The Interdependent, Overlapping, and Differential Roles of Type I and II IFNs in the Pathogenesis of Experimental Autoimmune Encephalomyelitis

Rodrigo Naves, Simer P. Singh, Kevin S. Cashman, Amber L. Rowse, Robert C. Axtell, Lawrence Steinman, John D. Mountz, Chad Steele, Patrizia De Sarno and Chander Raman

*J Immunol* published online 19 August 2013
http://www.jimmunol.org/content/early/2013/08/17/jimmunol.1300419

Supplementary Material

http://www.jimmunol.org/content/suppl/2013/08/21/jimmunol.1300419.DC1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
The Interdependent, Overlapping, and Differential Roles of Type I and II IFNs in the Pathogenesis of Experimental Autoimmune Encephalomyelitis

Rodrigo Naves,*1 Simer P. Singh,*2 Kevin S. Cashman,† Amber L. Rowse,† Robert C. Axtell,‡ Lawrence Steinman,‡ John D. Mountz,* Chad Steele,*† Patrizia De Sarno,§ and Chander Raman*§†

Type I IFNs (IFN-α and IFN-β) and type II IFN (IFN-γ) mediate both regulation and inflammation in multiple sclerosis, neuromyelitis optica, and in experimental autoimmune encephalomyelitis (EAE). However, the underlying mechanism for these Janus-like activities of type I and II IFNs in neuroinflammation remains unclear. Although endogenous type I IFN signaling provides a protective response in neuroinflammation, we find that when IFN-γ signaling is ablated, type I IFNs drive inflammation, resulting in exacerbated EAE. IFN-γ has a disease stage–specific opposing function in EAE. Treatment of mice with IFN-γ during the initiation phase of EAE leads to enhanced severity of disease. In contrast, IFN-γ treatment during the effector phase attenuated disease. This immunosuppressive activity of IFN-γ required functional type I IFN signaling. In IFN-α/β receptor–deficient mice, IFN-γ treatment during effector phase of EAE exacerbated disease. Using an adoptive transfer EAE model, we found that T cell–intrinsic type I and II IFN signals are simultaneously required to establish chronic EAE by encephalitogenic Th17 cells. However, in Th17 cells loss of either IFN signals leads to the development of a severe chronic disease. The data imply that type I and II IFN signals have independent but nonredundant roles in restraining encephalitogenic Th17 cells in vivo. Collectively, our data show that type I and II IFNs function in an integrated manner to regulate pathogenesis in EAE. The Journal of Immunology, 2013, 191: 000–000.

The IFNs are a family of related cytokines exerting an essential role in inflammation and autoimmunity. They are classified into two subtypes according to receptor specificity and sequence homology (1). Type I IFNs consist of IFN-β and several other members, whereas type II IFN has only a single member, IFN-γ. Types I and II IFNs bind distinct cell surface receptor complexes, the IFN-α/β receptor (IFNAR) and the IFN-γ receptor (IFNGR), respectively. IFNAR and IFNGR are comprised of two transmembrane glycoproteins: IFNAR1 and IFNAR2, and IFNGR1 and IFNGR2 (2, 3). A common feature of both type I and II IFNs is the employment of the JAK-STAT signal transduction pathway to regulate nuclear gene expression (1).

Although types I and II IFNs bind distinctive receptors and differentially regulate the expression of a variety of other cytokines, they develop synergistic functions priming macrophages for tumor cell killing (4), enhancing CTL responses to melanoma cell vaccines (5), and inhibiting viral replication (6). Recruitment and modulation of STAT1 are central elements in the cross-talk between types I and II IFNs triggered in response to viral infection (7, 8). However, the cooperative action of types I and II IFNs and the pathophysiological significance of this interaction in autoimmunity have been less studied.

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS and the leading cause of neurologic disability in young adults (9). There is compelling evidence supporting the individual role of types I and II IFNs in the pathogenesis of MS and its animal model, experimental autoimmune encephalomyelitis (EAE). The lack of either IFN-β or IFNAR results in a more severe and chronic EAE (10–12), and the systemic administration of IFN-β is, to date, the most commonly used therapy for MS providing longer periods of remission, reducing the severity of relapses, and decreasing the inflammatory lesion in the CNS (13–16). In contrast, controversial evidence has been reported in relationship to the role of IFN-γ in MS and EAE. A positive association between increased levels of IFN-γ and clinical manifestations attributed a pathological role of IFN-γ in MS (17–20). Subsequent studies have challenged the notion that IFN-γ is pathogenic, and they have even suggested a protective role for IFN-γ in EAE, and perhaps in some forms of MS (21–27). Furthermore, EAE can be ameliorated
after IFN-γ-treatment (24, 28–30), whereas MS patients treated with IFN-γ exhibited exacerbations of disease (31). Therefore, the role of IFN-γ in MS and EAE is still unresolved.

Th cells producing either IFN-γ (Th1) or IL-17 (Th17) have been shown to play a critical role in the immunopathogenesis of MS and EAE (32–35). Recently, we have demonstrated that IFN-β is very effective in the treatment of EAE induced by Th1 cells but it is ineffective and induces exacerbations when disease is led by Th17 lymphocytes (36). Remarkably, IFN-β treatment significantly worsened EAE in IFNGR-deficient mice, suggesting that immunosuppression by IFN-β required functional activity of IFN-γ (36). In this study we tested the hypothesis that IFN-β and IFN-γ act cooperatively to modulate autoimmune neuroinflammation. Our results revealed an intricate interaction between types I and II IFN signaling in the pathogenesis of EAE regulating the threshold of EAE susceptibility, the effector function of Th1 and Th17 cells, and the severity of disease. A functional and reciprocal interaction between types I and II IFN signaling was indispensable to promote a protective response.

