The Monocytopenia of Aplastic Anemia

By J. J. Twomey, C. C. Douglass, and O. Sharkey, Jr.

Eight out of 22 patients with aplastic anemia died from infections. All five patients with fewer than 100 granulocytes and 20 monocytes per cu mm of blood succumbed to overwhelming sepsis. The relationship that existed between absolute granulocyte and monocyte counts made it impossible to determine whether neutropenia or monocytopenia carried the greater risk. Monocytopenia correlated with reduced numbers of phagocytic cells in WBC cultures. This lack of phagocytes was associated with impairment of macrophage-dependent reactions, e.g., the mixed WBC reaction. The data suggest that aplastic anemia reduces the delivery of macrophage precursors into the circulation; this could ultimately deplete tissues of macrophages. Thus, monocytopenia, as well as neutropenia, may contribute to the risk from infection in patients with bone marrow failure.

Since first described by Ehrlich in 1888, it has become evident that the diagnosis of aplastic anemia carries an extremely grave prognosis. Hemorrhage due to thrombocytopenia was the most frequent cause of death in earlier series. Many patients also die from infectious complications. Susceptibility to infections has been largely attributed to neutropenia. The purpose of this report is to draw attention to the presence of monocytopenia in patients with aplastic anemia and to produce evidence from in vitro studies that monocytopenia, as well as neutropenia, may contribute to the risk of serious infections in these patients.

MATERIALS AND METHODS

The records of 22 patients with acquired aplastic anemia were reviewed. All patients had hypocellular bone marrow biopsies, 1500 or less PMNs plus bands and more than 1500 lymphocytes per cu mm of blood. In vitro studies were performed on six patients...
Table 1. Clinical Data on 22 Patients With Aplastic Anemia

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>(? Etiology</th>
<th>Months Followed</th>
<th>PMNs + Bands/ cu mm Blood</th>
<th>Infections</th>
<th>Hemorrhages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Recovered</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 20 M</td>
<td>—</td>
<td>2</td>
<td>25</td>
<td>Cellulitis</td>
<td>—</td>
</tr>
<tr>
<td>2. 25 M</td>
<td>—</td>
<td>2</td>
<td>800</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3. 58 M</td>
<td>Phenylbutazone</td>
<td>2</td>
<td>900</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4. 63 F</td>
<td>Phenylbutazone</td>
<td>10</td>
<td>1290</td>
<td>—</td>
<td>Petechiae</td>
</tr>
<tr>
<td>2. Survived</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. 50 M</td>
<td>—</td>
<td>&lt;50</td>
<td>200</td>
<td>Pneumonia, herpes simplex, cellulitis × 2, urinary tract, perirectal abscess</td>
<td></td>
</tr>
<tr>
<td>6. 16 F</td>
<td>—</td>
<td>5</td>
<td>490</td>
<td>Otitis externa, mild furunculosis × 3</td>
<td></td>
</tr>
<tr>
<td>7. 46 M</td>
<td>DDT</td>
<td>9</td>
<td>180</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8. 65 F</td>
<td>Phenylbutazone</td>
<td>5</td>
<td>200</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9. 43 M</td>
<td>—</td>
<td>13</td>
<td>485</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10. 71 M</td>
<td>Dilantin</td>
<td>1</td>
<td>1410</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3. Died from infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. 7 F</td>
<td>Chloramphenicol</td>
<td>1</td>
<td>20</td>
<td>Abdominal abscess, septicemia*</td>
<td></td>
</tr>
<tr>
<td>12. 71 M</td>
<td>Quinine</td>
<td>&lt;1</td>
<td>25</td>
<td>Pneumonia* Oral ulcer, phlebitis, urinary tract, pneumonia*</td>
<td></td>
</tr>
<tr>
<td>13. 20 F</td>
<td>Chlorothiazide</td>
<td>4</td>
<td>90</td>
<td>Petechiae Subarachnoid, GI</td>
<td></td>
</tr>
<tr>
<td>14. 21 M</td>
<td>—</td>
<td>3</td>
<td>185</td>
<td>Pharyngitis, cellulitis, urinary tract, pneumonia*</td>
<td></td>
</tr>
<tr>
<td>15. 50 M</td>
<td>—</td>
<td>1</td>
<td>210</td>
<td>Septicemia, nocardiosis*</td>
<td></td>
</tr>
<tr>
<td>16. 16 F</td>
<td>—</td>
<td>2</td>
<td>800</td>
<td>Cellulitis, septicemia*</td>
<td></td>
</tr>
<tr>
<td>17. 47 M</td>
<td>Benzene</td>
<td>8</td>
<td>1200</td>
<td>Cellulitis, pneumonia × 2, septicemia × 2*</td>
<td></td>
</tr>
<tr>
<td>18. 58 M</td>
<td>—</td>
<td>&lt;1</td>
<td>1500</td>
<td>Pneumonia*</td>
<td></td>
</tr>
<tr>
<td>4. Died from hemorrhage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. 7 F</td>
<td>Chloramphenicol</td>
<td>5</td>
<td>120</td>
<td>Furunculosis Pulmonary,* uterine</td>
<td></td>
</tr>
<tr>
<td>20. 68 M</td>
<td>Chloramphenicol</td>
<td>3</td>
<td>250</td>
<td>GI tract* uterine</td>
<td></td>
</tr>
<tr>
<td>21. 63 M</td>
<td>Chloramphenicol hepatitis</td>
<td>4</td>
<td>700</td>
<td>—</td>
<td>Cerebral*</td>
</tr>
<tr>
<td>5. Died from unrelated causes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22. 78 M</td>
<td>Insecticide</td>
<td>34</td>
<td>1680</td>
<td>Cystitis</td>
<td>—</td>
</tr>
</tbody>
</table>

