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A Transgenic Model of Central Nervous System Autoimmunity Mediated by CD4⁺ and CD8⁺ T and B Cells

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Experimental autoimmune encephalomyelitis (EAE) is a widely used model of multiple sclerosis. In NOD mice, EAE develops as a relapsing–remitting disease that transitions to a chronic progressive disease, making the NOD model the only mouse model that recapitulates the full clinical disease course observed in most multiple sclerosis patients. We have generated a TCR transgenic mouse that expresses the α- and β-chains of a myelin oligodendrocyte glycoprotein (MOG) 35–55–reactive TCR (1C6) on the NOD background. 1C6 TCR transgenic mice spontaneously generate both CD4⁺ and CD8⁺ T cells that recognize MOG and produce proinflammatory cytokines, allowing for the first time to our knowledge the simultaneous examination of myelin-reactive CD4⁺ and CD8⁺ T cells in the same host. 1C6 CD8⁺ T cells alone can induce optic neuritis and mild EAE with delayed onset; however, 1C6 CD4⁺ T cells alone induce severe EAE and predominate in driving disease when both cell types are present. When 1C6 mice are crossed with mice bearing an IgH specific for MOG, the mice develop spontaneous EAE with high incidence, but surprisingly the disease pattern does not resemble the neuromyelitis optica-like disease observed in mice bearing CD4⁺ T cells and B cells reactive to MOG on the C57BL/6 background. Collectively, our data show that although myelin-reactive CD8⁺ T cells contribute to disease, disease is primarily driven by myelin-reactive CD4⁺ T cells and that the coexistence of myelin-reactive T and B cells does not necessarily result in a distinct pathological phenotype. The Journal of Immunology, 2012, 188: 2084–2092.
that resembles neuromyelitis optica (NMO), a subtype of MS (19,
20). However, the disease phenotype that results when both CD4+ and CD8+ T cells as well as B cells reactive to myelin Ag are present in the same individual, a situation that likely best replicates the in vivo situation in MS patients, has never been examined. In this study, we describe a TCR transgenic model of EAE on the NOD background that allows for the first time to our knowledge the examination of CNS autoimmunity when both CD4+ and CD8+ T cells as well as B cells that recognize the same myelin Ag are present.

Materials and Methods
Generation of MOG 35–55–specific TCR transgenic mice on NOD background
The TCR α (Vα5Jα22) and β (Vβ7Dβ2Jβ2.7) regions of a CD4+ T cell clone (1C6) specific for MOG 35–55 (MEVGWYRSPFSRVVHLYRNGK) were amplified by PCR from genomic DNA and cloned into the PTα and PTβ genomic expression vectors (21). Linearized TCR-containing constructs were injected directly into the pronuclei of fertilized NOD oocytes. Transgenic founders were identified by PCR using primers specific for the Vα and Vβ regions. Three independent transgenic founders were identified and bred with NOD mice (Taconic Farms). Animals were housed in a specific pathogen-free facility and maintained in accordance with the guidelines established by the Committee on Animals of Harvard Medical School. IgH MOG mice on the C57BL/6 background (18) were back-crossed onto the NOD background for five generations prior to intercrossing with 1C6 TCR transgenic mice.

Flow cytometry
Single-cell suspensions from thymus, lymph node, or spleen were prepared and stained with the indicated Abs. All Abs were purchased from eBioscience, BD Biosciences, and BioLegend. All flow cytometry data were collected on a BD FACSCalibur or LSRII (BD Biosciences) and analyzed with FlowJo Software (Tree Star).

Proliferation assays and cytokine measurement
Splenocytes (4 × 10⁵/well) were cultured in triplicate in 96-well plates in the presence of the indicated concentration of Ag. CD4+ and CD8+ T cells (1 × 10⁵/well) were cultured in triplicate in 96-well plates in the presence of the indicated concentration of Ag and irradiated wild-type NOD splenic
APC (5 × 10^5). At 48 h, supernatants were collected for the measurement of cytokines by either ELISA or cytometric bead array (CBA) (BD Biosciences) according to the manufacturer’s protocol. Plates were then pulsed with 1[^H]Hthymidine and harvested 16 h later. Thymidine incorporation was measured using a β-scintillation counter (1450 Microbeta; PerkinElmer).

