

Short Communication

Bystander CD8 T-Cell-Mediated Demyelination is Interferon- γ -Dependent in a Coronavirus Model of Multiple Sclerosis

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Mice infected with the coronavirus mouse hepatitis virus, strain JHM (JHM) develop a disease that shares many histological characteristics with multiple sclerosis. We previously demonstrated that JHM-infected mice that only have CD8 T cells specific for an epitope not in the virus develop demyelination on specific activation of these cells. Herein we show that this process of bystander T-cell-mediated demyelination is interferon- γ (IFN- γ)-dependent. The absence of IFN- γ abrogated demyelination but did not change T-cell infiltration or expression levels of inflammatory cytokines or chemokines in the spinal cord. These results are consistent with models in which IFN- γ contributes to CD8 T-cell-mediated demyelination by activation of macrophages/microglia, the final effector cells in the disease process. (*Am J Pathol* 2004, 164:363–369)

The human disease multiple sclerosis (MS) is an immune-mediated, chronic inflammatory disease of the central nervous system (CNS) characterized histologically by multiple focal demyelinating lesions.^{1,2} Although T cells are found in the demyelinating lesions, the specificity of the majority of these T cells is largely unknown.¹ These cells do not generally appear to be autoreactive.

Intercurrent illnesses in patients with autoimmune disease, including keratitis and MS, often result in temporary worsening of disease.^{3,4} In some cases, intercurrent infection is the triggering event for the development of disease. Several mechanisms have been proposed to account for this phenomenon.^{5–7} The first of these is molecular mimicry, a phenomenon in which T-cell epitopes from a pathogen are sufficiently similar to a host

peptide to induce a misdirected T-cell response. Another possibility is the release of previously hidden self-epitopes to which T cells have not been tolerized, called epitope spreading. Alternatively, the cytokine milieu in the setting of infection might activate and induce the proliferation of nearby autoreactive T cells. Finally, T cells activated specifically against an unrelated infectious agent may migrate into areas of chronic inflammation. These cells could then interact with resident cells and soluble mediators at the site of inflammation to induce bystander pathology.

Mice infected with the neurotropic coronavirus mouse hepatitis virus, strain JHM (JHM) develop acute and chronic demyelinating disease with histopathological similarities to MS.^{8,9} The process of demyelination is immune-mediated, as RAG1^{-/-} or RAG2^{-/-} (mice deficient in recombination activation enzyme) or *scid* (mice with severe combined immunodeficiency) mice, which lack B and T lymphocytes, did not develop demyelination on infection with JHM^{10,11}; however, if RAG1^{-/-} mice were reconstituted with either JHM-immune CD4 or CD8 T cells, demyelination proceeded as in the immunocompetent mouse.¹¹ In either reconstituted RAG1^{-/-} or wild-type mice, JHM-specific T cells were detected in the infected CNS and constituted the majority of the infiltrating cells.^{11–13} Although T cells reactive to myelin or other CNS epitopes were not identified in JHM-infected mice, the specificity of a large fraction of these cells was not determined in those studies.

While some of these cells may target JHM-specific epitopes that are, as yet, unidentified, others are likely to be specific for neither JHM nor CNS antigens. Previously, we showed that CD8 T cells specific for either an epitope in lymphocytic choriomeningitis virus (LCMV) or vesicular

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stomatitis virus were able to mediate demyelination in infected RAG2^{-/-} mice.¹⁴ Demyelination was demonstrated most effectively when mice transgenic for T-cell receptors recognizing one of these epitopes were directly infected with JHM and T cells were activated by exposure to cognate antigen in adjuvant. This effect was limited to CD8 T cells because we showed that specific activation of non-JHM specific CD4 T cells did not result in bystander demyelination.¹⁵ Furthermore, mere activation of transgenic CD8 T cells with peptide in adjuvant, in the absence of JHM infection, did not result in demyelination.

Interferon- γ (IFN- γ) is a critical mediator of homeostasis and inflammation in MS and several of its rodent models, including experimental autoimmune encephalomyelitis (EAE) and demyelination mediated by Theiler's encephalomyelitis virus.¹⁶⁻¹⁹ Similarly, in JHM-infected mice, transfer of virus-specific, IFN- γ ^{-/-} CD8 lymphocytes to infected RAG1^{-/-} mice resulted in an 80% decrease in demyelination as compared to those mice receiving IFN- γ ^{+/+} CD8 T cells (4.8% vs. 26.3%).²⁰ This decrease was specific for the defect in IFN- γ as transfer of CD8 T cells deficient in tumor necrosis factor (TNF)- α did not result in a reduction in demyelination.

