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Independent and Interdependent Immunoregulatory Effects of IL-27, IFN-β, and IL-10 in the Suppression of Human Th17 Cells and Murine Experimental Autoimmune Encephalomyelitis

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IFN-β, IL-27, and IL-10 have been shown to exert a range of similar immunoregulatory effects in murine and human experimental systems, particularly in Th1- and Th17-mediated models of autoimmune inflammatory disease. In this study we sought to translate some of our previous findings in murine systems to human in vitro models and delineate the interdependence of these different cytokines in their immunoregulatory effects. We demonstrate that human IL-27 upregulates IL-10 in T cell-activated PBMC cultures and that IFN-β drives IL-27 production in activated monocytes. IFN-β–driven IL-27 is responsible for the upregulation of IL-10, but not IL-17 suppression, by IFN-β in human PBMCs. Surprisingly, IL-10 is not required for the suppression of IL-17 by either IL-27 or IFN-β in this model or in de novo differentiating Th17 cells, nor is IL-27 signaling required for the suppression of experimental autoimmune encephalomyelitis (EAE) by IFN-β in vivo. Furthermore, and even more surprisingly, IL-10 is not required for the suppression of Th17-biased EAE by IL-27, in sharp contrast to Th1-biased EAE. In conclusion, IFN-β and IL-27 both induce human IL-10, both suppress human Th17 responses, and both suppress murine EAE. However, IL-27 signaling is not required for the therapeutic effect of IFN-β in EAE. Suppression of Th17-biased EAE by IL-27 is IL-10–independent, in contrast to its mechanism of action in Th1-biased EAE. Taken together, these findings delineate a complex set of interdependent and independent immunoregulatory mechanisms of IFN-β, IL-27, and IL-10 in human experimental models and in murine Th1- and Th17-driven autoimmunity. The Journal of Immunology, 2013, 190: 3225–3234.

Interleukin-27 is a heterodimeric cytokine with pleiotropic functions, particularly in immunity. Originally regarded as a Th1-inducing cytokine, it is now recognized that IL-27 exerts potent anti-inflammatory effects in both infection and autoimmunity (1). IL-27 consists of a p28 subunit and an EBV-induced gene 3 subunit (2), the latter of which is shared with IL-35 (3, 4). Although produced primarily by activated APCs, a range of cell types has been shown to express IL-27, including astrocytes and microglia (5–9). IL-27 signals via the IL-27 receptor, which is composed of a WSX-1 subunit (also known as TCCR and IL-27R) and the gp130 subunit (IL-6R), which is shared by other cytokine receptors (10). The IL-27R is expressed on numerous cell types, including T cells, NK cells, B cells, monocytes, mast cells, dendritic cells (DCs), and endothelial cells (11). IL-27 activates multiple JAK/STAT signaling pathways depending on the cell type it targets, resulting in a wide range of cellular responses observed following IL-27R ligation. Some of the known anti-inflammatory effects of IL-27 include suppression of IL-2 (10), suppression of Th17 differentiation (9, 12), induction of SOCS3 (13), and up-regulation of IL-10 (14–17). Interestingly, IFN-β is also a key inducer of IL-10 and a suppressor of Th17 differentiation (18, 19).

IFN-β is a monomeric type I IFN with both antiviral and potent anti-inflammatory effects. IFN-β signaling is mediated through the common type I IFN receptor and proceeds through the classical JAK/STAT signaling pathway (20). Despite the fact that IFN-β was the first immunomodulatory therapy approved by the U.S. Food and Drug Administration for multiple sclerosis (MS) and is a first line of treatment for MS, its exact mechanism of action has yet to be elucidated. Furthermore, many patients do not respond to IFN-β or cease to show a clinical response after some time (21, 22). The major effects of IFN-β include inhibition of T cell proliferation and IFN-γ production, inhibition of MHC class II expression, inhibition of matrix metalloproteinase production and cell-associated adhesion molecule expression, induction of anti-inflammatory cytokines, inhibition of proinflammatory cytokines, induction of CD8 regulatory cell function, and inhibition of monoocyte activation (reviewed in Ref. 2). Of note, upregulation of IL-10 is positively predictive of a clinical response to IFN-β therapy in MS (23).

Since the initial characterization in 2005, Th17 cells have been implicated in a range of inflammatory diseases in several species and have become an attractive target for therapeutic intervention (24). Although the understanding of murine Th17 cell develop-
ment, regulation, and function has evolved at a significant pace, there is an understandable lag in the translation of such findings to human models. To target Th17 cells in human disease therapeutically, such translational studies are essential. Inhibition of Th17 cells by IL-27 and IFN-β was shown in a range of murine models both in vitro and in vivo (13, 25–27). However, the range of mechanisms of human Th17 cell inhibition by IL-27 and IFN-β are less well understood. In human experimental models studies have shown that IFN-β can directly inhibit Th17 responses in CD4+ T cells (28). Furthermore, IFN-β can impair the ability of DCs and B cells to promote Th17 differentiation by inhibiting IL-23p19 and IL-1β expression while promoting IL-12p35 and IL-27p28 expression by these cell populations (18, 29, 30).

