The Role of Peroxidase in the Bactericidal Activity of Human Blood Eosinophils

By Joseph S. Bujak and Richard K. Root

Utilizing cell preparations that contained greater than 90% eosinophils and sodium azide to inhibit the lysosomal enzyme peroxidase, studies of eosinophil bactericidal activity were undertaken. Eosinophils did not kill bacteria as well as neutrophils, principally because of a reduced phagocytic capacity. In contrast to neutrophils, eosinophils had high resting iodination activity but failed to increase this activity following phagocytosis. One millimolar sodium azide, which inhibits both eosinophil and neutrophil peroxidase as measured by the iodination of trichloroacetic acid precipitable protein, impaired neutrophil staphylocidal activity, but enhanced eosinophil killing of Staphylococcus aureus primarily by increasing phagocytic uptake. Bactericidal studies utilizing lysostaphin suggest that neutrophil peroxidase exerts its contribution to bactericidal activity only during the early postphagocytic period. Eosinophil peroxidase is genetically and biochemically distinct from neutrophil peroxidase, and appears to play no role in the bactericidal activity of intact eosinophils.

Hydrogen peroxide generation is required for normal neutrophil bactericidal activity, and in conjunction with the lysosomal enzyme myeloperoxidase (MPO), it provides an especially potent bactericidal system. Previous studies have documented that eosinophils generate more H$_2$O$_2$ and contain more peroxidase than neutrophils. The peroxidase located within the eosinophil granule is known to be genetically and biochemically distinct from neutrophil peroxidase, and to be discharged into the phagolysosome with the degranulation that follows phagocytosis. Eosinophils do not kill bacteria as well as neutrophils, apparently due to their diminished phagocytic capability rather than to any intracellular deficiency.

The interpretation of many of the previous studies of human eosinophils has been clouded by the inability to obtain eosinophil preparations free from contamination by other leukocytes. The advent of a technique for obtaining relatively pure preparations of human eosinophils prompted us to study aspects of eosinophil function that might contribute to an understanding of their bactericidal mechanisms.

Utilizing cell preparations containing greater than 90% eosinophils, we have investigated the role of peroxidase as it pertains to the bactericidal capabilities of these cells. The results suggest that eosinophil peroxidase may not be functionally involved in the killing of ingested staphylococci. This observation is in
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Duration of Eosinophilia</th>
<th>Presenting Symptoms and Signs</th>
<th>WBC</th>
<th>% Eosinophils</th>
<th>Diagnosis</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. M.</td>
<td>52</td>
<td>M</td>
<td>7 yr</td>
<td>Asymptomatic</td>
<td>13,600</td>
<td>44</td>
<td>Eosinophilia of unknown etiology</td>
<td>Remains well</td>
</tr>
<tr>
<td>D. P.</td>
<td>65</td>
<td>M</td>
<td>11 mo</td>
<td>Angioedema, hoarseness, malaise weakness, fever, myalgias, 10-lb weight loss, mucous diarrhea, salivary gland swelling, interstitial pneumonitis, myositis, proteinuria</td>
<td>30,000</td>
<td>60</td>
<td>Hypereosinophilic syndrome</td>
<td>Controlled on every other day prednisone; muscle biopsy revealed perivascular accumulation of eosinophils without vasculitis</td>
</tr>
<tr>
<td>J. T.</td>
<td>45</td>
<td>M</td>
<td>3 yr</td>
<td>Fatigue, weight loss, headache, shortness of breath, aortic valve dysfunction with emboli, left ventricular hypertrophy, congestive heart failure, proteinuria with decreased creatinine clearance</td>
<td>96,000</td>
<td>70</td>
<td>Loeffler’s endocarditis</td>
<td>Eosinophils appeared hypersegmented and with a paucity of granules, thrombocytopenia secondary to myelofibrosis therapy</td>
</tr>
<tr>
<td>H. E.</td>
<td>23</td>
<td>M</td>
<td>1 mo</td>
<td>Asymptomatic</td>
<td>8,700</td>
<td>11</td>
<td>S. haematobium infection</td>
<td>Peace Corps worker with asymptomatic S. haematobium infection; hookworm in stool</td>
</tr>
<tr>
<td>E. W.</td>
<td>43</td>
<td>M</td>
<td>1½ yr</td>
<td>Weakness, hypertension, hepatomegaly with abnormal liver function tests, congestive heart failure with cardiomyopathy, creatinine 2.3. Renal biopsy revealed eosinophilic nephritis with necrotizing arteriolitis</td>
<td>42,100</td>
<td>92</td>
<td>Loeffler’s endocarditis</td>
<td></td>
</tr>
<tr>
<td>G. M.</td>
<td>50</td>
<td>M</td>
<td>15 yr</td>
<td>Episodic pruritis with popular skin rash</td>
<td>25,900</td>
<td>80</td>
<td>Eosinophilia of unknown etiology</td>
<td>Totally well but for pruritis</td>
</tr>
<tr>
<td>D. C.</td>
<td>43</td>
<td>M</td>
<td>4½ yr</td>
<td>Cough, night sweats, transient skin rashes and dermatographism, fatigue, weight loss, splenomegaly, hilar and peri-aortic lymphadenopathy, frequent premature ventricular contractions, peripheral neuropathy, and neuritis</td>
<td>18,100</td>
<td>93</td>
<td>Probable Loeffler’s endocarditis, neuropathy of uncertain etiology</td>
<td></td>
</tr>
</tbody>
</table>
sharp contrast to the activity of neutrophil peroxidase, and serves to further distinguish the enzymes of these two cell types.

