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IL-11 Regulates Autoimmune Demyelination

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Current therapies for the autoimmune demyelinating disease multiple sclerosis (MS) target inflammation, but do not directly address neuroprotection or lesion repair. Cytokines of the gp130 family regulate survival and differentiation of both neural and immune cells, and we recently identified expression of the family member IL-11 in active MS plaques. In this study, we show that IL-11 regulates the clinical course and neuropathology of experimental autoimmune encephalomyelitis, a demyelinating model that mimics many of the clinical and pathologic features of MS. Importantly, the effects of IL-11 are achieved via a combination of immunoregulation and direct neuroprotection. IL-11Rα-null (IL-11Rα−/−) mice displayed a significant increase in clinical severity and neuropathology of experimental autoimmune encephalomyelitis compared with wild-type littermates. Inflammation, demyelination, and oligodendrocyte and neuronal loss were all exacerbated in IL-11Rα−/− animals. Conversely, wild-type mice treated with IL-11 displayed milder clinical signs and neuropathology than vehicle-treated controls. In cocultures of murine myelin oligodendrocyte glycoprotein35–55-specific CD4+ T lymphocytes and CD11c+ APCs, IL-11 treatment resulted in a significant decrease in T cell-derived effector cytokine production. This effect was generated via modulation of CD11c+ APC-mediated lymphocyte activation, and was associated with a decrease in the size of the CD11c+ cell population. Conversely, IL-11 strongly reduced apoptosis and potentiated mitosis in primary cultures of mouse oligodendrocyte progenitors. Collectively, these data reveal that IL-11 regulates inflammatory demyelination via a unique combination of immunoregulation and neuroprotection. IL-11 signaling may represent a therapeutic avenue to restrict CNS inflammation and potentiate oligodendrocyte survival in autoimmune demyelinating disease. The Journal of Immunology, 2009, 183: 4229–4240.

Multiple sclerosis (MS) is an inflammatory disease of the CNS primarily affecting young adults (1). Its pathology is characterized by leukocyte infiltration, demyelination, oligodendrocyte loss, axonal transection, and a reactive astrogliosis (2, 3). It is believed that early neurologic disability in MS is affected by conduction block in demyelinated axons, whereas axonal transection underlies the more permanent deficits observed later in the disease (4). Remyelination of nascent lesions has been observed and is associated with recovery of axonal conduction, but often fails with disease progression (5, 6). Therapies for MS have focused primarily on the immune system; however, novel approaches directed at CNS protection and regeneration, in concert with immunoregulation, are principal objectives of current research.

Analysis of cellular and molecular components of the MS lesion has led to significant advances in our understanding of disease pathogenesis. Notably, reactive astrocytes comprise the most prevalent cell type in MS plaques and have been shown to regulate both inflammation and CNS repair (7, 8). Recently, using a genomics-based approach, we examined gene expression patterns of human astrocytes treated with cytokines relevant to MS lesion formation (9, 10). In these studies, cytokines including IL-1β, IFN-γ, and TGF-β1 each elicited distinct changes in the astrocytic gene expression profile, suggesting functional outcomes. Significantly, both IL-1β and TGF-β1 induced genes of the gp130 cytokine family (10). Members of this family have previously been implicated as mediators of neural and immune cell differentiation, suggesting potential relevance to autoimmune demyelination (11, 12). IL-11 was one of the gp130 cytokines identified in our study, and it shares characteristics with both immunoregulatory (IL-6) and neuroprotective (LIF, ciliary neurotrophic factor (CNTF)) members of the family. IL-11 has been shown to regulate mononuclear phagocyte activation and also stimulates megakaryocytepoiesis and thrombopoiesis (10, 13–15). In the CNS, IL-11 has been shown to promote neuronal differentiation, and our experiments demonstrated that IL-11 also enhanced oligodendrocyte numbers and myelin formation in vitro (10, 16). In MS lesions, we found that IL-11 was expressed by reactive astrocytes, corroborating our genomics data (10).

In this work, we describe studies investigating the role of IL-11 in experimental autoimmune encephalomyelitis (EAE), a widely accepted animal model of MS. Using loss-of-function and gain-of-function approaches, we demonstrate that IL-11Rα−/− mice display exacerbated clinical and pathologic outcomes, whereas mice treated with exogenous IL-11 exhibit markedly milder disease. These effects are achieved via two distinct mechanisms.
IL-11 displays potent immunomodulatory effects on CD11c<sup>+</sup> APCs, and is also directly trophic for oligodendrocyte progenitors (OPCs). Thus, IL-11 exerts a unique spectrum of immunoregulation and neuroprotection. These effects suggest IL-11 signaling as a potential therapeutic avenue for inflammatory demyelinating disease.

**Materials and Methods**

**Antibodies**

A2B5 mouse hybridoma was obtained from American Type Culture Collection, and supernatant (IgM) was prepared by standard methods. Other Abs used for immunocytochemistry and immunohistochemistry are as follows: rat anti-CD11b (Invitrogen); hamster anti-CD11c (BD Pharmingen); rabbit anti-I-Ab (DakoCytomation); O4 mouse IgM (P. Davies, AECOM, Bronx, NY); mouse anti-2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) (IgG1), mouse anti-NeuN (IgG1), and rabbit anti-Olig2 (Millipore Chemicon); mouse anti-myelin basic protein (MBP) and mouse anti-neurofilament H nonphosphorylated (SMI 32; both Covance Sternberger); rabbit anti-myelin-associated glycoprotein (J. Salzer, New York University, New York, NY); sheep anti-Brdu (Novus); rabbit anti-Brdu (Immunology Consultants); goat anti-IL-11 (Santa Cruz Biotechnology); mouse anti-platelet-derived growth factor receptor α (IgG1), R&D Systems; mouse CC-1 and rabbit anti-CD3 (both Abcam); and mouse anti-actin (IgG1; Sigma-Aldrich). Fluorescently labeled Abs used for flow cytometry are as follows: hamster anti-CD11c, rabbit anti-CD86, hamster anti-CD80, rat anti-CD16/CD32, hamster IgG1, IL-11 (anti-trinitrophenyl), rabbit anti-IgG2a, 1 (anti-keyhole limpet hemocyanin), hamster IgG2k, 2 (anti-keyhole limpet hemocyanin), and I-A (all BD Biosciences). C57BL/6 mice do not express MHC class II (MHC II) I-A<sup>I</sup>/I-E<sup>E</sup>, rather just I-A<sup>I</sup> only; however, an I-A<sup>I</sup>/I-E Ab was used to identify the I-A epitope.

