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IL-13 Is a Susceptibility Factor for Leishmania major Infection

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Leishmania major infection is useful as an experimental model to define factors responsible for the development and maintenance of Th cell immune responses. Studies using inbred mouse strains have identified that the Th1 response characteristic of C57BL/6 mice results in healing, whereas BALB/c mice fail to control the infection due to the generation of an inappropriate Th2 response. We now demonstrate that IL-13 is a key factor in determining susceptibility to L. major infection. Overexpression of IL-13 in transgenic mice makes the normally resistant C57BL/6 mouse strain susceptible to L. major infection even in the absence of IL-4 expression. This susceptibility correlates with a suppression of IL-12 and IFN-γ expression. Furthermore, using BALB/c mice deficient in the expression of IL-4, IL-13, or both IL-13 and IL-4, we demonstrate that IL-13-deficient mice are resistant to infection and that there is an additive effect of deleting both IL-4 and IL-13.


Leishmaniasis is an important disease currently affecting 12 million people worldwide. Infection of various inbred mouse strains with the protozoan parasite Leishmania major provides a useful model for studying the immune response to disease. Such studies have identified that the differential development of Th1 cells (Th1-producing IFN-γ) or Th2 cells (Th2-producing IL-4, IL-13, IL-5, and IL-10) during experimental cutaneous leishmaniasis determines the outcome of infection (1). In resistant mouse strains (such as C57BL/6, CBA, and C3H/He), infection induces the onset of an IL-12-dependent parasite-specific Th1 response characterized by the enhanced expression of IFN-γ and results in the healing of the infection. In contrast, susceptible BALB/c mice develop a parasite-specific Th2 response characterized by the enhanced expression of IL-4, progressive lesion development, and eventual death.

Following infection with L. major, the relative balance of IL-4, IL-12, and IFN-γ has been proposed to be of primary importance in determining the outcome of the disease. Although a role for IL-4 in inducing susceptibility to infection has been demonstrated using mAb treatment and transgenic expression (2, 3), recent reports using IL-4-deficient mice have presented conflicting evidence for the importance of IL-4 in the disease process. Noben-Trauth et al. (4) demonstrated that two IL-4-deficient mice, on a BALB/c background, remained susceptible to L. major, whereas both Kopf et al. (5) and Mohrs et al. (6) showed that the same mouse strain was resistant. Recently, IL-4Rα-deficient animals were reported to be more resistant to L. major than IL-4-deficient mice (6, 7), highlighting the existence of further contributory factors promoting susceptibility to infection. Indeed, these and other reports have implied a role for IL-13 in regulating responses to L. major infection (6–8) although none have formally demonstrated a role for IL-13.

Genetic mapping studies have identified that the IL-4 gene lies within a genomic region associated with susceptibility in mouse (9–11). Significantly, this region also includes the IL-13 gene, and it is noteworthy that the IL-13 protein can also utilize the IL-4Rα (12). Furthermore, recent reports have highlighted the complex and integrated roles for IL-13 and IL-4 in the development of Th2 cell responses (13, 14).

To assess directly the contribution of IL-13 to the progression of L. major infection, we have examined the responses of IL-13-transgenic mice and mice deficient for either IL-13 alone or doubly deficient for both IL-4 and IL-13. Our results indicate that IL-13 is an important component for susceptibility to L. major.

Materials and Methods

Animals

IL-13-transgenic and littermate control mice were as described previously (15) and had been backcrossed onto C57BL/6 mice for six generations. IL-13-transgenic mice were crossed with IL-4−/− mice (16), which had been backcrossed for 10 generations onto C57BL/6. IL-13-deficient (17) and IL-4/13-deficient (14) mice were backcrossed for at least four generations with BALB/c. Wild-type littermates of the cytokine-deficient mice were also backcrossed with BALB/c and are referred to as crossbalb. IL-4-deficient (4) mice were generated on a BALB/c background. The mice were bred and maintained in the facility at the Medical Research Council Laboratory of Molecular Biology.

Parasites and Leishmania Ag

L. major (LV39) was maintained as described (18). Soluble Leishmania Ag (SLA)3 was prepared by rapidly freeze thawing a pellet of 5.6 × 10⁹ L. major promastigotes. Protein concentration was determined using the bicinchoninic acid protein assay kit (Pierce, Rockford, IL) according to the manufacturer’s instructions.

Infection

Eight- to 12-wk-old female mice were infected with two million metacyclic promastigotes in the hind footpad. Footpad thickness was determined by weekly measurement of the infected footpad with a Vernier caliper and subtracting the thickness of the contralateral uninfected footpad. Following infection, parasite numbers were determined in popliteal lymph nodes as described (19).

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Abbreviation used in this paper: SLA, soluble Leishmania Ag.

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Preparation of cells

Infected mice were sacrificed and the popliteal lymph nodes and blood were collected. Single-cell suspensions (4 \times 10^6 cells/ml) were prepared from pooled popliteal lymph nodes and cultured in RPMI 1640 with the following additions. LPS stimulations were performed for 24 h in the presence of 3 \mu g/ml LPS. Anti-CD3e Ab (0.25 \mu g/ml of clone 2C11; Becton Dickinson, Mountain View, CA) stimulations were performed for 48 h. Ag-specific responses were analyzed using 2.5 \mu g/ml SLA for 5 days. Serum was collected and tested for parasite-specific Ab content.

