Bone marrow of mice parasitized with *Nippostrongylus brasiliensis* showed increased numbers of eosinophils as early as 4 days after infection. By day 7, their bone marrow also contained elevated numbers of progenitors that form small eosinophil colonies (20 to 50 cells) in soft agar cultures supplemented with interleukin-5 (IL-5). However, when mice were infused with anti-IL-5 antibodies at the time of infection, the number of recognizable eosinophils present in bone marrow remained low and eventually dropped below normal levels. The antibody treatment also prevented increased generation of IL-5-responsive precursors capable of differentiating into mature eosinophils in liquid culture and inhibited the generation of progenitor cells capable of forming small eosinophil colonies or clusters in soft agar cultures. The results of these in vivo experiments directly show that IL-5 is an essential regulatory molecule required for the bone marrow-dependent phase of a parasite-induced eosinophilia.

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**In Vivo Administration of Antibody to Interleukin-5 Inhibits Increased Generation of Eosinophils and Their Progenitors in Bone Marrow of Parasitized Mice**

By Donna M. Rennick, LuAnn Thompson-Snipes, Robert L. Coffman, Brian W. P. Seymour, John D. Jackson, and Susan Hudak

**Parasitic Infection** is one of several disease states that is often characterized by an acute blood and tissue eosinophilia. Although eosinophils are the only cell type consistently found elevated in the blood of parasitized animals, tissues directly involved in the infection show an inflammatory response typified by large cellular infiltrates consisting of lymphocytes, mast cells, macrophages, and neutrophilic granulocytes, as well as eosinophils. The composition of these infiltrates together with the profound blood eosinophilia suggest that factors such as interleukin-5 (IL-5), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-3 (IL-3) may be involved.1,4

Recently, we assessed the in vivo role of IL-5 in a parasite-induced eosinophilia by giving a monoclonal anti–IL-5 antibody to mice infected with *Nippostrongylus brasiliensis* (Nb).3 Mice infected with this intestinal nematode develop a blood eosinophilia that peaks between 11 and 14 days. Because Nb larvae migrate through and molt in the lung during the first few days of infection, this organ becomes highly infiltrated with inflammatory cells, including large numbers of eosinophils. When Nb-infected mice were treated with anti–IL5 antibodies, they failed to develop the expected blood eosinophilia.5 Antibody treatment also inhibited the appearance of lung eosinophils without affecting infiltrating neutrophilic granulocytes and macrophages. This study clearly showed that IL-5 is essential in supporting increased numbers of mature tissue and blood-borne eosinophils during a parasite-induced eosinophilic response. Recently, similar results were obtained with mice infected with *Schistosoma mansoni*.6 In order to further elucidate the in vivo relevance of IL-5, we have used the same system to study precursor cell development in bone marrow of infected mice.

**Materials and Methods**

**Growth factors.** Purified recombinant mouse IL-3 (specific activity [sp act], 2 x 10⁶ U/mg) and IL-5 (sp act, 4 x 10⁶ U/mg) were provided by Drs Jolanda Schreurs and Janusz Wideman, respectively (DNAX, Palo Alto, CA). One unit of IL-3 or IL-5 is defined as that amount of factor that stimulates half-maximal incorporation of ³H-thymidine by an appropriate factor-dependent cell line. Each factor was used at 100 U/mL. This amount was found to be at least two times higher than that required to stimulate maximum colony formation after titration of each factor in semisolid bone marrow cultures.

**Antibodies.** Rat monoclonal antibodies (IgG2a) specific for IL-5 (TRFK-5) were prepared as previously described.7 Rat monoclonal antibodies (IgG2a) specific for *Escherichia coli* beta galactosidase (GL113) were purified by Verax Corp (Lebanon, NH). The purity of the antibody prepared by high-performance liquid chromatography (HPLC) was greater than 95%.

**Infected mice.** BALB/c mice, injected subcutaneously with third-stage Nb larvae, received either no antibodies, an injection of anti–IL-5 antibodies (TRFK-5), or an isotype-matched control (GL113). Antibodies were given intraperitoneally at 2 mg per mouse. This amount of anti–IL-5 antibody has been shown previously to suppress completely Nb-induced blood eosinophilia.5 At various time points, eosinophils were quantified by performing total cell counts and differentials on stained cytospin preparations from bone marrow, peritoneal cavity, and blood.

