Immune cell recruitment to and infiltration of the central nervous system (CNS) is central to the pathology of a variety of inflammatory neurological diseases, including infectious meningitis, multiple sclerosis, and cerebral ischemia (59, 60). Chemokines have been shown to be highly upregulated in both human diseases and animal models of neuroinflammation and are thought to be important mediators of immune cell entry into the CNS (59, 60). For example, during experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS), the chemokines CCL2 (monocyte chemotactactor protein 1 [MCP-1]), CCL3 (MIP-1α), and CCL5 (RANTES) in the cerebrospinal fluid (CSF). Mice deficient in IFN-γ had decreased CSF levels of CCL3, CCL5, and CXCL10 (IP-10), and decreased activation of both resident CNS and infiltrating antigen-presenting cells (APCs). The effects of IFN-γ signaling on macrophage lineage cells was assessed using transgenic mice, called “macrophages insensitive to interferon gamma” (MIIG) mice, that express a dominant-negative IFN-γ receptor under the control of the CD68 promoter. MIIG mice had decreased levels of CCL2, CCL3, CCL5, and CXCL10 compared to controls despite having normal numbers of LCMV-specific CD4+ T cells in the CNS. MIIG mice also had decreased recruitment of infiltrating macrophages and decreased activation of both resident CNS and infiltrating APCs. Finally, MIIG mice were significantly protected from LCMV-induced anorexia and weight loss. Thus, these data suggest that CD4+ T-cell production of IFN-γ promotes signaling in macrophage lineage cells, which control (i) the production of proinflammatory cytokines and chemokines, (ii) the recruitment of macrophages to the CNS, (iii) the activation of resident CNS and infiltrating APC populations, and (iv) anorexic weight loss.

IFN-γ is present in the CNS during autoimmunity and infection (7, 54, 69). Several studies suggest that IFN-γ can be a potent inducer of CNS chemokine expression. Adenoviral expression of IFN-γ in the CNS strongly induced CCL5 and CXCL10 mRNA and protein, and this induction was dependent on the presence of the IFN-γ receptor (50). In EAE and Toxoplasma infection, mice deficient in IFN-γ or the IFN-γ receptor demonstrated reduced expression of several chemokines, including CCL2, CCL3, CCL5, and CXCL10 (26, 69). However, given the near-ubiquitous expression of the IFN-γ receptor (44), the mechanisms by which IFN-γ regulates CNS chemokine production remain to be elucidated.

We studied neuroinflammation and immune-mediated disease using a well-studied mouse model of infection with lymphocytic choriomeningitis virus (LCMV). Intracranial (i.c.) infection of mice with LCMV results in seizures and death 6 to 8 days after inoculation. The onset of symptoms is associated with a massive influx of mononuclear cells into the cerebrospinal fluid (CSF), meninges, choroid plexus, and ependymal membranes (6, 8, 18), as well as the presence of proinflammatory cytokines (7, 38). The immune response is critical for disease, since infection of irradiated or T-cell-depleted mice leads to persistent infection with very high levels of virus in multiple tissues without the development of lethal meningitis (18, 34, 64). i.e. LCMV infection of β2-microglobulin-deficient mice (β2m−/− mice) also results in meningitis and production of proinflammatory cytokines and chemokines; however, meningitis occurs with a later onset and lower severity compared to wild-type mice (17, 24, 53, 57). Interestingly, i.e. LCMV infection of these mice also causes severe anorexia and weight loss (33, 38, 46, 52, 57) that is mediated by major histocompatibility
complex (MHC) class II-restricted, CD4⁺ T cells (17, 46, 53, 57). Anorexia and weight loss are also observed in wild-type mice, but they succumb to lethal meningoencephalitis shortly thereafter (33), making study of this particular aspect of disease difficult. LCMV-induced weight loss, similar to what we have observed in β₂m⁻/⁻ mice also occurs in perforin-deficient mice, which possess CD8⁺ T cells (37). Although some reports have observed weight loss after peripheral LCMV infection (11, 45), we note that these studies used high doses of the clone 13 strain of LCMV, in contrast to our studies which have used the Armstrong strain of LCMV and orders of magnitude less virus (33, 38, 46, 52, 57). Although we cannot exclude a contribution of peripheral cells to weight loss in our i.c. Armstrong infection model, we previously showed that this weight loss does not occur with peripheral infection with LCMV Armstrong (33, 38), indicating that interactions between the CNS and the immune system are contribute substantially to disease.

