Essential Role for IL-27 Receptor Signaling in Prevention of Th1-Mediated Immunopathology during Malaria Infection

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Successful resolution of malaria infection requires induction of proinflammatory immune responses that facilitate parasite clearance; however, failure to regulate this inflammation leads to immune-mediated pathology. The pathways that maintain this immunological balance during malaria infection remain poorly defined. In this study, we demonstrate that IL-27R-deficient (WSX-1<sup>−/−</sup>) mice are highly susceptible to Plasmodium berghei NK65 infection, developing exacerbated Th1-mediated immune responses, which, despite highly efficient parasite clearance, lead directly to severe liver pathology. Depletion of CD4<sup>+</sup> T cells—but not CD8<sup>+</sup> T cells—prevented liver pathology in infected WSX-1<sup>−/−</sup> mice. Although WSX-1 signaling was required for optimal IL-10 production by CD4<sup>+</sup> T cells, administration of rIL-10 failed to ameliorate liver damage in WSX-1<sup>−/−</sup> mice, indicating that additional, IL-10-independent, protective pathways are modulated by IL-27R signaling during malaria infection. These data are the first to demonstrate the essential role of IL-27R signaling in regulating effector T cell function during malaria infection and reveal a novel pathway that might be amenable to manipulation by drugs or vaccines.


It is well established that much of the pathology associated with blood-stage malaria infections is a result of excessive production of proinflammatory cytokines, including TNF-α, lymphotixin, and IFN-γ or insufficient production of anti-inflammatory cytokines, including IL-10 and TGF-β (reviewed in Refs. 1 and 2). Thus, it is necessary to define the pathways that regulate proinflammatory responses during malaria infection so that new therapeutic strategies and better vaccines can be developed. A recently identified regulatory cytokine, IL-27, has been shown to play an important role during a variety of infections (reviewed in Refs. 3 and 4), but its role during malaria infection has not yet been examined.

IL-27 is a heterodimeric cytokine composed of the IL-12p40–related molecule, EBV-induced gene 3, and p28, an IL-12p35–related subunit (5). IL-27 is produced by cells of the innate immune system, primarily macrophages and dendritic cells (DCs) (reviewed in Ref. 3). The IL-27R complex consists of the specific IL-27Rα subunit, WSX-1, a type I cytokine receptor, and gp130, the IL-6R subunit (6), and is expressed by numerous cells of the immune system, including CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells as well as monocytes, Langerhans cells, DCs, and NK cells (3, 7). IL-27R activates STAT1, STAT3, STAT4, and STAT5, with disparate downstream effector functions depending on the precise signaling pathway used (reviewed in Ref. 3).

IL-27 exerts both proinflammatory and suppressive effects on T cells, augmenting Th1 polarization by the induction of T-bet and increasing expression of ICAM-1 and responsiveness to IL-12 (4, 8–13), and suppressing CD4<sup>+</sup> T cell proliferation and effector function, for example, via suppressor of cytokine signaling 3-dependent downregulation of CD28-mediated IL-2 production (14, 15) or downregulation of the ROE/IL-17 pathway (16–19). IL-27 also promotes the production of IL-10 by various effector CD4<sup>+</sup> T cell populations, including Th1 and Th2 cells and CD4<sup>+</sup> T cells polarized under Th17-inducing conditions (20–25). In line with these disparate roles, IL-27 signaling is essential for the generation of early protective T cell responses during Leishmania major (9) and bacillus Calmette-Guérin (8) infections but is required for the suppression of Th1- and Th17-mediated inflammation during Toxoplasma gondii, Trypanosoma cruzi, Mycobacterium tuberculosis, Leishmania donovani, and nonhealing L. major infections (17, 26–31).

