Bystander CD8 T Cell-Mediated Demyelination After Viral Infection of the Central Nervous System

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Multiple sclerosis, a chronic inflammatory disease of the CNS, is characterized by immune-mediated demyelination. Many patients have a remitting-relapsing course of disease with exacerbations often following unrelated microbial illnesses. The relationship between the two events remains obscure. One possibility is that T cells specific for the inciting microbial pathogen are able to effect demyelination at a site of ongoing infection within the CNS. This possibility was examined in mice infected with mouse hepatitis virus, a well-described model of virus-induced demyelination. Using transgenic TCR/recombination activation gene 2−/− mice with only non-mouse hepatitis virus-specific T cells, we show that CD8 T cells are able to cause demyelination in the absence of cognate Ag in the CNS, but only if specifically activated. These findings demonstrate a novel mechanism for immune-mediated neuropathology and show that activated CD8 T cells may serve as important mediators of bystander demyelination during times of infection, including in patients with multiple sclerosis. The Journal of Immunology, 2002, 169: 1550–1555.

In certain autoimmune diseases, including diabetes, myocarditis, and herpes stromal keratitis, microbial infections often precede the onset of clinical disease (1–4), or correlate with relapses, most notably in the case of multiple sclerosis (MS) (5–8). MS, a common chronic inflammatory disease of the CNS, is characterized by periodic relapses of neurological disease (9). Several studies reveal a temporal correlation between microbial infections, most commonly upper respiratory tract infections, and subsequent or coincident relapse (5–8). Several possible mechanisms may explain this link between infection and aberrant immune responses (reviewed in Ref. 10). These include molecular mimicry, in which peptides derived from invading organisms cross-stimulate autoreactive T cells, and epitope spreading, in which tissue destruction results in presentation of previously sequestered self Ags to autoreactive T cells not specific for the foreign organism. Alternatively, autoimmune T cells may undergo bystander activation and proliferation by exposure to high levels of cytokines at sites of inflammation. A fourth possibility is that highly activated T cells responding to an unrelated infection may enter sites of chronic inflammation. Interactions between the activated T cells, resident cells, and soluble mediators at the site of inflammation may result in bystander pathology. Given all of these scenarios, the connection between microbial infections and relapses experienced by patients with MS remains a poorly understood phenomenon.

Immunocompetent mice infected with the neurotropic coronavirus mouse hepatitis virus (MHV), strain JHM, develop acute and chronic demyelinating diseases with clinical and pathological findings similar to those observed in patients with MS (11, 12). By contrast, mice that are immunodeficient, such as recombination activation gene (RAG) knockout mice or SCID mice, do not develop demyelination despite harboring large viral loads (13, 14). Adoptive transfer of T cells from C57BL/6 (B6) mice immunized with MHV to infected RAG 1−/− mice resulted in inflammation, activation of macrophages, and demyelination within 7 days post-transfer (15).

Accumulated evidence from multiple studies indicates that migration of T cells into the inflamed CNS is not Ag specific, and is not dependent on activation status (16–19). However, T cells that recognize Ags present in the CNS are preferentially retained (16). After splenocytes were transferred from B6 mice to MHV-infected RAG 1−/− mice, a large fraction of CD4 and CD8 T cells present in the CNS recognized MHV-specific epitopes (20–22). However, T cells with unidentified specificities were also present in the CNS after adoptive transfer, and in immunocompetent mice directly infected with MHV (15, 22). Although some may have been specific for novel CD4 or CD8 T cell epitopes from MHV, others were likely to recognize epitopes not present in MHV or the CNS. The participation of bystander T cells in demyelination has not been investigated. To address this possibility, we infected TCR transgenic/RAG 2−/− mice, which contain monospecific CD8 T cells that do not recognize MHV peptides. The results show that demyelination does occur, but requires specifically activated CD8 T cells. The data highlight a novel mechanism of immune-mediated pathology in the CNS and elucidate an explanation for the observed infection-related exacerbations in diseases such as MS.

Materials and Methods

Mice

(C57BL/6 × C57BL/10SgSnAi)- (transgenic) TCR lymphocytic choriomeningitis virus (LCMV) P14-(knockout) RAG 2 (P14 mice) and B6;129S6-RAG2−/−/TgN (N15 mice) mice were purchased from Taconic Farms (Germantown, NY). C3H/Bl-1/Bl6-RAG 1−/− mice were bred at the University of Iowa.

