The Ingestion of IgG-sensitized Erythrocytes by Abnormal Neutrophils

By A. Zipursky and E. J. Brown

The ingestion of IgG-sensitized erythrocytes was compared in monocytes and neutrophils. Normally neutrophils do not ingest IgG-sensitized erythrocytes, and it has been suggested that phagocytosis of IgG-sensitized erythrocytes is restricted to cells of the monocyte series. Neutrophils from 15 patients with acute leukemia and six patients with leukocytosis were studied in experiments with anti-D (IgG) sensitized erythrocytes. Normally these cells are not ingested by neutrophils, although monocyte erythrophagocytosis occurs. In contrast, neutrophils of patients with acute leukemia and leukocytosis ingested anti-D-sensitized erythrocytes following binding of these cells through a specific IgG receptor site. These results show that neutrophil function differs greatly, dependent on the pattern of myelopoiesis. Neutrophils formed during abnormal myelopoiesis may have erythrocyte binding and ingestion characteristics similar to that of the monocyte.

Monocytes and macrophages have surface receptors for immunoglobulin G (IgG) and for the third component of complement (C3). As a result, erythrocytes sensitized by IgG or C3 are ingested by these cells.

In contrast, several studies have shown that neutrophils ingest C3-sensitized, but not IgG-sensitized, erythrocytes. It had been suggested, therefore, that the IgG receptor site is relatively specific for the monocyte-macrophage series.

Messner and Jelinek recently reported that neutrophils can ingest certain IgG-sensitized bacteria and erythrocytes in the absence of complement activation. Furthermore, Ishizaka et al., using radiolabeled immunoglobulins, have detected IgG molecules and IgG receptors on the surface of neutrophils.

It is likely, therefore, that, although there are neutrophil IgG receptors, these cells are less effective phagocytes for IgG-sensitized erythrocytes than monocytes. In the present study, we confirm this in the case of normal neutrophils and show as well that neutrophils formed in disease have properties similar to the monocyte.

Materials and Methods

Leukocytes were obtained from heparinized blood (100 IU heparin sodium per 10 ml blood) by a 3% dextran sedimentation. Cells from the leukocyte-rich supernatant were spun out of the dextran plasma at 150 g for 8 min and were washed once in 20% fetal calf serum (FCS). Cells were resuspended in 30% FCS in Krebs-Ringer buffer and were allowed to attach to flying cover slips in Leighton tubes for 90 min at 37°C. The attached cell monolayer was washed twice (at 37°C) with 0.5 1.0 ml of 20% FCS in Krebs-Ringer, then the monolayer was overlaid with 1 ml of appropriate media: 30%, FCS Krebs-Ringer, or 30%, FCS Krebs-Ringer with added immuno-
globulin. Stock immunoglobulin was made of Cohn fraction II human gamma globulin and was used at a final concentration of 0.93 μM/liter.

Red cells of blood group A Rh-positive donors were washed three times in Krebs-Ringer buffer and resuspended to a 50%, hematocrit. To 0.04 ml of the erythrocyte suspension was added 0.4 ml of either Krebs-Ringer, 1 dilution of commercial (Dade) anti-A antisera in Krebs-Ringer, or a 1 dilution of commercial (Dade) anti-D antisera. The nonsensitized control cells and the anti-D-sensitized cells were incubated at 37°C for 1 hr. The anti-A-sensitized cells were sensitized for 1 hr at room temperature. Erythrocytes were washed three times and resuspended to 0.2 ml in Krebs-Ringer buffer. A Coombs test was done on the control and the anti-D-sensitized erythrocytes. Erythrocytes were added to Leighton tubes of attached leukocytes and incubated for 1 hr at 37°C. The preparation was agitated and washed as before with warm 20%, FCS Krebs-Ringer, the cover slips were removed, air dried, and stained with May-Grunwald-Giemsa stain.

Cells were evaluated by counting 100–200 monocytes or neutrophils and scoring these as cells with ingested erythrocytes or attached erythrocytes. If a cell had both ingested and attached red cells, it was scored as a cell with an ingested erythrocyte.

The appearance of neutrophils and monocytes on the cover slip is shown in Fig. 1A. The cells are easily distinguished from one another. In Fig. 1B, erythrophagocytosis is seen only in the monocyte and not in neutrophils. In Fig. 1C, both monocyte and neutrophil erythrophagocytosis is seen.

RESULTS

Normal Subjects

The peripheral blood monocytes and neutrophils of 16 normal subjects were studied. Anti-D-sensitized erythrocytes were exposed in suspension to monocytes and neutrophils adherent to glass cover slips. Under these conditions only ingestion of IgG-sensitized cells occurs, since complement is not activated.