**Cytokines**

Mouse and human IL-11 were purchased from PeproTech. Protein sequences of the two are 88.9% similar, and pilot experiments showed that both were equally effective in inducing responses in vivo and in all culture systems used in the project in vitro (data not shown). LPS from Salmonella minnesota was purchased from Alexis Biochemicals.

**Mice**

IL-11Ra<sup>-/-</sup> mice backcrossed onto a C57BL/6 background for at least 12 generations were purchased from The Jackson Laboratory and bred at Mount Sinai School of Medicine. This genotype was originally generated by H. Nandurkar, L. Robb, and C. Glenn Begley (Walter and Eliza Hall Institute, Melbourne, Australia) (17, 18). Genotyping was conducted according to the manufacturer’s instructions: Milliplex 9-plex, GM-CSF, IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α, and IL-17; Milliplex 6-plex, IL-1β, IL-6, IL-7, IL-12p40, IL-12p70, and TNF-α. Multiplex ELISA plates were read using a LumineX 100 multiplex plate reader (Lumexx).

**Flow cytometry**

At times stated in the text, unfixed cultures were stained with fluorescein-conjugated Abs, as previously reported, using combinations described. Flow cytometry was performed on a Cytomics FC 500 machine (Beckman Coulter) and analyzed using FlowJo software (Tree Star) (22).

**Immunocytochemistry**

OPC cultures were fixed 10 min with 4% paraformaldehyde, and processed for single or double immunostaining for A2B5 (1:500), O4 (1:250), CNPase (1:1000), Olig-2 (1:1000), and BrdU (1:1000), as previously described, using combinations described in the text (10). Immunostained cultures were examined and photographed using a Zeiss LSM 510 META laser-scanning confocal system attached to an Axiovert 200 inverted microscope (Zeiss MicroImaging). Apoptotic cells were counted in at least five fields per condition in each experiment by a blinded observer using ImageJ 1.30v software (National Institutes of Health). Results were expressed as percentage of total 4′,6-diamidino-2-phenylindole (DAPI)<sup>+</sup> cells, and as number of positive cells, and were compared by statistics (see below).

**Apoptotis assay**

Mitosis was quantified using a CellTiter96 Aqueous One Solution Cell Proliferation Assay (Promega), according to the manufacturer’s instructions.

**Multiplex ELISA**

Supernatants taken at times shown from cultures or cocultures treated as described in the text were assayed for the following cytokines using Miliplex mouse cytokine/chemokine multiplex ELISA panels (Millipore), according to the manufacturer’s instructions: Milliplex 9-plex, GM-CSF, IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α, and IL-17; Milliplex 6-plex, IL-1β, IL-6, IL-7, IL-12p40, IL-12p70, and TNF-α. Multiplex ELISA plates were read using a LumineX 100 multiplex plate reader (Lumexx).

**Tissue culture**

To establish cultures of CD11c<sup>+</sup> cells or CD4<sup>+</sup> cells, spleens from 8-wk male C57BL/6 mice were homogenized and incubated for 30 min in 2 ml of medium in 96-well plates. Splenocytes were suspended in MACS buffer (PBS (pH 7.2), 0.5% BSA, and 2 mM EDTA) and then subjected to magnetic cell sorting with mouse CD11c (N418) Microbeads (Milteny Biotech), or untouched magnetic cell sorting with mouse CD4<sup>+</sup> T Cell Isolation Kit MicroBeads (Miltenyi Biotech), according to the manufacturer’s instructions. Following isolation, cells were cultured in RPMI 1640, 10% FCS, 1 mM sodium pyruvate, 200 μM nonessential amino acids, 20 mM HEPES, 50 μM 2-ME, and 50 μM gentamicin. Purity of isolation for both cell types was confirmed as >90% by flow cytometry (see below). For isolation and culture of OPCs, cerebral cortices of P0–P2 rats were homogenized, and then C57BL/6 R2<sup>+</sup> cells were isolated by immunopanning and propagated or differentiated, as described (21).

**Coculture studies**

CD11c<sup>+</sup> cells (2 × 10<sup>5</sup>) were cocultured with 5 × 10<sup>4</sup> CD4<sup>+</sup> cells in 200 μl of medium in 96-well plates. Cocultures were pulsed with 50 μg/ml MOG<sub>35–55</sub>, 10 ng/ml mouse IL-11, 5 μg/ml PHA, or vehicle control for 24–72 h. In pretreatment experiments, CD11c<sup>+</sup> cells (2 × 10<sup>4</sup> cells in 100 μl of medium) or CD4<sup>+</sup> cells (5 × 10<sup>4</sup> cells in 1 ml of medium) were treated with IL-11 (10 ng/ml) for 24 h, and then washed three times with fresh medium. Treated CD11c<sup>+</sup> cells were then cocultured with vehicle-treated CD4<sup>+</sup> cells, and vice versa. Cocultures were treated with MOG<sub>35–55</sub> (50 μg/ml) upon plating, for 24–72 h.

**Apoptosis assay**

Cultures were fixed in 4% paraformaldehyde for 10 min, then washed and permeabilized in PBS 0.3% Triton X-100 for 10 min, and subjected to a TUNEL assay using the In Situ Cell Death Kit (Roche-Applied-Science), according to the manufacturer’s instructions. Cultures were imaged at ×20 magnification and photographed using a Zeiss LSM 510 META laser-scanning confocal system attached to an Axiovert 200 inverted microscope (Zeiss MicroImaging). Apoptotic cells were counted in at least five fields per condition in each experiment by a blinded observer using ImageJ 1.30v software (National Institutes of Health). Results were expressed as percentage of total 4′,6-diamidino-2-phenylindole (DAPI)<sup>+</sup> cells, and as number of positive cells, and were compared by statistics (see below).