**Degranulation assay**

Mice were immunized with 10 μg MOG 35–55 peptide emulsified in CFA (Difco) supplemented with 4 mg/ml Mycobacterium tuberculosis (H37RA; Difco). Each mouse also received 200 ng pertussis toxin (List Biological Laboratories) intravenously on days 0 and 2. Draining lymph nodes were harvested on day 7 and cultured in medium at 2.5 × 10^6/well in the presence of MOG 35–55 peptide (0–50 μg/ml), PMA (Sigma), and ionomycin (Sigma) or medium as an unstimulated control. LAMP-1 Ab (CD107a; BioLegend) and monensin (GolgiStop; BD Biosciences) were added to cultures containing PMA and ionomycin at the beginning of the incubation for 4 h at 37˚C. Cultures stimulated with MOG 35–55 were preincubated for 2 h before the addition of monensin and LAMP-1 Ab. Cells were then stained for cell surface expression of CD8 and intracellular expression of granzyme B, IFN-γ, IL-2, and TNF. Flow cytometric data were collected on a BD LSRII (BD Biosciences) and analyzed with FlowJo Software (Tree Star).

**Induction and assessment of EAE**

1C6 transgenic (Tg) mice and wild-type littermate controls were immunized with 10 μg MOG 35–55 emulsified in CFA (Difco) supplemented with 4 mg/ml M. tuberculosis (H37RA; Difco). Each mouse also received 200 ng pertussis toxin (List Biological Laboratories) intravenously on days 0 and 2. Clinical disease was assessed as follows: 0, no disease; 1, decreased tail tone; 2, hind limb paresis; 3, complete hind limb paralysis; 4, forelimb and hind limb paralysis; 5, moribund state.

**Adoptive transfer experiments**

Sorted 1C6 CD4+ or CD8+ T cells (1 × 10^6 to 1.75 × 10^6) were injected intravenously into NOD.Scid mice. After 21 d, mice were immunized with 25–50 μg MOG 35–55 emulsified in CFA and given 200 ng pertussis toxin intravenously on days 0 and 2 postimmunization. Clinical disease was assessed as described earlier.

**Histopathology**

When animals were moribund or at the end of the experiment, they were sacrificed, and brains and spinal cords were fixed in 10% formalin, processed for histologic analysis, and evaluated as described (22). Specifically, inflammatory foci (>10 perivascular mononuclear cells) were counted in leptomeninges and CNS parenchyma in Luxol fast blue–H&E–stained paraffin sections of the entire CNS of each mouse by a neuropathologist who was blinded to the clinical status and genotypes. Each sample counted contained representative 8-μm-thick sections of five levels of brain tissue and 15–20 sections of spinal cord tissue. Thus, both the sampling and total areas of CNS tissue analyzed were uniform for each mouse. In some analyses, the localization of parenchymal inflammatory foci in cerebrum, brain stem, cerebellum, and spinal cord were separately determined. Optic neuritis was scored as either present or absent for each mouse.

**FIGURE 2.** T cell responses in 1C6 TCR Tg mice. (A and B) Splenocytes from 1C6 Tg (line no. 1183) and wild-type littermates were stimulated with MOG 35–55 as indicated (A) and rMOG (B) at 50 μg/ml. (C) Sorted CD4+ and CD8+ T cells from 1C6 mice were stimulated with MOG 35–55 (100 μg/ml) and rMOG (50 μg/ml). Proliferation was determined by [3H]thymidine incorporation of triplicate wells. At 48 h, supernatants were harvested for measurement of production of the indicated cytokines by CBA. Data shown are representative of two to three independent experiments for each founder line. Error bars indicate SEM.
Isolation and analysis of cells from CNS
After perfusion, CNS tissue (brain and spinal cord) was digested with collagenase (2.5 mg/ml; Sigma) prior to centrifugation on a discontinuous Percoll gradient (GE Healthcare). Cells were then stimulated with PMA (50 ng/ml) and ionomycin (1 μg/ml) in the presence of a Golgi transport inhibitor for 3 h prior to staining with 7-amino-actinomycin-D and Abs against CD4 and CD8. After fixation, cells were permeabilized and stained with Abs against IFN-γ and IL-17. Flow cytometry data were collected on an LSRII (BD Biosciences).

