Macrophage–Eosinophil Interactions in the Inflammatory Response to Trichinella spiralis

By Ronald S. Walls, Peter Hersey, and Paul G. Quie

Little is understood of the reasons why eosinophils accumulate in vivo, although a tissue reaction to administered antigen has been shown to be a prerequisite for the response. The present experiments aim to understand the significance of the tissue reaction to parasitic larvae in the chain of events leading to eosinophilia. Trichinella spiralis larvae were injected intraperitoneally in rats, and the peritoneal exudate was examined at intervals. Eosinophils were closely associated with macrophages rather than with other cell types or with parasites. A striking collection of eosinophils around individual macrophages was noted 48 hr after injection, and in particular following a second challenge. Use of indirect immunofluorescence techniques showed that these coincided with presence of immunoglobulin on macrophage surfaces. Incubation of peritoneal cells in medium containing 0.25% trypsin reduced macrophage surface immunofluorescence and numbers of rosettes. Rosettes could be constituted in vitro by incubation of normal peritoneal cells in medium containing antigen and antibody. These findings suggest that in the tissue reaction to parasitic larvae a close association exists between eosinophils and macrophages.

Eosinophilia occurs frequently in association with parasitic infections, hypersensitivity diseases, and allergic disorders. Eosinophils are predominantly tissue cells. Their appearance in response to antigen depends upon an inflammatory reaction to the antigen in host tissue. They are present in blood during their passage from bone marrow to tissues, where they fulfill a function which continues to be a tantalizing unsolved mystery of cell biology.

An intense, short-lived, and reproducible eosinophilia follows a single intravenous injection of Trichinella spiralis larvae. A similar response occurs after intraperitoneal injection when eosinophils accumulate in peritoneal fluid. This report describes the cellular response in the peritoneal cavity. The sequence of cellular changes in peritoneal fluid can be studied quantitatively to an extent not possible in histologic sections. Eosinophils are prominent and a close association between them and macrophages has been demonstrated which these studies suggest may be mediated by antigen-antibody complexes on macrophage surfaces.

MATERIAL AND METHODS

Experimental Animals

Parasite-free Wistar rats, weighing 180–250 g were used for all experiments. The rats were obtained from the Medical Research Council Radiobiological Research Unit, Harwell, Berkshire, England.
Trichinella spiralis. The Culbertson strain of T. spiralis larvae was obtained originally from Professor G.S. Nelson at the London School of Tropical Hygiene and Medicine and was maintained by serial infection of a rat colony. For the stimulus to eosinophilia, 5000 muscle stage larvae were injected intraperitoneally. There was a 4-wk interval between successive injections. Methods of preparing and administering larvae have been described.²

Peritoneal lavage. Rats were killed with ether. A small volume of fluid was removed from the peritoneal cavity using a Pasteur pipette and was added to 1 ml of Parker 199 tissue culture medium (Burroughs Wellcome) with bicarbonate, antibiotics, and heparin, 1 U/ml. Ten milliliters of culture medium was then injected into the peritoneal cavity and the fluid was allowed to drain out through a fenestrated needle into a sterile 25-ml plastic universal container. Two further exchanges of 10 ml fluid were made. Of a total of 30 ml fluid injected, 27–30 ml could be retrieved on each occasion.

Examination of peritoneal fluid. Cell preparations were made on glass slides with a cytocentrifu (Shandon) from the first samples of peritoneal fluid removed by Pasteur pipette. The rest was added to the fluid retrieved from peritoneal washings. The volume was noted, and total white cell and eosinophil counts were made as described before.² Slides were fixed in methanol and stained with Giemsa. Four hundred cells were examined on two slides from each animal. To identify phagocytic cells, 0.1 ml of 1 : 10 dilution of carbon suspension (Pelikan) in sterile water was injected in 2 ml saline into the peritoneal cavity, 1 hr before cells were collected.

Immunofluorescent studies. Peritoneal exudate cells were washed three times with medium 199. A pellet of 2 × 10⁶ cells was incubated at 37°C with rabbit antirat immunoglobulin, and conjugated with fluorescein isothiocyanate (FITC) (Nordic Pharmaceuticals) for 30 min. Cells were then washed three times in medium 199 and mounted in 1 : 1 suspension of glycerol and phosphate-buffered saline (pH 7.5). Slides were examined using a fluorescent microscope (Zeiss) with quartz iodide 100-W direct light source and primary filters, a FITC-3 interference filter and a 2-mm B.G. 3.8 blue filter. After counting the cells with immunofluorescent surface staining, the same field was examined without the interference filter to obtain the total number of cells. Lymphocytes bearing immunoglobulin on their surface (B lymphocytes) were excluded from the count of immunofluorescent cells on the basis of their smaller, more regular size and the lightly staining "ring" pattern. Control slides were incubated first with unconjugated rabbit antirat immunoglobulin.