Because of the central role of IFN- γ in demyelination induced by JHM-specific CD8 T cells, we reasoned that this mechanism might be shared by non-JHM specific CD8 T cells. To test this hypothesis, we reconstituted RAG1^{-/-} mice with bone marrow cells derived from P14 transgenic IFN- γ ^{-/-} RAG2^{-/-} mice. These mice exhibited little demyelination after JHM infection when compared to mice reconstituted with bone marrow from P14 transgenic IFN- γ ^{+/+} RAG2^{-/-} mice, indicating that IFN- γ production is critical for bystander demyelination.

Materials and Methods

Mice

Pathogen-free P14 transgenic (P14) RAG2^{-/-} mice were purchased from Taconic Labs (Germantown, NY). These mice are transgenic for a TCR specific for residues 33-41 of the LCMV glycoprotein (epitope gp33). IFN- γ ^{-/-} mice were a gift from Dr. John Harty (University of Iowa). We bred P14 IFN- γ ^{-/-} RAG2^{-/-} mice from these strains at the University of Iowa animal care facility. RAG1^{-/-} mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were on the C57BL/6 background. All animal studies were approved by the University of Iowa Animal Care and Use Committee.

Virus and Infections

The 2.2-V-1 strain of JHM was kindly provided by Dr. John Fleming (University of Wisconsin, Madison, WI). Mice were infected with 1000 PFU of JHM intracranially and harvested at 14 days post-inoculation (p.i.). To activate T cells, 100 μ g gp33 peptide (KAVYNFATM) (Bio-Synthesis, Lewisville, TX) was emulsified in complete

Freund's adjuvant (CFA) and administered intraperitoneally. Virus was titered by a plaque-reduction assay as previously described.²¹

Bone Marrow Chimeras

RAG1^{-/-} mice were irradiated with 4.5 Gy and allowed to rest for 16 hours. Bone marrow was harvested from either P14 RAG2^{-/-} mice or from P14 IFN- γ ^{-/-} RAG2^{-/-} mice, and 1×10^6 cells were injected retro-orbitally into the recipient RAG1^{-/-} mice. Six weeks after transfer, peripheral blood mononuclear cells were stained by flow cytometry to ensure that P14 transgenic T cells were present.

Flow Cytometry

Staining for peptide-stimulated production of IFN- γ and TNF- α was done as previously described.¹¹ In brief, lymphocytes were harvested from the CNS using a 30% Percoll (Pharmacia, Uppsala, Sweden) gradient and incubated with peptide coated-EL-4 cells at a ratio of 50:1. P14 peptide or S510 peptide (the immunodominant CD8 T-cell epitope recognized in JHM-infected B6 mice; CSLWNGPHL^{22,23}) was used at a final concentration of 1 μ mol/L. CNS-derived lymphocytes were incubated with APCs and peptide for 5 hours in the presence of brefeldin A (BD Pharmingen, San Diego, CA). Cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD8 mAb and PE-conjugated anti-IFN- γ or TNF- α mAbs (BD Pharmingen).

Immunohistochemistry, Immunofluorescence, and Quantification of Demyelination

Spinal cords were removed from JHM-infected mice, fixed in zinc formalin (Labsco, Solon, OH) and embedded in paraffin. To determine areas of myelin damage, 8- μ m sections were stained with luxol fast blue (LFB) and counterstained with hematoxylin and eosin. Areas of total myelin and demyelination were quantified using NIH ImageJ software and the percentage of demyelination was determined. To stain for macrophages/microglia or virus, sections were incubated with antibodies directed against F4/80 (macrophages/microglia, Serotec, Oxford, UK), diluted 1:100 in normal goat serum or the nucleocapsid (N) protein of JHM (mAb 5B188.2; 1:10000; a gift from Dr. Michael Buchmeier, The Scripps Research Institute, La Jolla, CA). Secondary antibodies were biotinylated goat anti-rat (1:100; Vector Laboratories, Burlingame, CA) or anti-mouse IgG (1:100; Jackson Immunoresearch, West Grove, PA), respectively. The staining was visualized using streptavidin-peroxidase (Jackson Immunoresearch) and diaminobenzidine (σ -Aldrich, St. Louis, MO). Sections were counterstained with hematoxylin. Axons were detected with monoclonal antibody cocktail SMI-312 (1:1000; Sternberger Monoclonals, Lutherville, MD), followed by FITC-conjugated goat anti-mouse secondary antibody (1:100; ICN Biomedicals, Aurora, OH). All mi-

croscopy was done on a Leica DMB microscope and photographed using an Optronics digital camera.