During LCMV infection, there is biphasic production of IFN-γ: a small, early peak of IFN-γ (most likely produced by NK or NKT cells), followed by T-cell-mediated production of IFN-γ (23, 75). Further, both CD4⁺ T cells and CD8⁺ T cells produce large amounts of IFN-γ after LCMV infection and T-cell production of IFN-γ is critical for LCMV-induced weight loss (35). Chemokines, especially CXCL10, CCL5, and CCL2, and their receptors, are upregulated in the brain after i.c. LCMV infection (2, 13). Brain chemokine mRNA expression after i.c. LCMV infection is reduced in IFN-γ-deficient mice and relatively absent in athymic mice (2). However, the mechanism(s) by which T cells and IFN-γ mediate the effects on CNS chemokine expression, cellular infiltration into the CNS, and LCMV-induced anorexic weight loss remain unclear.

In the present study, we focused on two major questions. The first question concerned the role of IFN-γ on immune cell recruitment to and chemokine/cytokine production within the CNS? We found that macrophages and myeloid dendritic cells (DCs) require IFN-γ for their accumulation within the CNS. Second, since macrophages and myeloid DCs are the predominant cellular infiltrate, we sought to determine whether IFN-γ signaling on these cells was direct with regard to their recruitment and to chemokine/cytokine production. We found that IFN-γ signaling in macrophage lineage cells contributes significantly to their recruitment, to chemokine production in the CNS, and to anorexic weight loss. Together, these data suggest that much of the proinflammatory effects of IFN-γ in the CNS are mediated by the effects of IFN-γ on CD68-bearing cells.

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### MATERIALS AND METHODS

**Virus.** The Armstrong-3 strain of LCMV was kindly provided by Rafi Ahmed (Emory University, Atlanta, GA). Virus was grown in BHK-21 cells (American Type Culture Collection, Manassas, VA), and virus titers of supernatants were served weight loss after peripheral LCMV infection (11, 45), we previously showed that this weight loss does not occur with peripheral LCMV infection (2, 13). Brain chemokine mRNA expression after i.c. LCMV infection is reduced in IFN-γ-deficient mice and relatively absent in athymic mice (2). However, the mechanism(s) by which T cells and IFN-γ mediate the effects on CNS chemokine expression, cellular infiltration into the CNS, and LCMV-induced anorexic weight loss remain unclear.

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fluorescently tagged anti-CD44, anti-CD16/32, and anti-CD4 antibodies (eBioscience or BD Biosciences, San Jose, CA). The data were acquired on a FACSCalibur (BD Biosciences) and analyzed by using CellQuest Pro software (BD Biosciences).

For myc staining, brain cells were harvested from day 8 i.c. LCMV-infected mice and were stained with antibodies against CD11b, CD11c, CD45, and myc (clone 9E10, produced in house), and data were acquired by flow cytometry. For phospho-Stat1 staining, brain cells were harvested from uninfected MIG or C57BL/6 control mice and were stained with fluorescently labeled antibodies against CD11b, CD11c, and CD45 at 4°C and then washed and cultured for 15 min at 37°C with IFN-γ (5 ng/ml). Cells were then washed and fixed in 4% paraformaldehyde, washed twice, fixed again with 90% methanol, and then stained with anti-phospho-Stat1-Alexa-647 (BD Biosciences).