In this study we examine a complex network of independent and interdependent effects of IL-27, IFN-β, and IL-10 on Th17 cells and CNS autoimmune inflammation. We were surprised to find that despite suggestive in vitro findings to the contrary, IL-27R signaling is not required for experimental autoimmune encephalomyelitis (EAE) suppression by IFN-β. Furthermore, we identified that IL-27 suppresses Th17-biased EAE in an IL-10–independent manner, which is in contrast to its mechanism of suppression in Th1-biased EAE that we reported previously (16). Taken together, our findings show that IL-27 orchestrates multiple suppressive pathways in EAE dependent on the phenotype of disease pathogenicity and is not required for the suppressive effect of IFN-β in actively induced EAE.

Materials and Methods
Cell preparation and treatment
Whole venous blood was collected in EDTA-treated tubes from healthy donors with informed consent. PBMCs were isolated using Ficoll-Paque Plus density centrifugation; cord blood cells were obtained from All-Cells. CD4+ and CD14+ cells were purified by immunomagnetic separation using CD4+CD14+ microbeads and subsequent positive selection using a QuadroMACS separator. All human cells with the exception of monocytes were stimulated with anti-biotin MACS/Bead particles loaded with CD2, CD3, and CD28 Abs (T cell activation/expansion kit, Miltenyi Biotec) at a ratio of one bead particle for every two cells. Monocytes were stimulated with either LPS (20 μg/ml) or peptidoglycan (PGN; 1 μg/ml). Cells were cultured at a density of 5 × 10^5 cells/ml for PBMCs and CD4+ cells and 5 × 10^6 cells/ml for monocytes in 96-well U-bottom plates in serum-free X-VIVO 15 medium. Unless otherwise stated, PBMCs, cord blood cells, and CD4+ cells were incubated for 5 d whereas CD14+ monocytes were incubated for 48 h. To induce de novo Th17 differentiation, combinations of cytokines were used as described in the figure legends.

Media/reagents
Cells were prepared and cultured in X-VIVO 15 medium unless otherwise stated. LPS (Escherichia coli) and peptidoglycan (Staphylococcus aureus) were obtained from Sigma-Aldrich. Human IFN-β1a was obtained from EMD Serono. Recombinant human IL-21 and IL-23 were obtained from BioBioscience; recombinant human IL-27 and IL-1β were obtained from R&D Systems. TGF-β was obtained from PeproTech. Neutralizing anti-IL-27 and anti-IL-10 Abs were also obtained from R&D Systems.

Cytokine quantification
Supernatants were harvested and stored at −20°C at the termination of the indicated incubation periods. Cytokine levels were measured by ELISA according to the manufacturer’s instructions using DuoSet development kits (R&D Systems).

Flow cytometry
Cells were washed in staining buffer containing 1% FCS and 0.1% sodium azide in PBS and stained with appropriate Abs (PerCP-Cy5.5-conjugated anti-human IL-17A [BioBioscience]; allophycocyanin-Cy7 mouse anti-human CD4, allophycocyanin mouse anti-human IFN-γ, isotype controls [BD Pharmingen]). Stained cells were fixed and permeabilized with Caltag Fix/Perm reagents (Invitrogen). Data from labeled cells were acquired with a BD FACSaria flow cytometer and analyzed using FlowJo software (TreeStar).

Mice and EAE induction
Eight- to 12-wk-old wild-type (WT) female C57BL/6 mice were obtained from The Jackson Laboratory. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University. Actively induced EAE. Mice were immunized s.c. with 150 μg myelin oligodendrocyte glycoprotein (MOG) in CFA containing 5 mg/ml Mycobacterium tuberculosis (Difco Laboratories) at two sites on the back. Eleven days after immunization, mice were harvested from lymph nodes and spleens and cultured for 72 h in the presence of IL-23 (10 ng/ml). Purified CD4+ cells were injected i.v. into recipient mice (5 × 10^5/mouse) and mice were injected with 200 ng pertussis toxin in PBS on days 0 and 2 and were scored daily using an EAE clinical scale as detailed below.

Adoptively transferred EAE. Donor mice were immunized s.c. with 150 μg MOG in CFA containing 5 mg/ml M. tuberculosis (Difco Laboratories) at four sites on the back. Eleven days after immunization, mice were harvested from lymph nodes and spleens and cultured for 72 h in the presence of IL-23 (10 ng/ml). Purified CD4+ cells were injected i.v. into recipient mice (5 × 10^5/mouse) and mice were injected with 200 ng pertussis toxin in PBS on days 0 and 2 after transfer. Mice were scored daily using an EAE clinical scale as detailed below.

