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Schistosome Infection of Transgenic Mice Defines Distinct and Contrasting Pathogenic Roles for IL-4 and IL-13: IL-13 Is a Profibrotic Agent

Padraic G. Fallon, Emma J. Richardson, Grahame J. McKenzie, and Andrew N. J. McKenzie

Experimental Schistosoma mansoni infections of mice lead to a dynamic type 2 cytokine-mediated pathological process. We have used IL-4-deficient, IL-13-deficient, and IL-4/13-deficient mice to dissect the role of these cytokines in the development of immune response and pathology following S. mansoni infection. We demonstrate that while both of these cytokines are necessary to develop a robust Th2 cell-driven, eosinophil-rich granuloma response, they also perform disparate functions that identify novel sites for therapeutic intervention. IL-13-deficient mice demonstrated significantly enhanced survival following infection, which correlated with reduced hepatic fibrosis. In contrast, increased mortality was manifest in IL-4-deficient and IL-4/13-deficient mice, and this correlated with hepatocyte damage and intestinal pathology. Therefore, we demonstrate that during a dynamic type 2 cytokine disease process IL-13 is detrimental to survival following infection, whereas IL-4 is beneficial.

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of endotoxemia; implicating IL-4 as a protective cytokine in schistosome infection. The combined removal of both IL-4 and IL-13 demonstrated that the positive effects resulting from the ablation of IL-13 were over-ridden by the detrimental pathology resulting from IL-4 removal. Moreover, combined cytokine deletion resulted in a phenotype that was considerably more severe and deleterious than that observed in the IL-4-deficient line. These data identify the potential benefits of targeting IL-13 removal, but also highlight the dangers of blocking both IL-13 and IL-4 concurrently.

Materials and Methods

Mice and parasitology

The preparation of IL-4 (26), IL-13 (13) and IL-4/13 (14) gene-targeted mice has been described. All animals had been back-crossed on a BALB/c background at least four times. Animals were housed under standard conditions in a specific pathogen free facility. A Puerto Rican strain of S. mansoni was used for all experiments. Six- to 8 wk-old female mice were percutaneously infected with S. mansoni (27). In three separate experiments, mice were acutely infected (exposure to 100 cercariae) for 8 wk. In all acute infections, 6–12 mice were used per group. In separate studies, IL-13−/−, IL-4/13−/−, and homozygous (+/+) litter-mates on a 129 × C57BL/6 (F2) background were chronically infected (exposure to 25 cercariae) for 16 wk. For chronic infections, 14–15 mice were infected per group. Portal perfusion for worm counts, digestion of tissue for egg counts, and fecal egg counts were as described (27, 28).

Pathology studies

The methods used for measurement of pathological parameters have been described (29). In brief, liver or intestinal sections were stained with hematoxylin and eosin for granuloma detection and for eosinophil counts, or stained with Martius Scarlet Blue for examination of tissue fibrosis. All histological samples (histology slides, plasma) were numerically coded before analysis. The same individual measured all pathological parameters blind. The diameters of the granuloma surrounding individual eggs were measured using an ocular micrometer. For each mouse, the diameters of at least 21 individual egg granulomas were measured, with 6–12 mice examined (minimum of 126 individual granulomas measured per group in each of three separate experiments). The volume of the granuloma was calculated assuming a spherical shape. Tissue collagens were determined by differential staining of sections (three per mouse) on slides and was expressed as the percentage of tissue collagen. To determine hepatocyte damage, plasma aspartate aminotransferase levels were assayed (Sigma, Dorset, U.K.), LPS was measured in plasma using a commercial kit (COATEST Plasma-Endotoxin; Chromogenic AB, Molndal, Sweden); plasma samples were diluted in endotoxin-free water and assayed according to the manufacturers instructions. Intestinal eosinophils were determined using the eosinophil peroxidase assay, essentially as described (30). The numbers of eosinophils (expressed as 106 per gram of tissue) were interpolated from a standard curve prepared from eosinophils purified from the blood of Nippostrongylus brasiliensis-infected mice.

