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A Critical Role for IL-13 in Resistance to Intestinal Nematode Infection

Allison J. Bancroft, Andrew N. J. McKenzie, and Richard K. Grencis

Mice in which either the IL-4 or the IL-13 gene has been disrupted (IL-4 KO and IL-13 KO) were susceptible to infection with the intestinal nematode Trichuris muris, whereas their wild-type littermates were highly resistant and expelled the parasite. IL-4 KO mice showed diminished Th2-type responses with T. muris infection and also failed to produce parasite-specific IgG1 Abs. Although IL-13 KO mice made reduced Th2-type responses early in infection, they were capable of generating strong Th2-type responses at later time points and were unable to regulate the magnitude of their Ab isotype response. These results confirm the importance of IL-4 in resistance to T. muris and provide the first demonstration of an important role for IL-13 in resistance to helminth infection. The IL-13 KO mouse had a separate phenotype to that of the IL-4 KO mouse, suggesting that both IL-4 and IL-13 play important yet different roles in mediating immunity to intestinal helminths. The Journal of Immunology, 1998, 160: 3453–3461.

There are much data showing that in intestinal helminth infections protective immunity is associated with the activation of a strong Th2-type response (reviewed in Ref. 1). In particular, IL-4 plays an important role in resistance for a number of species of nematode including, Heligmosomoides polygyrus, Trichinella spiralis, and Trichuris muris infection in the mouse (2) (reviewed in Refs. 3 and 4). In all of these systems, abrogation or neutralization of the IL-4 activity by administration of anti-IL-4/anti-IL-4R mAbs or infection of IL-4 knockout mice compromises the expulsion of the parasite from the intestine.

There are exceptions, however; for example, expulsion of Nippostrongylus brasiliensis from the mouse does not require IL-4, as IL-4 KO mice expel the worms from the gut in a manner equivalent to wild-type littermates (5). The mechanism of expulsion is unknown but is unlikely to be mediated by a Th1 response, because in all systems studied, promotion of a Th2 response is associated with depression of the protective response and prolonged parasite survival. Indeed, in the T. muris system, induction of a Th1 response promotes chronic infection in a normally resistant strain of mouse (4, 6) and such a response becomes dominant in strains of mouse that naturally harbor a chronic infection (7, 8). Interestingly, in the latter system, neutralization of the protective IL-4 response in vivo could only be achieved with anti-IL-4R mAb (M1) (4), but not anti-IL-4 (either clones 11B11 or 1D11). Treatment with anti-IL-4 mAb did however significantly reduce IgE levels and mast cell numbers coincident with an increase in parasite-specific IgG2a levels. IL-13, another Th2 cytokine, has been shown to utilize the α-chain of the IL-4 receptor. This finding raises the distinct possibility that IL-13 may play an important role in the protective process.

IL-13 is situated in close proximity to IL-4 on mouse chromosome 11. Although the amino acid sequence homology between IL-4 and IL-13 is low (~30%), there is a high level of conservation of the tertiary structure between IL-4 and IL-13 (9). IL-13 shares many but not all of the biologic functions of IL-4. IL-13 up-regulates a number of surface markers (for example, MHC class II on monocytes and macrophages) while down-regulating their production of inflammatory cytokines (10) and has been shown to protect mice from LPS-induced lethal endotoxemia by down-regulation of IFN-γ (11). Unlike IL-4, IL-13 is not a T cell growth factor, although it may influence T cell development. 

Data presented here show that IL-13 does indeed play a critical role in resistance to T. muris. In the absence of IL-13, T. muris proceeds to a chronic infection. The IL-13 KO mouse is phenotypically different than the IL-4 KO mouse. Importantly, the data indicate that without IL-13, animals are unable to expel their worm burdens even in the presence of a strong Th2-type response. Furthermore, the data show that the absence of IL-13 interferes with the ability to regulate the magnitude of the Ag-specific isotype responses. These data do not negate an important role for IL-4 in resistance, as we clearly show that IL-4 KO mice are susceptible to T. muris. However, we believe this susceptibility is due to the inadequate production of Th2 cells and therefore the inability to produce Th2 cytokines, including IL-13.

