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The Inflammasome Sensor, NLRP3, Regulates CNS Inflammation and Demyelination via Caspase-1 and Interleukin-18

Sushmita Jha,1 Siddharth Y. Srivastava,2 W. June Brickey,1,3 Heather Iocca,1,3 Arrel Toews,4 James P. Morrison,6 Vivian S. Chen,1,5 Denis Gris,1 Glenn K. Matsushima,3,5 and Jenny P.-Y. Ting1,3,5

1Lineberger Comprehensive Cancer Center, 2Carolina Cardiovascular Biology Center, 3Department of Microbiology and Immunology, 4Department of Biochemistry and Biophysics, and 5Neuroscience Center, University of North Carolina, Chapel Hill, Chapel Hill, North Carolina 27599, and 6Charles River Pathology Associates, Durham, North Carolina 27703

Inflammation is increasingly recognized as an important contributor to a host of CNS disorders; however, its regulation in the brain is not well delineated. Nucleotide-binding domain, leucine-rich repeat, pyrin domain containing 3 (NLRP3) is a key component of the inflammasome complex, which also includes ASC (apoptotic speck-containing protein with a caspase-1 and procaspase-1. Inflammasome formation can be triggered by membrane P2X7R engagement leading to cleavage-induced maturation of caspase-1 and interleukin-1β (IL-1β)/IL-18. This work shows that expression of the Nlrp3 gene was increased 100-fold in a cuprizone-induced demyelination and neuroinflammation model. Mice lacking the Nlrp3 gene (Nlrp3−/−) exhibited delayed neuroinflammation, demyelination, and oligodendrocyte loss in this model. These mice also showed reduced demyelination in the experimental autoimmune encephalomyelitis model of neuroinflammation. This outcome is also observed for casp1−/− and IL-18−/− mice, whereas IL-1β−/− mice were indistinguishable from wild-type controls, indicating that Nlrp3-mediated function is through caspase-1 and IL-18. Additional analyses revealed that, unlike the IL-1β−/− mice, which have been previously shown to show delayed remyelination, Nlrp3−/− mice did not exhibit delayed remyelination. Interestingly, IL-18−/− mice showed enhanced remyelination, thus providing a possible compensatory mechanism for the lack of a remyelination defect in Nlrp3−/− mice. These results suggest that NLRP3 plays an important role in a model of multiple sclerosis by exacerbating CNS inflammation, and this is partly mediated by caspase-1 and IL-18. Additionally, the therapeutic inhibition of IL-18 might decrease demyelination but enhance remyelination, which has broad implications for demyelinating diseases.

Introduction

CNS inflammation (neuroinflammation) is a key component of many neurological diseases including multiple sclerosis (MS), Parkinson’s disease, and Alzheimer’s disease. MS results from neuroinflammation characterized by lymphocyte/macrophage infiltration, microglial and astrocytic activation, enhanced cytokine/chemokine production, demyelination, and axonal loss (Sospedra and Martin, 2005; Pittoc and Lucchetti, 2007). Understanding the mechanisms by which neuroinflammation affects demyelination is important for therapeutic development.

