Activated Eosinophils Are the Major Source of Th2-Associated Cytokines in the Schistosome Granuloma


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Activated Eosinophils Are the Major Source of Th2-Associated Cytokines in the Schistosome Granuloma


Eosinophils are a numerically dominant cell population within the schistosome granuloma. These granuloma eosinophils can produce a variety of cytokines, including IL-2, IL-4, IL-5, and IFN-γ. Therefore, eosinophils may play a key role in the determination of the unique cytokine microenvironment within the granuloma milieu. These studies investigated the potential role of eosinophils in the regulation of granuloma immunopathology. We have characterized spleen- and granuloma-derived eosinophils based on cellular activation and cytokine production during the development of murine schistosomiasis. Based on the criteria of hypodensity and CD69 expression, granuloma eosinophils were highly activated and very homogeneous at 7 and 11 wk postinfection. Splenic eosinophils were also activated at 7 wk postinfection, but were much more heterogeneous than their granuloma counterparts. By 11 wk postinfection, few hypodense splenic eosinophils were observed. Eosinophils represented the majority of cytokine-producing cells in the granuloma and were a dominant source of IL-4. Eosinophils also produced IL-2, IL-5, and IFN-γ, using the criteria of mRNA in situ hybridization and intracellular cytokine staining by FACS. Granuloma eosinophil activation and cytokine production were greatest at the time of maximum granuloma formation, i.e., 10–12 wk after initial cercarial exposure. Therefore, locally activated eosinophils, not Th2 lymphocytes, produce the majority of Th2 cytokines in the granuloma milieu and may be important determinants of immunopathology in schistosomiasis. The Journal of Immunology, 1999, 162: 1003–1009.

Schistosomiasis is a helminth-induced disease that affects an estimated 200 million individuals worldwide (1, 2). Pathology results primarily from the host’s immune response to parasite eggs and egg products that become trapped in host tissue (3, 4). The pathological immune response has two major components: granuloma formation around deposited eggs and hepatic portal fibrosis (5).

The mechanisms controlling granuloma formation and modulation have been the focus of intense research over the past 30 yr. Granulomas are generated and modulated by T cells and alterations in the cytokine milieu. Granuloma formation is known to be a CD4+-dependent, cell-mediated process, as evidenced by the suppression of hepatic granuloma formation in anti-CD4-treated mice (6, 7) and the formation of smaller granulomas in athymic mice (8). In addition, granuloma formation can be adoptively transferred by T cells (3).

The importance of Th1 and Th2 cytokines produced during granuloma formation is complex and controversial (9–11). The dominance of IL-4 and IL-5 as the disease progresses has largely been attributed to the effects of soluble egg Ag, which is produced with the onset of egg deposition. The majority of studies that have analyzed cytokine production have ascribed the cytokine patterns to the activities of T cell subpopulations and monocytes. However, these studies used in vitro activated cell cultures or tissue extraction techniques that could not identify with certainty the exact nature of the cells that actually produced the cytokines or quantify the production of these cytokines on a per cell basis.

Eosinophils are also a prominent cell within schistosome granulomas and are known to produce a variety of cytokines (12). Therefore, in the present study we have examined the activation of eosinophils and the production of specific cytokines and have combined this information with morphological characterization to precisely define the cellular source(s) of cytokine in the spleen as well as in hepatic granulomas. Our data show conclusively that eosinophils are the dominant source of IL-4 during Schistosoma mansoni infection and that eosinophils are the cell population most responsible for the cytokine profiles that had been previously attributed to a Th1 to Th2 shift.

Materials and Methods

Animals and infection

Female C57BL/6 (Harlan Sprague-Dawley, Indianapolis, IN) were used in all experiments. Mice (5–6 wk of age) were infected with 30 cercariae percutaneously by immersion of the tail for 1 h in water containing S. mansoni cercariae according to the method of Bruce and Radke (13). Infected snails were obtained from Fred Lewis at the Biomedical Research Institute (Rockville, MD).

Cell preparation

Spleens and livers were aseptically removed from infected animals, and cell suspensions were prepared as previously described (14).

