Drug-Induced Agranulocytosis: In Vitro Evidence for Immune Suppression of Granulopoiesis and a Cross-Reacting Lymphocyte Antibody

By Raymond Taetle, Thomas A. Lane, and John Mendelsohn

Two patients with agranulocytosis associated with diphenylhydantoin (DPH) therapy and clinical data suggesting suppression of granulopoiesis were investigated using in vitro culture techniques for committed granulocyte/macrophage precursors. Addition of DPH to cultures containing the patients' sera resulted in significant suppression of colony growth. Extensive studies on the acute serum from one patient revealed the drug-dependent inhibitory activity to be nondialyzable, resistant to chloroform extraction, heat stable, active in the presence of heat-inactivated fetal bovine serum, active against autologous as well as allogeneic cells, and absent from convalescent sera. Drug-dependent bone marrow colony-suppressing activity was removed by absorption on an anti-immunoglobulin-Sepharose column but not by IgG-Sepharose. The serum showed non-drug-dependent suppression of oxygen consumption by normal polymorphonuclear leukocytes engaged in phagocytosis and also showed evidence of ability to opsonize these cells. When the serum was incubated with mitogen-stimulated lymphocytes, suppression of \(^{3}H\)-thymidine uptake by autologous but not allogeneic cells was noted. Similarly, the serum suppressed short-term \(^{3}H\)-thymidine uptake by autologous but not allogeneic bone marrow. Absorption of the patients' sera with allogeneic polymorphonuclear leukocytes, autologous polymorphonuclear leukocytes, or autologous lymphocytes removed the drug-dependent inhibitory activity, but absorption with allogeneic lymphocytes did not. These data are most consistent with the presence of a noncomplement dependent antibody capable of suppressing granulopoiesis, mediating peripheral destruction of polymorphonuclear leukocytes, and cross-reacting with a lymphocyte antigen of limited population distribution.

Drug-Induced Leukopenia and agranulocytosis have been recognized as significant complications of drug therapy since the original reports implicating aminopyrine in the etiology of some cases of agranulocytosis.\(^1,^2\) Although prior studies have investigated possible mechanisms of induction of these drug reactions,\(^3,^8\) knowledge of the mode of induction of these reactions remains incomplete. Recently, we encountered two patients within a 6-mo period with a clinical syndrome of agranulocytosis associated with diphenylhydantoin (DPH) therapy. In vitro and clinical studies suggest antibody-mediated suppression of granulopoiesis as a major mechanism of induction of agranulocytosis in these two patients and cross-reaction with a lymphocyte antigen in one of them.
CASE HISTORIES

Patient 1

Patient 1 is a 26-yr-old white man who sustained a subdural hematoma secondary to head trauma on October 6, 1977. A small subdural hematoma was evacuated, which recurred 2 wk later and was treated in a similar manner. He returned with recurrent headaches and focal neurologic signs and had a third evacuation of a hematoma from the same site. Postoperatively, he was treated with DPH. His serum DPH level was 6 μg/ml after 2 wk of therapy, and his dose was increased. On the 19th postoperative day, he was noted to be febrile. The peripheral leukocyte count was 2900/mm³ with 24% segmented forms. His DPH was stopped, but the next day he was agranulocytic. A bone marrow aspirate showed a hypocellular specimen with greatly reduced early myeloid precursors and total absence of any forms more mature than a myelocyte. He recovered uneventfully (Fig. 1). Serum for study was drawn 3 days after onset of his fever.

Patient 2

Patient 2 was a 75-yr-old white man who was struck by a car while riding a motorcycle. He sustained an intracerebral hematoma that was evacuated surgically. Postoperatively, he was placed on DPH as a prophylaxis against seizures. On the 21st postoperative day, he was noted to be agranulocytic (Fig. 1) and febrile, and his DPH was stopped. A bone marrow aspirate showed complete absence of granulocytic precursors and normal numbers of erythroid forms and megakaryocytes. He subsequently developed polymicrobial sepsis and expired without any increase in his granulocyte count. Serum for study was drawn 3 days after onset of his fever.