Scoring. Mice were scored daily according to the following scale: 0, no sign of clinical disease; 1, paresis of the tail; 2, paresis of one hind limb; 3, paresis of both hind limbs; 4, paraparesis of the abdomen; 5, moribund/death.

Statistical analysis
For EAE studies, the area under the curve was calculated for each mouse, and values of experimental groups were compared for statistical significance using Instat software. Data were considered statistically significant where p < 0.05 in a two-tailed, unpaired, Student t test with Welch’s correction where appropriate.

Results
IL-27 upregulates IL-10 production in human T cells
We and others have shown in murine models that IL-27 drives the production of IL-10 by T cells (14–17). To examine whether this also occurs in human systems, we activated T cells in human PBMC cultures with anti-CD2/anti-CD3/anti-CD28 Abs in the presence or absence of exogenous IL-27. We found elevated IL-10 in the supernatant of IL-27–treated cultures, demonstrating that human IL-27 upregulates IL-10 production. To confirm that this was common to multiple donors we performed this study separately in PBMCs from seven individuals and consistently observed upregulation of IL-10 by IL-27, albeit to varying degrees in individual donors (Fig. 1A). Others have reported upregulation of IL-10 production by IL-27 in cultures of purified human CD4+ T cells (26); however, we did not observe a consistent effect of exogenous IL-27 on IL-10 production in purified CD4+ T cells in our culture system in five donors (Fig. 1B). This suggests that other cells or mediators may be involved in the optimal upregulation of IL-10 in CD4+ T cells by IL-27. However, one key difference between our culture system and that used by Murugaiyan et al. (26) is that our culture system used X-VIVO 15 medium equating to serum-free conditions. Therefore, we tested the effect of IL-27 on IL-10 production in culture systems with and without sera. FBS significantly increased basal IL-10 production in activated PBMC cultures, but human serum did not (Fig. 1C), suggesting that factors present in FBS potentiate IL-10 production. Although IL-27 showed a trend of upregulating IL-10 production in the presence of serum, results were highly variable between experiments and thus we opted to proceed with studies in serum-free conditions utilizing PBMCs to more specifically study the role of IL-27 in IL-10 upregulation.

To examine the kinetics of IL-10 production, we performed a time course analysis and observed maximal upregulation of IL-10 by IL-27 at 5 d after stimulation (Fig. 2A). To confirm that upregulation of IL-10 was specifically due to exogenous IL-27, we
added neutralizing anti–IL-27 Ab to the system and observed inhibition of IL-10 upregulation by exogenous IL-27 (Fig. 2B). Interestingly, we observed that levels of IL-10 in the presence of neutralizing anti–IL-27 Ab were in fact significantly lower than basal IL-10 levels, suggesting that endogenous IL-27 contributes to basal IL-10 production in anti–CD2/CD3/CD28-activated PBMCs. To confirm that endogenous IL-27 upregulates IL-10, we activated cultures in the presence or absence of neutralizing anti–IL-27 Ab. Strikingly, we observed consistent and extensive inhibition of IL-10 production in this setting, indicating a prominent role for endogenous IL-27 in basal IL-10 production in activated PBMCs (Fig. 2C). Others have reported that IFN-β potently upregulates IL-10 expression (29, 31, 32). To confirm this in our culture system, we performed this study in PBMCs from seven individuals and consistently observed significant upregulation of IL-10 by IFN-β (Fig. 2D). Taken together, these data show that both IL-27 and IFN-β upregulate IL-10 in PBMCs.

**IFN-β upregulates IL-27 production by human monocytes**

In MS patients, upregulation of IL-10 in peripheral blood is a positive indicator of responsiveness to IFN-β therapy (23). Additionally, upregulation of IL-10 by IL-27 is reminiscent of IFN-β bioactivity in murine systems (28). Given such similarity, it is plausible that IL-27 may mediate at least some of the immunomodulatory effects of IFN-β. This hypothesis was supported by the fact that IFN-β induced IL-27 expression in murine cells, and
in humans this has also been shown using in vitro–differentiated DCs (18, 33). Given that our culture system constituted fresh PBMCs, we sought to examine the effect of IFN-β on freshly isolated monocytes without prior in vitro differentiation. Toward this end, we purified monocytes from human PBMCs by immunomagnetic separation and activated cells with LPS in the presence or absence of IFN-β. As expected, LPS induced IL-27 production and this was augmented by IFN-β (Fig. 3A). PGN has recently been shown to drive Th17 development, and we have also observed this phenomenon (unpublished observations). Interestingly, we found that PGN also drove the production of IL-27 by human monocytes (Fig. 3B), suggesting that this microbial inflammatory signal invokes both inducing and suppressive pathways for Th17 cell development. We tested the effect of IFN-β in this model and found, consistent with the LPS-activated model, that IFN-β significantly augmented IL-27 production (Fig. 3B). To examine whether IL-27 played a role in the IFN-β/IL-10 axis, we cultured activated PBMCs with IFN-β in the presence or absence of neutralizing anti–IL-27 Ab. As expected, PBMCs cultured in the presence of IFN-β and IgG control Ab produced significantly elevated levels of IL-10 whereas PBMCs cultured with IFN-β in the presence of anti–IL-27 neutralizing Ab produced levels of IL-10 significantly lower than the basal production of activated PBMCs (Fig. 3C). These data demonstrate that IL-27 mediates upregulation of IL-10 by IFN-β in activated PBMCs.

