Granulocyte Precursor Cell Studies in *Schistosoma mansoni* Patients with Eosinophilia*†

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ABSTRACT

Eosinophilia is a common clinical presentation in patients with helminthic infections. A study was designed to determine the mechanism(s) for selective or preferential differentiation of precursor cells into mature eosinophils (eos). Thus, experiments were performed to delineate the frequency of colony forming units of eos (CFU-eos) in the peripheral blood of Egyptian patients with active *Schistosoma mansoni* infection with eosinophilia and normal healthy individuals. The number of CFU-eos among the nonadherent mononuclear cell population was assessed in a double layer soft agar culture with autologous unfractionated mononuclear cells serving as a source of colony stimulating factor(s).

Following 14 days of incubation, discrete colonies were distinguished morphologically as eosinophilic, neutrophilic, or mixed. Results indicated a two-fold increase in the total number of colonies per $10^6$ cultured nonadherent cells in patients with *S. mansoni* infection when compared to the number of colonies obtained with adult normal volunteers (57 ± 10 vs. 24 ± 4; $P < 0.025$). However, the frequency of CFU-eos and CFU-neut was similar in patients and normal individuals (66 ± 3 vs. 59 ± 8 percent CFU-eos; 30 ± 4 vs. 35 ± 6 percent CFU-neut). These data suggest that: (a) eosinophils may differentiate from progenitor cells at other anatomical sites; (b) there may be an increase in the half life of mature eosinophils in patients; (c) there is no strict correlation between the frequency of progenitor cells and the number of differentiating mature cells of this lineage at least as measured by this *in vitro* assay; and (d) the *in vitro* assay may not quantitatively reflect the *in vivo* differentiating capacity of progenitor cells.

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Introduction

The association between peripheral blood eosinophilia and mild to moderate neutropenia has long been observed in clinical *Schistosoma mansoni* infection. Data substantiating an effector role for eosinophils in immunity against *S. mansoni* have also been obtained from both *in vivo* and *in vitro* studies. The contribution of neutrophils and macrophages in mediating the *in vitro* killing of the migratory young stage of this trematode was not excluded.

During this infection, the factors responsible for the *in vivo* commitment of stem cells into specific differentiation lineages are poorly understood. Because of the alteration of the peripheral blood leukogram during schistosomiasis, eosinophil and neutrophil differentiation were investigated at the progenitor cell level. In an attempt to understand the mechanisms underlying altered granulopoiesis, the incidence of circulating colony forming cells of the eosinophil/neutrophilic lineages (CFU eos/neut) was determined in healthy and *S. mansoni* infected individuals, and the results of these studies constitute the basis of this report.

Materials and Methods

Study Population

Twelve *S. mansoni* infected and eight hematologically normal Egyptian males were selected for the study after providing informed consent. The patients were carefully selected from a hospitalized population after all the basic clinical laboratory parameters, such as complete bloodcounts, urinalysis, urine and stool examinations for helminthic infections, were performed. They were excreting variable numbers of only *S. mansoni* eggs in their stools ranging in numbers from 25 to 270 eggs per g stool (x ± S.D. = 162.7 ± 95). The patients were not previously infected with *W. bancrofti* or *T. spiralis* and were negative for intercurrent infections of salmonellosis, hepatitis B, and had no *S. hematobium* eggs in their urine samples. The control subjects were chosen from male laboratory workers who had no history of schistosomiasis and were free of other parasitic infections.

Cells

Venous blood was collected in preservative free heparin. The peripheral blood mononuclear cells (PBMC) were collected after 30 min centrifugation at 400 × g through a Ficoll-Hypaque density gradient. The PBMC were washed twice and suspended in complete alpha medium, supplemented with 15 percent heat-inactivated human AB serum, L-glutamine (4mM), penicillin (100 U per ml) and streptomycin (100 μg per ml). One aliquot of the PBMC (adjusted at 2 × 10⁶ per ml) was incubated in sterile plastic petri dishes at 37°C, 5 percent CO₂. After two 60 min-cycles of depletion of plastic adherent cells, the nonadherent cell population (NAC) and an aliquot of the whole PBMC were each suspended at 10 × 10⁶ per ml in complete alpha medium.