Materials and Methods

Mice

C57BL/6 (45.1 and 45.2) mice were purchased from Frederick Cancer Research. B6.129S7-Ifngr1m/mois (ifngr1−/−) mice were purchased from The Jackson Laboratory and backcrossed onto C57BL/6 (B6) background for 10–12 generations. The B6.ifnar1−/− mouse (37) was obtained from Dr. Jocelyn Demengeot (Instituto Gulbenkian de Ciência, Oeiras, Portugal). Ifngr1−/− mice were developed by crossing ifngr1−/− and ifngr1−/− mice. The Stat1m mice were provided to us by Dr. R. Lorenz (University of Alabama at Birmingham). All mice were maintained and bred at the University of Alabama at Birmingham in fully facility and treated in accordance with National Institutes of Health and the University of Alabama Animal Care and Use Committee guidelines.

Induction of EAE, scoring, and treatment

Active EAE was induced by immunizing wild-type (WT) and knockout mice 8–12 wk of age with a s.c. injection of MOGps55 peptide (MOGp55) (CPC Scientific) at the dose indicated as described previously (38). For treatment with IFN-γ, either 400 ng or 1 μg recombinant murine IFN-γ (Millipore) was administered i.p. Onset and classical clinical progression of EAE symptoms were monitored daily using a standard scale of 0–6 as described previously (38). Atypical EAE symptoms were scored as follows: 0, no clinical signs; 1, slight head tilt; 2, severe head tilt; 3, slight axial rotation/ staggered walking; 4, severe axial rotation/spinning; 5, moribund; 6, death.

CD4+ T cell differentiation and adoptive transfer of EAE

Single-cell suspensions were harvested from pooled spleens and lymph nodes (five mice per group) 11 d after immunization with MOGp in CFA. The cells were resuspended with MOGp under nonpolarizing (unpolarized) conditions or under Th1- or Th17-polarizing conditions as described previously (36, 39). The cell populations were characterized by flow cytometry for CD4+ T cell differentiation and adoptive transfer of EAE (32–35). Recently, we have demonstrated that IFN-b is ineffective and induces exacerbations when disease is led by Th17 lymphocytes (36). Remarkably, IFN-β treatment significantly worsened EAE in IFNGR-deficient mice, suggesting that immunosuppression by IFN-β required functional activity of IFN-γ (36). In this study we tested the hypothesis that IFN-β and IFN-γ act cooperatively to modulate autoimmune neuroinflammation. Our results revealed an intricate interaction between types I and II IFN signaling in the pathogenesis of EAE regulating the threshold of EAE susceptibility, the effector function of Th1 and Th17 cells, and the severity of disease. A functional and reciprocal interaction between types I and II IFN signaling was indispensable to promote a protective response.

Histology and immunohistochemistry

Cross-sections of brain stem, cerebellum, and spinal columns were obtained from mice at 20 d after EAE induction and were immersion-fixed in Bouin’s fixative. Histochemical and immunohistological analysis were performed as described previously (40). Inflammatory infiltrates and demyelination were analyzed histochemically by H&E and Luxol fast blue staining, respectively. Neutrophils (anti-myeloperoxidase; Lab Vision), CD4+ T cells (goat anti-CD4; R&D Systems), and activated microglia (anti–GS-IB4) were evaluated by immunohistochemical staining. Images were captured using a Zeiss LSM 710 confocal scanning microscope and Zen 2008 4.7.2 software (Carl Zeiss).

Statistical analysis

All EAE disease scores were analyzed using Mann–Whitney nonparametric test or one-way ANOVA Kruskal–Wallis with Dunn posttest using Prism 5 software (GraphPad Software). Mann–Whitney (with Tukey multiple comparisons) was used for all other analyses.

Results

Type I IFN signals exacerbate the effector phase of EAE in IFNGR-deficient mice

We reported that functional IFN-β signaling is necessary for IFN-β to be therapeutically effective in EAE (36). Thus, to test the hypothesis that type I and II IFNs cooperatively modulate pathogenesis in EAE, we examined the development and progression of EAE in Ifnar1−/−/Ifngr1−/− mice (Ifnar1−/−) and compared them to those in Ifngr1−/−, Ifnar1−/−, and WT mice. EAE in Ifngr1−/− mice was delayed in onset but more severe during the effector phase than that in WT and Ifnar1−/− mice (Fig. 1A, Table 1). In comparison, EAE in Ifngr1−/− mice was less severe than that in Ifngr1−/− mice but just as delayed in onset. In fact, the overall severity of disease in Ifnar1−/− mice was slightly but significantly lower than in Ifnar1−/− and WT mice (Fig. 1A). From these data we infer that 1) IFN-γ has a dual activity in the pathogenesis of EAE: pathogenic during the inductive phase, but protective during the effector phase; 2) type I IFN signals exacerbate EAE in the absence of IFN-γ signals, as suggested by the lowest EAE severity observed in Ifngr1−/− mice; and 3) during the initiation phase of disease, type II IFN signals are dominant over type I IFN.
In addition to classical EAE, mice with loss of IFNGR signals also develop an atypical form of disease (41, 42). When treated with 1 μg rIFN-γ beginning on the day of immunization developed exacerbated EAE with greater mortality than did those treated with PBS (Fig. 2A, Table III). rIFN-γ treatment did not alter the mean day of onset (Table III). A lower daily dose of rIFN-γ (400 ng) had no effect on disease severity or mortality. Most remarkably, initiation of treatment with either 1 μg or 400 ng rIFN-γ after the first sign of clinical symptoms (days 10–19) suppressed disease progression for the course of the treatment (Fig. 2B). Following cessation of treatment, EAE severity bounced to levels equivalent to those of PBS-treated mice. Reinitiation of rIFN-γ therapy moderately induced recovery (data not shown). The mean day of onset was also significantly delayed by both doses of rIFN-γ treatment (Table III).

Our results from active EAE (Fig. 1A) suggested that IFN-α/β signaling work in cooperation with IFN-γ signaling to modulate EAE severity. Therefore, we interrogated whether type I IFN signals participates in the disease amelioration induced by IFN-γ treatment. To test this hypothesis we induced EAE in Ifnar1−/− mice and on day 10 we initiated treatment with IFN-γ or PBS. Remarkably, EAE in Ifnar1−/− mice treated with IFN-γ was slight but significantly more severe than in PBS-treated mice (Fig. 2C, Table IV). This leads us to infer that type I IFN signals cooperate with IFN-γ signals to attenuate disease. Not surprisingly, IFN-γ treatment has no effect on EAE in mice lacking STAT1, the major STAT activated in response to engagement of both IFN-γ and IFN-α/IFN-β receptors (Fig. 2D, Table IV) (1).