MATERIALS AND METHODS

CFU-C Assay

Assay for marrow and peripheral blood granulocyte/macrophage precursors (CFU-C) was performed as described by Carbone et al.9 in 0.8% methyl cellulose, but without bovine serum albumin. Granulocyte/macrophage colony-stimulating activity was provided by (A) peripheral blood leukocyte feeders, as described by Pike and Robinson,10 (B) media conditioned by human embryonic kidney cells, as described by Carbone et al.9 or (C) media conditioned for 1 wk by 10⁶/ml normal peripheral blood Ficoll/Hypaque-purified mononuclear cells, stimulated with pokeweed mitogen (10 μl/ml, Grand Island Biological Co., Grand Island, N.Y.). The conditioned media was added at a final concentration of 10%.

Bone marrow specimens were obtained from the femur or iliac crest of hematologically normal individuals undergoing orthopedic procedures and on one occasion from patient 1, 5 mo after recovery. Specimens of 5–10 ml were aspirated into preservative free heparin. Peripheral blood (20–30 ml) was collected in a similar manner. The marrow aspirate or peripheral blood sample was pipetted to form a single-cell suspension and then underlayered with Ficoll/Hypaque and centrifuged at 1200 g for 20 min. The mononuclear cell fraction was removed, washed, and plated in quadruplicate at 1–2 x 10⁵ cells/ml (marrow) or 5 x 10⁵ cells/ml (peripheral blood). Cultures were incubated at 37°C in 7.5% CO₂ for 1 wk and aggregates of greater than 40 cells were scored as colonies. Colonies were aspirated by means of fine
Pasteur pipets, smeared on glass slides, fixed, and stained with Wright's stain for individual typing according to morphology.

**Sera**

Blood was collected in syringes or vacutainers, and the serum frozen at −20°C. Serum specimens were thawed, Millipore filtered, and added to bone marrow cultures at a concentration of 100 µl/ml. DPH (Dilantin®, Parke-Davis, Detroit, Mich.) was solubilized at 37°C and added to cultures at a concentration of 10 µg/ml just prior to plating.

In a separate set of experiments, 10⁶ Ficoll/Hypaque-separated bone marrow cells were incubated in McCoy’s 5A media with control or patient sera (10% final concentration) and DPH (10 µg/ml). The cells were then washed three times in McCoy’s 5A and plated over peripheral blood leukocyte feeders.

Only a small amount of serum from patient 2 was available for study. For some experiments, patient 1’s serum was subjected to (A) chloroform extraction, as described by Granstrom; (B) dialysis performed against phosphate-buffered saline, pH 7.42 (200 vol x 3, 72 hr, 4°C); or (C) heat inactivation carried out at 56°C x 2 hr, with the CFU-C assay performed in the presence of heat-inactivated fetal bovine serum. Convalescent sera from patient 1 were studied 3 and 5 mo after recovery.

Control sera were obtained from 7 normal individuals; 1 patient with idiopathic granulocytopenia and splenomegaly; 3 patients on chronic DPH therapy with serum levels of 10–20 µg/ml; and 1 patient who developed agranulocytosis over a 2-wk period while on DPH and multiple other drugs.

Absorption of serum with cells was accomplished using either mononuclear cells or a similar concentration of polymorphonuclear leukocytes from patient 1, a single normal donor, or pooled leukocytes from six normal donors. The mononuclear cells, purified on Ficoll/Hypaque, contained 80%-85% lymphocytes and 15%-20% monocytes, with less than 1% polymorphonuclear leukocytes. The polymorphonuclear leukocytes were prepared from the Ficoll/Hypaque pellet according to a modification of the technique described by Logue, employing 0.83% NH₄Cl lysis to remove red blood cells as described by Dioguordi. The polymorphonuclear leukocyte preparations contained <1% mononuclear cells (i.e., <1% lymphocytes and monocytes). One-half milliliter of absorbing cell suspension containing 30 x 10⁶ cells was incubated with 1 ml of a 1:10 dilution of patient 1’s serum at 37°C for 1 hr. The supernatant was then removed and subjected to a repeat absorption.

