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Disruption of Interleukin-27 Signaling Results in Impaired Gamma Interferon Production but Does Not Significantly Affect Immunopathology in Murine Schistosome Infection[▽]

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In schistosomiasis mansoni, parasite eggs cause hepatointestinal granulomatous inflammation and fibrosis mediated by CD4 T cells specific for egg antigens. The severity of disease varies extensively in humans and among mouse strains. Marked disease exacerbation induced in typically low-pathology C57BL/6 mice by immunization with schistosome egg antigens (SEA) in complete Freund's adjuvant (SEA/CFA) correlates with elevated production of the proinflammatory cytokines gamma interferon (IFN-γ) and interleukin-17 (IL-17), which are regulated by IL-12 and IL-23, respectively. Here we examined the effect on the schistosome infection of a third member of the IL-12 family of heterodimeric cytokines, IL-27, using SEA/CFA-immunized and unimmunized mice deficient in the IL-27 receptor chain WSX-1 (WSX-1$^{-/-}$). SEA-stimulated bulk mesenteric lymph node cells or CD4 T cells from 7-week-infected WSX-1$^{-/-}$ mice produced significantly less IFN-γ than did those from C57BL/6 mice, even though there was no difference between these mice in exacerbated hepatic egg-induced granulomatous inflammation or in the levels of IL-17 induced by immunization with SEA/CFA. A fraction of the cells in the granulomas stained positive for IL-27, but there were no significant differences between WSX-1$^{-/-}$ and BL/6 mice, nor were there differences in the number of CD4 T cells and eosinophils. A 24-week chronic infection resulted in markedly reduced levels of proinflammatory cytokines, including IFN-γ, in WSX-1$^{-/-}$ mice, but again the magnitude of immunopathology was not significantly different between the two groups. These findings indicate that despite the impaired IFN-γ production, IL-27 signaling has no significant effect on either the magnitude of egg-induced immunopathology or on its closest in vitro correlate, IL-17.

Schistosomiasis, caused by intravascular trematode helminths, is the second most prevalent potentially fatal tropical parasitic disease in the world, affecting more than 200 million people (10). The embolization of *Schistosoma mansoni* eggs in the host's liver results in pronounced granulomatous inflammation and fibrosis mediated by CD4 T cells sensitized against schistosome egg antigens (SEA). In the majority of affected individuals, the infection results in a relatively mild, chronic disease known as "intestinal" schistosomiasis; however, 5 to 10% of the patients suffer from a more severe form of the infection referred to as "hepatosplenic" schistosomiasis, characterized by hepatic fibrosis, portal hypertension, vascular shunting, ascites, gastrointestinal hemorrhage, and death (28). There is also marked heterogeneity in schistosome infection among different mouse strains; C3H and CBA mice develop severe disease, while C57BL/6 (BL/6) mice develop a milder form (7, 30, 31). Work with the mouse model has demonstrated that the host mounts an initial proinflammatory T helper 1 (Th1)-type immune response, which is superseded by a lasting anti-inflammatory Th2-type response (14, 28, 35); failure to down-regulate the proinflammatory response results in exacerbation of granulomatous inflammation, hepatocellular damage, and death. Aside from occurring naturally in the C3H and CBA strains, high pathology also develops in the absence of costimulatory signals or the anti-inflammatory cytokines interleukin-4 (IL-4) and IL-10 (17, 23, 27, 33, 34). Significantly, concomitant immunization with SEA emulsified in complete Freund's adjuvant (SEA/CFA) of normally low-pathology BL/6 mice causes marked exacerbation of pathology and death in a proinflammatory cytokine environment (30, 32).

The association of high pathology with a proinflammatory environment prompted investigation of the factors leading to such a response. IL-12 is a heterodimeric cytokine composed of IL-12p40 and IL-12p35 subunits, which has been identified as a key Th1-polarizing factor due to its ability to induce the production of gamma interferon (IFN-γ) (37, 38). Analysis of the role of IL-12 in murine schistosomiasis demonstrated that whereas mice deficient in the IL-12p40 subunit completely fail to up-regulate their immunopathology following immunization with SEA/CFA, IL-12p35-deficient mice exhibit exacerbated lesions similar to those seen in wild-type BL/6 mice (32). These findings, together with the high levels of IL-17 observed in the immunization-induced or natural high pathology in the CBA strain, suggested that IL-23 exerted far greater influence than IL-12 on the development of severe disease. IL-23, a novel cytokine composed of the same IL-12p40 subunit together with a specific IL-23p19 subunit (6), regulates IL-17, which is produced by a distinct subset of activated CD4 T cells with a memory phenotype (22, 26, 32). These Th17 cells have been shown to also play a role in the pathogenesis of autoimmune

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diseases, such as experimental autoimmune encephalomyelitis and collagen-induced arthritis (21, 25).