**IL-27 is not required for suppression of actively induced EAE by IFN-β**

IFN-β is a first line of therapy for MS and has been shown to suppress EAE in a range of models (34–37). We and others have shown that IL-27 suppresses EAE (5, 12, 38), and in a Th1-driven model of EAE we have shown that this suppression is dependent on IL-10 (16). Given the correlation between IL-10 levels and responsiveness to IFN-β therapy in human MS patients and the observation that IFN-β drives IL-10 through IL-27 (Fig. 3C), we hypothesized that IL-27 mediated the suppression of EAE by IFN-β. To test whether IL-27 mediated the therapeutic effect of IFN-β in vivo, we treated WT and IL-27 receptor-deficient mice, which had been immunized to develop EAE, with IFN-β i.p. daily from day 0 to day 19 after immunization. IFN-β significantly suppressed clinical disease in WT mice (Fig. 4A, 4C, 4D). Very surprisingly, we also observed suppression of EAE by IFN-β in IL-27R-deficient mice (Fig. 4B, 4D, 4D). Disease incidence in all groups was comparable (Table I). These data disprove our hypothesis and demonstrate that IL-27 signaling is not required for the suppression of MOG35–55-induced EAE by IFN-β. Given this surprising finding, we sought to further investigate the immunoregulatory mechanisms of IFN-β and IL-27.

**IL-27 and IFN-β inhibit human Th17 cells**

Given the pathogenic potential of Th17 cells in EAE and MS and previous reports of suppressive effects of IFN-β and IL-27 on these cells (5, 9, 25), we investigated this phenomenon in fetal and adult human T cell cultures. Development and regulation of human Th17 cells is currently a contentious topic. Thus we sought to examine whether IFN-β suppresses Th17 cells in a number of Th17-supportive conditions. Toward this end we first examined the effect of exogenous IFN-β on de novo differentiation of Th17 cells. Human naive CD4+ cells (CD45RA+) derived from cord blood were cultured in serum-free medium and activated with anti-CD2/anti-CD3/anti-CD28 Abs under Th17 polarizing conditions. Given the current disagreement in the optimal cytokine milieu for de novo human Th17 differentiation, we tested a range of cytokine combinations as described in Fig. 5A and in each case we tested the effects of IFN-β on IL-17 production. We consistently observed that IFN-β inhibited IL-17 production by CD4+ cultures regardless of the polarizing cytokine milieu, which was statistically significant in two of the three cytokine cocktails tested (Fig. 5A). We next investigated the effect of IFN-β on PBMCs from adult donors. In the presence of IL-23, IFN-β had a variable effect on IL-17 production (data not shown). However, IFN-β significantly suppressed IL-17 under basal conditions (Fig. 5B). We next examined the effect of IL-27 in the model of de novo Th17 differentiation described above. Similarly to IFN-β, IL-27 suppressed IL-17 production in this model, in agreement with recent reports, and this was statistically significant in the conditions that generated the most robust expression of IL-17 (TGF-β plus IL-1β plus IL-23) (Fig. 5C) (26, 33). Next, we investigated the effect of IL-27 in PBMCs cultured in the presence of IL-23, which supports differentiated Th17 cells. PBMCs from adult donors were cultured for 5 d in the presence of IL-23 and anti-
CD2/anti-CD3/anti-CD28 Abs in the presence or absence of IL-27. PBMCs were then analyzed by flow cytometry, and IL-27 treatment resulted in lower proportions of IL-17+ cells within the CD4+ population (23.1 versus 14.4%) (Fig. 5D, representative plot from a single, maximal response donor). Proportions of IL-17+ cells were highly variable across experiments and between donors; however, the proportion of CD4+ cells producing IL-17 decreased in all seven donors in the presence of IL-27 (Fig. 5E). Although not as striking as observed in de novo differentiating Th17 cells, PBMCs cultured with IL-23 in the presence of IL-27 had significantly less IL-17 detectable in the supernatant compared with control PBMC cultures with IL-23 alone (Fig. 5F). We repeated this study in seven donors and found mild but significant suppression of secreted IL-17 in five of seven donors (data not shown). Given the lower level of suppression of IL-17 by IL-27 in PBMC cultures compared with naïve CD4+ cell cultures, we hypothesized that endogenous IL-27 bioactivity may be exerting a suppressive effect on IL-17 production in control PBMC cultures that did not receive exogenous IL-27. To test this hypothesis we activated IL-23–driven PBMCs with anti-CD2/anti-CD3/anti-CD28 in the presence or absence of IL-27. Neutralization of endogenous IL-27 significantly enhanced IL-17 production in these cultures, suggesting that endogenous IL-27 inhibits IL-17 development in this model (Fig. 5G). We repeated this experiment in five donors and found that anti–IL-27 neutralizing Ab enhanced IL-17 production in all five donors (Fig. 5G).