As with CD4<sup>+</sup> T cells, IL-27 also positively and negatively regulates APCs. IL-27R–deficient DCs are hyperresponsive to LPS, with increased expression of CD80 and CD86 compared with wild-type (WT) DCs (32). In addition, administration of rIL-27 suppresses production of reactive oxygen intermediates, TNF-α and IL-12, by activated macrophages in vitro (30, 33). Although not responsible for the differentiation of alternatively activated macrophages, IL-27 may play an important role in the modulation of alternatively activated macrophage function (34). In contrast, IL-27 augments production of TNF-α, IL-12, IL-6, and IFN-γ by human monocytes/macrophages (35, 36).

The ability of IL-27 to both promote and inhibit inflammation suggested that it may play a role during malaria infection, where the outcome of infection is determined by the balance of pro- and
anti-inflammatory responses. Thus, we have compared the outcome of virulent *Plasmodium berghei* NK65 infection in WSX-1−/− mice and WT control mice. As expected, WT mice developed unremitting parasitemia and succumbed to infection after ~30 d. Interestingly, WSX-1−/− mice succumbed to infection much more rapidly than WT mice despite very low parasite burdens. The death of *P. berghei* NK65-infected WSX-1−/− mice was due to liver necrosis secondary to exacerbated Th-1 responses. This study is the first to demonstrate an essential role for IL-27 during malaria infection and affirms the pivotal role played by IL-27 in limiting Th1-mediated pathology in highly proinflammatory disease settings.

**Materials and Methods**

**Mice and parasites**

C57BL/6 and IL-10−/− mice were bred in-house or purchased from Harlan (Hillcrest, U.K.). Breeding pairs of IL-27R−deficient (WSX-1−/−) mice (9) were provided by Amgen (Thousand Oaks, CA). All transgenic strains were fully backcrossed to C57BL/6 mice. Animals were maintained under barrier conditions in individually ventilated cages. Cryopreserved parasites were passaged once through C57BL/6 mice before being used to infect experimental animals.

Six- to 10-wk-old mice (sex matched in each experiment) were infected by i.v. injection of 10^4 parasitized RBCs. Parasitemia was assessed every second day by the examination of Giemsa-stained thin blood smears. Body weight was assessed every second day and compared with preinfection weight. Mice were sacrificed on selected days postinfection (p.i.), and spleens and livers were removed. Single-cell suspensions from both organs were prepared by homogenizing through a 70-μm cell strainer (BD Biosciences, Franklin Lakes, NJ). Leukocytes were enriched from homogenized liver samples by resuspending cell pellets in a 32% Percoll-3% HBSS solution and centrifuging for 10 min to remove the floating hepatocytes. CD4^+ and CD8^+ T cells were purified by magnetic cell sorting (Miltenyi Biotec, Auburn, CA), according to the manufacturer’s instructions. Purity of sorted cells was routinely >95%. RBCs were lysed using BD RBC lysis buffer (BD Biosciences), and live cells were quantified by hemocytometer using trypan blue.

**Flow cytometry**

Phenotypic characterization of cell populations was performed by surface staining with anti-mouse CD4 (GK1.5), anti-mouse CD8 (H35-17.2), anti-mouse CD69 (H1.2F3), anti-mouse CD44 (IM7), and anti-mouse CD62L (MEL-14). For intracellular cytokine staining, splenocytes were stimulated in vitro for 5 h with PMA (200 ng/ml) and ionomycin (1 μg/ml) in the presence of brefeldin A. Cells were permeabilized with 1% saponin (Sigma-Aldrich, St. Louis, MO) and stained with anti-mouse IFN-γ (XMG1.2), anti-mouse TNF-α (MP6-XT22), or anti-mouse IL-17A (eBio17B7). All Abs were from eBioscience (Insight Biotechnology, Wembley, U.K.).

**Real-time PCR**

IL-2, IL-4, IL-10, IL-12p35, IL-17A, IL-22, IL-23p19, TNF-α, IFN-γ, RORc, Foxp3, GATA-3, and T-bet mRNA levels were quantified by real-time PCR (TaqMan) using validated gene expression assays from ABI Biosystems (Warrington, U.K.). RNA was extracted (RNeasy; Qiagen, Valencia, CA) and DNase I treated (Ambion/ABI, Austin, TX) prior to cDNA synthesis. cDNA expression for each sample was standardized using the housekeeping gene GAPDH. Cycling conditions were as follows: initialization 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.