Immunological assays

Animals were sacrificed on day 45 after infection, before substantial deaths in IL-4−/− and IL-13-deficient mice, for analysis of their immune responses. Plasma and blood were recovered. The spleen and mesenteric lymph node cells were aseptically removed. Single-cell suspensions were prepared as described (31). Cells (5 × 106/ml) were stimulated with parasite Ags (10 μg/ml or Escherichia coli LPS serotype 0127:B8 (1 μg/ml; Sigma), IL-4, IL-5, IL-10, IL-13, TNF-α, and IFN-γ were detected in culture supernatants by ELISA as described previously (14, 29). The Griess reaction (32) was used to quantify nitrite levels in the supernatants by ELISA.

FIGURE 1. Mortalities of IL-4−/−, IL-13-deficient, IL-4/13-deficient, and wild-type mice during S. mansoni infection. A, Mice were acutely infected with 100 S. mansoni cercariae, and mortalities were monitored until week 8 after infection. There was no statistical difference in the mortalities of IL-4-deficient or IL-4/13-deficient groups. IL-13-deficient mice had significantly fewer mortalities during an acute infection than wild-type mice (p < 0.05). Eleven to 14 mice were infected per group, and data are representative of three separate experiments. B, Following a chronic infection (25 cercariae), mortalities were followed until 16 wk postinfection. Fourteen to 15 mice were used per group. There was significantly greater (p < 0.05) survival of IL-13-deficient mice relative to wild-type animals.

Statistical analysis

Data from individual mice are presented as the group mean ± SD or SEM, as indicated. Statistical differences between groups was determined using ANOVA, and post hoc comparisons were done with Dunnett’s test. Differences between survival of mice were analyzed by Kruskal-Wallis test of ranked survival times. Values of p < 0.05 were considered significant.

Results

IL-13 deficiency enhances survival, while IL-4 deficiency induces mortality during schistosome infection

Following acute infection with S. mansoni (exposure to 100 cercariae), significant mortality was observed in the IL-4−/− and IL-4/13-deficient mice, with >80% expiration by day 56 (Fig. 1A). Death was preceded by progressive weight loss and cachexia (data not shown). In marked contrast, there were considerably fewer mortalities in wild-type animals (13%) and no deaths in the IL-13−/− mice (Fig. 1A). In three separate acute infections, IL-13−/− mice had significantly lower mortalities than wild-type animals (p < 0.05). A similar mortality profile was observed when animals were subjected to a chronic (exposure to 25 cercariae) infection (Fig. 1B). All IL-4/13-deficient mice (100% mortality) died by

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week 10, whereas by 16 wk of infection significantly fewer wild-type mice (36% mortality) and still fewer IL-13−/− mice (7% mortality) had expired (p < 0.05; n = 14–15 mice per group). Determination of parasite worm burdens and parasite fecundity demonstrated that the differences in mortality between groups were not simply due to variation in infectivity or fecundity of the parasite between the mouse lines (Table I). These data demonstrate that the absence of IL-13 was beneficial for infected animals, with IL-13−/− mice surviving longer than wild-type animals. Conversely, deficiency in IL-4 results in increased mortality, suggesting that IL-4, or IL-4-dependent responses, have a protective role in infection.

Granuloma formation and eosinophil infiltration are abrogated only in the combined absence of IL-4 and IL-13

During schistosome infection, the liver is the major organ affected, with eggs trapped in the liver parenchyma evoking type 2 cytokine-dependent granulomatous inflammation. This is characterized by the presence of numerous infiltrating eosinophils, but also by the deposition of collagen and a resultant fibrotic lesion. Histological analysis identified that while wild-type, IL-4−/−, and IL-13−/− mice had comparable hepatic granuloma formation, there was a striking reduction in granuloma development in the IL-4/13-deficient mice (Fig. 2a and Fig. 3A). Additionally, granulomas from the IL-4/13-deficient animals were virtually devoid of the characteristic eosinophil infiltration present in the granulomas of the other mouse lines (Fig. 3B). Instead, the limited granuloma response from the IL-4/13-deficient animals was comprised of macrophages (data not shown).

