouse H-2Kb/H-2Dab (28-8-6), mouse I-Ab (M5/114), and mouse CD80 were purchased from PharMingen (San Diego, CA). Recombinant dimers of the MHC class I (H-2Dab) molecule, recombinant human β2m, and synthetic peptide NP766–774 (ASNE METM) derived from influenza virus were also obtained from PharMingen.

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Active induction of EAE

Mice were immunized with pMOG35–55 peptide (200 µg per mouse) emulsified in CFA containing 0.6 mg Mycobacterium tuberculosis (H37Ra; Difco, Detroit, MI). After 48 h postimmunization, MOG-sensitized animals received a single i.p. dose of 400 ng of Pertussis toxin (Sigma, St. Louis, MO).

Adoptive transfer of T cells isolated from B6 mice with EAE actively immunized with pMOG35–55

To determine and characterize encephalitogenic T cells from pMOG35–55-immunized animals, T cells isolated from the draining lymph nodes and spleens of immunized animals were enriched by passage through nylon wool and restimulated in vitro with pMOG35–55 (20 µg/ml) presented by irradiated syngeneic spleen cells. After 3 days, activated T cell blasts were separated on a Ficoll gradient and injected (2 × 10⁶ i.p) into naive B6 or RAG-1⁻/⁻ mice.

T cell proliferation/inhibition assays

APC (irradiated syngeneic spleen cells, 2 × 10⁵/well) were preincubated in 96-well flat-bottom microtiter plates with an optimal dose (20 µg/ml) of pMOG35–55. After 1 h, enriched T cells (4 × 10⁵/well), prepared from lymph nodes or spleen by nylon wool adsorption, were seeded and further incubated for 48–72 h. The cultures were then pulsed with 1.0 µCi [³H]thymidine/well for 6 h and harvested, and the incorporated isotope was quantitated by liquid scintillation.

Depletion of T cell subsets by Ab-coated magnetic beads

Nylon wool-enriched splenic T cells were incubated with 10 µg/ml of either rat anti-mouse CD4 mAb (GK1.5) or normal rat Ig (control) for 30 min. Unbound Ab was removed by washing twice before the addition of magnetic biospheres coupled to goat anti-rat Ig (BioSource International, Camarillo, CA), incubating at 4°C, and agitating for 30 min. The tube of cells was then placed on a magnetic stand for 5 min, and cells free of magnetic particles were collected. The procedure was repeated 2–3 times as necessary.

Clinical evaluation of EAE

Animals were examined daily for weight loss and clinical signs. Animals were clinically graded as follows: 0 = no signs; 1 = partial loss of tail tonicity; 2 = loss of tail tonicity, difficulty in righting; 3 = unsteady gait and mild paralysis; 4 = hind-limb paralysis and incontinence; and 5 = moribund or death.

Flow cytometric detection of T cells binding to pMOG35–55 complexed with recombinant dimers of MHC class I (H-2Dβ) molecule

Dimeric MHC class I (H-2D β) used in this study is a fusion protein between mouse H-2D β and mouse IgG1. The recombinant protein was added to goat anti-rat Ig (BioSource International, Camarillo, CA), incubating at 4°C, and agitating for 30 min. The tubes of cells were then placed on a magnetic stand for 5 min, and cells free of magnetic particles were collected. The procedure was repeated 2–3 times as necessary.