Type I and II IFN signals are required to sustain Th1 EAE but not Th17 EAE

Both Th1 and Th17 cells contribute to the pathogenesis of EAE, albeit through different mechanisms (32, 43). To determine the T cell–intrinsic role of type I and II IFN signals in Th1 versus Th17 EAE, we generated nonpolarized, Th1-polarized, or Th17-polarized encephalitogenic T cells from WT mice and the different IFNR-deficient mice and transferred them into WT naive recipients. The donor cell populations from all groups of mice contained equal proportions of CD4+, CD8+, B220+, and regulatory T cells (CD4+CD25+Foxp3+), and no CD11b+ or CD11c+ cells at the time of transfer (Supplemental Fig. 1 and data not shown). Irrespective of the genotype of donor mice, restimulation cultures under nonpolarizing conditions contained Th1 cells, Th17 cells, and a small proportion of CD4+ T cells that coexpressed...
IFN-γ and IL-17 (ThIFN-γ'-IL-17') (Fig. 3). The proportion of Th1, Th17, and ThIFN-γ'-IL-17' cells in cultures from WT and Ifnar1−/− mice was similar. Likewise, nonpolarized cultures from Ifngr1−/− and Ifnagr1−/− mice contained equal proportions of the three Th effector subpopulations with respect to each other, but greater than Th effector subpopulations from WT or Ifnar1−/− mice (Fig. 3). The elevated numbers of Th17 cells in nonpolarized and Th17-polarized cultures from Ifngr1−/− mice are expected and reflect the inability of IFN-γ to suppress Th17 differentiation (44, 45). However, the finding that lack of IFNGR signaling led to several fold greater numbers of Th1 cells is unexpected because it opposes the dogma that IFN-γ is a feed-forward signal for Th1 differentiation (46).

Cells cultured under Th1- or Th17-polarizing conditions predominantly contained IFN-γ- or IL-17-expressing cells, respectively, independent of the genotype of the donor (Fig. 3). This reflects a good efficiency in the generation of encephalitogenic Th1 cells or Th17 cells.

We found that WT encephalitogenic T cells induced a more severe disease in naive recipients than did Ifnar1−/− donor T cells, but with similar onset (11 d) (Fig. 4A, Table V). Ifngr1−/− or Ifnagr1−/− donor encephalitogenic T cells induced disease with earlier onset and peak of severity than did disease induced by WT or Ifnar1−/− encephalitogenic cells (Table V). A feature common to recipients of Ifngr1−/− or Ifnagr1−/− cells is the partial remission of disease to a severity less than that induced by WT donors and equivalent to that induced by Ifnar1−/− donors (Fig. 4A).

Th1-polarized encephalitogenic T cells from Ifngr1−/− and Ifnagr1−/− mice induced a very rapid and acute disease followed by a nearly complete remission (Fig. 4B, Table V). In contrast, WT Th1-polarized cells induced a slightly delayed but a nonremitting chronic disease. This rapid remission in the recipients that received IFNGR-deficient Th1-polarized encephalitogenic T cells was impressive considering that the transferred cell population contained up to 6-fold greater IFN-γ-expressing CD4+ T cells than in the WT Th1-polarized population. We observed that Ifnar1−/− encephalitogenic Th1-polarized cells induced EAE with onset and peak of severity equivalent to WT Th1-polarized cells, but this was followed by a partial remission of disease (Fig. 4B). The results suggest that whereas there is a T cell–intrinsic requirement for type I IFN signals to maintain severity of Th1 EAE, type II IFN signals are essential for sustaining disease. The rapid recovery in recipients of IFNGR-deficient Th1-polarized cells does not represent increased numbers of regulatory T cells (Supplemental Fig. 1B), but it may reflect the ability of the innate cells to respond to IFN-γ stimulation. IFN-γ induces inducible NO synthase in innate cells and production of NO, leading to their death and consequently disease attenuation. This property is likely to be more pronounced in recipients of Ifngr1−/− Th1 cells because the donor populations contain greater numbers of Th1 cells expressing high levels of IFN-γ (Fig. 3). To test this possibility, we performed a converse experiment in which WT encephalitogenic Th1 cells were transferred into naive WT and Ifngr1−/− recipients. We observed that WT and Ifngr1−/− recipients of encephalitogenic WT Th1 cells induced nonremitting EAE that ultimately reached equal severity (Fig. 4C). The Ifngr1−/− recipients did exhibit a delay to reach peak of disease. We suggest that this delay in reaching peak of disease mechanistically reflects the delayed onset of active EAE in Ifngr1−/− mice (Fig. 1A); however, this requires additional investigation.

In opposition to Th1 EAE, recipients of Th17-polarized encephalitogenic T cells from all IFN receptor–deficient mice developed EAE that was significantly rapid in onset followed by a chronic phase showing no recovery during the 30 d of observation.

| Table II. Incidence of atypical EAE in IFNGR-deficient mice |
|-----------------|-----------------|-----------------|
| **Group of Mice** | **Incidence (%)** | **Group of Mice** | **Incidence (%)** |
| Standard MOG dose | | Low MOG dose | |
| WT | 0/10 (0) | WT | 0/14 (0) |
| Ifnar1−/− | 0/10 (0) | Ifnar1−/− | 0/13 (0) |
| Ifngr1−/− | 8/9 (89) | Ifngr1−/− | 12/14 (86) |
| Ifnagr1−/− | 6/8 (65) | Ifnagr1−/− | 8/11 (73) |

**These mice rapidly developed severe EAE, complicating the ability to resolve atypical from classical EAE. The percentage of atypical EAE is a conservative estimate; however, the actual incidence is most likely >90%.**

