The Cellular Composition of Inflammatory Exudates in Human Leukemias

By Dane R. Boggs

The nature of the defect in host defense mechanisms resulting in the increased incidence of bacterial infection in the leukemias is ill defined. Studies of antibody formation indicate that the patient with an acute leukemia or with chronic myelocytic leukemia has normal or near normal response to antigenic stimulation, whereas the patient with chronic lymphocytic leukemia frequently has depressed antibody response. The migration of leukocytes to inflammatory sites and their phagocytic function are of critical importance in bacterial defense. This study is concerned with the character and intensity of the cellular composition of an induced inflammation in human leukemias.

Materials and Methods

Rebuck has devised a simple procedure for observing the leukocytic infiltration in an area of inflammation. This technic, slightly modified, is as follows:

An area of skin, usually the volar surface of the forearm, is shaved and cleansed with alcohol. With use of a sterile scalpel blade, the epithelium is scraped until the papillary layer of the corium is reached in an area approximately 3 x 5 mm. A serosanguineous ooze is to be desired, but free bleeding should be avoided. One drop of diphtheria toxoid from a sterile 23 gage needle is applied to the area. A sterile round glass coverslip, 22 mm. or 15 mm. in diameter, is placed over the area in such a manner that the lesion is in the approximate center of the coverslip and this in turn is covered by a square of sterile cardboard slightly larger than the coverslip. Tape is then applied so that the glass is pressed firmly against the lesion and the edges of the cardboard are sealed to the skin.

A variety of irritants, including diphtheria toxoid, induce a similar pattern of cellular response. In subjects with systemic immunity to the protein antigen, the mononuclear response may be enhanced. Schick testing was employed in this study, and a questionable increase in mononuclear infiltration was observed in Schick-negative subjects. However, this difference was slight and is not further considered. The trauma of denuding the skin elicits a fairly brisk cellular response without addition of any irritant.

Three, 6, 12 and 24 hours after the initial application, the coverslip is changed without introducing further protein, and at 48 hours the coverslip is removed. These are air-dried, Cresa stained and mounted. The coverslip contains a single layer of cells approximately the size of the initial lesion.

Patients with very low platelet counts often had a significant number of red cells in the 3 hour exudate, but later slides were essentially free of erythrocytes. One hundred cell differential counts of the exudate and 200, 500 or 1000 cell counts of the blood were done when the total white cell count was less than 5,000, 5 to 20,000, or over 20,000, respectively. All counting was done by the author. Quantitation of the cellularity of the exudate is limited by the lack of uniformity in the size of the induced lesion. This was particularly true with normal subjects, when a layer of cells the size of the lesion was obtained. The six hour

I wish to thank Dr. James Stengle for his astute advice and criticisms in performing the study and preparing the paper, Dr. Emil Frei III for valuable suggestions in preparing the paper, Irene Clark for technical assistance, and especially Dr. John Rebuck, who kindly demonstrated his original technic used herein.

Submitted June 1, 1959; accepted for publication Oct. 15, 1959.
sample of the exudate best reflected the intensity of granulocytic response and was used for comparison of the quantity of cells in different subjects. Because of the above problem, comparison of large numbers of cells is probably meaningless, and counts were done on coverslips containing less than 1000 cells. Others were recorded as just containing more than 1000 cells.

The per cent of disintegrated cells was usually about 5 in the exudates from both normal and leukemic patients. The mononuclear cells seen in the exudate may be predominantly hypertrophied lymphocytes, but all mononuclear cells in the exudate will be referred to as macrophages.

Tests were performed on 4 patients with chronic myelocytic leukemia, one patient with a probable diagnosis of chronic monocytic leukemia, and one with a probable diagnosis of chronic eosinophilic leukemia. Fifteen tests were performed on 10 patients with chronic lymphocytic leukemia, 10 tests on 8 patients with acute myeloblastic leukemia and 13 tests on 10 patients with acute lymphoblastic leukemia. Seven healthy males, ages 27 to 32, served as controls, as did 6 patients with a variety of metastatic carcinomas but who were receiving no therapy and had normal blood leukocyte values.