Routine histology

For this, mice were anesthetized, bled, and perfused with 25 ml PBS and 10 ml 4% paraformaldehyde in buffered PBS. Brains and spinal cords were dissected out and fixed in 4% paraformaldehyde before embedding in paraffin. Paraffin sections were stained with hematoxylin/eosin and Luxol fast blue (for myelin). Immunocytochemistry was performed on 3-µm paraffin sections, using a biotin/avidin/peroxidase technique. For this, CNS tissue was examined from seven representative animals from the experimental groups (Table I). For detailed neuropathology, three mice were sampled during the acute phase of pMOG35–55-active EAE (19–24 days post immunization (dpi)) during the acute phase of the disease; two B6 mice with adoptive CD8⁺ pMOG35–55 EAE; and two RAG-1⁻/⁻ mice with adoptively transferred CD8⁺ pMOG35–55 EAE were studied during the chronic phase, 56 and 70 days post transfer (dpi). At the time of sampling, animals were anesthetized with ether and perfused through the heart with 20 ml of cold PO₄-buffered 2.5% glutaraldehyde. The brain and spinal cords were removed, and thin slices were taken from the cerebral hemispheres, cerebellum/brainstem, and spinal cord at C7, T2, L2, L5, S1, and S2. In addition, optic nerve and spinal nerve roots were taken. The tissue samples were then postosmicated in 1% osmium tetroxide, dehydrated, and embedded in epoxy resin. One-micrometer epoxy sections stained with toluidine blue were prepared from all levels for light microscope analysis.

Results

Increased activation of CD8⁺ encephalitogenic T cells

Splenic T cells were prepared by passing through a nylon wool column. CD4⁺ and CD8⁺ T cells were separated by negative depletion method using Ab-coated magnetic beads (see Materials and Methods). The CD4⁺ and CD8⁺ T cells thus obtained have the purity of >98%. When exposed to pMOG35–55 and APC, both cell fractions responded with proliferation (Fig. 1C). When the pMOG35–55-activated cells were cultured in IL-2-containing medium for 5–10 days, an overwhelming majority of the proliferating cells expressed a surface phenotype αβTcR⁺ CD4⁺ CD8⁻ (Fig. 1, A and B). To determine whether the dominant CD8⁻ cells were the major pathogenic effectors and that the disease was not the result of the minor CD4⁺ population, we depleted CD4 T cells from immunized animals using magnetic biospheres, and the remaining CD8⁻ T cell populations were stimulated with an optimal dose of pMOG35–55 and APCs. The results showed that depletion of CD4 T cells did not affect the pathogenic activity of enriched CD8⁺ T cells activated by pMOG35–55. Alternatively, we administered MOG-reactive T cells to β₂m⁻/- mice and compared them with B6 mice. Three separate experiments with a total of nine β₂m⁻/- mice and >100 B6 mice were conducted. All the β₂m⁻/- mice studied were resistant to EAE adoptively induced by CD8⁺ MOG-specific T cells (Fig. 2).

To determine whether the progressive disease induced by adoptive transfer of a few million CD8⁺ MOG-specific encephalitogenic T cells was related to the increased survival of such T cells, we also tried to detect and retrieve the injected T cells from recipient mice. Kinetic studies have shown that 6–8 mo after injection of a single dose of 2 × 10⁶ cells, CD8⁺ MOG-specific T cells could be detected (Fig. 3B) and re-isolated (Fig. 3C) from the recipient animals, indicating that such T cells may have an increased ability to survive in vivo. FACs determination of the MOG-specific T cells re-isolated from recipient animals’ spleens

Table I. Lesion pathology in 1-micron epoxy sections

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Induction (active/adoptive)</th>
<th>Mouse Strain</th>
<th>dpi</th>
<th>Days after Onset</th>
<th>Clinical Grade at Sampling</th>
<th>Inflammation</th>
<th>Demyelination</th>
<th>Remyelination</th>
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<tr>
<td>1</td>
<td>Active MOG/EAE</td>
<td>B6</td>
<td>24</td>
<td>5</td>
<td>2</td>
<td>++</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>2</td>
<td>Active MOG/EAE</td>
<td>B6</td>
<td>19</td>
<td>6</td>
<td>3</td>
<td>++</td>
<td>++</td>
<td>–</td>
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<tr>
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<td>Active EAE</td>
<td>B6</td>
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<td>6</td>
<td>2</td>
<td>++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Adoptive EAE</td>
<td>B6</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>+++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Adoptive EAE</td>
<td>B6</td>
<td>25</td>
<td>15</td>
<td>3</td>
<td>+++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Adoptive EAE</td>
<td>RAG-1⁻/⁻</td>
<td>54</td>
<td>37</td>
<td>3</td>
<td>±</td>
<td>±</td>
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<td>RAG-1⁻/⁻</td>
<td>76</td>
<td>66</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>±</td>
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</tbody>
</table>