FIGURE 2. Dual role of IFN-γ in EAE. Clinical scores from EAE in C57BL/6 mice that were treated with PBS, 400 ng or 1 μg IFN-γ daily from (A) day 1 to day 10 or (B) day 10 to day 19 after EAE induction (n = 9–13 mice/group). (C) Clinical scores from EAE in WT and Ifnar1−/− mice that were treated with PBS or 1 μg IFN-γ daily from day 10 to day 19 after EAE induction (n = 8 mice/group). (D) Clinical scores from EAE in WT and Stat1−/− mice treated with PBS or 1 μg IFN-γ daily from day 10 to day 19 after EAE induction (n = 7–12 mice/group). Results are pooled from two or three experiments. **p < 0.001 for comparisons between (A) 1 μg IFN-γ–treated WT mice versus PBS–treated WT and 400 ng IFN-γ–treated WT between days 16 and 30; (B) PBS-treated WT and 400 ng and 1 μg IFN-γ–treated WT mice between days 10 and 30; and (C) PBS-treated Ifnar1−/− mice versus IFN-γ–treated Ifnar1−/− between days 16 and 30.
in the recipients of WT or CD4+ T cells were determined 14 and 25 d following transfer. The numbers of infiltrating recipient CD4+ T cells were equivalent under nonpolarizing or Th1-polarizing conditions significantly in mice and the numbers of CNS (SC)-infiltrating recipient and donor T cells were necessary to restrain encephalitogenic Th1 cells from entering the brain.

**Th1 cells and not Th17 cells from mice lacking IFNGR hyperproliferate in response to Ag**

The rapid remission or development of chronicity may also reflect the differential ability of Th1 and Th17 cells from the IFNR-deficient mice to proliferate in response to Ag restimulation. To test for this possibility, we determined the proliferation of encephalitogenic T cells stimulated with MOGp and cultured under nonpolarizing, Th1-polarizing, or Th17-polarizing conditions. In the absence of stimulation or under Th17-polarizing condition, the proliferation was similar in T cells from all mice (Fig. 5A, 5B). In contrast, T cells from Ifngr1−/− and Ifnagr1−/− mice cultured under nonpolarizing or Th1-polarizing conditions significantly hyperproliferated in response to MOGp stimulation compared with WT and Ifnar1−/− cells (Fig. 5C, SD). Thus, the failure of type II IFN-γ-deficient Th1 cells to sustain disease is not due to T cell–intrinsic inability to proliferate in the recipient mice. We next determined whether Ifnagr1−/− Th1 cells are unable to persist in recipients. We transferred equivalent numbers of WT or Ifngr1−/− Th1-polarized cells (CD45.2+) into naive WT CD45.1 mice and the numbers of CNS (SC)-infiltrating recipient and donor CD4+ T cells were determined 14 and 25 d following transfer. The numbers of infiltrating recipient CD4+ T cells were equivalent in the recipients of WT or Ifngr1−/− Th1-polarized cells. In contrast, the SC of recipients that received IFNGR-deficient cells contained fewer donor cells than did that of WT recipients on day 14 (Fig. 5E). By day 28, <3500 IFNGR-deficient donor cells were recovered from the recipients. From these results we infer that the nearly complete remission of EAE in recipient mice that received IFNGR-deficient Th1 cells is, at least in part, due to a T cell–intrinsic requirement for IFNGR signaling for the persistence of encephalitogenic Th1 cells.

**IFNAR signaling does not affect IFN-γ–regulated migration to the brain**

The infiltration of mononuclear cells into the CNS is dependent on the Th1/Th17 encephalitogenic T cell ratio and is regulated by IFN-γ (47, 49). To determine whether IFNAR signaling altered IFN-γ–regulated migration of leukocytes into the CNS, we evaluated infiltration and demyelination in brain stem, cerebellum, and SC at the peak of disease (day 20). We observed detectable mononuclear infiltration and demyelination of brain stem and cerebellum only in sections from Ifnagr1−/− and Ifnagr1−/− mice (Fig. 6A, 6B). The levels of activated microglia/macrophages and the extent of infiltration of neutrophils and CD4+ T cells were similar in the CNS of Ifngr1−/− mice and Ifnagr1−/− mice. The results show that type I IFN signaling does not alter IFN-γ–dependent entry of inflammatory cells into the brain.

Although the clinical score was similar to WT (3.5) and Ifnar1−/− (3.5) mice, the SC sections from Ifngr1−/− mice (3.0) showed the greatest extent of mononuclear cell infiltration, demyelination, presence of neutrophils, CD4+ T cells, and activated microglia/macrophages (Fig. 6C). There was no difference between WT and Ifnar1−/− for all parameters evaluated. The extent of mononuclear cell infiltration and demyelination in SC from Ifnagr1−/− mouse was the least, and this correlated with the lower classical EAE score of 1.0. However, this mouse had severe atypical EAE (3.5).

### Table III. Disease stage specific activity of IFN-γ in EAE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day of Onset (Mean ± SD)</th>
<th>Maximum Score (Mean ± SD)</th>
<th>Time to Peak (d) (Mean ± SD)</th>
<th>Accumulative Score (Mean ± SD)</th>
<th>Incidence (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT PBS</td>
<td>16.1 ± 1.9</td>
<td>3.8 ± 0.7</td>
<td>19.5 ± 2.9</td>
<td>46.1 ± 7.0</td>
<td>13/13 (100)</td>
<td>1/13 (7.7)</td>
</tr>
<tr>
<td>WT 400 ng IFN-γ</td>
<td>15.8 ± 4.0</td>
<td>4.1 ± 0.2</td>
<td>21.8 ± 4.5</td>
<td>46.6 ± 11.5</td>
<td>5/5 (100)</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>WT 1 μg IFN-γ</td>
<td>16.4 ± 2.7</td>
<td>4.7 ± 1.1</td>
<td>20.6 ± 3.0</td>
<td>58.1 ± 26.6</td>
<td>9/9 (100)</td>
<td>3/9 (33.3)</td>
</tr>
<tr>
<td>After onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT PBS</td>
<td>14.5 ± 3.4</td>
<td>4.4 ± 1.2</td>
<td>21.6 ± 4.9</td>
<td>56.2 ± 26.9</td>
<td>10/10 (100)</td>
<td>3/10 (30)</td>
</tr>
<tr>
<td>WT 400 ng IFN-γ</td>
<td>24.3 ± 4.3</td>
<td>3.6 ± 0.5</td>
<td>26.5 ± 4.0</td>
<td>22.1 ± 17.2</td>
<td>4/4 (100)</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>WT 1 μg IFN-γ</td>
<td>19.4 ± 5.8</td>
<td>3.9 ± 0.5</td>
<td>27 ± 2.4</td>
<td>32.8 ± 12.1</td>
<td>9/9 (100)</td>
<td>0/9 (0)</td>
</tr>
</tbody>
</table>

*Moribund mouse that had to be sacrificed.