Cytokine ELISPOT assay

ELISPOT assays were performed based on the method described by Klinman and Nutman (15). Immunol II plates (Dynatech, Chantilly, VA) were coated overnight at 4°C with 1 μg of capture Ab (anti-IFN-γ clone HB170...
and anti-IL-4 clone 11B11, Pharmingen, San Diego, CA). Plates were washed three times with PBS/Tween-20 (0.5%) and blocked with 200 µl well of RPMI and 5% FCS for 2 h at 37°C. Single cell suspensions were prepared from spleen and granulomas as described above, except for the omission of RBC lysis. Cells were suspended in complete medium at a density of 1 x 10^6 leukocytes/ml. One hundred microliters of fivefold serial dilutions of cells were added to previously aspirated triplicate wells. Plates were incubated for 3 h at 37°C under 5% CO2. Following incubation, the plates were washed three times with PBS followed by three additional washes with PBS/Tween. Biotinylated anti-cytokine-detecting Abs (anti-IFN-γ clone XM6-6 and anti-IL-4 clone BV6D, Pharmingen) were diluted to 4 µg/ml in PBS/Tween, and 100 µl was added per well. Plates were incubated for 1 h at 37°C under 5% CO2 and then washed three times with PBS and three times with PBS/Tween. Streptavidin–alkaline phosphatase (Jackson ImmunoResearch Laboratories, West Grove, PA), diluted 1/2000 in PBS/Tween-20 and 5% FCS (100 µl/well), was added, and the plates were incubated 30 min at 37°C in 5% CO2. Plates were washed five times with PBS/Tween and 100 µl of BCIP (Sigma, St. Louis, MO) at 1 mg/ml in AMP buffer (0.15 M 2-amino-2-methyl-1-propanol) containing 0.6% agarose was added per well. The plates were allowed to stand until the agarose had set, were stored at room temperature overnight, and were scored the next day. All scoring was performed by a single investigator in a coded manner.

Flow cytometry

Surface and intracellular cytokine staining. Freshly prepared cell suspensions (1 x 10^6/ml) were incubated in RPMI 1640 and 10% FCS with 10 µg/ml brefedulin A (Sigma) for 3 h at 37°C in 5% CO2. Following incubation, the cells were harvested, surface stained (Pharmingen α CD69 clone H1.2F3), and fixed overnight in 2.5% paraformaldehyde. The following day, cells were stained for intracellular cytokines as previously described (14) using FITC-conjugated anti-cytokine mAbs (Pharmingen; α IL-2 clone 5B4, α IL-4 clone 11B11, and α IFN-γ clone XM61.2). After staining, the samples were immediately analyzed by FACS. All data were acquired and analyzed using a FACScan or FACScanLaser instrument equipped with CellQuest software (version 3.0.1) or FACStar Plus equipped with LYSIS II software (Becton Dickinson, Mountain View, CA).

Cell sorting. Unlabeled cell suspensions prepared from spleen or granulomas were sorted on a FACStar Plus instrument. Lymphocyte and eosinophil gates were defined by forward and side light scatter parameters, and sorted cells were stained with Briebrich Scarlet and hematoxylin to assess cell phenotype and purity in each gate. Sorted cells were also used in mRNA experiments in situ hybridization.