**Proliferation assay**

At times stated in the text, fixed cultures were stained with fluorescein-conjugated Abs, as previously reported, using combinations described. Flow cytometry was performed on a Cytomics FC 500 machine (Beckman Coulter) and analyzed using FlowJo software (Tree Star) (22).
without EAE were sacrificed by anesthetic overdose and perfused with 4% paraformaldehyde. Brain and spinal cord were removed and processed for cryostat or paraffin embedding. For immunostaining, 20-μm sections of lumbar, thoracic, and cervical spinal cord and cerebral cortex were rehydrated with PBS for 10 min, and then immunostained using protocols previously described (23), and counterstained with DAPI. Immunostained sections were examined and photographed, as above, and Z-series stacks were collected using 1 μm on the z-axis and assembled into projections using Zeiss LSM Image Browser software. The number of cells positive for lineage and differentiation markers was counted in Z-series stacks at ×20 magnification by a blinded observer using ImageJ version 1.30 software in lumbar, thoracic, and cervical spinal cord sections, at least five fields at 20× magnification per animal, three animals per genotype or condition per time point, in at least three independent experiments per study. Data were expressed as number of cells positive for each marker per unit area (100 μm²). To quantify demyelination, the MBP⁺ area in each section was measured in Z-series stacks at ×10 magnification using ImageJ software version 1.30, and expressed as absolute area and as percentage of entire section area. Results from different genotypes or conditions were compared using statistics (see below).

Histopathology

Paraffin-embedded 20-μm sections of lumbar, thoracic, and cervical spinal cord from animals sacrificed as described above were rehydrated with PBS for 10 min and stained with H&E using standard protocols, and examined and photographed on a Zeiss Axiosplan microscope (Zeiss MicroImaging) with a Q-Imaging MP3.3 RTV color camera and QED Capture software. Within each section, the area occupied by inflammatory infiltrates (defined as hypercellular perivascular or subpial accumulations of small mononuclear cells) was measured at ×10 magnification by a blinded observer using ImageJ software version 1.30. Results were expressed as absolute area and as percentage of entire section area. Results from different genotypes or conditions were compared using statistics (see below).

Statistical analysis

Analyses were performed using Prism software v4.0 (GraphPad). Datasets were tested to confirm a Gaussian distribution using D’Agostino and Pearson normality test. For multiple comparisons, one-way ANOVA, followed by Bonferroni posttest was used. Student’s t test was used to compare two groups of matched samples. Tests used for each experiment are specified in figure legends. In all cases, p < 0.05 was considered significant.

Results

IL-11 signaling regulates the clinical severity and neuropathology of EAE

To investigate the role of IL-11 signaling in autoimmune demyelination, we induced EAE in mice with a selective deletion of IL-11Rα (IL-11Ra⁻/⁻) and littermate controls (Figs. 1 and 2). When unchallenged by MOG35-55 sensitization, IL-11R-null mice were healthy and showed no differences in CNS or peripheral immune system composition when compared with wild-type animals. In particular, IL-11Ra⁻/⁻ and IL-11Ra⁺/+ animals had similar numbers of Olig2⁺ oligodendrocyte lineage cells, and both groups exhibited a normal pattern of CNS immunoreactivity for the myelin marker MBP (data not shown). No differences were observed in the number or activation of CD11c⁺ cells or CD4⁺ lymphocytes in spleens of mutant animals. However, upon EAE induction, we observed significant contrasts between receptor-null and wild-type cohorts (Fig. 1). Following sensitization with MOG35-55, mice were monitored daily for weight loss and clinical signs, which consisted of an ascending flaccid paralysis and were evaluated based upon a widely used five-point scoring paradigm (20). The resulting clinical profiles revealed that both IL-11Ra⁻/⁻ mice and IL-11Ra⁺/+ heterozygotes exhibited neurological signs starting at approximately the same time post sensitization as wild-type littermates, but that their disease was significantly more severe (Fig. 1a; days 16–21 and 23–28, p < 0.05, ANOVA followed by Bonferroni posttest). This divergence in clinical scores was evident at 16 days post sensitization and endured through the end point of the experiment (30 days; Fig. 1a). Together with a worsened clinical profile, IL-11Ra⁻/⁻ and IL-11Ra⁺/+ mice exhibited more extensive weight loss than wild-type littermate controls (Fig. 1b; days 16–17, p < 0.05). Additionally, we compared the highest clinical score for each animal in all three genotypes during the course of the disease (Fig. 1c). Notably, we found that mice within the wild-type control group displayed a mean peak clinical score of 2.33, indicating hindlimb weakness, whereas heterozygotes and mutants scored 4.28 and 3.86, respectively, indicating severe paralysis (Fig. 1c; ANOVA plus Bonferroni posttest, IL-11Ra⁻/⁻, p < 0.01; IL-11Ra⁺/+, p < 0.01, at least seven animals per group per experiment).

To investigate the pathology associated with these contrasting clinical deficits, the most severe animals from each cohort were sacrificed during the acute clinical episode of disease at 18- to 22-day post sensitization. Sections of thoracic and lumbar spinal cord from animals in each group were subjected to immunohistochemistry for markers of leukocyte infiltration and CNS inflammation (CD3, CD11b, I-A, and fibrinogen). Sections were also immunostained for myelin (MBP), oligodendrocyte lineage cells (Olig2), neurons (NeuN), and SMI-32, a marker of axonal dystrophy (24). Stained sections were imaged using confocal microscopy, and results were quantified by computer-assisted morphometry (see Materials and Methods; at least three animals per genotype, at least three sections per animal, and five representative image fields per section).

Typical pathology of EAE was observed in IL-11Ra⁻/⁻ and IL-11Ra⁺/+ CNS tissue, including perivascular infiltration of mononuclear inflammatory cells (Fig. 1d) and CNS demyelination (see Fig. 2). Quantitative analysis of immunostained sections revealed that inflammation was significantly more extensive in IL-11Ra⁻/⁻ mice and heterozygotes than in wild-type littermates (Fig. 1, d–j). In mutant and heterozygote spinal cord sections, infiltration of CD3⁺ T lymphocytes was more pronounced than in controls (Fig. 1, e and f; p < 0.05, ANOVA plus Bonferroni posttest). Activated mononuclear phagocytes (CD11b⁺) were also more numerous in both IL-11Ra⁻/⁻ and IL-11Ra⁺/+ spinal cord than in controls (Fig. 1, g and h; mutant, p < 0.001; heterozygote, p < 0.01). Additionally, increased numbers of MHCh-expressing cells (assessed by I-A immunoreactivity), which are most likely (although not conclusively) activated APCs, were observed in mutant sections (Fig. 1, i and j; p < 0.05). A trend toward increased I-A immunoreactivity was also observed in heterozygotes, but did not reach significance (Fig. 1i). Collectively, these studies revealed exacerbation of CNS inflammation in animals with defective IL-11 signaling. These changes included elements of both the innate and adaptive arms of the immune system.