*A higher mortality was observed within the PBS treated group, perhaps an outcome of the stress induced by daily injections.

*p < 0.05, comparison between IFN-γ treatment and PBS treatment.

### Table IV. Activity of IFN-γ is dependent of type I IFN signaling and STAT1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day of Onset (Mean ± SD)</th>
<th>Maximum Score (Mean ± SD)</th>
<th>Time to Peak (d) (Mean ± SD)</th>
<th>Accumulative Score (Mean ± SD)</th>
<th>Incidence (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 2C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT PBS</td>
<td>12.4 ± 2.7</td>
<td>4.3 ± 0.7</td>
<td>14.9 ± 3.8α</td>
<td>65.9 ± 20.4</td>
<td>8/8 (100)</td>
<td>1/8 (12.5)</td>
</tr>
<tr>
<td>WT IFN-γ</td>
<td>15.6 ± 3.2</td>
<td>4.2 ± 0.4</td>
<td>22.3 ± 3.2</td>
<td>46.8 ± 15</td>
<td>8/8 (100)</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>Ifnar1−/− PBS</td>
<td>11.5 ± 0.9</td>
<td>3.8 ± 0.3</td>
<td>14.1 ± 1.9</td>
<td>62.3 ± 7.0</td>
<td>8/8 (100)</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>Ifnar1−/− IFN-γ</td>
<td>12.1 ± 1.0</td>
<td>4.4 ± 0.7</td>
<td>16.3 ± 2.4</td>
<td>70.5 ± 16.5</td>
<td>8/8 (100)</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>Fig. 2D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT PBS</td>
<td>11.7 ± 2.6α</td>
<td>3.8 ± 1.1</td>
<td>13.7 ± 2.9α</td>
<td>55.7 ± 25.5α</td>
<td>10/10 (100)</td>
<td>1/10 (30)</td>
</tr>
<tr>
<td>WT IFN-γ</td>
<td>18.9 ± 3.5</td>
<td>4.4 ± 0.8</td>
<td>25 ± 4.0</td>
<td>34.1 ± 12</td>
<td>7/7 (100)</td>
<td>1/7 (14.2)</td>
</tr>
<tr>
<td>Stat1−/− PBS</td>
<td>13.6 ± 4.2</td>
<td>4.6 ± 0.5</td>
<td>18.9 ± 5.4</td>
<td>69.3 ± 25.4</td>
<td>8/8 (100)</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>Stat1−/− IFN-γ</td>
<td>14.9 ± 3.6</td>
<td>5.1 ± 0.9</td>
<td>21.9 ± 3.5</td>
<td>65.5 ± 20.5</td>
<td>7/7 (100)</td>
<td>3/7 (42.9)</td>
</tr>
</tbody>
</table>

*p < 0.05, comparison between treatments in WT mice.
IFNAR-deficient mice than in WT mice, but this difference was not significantly different from any of the IFNR-deficient mice (Fig. 7C). IL-13 deletion of IFNAR (Fig. 7B). WT mice had the highest levels of IL-13 in comparison with any of the IFNGR-deficient mice (Table I). Notably, IFN-γ signaling on the expression of cytokines and chemokines in the CNS, we evaluated the expression of 31 cytokines and chemokines at the peak of disease (day 20) in brain and SC. IFN-γ expression was elevated to equivalent levels in SC of WT and IFNAR-deficient mice (Fig. 7A). Small amounts of IFN-γ were detected in brains of some WT and IFNAR-deficient mice, but overall the levels were not significantly different from unimmunized mice (Supplemental Table I). Notably, IFN-γ was expressed at significantly higher levels in brain and SC of Ifnagr1−/− and Ifngr1−/− mice than in WT and Ifnar1−/− mice (Fig. 7A, Supplemental Table I). In both CNS tissues, there was a trend for lower IFN-γ in mice with combined lack of IFNAR and IFNGR compared with IFNGR-only-deficient mice; however, the difference was not statistically significant within this sample size. This elevated level of IFN-γ in the absence of IFNGR signaling was not associated with increased expression of Tbet as determined by real-time PCR or IL-12p70 (data not shown). IL-17 was detected in all mice with EAE in both SC and brain (Fig. 7B). We observed significantly higher levels of IL-17 in the SC, but not in the brain, of Ifnar1−/− mice compared with WT mice. The CNS of IFNGR-deficient mice contained greatly elevated levels of IL-17 that were not altered by the deletion of IFNAR (Fig. 7B). WT mice had the highest levels of IL-13 in comparison with any of the IFNR-deficient mice (Fig. 7C). IL-13 was essentially absent when IFNAR signaling was ablated.

In both the SC and brain the levels of CXCL10 (IP10) was lower significant only in the brain (Fig. 7D). IP-10 levels were further significantly reduced in CNS of IFNGR-deficient mice, and mice lacking both IFN receptors had the lowest levels of the chemokine. In fact, the expression of CXCL10 in Ifngr1−/− mice was comparable to that in unimmunized mice. The results indicate that both IFNAR and IFNGR independently induce CXCL10, and combined loss of both IFN receptors has an additive effect. Mice lacking only IFNAR had levels of CXCL9 (MIG) or CCL5 (RANTES) similar to WT mice (Fig. 7E, 7F). However, ablation of IFNGR resulted in complete absence of CXCL9. IFNGR-deficient mice also had dramatically reduced levels of CCL5 that were decreased to levels equivalent to those in unimmunized mice when both IFNGR and IFNAR were absent (Fig. 7F). IFNAR signaling-depending expression of CCL5 has been observed previously (50), and our data suggest that this occurs only in the absence of IFN-γ signaling.

