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Proapoptotic and Antiapoptotic Actions of Stat1 versus Stat3 Underlie Neuroprotective and Immunoregulatory Functions of IL-11


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Proapoptotic and Antiapoptotic Actions of Stat1 versus Stat3 Underlie Neuroprotective and Immunoregulatory Functions of IL-11


Current therapies for multiple sclerosis target inflammation but do not directly address oligodendrocyte protection or myelin repair. The gp130 family cytokines ciliary neurotrophic factor, leukemia inhibitory factor, and IL-11 have been identified as oligodendrocyte growth factors, and IL-11 is also strongly immunoregulatory, but their underlying mechanisms of action are incompletely characterized. In this study, we demonstrate that these effects of IL-11 are mediated via differential regulation of apoptosis in oligodendrocytes versus Ag-presenting dendritic cells (DCs), and are dependent on lineage-specific activity of the transcription factors Stat1 versus Stat3. Focal demyelinating lesions induced in cerebral cortices of IL-11−/− mice using stereotactic microinjection of lysolecithin were larger than in controls, and remyelination was delayed. In IL-11Rα2−/− mice, lesions displayed extensive oligodendrocyte loss and axonal transection, and increased infiltration by inflammatory cells including CD11c+ DCs, CD3+ lymphocytes, and CD11b+ phagocytes. In oligodendrocyte progenitor cell (OPC) cultures, IL-11 restricted caspase 9 family, suggesting avenues to enhance oligodendrocyte viability and restrict CNS inflammation in multiple sclerosis. The Journal of Immunology, 2011, 187: 1129–1141.

Multiple sclerosis (MS) is a presumed autoimmune demyelinating disease of the CNS characterized by inflammation, loss of myelin and oligodendrocytes, axonal transection, and reactive astrogliosis (1). Conduction block is believed to underlie early neurologic dysfunction (2), whereas axonal transection is responsible for more permanent deficits later (3). Remyelination occurs in early lesions and is associated with functional recovery (4), but fails with disease progression (5). Notably, existing therapies focus on immunoregulation and reduce the frequency of clinical relapse, but do not directly address protection of oligodendrocytes and neurons or myelin repair (6).

Members of the gp130 family of neurotrophic cytokines, including ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and IL-11, have been identified as survival factors for oligodendrocytes (7–9) and as potential therapeutic avenues for MS (10–12). The family also encompasses IL-6, IL-27, ciliary neurotrophic factor-1 (CT-1), and oncostatin M. All members bind to receptors containing the common gp130 subunit and activate downstream Jak-Stat signaling and Stat-independent pathways (13). Each member shows a distinct spectrum of activity. CNTF−/− mice with experimental autoimmune encephalomyelitis (EAE), a model of MS, display more severe disease than do controls, with increased oligodendrocyte apoptosis and axonal transection (10), LIFRβ−/−Δgp130−/− and LIF−/− mice exhibit a similar phenotype both in EAE and in cuprizone-induced demyelination (11), although LIF−/− mice also show attenuated later disease (14). In a third pattern, we recently found that IL-11 is expressed by reactive astrocytes in MS plaques (9) and that mice deficient for IL-11 receptor α (IL-11Rα−/−) display exacerbated EAE, associated with increased size of demyelinated lesions and also with potentiated CNS inflammation (12). Our studies confirmed that IL-11 enhances oligodendrocyte lineage cell number in vitro (9), but revealed that IL-11 limits the function of Ag-presenting CD11c+...
dendritic cells (DCs), restricting activation of encephalitogenic T lymphocytes (12).

Thus, IL-11 signaling is both neuroprotective and immunoregulatory, but the mechanisms underlying these effects are unknown. In a wider context, the processes determining the distinct actions of gp130 cytokines are incompletely characterized. In this report, we identify differential regulation of apoptosis in Olig2\(^+\) oligodendrocytes versus CD11c\(^+\) DCs as an important mechanism underlying the effects of IL-11 in inflammatory demyelinating disease. These effects depend on lineage-specific activity of the transcription factors Stat1 and Stat3. In both cell types, Stat3 is antiapoptotic, whereas Stat1 promotes cell death. However, activity of Stat3 after IL-11 treatment predominates over its relative proapoptotic effects of Stat1 prevalent in DCs. Collectively, these data reveal novel mechanisms underlying the actions of a neuroprotective and immunoregulatory member of the gp130 cytokine family. Our findings suggest new avenues to enhance oligodendrocyte survival and restrict CNS inflammation in MS.

Materials and Methods

Abs

The A2B5 hybridoma was obtained from the American Type Culture Collection (Manassas, VA), and supernatant (IgM) was prepared. Other Abs and dyes were: rat anti-CD11b, rat anti-glial fibrillary acidic protein (GFAP), and fluoromyelin (Invitrogen, Carlsbad, CA); hamster anti-CD11c (eBioscience, San Diego, CA); O4 mouse IgM (Dr. Peter Davies, Albert Einstein College of Medicine); mouse anti-CNPase (IgG1) and rabbit anti-Olig2 (Millipore Chemicon, Temecula, CA); mouse anti-myelin basic protein (MBP) and mouse anti-neurofilament H nonphosphorylated (SMI 32; Covance Sternberger, Berkeley, CA); sheep anti-BrdU (Novus, Littleton, CO); mouse anti-PDGFR\(\alpha\) (R\&D Systems); rabbit anti-CD3 (Abcam, Cambridge, MA); mouse anti-actin (Santa Cruz Biotechnology, Santa Cruz, CA); and rabbit caspase 9, cleaved caspase 3, PI3 kinase p85, phospho-Stat1(Tyr\(\beta\)), Stat1, phospho-Stat3(Y705), Stat3, phospho-Stat5p (Y580), Shp2, phospho-p44/42(T202/Y204), and phospho-Gsk3(S21/9) (Cell Signaling, Beverly, MA).

Cytokines

Mouse IL-11 was from PeproTech (Rocky Hill, NJ) and was used at 1–100 ng/ml, most commonly 100 ng/ml based on our previous dose-response data (9, 12). Previous work in our laboratory has confirmed that mouse IL-11 is equally effective in mouse and rat cultures, and the mouse and rat IL-11 protein sequences are 97.5% similar (NCBI database). Rat TNF-\(\alpha\) was obtained from PeproTech and was used at 10 ng/ml. Salmonella minnesota LPS was obtained from Alexis Biochemicals (Plymouth Meeting, PA). CpG was obtained from Coley Pharmaceuticals (Wellesley, MA).

Inhibitors

A cell-permeable analog of the Stat3-SH2 domain-binding phosphopeptide containing a C-terminal membrane translocating sequence (Stat3\(C_{3}P_{1}C\)) was obtained from EMD (Gibbstown, NJ) and used at 100 \(\mu\)M based on previous studies (15). Inactive nonphosphorylated control was from the same source. Pilot studies confirmed that 100 \(\mu\)M Stat3\(C_{3}P_{1}C\) restricted activation of Stat3, but not Stat1 in CD11c\(^+\) DCs (data not shown).