**Eosinophil progenitor cell assays.** On days 4, 7, and 11 postinfection, four mice from each experimental group and four normal mice were sacrificed. Bone marrow was harvested from their femurs, with an average cell recovery of 1.8 x 10⁷ nucleated cells per femur. Single cell suspensions were prepared individually from one femur of each mouse and seeded at 2 x 10⁵ cells into triplicate 1 ml cultures containing modified Iscove’s medium (GIBCO, Grand Island, NY), 20% fetal calf serum, 50 μmol/L 2-mercaptoethanol, 0.3% (wt/vol) IL-3 (100 U/mL) or IL-3 (100 U/mL). After 6 days of incubation, the agar cultures were fixed with 2.5% glutaraldehyde, washed with water, transferred to gelatin-coated glass slides, and air dried. The slides were then stained with 0.1% Luxol Blue and counterstained with Mayer’s Hematoxylin. Preliminary experiments showed that 5 to 6 days of incubation was optimal for evaluating eosinophil colony formation. Upon further incubation, colonies that contained greater than 50 cells at day 6 did not increase in number, although some continued to increase in size. The smaller colonies began to disintegrate or the cells migrated into the surrounding agar leading to a diffuse background of Luxol Blue positive cells.

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Precursors in bone marrow differential counts were done from normal mice and from mice cultures contained mL liquid cultures of the basic medium described above. The larvae. The cells were suspended at 1 x 10⁶ cells/mL of parasitized mice (Fig 1A). These results showed that the absence of a blood and tissue eosinophilia was not simply due to the defective release of mature eosinophils into circulation. Instead, it appeared that bone marrow precursors had failed to differentiate into mature forms in parasitized animals made IL-5-deficient.

If this were the only effect of neutralizing IL-5 in parasitized mice, we reasoned that their eosinophil precursor levels should still be similar to or even higher than that of the parasitized controls. To test this directly, the number of precursors in infected, anti-IL-5-treated hosts was compared with normal mice and with infected mice left untreated or treated with control antibodies. Precursor levels were roughly equated to the capacity of bone marrow from the various experimental groups to give rise to mature eosinophils in cultures supplemented with IL-5. The number of eosinophils contained in the original cell inoculum (10⁶ cells per culture) was determined to be 2.8 x 10⁵ for normal mice, 14.8 x 10⁴ for parasitized mice, 11.2 x 10⁴ for parasitized mice treated with anti-β-gal antibodies, and 2.6 x 10⁴ for parasitized mice treated with anti-IL-5 antibodies. It was found that all suspension cultures stimulated with IL-5 showed an absolute increase in the total number of eosinophils detected after 7 days of culture with IL-5 (Fig 2). Moreover, cultures established with bone marrow of infected mice (+control antibody treatment) produced 3 to 4 times as many eosinophils as cultures established with normal bone marrow (Fig 2). In contrast, cells from parasitized mice injected with anti-IL-5 antibodies produced the same number of eosinophils as cells from normal mice, a value well below that observed for parasitized mice receiving no antibodies or control antibodies (Fig 2). Therefore, it appears that the elevated number of undifferentiated precursors associated with parasitic infection is directly regulated by IL-5.

Liquid suspension cultures. Bone marrow cells were harvested from normal mice and from mice 7 days post-infection with Nb larvae. The cells were suspended at 1 × 10⁶ cells/mL in triplicate 10 mL liquid cultures of the basic medium described above. The cultures contained no growth factor, or were supplemented with IL-5 (100 U/mL) or IL-3 (100 U/mL). After 5 days of incubation, the total number of viable cells were counted using dye exclusion and differential counts were done on Wright-Giemsa-stained cytospin preparations.

RESULTS

Administration of anti-IL-5 antibodies suppressed development of mature eosinophils and their undifferentiated precursors in bone marrow of Nb-infected mice. On average, the number of recognizable eosinophils in normal bone marrow was 2.8% of all nucleated cells. The total number of eosinophils per femur was found to be 5 x 10⁵ (Fig 1A, dashed line). After 4 days of infection with Nb larvae, mature and immature eosinophils increased almost sixfold in bone marrow of parasitized mice (Fig 1). The bone marrow-associated eosinophilia peaked by day 7, several days earlier than the maximum eosinophilic response occurring in the blood and peritoneal cavity (Fig 1B and C). When Nb-infected mice received anti-IL-5 antibodies, they not only failed to develop a peripheral eosinophilia, but they also failed to develop the expected numbers of maturing eosinophils in their bone marrow (Fig 1A). These results showed that the absence of a blood and tissue eosinophilia was not simply due to the defective release of mature eosinophils into circulation. Instead, it appeared that bone marrow precursors had failed to differentiate into mature forms in parasitized animals made IL-5-deficient.

