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Inflammation in the CNS and Th17 Responses Are Inhibited by IFN-γ–Induced IL-18 Binding Protein

Jason M. Millward,*†,1 Morten Løbner,† Rachel D. Wheeler,* and Trevor Owens*,†

Inflammatory responses are essential for immune protection but may also cause pathology and must be regulated. Both Th1 and Th17 cells are implicated in the pathogenesis of autoimmune inflammatory diseases, such as multiple sclerosis. We show in this study that IL-18–binding protein (IL-18bp), the endogenous inhibitor of the Th1-promoting cytokine IL-18, is upregulated by IFN-γ in resident microglial cells in the CNS during multiple sclerosis-like disease in mice. Test of function by overexpression of IL-18bp in the CNS using a viral vector led to marked reduction in Th17 responses and robust inhibition of incidence, severity, and histopathology of disease, independently of IFN-γ. The disease-limiting action of IL-18bp included suppression of APC-derived Th17-polarizing cytokines. IL-18bp thus acts as a sensor for IFN-γ and can regulate both Th1 and Th17 responses in the CNS. The Journal of Immunology, 2010, 185: 000–000.

Inflammatory diseases of the CNS, such as multiple sclerosis and its animal model experimental encephalomyelitis (EAE), have been associated with the influx of both Th1 cells producing IFN-γ and Th17 cells producing IL-17. Results from many laboratories point to critical roles for both cytokines (1, 2), although neither is absolutely required for EAE (3, 4), and instead, Ifng−/− mice develop more severe disease (5, 6). Individual T cells in EAE and MS can produce both cytokines (7), and current thinking suggests that the balance between IL-17 and IFN-γ determines the outcome of disease (8, 9). It is therefore necessary to better understand how these cytokines are controlled.

One such control mechanism is IL-18, a member of the IL-1 family that synergizes with IL-12 to induce Th1 polarization and IFN-γ production. IL-18 is upregulated during EAE (10), and microglia have been identified as a cellular source of IL-18 in the CNS (11). Treatment with anti–IL-18 Abs inhibited EAE (12). Although it has been shown that IL-18 itself is not required for Th1 responses or induction of EAE, signaling through the IL-18Rα was necessary for the generation of Th17 cells, and was essential for EAE (13). Whether this signaling is via IL-18 or an alternative ligand, the IL-18 system is important for the regulation of Th17 development and CNS inflammation.

IL-18 is itself regulated by IL-18–binding protein (IL-18bp), an endogenous inhibitor that has sequence homology to the IL-1 type II receptor (14). IFN-γ is the principal inducer of IL-18bp, thus establishing an immunoregulatory feedback loop in which IFN-γ is induced by IL-18, and subsequently limits IL-18 activity via IL-18bp (15, 16). It has been shown that IL-18bp can inhibit IL-18R signaling via an IL-18–independent interaction with IL-18Rβ subunit (17). Several studies have demonstrated that administration of exogenous IL-18bp can have a therapeutic effect in a variety of inflammatory diseases (18–21), and IL-18bp improved recovery in a model of closed head trauma (22), although there is a paucity of studies on the role of IL-18bp in the CNS.

Given this we examined the expression and role of IL-18bp in CNS inflammation. We show that its expression is IFN-γ–dependent, and that it is expressed by resident microglia and infiltrating macrophages in EAE. This suggested to us that IL-18bp could act within the CNS to influence Th1 versus Th17 cytokine regulation, as had previously been reported for IL-23 (23). We therefore targeted an IL-18bp–encoding adenoviral vector specifically to the CNS by intrathecal administration, in mice that were already immunized for EAE. Strikingly, this adenoviral vector encoding IL-18bp (AdIL-18bp) selectively blocked the induction of Th17, but not Th1, in the CNS, and reduced the incidence and severity of EAE. AdIL-18bp reduced expression of Th17 but not Th1-polarizing cytokines, and by targeting the intrathecal compartment, this effect was ideally positioned to influence CNS APCs and affect disease outcome. The disease-limiting effects of AdIL-18bp were robust, occurring in both C57BL/6 and SJL/J mice, independently of IFN-γ. These results show that IL-18bp can influence Th17 as well as Th1 responses, and act as a bridge between them in the regulation of CNS inflammation.