Given the profound impact of the IL-23/IL-17 pathway on the immunopathology of schistosomiasis, the current work was performed to assess the role of yet another recently identified member of the IL-12 family of cytokines, IL-27. Like IL-12 and IL-23, IL-27 is a heterodimeric protein consisting of an IL-12p40-like subunit, Epstein-Barr virus induced gene 3 (EBI3), and an IL-12p35-like subunit, p28. The IL-27 receptor (IL-27R) exhibits sequence homology with the IL-12 and IL-23 receptors and is composed of a signaling chain, gp130, and a cytokine binding chain, WSX-1 (19). IL-27 is produced primarily by macrophages and dendritic cells, whereas the IL-27R is expressed by a wider range of cells, which may explain the pleiotropic role of IL-27 (41). Upon binding of IL-27 to its receptor, several Jak/STAT signaling cascades are activated, including Jak1, STAT1, STAT3, STAT4, and STAT5. It has been postulated that IL-27 synergizes with IL-12 to launch an optimal Th1 response by priming naïve CD4 T cells for IL-12 recognition via up-regulation of the IL-12Rβ2 chain (19). Interestingly, IL-27 has been shown to significantly and differentially affect the course of, and the host immune responses to, several parasitic infections (20), and it was therefore of interest to assess its possible role in schistosomiasis. For this purpose, we used mice lacking the IL-27 binding chain of the IL-27R, WSX-1 (WSX-$1^{-/-}$ mice). Since the WSX-$1^{-/-}$ mice are on a low-pathology BL/6 background, they were either infected with schistosomes alone or infected with schistosomes in combination with SEA/CFA immunization, as this protocol allowed assessment of potential enhancing or dampening effects that IL-27 could have on the outcome of disease. The salient findings of our study indicate that the absence of IL-27 signaling results in impairment of IFN-γ production but does not significantly affect the severity and course of the experimental schistosome infection or the levels of its immediate in vitro correlate IL-17.

MATERIALS AND METHODS

Mice, infection, and treatments. WSX-$1^{-/-}$ and control BL/6 mice (both H-2^b) were used in this study. The WSX-$1^{-/-}$ mice were provided by Christiaan Saris, Amgen Inc. (Thousand Oaks, CA), and were bred in house. Five- to six-week-old BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained in the Animal Facility at Tufts University School of Medicine in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care guidelines. Mice were infected by intraperitoneal injection of 80 cercariae of *S. mansoni* (Puerto Rico strain), which were obtained from infected *Biomphalaria glabrata* snails provided by Fred Lewis of the Biomedical Research Institute (Rockville, MD). Some mice were immunized by subcutaneous injection of 50 μg of SEA/CFA, as described previously (30). Treatment of BL/6 mice with SEA/CFA causes marked exacerbation of egg-induced immunopathology; either SEA or CFA by itself is ineffective (30). Both immunized and unimmunized mice were examined after 7 weeks of infection; additional studies after a chronic 24-week infection were performed only with unimmunized mice due to the fact that SEA/CFA immunization causes early death around 7 to 8 weeks postinfection (30). SEA from *S. mansoni* was obtained from the Biomedical Research Institute and was prepared as described previously (5).

Cell preparations and cytokine determinations. Mesenteric lymph node cells (mLNC) were isolated from 7- and 24-week-infected WSX-$1^{-/-}$ and control BL/6 mice, as previously described (30). Cell preparations were >95% viable as determined by trypan blue exclusion. Bulk mLNC suspensions (5×10^6 cells/ml) were incubated in the presence or absence of 15 μg/ml of SEA. Additionally, mLNC suspensions were enriched for CD4 T cells using CD4 microbeads and positive selection columns (Miltenyi Biotec, Auburn, CA) and were cocultured at a concentration of 1×10^6 cells/ml together with 4×10^6 homologous irradiated splenic antigen-presenting cells in the presence or absence of 15 μg/ml of SEA. In all cases cultures were incubated for 48 h, and then the supernatants were tested for the presence of the cytokines IFN-γ, IL-17, IL-5, IL-10, IL-4, and IL-13 by enzyme-linked immunosorbent assays (ELISA). For IFN-γ, IL-5, IL-10, and IL-4 monoclonal antibodies, standard cytokines and protocols were obtained from BD-PharMingen (San Diego, CA), and for IL-17 and IL-13 monoclonal antibodies, standard cytokines and protocols were obtained from R&D Systems, Inc. (Minneapolis, MN).

Hepatic immunopathology and immunocytochemistry. Sections of liver samples fixed in 10% buffered formalin and processed by a routine histopathologic technique were stained with hematoxylin and eosin and examined by optic microscopy. The sizes of the granulomatous lesions were determined by computer-assisted morphometric analysis, as described previously (30). The cellularity of the granulomas was determined manually using the same sections. Using the same computer software, liver fibrosis was measured by automated colorimetric analysis of consecutive 5-μm tissue sections stained with picrosirius red (11). Taking advantage of the affinity of picrosirius red for collagen, this technique is based on the notion that positively staining areas accurately reflect collagen deposits in the tissues. Hepatic fibrosis in liver sections was determined by calculating the percentage of the liver area staining positive with picrosirius red. Eight to 15 fields at a magnification of ×40 were analyzed for each liver. Additionally, liver sections were frozen in OCT embedding medium, 5-μm cryostat sections were fixed in cold acetone for 10 s and washed with phosphate-buffered saline, and endogenous peroxidase was quenched with 0.3% H_2O_2. Prior to application of primary antibodies, tissue sections were blocked with 1.5% normal rabbit serum. The primary monoclonal antibodies, rat anti-mouse eosinophil major basic protein (MBP) provided by James Lee (Mayo Clinic, Scottsdale, AZ), rat anti-mouse CD4 (clone L3T4; BD Pharmingen, San Diego, CA), and goat anti-mouse IL-27p28 (R&D Systems Inc., Minneapolis, MN), were diluted 1:500, 1:100, and 1:20, respectively. Unbound primary antibody was removed with additional washes with phosphate-buffered saline, and samples were incubated with 1:100-diluted biotinylated anti-rat immunoglobulin G or anti-goat immunoglobulin G secondary antibodies (Vector Laboratories, Burlingame, CA). MBP-, CD4-, and IL-27-expressing cells were detected using an avidin-biotin-horseradish peroxidase complex (Vectastain Elite ABC; Vector Laboratories) and diaminobenzidine. The sections were counterstained with 0.1% methyl green and mounted with Vectashield (Vector Laboratories). IL-27-, MBP-, and CD4-positive cells in granulomas were counted manually.