Given similar suppressive profiles of IFN-β and IL-27 in human Th17 cultures, we hypothesized that IL-27 mediates the suppression of IL-17 by IFN-β in this model. When PBMCs were activated in the presence of IFN-β, IL-17 production was significantly suppressed; however, anti–IL-27 Ab did not abrogate this suppressive effect (Fig. 5H). These data suggest that contrary to our hypothesis, IL-27 is not required for the suppression of IL-17 by IFN-β in PBMC cultures. Thus, IFN-β and IL-27 both inhibit human Th17 responses; however, these data suggest distinct regulatory pathways for each cytokine, at least in the context of direct effects on PBMCs activated via TCR and costimulation.

IFN-β and IL-27 suppress human Th17 differentiation independent of IL-10

To further investigate these complex immunoregulatory pathways, we investigated whether IL-10 is involved in the suppression of IL-17 by either IL-27 or IFN-β. To test this in human cells we activated naïve CD4+ cells from human cord blood in Th17 polarizing conditions using combinations of cytokines as described. Cultures were treated with IL-27 or IFN-β in the presence or absence of neutralizing anti–IL-10 Ab. We did not see any loss of suppression of IL-17 when IL-10 was neutralized in samples that were treated with either IFN-β (Fig. 6A) or IL-27 (Fig. 6B). This suggests that IFN-β and IL-27 suppress de novo Th17 differentiation independent of IL-10.

IL-10 is not required for suppression of Th17-biased EAE by IL-27

Previously we have shown that IL-10 is required for the suppression of EAE by IL-27, but this was in an IL-12–driven Th1-biased model of adoptively transferred EAE (16). Given that we did not observe a role for IL-10 in suppression of IL-17 by IL-27 in

Table I. Disease incidence in experiments of WT and IL-27R−deficient mice immunized to develop EAE and treated with IFN-β daily from day 0 to 19 after immunization (see Fig. 4)

<table>
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<tr>
<th>Experiment</th>
<th>WT Control</th>
<th>WT IFN-β</th>
<th>il27ra−/− Control</th>
<th>il27ra−/− IFN-β</th>
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<tbody>
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<td>1</td>
<td>8/8 (100%)</td>
<td>6/6 (100%)</td>
<td>6/6 (100%)</td>
<td>7/8 (87.5%)</td>
</tr>
<tr>
<td>2</td>
<td>8/8 (100%)</td>
<td>7/8 (87.5%)</td>
<td>4/5 (80%)</td>
<td>4/5 (80%)</td>
</tr>
<tr>
<td>Total</td>
<td>16/16 (100%)</td>
<td>13/14 (92.9%)</td>
<td>9/10 (90%)</td>
<td>11/13 (94.6%)</td>
</tr>
</tbody>
</table>

FIGURE 4. IFN-β inhibits clinical EAE independent of IL-27 signaling. WT and IL-27R−deficient mice were immunized to develop EAE and treated with IFN-β (50,000 U/mouse/day i.p.) from day 0 to 19 after immunization. IFN-β effectively suppressed clinical EAE in WT (A, n = 8) and il27ra−/− (B, n = 6) mice. Cumulative (C) and mean (D) clinical scores were significantly reduced in WT mice treated with IFN-β, and a similar but not quite significant trend in il27ra−/− mice was observed. Data are representative of two independent experiments; n = 6–8 mice/experiment.
human naive CD4+ cells in any of the polarizing conditions tested (Fig. 6B), we questioned the relevance of the IL-27/IL-10 axis in the Th17 pathologic setting. To address this question in vivo, we established an IL-23–driven Th17-biased model of adoptively transferred EAE using MOG35–55-immunized C57BL/6 and il10−/− donors and an in vitro culture phase of encephalitogenic cells reactivated with Ag in the presence of IL-23. In agreement with our previous findings in an SJL/proteolipid (PLP) model of IL-23–driven EAE (5), we observed significant suppression of disease when MOG35–55-reactive cells were treated with IL-27 prior to transfer (Fig. 6C). Interestingly, we also observed suppression when IL-10−/− donor cells were treated with IL-27 and injected into WT recipients (Fig. 6D). Suppression of clinical disease by IL-27 was slightly more pronounced in mice receiving WT cells than in mice receiving il10−/− cells, which may point toward a minor role for IL-10; however, this may also simply be due to slightly enhanced disease severity in mice receiving il10−/− cells. IL-27 suppressed the mean, maximal, and cumulative clinical scores (Fig. 6E–G) and the incidence (Table II) in both genotypes. These data demonstrate that IL-27 suppresses IL-23–driven EAE independently of IL-10, contrasting with the requirement of IL-10 for suppression of Th1-biased EAE by IL-27 (16).