RESULTS

The cellular response of controls was fairly uniform (table 1) and corresponds with the normal response observed by Rebuck. Segmented neutrophils were predominant in the 3 and 6 hour exudates (figs. 1 and 2). A few metamyelocytes, occasional eosinophils and rare basophils were seen. Macrophages were scarce in the 3 hour exudate and were present in small but significant numbers by 6 hours. The 12 hour inflammation (fig. 3) varied but was usually composed of approximately equal numbers of polymorphonuclear and mononuclear cells. By 24 hours (fig. 4), the mononuclear was predominant. The change in cellular content from the 24 to 48 hour exudate was minor, and the 48 hour observation was abandoned in later portions of the study.

The major abnormalities were found in the patients with an acute leukemia (table 1). Acellular exudates were frequently encountered. The leukemic lymphoblast or myeloblast was rarely seen in the inflammatory exudate. Metamyelocytes were seen in the exudate, and the percentage of band forms was greater than in the controls. No differences were noted in the granulocytic response between acute lymphoblastic leukemia and acute myeloblastic leukemia. The macrophage response was particularly deficient in acute lymphoblastic leukemia, for in all but one instance the percentage of granulocytes still exceeded macrophages in the 24 hour preparation. The percentage macrophage response more closely approximated control observation in acute myeloblastic leukemia. One patient responded almost exclusively with macrophages on two tests (fig. 5).

There was no ready explanation for this difference with reference to circulating elements. Typical-appearing normal lymphocytes were as frequent in the circulation of patients with acute lymphoblastic as those with acute myeloblastic leukemia. Monocytes were infrequent in both diseases.

Apparently it was not the presence of leukemic cells in the circulation that influenced the cellular response to inflammation. The granulocytic content of the exudate paralleled the number of mature granulocytes in the circulation (fig. 6). Acellular exudates were found only with extreme depression of circulating granulocytes. Marked variation in the absolute number of circulating abnormal cells per se did not influence the exudate.
Table 1.—Cellular Character of the Exudates

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of tests</th>
<th>3 hour</th>
<th>6 hour</th>
<th>12 hour</th>
<th>24 hour</th>
<th>Mature granulocytes per cu.mm. of blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PMN*</td>
<td>MET*</td>
<td>MON*</td>
<td>PMN</td>
<td>MET</td>
</tr>
<tr>
<td>Controls</td>
<td>7</td>
<td>95</td>
<td>90</td>
<td>52</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>6</td>
<td>96</td>
<td>89</td>
<td>56</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>13</td>
<td>62</td>
<td>67</td>
<td>59</td>
<td>62</td>
<td>17</td>
</tr>
<tr>
<td>Acute myeloblastic leukemia</td>
<td>10</td>
<td>60</td>
<td>48</td>
<td>50</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>15</td>
<td>89</td>
<td>82</td>
<td>64</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Chronic myelocytic leukemia</td>
<td>4</td>
<td>60</td>
<td>70</td>
<td>56</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Chronic monocytic leukemia †</td>
<td>1</td>
<td>89</td>
<td>65</td>
<td>18</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Chronic eosinophilic leukemia †</td>
<td>1</td>
<td>97</td>
<td>74</td>
<td>50</td>
<td>14</td>
<td>0</td>
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</table>

*PMN represents polymorphonuclear, MET represents metamyelocyte, MON represents mononuclear cells other than granulocytes.
†Polymorphonuclears and metamyelocytes.
‡Calculated from slides with at least 100 cells. If bacteria were evident on the slide, it was not included in calculation.
Figs. 1-4.—Normal sequence of cellular content of the exudate at 3, 6, 12 and 24 hours.
Fig. 5.—Three hour exudate from the patient with acute myeloblastic leukemia who had macrophage infiltration almost exclusively.

Fig. 6.—Relation of circulating mature granulocytes to the number in the 6 hour exudate in acute leukemia.

Two 24 hour slides were grossly contaminated with bacteria, although no evidence of infection was present in the wound, and in these a secondary granulocytic response was elicited.

Patients with chronic lymphocytic leukemia generally had a normal response. One initially responded with a striking 3 hour mononuclear infiltration which
Faded but returned by 24 hours. The early cells had the appearance of small lymphocytes (fig. 7). This was not reduplicated in a test one week later in the same patient. Metamyelocytes were present in a higher percentage than in control inflammations, but the increase was less striking than in the acute leukemias or myelocytic leukemia.

