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IL-27 Regulates IL-10 and IL-17 from CD4\(^+\) Cells in Nonhealing Leishmania major Infection\(^1\)

Charles F. Anderson,* Jason S. Stumhofer,† Christopher A. Hunter,† and David Sacks\(^2\)*

Control of infection caused by Leishmania major requires the development of IFN-γ\(^+\)CD4\(^+\) lymphocytes for the induction of microbicidal activity in host macrophages. We recently reported on the inability of conventionally resistant C57BL/6 mice to successfully resolve infection by an isolate of L. major, despite a strong IFN-γ response by the host. Susceptibility was caused by Ag-specific IL-10 from CD4\(^+\) cells that were also producing IFN-γ. In the present studies, we have explored the role for IL-27 in the regulation of IL-10 from Th1 cells in leishmaniasis. Cytokine analysis of CD4\(^+\) cells in the lesions and draining lymph nodes of infected IL-27R-deficient (WSX-1\(^{-/-}\)) mice revealed diminished IL-10 from IFN-γ\(^+\) CD4\(^+\) cells, which was accompanied by a reduction in total IFN-γ\(^+\)CD4\(^+\) cells and an increase in IL-4. Despite the inhibition of IL-10 from CD4\(^+\) cells, no significant change in parasite numbers was observed, due both to the shift in the Th1/Th2 balance and to residual levels of IL-10. Strikingly, infected WSX-1\(^{-/-}\) mice developed more severe lesions that were associated with the appearance of IL-17\(^+\) CD4\(^+\) cells, demonstrating a function for IL-27 in blocking the development of inappropriate Th17 cells during L. major infection. The results demonstrate the pleiotropic effects that IL-27 has on L. major-driven Th1, Th2, and Th17 development, and reinforce its function as a key regulatory cytokine that controls the balance between immunity and pathology. The Journal of Immunology, 2009, 183: 4619 – 4627.

The intracellular protozoan Leishmania can produce a spectrum of clinical diseases, ranging from a single cutaneous ulcer that spontaneously heals, to chronic cutaneous or mucocutaneous lesions that are nonhealing or slow to resolve, to visceral disease that is generally fatal in the absence of treatment. It is well established that successful control of infection caused by Leishmania major requires the development of IFN-γ\(^+\) lymphocytes for the induction of microbicidal activity by parasitized macrophages. Experimental models for cutaneous leishmaniasis have historically relied upon the disparate disease phenotypes displayed by C57BL/6 and BALB/c mouse strains to identify immunological mechanisms underlying, respectively, host resistance and susceptibility (1). However, the key conclusion that Th2 dominance controls susceptibility has failed to adequately explain nonhealing or reactivated forms of cutaneous or visceral leishmaniasis in humans.

IL-10 has pleiotropic, primarily anti-inflammatory properties that include suppression of dendritic cell functions and rendering macrophages unresponsive to activation signals (reviewed in Ref. 2). Although its up-regulation is considered a homeostatic mechanism, IL-10 has pleiotropic, primarily anti-inflammatory properties (3–6). In human visceral leishmaniasis, elevated levels of IFN-γ mRNA have been found in target organs, such as the spleen and bone marrow, accompanied by increased levels of IL-10 (7–9), the predominant source of which is Foxp3\(^+\)CD25\(^+\)CD3\(^+\) cells (10). Accumulating evidence from mouse models of nonhealing or disseminating forms of leishmaniasis has reinforced pathogenetic mechanisms that take into account the presence of parasite-driven Th1 responses that are suppressed either in magnitude or function by IL-10 (reviewed in Ref. 11).

We have introduced a model of nonhealing L. major in conventionally resistant C57BL/6 mice, in which IL-10 functions in a Th1-polarized setting to prevent clinical cure, and have argued that this model better reflects the conditions underlying nonhealing forms of clinical disease (12). A notable feature of this infection is the presence of IFN-γ\(^+\) CD4\(^+\) cells that also produce IL-10. IL-10 from this cellular source was necessary and sufficient to mediate susceptibility, because specific ablation of IL-10 from this subset resulted in enhanced clearance of infection (13). The factors that regulate IL-10 production by Th1 cells in this setting are unknown.