In situ mRNA hybridization

Oligonucleotide probes were labeled with digoxigenin (DIG)-11-2′-3′-dideoxy-UTP using a Genius 3 Kit (Boehringer Mannheim, Indianapolis, IN). The sequences of the antisense probes used were as follows: IL-2, 5′-GAGTCATAAATCCGACATTGC-3′; IL-4, 5′-AACGCTACACTGCACTGAACT-3′; IL-5, 5′-GACTCTTGCACTGTTG-3′; IFN-γ, 5′-GACTCTAAAGAAGTCTGAGGT-3′. Sorted cells (0.1 x 10^6) were cytospun and mounted on SuperFrost Plus-precoated slides (Fisher, Pittsburgh, PA). The slides were fixed in 4% paraformaldehyde (pH 7.4) for 20 min, washed once with diethylpyrocarbonate/PBS, and incubated with acetylation solution (0.1 M triethanolamine and 0.25% acetic anhydride) for 20 min at room temperature in a humid chamber. The slides were then washed and incubated in 70% ethanol for 1 h at 4°C before two washes with 2X SSC. Prehybridization solution was added (per 1 l: 50 mg of dex- tran sulfate, 100 ml of 20X SSC, 50 ml of deionized formamide, 2 ml of 0.5 M EDTA (pH 8.0), 1 ml of Tris (pH 8.0), 100 mg of denatured sheared salmon sperm DNA, and 20 ml of 50X Denhardt’s solution), and the slides were incubated for 1 h at 37°C in a humidified chamber. Hybridization solution with prehybridization solution plus DIG-labeled probe) was then added, and the samples were incubated overnight at 45°C in a humid chamber. Slides were washed three times with 2X SSC and then washed four times with 0.1X SSC, incubating each wash for 15 min at 37°C in a humid chamber, followed by one additional 15-min wash in double distilled-H2O. Blocking buffer (1% blocking reagent, 0.1 M maleic acid, and 0.15 M NaCl, pH 7.5) was added to each slide, and the slides were incubated for 1 h at room temperature in a humid chamber followed by addition of alkaline phosphatase-conjugated anti-DIG (1/1000; Genius 3 Kit, Boehringer Mannheim) and incubation overnight at 4°C in a humid chamber. The slides were washed three times for 10 min each at room temperature in buffer 3 (100 mM Tris-HCl, 100 mM NaCl, and 50 mM MgCl2, pH 9.5) and dried. Slides were covered with 4-nitro blue tetrazolium chloride/BCIP substrate (3.5 µl BCIP, 4.5 µl 4-nitro blue tetrazolium chloride, and 1 ml buffer 3), incubated in the dark for 2–4 h at room temperature until color developed, and washed with H2O. Nuclei were counterstained with methyl green, and the slides were mounted with Permoun (Fisher). Negative controls included cytospun samples incubated with either no probe or irrelevant antisense probes.

Determination of eosinophil density

Eosinophil density was determined using the method of Hua et al. (16). Cells were isolated from the spleens and granulomas of 7- or 11-wk infected animals as described above for spleen cell preparation without collagenase treatment. Discontinuous Percoll (Sigma) density gradients were prepared by mixing heavy and light solutions (pH 7.4) and weighing 2 ml of individual solutions as described by Gartner (17) to determine the actual density of each solution. Gradients were composed of 1.5 ml of each Percoll solution, layered sequentially in a 15-ml polypropylene tube at densities of 1.09, 1.085, 1.08, 1.075, 1.07, 1.065, and 1.06 g/ml. Cells (0.5–5 x 10^7) were suspended in 1 ml of Percoll (1.055 g/ml) and layered on top of each gradient. After centrifugation in a fixed angle rotor at 2500 x g for 30 min at room temperature, cells were removed from each interface of the gradient and washed twice. Eosinophils were stained with Hinkelmann’s solution (0.5% eosin Y, 0.5% formalin, and 95% ethanol) and enumerated in a hemacytometer.

Results

Total cytokine production by ELISPOT

We initially wished to determine the overall profile of total cytokine-producing cells in the spleens and granulomas of S. mansoni-infected mice and to compare that profile to the actual kinetics of granuloma formation. The numbers of ELISPOTs per 100,000 spleen- or granuloma-derived cells harvested from animals 6–17 wk postinfection are shown in Figs. 1 and 2, respectively. The numbers of IFN-γ-producing ELISPOTs per 100,000 cells were similar for spleen- and granuloma-derived cells obtained from acutely infected animals (~20/10^7). A slight decline in the number of IFN-γ-producing cells and a slight increase in the number of IL-4-producing cells were noted in spleen populations as the disease progressed to 15–17 wk postinfection. Conversely, dramatic differences were observed in the numbers of IL-4-producing ELISPOTs per 100,000 cells when spleen and granuloma cells were compared. Very few IL-4 ELISPOTs were observed in the spleen (<20/100,000 cells) at all times of infection examined. In contrast, very large numbers of IL-4-producing ELISPOTs were obtained with granuloma cells at all periods of infection (note the change in scale). In addition, the number of cytokine-producing cells, as a
function of weeks postinfection, correlated well with our previously published kinetics of granuloma formation (18). The number of IL-4-producing ELISPOTs peaked around 6,500/100,000 at 13 wk postinfection. This frequency per se exceeded the total frequency of all lymphocytes in the granulomas (4.03 ± 3.08%). Additional studies had previously shown that only a minority of lymphocytes in the granuloma produced IL-4 (14). These findings suggested that Th2 cytokines were being produced by large numbers of granuloma cells that were not lymphocytes.