Statistical analysis. Analysis of variance and Student's *t* tests were used to determine the statistical significance of the differences among groups. A P value of <0.05 was considered significant. Each individual experiment was conducted with groups of three to six mice, and 6 to 10 granulomas per liver section were evaluated for morphometric, cellularity and immunocytochemical analyses.

RESULTS

IFN-γ production, but not IL-17, IL-5, or IL-10 production, by SEA-stimulated bulk mLNC and CD4 T cells is significantly reduced in 7-week-schistosome-infected, SEA/CFA-immunized WSX-$1^{-/-}$ mice. Concomitant immunization of schistosome-infected BL/6 mice with SEA/CFA induces a severe form of schistosomiasis characterized by pronounced hepatic granulomatous inflammation with enhanced parenchymal mononuclear cell infiltration and death in a milieu marked by significant elevation of the levels of proinflammatory cytokines IFN-γ and IL-17 (30, 32). We have recently shown that liver immunopathology in schistosomiasis strongly correlates with the levels of IL-17, thus implicating this cytokine as a pathogenic factor in the development of the disease (32). Since the up-regulation of these two cytokines is controlled by IL-12 and IL-23, we investigated the potential role of yet another member of the IL-12 family, IL-27, using mice that lack the cytokine binding chain of the IL-27R, WSX-1. WSX-$1^{-/-}$ mice are incapable of binding IL-27 and cannot perpetuate the proper downstream signaling cascade. In WSX-$1^{-/-}$ and BL/6 mice, immunization with SEA/CFA sharply enhanced the SEA-stim-