Materials and Methods

Mice

Female age-matched C57BL/6 mice in groups of four, which had targeted disruption of the IL-4 gene (IL-4 KO), were purchased from B&K Universal (Hull, U.K.) as IL-4T. These mice were generated as previously described (12). The mice were originally produced on a 129 × C57BL/6 background, but have been subsequently backcrossed onto C57BL/6 six times. Age- and sex-matched C57BL/6 mice were used as wild-type controls.

129 mice that had a disrupted IL-13 gene (IL-13 KO mice) were generated as described in McKenzie et al.4 Age- and sex-matched IL-13 KO mice were used in groups of four to five. Wild-type 129 littermates were also used as wild-type controls.

Parasites

_T. muris_ was maintained as described (13). Mice were killed at various time points after infection, and worm burdens were assessed as previously described (14).

Experimental protocol

Mice were infected on day 0 with approximately 300 _T. muris_ eggs. On day 10, the viability of the infection was assessed. On days 14 or 21 postinfection (p.i.), 3 mesenteric lymph node cells from infected and uninfected control IL-4 and IL-13 KO (and their wild-type littersmates) were removed and set up in culture as described below. The mice were exsanguinated, and serum was prepared for Ab analysis. The cecum and adjacent colon were also removed to determine worm burdens. On day 35 p.i., only serum and parasitologic samples were taken.

Parasite Ag

Adult worms were cultured in RPMI 1640 (Gibco Life Technologies, Paisley, U.K.) and excretory-secretory Ag (ES) was collected over the first 4 h of culture. The ES was pelleted to remove parasite eggs, concentrated using Minicon-B15 concentrator (Amicon, Gloucester, U.K.), and then dialyzed against PBS. Protein concentration was determined using a Lowry assay (15) and added to cultures at a predetermined optimal concentration of 50 μg/ml.

Cytokine analysis

Mesenteric lymph node cells (MLNC) were removed from infected and control uninfected mice. MLNC were resuspended in RPMI 1640/10% FCS/2 mM t-glutamine, 1000 U/ml penicillin, and 1000 μg/ml streptomycin. They were stimulated with either 2.5 μg/ml of Con A or 50 μg/ml of parasite ES. Anti-IL-4 mAb (M1) was added to all cultures at a concentration of 5 μg/ml to increase the sensitivity of IL-4 detection. MLNC were cultured at 37°C and 5% CO₂. Supernatants were collected at 24 h, pelleted to remove parasite eggs, concentrated using Minicon-B15 concentrator (Amicon, Gloucester, U.K.), and then dialyzed against PBS. Protein concentration was determined using a Lowry assay (15) and added to cultures at a predetermined optimal concentration of 50 μg/ml.

Ab analysis

Parasite-specific IgG1 and IgG2a were conducted by ELISA as described (16). Briefly, Immulon IV plates (Dynatech, Denkendorf, Germany) were coated overnight with 5 μg/ml of ES in carbonate/bicarbonate buffer, pH 9.6, at 4°C. Following blocking (PBS/0.05% Tween 20, 3% BSA), serial twofold dilutions of serum were added from 1/20 to 1/2560 or 1/200 to 1/2560. Parasite-specific IgG1 and IgG2a Abs were detected using biotinylated rat anti-mouse IgG1 (Serotec) and biotinylated rat anti-mouse IgG2a (PharMingen).

Total serum IgE was determined also as described (16). Briefly, ELISA plates were coated with an anti-mouse IgE mAb (Serotec, Oxon, U.K.) and detected using a goat anti-mouse IgE polyclonal Ab conjugated to horseradish peroxidase (Nordic, Tilberg, The Netherlands). Quantification was made by reference to an IgE anti-dinitrophenol mAb (ICN, Buck, U.K.).

Histology

At autopsy, the cecal tip was removed and fixed in Carnoy’s fixative before paraffin embedding. The embedded cecal tip was sectioned and stained for mast cells using 0.5% toluidine blue, pH 0.3. Numbers of mast cells were counted and expressed per 20 cecal crypt units.