**MATERIALS AND METHODS**

**Patients**

Patients with a circulating eosinophilia of greater than 15% were selected for study. A variety of primary diagnoses were associated with the eosinophilia, including one patient with schistosomiasis and two healthy patients with long-standing eosinophilia for which no cause could be found (Table I). Studies in seven patients confirmed previous work that, regardless of the primary diagnosis, the findings were consistent from individual to individual. For this reason, the data obtained from the different patients were grouped together and can be presumed to indicate the function of eosinophils per se, rather than a particular manifestation of their individual diseases. The data on bactericidal function and peroxidase activity was obtained using cells from patients DP, DC, and EW only. Controls consisted of healthy laboratory personnel.

**Leukocytes**

Patient and control leukocytes were separated from heparinized peripheral blood by dextran sedimentation using previously reported methods. Hypotonic lysis was routinely employed to reduce erythrocyte contamination to less than one red cell per leukocyte in all cell suspensions.

To obtain leukocyte suspensions with greater than 90% eosinophils, the heparinized peripheral blood of the patients was processed according to the method of Day. In this technique, the leukocyte-rich supernatant obtained after dextran sedimentation of red cells is layered on Hypaque and the eosinophils separated by density gradient centrifugation. Eosinophils prepared in this manner were not functionally different from those obtained by dextran sedimentation alone. This technique was not necessary when blood was obtained from patients with >90% eosinophilia. After twice washing the cells in divalent cation-free modified Hank's solution, the number of leukocytes was enumerated with a Coulter Counter and the differential counts obtained by examining Wright's stained smears. The suspensions were adjusted to contain 10 phagocytes per milliliter in Hank's balanced salt solution (HBSS). In all studies, eosinophils comprised greater than 90% of the final cell suspension, and neutrophils greater than 85% of the control preparations. The neutrophil content of the eosinophil preparations averaged less than 5% and that of eosinophils in control preparations less than 5%.

**Organism**

A strain of coagulase positive *Staphylococcus aureus* phage type 29 and 42B was used throughout.

**Serum**

A single normal donor (AB+) was used throughout these studies to provide serum for opsonization of organisms. Peripheral blood was allowed to clot for 1-2 hr at room temperature, the serum separated, aliquoted, and stored at -70°C until used. All samples were used within 1 mo, and over this time period there was no loss in opsonic activity.