Virus

The neuroattenuated variant of MHV-JHM, strain J2.2-V-1, was kindly provided by J. Fleming (University of Wisconsin, Madison, WI). In all experiments, mice were infected by intracranial inoculation with \( 1 \times 10^3 \) PFU MHV-J2.2-V-1 in 30 μl DMEM plus 15 mM HEPES (pH 7.0). In
some experiments, P14 mice were infected with $1 \times 10^5$ PFU Armstrong strain of LCMV i.p., generously provided by J. Harty (University of Iowa).

**Peptide injections**

Three days before infection with MHV, some mice received one i.p. injection of 100 μg peptide emulsified in CFA (Sigma-Aldrich, St. Louis, MO). Peptides used were LCMV gp33 (KVAYNFAQT) (provided by M. Buchmeier, The Scripps Research Institute, La Jolla, CA) or vesicular stomatitis virus nucleocapsid protein 52-59 (N52) (RGGYVYQGL) (Biosynthesis, Lewisville, TX).

**RNase protection assays (RPAs)**

RNA was isolated using Tri Reagent, according to the specifications of the manufacturer (Molecular Research Center, Cincinnati, OH). RPAs were performed, as previously described (23), using the probes indicated in Fig. 1 (provided by I. Campbell, The Scripps Research Institute). Templates for the probes were linearized with EcoRI and 32P-labeled antisense RNA probes synthesized using T7 RNA polymerase. We used 5 μg spinal cord RNA for each hybridization reaction. A probe for L32 was included in each assay as an internal control. After hybridization and digestion with RNase, samples were analyzed on denaturing polyacrylamide gels. Gels were then exposed to a phosphor imaging screen.

**Histology**

Spinal cords were harvested, fixed in zinc Formalin, and embedded in paraffin. For myelin examination, 8-μm sections were stained with luxol fast blue and counterstained with H&E.

**Immunohistochemistry**

Spinal cord sections were deparaffinized, rehydrated, and permeabilized with 0.1% Triton X-100 before blocking with CAS block (Zymed Laboratories, San Francisco, CA). Following blocking, sections were incubated with rat anti-mouse F4/80 Ab (C57/10H2A5; Serotec, Oxford, U.K.) or rat Ig (all purchased from BD PharMingen). To detect intracellular IFN-γ, some samples, PE-conjugated anti-mouse CD44 (IM7) or PE-conjugated rat Ig (all purchased from BD PharMingen) were washed and incubated with biotinylated goat anti-rat Ab (Vector Laboratories, Burlingame, CA). Following incubation with streptavidin-HRP (Jackson ImmunolResearch Laboratories, West Grove, PA), Ag was detected using 3,3′-diaminobenzidine (Sigma-Aldrich). Sections were counterstained with hematoxylin.

**Imaging**

Images of spinal cords were digitized using an Optronics camera attached to a Leitz diplan light microscope. Demyelination was quantified, as described previously, using Vtrain software (Image Analysis Facility, University of Iowa) (24).

**FACS analysis**

Lymphocytes were prepared from brains, as previously described (20). For intracellular staining, lymphocytes were incubated with IL-4, which served as APCs, and 1 μM peptide in the presence of monensin (Golgistop; BD PharMingen) for 4–5 h at 37°C. Samples were washed and incubated in blocking buffer (anti-mouse CD16/CD32 Ab (24G.2) in 10% rat serum). After incubation and washing, cells were analyzed using a FACScan flow cytometer (BD Biosciences, San Jose, CA). The number of lymphocytes harvested from each brain ranged between $2 \times 10^6$ and $8.4 \times 10^6$. CNS-derived lymphocytes from individual animals were analyzed in these experiments.

**Quantification of Ag-specific lymphocytes**

Numbers of gp33- or N52-specific lymphocytes were calculated as follows: total number of CNS-derived lymphocytes $\times$ the percentage of CD8 T cells $\times$ (the percentage of peptide-specific T cells $-$ the percentage of cells that expressed IFN-γ in response to irrelevant peptide).