Ribonuclease Protection Assay

RNA was extracted from the spinal cords of mice using Tri-Reagent (Molecular Research Center, Cincinnati, OH). We performed RNase protection assays (RPA) as previously described,¹⁴ using probes for the cytokines lymphotoxin- α , TNF- α , interleukin (IL)-4, IL-12 p40, IL-6, and IL-1 β ; and the chemokines CCL2 (MCP-1), CCL3 (MIP-1 α), CCL4 (MIP-1 β), CCL5 (RANTES), CCL7 (MCP-3), CXCL10 (IP-10), and MIP-2. These probes were generously provided by Dr. Iain Campbell (The Scripps Research Institute). Templates for the probes were linearized with *EcoRI* and ³²P-labeled antisense probes were synthesized using T7 RNA polymerase. Ten micrograms of spinal cord RNA was used for each hybridization reaction. Samples were hybridized to probe, treated with RNase and run on a denaturing 10% polyacrylamide 7 mol/L urea gel. Radioactivity was measured using phosphorimager and quantified using NIH Image software.

Statistics

Statistical significance was determined by unpaired *t*-tests. All results are expressed as means \pm SEM. Values of *P* < 0.05 were considered statistically significant.

Results

Development of Bone Marrow Chimeras

Given the key role that IFN- γ has in demyelination mediated by JHM-specific CD8 T cells, we investigated whether IFN- γ produced by bystander cells was also important for their ability to cause myelin destruction. For this purpose, we created bone marrow chimeras in which IFN- γ was only deficient in the T-lymphocyte compartment. The resulting chimeras had only transgenic CD8 T cells deficient in the ability to produce IFN- γ , but other cells of hematopoietic lineages were derived, in part, from the recipient bone marrow, ensuring that such sources of IFN- γ as NK cells were present.²⁴ In all experiments, we used chimeras created with bone marrow from P14 IFN- γ ^{+/+} RAG2^{-/-} mice as controls.

IFN- γ Was Required for Bystander Demyelination and Macrophage/Microglia Infiltration into the White Matter

Chimeras with P14 transgenic T cells deficient or sufficient for IFN- γ were infected with JHM. To activate the transgenic T cells, we injected these mice on the day of infection with 100 μ g LCMV gp33 peptide in CFA (CFA: gp33). This amount of peptide was previously shown to provide optimal stimulation of the gp33-specific TCR

transgenic cells.¹⁴ These mice were monitored for the development of signs of demyelination, including hind-limb paralysis, in the 2 weeks following infection. CNS tissue was harvested from mice on day 14 p.i.

We stained the spinal cords from JHM-infected, CFA: gp33-treated, IFN- γ -sufficient or -deficient chimeras with LFB to determine the amount of bystander demyelination in these mice (Figure 1, A and E). Chimeras with P14 transgenic T cells deficient for IFN- γ did not develop demyelination substantially above the background level (Table 1) seen in RAG1^{-/-} or RAG2^{-/-} mice infected with JHM.¹⁴ As such, this 70% reduction represented a nearly complete abrogation in demyelination in chimeras reconstituted with IFN- γ ^{-/-} T cells.

Wild-type mice infected with JHM develop demyelination that is largely primary demyelination with relative sparing of axons.⁸ To test whether JHM infection in our P14 chimeras similarly resulted in primary demyelination, we stained spinal cord sections with an antibody cocktail that recognizes all axons, as described in Materials and Methods. Areas of demyelination in chimeras containing IFN- γ ^{+/+} T cells all demonstrated positive staining for axons, indicating that the demyelinating process in these mice, as in wild-type mice, is primary (Figure 1D). There was no evidence for axonal loss in IFN- γ ^{-/-} chimeras (Figure 1H), reflecting the minimal amount of demyelination observed in these animals.