*Caused death.
(Table 1, patients 1, 5, 6, 9, 13, and 14) who had not been transfused during the 2 wk prior to study. Patients 5 and 13 were mentioned briefly in an earlier publication. Control blood donors without known immunologic disease were awaiting minor surgery.

About 90 ml of heparinized blood for cultures and 5 ml of oxalated blood for routine blood counts were collected after an overnight fast. Leukocyte-rich plasma was harvested after spontaneous red cell sedimentation. Aliquots of WBC were exposed to 1250 R of x-irradiation at 154 R/min, so as to inhibit their capacity for nucleotide synthesis. Other aliquots of WBC were incubated in cotton-packed syringes; greater than 96% of the cells recovered were lymphocytes (henceforth, these preparations will be called lymphocytes). Greater than 98% of all cell preparations excluded trypan blue.

The culture media included Eagle’s spinner media fortified with l-glutamine and 20% decomplemented human plasma. Each culture contained $1 \times 10^6$ lymphocytes/ml and 2 ml total volume. The number of WBC per ml was adjusted so that each ml contained $1 \times 10^8$ lymphocytes. Equal numbers of lymphocytes were added from each donor in mixed cell cultures. Cultures were prepared in plastic Petri dishes and were incubated for 7 days in a humidified CO$_2$-enriched incubator. Incorporation from 2 $\mu$Ci of $^3$H-thymidine during the last 24 hr of culture was measured in a Packard Tricarb liquid scintillation counter.

Cultures of WBC were stimulated with 0.1 ml of phytohemagglutinin (PHA)-M (Difco), 0.05 mg of concanavallin-A (Calbiochem), 0.1 ml of a 1:100 dilution of Candida antigen (Hollister-Stier), and 0.1 ml of a 1:10 dilution of mumps antigen (Lily). The protocol for the adapted mixed leukocyte culture test (AMLCT) was as follows: (1) unstimulated lymphocytes, (2) mixed patient and control irradiated WBC (WBC$_x$), (3) mixed patient and control lymphocytes, (4) control WBC$_x$ stimulating ($\rightarrow$) patient lymphocytes, (5) patient WBC$_x$ $\rightarrow$ control lymphocytes, and (6) patient WBC$_x$ $\rightarrow$ control WBC. Patient plasma was tested for an inhibitor of the mixed leukocyte reaction (MLR) by comparing $^3$H-thymidine incorporation in mixed WBC cultures between pairs of control donors first with 20% patient plasma and then with 20% control plasma in the media.