**Multiplex analysis of CSF.** Detection of granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), IL-1α, IL-1β, IL-10, CCL2, CCL3, CCL5, CXCL10, and IFN-γ in diluted CSF was performed by using a Mouse Cytokine/Chemokine LINCOplex kit (Millipore, St. Charles, MO) according to the manufacturer’s instructions. Concentrations of analytes were calculated as follows: (the observed concentration) × [the amount CSF harvested/25 μl]. Samples in which analytes were below the limit of detection were given a value of zero. Samples in which CXCL10 was described above the limit of detection were given a value of (amount CSF harvested/25 μl) × 10,000 pg/ml (the topmost value on the standard curve).

**Statistical analyses.** Statistical analyses were performed by using a Student t test using either Excel or Minitab for Windows software, release 14 (Minitab, Inc., State College, PA). For weight loss studies, a repeated-measures analysis of variance was performed. Calculated P values of ≤0.05 were considered significant.

**RESULTS**

**T cells are required for CNS immune cell recruitment and chemokine production.** Previous studies have shown that CD4+ T cells and IFN-γ are necessary to cause anorexia and weight loss in β2-m−/− mice after i.c. LCMV infection (53, 57). However, the mechanism(s) through which T cells and IFN-γ mediate disease are still unknown. Before examining the effects of T cells on the CNS immune response to LCMV, we first identified resident CNS and immune cell populations in the brain after i.c. LCMV infection. We identified five populations of cells, as defined in Fig. 1. Resident and infiltrating cells of the CNS can be differentiated based on their staining for the leukocyte common antigen CD45, with resident cells displaying intermediate staining (CD45int) and infiltrating cells having high-intensity staining (CD45high) (22, 67). Within the CD45int cells, we identified two subsets of microglia based on their expression of CD11c, as previously described by others (22, 62), and within the CD45high cells, we identified four populations of cells (Fig. 1). Additional stains indicated that most of the CD11b+ CD11c− population represents macrophages and that the majority of the CD11b+ CD11c+ (DC subset 1 [DC1]) and the CD11b− CD11c+ populations (DC2) are myeloid and plasmacytoid DCs, respectively (data not shown) (39, 62). Although this analysis does not reflect the heterogeneity that exists within these populations, it does represent major APC populations. Other stains also indicated that over half of the cells that are CD11b+ CD11c+ (Lym) are CD4+ T cells and the remaining cells are largely B cells (data not shown).

Having roughly defined brain APC populations, we investigated the effect of T cells on APC number, APC activation, and cytokine and chemokine production in response to i.c. LCMV infection in CD4+ T cell-depleted β2-m−/− mice. CD4+ T cells were depleted from β2-m−/− mice prior to infection via injection of a CD4-depleting monoclonal antibody; at least 98% depletion of CD4+ T cells was achieved (data not shown). At day 8 after infection, CD4-depleted β2-m−/− mice had ~5-fold lower numbers of CD45high cells in the brain than nondepleted controls, while there were no differences in the number of CD45int cells or in the distribution of cells within the CD45int population (Fig. 2A and B). The major effect of CD4 depletion was a significant loss of CD45high APC recruitment into the brain, which largely affected macrophages, although both subsets of DCs and lymphocytes themselves were decreased (Fig. 2A and C). Interestingly, depletion of CD4+ T cells dramatically affected activation of microglial, but not hematopoietic APC populations, at least as assessed by MHC class II expression (Fig. 2D and E). Perhaps the few cells that were able to migrate to the CNS were activated by CD4+ T-cell-independent mechanisms. Together, these data suggest that T cells are necessary for the recruitment of most hematopoietic cells to the CNS. Conversely, the lack of CD4+ T cells did not affect the numbers of microglia but did affect their activation.

To assess the contribution of T cells to the CSF cytokine/chemokine milieu, CSF was harvested from infected CD4-depleted and nondepleted β2-m−/− mice at day 8 after infection and subjected to multiplex analysis. We observed a difference...
in the abundance of cytokines and chemokines, with one group present in the CSF in picogram amounts, while another group was present in nanogram amounts (Fig. 2F and G). CD4-depleted mice had significantly lower levels of the chemokines CCL2 (MCP-1), CCL3 (MIP-1), and CCL5 (RANTES), and the cytokines IL-6 and IFN- compared to nondepleted controls (Fig. 2F and G). A trend toward decreased levels of CXCL10 (IP-10) was also observed (Fig. 2G). No significant differences were seen in the levels of TNF- or GM-CSF (Fig. 2F) or in the levels of IL-1 and IL-1 (data not shown). Thus, CD4 T cells are critical for immune cell infiltration and production of certain cytokines and chemokines in the CNS after viral infection.