Materials and Methods

Animals and EAE induction

Female C57BL/6 wild-type (WT) mice were purchased from Taconic Farms, Europe (Ry, Denmark), and C57BL6 Ifng−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Ifng−/− SJL/J mice were bred and maintained at the Montreal Neurological Institute as described previously (24). C57BL6 IL-18−/− mice were bred at the University Hospital Zurich.
and RNA extracted with TRIzol. (TreeStar, Ashland, OR). Cell sorting was done on a FACSVantage-DiVa (Becton-Dickinson, CA). Staining was detected using FACSCalibur or LSRII flow cytometers.

Josephine Nalbantoglu (Montreal Neurological Institute, McGill University).

GolgiPlug, Broendby, Denmark). Cells were harvested and incubated with

The qPCR was performed with 1 μl TaqMan PCR Master Mix (Applied Biosystems), using an ABI Prism 7300 Sequence Detection System (Applied Biosystems). Cycle threshold values were converted to arbitrary units using standard curves generated by serial dilutions of cDNA from one sample for each gene analyzed. Data are reported as the ratio of target gene expression over expression of 18 s rRNA, which served as an endogenous reference. cDNA was diluted 1:1000 for 18 s rRNA analysis. Primer and probe sequences for all targets are available online at: www1.sdu.dk/multi/oak/index.php?page=milward-et-sequences

Flow cytometry

Intrathecal injections

To evaluate how IL-18, IL-18bp, and IFN-γ interactions are implicated in CNS inflammation, we measured IL-18bp mRNA in spinal cords of WT, Ifng−/−, and IL-18−/− mice. IL-18bp expression increased dramatically above baseline in WT mice with EAE induced by immunization with MOG35–55. This increase was dependent on IFN-γ, as IL-18bp expression did not increase above baseline in Ifng−/− mice with equivalent severity of EAE. IL-18bp expression in EAE was not dependent on IL-18, as expression in IL-18 knockout mice that also showed severe EAE was equivalent to that of WT mice with EAE (Fig. 1A). To identify the cellular source of IL-18bp in the CNS, populations of cells were sorted by flow cytometry from spinal cords of WT mice with EAE, on the basis of CD45 and CD11b expression (24, 25). The major cellular sources of IL-18bp in the CNS were the CD11b+CD45high (includes macrophages and microglia) populations (Fig. 1B). Both of these populations expressed significantly greater IL-18bp mRNA than CD11b−CD45low (resident microglia) populations (Fig. 1B). Thus, macrophages and microglia produce elevated levels of IL-18bp in the CNS during EAE.

AdIL-18bp reduces incidence and severity of EAE independently of IFN-γ and IL-18

To investigate the role of this elevated IL-18bp in EAE, we cloned the murine IL-18bp isoform C gene into a replication-defective adenoviral vector (AdIL-18bp) (26). Using an IFN-γ-based IL-18bp bioassay we verified that the cells infected with the AdIL-18bp vector produced bioactive IL-18bp, and produced immunoreactive protein of the expected size (27) (Supplemental Fig. 1). We administered the AdIL-18bp vector to WT mice that were immunized with MOG35–55 to induce EAE. AdIL-18bp was administered by intrathecal injection into the cisterna magna, which

Histology

Spinal cords were removed from PBS perfused anesthetized mice and cut into eight cross-sectional segments. The segments were embedded together in Cryo-Embed (Ax-laboratory), frozen in a methylbutane/dry ice mixture, then 12 μm sections were cut on a cryostat. H&E-stained sections were examined at high power for the presence of inflammation. Semi quantitative assessment was done by counting the number of quadrants that contained inflammatory infiltrates for each of the eight segments. Quadruplicate sections for each mouse were assessed (blinded to treatment group) and data were presented as the percentage of total quadrants positive for inflammatory infiltrates.