*In addition, >50 mice were studied by routine histology.
showed that the re-isolated T cells were predominantly CD8$^+$ (Fig. 3C). We also determined the number of pMOG$_{35-55}$-reactive T cells that were restricted by MHC class I Ags by their binding ability to recombinant H-2D$^b$ dimers. Thus, 20 days after the immunization of pMOG$_{35-55}$/CFA, the animals were sacrificed, and the splenic T cells were enriched by passing through nylon wool. Such T cells were incubated with irradiated spleen cells (APC) and 20 μg/ml pMOG$_{35-55}$ for 48 h, separated on a Ficoll gradient, and cultured in IL-2-containing medium. The proliferating cells were stained with MOG$_{35-55}$-H-2D$^b$. The percentage of positive cells was quite low before the cells were expanded in IL-2-containing medium. However, 20–60% of the total T cells were stained by MOG$_{35-55}$-H-2D$^b$, but not the same class I molecules (H-2D$^b$) bound by a control peptide NP$_{366-374}$ derived from influenza virus and known to bind H-2D$^b$ (29, 30) after a 7- to 10-day culture in IL-2-containing medium (Fig. 4).

We also compared the disease-inducing effect of CD4$^+$, CD8$^+$ MOG-specific T cells alone and cotransfer of both encephalitogenic T cells. Our results showed that transfer of CD8$^+$ MOG-specific T cells alone and cotransfer of both populations of MOG-specific T cells had comparable disease-inducing activity in that less than one million CD8$^+$ MOG-specific T cells consistently induced permanent paralysis. The severity of disease induced by 5 × 10$^6$ CD4$^+$ MOG-specific T cells was comparable to that induced by 1 × 10$^6$ CD8$^+$ MOG-specific T cells and the recipient mice recovered earlier (data not shown).

Massive CNS inflammation and overinfiltration of macrophage/microglia in the CNS

Most, if not all, B6 mice developed a chronic, relapsing EAE after immunization with ~200 μg of synthetic peptide pMOG$_{35-55}$ emulsified in CFA (24). The duration of the disease varied, and this was closely related to the dose of M. tuberculosis (H37RA). In most cases, mice immunized with a similar dose of pMOG$_{35-55}$ but <0.6 mg of H37RA, had a shorter duration of disease, differing from 20 to 60 days. Remarkably, both clinical signs and pathologic changes induced by adoptive transfer of MOG-specific T cells were much more severe than those induced by active immunization with peptide. Less than 1 × 10$^6$ MOG-specific T cells were found to cause a severe paralytic disease in naive B6 and RAG-1$^{-/-}$ mice (Table I). Examination by routine histology revealed that massive CNS infiltration, myelin degeneration, and demyelination were consistently identified in animals with adoptively transferred EAE (Fig. 5B), features which in most cases were

FIGURE 1. MOG-reactive T cells are predominantly CD8$^+$. Nylon wool-enriched cells from pMOG$_{35-55}$-immunized B6 mice remained untreated (A) or they were stimulated (B) with pMOG$_{35-55}$ and APC for 48 h. Activated T cells were separated on a Ficoll gradient and cultured in IL-2-containing medium for 7 days before staining with FITC-labeled mAbs specific for mouse αβ TCR (H57-597) and PE-labeled mAbs specific for mouse CD8 (53-6.7). C, Nylon wool-enriched splenic T cells were separated using magnetic beads into CD4 and CD8 T cell subsets. Triplicate cultures were set in 96-well plates in which the separated cell populations (4 × 10$^5$/well) were tested for proliferation against irradiated APC (2 × 10$^5$/well) and pMOG$_{35-55}$ or pMOG$_{44-53}$ (20 μg/ml), a truncated peptide of pMOG$_{35-55}$.