An average of 64% of the monocytes ingested anti-D-sensitized cells, compared to 0.5% of the neutrophils (range, 0%–4%) (Fig. 2). The ingestion of anti-D-sensitized cells by neutrophils was not significantly greater than the ingestion of nonsensitized erythrocytes. That the neutrophils were viable is shown by their ingestion of anti-A-sensitized cells, which occurs through a complement-dependent reaction.7 The addition of nonspecific gamma globulin (final concentration 0.93 μM/liter) to the incubation suspension completely inhibited phagocytosis of anti-D-sensitized cells, suggesting that binding had occurred through a receptor site for IgG. The ingestion of anti-A-sensitized cells was
Fig. 2. Phagocytosis of anti-D- and anti-A-sensitized erythrocytes by monocytes (M) and neutrophils (N) of normal subjects. Monocytes and neutrophils were attached to cover slips in Leighton tubes. These cells were then incubated with nonsensitized (control), anti-D-sensitized, or anti-A-sensitized erythrocytes. The percentage of neutrophils and monocytes containing erythrocytes was then counted.

not inhibited by the gamma globulin, in keeping with the complement-mediated erythrophagocytosis in this system.7

Acute Leukemia

The patients studied included ten with myeloblastic, one with monocytic, and four with lymphoblastic leukemias. All studies were performed when the patients were in relapse. Many of the patients were studied more than once; the

Fig. 3. Phagocytosis of anti-D-sensitized erythrocytes by neutrophils of subjects with acute leukemia. Patients 1–10 were acute myeloblastic or myelomonocytic leukemia, patient 11 was acute monocytic leukemia, patients 12–15 were acute lymphoblastic leukemia. Each number on the graph represents one patient, and the results for each time they were tested are shown.
results of all studies are shown in Fig. 3. The majority of cases showed neutrophil erythrophagocytosis greater than normal; however, it should be observed that there was considerable variation within the group and with repeat testing of the same patient. In each study, erythrophagocytosis of nonsensitized erythrocytes was less than 1%. Also, the addition of gamma globulin to the incubation medium in a final concentration of 0.93 μmole/liter reduced neutrophil erythrophagocytosis to less than 1%.

The results bore no relationship to total leukocyte or neutrophil count, or to the type of treatment the patient had received. Neutrophil morphology was normal in all cases. The highest counts were in one patient with acute monocytic leukemia.

Subjects With Leukocytosis

Six patients with neutrophilic leukocytosis associated with severe infections were studied (three on one occasion and three twice) (Table 1). Neutrophilic erythrophagocytosis of anti-D-sensitized cells was above normal in all but one test. In all studies the addition of IgG to the incubation suspension reduced erythrophagocytosis to less than 1%. Erythrophagocytosis of nonsensitized erythrocytes was also less than 1%. There did not appear to be any relation between the degree of erythrophagocytosis and the total neutrophil count.

DISCUSSION

These studies demonstrate that neutrophils formed during disease have properties that differ from normal. In our experimental system, normal neutrophils do not ingest anti-D-sensitized erythrocytes, in contrast to monocytes where erythrophagocytosis is active. In disease, however, neutrophil phagocytosis of anti-D-sensitized erythrocytes was similar to that of monocytes. In both cells, ingestion seemed to occur through binding to a surface IgG receptor, since the phenomenon was completely inhibited by nonspecific immunoglobulin in the incubation suspension.

It has been suggested that the IgG receptor is characteristic of the monocyte-macrophage series. Certainly under normal conditions monocytes bind and ingest IgG-sensitized erythrocytes, in contrast to neutrophils which display virtually no binding or ingestion.

Table 1. Neutrophil Phagocytosis of Anti-D-Sensitized Erythrocytes

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Neutrophil Count (per cu mm)</th>
<th>Per Cent Neutrophils With Ingested Erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombophlebitis</td>
<td>21,200</td>
<td>6</td>
</tr>
<tr>
<td>Prostatitis</td>
<td>20,000</td>
<td>5.5</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>20,000</td>
<td>50</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>19,500</td>
<td>21.5</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>14,000</td>
<td>13</td>
</tr>
<tr>
<td>30,000</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>8,300</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>14,500</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ruptured appendix</td>
<td>17,000</td>
<td>5</td>
</tr>
<tr>
<td>Control subjects (15)</td>
<td>0–4</td>
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Why, then, in association with leukemia or leukocytosis, do neutrophils ingest IgG-sensitized cells? It would seem most likely that these cells possess more functional or exposed IgG receptors than the normal, permitting binding and ingestion of sensitized erythrocytes.

Whatever the explanation, it is clear that neutrophils of patients with acute leukemia or leukocytosis differ, qualitatively, from normal. That this is not simply a function of neutrophil age is supported by the finding that normal bone marrow neutrophils do not ingest anti-D-sensitized cells. Furthermore, it has been shown that the life span (and therefore mean age) of blood granulocytes in acute myeloid leukemia is greater than normal.

There are other studies suggesting that leukocyte function in acute leukemia is abnormal. Abnormalities have been found in neutrophil migration into skin windows, phagocytosis, morphology, and cytochemistry. It has been postulated that such abnormalities are evidence that these neutrophils are the product of a leukemic line of myeloid cells. If so, the abnormal neutrophil function reported in this paper may also be characteristic of a "leukemic neutrophil." This abnormality is not restricted to "leukemic neutrophils," since it is also found in the neutrophils of patients with acute lymphoblastic leukemia and in association with the neutrophilia of acute infections.

Neutrophil abnormalities during acute infections have been reported by several groups. These include abnormal granulation and reduced chemotactic response, decreased bactericidal activity, and abnormal particle-binding characteristics.

It is clear, therefore, that neutrophil function and physiology can differ greatly, depending on the pattern of myelopoiesis. This may be similar to red cell production, where the characteristics of the red cells produced can differ greatly, depending on the pattern of erythropoiesis.

Finally, it should be pointed out that the phenomenon of phagocytosis of IgG-sensitized erythrocytes is not a property restricted to the monocyte-macrophage system. Neutrophils formed as a result of abnormal myelopoiesis may have erythrocyte-binding and -ingestion characteristics similar to that of the monocyte.

REFERENCES

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