IFN- activates resident and infiltrating APCs and promotes CNS chemokine production. Of the cytokines influenced by T cells at day 8 after infection, IFN- is the only T-cell-dependent cytokine in Fig. 2 to have been shown to play a role in LCMV-induced anorexia and weight loss (38). Therefore, we sought to determine whether IFN- was required to drive APC activation and CNS cytokine and chemokine production. We examined immune cell populations in the brain and CSF cytokines and chemokines in IFN- mice 8 days after i.c. LCMV infection. Because CD8 T cells kill 100% of wild-type mice by day 8 after i.c. LCMV infection (6, 18), we depleted animals of CD8 T cells by injection of a CD8-depleting monoclonal antibody prior to i.c. LCMV infection.

No significant differences were seen in the total numbers of CD45 and CD45 cells in the brain between IFN- mice (Fig. 3A). In the CD45 compartment, the numbers of CD1 and DC1 cells were significantly decreased in IFN- mice, whereas DC2 cells were unaffected and lymphocytes were increased in IFN- mice (Fig. 3C). The percentages of MHC class II-positive microglia and infiltrating macrophages and DCs were significantly lower in IFN- mice than C57BL/6 mice (Fig. 3D and E). Thus, IFN- is necessary for the activation of both resident microglia and infiltrating APCs after i.c. LCMV infection. These data also suggest that IFN- contributes to macrophage and DC recruitment while decreasing lymphocyte recruitment to the CNS.

To assess the contribution of IFN- on CNS cytokine and chemokine production, CSF was harvested from CD8-depleted IFN- and C57BL/6 mice at day 8 of LCMV infection and
analyzed by multiplex assay. IFN-γ−/− mice had significantly lower levels of TNF-α, CCL3, CCL5, and CXCL10 in the CSF compared to C57BL/6 mice, with a similar trend in CCL2 (Fig. 3F and G). IFN-γ did not appear to be required for CNS production of GM-CSF or IL-6 (Fig. 3F and G) nor for IL-1α, IL-1β, or IL-10 (data not shown). Thus, both CD4+ T cells and IFN-γ are critical for CNS production of CCL2, CCL3, CCL5, and CXCL10.

IFN-γ signaling in macrophage lineage cells promotes their activation and chemokine production in the CSF. IFN-γ affects multiple immune cells. However, we noticed particularly strong effects of T cells and IFN-γ on macrophages and macrophage-derived proinflammatory mediators. To more precisely determine the role of IFN-γ on macrophages and macrophagelike cells, we generated transgenic mice expressing a dominant-negative form of the IFN-γ receptor (dnIFN-γR) under the control of the CD68 promoter, that we refer to as MIIG mice. We first verified expression of the transgene by isolating macrophages from multiple tissues of MIIG mice and assessing their cell surface expression of the myc-tagged transgene using flow cytometry. Although the level of the myc staining varied somewhat from one tissue to the next, macrophages in all tissues analyzed from MIIG mice (e.g., blood, spleen, liver, bone marrow, peritoneum, and lung) stained positively for myc, whereas those isolated from wild-type mice did not stain with the myc transgene (data not shown). Consistent with the activity of the CD68 promoter, we found that the dnIFN-γR was expressed on microglial MG1 and MG2 cells and on infiltrating macrophages and DC1 cells but not on DC2 cells or on lymphoid cells (Fig. 4A). We consistently observed that expression of the dnIFN-γR was slightly higher on macrophages and DC1 cells than it was on microglial cells (Fig. 4A). We also found that the dnIFN-γR prevented the IFN-γ-driven induction of MHC class II on resident (CD45int) CNS cells (Fig. 4B). Finally, we assessed the functionality of the dnIFN-γR by measuring the IFN-γ-induced phosphorylation of Stat1 by flow cytometry. Although IFN-γ-induced phosphorylation of Stat1 by flow cytometry. Although IFN-γ induced phospho-Stat1 readily in wild-type MG1 and MG2 cells, it was significantly impaired from doing so in MG1 and MG2 cells from MIIG mice (Fig. 4C and D). Thus, the dnIFN-γR is expressed on macrophage lineage cells and markedly impairs their ability to phosphorylate Stat1 and increase MHC class II expression in response to physiologic amounts of IFN-γ.