FIGURE 2. CD8$^+$ pMOG$_{35-55}$-specific T cells fail to induce EAE in β$_2$m$^{-/-}$ mice. Nylon wool-enriched splenic T cells prepared from pMOG$_{35-55}$-immunized B6 mice were stimulated in vitro with 20 μg/ml of pMOG$_{35-55}$ and irradiated spleen cells (APC) for 3 days. The activated lymphoblasts were separated on a Ficoll gradient. T cells (2 × 10$^5$) were injected i.p. into B6 or β$_2$m$^{-/-}$ mice. Each line represents a pooled result from three tested mice. Note the failure of β$_2$m$^{-/-}$ mice to develop EAE.
milder in actively induced disease. Immunohistochemical studies showed that an overwhelming majority of the CNS infiltrating cells were reactive with isolectin B4 (Fig. 5C), a marker of microglia and inflammatory macrophages (31, 32). The ratio between isolectin B4- and CD3-stained cells was 10:1. Kinetic studies revealed that CD3 cells disappeared much earlier in comparison to isolectin B4-staining cells from the CNS, even though the disease remained relapsing or progressive (Fig. 5C). Pathologic expression in chronic phases of actively induced disease varied significantly in that both the duration and relapse rate of the disease were not predictable (data not shown). We have also determined CD4 and CD8 cells in brain lesions, using immunocytochemical staining; however, we could not show a significantly increased infiltration of CD8 cells in the CNS inflammation (data not shown).

pMOG35–55 T cells induce a more progressive, destructive type of CNS lesion

Histopathologic analysis of 1-μm epoxy sections showed that lesions within the CNS of pMOG35–55 actively and adoptively induced EAE differed markedly (Table I). In the actively induced form at initial onset of signs in B6 mice, lesions occurred mostly in the spinal cord and were diffusely distributed in subpial regions. They displayed an infiltrate comprising large mononuclear cells,
small lymphocytes, and a modest number of neutrophils (Fig. 6A). Demyelinated axons were common, and around the margins of affected areas, occasional fibers undergoing Wallerian degeneration (vacuolation of myelin sheath and disintegration or swelling of the axon) were in evidence. By 6 days after onset of actively induced EAE in B6 wild-type mice, lesions were well demarcated, the CNS infiltrate comprised small lymphocytes, large mononuclear cells, and a few plasma cells, but neutrophils were rare to absent. Demyelinated axons were present, as was a narrow zone of fibers undergoing Wallerian degeneration at the lesion edge (Fig. 6B). This zone contained large myelin-bound vacuoles, some of which surrounded much enlarged dystrophic and degenerating axons.

B6 and RAG-1−/− B6 mice with EAE adoptively transferred by CD8+ pMOG35–55-responsive T cell lines displayed more widespread and more severe CNS lesion activity during the acute phase. Within cerebral white matter 2 days after clinical onset, diffuse inflammatory activity, demyelination, and Wallerian degeneration were apparent (data not shown). A significant percentage of the infiltrating cells (>30%) were neutrophils. A similar pattern of destruction was seen in the brainstem (Fig. 6C) where many neutrophils and monocytes, plus the occasional small lymphocyte, constituted the CNS infiltrate. Lesions in the spinal cord of CD8+ pMOG35–55 mice at the same interval (after 2 days of signs) were also more extensive than in their actively induced counterparts, being extensively infiltrated, particularly by neutrophils, and displayed a broader zone of nerve fiber damage. Large mononuclear cells and neutrophils percolated throughout the adjacent myelinated white matter, and small lymphocytes were rare (Fig. 6D). pMOG35–55 adoptively transferred EAE did not remit, and 15 days after onset, large areas of ongoing nerve fiber destruction rich in infiltrating cells were still in evidence within the CNS parenchyma (Fig. 7A). Within the leptomeningeal space, an extensive, closely packed population of infiltrating cells, many of them macrophages laden with lipid droplets and myelin debris, was also seen. This extensive, later involvement of macrophages probably coincided with the enhanced B4 (isolecitin) positivity seen by immunocytochemistry (see above). At higher magnification, demyelinated axons could be discerned (Fig. 7B), but these were usually overshadowed by the extensive Wallerian degeneration.