RESULTS

Peritoneal Fluid Cytology

Total white cell counts in peritoneal fluid increased fourfold after one injection of larvae (Table 1) and sixfold after a second injection (Table 2). The increase in eosinophils was proportionately greater; sixfold after the first injection.
INFLAMMATORY RESPONSE TO TRICHINELLA SPIRALIS

Table 2: Cellular Composition of Peritoneal Fluid After Second Injection of Trichinella Larvae

<table>
<thead>
<tr>
<th>Time After Injection of Larvae (Mean ± 1 SE)</th>
<th>Number of Animals Examined</th>
<th>Total Eosinophils x 10⁶</th>
<th>Total White Cell Count x 10⁶</th>
<th>Per Cent Cells Counted on Smear (Mean ± 1 SE)*</th>
<th>Eosinophils</th>
<th>Neutrophils</th>
<th>Mononuclear Cells</th>
<th>Mast Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>3.53 ± 0.95</td>
<td>19.24 ± 4.45</td>
<td>30.13 ± 3.66</td>
<td>0.06 ± 0.04</td>
<td>61.78 ± 3.52</td>
<td>8.03 ± 2.19</td>
<td></td>
</tr>
<tr>
<td>6 hr</td>
<td>6</td>
<td>2.46 ± 0.74</td>
<td>31.23 ± 4.47</td>
<td>6.54 ± 0.99</td>
<td>57.17 ± 4.33</td>
<td>34.5 ± 4.06</td>
<td>1.71 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>24 hr</td>
<td>6</td>
<td>3.73 ± 0.83</td>
<td>28.82 ± 4.74</td>
<td>13.48 ± 3.75</td>
<td>28.96 ± 3.19</td>
<td>61.08 ± 4.11</td>
<td>0.33 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>48 hr</td>
<td>4</td>
<td>12.44 ± 3.71</td>
<td>38.08 ± 2.63</td>
<td>40.21 ± 4.69</td>
<td>2.01 ± 1.22</td>
<td>57.78 ± 3.75</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td>5</td>
<td>39.88 ± 6.82</td>
<td>117.24 ± 15.20</td>
<td>39.6 ± 3.02</td>
<td>0.3 ± 0.09</td>
<td>59.35 ± 2.93</td>
<td>0.75 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>4</td>
<td>30.35 ± 2.64</td>
<td>76.55 ± 15.61</td>
<td>38.81 ± 4.30</td>
<td>0</td>
<td>59.63 ± 4.33</td>
<td>1.56 ± 0.36</td>
<td></td>
</tr>
</tbody>
</table>

*Absolute cell counts x 10⁶ computed from total white cell and differential counts given in parenthesis.

A discrepancy was noted between total eosinophils counted by the direct method and numbers computed from differential and total white counts when they were present in low numbers (0 hr) or at 48 hr, possibly related to their tendency to associate with macrophages. These conclusions remain valid, however, whether direct eosinophil counts or numbers computed from total and differential counts are considered (Tables 1 and 2). The mononuclear cell response showed a similar time pattern to that of eosinophils. Neutrophils, on the other hand, were most numerous 6 hr after injection. Their numbers dropped rapidly thereafter (Tables 1 and 2). Mast cells became less numerous after injection. *Trichinella* larvae were sometimes seen in peritoneal fluid. They were surrounded by a heavy coating of cells, mostly neutrophils at 6 hr, and mononuclears thereafter, but at no stage were eosinophils in direct contact with the larvae.

**Eosinophil-Macrophage Rosettes**

A striking feature of the inflammatory response to larvae was the association between eosinophils and macrophages. This was demonstrated most dramati-

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*Fig. 1. Peritoneal fluid 48 hr after second injection of larvae. Numerous rosettes are formed by clustering of eosinophils around large mononuclear cells. Giemsa stain. x 140.*
cally by the formation of rosettes after a second injection of larvae (Figs. 1 and 2). Peripheral cells in most cases were exclusively eosinophils, but sometimes there were other mononuclear cells in addition. In the center were macrophages, 99% of which phagocytized carbon particles. Other cell types did not form clusters in this way. Rosettes were found in largest numbers 48 hr after a second injection of larvae. They were not present at 24 hr; only rarely were they seen at 72 hr and not at all later. As shown in Table 3, approximately 50% of mononuclear cells counted at 48 hr formed the central cells of rosettes.