Mice

All work was approved by the Institutional Animal Care and Use Committee. IL-11R\(^{+/−}\) mice backcrossed onto C57BL/6 background for >12 generations were purchased from Jackson Laboratories (Bar Harbor, ME) and bred at Mount Sinai School of Medicine (MSSM) (12). This genotype was generated by Drs. Lorraine Rombb and C. Glenn Begley (Walter and Eliza Hall Institute, Melbourne, Australia) (16). Genotyping was by PCR as on the JAX Web site (http://jaxmice.jax.org) using primers: IL11R\(F_{3}\) 5’-GGCTCCGGCTACATCACTACA-3’, IL11R\(R_{3}\) 5’-AGACGTCTACCCGGCTACA-3’, IL11R\(P_{3}\) 5’-CTCTTGGTACGCGACGCTG-3’. Stat1\(^−/−\) mice (129S6/SvEv-Stat1\(^+/−\)) were generated by Dr. Robert Schreiber (Washington University, St. Louis, MO) and purchased from Taconic (Germantown, NY). Olig2\(^+\) cell numbers were similar in the CNS of unchallenged IL-11R\(^{+/−}\) mice and wild type (WT) littermates (Fig. 1E), and both groups exhibited normal CNS MBP immunoreactivity as shown previously (12). Light microscopic analysis of resin-embedded adult (12 wk) corpus callosal samples without lesions (four per genotype) confirmed that myelin thickness and the ratio of axon to fiber diameter (G ratio) (17) were similar in IL-11R\(^{−/−}\) mice and WT littermates (Table I). Unchallenged IL-11R\(^{−/−}\) mice showed no differences in CNS or peripheral immune system compared with WT controls, and no abnormalities in CD3\(^+\) or CD11c\(^+\) cell populations in splenic samples (12).

Stereotactic microinjection

One percent lysolceithin (1.5 \(\mu\)l; Sigma) was delivered into the corpus callosum of 12-wk-old mice by stereotactic microinjection, as described previously (18). Injected animals included IL-11R\(^{−/−}\) mice and littermate WT controls (at least five per genotype per time point). Mice were perfused at 7–28 days postinjection (dpi) with 4% paraformaldehyde or glutaraldehyde, and brains were processed for cryostat or epoxy embedding.

Immunohistochemistry

Sections of cerebral cortex (20 \(\mu\)m) were immunostained using described protocols (12). Stained sections were examined using a Leica TCS5 confocal microscope, and stacks were collected using 1 \(\mu\)m on the Z-axis and assembled into projections. To quantify demyelination, the cross-sectional MBP\(^+\) area was measured in stacks at \(\times10\) magnification using ImageJ software (National Institutes of Health), and the rostrocaudal size of the MBP\(^+\) area was estimated in serial stacks. Cells positive for lineage and differentiation markers were quantified within the hypercellular lesion area by a blinded observer, at least five fields at \(\times20\) magnification per animal, at least five animals per time point.

Histopathology

Epoxy sections of corpus callosum (1 \(\mu\)m) were stained with toluidine blue and photographed on a Zeiss Axioplan2 microscope. For electron microscopy, serial sections were placed on 200 mesh copper grids, contrasted with lead citrate and uranyl acetate and scanned in a Hitachi HS600 microscope. For quantitation of myelin thickness and G ratio, random fields of lysolceithin lesion border (see Results) or corpus callosum without lesions were captured by electron microscopy (at least five fields per animal, at least four mice per genotype at 14 dpi), and axon and fiber diameters were measured for 10 random axons per field by a blinded observer using ImageJ software. Myelin thickness was calculated as (fiber diameter − axon diameter)/2. G ratio was calculated as (axon diameter)/ (fiber diameter) (17, 18).

Oligodendrocyte progenitor cell monocultures

Oligodendrocyte progenitor cells (OPCs) were purified from cerebral cortices of P2 Sprague Dawley rats as described previously (18). OPCs were plated onto poly-d-lysine coated focal dishes (Mat-Tek, Ashland, MA). After 24 h, growth factor-free medium with 1–100 ng/ml human IL-11 or vehicle was added and cells were grown for 5 d. At plating, these cultures are >95% Olig2\(^+\) A2B5\(^+\) PDGFR\(\alpha\)\(^+\) CNPase\(^+\) MBP\(^+\) OPCs (12, 18). Oligodendrocyte lineage cells express IL-11Ra at all stages of differentiation (9). For siRNA studies, 5 nM Stat1, Stat3, Shp2, or PI3 kinase siRNA (Dharmacon, Lafayette, CO) was nucleofected using an Amaxa Rat Nucleofector Kit (Gaithersburg, MD). Controls included nontargeting siRNA and sham, and specificity was assessed by immunoblotting.

Modeling

To separate IL-11 effects on proliferation, apoptosis, and differentiation, we adapted a previously described mathematical model to our OPC culture system (19). The model quantitates Olig2\(^+\) cells positive or negative for BrdU at 12-h time points and separates the proliferation rate of Olig2\(^+\) MBP\(^+\) OPCs (\(\alpha\)) and mature Olig2\(^+\)MBP\(^+\) oligodendrocytes (\(\gamma\)), and the differentiation rate (\(\beta\)) from one to the other (Fig. 3F). The model also measures the rate of apoptosis using TUNEL immunostaining. Apoptosis cannot be separated experimentally into rates for OPCs (\(\beta\)) and mature cells (\(\gamma\)); therefore, the model is bounded by the possibilities that all cell death is from only OPCs or only mature cells or that the death rates for both are equal (19). The model uses six equations to generate coefficients \(\alpha\) through \(\epsilon\).

Change in OPC number (\(N_{OPC}\)) over time:

\[
\frac{dN_{OPC}}{dt} = (\alpha - \beta)N_{OPC} - BK_{OPC}.
\]

(1)

Changes in mature cell number (\(N_{MBP}\)) over time:

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confirmed that CD11c+ DCs express IL-11R. Purity was confirmed by immunostaining for CD11c. We have previously shown that DC cultures, myelin formation was quantified in stacks immunostained for MBP and neurofilament (NF). Myelin segments were identified as MBP+ linear profiles extending along NF+ axons, and their number and length were quantified by a blinded observer in at least five stacks per experiment (9, 18).

Statistics

For multiple comparisons, one-way ANOVA followed by Bonferroni posttest was used. Two-way ANOVA plus Bonferroni posttest was used to compare two treatment groups over multiple time points. Student t test was used to compare two groups of matched samples. A p value <0.05 was considered significant.

Results

Previously, we demonstrated that IL-11 receptor-deficient mice (IL-11R−/−) display exacerbated disease in myelin oligodendrocyte gp35–55-induced EAE (12). Compared with WT controls, IL-11R−/− mice exhibited a worsened clinical course, more extensive demyelinated lesions, and increased CNS inflammation (12). To clarify the roles of IL-11 in restricting myelin loss versus facilitating repair, we used a focal model of demyelination and remyelination in vivo (Figs. 1, 2, Table I, Supplemental Fig. 1). We then defined the mechanism of action of IL-11 in oligodendrocyte progenitors versus inflammatory cells in complementary studies in primary cultures (Figs. 3–6, Supplemental Figs. 2, 3).

Demyelination and oligodendrocyte death are exacerbated in IL-11R−/− mice

We used stereotactic microinjection of lysolecithin (18) to induce focal demyelinating lesions in the corpus callosum of 12-wk-old IL-11R−/− mice and WT littermates, and then followed the time course of lesion formation and resolution over a 4-wk period using confocal imaging (Fig. 1, Supplemental Fig. 1) and light and electron microscopy (Fig. 2, Table I). In confocal imaging experiments, we stained serial 20-µm sections of cerebral cortices at 7, 14, and 28 dpi for myelin (MBP and fluoromyelin) and the astrocyte gp35–55-induced EAE (12). Compared with WT controls, IL-11R−/− mice exhibited a worsened clinical course, more extensive demyelinated lesions, and increased CNS inflammation (12). To clarify the roles of IL-11 in restricting myelin loss versus facilitating repair, we used a focal model of demyelination and remyelination in vivo (Figs. 1, 2, Table I, Supplemental Fig. 1). We then defined the mechanism of action of IL-11 in oligodendrocyte progenitors versus inflammatory cells in complementary studies in primary cultures (Figs. 3–6, Supplemental Figs. 2, 3).