**Fibrosis is significantly diminished in the absence of IL-13**

Because hepatic fibrosis is a major clinical manifestation of schistosomiasis, we examined collagen deposition in the livers of infected mice. Histological analysis and collagen quantification identified that there was negligible hepatic fibrosis in the IL-13-deficient or IL-4/13-deficient mice when compared with wild-type or IL-4-deficient mice (Fig. 2b and Fig. 3C). Although schistosome-infected mice do not develop portal fibrosis to the same extent as schistosome-infected humans (34), it is noteworthy that IL-4/13-deficient mice also displayed reduced periportal fibrosis compared with the collagen deposition in wild-type animals (Fig. 2b).

**IL-4 protects against liver damage**

To determine whether the alterations in the liver pathology generated in the different mouse strains affected hepatocyte integrity, we measured plasma aspartate aminotransferase levels as an indicator of cell damage. IL-13-deficient mice had similar transaminase levels to wild-type mice (Fig. 3D). In contrast, although IL-4-deficient mice demonstrated normal granulomatous responses, these animals had ~5-fold greater levels of circulating transaminase than wild-type mice. Hepatocyte damage was even more markedly exacerbated in IL-4/13-deficient mice, with transaminase levels 10-fold higher than wild-type mice (Fig. 3D). Elevated plasma transaminase levels and impaired hepatic granuloma responses in schistosome-infected mice have been associated with steatohepatitis and extensive microvesicular steatosis (29, 35). However, there was no microvesicular damage in IL-4/13-deficient mice surviving longer than wild-type animals. Conversely, deficiency in IL-4 results in increased mortality, suggesting that IL-4, or IL-4-dependent responses, have a protective role in infection.

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** Hepatic pathology in IL-4-deficient, IL-13-deficient, IL-4/13-deficient, and wild-type mice during *S. mansoni* infection. *a*, Haematoxylin-and eosin-stained sections of livers from infected mice showing the granulomatous response surrounding a parasite egg (arrow). Original magnifications, ×100. *b*, Martius Scarlet Blue staining of sections of livers demonstrates the absence of fibrosis (blue staining) in IL-4/13-deficient mice compared with wild-type mice. The large arrow identifies a single egg and the small arrow identifies deposition of collagen around portal tracts. Original magnifications, ×40.
mice, although there were sporadic polymorphonuclear leukocyte infiltrations (primarily neutrophils) and foci of necrosis throughout the hepatic parenchyma (data not shown).

**IL-4 but not IL-13 has a distinct role in intestinal function during schistosome infection**

Intestinal pathology is a feature of schistosome infections of humans and mice. During *S. mansoni* infection, the intestine is subject to the insult of eggs laid by the adult worms in the mesenteric venules. These eggs must then translocate across the intestinal wall to the gut lumen for excretion in the feces. Examination of the gastrointestinal tracts from the infected mice revealed marked dis-tension and inflammation of the ileum, but not the colon, in the IL-4/13-deficient and IL-4-deficient mice (data not shown). In contrast, the intestines of IL-13-deficient mice were comparable to wild-type animals. The intestinal inflammation observed in the IL-4/13-deficient and IL-4-deficient mice correlated with dramatically impaired parasite egg excretion from the intestine (Fig. 4A), leading to an accumulation of eggs in the intestinal wall (Table I). The limited granulomatous response surrounding eggs in the ileums of IL-4/13-deficient and IL-4-deficient mice was characterized by limited eosinophil infiltration, <1 × 10⁶ eosinophils per gram of intestine; whereas in both wild-type and IL-13-deficient mice there was marked intestinal eosinophilia, 10 × 10⁶ eosinophils per gram.

Alterations in the physiological functions of the intestine may represent a pathological consequence of intestinal inflammation. In particular, the integrity of the gut as a barrier may become compromised leading to systemic leakage of intestinal contents and endotoxemia. Analysis of LPS in the plasma of the infected mice demonstrated ~20-fold greater levels of LPS in the circulation of IL-4-deficient and IL-4/13-deficient mice compared with levels in wild-type and IL-13-deficient mice (Fig. 4B). The levels of systemic LPS in the IL-4-deficient or IL-4/13-deficient mice are comparable to circulating endotoxin levels in mice suffering from acute endotoxemia (36). In support of this conclusion, proliferation responses to cecal bacteria, by splenocytes or mesenteric lymph node cells derived from these mice, were substantially elevated (data not shown). Furthermore, splenocytes from IL-4-deficient and, even more markedly from IL-4/13-deficient mice, produced highly elevated levels of TNF-α and NO in response to LPS (Fig. 4C). Thus, as reported previously, IL-4 represents an important factor in the response to *S. mansoni* infection (37). However, the additional absence of IL-13 exacerbated the intestinal pathology, the associated elevation in systemic LPS leakage, and the increase in TNF-α and NO production (Fig. 4, B and C), indicating that IL-13 can also modify this immune response.