The IL-12-related cytokine, IL-27, is a heterodimer composed of EBI3 and p28, and is produced by innate cells, such as macrophages and dendritic cells (14). The receptor for this cytokine is composed of gp130, a subunit used by other growth factors, in-
(for a review, see Ref. 14). Several recent studies have shown IL-27 to mediate anti-inflammatory activity through its ability to suppress Th17 cells (17, 18), and through the induction of IL-10 from activated CD4+ cells. Under neutral in vitro culture conditions, exogenous IL-27 exclusively induced IL-10 from naïve CD4+ cells, and enhanced IL-10 production when cells were activated in the presence of Th1- or Th2-polarizing cytokines (19–21). In vivo, CD4+ cells from WSX-1−/− mice infected with Toxoplasma gondii or Listeria monocytogenes produced less IL-10 than wild-type counterparts (19, 21). In the mouse model for multiple sclerosis, exogenous IL-27 suppressed experimental autoimmune encephalomyelitis induced by adoptive transfer of pathogenic CD4+ cells in an IL-10-dependent manner (20). The present studies were designed to assess the function of IL-27 in the regulation of IL-10 by CD4+ effector cells responding in an IL-10-dependent, noncurcuring model of L. major infection, and how this regulation affects parasite control and pathology.

Materials and Methods

Mice and reagents

C57BL/6 mice were purchased from Taconic Farms, maintained in the National Institute of Allergy and Infectious Diseases animal care facility under specific pathogen-free conditions, and used under a study protocol approved by the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee. IL27Ra−/− (WSX-1−/−) mice, backcrossed more than nine generations onto C57BL/6 mice (15), were provided by C. Saris (Amgen, Thousand Oaks, CA) and were housed and bred in specific pathogen-free facilities in the Department of Pathobiology at the University of Pennsylvania in accordance with institutional guidelines. IL27Ra−/− and IL27Ra+/+ mice, each backcrossed at least 10 generations onto C57BL/6 mice (22), were also supplied by Genentech, and were housed and bred in specific pathogen-free conditions in the National Institute of Allergy and Infectious Diseases animal care facility.

Infections and in vivo treatments

L. major strain NIH/Sd (MHOM/SON/74/SD) was cultured in medium 199 with 20% heat-inactivated FCS (Gemini Bio-Products), 100 µg/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, 40 mM HEPES, 0.1 mM adenine (in 50 mM HEPES), 5 mg/ml hemin (in 50% triethanolamine), and 1 mg/ml 6-biotin (in 50 mM HEPES). Infective-stage metacyclic promastigotes of L. major were coadministered beginning 6 wk after infection, with mAb/injection) were coadministered beginning 6 wk after infection, with IL-10

Lymphocyte isolation and in vitro restimulation

To characterize leukocytes in the inoculation site, the ears were collected, and the ventral and dorsal dermal sheets were prepared, as described above. Following preparation, cells were analyzed for surface phenotype by flow cytometry. For in vitro restimulation of dermal lymphocytes, cells were stimulated with 10 ng/ml PMA and 500 ng/ml ionomycin in the presence of monensin (GolgiStop; BD Biosciences) for 4 h. The cells were then analyzed for surface markers and intracytoplasmic staining for cytokines.

In vitro restimulation of draining lymph node (dLN)3 cells, CD4+ cells from cervical dLN were purified by negative selection (Miltenyi Biotec) and cocultured with CD11c+ splenic APCs (Miltenyi Biotec) isolated from naive C57BL/6 mice at a T cell:APC ratio of 4:1 in 200 µl of RPMI 1640 containing 10% FCS, 10 mM HEPES, glutamine, and penicillin/streptomycin in round-bottom 96-well plates with or without 50 µg/ml freeze-thaw Leishmania Ag prepared from NIH/Sd stationary-phase promastigotes. Where indicated, rIL-27 (R&D Systems) was added at a final concentration of 20 ng/ml. After 72 h, culture supernatants were collected for ELISA measurements, or cells were stimulated with PMA and ionomycin in the presence of monensin for 4 h before intracellular cytokine staining.