JHM infection resulted in similar clinical disease in the two groups of mice with CFA:gp33-stimulated T cells. Signs were largely those of encephalitis (hunching, ruffled fur, lethargy) rather than demyelination (hindlimb paralysis, righting inability), probably as a result of the low amount of demyelination observed.⁸ Additionally, to determine whether differences in virus clearance contributed to the differences in demyelination, we measured viral titers in the brain at day 14 p.i. (Table 1). There was not an appreciable difference in viral titers between the two groups.

Macrophages/microglia are likely the terminal effectors of demyelination in JHM-infected mice.²⁵ As such, we reasoned that the lack of IFN- γ produced by activated, non-JHM-specific T cells would result in a decrease in macrophage/microglia infiltration into the white matter of the spinal cord. We used the macrophage/microglia marker F4/80 to identify the location and number of these cells in the white matter of chimeras with IFN- γ -sufficient or -deficient T cells. Consistent with previous studies of JHM-induced demyelination,^{20,25,26} IFN- γ -sufficient chimeras had infiltration of macrophages/microglia into the white matter in areas of demyelination (Figure 1, A and B). IFN- γ -deficient chimeras, however, had limited macrophage/microglia infiltration into the spinal cord. These areas may represent sites of incipient myelin destruction, although little frank demyelination was detected (Figure 1, E and F). Demyelination occurred in the vicinity of virus-infected cells. However, virus antigen was not found in areas of demyelination, suggesting that myelin destruction occurred during the process of virus clearance. (Figure 1, C and G).