**Phagocytic Studies**

The phagocytic uptake of 14C radiolabeled *S. aureus* was measured utilizing a previously reported method and a ratio of 10 bacteria per phagocyte. Each experiment was run in triplicate and the per cent uptake calculated as

\[
\frac{\text{Average cell-associated CPM}}{\text{Total CPM added}} \times 100.
\]

Previous studies have shown that the radioactivity associated with the washed cells agrees precisely with microscopic determinations of phagocytosis.
Bactericidal Assay

The bactericidal assays were performed in duplicate by the method of Hirsch and Strauss, with modifications as previously described. A ratio of three to five resting-phase organisms per phagocyte was employed. Studies of the killing of intracellular S. aureus were carried out as follows: 5 x 10^6 phagocytes in HBSS and 10% AB serum were preincubated at 37°C for 10 min, and then approximately 2 x 10^7 resting-phase organisms were added (final volume 1 ml). The capped tubes were returned to the incubator and vertically tumbled 12 times per minute (Fischer Roto Rack) for 20 min to allow phagocytosis to occur. The tubes were then sampled to assess total killing at 20 min, and 0.1 ml lysostaphin (Schwarz/Mann, Orangeburg, N.Y.) in HBSS was added (final concentration 10 u/ml) to kill extracellular staphylococci. Further samples were plated at 30, 90, and 120 min to assess survival of intracellular bacteria at these times. Since lysostaphin destroyed all extracellular organisms by 10 min, the percent of viable intracellular organisms could be determined and compared to the total viable number at 20 min.

Iodination

Iodination of protein by resting and phagocytizing leukocytes was measured by previously described methods. Heat-killed S. aureus in a ratio of 100 organisms per phagocyte was utilized as the phagocytic particle.

In some experiments, sodium azide (final concentration 1 mM) was added to inhibit iodination and to test its effect on phagocytosis and killing of bacteria.

RESULTS

Phagocytosis of ^14C S. aureus

Eosinophils exhibited significantly less phagocytic activity than neutrophils during kinetic studies over a 20-min period (Fig. 1). By 20 min, the uptake by eosinophils averaged 74.5% of that by the control neutrophils (Table 2). The addition of 1 mM sodium azide to the cell preparations stimulated phagocytosis.

![Fig. 1. Phagocytic uptake of ^14C-labeled heat-killed S. aureus by neutrophils (five normal laboratory personnel) and eosinophils (patients D. P., L. M., E. W., D. C., and H. E.). The differences between each of the three points plotted are statistically significant with a p value < 0.01 (paired sample t test). Each line represents the mean of the numbers of experiments shown by the numbers in parentheses, and the brackets the standard error of the mean.](image-url)
Table 2. Phagocytosis of $^{14}$C S. aureus by Eosinophils* and Neutrophils and Effect of Sodium Azide

<table>
<thead>
<tr>
<th>Preparation</th>
<th>% Uptake at 20 min</th>
<th>% of Neutrophil Activity</th>
<th>p Value</th>
<th>% of Non-azide Treated Activity</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils</td>
<td>45.8 ± 1.3</td>
<td>74.5 ± 6.4</td>
<td>&lt;0.05</td>
<td>112.8 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Eosinophils and azide</td>
<td>51.6 ± 1.4</td>
<td>83.9 ± 7.0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>64.3 ± 6.7</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils and azide</td>
<td>67.6 ± 7.3</td>
<td>—</td>
<td></td>
<td>107.2 ± 6.7</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

*Assays performed using eosinophils from patients E. W., D. C., and D. P.
†Per cent of bacterial inoculum phagocytized in six experiments.
‡Paired sample t test comparing the mean differences between the phagocytic activity of each preparation as indicated.
§Per cent of the activity of azide-treated neutrophils.

by neutrophils slightly (a mean 7.2%); however, this increase was not statistically significant. In contrast, azide produced a greater and statistically significant stimulation of eosinophil phagocytic activity (a mean 12.8%). Despite this stimulation, the resulting phagocytic activity of eosinophils still averaged only 81.0%–83.9% of that of neutrophils (Table 2).

Iodination

The average iodination activity of resting eosinophils was 10.7 times greater than resting neutrophils (Table 3); however, neutrophils increased this activity almost sixfold following phagocytosis, while eosinophil activity remained essentially unchanged. Azide treatment reduced the level of iodination in phagocytizing eosinophils and neutrophils to below their respective resting values.