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Lysolecithin microinjection into the left side of the corpus callosum (Fig. 1A) (18) resulted in demyelination in both genotypes, with lesions larger at 7 dpi than at 14 dpi (Fig. 1B–D). Importantly, the maximal cross-sectional MBP/fluoromyelin negative demyelinated area was significantly larger in the brains of IL-11R−/− mice than in controls at both time points (Fig. 1D). Rostrocaudal lesion size was also increased in IL-11R−/− mice, at both 7 dpi (IL-11R−/− mice) and at 14 dpi (IL-11R−/− mice) versus WT 455 µm, p < 0.05. Student t test) and 14 dpi (IL-11R−/− mice) versus WT 280 µm, p < 0.0001). Moreover, numbers of Olig2+ cells were reduced to a much greater extent in lesions in IL-11R−/− mice than in controls at both time points (Figs. 1E, 1F). The extent of GFAP+ reactive astrogliosis was also increased (Figs. 1G–I) and neuronal damage was exacerbated, as shown by increased density of SMI-32+ transected axons in lesions in IL-11R−/− mice (Figs. 1G, 1H). Lesions had resolved in both genotypes by 28 dpi (data not shown).

CNS inflammation was also increased in lysolecithin lesions in IL-11R−/− animals (Fig. 1J–O). In IL-11R−/− mice, inflammatory infiltrates extended beyond the borders of the demyelinated area (Supplemental Fig. 1) and contained activated CD11b+ phagocytes (Fig. 1J, 1K) and smaller numbers of inflam-
treating CD3+ T lymphocytes and CD11c+ Ag-presenting DCs (Fig. 1M, 1N), which further analysis identified as CD11b+ CD11c+ myeloid DCs (Fig. 1J, 1K). In receptor null mice, the inflammatory lesion was markedly expanded, and infiltration of all three cell types was much more extensive than in controls at both 7 and 14 dpi (Fig. 1L, 1O).

**FIGURE 1.** IL-11Rα2/2 mice display exacerbated demyelination and inflammation in lysolecithin lesions. A, IL-11Rα2/2 mice and WT littermates (12 wk) were stereotactically microinjected with 1.5 μl 1% lysolecithin into the left corpus callosum at 5.5 mm anterior to λ, 1 mm lateral to bregma and 2 mm deep, and sacrificed at 7–28 dpi. Serial 20 μm sections were stained for myelin (MBP), Olig2, GFAP, SMI-32 (transected axons) CD11b, CD11c and CD3, and morphometric data quantitated from Z-series projections (see Materials and Methods). B, C, H, K, and N show entire lesions. Higher magnification images of IL-11Rα2/2 and WT littermate control samples are shown in E, G, J, M, and morphometric data is shown in D, F, I, L, O. Data in I and O are from 14 dpi. B–D, Lysolecithin induced demyelination in the corpus callosum, with lesions larger at 7 dpi (maximal demyelination) than 14 dpi (onset of repair). Maximum cross-sectional MBP2 area was greater in IL-11Rα2/2 mice than controls at both 7 and 14 dpi (D). Oligodendrocyte loss in lesions was increased in IL-11Rα2/2 mice over WT littermates (E, F). Reactive astrogliosis was also more extensive (H, I) and the density of SMI-32+ transected axons higher in lesions in IL-11Rα2/2 mice (G, I). J–O, In IL-11Rα2/2 animals, the inflammatory lesion was also markedly expanded, and infiltration of CD11b+ mononuclear phagocytes (J–L) and CD3+ T lymphocytes and CD11c+ DCs (M–O) was significantly increased. The latter were CD11c+CD11b+ myeloid DCs (J). Results are from five animals per genotype per condition per time point, five fields per animal (original magnification ×20). Scale bars, 90 μm (B, C, H, K, N) and 15 μm (E, G, J, M). *p < 0.05, **p < 0.01, Student t test (D, I, L, O); *p < 0.05, ANOVA plus Bonferroni post test (F).
Collectively, these results showed that demyelination, oligodendrocyte loss, and axonal transection were all markedly exacerbated in lysolecithin lesions in IL-11Rα2/2 mice. Moreover, CNS infiltration of inflammatory cells including CD11b+ mononuclear phagocytes, CD3+ T lymphocytes, and CD11c+ DCs was strongly increased. Because myelin repair in lysolecithin lesions is known to begin at 10–14 dpi (18, 23), the data from 7 dpi implicated increased demyelination as a significant factor in determining lesion size in IL-11Rα2/2 animals.

Remyelination is delayed in lysolecithin lesions in IL-11Rα2/2 mice
To investigate remyelination, we analyzed epoxy-embedded samples by light and electron microscopy (Fig. 2). Because myelin repair is first observed in lysolecithin lesions at 10–14 dpi (18, 23), we focused on 14-dpi samples in our analyses. These studies revealed that remyelination was delayed in IL-11Rα2/2 mice.

FIGURE 2. Delayed remyelination in IL-11Rα2/2 mice. A, Light microscopy of epoxy sections stained with toluidine blue, showing representative pathology from 14 dpi lysolecithin lesions in an IL-11Rα−/− mouse (lower panel) and WT littermate (upper panel). Individual cell types or details of pathology are illustrated at higher magnification in B–G (all IL-11Rα−/− genotype). A, At 14-dpi, the lesion center in both genotypes was hypercellular and demyelinated (right panels), and contained hypertrophic astrocytes (marked “A”, example shown in B), lipid-containing mononuclear phagocytes (C, marked “M”), and dystrophic transected axons (marked “D”, example shown in D). The lesion center was edematous in IL-11Rα−/− mice compared with controls. In agreement with data from samples immunostained for GFAP and SMI-32, reactive astrogliosis was more pronounced and dystrophic axons more numerous (A, lower panel). E, Normal myelin and oligodendrocytes (O) were present outside the lesion in both genotypes (see Table I). Adjacent to the lesion center in both genotypes was a distinct border (A, left panels) containing axons wrapped by disproportionately thin myelin characteristic of remyelination (examples shown in F, compare with normal myelin in G). H–K illustrate remyelination at the lesion border in both genotypes using electron microscopy of serial grids. H compares the lesion border at 14 dpi in an IL-11Rα−/− mouse (lower panel) and WT littermate (upper panel), showing demyelinated (asterisks) and remyelinated axons (arrowheads). Remyelinated axons were less abundant in IL-11Rα−/− samples than controls. Calculations of G ratio (axon diameter versus fiber diameter, a measurement of myelin thickness) for individual fibers within the lesion border in IL-11Rα−/− and WT mice (see Materials and Methods) confirmed that a significantly smaller percentage of axons were remyelinated (G ratio > 0.7) in IL-11Rα−/− mice than in WT littermates (IL-11Rα−/− 12.5 ± 1.6 versus WT 20.4 ± 1.9, p = 0.011, Student t test). Demyelinated and remyelinated axons are highlighted in lesion border (WT) in J and are compared in J and K, in transverse (J) and longitudinal section (K, higher magnification inset). Images are representative of data from four animals per genotype per condition, five fields per animal at ×2000, 10 axons per field. Original magnification ×300 (A), ×750 (B–G), ×2000 (H, I), ×5000 (J, K), ×10,000 (inset).
Table I. Normal myelin thickness and G ratio in corpus callosum of IL-11Rα<sup>−/−</sup> mice