Phagocyte counts were performed on 7-day unstimulated WBC cultures. Polystyrene particles, 2 $\mu$ in diameter, were added to these cultures 24 hr before they were terminated. Giemsa-stained smears were prepared from the harvested cells, and the per cent phagocytes was determined from counts of 500 cells. Phagocytes were arbitrarily defined as noneosinophilic cells that had engulfed five or more polystyrene particles.

RESULTS

Clinical Observations

The 22 patients were divided into five groups (Table 1): Group 1, four patients who achieved complete remissions; group 2, six patients who survived through follow-up without hematologic recovery; group 3, eight patients who died from infections; group 4, three patients who died from hemorrhage; and group 5, one elderly man who died from causes unrelated to his unresolved aplastic anemia.

Age and sex did not influence over-all prognosis. Thirteen patients had been exposed to drugs, most frequently chloramphenicol or phenylbutazone, or chemicals that are potentially toxic to bone marrow within 2 mo before aplastic anemia was diagnosed. Survival was more frequent with phenylbutazone-related than with chloramphenicol-related bone marrow hypoplasia. The eventual outcome was usually decided within 4 mo after diagnosis; all four recoveries and nine of the 11 fatalities from complications of bone marrow failure took place within this interval. No patient was treated with methenolone or oxymetholone.

Most patients were severely thrombocytopenic (less than 50,000 platelets/cu mm of blood); only patient 1 maintained a normal platelet count.
Although abnormal bleeding was usually associated with a poor prognosis, more of these patients died from infection than from hemorrhage. Infections tended to recur in the same patients and were associated with a high mortality. The initial hemograms were of little value in predicting prognosis. The average number of PMNs plus bands per cu mm of blood at diagnosis was 754 for group 1, 490 for group 2, 504 for group 3, and 357 for group 4; there was considerable overlap.

The relationship between total monocyte counts and total PMN plus band counts per cu mm of the same collections of blood was studied. These counts were recorded when patients presented with fatal infections or at diagnosis in patients who did not die from infections. Since thrombocytopenic bleeding appeared to influence prognosis, patient 1—who maintained a normal platelet count was not included. Counts on 40 control patients without hematologic disease were also compared.

There was a significant correlation between total monocyte and total

**Table 2. In vitro Responses by WBC**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Baseline</th>
<th>PHA</th>
<th>Concanavalin-A</th>
<th>Mumps</th>
<th>Candida</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7</td>
<td>5</td>
<td>200</td>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>185</td>
<td>89</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>6</td>
<td>0.7</td>
<td>260</td>
<td>205</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>0.3</td>
<td>331</td>
<td>198</td>
<td>8.8</td>
<td>2.9</td>
</tr>
<tr>
<td>13</td>
<td>2.2</td>
<td>107</td>
<td>107</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Normal</td>
<td>&gt;25</td>
<td>&gt;20</td>
<td>&gt;2.5*</td>
<td>&gt;2.5*</td>
<td></td>
</tr>
</tbody>
</table>

*Plus at least five times greater than baseline incorporation.*
PMN plus band counts, both in health and with aplastic anemia ($r= 0.75$, $p <0.001$) (Fig. 1). The only exception to this correlation was patient 3 who recovered hematologically shortly afterward. He had relatively more monocytes than granulocytes, which illustrates the monocytosis that often precedes recovery from depressed granulopoiesis. Both absolute monocyte and PMN plus band counts on the other 20 patients with aplastic anemia were clearly lower than on the control patients. Patient values fell into three groups: (1) Five patients with less than 20 monocytes and less than 100 PMNs plus bands per cu mm of blood; they all succumbed rapidly to overwhelming infections. (2) Eight patients with 20–75 monocytes and 100–400 PMNs plus bands per cu mm of blood; one died from pneumonia, another from sepsis, and two others from hemorrhage. (3) Eight patients with greater than 75 monocytes and more than 400 PMNs plus bands per cu mm of blood. These had a considerably better prognosis; three recovered completely, one died from pneumonia, and another died from intracranial hemorrhage.