Immunolabeling, flow cytometry analysis, and cytokine measurements

The following Abs used for immunophenotyping were purchased from BD Biosciences: FITC anti-mouse TCR β-chain (H57-597), PE-Cy7 anti-mouse CD4 (L3T4; RM4-5), allopseudocyanin anti-mouse F4/80 (BM8), PE anti-mouse Ly6G and Ly-6C (GR-1; RB6-8C5), allopseudocyanin anti-mouse IFN-γ (XMG-1.2), PE or allopseudocyanin anti-mouse IL-10 (JES5-16E3), and PE anti-mouse IL-17A (TC11-18H10). FITC anti-Foxp3 staining was performed using eBioscience reagents, according to manufacturer’s protocol. The isotype controls used (all from BD Pharmingen) were rat IgG2a (A95-1), rat IgG2a (R35-95), and hamster IgG, group 2 (Hu/Ig). Before staining, lymph node or dermal cells were incubated with an anti-FcRIIa/I1 mAb (2–4G1) in PBS containing 0.1% BSA and 0.01% NaN3. The staining of surface and intracytoplasmic markers was performed sequentially, as follows: the cells were stained first for their surface markers, followed by fixation/permeabilization and staining for IFN-γ, IL-10, and IL-17A, and for each sample, 400,000 cells were collected for analysis. The data were acquired using a BD FACSCanto II flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star). The lymphocytes from ear cells were identified by characteristic size (forward light scatter) and granularity (side light scatter), and by lymphocyte surface phenotype. ELISA measurements for IFN-γ, IL-10, IL-4, and IL-17A were performed using eBioscience kits, according to manufacturer’s instructions.

Real-time PCR

For analysis of gene expression, ears were removed and immediately placed in RNAlater (Qiagen). Ear tissue was then disrupted mechanically using liquid nitrogen and a mortar and pestle. Homogenates were then passed through Qiashredder columns, and RNA was purified using RNAeasy mini kit (Qiagen), according to the manufacturer’s protocol. Reverse transcription was performed using Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies). Real-time PCR was performed on an ABI Prism 7900 sequence detection system (Applied Biosystems). PCR primer probe sets were predesigned for Applied Biosystems, and the quantities of products were determined by the comparative threshold cycle method using the equation 2–ΔΔCt (where Ct represents cycle threshold) to determine the fold increase in product. Each gene of interest was normalized to the 18s rRNA endogenous control, and the fold change in expression is displayed as relative to naïve controls.

Statistical analyses

Values of p were determined using unpaired, two-tailed Student’s t tests with Welch’s correction.

Results

IL-27 enhances IL-10 production from Foxp3− IFN-γ− CD4+ cells

We previously demonstrated the presence of Foxp3+ CD4+ cells that produced Ag-specific IL-10 in a Th1-polarized setting during

3 Abbreviation used in this paper: dLN, draining lymph node.
a nonhealing *L. major* infection in C57BL/6 mice. Following the recent findings regarding IL-27 regulation of IL-10 production by naive and activated T cells, the expression of IL-27 during *L. major* NIH/Sd infection and its influence on the evolution of the nonhealing phenotype were explored. A kinetic analysis of IL-27 mRNA in the infection site revealed a progressive increase over the first 8 wk in transcripts for both IL-27 subunits, with p28 showing up-regulated expression by week 2, whereas elevated EBI3 was not detectable until week 5 (Fig. 1). The absolute cycle threshold values for p28 and EBI3 in naive ears were 17.26 ± 0.55 and 14.39 ± 0.95, respectively. The increase of IL-27 correlated with the up-regulation of IL-10. To determine whether IL-27 had the potential to regulate IL-10 in this infection, purified CD4<sup>+</sup> T cells from dLN of chronically infected mice were cocultured with splenic CD11c<sup>+</sup> APCs from naive mice and restimulated with parasite Ag alone, or in combination with exogenous IL-27. Intracellular cytokine staining of CD4<sup>+</sup> cells (3.5 vs 5.2%) and IL-10 secretion as measured by ELISA, and concomitantly decreased IFN-γ secretion (Fig. 2B). The latter effect was due to increased IL-10, because IFN-γ levels were restored by Ab blockade of IL-10R. Therefore, IL-27 signaling in Ag-primed, Foxp3<sup>+</sup> CD4<sup>+</sup> cells enhanced IL-10 production. Because restimulation with Ag alone efficiently induced the CD4<sup>+</sup> T cells to produce IL-10, the requirement for endogenous IL-27 in these cultures was investigated using anti-IL-27 Abs. Although no effect of IL-27 neutralization was observed (data not shown), the possibility remained that IL-27 plays an instructional role during the priming stage for development of Th1 cells capable of IL-10 secretion upon subsequent encounter with Ag.

