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Injury-Induced Type I IFN Signaling Regulates Inflammatory Responses in the Central Nervous System

Reza Khoroooshi and Trevor Owens

Innate glial response is critical for the induction of inflammatory mediators and recruitment of leukocytes to sites of the injury in the CNS. We have examined the involvement of type I IFN signaling in the mouse hippocampus following sterile injury (transsection of entorhinal afferents). Type I IFNs signal through a receptor (IFNAR), which involves activation of IFN regulatory factor (IRF)9, leading to the induction of IFN-stimulated genes including IRF7, that in turn enhances the induction of type I IFN. Axonal transsection induced upregulation of IRF7 and IRF9 in hippocampus. Induction of IRF7 and IRF9 mRNAs was IFNAR dependent. Double-labeling immunofluorescence showed that IRF7 selectively was induced in Mac-1/CD11b+ macrophages/microglia in hippocampus after axonal transection. IRF7 mRNA was also detected in microglia sorted by flow cytometry. Lack of type I IFN signaling resulted in increased leukocyte infiltration into the lesion-reactive hippocampus. Axonal lesion-induced CXCL10 gene expression was abrogated, whereas matrix metalloproteinase 9 mRNA was elevated in IFNAR-deficient mice. Our findings point to a role for type I IFN signaling in regulation of CNS response to sterile injury. The Journal of Immunology, 2010, 185: 1258–1264.

Injury to the CNS induces an innate glial response, in which microglia and astrocytes become activated. This response is essential for the induction of cytokines and chemokines, which initiate a variety of cellular responses including the migration of peripheral immune cells into the damaged tissue. The activation of glial cells and the entry of leukocytes may be beneficial for repair and regeneration processes in the damaged CNS but may also exacerbate neurodegeneration (1, 2).

The molecular basis for glial activation is poorly understood. The initial glial response involves TLR signaling, which can induce proinflammatory cytokines (3, 4), among them type I IFNs. Type I IFNs (IFN-α and IFN-β) play a critical role in the innate immune response against viral infection (5). The signaling pathway for both IFN-α and IFN-β involves a common cell surface receptor, IFNAR. IFNAR is associated with STAT1 and STAT2, which together with IFN regulatory factor (IRF)9 form a complex that binds to IFN-stimulated response element in promoter regions and stimulates type I IFN-dependent gene transcription including IRF7 (6, 7). IRF7 has been described as a master regulator of type I IFN signaling (8, 9).

Type I IFN signaling is normally associated with antiviral immune responses (10). IFN-β is also used in the treatment of multiple sclerosis, and mice with defective type I IFN signaling develop more severe experimental autoimmune encephalomyelitis (EAE) (11, 12), indicating the involvement of type I IFN signaling in the regulation of CNS inflammation. Involvement of type I IFN in a noninfectious injury-induced glial response has not been studied. A well-established and widely used model to study the glial response after brain injury is an entorhinal axonal lesion. The transsection of projections from entorhinal cortex to the hippocampus leads to anterograde axonal degeneration and loss of synapses in the outer molecular layer of the dentate gyrus, followed by glial activation, leukocyte infiltration, and sprouting (13–20). Involvement of TLR signaling as well as cytokines (TNF-α and IL-1β) and chemokines (CCL2 and CXCL10) in glial response and recruitment of leukocytes in the lesion-reactive hippocampus have been described previously (15, 17, 21, 22). We previously described glial upregulation of STAT1 and STAT2 (21), which suggested the involvement of type I IFN signaling.

The aim of this study was to examine the involvement and functional significance of type I IFN signaling in lesion-induced glial responses. We show that sterile axonal lesion induces IRF7 and IRF9 gene expression and that this is IFNAR dependent. Immunohistochemical staining and flow cytometry localized IRF7 to Mac1/CD11b+ macrophages/microglia in lesion-reactive hippocampus at 1 d postlesion. Leukocyte infiltration was increased in IFNAR-deficient mice. Unlike wild-type (WT) mice, axonal lesion did not induce an increase in CXCL10, but it increased an increase in matrix metalloproteinase (MMP)9 gene expression in IFNAR-knockout (KO) mice. These findings identify a role for type I IFN in regulating innate responses to CNS injury.