**In vivo Ab treatments**

A total of 500 μg anti-CD4 (GK1.5) or anti-CD8 (53.6.72) Abs were injected i.p. every 3 d starting on day 7 p.i. In separate experiments, 250 μg anti–IFN-γ (XMG1.2) and anti–TNF-α (XT3.11) Abs were injected i.p. every 2 d starting on day 7 p.i. All Abs were obtained from Bio-Xcell (formerly Bio-Express, West Lebanon, NH). A total of 500 ng IL-10 or 100 ng rIL-27 (both R&D Systems, Abingdon, U.K.) were injected i.p. daily starting on the day of infection.

![FIGURE 1. IL-27R signaling significantly modulates the outcome of *P. berghei* NK65 infection. C57BL/6 and WSX-1−/− mice were infected i.v. with 10^4 *P. berghei* NK65 parasites and the course of infection was followed by monitoring parasitemia (A), percentage weight loss (B), and percentage survival (C, D). The level of liver pathology was examined in WT and WSX-1−/− mice on days 7 and 14 of infection (original magnification ×20). *p < 0.05 between WT and WSX-1−/−.](http://www.jimmunol.org/doi/abs/10.4049/jimmunol.1600224)
Histopathology

Tissues were removed on selected days p.i. and fixed in 10% formalin saline. Fixed tissues were paraffin embedded and sectioned before being stained with H&E (Independent Histological Services, London, U.K.). Slides were examined under ×20 magnification.

Statistical analysis

Statistical significance was determined, unless otherwise stated, using Student t test, with p < 0.05 taken as indicating a significant difference.

Results

IL-27R–deficient (WSX-1−/−) mice control infection with P. berghei NK65 but develop severe liver pathology

To determine the role of IL-27R signaling during malaria infection, we compared the outcome of P. berghei NK65 infection in WSX-1−/− (IL-27R–deficient) and C57BL/6 (WT) mice (Fig. 1). As expected, WT mice developed unremitting parasitemia and anemia, succumbing to infection after ~30 d with parasitemia in excess of 60%. Ablation of IL-27 signaling clearly modulated the course of P. berghei NK65 infection (Fig. 1), and WSX-1−/− mice displayed significantly reduced parasite burdens throughout the course of infection (Fig. 1A). Importantly, WSX-1−/− mice—despite reduced parasite burdens—exhibited significantly elevated and accelerated weight loss and succumbed to infection more rapidly than WT controls (Fig. 1B, 1C). Because mice normally tolerate parasite burdens of 5–10% without any significant clinical signs, we looked for alternative explanations for the deaths of the WSX-1−/− mice. Histological examination of the livers of WT mice 14 d p.i. revealed only minor abnormalities, including pigmented and hypertrophic Kupffer cells and some periportal inflammation. By contrast, at the same time point, livers of WSX-1−/− mice contained many large areas of hepatocellular necrosis with extensive infiltration by inflammatory leukocytes, including macrophages and lymphocytes (Fig. 1D).

IL-27R–deficient mice make exacerbated proinflammatory cytokine responses during P. berghei NK65 infection

The ability of WSX-1−/− mice to very effectively limit parasite replication at the expense of developing liver necrosis was consistent with the notion that IL-27 signaling is required to regulate proinflammatory immune responses during P. berghei NK65 infection. To test this hypothesis, circulating cytokine concentrations and hepatic cytokine gene expression profiles were compared between WSX-1−/− and WT mice (Fig. 2). Although cytokine expression profiles were broadly similar in WT and WSX-1−/− mice on day 7 of infection (Fig. 2A), expression levels of both IFN-γ and IL-12 were significantly higher in livers of WSX-1−/− mice than WT mice on day 14 p.i. (Fig. 2B). By contrast, expression of IL-17A, TNF-α, IL-10, IL-22, and IL-23 was similar in livers of WSX-1−/− and WT mice on days 7 and 14 p.i. (Fig. 2A, 2B). In accordance with the gene expression levels, significantly higher levels of serum IFN-γ were observed in WSX-1−/− mice than in WT mice on day 14 of infection (Fig. 2C). Serum IL-17A levels were also slightly, but significantly, higher in WSX-1−/− mice than WT mice on day 14 p.i. (Fig. 2D).