In Vitro Studies

Both PHA and concanavallin-A stimulated normal incorporation by WBC from four patients (Table 2); cells from patient 1 responded poorly to PHA but normally to concanavallin-A. Mumps or Candida antigens stimulated at least 2.5 mCi and five times baseline $^3$H-thymidine incorporation by WBC from 24 out of 25 unselected control donors. Only WBC from patient 9 responded normally to these antigens. The difference between patient and control responses to mumps and Candida antigens was statistically significant ($p <0.01$).

The AMLCT results are listed in Table 3. The low incorporation in patient plus control lymphocyte cultures was comparable to the incorporation in unstimulated patient lymphocyte cultures; incorporation was much more when macrophage-rich control WBC were cultured with patient cells. This indicated that macrophages were removed during lymphocyte purification to concentrations that were insufficient to mediate an MLR in our system and permitted assessment of macrophage function in WBCx components of WBCx → lymphocyte cultures. Incorporation was also minimal in patient WBCx → lymphocyte cultures.

<table>
<thead>
<tr>
<th>Culture Contents</th>
<th>Patient 1</th>
<th>5</th>
<th>13</th>
<th>14</th>
<th>9</th>
<th>Controls (N=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient lymphocytes</td>
<td>0.4</td>
<td>0.1</td>
<td>0.4</td>
<td>0.9</td>
<td>0.5</td>
<td>0.1 &lt; 1.7</td>
</tr>
<tr>
<td>Patient lymphocytes + control</td>
<td>—</td>
<td>0.1</td>
<td>0.6</td>
<td>0.7</td>
<td>0.2</td>
<td>0.6 &lt; 2.7</td>
</tr>
<tr>
<td>Patient WBCx + control WBC</td>
<td>1.1</td>
<td>0.1</td>
<td>0.4</td>
<td>0.3</td>
<td>1.0</td>
<td>0.5 &lt; 1.6</td>
</tr>
<tr>
<td>Control WBCx → patient lymphocytes</td>
<td>15.0</td>
<td>18.6</td>
<td>23.3</td>
<td>13.0</td>
<td>40.9</td>
<td>22.5 &gt; 10.0</td>
</tr>
<tr>
<td>Patient WBCx → control lymphocytes</td>
<td>3.9</td>
<td>0.1</td>
<td>0.8</td>
<td>1.7</td>
<td>5.6</td>
<td>24.8 &gt; 6.0*</td>
</tr>
<tr>
<td>Patient WBCx → control WBC</td>
<td>—</td>
<td>53.0</td>
<td>20.5</td>
<td>29.8</td>
<td>12.6</td>
<td>13.3 &gt; 5.0*</td>
</tr>
<tr>
<td>Patient WBC: % macrophages</td>
<td>0.4</td>
<td>&lt;0.1</td>
<td>0.5</td>
<td>0.9</td>
<td>1.6</td>
<td>2.4 &gt; 4.0</td>
</tr>
</tbody>
</table>

*95% confidence limit.
plus control WBCs cultures. This assured one-way stimulation in WBCs → lymphocyte cultures. Incorporation in mixed WBC cultures between control donor pairs was not inhibited by patient plasma. Lymphocyte responses in the AMLCT normally exceed 10 mCi of 3H-thymidine incorporation.11 Lymphocytes from all six patients with aplastic anemia responded normally to allogeneic WBCs in this test.

The WBCs provide the MLR stimulus and macrophages in WBCs → lymphocyte cultures. Our experience with WBCs performance in the AMLCT on 50 control donors is shown in Fig. 2. It is evident that WBCs from patients 1, 5, 13, and 14 did not elicit normal incorporation by lymphocytes from control donors. The response by control lymphocytes to WBCs from patient 6 was within the lower second percentile of our normal range; WBCs from patient 9 stimulated normally. In contrast to the responses by control lymphocytes, macrophage-rich control WBC responded normally to WBCs from all six patients (Table 3).

The concentration of phagocytic cells in WBC cultures on all six patients was greatly reduced (normal: 4%–28%, N = 25).11 Absolute monocyte counts and the concentration of phagocytes in WBC cultures on the same collections of blood were compared (Fig. 3). There was a significant over-all correlation between these counts, both on control donors (N = 20) and on the patients with aplastic anemia (r = 0.87, p < 0.001).