Agar Culture

All cultures were performed in 35 × 10 mm gridded plastic culture dishes. The details of the double agar layer technique for growing and staining eosinophilic/neutrophilic colonies are described elsewhere. Briefly, feeder underlayers of 0.5 percent agar in complete medium containing 10⁶ unseparated PBMC served as a source of colony stimulating factor (CSF). Overlays...

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were then prepared containing $10^6$ non-adherent cells in 0.3 percent agar. Triplicate cultures were incubated in a 5 percent CO$_2$, 37°C humidified atmosphere. After 14 days, the overlayers were carefully separated from feeder layers and mounted on bovine serum albumin-coated 75 × 50 mm glass slides and allowed to dry overnight at room temperature. The slides were stained for two hours with 0.1 percent Luxol Fast Blue MBS* in 70 percent urea-saturated ethanol and counterstained with hematoxylin.* Aggregates of >40 cells were counted as colonies. The majority of eosinophilic colonies were compact and were easily distinguished from neutrophil colonies by their aquamarine granules in >90 percent of the cells. In contrast, neutrophil colonies tended to spread out in the agar matrix and were characterized by a pale gray-blue cytoplasm.4

**Results**

**Clinical Findings**

The clinical features of the study groups are summarized in table I. All patients were treated between three and 40 days prior to the day of the assay with a single dose of the schistosomicidal drug Praziquantel (30 mg per kg body weight). This treatment, however, did not significantly diminish the level of the peripheral blood (PB) eosinophilia for up to eight weeks after administration (unpublished observation).

**Frequency of Circulating Progenitor Cells**

As shown in table II, the levels of mature eosinophils or neutrophils in the individual peripheral blood samples of schistosomiasis patients did not correlate with the observed progenitor cell frequency as judged by the number of *in vitro* developed respective colonies. Linear regression analyses of the incidence of CFU-eos and CFU-neut in relation to the respective PB cell population revealed insignificant and negative correlations (r values = −0.0141 and −0.247, respectively), and p values >0.05 for both cases.

In this study, more than a two-fold increase (p < 0.025) in the incidence of the total CFU-eos and CFU-neut was detected in the PB of patients as compared with normal individuals (table III).

**TABLE I**

Clinical Features of Schistosomiasis Patients and Healthy Control Individuals as Analyzed on Day of Experiment

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>S. mansoni Patients</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Range</td>
<td>Mean ± S.E.M.</td>
</tr>
<tr>
<td>11 - 21 yrs</td>
<td>18.7 ± 2.9</td>
<td>26.1 ± 2.3</td>
</tr>
<tr>
<td>WBC/mm$^3$</td>
<td>6,700 - 22,000</td>
<td>11,696 ± 1.3</td>
</tr>
<tr>
<td>% Eosinophils</td>
<td>7 - 46*</td>
<td>20.5 ± 3.0</td>
</tr>
<tr>
<td>% Neutrophils</td>
<td>16 - 54</td>
<td>38.6 ± 3.6</td>
</tr>
<tr>
<td>Liver involvement</td>
<td>Palpable - 8 cm BCM†</td>
<td>5,400 - 10,500</td>
</tr>
<tr>
<td>Spleen involvement</td>
<td>6 - 8 cm BCM</td>
<td>Normal spleens</td>
</tr>
<tr>
<td>Treatment</td>
<td>Praziquantel; 3-40 days prior to inclusion into study</td>
<td>Occasional “over the counter” medications</td>
</tr>
</tbody>
</table>

*Eosinophilia persisted for more than 30 days after study.
†Below costal margin.
Although the increase was attributed to the significant rise in the number of PB CFU-eos and not CFU-neut, \( p < 0.01 \), the ratio of CFU-eos or CFU-neut to the total number of colonies was similar in both normal and parasitized individuals.