Results
Generation of 1C6 TCR Tg mice and selection of 1C6 TCR Tg T cells
We isolated a CD4+ T cell clone from an NOD mouse immunized with MOG 35–55. This clone called 1C6 expressed a TCR composed of Vα5 and Vβ7. The rearranged TCRα and TCRβ chains from the 1C6 clone were isolated and cloned into expression cassettes and microinjected into NOD oocytes. We then screened progeny for the presence of the Tg Vα and Vβ and successfully identified three independent founders (data not shown).

We have analyzed all three 1C6 founder lines and have not observed any gross differences in thymic cellularity in 1C6 Tg mice relative to wild-type littermate controls (data not shown). When we analyzed CD4, CD8, and Vβ7 expression in thymocytes, we were surprised to find that there was not a strong bias toward CD4 single-positive T cells in 1C6 Tg mice relative to wild-type littermate controls and that there was prominent expression of Vβ7 on both CD4SP and CD8SP thymocytes in 1C6 Tg mice (Fig. 1).

Accordingly, we observed similar CD4/CD8 ratios in the spleen and lymph nodes of 1C6 Tg mice relative to wild-type littermate controls as well as expression of Vβ7 on both CD4+ and CD8+ T cells in the spleen and lymph nodes of 1C6 TCR Tg mice (Fig. 1 and data not shown). Indeed, we have observed the presence of Vβ7-positive CD8+ T cells in three independent Tg lines (data not shown), indicating that the presence of CD8+Vβ7+ cells is not due to an integration artifact. We next addressed whether the CD8+ T cells from 1C6 Tg mice expressed a rearranged Vα5 gene. Indeed, we found that 1C6 CD8+ T cells express a rearranged Vα5 gene and thus should have specificity for MOG 35–55 (Supplemental Fig. 1). We further examined the extent of allelic exclusion at the TCR α locus and found that 1C6 CD8+Vβ7+ T cells exhibit rearrangement of some endogenous α-chains but not others (Supplemental Fig. 1). Lastly, we examined whether the frequency of regulatory T cells was altered by the presence of the 1C6 transgene and found that the frequency of CD4+Foxp3+ cells is the same in wild-type and 1C6 Tg mice (Supplemental Fig. 2).

Responses to MOG in 1C6 TCR Tg mice
Next, we tested responsiveness of spleen cells from 1C6 Tg mice to MOG 35–55 peptide and recombinant MOG (rMOG) protein (consisting of the extracellular domain). Splenocytes from 1C6 Tg mice showed a strong proliferative response to both MOG 35–55 peptide and rMOG (Fig. 2A, 2B). In terms of cytokine, the response was dominated by production of IFN-γ and IL-17. Other cytokines such as TNF, IL-6, and IL-10 were variable and were
Collectively, these data demonstrate that 1C6 CD4+ and CD8+ cytokine, the response was again dominated by IFN-γ CD8+ cells (14, 15). We isolated draining lymph nodes and CFA has been shown previously to recruit MOG 35–55–reactive MOG 35–55 in CFA. Indeed, immunization with MOG 35–55/2C). We also addressed the MHC restriction of 1C6 CD8+ T cells and found that they can recognize MOG 35–55 in the context of both MHC class I and class II (Supplemental Fig. 3). In terms of cytokine, the response was again dominated by IFN-γ and IL-17. Collectively, these data demonstrate that 1C6 CD4+ and CD8+ T cells can develop spontaneous disease, they do not do so at an appreciable frequency.

Given that 1C6 Tg mice harbor both CD4+ and CD8+ T cells that respond to MOG, we sought to address the relative contribution of CD4+ and CD8+ T cells to disease. We therefore isolated highly purified populations of CD4+ and CD8+ 1C6 T cells from unimmunized mice and transferred them either alone or together into NOD.Scid recipients. We allowed 21 d for reconstitution of the peripheral lymphoid compartment. None of the mice developed spontaneous disease during this time. Therefore, we immunized mice with MOG 35–55/CFA. Mice that received 1C6 CD4+ T cells alone developed typical EAE with almost 100% incidence and good severity (Fig. 4, Table I). Mice that received both 1C6 CD4+ and CD8+ T cells developed disease with nearly identical clinical courses as mice that received CD4+ T cells alone. In contrast, mice that received 1C6 CD8+ T cells alone developed mild disease with a much lower clinical incidence. Histopathologic analysis revealed predominance of spinal cord lesions in all three groups (data not shown). Notably, there was a large discordance between incidence of clinical disease and histological disease (48% versus 70%, respectively) in recipients of 1C6 CD8+ T cells alone (Table I). Also noteworthy is that there was a trend toward a higher incidence of optic neuritis in mice that received both 1C6 CD4+ and CD8+ T cells relative to mice that received either 1C6 CD4+ T cells or 1C6 CD8+ T cells alone; however, this did not reach statistical significance.