Bactericidal Activity

Eosinophils killed significantly fewer bacteria than neutrophils over a 2-hr incubation period (Fig. 2). As shown in Table 4, killing by eosinophils averaged 56.7% of that of neutrophils at 20 min and 69.6% at 120 min. The addition of

Table 3. Iodination of Trichloroacetic Acid Precipitable Protein by Resting Eosinophils and Neutrophils and those Phagocytosing Heat-Killed S. aureus With and Without 1 mM Sodium Azide

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>No. Subjects</th>
<th>No. Experiments</th>
<th>Protein Iodination (nmole/5 x 10^6 cells/hr) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting neutrophils</td>
<td>4</td>
<td>5</td>
<td>0.095 ± 0.036</td>
</tr>
<tr>
<td>Phagocytosing neutrophils</td>
<td>4</td>
<td>5</td>
<td>0.408 ± 0.115</td>
</tr>
<tr>
<td>Phagocytosing neutrophils plus 1 mM azide</td>
<td>2</td>
<td>2</td>
<td>0.019 (0.012–0.026)*</td>
</tr>
<tr>
<td>Resting eosinophils</td>
<td>5</td>
<td>6</td>
<td>1.019 ± 0.045</td>
</tr>
<tr>
<td>Phagocytosing eosinophils</td>
<td>5</td>
<td>6</td>
<td>1.111 ± 0.046</td>
</tr>
<tr>
<td>Phagocytosing eosinophils plus 1 mM azide</td>
<td>2</td>
<td>2</td>
<td>0.019 (0.012–0.026)*</td>
</tr>
</tbody>
</table>

*Range of two experiments.
†Cells obtained from patients D. P., D. C., J. T., H. E., and E. W. (twice).
Fig. 2. Killing of *S. aureus* by eosinophils and neutrophils with and without the addition of 1mM sodium azide. The points plotted at 20 min are the mean of four experiments, and those plotted at 60 and 120 min are the mean of six experiments. The brackets represent the standard error of the mean. These data were obtained using cells from patients E. W., D. C., and D. P., and six normal laboratory personnel.

Table 4. Bactericidal Activity of Neutrophils and Eosinophils and the Effect of Sodium Azide

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Time</th>
<th>% Killing Mean ± SE</th>
<th>% of Neutrophil Activity Mean ± SE</th>
<th>% of Nonazide-Treated Activity Mean ± SE</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils</td>
<td>20 min</td>
<td>46.7 ± 6.1</td>
<td>56.7 ± 6.0</td>
<td>&lt;0.01</td>
<td>—</td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td>67.5 ± 4.7</td>
<td>82.2 ± 2.8</td>
<td>&lt;0.01</td>
<td>148.6 ± 12.5</td>
</tr>
<tr>
<td>+ azide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td>82.0 ± 3.9</td>
<td>100.0</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>+ azide</td>
<td>53.5 ± 8.4</td>
<td>—</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>120 min</td>
<td>64.5 ± 4.0</td>
<td>69.6 ± 3.7</td>
<td>&lt;0.001</td>
<td>121.4 ± 12.6</td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td>76.0 ± 4.6</td>
<td>82.4 ± 5.5</td>
<td>&lt;0.05</td>
<td>121.4 ± 12.6</td>
</tr>
<tr>
<td>+ azide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td>92.5 ± 1.4</td>
<td>100.0</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>+ azide</td>
<td>77.1 ± 4.9</td>
<td>—</td>
<td></td>
<td>—</td>
</tr>
</tbody>
</table>

*Paired sample t test comparing the mean differences for the bactericidal activity of each preparation as indicated.

†Parentheses indicate number of experiments.

§Per cent of the activity of azide-treated neutrophils.

Although the data on the six experiments shown failed to demonstrate statistical significance, because the data was suggestive, two additional experiments comparing azide-treated eosinophils to untreated controls were done. The mean ± SE over the eight experiments were 74.4 ± 4.8 and 57.5 ± 5.4, respectively, and the difference statistically significant p < 0.05.
Table 5. The Effects of 1 mM Sodium Azide on the Bactericidal Activity of Eosinophils and Neutrophils as Measured Following the Addition of Lysostaphin

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Viable Intracellular S. aureus at 30 min</th>
<th>% Reduction in Viability at 60 min</th>
<th>% Reduction in Viability at 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>±SE</td>
<td>p Value</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.2</td>
<td>1.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Neutrophils plus azide</td>
<td>7.7</td>
<td>1.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1.4</td>
<td>3.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Eosinophils plus azide</td>
<td>4.8</td>
<td>3.4</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Lysostaphin in a final concentration of 10 U/ml was added at 20 min, and, in the subsequent 10 min, killed all S. aureus in cell-free control. (The number of viable organisms at 30 min equals 100%.

sodium azide impaired the bactericidal activity of neutrophils to 64.3% of the untreated control at 20 min and 83.3% at 120 min. In contrast, the bactericidal activity of azide-treated eosinophils improved relative to untreated cells. Killing became equivalent to that of azide-treated neutrophils, but was still below that of untreated neutrophils.