Cell sorting and purity

We next wished to determine the exact nature of the cells that were responsible for the production of cytokines. Spleen and granuloma cells from acutely infected animals were sorted by forward and side light scatter characteristics (Fig. 3). The cellular gates defined by forward and side light scatter characteristics were the same as those used for cell sorting and intracellular cytokine staining experiments. Sorted cells were stained with hematoxylin and Biebrich Scarlet, which specifically binds the arginine-rich proteins of eosinophil granules, but not neutrophil granules (19, 20).

![FIGURE 3](image-url)

**FIGURE 3.** Typical flow cytometric lymphocyte and eosinophil gates based on forward and side light scatter characteristics. Cells were isolated from hepatic granulomas of *S. mansoni*-infected mice 9 wk after cercarial exposure as described in *Materials and Methods* and were analyzed by flow cytometry.

<table>
<thead>
<tr>
<th>Spleen lymphocyte gate</th>
<th>98.1 ± 1.04</th>
<th>30.33 ± 0.41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen eosinophil gate</td>
<td>237.3 ± 3.46</td>
<td>76.3 ± 3.46</td>
</tr>
<tr>
<td>Granuloma lymphocyte gate</td>
<td>89.3 ± 4.72</td>
<td>2.63 ± 1.58</td>
</tr>
<tr>
<td>Granuloma eosinophil gate</td>
<td>2.27 ± 1.51</td>
<td>85.2 ± 8.70</td>
</tr>
</tbody>
</table>

*Cells were sorted by flow cytometry using gates defined by forward and side light scatter properties and stained with Biebrich scarlet and hematoxyline. Data shown represent the average purity of cells from three separate sorting experiments ± SEM.*

to assess purity and morphology. The results are shown in Table I. Cells recovered from the lymphocyte gates in the spleen and granuloma were 98 and 89% pure small mononuclear lymphocytes, respectively. Eighty-five percent of the cells within the granuloma eosinophil gate were eosinophils as evidenced by Biebrich Scarlet staining and the presence of a donut-shaped nucleus, which is highly characteristic of murine eosinophils. Seventy-six percent of the splenic eosinophil-gated cells were eosinophils by morphological and staining criteria. The lower percent purity observed in the splenic eosinophil gate was due to the presence of polymorphonuclear cells and large mononuclear cells with extensive cytoplasm, most likely monocytes or macrophages. The number of contaminating lymphocytes in the eosinophil gate was <5%.

The absolute numbers of cells per 10,000 FACS counted events from the spleen and granulomas of acutely infected animals that fell in each of the light scatter-defined gates are shown in Table II. At 8–12 wk postinfection, the spleen cell preparation was rich in lymphocytes, and eosinophils constituted less than 20% of the total cells. In contrast, the granuloma cell preparations from these acutely infected animals were composed of approximately 90% eosinophils. The striking difference in the cellular compositions of the spleen and granulomas in *S. mansoni*-infected mice along with ELISPOT data showing high numbers of IL-4-producing ELISPOTs in the granuloma suggested that eosinophils might be the dominant numerical source of IL-4-producing cells. Therefore, we studied the characteristics of these eosinophils in more detail.