Several families of innate immune receptors or sensors have been identified, with the nucleotide-binding domain, leucine-rich repeat containing (NLR) family receiving significant attention because of its genetic linkage to human immunologic diseases and its role in immune regulation (Harton et al., 2002; Ting et al., 2006). NLR family, Pyrin-domain containing 3 (NLRP3) (also Cryopyrin, NALP3, PYPAP1, CIAS1) represents a core component of a caspase-1-activating inflammasome complex, composed of an NLR protein, the adaptor molecule apoptotic speck-containing protein with a caspase-1 and procaspase-1 (Sutterwala et al., 2006a,b). Activated caspase-1 in turn cleaves >70 substrates including the proinflammatory cytokines interleukin-1β (IL-1β) and IL-18 (Shao et al., 2007; Keller et al., 2008). NLRP3 forms an inflammasome in response to bacterial RNA and toxins (Kanneganti et al., 2006a,b), viruses (Allen et al., 2009; Thomas et al., 2009), ATP (Mariathasan et al., 2006), uric acid (Martinon et al., 2006), hyaluronan (Yamasaki et al., 2006), amyloid-β (Halle et al., 2008), asbestos, silica (Dostert et al., 2008; Hornung et al., 2008), and alun (Hornung et al., 2008; Li et al., 2008). NLRP3 gene mutations have been identified in dominantly inherited autoinflammatory syndromes collectively referred to as Cryopyrin-associated periodic syndromes (CAPS) (Neven et al., 2004; Ting and Davis, 2005; Jha and Ting, 2009) characterized by hyperactivation of the inflammasome complex and increased IL-1β (Neven et al., 2004; Goldbach-Mansky et al., 2006). CAPS symptoms are treatable with the IL-1...
IL-18 (University of Alabama, Birmingham, AL) (Shornick et al., 1996), re-
directed through a graded series of alcohol washes and stained with Luxol
dye (supplemental material), ruffled hair, and altered gait as described
in vivo.

Materials and Methods

**Mice.** Nlrp3−/− mice were provided by Millennium Pharmaceuticals
through Drs. Fayaz Sutterwala and Richard Flavell (Yale University, New
Haven, CT) and were further backcrossed to C57BL/6 mice for a total of
nine generations. Casp1−/− and IL-1β−/− mice were kindly provided by Dr.
Richard Flavell (Yale University, New Haven, CT) and Dr. David Chaplin
(University of Alabama, Birmingham, AL) (Shornick et al., 1996), re-
spectively. C57BL/6 mice [wild type (WT)] were purchased from the
National Cancer Institute (Bethesda, MD) and The Jackson Laboratory.

**Cuprizone treatment.** Eight- to 10-week-old male mice were fed
0.2% cuprizone [oxalic bis(cyclohexyldenediyhydrazide)] (Sigma-Aldrich)
mixed into ground chow ad libitum for 6 weeks to induce progressive
demyelination. Untreated control mice were maintained on a diet of
normal pellet chow. During cuprizone treatment, mice showed lethargic
movement, weight loss (supplemental Fig. 1, available at www.jneurosci.
m.org as supplemental material), ruffled hair, and altered gait as described
in vivo. We found a significant role for the NLRP3 inflammasome pathway in the activation of neuroinflammation. In addition, we also describe a role for IL-18 in demyelination and remyelination.

**Immunohistochemistry.** Immunohistochemistry was performed on 5
μm paraffin embedded sections that were deparaffinized and rehydrated
through alcohol as described previously (Arnett et al., 2002). For the
detection of mature oligodendrocytes, the sections were processed by
boiling in antigen unmasking solution (1:100; Vector) for 13 min in a
microwave. These sections were permeabilized with 0.1% Triton/PBS
for 20 min and incubated with 2% normal goat serum (NGS) in 0.1%
Triton/PBS for 20 min at RT. Subsequently, the sections were incu-
bated with rat anti-mouse polyclonal antibody, anti-γ-glutathione
S-transferase π subunit (GSTπ) (1:500; Assay Designs), at 4°C overnight.
Sections were then washed in PBS and incubated with the appropriate
biotinylated antibody against primary antibody (1:100; Vector) and
Texas Red-conjugated avidin (1:500; Vector). To detect astrocytes, sec-
tions were incubated with 5% NGS in 0.1% Triton/PBS for 20 min at RT.
Subsequently, the sections were washed and incubated with rabbit anti-
cow monoclonal antibody to glial fibrillary acidic protein (GFAP) (1:100;
Dako) and goat anti-rabbit fluorescein-conjugated secondary antibody
(1:100; Vector). To detect microglia, sections were processed by boiling
in antigen unmasking solution (1:100; Vector) for 13 min in a micro-
wave. These sections were permeabilized with 0.1% Triton/PBS for 10
min at RT. Subsequently, the sections were washed and incubated with
goat anti-mouse polyclonal antibody to ionized calcium binding adaptor
molecule-1 (Iba-1) (1:100; Abcam) and rabbit anti-goat biotin-conju-
gated secondary antibody (1:500; Vector). After PBS washes, the sections
were incubated with Alexa 594-conjugated avidin (1:500; In-
vitrogen) for 1 h at RT. For the detection of 2’,3’-cyclic nucleotide phos-
phodiesterase (CNPase), the sections were processed by boiling in
antigen unmasking solution (1:100; Vector) for 13 min in a microwave.
These sections were permeabilized with 0.1% Triton/PBS for 20 min and
incubated with 5% NGS in 0.1% Triton/PBS for 20 min at RT. Subse-
quently, the sections were incubated with chicken polyclonal antibody
to CNPase (1:500; Millipore Bioscience Research Reagents) at RT for 1 h.
Sections were then washed in PBS and incubated with rabbit polyclonal
to chicken IgY biotin (1:500; Abcam) for 1 h at RT. After PBS washes,
the sections were incubated with Alexa 594-conjugated avidin (1:500;
In-vitrogen) for 1 h at RT. Immunopositive cells with an observable
DAPI-stained nucleus were counted blindly twice. Cell counts are averages of at
least 9 and up to 14 mice per time point.

**Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick
end labeling.** To identify apoptotic cells in the brain, the terminal deox-
ynucleotidyl transferase-mediated biotinylated UTP nick end labeling
(TUNEL) assay was used. Briefly, 5 μm paraffin embedded sections were
rehydrated and permeabilized as described previously. TUNEL was per-
formed using the in situ cell death detection kit (Roche). For the colocal-
ization of TUNEL with GSTπ+ or RCA+ cells, sections were stained for
GSTπ or RCA before TUNEL staining.

**Imaging.** All cell counts were taken from the midline of the corpus
callosum, confined to an area of 0.033 mm² taken with a 50× oil-
immersion objective. An Olympus BX-40 microscope with camera
(Optronics Engineering) and Scion Image acquisition software was used for
taking images.

**Reverse transcription-PCR and quantitative real-time reverse transcription-
PCR.** Total RNA was isolated from a dissected region of the brain
containing the corpus callosum of wild-type and Nlrp3−/− mice at several
points during and after cuprizone treatment. RNA isolation was performed using Trizol reagent (Invitrogen) under RNase-free
conditions.

**Protein analysis.** Total protein was extracted from the forebrains of
cuprizone-treated and untreated control C57BL/6 mice. Briefly, corpus
callosi were homogenized on ice in 600 μL of radioimmunoprecipitation
assay buffer containing protease inhibitors. The homogenate was centri-
Results

Nlrp3 expression is increased in the cuprizone model of demyelination

Nlrp3 transcript expression in the CNS of cuprizone-treated C57BL/6 mice was examined by real-time quantitative PCR amplification. Nlrp3 expression was found to increase progressively to >120-fold by the 4 week time point of a course of cuprizone-induced demyelination (Fig. 1a). This increase coincided with the peak of inflammatory cell infiltration, demyelination, and mature oligodendrocyte death. Protein lysates from corpus callosi of cuprizone-treated Nlrp3−/− and control mouse brains analyzed by IL-1β ELISA showed a similar increase in IL-1β protein as Nlrp3 mRNA, supporting a functional increase in NLRP3 activity (Fig. 1b). This corroborates results from a previous study that quantified the number of IL-1β+ cells during cuprizone-induced demyelination and remyelination (Mason et al., 2001). Baseline cytokine levels were similar in Nlrp3−/− and control mouse brains as measured by IL-1β and TNF-α ELISA (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). The lack of a difference in IL-1β levels between Nlrp3−/− and control mice is not surprising as this has been reported previously by others (Mayer-Barber et al., 2010). Many proteases can cleave pro-IL-1β, such as by Fas ligand (Miwa et al., 1998), human mast cell chymase (Omoto et al., 2006), trypsin and plasmin (Coeshott et al., 2006), trypsin and plasmin (Matsusawa et al., 1998), human mast cell chymase (Omoto et al., 2006), trypsin and plasmin (Matsusawa et al., 1998), human mast cell chymase (Omoto et al., 2006), and matrix metalloproteinases (Schönbeck et al., 1998). However, the use of IL-1β−/− mice indicates that this cytokine is not a determining factor in the demyelination phase of the cuprizone model, which supports a previous publication (Mason et al., 2001). A lack of a difference in TNF-α is typical of NLRP3-deficient mice (Mariathasan et al., 2006; Sutterwala et al., 2006a).
Recruitment of microglia and astrocytes is delayed in Nlrp3<sup>−/−</sup> mice