To determine if this enhancement by azide was due to an increase in the rate of killing of intracellular bacteria in addition to its stimulatory effect on phagocytosis, lysostaphin was utilized to kill nonphagocytized extracellular bacteria after 20 min, and the number of viable counts followed as described in Materials and Methods. As shown in Table 5, more intracellular organisms were recovered from both eosinophils and neutrophils at 30 min (10 min after lysostaphin addition) when incubated in the presence of azide. Thereafter, the rate of decline of viable bacteria (expressed in terms of the per cent survival of organisms present at 30 min) was similar in both cell types and was not altered by azide.

DISCUSSION

As noted in previous studies and confirmed in the present investigation, eosinophils do not kill S. aureus as effectively as neutrophils. In these earlier studies, microscopic methods and late (30 min) time periods were used to assess the phagocytic activity of the different cell preparations. A diminished phagocytic capacity of eosinophils compared to neutrophils was reported and was thought to be primarily responsible for their reduced bactericidal activity. Our findings support these conclusions and more precisely define the kinetics of phagocytosis in relation to killing by both cell types. As suggested by others, the major rate-limiting step for neutrophil bactericidal activity in the initial stages of contact between staphylococci and cells is phagocytosis, since phagocytic and killing rates were almost identical. The discrepancy between the per cent of organisms taken up at 20 min as calculated with 14C S. aureus (64.3% ± 6.7%) versus the number killed (82.0% ± 3.9%) in the same time period could be explained by differing bacteria to cell ratios in the two assays (10:1 for phagocytosis versus 4:1 for bactericidal measurements), or, more likely, that the pour plate method used to measure bactericidal activity may give falsely low readings for viable cell-associated bacteria due to clumping of...
organisms. Like neutrophils, eosinophil phagocytic and bactericidal rates were similar over the first 20 min (45.8% ± 1.3% phagocytized, 46.7% ± 6.1% killed) and averaged 74.5% ± 6.4% and 56.7% ± 6.0%, respectively, of similar activities for neutrophils. In both neutrophils and eosinophils, intracellular bacteria, distinguished with the use of lysostaphin, were killed at similar rates after 20 min.

While such data suggest that the bactericidal mechanisms of neutrophils and eosinophils might be basically similar, the observations on the in situ operation of the myeloperoxidase-H$_2$O$_2$-halide antimicrobial systems within the two cell types suggest otherwise. As shown by Klebanoff, myeloperoxidase from neutrophils is capable of fixing free iodide to protein in the presence of a suitable source of peroxide to act as substrate, and intact phagocytizing neutrophils show increased iodination. Sodium azide inhibits both iodination and intracellular killing by neutrophils, by inhibition of myeloperoxidase. In the present investigation, eosinophils were found to be capable of converting iodide to a trichloroacetic acid precipitable form, and, like the control neutrophil preparations, this activity was azide inhibitable. Beyond this, however, the resemblance between the two cell types with respect to iodination and azide responses differed significantly. Eosinophils were found to have over tenfold greater iodination activity than neutrophils during the resting phase. This parallels previous observations of the hypermetabolic state of these cells with respect to oxygen consumption, glucose oxidation, and H$_2$O$_2$ production. Furthermore, eosinophils failed to increase protein iodination with phagocytosis of bacteria, whereas this activity was increased sixfold by neutrophils ingesting bacteria. Finally, azide did not inhibit killing of staphylococci by eosinophils, but in fact stimulated it.