APC cytokine assay

Spleens were collected from unmanipulated WT mice, homogenized through a 70-μm filter, and depleted of RBCs by incubation in 0.83% NH4Cl for 10 min at room temperature. CD11c+ cells were isolated by magnetic separation using CD11c (N418) MicroBeads (Miltenyi Biotec, Auburn, CA). After separation, the CD11c+ cells were cultured at 2 × 105 cells per well in RPMI 1640 with 10% FBS. Cultures were stimulated with LPS (2 μg/ml, Sigma-Aldrich) and AdIL-18bp or AdLacZ were applied at a multiplicity of infection of 50. After 5 d in culture supernatants were collected, and p40 protein measured using the eBioscience total p40 ELISA kit. The limit of detection for the ELISA was 2 pg/ml.

Statistical analysis

Interval-scale data (e.g., qPCR, flow cytometry) were analyzed with an unpaired t test or one-way ANOVA (two-tailed), with the Tukey post hoc test, as appropriate. Gaussian distributions were verified with the Kolmogorov-Smirnov test. Noninterval data (e.g., EAE clinical scores, histopathology scores) were analyzed with the nonparametric Mann-Whitney U test. Disease incidence was analyzed with Fisher exact test. The p values <0.05 were considered significant. GraphPad Prism 4 (GraphPad Software, San Diego, CA) was used for analysis.

Results

IL-18bp is upregulated in CNS during EAE in WT and IL-18−/− but not Ifng−/− mice

Intrathecal injections

Injections were made in anesthetized mice under sterile conditions, as described (24). A 30-gauge needle (bent at a 45° angle 3 mm from the tip) was attached to a 50 μl Hamilton syringe and inserted into the intrathecal (subarachnoid) space of the cisterna magna (cerebellomedullary cistern). A 10-μl volume of sterile PBS containing the viral vector (107 infectious units) was slowly injected over a period of 10 min at room temperature. Culture medium (CMV promoter, multiple cloning site, IRES, DsRed2, and SV40pA sequences using the AdEasy method, as described (26). In brief, the combined CMV promoter, multiple cloning site, IRES, and DsRed2, and SV40pA sequences were excised from pShuttle-DrRed2 (Clontech Laboratories, Palo Alto, CA), and inserted into pShuttle. A plasmid encoding murine IL-18bp was purchased from Invitrogen (Eston clone [IMAGE] ID 4007711) (Carlsbad, CA). The IL-18bp sequence was released from this plasmid and inserted into the multiple cloning site between the CMV promoter and IRES. The pShuttle/IRES/IL-18bp plasmid was then combined with AdEasy by homologous recombination in BJ5183 cells after electroporation. The resulting AdEasy-IL18bp construct was transfected into the complementing HEK293A cell line, to permit the production of virus particles. Virus was propagated in HEK293A cells, and purified by CsCl centrifugation. pShuttle, pAdEasy, and BJ5183 and HEK293A cells, as well as a control adenoviral vector encoding the β galactosidase gene (AdLacZ) were generously provided by Dr. Josephine Nalbantoglu (Montreal Neurological Institute, McGill University).