To assess IL-11Rα expression in these animals, we performed immunocytochemistry on immune cells from IL-11Ra⁻/⁻, IL-11Ra⁺/+ and IL-11Ra⁻/⁻ mice. CD11c⁺ APCs isolated from spleens of unchallenged 8-wk-old male mice of each genotype were stained with I-A and DAPI (supplemental Fig. 1). IL-11Rα immunoreactivity localized to the surface of CD11c⁺ cells from wild-type mice (supplemental Fig. 1a). Conversely, compatible with previous reports, cells from heterozygotes displayed lower level expression of the receptor (supplemental Fig. 1b), and IL-11Rα immunoreactivity was absent from IL-11Ra⁻/⁻ CD11c⁺ APCs (supplemental Fig. 1c) (17, 25). Cell surface expression of I-A was similar in all three genotypes (supplemental Fig. 1, a–c).

Analysis of sections stained for lineage-specific and myelin markers revealed that the increase in CNS inflammation observed
FIGURE 1. IL-11 signaling regulates clinical severity and neuroinflammation in EAE. 

*a*, IL-11R<sup>−/−</sup> and IL-11R<sup>+/−</sup> mice and wild-type C57BL/6 littermates were sensitized to MOG<sub>35–55</sub>, as described in Materials and Methods. Mice developed clinical signs of EAE starting at 10-day postsensitization, characterized by an ascending flaccid paralysis, and were scored with widely used 5-point paradigm, as described in Materials and Methods. The severity of disease in both IL-11R<sup>−/−</sup> and IL-11R<sup>+/−</sup> mice was significantly more severe than in controls (16–21 days and 23–28 days; *p* < 0.05, ANOVA followed by Bonferroni posttest). 

*b*, Body weight was also measured daily. Mutants and heterozygotes exhibited greater weight loss during the course of disease when compared with wild-type controls (mutant, 16–17 days; *p* < 0.05). 

*c*, Mutant and heterozygous animals also displayed mean peak clinical scores of EAE significantly higher than controls (IL-11R<sup>−/−</sup>, *p* < 0.01; IL-11R<sup>+/−</sup>, *p* < 0.01, at least seven animals per group per experiment).

*d*, H&E-stained section of lumbar spinal cord from 11-wk wild-type C57BL/6 mouse 22 days postinduction of EAE with MOG<sub>35–55</sub> peptide. Typical perivascular mononuclear inflammatory infiltrates are observed in white matter areas, illustrated at higher magnification below.

*e–j*, Confocal Z-series projections of matched ventrolateral thoracic and lumbar spinal cord sections from 11-wk IL-11R<sup>−/−</sup> animals with EAE 18–22 day postinduction (*f*, *h*, and *j*, right panels), heterozygotes, or wild-type littermate controls (*left panels*), immunostained for CD3 (*f*), CD11b (*h*), or I-A (*j*), and quantitated by morphometric analysis, as described in Materials and Methods (*e*, *g*, and *i*). Sections from IL-11R<sup>−/−</sup> animals display more extensive inflammation than controls, including potentiated infiltration by CD3<sup>+</sup> lymphocytes (*e* and *f*), and enhanced parenchymal immunoreactivity for the mononuclear phagocyte marker CD11b (*g* and *h*) and I-A (MHCII) (*i* and *j*). 

*a*, *b*, *e*, *g*, and *i*, *p* < 0.05; ***, *p* < 0.001; ANOVA followed by Bonferroni posttest. Results shown are from three animals per condition, at least six ×20 fields per animal, and are representative of at least five independent experiments. Scale bars (*f*, *h*, and *j*), 70 μm.
in IL-11Ra−/− and heterozygote animals was associated with exacerbated demyelination and oligodendrocyte loss, together with axonal transection and loss of neurons (Fig. 2). Whereas wild-type thoracolumbar spinal cord contained distinct areas of myelin loss (as assessed by immunostaining for MBP) in subpial and perivascular white matter, spinal cord tissue from mutant and heterozygous animals exhibited more confluent demyelination (Fig. 2, a and b), which was typically observed in areas of hypercellularity corresponding to inflammatory cell infiltration. IL-11Ra−/− samples also exhibited more extensive oligodendrocyte loss than wild-type littermates (c and d), which similarly corresponded to hypercellular regions (c, arrows). Quantification of axonal dystrophy using SMI-32 immunostaining demonstrated that spinal cord sections from mutant and heterozygous animals displayed greater axonal damage than controls (e). Immunostaining for the neuronal marker NeuN also revealed a significant decrease in neuronal numbers in spinal cord tissue of IL-11Ra−/− and IL-11Ra+/− samples when compared with wild-type controls (f and g). b, d, e, g, *p < 0.05; **, p < 0.01; ***, p < 0.001; ANOVA followed by Bonferroni post test. Results shown are from three animals per condition, at least six ×20 fields per animal, and are representative of at least five independent experiments. Scale bars: a, 200 μm; c, 50 μm; f, 100 μm.

**FIGURE 2.** IL-11Ra−/− mice with EAE display exacerbated demyelination, oligodendrocyte, and neuronal loss. Confocal Z-series projections of spinal cord sections from the same IL-11Ra−/−, IL-11Ra+/−, and wild-type control mice with EAE described in Fig. 1 were immunostained for MBP (myelin), Olig2 (oligodendrocyte lineage), NeuN (neurons), and SMI-32 (axonal dystrophy). Projections were quantitated by morphometry, as described in Materials and Methods (b, d, e, and g). Sections from IL-11Ra−/− and heterozygous mice display more extensive demyelination than controls (a and b). Spinal cord sections from wild-type controls contained distinct areas of myelin loss, whereas mutant and heterozygous animals exhibited more confluent demyelination (a, arrows), which was typically observed in areas of hypercellularity corresponding to inflammatory cell infiltration. IL-11Ra−/− samples also exhibited more extensive oligodendrocyte loss than wild-type littermates (c and d), which similarly corresponded to hypercellular regions (c, arrows). Quantification of axonal dystrophy using SMI-32 immunostaining demonstrated that spinal cord sections from mutant and heterozygous animals displayed greater axonal damage than controls (e). Immunostaining for the neuronal marker NeuN also revealed a significant decrease in neuronal numbers in spinal cord tissue of IL-11Ra−/− and IL-11Ra+/− animals compared with wild-type controls (Fig. 2, f and g; mutant, p < 0.01; heterozygote, p < 0.05). Staining for the neuronal marker NeuN also revealed a significant decrease in neuronal numbers in spinal cord tissue of IL-11Ra−/− and IL-11Ra+/− animals compared with wild-type controls (Fig. 2). Collectively, these findings show that loss of IL-11 signaling is associated with intensified clinical deficits, heightened CNS inflammation, and more extensive demyelination and neurodegeneration.