Materials and Methods

Animals

Adult female mice deficient in STAT1 (STAT1-KO) 129s6/SvEv background (23) were purchased from Taconic Farms (Germantown, NY), IFNAR-KO on 129s6/SvEv background were purchased from B&K Universal Limited (Hull, U.K.), IRF7-KO on C57BL/6 background were purchased from Riken BRC (Tsukuba, Japan) with agreement from Dr. T. Taniguchi (Department of Immunology, University of Tokyo, Tokyo, Japan) (9), and IFNAR-KO on C57BL/6 background (11) were provided by Dr. M. Prinz (Department of Neuropathology, University of Freiburg, Germany). WT mice (C57BL/6 and 129s6/SvEv) were purchased from Taconic (Taconic Europe, Ry, Denmark). These experiments were conducted according to the guidelines of the National Danish Animal Research Committee.

Entorhinal cortex lesioning and tissue preparation

Under anesthesia, adult female (18–20 g) mice were placed in a Kopf stereotactic apparatus (Kopf Instruments, Tujunga, CA). The entorhino-
dentate perforant path projection was transected as described previously (21).

For immunohistochemical analysis, mice were perfused transcardially with PBS, followed by 4% paraformaldehyde (Sigma-Aldrich, Broendby, Denmark) in PBS. The brains were removed and postfixed in PBS containing 4% paraformaldehyde for 2 h on ice, then immersed in 30% sucrose in PBS overnight at 4°C, frozen with C02-snow, and stored at −20°C until sectioning. For flow cytometry and quantitative real-time RT-PCR analysis, mice were perfused transcardially with PBS, and brains were removed aseptically.

Single- and dual-labeling immunohistochemistry

Immunohistochemical staining was performed as described previously (21). In brief, after blocking endogenous peroxidases, the sections were incubated overnight with rabbit anti-IRF7 Ab (SC-9083; Santa Cruz Biotechnology, Santa Cruz, CA), rat anti-mouse Mac-1/CD11b (MC7111; Serotec, Oxford, U.K.) (to identify microglia/macrophages), or rabbit anti-iGFP (20334; DakoZ) (to identify astrocytes), and in the following day, the sections were incubated with secondary biotinylated Ab (Amersham Biosciences, Little Chalfont, U.K.). The sections were finally incubated with streptavidin-HRP (P0397; DakoCytomation) and developed with 0.5 mg/ml diaminobenzidine (Sigma-Aldrich).

For dual-labeling immunofluorescence, sections were incubated with rabbit anti-iGFP and with rat anti-mouse Mac-1/CD11b or monoclonal mouse anti–GFAP (Sigma-Aldrich) Ab followed by incubation with fluorescent secondary Abs, Alexa 488-labeled goat anti-rabbit (A11034; Invitrogen, Taastrup, Denmark) and Alexa 568-labeled goat anti-rabbit (A11077; Invitrogen), as described previously (21). Images were acquired using either an Olympus BX51 microscope (Olympus, Ballerup, Denmark) connected to an Olympus DP71 digital camera and a Confocal microscope (Olympus FV1000; Olympus, Hamburg, Germany). Images were combined using Adobe Photoshop CS version 8.0 to visualize double-labeled cells. To verify Ab specificity, control sections were treated without primary Ab or with isotype-matched primary Abs. Control sections displayed no staining comparable with that seen with primary Abs (data not shown).