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<th>WT</th>
<th>IL-11Rα&lt;sup&gt;−/−&lt;/sup&gt;</th>
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<tr>
<td>G ratio (axon/fiber diameter)</td>
<td>0.64 ± 0.01</td>
<td>0.62 ± 0.01</td>
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<tr>
<td>Myelin thickness (nm)</td>
<td>152.3 ± 8.3</td>
<td>174.2 ± 11.1</td>
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Random fields from epoxy-embedded sections of unlesioned corpus callosum from 12-wk-old IL-11Rα<sup>−/−</sup> mice and WT littermates were captured by electron microscopy (at least five fields per animal, at least four mice per genotype), and axon and fiber diameter were measured for 10 random axons per field by a blinded observer. Myelin thickness was calculated as (fiber diameter − axon diameter)/2. G ratio (myelin thickness relative to axon diameter) was calculated as (axon diameter)/(fiber diameter). Data were compared using Student’s t test; p values were not significant. These parameters were similar in both genotypes, and no differences in myelin architecture were detected in samples without lesions by light or electron microscopy.

and contained hypertrophic astrocytes (Fig. 2B), lipid-containing mononuclear phagocytes (Fig. 2C), and dystrophic transected axons (Fig. 2D). The lesion center was edematous in IL-11Rα<sup>−/−</sup> mice compared with controls, and compatible with data from samples stained for GFAP and SMI-32, reactive astrogliosis was more pronounced and dystrophic axons were more numerous (Fig. 2A). Outside the lesion were oligodendrocytes and myelin (Fig. 2E), and myelin thickness and the ratio of axon to fiber diameter (G ratio) were similar in normal-appearing white matter in both genotypes (Table I). In both IL-11Rα<sup>−/−</sup> mice and controls, between the lesion center and normal-appearing white matter was a distinct border containing myelinated and demyelinated axons (Fig. 2A) and axons wrapped by disproportionately thin myelin, characteristic of remyelination (Fig. 2F–K). The presence of remyelinated axons was confirmed at the lesion border in electron micrographs of serial grids from both IL-11Rα<sup>−/−</sup> and WT samples (Fig. 2H–K), and importantly fewer remyelinated axons were observed in IL-11Rα<sup>−/−</sup> samples than in controls (Fig. 2H).

To quantify numbers of remyelinated axons, we calculated the G ratio as a measurement of relative myelin thickness for individual fibers within the lesion border in IL-11Rα<sup>−/−</sup> and WT mice (see Materials and Methods) (17). In both genotypes, the border contained axons with G ratio values above the normal range (G ratio >

FIGURE 3. IL-11 potentiates oligodendrocyte progenitor survival and differentiation. A–E. Rat P2 OPCs grown in the presence of 100 ng/ml IL-11 or vehicle for up to 120 h were stained for Olig2, MBP, and BrdU or TUNEL. A–C. In all cultures, the percentage of mature Olig2<sup>+</sup>MBP<sup>+</sup> oligodendrocytes increased over time, while immature Olig2<sup>+</sup>MBP<sup>−</sup> OPCs declined. These changes were more pronounced in IL-11–treated cultures than in controls, and reached significance from 84 h (A, B). D. The percentage of TUNEL<sup>+</sup> apoptotic cells rose throughout the experiment in controls, but remained stable in IL-11–treated cultures. This difference was significant at 12 h and became more pronounced toward the end of the study. E. In contrast, IL-11 had no effect on proliferation. F. To understand the mechanism underlying these effects, we used a mathematical model to generate rates of proliferation for Olig2<sup>+</sup>MBP<sup>−</sup> OPCs (α) and mature Olig2<sup>+</sup>MBP<sup>+</sup> cells (γ), differentiation from one to the other (β), and apoptosis for OPCs (δ) and mature oligodendrocytes (ε; see Materials and Methods). G and H. IL-11 greatly reduced the rate of apoptosis (δ + ε) in OPC cultures (G), and increased the rate of differentiation (β) (H). The reduction in rate of apoptosis was strongest within the first 36 h (G), whereas the increase in differentiation rate was significant from 72 h onward (H). Scale bars, 20 μm. Data are representative of three independent experiments on separate cultures. *p < 0.05, **p < 0.01, ***p < 0.001, ANOVA plus Bonferroni post test.
0.7), indicative of remyelination (Fig. 2I–K). Notably, a smaller percentage of axons was remyelinated in IL-11Rα2/2 mice than in WT littermates, and this difference was statistically significant (IL-11Rα2/2 12.5 ± 1.6 versus WT 20.4 ± 1.9, p = 0.011, Student t-test). Thus, remyelination was delayed in IL-11Rα2/2 animals.

IL-11 potentiates oligodendrocyte progenitor survival and differentiation

The results of these experiments in vivo revealed roles for IL-11 in limiting demyelination and oligodendrocyte loss and in facilitating myelin repair. Moreover, these data also implicated IL-11 as immunoregulatory, restricting inflammation in demyelinated lesions. To investigate the mechanisms underlying these effects, we used mechanistic studies in primary cell cultures (Figs. 3–6, Supplemental Figs. 2 and 3).

Initially, we used modeling to analyze the effect of IL-11 on rates of proliferation, differentiation, and apoptosis in primary cultures of OPCs (Figs. 3–5). These cells were purified from P2 rat cerebral cortices (18) then grown in the presence of 1–100 ng/ml IL-11 or vehicle (most commonly 100 ng/ml; see Materials and Methods) and harvested at 12-h intervals for up to 120 h (5 d). Cultures were stained for Olig2 and the differentiation markers PDGFRα and A2B5 (OPCs), and CNPase and MBP (mature cells). TUNEL and BrdU labeling were used to identify apoptotic and mitotic cells. These experiments showed that IL-11 enhanced oligodendrocyte maturation and restricted apoptosis. All cultures, both IL-11-treated and controls, became progressively more differentiated...
inhibited induction of all of these transcripts. IL-11 reduced the rate of apoptosis (\( \alpha \)) of OPCs (Fig. 3A) and OPCs (Olig2\(^+\)MBP\(^+\)) declining (Fig. 3B). However, these changes were much more pronounced in IL-11–treated cultures than in controls, and these differences were significant from 84 h onwards (Fig. 3A–C). Apoptosis was also strongly reduced in IL-11–treated cultures from as early as 12 h, and this effect became more pronounced from 96 h (Fig. 3D, p values shown). IL-11 had no effect on proliferation (Fig. 3E). Similar trends were seen using lower IL-11 concentrations, and data were the same in cultures stained for alternate markers for OPCs (PDGFR\(^\alpha\)CNPase\(^-\)) and mature cells (PDGFR\(^\alpha\)CNPase\(^-\), data not shown).

To determine whether the shift toward differentiation in IL-11–treated cultures was due to enhanced maturation versus reduced apoptosis of mature cells, we adapted a previously described mathematical model (Fig. 3F–H) (19). The model uses ordinary differential equations to define the rates of proliferation of Olig2\(^+\)MBP\(^-\) OPCs (\( \alpha \)) and mature Olig2\(^+\)MBP\(^+\) oligodendrocytes (\( \gamma \)), and the rate of differentiation of one to the other (\( \beta \)) (Fig. 3F and see Materials and Methods). The model also generates the combination of supportive and instructive actions, enhancing both survival and maturation.