**IL-11 reduces apoptosis and potentiates mitosis in OPC cultures**

To define the mechanisms underlying these effects, we performed parallel studies in vitro. Because demyelination and oligodendrocyte loss were potentiated in IL-11Ra−/− mice with EAE induced by MOG35−55 sensitization, we investigated whether IL-11 is directly protective for Olig2+ cells using primary cultures of OPCs...
We also examined immunoregulatory effects of IL-11 in cocultures of MOG35–55-specific CD4+/H11001 lymphocytes and CD11c+/H11001 APCs, because spinal cord infiltration of these cell types was enhanced in IL-11R+/H9251/H11002 mice with EAE (Fig. 4).

In experiments investigating effects on oligodendrocyte lineage cells, primary cultures of A2B5+/H11001 Ran2+/H11002 OPCs were purified from P1 mouse cortex, as previously described, then left to differentiate in serum-free medium in the presence of rIL-11 (1–100 ng/ml, most commonly 10 ng/ml) or vehicle control for up to 5 days (21) (Fig. 3). Previous work in our laboratory has shown that IL-11R is expressed by both rodent and human oligodendrocyte lineage cells, localizing primarily to A2B5+/H11001 OPCs (10). In initial studies, IL-11-treated cultures and controls were immunostained for the stage-specific differentiation markers A2B5 (OPCs), CNPase (oligodendrocytes), and MBP (mature oligodendrocytes), and imaged using confocal microscopy. Random ×20 Z-series stacks were captured (at least five per condition), and cells positive for each marker were counted and compared (see Materials and Methods).

These studies showed that after differentiation for 5 days, vehicle-treated control cultures contained more oligodendrocyte lineage cells of all differentiation stages than controls, including A2B5+ OPCs (a and c), CNPase+ arborized oligodendrocytes (b and c), and mature MBP+ cells (c). *, p < 0.05; **, p < 0.01; ***, p < 0.001; Student’s t test. d–f Rat P1 OPCs were purified as above, then left to differentiate for 4, 6, or 8 days in the presence of 10 ng/ml IL-11 or vehicle control. Cultures were then fixed, apoptosis was quantified by TUNEL immunostaining (d and e), and proliferation was quantified using BrdU labeling in parallel studies on the same cultures (d and f). Data shown in d are from 6 days. In control cultures, at 4 days few cells were TUNEL+ (e), whereas large numbers of cells were mitotic, as assessed by BrdU labeling (f). Conversely, at 6 and 8 days, apoptosis was more prevalent, whereas proliferation was less extensive (e and f). Treatment with IL-11 was associated with significantly reduced apoptosis and potentiated mitosis at both 6 and 8 days (e, 6 and 8 days, p < 0.001; f, 6 and 8 days, p < 0.01; Student’s t test). Scale bars (a, b, and d), 40 μm. Data for all panels are representative of findings from at least three independent experiments using separate cultures.

(Relevant Figures and Tables)
Proliferation was quantified using BrdU labeling in parallel studies on the same cultures (Fig. 3, d and e). These studies showed that, at 4 days, control cultures contained very few TUNEL+/H11001 cells (Fig. 3e), whereas large numbers of cells were mitotic, as assessed by BrdU labeling (Fig. 3f). Previous work has shown that A2B5+ OPCs comprise the mitotic population in these cultures (26). Conversely, at 6 and 8 days, apoptosis was more prevalent, whereas proliferation was less extensive (Fig. 3, e and f). Treatment with IL-11 was associated with significantly reduced apoptosis and potentiated mitosis at both 6 and 8 days (Fig. 3, e and f, p < 0.01; Fig. 3, 6 and 8 days, p < 0.01; Student’s t test).
Collectively, these experiments reveal that IL-11 is directly trophic for the oligodendrocyte lineage. IL-11 slows the increase in apoptosis normally observed over time in OPC cultures, and results in a significant increase in the number of cells of all maturation stages, including proliferating A2B5+ OPCs and mature MBP+ oligodendrocytes.

**IL-11 reduces CD4+ lymphocyte activation via inhibitory effects on CD11c+ cells**

In parallel with studies on cultured oligodendrocytes, we conducted mechanistic in vitro experiments focused on immune cells relevant to autoimmune demyelination (Fig. 4). CD4+ Th1/Th17 lymphocytes are known to play a prominent role in the pathogenesis of EAE and MS, and the importance of CD11c+ APCs in autoimmunity is underscored by their capacity to prime, activate, and even induce tolerance in the CD4+ lymphocyte population (27–30). We cocultured murine MOG35–55-specific CD4+ lymphocytes with MOG35–55-pulsed CD11c+ APCs, and investigated the effects of IL-11 treatment. CD4+ lymphocytes and CD11c+ cells were isolated from spleens of sensitized and unchallenged 8-wk C57BL/6 mice, respectively (see Materials and Methods) and cocultured in the presence of MOG35–55 and 10 ng/ml IL-11 or vehicle control for 48–72 h. Cytokine production, proliferation, and surface molecule expression were then quantified.

We initially examined the cytokine content of coculture supernatants using a multiplex ELISA system (see Materials and Methods), focusing on a panel of Th1/Th17 and Th2 effector cytokines (Fig. 4a). Sensitization with MOG35–55 in CFA results in a delayed-type hypersensitivity response, the production of Th1/Th17 cytokines, and induction of EAE. Thus, these studies showed that, compared with unstimulated controls, MOG35–55-treated cultures exhibited a significant increase in the production of IL-17 and the Th1 cytokines GM-CSF, IL-2, and IFN-γ (Fig. 4a). Also assayed were IL-10 and the Th2 cytokines IL-4 and IL-5; however, as expected, we found no significant induction of these factors in MOG35–55-pulsed cultures (Fig. 4a). Importantly, in the presence of treatment with 10 ng/ml IL-11, production of all Th1/Th17 cytokines assayed (IL-17, GM-CSF, IL-2, and IFN-γ) was reduced by ~25–50% (Fig. 4a).

