Interleukin-5 Transgenic Mice Show Enhanced Resistance to Primary Infections with *Nippostrongylus brasiliensis* but Not Primary Infections with *Toxocara canis*

LINDSAY A. DENT,∗ CHRISTINE M. DALY, GRAHAM MAYRHOFER, TRUDY ZIMMERMAN, ANN HALLETT, LEON P. BIGNOLD, JENETTE CREANEY, AND JIM C. PARSONS

Departments of Microbiology and Immunology and Pathology, University of Adelaide, Adelaide, South Australia, and Victorian Institute of Animal Science, Atwood, Victoria, Australia

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In this study, interleukin-5 (IL-5) transgenic mice with lifelong eosinophilia were assessed for resistance to primary infections with two tissue-invading nematodes, *Nippostrongylus brasiliensis* and *Toxocara canis*. Relative to nontransgenic littermates, three lines of IL-5 transgenic mice with varying degrees of eosinophilia all displayed enhanced resistance to *N. brasiliensis*. Although the timing of final worm expulsion was similar in transgenic and nontransgenic hosts, intestinal worms in transgenic mice were fewer in number throughout infection, failed to increase in size over the course of the infection, and were much less fecund. In contrast, *T. canis* larvae were recovered in similar numbers from tissues of transgenic mice with “low” or “high” eosinophilia and from nontransgenic mice. These results and other data suggest that eosinophils can contribute to host resistance to some parasite species. Parasite transit time through the host may correlate with relative sensitivity to eosinophils.

Tissue-invasive helminth species often induce intense tissue and peripheral blood eosinophilia. It has long been argued that eosinophils may kill some species of parasites or at least impede larval migration and development. Early studies suggested that depletion of eosinophils with polyclonal antieosinophil antibodies impaired immunity to *Schistosoma mansoni* (13) and *Trichinella spiralis* (7). More recent studies have shown that while monoclonal anti-interleukin-5 (IL-5) antibodies can decrease eosinophilia induced by infections with these parasite species, in all cases the parasite burden remained unchanged (8, 18, 19). Our own data suggest that in IL-5 transgenic mice, eosinophils may even be counterproductive in primary infections with both *S. mansoni* and *T. spiralis* (2, 3). Nevertheless, depletion studies with anti-IL-5 antibody suggest that IL-5 and/or eosinophils are protective against some helminth species, including *Strongyloides venezuelensis* and *Angiostrongylus cantonensis* (11, 17).

This study addresses the importance of eosinophils in resistance to two tissue-invasive intestinal helminths. *Nippostrongylus brasiliensis* was chosen because it has a very short transit time through the host, and *Toxocara canis* was selected because it remains in the host for extended periods of time. We used two lines of IL-5 transgenic mice (Tg5C1 and Tg5C2) which have been described elsewhere (2–4, 20) and another previously unreported but related IL-5 transgenic line, Tg5C3 (44 transgene copies). These IL-5 transgenic lines can be divided into “low-” (Tg5C1) and “high-level” (Tg5C2 and Tg5C3) eosinophilia categories, having approximately two (Tg5C1) to eight times (Tg5C2 and Tg5C3) more peripheral blood eosinophils than nontransgenic mice infected with the helminth *Mesocestoides corti*, a known inducer of eosinophilia (4). Since these lines of IL-5 transgenic mice have proven difficult to establish as homozygotes, all transgenic animals described were heterozygotes, and the nontransgenic control animals were generated from the same litters. *N. brasiliensis* and *T. canis* were passaged, cultured, and enumerated using conventional techniques (9, 16). In all experiments mice either were injected subcutaneously (s.c.) at the base of the neck with approximately 500 *N. brasiliensis* L3 infective larvae or were given 500 infective *T. canis* eggs by gavage. Statistical significance of the data was assessed by two-tailed Student's *t* test using Excel for Macintosh 4.0 (Microsoft), where *P* < 0.05 was considered significant.

*N. brasiliensis* egg production. Eggs produced by *N. brasiliensis* infecting nontransgenic CBA/Ca mice were released in large numbers in the feces from six to eight days postinfection (PI) but were never detected outside of this period (Fig. 1). In contrast, worms infecting IL-5 transgenic CBA/Ca mice produced few eggs and when detected, peak production tended to be delayed by one to two days (Fig. 1). Counts of eggs in feces remained very low throughout the course of infection in Tg5C1 and Tg5C2 transgenic mice (Fig. 1) and in experiments with Tg5C3 mice whose results are not presented. While egg production in low-eosinophilia Tg5C1 mice was greater than in high-eosinophilia Tg5SC2 animals, it failed to reach statistical significance. However, a clear difference between the lines was the absence of eggs in any of the Tg5C2 mice on Day 6 PI.

Intestinal *N. brasiliensis* worms. Large numbers of worms were found in the small intestines of nontransgenic mice as early as 3 days PI, and this level of infection was maintained for at least another 2 days (Fig. 2). In contrast, worm numbers were much lower in both low- and high-eosinophilia IL-5 transgenic lines. Intestinal worm numbers in IL-5 transgenic mice in this and other experiments peaked later than in nontransgenic controls.