Higher levels of G-CSF were observed in SC and brains of mice lacking IFNGR compared with IFNAR-deficient mice (Fig. 7G). This observation is consistent with the elevated infiltration of neutrophils in the CNS of Ifngr1−/− mice (Fig. 6). In the SC, the levels of G-CSF were significantly higher in IFNAR-deficient mice compared with WT mice, but this was not associated with any significant increase in neutrophil infiltration (Fig. 7G). We found no significant effect of IFN receptor deletion on the remaining 24 cytokines (see Materials and Methods and data not shown).

Type I and II IFN signals regulate threshold of response to development of EAE

Two previous studies using the same Ifnar1−/− mouse as this study showed that loss of IFNAR signaling exacerbates EAE, a finding that differs from our result (Fig. 1A) (10, 11). We noticed that in both of these reports EAE was induced with a greater amount of MOGp than used in our protocol. To determine whether the dif-
ference in Ag dose explains the divergence of results, we im-
munized the different IFNRF-deficient mice and WT mice with 300 μg
MOGp (high dose) and evaluated the development and progression of
EAE. At this higher immunization dose of MOGp, EAE in
Ifnar1−/− mice as well as in Ifngr1−/− and Ifnagr1−/− mice was
more severe that in WT mice, thus reproducing the published
reports (Fig. 8A, Table I) (10, 11). However, increasing the MOGp
immunization dose from 150 to 300 μg led to a decrease in disease
severity in WT mice (Table I). This decrease in disease severity
with increase in immunization dose might reflect the phenomenon
of high dose tolerance (51, 52). Such a decrease in disease severity
was not observed in Ifnar1−/− mice. In Ifngr1−/− mice the increase
in peptide immunization dose did lead to significant increase in
disease severity and mortality (Supplemental Fig. 2C, Table I).

The above results suggest that type I and II IFNs have a role
in regulating the threshold or response to immunization dose and
consequently affecting EAE disease course. We therefore examined
the effect of low-dose MOGp (50 μg) immunization on de-
velopment of EAE in all groups of mice. The severity of EAE
in all of the IFNRF-deficient mice was similar to each other but
greater than that in WT mice (Fig. 8B, Table I). Importantly,
immunization with lower amounts of peptide did not significantly
alter onset, incidence, or severity of disease in any of the IFNRF-
deficient mice. In contrast, in WT mice immunized with 50 μg
MOGp, EAE was significantly less severe than in mice immunized
with 150 μg MOGp (Supplemental Fig. 2, Table I). The severity of
atypical EAE in Ifngr1−/− and Ifnagr1−/− mice was not
significantly altered with 300 μg peptide immunization (Supple-
mental Fig. 3). However, in mice immunized with 50 μg peptide,
Ifngr1−/− mice exhibited lower atypical disease severity than did
Ifnagr1−/− mice. Overall the results show that type I (IFN-α and/or
IFN-β) and IFN-γ signals are each independently involved in
setting the thresholds for susceptibility to EAE.

Discussion
This study reveals a remarkable cooperative relationship between
type I and II IFNs in regulating pathogenesis of EAE. We
show that the exacerbated disease observed in IFNRF-deficient
mice is reversed to levels observed in WT mice when there is
concomitant ablation of type I IFN signaling. IFN-β is
highly expressed by brain-resident glial cells and possibly infiltrating
dendritic cell subsets in the CNS of mice during active EAE (11,
53–55). In fact, we recently showed that endogenous IFN-β
continues to be highly expressed in the CNS of mice lacking
IFNGR (56). This local expression of IFN-β is a protective re-
sponse in EAE and is the principle behind using it as therapy for
the treatment of MS (10–12, 57). Our results now show that in
the absence of IFN-γ cosignaling, endogenous IFN-α/β drives in-
flammation rather than immunosuppression. This is consistent
with our recent finding that administration of IFN-β to IFNRF-
deficient mice with EAE further exacerbates disease (36). This
Janus-like property of IFNAR signaling is also evident for IFNGR
signaling. During the initiation phase of EAE, IFN-γ drives path-
ogenesis, as suggested by the delayed onset of disease in IFNRF-
deficient mice. Indeed, we found that administration of IFN-γ
during this period leads to exacerbation of disease. In contrast, type I
IFN signaling seems to have a limited role in disease initiation.
In comparison with EAE in WT mice, the lack of IFNAR or IFN-β
do not alter disease onset (10–12). The disease onset in mice with
combined absence of IFNAR and IFNGR was not different from
those lacking only IFNGR, indicating that type I IFN signaling
begins to participate in EAE at a time point later than IFN-γ sig-
aling. In fact, IFN-β in EAE is first detected in the CNS only after
onset of clinical symptoms (11).

IFN-γ treatment controls the progression of experimental au-
toimmune myocarditis (58). In rodents, IFN-γ treatment amelo-
rated EAE in some studies but induced encephalomyelitis in
others (24, 28–31, 59, 60). In MS, IFN-γ treatment led to exac-
erbations in some patients and no benefit in others, an outcome
recapitulated to some extent in a novel marmoset model of EAE
(31, 61). Overall, these studies led to ambiguity regarding the role
of IFN-γ in EAE and MS (17–26). The present study contributes
to clarify this paradoxical evidence and provides insight into the
complex role of IFN-γ during EAE pathogenesis. Our results in-
dicate that the previous mixed outcome likely reflects disease
stage-specific Janus-like function of IFN-γ. We found that IFN-γ
treatment during the initiation phase promoted pathogenesis; in
contrast, such treatment during the effector phase was immuno-
suppressive in EAE. However, this immunosuppressive activity of
IFN-γ required functional type I IFN signaling. The composite
developing picture reinforces the model that integration of type I
and II IFN signaling pathways are necessary to regulate patho-
genesis in EAE. Imbalance of either pathway drives pathogenesis.