**Results**

**Up-regulation of chemokine and cytokine expression in the CNS of MHV-infected RAG 1−/− mice**

MHV-induced inflammatory cytokines and chemokines are likely to be critical for the rapid recruitment of Ag-specific T cells and macrophages/microglia into the CNS, and likewise may be important in recruitment of bystander T cells. In initial experiments, we analyzed the expression of proinflammatory cytokines and chemokines in the CNS of 10-day MHV-infected, or uninfected RAG 1−/− mice by RPA. mRNA for lymphotixin α (TNF-α), IL-12 p40, IL-6, and IL-1β, but not IL-4, was detected in the MHV-infected RAG 1−/− CNS. We detected low levels of mRNA for lymphotixin α and TNF-α in the CNS of naive RAG 1−/− mice (Fig. 1A), in agreement with previous reports (25, 26). Infection with MHV resulted in up-regulation of several chemokines, including CCL2/macrophage chemoattractant protein-1 ( MCP-1), CCL7/MCP-3, macrophage-inflammatory protein-2 (MIP-2), CXCL10/IP-10, and CCL5/RANTES (Fig. 1B). This profile is identical with the profile of chemokines expressed in the CNS of immunocompetent C57BL/6 (B6) mice after MHV infection (27). Astrocytes are the source for cytokines such as TNF-α and IL-1β, and chemokines such as CXCL10/IP-10, in B6 mice chronically infected with MHV (27, 28). Similarly, astrocytes were also the source for a representative cytokine, TNF-α, in MHV-infected RAG 1−/− mice (Fig. 1C). These results show that a proinflammatory milieu existed in the CNS of MHV-infected RAG 1−/− mice in the absence of demyelination.

**Non-MHV-specific CD8 T cells caused demyelination if specifically activated before infection**

To investigate whether CD8 T cells specific for Ags unrelated to MHV- or CNS-derived Ags were capable of causing demyelination, we infected P14 and N15 transgenic mice crossed onto a RAG 2−/− background. The only T cells in these mice are CD8 T cells specific for residues 33–41 of the LCMV gp33 (P14 mice) or for residues 52–59 of the vesicular stomatitis virus nucleoprotein (N52) (N15 mice). Both P14 and N15 transgenic mice infected with MHV were asymptomatic until days 12–14 postinoculation (p.i.), at which time they developed signs of severe encephalitis.
(ruffled fur, hunched posture, lethargy) and rapidly became moribund. This disease course was identical with that observed in infected nontransgenic RAG 1−/− mice (13). As a control for the experiments described below, uninfected mice were treated with LCMV gp33 emulsified in CFA (CFA:peptide gp33). After this treatment, mice remained asymptomatic throughout the experiment. Histologically, spinal cords harvested from uninfected peptide gp33-treated P14 mice and MHV-infected P14 and N15 mice showed little evidence of demyelination (Fig. 2, A and C, and data not shown) or macrophage infiltration (Fig. 2, B and D, and data not shown). The amount of demyelination in MHV-infected P14 or N15 mice ranged from 0.41 to 1.77% and was not greater than that observed in nontransgenic, infected RAG 1−/− mice (13) (Fig. 3 and Table I).

In marked contrast, P14 mice that had been given a single i.p. injection of CFA:peptide gp33, or N15 mice that had been injected with CFA:peptide N52 i.p. 3 days before infection with MHV exhibited significant amounts of demyelination. CFA:peptide-treated, MHV-infected transgenic mice were asymptomatic until days 12–14 p.i., at which time they developed signs of both encephalitis and hind limb weakness or paralysis (ruffled fur, limp tail, wobbly gait, hind limb weakness, and wasting). Luxol fast blue staining of spinal cord sections from peptide-treated, infected mice revealed multiple focal lesions of myelin destruction (Fig. 2E). Colocalized with these areas of demyelination were large numbers of activated F4/80+ macrophages/microglia (Fig. 2F). Quantification of demyelination in peptide-treated, infected P14 and N15 mice yielded averages of 10.4% (range 5.0–16.5%) and 6.7% (range 4.6–10.5%) for each group, respectively (Fig. 3 and Table I). When we treated P14 transgenic mice with CFA alone, or immunized P14 transgenic mice with CFA:peptide N52 3 days before infection with MHV, we observed no significant demyelination indicating that the ability of non-MHV-specific T cells to mediate demyelination was dependent on peptide-specific activation (Table I). For comparison, MHV-infected RAG 1−/− mice that received adoptive transfer of MHV-immune CD8 T cell-enriched populations exhibited 25% demyelination at this same time p.i. (day 14 p.i.) (15).