**Eosinophil characterization**

**Eosinophil activation.** First, we examined the relative state of activation of splenic and granuloma eosinophils. To measure one criteria of physiological activation, we determined the cellular densities of splenic and granuloma eosinophils. One characteristic of activated eosinophils is a decrease in relative density due to a combination of cellular enlargement and loss of intracellular granules secondary to degranulation (16, 21). The density of blood eosinophils isolated from normal, noninfected mice is between 1.070–1.080 g/ml (16). Fig. 4A displays the density profile of splenic and granuloma eosinophils isolated 7 wk postinfection during the earliest phase of granuloma formation. The broader, higher density curve observed for splenic eosinophils indicated that these cells were very heterogeneous with regard to cellular density and

![Density Profile](image-url)

**FIGURE 2.** High frequency of IL-4-producing cells in schistosome granulomas. Granuloma cells were isolated from 8-wk (solid black bars), 13-wk (open bars), 15-wk (hatched bars), and 17-wk (solid gray bars) infected animals, and ELISPOT assays were performed as described in *Materials and Methods*. Note scale of the y-axis (0–9000) compared with that of the y-axis (0–35) used in Fig. 1.
were nonactivated. Approximately 80% of the splenic eosinophils were recovered from the density interfaces between 1.065, 1.070, and 1.075 g/ml. The granuloma eosinophils were hypodense and were more homogeneous, with 70% of the eosinophils accumulating at the 1.065 g/ml interface. The density determinations were repeated at 11 wk postinfection, during the time of peak granuloma formation. As shown in Fig. 4B, the splenic eosinophils were of normal density and were much more homogeneous at 11 wk postinfection than at 7 wk postinfection. Over 50% of the total eosinophils accumulated at the 1.075 g/ml interface. Similar to the 7 wk point, the granuloma-derived eosinophils were highly activated, i.e., homogeneous and of lower density than splenic eosinophils.

Next we confirmed that these eosinophils were activated using the criteria of expression of the early activation marker, CD69. We examined CD69 on granuloma and splenic eosinophils from 7–14 wk postinfection. At all time points examined, CD69 expression was greater on granuloma eosinophils than on splenic eosinophils (data not shown). These data confirmed that granuloma eosinophils were more highly activated than splenic eosinophils.

Eosinophil cytokine production. Previous studies and our initial observation had indicated that high levels of cytokines are produced in the granulomas. We wished to assess the exact source of these cytokines with specific reference to eosinophils.

Detection of eosinophil cytokine mRNA by in situ hybridization. We first directly demonstrated that eosinophils potentially could produce cytokines by measuring cytokine mRNA in these cells. Sorted spleen and granuloma eosinophils from 7-, 9-, 11-, and 17-wk-infected animals were cytospun, and IL-2, IL-4, IL-5, and IFN-γ mRNA were detected by in situ hybridization. Negative controls shown in Fig. 5 were incubated with no probe. Additional samples were incubated with irrelevant antisense oligo probes and were also negative for cytokine mRNA (data not shown). Splenic and granuloma eosinophils obtained from mice 7 wk postinfection stained brightly for mRNA encoding IL-2, IL-5, and IL-4 (Fig. 5, A and B). IFN-γ mRNA staining was less intense at 7 wk postinfection, particularly in granuloma eosinophils. At 11 wk postinfection, the intensity of IL-2 and IFN-γ mRNA staining in splenic eosinophils increased, and staining of IL-4 and IL-5 mRNA remained strong (Fig. 5C). mRNA staining for IL-2, IL-4, and IL-5 was slightly less intense in granuloma eosinophils at 11 wk postinfection (Fig. 5D) than at 7 wk, while IFN-γ mRNA staining increased.

Detection of eosinophil intracellular cytokines by flow cytometry. mRNA staining demonstrated unequivocally that eosinophils had the potential to produce cytokines. However, these initial in situ studies were not quantitative and did not prove that the eosinophils actually spontaneously produced cytokine proteins in vivo. Therefore, we measured intracellular cytokine protein production by freshly isolated cells using flow cytometry. Fig. 6 shows typical histogram profiles for eosinophils isolated from spleens and granulomas of S. mansoni-infected mice, 9 wk after cercarial exposure. The cells were cultured for 3 h without the addition of any in vitro activation agents but in the presence of brefeldin A. Brefeldin A interferes with protein secretion; therefore, any cytokine protein that was being produced in vivo, just before isolation of the cells, accumulates within the cell and can be detected by flow cytometric analysis.