IL-27R signaling regulates IFN-γ and IL-10 production by splenic CD4+ T cells during malaria infection

To identify the source of proinflammatory cytokines during P. berghei NK65 infection, the activation status and cytokine production capacity of splenic lymphocytes were compared between

![FIGURE 2. IL-27R signaling suppresses IFN-γ production during P. berghei NK65 infection. A and B, The expression level of various genes was determined in the liver of WT and WSX-1−/− mice on days 7 (A) and 14 (B) of infection by real-time PCR. C and D, The levels of IFN-γ (C) and IL-17A (D) were determined in the plasma of WT and WSX-1−/− mice on day 14 of infection by ELISA. Results are representative of four separate experiments with three to five mice per group. *p < 0.05; **p < 0.01 between WT and WSX-1−/−.](http://www.jimmunol.org/issue/14/5/9/1/2484/Fig2.png)
infected WSX-1−/− and WT mice. Proportions and total numbers of splenic CD4+ and CD8+ T cells were comparable in WT and WSX-1−/− mice on both days 7 and 14 of infection, although the proportion of activated (CD44+) CD4+ T cells was slightly lower in WSX-1−/− mice than in WT mice on day 7 (Supplemental Fig. 1).

Splenic CD4+ T cells from infected WSX-1−/− mice (14 d p.i.) were, however, hyperresponsive to in vitro mitogen stimulation, with both the proportion of cells producing IFN-γ and the mean fluorescence intensity of IFN-γ staining being significantly higher in cells from WSX-1−/− mice than in WT CD4+ T cells (Fig. 3A, 3B). In confirmation, ex vivo IFN-γ gene expression was significantly higher in splenic CD4+ T cells from infected WSX-1−/− mice than in cells from WT mice (Fig. 3C). By contrast, WSX-1−/− and WT CD4+ T cells had similar capacity to produce TNF-α and IL-17A (Fig. 3), and enhanced IFN-γ production was restricted to CD4+ T cells as WSX-1−/− and WT CD8+ T cells produced similar amounts (data not shown). Notably, the frequency of IL-17A–producing CD4+ T cells in spleens of infected WT and WSX-1−/− mice was extremely low, indicating that P. berghei NK65 infection does not induce strong Th17 responses. Although we could not detect IL-10 production by either WT or WSX-1−/− CD4+ T cells by intracellular staining (results not shown), IL-10 gene expression was significantly lower in splenic CD4+ T cells from infected WSX-1−/− mice than in the corresponding cells from WT mice (Fig. 3C). There was no significant difference in splenic CD4+ T cell expression of T-bet between infected WSX-1−/− and WT mice, although expression of Foxp3, GATA-3, and IL-4 were significantly lower in WSX-1−/− CD4+ T cells, suggesting that splenic natural regulatory T cell and Th2 responses are attenuated during malaria infection in the absence of WSX-1 signaling. Surprisingly, the expression of IL-2 was significantly lower in WSX-1−/− CD4+ T cells compared with WT CD4+ T cells. Although it is possible that the lower parasite burdens in WSX-1−/− mice, rather than a direct result of the lack of WSX-1 signaling, contributed to differences in immunity, the increase in IFN-γ production in WSX-1−/− mice is highly suggestive that IL-27 exerts suppressive functions during infection.