At day 8 after i.c. LCMV infection, MIIG mice had significantly higher numbers of CD45int cells in the brain compared to littermate controls (Fig. 5A and B). However, compared to naive control mice the numbers of CD45int cells were decreased which was largely due to decreases in the numbers of
MG1 cells (data not shown). Compared to MG1 cells in naive control mice, in infected mice MG1 cells were reduced by ~50% in MIIG mice and by ~75% in control mice. Interestingly, the numbers of macrophages were significantly decreased in MIIG mice (Fig. 5C), whereas the numbers of lymphocytes were significantly increased, similar to the previous results in IFN-γ−/− mice (Fig. 3). DC1 cells were slightly, but not significantly (P < 0.08) decreased in MIIG mice, while DC2 cells were not changed in MIIG mice (Fig. 5C). MIIG mice had threefold lower percentages of MHC class II-positive microglia and MHC class II-positive macrophages in the brain compared to littermate controls (Fig. 5D and E). The percentages of class II-positive DC1 and DC2 cells were more subtly, albeit significantly decreased in MIIG mice (Fig. 5E). Together, these data suggest that IFN-γ receptor signaling on macrophage lineage cells is critical for the normal recruitment of macrophages and lymphocytes to the CNS.

To determine the effect of macrophage and microglial IFN-γ receptor signaling on CNS cytokine and chemokine production, CSF was harvested from MIIG and C57BL/6 mice 8 days after i.c. LCMV infection and analyzed by multiplex assay. MIIG mice had significantly lower levels of CCL2, CCL3, CCL5, and CXCL10 in the CSF compared to littermate controls (Fig. 5F and G). No significant differences in the levels of GM-CSF, TNF-α, or IL-6 were observed (Fig. 5F and G), nor were there significant differences in the levels of IL-1α, IL-1β, or IL-10 (data not shown). Since differential levels of virus could potentially explain some of these results, we assessed viral load in MIIG compared to wild-type mice. CD8-depleted MIIG mice and BL/6 controls had indistinguishable viral levels, with both having ~10^8 PFU/gm in the spleen and ~10^7 PFU/gm in the brain at 8 days after i.c. LCMV infection (data not shown). Together with the data from Fig. 2 and 3, these data suggest that IFN-γ from T cells requires IFN-γ signaling in macrophage lineage cells to promote production of CCL2, CCL3, CCL5, and CXCL10 within the CNS.

IFN-γ receptor signaling in macrophage lineage cells does not affect the magnitude of the T-cell response. Previous studies have suggested that IFN-γ can control the generation of CD4+ T-cell responses (77). Therefore, we examined whether the lack of IFN-γ signaling in macrophage lineage cells would affect the LCMV-specific CD4+ T-cell response and their recruitment to the CNS after i.c. LCMV infection by tracking the cells with MHC class II tetramers. There was a slight increase in the frequency and total numbers of LCMV-sp. CD4+ T cells in MIIG mice compared to littermate controls in both the brain and cervical LN at day 8 after infection (Fig. 6A and B). The difference in LCMV-specific CD4+ T cells in MIIG mice compared to controls appeared to be greater in the brain than in the LN, although the data were not significant (P < 0.07). Further, these effects on the T-cell response were not consistent across several experiments. There were also no differences observed in the numbers of LCMV-specific T cells between IFN-γ−/− mice and wild-type controls (data not shown). Together, these data suggest that IFN-γ signaling in macrophage lineage cells does not decrease the magnitude of the T-cell response after i.c. LCMV infection.