**Discussion**

These studies were initiated to translate previous findings in murine systems to human experimental models. In agreement with the findings of our group and those of others, demonstrating that exogenous IL-27 drives IL-10 expression in murine T cells (9, 14–16), we confirm in this study that this also occurs in activated human T cells. This is in agreement with recent reports by other groups (26, 39, 40). However, a key difference in our system is that we did not observe a consistent direct effect of IL-27 on purified CD4+ T cells, suggesting that influence of other mononuclear cells in our culture system potentiates the effect of IL-27 on IL-10 production by CD4+ T cells. Furthermore, separate studies by
Stumhofer et al. (17) and Awasthi et al. (14) showed that TGF-β potentiates IL-10 upregulation by IL-27 in murine cultures. A potential explanation for the difference between our findings in human T cells and those of Murugaiyan et al. (26) is the absence of serum in our culture conditions, as serum would contain appreciable levels of TGF-β, and indeed we observed significantly enhanced IL-10 production in the presence of serum (Fig. 1C). Given the potent induction of IL-10 by TGF-β (14, 17) we chose to perform all cell culture experiments in serum-free conditions. We identified powerful bioactivity of endogenous IL-27 in driving IL-10 production in PBMC cultures as exemplified by a lack of IL-10 production in the presence of neutralizing anti–IL-27 Ab. This confirmed that upregulation of T cell IL-10 production by exogenously added IL-27 reflected a function of endogenous IL-27.

IL-27 expression can be upregulated by type I IFN signaling (38), and IFN-β is a first line of therapy for MS. Furthermore, there is a positive correlation between a clinical response to IFN-β therapy and IL-10 upregulation in serum (23). Based on these relationships we developed a working hypothesis that IL-27 may mediate the clinical effect of IFN-β in MS and that this may involve IL-10 upregulation. We first confirmed upregulation of human IL-10 by IFN-β in our experimental PBMC model and then examined IL-27 production in response to IFN-β. To maximize similarity to an in vivo scenario we chose a model of freshly isolated monocytes from adult peripheral blood, immediately activated cells with TLR stimuli in the presence or absence of IFN-β, and measured heterodimeric IL-27 protein secreted into culture supernatants. In this model, IFN-β indeed upregulated IL-27 expression by type I IFN signaling (38), and IFN-β is a first line of therapy for MS. Furthermore, there is a positive correlation between a clinical response to IFN-β therapy and IL-10 upregulation in serum (23). Based on these relationships we developed a working hypothesis that IL-27 may mediate the clinical effect of IFN-β in MS and that this may involve IL-10 upregulation. We first confirmed upregulation of human IL-10 by IFN-β in our experimental PBMC model and then examined IL-27 production in response to IFN-β. To maximize similarity to an in vivo scenario we chose a model of freshly isolated monocytes from adult peripheral blood, immediately activated cells with TLR stimuli in the presence or absence of IFN-β, and measured heterodimeric IL-27 protein secreted into culture supernatants. In this model, IFN-β indeed upregulated IL-27 production in a dose-dependent manner.
protein production, and we went on to identify that IL-27 was required for IL-10 upregulation by IFN-β. These data lent support to our working hypothesis and the conclusions of a recent study by Sweeney et al. (33) that IL-27 mediated the suppressive effect of IFN-β in MS. However, to categorically test this hypothesis in CNS autoimmune inflammation, we returned to the mouse model of MOG35–55-induced EAE. Very surprisingly, in two separate experiments, IFN-β suppressed EAE both in WT mice and in mice lacking the IL-27 receptor (wvx1/–).