We activated P14 transgenic T cells in an alternative way to explore further the relationship between T cell activation and the ability to cause bystander pathology. We infected P14 transgenic mice i.p. with the Armstrong strain of LCMV, followed 6 or 8 days later by intracranial infection with MHV. Demyelination was observed in these dually infected mice; however, it was not as consistent as the demyelination that occurred after CFA:peptide gp33 treatment. The range of demyelination values was 1.9–18.2% with an average of 5.8% (Fig. 3 and Table I). One explanation for this finding is that LCMV infection is almost cleared from the P14 mice at the time of MHV infection (data not shown), and T cells are consequently in the contraction phase (29).

To address the possibility that uptake of CFA:peptide gp33 by APCs and subsequent transport to the CNS contributed to the development of demyelination, adoptive transfer experiments were performed. Initially, splenocytes were harvested from P14 transgenic mice 3 days after treatment with CFA:peptide gp33 or from mice 8 days after infection with LCMV. A total of 5–10 × 10⁶ splenocytes was transferred into RAG 1−/− mice on the same day that they were infected with MHV. Both sets of recipient mice inconsistently developed demyelination (CFA:peptide gp33, range 0.6–7.0%, average 2.6 ± 1.1%; LCMV infected, range 1.2–4.8%, average 2.7 ± 0.6%) (data not shown), presumably due to the extended time that the transgenic T cells were separated from cognate Ag.

To minimize the time that transgenic T cells were separated from cognate Ag, splenocytes were harvested from P14 mice 4 days after treatment with CFA:peptide gp33 and transferred into RAG 1−/− mice, which had been infected with MHV 9 days earlier. These mice consistently developed demyelination by the time they were harvested at day 15 p.i./day 6 posttransfer (average...

**FIGURE 2.** Demyelination in MHV-infected P14 transgenic mice after treatment with CFA:peptide gp33. We analyzed spinal cord sections from P14 transgenic mice that were treated with CFA:peptide gp33 (A and B), MHV (C and D), or CFA:peptide gp33 + MHV (E and F) for myelin damage (luxol fast blue staining) (A, C, and E), or for the presence of activated macrophages/microglia (F4/80 staining) (B, D, and F). We observed normal appearing white matter in mice treated with CFA:peptide gp33 (A) or infected with MHV (C), MHV-infected mice also treated with CFA:peptide gp33 (E) had multiple demyelinating lesions. No activated macrophages/microglia (F4/80+) cells were observed in sections from CFA:peptide gp33-treated mice (B). Scattered F4/80+ cells were detected in the gray matter of MHV-infected mice (D), whereas large numbers of these cells colocalized with areas of demyelination in MHV-infected transgenic mice treated with CFA:peptide gp33 (F). Magnification bar is equal to 200 μm.

**FIGURE 3.** Quantification of demyelination in transgenic mice. We quantified demyelination in P14 (columns 1–5) or N15 (columns 6 and 7) transgenic mice, as described in Materials and Methods. We detected background levels of demyelination in RAG 1−/− (column 1) or transgenic mice (columns 2 and 6) infected with MHV only or in uninfected P14 transgenic mice treated with CFA:peptide gp33 (column 3). Demyelination was most extensive in MHV-infected mice in which transgenic cells were activated with CFA:peptide gp33 (column 4) or CFA:peptide N52 (column 7) or by infection with LCMV (column 5). These data are summarized in Table I.
4.7 ± 0.9%, p < 0.0001 compared with nontransgenic RAG 1−/− mice infected with MHV (data not shown). This result indicated that recently activated bystander CD8 T cells were capable of causing bystander demyelination even when removed from cognate Ag. This experiment also showed that CFA:peptide gp33 did not directly contribute to demyelination in MHV-infected transgenic mice. In support of this conclusion, demyelination was not detected in MHV-infected CD4 TCR transgenic mice (TCL1/RAG 1−/−) (30) that were treated with CFA:cognate peptide (manuscript in preparation). If CNS entry of CFA:peptide-containing APCs contributed to demyelination, these mice should have developed demyelination because MHV-specific CD4 T cells are efficient mediators of demyelination in infected RAG 1−/− mice (15).