Western blotting

Western blot analysis was performed as described previously (21). Both contralateral and ipsilateral hippocampi were isolated from PBS-perfused mice and lysed in lysis buffer. Cell lysate was mixed with sample buffer, denatured by boiling, and separated by electrophoresis in an 8–10% polyacrylamide gel. The separated proteins were transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA). The membranes were blocked for 1 h at room temperature in 5% nonfat dry milk in PBS containing 0.1% Tween 20 (PBST). The membranes were then incubated with rabbit anti-IRF7 and/or rabbit anti-actin (A5060; Sigma-Aldrich) Abs at 4°C overnight. After washing in PBST, the membranes were incubated with goat anti-rabbit IgG HRP conjugate (Sigma-Aldrich) for 1 h at room temperature. Staining was detected using chemical luminence methodology (GE Healthcare U.K., Amersham Place, U.K.). The intensities of IRF7 and actin were calculated using an image analyzing system (ImageJ; National Institutes of Health, Bethesda, MD). The relative levels of IRF7 were normalized to densitometric values from lesion-reactive hippocampi divided by the values from contralateral to calculate the fold induction of IRF7.

Fluoro-Jade staining

Fluoro-Jade (FJ) is used as a marker to identify degenerating neuronal tissue (24). To examine whether lack of IFNAR signaling may affect FJ staining in the outer molecular layer of dentate gyrus, brain sections were stained with FJ (Histo-Chem, Jefferson, AK). Sections were immersed in 100% ethanol for 3 min in 70% ethanol for 1 min and rinsed in H2O for 1 min before being incubated for 15 min in 0.06% KMnO4. Thereafter, tissue was rinsed for 1 min in H2O and stained for 45 min in 0.001% FJ solution diluted in 0.1% acetic acid. Sections were then rinsed in distilled water, air-dried, dipped in xylene, coverslipped, and mounted with Depex.

Quantitative real-time PCR

As previously described (21), RNA was isolated from the contralateral, ipsilateral, and uninmanipulated hippocampi and converted to cDNA. Quantitative real-time PCR was performed using an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA) (21). 18S rRNA was measured as a control and used to normalize gene expression (Applied Biosystems). Primer and probe sequences were as follows: CCL2 (forward, TCT GGG CCT GCT CCTA C; reverse, CCTACTCATT-GAGGCACCATTAGAGA; reverse, CACCACTCGGCCACCATAG; probe, FAM-AGC TGG AGAGTGTGGAT-MGB); TNF-α (forward, GCCCGTACTTTCCTGCTAT; reverse, TCTCCCTGTTGGTTTGC; probe, FAM-CTACACTCATGATC-MGB); CXCL10 (forward, GCCCGTACTTTCTGCTAT; reverse, GGGCCGCATTGATG; probe, FAM-GGACTCAAGGGTGTC-MGB); IL-1β (forward, CTTGGGCTCAAAGGAAAGAA; reverse, AAGACAAACCGTTTTCTCACT-T; probe, FAM-AGC TGG AGAGTGTGGAT-MGB); IFN-γ (forward, CACCCTCTTCGCTACTA; reverse, CAAAACCAAGGTGATAGGTGA; probe, FAM-CACTTTTCCCAGAATCCT-MGB); IRF9 (forward, AACACT TGAGCACCATTAGA; reverse, CACCCCTGCCACCATAD; probe, FAM-TGAACCTCAGACTCTGCT-MGB); IRF3 (forward, CACCCCAA-GAAAATCCACTA; reverse, AGGGCGTACTCCTGAACT; probe, FAM- TACCTGAGAACAAGT-MGB); and MMP9 (forward, CGAACCTTGACA TAGAAAGG; reverse, GCCACCTGGAATGCTACAAG; probe, FAM-TGC TTGCCAGACCGTGACCCGGTTC-MGB).