**Mice lacking the IL-27R have decreased IL-10 and increased IL-17**

To determine whether an absence of IL-27 signaling during Th1 development results in reduced IL-10 production by IFN-γ<sup>+</sup> CD4<sup>+</sup> cells during infection, thereby increasing host resistance, IL-27R-deficient (WSX-1<sup>−/−</sup>) mice were infected intradermally with a physiological dose of *L. major* NIH/Sd. Following inoculation, WSX-1<sup>−/−</sup> mice developed more severe lesions that were evident at 6 wk after infection, and necessitated euthanasia by 11 wk due to extensive necrosis and dermal erosion (Fig. 3A). Despite the increased pathology, there were no differences in the lesional parasite burdens comparing C57BL/6 and WSX-1<sup>−/−</sup> mice at either time point (Fig. 3B), suggesting a role for IL-27 in restraining immunopathology. CD4<sup>+</sup> cells isolated from the lesions of C57BL/6 and WSX-1<sup>−/−</sup> mice were compared for intracellular IFN-γ and IL-10 production, following ex vivo restimulation with PMA and ionomycin. WSX-1<sup>−/−</sup> mice had a reduced percentage
of CD4+ cells simultaneously producing IFN-γ and IL-10 (6.3 vs 1.6%), which was accompanied by a reduction in cells producing IFN-γ alone (34.0 vs 18.3%) (Fig. 4A). Because WSX-1−/− mice had increased pathology compared with C57BL/6 mice, and because IL-27 has been demonstrated to inhibit the development of pathological IL-17+CD4+ cells, intracellular cytokine analysis for IL-17A was performed. This revealed a large percentage of CD4+ cells producing IL-17A in WSX-1−/− mice that was nearly absent in C57BL/6 mice (17.5 vs 2.3%). This population did not coproduce IFN-γ (Fig. 4A) or IL-10 (data not shown). There were no differences between the two strains in the proportion of CD4+ cells that expressed Foxp3, and IL-10 production from Foxp3+ CD4+ cells from the lesions was comparable (Fig. 4B). The severe pathology and IL-17+CD4+ cells were also associated with an increase in cellular infiltrate-expressing macrophage and neutrophil markers (Fig. 4C). In agreement with the data from lesionsal cells, Ag-restimulated dLN CD4+ cells from WSX-1−/− mice had a reduced frequency of IL-10+IFN-γ+ producers (1.4 vs 0.3%) and reduced IL-10+IFN-γ+ producers (4.0 vs 2.0%) when compared with wild-type mice (Fig. 5A). IL-17A was also detected in WSX-1−/− mice, but not from wild-type mice. Further analysis of lymph node cells revealed that IL-17A was not detected from non-CD4+ cells (data not shown). To confirm that the cytokines analyzed at the single cell level corresponded to total amounts of cytokines produced, the supernatants of Ag-restimulated dLN CD4+ cells were analyzed by ELISA. IFN-γ was secreted in high concentration by cells from C57BL/6 mice, and modestly, although significantly reduced by cells from WSX-1−/− mice (Fig. 5B). IL-17 was secreted in concentrations comparable with IFN-γ in WSX-1−/− mice, although nearly absent in C57BL/6 mice. IL-10 was significantly reduced in WSX-1−/− mice, but not absent, revealing the presence of an IL-27-independent pathway for the induction of IL-10. Finally, IL-4 was produced at higher amounts in WSX-1−/− mice, consistent with the known ability of IL-27 to suppress Th2...
IL-10-mediated control, demonstrated that an early and transient burst of IL-4 was subject to suppression by IL-27, thereby dictating the requirement for IL-27 in early Th1 development. Neutralization of IL-4 early in the infection, or the absence of IL-4 at later stages in the infection, rendered IL-27 dispensable (16). To determine whether the increased IL-4 in WSX-1-/- mice infected with L. major NIH/Sd was responsible for preventing the increased parasite killing that was expected to occur as a consequence of reduced IL-10, anti-IL-4-neutralizing Ab was administered during the first 5 wk of infection. When analyzed at 11 wk postinfection, the anti-IL-4-treated WSX-1-/- mice had a slight increase in the proportion of CD4+ cells producing IFN-γ at the infection site compared with control-treated mice (Fig. 6A), although still reduced compared with C57BL/6 mice. Importantly, the increase in IFN-γ was not associated with a concomitant increase in IL-10. The treatment also resulted in an increase in IL-17+CD4+ cells in WSX-1-/- mice, indicating that IL-4 was also suppressing development of Th17 cells. The proportion of IFN-γ+CD4+ cells in dLN of the anti-IL-4-treated WSX-1-/- mice was not increased over that in control-treated mice, and was again lower than that in wild-type mice (Fig. 6B). Most importantly, the treatment resulted in a modest, although statistically significant, reduction in parasite burdens in WSX-1-/- mice (Fig. 6C). By contrast, the wild-type mice showed no change in IFN-γ and IL-10 following IL-4 neutralization, and no reduction in parasite burdens, consistent with previous data demonstrating that IL-4 was inconsequential to the noncure phenotype of C57BL/6 mice (12). Taken together, the results show that, in the absence of IL-4, the increased parasitic control resulting from decreased IL-10 in WSX-1-/- mice can be revealed. However, the increase in IL-4 in WSX-1-/- mice is only partly responsible for suppressing Th1 responses, and the role for IL-27 in promoting Th1 development during a chronic infection is not restricted to its regulation of IL-4 in the initial weeks of infection.