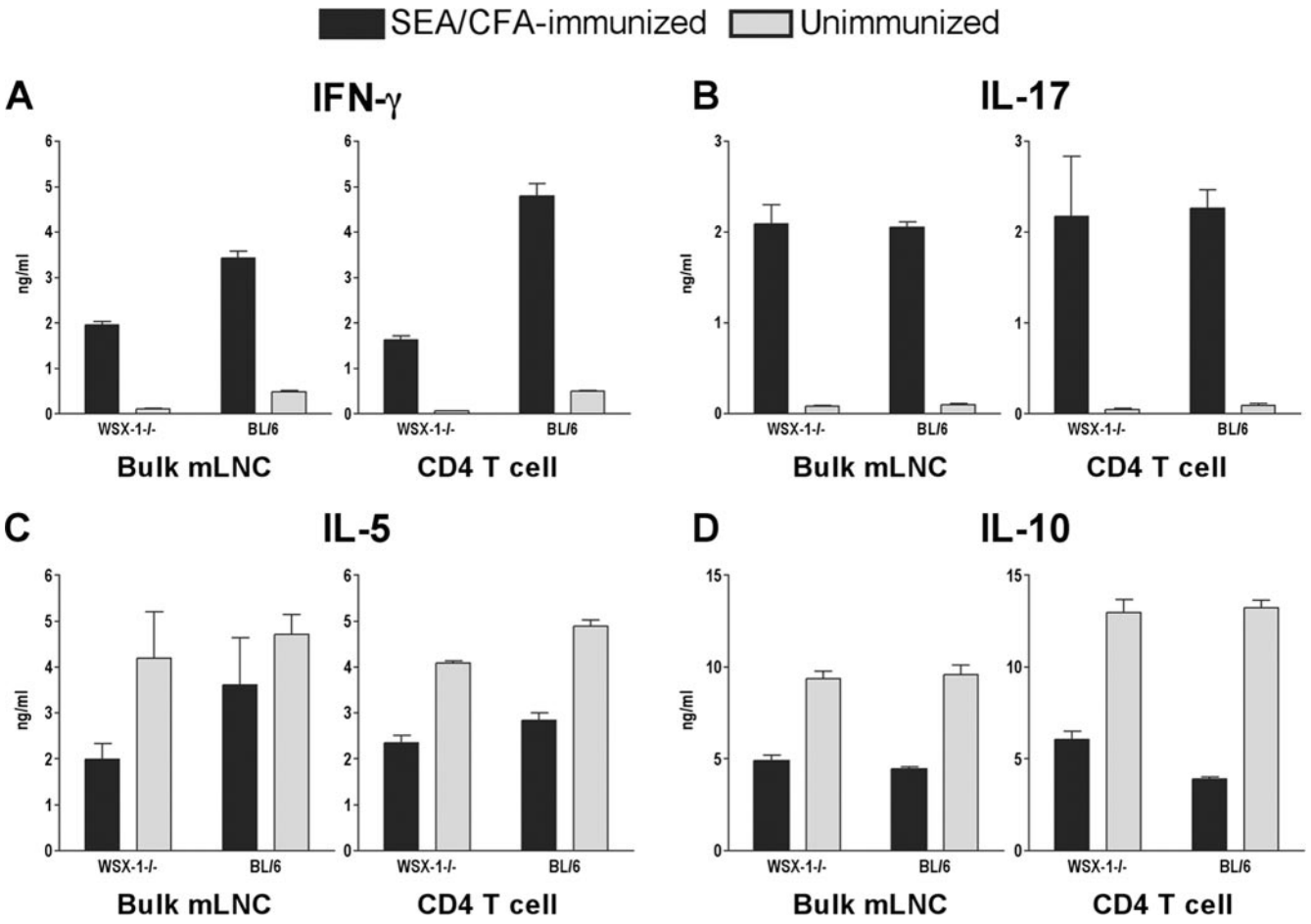

FIG. 1. SEA-induced cytokine production by bulk mLNC and CD4 T cells isolated from 7-week-schistosome-infected, SEA/CFA-immunized and unimmunized WSX-1$^{-/-}$ and BL/6 mice. Cytokine levels in 48-h supernatants from SEA-stimulated cell preparations were determined by ELISA as described in Materials and Methods. (A) IFN-γ production by bulk mLNC and CD4 T cells is significantly greater in SEA/CFA-immunized WSX-1$^{-/-}$ and BL/6 mice than in unimmunized WSX-1$^{-/-}$ and BL/6 mice ($P < 0.001$). However, both cell populations from the WSX-1$^{-/-}$ mice produce significantly less IFN-γ than the cell populations from the BL/6 mice ($P < 0.001$ for SEA/CFA-immunized groups and $P < 0.01$ for unimmunized groups). (B) IL-17 production by bulk mLNC and CD4 T cells is significantly greater in SEA/CFA-immunized WSX-1$^{-/-}$ and BL/6 mice than in unimmunized WSX-1$^{-/-}$ and BL/6 mice (both $P < 0.001$), but there are no differences in IL-17 production between the WSX-1$^{-/-}$ and BL/6 mice. (C and D) IL-5 (C) and IL-10 (D) production by bulk mLNC and CD4 T cells is lower in SEA/CFA-immunized WSX-1$^{-/-}$ and BL/6 mice than in unimmunized WSX-1$^{-/-}$ and BL/6 mice, and there are no differences in cytokine production by any of the cell populations between the two mouse groups. The cytokine values are representative of four independent experiments and are means ± standard deviations of triplicate determinations; background levels from unstimulated cell preparations were subtracted.

ulated production of IFN-γ by bulk mLNC, as well as CD4 T cells; however, in the WSX-1$^{-/-}$ mice this response was significantly diminished (Fig. 1A). By comparison, dramatic increases in IL-17 production by bulk mLNC and CD4 T cells were similar in the two mouse groups (Fig. 1B). The level of IFN-γ and IL-17 production was very low in unimmunized mice; again, the level of IFN-γ was lower in WSX-1$^{-/-}$ mice, and IL-17 was virtually absent in both groups (Fig. 1A and B). In contrast to the results obtained for IFN-γ and IL-17, SEA/CFA immunization caused reductions in the levels of the anti-inflammatory cytokines IL-5 and IL-10 in both the WSX-1$^{-/-}$ and BL/6 mice (Fig. 1C and D). Although not statistically significant, the decrease in the IL-5 level was more pronounced in the WSX-1$^{-/-}$ mice, whereas no difference was noted for IL-10. The levels of IL-4 production were also similar in WSX-1$^{-/-}$ and BL/6 mice (data not shown). These findings suggest that despite having a profound effect on IFN-γ levels, the absence of WSX-1 does not significantly alter the production of the pathogenic cytokine IL-17 or the anti-inflammatory cytokines IL-5, IL-10, and IL-4.

Absence of IL-27 signaling does not significantly affect the outcome of the immunopathology in a 7-week acute schistosome infection. Cytokine analysis demonstrated that there was significant impairment of IFN-γ production by WSX-1$^{-/-}$ mice (Fig. 1A), whereas the levels of IL-17, which have been shown to correlate with the intensity of egg-induced immunopathology (32), were no different from those measured in BL/6 mice (Fig. 1B). Histological analysis of the livers after 7 weeks of infection indeed demonstrated that there were no quantitative differences in granulomatous lesions between WSX-1$^{-/-}$ and BL/6 mice; in both cases there were similar marked increases in granuloma size caused by immunization with SEA/