Flow cytometry

To measure leukocyte entry in response to axonal injury, hippocampi were homogenized and incubated with blocking solution to block nonspecific staining as described previously (21). Cells were then stained with rat anti-mouse CD45-conjugated PE, rat anti-CD11b-conjugated PerCP, and hamster anti-mouse TCR-β conjugated APC Abs (BD Biosciences, Erembodegem, Belgium) to detect microglia/macrophages or T cells, respectively (15, 17). Data were collected on a FACS Calibur (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (Tree Star, Ashland, OR) (21).

To sort microglia and macrophages, hippocampi were pooled from six mice, and a similar experiment was performed as described above with few modifications. Homogenates were enriched for mononuclear cells by centrifugation through 35% isotonic Percoll prior to blocking the non-specific staining. After staining with respective Abs, cells were sorted by a FACSVantage/Divia Sorting Flow Cytometer (BD Biosciences). Total RNA was extracted using TRIZol reagent as previously described (21) and converted to cDNA, and quantitative real-time PCR was performed as described above. ∆CT values for IRF7 gene expression were then calculated. ∆CT is the difference in CT between IRF7 gene and the internal reference control gene, 18S rRNA.

Data analysis

Results were analyzed by two-tailed paired/unpaired t test or one-way ANOVA with Bonferroni’s postest using GraphPad Prism software (GraphPad Software, San Diego, CA). A value of p < 0.05 was considered to be statistically significant. Data are presented as mean ± SEM.

Results

Lesion-induced IRF7 gene expression was STAT1 and IFNAR dependent

IRF7 mRNA was constitutively expressed at low levels in unmanipulated hippocampi from WT, IFNAR-KO, and STAT1-KO mice (data not shown). Levels of IRF7 increased significantly in lesion-reactive hippocampi of WT mice at 1, 3, and 5 d (Fig. 1A). In contrast, axonal lesion did not induce a significant increase in IRF7 gene expression in IFNAR-KO and STAT1-KO mice (Fig. 1A). Whether or not mice were on 129s6/SvEv background had no effect on these events.

Like IRF7, IRF9 mRNA was constitutively expressed at low levels in unmanipulated hippocampi from both WT and IFNAR-KO mice (data not shown). IRF9 mRNA levels increased in the lesion-reactive hippocampi at 1 d postlesion, and this was also IFNAR dependent (Fig. 1C).

In addition to IRF7 and IRF9, we also examined IRF3 gene expression. IRF3 is involved in the early induction of type I IFNs in antiviral immune response (25). IRF3 was constitutively expressed in unmanipulated hippocampi in both WT and IFNAR-KO mice. Unlike IRF7 and IRF9, there was no induction of IRF3 at 1 d after lesion (data not shown).

IRF7 was expressed by microglia/macrophages in the outer molecular layer of dentate gyrus

We next examined IRF7 distribution and cellular localization in the denervated outer molecular layer of the dentate gyrus at 1 d postlesion. IRF7 immunoreactivity was not evident in unmanipulated...
FIGURE 1. Injury-induced increase in IRF7 and IRF9 gene expression was IFNAR and STAT1 dependent. A. IRF7 mRNA increased significantly in lesion-reactive hippocampi of 129s6/SvEv WT but not in IFNAR-KO and STAT1-KO mice at 1, 3, and 5 d postaxonal lesion. B. IRF7 mRNA increased significantly in lesion-reactive hippocampi of C57BL/6 WT but not in IFNAR-KO mice on C57BL/6 background. C. IRF9 gene expression was increased in lesion-reactive hippocampi of WT but not in IFNAR-KO mice at 1 d postlesion. Groups of matched contralateral and lesion-reactive hippocampi were analyzed by two-tailed paired Student t test. *Statistically significant differences versus contralateral as follows: A, 1 day postlesion (dpl), p = 0.009; 3 dpl, p = 0.031; 5 dpl, p = 0.031; B, WT, p = 0.0039; and C, WT, p = 0.001. Error bars represent SEM. C, contralateral; L, lesion-reactive hippocampi.