Next examined active induction of disease in 1C6 Tg mice. To do this, we immunized 1C6 Tg mice with a low dose of MOG 35–55. With this dose of MOG 35–55, 1C6 Tg mice developed disease by day 9, whereas none of the wild-type littermates developed disease. Disease consisted of an initial wave of mild severity that resolved completely. This was then followed by a relapse of greater severity that did not resolve completely. After the second wave of disease, 1C6 Tg mice developed chronic disease (Fig. 5A). Thus, 1C6 Tg mice exhibit the progression of relapsing–remitting chronic EAE typically observed in NOD mice. Examination of the CNS of 1C6 Tg mice with EAE showed the presence of both CD4+ and CD8+ T cells, although CD4+ T cells outnumbered CD8+ T cells by ~30-fold (Fig. 5B). The CD4+ T cells in the CNS of 1C6 Tg mice consisted of cells producing IFN-γ, IL-17, and IFN-γ plus IL-17 (Fig. 5C). IL-17–producing cells appeared to be the most abundant population of CD4+ T cells; hence, we used this subset of cells to study effector function in disease.

Disease in 1C6 Tg mice

MOG 35–55 TCR Tg mice on the C57BL/6 background develop spontaneous optic neuritis as well as EAE, albeit at very different frequencies (>30% versus 4%, respectively) (8). We therefore followed 1C6 Tg mice for the development of spontaneous disease. We have observed >400 mice and found only 5 mice with spontaneous optic neuritis and 4 with spontaneous EAE. These cases were confirmed by histopathology and occurred in both male and female Tg mice (data not shown). Thus, whereas 1C6 Tg mice can develop spontaneous disease, they do not do so at an appreciable frequency.

We next examined active induction of disease in 1C6 Tg mice. To do this, we immunized 1C6 Tg mice with a low dose of MOG 35–55. With this dose of MOG 35–55, 1C6 Tg mice developed disease by day 9, whereas none of the wild-type littermates developed disease. Disease consisted of an initial wave of mild severity that resolved completely. This was then followed by a relapse of greater severity that did not resolve completely. After the second wave of disease, 1C6 Tg mice developed chronic disease (Fig. 5A). Thus, 1C6 Tg mice exhibit the progression of relapsing–remitting chronic EAE typically observed in NOD mice. Examination of the CNS of 1C6 Tg mice with EAE showed the presence of both CD4+ and CD8+ T cells, although CD4+ T cells outnumbered CD8+ T cells by ~30-fold (Fig. 5B). The CD4+ T cells in the CNS of 1C6 Tg mice consisted of cells producing IFN-γ, IL-17, and IFN-γ plus IL-17 (Fig. 5C). IL-17–producing cells appeared to be the most abundant population of CD4+ T cells.

Table I. Disease induced by 1C6 CD4+ and CD8+ T cells

<table>
<thead>
<tr>
<th>Clinical Disease</th>
<th>Histological Disease: Inflammatory Foci</th>
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<tbody>
<tr>
<td></td>
<td>Incidence (%)</td>
</tr>
<tr>
<td><strong>Group</strong></td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>23/24 (96)</td>
</tr>
<tr>
<td>CD8</td>
<td>11/23 (48)</td>
</tr>
<tr>
<td>CD4 + CD8</td>
<td>16/19 (84)</td>
</tr>
</tbody>
</table>

Data are pooled from six independent experiments.

*Values are mean ± SEM.
however, this was only significant when compared with cells producing both IFN-\(\gamma\) and IL-17. Although CD8\(^+\) T cells were low in frequency, a large fraction of these cells was producing IFN-\(\gamma\) (Fig. 5C). Lastly, histopathologic analysis showed the presence of typical EAE lesions (data not shown). Collectively, our adoptive transfer and active disease induction data indicate that myelin-reactive CD8\(^+\) T cells can induce optic neuritis and mild clinical disease on their own and can contribute to disease after immunization for EAE; however, myelin-reactive CD4\(^+\) cells appear to have the dominant role in driving disease.