These in vivo experiments disproved our working hypothesis and demonstrated that IL-27 signaling is not required for the therapeutic suppressive effect of IFN-β in actively induced EAE. These studies cannot rule out a role for IL-27 in the suppression of EAE by IFN-β in a WT system; however, our results suggest that if this were the case, other mechanisms of IFN-β would prevail in the absence of IL-27 to orchestrate EAE suppression in an IL-27–independent manner. There are significant differences both in the IFN signaling system between mice and humans and between EAE and MS. Indeed, we only tested one type of EAE model owing to restrictions of the genetic background of wvx1/– mice; however, these findings call into question the functional relevance of IL-27 in clinical responses to IFN-β therapy at least in murine models and suggest that IL-27 upregulation by IFN-β in MS therapy may be merely associative.

These surprising findings led us to examine other aspects of immunosuppression by IFN-β and IL-27. Because Th17 cells are regarded as pathogenic effectors of autoimmune inflammation in many experimental models, as well as in MS, we sought to investigate the effects of IFN-β and IL-27 on human Th17 cells. Differentiation and stabilization of human Th17 cells is a controversial topic at present and a number of recent studies have shown direct and indirect suppressive effects of IFN-β and IL-27 on IL-17/Th17 responses in different models (18, 26, 28, 29, 32, 33). We chose to investigate de novo human Th17 differentiation using truly naïve CD4+ T cells from cord blood, differentiated in three different cocktails of Th17-promoting cytokines. We consistently observed suppression of Th17 differentiation by IFN-β and by IL-27. We then moved on to PMBCs from adult blood, which are more representative of clinical samples. Although we did not observe human Th17 expansion in response to the cytokines that drove de novo Th17 differentiation, we did observe Th17 expansion in response to IL-23, which was suppressed by IL-27. Because IFN-β suppressed IL-17 and upregulated IL-27 in our models, which is in agreement with other reports (18, 33), we tested whether IL-27 mediated the suppressive effect of IFN-β on IL-17. Again, to our surprise, we discovered that IL-27 was not required for the suppressive effect of IFN-β on IL-17 production in our system of PBMCs activated via CD3 and CD28, as demonstrated by comparable suppression in the presence of neutralizing anti–IL-27 Ab. This contrasts with a recent report (33) that showed that IL-27 mediated the suppression of human Th17 development by IFN-β in a different model. The model used initially differentiated human DCs in vitro, which were then activated with zymosan in the presence or absence of IFN-β and neutralizing anti–IL-27 Ab. Subsequently, supernatants from these cultures were added to CD4+ T cells activated with irradiated allogeneic PBMCs to drive Th17 differentiation. In contrast, to be somewhat representative of therapeutic administration of IFN-β, we treated whole PBMCs with IFN-β and did not observe any loss of IL-17 suppression by IFN-β when neutralizing anti–IL-27 was included.

The findings of these two studies likely differ due to the different IFN-β–responsive cells in the separate experimental models and different activation stimuli used and suggest differential requirements for IL-27 in the suppression of Th17 development by IFN-β. To add further complexity to the range of IFN-β–responsive cell types that regulate Th17 development, other studies by Ramgolam et al. (18, 29) and Zhang et al. (30) have shown that supernatants from both DCs and B cells treated with IFN-β exhibit impaired capacity to drive Th17 differentiation and this was associated with decreased IL-23p19 and IL-1β and increased IL-12p35 and IL-27p28 expression. Taken together, all of these studies reveal multiple inhibitory direct and indirect effects of IFN-β on Th17 responses, and the requirement of IL-27 for IFN-β–mediated suppression of human Th17 cells in vivo likely depends on the cellular microenvironment.

Given that IL-10 upregulation by IFN-β was downstream of IL-27 in our earlier studies (Fig. 3C), we asked whether IL-10 was required for the suppressive effect of IFN-β or IL-27 on de novo human Th17 differentiation. In all three de novo Th17 differentiating conditions tested, IL-10 was not required for the suppressive effect of either IFN-β or IL-27. These data added further complexity to the divergent immunosuppressive pathways of IFN-β and IL-27 on Th17 cells and CNS autoimmune inflammation. We were particularly perplexed that IL-10 did not influence suppression of de novo human Th17 cell differentiation by IL-27, as we had previously observed a requirement of IL-10 for suppression of murine IL-17 by IL-27 in nonpolarized conditions and a partial role in Th17 supportive conditions (16). We had also identified that IL-10 was required for suppression of adoptively transferred EAE by IL-27; however, this was in an IL-12–driven Th1-biased model of disease (16). We had previously shown that IL-27 also suppresses IL-23-driven, Th17-biased EAE in SJL/PLP139–151 model and suppression of Th17 cells was a central mechanism (5). Thus, given that IL-10 was not required for human Th17 suppression by IL-27, we asked whether IL-10 was required for suppression of Th17-biased EAE by IL-27. Given the genetic background of il10 /– mice, we performed these studies using MOG35–55 and C57BL/6 mice rather than using PLP139–151 and SJL mice. In this model, donor cells were exposed to exogenous IL-27 during the in vitro phase of culture and, as expected, this resulted in a significant reduction in clinical disease in recipient mice. Surprisingly, IL-27 also suppressed the pathogenicity of il10 /– cells to a similar degree. These findings suggest that IL-27 suppresses Th17-biased CNS autoimmune inflammation inde-
ependently of IL-10, which is in stark contrast to its mechanism of action in Th1-driven disease (16). Thus, our data suggest that IL-27 utilizes distinct mechanisms of action to inhibit autoimmune inflammation depending on the central pathogenic effectors mediating disease.