ELISA assays

ELISAs were conducted upon Maxisorb 96-well immunoplates (Nunc, Naperville, IL) using standard Becton Dickinson ELISA protocols as described previously. Parasite-specific ELISAs were performed by coating 96-well plates with SLA at 2.5 \mu g/ml; bound Ig of diluted serum samples was detected using biotinylated monoclonal anti-Ig isotype detection Abs (Becton Dickinson). Cytokine ELISAs were as described elsewhere (13).

Results

Overexpression of IL-13 promotes susceptibility in otherwise resistant mice

IL-13-Tg (15) and control C57BL/6 mice were infected in the rear footpad with L. major LV39 promastigotes (9, 18). Following infection, footpad inflammation was monitored as an indicator of disease progression (9, 18). As expected, C57BL/6 mice displayed an initial period of footpad swelling lasting ~35 days, but thereafter the swelling decreased to near normal by 91 days (Fig. 1). In contrast, the footpads of IL-13-transgenic mice continued to increase in size before finally reaching a plateau by 49 days, and these animals were unable to resolve the parasite infection even by 91 days postinfection (Fig. 1). At this time, there was severe inflammation along the length of the limb combined with ulceration and marked deterioration of the integrity of the foot.

IL-13-induced susceptibility is IL-4 independent

Because IL-4 has been identified as a major factor in the generation of susceptibility to L. major infection, we determined the ability of IL-13 to promote susceptibility in the absence of IL-4. The IL-13-transgenic mice were bred with IL-4-deficient mice on a C57BL/6 background (IL-4 ^/-/^- (B6) IL13Tg) and infected with L. major alongside IL-4-deficient mice (IL-4 ^/-/^- (B6)) and wild-type controls. By day 60 postinfection, the footpads of both IL-4 ^/-/^- (B6) and wild-type mice had returned to normal (Fig. 2). However, despite the absence of IL-4, the IL-4 ^/-/^- (B6) IL13Tg mice failed to control the parasite infection (Fig. 2). Furthermore, the parasite burden was 1000-fold higher in IL-13-transgenic mice at 70 days postinfection than in controls \(10^4 - 10^5\) parasites/\(1 \times 10^6\) transgenic lymph node cells vs \(10^2 - 10^3\) parasites/\(1 \times 10^6\) wild-type lymph node cells (19). Thus, the ability of IL-13 to induce susceptibility to infection is not dependent on IL-4.

FIGURE 1. Footpad swelling following infection of IL-13-transgenic mice with L. major. IL13Tg (●) mice or C57BL/6 (△) littermate controls were infected in the rear hind footpad with \(2 \times 10^6\) promastigotes, and their footpad swelling was measured. Data are representative of four experiments with five to eight animals per group. Data represent means plus SD.

FIGURE 2. Footpad swelling following infection of IL-4 ^/-/^-IL13Tg mice with L. major. IL-4 ^/-/^-IL13Tg (●) mice, IL-4 ^/-/^- (B6) (△), and C57BL/6 (△) mice were infected in the rear hind footpad with \(2 \times 10^6\) promastigotes, and their footpad swelling was measured. Six animals per group were used. Data represent means plus SD.

FIGURE 3. Cytokine expression following infection of IL-13-transgenic mice with L. major. Popliteal lymph node cells were isolated from wild-type (□) and IL-13-transgenic (●) mice, stimulated, and analyzed using ELISA. A, IL-12 levels following stimulation with LPS (3 \mu g/ml) for 24 h. B, IFN-γ levels following stimulation with 2.5 \mu g/ml of SLA for 5 days. C, IL-5 levels following stimulation with anti-CD3e Ab (0.25 \mu g/ml) for 48 h. After stimulation with SLA, the levels of IL-4 and IL-5 were below the levels of detection (20 pg/ml). Data represent means plus SD from triplicate cultures.
IL-13 IS A SUSCEPTIBILITY FACTOR FOR L. major INFECTION

Suppression of Th1 responses in infected IL-13 transgenics

Th1-like responses have been shown to promote healing of infection (20–22). Therefore, we examined whether the overexpression of IL-13 had perturbed the generation of a Th1 cytokine response to L. major. The cytokine production from popliteal lymph node cells derived from infected mice was analyzed. IL-12 production was reduced in cell cultures from transgenic mice as compared with those from C57BL/6 controls (Fig. 3A). This decrease was mirrored by substantially reduced levels of Ag-specific IFN-γ production by the IL-13-transgenic mice (Fig. 3B). Significantly, cultures from IL-13 transgenics produced elevated levels of IL-5 as compared with wild-type (Fig. 3C). These data demonstrate that the Th1-like cytokine response in the IL-13-transgenic mice is suppressed.