Bone marrow cells from each experimental group were also established in suspension cultures containing IL-3. There were no significant differences in the production of eosinophils when comparing cultures established with bone

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**Fig 1.** Administration of anti-IL-5 antibody dramatically reduces the number of eosinophils in bone marrow (A) as well as peritoneal cavity (B) and blood (C) of parasitized mice. Female 12-week-old BALB/c mice were injected subcutaneously with 750 third-stage Nb larvae. On the same day (day 0), mice were injected intraperitoneally with either anti-IL-6 (2 mg per mouse) or an isotype-matched control (10 mg per mouse). Animals were killed on days indicated, and the eosinophil numbers were determined in bone marrow, peritoneal cavity, and blood. Data represent mean values with standard deviations for groups of four mice. ○—○, Nb-infected mice treated with anti-IL-3 antibodies; ○—O, Nb-infected mice treated with anti-β-gal antibodies. The dashed line in each graph represents the average number of eosinophils observed in normal mice.
Fig 2. Nb-infected mice treated with anti-IL-5 antibodies have reduced numbers of eosinophil precursors that differentiate to mature cells in suspension cultures containing IL-5. Suspension cultures containing IL-5 were established with a total of 10^6 bone marrow cells from normal mice and from mice infected 7 days earlier with Nb larvae. Some of the infected mice were treated with anti-IL-5 or isotype-matched control antibodies as previously described. Data represent mean values with standard deviations for groups of four mice.

Effects of anti-IL-5 antibody administration on generation of eosinophil colony-forming cells in bone marrow of Nb-infected mice. The numbers of eosinophil colony-forming cells (Eo-CFCs) present in bone marrow of normal and Nb-infected mice (+ anti-IL-5 antibody treatment) were analyzed. These experiments were designed to determine what effect neutralizing anti-IL-5 antibodies would have on the generation of eosinophil progenitors during the early phase of infection. Progenitors were enumerated by their ability to form eosinophil colonies in soft agar cultures containing an appropriate growth factor. We found that bone marrow from normal mice and from mice 4 days post-infection contained equivalent numbers of Eo-CFCs when assayed in IL-5-stimulated cultures (data not shown). However, bone marrow harvested from mice 7 days post-infection showed a significant increase in Eo-CFCs capable of forming small colonies (20 to 50 cells) in response to IL-5 (Fig 3). Treatment of parasitized mice with anti-IL-5 antibodies reduced this number to that obtained with normal mice.

In contrast, normal and Nb-infected bone marrow (+ anti-IL-5 antibody treatment) showed no difference in the numbers of Eo-CFCs capable of forming large IL-5-dependent colonies (greater than 50 cells). These results show that the generation of these particular Eo-CFCs are not affected by infection. Similarly, no differences between normal and Nb-infected bone marrow (+ anti-IL-5 antibody treatment) were observed with respect to numbers of IL-3-responsive Eo-CFCs or Eo/mixed-CFCs (Table 1).

![Graph showing eosinophil colony counts](image)

Fig 3. Effects of anti-IL-5 antibodies on in vivo generation of eosinophil progenitors in bone marrow of Nb-infected mice. Seven days post-infection, bone marrow cells from each animal in each experimental group were plated at 2 x 10^5/mL in triplicate soft agar cultures containing IL-5. Eosinophil colonies were identified by the presence of Luxol Blue staining cells and were scored according to size. Values reported are mean + SD of four mice per group. Results are typical of two independent experiments. Cultures not supplemented with growth factor failed to develop any eosinophil colonies.
Table 1. Effect of Anti-IL-5 Antibodies on In Vivo Generation of Eosinophil Progenitors in Nb-Infected Mice

<table>
<thead>
<tr>
<th>Donor</th>
<th>Treatment</th>
<th>IL-3-Stimulated Colonies Containing:</th>
<th>Eo/Mixed</th>
<th>&gt;50 Cells</th>
<th>20-50 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td>3.0 ± 1.0</td>
<td>5.4 ± 1.3</td>
<td>20.7 ± 2.9</td>
</tr>
<tr>
<td>Nb</td>
<td></td>
<td></td>
<td>3.0 ± 1.1</td>
<td>5.2 ± 2.0</td>
<td>22.1 ± 7.3</td>
</tr>
<tr>
<td>Nb + anti-IL-5</td>
<td></td>
<td></td>
<td>4.4 ± 1.9</td>
<td>7.3 ± 2.3</td>
<td>18.7 ± 10.0</td>
</tr>
<tr>
<td>Nb + anti-β-gal</td>
<td></td>
<td></td>
<td>3.3 ± 0.8</td>
<td>4.9 ± 0.5</td>
<td>18.7 ± 2.4</td>
</tr>
</tbody>
</table>

Results show number of eosinophil colonies x 10^2/femur and are expressed as mean ± 1 SD for groups of four mice. Data are representative of two separate experiments.