Low egg counts in transgenic mice were not simply due to a paucity of worms. In a separate experiment, a mean of 27.7 (standard error of the mean, 5.9) worms in nontransgenic mice produced 14.3 eggs/fecal sample, whereas a similar number of worms (28.3 ± 2.3) in Tg5C2 mice did not produce eggs at a level detectable with the same techniques. Thus, while it is likely that few parasites survived migration from the s.c. site of inoculation in IL-5 transgenic mice, those that did reach the
gut also appeared to be less fecund. Neither was this result due to an absence of female worms in transgenic mice. In a typical experiment, worm sex ratios of 213 females to 108 males (i.e., 66% female) and 30 females to 14 males (i.e., 69% female) for nontransgenic and transgenic mice, respectively, were determined.

Worms were detected in the gut 3 days after s.c. injection of L3 larvae into nontransgenic mice and typically, they could be detected for 7 to 9 days PI. During this period of residence in the gut of nontransgenic hosts, worms mature (9) and increase in length (Fig. 3). The mean lengths of worms recovered from the lower gastrointestinal tracts of each of the three IL-5 transgenic lines were significantly less ($P < 0.003$) than those of worms found in nontransgenic mice at all three time points (Fig. 3). Male worms are smaller than female worms (9), but since worm sex ratios were approximately the same in transgenic and nontransgenic mice, respectively, were determined.

Worms recovered from transgenic mice were often pale in appearance, suggesting that they were malnourished. Worms also failed to localize in the preferred anterior third of the gut in each of the IL-5 transgenic mouse lines tested (data not shown), and this may adversely affect worm nutrition. The reduced fecundity routinely observed in infections in IL-5 transgenic mice may be directly related to poor growth and development of intestinal worms, but this is reversible. When transferred surgically to naive hosts, intestinal worms recovered from transgenic mice developed sufficiently to produce eggs (unpublished results).

Other studies with IL-5-overexpression transgenic mice and with IL-5 receptor α-chain knockout mice show that animals with high levels of IL-5 and eosinophilia also have enhanced resistance to the nematode *A. cantonensis* (22). Although the IL-5-overexpression transgene construct and background strain employed to generate the mice (24) used by these workers were different from those used in our study, the results support the hypothesis that eosinophilia can impair the migration of some tissue-invasive parasite species and reduce their reproductive success. Treatment of mice with anti-IL-5 antibodies can abolish eosinophilia induced by *N. brasiliensis* (1), and it has been shown to result in increased parasite load in mice infected with *S. venezuelensis* (11), *A. cantonensis* (17), and *Onchocerca* spp. (6, 12), adding further support to this hypothesis.

Our results and those of another study in which eosinophilia was prevented by treating animals with anti-IL-5 antibodies (10), suggest that IL-5 and eosinophils may not influence greatly the expulsion of adult *N. brasiliensis* worms. Rather,
Eosinophils appear to be important in the killing of larvae either at the site of the initial infection or elsewhere during their passage to the gut. Other data (1a) suggest that eosinophils are recruited to the site of inoculation of *N. brasiliensis* larvae within 6 h and, most significantly, that this is a major phase in which the migration of the parasites is inhibited. However it also seems likely that viable larvae which complete migration to the gut in IL-5 transgenic mice experience further damage or maturational inhibition once they reach this site. The worms do not increase in length from days 3 to 7 PI in IL-5 transgenic hosts, but it has yet to be determined whether egg production is suppressed due to a general effect on nutrition or development or whether it is a more specific effect on mating or fecundity.

**Blood and tissue eosinophilia in *N. brasiliensis*-infected mice.** Leukocytes in samples of tail blood were enumerated with a Coulter ZF automated cell counter (Coulter Electronics, Harpenden, England). Differential cell counts were performed on methanol-fixed blood films stained with Giemsa. Mean peripheral blood eosinophil counts in uninfected Tg5C2 mice were approximately 100 times greater than those in nontransgenic mice (Fig. 4). Eosinophils represented 72 and 4% of total peripheral blood leukocytes for Tg5C2 and nontransgenic groups, respectively. Tg5C2 transgenic mice infected with *N. brasiliensis* showed a 35 to 50% decline in peripheral blood eosinophilia in the early stages of infection (days 1 to 9 PI), but total numbers of eosinophils returned to preinfection levels by 15 days PI, while those in the small intestines of transgenic mice remained elevated at this time.

While we cannot exclude the possibility that overexpression of IL-5 may mediate resistance through other mechanisms, our results are consistent with the hypothesis that the development and fecundity of adult worms may be affected adversely by the presence of large numbers of eosinophils in the lamina propria of the gut. It is possible that local production of IL-5 enhances activation of eosinophil functions that are deleterious to *N. brasiliensis* intestinal worms, since expression of IL-5 in gut tissue has been shown to correlate with differential resistance to *N. brasiliensis* in BALB/c and C57BL/6 mice (25).