To explore whether NLRP3 has a role during cuprizone-induced inflammation, we used mice lacking the Nlrp3 gene. WT and Nlrp3<sup>−/−</sup> mice showed similar reduction in weight during the course of cuprizone treatment (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). We first examined whether deletion of this gene in mice had an effect on microglial accumulation and astrogliosis (Fig. 1c–f). Microglia are resident immune cells of the CNS (Hаниsh and Ketenmann, 2007). Activated microglia can phagocytose cellular debris, present antigens to T-cells, and release cytokines and chemokines (Hаниsh and Ketenmann, 2007; Napoli and Neumann, 2009). Activated microglia and astrocytes perform several overlapping roles during neuroinflammation (Dong and Benveniste, 2001). Microglial populations at the corpus callosum were identified by RCA-1 lectin (Fig. 1c) and Iba-1 staining (Fig. 1g). Astroglial populations at the corpus callosum were identified by GFAP staining (Fig. 1d). Untreated, age-matched (0 week) Nlrp3<sup>−/−</sup> mice and C57BL/6 (WT) controls showed no difference in the quantitation of microglia and astrocytes at the corpus callosum (Fig. 1c–h). A histological representation of these data is shown in Figure 1, c, d, and g. Quantitation is shown in Figure 1, e, f, and h. At 3, 3.5, and 4 weeks of cuprizone treatment, there was a progressive and significant (p = 0.02 at 3 weeks, p = 0.05 at 3.5 weeks, p = 0.01 at 4 weeks) reduction in microglial infiltration in Nlrp3<sup>−/−</sup> mice relative to WT controls as measured by RCA staining (Fig. 1e). Similarly, there was a progressive and statistically significant reduction in astrogliosis in the Nlrp3<sup>−/−</sup> mice at weeks 3 and 4 of cuprizone treatment (Fig. 1f) (p = 0.03 at 3 weeks, p = 0.001 at 4 weeks). These results indicate that NLRP3 enhances microglia accumulation and astrogliosis in the affected tissues. After 5 weeks of cuprizone treatment, microglial accumulation and astrogliosis between Nlrp3<sup>−/−</sup> and C57BL/6 control animals were similar (Fig. 1e,f). This is consistent with other studies of the cuprizone model wherein the removal of inflammatory genes has not affected neuropathology after this time point (Arnett et al., 2001; Mason et al., 2001). As an additional assessment of microglial cells, Iba-1 staining at 3.5 weeks of cuprizone treatment showed a similar reduction in microglial infiltration as RCA in Nlrp3<sup>−/−</sup> mice relative to WT controls (Fig. 1h) (p = 0.04 at 3.5 weeks). In addition to the midline corpus callosum, microglial infiltration and astrogliosis were also studied in two separate fields lateral to the midline corpus callosum as outlined by gray boxes in the schematic in Figure 2a. Both microglial infiltration and astrogliosis were significantly reduced in Nlrp3<sup>−/−</sup> mice compared with C57BL/6 controls, similar to the reduction observed at the midline corpus callosum (Fig. 2b–i).