**Increased number of activated T cells in the CNS of mice with demyelination**

To begin to investigate the relationship between T cell activation and subsequent steps in the pathological process, we determined whether there was an increased number of activated transgenic T cells in the CNS during times when demyelination was observed. Very few P14 transgenic T cells trafficked to the CNS after MHV infection only (Fig. 4 and Table I), and these cells expressed a variable level of CD44 (Fig. 4B). However, they were not functionally activated based on the lack of IFN-γ production after cognate peptide stimulation (Fig. 4, F and J). We also detected few P14 transgenic T cells in the CNS after CFA:peptide gp33 treatment without subsequent infection with MHV (Fig. 4, A, E, and I, and Table I) or after infection of mice treated with CFA:peptide N52 or CFA alone (Table I). In striking contrast, prior immunization with CFA:peptide gp33 resulted in a significant increase in the number of activated P14 transgenic T cells in the MHV-infected CNS (Fig. 4, C, G, and K, and Table I). These cells were >95% CD44+ (Fig. 4C), and approximately 70% made IFN-γ when stimulated with peptide gp33 (Fig. 4, G and K). Similar results were obtained when we analyzed the CNS of MHV-infected N15 transgenic mice for activated T cells (Table I). Together, these results show a correlation between increased numbers of activated T cells in the CNS at the time of peak disease and the occurrence of significant demyelination.

We isolated the greatest number of activated transgenic T cells from the CNS of mice infected with MHV and LCMV, although less demyelination was observed than in mice treated with CFA:peptide gp33. These cells appeared to be less effective on a per cell basis in initiating demyelination, perhaps because they were in the process of undergoing contraction as viral titers decreased. It has previously been shown that areas of demyelination are associated with increased numbers of activated macrophages/microglia (13). Consistent with the disease phenotype observed in infected, CFA:peptide gp33-treated P14 transgenic mice and the data presented in Fig. 1, macrophage infiltration into the spinal cords of these mice was significantly increased compared with untreated P14 mice infected with MHV. To quantify these results, we counted all of the F4/80+ cells in 1.25-mm-wide cross sections at eight different levels within spinal cords from three mice in each group. Significantly more macrophages/microglia were present in MHV-infected CFA:peptide gp33-treated mice when compared with infected mice not treated with peptide (119 ± 12 in the infected, CFA:peptide gp33-treated group vs 15 ± 2 in the MHV-only group, p < 0.0001). This difference was entirely due to increased numbers of cells in the white matter. MHV-infected, CFA:peptide gp33-treated P14 transgenic mice had on average 113 ± 11 macrophages/microglia in the white matter per cross sectional area compared with 2 ± 1 in the white matter of MHV-infected P14 transgenic mice (p < 0.0001). Low numbers of macrophages/microglia were detected in the gray matter of both groups (5 ± 1 for the peptide-treated MHV-infected group compared with 13 ± 2 for the virus only group, p < 0.002).

To investigate whether the observed increased numbers of macrophages/microglia in the spinal cords of infected, CFA:peptide gp33-treated P14 transgenic mice correlated with increased expression of macrophagetropic and other chemokines, we performed RPA on spinal cord RNA isolated from P14 transgenic mice infected with MHV and from infected, CFA:peptide gp33-treated mice. mRNAs for lymphotoxin, CCL5/RANTES, CCL4/MCP-1β, CCL3/MIP-1α, MIP-2, CXCL10/IP-10, and CCL2/MCP-1 were assayed in these experiments. Levels of all of these chemokines were nearly the same in both groups (data not shown). Thus, these assays did not imply any specific chemokine in bystander demyelination.

**Discussion**

Our results provide one explanation for the well-established relationship between intermittent microbial infections and exacerbations in diseases such as MS (5–8). Using TCR transgenic/RAG 2−/− mice infected with MHV, we show that CD8 T cells that were not specific for MHV- or CNS-derived Ags were able to cause significant demyelination if they were activated with cognate Ag (Table I). Cells that were not activated with cognate Ag, such as those exposed to adjuvant only or to an irrelevant peptide, were unable to reproducibly induce demyelination.