Quantitative real-time PCR

RNA was extracted from spinal cord using the TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. The RNA was reverse transcribed, and quantitative PCR (qPCR) carried out as described (25). The qPCR was performed with 1 μl sample cDNA in a 25-μl reaction volume containing 12.5 μl TaqMan PCR Master Mix (Applied Biosystems, Foster City, CA), forward and reverse primers (900 nM), and TaqMan probe (200 nM) (Applied Biosystems), using an ABI Prism 7300 Sequence Detection System (Applied Biosystems). Cycle threshold values were converted to arbitrary units using standard curves generated by serial dilutions of cDNA from one sample for each gene analyzed. Data are reported as the ratio of target gene expression over expression of 18 s rRNA, which served as an endogenous reference. cDNA was diluted 1:1000 for 18 s rRNA analysis. Primer and probe sequences for all targets are available online at: www1.sdu.dk/multi/oak/index.php?page=milward-et-sequences

Flow cytometry

The preparation of spinal cord cell suspensions, and intracellular cytokine staining was done as previously described (25). Cell suspensions were cultured with or without anti-TCR/β for 5 h with 1 μg/ml brefeldin A (BD GolgiPlug, Broendby, Denmark). Cells were then washed, incubated with anti-FcR (24G2) before staining for extracellular markers with anti-CD45 (FITC), anti-CD4 (BD-HorizonV500), and anti-CD11b (PerCP). Cells were permeabilized using the BD Cytofix/Cytoperm kit, according to the manufacturer’s instructions, and then incubated with anti-IL-17 (PE) or anti–IFN-γ (PE). All Abs were purchased from BD Pharmingen (San Diego, CA). Staining was detected using FACSCalibur or LSRII flow cytometers (BD Biosciences, CA), and analyzed with FlowJo version 7.1.3 (TreeStar, Ashland, OR). Cell sorting was done on a FACSVantage-DeVl flow cytometer (BD Biosciences). Sorted cells were collected into HBSS, and RNA extracted with TRIzol.
is a minimally invasive and effective way to deliver viral vectors to the CNS, without inducing peripheral effects (24, 28). Mice that received a single dose of 10^7 infectious units of AdIL-18bp on day 5 postimmunization showed a significantly reduced incidence of EAE, compared with those that received a control adenoviral vector (AdLacZ) (Table I). AdIL-18bp–treated mice also showed a sustained and significant reduction in clinical severity, as indicated by reduced cumulative disease activity [calculated as the area under the curve of clinical score plots for each individual animal (29)] (Table I), as well as reductions in the median clinical score that were statistically significant from day 16 until termination of the experiment at day 28 postimmunization, as compared with mice given the control vector (Fig. 2). Administration of AdIL-18bp to the periphery by i.p. injection had no effect on disease outcomes, even at doses 20-fold higher than those used in the CNS (AdIL-18bp versus AdLacZ, cumulative disease activity, p = 0.959; data not shown).

We then asked whether a similar effect would be seen in Ifng^−/− mice, which develop more severe EAE than WT mice and do not upregulate endogenous IL-18bp during EAE. Just as in WT mice, AdIL-18bp treatment led to significantly reduced disease incidence, and a sustained and significant amelioration of EAE clinical severity, compared with the control AdLacZ treatment (Fig. 2, Table I). A similar disease-limiting effect of AdIL-18bp was also seen in both WT and Ifng^−/− SJL/J mice with proteolipid protein–induced EAE (Fig. 2).

There have been conflicting reports about the susceptibility of IL-18^−/− mice to EAE. Although Shi et al. (30) reported that IL-18^−/− mice were resistant to EAE, Gutcher et al. (13) reported that the same mice were just as susceptible to EAE as the WT. Our results indicate these IL-18^−/− mice are susceptible to EAE. Furthermore the disease-limiting activity of AdIL-18bp treatment was also observed. There was a clear, significant reduction in disease incidence, cumulative disease activity and severity of clinical scores in IL-18^−/− mice receiving AdIL-18bp compared with the control vector (Fig. 2, Table I).

The disease-limiting activity of AdIL-18bp was not due to interference with the generation of a specific T cell response to myelin Ag. T cells from peripheral lymph nodes of MOG-immunized mice given AdIL-18bp or AdLacZ proliferated equivalently in response to antigenic peptide in vitro (Fig. 3A). In addition, expression of the regulatory T cell–associated transcription factor Foxp3 in spinal cord of immunized mice did not differ between AdIL-18bp and AdLacZ–treated mice for each of the three strains examined (Fig. 3B). Expression of the anti-inflammatory cytokine IL-10 was also not affected (Fig. 3C).