In many models of gastrointestinal inflammation, there is an alteration in the normal cytokine repertoire of the intestine (38). To examine the cytokine profile elicited in the intestines of the cytokine-deficient animals, ileum-derived CD4⁺ lamina propria T cells from infected mice were examined using intracellular cytokine staining. Schistosome-infected wild-type and IL-13-deficient mice had 10–15% IFN-γ-positive CD4⁺ lamina propria T cells, with <5% of cells producing IFN-γ, indicative of a Th2-like phenotype in the intestines of infected mice (Fig. 4D). In contrast, CD4⁺ T cells from the lamina propria of both the IL-4-deficient and IL-4/13-deficient mice had 3-fold higher frequencies of IFN-γ-producing cells, with no IL-5-positive CD4⁺ T cells detected (Fig. 4D), typical of a Th1-like response. The absence of Th2 cells in the lamina propria of IL-4- and IL-4/13-deficient mice was reflected by the virtual absence of eosinophils in the ileums of these mice (see above).

**Systemic responses**

To determine how the absence of IL-4, IL-13, or IL-4 and IL-13 together influenced the development of cytokine responses to infection, we isolated splenocytes from infected mice 45 days postinfection (before high mortality in the IL-4/13-deficient population) and assessed cytokine production following restimulation in vitro with soluble schistosome egg Ags. Wild-type animals exhibited a type 2 cytokine response with elevated IL-4, IL-5, IL-10, and IL-13 production and limited expression of IFN-γ (Fig. 5). A similar profile was observed in the analysis of IL-13-deficient mice, with the exception that IL-13 was not detected (Fig. 4). IL-4-deficient mice developed a diminished type 2 cytokine response, with limited IL-5, IL-10, and IL-13 being produced and a marginal (~2-fold) increase in IFN-γ production compared with wild-type animals (Fig. 5). In contrast to the single cytokine-deficient animals, IL-4/13-deficient mice developed a type 1 cytokine dominated response, with a 10-fold increase in the secretion of IFN-γ and negligible production of type 2 cytokines (Fig. 5).

While IL-13-deficient mice had normal IgE and IgG1 isotype responses to infection, IL-4-deficient mice exhibited lower levels of IgE and IgG1 and increased IgG2a (data not shown). IL-4/13-deficient mice had undetectable levels of parasite Ag-specific IgE but highly elevated production of IgG2a (data not shown), in keeping with their enhanced expression of IFN-γ.
**Discussion**

This study demonstrates clear differences in the functions of these closely related cytokines in mediating Th2 cell responses to *S. mansoni* infection. The enhanced survival exhibited by the IL-13-deficient mice indicates that removal of IL-13 function can produce a beneficial effect on the outcome of infection, while the absence of IL-4 is detrimental. The improved prognosis in the IL-13-deficient mice correlated with a reduction in the expression of collagen and a resultant decrease in hepatic fibrosis. These data demonstrate that IL-13 has a unique role in hepatic fibrosis. A profibrotic role for IL-13 has also been shown in mice with targeted pulmonary expression of IL-13 transgenes, with these animals developing subepithelial airway fibrosis (39). More recently, a direct role for IL-13 in hepatic fibrosis during murine schistosome infection has also been demonstrated (25).