### Table I. Quantification of activated T cells and demyelination in the CNS

<table>
<thead>
<tr>
<th>Expt. Group (No.)</th>
<th>Total</th>
<th>CD8+</th>
<th>Activated</th>
<th>% Demyelination</th>
</tr>
</thead>
<tbody>
<tr>
<td>P14 + MHV (5)</td>
<td>2.9 ± 0.5 × 10⁶</td>
<td>4.1 ± 0.8 × 10⁵</td>
<td>2.0 ± 0.6 × 10⁵</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>P14 + CFA + MHV (4)</td>
<td>2.4 ± 0.1 × 10⁶</td>
<td>2.6 ± 0.6 × 10⁵</td>
<td>6.4 ± 2.3 × 10⁵</td>
<td>2.0 ± 1.2</td>
</tr>
<tr>
<td>P14 + CFA:gp33 (3)</td>
<td>19.0 ± 2.0 × 10⁵</td>
<td>9.8 ± 1.4 × 10⁵</td>
<td>1.1 ± 0.4 × 10⁵</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>P14 + CFA:N52 + MHV (3)</td>
<td>31.3 ± 0.3 × 10⁵</td>
<td>19.0 ± 0.6 × 10⁵</td>
<td>3.8 ± 0.7 × 10⁵</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>P14 + CFA:gp33 + MHV (7)</td>
<td>5.1 ± 0.8 × 10⁵</td>
<td>1.3 ± 0.2 × 10⁴</td>
<td>4.5 ± 1.0 × 10⁻⁶</td>
<td>10.4 ± 1.8⁻⁶</td>
</tr>
<tr>
<td>P14 + LCMV + MHV (7)</td>
<td>5.1 ± 0.4 × 10⁵</td>
<td>1.4 ± 0.3 × 10⁵</td>
<td>9.4 ± 2.2 × 10⁻⁶</td>
<td>5.8 ± 2.3⁻⁶</td>
</tr>
<tr>
<td>N15 + MHV (8)</td>
<td>3.3 ± 0.6 × 10⁵</td>
<td>8.7 ± 2.3 × 10³</td>
<td>1.6 ± 0.8 × 10⁴</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>N15 + CFA:N52 + MHV (10)</td>
<td>4.7 ± 0.5 × 10⁵</td>
<td>1.6 ± 0.3 × 10⁴</td>
<td>6.1 ± 1.8 × 10⁷</td>
<td>6.7 ± 0.7⁻⁶</td>
</tr>
</tbody>
</table>

* Number of mice.
* Number of activated gp33- or N52-specific T cells calculated as described in Materials and Methods.
* Number of activated cells is statistically different (p < 0.02) from the number of activated cells in the P14 + MHV group.
* Percent demyelination is statistically different (p < 0.02) from the percent demyelination in the P14 + MHV group.
* Percent demyelination compared with percent demyelination in the P14 + MHV group (p = 0.11).
* Number of activated cells is statistically different (p < 0.0001) from the number of activated cells in the N15 + MHV group.
* Percent demyelination is statistically different (p < 0.0001) from the percent demyelination in the N15 + MHV group.
A likely key component of the demyelinating process observed in MHV-infected RAG 1−/− mice was the presence of an inflamed CNS. Viral infection induced the expression of proinflammatory cytokines and chemokines that are chemoattractants for T cells and for macrophages (Fig. 1). CCL5/RANTES, CXCL9/monokine induced by IFN-γ, and CXCL10/IP-10 are all critical for induction of the inflammatory response or demyelination in mice infected with MHV (31–33). Similarly, in MS patients experiencing relapses, the CNS is in a state of chronic inflammation, characterized by elevated levels of proinflammatory cytokines and chemokines such as TNF-α, IFN-γ, CXCL9/IP-10, CXCL9/monokine induced by IFN-γ, and CCL5/RANTES (34, 35). In addition, analysis of brain tissue from MS patients revealed the presence of lymphocytes expressing CXCR3, a CXCL9/CXCL10 receptor, and CCR5, a CCL5 receptor, in active lesions (35).

There is precedence for bystander T cells causing pathology in other disease models, most notably in mice with herpes stromal keratitis (36). In these studies, CD4 T cells specific for OVA were demonstrated to traffic to the eye after corneal infection with HSV-1 and were able to cause lesions in the absence of any HSV-specific CD4 or CD8 T cells. Lesions were more severe if CD4 T cells were activated before infection. Our study showed that non-virus-specific CD8 T cells were also able to cause bystander pathology in the CNS, but unlike the above-mentioned experiments, we showed that it was a prerequisite that the CD8 T cells be recently activated. Furthermore, we showed that the transgenic cells could be activated by infection with LCMV (Table I). Both of these features mimic the pathological process in MS patients who develop an infection.