FIGURE 5. Proapoptotic effects of IL-11 on DCs. A and B, Mouse CD11c\(^+\) DCs were treated with 100 ng/ml IL-11 or vehicle for 24 h, then exposed to 5 ng/ml LPS or vehicle for 6 h. RNA was collected for quantitative PCR analysis (A), and supernatants for multiplex ELISA (B). See also Supplemental Figure 3A. A, LPS strongly induced mRNA for cytokines or their subunits including IL-23 p19, IL-12 p35, IL-12/23 p40, IL-6, IFN-\( \gamma \), and IL-10, and costimulatory molecules including C800. *p < 0.05, **p < 0.01, ***p < 0.001, ANOVA plus Bonferroni post test. IL-11 pretreatment strongly inhibited induction of all of these transcripts. B, Multiplex ELISA confirmed similar findings at the protein level. C and D, CD11c\(^+\) cells were exposed to IL-11 or vehicle for 72 h, and then total cells and the percentage of TUNEL\(^+\) apoptotic cells and BrdU\(^+\) proliferating cells were quantified. CD11c\(^+\) DCs were reduced by >60% in IL-11–treated cultures at 72 h (C). Moreover, the percentage of apoptotic cells in treated cultures doubled to almost 40%, while the parameter of proliferating cells was halved to 15% (D). E–G, CD11c\(^+\) DCs were exposed to IL-11 or vehicle for 72 h and then immunoblotted for caspase 9 and cleaved caspase 3 (E). Blots were quantified by densitometry, and data from multiple experiments were compared (F, G). Cleavage of both caspases was upregulated in IL-11–treated cultures at both time points tested, 36 h and especially 72 h. Data are representative of three independent experiments on separate cultures. *p < 0.05, **p < 0.01, Student t test.

The reduction in apoptosis (\( \delta + \epsilon \)) was strongest within the first 36 h of the experiment (Fig. 3G), whereas the increase in differentiation rate (\( \beta \)) reached statistical significance later, from 72 h onward (Fig. 3H). No effects were observed on other parameters (data not shown). Collectively, these findings show that IL-11 acts on OPCs via a combination of supportive and instructive actions, enhancing both survival and maturation.

Differential actions of Stat1 and Stat3 underlie the effects of IL-11 on OPC cultures

To define the signaling events responsible for these effects, we used siRNA (Fig. 4, Supplemental Fig. 2). Gp130 family members signal via the transcription factors Stat1 and Stat3, and the Stat-independent SHP2 pathway through Ras-Erk and PI3 kinase-akt (Fig.4A) (13). Immunoblotting with phosphospecific Abs showed that in OPCs, IL-11 treatment resulted in phosphorylation of Stat1a/β at Tyr701 and Stat3α/β at Tyr705 (Supplemental Fig. 2A, 2B). Phosphorylation of Shp2 (Tyr580) and induction of Erk signaling (phospho-p44/p42 at Thr202/Tyr204) were also seen, but no changes in PI3 kinase-Akt pathway activation (phospho-GSK3 Ser21/9). Thus, IL-11 activated both Stat-dependent and independent signaling.

SiRNA-mediated definition of the roles of these pathways in the effects of IL-11 produced striking findings (Fig. 4B–D, Supplemental Fig. 2C). OPCs were nucleasefected with siRNA for Stat1, Stat3, Shp2, PI3 kinase, or nontargeting (NT) control, or without siRNA (sham), then treated with IL-11 or vehicle and harvested at
The presence of Stat1 knockdown (Fig. 4) data were also observed in the presence of PI3 kinase silencing. Strikingly, IL-11 treatment of Stat3-silenced cultures was also especially in the presence of IL-11 treatment (Fig. 4B–D). DC cultures from Stat1<sup>−/−</sup> mice or WT littermates were exposed to IL-11 or vehicle for 72 h, and cleavage of caspases 9 and 3 were assayed by immunoblotting and densitometry as above. The IL-11–induced upregulation of caspase cleavage seen in WT cultures was abrogated in cultures from Stat1<sup>−/−</sup> mice. Data are representative of three independent experiments on separate cultures. *p < 0.05, Student t test.

4–7 d for immunoblotting (Fig. 4B). Blots were quantified by densitometry (see Materials and Methods), and data from multiple experiments were compared (Fig. 4C, 4D). Suggesting interaction of different gp130 signaling pathways, Stat3 silencing induced a 6-fold increase in Stat1 expression, whereas Shp2 silencing was associated with a 5-fold increase in Stat3 in IL-11–treated cultures (Fig. 4B). Confirming effects of IL-11 on oligodendrocyte survival and maturation, IL-11 treatment increased expression of the differentiation marker MBP and reduced levels of cleaved caspase 9, indicative of decreased apoptosis, in sham and NT-nucleofected cultures or in the presence of Shp2 silencing (Fig. 4B–D). Similar data were also observed in the presence of PI3 kinase silencing (Supplemental Fig. 2C). Notably, both effects were enhanced in the presence of Stat1 knockdown (Fig. 4B–D). Conversely, in Stat3-silenced cultures, caspase cleavage was markedly increased, especially in the presence of IL-11 treatment (Fig. 4B, 4D). Most strikingly, IL-11 treatment of Stat3-silenced cultures was also associated with loss of detectable MBP (Fig. 4B, 4C).

To validate these effects, we used TUNEL immunostaining (Fig. 4E). These studies confirmed that in OPC cultures nucleofected with nontargeting siRNA—or siRNA for Shp2, or Stat1, IL-11 treatment—reduced the percentage of TUNEL<sup>+</sup> apoptotic cells. Conversely, IL-11 increased the percentage of apoptotic cells in Stat3-silenced cultures (Fig. 4E).

These results revealed that Stat1 and Stat3 exerted differential effects in IL-11–treated OPC cultures. Stat3 restricted apoptosis and enhanced maturation, and its activity prevailed over that of Stat1, producing a net positive outcome. Silencing of Stat3 allowed the effects of Stat1 to become visible, converting IL-11 from a survival and differentiation factor into a proapoptotic signal for OPCs.

To determine whether IL-11 also protected OPCs against apoptosis induced by inflammatory factors, we tested its effects in cultures exposed to TNF-α, which has been implicated as an inducer of oligodendrocyte cell death in the inflamed CNS (25) (Supplemental Fig. 2D). Cells were treated with 10 ng/ml TNF-α plus IL-11 or vehicle control and then harvested at 5 d for immunoblotting as described above. In cultures exposed to TNF-α, caspase cleavage was markedly increased, but this effect was rescued in the presence of IL-11 cotreatment.

**IL-11 increases numbers rather than percentage of myelinating cells in CNS cocultures**

This work showed that IL-11 treatment of OPCs reduced apoptosis and enhanced maturation, with the net outcome determined by a balance in activity of Stat3 and Stat1. To define the roles of these mechanisms in the capacity for myelin formation, we examined cocultures of OPCs and DRG neurons (Fig. 4F–H). Previously, we found that IL-11 treatment increased numbers of myelin segments in vitro (9) but the mechanism was uncharacterized, although it was almost certainly oligodendrocyte-specific because >50% of OPCs express IL-11Rα, versus only 4.4% of DRG neurons (9).