Adoptively transferred pMOG35–55 EAE animals sampled at later chronic time points displayed an abundance of lesions at different stages, some showing stable chronic disease, some ongoing relapsing disease, and others even displaying CNS repair. At 54 dpt, large well-defined areas of chronic demyelination, residual damage to nerve fibers represented by an abundance of collapsed myelin sheaths, and a low grade CNS infiltrate were seen in the spinal cord (Fig. 7C). The CNS infiltrate contained the occasional neutrophil and plasma cell but mainly comprised large mononuclear cells and macrophages (Fig. 7, C and D). Apparently well-preserved, demyelinated axons existed alongside fibers that had undergone Wallerian degeneration (Fig. 7D). In one adoptive EAE animal sampled at 76 dpt, both active and reparatory lesions could be seen. Active lesions probably reflected relapsing lesion activity and presented as areas of ongoing demyelination intensively infiltrated by large mononuclear cells and a few small lymphocytes, neutrophils, and plasma cells (Fig. 7E). Again, demyelinated axons coexisted with fibers displaying early and advanced Wallerian degeneration and macrophages laden with myelin debris (Fig. 7F). Elsewhere in the same spinal cord at 70 dpt, areas of longer-standing disease displayed CNS remyelination and fibrous astroglisisis (scarring) (Fig. 7G). Even in these areas of myelin repair, low

FIGURE 6. Neuropathology of the acute phase of actively induced (A and B) and adoptively transferred (C and D) pMOG35–55 EAE (1-μm epoxy sections, stained with toluidine blue). A. Actively induced pMOG35–55 EAE in a B6 mouse; 19 days post inoculation, 6 days after onset. An acutely inflamed lesion is seen in the subpial region of the lower lumbar spinal cord. Note the scattered neutrophils in the infiltrate (short arrows), several fibers undergoing Wallerian degeneration (asterisks), and demyelinated axons (long arrows) (magnification, ×750). Bar = 10 μm. B. Actively induced pMOG35–55 EAE in a B6 mouse; paralysis for 5 days; 24 days post inoculation. A similar lesion from the lower lumbar cord shows an infiltrate comprising large mononuclear cells and a few small lymphocytes, but no neutrophils. Some proliferation of fibrous astrocytes (left, middle) is seen within the parenchyma. Demyelinated axons (arrows) and fibers undergoing Wallerian degeneration are also apparent (magnification, ×750). Bar = 10 μm. C. Adoptively transferred pMOG35–55 EAE in a B6 mouse; paralysis for 2 days; 10 dpt. An area of cerebellar white matter displays widespread Wallerian degeneration (asterisks) and a few demyelinated axons (arrows). The CNS infiltrate is rich in neutrophils (lower right and upper left) (magnification, ×750). Bar = 10 μm. D. Adoptively transferred pMOG35–55 EAE, same animal as in C. An area of thoracic spinal cord shows an acute lesion containing a nest of neutrophils adjacent to a fiber undergoing Wallerian degeneration (center). Demyelinated axons are present among the neutrophils but are difficult to discern. A few extravasated red blood cells are also in evidence (arrows) (magnification, ×750). Bar = 10 μm.
grade inflammatory actively (mainly by large mononuclear cells) was in evidence. In general, the blood-brain barrier remained structurally intact and widespread red cell extravasation (hemorrhage) was not a marked feature of either the active or adoptive forms of pMOG35–55 EAE. However, in a few lesions from animals sampled during the acute phase of adoptive disease, an occasional extravasated red cell was detected (Fig. 6D), indicating that this form of the disease appeared to have a greater effect upon the blood-brain barrier.