To determine whether this change resulted from IL-11-mediated effects on CD11c+ cells or CD4+ lymphocytes, we repeated these studies using a pretreatment paradigm (Fig. 4b). CD4+ lymphocytes and CD11c+ cells isolated, as above, were independently treated with 10 ng/ml IL-11 or vehicle control for 24 h. Each population was then washed and resuspended in fresh medium before being cocultured in the presence of MOG35–55. The design of these experiments provided a means for us to study the effects of IL-11 signaling on cocultures in the context of lymphocyte vs CD11c+ APC exposure. We observed generally lower cytokine induction in these experiments, presumably due to the pretreatment paradigm. However, these studies clearly showed that both CD11c+ cell pretreatment and CD4+ lymphocyte pretreatment resulted in reduced production of IL-17, GM-CSF, IL-2, and IFN-γ compared with vehicle-treated controls (Fig. 4b). Notably, the CD11c+–pretreated cultures exhibited a much more pronounced reduction (50–80%) of inflammatory cytokines than the CD4+ lymphocyte-pretreated cultures (Fig. 4b). The effect of IL-11 on CD11c+ pretreatment reached significance for all four of the aforementioned cytokines (GM-CSF, p < 0.001; IFN-γ, p < 0.001; IL-2, p < 0.05; IL-17, p < 0.05; ANOVA plus Bonferroni post-test). By contrast, CD4+ pretreatment reached significance only in the case of IFN-γ (p < 0.01; Fig. 4b). Neither CD11c+ pretreatment nor CD4+ pretreatment resulted in effects on the cytokines IL-4, IL-5, and IL-10 (data not shown).

To investigate whether these effects were associated with changes in lymphocyte proliferation, we conducted 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reduction assays in cocultures treated with IL-11 using both cotreatment and pretreatment paradigms, as described above (Fig. 4c). CD11c+ APCs are terminally differentiated nonproliferating cells; thus, increases in cell number in these cocultures were attributable to clonal expansion of MOG35–55–specific CD4+ lymphocytes (31). In the absence of IL-11 treatment, MOG35–55–pulsed cocultures displayed a significant increase in cell number after 72 h compared with unstimulated controls (Fig. 4c). Interestingly, although pretreatment with IL-11 was associated with reduced production of Th1/Th17 cytokines, we detected no differences in proliferation in cocultures treated according to this paradigm. Conversely, IL-11 pretreatment of CD11c+ APCs and CD4+ lymphocytes generated differential effects on cell number. When compared with MOG35–55–stimulated untreated cocultures, IL-11 pretreatment of CD11c+ cells resulted in significantly decreased proliferation (Fig. 4c; p < 0.01, ANOVA plus Bonferroni posttest), compatible with data from our multiplex ELISA studies. Interestingly, pretreatment of CD4+ lymphocytes resulted in an additional increase in cell number compared with MOG35–55–pulsed controls (Fig. 4c; p < 0.05), despite its subtle down-regulatory effects on inflammatory cytokine production (Fig. 4, compare b and c).

To define the nature of IL-11–signaling effects on CD11c+ cells, we repeated CD11c+ pretreatment experiments and analyzed the resulting cocultures after 48 h using flow cytometry (Fig. 4, d and e). Cocultures were stained for CD11c, I-A (MHCII), and the costimulatory molecules CD80 and CD86, and then subjected to analysis by flow cytometry (see Materials and Methods). In MOG35–55–pulsed cultures, CD11c+ cells made up a smaller percentage of the total cell population (12.4 vs 21.3%), due to clonal expansion of MOG35–55–specific memory T lymphocytes (Fig. 4d). However, we found that IL-11 pretreatment of CD11c+ cells was associated with a further decrease in the relative number of CD11c+ cells (7.9 vs 12.4%), even though proliferation was reduced in these cultures compared with MOG35–55–pulsed controls (Fig. 4, compare d with c). In cocultures absent of MOG35–55–IL-11 pretreatment of CD11c+ APCs also resulted in a significant reduction in the relative number of CD11c+ cells (8.7% of coculture cells) compared with untreated controls (21.3%; Fig. 4d). Upon analyzing I-A expression in these cocultures, we observed a similar pattern (Fig. 4e). We found that IL-11 pretreatment of CD11c+ cells was associated with a decrease in the relative number of I-A+ CD11c+ cells, and this finding was observed in both unstimulated and MOG35–55–pulsed cultures (Fig. 4e). Interestingly, although the percentage of I-A+ cells was reduced in cultures exposed to IL-11, we did not detect significant alterations in I-A levels in expressing cells (data not shown). Similar results were observed for the costimulatory molecules CD80 and CD86 (data not shown).

Collectively, these findings demonstrate that IL-11 signaling has potent effects on both CD4+ lymphocytes and CD11c+ APCs. Most notably, IL-11 regulates production of Th1/Th17 effector cytokines, via both direct and indirect pathways. The indirect pathway is the more effectual of the two and results from effects on CD11c+ APCs.

**IL-11 treatment mitigates disease severity in EAE**

To investigate effects of IL-11 gain-of-function in vivo, we used a treatment paradigm in which rIL-11 was administered to 8-wk C57BL/6 mice sensitized with MOG35–55 (Fig. 5). Mice were injected i.p. with IL-11 (25 or 50 μg/kg/day) or vehicle control daily for 10 days (days 10–19) starting at clinical onset
of EAE. Clinical disease and weight loss were evaluated daily, as above. These studies showed that treatment with 50 µg/kg/day IL-11 was associated with significantly reduced disease severity, as assessed by motor deficit, compared with vehicle-treated littermate controls (days 19–26, p < 0.05; Student’s t test). Noticeable therapeutic effects of IL-11 were observed from day 17 (day 9 of treatment), and these effects endured to day 26 (7 days following cessation of treatment). Noticeable therapeutic effects of IL-11 were observed from day 17 (day 9 of treatment), and these effects endured to day 26 (7 days following cessation of treatment). At day 27 (8 days following cessation of treatment), the mean score of the IL-11-treated group began to increase and converge with that of the control group, suggesting that IL-11 exerts its protective effects in a treatment-dependent fashion (Fig. 5a). Lower doses of IL-11 did not produce significant protection (data not shown).