**IL-27-independent IL-10 in WSX-1-/- is sufficient to inhibit the effector response**

Although IL-10 from T cells was significantly reduced in WSX-1-/- mice, its production may still have been sufficient to inhibit the effector response and prevent parasite clearance. To determine this, infected mice were treated with blocking Ab against the IL-10R for a period of 3 wk, beginning at 6 wk postinfection, and examined at 10 wk. As previously described (12), anti-IL-10R-treated C57BL/6 mice exhibited a significantly lower parasite burden (~100-fold) compared with control-treated mice (Fig. 7A). WSX-1-/- mice receiving the same regimen also had a significantly reduced parasite burden, although not as great as C57BL/6 mice (~10-fold). Additionally, anti-IL-10R-treated WSX-1-/- mice had an increase in lesion pathology compared with the control group (Fig. 7B), revealing that the IL-10 in WSX-1-/- mice can function to suppress pathology independently of IL-27. Treated C57BL/6 mice did not display increased pathology compared with controls. Cytokine analysis of WSX-1-/- lesions revealed an increase in the proportion of CD4+ cells producing IFN-γ (22.5 vs 39.9%) (Fig. 7C), demonstrating that the IL-10 produced in the WSX-1-/- mice, although significantly reduced compared with C57BL/6 mice, was still sufficient to inhibit the effector response, particularly in the context of the shift in the Th1/Th2 balance that results from the absence of IL-27 signaling. Importantly, the frequency of IL-17+CD4+ cells in WSX-1-/- mice was unchanged following treatment, suggesting that, in the context of a mixed Th1/Th17 response, IL-10 functions preferentially to suppress Th1 cells.
To determine whether the inability of the anti-IL-10R treatment to more efficiently clear parasites from the chronic lesions in the WSX-1<sup>−/−</sup> mice was due to the continued influence of up-regulated IL-4, IL27Ra<sup>−/−</sup> mice were coinjected with anti-IL-10R and anti-IL-4 Abs beginning at 6 wk postinfection. Of note, the IL-27R-sufficient mice used in this experiment were generated from IL27Ra<sup>+/+</sup> littermates backcrossed to C57BL/6 mice, rather than the C57BL/6 wild-type mice used in the previous experiments. The comparison of lesion scores again revealed significant exacerbation of the dermal pathology in the IL27Ra<sup>−/−</sup> mice compared with IL27Ra<sup>+/+</sup> mice beginning at 4 wk postinfection (Fig. 8A).