Rat OPCs were plated onto ED 16.5 DRG neurons, and myelination was initiated with 1 μg/ml TrkA-Fc (9, 20). IL-11 was added and left in the medium during myelination, which occurs over 10–14 d (20). Next, cells were fixed and stained for neurofilament (NF, axons), MBP, and Olig2 (Fig. 4F–H and see Materials and Methods). At 14 d, all cultures contained mature MBP<sup>+</sup> oligodendrocytes, and in those exposed to TrkA-Fc, MBP<sup>+</sup> processes colocalized with NF<sup>+</sup> axons, forming linear MBP<sup>+</sup>NF<sup>+</sup> profiles that correspond to compact myelin segments (Fig. 4F) (9, 18, 20). Cultures exposed to IL-11 contained more segments per field and per cell than did controls (Fig. 4G), and segment length was increased (23 ± 2.8 μm versus 14 ± 1.5 μm in controls; Student t test, p = 0.013). Suggesting an underlying mechanism, IL-11 treatment increased numbers of Olig2<sup>+</sup> cells at all stages of differentiation, including mature MBP<sup>+</sup> cells and myelinating cells (MBP<sup>+</sup> with myelin segments), as well as immature Olig2<sup>+</sup> MBP<sup>+</sup> OPCs (Fig. 4H). Mature and myelinating cells were not increased as a percentage of the total Olig2<sup>+</sup> population in treated cocultures, and myelin segments were not observed any earlier than in controls (data not shown).

These data suggested that the effect of IL-11 on myelination was likely the result of an overall increase in oligodendrocyte lineage cell viability in treated cultures. A smaller maturational effect could...
not be discounted, because treated cultures also displayed more segments per myelin-forming cell.

**Stat1 activity underlies the proapoptotic effects of IL-11 on dendritic cell cultures**

To investigate the mechanism of action of IL-11 in limiting CNS inflammation, we used primary mononuclears of CD11c⁺ DCs and CD11b⁺ macrophages (Figs. 5, 6, Supplemental Fig. 3). Potential effects on CD3⁻ CD4⁻ T lymphocytes were discounted because we demonstrated previously that IL-11 exerts minimal or no direct actions on these cells (12). We examined cytokine and costimulatory molecule expression in DC cultures and compared proliferation and apoptotic activity in DCs in CD11b⁺ macrophages (Fig. 5, Supplemental Fig. 3). We then characterized the roles of Stat transcription factors in these events (Fig. 6). Importantly, these studies revealed that IL-11 is strongly proapoptotic for Ag-presenting CD11c⁺ DCs, and that its differential actions on these cells versus Olig2⁺ oligodendrocytes are mediated via lineage-specific activity of Stat1 versus Stat3.

Mouse CD11c⁺ DCs (22) were treated with 1–100 ng/ml IL-11 (most commonly 100 ng/ml) or vehicle for 24 h and then exposed to TLR4 activation using 5 ng/ml LPS for 6–24 h. Quantitative PCR analysis showed that LPS strongly induced mRNA for cytokines implicated in Th1 (IL-23 p19, IL-6) and Th1 (IL-12 p35) differentiation, plus the shared IL-12/23 p40 subunit and other cytokines, including TNF-α, IL-1α, IFN-β and IL-10, but not TGF-β1 (Fig. 5A). Importantly, pretreatment with IL-11 strongly inhibited induction of almost all cytokines tested, including IL-23 p19, IL-12 p35, p40, IL-6, IL-1α, TNF-α and IFN-β mRNA (Fig. 5A, 5B). Multiplex ELISA demonstrated similar findings at the protein level (Fig. 5B). IL-11 also strongly restricted induction of IL-10 protein (Fig. 5B), although effects on IL-10 mRNA did not reach significance (data not shown). Moreover, IL-11 inhibited LPS-induced expression of the costimulatory molecules CD80 and CD86 in CD11c⁺ DC cultures (Fig. 5A). Similar effects of IL-11 were observed in cultures subjected to TLR9 activation using 5 ng/ml CpG (Supplemental Fig. 3A).

Results of subsequent experiments suggested that these effects of IL-11 were due to increased apoptosis in treated DC cultures, as opposed to a phenotypic shift. CD11c⁺ cells were treated with IL-11 or vehicle for 24–72 h, and then total cells and TUNEL⁺ apoptotic and BrdU⁺ proliferating cells were counted (Fig. 5C, 5D). In marked contrast to our data from OPCs, numbers of CD11c⁺ DCs were reduced by >60% in IL-11–treated cultures at 72 h (Fig. 5C). Importantly, the percentage of apoptotic cells in treated cultures doubled to 40%, while the percentage of proliferating cells was halved (Fig. 5D). Moreover, this effect appeared specific; treatment of primary CD11b⁺ CD11c⁻ mouse peritoneal macrophages with 100 ng/ml IL-11 for up to 7 d had no effect on cell number or apoptosis (data not shown).

These results suggested proapoptotic effects of IL-11 on CD11c⁺ DCs as a significant mechanism underlying its immunoregulatory actions. To further test this hypothesis, we harvested protein extracts from IL-11–treated DC cultures and vehicle controls over 72 h, which were immunoblotted for caspase 9 and cleaved caspase 3, and then quantified the data by densitometry (Fig. 5E–G). Cleavage of both caspses was significantly potentiated in IL-11–treated cultures at both time points tested, 36 h and especially 72 h, confirming that apoptotic activity was increased in IL-11–treated DC cultures (Fig. 5E–G).

Next, we examined the roles of Stat1 and Stat3 in these effects (Fig. 6, Supplemental Fig. 3). Immunoblotting with phosphospecific Abs confirmed that IL-11 activated both transcription factors in CD11c⁺ DC cultures at 30 min, declining by 120 min (Supplemental Fig. 3B). To inhibit Stat3 activity, we used a Stat3 cell-permeable inhibitor (CPI, 100 μM; Fig. 6A–C) (15). Cultures were pretreated with Stat3CPI or inactive analog for 1 h and then exposed to IL-11 for up to 72 h. Importantly, IL-11–induced cleavage of caspases 9 and 3 was exacerbated in Stat3CPI-treated cultures (Fig. 6A–C). Stat3CPI also caused a trend toward increased caspase cleavage even in the absence of IL-11 (Fig. 6A–C).

Compatible with our results from oligodendrocyte cultures, inhibition of Stat1 activity produced the opposite effect. To investigate the role of Stat1 in IL-11–induced apoptosis, we examined caspase cleavage in DC cultures from Stat1⁻/⁻ mice (Fig. 6D–F). Importantly, whereas the cleaved forms of caspases 9 and 3 were both strongly upregulated at 72 h in IL-11–treated WT DCs compared with vehicle control, this increase was abrogated in Stat1⁻/⁻ cultures (Fig. 6D–F). Thus, the proapoptotic effect of IL-11 in DC cultures was Stat1-dependent.

Collectively, our studies in vitro revealed that IL-11 differentially regulated apoptosis in Olig2⁺ oligodendrocytes versus CD11c⁺ DCs, and showed that the net outcome of IL-11 signaling in both cell types depended on activity of Stat1 versus Stat3 (Fig. 7). In both lineages, Stat3 was antiapoptotic, whereas Stat1 was proapoptotic. IL-11 treatment resulted in Stat3-dependent enhancement of survival in OPC cultures, but Stat1-mediated apoptosis in CD11c⁺ DCs. Our findings from lysolecithin lesions suggested the relevance of these results to inflammatory demyelinating disease, implicating these differential effects of IL-11 as significant mechanisms underlying its neuroprotective and immunoregulatory actions in vivo.