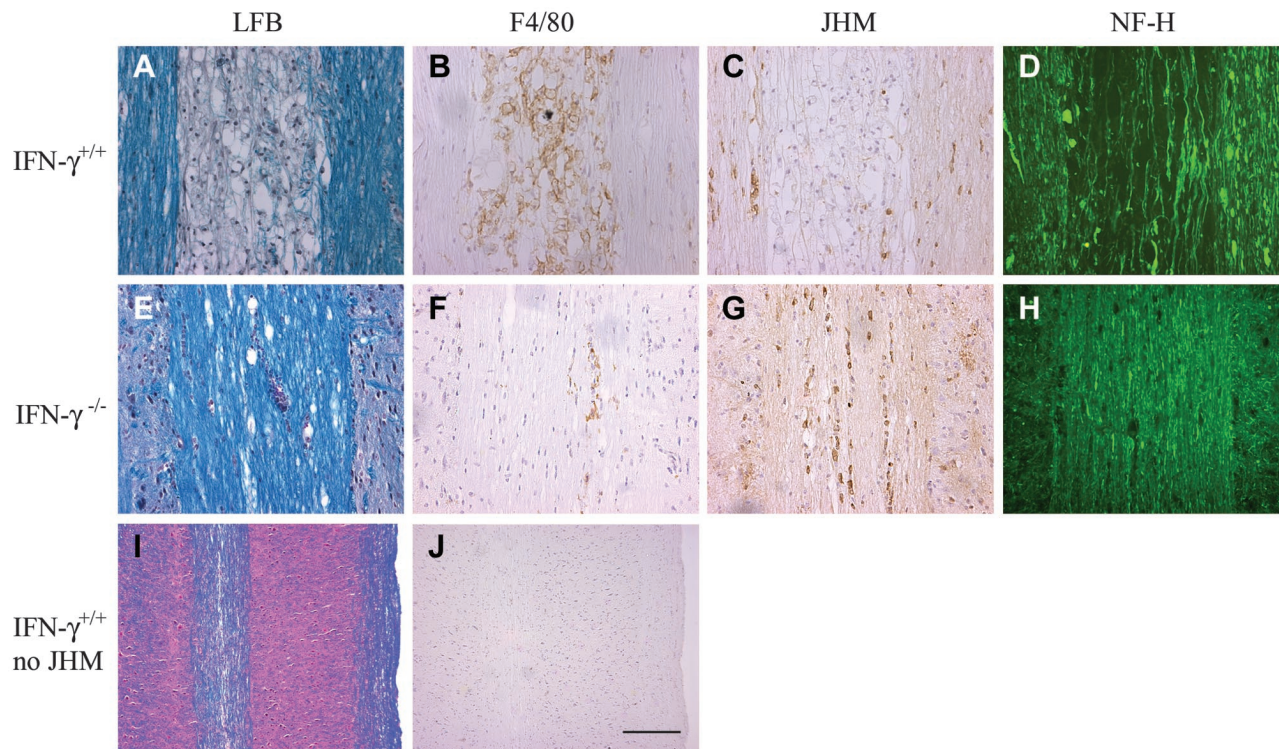


Figure 1. Absence of IFN- γ results in minimal demyelination. Serial, 8- μ m midsagittal sections of spinal cords from IFN- γ -sufficient (**A to D, I, J**) or -deficient (**E to H**) chimeras were stained for myelin (**A, E, I**), macrophages/microglia (**B, F, J**), viral antigen (**C and G**), or neurofilament H (**D and H**). Demyelination in both types of JHM-infected mice was focal, although it was uniformly more extensive in IFN- $\gamma^{+/+}$ chimeras (**A**) than in IFN- $\gamma^{-/-}$ chimeras (**E**). Demyelination in IFN- $\gamma^{+/+}$ chimeras correlated with increased macrophage infiltration into the white matter (**B**) as compared to IFN- $\gamma^{-/-}$ chimeric mice (**F**). In both cases, viral antigen was abundant throughout the white matter (**C and G**) although antigen could not be detected in areas of robust demyelination (**A and C**). Axons were detected traversing an edematous, demyelinated area, consistent with primary loss of myelin (**D**). No axonal loss was detected in IFN- $\gamma^{-/-}$ chimeras, consistent with the lack of demyelination (**H**). In chimeras with activated T cells but no JHM infection, there was neither demyelination (**I**) nor macrophage/microglia activation (**J**). Scale bar, 50 μ m for **A to H**; 100 μ m for **I and J**.

Activated T Cells Infiltrated the CNS in Similar Numbers

An explanation for the lack of demyelination and macrophage/microglia infiltration in chimeras containing IFN- $\gamma^{-/-}$ lymphocytes is a reduction in T-cell activation or infiltration into the CNS, or both. To address this possibility, we used flow cytometric analysis to determine the number of lymphocytes in the CNS and their activation status. There were similar influxes of CD8 transgenic T cells in the CNS (Table 1), with approximately 9.6×10^3 cells in the CNS of IFN- $\gamma^{+/+}$ chimeras, and 8.8×10^3 cells in the CNS of IFN- $\gamma^{-/-}$ chimeras.

To determine whether these CNS-derived, transgenic T cells were functionally activated, we stained for the intracellular production of cytokines IFN- γ or TNF- α (Fig-

ure 2, A to H). These cytokines are produced by T cells in response to specific activation through recognition of cognate peptide by the TCR.²⁷ As expected, IFN- γ was expressed only by transgenic CD8 T cells harvested from recipients of IFN- $\gamma^{+/+}$ bone marrow (Figure 2, A and E). There was a similar frequency and number of activated cells in both IFN- γ -sufficient and -deficient chimeras as measured by TNF- α staining (Figure 2, B and F; Table 1). Therefore, we conclude that the production of IFN- γ by specifically activated cells, and not merely activation of T cells, is responsible for bystander demyelination in this system. Additionally, there were no CD8 T cells specific for the immunodominant epitope of JHM (residues 510–518 of the spike glycoprotein) in any mice tested (Figure 2, C, D, G, H). A recent study²⁸ demonstrated that, even in the absence of cognate antigen in the CNS, activated

Table 1. Demyelination, Viral Titer, and CD8 T-Cell Infiltration in the CNS of JHM-Infected IFN- $\gamma^{+/+}$ and IFN- $\gamma^{-/-}$ Chimeras

Mice	Demyelination (N)*	Viral titer log ₁₀ PFU/g	CD8 lymphocytes per CNS		
			Total	Activated (TNF- α)	Activated (IFN- γ)
IFN- $\gamma^{+/+}$ chimeras	3.30% \pm 0.60 [†] (7)	5.27 \pm 0.02	9.6 \pm 0.47 $\times 10^3$	2.61 \pm 0.30 $\times 10^3$	3.84 \pm 0.39 $\times 10^3$
IFN- $\gamma^{-/-}$ chimeras	0.72% \pm 0.17 (6)	5.51 \pm 0.11	8.8 \pm 0.53 $\times 10^3$	2.0 \pm 0.24 $\times 10^3$	0

*The number of mice analyzed per group is shown in parenthesis.

[†]This value is significantly different than that observed in IFN- $\gamma^{-/-}$ chimeras; $P < 0.002$.