**FIGURE 3.** IL-27R signaling is required to regulate splenic CD4+ T cell effector function during *P. berghei* NK65 infection. A and B, Representative dot plots and graphs showing the mean frequencies of IFN-γ, TNF-α, and IL-17A production by splenic CD4+ T cells derived from WT and WSX-1−/− mice on days 0 and 14 p.i. following in vitro restimulation with PMA and ionomycin. C, The expression level of various genes in WSX-1−/− splenic CD4+ T cells relative to WT splenic CD4+ T cells obtained on day 14 of *P. berghei* NK65 infection. Results are representative of four separate experiments with three to five mice per group. *p < 0.05 between WT and WSX-1−/−.
Accumulation of IFN-γ–producing CD4+ T cells in the livers of infected WSX-1−/− mice

Because the major pathology of malaria-infected WSX-1−/− mice was hepatic necrosis and inflammation, we next examined the frequencies, numbers, and effector functions of CD4+ and CD8+ T cells in the liver on day 7 (prior to onset of pathology) and day 14 (fulminant liver pathology in WSX-1−/− mice) (Fig. 4). There were no significant differences between the strains on day 7, suggesting that lack of IL-27R signaling does not affect the induction phase of the CD4+ T cell response (Fig. 4A–D). Similarly, the frequency, numbers and activation status of CD8+ T cells infiltrating the liver were also comparable between WT and WSX-1−/− mice on day 14 p.i. (Fig. 4E,4F). However, the proportion of hepatic leukocytes that were CD4+ T cells was significantly higher in WSX-1−/− mice than in WT mice on day 14 p.i. (Fig. 4A), and the total number of hepatic CD4+ T cells—as well as the numbers of activated (CD44+, CD62Llow, and CD69+) CD4+ T cells—was also significantly higher in WSX-1−/− mice (Fig. 4B,4F; results not shown). Thus, lack of IL-27R signaling leads to over-accumulation of activated CD4+ T cells in the liver during the second week of P. berghei infection.

As previously described for the spleen, CD4+ T cells purified from livers of WSX-1−/− mice on day 14 of infection were hyper-responsive to mitogenic stimulation, with significantly higher proportions of WSX-1−/− than WT CD4+ T cells producing IFN-γ and TNF-α (Fig. 5A, 5B). By way of confirmation, IFN-γ gene expression was also significantly higher in WSX-1−/− than WT CD4+ T cells when measured by real-time PCR (Fig. 5C). A significantly higher proportion of CD4+ T cells within the liver of naive WT and WSX-1−/− mice exhibited characteristics of memory or effector cells compared with CD4+ T cells within the spleen, leading to rapid production of cytokines by hepatic CD4+ T cells following ex vivo stimulation (Fig. 5A, 5B). Nevertheless, the frequencies of activated CD4+ T cells (of total CD4+ T cells) were comparable in the livers of WT and WSX-1−/− mice. Further staining with anti-CD3, anti-γδ TCR, and anti-NK1.1 confirmed that the major sources of IFN-γ in the liver of infected mice were CD4+ γδ T cells and not NK, NKT, or γδ T cells (data not shown). Although IL-17A gene expression was significantly lower in WSX-1−/− than WT CD4+ T cells (Fig. 5C), the frequency of IL-17A+ cells was extremely low in livers of both mouse strains (Fig. 5A, 5B), confirming the lack of evidence for a role for Th17 cells in liver pathology. Importantly, IL-10 gene expression was lower in WSX-1−/− than WT CD4+ T cells, showing that—as in the spleen—IL-27 is required for optimal CD4+ T cell production of IL-10 during malaria infection. Although IL-4 gene transcription levels were lower in hepatic CD4+ T cells of WSX-1−/− mice compared with WT mice, there were no significant differences in expression of the lineage-specific transcription factors GATA3, T-bet, Foxp3, or RORγt, suggesting that there were no major differences in Th polarization of liver accumulating CD4+ T cells in WSX-1−/− mice (Fig. 5C).