**Discussion**

Members of the gp130 cytokine family have important functions in the hematopoietic, immune, reproductive, and cardiovascular systems as well as in the CNS (13). IL-6 is immunoregulatory (26) and acts with TGF-β1 in driving Th17 effector cell differentiation (27), whereas IL-27 exerts both proinflammatory Th1-enhancing activity and anti-inflammatory functions (28). IL-6 and LIF reg-
ulate hematopoiesis, and hematopoietic progenitors are reduced in IL-6−/− and LIF−/− mice (29, 30), while IL-11 and IL-6 stimulate megakaryocytopenesis (31, 32). LIF and IL-11 are important in pregnancy, and uterine decidualization or implantation are defective in IL-11Rα−/− or LIF−/− animals (16, 33). Although these family members all signal in part via the common gp130 receptor subunit, the mechanisms responsible for their distinct patterns of activity are incompletely characterized.

Importantly, several members of the gp130 family are strongly implicated as neuroprotectants. Mice deficient in CNTF or CNTFR, and CT-1−/− mice, show loss of motoneurons (34, 35), and CNTF is protective in neurodegenerative primate models (36). CNTF and LIF also promote oligodendrocyte viability, and CNTF−/− or LIFR−/− gp130−/− mice with EAE display exacerbated disease and oligodendrocyte apoptosis, although LIF−/− mice show attenuated signs later (10, 11, 14). Recently, we demonstrated that IL-11Rα−/− mice also display exacerbated clinical signs and demyelinated lesions in EAE, but that in addition, they exhibit increased CNS inflammation (12). We found that IL-11 is trophic for oligodendrocytes (9), and our studies also revealed that IL-11 limits Ag-presenting CD11c+ DC function and thus encephalitogenic T cell activation (12). However, the mechanisms underlying these neuroprotective and immunoregulatory effects remained unknown.

Our data reveal that these actions of IL-11 are mediated via differential regulation of apoptosis in Olig2+ oligodendrocyte lineage cells versus CD11c+ DCs, and the actions depend on cell type-specific activity of the transcription factors Stat1 and Stat3. In primary OPC cultures, IL-11 restricted caspase 9 cleavage and apoptosis and enhanced maturation, resulting in increased oligodendrocyte numbers and myelin formation in OPC-DRG cocultures (Figs. 3, 4, Supplemental Fig. 2). Notably, siRNA silencing of Stat1 augmented the effects of IL-11 in OPC cultures, whereas IL-11 induced apoptosis in the presence of Stat3 knockdown (Fig. 4). Conversely, in CD11c+ DCs, IL-11 treatment potentiated caspase activation and increased cell death (Fig. 5, Supplemental Fig. 3). The same preparation of IL-11 was used in both OPC and DC cultures, ruling out nonspecific toxicity as a potential cause of the effects observed in the latter cell type. Moreover, the proapoptotic effects of IL-11 in DC cultures were increased by Stat3 inhibition, but were ablated in Stat1−/− DC cultures (Fig. 6). Suggesting relevance to MS, demyelination and inflammation were exacerbated in the lysocetin model in IL-11Rα−/− mice (Fig. 1, Supplemental Fig. 1), and remyelination was delayed (Fig. 2, Table I). Lesions in IL-11Rα−/− mutants showed increased oligodendrocyte loss and axonal transection, and the expanded infiltration of CD11b+ mononuclear phagocytes, CD3+ T lymphocytes, and CD11c+ DCs. IL-11 effects on CD11c+ DCs were likely significant in determining the latter phenotype, because IL-11 did not display strong effects in CD11b+ macrophage cultures and has minimal direct impact on encephalitogenic CD3+ T cells (12). Collectively, our findings suggest that distinct effects of IL-11 on oligodendrocytes versus DCs are critical in the pathogenesis of CNS disorders. Members of the Stat family (Stats1–6) are selectively activated by different cytokines via phosphorylation of conserved C-terminal tyrosine and serine residues by JAK and MAPKs, inducing dimerization and nuclear translocation (13). Structure is conserved, particularly for Stat1 and Stat3, but the functions of the two are distinct. Stat1−/− mice are susceptible to infection with microbial pathogens because of defects in IFN signaling (37). Stat1 has also been implicated in apoptotic cell death, and Stat1-deficient cell lines are less susceptible to TNF-α–induced apoptosis than are controls (38). Conversely, Stat3 has antiapoptotic functions, in healthy cells and especially in malignancies where it is constitutively activated (39). Stat3 has been shown to transform fibroblasts and cause tumors in nude mice, whereas dominant negative or antisense Stat3 constructs induce apoptosis in tumor cell lines (40). Despite structural similarity, in some contexts Stat1 and Stat3 differentially regulate viability. A precedent for differential roles in the same cell type has also been reported. For example, although type I IFNs are antiapoptotic in WT CD4+ or CD8+ T cells, they are mitogenic and antiapoptotic in Stat1−/− mice, and both effects are Stat3 dependent (41). Importantly, our data reveal that Stat1 and Stat3 mediate distinct outcomes of the same cytokine in different lineages, and moreover they suggest the relevance of these differential actions to the same inflammatory demyelinating disorder in vivo. Interestingly, we also observed increased Stat1 protein in the presence of Stat3 silencing, compatible with previous findings suggesting reciprocal inhibition by Stat1 and Stat3 of one another’s activation or expression, or both (42, 43).

We are investigating mechanisms determining the discordant outcomes of IL-11–driven Stat1/3 activation in OPCs versus DCs, and we are examining the roles of the suppressors of cytokine signaling Socs1 and Socs3 in these effects. Socs family members have been implicated in fine control of Stat activation by IFNs and gp130 cytokines, and Socs1 and Socs3 have reciprocal functions in IL-6 and IFN-γ regulation (44). Socs3 deficiency results in prolonged activation of Stat1 and Stat3 by IL-6, but normal Stat1 activation after IFN-γ treatment (45). Conversely, whereas IL-6 stimulation of Stat1 and Stat3 is normal in Socs3−/− deficient cells, Stat1 activation by IFN-γ is prolonged (45). We are currently testing whether members of the Socs family also define cell type-specific outcomes of gp130 cytokine signaling.

Downstream of Stat activation, we are also examining effectors determining proapoptotic versus antiapoptotic outcomes. Stats regulate programmed cell death through both transcription-dependent and independent means. Transcriptional mechanisms include induction or suppression of caspase, Bcl/Bax, and cell cycle arrest genes, whereas transcription-independent events encompass interactions with molecules such as p53 and histone deacetylase (46). Previous reports have shown that different Stats have opposite effects on activity of antiapoptotic members of the Bcl family, such as Bcl-2 and Bcl-XL, versus proapoptotic molecules such as Bax and Bid (46). The latter molecules form pores in the outer mitochondrial membrane resulting in leakage of cytochrome c into the cytoplasm, binding to Apaf-1 and caspase 9, and initiation of apoptosis. Bcl-2 and Bcl-XL antagonize Bax, and a shift toward their expression restricts programmed cell death (47). Importantly, in some systems Stat1 negatively regulates the Bcl-XL promoter, whereas Stat3 positively modulates it (47). In addition, Stat3 activation has been shown to upregulate Bcl-XL and reduce apoptosis in CNS neurons (48). Although our data demonstrate the relevance of IL-11 signaling to demyelination and remyelination, defining contributions of neuroprotective versus immunoregulatory outcomes to inflammatory demyelinating disease will require inducible lineage-specific models, such as using oligodendrocyte- versus dendritic cell-specific activation or inactivation of Stat1 or Stat3. Because the signaling events underlying the exacerbated EAE phenotypes of CNTF and LIF mutants have also not yet been fully characterized (10, 11), lineage-specific targeting of individual pathways is likely to contribute to our wider understanding of the neuroprotective roles of gp130 cytokines in vivo. Interestingly, selective improvement of oligodendrocyte viability may also allow investigation of the extent to which axonal integrity depends on an intact myelinating oligodendrocyte, and whether neuronal and oligodendrocyte pathology can be uncoupled.
Directly targeting oligodendrocyte protection or myelin repair represents a therapeutic approach for MS that could be used in combination with available immunomodulatory treatments, for additive or perhaps synergistic clinical outcomes. The gp130 cytokine family has been widely suggested as a potential avenue for neuroprotection in a variety of conditions, including MS, and our data reveal novel mechanisms underlying the actions of a family member that is both neuroprotective and anti-inflammatory. These findings increase our understanding of the properties of IL-11, and of the gp130 family more widely. The findings suggest that cell type-specific manipulation of the component pathways of gp130 signaling represents a means to enhance oligodendrocyte survival and restrict CNS inflammation in MS.

