Role of Interleukin-6 in the Control of Acute and Chronic *Giardia lamblia* Infections in Mice

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In this study, we investigated the role of interleukin-6 (IL-6) in *Giardia lamblia* infections in mice. Elevated IL-6 expression was found in wild-type mice 15 days postinfection. Furthermore, IL-6-deficient mice controlled infections only slowly although normal immunoglobulin A production was observed. Thus, IL-6 is necessary for early control of acute *G. lamblia* infections.

*Giardia lamblia* infects many species of mammals, including humans. Infections can result in severe cramps and diarrhea, although asymptomatic infections are common (1, 18). Most individuals control acute infections within a few weeks. However, some individuals, particularly those with hypogammaglobulinemia, develop chronic infections with this parasite, either with or without symptoms. The underlying explanation for the ability or failure of some individuals to control these infections remains undefined.

Two model systems are commonly used for the study of *Giardia* infection in adult mice, infection with *Giardia muris* and, more recently, infection with the *G. lamblia* isolate GS/M (2). In both models, depletion of CD4+ T cells prevents the control of acute infections with *Giardia* (7, 15) and T-cell-receptor β-gene-targeted mice have a severe defect in the control of *G. lamblia* infection (15). Parasite-specific antibody production, especially that of immunoglobulin A (IgA), also plays a role in controlling *Giardia* infection (6, 9). However, using the *G. lamblia* model, it was recently shown that B-cell-deficient (μMT) mice control acute infections with *G. lamblia* as well as wild-type mice (15). While μMT mice can generate some IgA responses (10), they do not produce parasite-specific IgA during *G. lamblia* infections (17).

While T cells are important, no single cytokine has been shown to be required for the control of infections. It was previously shown that gamma interferon (IFN-γ), interleukin-4 (IL-4), IL-4Rα, and STAT-6-deficient mice all control *G. lamblia* infections in a manner similar to that of wild-type mice (15). Other studies have found an increased production of IL-4, IL-5, and/or IFN-γ by using Peyer’s patches or mesenteric lymph node cells following *G. muris* infection (reviewed in reference 6). However, to our knowledge, no experimental infections have been performed in mice genetically deficient in cytokines other than IFN-γ or IL-4. Furthermore, while anti-IFN-γ treatment mildly exacerbated *G. muris* infection in C57BL/10 mice, it had no effect in BALB/c mice (19).

Role for IL-6 in the control of infections. Because IL-6 is known to be a switch factor for IgA production (8), we determined whether IL-6 was produced during *G. lamblia* infection of wild-type mice. RNA was collected from 0.5-cm-thick fragments of the small intestine near the duodenal-jejunal border, and IL-6 and hypoxanthine phosphoribosyltransferase (HPRT) mRNA levels were determined by reverse transcription (RT)-PCR as previously described (12). Little difference in the IL-6 mRNA levels was seen between uninfected mice and mice that had been infected for 5 days (Fig. 1). However, elevated levels of IL-6 mRNA were clearly seen 15 days postinfection (Fig. 1). In a separate experiment, IL-6 mRNA levels in four uninfected mice and four mice infected for 11 days were measured by competitive RT-PCR to quantitate the differences in expression (12). In this experiment, there was an average of nine times more IL-6 mRNA in the infected mice than in the uninfected controls (data not shown). Scott et al. previously found a roughly twofold decrease in the levels of IL-6 protein in jejunal homogenates of *G. muris*-infected mice compared to those in uninfected mice 6 days postinfection (14). Our data are consistent with this result but show an increase in IL-6 expression that correlates with the control of the infection at day 15 but not day 5 (Fig. 2).

To determine if the production of IL-6 was important for control of the infection, we infected IL-6-deficient mice with *G. lamblia* and determined parasite loads at 5, 15, 28, and 60 days postinfection. The IL-6-deficient mice were severely impaired in their ability to eliminate parasites compared to the wild-type mice (Fig. 2). The IL-6 knockout mice had much greater parasite numbers at 5, 15, and 28 days postinfection. Significantly, 28 days postinfection, the IL-6-deficient mice all still carried large numbers of parasites while the wild-type mice had cleared their infections. However, the number of parasites at day 28 in IL-6-deficient mice was fewer than that recovered early during the infections and by day 60, parasite numbers were reduced below the limit of detection (<10⁴ parasites/mouse) in the IL-6-deficient mice. Thus, IL-6 is required early in infection to control parasites but not to control infections later on.

Normal IgA production in IL-6-deficient mice. To determine if a lack of IgA was involved in the inability of IL-6-deficient mice to control infections, we measured anti-parasite IgA at
different times postinfection by indirect immunofluorescence (16) with an IgA-specific secondary antibody (Southern Biotechnology Associates, Birmingham, Ala.). Intestinal washes were collected from 10-cm-long segments of the proximal jejunum immediately adjacent and distal to the segments where parasite numbers were determined. Both wild-type and IL-6-deficient mice began to produce anti-parasite IgA by 15 days postinfection (Fig. 3). Interestingly, in both wild-type and IL-6-deficient mice, the IgA in intestinal washes at days 15 and 28 postinfection always reacted with a subset (5 to 10%) of the trophozoites present in our cultures. Serial dilution of intestinal washes showed no difference in the amounts of IgA produced by wild-type and IL-6-deficient mice (data not shown). The IgA present in intestinal washes at day 60 postinfection, however, always stained every trophozoite in the population. This is consistent with earlier results by Muller et al. showing that IgA responses to *G. lamblia* infection are initially specific for a single variant-specific surface protein but eventually diversify to recognize the full repertoire of variant-specific surface proteins in the parasite population (11). Together with our data, this suggests that an IL-6-dependent pathway contributes to the early control of infections and that IgA becomes more important as the infection progresses.

In addition to its role in IgA production, IL-6 has been shown to promote neutrophil responses in several infection models (3–5, 13). However, neutrophils were not observed during *G. lamblia* infection of wild-type mice (2), suggesting that IL-6 has some other role in helping to control *G. lamblia* infections. The precise role of IL-6 in the control of *Giardia* parasites remains to be defined.

**Distinct mechanisms for the control of acute and chronic *G. lamblia* infections.** Our data show that control of the early infection with *G. lamblia* requires T cells and IL-6 but not the production of antibodies. However, in the absence of IL-6, antibodies are eventually able to eliminate the majority of parasites, preventing a chronic infection. This dichotomy between the early and late control of *G. lamblia* infection in mice is strongly parallel to the various clinical outcomes of giardiasis in humans, suggesting the existence of multiple pathways to control this parasite in humans as well. This *G. lamblia* mouse
model thus offers an excellent opportunity to investigate both pathways of the immune control of this important human pathogen.

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