IL-13-deficient BALB/c mice are resistant to L. major

Because overexpression of IL-13 promoted susceptibility to L. major infection, we addressed whether ablation of IL-13 expression would confer resistance on normally susceptible BALB/c mice. IL-13-deficient (IL-13−/−) and doubly IL-4/13-deficient (IL-4−/− IL-13−/−) mice backcrossed onto a BALB/c genetic background (13, 14) were infected with L. major LV39. In addition, IL-4-deficient (IL-4−/−) mice generated on a BALB/c background (4) were also infected, and footpad swelling was measured at 7-day intervals. BALB/c mice and backcrossed (crossbalb) control mice failed to control parasite infection, and, by 35 days postinfection, these animals were sacrificed due to the extent of footpad inflammation and ulceration (Fig. 4). Importantly, the IL-13−/− mice were able to control infection and by day 56 had normal footpads (Fig. 4). Similarly, IL-4-deficient mice were also found to be resistant to infection (Fig. 4). In both cases, the severity of infection of IL-4-deficient or IL-13-deficient mice was similar to that of C57BL/6 mice (Fig. 4). Interestingly, the double IL-4/13-deficient animals had the least severe footpad swellings of any group and resolved the infection more quickly (Fig. 4).

In contrast to the experiment using IL-13-transgenic animals, analysis of cytokine production from the gene-targeted animals failed to demonstrate any significant correlation with disease progression. As expected, BALB/c mice had relatively high levels of IL-4 expression in comparison to other groups. However, levels of IL-4 expression at 21- and 35-day time points did not correspond to a more susceptible phenotype (Fig. 5A). For example, IL-13−/− mice had elevated IL-4 levels in comparison to C57BL/6 but were nevertheless able to control parasite infection. Similarly, there was no significant correlation between IFN-γ expression and resistance to infection, although there were slightly enhanced levels of IFN-γ production in both IL-4−/− and IL-4−/− IL-13−/− mice (Fig. 5A). IL-12 expression levels also failed to correlate with a resistant phenotype (Fig. 5C). In addition, there was no correlation between Ab response and resistance to infection in the various cytokine-deficient mice (Fig. 6).

Discussion

Our results formally demonstrate for the first time that IL-13 is an important component for the generation and maintenance of immune responses to L. major infection. The overexpression of IL-13 resulted in C57BL/6 mice becoming susceptible to L. major, and significantly, this was independent of IL-4 expression. Conversely, IL-13-deficient BALB/c mice were resistant to L. major. We also noted that BALB/c mice doubly deficient for IL-4 and IL-13 were significantly more resistant to L. major infection than either of the
IFN-g contains the Th1 phenotype. Consequently, suppression or ablation of these alternative pathways are not mutually exclusive and would represent an integrated immune response to intestinal helminth infection and synchronous pulmonary granulomas (13, 14). Equally, IL-13 may be acting directly on lung epithelial cells such as IL-5. These data are in keeping with previous studies in mice which have demonstrated that IL-13 plays an important positive role in the onset and magnitude of Th2-like responses to gastrointestinal helminth infection and synchronous pulmonary granuloma production (13, 14). Equally, IL-13 may be acting directly on macrophages by inhibiting parasite killing mechanisms. Thus, these alternative pathways are not mutually exclusive and would represent an integrated immune response to L. major infection. In addition, it is also possible that in the case of the IL-13-transgenic mice, the observed decrease in IL-12 production may be due to the increased parasite load suppressing normal macrophage function. Genetic mapping and functional studies have implicated a number of loci that may influence susceptibility to L. major infection.

One such susceptibility locus has been mapped to the gene cluster on mouse chromosome 11 containing both IL-4 and IL-13 (10, 11). Although previous investigations have linked IL-4 with susceptibility, we now demonstrate conclusively that both IL-4 and IL-13 are factors that can induce susceptibility to L. major infection and that IL-13 can mediate this role even in the absence of IL-4 expression. Furthermore, our studies also help clarify the inconclusive results obtained using mouse strains with gene disruptions in IL-4 and/or IL-13-signaling pathways, including IL-4R-deficient mice (6, 7), STAT6, and BCL6-deficient mice (8).

Reports examining the responses of IL-4-deficient mice to L. major have produced conflicting results. Noben-Trauth et al. (4) initially reported that IL-4-deficient mice generated on a BALB/c background remained susceptible to L. major (LV39). However, Mohrs et al. (6), and our results shown above, found that the same IL-4-deficient BALB/c mice were resistant to L. major (LV39). It is difficult to identify the reason for these conflicting results although it is well documented that the virulence of L. major differs markedly between strains. Therefore, one explanation for these differing results might be strain differences of LV39 between laboratories.

Our experiments illustrate a novel and previously unreported role for IL-13 in the induction of susceptibility to L. major infection. Importantly, our results also demonstrate that IL-13 acts independently of IL-4 and thus identifies IL-13 as a major component of the immune response to L. major. In addition, the finding that IL-4/13-deficient animals have enhanced resistance to infection reinforces the notion that these cytokines act in combination to produce robust Th2 responses and inhibit Th1 differentiation and parasite killing.

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