Persistence of MOG-specific T cells in animals actively immunized with pMOG35–55 or in recipient B6 mice adoptively transferred with MOG-specific T cells

In parallel with chronic disease expression, MOG-specific T cells could be detected in the draining lymph nodes and the spleen of immunized animals for >6–8 mo after single immunization. This contrasted significantly with MBP-induced EAE in the Lewis rat in which MBP-reactive T cells became undetectable following the acute phase of the disease (9–20 dpi). pMOG35–55-specific T cells were readily detectable in both spleen and lymph node >240 days after immunization (Fig. 3A). After activation, splenic T cells from MOG-immunized mice remained fully encephalitogenic after >240 dpi, when tested by adoptive transfer into naive recipients (data not shown).

Delayed onset of adoptively transferred EAE in RAG−/− mice

The onset of transferred disease in B6 mice regularly started at 7–8 days after cell injection. In contrast, initiation of a transferred disease in RAG−/− mice varied greatly. In four separate experiments, a total of 15 RAG−/− mice were studied. All mice had delayed onset, varying from weeks to months. It was not unusual to observe recipient RAG−/− mice apparently healthy for 60–80
FIGURE 8. MOG-specific T cells induce progressive EAE in both B6 and RAG-1−/− mice; however, RAG-1−/− mice have a severely delayed onset. B6 and RAG-1−/− mice were i.p. administered with 2 × 10^6 newly activated MOG-specific T cells. The animals were recorded daily for weight loss and clinical signs for >90 consecutive days.

Discussion

Immunization of rodents with MBP or PLP consistently stimulates autoreactive T cell subsets that are CD4^+αβTCR^+ cells, which, upon transfer to syngeneic naive animals, cause paralytic disease. This has led to the generally held view that effector cells in EAE are invariably CD4^+αβTCR^+ cells. Recent studies have shown that T cell subsets reactive to myelin PLP contain CD8^+ T cells (35). It was also observed that T cells infiltrating active MS lesions are dominated by CD8^+ T cells (36). Here, we provide strong evidence that a synthetic peptide of MOG has the unexpected ability to induce CD8^+ encephalitogenic T cells. Adoptive transfer of MOG-specific T cells caused a much more severe and progressive form of EAE (particularly histopathologically) than active immunization of mice for disease induction, and such T cells could be consistently re-isolated from recipient animals.