IFN-γ receptor signaling in macrophage lineage cells contributes to LCMV-induced anorexia and weight loss. Since previous data have implicated IFN-γ in LCMV-induced weight loss (35), we next inquired whether IFN-γ signaling in macrophage lineage cells was critical for such weight loss. To test
this, we infected groups of MIIG and wild-type control mice i.c. with LCMV and monitored them daily for food intake and weight loss. Weight loss began at the same time in both MIIG and control mice. MIIG mice initially lost weight similarly to controls; however, at day 9 after infection, MIIG mice were protected from further weight loss, whereas control mice progressed to lose more body weight (Fig. 7A). Weight loss was concurrent with a sharp decrease in food intake, which persisted in controls but not in MIIG mice (Fig. 7B). Thus, IFN-γ signaling in macrophage lineage cells is not necessary for the initiation of anorexic weight loss but instead is critical for the magnitude of such weight loss.

**DISCUSSION**

The infiltration of mononuclear cells, especially T cells, to the CNS is clearly central to the pathogenesis of LCMV-induced disease (17, 18, 46, 53, 57), but the mechanisms through which T cells mediate disease remain unclear. Here, we further investigated the role of CD4+ T cells and IFN-γ on immune cell infiltration and cytokine and chemokine production in the CNS after i.c. LCMV infection. Previous studies in this model have shown that at the onset of disease, IFN-γ is produced mainly by T cells and that deficiency in IFN-γ lessens LCMV-induced anorexia and weight loss (38). However, the IFN-γ receptor is expressed on both immune and nonimmune cells in all tissues (44), making it possible for IFN-γ to exert effects through a number of mechanisms.

In this report, we show that CD4+ T cells and IFN-γ, acting on macrophage lineage cells, promote the CNS production of inflammatory chemokines and the upregulation of MHC class II on resident APCs. These findings are consistent with earlier studies in which detection of chemokine mRNA was severely diminished in brains of athymic or IFN-γ-deficient mice infected with LCMV (2). We found here that CD4+ T-cell-depleted βm−/− mice had lower levels of CCL2, CCL3, CCL5, and CXCL10 in the CSF than did nondepleted controls. Deficiency in IFN-γ also affected levels of these same chemokines after LCMV infection. Since previous reports showed a role for IFN-γ in promoting optimal CD4+ T-cell responses (77), lower chemokines in IFN-γ−/− mice could be potentially explained by a decreased CD4+ T-cell response. However, the numbers of LCMV-specific T cells in the cervical LN or brain of IFN-γ−/− mice were not different from those of C57BL/6 controls (data not shown).

Our observation of normal numbers of LCMV-specific
we studied the immune response to LCMV during intracranial infection, whereas Whitmire et al. studied i.p. infection. It is possible that the requirements for IFN-γ signaling in the expansion of LCMV-specific CD4⁺ T cells are different depending on the route of infection. Second, Whitmire et al. assessed the role for IFN-γ receptor signaling using either an adoptive-transfer approach with T-cell-receptor transgenic T cells or by assessing intracellular cytokine production by endogenous CD4⁺ T cells (77). Adoptive transfer of T-cell-receptor transgenic T cells in LCMV infection can lead to opposing results depending on precursor frequency (76); thus, whether it mimics the endogenous response is unclear. Although Whitmire et al. also showed a decreased frequency of endogenous IFN-γ-producing CD4⁺ T cells in IFN-γ receptor-deficient mice (77), this does not necessarily identify all of the LCMV-specific T cells. Further, another group has not observed dramatic effects of IFN-γ receptor signaling on LCMV-specific CD4⁺ T-cell expansion (30). Here, we used LCMV-specific tetramers to identify I-A<sup>b</sup> gp61-80 specific T cells irrespective of cytokine production, and our data are more consistent with the latter study. Alternatively, if IFN-γ potentiates its own production, as shown in nonimmune cells (58), a lack of IFN-γ signaling to T cells could decrease the frequency of IFN-γ-producing cells, but not necessarily their overall abundance. Indeed, we found a decreased frequency of IFN-γ-producing CD4⁺ T cells in the brains of LCMV-infected MIIG mice compared to wild-type controls (data not shown). In addition, as an in vivo readout of CD4⁺ T-cell-dependent IFN-γ production, MIIG mice have slightly, albeit significantly less IFN-γ in their CSF compared to wild-type controls (Fig. 5F). Thus, it is possible that IFN-γ signaling does not affect the generation of an antiviral T-cell population; rather, IFN-γ signaling may reinforce T-cell production of IFN-γ and commitment to a Th1 response.