The major effect of azide on enhancing eosinophil bactericidal activity appeared to be related to a stimulation of phagocytosis. Azide-treated eosinophils phagocytized a mean 51.6% ± 1.4% of the $^{14}$C $S$. aureus inoculum at 20 min and killed 67.5% ± 4.7%, representing a stimulation of 12.8% and 48.6% of both activities, respectively, above nontreated cells. The increases for both functions were statistically significant, and, as mentioned above for neutrophils, the discrepancies between the values for ingestion and killing have at least two possible explanations. The findings of greater numbers of bacteria at 30 min within eosinophils treated with azide, in the presence of less total surviving bacteria, is consistent with the enhanced phagocytic activity. Whether azide directly stimulates phagocytosis itself or the increased uptake reflects an improved rate of "processing" of intracellular bacteria cannot be precisely answered by our data. Regardless of the mechanism, the findings stand in sharp contrast to those with neutrophils.

Azide-treated neutrophils displayed slightly, but not significantly, increased phagocytic rates and significantly depressed intracellular killing, as previously reported. Of note was the observation that killing rates in both untreated and azide-treated neutrophils were similar after 30 min, suggesting that the major contribution of myeloperoxidase to the bactericidal activity of neutrophils is within the early time periods after phagocytosis. This is consistent with other data that indicate that myeloperoxidase is rapidly delivered into phagocytic
vacuoles after ingestion and that patients totally lacking in myeloperoxidase or with defective intraphagosomal delivery of this enzyme have impairments in killing of intracellular organisms that are most pronounced in the early phases of phagocytosis.

These findings raise several important questions about the biological role of myeloperoxidase within eosinophils. The operation of this system within neutrophils has been shown to be dependent upon several factors, including post-phagocytic increments in H₂O₂ production, the number and type of particles ingested, and the simultaneous appearance of the reactants within the phagocytic vacuole. What accounts for the very high levels of resting eosinophil iodination activity is not apparent. In part, this must be related to the increased peroxide production of these cells, but what protein is being iodinated in the absence of phagocytized particles? Secondly, why is there no significant increase in iodination after phagocytosis? Such results could be due to a failure of eosinophils to produce significantly increased amounts of peroxide after particle ingestion; however, previous studies have indicated that another peroxide-dependent reaction mediated by catalase, formate oxidation, increases when eosinophils phagocytize. A third possibility is that eosinophil peroxidase may not be delivered into the phagocytic vacuole with the ingested recipient bacteria, a possibility that seems ruled out by studies showing that eosinophil lysosomes degranulate following phagocytosis. Finally, it is conceivable that recipient proteins within the eosinophils, other than bacteria, compete with the ingested organisms for myeloperoxidase-H₂O₂ catalyzed iodination.

Whatever the explanation, the present investigations indicate that beyond its basic peroxidative activities, eosinophil peroxidase functionally resembles neutrophil peroxidase only to the extent that the iodinating activities of both enzymes within intact cells are inhibited by azide. Whereas neutrophil myeloperoxidase is integrally involved in the activity of a major bactericidal system within the cell, the failure of azide to inhibit killing by eosinophils suggests that it does not play a role in the intracellular killing mechanisms of these cells. Thus, in addition to the antigenic, genetic, and biochemical distinctions between eosinophil and neutrophil peroxidases previously reported, our studies appear to indicate that they are functionally distinct within the intact cell as well. The crucial question of whether or not this functional distinction would hold true in an in vitro system to assess bactericidal activity outside intact cells employing peroxidases from both cells remains to be answered by future investigations.

Since eosinophils lack other antibacterial agents present in neutrophils, such as lysozyme and phagocytin, how does the eosinophil kill ingested organisms if myeloperoxidase does not participate in this activity? Klebanoff has shown that neutrophils genetically deficient in myeloperoxidase and azide-treated normal neutrophils generate increased levels of H₂O₂ as compared to controls. He has suggested that this is the result of diminished catabolism of H₂O₂ by the deletion of the MPO pathway and, in the case of hereditary MPO deficiency, that the increased levels of peroxide that result may explain the relatively normal bactericidal activity of these cells. Whether or not an analogous mechanism exists within eosinophils remains to be shown. Finally, considerable data
are being accumulated to indicate that neutrophils contain bactericidal mechanisms that do not involve H$_2$O$_2$ and MPO. It seems quite possible that eosinophils may possess some or all of the elements of the systems, and this provides an area for further study.

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REFERENCES

11. Bujak JS: Unpublished observations
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