Following five biweekly injections, the parasite burdens in the lesions failed to reveal an additive effect of the combined anti-IL-4/IL-10R treatment, with each treatment group showing a comparable 10- to 20-fold reduction in the number of parasites in the site relative to the control-treated mice (Fig. 8B). Of note, the reduced

**FIGURE 6.** Neutralization of IL-4 in WSX-1<sup>−/−</sup> mice early in infection enhances resistance. Mice infected intradermally with L. major NIH/Sd were given weekly injections of neutralizing Ab to IL-4, beginning 1 day before infection and then weekly during the first 5 wk of infection. After 11 wk of infection, mice were sacrificed and CD4<sup>+</sup> cells from ear lesions were restimulated with PMA and ionomycin and analyzed by intracellular staining (A and B), and parasite burdens were measured (C). Burden data show individual ears and geometric mean parasite loads per ear. The experiment was repeated twice with similar results.

**FIGURE 7.** IL-10 produced independently of IL-27 is sufficient to inhibit microbicidal activity. Chronically infected mice were treated with anti-IL-10R Ab once per week for a period of 3 wk beginning at 6 wk postinfection, and then sacrificed 1 wk after the final treatment. A, Parasite burdens were determined by limiting dilution analysis. Parasite burdens per individual ear and geometric mean parasite burdens are shown. B, Lesion pathology was measured at the time of sacrifice. C, CD4<sup>+</sup> cells from lesions were isolated, pooled, and restimulated with PMA and ionomycin, and analyzed by intracellular cytokine staining. Plots are gated on CD4<sup>+</sup> cells. Quadrant values are the percentage of total CD4<sup>+</sup> cells. The experiment was repeated twice with similar results.
parasite loads in infected IL27Ra−/− mice, was associated with severe pathology at the infection site. Thus, by its ability to promote T-bet induction (25), and its inhibitory effects on parasite-driven Th2 and Th17 development, the findings illustrate the multifaceted role for IL-27 as a key instructional cytokine for Th1 polarization, and its subsequent modulation of this response via a mechanism of IL-10-mediated feedback control.

One possible explanation for the reduction in IL-10+ IFN-γ+ cells in WSX-1−/− mice is that the population of CD4+ cells producing IL-10 is directly proportional to IFN-γ, possibly due to intrinsic programs of cytokine gene expression during Th1 development, and/or direct effects of IFN-γ on IL-10 induction. The latter possibility is consistent with the observations that IL-12, or IL-12-induced IFN-γ, can activate Th1 cells to produce IL-10 (26–28). In the current studies, the increase in IFN-γ following anti-IL-4 or anti-IL-10R treatment in the WSX-1−/− mice did not result in a concomitant increase in IL-10, arguing for an IL-27-mediated mechanism of IL-10 induction that is independent of its effects on Th1 development.

The most striking observation of the current study is the development of severe pathology that correlates with both the reduced levels of IL-10 and the appearance of IL-17+ CD4+ cells in the absence of IL-27 signaling. Th17 cells appear to be involved in host defense against certain extracellular bacterial and fungal pathogens, but they also mediate severe immunopathologies (29). There is limited information on the role of Th17 cells in experimental leishmaniasis. Local injection of IL-1β following L. amazonensis infection accelerated disease progression that was associated with, among other changes, increased activation of Th17 cells (30). IL-17-deficient BALB/c mice developed dramatically smaller lesions despite only a modest reduction in parasite loads (31), consistent with our observations that the elevated IL-17 conferred no beneficial effect in controlling parasite replication, but contributed to lesion pathology. The abundance of neutrophils in the lesion is most likely due to the ability of IL-17 to regulate granulopoiesis, through the induction of G-CSF, and neutrophil recruitment, through regulation of CXC chemokines (32).