Our data show that IFN-γ acts directly on macrophage lineage cells (including microglia, macrophages, and myeloid DCs) to promote the production of chemokines in the CNS. However, it is not clear whether this is a direct effect of IFN-γ on microglial/macrophage/DC production of chemokines or whether IFN-γ signaling in microglia/macrophages/DCs induces these cells to secrete a factor to stimulate chemokine production from other cell types. Asensio and Campbell showed that CCL2, CCL5, and CXCL10 mRNA is detected in the brain is detected as early as 3 days after i.c. LCMV infection, prior to the infiltration of mononuclear cells (2). This finding suggests that resident cells, such as microglia and astrocytes, may produce these chemokines in response to infection. The capacity of these cells to secrete CCL2, CCL5, and CXCL10 in response to various stimuli, including IFN-γ, has been shown in several studies (4, 31, 36, 69, 74). Chemokine production could also be induced by LCMV stimulation of Toll-like receptors (TLR); astrocytes have been shown to preferentially express TLR3 and produce CCL2, CCL5, and CXCL10 response to various stimuli, including IFN-γ (20). However, without T cells, chemokine/cytokine levels in the CSF remain low. Later in i.c. LCMV infection, when mononuclear cell (particularly T-cell) infiltration to the CNS occurs, CCL3 mRNA is detected in the brain, and CCL2, CCL5, and CXCL10 mRNA is increased compared to day 3 (2). It is unclear whether the cellular infiltrate amplifies chemokine production from resident cells, or whether infiltrating
cells themselves produce chemokines. Nonetheless, early in infection, resident CNS production of chemokines may initiate hematopoietic cell infiltration, whereas later in infection, our data suggest that T-cell production of IFN-γ drives IFN-γ receptor signaling in macrophage lineage cells that strongly enhances chemokine expression in the CNS.

The chemokines CCL2, CCL3, CCL5, and CXCL10 are decreased in the CSF of mice with deficiencies in T cells, IFN-γ, or IFN-γ receptor signaling in macrophage lineage cells. CCL5 is a chemokine shown to be important for T-cell entry to the CNS (29, 42); however, we failed to observe a significant role for CCL5 or CCR5 in the recruitment or activation of LCMV-specific CD4+ T cells or APCs within the CNS (data not shown). Deficiency in CXCL10 and its receptor CXCR3 have been shown to decrease susceptibility of mice to fatal meningitis after i.c. LCMV infection due to decreased migration of CD8+ T cells into the brain parenchyma from the meninges (9, 13). Whether the same occurs for CD4+ T cells is unclear. It is possible that recruitment of LCMV-specific T cells to the CNS relies on several chemokines, which may have redundant functions. Future experiments will determine the pattern of chemokine receptor expression on CD4+ T cells and how IFN-γ affects these patterns, as well as the effect of CXCL10/CXCR3 deficiency on the recruitment of CD4+ LCMV-specific T cells and LCMV-induced anorexia and weight loss.