In a separate experiment, we found that bone marrow harvested 14 days after mice were infected (+ anti-IL-5 antibodies) gave the same results as bone marrow sampled 7 days after infection (+ anti-IL-5 antibodies) (data not shown).

**DISCUSSION**

We have previously shown that IL-5 plays an essential role in sustaining the blood and tissue eosinophilic response of mice infected with *Nippostrongylus brasiliensis*. In this study, the importance of IL-5 to eosinophil development in bone marrow of parasitized mice was evaluated. We found increased numbers of maturing eosinophils in bone marrow 4 days after Nb infection (Fig 1). The bone marrow eosinophilia peaked by day 7, several days earlier than that occurring in the periphery. By day 7, it was determined that bone marrow of Nb-infected mice also contained higher than normal numbers of IL-5-responsive eosinophil precursors as identified by their ability to rapidly differentiate into mature forms in culture (Fig 2). Similar results have been reported using mice infected with other helminths. When Nb-infected mice were treated with anti-IL-5 antibodies, there was a dramatic drop in precursor levels and in the number of recognizable eosinophils. In fact, the absolute numbers of maturing eosinophils present in the bone marrow of these treated mice dropped below the levels detected in bone marrow of normal mice (Fig 1). This result suggested that IL-5 may be necessary for terminal cell differentiation, an activity of IL-5 documented by numerous in vitro studies. However, our results further suggested that IL-5 is necessary for the increased generation of precursor cells usually found in bone marrow of parasitized mice (Fig 2). Thus, IL-5 not only supports cell maturation but also regulates the size of the undifferentiated precursor cell pool.

To continue this line of investigation, we next determined what effect anti-IL-5 antibodies would have on the ability of parasitized mice to generate increased numbers of Eo-CSFs. Results of clonal assays showed that giving neutralizing antibodies decreased Eo-CFC levels to that observed in uninfected mice. Specifically, anti-IL-5 antibody-treated mice showed reduced numbers of IL-5-responsive progenitors capable of forming small colonies or clusters (20 to 50 cells) (Fig 3). Presumably, these are relatively mature cells compared with those that form large colonies (greater than 50 cells) in response to IL-5. Interestingly, the number of large IL-5-responsive colony forming cells was not significantly elevated during infection, nor was it affected by treating parasitized mice with anti-IL-5 antibodies (Fig 3). Although the relatively immature large colony forming cells have acquired IL-5 responsiveness in vitro, apparently their in vivo generation from an even earlier progenitor was not dependent on IL-5. It was also found (Table 1) that giving anti-IL-5 antibodies to parasitized mice did not affect the in vivo generation of IL-3-responsive eosinophil progenitors representing an even earlier stage in the developmental pathway. Both of these results are in agreement with those of in vitro studies, showing that IL-5 regulates only late stages of eosinophil development.

In summary, the present studies clearly show that IL-5 is sufficient and necessary to sustain the bone marrow eosinophilic response associated with helminth infection. During a parasite-induced response, IL-5 functions as more than just a maturation and survival factor; it also expands the size of the eosinophil precursor compartment by stimulating the increased generation of mature progenitor cells with a limited proliferative potential (20 to 50 cells per colony). Thus, IL-5 augments the generation of its own final targets, those exhibiting dependence on IL-5 for terminal differentiation (summarized in Fig 4). These results are consistent with the predicted physiologic actions of IL-5 based on many informative in vitro studies.

Although the present experiments show that IL-5 is required for the eosinophilia manifested by parasitized mice, they do not rule out a role for IL-3 or GM-CSF. The ability of these two factors to induce a profound blood and tissue eosinophilia has been documented in other experimental systems. The possibility that IL-3 and/or GM-CSF are involved in earlier stages of a parasite-induced eosinophilia may be resolved by using specific neutralizing antibodies in studies similar to those described here.

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