**Gel Filtration**

One milliliter of patient 1’s serum was applied to a sepharose column equilibrated with veronal-buffered saline, pH 7.1, to which rabbit anti-IgG with anti-κ and anti-λ activity had been coupled (described elsewhere and kindly provided by Dr. Robert Schreiber, Scripps Clinic and Research Foundation, La Jolla, Calif.). The immunoglobulin fraction was then recovered. The column flow-through and eluate were dialyzed against phosphate-buffered saline, concentrated to their original volume, and both fractions tested. All immunoglobulins were retained by the column as determined by immunodiffusion in Ouchterlony plates. As a control, serum was also passed through an IgG-sepharose column, dialyzed, concentrated, and tested separately.

**Effects of Serum on Mature Polymorphonuclear Leukocytes**

The effect of patient 1’s serum on mature polymorphonuclear leukocytes was assessed by measuring oxygen consumption during phagocytosis. Purified polymorphonuclear leukocytes (4 x 10⁶/ml) in Eagle’s medium were incubated with opsonized zymosan particles and control levels of oxygen consumption were measured over a 10-min period in a Yellow Springs Instruments biological oxygen monitor. Normal serum or patient 1’s serum were then added and changes in oxygen consumption assayed.

The ability of patient 1’s serum to opsonize polymorphonuclear leukocytes inactivated by 2-deoxyglucose was examined by a modification of the technique of Boxer et al. Purified polymorphonuclear leukocytes (10⁷/ml) were incubated with 2-deoxyglucose (15 µl/ml of a 20% solution) and normal or patient 1’s serum (150 µl/ml). One milliliter of inactivated and opsonized polymorphonuclear leukocytes and 1 ml (10⁷/ml) of untreated responder polymorphonuclear leukocytes were mixed and oxygen consumption measured in the biologic monitor for 15 min.
Lymphocyte Stimulation

Lymphocyte stimulation with phytohemagglutinin (PHA) was performed using previously described techniques. Ficoll/Hypaque separated mononuclear cells from patient 1 and 8 normal donors were incubated in 1 ml Dulbecco's MEM plus 10% fetal calf serum with 1 μg of PHA-P (Burroughs-Wellcome, Research Triangle Park, N.C.) and pulsed with 2 μCi/ml ³H-thymidine (6 Ci/mM) for 2 hr at 37°C on day 5. Sera from normal donors or patient 1’s acute or convalescent sera were added at initiation of the culture at a final concentration of 10%. DPH at a final concentration of 10 μg/ml was added to some cultures.

Bone Marrow ³H-Thymidine Incorporation

Bone marrow ³H-thymidine incorporation was measured by incubating 10⁶ human bone marrow cells in 1 ml for 2 hr with 10 μCi of ³H-thymidine, and counts were recovered on filter discs for liquid scintillation counting.

Statistics

Statistical analysis was accomplished by use of the Student’s t test for paired or nonpaired observations.

Human Subjects

Specimens obtained in this investigation were obtained in a manner reviewed by the Human Subjects Committee, University of California, San Diego, Calif.

RESULTS

The effect of addition of DPH to cultures of normal bone marrow was first evaluated in the presence of control sera (Table 1). In the presence of control sera and DPH, no inhibition was noted. Drug-dependent inhibition was not observed in the presence of sera from patients on chronic DPH therapy, either control patient with agranulocytosis, or patient 1’s convalescent sera. The presence of patient 1’s acute serum significantly and consistently increased colony numbers. In contrast to control sera, addition of DPH to bone marrow cultures in the presence of patient 1’s or patient 2’s acute serum resulted in significant depression of bone marrow colony formation below the level without drug (Table 1). Inhibition ranged from 38% to 93% in 9 of 10 marrows tested. No change in colony size or morphological composition was noted in the presence of DPH.