Our results are unlikely to result from cross-reactivity between the transgenic T cells and MHV or CNS Ags for several reasons. First, we observed that both N15 and P14 transgenic T cells caused demyelination, even though they recognize unrelated peptide sequences. Second, we detected no potentially cross-reactive sequences between MHV and peptides gp33 and N52 by computer-based sequence comparisons. Third, infection with MHV in the absence of CFA:peptide treatment did not cause demyelination, consistent with a lack of cross-reactivity between MHV and peptides gp33 or N52. Fourth, it was possible that MHV infection exposed previously hidden CNS epitopes that cross-reacted with epitopes gp33 and N52. However, this is unlikely because MHV infection, without concomitant CFA:peptide treatment, did not result in demyelination, even though these putative CNS epitopes should be exposed and available to activate transgenic T cells under these conditions.

The mechanism of bystander demyelination remains to be determined. Direct cytolysis is very unlikely given the absence of specific epitope presentation in the CNS. In contrast, IFN-γ is critical for experimental autoimmune encephalomyelitis mediated by CD8 T cells (37) or CD8 T cell-mediated demyelination in

**FIGURE 4.** Quantification of activated P14 transgenic T cells harvested from the brain. We isolated lymphocytes from brains of P14 transgenic mice that were treated with CFA:peptide gp33 only (A, E, and I), infected with MHV (B, F, and J), or treated with CFA:peptide gp33 and infected with MHV (C, D, G, H, and K) and performed surface and intracellular cytokine staining. Few T cells were isolated from the brains of CFA:peptide gp33-treated or MHV-infected P14 transgenic mice. The cells we obtained expressed a range of CD44 (A and B), but did not make IFN-γ when specifically stimulated (E and F) with cognate peptide, or when stimulated with an irrelevant peptide (peptide SS10, derived from MHV) (I and J). We isolated more activated P14 transgenic T cells from the brains of infected, CFA:peptide gp33-treated transgenic mice. These cells were mostly CD44+ (C), and a large portion made IFN-γ when specifically stimulated (G), but not when stimulated with irrelevant peptide (K). Background staining with isotype-matched control Abs is shown in D and H. Similar results were obtained when FACS analysis was performed on lymphocytes isolated from three CFA:peptide gp33-treated, five MHV-infected, and seven CFA:peptide gp33-treated, MHV-infected P14 transgenic mice.
MHV-infected mice (38). Of note, IFN-γ is rapidly down-regulated when T cells are removed from Ag (39). Consequently, activated P14 or N15 CD8 T cells should no longer express IFN-γ by the time that they traffic to the CNS. Thus, it is unlikely that direct effects of IFN-γ on MHV-infected cells would be the primary mechanism of bystander pathology. However, IFN-γ has an important role in the induction of chemokines (40), and tight Ag-specific regulation of chemokine expression has not been demonstrated. Thus, highly activated CD8 T cells, including the peptide-activated transgenic T cells in our study, or T cells responding to an unrelated infection in patients with MS, may secrete chemokines for a short time after Ag contact is broken. We were unable to identify a macrophagotropic chemokine that was up-regulated in CFA:peptide gp33-treated mice. These results are similar to those obtained after transfer of splenocytes from MHV-immune B6 to MHV-infected RAG 1−/− mice. In these experiments, extensive demyelination is observed by 7 days posttransfer (13, 15); however, even in these mice, levels of CCL2/MCP-1, CCL7/MCP-3, CCL3/MIP-1α, and CCL4/MIP-1β were not significantly changed compared with mice not receiving transferred cells. A chemokine that did increase was RANTES (data not shown). This was consistent with the large increase in lymphocytes observed in the CNS of recipients of MHV-immune splenocytes because RANTES is largely produced by T cells. No differences in the levels of RANTES were observed in the present experiments examining bystander T cells, probably because there was only a modest increase in the number of T cells after treatment with CFA:peptide gp33 (Table I). However, it is still possible that expression of a critical chemokine by activated gp33-specific CD8 T cells causes localized changes in the spinal cord, resulting in focal areas of demyelination.

These studies provide one explanation for the observed link between illnesses and relapses in MS patients and other diseases with an activated gp33-specific T cell response in the central nervous system of coronavirus-infected mice. It is possible that expression of a critical chemokine by activated gp33-specific CD8 T cells causes localized changes in the spinal cord, resulting in focal areas of demyelination.

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References


References