FIGURE 2. IRF7 immunoreactivity increased in lesion-reactive hippocampi at 1 d postlesion. A. IRF7 immunoreactivity was not evident in contralateral hippocampus. B. Axonal lesion-induced increase IRF7 immunostaining in the outer molecular layer in dispersed cells that had morphologic features resembling activated microglia or macrophages (Fig. 2B, 2C). Double labeling with Mac-1/CD11b and GFAP showed that IRF7 colocalized with Mac-1/CD11b (Fig. 2D–G) and not with GFAP (Fig. 2H–K), indicating expression in microglia/macrophages. IRF7 protein was also detected by Western blotting (data not shown). We identified relevant bands on the basis of their absence from Western blots of hippocampal lysates from IRF7-KO mice; this verified the specificity of IRF7 Ab (data not shown). The level of IRF7 protein did not increase at 1 d postlesion in lesion-reactive hippocampi compared with contralateral from either WT or IFNAR-KO mice (data not shown).

IRF7 gene expression in sorted CD45dimCD11b+ microglia

The expression of IRF7 protein in Mac-1/CD11b microglia/macrophages was confirmed by analysis of IRF7 gene expression in microglia sorted by flow cytometry (Fig. 3). IRF7 gene expression could be detected in sorted CD45dimCD11b+ microglia at 1 d postaxonal lesion (Fig. 3). ΔCT values for IRF7 gene expression in CD45dimCD11b+ microglia were ~16 (Fig. 3B). Because of the limited number of CD45dimCD11b macrophages sorted by flow cytometry from hippocampi, we were not able to detect IRF7 gene expression in macrophages (Fig. 3).

Lesion-induced leukocyte infiltration was increased in IFNAR-KO mice

The upregulation of downstream components of type I IFN signaling indicates involvement in axonal lesion-induced glial response. Low levels of type I IFN gene expression could be detected in hippocampal samples by quantitative real-time PCR (data not shown). However, expression was difficult to detect, and it was not possible to reliably determine whether axonal-lesion influenced type I IFN induction, consistent with findings by Ousman et al. (10), in which the levels of type I IFN within the CNS were at the limit of detection even after several days postvirus infection.

To examine the functional significance of type I signaling in this model, leukocyte recruitment to the lesion-reactive hippocampi was analyzed by flow cytometry, measuring the proportion of CD45high cells (Fig. 4A). Proportions of blood-derived leukocytes were similarly low in unmanipulated hippocampi from WT and IFNAR-KO mice, regardless of strain background. The proportion of CD45high cells was significantly increased in lesion-reactive hippocampi of both WT and IFNAR-KO mice compared with the contralateral hippocampus of each mouse, regardless of whether WT and IFNAR-KO mice were on C57BL/6 (data not shown) or 129s6/SvEv (Fig. 4B) backgrounds. However, the proportions of...
infiltrating CD45<sup>high</sup> cells were significantly higher in IFNAR-KO than in WT mice (Fig. 4B) at 1 d. Lesion-induced increase in leukocyte infiltration in IFNAR-KO mice compared with WT was transient and was not seen at 3 and 5 d postlesion (Fig. 4B), although the proportion of infiltrating CD45<sup>high</sup> cells were increased by lesion in both WT and IFNAR-KO mice at these time (Fig. 4B). The fold increase in leukocyte infiltration in lesion-reactive versus contralateral hippocampus at 1 d in IFNAR-KO mice was 3-fold higher than WT (Fig. 4C).

Leukocyte infiltration to lesion-reactive hippocampus includes both T cells and macrophages (15, 17). To ask whether IFNAR deficiency differentially affected T cell and macrophage infiltration, we counted proportions of CD11b<sup>+</sup> and TCR-β<sup>-</sup> cells. Whereas the proportion of macrophages (CD45<sup>dimCD11b<sup>+</sup></sup>) doubled in lesioned IFNAR-deficient hippocampus compared with WT, the lesion-induced increase in T cells (CD45<sup>dimCD11b<sup>+</sup></sup>TCR-β<sup>-</sup>) was not affected by IFNAR deficiency (data not shown). There was no significant difference between the proportions of CD45<sup>dimCD11b<sup>+</sup></sup> microglia in lesion-reactive hippocampi of WT and IFNAR-KO mice (data not shown).