The 4 patients with chronic myelocytic leukemia had normal response, with the exception of a striking increase in metamyelocytes of all ages. All of these patients had significant numbers of circulating myeloblasts, promyelocytes and myelocytes, but with rare exception these cells were not observed in the exudate.

The patient with a probable diagnosis of chronic monocytic leukemia had a white count of 68,000 with approximately 50 per cent mature monocytes and 50 per cent mature granulocytes. Her response differed from the controls in that the macrophage infiltration appeared earlier.

Eosinophils were infrequent in the exudate of the patient with eosinophilic leukemia despite 6,200/cu.mm. in the blood.

The presence of metamyelocytes in the inflammatory exudate generally paralleled their number in the blood. However, when a normal number of polymorphonuclears were in the circulation, the polymorphonuclear to metamyelocyte ratio in the inflammation was greater than that in the blood. The reverse was frequently seen in the 3 hour exudates in subjects having acute leukemias with markedly reduced granulocytes in the circulation.

It was evident that the quantity of cells in exudates of patients with the acute leukemias was markedly reduced (table 2). The patients with the chronic leukemias usually did not vary from the controls, but three patients with chronic lymphocytic leukemia and reduced numbers of circulating granulocytes had mild reductions. Two patients with acute leukemia in a partial remission and 1 with very stable disease and normal numbers of mature granulocytes had comparable numbers of cells in the exudate to the controls.

Most patients were receiving some form of chemotherapy. Two patients receiving 6-mercaptopurine for acute leukemia were tested during an active phase and again when remission had been achieved. In both instances their response during remission approached normal. Similarly, progressing disease with continuing chlorambucil therapy was associated with less intense cellular
Table 2.—Total Number of Cells on the Slide from the 6 Hour Exudate

<table>
<thead>
<tr>
<th>No. of cells</th>
<th>No. of tests</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>7</td>
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<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3.—The Relation of Bacterial Infection to Granulocytic Response in Patients with the Acute Leukemias

<table>
<thead>
<tr>
<th>No. of granulocytes in the 6 hour exudate</th>
<th>No. of tests</th>
<th>No. of patients with bacterial infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than 200</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>more than 200</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

response. Prednisone therapy (usually 1 mg./Kg. per day) did not alter the character of the exudate in patients with chronic lymphocytic leukemia. No consistent differences in the cellular character of the exudate were found in testing patients before, during and after a course of prednisone therapy.

Infection was more frequent in patients with acute leukemia and decreased granulocytic response (table 3). Twelve of the 23 tests on patients with acute leukemia were done at a time when the granulocyte count was less than 500/ cu.mm. of blood and resulted in less than 200 granulocytes in the 6 hour exudate sample. Bacterial infection was significantly more frequent (P = <0.05 by Chi square) in these patients than in the 11 tests in patients with more than 500 granulocytes per cu.mm. of blood.

DISCUSSION

The granulocyte content of the induced inflammation correlates well with the number of polymorphonuclears and metamyelocytes in the circulation. This is in agreement with the observations of Page and Good on a patient with cyclic neutropenia. It appears that the polymorphonuclear cell responds preferentially to the stimulus. However, as the absolute number of circulating polymorphonuclears decreases, the immature to mature granulocyte ratio in the exudate may exceed the ratio in the circulation. The metamyelocyte was the youngest granulocyte seen in the exudates.

Page and Good feel that the macrophage response is somehow conditioned by the preceding granulocytic response. Although no differences were noted
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in the granulocytic response of patients with acute lymphoblastic leukemia and acute myeloblastic leukemia, their macrophage response differed. Patients with acute lymphoblastic leukemia often had more adequate granulocyte than macrophage responses, while the opposite was true in acute myeloblastic leukemia. This could not be explained by the number of normal-appearing circulating lymphocytes or monocytes. One patient with acute myeloblastic leukemia responded almost entirely with macrophages.

The increased numbers of bands and metamyelocytes in the exudate in chronic leukemia, the lack of eosinophils in the exudate despite the tremendous number in the circulation, the general lack of increased lymphocytic infiltration in chronic lymphocytic leukemia and the possible early macrophage infiltration in the case of chronic monocytic leukemia are of interest. However, the most striking finding is what appears to be a morphologically normal cellular exudate in patients with chronic forms of leukemia. Such patients with neutropenia of the degree encountered in the acute leukemias might respond in a less adequate fashion.