FIGURE 5. IL-11 treatment mitigates disease severity in EAE. a, C57BL/6 mice, sensitized with MOG35–55 at 8 wk, were injected i.p. with IL-11 (50 µg/kg/day) or vehicle control daily for 10 days (days 10–19) starting at clinical onset of EAE. Clinical deficits were evaluated based upon a widely used scoring paradigm detailed in Materials and Methods. Treatment with 50 µg/kg/day IL-11 was associated with significantly reduced disease severity, as assessed by neurologic deficit, compared with vehicle-treated littermate controls (days 19–26, p < 0.05; Student’s t test). Noticeable therapeutic effects of IL-11 were observed from day 17 (day 9 of treatment), and these effects endured to day 26 (7 days following cessation of treatment). b, Animals treated with IL-11 also exhibited significantly improved retention of body mass during the course of disease when compared with vehicle-treated controls (days 17–19 and 21–23, p < 0.05; day 20, p < 0.01). Animals from each cohort were sacrificed during the acute clinical episode (20–22 day postinduction), and immunohistochemistry was performed on spinal cord tissue sections as in Fig. 1 and quantitated by morphometry described in Materials and Methods. IL-11 treatment was associated with a striking reduction in the extent of demyelination in MBP-immunostained sections when compared with vehicle-treated controls (c and d, p < 0.001; Student’s t test). IL-11-treated animals also displayed a minor, but significant decrease in the loss of Olig2+ oligodendrocyte lineage cells in the spinal cord (e). f–h, The observed changes in neuropathology accompanied significantly attenuated inflammation in samples from IL-11-treated mice. The presence of I-A+ APCs was significantly reduced in spinal cord sections of IL-11-treated mice compared with vehicle-treated controls (f and g; p < 0.001). Numbers of CD3+ lymphocytes were also significantly decreased (h; p < 0.001). Results shown are from three animals per condition, at least six ×20 fields per animal, and are representative of at least five independent experiments. Scale bars: c, 150 µm; f, 70 µm.
To investigate the effects of IL-11 treatment on autoreactive inflammation and CNS pathology, animals from each cohort were sacrificed during the acute clinical episode (20- to 22-day post-sensitization), and immunohistochemistry was performed on spinal cord tissue sections, as above. As suggested by our functional studies in vitro, we found a striking reduction in the extent of demyelination per section in IL-11-treated animals compared with vehicle-treated controls (Fig. 5, c and d; p < 0.001; Student’s t test). This was associated with a small, but significant effect on the number of Olig2+ oligodendrocyte lineage cells, which were observed in slightly higher numbers in spinal cord from IL-11-treated animals (Fig. 5e). These changes accompanied a significant decrease in inflammation in samples from IL-11-treated mice with EAE (Fig. 5, f–h). The presence of I-A+ APCs was significantly reduced in spinal cord sections of IL-11-treated mice compared with vehicle-treated controls (Fig. 5, f and g; p < 0.001). Numbers of CD3+ lymphocytes were also significantly decreased (Fig. 5h; p < 0.001). Collectively, the findings of these gain-of-function studies in vivo are compatible with the results of our experiments in vitro. They demonstrate that administration of IL-11 to mice with EAE is associated with amelioration of neurological disease, and diminished inflammation and CNS demyelination.

Discussion

In this study, we have investigated the functional significance of IL-11 signaling in the context of autoimmune demyelinating disease. Our data establish that IL-11 exerts protective effects in EAE via immune system modulation as well as trophic effects on oligodendrocytes. MOG35-55-sensitized IL-11Rα-/- mice displayed significantly worsened clinical signs of EAE compared with IL-11Rα +/- littermates (Fig. 1). This exacerbated clinical outcome was accompanied by heightened CNS inflammation and neuropathology (Figs. 1 and 2). Conversely, IL-11 (50 µg/kg/day) treatment of wild-type MOG35-55-sensitized animals resulted in the amelioration of clinical EAE and pathology consistent with disease attenuation (Fig. 5). Our in vitro studies revealed that OPCs treated with IL-11 exhibited reduced apoptosis and potentiated mitosis (Fig. 3). In contrast to these trophic effects on oligodendrocytes, we found that IL-11 treatment of CD11c+ APCs, when cocultured with MOG35-55-specific CD4+ lymphocytes, resulted in diminished lymphocyte proliferation and reduced production of Th1/Th17 effector cytokines. This inverse relationship, supportive effects on oligodendroglia vs inhibitory effects on leukocytes, positions IL-11 as a potentially protective factor in inflammatory disease of the mammalian CNS.

IL-11 is a member of the gp130 family, a pleiotropic group of cytokines known to modulate proliferation, maturation, and survival in multiple cell lineages (32). Members of the family include IL-6, IL-11, LIF, CNTF, and oncostatin M, and all bind to cell surface receptors containing the common signal-transducing subunit gp130 (33). Family members have pro- as well as anti-inflammatory properties and are important regulators of hematopoiesis, as well as innate and adaptive immune responses (32). For example, IL-6 has a mixed history of both promoting and inhibiting inflammation, and is also known as an acute-phase reactant that produces fever (34–36). In addition, recent work has shown that IL-6 acts in combination with TGF-β1, a cytokine previously believed to be predominantly anti-inflammatory, to induce Th17 effector cells (37, 38). Although IL-6 is known for its potent effects on cells of the immune system, reports of gp130-driven neuroprotection have further encouraged research focus on this family of mediators. CNTF has been shown to promote survival in oligodendrocytes and neurons in addition to preventing blood-brain barrier (BBB) disruption (12, 39), and LIF is also known to enhance oligodendrocyte viability in animal models of autoimmune demyelination (11).