Eosinophils isolated from spleen and granulomas produced IL-2 and showed similar levels of cytokine staining (Fig. 6, A and B). Low levels of IFN-γ protein were detected in both splenic and granuloma eosinophils in this experiment (Fig. 6, C and D). In replicate experiments, IFN-γ staining was slightly more intense. However, IFN-γ staining was consistently less than IL-2 staining in every experiment. This low level of IFN-γ expression was consistent with the numbers of IFN-γ ELISPOTs detected (Fig. 1). Splenic eosinophils were only slightly positive for IL-4. Granuloma eosinophils, on the other hand, were highly positive for IL-4 protein as indicated by the shift of nearly an entire log unit in the mean channel fluorescence intensity of the entire eosinophil population in the IL-4-stained sample compared with the isotype control (Fig. 6, E and F).

Splenic and granuloma lymphocyte cytokine production were compared. IL-4 data are presented in Fig. 6. Splenic lymphocytes produced virtually no IL-4 protein based on similar isotype and IL-4 cytokine fluorescence histograms (Fig. 6G). A small percentage of granuloma lymphocytes (~5%) produced modest amounts of IL-4 (Fig. 6H). Since the same concentration of fluorochrome-labeled Ab was added to each sample, and all other conditions were held constant, the relative change in fluorescence intensity of cytokine-stained samples compared with isotype controls is a semiquantitative, but proportional, measurement of intracellular cytokine concentration. Based on the high percentages of eosinophils producing IL-4 and their numerical dominance in the granuloma (~90% of the granuloma cell preparation), these data demonstrated that granuloma lymphocytes were producing a small
amount of IL-4 and that the eosinophils were the dominant quantitative source of IL-4 in the granuloma.

Discussion

Cytokines play an important role in regulation of the inflammatory, granulomatous response in schistosomiasis. Both Th1 and Th2 cells are important for the generation of granulomas in vitro and increase granuloma formation in vivo (22–24). Early granulomas are rich in IL-2 and IFN-γ, while IL-4 and IL-5 are the dominant cytokines in the largest granulomas (25–27). The majority of studies to date have analyzed cytokine production by ELISA or immunocytochemical techniques after in vitro stimulation. Although these studies were useful in characterizing the general cytokine responses elicited as a result of *S. mansoni* infection, they provided limited information about the nature of the cell population(s) that was specifically producing cytokines in vivo. In addition, the studies introduced a potential confounding variable, i.e., the effects of in vitro incubation with lectins or Ag. We chose to examine in greater detail the cell types responsible for the observed Th2 cytokine response and in particular to focus on eosinophils immediately after their isolation from infected mice.

Our initial ELISPOT studies with spleen and granuloma cells showed large numbers of IL-4-producing cells in the granuloma compared with those in the spleen. Several types of cells could contribute to cytokine production. A recent study suggested that non-CD4⁺ peritoneal exudate cells produced IL-4 following i.p. injection of *S. mansoni* eggs. Immunocytochemical analyses of these cells suggested that they were eosinophils (28). In the present study we assessed the nature of the cells responsible for IL-4 production within hepatic granulomas.

CD4⁺ T cells are required for granuloma formation and have been shown to produce cytokines within granulomas (14). For example, both spleen and granuloma lymphocytes produce IL-4 (Fig. 6, *G* and *H*). However, in this study we show that eosinophils constitute approximately 90% of the cells in acute granulomas and are highly positive for IL-4. Therefore, eosinophils represent the dominant source of IL-4 in granulomas, and eosinophils may be the actual source of the observed Th1 to Th2 cytokine shift seen in schistosomiasis.

Eosinophils are thought to play a major role in a variety of human diseases, including allergic inflammation, malignancy, and host defense against helminth infections (29). However, the exact role(s) of eosinophils in schistosomiasis relative to immunopathology remains unclear.