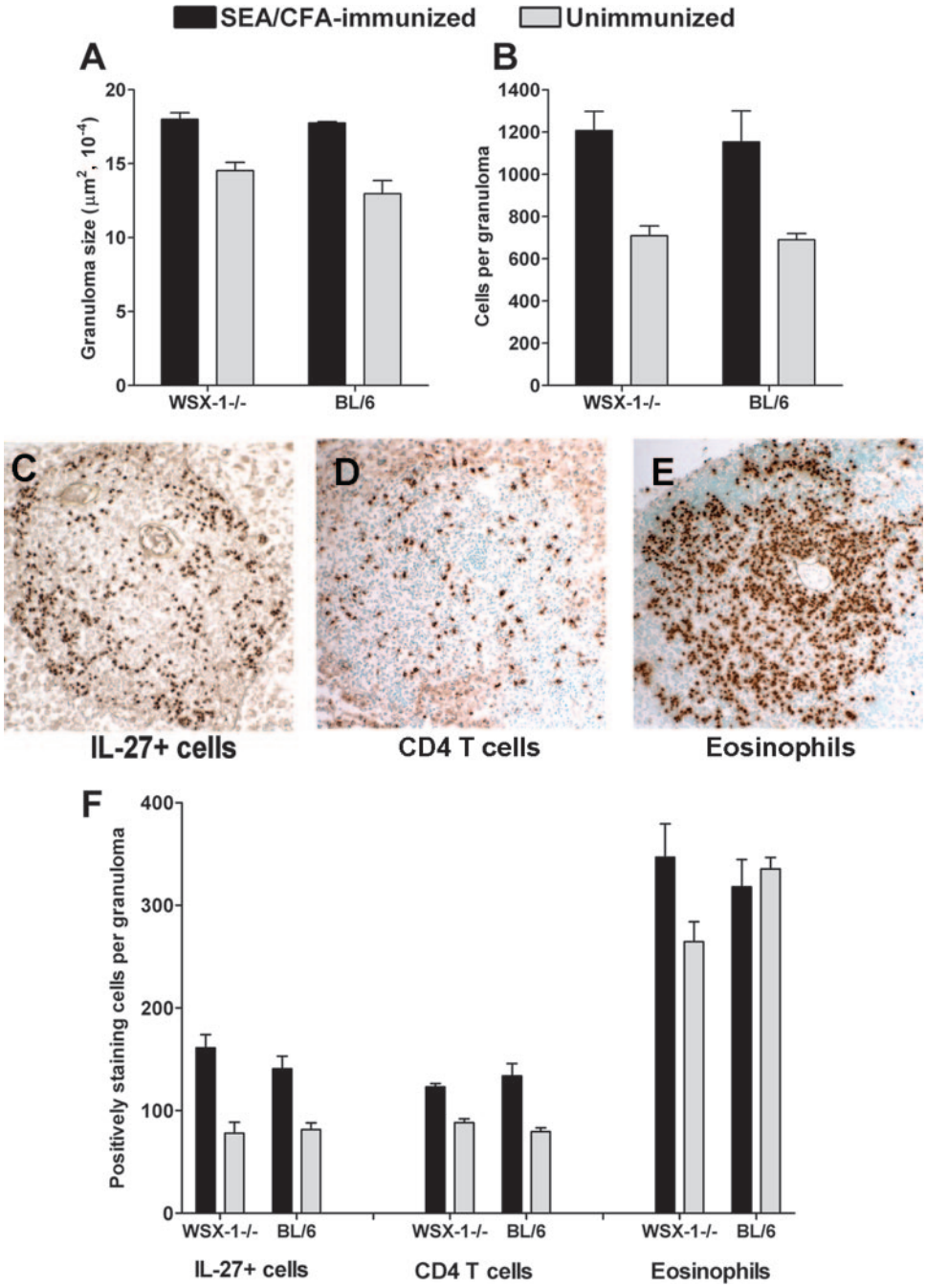

FIG. 2. Egg-induced immunopathology and cellular composition of granulomatous lesions in 7-week-schistosome-infected, SEA/CFA-immunized and unimmunized WSX-1$^{-/-}$ and BL/6 mice. Liver samples were obtained and granuloma size was measured using morphometric analysis. The cellularity was assessed by manual counting. The numbers of IL-27-producing cells, CD4 T cells, and MBP-expressing eosinophils within the granulomas were determined by immunocytochemistry as described in Materials and Methods. (A) Immunization with SEA/CFA results in a significant increase in granuloma size in both WSX-1$^{-/-}$ mice ($P < 0.01$) and BL/6 mice ($P < 0.001$), but there are no statistically significant differences between the two groups of mice. (B) Immunization with SEA/CFA results in a significant increase in granuloma cellularity in both WSX-1$^{-/-}$ and BL/6 mice (both $P < 0.01$), but there are no statistically significant differences between the two mouse groups. (C to E) IL-27-positive cells (C), CD4 T cells (D), and MBP-positive eosinophils (E) in representative granulomas from SEA/CFA-immunized WSX-1$^{-/-}$ mice. Magnification, ×100. (F) Quantitative analysis of positively staining cells in granulomas. Immunization with SEA/CFA results in an increase in IL-27-positive cells and CD4 T cells, but in no case is there a significant difference between the WSX-1$^{-/-}$ and BL/6 mouse groups.

CFA (Fig. 2A). Likewise, total cell counts for the granulomas revealed no significant difference in their cellularity. Thus, in SEA/CFA-immunized WSX-1$^{-/-}$ mice, the granulomas contained 1,206.3 ± 90.90 cells (mean ± standard error), and in the BL/6 mice the granulomas contained 1,152.4 ± 207.76 cells; in the unimmunized WSX-1$^{-/-}$ mice the cellularity was 707.8 ± 48.09, and in the unimmunized BL/6 mice the cellularity was 689.7 ± 41.40 (Fig. 2B). These findings, in conjunction with

our previous studies, support the idea that the severity of hepatic damage associated with schistosomiasis develops independent of IFN-γ production and emphasizes the importance of IL-17 in the development of pathology (32). Taken together, these data suggest that in an acute schistosome infection, egg-induced liver immunopathology is not regulated by IL-27.

Expression of IL-27 and the cellular composition of granulomatous lesions are not significantly different in WSX-1$^{-/-}$ and BL/6 mice. Immunocytochemical staining of liver sections obtained from 7-week-infected, SEA/CFA-immunized and unimmunized WSX-1$^{-/-}$ and BL/6 mice demonstrated the presence of IL-27-expressing cells in granulomas. These cells tended to be more frequent toward the periphery of the granulomas, although their precise phenotype could not be established (Fig. 2C). Treatment with SEA/CFA resulted in an increase in IL-27-positive cells; however, there were no significant differences in the numbers of these cells in granuloma sections between WSX-1$^{-/-}$ and BL/6 mice (Fig. 2F). CD4 T cells were detected in the granulomas, confirming observations made in a previous study (18). They also tended to congregate in the periphery of the granulomas (Fig. 2D) and appeared to be more numerous in the SEA/CFA-immunized mice. However, the differences in CD4 T cells between the WSX-1$^{-/-}$ and BL/6 groups were not significant (Fig. 2F), nor was their level of expression of the CD69 cell activation marker (data not shown). Lastly, the stain for MBP indicated that numerous, more evenly distributed eosinophils were present in the granulomas (Fig. 2E), but again, there were no significant differences between the SEA/CFA-immunized and unimmunized WSX-1$^{-/-}$ and BL/6 mouse groups (Fig. 2F).

Based on the total cellularity of the granulomas (Fig. 2B), the overall frequencies of IL-27-, CD4- and MBP-expressing cells in the SEA/CFA-immunized and unimmunized mouse groups were calculated to be 11.30 to 13.97, 10.67 to 12.81, and 27.58 to 48.65%, respectively. These values indicate that cell number largely correlated with granuloma size.