Because the above finding is in marked contrast to our earlier studies using dozens of peptides derived from other myelin Ags such as MBP and PLP, which elicited reactive populations overwhelmingly dominated by cells of the CD4^+αβTCR^+ phenotype, we made a special effort to verify that CD8^+ MOG-specific T cells were indeed pathogenic. The encephalitogenic activity observed was not due to the presence of a small fraction of CD4^+ MOG-specific T cells in the T cell lines. First, transfer of as few as 0.5 × 10^6 CD8 T cells to naive B6 or RAG-1−/− mice readily induced paralysis in syngeneic recipients, but not in isogenic β2m−/− mice. Presumably, due to the failure of these latter mice to express intact MHC class I molecules, CD8^+ effector T cells did not remain functionally intact (37). Second, ablation of CD4^+ cells from the transferred T cell population had no effect on the development of EAE in recipients. Indeed, in adoptively transferred mouse EAE, the minimal number of CD4^+ cells needed to induce disease is in the range of 5 × 10^6; it is unlikely that a small percentage of CD4^+ cells from a total of 0.5 × 10^6 CD8-dominated cells would account for the disease activity. Third, an increased number of
pMOG<sub>35–55</sub>-activated T cells binds to recombinant class I molecules (H-2D<sup>b</sup>) bound by pMOG<sub>35–55</sub>, but not to the same MHC molecules bound by a control peptide NP<sub>366–374</sub>. It is necessary to point out that our conclusion that CD8<sup>+</sup> MOG-specific T cells played a major pathogenic role in EAE did not exclude a patho-
genic role for CD4<sup>+</sup> MOG-specific T cells, because CD4<sup>+</sup> cells may well be required for the efficient recruitment of naive auto-
mobile T cells in vivo. For example, we could have shown that MHC class II<sup>-/-</sup> mice are susceptible to adoptively transferred disease induced by CD8<sup>+</sup> MOG-specific T cells, but they are re-
sistant to actively induced disease (data not shown). These obser-
vations indicate that CD4<sup>+</sup> cells are essential for the initiation of disease, and/or development of CD8<sup>+</sup> effector cells may need the
help of CD4<sup>+</sup> T cells; however, once activated, CD8<sup>+</sup> encephalito-
genic T cells can cause disease in the absence of CD4 T cell help. Studies are underway to determine whether the two MOG-
specific T cell subsets examined herein have other synergistic ef-
effects in the pathogenesis of EAE and whether blockade of the
progression of EAE will be more effective if CD8<sup>+</sup> encephalito-
genic T cells are targeted. Although MBP is the most commonly used Ag in the induction of EAE, recent studies have demonstrated encephalitogenic activ-
ity in a much broader range of myelin Ags, including PLP, myelin-
associated glycoprotein, MOG, and myelin oligodendrocyte basic protein. Moreover, individual myelin proteins may exist as multi-
ple isoforms and isomers generated by alternative exon use or
posttranslational modification (38–40), thus forming “new autoan-
tigens” that induce EAE by eliciting distinct or different subsets of
pathogenic T cells (41). It remains to be determined whether the
pathogenesis of the human disease, MS, involves more than one
myelin protein, depending on the genetic makeup of the patients
and environmental factors predisposing disease development. It is
also important to determine whether different myelin proteins, or
different isomers of a given myelin protein, induce similar disease
by eliciting different immune responses, perhaps involving distinct
pathogenic mechanisms.

Due to the fact that growth factors promoting CD8<sup>+</sup> T cell ex-
pansion in vitro are less well characterized than those promoting CD4<sup>+</sup> cells, and that CD8<sup>+</sup> encephalitogenic T cell lines are as yet
unavailable, most treatment regimens of EAE have been linked to
the study of pathogenic CD4<sup>+</sup> T cells and have targeted CD4<sup>+</sup>
cells. For example, the identification of factors important for pro-
moting or inhibiting the activation of CD4<sup>+</sup>-autoreactive T cells has
been the subject of many reports. It is reasonable to assume
that these same identified factors may not have relevance in the
activation of CD8<sup>+</sup>-autoreactive T cells. Indeed, our preliminary
results have also demonstrated that not only are the recognition or
function of CD8<sup>+</sup>-pathogenic T cells restricted by different MHC
molecules, but that inflammatory molecules, such as TGF-β1 and
IL-15, have distinct effects on CD4<sup>+</sup>- and CD8<sup>+</sup>-autoreactive T
cells (data not shown).