**AdIL-18bp treatment inhibits Th17 but not Th1**

Intrathecal administration of AdIL-18bp was associated with a significant reduction in the proportion of IL-17–producing cells, as detected by intracellular cytokine staining of infiltrating CD4^+ cells from spinal cord of immunized mice (Fig. 4A). It was shown by gating on CD4^+CD45^high CD4^+ cells from spinal cord of immunized mice (Fig. 4A). It was shown by gating on CD45^high CD4^+ cells that proportions of Th17 (CD4^+ IL-17^+ cells) were also reduced by this treatment (Supplemental Fig. 2). This finding was corroborated by a significant reduction in message for both IL-17, and the Th17-associated transcription factor retinoid-related orphan receptor-γ (RORγT) in whole spinal cord of WT, Ifng^−/− and IL-18^−/− mice (Fig. 4B). In contrast, the proportion of IFN-γ–producing cells in spinal cord of immunized mice was not decreased by AdIL-18bp treatment, but was in fact significantly greater than in mice receiving the control vector (Fig. 4C). In line with this result is the observation that AdIL-18bp did not lead to a reduction in IFN-γ message, or in message for the Th1–associated transcription factor T box.

### Table I: Effects of AdIL-18bp on EAE

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Incidence</th>
<th>p Value</th>
<th>Cumulative Disease Activity</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>AdIL-18bp</td>
<td>8/19 (42)</td>
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<td>7.75</td>
<td>0.0021</td>
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<td></td>
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<td>AdIL-18bp</td>
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<td>3.25</td>
<td>0.0006</td>
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<tr>
<td></td>
<td>AdLacZ</td>
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<td></td>
<td>31.50</td>
<td></td>
</tr>
<tr>
<td>IL-18^−/−</td>
<td>AdIL-18bp</td>
<td>10/17 (58)</td>
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<td>7.00</td>
<td>0.0037</td>
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<tr>
<td></td>
<td>AdLacZ</td>
<td>16/16 (100)</td>
<td></td>
<td>28.00</td>
<td></td>
</tr>
</tbody>
</table>

*a, n percent. 
*bComparison between AdIL-18bp and AdLacZ; p value from Fisher’s exact test. 
*cMedian value of cumulative disease activity, calculated from area under the curve for each individual sample. 
*dComparison between AdIL-18bp and AdLacZ; p value from Mann-Whitney U test.
expressed in T cells (T-bet) (Fig. 4D). IFN-γ message was significantly increased in IL-18−/− mice given AdIL-18bp, and AdIL-18bp increased T-bet expression in both the WT and IL-18−/− strains. Thus, the relative proportion of Th17 versus Th1 cells in EAE CNS was reduced by AdIL-18bp treatment. This effect was confined exclusively to the CNS, as Th17 cells in lymph nodes from the same animals were not affected by intrathecal AdIL-18bp treatment (data not shown).

AdIL-18bp treatment reduces the extent and distribution of histopathology

In addition to the robust effect on clinical symptoms of EAE and Th17/Th1 ratios, AdIL-18bp treatment also affected CNS histopathology. Analysis of spinal cord sections revealed a significant reduction in inflammatory pathology, defined as the percentage of spinal cord segments that contained inflammatory infiltrates (Fig. 5). This reduction in total pathology was seen in WT, Ifng−/− and IL-18−/− mice treated with AdIL-18bp. The number of CD11b+ (myeloid) cells in spinal cord of mice with EAE was reduced in mice treated with AdIL-18bp, but this did not achieve statistical significance (Supplemental Fig. 3). In addition, AdIL-18bp influenced the parenchymal dissemination of infiltrating cells in WT and Ifng−/− spinal cord. In these strains, AdIL-18bp treatment led to a significant reduction in the percentage of parenchymally diffused inflammatory lesions, and a shift to more constrained lesions, at all levels of the spinal cord (Fig. 5). This difference was not as pronounced in IL-18−/− spinal cords.