Overall SEA-stimulated bulk mLNC and CD4 T-cell cytokine production is down-modulated in 24-week-schistosome-infected mice, with IFN-γ levels again lower in WSX-1$^{-/-}$ mice. The balance between a proinflammatory and an anti-inflammatory immune response is a key factor influencing the outcome of disease in murine schistosomiasis (35). IFN-γ is typically regarded as a marker of a proinflammatory cytokine environment, but it has also exhibited antifibrotic activity by interfering with transforming growth factor β signaling and directly down-regulating collagen synthesis (3, 9, 12). Because the evolving, chronic schistosome infection results in incremental liver fibrosis (15) and there is significantly less IFN-γ production in WSX-1$^{-/-}$ mice, we examined 24-week-infected WSX-1$^{-/-}$ and BL/6 mice for possible differences in overall immunopathology and cytokine production, including production of IL-13, which is a mediator of fibrosis (8, 13). Early death of SEA/CFA-immunized mice at 7 to 8 weeks postinfection, which was not significantly different for WSX-1$^{-/-}$ and BL/6 mice, restricted the study to the unimmunized groups. After 24 weeks of infection, SEA stimulation of bulk mLNC and CD4 T cells elicited lower levels of cytokine production, an observation in agreement with the well-known down-modulation of the immunopathogenic response ("immunomodulation") observed in chronic schistosome infection (4, 28, 35). Thus, as shown in Fig. 3A and B, both proinflammatory IFN-γ and IL-17 levels produced by bulk mLNC and CD4 T cells were approximately 10-fold lower than the levels in the 7-week-infected mice and up to 100-fold lower than the levels in the 7-week-infected, SEA/CFA-immunized mice. Nevertheless, there was less IFN-γ secretion in the WSX-1$^{-/-}$ mice. There also appeared to be less IL-17 in this group, although the exceedingly low measured levels were at the limit of assay sensitivity and, in the case of CD4 cells, not significantly different. In comparison with the IFN-γ and IL-17 results, the reduction in the secretion of the anti-inflammatory cytokines IL-5 and IL-10 in the 24-week infection was smaller (Fig. 3C and D); this uneven down-regulation was not unexpected because in chronic infections with low-pathology strains the proinflammatory immune response is largely superseded by a predominantly Th2 cytokine environment (14, 28, 35). Interestingly, there was less IL-5 production by WSX-1$^{-/-}$ mice but no difference in IL-10 production; IL-13 production was also reduced in the WSX-1$^{-/-}$ mice (Fig. 3E).

Down-regulation of granulomatous inflammation in a 24-week schistosome infection is no different in WSX-1$^{-/-}$ and BL/6 mice, but WSX-1$^{-/-}$ mice develop slightly less liver fibrosis. Consistent with the marked inhibition of proinflammatory cytokines by SEA-stimulated mLNC and CD4 T cells after 24 weeks of infection, there was also down-regulation of the size of newly forming hepatic egg granulomas; however, the extent of immunomodulation was not significantly different between the WSX-1$^{-/-}$ and BL/6 mice (Fig. 4A). In the chronic infection, there were reduced inflammatory cell infiltrates with numerous plasma cells. There also was increased schistosome pigment deposition (36) and significant branching and anastomosing periportal fibrous scarring. The collagen component in the fibrotic areas, which stained strongly positive with picrosirius red, was measured by automated colorimetric analysis. Surprisingly, despite the similarity in the granulomatous lesions, the WSX-1$^{-/-}$ mice exhibited slightly less fibrosis than the BL/6 mice, although the difference was not significant (Fig. 4B).

DISCUSSION

In the murine model of schistosomiasis, severe egg-induced immunopathology is a consequence of a persistent proinflammatory state. The IL-12 family of cytokines plays a vital role in the induction of proinflammatory responses. In particular, high levels of IL-17, which can be induced in a proinflammatory milieu in the presence of transforming growth factor β and IL-6 and are sustained by IL-23 (39), correlate best with high pathology (32). IL-27, the newest member of the IL-12 family, is thought to play a role in the early Th1 response by priming naïve CD4 T cells for IL-12 recognition by inducing up-regulation of the IL-12Rβ2 chain (19), a function that becomes most apparent in the presence of IL-4 (1). Due to its obvious influence on the host immune response, the role of IL-27 has already been analyzed using several parasitic disease models (20), and it was therefore of great interest to examine its role in experimental murine schistosomiasis.

Our study involved the use of mice incapable of utilizing IL-27 because they lack the cytokine binding chain of the IL-27R, WSX-1. Since the WSX-1$^{-/-}$ mice are on a BL/6