We found that encephalitogenic Th1 cells require type II IFN
signals for inducing persistent and chronic EAE. In comparison,
IFN-α/β signals are not necessary for chronicity but are important
for disease severity. As reported in this study and shown previ-

Table V. Type I and II IFNs are required to sustain Th1 EAE and restrain Th17 EAE

<table>
<thead>
<tr>
<th>Group of Mice</th>
<th>Day of Onset (Mean ± SD)</th>
<th>Maximum Score (Mean ± SD)</th>
<th>Time to Peak (d) (Mean ± SD)</th>
<th>Accumulative Score (Mean ± SD)</th>
<th>Incidence (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpolarized T cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>10.8 ± 2.2</td>
<td>4.0 ± 0.0</td>
<td>13 ± 1.7</td>
<td>72.3 ± 5.2</td>
<td>10/10 (100)</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>Ifnar1−/−</td>
<td>11.1 ± 4.5</td>
<td>3.8 ± 0.4</td>
<td>13.7 ± 4.8</td>
<td>52.8 ± 19.5</td>
<td>7/7 (100)</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>Ifngr1−/−</td>
<td>7.2 ± 0.5a</td>
<td>4.4 ± 0.7</td>
<td>10.1 ± 0.8a</td>
<td>68.6 ± 21.9</td>
<td>8/8 (100)</td>
<td>1/8 (12.5)</td>
</tr>
<tr>
<td>Ifnagr1−/−</td>
<td>7.0 ± 0.0a</td>
<td>3.8 ± 0.7</td>
<td>9.5 ± 1.7a</td>
<td>62.1 ± 31.8</td>
<td>8/8 (100)</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>Th1 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>8.5 ± 1.1</td>
<td>4.0 ± 0.0</td>
<td>10.8 ± 1.7</td>
<td>80.9 ± 4.9</td>
<td>10/10 (100)</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>Ifnar1−/−</td>
<td>8.0 ± 0.8</td>
<td>3.9 ± 0.2</td>
<td>9.7 ± 1.1</td>
<td>56.6 ± 26.3</td>
<td>7/7 (100)</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>Ifngr1−/−</td>
<td>6.2 ± 0.4b</td>
<td>3.5 ± 0.4a</td>
<td>8.5 ± 0.7a</td>
<td>31.4 ± 14.3b</td>
<td>11/11 (100)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>Ifnagr1−/−</td>
<td>6.0 ± 0.0a</td>
<td>3.5 ± 0.2a</td>
<td>8.1 ± 0.7a</td>
<td>43.1 ± 17.5b</td>
<td>11/11 (100)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>Th17 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>17.1 ± 2.1</td>
<td>3.6 ± 0.9</td>
<td>19.7 ± 1.6</td>
<td>40 ± 15</td>
<td>7/7 (100)</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>Ifnar1−/−</td>
<td>11.4 ± 2.8a</td>
<td>4.0 ± 1.1</td>
<td>14.4 ± 1.5a</td>
<td>75.8 ± 28</td>
<td>5/5 (100)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Ifngr1−/−</td>
<td>10.8 ± 1.1b</td>
<td>4.4 ± 0.2a</td>
<td>14.5 ± 1.1b</td>
<td>77.5 ± 8.2b</td>
<td>6/6 (100)</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>Ifnagr1−/−</td>
<td>8.8 ± 3.4a</td>
<td>4.5 ± 0.0a</td>
<td>17.2 ± 2.5a</td>
<td>74.7 ± 14.5a</td>
<td>6/6 (100)</td>
<td>0/6 (0)</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.001, comparison of each IFNRF-deficient mice versus WT mice with a similar experimental paradigm.

One moribund mouse was sacrificed.

Downloaded from http://www.jimmunol.org/ by guest on April 16, 2017
viously, MOGp-induced EAE in IFN-γ signaling–deficient mice is severe and chronic, and this seems to conflict with the self-limiting EAE induced by IFNGR-deficient Th1 cells (21–23, 27). We suggest this difference reflects role of IFNGR signaling in Th1 cells versus other effector cells. IFN-γ signaling is needed 1) to restrain Th17 differentiation and expansion (44, 45), 2) for efficient IL-27 induction by dendritic cells and macrophages (62, 63), and 3) for induction of inducible NO synthase and consequently production of NO (23, 28). Loss of these key protective mechanisms would lead to exacerbation of disease.

The absence of persistent EAE induced by IFN-γ signaling–deficient mice is severe and chronic, and this seems to conflict with the self-limiting EAE induced by IFNAR-deficient Th1 cells (21–23, 27). We suggest this difference reflects role of IFNAR signaling in Th1 cells versus other effector cells. IFN-γ signaling is needed 1) to restrain Th17 differentiation and expansion (44, 45), 2) for efficient IL-27 induction by dendritic cells and macrophages (62, 63), and 3) for induction of inducible NO synthase and consequently production of NO (23, 28). Loss of these key protective mechanisms would lead to exacerbation of disease.

The absence of persistent EAE induced by IFN-γ signaling–deficient Th1 donor cells can also be attributed to the protective effects of IFN-γ. Th1-polarized cell cultures from IFNAR-deficient mice contain several fold greater proportion of IFN-γ–expressing cells and these cells also express higher levels of IFN-γ compared with cells from WT or IFNAR-deficient mice. The high levels of IFN-γ can initially promote pathogenesis by promoting activation of APCs and other accessory cells such as upregulation of MHC class II, costimulatory molecules, and adhesion molecules (64). Subsequently, this higher level of IFN-γ can suppress CNS inflammation by inducing expression of NO in activated macrophages (65), inducing apoptosis of CNS-infiltrating lymphocytes and other mononuclear cells (28) and/or production of anti-inflammatory or neuroprotective cytokines/chemokines such as IL-13 and LIF (66–69). Indeed, we did observe that both IL-13 and LIF were barely induced in the CNS of IFNAR-deficient mice with EAE.

In opposition to Th1 cells, type I and II IFN signals restrain encephalitogenic Th17 cells and do so independently of each other. These observations recapitulate in vivo our previous in vitro finding of the ability of IFN-β to directly suppress Th17 cells but not Th1 cells (36). The direct suppression of Th17 cell differentiation by IFN-γ or IFN-β in vitro has been reported in several studies (10, 44, 45, 70).