**DISCUSSION**

Survival with aplastic anemia has improved since the introduction of new anabolic steroids, antibiotics, and transfusion techniques; 35% of 46 patients diagnosed after 1962 survived throughout follow-up, compared to only 24% of 38 patients diagnosed before 1959 (Table 4).3,4,9 While platelet transfusions have reduced hemorrhagic fatalities, abnormal bleeding still suggests a poor prognosis. Less progress has been made in the management of leuko-
penia; 80% of deaths in the two more recent series were related to infections, as opposed to 24% in patients diagnosed before 1959. Our experience re-emphasizes the importance of the first few months after diagnosis to long-term survival.9

What degree of neutropenia is clinically significant? The prevalence of infections is increased among acute leukemic patients with fewer than 500 mature granulocytes/cu mm of blood but not when these counts exceed 1500/cu mm.13,14 However, factors other than neutropenia also make leukemic patients more susceptible to infections: (1) Leukemia itself may impair immunologic reactivity.15 (2) Antileukemic therapy is immunosuppressive.15 (3) Some leukemic blast cells may be able to mature morphologically;16,17 the functional capacity of such cells is open to question. Neutropenia can be assessed more directly in patients with aplastic anemia. All of our patients with fewer than 100 PMNs plus bands/cu mm of blood died from overwhelming infections; slightly higher counts were often tolerated without complications. Counts recorded during severe infections may have been lowered further by the infections.16,17

It is generally believed that tissue macrophages are derived from blood

Table 4. Infections in Recent and Earlier Series of Patients With Aplastic Anemia

<table>
<thead>
<tr>
<th>Period of study</th>
<th>Scott et al.3</th>
<th>Hasselback and Thomas*</th>
<th>Davis and Rubin*</th>
<th>Present Series</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>19</td>
<td>19</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Survived</td>
<td>7</td>
<td>2</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Died</td>
<td>12</td>
<td>17</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Infection major factor in cause of death</td>
<td>5</td>
<td>2</td>
<td>16*</td>
<td>8</td>
</tr>
</tbody>
</table>

*Listed as “life-threatening” infections; all died.
monocytes. These cells are normally replenished from a pool of pro-monocytes in the bone marrow. It is, therefore, not surprising that aplastic anemia is associated with monocytopenia. All of our patients with fewer than 20 monocytes/cu mm of blood died from infections. However, it was not possible to determine whether monocytopenia or neutropenia was the greater risk factor in these patients because of the relationship between absolute monocyte and granulocyte counts. This relationship, both in health and with aplastic anemia, supports other evidence that monocytopoiesis and granulopoiesis are closely related to one another.

Although lymphocytes in certain circumstances are capable of some phagocytosis in vitro, most of the phagocytic cells harvested from our unstimulated WBC were probably macrophages. Our data show that blood from patients with aplastic anemia contains reduced numbers of macrophage precursors and illustrate the relationship between monocytes and macrophages.

This study and the report of Flad et al. indicate that lymphocytes from patients with aplastic anemia usually respond normally to various mitogens in vitro. However, WBC cultured from our severely monocytopenic patients were hyporesponsive to soluble antigens and were unable to stimulate allogeneic lymphocytes in the AMLCT. A relationship was evident between $^3$H-thymidine incorporation in these macrophage-dependent reactions and the concentration of macrophages in patient WBC cultures. (Macrophage concentrations are reduced to about 50% in the AMLCT when patient WBCs are mixed in equal parts with macrophage-poor control lymphocytes.)

This study points out that both neutropenia and monocytopenia occur with aplastic anemia. The monocytopenia was sufficient in some instances to impair macrophage-dependent in vitro reactions. Reduced numbers of circulating macrophage precursors may have clinical significance by interfering with replenishment of tissue macrophages. Perhaps exhaustion of tissue macrophage reserves may have contributed to the increased prevalence of cutaneous anergy to tuberculin reported by Libansky in pancytopenic patients.

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REFERENCES

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