AdIL-18bp treatment reduces expression of Th17-polarizing cytokines

We then determined whether levels of Th17-polarizing cytokines were affected by AdIL-18bp. There were significant reductions in the expression of TGF-β, IL-6, IL-23p19, and TNF-α (Fig. 6), cytokines which promote the development of Th17 cells, in spinal cords of AdIL-18bp-treated WT mice. These results were replicable in Ifng−/− mice (Fig. 6). In IL-18−/− mice, TGF-β was reduced by AdIL-18bp treatment, but IL-23p19, IL-6, and TNF-α expression were not significantly affected (Fig. 6). Expression of Th2-associated cytokines (IL-4, IL-10) was not affected by AdIL-18bp treatment (Fig. 3C, and not shown). There was no effect of AdIL-18bp on expression of Th1-polarizing IL-12p35, or of IL-1β (Supplemental Fig. 4). AdIL-18bp was also shown to have a direct effect on the production of a T cell-polarizing cytokine by APCs in vitro. Infection of LPS-stimulated CD11c+ splenic dendritic cells with AdIL-18bp led to a significant reduction in their production of IL-12/IL-23 p40 protein, compared with cultures infected with AdLacZ (Fig. 6B).

Discussion

We show in this study that IL-18bp is upregulated during EAE, and is expressed by CNS resident microglia. This may reflect an immunoregulatory process selected to restrict inflammation in the CNS. Consistent with this interpretation, we observed that IL-18bp was not induced in Ifng−/− mice that develop severe EAE. Furthermore, overexpression of IL-18bp specifically in the CNS led to a robust inhibition of EAE. This was associated with a shift toward reduced Th17 response in the CNS, with no inhibition of Th1 responses, and thus reveals a novel regulatory role for IL-18bp in bridging Th1 and Th17 and directing T cell responses during CNS inflammation.

The observation of IL-18bp expression by CNS-resident glial cells raises the possibility of a role for this mediator in normal CNS physiology. The experimental approach of overexpression of this endogenous protein has allowed us to investigate its role in CNS inflammation. Examination of other roles, such as in CNS homeostasis or development, remain to be addressed and would require gene targeting among other approaches.

Our data suggest that AdIL-18bp treatment led to a shift in the balance between Th17 and Th1. The impact of such a shift would not be affected by whether it reflected a change in the phenotype of individual cells or of different T cell populations. We showed that both total IL-17−producing CD45high cells as well as CD4+ Th17 cells specifically were reduced in CNS by AdIL-18bp. AdIL-18bp treatment led to a reduction in IL-17 protein and message, and reduced expression of the Th17-associated transcription factor RORγT. However, IFN-γ and the Th1-associated transcription factor T-bet were not reduced by AdIL-18bp, and were...
of regional redistribution, we find that the most prominent histological correlate of the Th1-Th17 shift was a more spatially confined perivascular infiltration.