Lesion-induced CXCL10 and MMP9 gene expression were IFNAR regulated

Our findings indicate that IFNAR deficiency leads to increased leukocyte infiltration in lesion-reactive hippocampus. The involvement of cytokines (including TNF-α and IL-1β) and chemokines (including CCL2 and CXCL10) in leukocyte entry to CNS has been shown previously (21, 26, 27). In addition, MMPs are known to degrade extracellular matrix and to facilitate leukocyte infiltration to the CNS (28). Interestingly, type I IFN signaling regulates MMP9 expression (29). We next examined whether increased infiltration correlated with changes in expression of lesion-induced CCL2, CXCL10, TNF-α, IL-1β, and MMP9. As previously shown (17, 21), axonal injury induced upregulation of CXCL10 (Fig. 5A), CCL2 (Fig. 5B), TNF-α (Fig. 5C), and IL-1β (Fig. 5D) in WT mice. In IFNAR-KO mice, axonal injury also induced a significant increase in CCL2 (Fig. 5B), TNF-α (Fig. 5C), and IL-1β (Fig. 5D) but not in CXCL10 gene expression (Fig. 5A). Unlike WT mice, MMP9 gene expression increased significantly in lesion-reactive hippocampi from IFNAR-KO mice (Fig. 5E).

FJ and glial staining in response to axonal lesion

To examine whether lack of type I IFN signaling may influence axonal degeneration and glial response in the outer molecular layer of dentate gyrus, we performed FJ, Mac-1/Cd11b, and GFAP staining on brain sections from WT and IFNAR-KO mice at 1, 3, and 5 d.

Discussion

Our findings show the involvement of type I IFN signaling in response to sterile injury in the CNS. Type I IFN signaling in the CNS is normally associated with antiviral immune responses (10, 30).
Type I IFNs exert a wide range of effects on inflammatory processes in the CNS during infection and in EAE, in both of which pathogen-associated signals may play a role. The functional significance of type I IFN in sterile injury-induced response in the CNS is therefore of interest. Similar to findings in viral infection models (10, 30), the expression of IRF7 and IRF9 gene increased following axonal lesion, and this increase was IFNAR dependent (10). Other studies have shown a role for type I IFN in regulating leukocyte infiltration to the CNS in EAE models (11, 12, 31), and we have shown a similar effect in response to sterile injury. Our results support an innate signaling role for type I IFN in the CNS.

IRF7 and IRF9 belong to a family of transcription factors with diverse functions including host defense, regulation of cell growth, apoptosis, and immune cell development. However, mice deficient in IRF7 and IRF9 develop normally and have no obvious differences in size and behavior compared with WT littermates (9, 32). Most importantly, these two reports emphasize the essential role of IRF7 and IRF9 in the regulation of type I IFN signaling and antiviral defense. The CNS is susceptible to infection, and constitutive expression of IRF7 and IRF9 may therefore be necessary for initiation of type I IFN response to infection. In the current study, we showed CNS upregulation of IRF7 and IRF9 in response to injury, which suggest their involvement in noninfectious responses as well.