IL-4-deficient animals had normal levels of hepatic collagen deposition, whereas the doubly deficient mice exhibited a more profound reduction in collagen levels than the IL-13-deficient animals, indicating an additive effect of IL-13 and IL-4. The lower levels of hepatic collagen in IL-4/13−/− mice relative to IL-13−/− animals may reflect the elevated IFN-γ in double-deficient mice; IFN-γ has been shown to reduce hepatic fibrosis in murine schistosome infection (40). However, it is evident that IL-13 is the primary cytokine responsible for fibrosis. These data clarify the results reported for schistosome infections of STAT6- and IL-4R-deficient animals in which collagen deposition was found to be...
The role of type 2 responses is particularly apparent in the intestines of infected mice. During infections of normal mice, the process of egg translocation through the intestinal wall is associated with local granulomatous inflammation, eosinophilia, and a Th2 response (Fig. 4D, above). In contrast, in mice that fail to excrete parasite eggs (Refs. 29 and 35, this study), there is intestinal inflammation. The intestinal pathology in these mice is associated with a Th1-dominated response in the ileum and impaired recruitment of eosinophils to intestine. This implicates that type 2 cytokine responses, in particular intestinal eosinophilia, reduce intestinal damage elicited during schistosome infection.

In this study, cytokine-deficient animals have enabled us to decipher the individual effects of IL-4 and IL-13 and to assess the result of combined IL-4/13 disruption on the pathology of infection. These results are summarized in Table II, and demonstrate a clear demarcation of function for IL-4 and IL-13 in the development of pathology. It is clear that inhibiting IL-13 results in an improved prognosis that is characterized by the generation of a normal Th2 response, but an attendant decrease in hepatic fibrosis and an associated reduction in mortality. In contrast, IL-4-deficient mice display depressed Th2 responses, continue to deposit hepatic collagen, and succumb to severe intestinal inflammation resulting in endotoxemia and death. Analysis of the doubly deficient animals shows that combined blocking of both IL-4 and IL-13 is extremely detrimental, resulting in an even more severe phenotype than that displayed by the IL-4-deficient mice. IL-4/13-deficient animals demonstrate that the beneficial effects of removing IL-13 in isolation are overcome by the pathological consequences of removing IL-4 and highlight the additive roles that both of these cytokines play in suppressing the development of Th1 responses. These results indicate that IL-13 is a key target for therapeutic intervention.

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References


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Table II. Summary of the consequence of S. mansoni infection of wild type, IL-13-deficient, IL-4-deficient, and IL-4/13-deficient mice

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<tr>
<th>Liver</th>
<th>Intestine</th>
<th>Systemic</th>
</tr>
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<tbody>
<tr>
<td>Gr*</td>
<td>Eo</td>
<td>Fibrosis</td>
</tr>
<tr>
<td>Wild type</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-13−/−</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>IL-4−/−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-4/IL-13−/−</td>
<td>–</td>
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*Gr. Granulomatous inflammation.
*Eo. Tissue eosinophilia.
*Mortalities are expressed as percentage (ranges) of mice dead by 56 days after an acute infection.

Table II: Summary of the consequence of S. mansoni infection of wild type, IL-13-deficient, IL-4-deficient, and IL-4/13-deficient mice

- In the absence of IL-4, the cytokine response during schistosome infection is changed from a type 2 phenotype to a more type 1-dominated response. However, as reported previously, IL-4−/− mice do produce type 2 cytokines during infection (23, 37, 41,46). However, while IL-13-deficient mice retain a Th2 phenotype in response to infection, the doubly deficient animals verify that IL-13 does play an additive role with IL-4 in suppressing the emergence of a type 1 response. Thus, the IL-4/13-deficient animals display a highly polarized Th1-like phenotype. However, it is clear that IL-4 is the dominant factor in this process.

- Despite the formation of the schistosome granuloma being type 2 cytokine dependent, it has recently been highlighted that type 2 cytokine responses have a protective role in schistosome infection (34). Thus, in a number of different mouse models (IL-4-deficient mice; schistosome egg-tolerised mice; CD4+ T cell-depleted mice) there are marked mortalities during schistosome infection that are associated with diminished type 2 cytokine responses (29, 35, 37). In this study, we further demonstrate that the protective role of type 2 responses is particularly apparent in the intestines of infected mice. During infections of normal mice, the process of egg translocation through the intestinal wall is associated with local granulomatous inflammation, eosinophilia, and a Th2 response (Fig. 4D, above). In contrast, in mice that fail to excrete parasite eggs (Refs. 29 and 35, this study), there is intestinal inflammation. The intestinal pathology in these mice is associated with a Th1-dominated response in the ileum and impaired recruitment of eosinophils to intestine. This implicates that type 2 cytokine responses, in particular intestinal eosinophilia, reduce intestinal damage elicited during schistosome infection.

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