Our findings from these collective studies reveal a complex and somewhat paradoxical network of immunoregulatory functions of IFN-β, IL-27, and IL-10. Most strikingly, we have determined that IL-27 signaling is not required for suppression of EAE by IFN-β, likely having important implications for the mechanism of action of IFN-β in MS. We have also identified that IL-10 does not mediate the suppressive effect of IL-27 in a Th17-biased model of EAE. This presents a paradigm whereby IL-27 utilizes different mechanisms to suppress Th1-driven pathology versus Th17-driven pathology, with the former being dependent on IL-10 (16) and the latter being IL-10-independent (Fig. 6C, 6D).

It is important to recognize that EAE is a collection of models that, taken together, reflect many aspects of the heterogeneous disease that is human MS far more accurately than any single model of EAE or indeed single viral- or toxin-induced models of CNS demyelination alone. Encouragingly, we have now demonstrated that exogenous IL-27 suppresses clinical EAE in four separate models: actively induced EAE using MOG35-55 Ag in C57BL/6 mice (5), Th17-biased (IL-23-driven) adoptively transferred EAE using PLP139–151 Ag in the SJL mouse model (5), Th1-biased (IL-12-driven) adoptively transferred EAE using MOG35-55 Ag in C57BL/6 mice (16), and, in this study, in a Th17-biased (IL-23-driven) model of adoptively transferred EAE using MOG35-55 Ag in C57BL/6 mice. Furthermore, Guo et al. (38) have also shown that exogenous IL-27 suppresses highly severe EAE observed in ifnar1−/− mice. It is likely that multiple cellular and molecular mechanisms mediate the effects of IL-27 in these models, many of which may remain to be elucidated. Although it is tempting to speculate that IL-27 may be a potential therapeutic for inflammatory diseases such as MS, the potent suppression of de novo Th17 differentiation that is observed in mouse and human systems (9, 12, 26, 41) (Fig. 5) and indeed the suppressive effects on Th1 and Th2 responses also are a concern for maintenance of general immune function. Thus, understanding the mechanisms of IL-27 in suppression of clinical disease is of prime importance such that targeted therapeutics that minimize broad immunosuppression can be designed. It is also imperative to investigate in depth the effects of IL-27 in a wide range of models of inflammatory disease. Of particular interest is a recent report by Axtell et al. (34) that demonstrated both suppressive and exacerbating effects of IFN-β on Th1- and Th17-biased models of EAE, respectively. Given the close relationship between the functions of IFN-β and IL-27, it is important to identify the shared as well as distinct mechanisms of action in experimental models. Studies have demonstrated anatomically and pathologically distinct models of EAE based on the proportions of encephalitogenic Th1 and Th17 cells (42), and pathogenic Th9 cells have also been implicated (43). Clinical MS varies greatly, not only in the type of lesions that develop but also in the anatomical sites that are affected and the frequency of attacks. Distinct helper T cell subsets are now implicated in MS, and B cell subsets are also prominently implicated as drivers of pathogenesis (44). Such heterogeneity likely contributes to the selective response of patient subsets to immunomodulatory therapies such as IFN-β. Broad immunosuppression is not the optimal solution to treating inflammatory diseases such as MS, and thus it is crucial to elucidate specific mechanisms of clinical disease suppression to improve therapeutic options.

Our findings in this study reveal that in human experimental models derived from PBMCs, IFN-β and IL-27 both upregulate IL-10; IFN-β upregulates IL-27, and IL-27 is required for IL-10 upregulation by IFN-β. However, whereas both IFN-β and IL-27 suppress Th17 responses, this suppression is independent of IL-10, and suppression of Th17 cells by IFN-β is independent of IL-27 in the models we tested. In murine models of EAE we found that IL-27 signaling is not required for suppression of EAE by IFN-β and that IL-10 is not required for suppression of Th17-biased EAE by IL-27, which is in stark contrast to suppression of Th1-driven EAE by IL-27 (16). Taken together, these findings present distinct regulatory mechanisms of IFN-β and IL-27 in Th1- and Th17-driven inflammation.

Disclosures

The authors have no financial interests of conflict.

References