IL-11 was originally isolated from bone marrow stromal cells, and early studies revealed its potent effects on hematopoiesis (40, 41). In particular, IL-11 stimulates megakaryocytepoiesis and thrombopoiesis, which has resulted in its therapeutic use in patients exhibiting chemotherapy-driven thrombocytopenia (13, 15, 42). In related studies, overexpression of IL-11 in transgenic mice resulted in stimulation of long bone formation with increased cortical thickness and decreased bone loss over time (43). Additional reports have revealed the anti-inflammatory properties of IL-11 in conditions such as mucositis and periodontitis (44, 45). Work by Robb et al. (18) has also demonstrated that IL-11 signaling is required for normal uterine decidualization, and that female mice lacking IL-11Rα are infertile. Finally, research into CNS neurogenesis has shown that neuronal progenitors within the hippocampus differentiate in response to IL-11 treatment in vitro. This collection of studies, among others, illustrates the pleiotropy of IL-11 signaling in mammalian biology.

Using functional genomics data, we recently discovered that expression of IL-11, along with other gp130 cytokines, was strongly induced in cultures of human astrocytes in response to IL-1ß or TGF-ß1 (10). Both of these cytokines have previously been implicated in lesion formation in inflammatory CNS diseases, including MS (46, 47). We therefore examined brain tissue samples from MS patients and controls, and discovered that IL-11 was present at the border of both chronic active and silent MS lesions and that IL-11 immunoreactivity localized to reactive astrocytes in vivo. We also found IL-11Rα expression to colocalize with oligodendrocyte markers in the same tissue sections (10). In the current study, we have examined the functional relevance of these findings in the context of autoimmune demyelinating disease in vivo. Our results are compatible with our initial in vitro findings, which showed that IL-11 potentiates oligodendrocyte survival and maturation in human cultures (10). However, our current data also clearly demonstrate a role for IL-11 in functional regulation of leukocytes relevant to autoreactive inflammation of the CNS. Of particular interest are our results showing modulation of CD11c+ APC function, which support and extend a previous report that IL-11 exerts anti-inflammatory effects on cells of the innate immune system (14).

The mechanism underlying the effects of IL-11 on CD11c+ APCs is presently an area of active research in our laboratory. Although the direct impact of IL-11 on CD4+ lymphocyte cytokine production in our experiments was subtle, pretreatment of CD11c+ APCs evoked much more potent suppression of lymphocyte cytokine production in cocultures. To define the changes elicited by IL-11 on CD11c+ function, we are currently examining three distinct hypotheses. First, our present findings show a reduction in the size of the CD11c+ population in cocultures containing IL-11-pretreated CD11c+ cells. This may result from directly apoptotic effects of the cytokine, or from the observed reduction in survival factors, such as GM-CSF, in these experiments. Second, IL-11 may suppress activation of CD11c+ APCs, resulting in altered production of cytokines required for amplification of effector CD4+ lymphocytes. Studies to test this hypothesis are planned. Finally, IL-11 may regulate expression of stimulatory, costimulatory, or adhesion molecules relevant to efficient Ag presentation by CD11c+ cells. These hypotheses are currently under investigation in our laboratory.

The neuropathologic phenotype of IL-11Rα–/– mice with EAE was also of particular interest to us in the current work. Loss of IL-11Rα expression and signaling was associated with a significant increase in CNS demyelination, potentiated loss of Olig2+...
oligodendrocytes, increased axonal dystrophy, and extensive neurodegeneration. Whereas loss of myelin in wild-type littermates occurred in discrete perivascular and subpial white matter areas, in IL-11Ra−/− animals we observed more confluent areas of demyelination. Notably, deficiency of just one allele of IL-11Ra in heterozygotes was sufficient to generate comparable effects to those observed in receptor-null mutants, suggesting that partial loss of IL-11 signaling may be nearly as deleterious as complete inactivation. Defining the relative contributions of immunoregulatory vs directly protective effects of IL-11 to the phenotype of IL-11Ra−/− mice and heterozygotes will require refinement of our current experimental paradigm to include the use of cell type-specific loss-of-function studies, and we are currently pursuing this approach.

A cell type-specific approach would also contribute to our understanding of the results of our gain-of-function studies in vivo, which were compatible with, but not the exact converse of the phenotype observed in IL-11Ra−/− animals. Specifically, whereas we observed a striking reduction in demyelination in mice with EAE that received IL-11 treatment, protection of oligodendrocyte lineage cells in these animals only appeared partial, albeit statistically significant. Although at 19 kDa IL-11 would not be expected to pass freely through the BBB, mice sensitized to develop EAE using our chosen protocol are known to display extensive histamine-driven BBB disruption (48), providing peripheral factors a potential means of entry into the CNS parenchyma. However, it is difficult to estimate the extent to which systemically administered cytokine entered the CNS in our gain-of-function study. Thus, efficient delivery of therapies based on the IL-11 pathway may require a small m.w. agonist designed to emulate the way may require a small m.w. agonist designed to emulate the

Potential therapeutic strategies for MS can be divided into two main areas. In the first are immunomodulatory treatments designed to reduce or prevent the formation of new CNS lesions, and hence, new clinical episodes. In the second are neuroprotective or regenerative therapies aimed at repairing pre-existing damage. Avenues combining both approaches may represent novel and efficacious treatments for inflammatory CNS demyelination. Our results show that IL-11 has potent anti-inflammatory properties and also acts to support oligodendrocyte populations directly, a spectrum of biological action clearly relevant to the clinical course and neuropathology of EAE. On the basis of our previous findings and the work described in the current manuscript, we suggest that manipulation of IL-11 signaling may represent a potential means of therapy for inflammatory diseases of the CNS, including MS.

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Disclosures

The authors have no financial conflict of interest.

References


38. Veldhoen, M., R. J. Hocking, C. J. Atkins, R. M. Locksley, and B. Stockinger. 2006. TGF-$
\beta$ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 24: 179 –189.


Supplementary Figure 1. IL-11Rα expression on splenic CD11c+ APCs. Spleens were taken from unchallenged male 8wk IL-11Rα−/−, IL-11Rα+/− and IL-11Rα+/+ mice. CD11c+ APCs were isolated from splenocytes and immunostained for IL-11Rα, I-A, and DAPI (a-c). CD11c+ cells from wildtype animals displayed cell surface expression of both IL-11Rα and I-A (a). Cells from heterozygotes exhibited a lower level of IL-11Rα expression than wildtype mice (b). IL-11Rα was absent from the surface of CD11c+ cells from IL-11Rα−/− mice (c). Scalebars, 5µm.