FIGURE 4. Increased accumulation of CD4+ T cells in the liver of WSX-1−/− mice during malaria infection. Frequency (A) and absolute numbers (B) of total liver accumulating CD4+ and CD8+ T cells on days 7 and 14 of P. berghei NK65 infection. C–F, Frequencies (C, E) and absolute numbers (D, F) of CD44highCD4+ and CD8+ T cells on day 7 (C, D) and day 14 (E, F) of infection. Results are representative of four separate experiments with three to five mice per group. *p < 0.05 between WT and WSX-1−/−.
Depletion of CD4+ T cells during infection prevents liver pathology in WSX-1−/− mice

To determine whether the influx of IFN-γ– and TNF-α–secreting CD4+ T cells into the livers of WSX-1−/− mice was the direct cause of liver damage or was a consequence of liver damage caused by other aspects of the infection, P. berghei NK65-infected WSX-1−/− mice were treated with depleting anti-CD4 Abs, and the course of infection and severity of liver damage was assessed (Fig. 6). As a control and to confirm the apparent lack of involvement of CD8+ T cells in the disease, another group of infected mice was treated with depleting anti-CD8 Abs. Ab treatment effectively depleted CD4+ and CD8+ T cells, respectively, in the spleen (data not shown) and liver (Fig. 6A, 6B).

Depletion of CD4+ T cells, but not CD8+ T cells, abrogated the very effective parasite clearance that was observed in untreated WSX-1−/− mice such that CD4+ T cell-depleted WSX-1−/− mice developed parasite burdens that were very similar to those seen in intact WT mice (Fig. 6C). Importantly, the livers of CD4+ T cell-depleted WSX-1−/− mice were indistinguishable from those of infected WT mice, with only small foci of leukocyte infiltration, whereas CD8+ T cell-depleted WSX-1−/− mice developed widespread hepatocellular necrosis that was indistinguishable from the pathology in intact WSX-1−/− mice (Fig. 6D). These data demonstrate that WSX-1 signaling is required to limit CD4+ T cell migration to and accumulation within the liver during this infection and that this regulatory pathway limits immune-mediated liver pathology during malaria.

Neutralization of IFN-γ or TNF-α fails to prevent liver pathology in WSX-1−/− mice

Having demonstrated that CD4+ T cells are directly responsible for initiation of liver pathology in WSX-1−/− mice during P. berghei NK65 infection, we next addressed the mechanism of T cell-mediated pathology. Because IL-17 production was comparable in infected WSX-1−/− and WT mice, and similar levels of IL-17 were produced by infection-derived WSX-1−/− and WT CD4+ T cells (Figs. 3, 5), Th17 cells were not expected to be involved in liver damage in this model. Consequently, we examined whether hepatic pathology in WSX-1−/− mice was induced via the overproduction of IFN-γ or TNF-α. Administration of neutral-
izing Abs to either IFN-γ or TNF-α to WSX-1−/− mice had no effect on parasitemia during the first 14 d of infection—indicating that parasite control in WSX-1−/− mice is independent of TNF-α and IFN-γ during the early stages of infection—but from day 16 p.i. onward, parasitemia was higher in anti–IFN-γ and in anti–TNF-α–treated animals (Fig. 7A). However, neither the neutralization of IFN-γ or TNF-α reduced the accumulation of CD4+ T cells in the liver of WSX-1−/− mice (Fig. 7B) or reduced the severity of liver pathology; widespread areas of hepatocellular and bridging necrosis and cellular infiltration were observed in the livers of control and Ab-treated mice (Fig. 7C). These data indicate that neither IFN-γ nor TNF-α are required for the development of liver pathology—or for the hepatic accumulation of CD4+ T cells—in WSX-1−/− mice during P. berghei NK65 infection.

IL-10−/− and WSX-1−/− mice develop severe hepatic immunopathology during P. berghei NK65 infection through distinct immunological processes

It has recently been reported that IL-27 can induce and/or enhance IL-10 production by CD4+ T cells (20–25). Accordingly, our observation of lower IL-10 gene expression in CD4+ T cells in spleens and livers of infected WSX-1−/− mice when compared with WT mice (Figs. 3, 5) suggests that IL-27 signaling is also required for optimal IL-10 production by CD4+ T cells during malaria infection. As we have previously shown an essential role for CD4+ T cell-derived IL-10 in limiting liver pathology during Plasmodium yoelii infection (37), we hypothesized that the severe liver pathology observed in WSX-1−/− mice was due to insufficient production of IL-10 by CD4+ T cells. Thus, to determine the importance of IL-27–driven IL-10 secretion during P. berghei NK65 infection, we compared the outcome of P. berghei NK65 infection in WT, WSX-1−/−, and IL-10−/− mice and examined the effect of treatment of WSX-1−/− mice with rIL-10 (Fig. 8).