Results

IL-4 KO mice are susceptible to _T. muris_ infection

Figure 1 shows worm burden data at day 35 p.i. from IL-4 KO and wild-type C57BL/6 mice. It can clearly be seen that IL-4 KO mice were susceptible to infection with _T. muris_, whereas the wild-type C57BL/6 mice have expelled the parasite. On day 32 p.i., female worms start to produce eggs, which are shed in the feces, and then parasite transmission can take place. Previous experiments have shown that later stages of the parasite promote their own survival, and when the parasite has reached adulthood it is not expelled from the gut without immune intervention or anthelmintic treatment (17). Mice that have worms on day 35 p.i. are therefore termed susceptible and said to have a chronic infection.

IL-4 KO mice make reduced levels of Th2-type cytokines

Figure 2 shows cytokine production from infected and uninfected IL-4 KO and wild-type C57BL/6 mice. MLNC removed at day 21 p.i. were stimulated with 2.5 μg/ml of Con A. This protocol, which has previously been used to analyze cytokine production in strains of mouse that are resistant and susceptible to _T. muris_, yields highly reproducible results (7, 8, 16). Mice that lacked the IL-4 gene, in addition to making no IL-4 (Fig. 2A), made significantly reduced levels of other Th2-type cytokines (IL-5, IL-9, and IL-13) (Fig. 2, B–D) in comparison with infected C57BL/6 mice (_p_ < 0.05). This confirms that IL-4 is necessary to make the Th2-type response associated with _T. muris_ infections in this strain of mouse. In contrast, IL-4 KO mice made high levels of IFN-γ, a Th1-type cytokine (Fig. 2E), whereas infected (and resistant) C57BL/6 mice made much lower levels of this cytokine.

IL-4 KO mice make undetectable parasite-specific IgG1 and high levels of parasite-specific IgG2a

Figure 3, A and B, shows parasite-specific IgG1 and IgG2a, respectively. Figure 3A shows a level of parasite-specific IgG1 in infected C57BL/6 mice typical of a resistant strain, whereas IL-4 KO mice were unable to generate a detectable parasite-specific IgG1 response to infection. This is different from normally susceptible strains of mouse, which usually generate a strong Th1 response (high IgG2a) and a low but measurable parasite-specific IgG1 response (4). Figure 3B shows that IL-4 KO mice made high levels of IgG2a. This finding correlates well with the elevated levels of IFN-γ observed after in vitro stimulation (Fig. 2E), as the
isotype switch to IgG2a is under the control of this cytokine (18). The levels of parasite-specific IgG2a were comparable to those seen in normally susceptible mouse strains. In comparison, infected C57BL/6 mice, which are resistant, made much lower levels of this Ab isotype.

**IL-13 KO mice are susceptible to T. muris infection**

Figure 4 shows worm burden data from IL-13 KO mice and wild-type 129 littermates at day 35 p.i. Mice that lack IL-13 were completely susceptible to infection, whereas wild-type 129 mice had expelled their worms. 129 Wild-type mice readily expel their worms; 43.85% of the established worm burden was expelled between days 10 and 14 p.i., and a further 32.67% was lost between days 14 and 21 p.i. (data not shown). By day 21, the majority of the animals had completely expelled their parasites.

**Th2-type responses in IL-13 KO mice**

Figure 5 shows cytokine production from infected IL-13 KO mice and infected 129 wild-type mice. MLNC were taken at day 14 p.i. and stimulated with 2.5 μg/ml Con A. Figure 5, A through D, demonstrates that, in addition to making no IL-13, IL-13 KO mice made significantly reduced levels of IL-4, IL-5, and IL-9 ($p < 0.05$) in comparison with 129 wild-type mice. In contrast, IL-13
KO mice appeared to make higher levels of IFN-γ (Fig. 5E) than 129 mice, although this was not statistically significant.