Demyelination is delayed in cuprizone-treated Nlrp3<sup>−/−</sup> mice

A second component of the cuprizone model that is relevant to human diseases is the demyelination process. To assess whether NLRP3 plays a role in demyelination and in the loss of mature oligodendrocytes, Nlrp3<sup>−/−</sup> mice along with age-matched control mice were treated with cuprizone for 3, 3.5, 4, and 5 weeks. Representative scoring of the extent of demyelination as measured by LFB-PAS staining is shown in Figure 3a. Slides were...
The above results indicate the importance of NLRP3 in exacerbating demyelination disease severity and demyelination is found in another disease model, we used EAE, a model of T-cell-mediated demyelination disease severity and demyelination is found in an- weeks). To assess whether the role of NLRP3 in exacerbating demyelination in Nlrp3 mice showed delayed loss of myelin in Nlrp3/mice (open circles) show delayed demyelination compared with WT controls (filled circles). Demyelination was quantitated by LFB-PAS staining. Each circle represents the averaged observed LFB score from three readers for one mouse. The mean value of each data set is depicted by a red line (p = 0.003 at 3 weeks, p = 0.012 at 3.5 weeks, p = 0.025 at 4 weeks). c. Clinical scores for EAE showed significantly reduced disease in Nlrp3 mice relative to WT controls. d. CNPase staining of paraffin-embedded spinal cord sections from Nlrp3 mice and WT control mice during EAE showed delayed loss of myelin in Nlrp3 mice. *p < 0.05, **p < 0.005. Error bars indicate SEM.

Figure 3. NLRP3 exacerbates demyelination in the corpus callosum during cuprizone-induced demyelination and experimental autoimmune encephalomyelitis. a. The schematic depicts the scale for scoring of demyelination. Each slide was scored by three blinded readers on a score of 0 (no demyelination) to 3 (complete demyelination). All scores are restricted to the midline corpus callosum (boxed area). b. Nlrp3/mice showed delayed demyelination compared with WT controls (filled circles). Demyelination was quantitated by LFB-PAS staining. Each circle represents the averaged observed LFB score from three readers for one mouse. The mean value of each data set is depicted by a red line (p = 0.003 at 3 weeks, p = 0.012 at 3.5 weeks, p = 0.025 at 4 weeks). c. Clinical scores for EAE showed significantly reduced disease in Nlrp3 mice relative to WT controls. d. CNPase staining of paraffin-embedded spinal cord sections from Nlrp3 and WT control mice during EAE showed delayed loss of myelin in Nlrp3 mice. *p < 0.05, **p < 0.005. Error bars indicate SEM.

LFB is a screening assay for myelin that requires additional analysis of oligodendrocytes and is used to assess differences in cell death after 3 weeks of cuprizone-induced demyelination. Nlrp3 mice showed relatively less apoptotic cells compared with C57BL/6 controls at the midline corpus callosum (Fig. 4c, inset). These apoptotic cells were identified as mature oligodendrocytes by colocalization with the oligodendrocyte marker GST (Fig. 4c). Double staining for apoptotic TUNEL+ cells with the microglial marker RCA showed that microglia were not undergoing apoptosis (Fig. 4d) as described previously (Arnett et al., 2001). As an additional analysis of oligodendrocytes and myelination, a CNPase stain was performed and showed similar results as the LFB and GST (Fig. 4e).

Demyelination, mature oligodendrocyte death, astrogliosis, and microglial infiltration during cuprizone-induced demyelination are independent of IL-1β

The above results indicate the importance of NLRP3 in pathology associated with the cuprizone model; hence we examined IL-1β in the cuprizone model; hence we examined the role of an inflammasome end product (i.e., IL-1β) in this model. A previous study of IL-1β−/− mice showed delayed re-myelination but no difference in demyelination in the cuprizone model, although this latter issue was only peripherally addressed in that report (Mason et al., 2001). To elaborate on these results, we performed a more extensive analysis of cuprizone-induced demyelination in IL-1β−/−mice. IL-1β−/−mice showed no difference in demyelination (Fig. 5a) (p = 0.07 at 3 weeks, p = 0.55 at 4 weeks), loss of GST mature oligodendrocyte (Fig. 5b) (p = 0.29 at 3 weeks, p = 0.16 at 4 weeks), accumulation of microglia (Fig. 5c) (p = 0.56 at 3 weeks, p = 0.15 at 4 weeks) or astrogliosis (Fig. 5d) (p = 0.80 at 3 weeks, p = 0.26 at 4 weeks) during demyelination. This indicates that all of the measured neuropathology observed in the cuprizone model is IL-1β independent.