1C6 \(\times\) IgH\(_{\text{MOG}}\) Tg mice

We have next crossed IgH\(_{\text{MOG}}\) knock-in mice (18) onto the NOD background so that in conjunction with 1C6 Tg mice we could study for the first time to our knowledge the immune response and development of disease in hosts that have CD4\(^+\) and CD8\(^+\) T cells as well as B cells that recognize the same myelin Ag, MOG. We first examined responses to MOG 35–55 and rMOG in 1C6 \(\times\) IgH\(_{\text{MOG}}\) mice. Spleen cells from 1C6 \(\times\) IgH\(_{\text{MOG}}\) mice proliferate better in response to stimulation with both MOG 35–55 and rMOG than spleen cells from 1C6 Tg mice (Fig. 6A, 6B). Again, we observed that the cytokine response was dominated by production of IFN-\(\gamma\) and IL-17 with spleen cells from 1C6 \(\times\) IgH\(_{\text{MOG}}\) mice producing more IL-17 than spleen cells from 1C6 Tg mice in response to rMOG (Fig. 6C).

Next, we examined both the clinical and pathological phenotype of disease in 1C6 \(\times\) IgH\(_{\text{MOG}}\) mice. We were particularly interested in the disease phenotype of these mice given that when MOG 35–55-reactive CD4\(^+\) T cells (from 2D2 TCR Tg) and MOG-reactive B cells (from IgH\(_{\text{MOG}}\) knock-in) coexist on the C57BL/6 background, the mice develop spontaneous disease with 60% incidence and exhibit a distinct pathological pattern in which lesions are localized mostly in the spinal cord and optic nerve, thus resembling NMO, a subtype of MS most commonly observed in Asian populations (19, 20). Moreover, those mice exhibit development of ectopic lymphoid follicle-like structures in the spinal meninges (19).

In contrast to 1C6 Tg mice in which we observed only rare cases of spontaneous disease, 1C6 \(\times\) IgH\(_{\text{MOG}}\) mice developed spontaneous disease with high incidence: 45% among males and 79% among females with an average age of onset of 15 wk (Table II).
Examination of the CNS of 1C6 × IgHMOG mice with spontaneous disease revealed the presence of both CD4\(^+\) and CD8\(^+\) T cells. In comparison with 1C6 Tg mice with EAE, 1C6 × IgHMOG mice exhibited more CD4\(^+\) and CD8\(^+\) T cells in the CNS, and CD4\(^+\) T cells only outnumbered CD8\(^+\) T cells by ∼7-fold (Fig. 7A). Similar to 1C6 Tg mice with EAE, CD4\(^+\) T cells in 1C6 × IgHMOG CNS also consisted of IFN-γ–producing, IL-17–producing, and IFN-γ plus IL-17–producing cells. Whereas in 1C6 Tg mice there was a trend toward IL-17–producing cells being the most abundant population of CD4\(^+\) T cells, in 1C6 × IgHMOG mice there was a trend toward IFN-γ–producing cells being the most abundant population of CD4\(^+\) T cells in the CNS. However, this was only significant when compared with IFN-γ plus IL-17–secreting cells (Fig. 7B). As observed in 1C6 Tg mice, the CD8\(^+\) T cells in the CNS of 1C6 × IgHMOG mice predominantly produce IFN-γ (Fig. 7B). Collectively, these data indicate that relative to 1C6 Tg mice with EAE, 1C6 × IgHMOG mice accumulate more CD4\(^+\) (3-fold) and CD8\(^+\) (14-fold) T cells in the CNS and that the majority of these cells produce IFN-γ. Given the disproportionate increase in CD8\(^+\) T cells in the CNS of 1C6 × IgHMOG mice, it is interesting to speculate whether the increased involvement of CD8\(^+\) T cells, at least in part, underlies the high frequency of spontaneous disease observed in these mice relative to 1C6 Tg mice.

In sharp contrast to 2D2 × IgHMOG mice, 1C6 × IgHMOG mice do not exhibit NMO-like lesion distribution. Although the majority of lesions was restricted to the spinal cord, less than half of the mice (40%) had optic nerve lesions (Tables II and III); there was also no evidence of ectopic follicle-like structures in the CNS. These data indicate that the coexistence of myelin-reactive T and B cells does not necessarily result in an NMO-like disease phenotype. Rather, the genetic background in conjunction with T and B cell reactivity appear to influence the lesion distribution in the CNS and the development of ectopic follicle-like structures in this disease.