It would appear that the chemotherapeutic agents do not per se depress response. When leukopenia of marked degree attends their use, the cellularity of the exudate presumably would be depressed. ACTH, administered in a single dose, moderately delays the macrophage reaction, but significant evidence of delay or depression in patients receiving relatively long-term prednisone therapy was not evident in this study.

Patients with carcinoma and bacterial infection frequently have their initial site of infection in an area of actual tumor invasion. Presumably the necrosis, stasis and blockage produced provides a local environment favorable to the growth of bacteria. Systemic spread of infection occurs in carcinoma but with less frequency than in the lymphomas and leukemias, and the incidence of infection is greater in the latter diseases. Some infection in leukemia apparently originates in tumor infiltration, but in most instances does not. Thus one is impressed that a deficit or deficits of the host's systemic bacterial defense mechanisms must exist.

Antibody formation is grossly deficient in many cases of chronic lymphocytic leukemia, and this deficiency may also be represented in the frequency with which significant reduction of gamma globulins is encountered. In contrast, antibody response to antigenic stimulation in the acute leukemias is normal or near normal, and the gamma globulins are rarely decreased.

Jaffe observed virtually absent granulocytic response in 2 of 6 patients with acute leukemia dying with infection. Silver et al. failed to correlate incidence of infection with the actual numbers of granulocytes in circulation but found that a fall in mature granulocytes frequently preceded bacterial infection. Granulocyte counts in acute leukemia at onset of fever due to infection are significantly lower than at onset of fever of undetermined etiology. The induced inflammation is accompanied by a sequence of cellular infiltration that approximates cellular response to most bacterial infections. The results of the present study in patients with acute leukemia are in agreement with the aforementioned evidence that deficient normal phagocyte production is at least one of the defects leading to depressed bacterial defense. The clinical
implication of cause and effect is difficult to document, but there was a significantly higher number of infections in the patients with acute leukemia with markedly depressed inflammatory response.

Chronic lymphocytic leukemia, though pursuing a longer course, is probably associated with as many if not more instances of infection per patient as acute leukemia. Infection in these patients was fairly frequent despite their normal inflammatory response.

The presence of morphologically normal cells in the inflammatory exudate does not assure functional integrity. The phagocytic ability of the mature granulocyte of chronic myelocytic leukemia is in dispute, while the mature granulocytes in the acute leukemias have intact phagocytic ability.

These demonstrations of specific host defense deficit are not of necessity the total deficit. Much remains obscure, particularly in the area of innate host resistance, and further abnormalities may be demonstrated. Nevertheless, alterations of sufficient magnitude to explain an increased susceptibility to bacterial infection are evident in acute myeloblastic leukemia, acute lymphoblastic leukemia and chronic lymphocytic leukemia.

**Summary**

An induced inflammation in patients with the leukemias was studied with reference to the cellular character of the exudate. Patients with the chronic forms of leukemia generally exhibited a normal cellular response. The granulocytic character of the exudate was proportional to the number of circulating mature neutrophils and metamyelocytes. Immature leukemic cells were not found in the exudates. The patients with acute leukemia with markedly reduced circulating mature granulocytes responded with acellular exudates or with marked qualitative and quantitative alteration in cellular composition. Bacterial infection was most frequent in the patients with few or no cells in the exudate.

The nature of the defect or defects in leukemia relating to increased susceptibility to bacterial infection are reviewed.

**Summario in Interlingua**

Un inflammation inducite in patientes con un leucemia esseva studiate con referentia al character cellular del exsudato. Patientes con le formas chronic de leucemia exhibiva generalmente un responsa cellular normal. Le character granulocytic del exsudato esseva proportional al numero del circulante neutrophilos matur, bandas, e metamyelocytos. Immature cellulas leucemic non esseva trovate in le exsudatos. Le patientes con acute leucemia con marcate mente reducite contos de circulante granulocytos matur reageva con exsudatos acellular o con marcate alterationes qualitative e quantitativa in le composition cellular del exsudato. Infectiones bacterial esseva le plus frequente in le patientes con pauc o nulle cellulas in le exsudato.

Es revistate le natura del defectos o defectos in leucemia que es relationate al augmento del susceptibilitate pro infectiones bacterial.
REFERENCES

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