IRF7 gene expression is specifically of interest, because it positively regulates the induction of type I IFN, which in turn regulates IRF7 induction. The cellular expression of IRF7 may therefore be indicative of the source of type I IFN. We have been able to show induction of IFN-α and IFN-β by RT-PCR in lesion-reactive hippocampus, but this has been difficult to reproduce, and we have been unable to localize the cellular source of IFN I. This is probably due to a combination of low expression level and that current methods are not sensitive enough to detect the differences in type I IFN induced by injury in this model. Identification and localization of type I IFN in the CNS is of interest, particularly in response to viral infection. To our knowledge, only two studies have identified the source of type I IFN in the CNS. Delhaye et al. (30) identified that neurons produced IFN-α in response to infection, and Teige et al. (12) found that during EAE, both infiltrating cells and cells with the morphology of microglia expressed IFN-β. Otherwise, surrogate markers of downstream signaling associated with type I IFN signaling have been used to identify type I IFN-producing cells within the CNS (10, 30).

Our results pointed to upregulation of IRF7 in Mac-1/CD11b+ microglia/macrophages, although absence of IRF7 immunoreactivity in other cell types does not exclude their ability to produce type I IFN. IRF7 protein expression could be detected in unmanipulated hippocampal samples as well as in contralateral and lesion-reactive hippocampi. Furthermore, both PCR and Western blot analysis showed that the level of IRF7 protein expression was higher in WT mice compared with IFNAR-KO mice, although not significantly. The immunostaining of sections with IRF7 Ab showed increased but dispersed IRF7+ cells in the outer molecular layer of dentate gyrus at 1 d postaxonal lesion. Levels of FJ staining increased in the outer molecular layer of dentate gyrus at 3 d after axonal lesion of both WT (inset in A) and IFNAR-KO (inset in B). No differences in FJ staining were observed between IFNAR-KO and WT mice. Axonal lesion caused an increase GFAP (C, D, arrows) and Mac-1/CD11b immunoreactivity (E, F, arrows) in the outer molecular layer of dentate gyrus. However, there were no differences in GFAP and Mac-1/CD11b immunoreactivity between IFNAR-KO and WT mice. DG, dentate gyrus; OML, outer molecular layer. Original magnification ×10 (A, B, insets), ×40 (C–F). Scale bars, 20 μm.

FIGURE 5. Lesion-induced CXCL10 and MMP9 gene expression was IFNAR dependent. Whereas CXCL10 gene expression (A) did not increase significantly in lesion-reactive hippocampi of IFNAR-deficient mice, CCL2 (B), TNF-α (C), and IL-1β (D) were significantly increased in lesion-reactive hippocampi of both WT and IFNAR-KO mice at 1 d postaxonal lesion. (E) In contrast to WT, lesion induced a significant increase in MMP9 gene expression in IFNAR-deficient mice at 1 day postaxial lesion. Data were analyzed using one-way ANOVA with Bonferroni’s posthoc analysis. Error bars represent SEM. Statistically significant differences versus contralateral. *p < 0.05; **p < 0.01; ***p < 0.001. †Not significantly different between contralaterals for each message. C, contralateral; L, lesion-reactive hippocampi.

FIGURE 6. FJ, GFAP, and Mac-1/CD11b staining in the outer molecular layer of dentate gyrus in response to axonal lesion. A and B. Images showing FJ staining in lesion-reactive hippocampi of WT versus IFNAR-KO at 1 d. FJ staining was present at very low level in the outer molecular layer of dentate gyrus at 1 d postaxonal lesion. Levels of FJ staining increased in the outer molecular layer of dentate gyrus at 3 d after axonal lesion of both WT (inset in A) and IFNAR-KO (inset in B). No differences in FJ staining were observed between IFNAR-KO and WT mice. Axonal lesion caused an increase GFAP (C, D, arrows) and Mac-1/CD11b immunoreactivity (E, F, arrows) in the outer molecular layer of dentate gyrus. However, there were no differences in GFAP and Mac-1/CD11b immunoreactivity between IFNAR-KO and WT mice. DG, dentate gyrus; OML, outer molecular layer. Original magnification ×10 (A, B, insets), ×40 (C–F). Scale bars, 20 μm.
response to toxin-induced demyelination, although there appeared to be no role for type I IFN signaling, and the cell source was not identified (33). In virally infected brain, IRF7 gene expression was detected in astrocytes, microglia, neurons, and infiltrating CD3+ T cells (10). Another study showed that viral infection induced upregulation of IRF7 and type I IFN in neurons as well as in Mac-1/CD11b+ microglia/macrophages (30). It is also evident that all cells in the CNS can respond to type I IFN (30, 34–36). IRF7 alone or together with IRF3 can induce transcription of type I IFN (8, 25). Hippocampal IRF3 gene expression was unaltered in response to axonal lesion in both WT and IFNAR-KO after 1 d, suggesting that IRF3 may not be involved in this case. Similarly, viral infection in the CNS did not induce changes in IRF3 gene expression, even after several days (10).