Cuprizone-induced microglial accumulation, astrogliosis, and demyelination are partially dependent on caspase-1

NLRP3 is required for the processing of caspase-1, which in turn cleaves pro-IL-1β and IL-18 to their mature active forms (Sutterwala et al., 2006b). To establish whether NLRP3-dependent CNS inflammation and demyelination are caspase-1 dependent, we studied casp1−/−mice. Casp1−/−mice showed a statistically significant difference when compared with WT controls, in the extent of demyelination as measured by LFB (Fig. 6a) (p = 0.02 at 3 weeks, p = 0.07 at 4 weeks) and in the number of mature oligodendrocyte as detected by GST immunostaining (p = 0.001 at 3 weeks, p = 0.45 at 4 weeks, and p = 0.39 at 5 weeks) after cuprizone treatment (Fig. 6b). Casp1−/− mice also showed...
showed that by the absence of this suggests that remyelination does not appear to be influenced described previously. and demyelination were studied as de- tor molecule for caspase-1-induced de-

IL-18 is one of the several caspase-1 cleav-

demyelination are IL-18 dependent

Remyelination in the cuprizone model

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Demyelination, mature oligodendrocyte death, astrogliosis, and microglial infiltration during cuprizone-induced demyelination are IL-18 dependent

IL-18 is one of the several caspase-1 cleavage substrates and since demyelination is IL-1β independent, we assessed its role as a candidate effector. To identify an effector molecule for caspase-1-induced demyelination in the cuprizone model, we used IL-18-deficient (IL-18−/−) mice. Microglial infiltration, astrogliosis, mature oligodendrocyte (ODG) depletion, and demyelination were studied as described previously. IL-18−/− and WT controls showed delayed demyelination (Fig. 7a) (p = 0.04 at 3 weeks) and mature oligodendrocyte depletion after 3 weeks of cuprizone treatment (Fig. 7b) (p = 0.00023 at 3 weeks). IL-18−/− mice showed significantly reduced microglial infiltration (Fig. 7c) (p = 0.019 at 3 weeks) and astrogliosis (Fig. 7d) (p = 0.004 at 3 weeks). These results indicate that IL-18 exacerbates demyelination and cellular infiltration in the cuprizone model and can partially attribute to the Nlrp3−/− phenotype.

Remyelination in the cuprizone model is IL-18 dependent

Regulation of oligodendrogenesis by progenitors is a potential therapeutic intervention for the functional loss after demyelination in MS. Previous studies in the cuprizone model have shown that remyelination is dramatically reduced in IL-1β−/− mice (Mason et al., 2001); thus, we investigated whether NLRP3 via its regulation of IL-1β processing may regulate oligodendrogenesis and remyelination. To assess the role of NLRP3 in remyelination, Nlrp3−/− mice along with age-matched C57BL/6 control (WT) mice were treated with cuprizone for 6 weeks and then returned to regular chow to allow for remyelination. Surprisingly, at 8, 10, and 12 weeks of this regimen when remyelination was allowed to occur for 2, 4, and 6 weeks, there was no difference in mature ODG repopulation (Fig. 8b) (p = 0.99 at 8 weeks, p = 0.74 at 10 weeks, and p = 0.56 at 12 weeks) and myelin staining (Fig. 8a) (p = 0.99 at 8 weeks, p = 0.36 at 10 weeks, and p = 0.89 at 12 weeks) of the corpus callosum in Nlrp3−/− mice relative to C57BL/6 controls. This suggests that remyelination does not appear to be influenced by the absence of Nlrp3. However, a more in-depth investigation showed that IL-18−/− mice exhibited a faster remyelination as measured by LFB-PAS staining (Fig. 8c) (p = 0.00015 at 8 weeks, p = 0.014 at 10 weeks) and CNPase staining (Fig. 8d) for myelin. These results reveal a previously unknown detrimental role for IL-18 in remyelination that requires more in-depth analysis that is beyond this report. We hypothesize that this harmful role of IL-18 during remyelination might negate the beneficial role of IL-1β described in the literature. This provides a feasible explanation for why Nlrp3−/− mice that lack both cytokines do not show a difference from wild-type controls.