As expected, both WSX-1−/− and IL-10−/− mice were significantly more able to control their infections than WT mice (Fig. 8A). However, weight loss in the latter stages of infection was less severe in IL-10−/− mice than in WSX-1−/− mice (Fig. 8B). This observation suggested that the pathology associated with loss of IL-27R signaling was not entirely IL-10 mediated. Also, although liver pathology was similar, in form and severity, in WSX-1−/− and IL-10−/− mice, with widespread areas of hepatocellular necrosis observed in both strains on day 14 of infection (Fig. 8C), there...
were differences in the CD4+ T cell response between the two strains of mice. Thus, although IL-10<sup>−/−</sup> mice had significantly higher numbers of total splenic CD4+ T cells than either WT or WSX-1<sup>−/−</sup> mice (Fig. 8D), numbers of liver-infiltrating CD4+ cells were significantly lower on day 14 p.i. in IL-10<sup>−/−</sup> mice than in WSX-1<sup>−/−</sup> mice (Fig. 8E). Frequencies of splenic IFN-γ<sup>+</sup>CD4<sup>+</sup> T cells did not differ between IL-10<sup>−/−</sup> and WSX-1<sup>−/−</sup> mice on day 14 of infection but were significantly higher in both strains than in WT mice (Fig. 8F).

Taken together, these data suggest that IL-27 and IL-10 may play distinct roles in the regulation of CD4<sup>+</sup> T cell migration and accumulation within the liver during <i>P. berghei</i> NK65 infection, with IL-27 signaling directly limiting CD4<sup>+</sup> T cell migration to, or accumulation in, the liver and IL-10 signaling controlling the activation and expansion of CD4<sup>+</sup> T cells within the spleen. Consequently, although treatment of <i>P. berghei</i> NK65-infected WSX-1<sup>−/−</sup> mice with rIL-10 led to higher parasitemia in the later stages of infection (Fig. 8G), and reduced the numbers of IFN-γ<sup>+</sup>CD4<sup>+</sup> T cells in the spleen (Fig. 8H), rIL-10 treatment did not prevent accumulation of CD4<sup>+</sup> T cells in the livers of WSX-1<sup>−/−</sup> mice and was not able to prevent liver pathology (Fig. 8I, 8J). Importantly, in control experiments within our laboratory using an equivalent dose and administration regimen, rIL-10 treatment led to increased parasite levels in IL-10<sup>−/−</sup> mice during <i>P. berghei</i> NK65 infection and in WT mice during lethal <i>P. yoelii</i> (XL) infection (data not shown). Thus, our results strongly point to IL-10–independent regulatory effects of IL-27 in the limitation of hepatic immunopathology during <i>P. berghei</i> NK65 infection.

**Discussion**

In this study, we have shown, for the first time, an essential role for IL-27R signaling in the regulation of proinflammatory Th1 responses and suppression of immune-mediated pathology during malaria. In the absence of IL-27 signaling, hepatic accumulation of effector CD4<sup>+</sup> T cells in <i>P. berghei</i> NK65-infected mice was exacerbated, leading to severe liver necrosis in the second half of
their infections. Importantly, despite evidence of exaggerated proinflammatory cytokine responses in WSX-1^{-/-} mice, our results suggest that the capacity of CD4^{+} T cells to migrate to and accumulate within the liver, rather than their ability to secrete proinflammatory cytokines such as IFN-\(\gamma\) and TNF-\(\alpha\), is the fundamental underlying cause of liver pathology. A crucial role for IL-27 in dampening immunopathology was also observed during nonlethal \(P. yoelii\) infection but not during acute \(P. berghei\) ANKA
infection (results not shown), indicating that IL-27 is a wide-ranging negative regulator of T cell function during long-term, chronic, malaria infections.