Our findings extend to the sterile injury response observation of type I IFN regulation of leukocyte entry to the CNS. The proportion of CD45highCD11b+ macrophages but not of CD45highTCR-β+ T cells doubled in IFNAR-KO in comparison with WT animals at 1 d postaxonal lesion. Analogous effects of type I IFN signaling on infiltration of macrophages to the CNS have been demonstrated in EAE (11, 12, 31). Increased clinical scores of EAE in IFNAR-KO mice were associated with elevated number of MAC-3+ macrophages (11), and treatment with IFN-β reduced both the clinical scores and the number of ED1+ macrophages in rats (31). In addition, it was reported that the CNS of IFN-β-KO mice contained more activated macrophages than in WT mice (12). Lack of type I IFN signaling had no effect on T cell infiltration in the two of these studies, in which it was examined (11, 12).

Unlike in WT mice, CXCL10 gene expression was not elevated in lesioned IFNAR-KO mice. In contrast, CCL2, TNF-α, and IL-1β were similarly upregulated in both IFNAR-KO and WT mice. This contrasts with findings in EAE, in which elevated macrophage infiltration correlated with increased levels of TNF-α, CCL2, and CXCL10 expression in IFNAR- and IFN-β-KO compared with WT mice (11, 12). As shown by others and ourselves in entorhinal cortex lesion and other models of brain injury, glial cells represent a major source for many of these mediators. Previous studies identified microglia and astrocytes as a prominent source of CCL2 (17). IL-1β and TNF are also glial derived (37), and we must assume that the leukocyte contribution was not significant in this case. The differences in effect on CXCL10 may reflect involvement of adjuvant in the EAE model or other differences between these systems. It has been demonstrated that an IFN-stimulated response element is contained in the CXCL10 promoter (39, 40). Type I IFN is capable of stimulating CXCL10 production by hepatocytes in vitro, and ischemia/reperfusion-triggered intrahepatic CXCL10 expression was diminished in IFNAR-KO mice (41). The induction of CXCL10 has been used as biomarker to measure type I IFN activity (42). Our study suggests that type I IFN signaling normally either allows or directly stimulates CXCL10 production in the CNS in response to axonal injury. Our findings further suggest that type I IFN signaling may regulate leukocyte entry through this or other mechanisms involving disruption of blood-brain barrier (29). MMP9 is involved in degradation of extracellular matrix and facilitating the recruitment of leukocytes (28, 43). In the CNS, both astrocytes and microglia express MMP9 (28), and it is also expressed by infiltrating macrophages (44). It has been shown that type I IFN inhibits MMP9 expression in astrocytes and microglia and reduces leukocyte infiltration to the CNS (29, 43, 45–47). We find that in the absence of type I IFN signaling, MMP9 was significantly increased. This could both result from and contribute to the increased leukocyte entry into the hippocampus of IFNAR-deficient mice in response to axonal lesion.

These findings of innate expression and response to type I IFN in the CNS likely have relevance to understanding the effectiveness of IFN-β as a therapeutic for multiple sclerosis. Innate signaling pathways in the CNS offer new potential targets for therapy for neuroinflammatory diseases.

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Disclosures

The authors have no financial conflicts of interest.

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