Eosinophils may participate in Ab-dependent protective immune responses (30, 31). Human eosinophils express IgE receptors

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**FIGURE 5.** Splenic and granuloma eosinophils produce IL-2, IL-4, IL-5, and IFN-γ mRNA. In situ mRNA staining for IL-2, IL-4, IL-5, and IFN-γ in sorted splenic and granuloma eosinophils from *S. mansoni*-infected mice 7 and 11 wk postinfection was performed as described in Materials and Methods.
suggested partial activation of eosinophils in the spleen and augmented eosinophil activation in the granuloma, perhaps due to greater Ag interactions in the granuloma.

Granuloma eosinophils produce a variety of cytokines. Of special interest is the production of the Th2-type cytokines IL-4 and IL-5 by granuloma eosinophils. Several investigators have shown that a Th1 to Th2 shift has profound effects on the ability of the host to respond to other infectious diseases, specific Ags, and allergens (36–38). Flow cytometry data for intracellular IL-5 production, a more stringent marker of the Th2 response, were not presented in this report because we had technical difficulty detecting intracellular IL-5 by flow cytometry. This was most likely due to the fact that we did not stimulate freshly isolated splenic and granuloma cell suspensions in vitro. However, we did demonstrate by in situ hybridization that sorted splenic and granuloma eosinophils had high levels of IL-5 mRNA expression. Anti-IL-5 treatment has been shown to specifically deplete eosinophil accumulation in granulomas (39), further supporting the importance of IL-5 in eosinophil recruitment. Ongoing RNase protection studies using sorted splenic and granuloma lymphocytes and eosinophils will further define which cells are producing specific cytokine and chemokine RNAs. The kinetics of cytokine and chemokine production will help determine whether expression is being regulated in an autocrine, paracrine, or juxtacrine manner. The identification of eosinophils as the dominant contributors to the Th2 response in S. mansoni infection suggests that other studies addressing Th1 and Th2 responses may need to be reinterpreted to determine the cellular source(s) of cytokines and the basis of systemic perturbations of immunity.

Of particular interest is why eosinophils achieve such numerical dominance in granulomas and what is the implication of that dominance. Eosinophils may only reach the granuloma as a result of their response to factors produced by Ag-specific T cells. Indeed, anti-IL-5 treatment will prevent the accumulation of eosinophils in granulomas but does not grossly affect granuloma formation per se (39). Conversely, once the eosinophils have reached the granulomas, they may produce cytokines that adversely affect T cells, especially pathogenic Th1 cells, but that augment their own survival. Our preliminary data suggest that, unlike lymphocytes, eosinophils do not undergo apoptosis in the granulomas. Perhaps this survival is augmented by the presence of large amounts of IL-4 and IL-5, which have been shown to promote eosinophil survival in vitro. Finally, eosinophils have been shown to produce a number of proteases, peroxidases, histamines, etc., which may directly affect local inflammation and thereby effect pathology. The mechanisms of cytokine-regulated survival and apoptosis by granuloma subpopulations has profound implications vis-à-vis pathology and are the subject of current study in our laboratory.

To further define the role of murine eosinophils in the pathology of S. mansoni infection, we examined several parameters, including activation and cytokine production. During the acute phase of infection, we showed selective degranulation of granuloma eosinophils and very heterogeneous splenic eosinophils. As disease progressed, granuloma eosinophils remained hypodense, and splenic eosinophils became much more homogeneous and normal. In additional experiments, we examined expression of the early activation marker CD69 on splenic and granuloma eosinophils. Based on greater mean channel fluorescence intensities, we detected enhanced levels of CD69 expression on granuloma eosinophils compared with splenic eosinophils (data not shown). These findings that participate in an IL-5-dependent Ab-dependent cell-mediated cytotoxicity reaction against schistosomula in vitro (32–35). Eosinophils also mediate the destruction of miracidia and schistosome eggs (30). Murine eosinophils do not express surface IgE receptors, but do express IgG receptors. An oxidative burst can be triggered through these IgG receptors (30), suggesting that murine eosinophils may use an alternate mechanism to eliminate schistosomula.

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References