Figure 6 shows Ag-specific cytokine production from infected and uninfected IL-13 KO mice taken at day 14 p.i. and stimulated with 50 μg/ml of parasite ES Ag. Cytokine production mirrored that seen after Con A stimulation, although absolute levels of cytokine were lower after specific stimulation. It could again be seen that the IL-13 KO mice made significantly reduced levels of Th2-type cytokines in comparison with 129 wild-type mice. Levels of Ag-specific IFN-γ were not significantly different in infected IL-13 KO mice (mean, 47.15 ± 9.7 U/ml) and infected 129 wild-type mice (mean, 31.3 ± 11.4 U/ml).

Figure 7 shows Ag-specific cytokine production at day 21 p.i. in infected IL-13 KO mice. Although IL-13 KO mice produce no IL-13 (Fig. 7D), IL-4 and IL-5 production (Fig. 7, A and B) was similar in infected IL-13 KO mice and wild-type mice. Indeed, IL-9 production was actually greater in IL-13 KO mice than in wild-type mice at this time point (Fig. 7C). Interestingly, IFN-γ production was significantly higher in infected IL-13 KO mice (131.24 ± 22.41 U/ml) than in infected wild-type mice (68.94 ± 23.39 U/ml) at this time point.

Moreover, on day 35 p.i., IL-13 KO mice had a significant elevation in mast cell numbers in the cecum (mean, 118.43 ± 55.0), which was comparable to levels in infected wild-type mice (mean, 124.6 ± 8.87) although somewhat more variable. Uninfected IL-13 KO mice and uninfected 129 wild-type mice had negligible numbers of mast cells (mean, 10.60 ± 1.29 and 5.75 ± 0.48, respectively) in the cecum. Although IL-13 KO mice produced lower levels of total serum IgE than wild-type mice, levels of IgE were raised in comparison with uninfected IL-13 KO mice (Fig. 8). Both mast cell production and IgE production are associated with the production of a Th2-type response (19, 20).

IL-13 KO mice have an altered Ab isotype response

Figure 9, A and B, demonstrates the parasite-specific IgG1 and IgG2a Ab response, respectively, in both infected and uninfected IL-13 KO and 129 mice. In contrast to the IL-4 KO mice, IL-13 KO mice made a high level of parasite-specific IgG1 (Fig. 9A). The levels of parasite-specific IgG1 seen in the infected 129 mice were typical for resistant mice. The levels of parasite-specific IgG1 seen in the KO mice were extremely high and did not titrate out until a dilution of 1/25000 (data not shown). Figure 9B shows that IL-13 KO mice also had very high levels of parasite-specific IgG2a. The 129 wild-type mice had low levels of parasite-specific IgG2a with infection, typical of this resistant phenotype. The levels of parasite-specific Ab (IgG1 and IgG2a) observed in the IL-13 KO mice exceeds that seen in previous experiments with *Trichuris*.
When total Ab isotypes were analyzed in serum at day 35 p.i. (IgM, IgG1, IgG2a, IgG2b, and IgA), there was no significant difference in the levels of IgM, IgG1, and IgA between IL-13 KO and wild-type mice with infection. IgG2b levels were 5-fold higher in infected IL-13 KO mice than in infected 129 mice, with the greatest difference seen with IgG2a, in which there was a 45-fold increase in IL-13 KO mice with infection compared with wild-type mice (data not shown).

Discussion

These results provide the first demonstration of a critical role for IL-13 in resistance to helminth infection and demonstrate that both IL-4 and IL-13 are important in mediating immunity to *T. muris*. Both IL-4 and IL-13 are Th2 cytokines and share many biologic functions (10). It is clear from the present data, however, that the IL-4 and IL-13 KO mouse are phenotypically different, and in the context of *T. muris* infection, these cytokines must have separate and distinct roles.