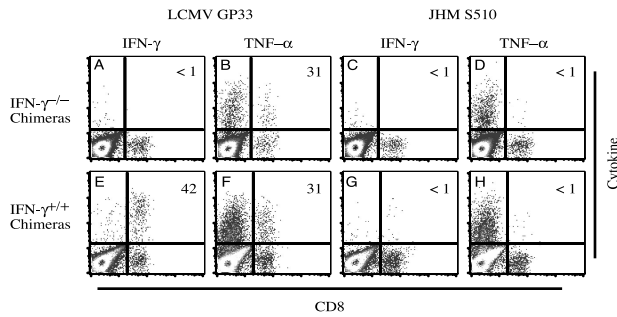


Figure 2. T cells in the CNS are similarly activated in IFN- γ -sufficient and -deficient chimeras. To determine the activation state of T cells in the CNS of JHM-infected IFN- $\gamma^{+/+}$ (A to D) or IFN- $\gamma^{-/-}$ (E to H) chimeras, we stained cells for flow cytometry with anti-CD8 and anti-IFN- γ or anti-TNF- α mAbs after incubation of these cells in the presence of antigen-presenting cells and either LCMV gp33 or JHM S510 peptides. IFN- $\gamma^{+/+}$ and IFN- $\gamma^{-/-}$ chimeras exhibited similar frequencies of activated CD8 T cells as measured by TNF- α intracellular staining (B and F). This value was slightly lower than that seen in IFN- $\gamma^{+/+}$ chimeric mice after IFN- γ staining (E). Notably, there were no cells specific for the JHM-specific S510 epitope in either mouse (C, D, G, H).

CD8 T cells could infiltrate the CNS, with subsequent activation of microglia. We were able to detect activated P14 T cells in the CNS of CFA:gp33-treated mice, in the absence of JHM infection, although there were roughly six-fold fewer T cells in the CNS of uninfected mice than in their JHM-infected counterparts (data not shown). However, in this model system, we were unable to find histological evidence of macrophage/microglia activation or demyelination in the absence of JHM infection (Figure 1, I and J).

No Differences in Cytokine or Chemokine Production Were Detected in Recipients of IFN- γ -Sufficient or -Deficient Cells

We next asked whether the difference in IFN- γ production by activated lymphocytes in the CNS would result in differential expression of other cytokines or chemokines within the CNS. To answer this question, we extracted RNA from whole spinal cords of chimeras with IFN- γ -sufficient and -deficient lymphocytes, and quantified the mRNA levels of eight chemokines and six cytokines by RPA, as described in Materials and Methods. TNF- α was the only cytokine detected of the six assayed and it was present at similar levels in both groups of mice (data not shown).

Message levels of the chemokines tested were similar in the two experimental groups (Figure 3). There was a trend toward higher levels of CXCL10 and CCL5 in those mice containing IFN- $\gamma^{+/+}$ lymphocytes, although this did not reach statistical significance. In mice treated with CFA:gp33 but not infected with JHM, we could not detect significant amounts of any chemokine by RPA, consistent with our finding that macrophages/microglia were not activated in this setting (Figure 3 and Figure 1J).

Discussion

A key element of the present study was the use of bone marrow chimeras to address the role of IFN- γ in by-

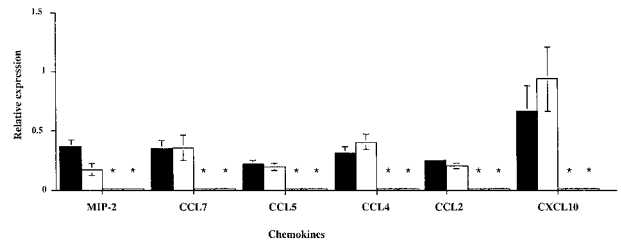


Figure 3. Analysis of chemokines within the CNS. We used RPA to identify differences in chemokines within the spinal cords of JHM-infected, CFA:gp33-treated, IFN- $\gamma^{+/+}$ (open bars) or IFN- $\gamma^{-/-}$ (closed bars) chimeras. We also measured cytokine and chemokine levels for uninfected, but CFA:gp33-treated, IFN- $\gamma^{+/+}$ (hatched bars) and IFN- $\gamma^{-/-}$ (shaded bars) chimeras. *, No mRNA for this chemokine was detectable in these mice. Values are normalized to expression of the housekeeping gene L32. There were no statistically significant differences in measured chemokine levels between JHM-infected IFN- $\gamma^{-/-}$ and IFN- $\gamma^{+/+}$ chimeras or between uninfected IFN- $\gamma^{-/-}$ and IFN- $\gamma^{+/+}$ chimeras. We analyzed 7 IFN- $\gamma^{+/+}$ and 5 IFN- $\gamma^{-/-}$ JHM-infected, CFA:gp33-treated chimeras, and 3 mice in each group of the uninfected limb.