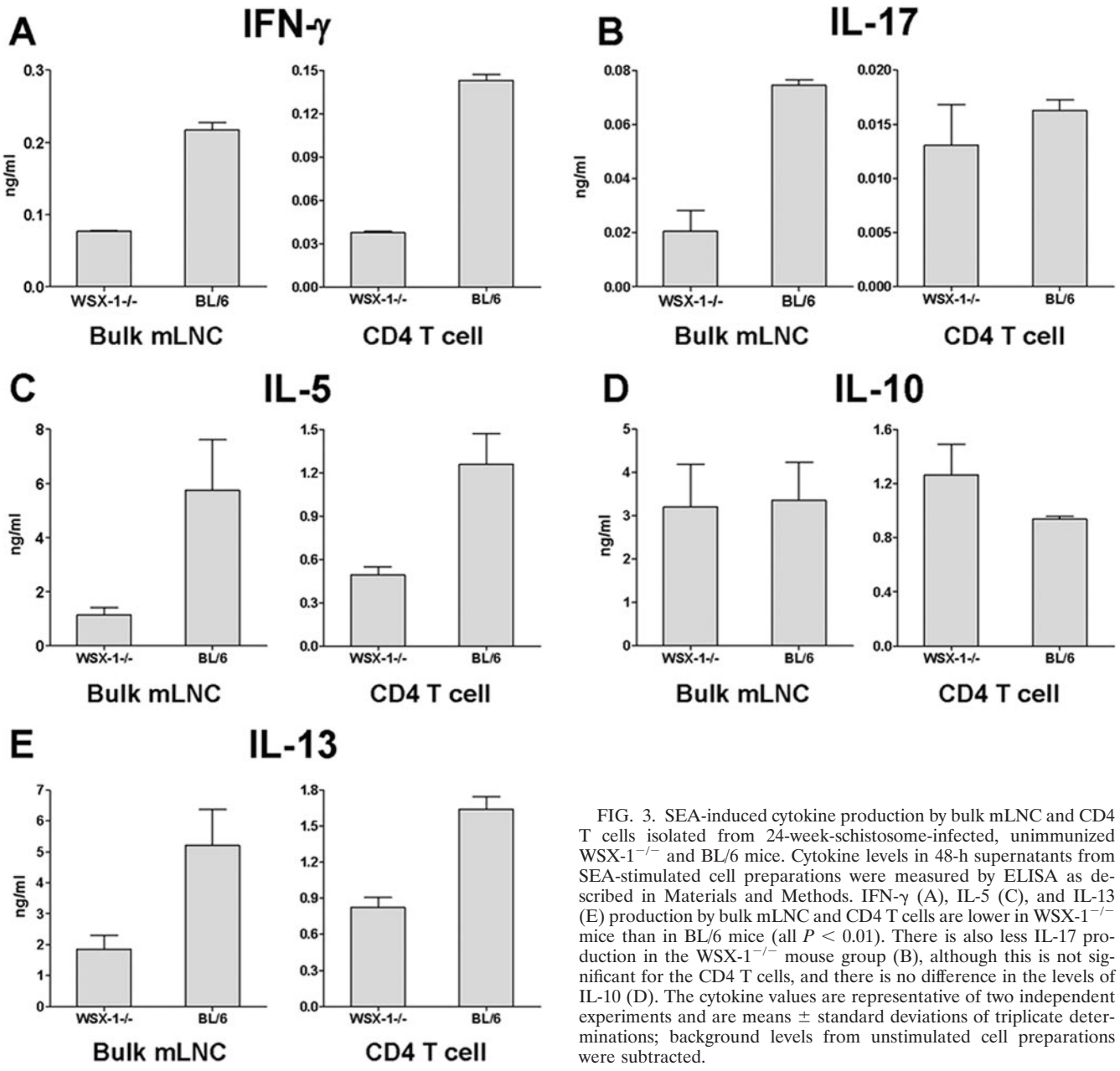

FIG. 3. SEA-induced cytokine production by bulk mLNC and CD4 T cells isolated from 24-week-schistosome-infected, unimmunized WSX-$1^{-/-}$ and BL/6 mice. Cytokine levels in 48-h supernatants from SEA-stimulated cell preparations were measured by ELISA as described in Materials and Methods. IFN-γ (A), IL-5 (C), and IL-13 (E) production by bulk mLNC and CD4 T cells are lower in WSX-$1^{-/-}$ mice than in BL/6 mice (all $P < 0.01$). There is also less IL-17 production in the WSX-$1^{-/-}$ mouse group (B), although this is not significant for the CD4 T cells, and there is no difference in the levels of IL-10 (D). The cytokine values are representative of two independent experiments and are means ± standard deviations of triplicate determinations; background levels from unstimulated cell preparations were subtracted.

(H-2^b) low-pathology background, they were infected with schistosomes in the absence or presence of pathology-exacerbating immunization with SEA/CFA (30), thereby making it possible to determine whether IL-27 signaling has an enhancing or ameliorating effect on the disease. In the acute 7-week schistosome infection, the salient finding was the significant impairment of SEA-stimulated IFN-γ production by bulk mLNC and CD4 T cells in either SEA/CFA-immunized or unimmunized WSX-$1^{-/-}$ mice, whereas there were no significant differences in the production of IL-17 or in the production of the anti-inflammatory cytokines IL-5, IL-10, and IL-4 between the homologous WSX-$1^{-/-}$ and BL/6 mouse groups. A possible explanation for the lower levels of IFN-γ versus IL-17 in the WSX-$1^{-/-}$ mice is that IL-27 specifically upregulates CD4 T-cell expression of IL-12Rβ2, which is required for the binding of IL-12 and consequently the stimulation of IFN-γ production (19). By comparison, normal levels of IL-17 are likely due to the fact that this cytokine is regulated by IL-23, which binds to and signals via the IL-12Rβ1 common and IL-23-specific receptors and is therefore independent of IL-12Rβ2.

Analysis of liver immunopathology in the 7-week-infected WSX-$1^{-/-}$ and BL/6 mice revealed comparable granulomatous inflammation, both quantitative and qualitative, despite the difference in IFN-γ production; this observation is consistent with the notion that IL-17, but not IFN-γ, best correlates with the magnitude of disease (32). Immunohistochemical studies demonstrated the presence of IL-27-expressing cells in