In vitro cytokine analysis of IL-4 KO mice revealed that upon infection these animals have no IL-4 and very low levels of IL-5, IL-9, and IL-13 as well as an elevated level of IFN-γ. This was reflected in parasite-specific isotype responses in vivo. The present data confirm that IL-4 is important for the generation of Th2-mediated immunity in response to *T. muris* and are consistent with studies of other intestinal nematode infections in which this cytokine mediates protective immunity (3). They also support the view...
that in the absence of expansion of Th2 cells, levels of Th2 cytokines (including IL-13) are inadequate to generate a protective response. IL-4, unlike IL-13, influences T cell proliferation having an autocrine effect on Th2 cell expansion (21). It has been proposed that IL-13 plays a role in Th2 cell differentiation. This may suggest that even if IL-13-directed Th2 cell differentiation could take place in IL-4 KO mice, expansion of a Th2 cell population would be unlikely to occur.

The inability of IL-13 KO mice to expel *T. muris* demonstrates an important role for IL-13 in resistance to intestinal helminths, and although as yet we do not know the precise mechanism by which it acts, there are a number of possibilities. It has been proposed that IL-13 affects the differentiation of T cells along the Th2 pathway. It has previously been shown that the addition of exogenous IL-4 to in vitro cultures of T cells from IL-4 KO mice restored the production of IL-5 and IL-10 to levels comparable

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**FIGURE 6.** Cytokine analysis of IL-13 KO mice and 129 wild-type (WT) controls. MLNC from infected (Inf) and uninfected (Uninf) mice were removed at day 14 p.i. Cultures were stimulated in vitro with 50 μg/ml ES. Supernatants were harvested at 24 h and analyzed by sandwich ELISA for: A, IL-4; B, IL-5; C, IL-9; and D, IL-13. The results are the mean values ± SEM.
with those in wild-type mice (22). In similar experiments, however, using T cells from IL-13 KO mice, the addition of IL-4 had no such effect, suggesting that IL-13 may act upstream of IL-4 in Th2 cell development. Thus, the observed delay in the onset of cytokine responses (namely IL-4, IL-5, and IL-9 production) following T. muris infection in IL-13 KO mice is supportive of an important early role for IL-13 in the promotion of Th2-type responses. Eventual potentiation of the Th2-type response in IL-13 KO mice may reflect the action of additional non-T cell sources of IL-4, for example, mast cells. Certainly, bone marrow-derived mast cells from IL-13 KO mice have been shown to produce levels of IL-4 and IL-5 similar to those in wild-type mice mast cells upon stimulation in vitro. Indeed, our data confirm the ability of IL-13 KO mice to generate an intestinal mastocytosis coincident with an

FIGURE 7. Cytokine analysis of IL-13 KO mice and 129 wild-type controls. MLNC from infected and uninfected control mice were removed at day 21 p.i. They were stimulated in vitro with 50 μg/ml ES. Supernatants were harvested at 24 h and analyzed by sandwich ELISA for: A, IL-4; B, IL-5; C, IL-9; and D, IL-13. The results are the mean values ± SEM.
elevation of the Th2-type cytokine response, which can be seen most clearly by comparing in vitro cytokine production on day 21 p.i. in infected IL-13 KO mice. In vitro cytokine responses were reflected in vivo by elevated mast cell numbers, total IgE, and most strikingly, parasite-specific IgG1, which is in complete contrast to such responses in IL-4 KO mice.

The mechanism of IL-13-mediated protection remains to be defined, but suggestions have been made in other systems. In a study in which rIL-13 was administered to mice given a lethal dose of LPS and protected from endotoxic shock, there was a down-regulation of TNF-α, IL-12, and IFN-γ (11). Ablation of both the IL-12 and the IFN-γ response in mice normally susceptible to T. muris induces resistance through the induction of a Th2 response (our unpublished observations; and Ref. 4). Our present findings are consistent with the possibility that IL-13 may act by down-regulating cytokines, including IFN-γ and IL-12, following helminth infection.

It has also been suggested that IL-13 may contribute to the replenishment of effector cells during strong Th2 responses, as IL-13 has been shown to promote extramedullary hemopoiesis in mice similar to the responses observed when mice were infected with N. brasiliensis (23).

In contrast, IL-13 has been shown to enhance resistance to murine listeriosis (24), which shows a typical Th1-dominated immune response. More specifically, in that study it was demonstrated that IL-13 stimulated the production of IL-12 p40. Another study examined cytokine gene expression by in situ hybridization in patients with chronic atopic dermatitis and demonstrated coincident high levels of IL-12 and IL-13 (25). Nevertheless, most of the literature shows an inverse relationship between IL-12 and IL-13 production (11, 26).