Differentiation and maintenance of Th17 cells requires specific cytokines (31–34). We examined TGF-β, IL-6, IL-23, and TNF-α. AdIL-18bp treatment was associated with reduced expression of these Th17-polarizing cytokines in the CNS of Ifng−/− and WT mice. It is noteworthy that the effects of IL-18bp were seen in the CNS and that we did not detect effects on peripheral Th17. Although Th1 and Th17 differentiation occurs in peripheral lymphoid tissue, the infiltrating Th1 or Th17 cells are subject to subsequent redirection in the CNS. Cua et al. (23) used intracerebroventricularly administered adenoviral vectors encoding IL-23 p19 to redirect T cell responses in the CNS of mice and restore EAE susceptibility. We tested whether IL-18bp could affect cytokine redirection by using the intrathecal route of administration. This approach permits the infection of meningeal and ependymal cells, that in turn secrete the exogenous gene product into the cerebrospinal fluid (CSF) (24, 35). It is known that this has minimal peripheral effects (24, 35), which we confirmed. Thus, IL-18bp in the CSF is in the right location to affect perivascular APC in the CNS, such as dendritic cells, which can subsequently direct the polarization of infiltrating T cells and influence disease outcomes (36, 37). The observation that AdIL-18bp could have a direct effect on the production by APCs of the T cell polarizing cytokine IL-12/23 p40 in vitro is consistent with such a mechanism.

The dramatic reduction in incidence and severity of EAE in Ifng−/− mice given AdIL-18bp aligns with the notion that lack of IL-18bp-mediated regulation may contribute to the more severe EAE phenotype of the Ifng−/−. In addition, the reduction in IL-17 and Th17-polarizing cytokines implies that AdIL-18bp was acting via a similar mechanism in the Ifng−/− as in the WT. Clearly IL-18bp, although dependent on IFN-γ for its upregulation, can mediate its effects independently of IFN-γ. The fact that AdIL-18bp treatment did not suppress Th1 responses in vivo reflects redundancy in the process of Th1 promotion, consistent with the fact that Th1 responses can occur in IL-18−/− mice (13).

Interestingly, the induction of Th17 responses has also been shown to be independent of IL-18 (13), and we show that inhibition of Th17 responses by AdIL-18bp was also independent of IL-18. This is intriguing because IL-18 is also suggested to play a role in Th17 induction. Th17 cells polarized by TGF-β and IL-6 without IL-23− produced IL-17 when subsequently stimulated by both IL-23 and IL-18, but not by IL-23 alone (38–40), suggesting that IL-18 can act to enhance IL-17 production in already committed Th17 cells in synergy with IL-23, analogous to the IL-18 synergy with IL-12 in the promotion of Th1 cells and IFN-γ (41). The IL-18 system has recently come under scrutiny on account of discrepancies in the phenotype between cytokine and receptor knockout mice. Results from Gutcher et al. (13) indicate the existence of a ligand other than IL-18 for the IL-18Rα. They reported IL-18−/− mice are susceptible to EAE, whereas IL-18Rα−/− mice were resistant, and when IL-18−/− mice with EAE were treated with anti–IL-18Rα Abs, EAE was inhibited. In addition, in the human system it is known that IL-18bp binds to IL-1F7 (41), and although there is no mouse homolog for this ligand, it suggests the possibility of alternative interaction partners for IL-18bp. Thus neither induction of IL-18bp nor its Th17 immunoregulatory effects are dependent on IL-18.

In the IL-18−/− in which AdIL-18bp treatment ameliorated EAE and reduced Th17 response in CNS, TGF-β expression was also reduced. The fact that levels of IL-23, IL-6, and TNF-α were unaffected suggests alternative routes toward promotion of Th17 cells in the IL-18−/− (42, 43). AdIL-18bp treatment reduced the...
overall extent of histopathology in spinal cord of WT, Ifng<sup>-/-</sup>, and IL-18<sup>-/-</sup> strains. Although the proportion of diffuse inflammatory lesions was reduced in the WT and Ifng<sup>-/-</sup> spinal cords, this change did not reach statistical significance in the IL-18<sup>-/-</sup>. Thus, although it seems that IL-18bp suppresses EAE via reduction in Th17 cells in the CNS, this reduction can be achieved via a mechanism independent of IL-23p19 or IL-6 expression by CNS APC in the special case of IL-18 deficiency.