While IL-5 is also a growth and differentiation factor for B lymphocytes and promotes immunoglobulin A (IgA) production in the mouse, the very short course of a primary infection makes unlikely any contribution from the humoral arm of the adaptive immune response.

**T. canis infection in IL-5 transgenic mice.** *T. canis* infections induce very substantial blood eosinophilia, and the granulomas which form around larvae are rich in eosinophils. Eosinophilia is suppressed in *T. canis*-infected pregnant and lactating dogs, and it has been suggested that this may facilitate parasite transmission to newborn offspring. Treatment of infected mice with anti-IL-5 antibodies suppresses both blood and tissue eosinophilia induced by this parasite but does not seem to influ-

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**FIG. 4.** Mean total peripheral blood eosinophils in IL-5 transgenic (closed box) and nontransgenic (open circle) mice infected with *N. brasiliensis* (five mice/group). Asterisks represent statistically significant differences from values for the corresponding uninfected (day 0) mice ($P < 0.05$).

**FIG. 5.** Tissue eosinophils in histological sections of the small intestines of (IL-5 Tg5C2 transgenic [grey bars] and nontransgenic [NTg] [open bars]) mice infected with *N. brasiliensis*. Each value is a group mean for 3 or 4 mice and was calculated from means of counts on 10 or 20 villus crypt units (VCU) for each mouse in a group. Asterisks represent a statistically significant difference from values for the corresponding nontransgenic mice on the days indicated ($P < 0.05$).
ence the number of larvae subsequently recovered from the liver (15). Our experiments were designed to assess the significance of preexisting eosinophilia on the survival and migration of *T. canis* larvae. Twenty-eight days postinfection, the numbers of larvae recovered from the brain, liver, and muscles were similar in TgS5C1 (low eosinophilia) and TgS5C2 (high eosinophilia) IL-5 transgenic mice and in nontransgenic CBA/Ca mice (Table 1). At this time, peripheral blood eosinophil counts in nontransgenic mice had risen approximately 20-fold, whereas in infected transgenic animals, eosinophils were prominent in liver granulomas found in both transgenic and nontransgenic mice, suggesting that recruitment of these leukocytes to areas of larval deposition was unimpaired in all lines (data not shown).

Sugane and colleagues (21) studied *T. canis* infections in mice expressing a different IL-5 transgene construct (24) and found that overexpression of IL-5 and eosinophilia did not influence the number of larvae recoverable from the lungs within the first 2 weeks of exposure to the parasite. The present study supports and extends both this observation and our earlier studies (15). Even late in infection (i.e., 28 days PI) we could find no effects of eosinophilia on larval numbers recovered from either eosinophil-rich tissues, such as liver and muscle, or from relatively eosinophil-poor brain tissue. Our observations are also consistent with the report that mice unable to mount an eosophilic response due to disruption of IL-5 genes (23) carry numbers of larvae similar to those of wild-type mice. In our experimental model, unlike natural infections in pregnant and lactating dogs, eosinophilia cannot be dramatically down-regulated during *T. canis* infections. Our findings suggest therefore, that the suppression of eosinophilia seen during pregnancy in dogs may not be essential for *T. canis* larval migration and transmission to offspring.

This study provides evidence that a preexisting state of eosinophilia enhances resistance to primary infections with *N. brasiliensis* but does not promote clearance of *T. canis* larvae. On first appraisal, IL-5 transgenic mice may seem an artificial model from which to draw conclusions about immunity to parasites in natural infections. However, many humans and other animals are exposed to tissue-invasive parasites for much of their lives. Although initially generated as part of a parasite-specific response, a state of constant eosinophilia may contribute nonspecifically to immunity to newly encountered helminth species. The resistance of IL-5 transgenic mice to infection with *N. brasiliensis*, without preexisting specific immunity, provides evidence to support the hypothesis that eosinophilia alone can confer at least partial resistance. Preexisting eosinophilia, especially in the tissues, might be expected to be more beneficial for resistance to parasites which normally spend only a short period of time in tissues of the host, i.e.,

where only an early response can be effective in preventing passage of larvae to definitive sites, such as the gut.

It seems likely that although infections with many parasite species induce eosinophilia, not all helminths will be susceptible to these leukocytes. In contrast to organisms such as *N. brasiliensis*, helminths which parasitize the host for long periods are likely to have evolved strategies which make them resistant to the actions of eosinophils. Treatment with anti-IL-5 antibody does not seem to alter parasite burdens in mice infected with a range of other parasite species, including *S. mansoni* (18, 19) and *T. spiralis* (8). Our earlier studies with other parasite species, including *S. mansoni* (3) and *T. spiralis* (2), suggest that chronic eosinophilia and/or overexpression of IL-5 may, by mechanisms yet to be determined, actually be detrimental to host resistance against some infections. In contrast, in this study we have shown that *T. canis* does not seem to be either advantaged or disadvantaged in eosinophilic IL-5 transgenic mice. Therefore, eosinophilia may not be a universally beneficial response to all invasive helminths. These results provide support for a reassessment of the importance of innate immune mechanisms (5), particularly those operating in infections with metazoan parasites.

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