Previous studies using EAE models induced by MBP have
shown that the dominant autoimmune cells are CD4<sup>+</sup> (42, 43),
and that the recognition of CD4<sup>+</sup> T cells requires a trimolecular
interaction among TCR, antigenic peptide, and MHC class II. It
has been hypothesized that autoaggressive cells in EAE may attack
parchymal cells with the potential to express MHC class II mol-
ecules (44). In this study, we have shown that CD8<sup>+</sup> MOG-spe-
cific T cells had a wide range of cytotoxic activity. They have been
shown to be cytolytic for astrocytes, oligodendrocytes, and a num-
ber of tumor target cell lines, and the effect is not Ag dependent
(data not shown). It appears that the cytolytic activity of CD8<sup>+</sup>
MOG-specific T cells is less restricted than that of CD4<sup>+</sup>-enceph-
alitogenic T cells, the latter only able to induce killing when target
cells (e.g., astrocytes) are reinduced for expression of MHC class
II. The underlying mechanism for the enhanced pathology encoun-
tered in adoptively transferred animals was perhaps related to an
increased, nonspecific cytotoxic effect exerted by CD8<sup>+</sup> T cells
that led to a progressive bystander destructive effect upon nerve
fibers, rather than a specific demyelinating process. Thus, although
reparatory processes were in place, they were overridden by on-
going destruction. Moreover, the prolonged involvement of neu-
rophils in adoptive EAE may be of pathogenetic significance.

In acute EAE, polymorphonuclear cell invasion is brief, transient,
and precedes macrophage entry (45). Thus the more severe and
protracted involvement of polymorphonuclear cells and macro-
phages in the present CD8 paradigm may bespeak widespread cy-
totoxicity and may belie the observed extensive and long-lasting
isletin reactivity in lesion areas. We have also determined the
infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> cells in brain lesions; however,
we could not show a significantly increased infiltration of CD8<sup>+</sup>
cells in the CNS inflammation. Conceivably, this has been due to the
fact that >95% of infiltrating cells in the CNS inflammation are
nonspecifically recruited as previously reported (46, 47). One un-
expected observation from this study was that adoptive transfer of
CD8<sup>+</sup> pMOG<sub>35–55</sub>-reactive T cells from B6 donors into RAG-
1<sup>-/-</sup> mice caused a delayed onset of disease. We are currently
investigating whether such a unique disease pattern was due to a
lack of regulatory T cells (48, 49), functionally antagonistic to
autoreactive cells in RAG-1-deficient mice. Previous studies have
shown that MBP-reactive T cells have a skewed TCR usage, fre-
quently favoring the expression of Vβ8.2. However, our analysis
of three pMOG<sub>35–55</sub>-specific T cell lines did not reveal any similar
skewing.

We have shown that an increased number of MOG<sub>35–55</sub>-stimu-
lated T cells from MOG-immunized animals were specifically
stained by MOG<sub>35–55</sub>-H-2Db (30) (Fig. 4). Such an observation
supports the notion that MOG<sub>35–55</sub> activates an increased number
of CD8<sup>+</sup> T cells. However, pMOG<sub>35–55</sub> is a 20-mer, which is
much longer than the theoretical peptide length necessary for fit-
ing the MHC class I groove and for stimulation of CD8<sup>+</sup> T cells
(50, 51). In contrast, a recent report has shown that longer peptides
can be accommodated in the MHC class I binding site by a pro-
trusion mechanism (52), a mechanism that may explain our own
observation. We are currently determining whether truncated
pMOG<sub>35–55</sub> peptides containing essential amino acid sequences
will fit better into the binding groove of MHC class I molecules,
show more intense staining by FACs analysis, and exert stronger
stimulatory effects on MOG-specific T cells. Our preliminary
studies indicate that truncated pMOG<sub>40–54</sub> peptides have increased
stimulating activity for CD8<sup>+</sup>, but not CD4<sup>+</sup>, MOG-specific T
cells. Such a study should allow us to determine whether activation
of CD8 MOG-specific T cells requires a specific CD4 response and
whether disease progression requires interactive CD4 and CD8
responses.

In summary, CD8<sup>+</sup>αβ<sup>-</sup> cells comprise a large fraction of the
encephalitogenic T cells in MOG-induced EAE. This newly es-
tablished experimental paradigm should allow us to explore the
mechanism by which CD8<sup>+</sup>-autoreactive T cells mediate this dis-
ease, and to determine immune perturbations elicited by CD8<sup>+</sup>-
and CD8<sup>+</sup>-pathogenic T cells, respectively.

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