stander demyelination mediated by CD8 T cells. The production of these chimeras allowed us to address the hypothesis that IFN- γ produced by CD8 T cells, and not from other sources, was the critical element in mediating bystander demyelination. Furthermore, this approach did not compromise IFN- γ production by cells such as NK cells and dendritic cells, thereby preserving the innate immune response to the virus.^{24,29} The results showed that IFN- γ produced by these innate cells was unable to initiate the demyelinating process, even in the context of activated CD8 T cells lacking only the ability to produce IFN- γ .

Our results show that bystander demyelination mediated by CD8 T cells is IFN- γ -dependent and thereby is similar to that mediated by JHM-specific CD8 T cells. These results highlight the important role that CD8 T cells have in demyelination in JHM-infected mice. IFN- γ has also been shown to be critical in other models of demyelination and in MS. In rodents with EAE induced by CD8 T cells specific for a CNS antigen, demyelination is greatly diminished in the absence of IFN- γ .³⁰ In all cases, the presence of CD8 T-cell-produced IFN- γ results in increased macrophage/microglia infiltration into the white matter, with subsequent demyelination. Similarly, CD8 T cells are abundant in MS lesions³¹ and treatment with anti-IFN- γ antibody appeared to reduce disability in a small ($n = 15$ patients receiving treatment) clinical trial.³² Consistent with these data, administration of IFN- γ to patients with MS resulted in disease exacerbation.³³ In patients with MS, the source of the IFN- γ that leads to disease exacerbation is unknown, but based on our results, it may be from CD8 T cells responding to an intercurrent infection. From our results, it appears that IFN- γ and not any other cytokine or chemokine was the key mediator of bystander demyelination (Figure 3). However, because our screen for chemokines and cytokines was not exhaustive, it is possible that other soluble factors were affected by the presence or absence of CD8 T-lymphocyte-produced IFN- γ . Alternatively, differences in local concentration of chemokines or cytokines may be critical in the demyelinating process and might not be evident in this assay. It is also possible that IFN- γ affects

the functions of non-soluble molecules that may be involved in macrophage/microglia activation.

The amount of demyelination observed in these JHM-infected bone marrow chimeras was substantially less than that seen in infected, RAG1^{-/-} mice reconstituted with JHM-immune splenocytes.¹¹ This relatively low level of demyelination was also seen in our previous study using nonchimeric mice.¹⁴ Because the P14 T cells are not specific for JHM, this suggests that nonspecific T cells are substantially less efficient at mediating demyelination than are virus-specific cells, probably because cognate antigen-TCR interactions do not take place within the CNS in this model.¹⁴ Analogously, such a low but significant level of demyelination would be consistent with a role for activated, bystander T cells in clinical exacerbations in patients with MS.

In contrast to the results obtained with CD8 T cells, IFN- γ is a negative regulator of demyelination in EAE mediated by CD4 T cells.^{16,18,19} In IFN- γ ^{-/-} or IFN- γ R^{-/-} mice with EAE, disease was more severe and greater numbers of CD4 T cells were detected in the CNS than in wild-type mice with EAE. Most strikingly, a neutrophilic infiltration into the CNS occurred in the absence of IFN- γ and chemokine production was greatly altered. Similarly, in JHM-infected RAG1^{-/-} mice, adoptive transfer of JHM-immune CD4 T cells from IFN- γ ^{-/-} donors resulted in more severe disease and enhanced demyelination when compared to recipients of wild-type CD4 T cells.³⁴ Thus, in the context of CD4 T cells, IFN- γ acts, at least in part, to down-modulate the immune response, resulting in less myelin destruction.

Collectively, these results highlight the importance of CD8 T cells in demyelinating diseases, including MS. The results of the study presented here also emphasize the central role that IFN- γ has in this process, both in the context of CD8 T cells specific for an antigen in the CNS and those that may be activated by an unrelated antigen.

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