Discussion

The NLR genes, particularly those associated with the inflamma-
some function, have secured much interest as sensors of PAMPs (pathogen-associated molecular patterns) as well as DAMPs.
IL-1β does not appear to be required in demyelination, astrogliosis, microglial infiltration, and mature oligodendrocyte depletion. a, IL-1β−/− mice exhibit no difference in demyelination. Each circle represents the averaged observed LFB score from three readers for one mouse. Demyelination was quantitated by LFB-PAS staining. IL-1β−/− mice (open circles) show no difference in demyelination compared with WT controls (filled circles) (p = 0.07 at 3 weeks, p = 0.55 at 4 weeks). Mature oligodendrocytes were measured by the GST+ stain at the corpus callosum. b, IL-1β−/− mice (white bars) exhibit no difference in mature oligodendrocyte number compared with age-matched WT controls (black bars) (p = 0.29 at 3 weeks, p = 0.16 at 4 weeks). Mature oligodendrocytes were measured by the GST+ stain at the corpus callosum. c, IL-1β−/− mice (white bars) exhibit no difference in microglial infiltration compared with age-matched WT controls (black bars) (p = 0.56 at 3 weeks, p = 0.15 at 4 weeks). Microglia were measured by RCA staining at the corpus callosum after 3 and 4 weeks of cuprizone treatment. d, IL-1β−/− mice (white bars) exhibit no difference in astrogliosis when compared with age-matched WT controls (black bars) (p = 0.80 at 3 weeks, p = 0.26 at 4 weeks). Astrocytes were measured by the GFAP+ stain at the corpus callosum after 3 and 4 weeks of cuprizone treatment. GFAP was used to detect astrocyte accumulation in the corpus callosum. RCA− or GFAP− cells with an observable DAPI-stained nucleus were counted blindly twice. Cell counts for b–d are averages of between 6 and 10 mice per time point. Error bars indicate SEM.

IL-18 exacerbates demyelination, mature oligodendrocyte depletion, microglial infiltration, and astrogliosis. a–d. Compared with WT controls (filled circles or bars), IL-18−/− mice (open circles or bars) showed reduced demyelination as determined by LFB (p = 0.04 at 3 weeks) (a), delayed mature oligodendrocyte depletion as determined by GST+ cells (p = 0.00023 at 3 weeks) (b), reduced microglial infiltration (RCA+ cells) (p = 0.019 at 3 weeks) (c), and reduced astroglia (GFAP+ cells) at the corpus callosum (p = 0.004 at 3 weeks) (d). *p < 0.05, **p < 0.005, ***p < 0.0005. Error bars indicate SEM. Cell counts for b–d are the averages of six to eight mice per time point.
correlate with disease susceptibility, severity, and progression (Kantarci et al., 2000; de Jong et al., 2002). IL-1β immunoreactivity has been found in activated microglia and macrophages during EAE in rats (Bauer et al., 1993). Treatment with either sIL-1R (soluble IL-1 receptor) or IL-1Ra (IL-1 receptor antagonist) reduces clinical signs of EAE in rats (Jacobs et al., 1991; Badovinac et al., 1998). IL-1β is cytotoxic to mature oligodendrocytes both in vivo and in vitro, while stimulating proliferation of both microglia and astrocytes (Takahashi et al., 2003). However, IL-1β−/− mice also display delayed remyelination, indicating a reparative role of IL-1β (Mason et al., 2001). Our study of IL-1β−/− mice in the cuprizone model showed no differences in demyelination, microglial infiltration, astrogliaosis, or mature oligodendrocyte death after 0, 3, and 4 weeks of cuprizone treatment. This indicates that the demyelination observed in the cuprizone model is IL-1β independent even though IL-1β levels increase concurrent with inflammatory cell infiltration during demyelination (Mason et al., 2001).