A previous study has suggested that IFN-γ contributes to anorexic weight loss during LCMV infection via the upregulation of MHC class II in the brain, which further drives the T-cell response (38). In the present study, the authors sug-
gested that in the absence of IFN-γ, the lack of MHC class II induction suppressed CD4⁺ T-cell responses and, by doing so, inhibited weight loss. However, we found here, using MIIG mice, that IFN-γ contributes to anorexic weight loss through its effects on macrophage lineage cells despite seemingly normal (or even slightly enhanced) T-cell responses. Thus, our data suggest that macrophage lineage cells responding to IFN-γ are cellular intermediates between T cells and anorexic weight loss, controlling the magnitude of the weight loss. In this manner, IFN-γ may induce the production of factors from macrophage lineage cells that then act in the hypothalamus, a region of the brain heavily involved in regulation of food intake (66), to decrease feeding. IL-1β can be produced by macrophages and microglia during CNS inflammation (5) and has been previously shown by us and others to be involved in LCMV-induced anorexia and weight loss (33, 38). However, the levels of CSF IL-1β were not affected by loss of IFN-γ or IFN-γ signaling (data not shown). Chemokines may be involved in LCMV-induced weight loss; CXCL10, CCL2, and CCL5 have been shown to decrease food intake after intracerebroventricular administration (56), and chemokine receptors are found in the hypothalamus (3). Thus, in addition to their effects on cellular recruitment, chemokines may also act downstream of IFN-γ signaling to promote and/or sustain weight loss during LCMV infection. Alternatively, we cannot exclude a potential metabolic effect of IFN-γ signaling as contributing to weight loss. We know that the initial weight loss is due to anorexia, since we have previously shown that uninfected mice, when pair-fed the amount of food eaten by infected mice, underwent a very similar weight loss (31). Any potential metabolic effect might be uncovered later in disease, when mice resume eating. We cannot exclude the possibility that IFN-γ drives increased metabolism that sustains weight loss and/or prevents weight gain.

Interestingly, IFN-γ signaling appears to affect microglial proliferation and/or survival. MIIG mice had significantly increased numbers of CD45⁺ cells in the brain compared to wild-type controls, and similar trends were seen in IFN-γ mice. The CD45⁺ population mostly affected was the CD11b⁺ CD11c⁻ MG1 microglia, which are thought to be CNS-derived microglia as opposed to the bone marrow-derived CD11b⁺ CD11c⁺ MG2 microglia (63, 68). In general, MG1 cells are lost after infection, since infected mice have consistently less MG1 cells compared to uninfected mice (data not shown). MG1 microglia (as well as MG2 microglia) express the dominant-negative transgene (Fig. 4A), suggesting that IFN-γ can act directly on these cells to promote apoptosis and/or inhibit proliferation. Preliminary experiments in MIIG mice suggest that IFN-γ signaling in MG1 microglia does not affect their expression of the proapoptotic molecule Bim but does slightly impair their proliferation (data not shown). Thus, IFN-γ may inhibit proliferation of microglia, since it has been shown to inhibit proliferation of a wide variety of immune and nonimmune cell types (51, 61, 70). The effect of IFN-γ on microglial numbers may be important for the progression of LCMV-mediated disease. Microglia have been shown to secrete anti-inflammatory molecules such as IL-10, transforming growth factor β, and indoleamine 2,3-dioxygenase (47, 80) that could dampen the immune response and lead to the decreased anorexia and weight loss seen in MIIG mice compared to wild-type controls. Future experiments will examine the potential immunosuppressive role of MG1 microglia in this model.

In conclusion, our results demonstrate that IFN-γ, most likely produced by CD4⁺ T cells, acts directly on macrophage lineage cells in vivo, leading to their upregulation of MHC class II and CNS production of inflammatory chemokines, which can contribute to further activation of T cells and progression of disease. These observations are intriguing given the purported role of IFN-γ in the pathogenesis of several diseases, such as multiple sclerosis, sepsis, human immunodeficiency virus-associated dementia, and Alzheimer’s (14, 19, 32, 79, 81). The results of the present study can lend insight into results from current clinical trials involving the administration of IFN-γ as adjuvant therapy (49) and possibly the design of future trials involving IFN-γ blockade. The present study also provides a basis for further investigation of the effects of IFN-γ in immunopathology and neuroimmunology, as well as the effects of IFN-γ on infection-induced anorexia, all of which may have significant implications in the development of therapies for neuroinflammatory and immune-mediated diseases.

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