Although IL-27 is known to inhibit Th17 development (17, 18), there are diseases in which they do not develop in WSX-1−/− mice (33, 34). It is therefore likely that L. major infection induces factors, such as IL-6 and TGF-β, that in the absence of IL-27 signaling, promote the development of Th17 cells. And whereas Th17 development can be inhibited by IFN-γ and IL-4 (35, 36), the mixed Th1/Th2/Th17 response seen in the L. major-infected WSX-1−/− mice suggests that Th17 cells are able to develop in the presence of IFN-γ and IL-4. The current data also do not support a role for IL-10 in the inhibition of IL-17 from CD4+ cells, as has been reported (20, 37), because following IL-10R blockade initiated during chronic infection, the amount of IL-17 that was measured in the dLN was decreased relative to controls (data not shown), and the proportion of IL-17+ CD4+ cells in the lesions was unchanged. It is possible that the increased IFN-γ that resulted from the treatment was itself inhibitory to the Th17 response, or that the effect of IL-10 on Th17 development is confined to the initial priming of naive cells.

The predominant function of IL-27 in promoting early Th1 development vs suppressing an exuberant inflammatory response is
likely to be contextual. In *T. gondii* infection, normal Th1 development and parasite clearance occurred in the absence of IL-27 signaling. The infection, however, resulted in a lethal immunopathology (38). *Trypanosoma cruzi* infection in WSX-1 mice resulted in heightened Th2 and Th1 responses, the former responsible for high parasitemia, the latter producing more severe pathology (39). Experimental tuberculosis in WSX-1 mice resulted in normal Th1 development, decreased bacterial burdens, and increased pathology (40, 41). Similarly, *Leishmania donovani* appeared not to require IL-27 for normal Th1 development, because TCCR+/− mice controlled parasite growth better than the wild-type mice. The mice, however, developed more severe liver pathology (42). In the current studies, as in the prior studies involving *L. major*, IL-27 signaling was required for normal early Th1 development. The difference, particularly in reference to *L. donovani*, might be the early burst of IL-4 following *L. major*, but not *L. donovani* infection in mice, requiring IL-27-mediated suppression for normal Th1 development in the former, but not the latter. In the present studies, a requirement for IL-27 in instructing early Th1 development appeared not to be limited to its suppression of IL-4, because neutralization of IL-4 did not restore full Th1 development. One explanation for this may be the comparatively low levels of IL-12 induced by *L. major*, independent of IL-4 (43), which, in contrast to other infectious agents, e.g., *T. gondii* and *Mycobacterium tuberculosis*, dictates the early requirement for IL-4, because neutralization of IL-4 did not restore full Th1 expression for normal Th1 development in the former, but not the latter. In the current studies, as in the prior studies involving *L. major*, IL-27 signaling was required for normal early Th1 development. The difference, particularly in reference to *L. donovani*, might be the early burst of IL-4 following *L. major*, but not *L. donovani* infection in mice, requiring IL-27-mediated suppression for normal Th1 development in the former, but not the latter. In the present studies, a requirement for IL-27 in instructing early Th1 development appeared not to be limited to its suppression of IL-4, because neutralization of IL-4 did not restore full Th1 development. One explanation for this may be the comparatively low levels of IL-12 induced by *L. major*, independent of IL-4 (43), which, in contrast to other infectious agents, e.g., *T. gondii* and *Mycobacterium tuberculosis*, dictates the early requirement for IL-27 as a cofactor to increase responsiveness to IL-12. This requires early IL-12 production, because IL-4 did not restore full Th1 development. One explanation for this may be the comparatively low levels of IL-12 induced by *L. major*, independent of IL-4 (43), which, in contrast to other infectious agents, e.g., *T. gondii* and *Mycobacterium tuberculosis*, dictates the early requirement for IL-27 as a cofactor to increase responsiveness to IL-12. This requirement may explain why even the combined treatment with anti-IL-10R and anti-IL-4 failed to promote more efficient resistance in the WSX-1/− mice, because strong Th1-inducing signals were still absent in these mice. Although the influence of IL-27 signaling on Th1 development may represent a point of departure for these various pathogens, they all converge with respect to the role of IL-27 in limiting inflammation. The present data reinforce the critical role of IL-27 in inducing IFN-γ and IL-10 from CD4+ T cells, and in suppressing inappropriate Th17 subset development, to achieve a balance between protective immunity and pathology in response to an intracellular parasitic infection.

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Disclosures

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