In addition to the lack of NMO-like disease, we also made a surprising observation in 1C6 × IgHMOG mice that did not develop signs of classical EAE. These mice were sacrificed for use as controls for experiments or because they had reached greater than 20 wk of age and did not exhibit any overt signs of neurologic disease at the time of sacrifice. Upon histopathological examination of the CNS, we observed the presence of CNS lesions in more than half of these mice (Table III). Of these, 75% exhibited lesions localized exclusively to the cerebellum, indicating the presence of a pathologically distinct neurologic disease.

**Discussion**

Whereas EAE shares many clinical and histological features with MS, it has been argued that EAE is not an adequate model for MS. In most EAE models, disease is induced by immunization with MHC class II-restricted myelin Ags. Consequently, disease is driven largely by CD4\(^+\) T cells. In MS, however, there is considerable evidence that other lymphocytes such as CD8\(^+\) T cells and B cells likely play important pathogenic roles. In the case of CD8\(^+\) T cells, it is known that CD8\(^+\) T cells outnumber CD4\(^+\) T cells in MS lesions and exhibit oligoclonal expansion (9–11). Although the presence of CD8\(^+\) T cells in CNS lesions is well established, it has been debated whether the CD8\(^+\) T cells are playing a pathogenic role or protective role. Myelin Ag-specific CD8\(^+\) T cells have been shown to induce EAE (13–15), but studies of EAE in CD8-deficient mice suggest that CD8\(^+\) T cells may also have a protective role (23, 24).

Recently, some TCR Tg models of CD8\(^+\) T cell-driven EAE have been generated. Two of these rely on expression of CD8\(^+\) Tg TCRs specific for MBP. These models have been useful for the study of how MBP-specific CD8\(^+\) T cells undergo tolerance (25, 26). A unique feature of one of these models is the fact that disease can be induced by both control and MBP-expressing vaccinia virus. The disease induced by the two different viruses is indistinguishable and is characterized by weight loss, ataxia, and tail weakness (27). The induction of disease by control vaccinia virus

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**Table II. Spontaneous classical EAE in 1C6 × IgHMOG mice**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Incidence (%)</th>
<th>Mean Onset ± SEM, d</th>
<th>Mean Maximum Score ± SEM</th>
<th>Meninges(^a)</th>
<th>Parenchyma(^a)</th>
<th>Total (n)(^b)</th>
<th>Optic Neuritis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>5/11 (45)</td>
<td>105 ± 21.1</td>
<td>2.3 ± 0.2</td>
<td>18.3 ± 5.8</td>
<td>32 ± 13.1</td>
<td>50.3 ± 15.5 (3)</td>
<td>2/3 (67)</td>
</tr>
<tr>
<td>Female</td>
<td>11/14 (79)</td>
<td>105.7 ± 11.4</td>
<td>2.55 ± 0.18</td>
<td>54.9 ± 23.6</td>
<td>53.3 ± 27.6</td>
<td>108.1 ± 51.0 (7)</td>
<td>2/8 (25)</td>
</tr>
<tr>
<td>Total</td>
<td>16/25 (64)</td>
<td>105.6 ± 9.9</td>
<td>2.47 ± 0.14</td>
<td>23.6 ± 5.8</td>
<td>32 ± 13.1</td>
<td>51.0 ± 7.1 (5)</td>
<td>4/11 (36)</td>
</tr>
</tbody>
</table>

\(^a\)Values are mean ± SEM.

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**FIGURE 7.** Disease in 1C6 × IgHMOG mice. (A and B) Mononuclear cells were isolated from the CNS of 1C6 and 1C6 × IgHMOG mice with EAE (score 2–2.5). Cells were stimulated with PMA/ionomycin prior to staining with Abs against CD4 and CD8 and the examination of intracellular cytokine production by flow cytometry. Data shown are the mean of three independent experiments. Error bars indicate SEM. Data shown are representative of two to three independent experiments for each founder line. \(^*p = 0.0002\) (unpaired t test), \(**p = 0.014\) (unpaired t test), \(***p < 0.05\) (one-way ANOVA, Tukey’s multiple comparison test).
is due to the activation of Tg T cells through endogenously rearranged TCR chains, thus supporting a role for dual-TCR-expressing cells in disease induction. A third model of CD8+ T cell-induced CNS autoimmunity consists of a human myelin proteolipid protein-reactive TCR expressed in an HLA-A3 Tg mouse (28). In these mice, there is little spontaneous disease, but after immunization, ~70% of mice develop mild weakness and 25% go on to develop more severe disease that includes hind limb paralysis. Notably, the development of hind limb paralysis was found to be dependent on the recruitment of endogenous CD4+ T cells reactive to other myelin epitopes. This observation is reminiscent of our data showing that whereas CD8+ T cells can induce mild disease and optic neuritis on their own, CD4+ T cells are the main drivers of disease.