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Disclosures
The authors have no financial conflicts of interest.

References
13. Ernst, M., and B. J. Jenkins. 2004. Acquiring signalling specificity from the gp130 family more widely. The findings suggest that cell type-specific manipulation of the component pathways of gp130 signaling represents a means to enhance oligodendrocyte survival and restrict CNS inflammation in MS.


Supplementary Figure Legends

Suppl. Figure 1. Expansion of demyelination and inflammation in lysolecithin lesions in IL-11Ra<sup>−/−</sup> mice. IL-11Ra<sup>−/−</sup> mice and wildtype littermates (12wk) were microinjected stereotactically with lysolecithin into the left corpus callosum, then sacrificed at 14dpi. Serial 20μm sections were stained for myelin (MBP) and for GFAP, CD11b and CD11c, and morphometric data quantitated from Z-series projections (see Methods section). Results shown here are from panels (d,i,l and o) of Figure 1, arranged to compare the extent of demyelination and inflammation in both genotypes. Statistical analyses of these data are presented in Figure 1, and are not shown here. Lysolecithin induced demyelination in the corpus callosum, and maximum cross-sectional MBP<sup>+</sup> area was greater in IL-11Ra<sup>−/−</sup> mice than controls. In both IL-11Ra<sup>−/−</sup> and wildtype mice, inflammatory infiltrates extended beyond the borders of the demyelinated area, and included reactive GFAP<sup>+</sup> astrocytes, CD11c<sup>+</sup> dendritic cells, and activated CD11b<sup>+</sup> phagocytes. In receptor null mice, the inflammatory lesion was also expanded, and the area of infiltration of all three cell types was more extensive than in wildtype controls. Results are from 5 animals per genotype per condition, 5 20x fields per animal.

Suppl. Figure 2. Impact of IL-11 treatment on OPC cultures. (a) IL-11 ligand-receptor binding activates STAT-dependent signaling via Stat1 and Stat3, and the Stat-independent SHP2 pathway, which stimulates Ras-Erk and PI3 kinase-Akt signaling. (b) Protein extracts from rat OPCs exposed to 100ng/ml IL-11 or vehicle for 30min were immunoblotted with phospho-specific antibodies for activated signaling pathway components. IL-11 induced phosphorylation of Stat1α/β at Tyr701, Stat3α/β at Tyr705, Shp2 at Tyr580, and p44/42 at Thr202/Tyr204, but had no effect on activation of Gsk3, a downstream effector of the PI3 kinase-Akt pathway. These data suggested that the effects of IL-11 on oligodendrocytes might be mediated via Stat-
dependent or MAP kinase signaling, as opposed to the PI3 kinase-Akt pathway. (c) Silencing of PI3 kinase has no impact on effects of IL-11 on OPC cultures. OPCs were nucleofected with siRNA for PI3 kinase, or nontargeting (NT) control, or without siRNA (sham), then exposed to 100ng/ml IL-11 or vehicle for 4-7d and protein extracts subjected to immunoblotting. Blots were quantified by densitometry. In sham and NT cultures, IL-11 increased MBP expression and decreased caspase 9 cleavage. Similar data were observed in the presence of PI3 kinase silencing. Thus, compatible with data in panel (b), and in contrast to effects observed in the presence of siRNA for Stat1 or Stat3 (see Figure 4), silencing of PI3 kinase had no detectable effect on markers of oligodendrocyte maturation or apoptotic activity. (d) IL-11 inhibits TNFα-induced cell death in OPC cultures. OPCs were treated with TNFα (10ng/ml) plus IL-11 or vehicle control, then harvested at 5d and protein extracts subjected to SDS-PAGE and immunoblotting for cleaved caspase 9 and caspase 3. Blots were quantified by densitometry. In cultures exposed to TNFα, caspase cleavage was increased over controls, but this effect was rescued in the presence of IL-11 co-treatment. Thus, IL-11 reduces actively-induced cell death in OPC cultures, in addition to its anti-apoptotic effects in cells exposed to growth factor withdrawal (see Figure 4). Data in all panels are representative of three independent experiments on separate cultures.

Suppl. Figure 3. IL-11 inhibits cytokine production by CD11c+ dendritic cells. (a) Mouse CD11c+ DCs were treated with 100ng/ml IL-11 or vehicle for 24h, then exposed to 5ng/ml LPS or CpG or vehicle control for 6h. RNA was collected for QPCR. Activation of TLR4 with LPS or of TLR9 using CpG both strongly induced mRNA for cytokines or subunits including IL-23 p19, IL-12 p35, IL-12/23 p40, IL-6, and IFNβ. IL-11 pretreatment inhibited induction of all of these transcripts. (b) Protein extracts from mouse DCs exposed to 100ng/ml IL-11 or vehicle for
30 min or 120 min were immunoblotted with phospho-specific antibodies for Stat1α/β (Tyr701) or Stat3α/β (Tyr705). IL-11 activated both transcription factors in CD11c⁺ DC cultures at 30 min, declining by 120 min. (a) ANOVA plus Bonferroni post test, *P<0.05, **P<0.01, ***P<0.001. Data are representative of three independent experiments on separate cultures.
Supplementary Figure 1

Maximal area (x10^4 μm^2)

- Demy. (MBP^+)
- CD11c^+
- GFAP^+
- CD11b^+  

+/+  -/-

14dpi
Supplementary Figure 2

a. Diagram showing the interaction of IL-11, IL-11Rα, gp130, Stat1, Stat3, Ras-Erk, and Akt.

b. Table showing protein levels under different conditions:
- pStat1: 91
- pStat3: 86/79
- pShp2: 72
- pErk: 44/42
- pGsk3: 51
- Actin: 42

Veh IL-11

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c. Western blot showing protein levels under different treatments:
- MBP: Sham 22, NT 17, PI3K 85
- Cleaved Casp9: Sham 17, NT 17, PI3K 42

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</table>


d. Table showing protein levels under different conditions:
- TNFα: - - + +
- IL-11: - + - +
- Cleaved Casp9: - 17
- Cleaved Casp3: - 17
- Actin: - 42

<table>
<thead>
<tr>
<th></th>
<th>Cleaved Casp9/Actin</th>
<th>Cleaved Casp3/Actin</th>
<th>PI3K/Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα: - - + +</td>
<td>0.01 0.75 0.08</td>
<td>0.28 0.13 0.81 0.18</td>
<td></td>
</tr>
<tr>
<td>IL-11: - + - +</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>