Very little is known about the precise effector mechanisms involved in immunity to T. muris. A consistent finding is an important role for CD4+ T cells. The Th2-controlled eosinophilia and intestinal mastocytosis associated with resistance, however, are not essential but rather reflect the activation of a Th2-type response (reviewed in Ref. 27). Other candidate mechanisms potentially controlled by CD4+ T cells include intestinal goblet cell responses and changes in turnover of intestinal epithelium. Certainly there are dramatic changes in both responses during Trichuris infection (D. Artis and W. I. Khan, unpublished observations), although the particular role that IL-13 plays remains to be explored.

The role of Ab in protection to T. muris is unclear. Adoptive transfer studies have shown protective immunity can be generated in SCID mice reconstituted with CD4+ T cells in the complete absence of a B cell response (28). Furthermore, the data presented here show that IL-13 KO mice are unable to expel their parasites despite having extraordinarily high levels of parasite-specific Ab

**FIGURE 8.** Total serum IgE in ng/ml for infected (Inf) and uninfected control (Uninf) IL-13 KO and 129 wild-type mice. Serum was obtained from individual mice at day 35 p.i., and the results are the mean ± SEM for each group. Quantification of IgE was made by reference to an IgE anti-dinitrophenol mAb (ICN).

**FIGURE 9.** Serum was obtained at day 35 p.i. from individual infected (Inf) and uninfected (Uninf) IL-13 KO mice and 129 wild-type mice. Mean level ± SEM of parasite-specific IgG1 (A) and IgG2a (B) tested by ELISA for each group. Serial twofold dilutions of serum were conducted from 1/20 to 1/2560 and are expressed on the x-axis as a log scale.
in their serum. Indeed, these results indicate a possible role for IL-13 in regulation of isotype responses in mice. Whether this is an indirect effect through lack of control of other cytokines (e.g., IFN-γ, IL-4, IL-6) or whether this is a direct effect on B cells is unknown. This phenomenon may be related to a regulatory role of IL-12 on IL-12 production, as reported by others (29). Previous work in mice has demonstrated a significant increase in serum IgG1 and IgG2a levels following in vivo administration of IL-12 (30). We have also observed similar effects on T. muris-specific IgG2a and IgG1 responses following in vivo treatment with IL-12 (6). Importantly, resistance to T. muris can be induced in normally susceptible mice following in vivo treatment with anti-IL-12 mAb (our unpublished data), suggesting an important role for IL-12 in the natural progression to chronic infection.

Finally, different inbred strains of mouse differ in their capacity to expel T. muris (31) and their ability to mount Th1 or Th2 responses (32, 33). Recent data have highlighted the effects of genetic backgrounds upon immune responses in KO mice (34, 35). Wild-type 129 expel T. muris rapidly and C57BL/6 at a slower rate, although both expel their parasites efficiently. We feel, therefore, that background strain considerations do not detract from the present data. We would suggest that IL-4 KO mice are susceptible because they cannot make a sufficient Th2 response; most importantly, they cannot make enough IL-13. IL-13 KO mice show that even in the presence of strong Th2-type responses, if no IL-13 is present, parasite expulsion is unlikely to take place. We cannot, however, rule out the possibility that IL-4 does play an important early effector role, as the IL-13 KO mice show a defect in early IL-4 production. It is clear, nevertheless, that IL-4 and IL-13 play important and distinct roles in resistance to T. muris.

Further evidence supporting an important role for IL-13 in resistance to intestinal helminths comes from N. brasiliensis infections in STAT 6 KO mice (3). IL-4 and IL-13 both signal through STAT 6, and unlike IL-4 KO mice, STAT 6 KO mice show a delayed expulsion of the parasite from the gut. The IL-13 KO mouse clearly reveals a unique phenotype and provides the ideal tool to further investigate the role of IL-13 in host parasite interactions and models of allergic inflammation.

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