The disease-limiting action of AdIL-18bp was a robust finding that was reproduced in EAE induced by two different encephalitogens in two strains of mice, and was observed in two separate animal facilities. IL-18bp has beneficial effects in a variety of model
system of inflammatory disease, including contact hypersensitivity (20), diabetes (21), and models of inflammation-induced liver disease (18, 19). In the CNS, IL-18bp was shown to have a beneficial effect on recovery from closed head trauma (22), and MBP-specific Th2 cell lines engineered to express IL-18bp had a beneficial effect on EAE (44).

There is a precedent in nature for a role for exogenous IL-18bp in limiting immune responses. Several human poxviruses, including

![FIGURE 5. AdIL-18bp treatment affects the magnitude and distribution of CNS histopathology. Representative H&E stained spinal cord sections show AdIL-18bp treatment reduced inflammatory histopathology in WT, Ifng<sup>−/−</sup>, and IL-18<sup>−/−</sup> mice, compared with mice from each strain that received the control AdLacZ vector. There was a significant reduction in the extent of histopathology, measured as the median percentage of quadrants of spinal cord segments with inflammatory infiltrates. Of those segments showing inflammation, the percentage that included diffuse (arrow, top left) as opposed to exclusively constrained lesions (arrowhead) was lower in mice treated with AdIL-18bp compared with AdLacZ-treated mice. This was statistically significant in the WT and Ifng<sup>−/−</sup> strains (analyzed by the Mann-Whitney U test). Scale bar, 500 μm (original magnification ×100).](http://www.jimmunol.org/)

![FIGURE 6. AdIL-18bp treatment reduced expression of Th17-polarizing cytokines.](http://www.jimmunol.org/)
vaccinia virus, encode proteins that have sequence similarity to human IL-18bp, and show IL-18bp activity, including blunting of IFN-γ induction (45, 46). It is understood that production of these IL-18–binding proteins is part of the viral immune evasion strategy, to restrict the actions of IL-18, particularly the induction of IFN-γ, which suggests the possibility that IL-18bp might be used as a therapeutic agent. In addition to viral IL-18bp, however, poxviruses also encode an inhibitor of caspase-1, which is required for cleavage of pro–IL-18 and pro–IL-1β to the active forms of these cytokines (47). The vaccinia virus protein C12L has homology to IL-18bp and inhibits IFN-γ production, although vaccinia virus produces numerous additional immunomodulators, including proteins that bind to IFN-γ, IFN-α/β, complement factors, and TNF-α [summarized in (46)]. Thus, the IL-18 inhibition during infection with vaccinia virus or poxviruses is merely one component of a multifactorial immune evasion strategy. In the current study, we introduced exclusively IL-18bp, and this was not in the context of an active viral infection. Consequently, differences between the effects seen in our study and effects during viral infections are to be expected.

In this study, we have identified a novel capacity for IL-18bp to influence the pathogenesis of CNS inflammation by inhibiting Th17 responses. This complements its role in the Th1 system, and positions IL-18bp to affect the balance of Th1 and Th17 responses, and thereby influence the outcome of CNS inflammation and disease.

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Disclosures
The authors have no financial conflicts of interest.

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9. Lees, J. R., P. T. Golombek, J. Sim, D. Dorsey, and J. H. Russell. 2008. Regional differences in IFN-γ production, although vaccinia virus produces numerous additional immunomodulators, including proteins that bind to IFN-γ, IFN-α/β, complement factors, and TNF-α [summarized in (46)]. Thus, the IL-18 inhibition during infection with vaccinia virus or poxviruses is merely one component of a multifactorial immune evasion strategy. In the current study, we introduced exclusively IL-18bp, and this was not in the context of an active viral infection. Consequently, differences between the effects seen in our study and effects during viral infections are to be expected.

In this study, we have identified a novel capacity for IL-18bp to influence the pathogenesis of CNS inflammation by inhibiting Th17 responses. This complements its role in the Th1 system, and positions IL-18bp to affect the balance of Th1 and Th17 responses, and thereby influence the outcome of CNS inflammation and disease.

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Disclosures
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References