Since IL-1β−/− and WT control mice showed no difference in demyelination, we explored the role of caspase-1 in demyelination. Caspase-1 has been implicated in both human and mouse neuroinflammation. Caspase-1 levels are significantly increased in peripheral blood mononuclear cells from MS patients (Huang et al., 2004). Moreover, caspase-1 is known to contribute to the pathology of EAE (Furlan et al., 1999; Ahmed et al., 2002). Our studies with casp1−/− mice showed delayed microglial infiltration, astrogliaosis, a reduction in mature oligodendrocyte depletion, and a decrease in demyelination. This suggests a caspase-1/NLRP3-dependent but IL-1β-independent mechanism that leads to increased demyelination and the loss of mature oligodendrocytes. Several studies have shown that caspase-1 has numerous targets besides IL-1β and IL-18, with upward of 70 substrates (Shao et al., 2007; Keller et al., 2008). It is likely that the activation of these other substrates is responsible for the role of NLRP3 and caspase-1 in demyelination.

An alternate cytokine that might explain our data is IL-18. IL-18 is an 18 kDa member of the IL-1 family of cytokines. IL-18 is produced by several immune and nonimmune cells including monocytes, splenocytes, keratinocytes, microglia, macrophages, and astrocytes (Conti et al., 1999; Prinz and Hanisch, 1999; Sugama et al., 2002). In the CNS, IL-18 induces microglial production of proinflammatory cytokines such as IL-1β and TNFα and MMPs (matrix metalloproteinases). Extravasation of PMNs (polymorphonuclear leukocytes) and monocytes/macrophages is amplified by IL-18-dependent upregulation of ICAM-1 (intercellular adhesion molecule-1) on endothelial cells. IL-18 levels are elevated in demyelinating cerebral lesions of MS patients (Balashov et al., 1999; Losy and Niezgoda, 2001; Nicoletti et al., 2001). Moreover, expression of IL-18 and its receptor on oligodendrocytes is greater in brain tissue from patients with active MS than in patients with silent MS or from neuropathologically normal subjects (Cannella and Raine, 2004). In EAE, a murine model of MS in which the induction of MBP (myelin basic protein)-specific CD4+ T-cells secreting cytokines, particularly IFN-γ and TNF-α, results in limb paralysis, elevated IL-18 mRNA is seen in the CNS of EAE rats at onset and during the disease (Jander and Stoll, 1998; Wildbaum et al., 1998). However, EAE studies with mice deficient in IL-18 (IL-18−/−) have not always been consistent. Whereas Shi et al. (2000) reported that IL-18−/− mice are resistant to EAE, Gutcher et al. (2006) reported that IL-18−/− mice are susceptible to EAE but IL-18 receptor α-deficient mice (IL-18Rα−/−) are resistant to EAE indicating the role of a ligand other than IL-18 acting via IL-18R of 0.56 at 12 weeks). The results show that the neuroinflammatory component of the disease model is mediated by an NLRP3−/−, caspase-1−/−, and IL-18−/− pathway. However, remyelination is NLRP3 independent and IL-18 dependent. Thus, the positive effects of IL-1β and the negative effects of IL-18 on remyelination might negate each other in Nlrp3−/− mice. Such a possibility is not without precedence. For example, another study has found that IL-18 negated the febrile effect associated with IL-1β (Gatti et al., 2002). In summary, the role of IL-18 in exacerbating neuroinflammation along with its inhibitory role in remyelination indicate that inhibition of IL-18 may prove to be a valuable therapeutic approach for demyelinating diseases such as MS. This finding has broad implications for inflammation in other acute
and chronic neuroinflammatory diseases, including stroke and Alzheimer’s disease.

References