Other models using adoptive transfer of MOG 35–55–reactive CD8+ T cells or MBP-reactive T cell clones into lymphopenic hosts also support a pathogenic role for CD8+ T cells in disease (13–15); however, the study by York et al. (29) found that adoptive transfer of MOG 35–55–reactive CD8+ T cells alone does not induce clinical disease. Unfortunately, these recipient mice were not examined histopathologically for the presence of subclinical disease and/or optic neuritis as we have observed in the majority of mice receiving CD8+ T cells alone in our study. In contrast, the study by York et al. (29) shows that adoptive transfer of MOG 35–55–reactive CD8+ T cells into mice at the peak of disease or into mice subsequently immunized for EAE can ameliorate disease. These findings are in line with the studies in CD8-deficient mice that suggest a protective/regulatory role for CD8+ T cells in EAE (23, 24). Our data showing the presence of IFN-γ-secreting CD8+ T cells in the target organ of 1C6 Tg mice with both active disease induction and spontaneous disease further support a pathogenic role for CD8+ T cells in EAE. Thus, the majority of studies all using different methodologies (adoptive transfer of in vitro-activated CD8+ T cells, immunization of mice that receive CD8+ T cells alone, examination of CD8+ T cells in situ during induced and spontaneous disease) support a pathogenic role for CD8+ T cells in EAE. Although these data do not exclude a protective/regulatory role for CD8+ T cells in CNS autoimmunity, it seems that such a role may be more the exception than the rule and may only manifest in specific circumstances.

As in MS, genetics has major effects on susceptibility and manifestations of EAE. Accordingly, genetic studies of EAE have identified a number of disease-modifying loci. Notably, many of these overlap with loci that have been identified in studies of type 1 diabetes in the NOD mouse, giving rise to the concept that there are “common autoimmune genes” (1–3). In the NOD mouse, many congenic strains in which type 1 diabetes susceptibility loci (Idd) have been introgressed from resistant strains onto the NOD strain have been generated. The 1C6 TCR Tg mouse now provides a tool that can be used in conjunction with NOD.Idd congenic mice to study the effects of Idd loci on both the selection and effector function of myelin-reactive CD4+ and CD8+ T cells.

The examination of 1C6 × IgHMOG mice reveals that there is a gender bias in the development of spontaneous disease. This was not observed in the 2D2 × IgHMOG mice on the C57BL/6 background. Moreover, on the NOD background, the mice do not develop NMO-like disease nor are any ectopic follicle-like structures seen in the CNS. Whether these differences are due to genetic differences in the NOD background or the presence of MOG-reactive CD8+ T cells in 1C6 × IgHMOG mice remains to be determined. Collectively, these data indicate that the simple coexistence of myelin-reactive CD4+ T cells and B cells with the same Ag specificity does not result in an NMO-like disease phenotype.

Why some 1C6 × IgHMOG mice developed classical EAE and others developed exclusive cerebellar inflammation is unclear. Whether differential involvement of the CD4+ T, CD8+ T, and B cell response is responsible for these two different disease pathologies requires further examination. To accomplish this, it would be necessary to identify mice with cerebellar inflammation antemortem; for example, using additional tests of cerebellar function, such as the rotarod and treadmill/gait tests. In summary, we have developed a novel TCR Tg model of EAE that capitalizes on the unique features of disease and the tools available on the NOD background. Serendipitously, our model spontaneously generates both CD4+ and CD8+ T cells that express the Tg TCR and recognize MOG 35–55, allowing for the first time to our knowledge the examination of the relative roles in disease of these two important cell types both in isolation and together. We have found that whereas CD8+ T cells alone can induce mild CNS disease, CD4+ T cells are the main drivers of CNS autoimmunity. Additionally, we have found that the simple presence of CD4+ T cells and B cells with the same myelin Ag specificity does not necessarily result in the induction of NMO-like disease.

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### Disclosures

The authors have no financial conflicts of interest.

### References