The precise mechanism of CD4+ T cell-mediated liver pathology in this model remains to be elucidated. Although the regulatory function of IL-27 is believed to be mediated in part via the induction of IL-10 secretion from CD4+ T cells (20–25), and although we have shown that CD4+ T cell-derived IL-10 is essential for the prevention of severe liver pathology during P. yoelii infection (37), it is clear from the studies presented in this paper that IL-27 does not mediate its effects during malaria infection (both P. berghei and P. yoelii; data not shown) simply by inducing IL-10. Thus, although the outcome of P. berghei NK65 infection in IL-10–/– and WSX-1–/– mice is superficially very similar, their immune responses are quite different. Although the administration of rIL-10 to WSX-1–/– mice cannot completely mimic endogenous IL-27–induced IL-10 production, rIL-10 treatment did affect T cell activation within the spleen and liver, demonstrating the effectiveness of the treatment regimen. As such, the inability of rIL-10 to prevent CD4+ T cell accumulation within the liver or ameliorate liver pathology in WSX-1–/– mice is highly indicative that IL-27 may function through additional IL-10–independent processes. These data suggest that IL-10–independent processes are in agreement with previous work published showing that WSX-1–/– and IL-10–/– mice behave differently following Trichuris muris infection and in the development of colitis (38). Furthermore, although IL-10 expression in splenic and liver accumulating CD4+ T cells was lower in infected WSX-1–/– mice than in WT mice, total IL-10 gene expression was not significantly reduced in the livers or spleens of WSX-1–/– mice, suggesting that, in the absence of IL-27R signaling, reductions in IL-10 production by CD4+ T cells may be compensated for by IL-10 production from other cells. However, this compensation fails to prevent accumulation of CD4+ T cells in the liver or the development of immunopathology, indicating that IL-27 suppresses liver pathology through IL-10–independent mechanisms, possibly by direct modulation of T cell function.

During inflammatory diseases and other protozoan infections, such as T. gondii and virulent strains of L. major, one function of IL-27 appears to be suppressing Th17 responses (16–19, 31). It is perhaps surprising, therefore, that we observed no differences in IL-17 responses, or in expression of RORγt, between WT and WSX-1–/– mice during P. berghei NK65 infection. Importantly, however, there was no indication that Th17 responses developed in either strain of mice during P. berghei NK65 infection, and we have previously been unable to identify any role for IL-17 or Th17 cells in mice infected with the related parasite P. berghei ANKA (R. Greig and K.N. Couper, unpublished observations); indeed, at present, there is no evidence that IL-17 contributes to the outcome of any murine malaria infection. On the contrary, given that there is abundant evidence that the pathology of malaria infections is mediated by inflammatory cytokine such as IFN-γ and TNF-α (reviewed in Refs. 1 and 2), it was very surprising that singular or combined neutralization of IFN-γ or TNF-α had no effect on the liver pathology of infected WSX-1–/– mice, although this is in agreement with a previous study where administration of anti–IFN-γ did not affect the outcome of T. gondii infection (15). Alternatively, WSX-1–/– CD4+ T cells may mediate their damaging effects on hepatocytes directly via FAS–FAS ligand or CD154–CD40 interactions and/or by the production of proteases (39–41). Experiments are under way to test these hypotheses and to elucidate the IL-27–dependent pathways controlling migration to and accumulation of effector CD4+ T cells within the liver during malaria infection.

In conclusion, we have described an essential role for IL-27 in limiting both the effector function of CD4+ Th1 cells and the migration and accumulation of these cells in peripheral organs such as the liver during malaria infection. Furthermore, we have shown that it is the failure to regulate T cell accumulation—rather than any defects in inflammatory or anti-inflammatory cytokine production—that most closely correlates with liver disease in IL-27R–deficient mice. These data add significantly both to our understanding of the pathways that regulate inflammation during malaria infections and to our understanding of the pathways by which IL-27 can modulate inflammation and tissue damage in different disease settings.

Disclosures
The authors have no financial conflicts of interest.

References