FIGURE 5. IFNGR-deficient encephalitogenic Th1 cells but not Th17 cells hyperproliferate but do not persist in vivo. [3H]Thymidine cell proliferation of donor cells restimulated in vitro in (A) absence (control media) or (B–D) presence of 10 μg/ml MOGp in (B) nonpolarizing (Non), (C) Th1-polarizing (Th1), or (D) Th17-polarizing (Th17) conditions. Results are expressed in cpm. Error bars represent means ± SEM values from three independent experiments. *p < 0.05. (E) IFNGR1-deficient Th1-polarized encephalitogenic T cells do not persist. CD45.2 WT or Ifngr1<sup>−/−</sup> Th1-polarized encephalitogenic cells were transferred into naïve WT CD45.1 recipients. On days 14 and 25 posttransfer, the number of infiltrating recipient (CD45.1<sup>+</sup>) CD4<sup>+</sup> T cells or donor (CD45.2<sup>+</sup>) CD4<sup>+</sup> T cells was determined by flow cytometry. Cell number ± SD at each time point represents mean from two pools of three mice each.

FIGURE 6. IFN-γ signaling determines the distribution of CNS cell infiltration independent of type I IFN signaling. Histology of (A) brain stem, (B) cerebellum, and (C) SC sections from uninduced WT mice and EAE-induced WT, Ifnar1<sup>−/−</sup>, Ifngr1<sup>−/−</sup>, and Ifnagr1<sup>−/−</sup> mice after 20 d of induction. Tissue sections were stained to evaluate cell infiltration (H&E), demyelination (Luxol fast blue, LFB), neutrophil infiltration (myeloperoxidase, MPO), microglia/macrophage activation (GS-IB4), and CD4<sup>+</sup> T cell infiltration. Arrows mark areas of cell infiltration or demyelination. Representative sections from two serial sections per mouse from two mice per group are shown.
Prinz et al. (11), using Ifnar−/− conditional mice, demonstrated that development and severity of MOGp-induced EAE were not altered in mice with selective loss of IFNAR in CD4+ T cells. On first examination, our result is inconsistent with their findings. Importantly, however, note that the previous study represents active EAE and therefore the combined result of Th1 and Th17 cells. Our study dissects the relative role of IFNAR in Th1 cells versus Th17 cells. Because loss of IFNAR signaling has opposing outcomes in Th1 and Th17 EAE, the combined effect could be null and therefore it reconciles the inconsistencies between the two studies.

The higher levels of IFN-γ and IL-17 in brains and SC of IFNGR−/− mice are expected because IFN-γ signals inhibit IL-17 gene expression (44, 45). However, the expanded numbers of Th1 cells induced in the absence of IFNAR is counterintuitive when considering the paradigm that IFN-γ provides feed-forward signals for the expression of Tbet (46). This suggests that IFN-γ may also have regulatory effects in Th1 cells. IFNAR-deficient mice showed significantly lower concentration of CXCL10 and IL-13 in brain, whereas higher levels of G-CSF and CCL11 were detected in SC with respect to WT mice. Remarkably, the absence of IFN-γ signaling resulted in a similar effect on these cytokines. These results suggest that the expression of CXCL10, IL-13, G-CSF, and CCL11 is independently regulated by both type I and II IFN signaling and in a tissue-specific manner. The CNS of mice lacking both IFNAR and IFNGR contained lower levels of CXCL10 and higher levels of IL-17 than did the CNS of mice that only lacked IFNAR. This indicates modulation by type I IFN signaling on those IFN-γ-regulated cytokines. The brain and spinal cords of IFNGR-deficient mice with EAE contained high levels of G-CSF and very low expression of the IFN-γ-regulated chemokines CXCL9, CXCL10, and CCL5. The elevated G-CSF might contribute to the enhanced numbers of neutrophils detected in the CNS of IFN-γ signaling–deficient mice. CXCL9 and CXCL10 are chemokines that restrain the entry of leukocytes into the brain, and their downregulation is associated with atypical EAE (47, 48), whereas CCL5 is an important chemokine for firm adhesion of leukocytes to the vasculature in the brain (71). Collectively, these results reveal the independent and dependent action of type I and II IFNs in the CNS-specific modulation of key cytokines and chemokines in the pathogenesis of EAE.

As previously reported, we found high levels of IFN-γ and IL-17 in the brains of IFNGR-deficient mice as they developed atypical EAE (35, 47). Mice with atypical EAE have much greater infiltration of encephalitogenic effector cells into the brain and brain stem than do those with classical EAE (47). Our finding that IFNAR-deficient mice do not develop symptoms or pathological features of atypical EAE indicates that IFN-γ signaling, but not IFNAR signaling, is necessary to restrain encephalitogenic cells from entering the brain. None of the mice receiving IFNGR-deficient Th1 or Th17 cells developed clinical symptoms of atypical EAE. This implies that loss of IFNGR signals in T cells is insufficient to induce atypical EAE and suggests a role of other cells such as macrophages, epithelial cells, and glial cells in this process.

In summary, our data reveal that cooperative signaling from type I and II IFNs is necessary to restrain the pathogenesis in EAE. Loss and perhaps imbalance of either IFN signal aggravates inflammation and results in exacerbated disease. This study also demonstrates that response to IFN signals by the different effector populations involved in the pathogenesis of EAE varies and can have opposite outcomes. Elucidating these mechanisms for the different innate and
adaptive effector cells that contribute to the pathogenesis of MS will enable the development of effective therapeutics that selectively target arms of the IFN signaling pathway.

Acknowledgments

We thank the following University of Alabama at Birmingham core facilities: the Neuroscience facility for histology and immunohistochemistry, the X-Radia facility, and the Flow Cytometry and Analytic and Immunoreagent cores of the Comprehensive Arthritis Musculoskeletal and Autoimmunity Center. We also thank Dr. Robin Lorenz (Department of Pathology, University of Alabama at Birmingham) for technical assistance with the multiplex cytokine assay, and PingAr Yang and Qi Wu (Department of Medicine, University of Alabama at Birmingham) for technical assistance with mice.

Disclosures

The authors have no financial conflicts of interest.

References