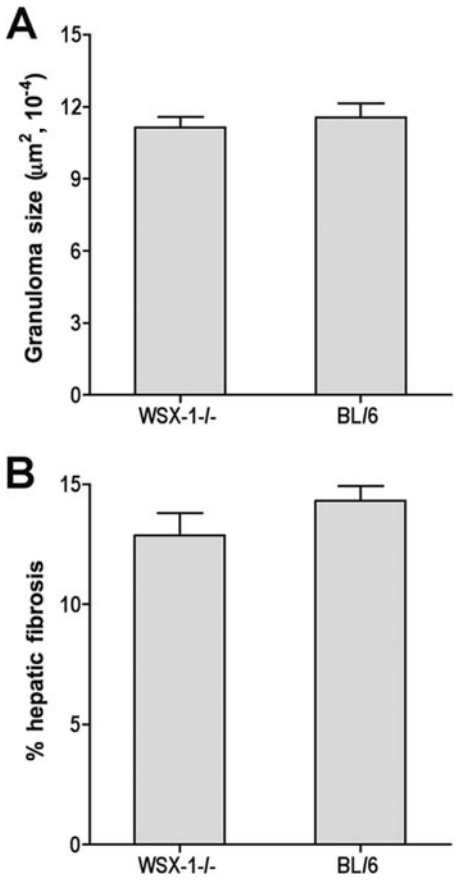

FIG. 4. Egg-induced hepatic granulomatous inflammation and fibrosis in 24-week-schistosome-infected, unimmunized WSX-1$^{-/-}$ and BL/6 mice. (A) Granulomatous inflammation in 24-week-infected WSX-1$^{-/-}$ and BL/6 mice was measured using morphometric analysis as described in Materials and Methods. There was no significant difference in the size of egg-induced granulomas between WSX-1$^{-/-}$ and BL/6 mice. (B) Hepatic fibrosis in picrosirius red-stained liver sections was measured by automated colorimetric analysis as described in Materials and Methods. There was no statistically significant difference between the two mouse groups. The data are representative of two independent experiments.

the granulomas, indicating their recruitment and participation in these schistosome egg-induced inflammatory lesions. However, the absence of the WSX-1 chain did not significantly impact the number of these cells in the granulomas. Further inquiry into possible qualitative disparities in the cellular makeup of the granulomas revealed no significant differences in CD4 T cells and eosinophils between the WSX-1$^{-/-}$ and BL/6 mouse groups.

Given the significantly impaired production of IFN-γ observed in WSX-1$^{-/-}$ mice, it was of interest to explore how this deficiency was reflected in the chronic schistosome infection, which in low-pathology BL/6 mice is typically associated with decreased overall (but chiefly proinflammatory) T-cell cytokine production in response to SEA stimulation, downsizing of granulomatous inflammation to newly deposited parasite eggs, and a moderate but progressive increase in hepatic fibrous scarring. However, because of their expected death during the acute phase of the disease (30), this study could not be conducted with infected mice concomitantly immunized with SEA/CFA.

Our results showed that after 24 weeks of infection, cells from both WSX-1$^{-/-}$ and BL/6 mice produced vastly reduced amounts of the proinflammatory cytokines IFN-γ and IL-17 and that IFN-γ production, but not IL-17 production, remained significantly depressed in the WSX-1$^{-/-}$ group. The levels of IL-5 and IL-13 were also reduced in the WSX-1$^{-/-}$ mice, whereas the level of IL-10 was not significantly different from that in the BL/6 mice. Similarly, histopathological analysis of chronic liver disease revealed comparable down-modulation of granuloma size in both groups, but determination of liver fibrosis, which is a key component of the pathology in chronic schistosomiasis, demonstrated that there was slightly less hepatic fibrosis in the WSX-1$^{-/-}$ mice, although the difference was not significant. This finding correlated well with the reduced levels of the fibrogenic cytokine IL-13 (8, 13), even though the decrease in IL-13 is not consistent with observations for WSX-1$^{-/-}$ mice infected with the intestinal nematode helminth *Trichuris muris* (1). Moreover, if indeed the reduction in liver fibrosis was mostly due to lower IL-13 levels, it was seemingly unaffected by the drop in IFN-γ, which has been reported to be an antagonist of fibrosis (3, 9, 12). It is conceivable that the exceedingly low levels of IFN-γ and IL-17 in the chronic infection were below the threshold for biological activity.

Examination of the role of IL-27 in regulating disease resistance or susceptibility to parasitic infections has revealed surprising and unpredictable diversity (20). For example, in the acute stage of *Leishmania major* infection, WSX-1$^{-/-}$ mice produce lower levels of IFN-γ and increased amounts of the Th2 cytokine IL-4, leading to increased disease susceptibility due to higher parasite replication and enhanced lesion development. However, in the later stages of leishmaniasis, although IL-4 production remains elevated, IFN-γ production is similar to wild-type levels, allowing normal healing of parasite-induced cutaneous lesions and control of parasite replication (42). In *Trypanosoma cruzi* infection, WSX-1$^{-/-}$ mice display a more robust Th2 cytokine response, but regardless of an increase in the proinflammatory cytokines IL-6, tumor necrosis factor alpha, and IFN-γ, the mice exhibit elevated parasitemia, enhanced liver damage, and accelerated mortality (16). By comparison, in visceral leishmaniasis caused by *Leishmania donovani*, WSX-1$^{-/-}$ mice develop heightened resistance to disease together with enhanced liver pathology (29), and after infection with *Toxoplasma gondii*, they also mount protective immunity but are unable to down-regulate deleterious T-cell hyperactivation characterized by increased proliferation and IFN-γ production (40). Lastly, in *T. muris* infection, WSX-1$^{-/-}$ mice also display increased resistance as a consequence of a lower worm burden and accelerated worm expulsion by virtue of a stronger Th2-type immune response (1, 2); however, such an enhanced Th2 response exacerbates asthma (24). The remarkable discrepancies in the responses reflect unusually disparate consequences of IL-27 signaling, in terms of both regulation of the cytokine environment and, most importantly, host resistance or susceptibility to a given pathogen.

In conclusion, our findings describe the role of IL-27 in murine *S. mansoni* infection and its impact on cytokine regulation and the development of egg-induced immunopathology.

As observed in both the acute and chronic stages of infection, the absence of IL-27 signaling markedly reduced the levels of IFN-γ but did not alter the levels of IL-17 or the overall nature and course of the immunopathology. Taken together, these findings suggest that IL-27 function is necessary for optimal IFN-γ production but minimally affects the outcome of disease in schistosomiasis.

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