**Discussion**

The present study, utilizing *in vitro* methods to culture, stain, and identify myeloid progenitor cells, has demonstrated a two-fold increase in the total number of colonies per \( 10^6 \) cultured PB-nonadherent cells in *S. mansoni* infected individuals as compared to normal individuals, without a significant difference in both the spontaneous (unstimulated) cloning efficiency and colony size. The wide variation in the yield of colonies observed between some patients (table II) could not be due to a possible toxic effect of the density gradient or the culture medium on the progenitor cells, since high and low yields were obtained from cell populations set up simultaneously for culture under the same experimental conditions (e.g., patients

**TABLE III**

Incidence of Circulating Eosinophilic and Neutrophilic Colony Forming Units (CFU-eos/neut) in Normal and *S. mansoni* Infected Individuals

<table>
<thead>
<tr>
<th>Peripheral Blood NAC</th>
<th>Total Number of Colonies/( 10^6 ) NAC*</th>
<th>CFU-eos Number % of Total</th>
<th>CFU-neut Number % of Total</th>
<th>CFU-mix Number % of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients†</td>
<td>56.9 ± 10.5</td>
<td>19.9 ± 5.9</td>
<td>34.9 ± 4.0</td>
<td>62.4 ± 3.4</td>
</tr>
<tr>
<td>Normals‡</td>
<td>23.9 ± 3.5</td>
<td>6.8 ± 2.2</td>
<td>36.8 ± 6.2</td>
<td>57.3 ± 7.8</td>
</tr>
</tbody>
</table>

*Student's "t" test*  
\( p < 0.025 \)  | N.S.§  | N.S.  | p < 0.01  | N.S.  | N.S.  | N.S.  |

*Mean ± standard error of mean per \( 1 \times 10^6 \) plastic nonadherent cells (NAC) plated.  
†Ratio of circulating eosinophils to total leucocytes is 1 : 5.  
‡Ratio of circulating eosinophils to total leucocytes is 1 : 100.  
§Not significant (\( p > 0.05 \)).
The results showed that the relative frequency of CFU-eos or CFU-neut to the total number of colonies per study cell population was similar in both the normal and parasitized individuals.

Kern & Dietrich monitored the circulating and bone marrow myeloid progenitor cells in patients with persistent eosinophilia during helminthic infections. In their study, no difference in the total colony numbers was observed when PBMNC were cultured from patients or control individuals. The discrepancy between these two studies could be due to differences in their control individuals who were selected from patients without eosinophilia, or to the source of exogenous CSF used in their assays. In our study, PB samples from healthy individuals were used for comparison, and autologous PBMNC populations were the source of CSF in order to mimic the in vivo situation. The effect of CSF on the in vitro response of progenitor cells from PBMC of patients was examined in two cases. Using nonadherent mononuclear cells (MNC) from two patients (JK and TU), no significant change in total CFU eos/neut yield was seen (84 ± 5.3 and 45.6 ± 6.2, respectively) when normal allogeneic PBMNC feeder layers replaced the autologous CSF source (data not shown). Similar findings were obtained when normal NAC were cultured over PBMNC underlayers of these patients.

From these data, it is evident that the high numbers of eosinophils in the PB of schistosomiasis patients (20 times the value of normal PB) are not reflected in vitro at the level of committed eosinophil stem cells. If one assumes that the in vitro progenitor cell assay is a true measurement of the frequency of committed progenitor cells of a specific lineage in vivo, then these data appear to indicate that other factors are involved in disease states. There are several possibilities. First, progenitor cells may differentiate at other anatomical site(s) and then migrate into the circulation. The bone marrow is a possible site, although it has been previously shown that more circulating stem cells (47 percent) differentiate in vitro into the eosinophilic lineage than do bone marrow stem cells (14 percent). Second, enhanced granulopoietic activity under this parasitic stress, as evidenced by histologic and anatomicopathologic examinations, may occur in extramedullary tissues with hematopoietic potential, such as the liver or spleen. Active eosinophiloiesis in such compartments could be attributed to a factor, similar to the eosinophil differentiation factor (EDF), being elaborated by antigen (e.g., eggs)-reactive T lymphocytes. Third, in PB of schistosomiasis patients, the eosinophils may accumulate as a result of either a prolonged half-life, as seen in cases of patients with the hypereosinophilic syndrome or a shortening of cell cycle time of eosinophilic precursors and a reduction of bone marrow transit time.

Prompted by this current investigation and previous work on accelerated eosinophilia during metazoan infections, further experiments are in progress to demonstrate the influence of schistosomal antigens and/or immune cellular elements on the regulation of granulopoiesis during human helminthic infection.

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References