Nature of Eosinophil-Macrophage Interaction

Fluorescent techniques were used to investigate the presence of antibody on the surface of macrophages and eosinophils. Peritoneal cells from normal rats and from those injected 48 hr previously for the second time with larvae (sensitized cells) were examined as described above. Fluorescence was observed on approximately 35% of sensitized mononuclear cells, but on only 3% of mononuclear cells from normal rats. Incubation with unconjugated rabbit antirat immunoglobulin prior to incubation with conjugated immunoglobulin quenched fluorescence completely. Eosinophils showed mson autofluores-

Table 3. Cells in Peritoneal Harvest 48 Hr After Second Injection of Trichinella Spiralis Larvae

<table>
<thead>
<tr>
<th>Rat</th>
<th>Eosinophils</th>
<th>Mononuclear Cells</th>
<th>Mononuclear Cells Forming Rosettes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>19</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>38</td>
<td>42</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>41</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>39</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>21</td>
<td>66</td>
</tr>
<tr>
<td>6</td>
<td>26</td>
<td>12</td>
<td>62</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>21</td>
<td>64</td>
</tr>
<tr>
<td>Mean</td>
<td>19.1</td>
<td>27.3</td>
<td>55.0</td>
</tr>
<tr>
<td>SE</td>
<td>8.5</td>
<td>11.7</td>
<td>14.3</td>
</tr>
</tbody>
</table>
Inflammarory Response to Trichinella spiralis

In three separate experiments, sensitized peritoneal cells were incubated with 0.25% trypsin in medium 199 for 1 hr at 37°C. This had the following effects:

1. It reduced the number of macrophages with fluorescence from 35% to 15%.
2. It also reduced the number of rosettes from a mean of 66 per 100 mononuclear cells counted to a mean of 17, and this was associated with an increasing number of free eosinophils in the peritoneal sample. Incubation of cells for 1 hr in medium 199 without trypsin did not affect the rosette phenomenon.

Clustering of macrophages and eosinophils was reproduced in vitro by incubating peritoneal cells from normal rats with the supernatant of the peritoneal harvest from sensitized rats, or with larval antigen and antiserum. Larval antigen consisted of a saline extract of T. spiralis larvae, prepared according to the method of Kagan. Antiserum consisted of the pooled serum from rats collected on day 5 of a second intraperitoneal injection of T. spiralis larvae. In one such experiment, incubation of normal cells with supernatant from sensitized cells resulted in 24 rosettes per 100 mononuclear cells, with a corresponding drop in the number of free mononuclears and eosinophils. This was associated with an increase in the number of mononuclear cells with fluorescence from 3% to 26%. Incubation of normal peritoneal cells with antigen alone or antiserum alone did not result in rosette formation. Other antigen-antibody combinations were not tested.

Discussion

A single intraperitoneal injection of T. spiralis larvae produced an accumulation of eosinophils in peritoneal fluid disproportionate to the total cellular response. A second injection resulted in an augmented response as well as eosinophils in peripheral blood. In most other studies it has been necessary to give multiple injections of antigens to elicit an eosinophil response.

A striking feature of the reaction was the association of eosinophils with macrophages, and this is emphasized by their organization into rosettes. Speirs and Speirs noted clusters of neutrophils, eosinophils, and lymphocytes around large mononuclear cells in peritoneal fluid of mice injected with 1H tetanus toxin administered with antitoxin, or in mice previously immunized with toxoid. However, the emphasis of the present report on eosinophil-macrophage rosettes was not apparent.

Immunofluorescent studies suggested that eosinophil-macrophage rosettes were mediated by the presence of immunoglobulin on the surface of macrophages. Additional evidence for the role of immunoglobulin was the observation that numbers of rosettes were reduced by incubation with trypsin, which alters surface properties of cells and may remove cytophilic antibody.7 In addition, clustering of mononuclear cells and eosinophils could be reproduced in vitro by adding to normal peritoneal cells a mixture of immune serum and larval antigen. This suggests that both antibody and antigen are required to produce this phenomenon, possibly through the presence of immune complexes...
on the surface of macrophages. This would be in keeping with previous observations that eosinophils are attracted to and ingest antigen-antibody complexes.\(^8\) The reason for the formation of rosettes at a particular time in the response is not explained by these experiments, but may be associated with the class of antibody produced or the size of complexes formed.

We feel that eosinophil-macrophage rosettes are a real phenomenon with physiologic significance. They did not appear to be merely the result of physical approximation of increasing numbers of eosinophils and macrophages since both continued to increase in peritoneal fluid after the time of maximum rosette formation. Nor is it likely that they represent nonspecific ingestion by macrophages of effete cells. The majority of rosettes were formed by eosinophils and not randomly by mixed cell types, and macrophages were not seen to contain eosinophils or eosinophil granules after the time of maximal rosette formation. Certainly a parallel is seen in the requirement for clustering of lymphoid cells in antibody production during the immune response in vitro.\(^9,10\)

ACKNOWLEDGMENT

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REFERENCES

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