When bone marrow cells were exposed to the patient’s sera for 1 hr in the presence of DPH and then washed, suppression of bone marrow colony formation

<table>
<thead>
<tr>
<th>Table 1. Alloimmune Bone Marrow Granulocyte/Macrophage Colonies in the Presence of Sera and DPH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Additions</strong></td>
</tr>
<tr>
<td><strong>Sera</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Patient 1</td>
</tr>
<tr>
<td>Patient 1</td>
</tr>
<tr>
<td>Patient 2</td>
</tr>
<tr>
<td>Patient 2</td>
</tr>
</tbody>
</table>
Table 2. Effects of Short-Term (1 hr) Exposure to Sera and DPH on Allogeneic Bone Marrow Granulocyte/Macrophage Colonies

<table>
<thead>
<tr>
<th>Additions</th>
<th>Colonies/10^5 Cells (± SEM)</th>
<th>Number of Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sera</td>
<td>DPH</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>124 ± 49</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>114 ± 32</td>
</tr>
<tr>
<td>Patient 1</td>
<td>+</td>
<td>109 ± 34</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>54 ± 28</td>
</tr>
<tr>
<td>Patient 2</td>
<td>-</td>
<td>118 ± 46</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>58 ± 32</td>
</tr>
</tbody>
</table>

was also observed (Table 2). These data indicate that the sera contained factors capable of damaging committed granulocyte/macrophage precursors as well as the more mature forms arising in the colonies.

Table 3 shows the growth of autologous bone marrow and peripheral blood colonies from patient 1 in the presence of his acute and convalescent sera and DPH. Both bone marrow and peripheral blood colonies showed significant drug-dependent suppression in the presence of his acute serum. Colony growth actually appeared to be increased in the presence of his convalescent sera and DPH. Although this effect did not achieve statistical significance, it was observed on the one occasion when the patient’s marrow was tested, and on two separate occasions when his peripheral blood colony-forming units were cultured.

Drug-dependent colony inhibitory activity persisted in patient 1’s acute serum and patient 2’s serum after chloroform extraction, and in patient 1’s acute serum after dialysis and heat inactivation (see Materials and Methods). Passage of patient 1’s acute serum through an immunoabsorbent column resulted in removal of IgM, IgG, and IgA and removal of the drug-dependent colony-inhibitory activity (Table 4). Drug-dependent colony-inhibiting activity was recovered by elution of

Table 3. Autologous Granulocyte/Macrophage Colonies in the Presence of Autologous Sera and DPH

<table>
<thead>
<tr>
<th>Cells</th>
<th>Serum</th>
<th>DPH</th>
<th>Colonies/2 x 10^3 Cells (± SEM)</th>
<th>Number of Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>-</td>
<td>77.5 ± 5</td>
<td>p &lt; 0.05</td>
<td>1</td>
</tr>
<tr>
<td>Acute</td>
<td>+</td>
<td>26 ± 4</td>
<td>p &lt; 0.05</td>
<td>1</td>
</tr>
<tr>
<td>Convalescent</td>
<td>-</td>
<td>56 ± 4</td>
<td>p &lt; 0.1</td>
<td>1</td>
</tr>
<tr>
<td>Convalescent</td>
<td>+</td>
<td>109 ± 8</td>
<td>p &lt; 0.1</td>
<td>1</td>
</tr>
<tr>
<td>Peripheral Blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>-</td>
<td>32 ± 1</td>
<td>p &lt; 0.05</td>
<td>2</td>
</tr>
<tr>
<td>Acute</td>
<td>+</td>
<td>15 ± 2</td>
<td>p &lt; 0.05</td>
<td>2</td>
</tr>
<tr>
<td>Convalescent</td>
<td>-</td>
<td>5.0 ± 1</td>
<td>p &lt; 0.1</td>
<td>2</td>
</tr>
<tr>
<td>Convalescent</td>
<td>+</td>
<td>16 ± 1</td>
<td>p &lt; 0.1</td>
<td>2</td>
</tr>
</tbody>
</table>
the gamma globulins. Passage of the serum through a sepharose column containing IgG but without antiimmunoglobulin activity did not remove the drug-dependent colony-inhibitory activity. These data are most consistent with mediation of the drug-dependent colony inhibition by a non-complement-dependent immunoglobulin.

Table 5 shows the results of two separate assays designed to examine the effect of patient 1’s acute serum on mature polymorphonuclear leukocytes. The effect of the serum on oxygen consumption of leukocytes engaged in phagocytosis was first evaluated. Patient 1’s serum inhibited the oxygen uptake of the polymorphonuclear leukocytes compared with control serum from a normal donor. These results may indicate an effect on leukocyte function by patient 1’s serum, but should be interpreted with caution, since the drug can inhibit cell function in this assay and trace amounts of DPH were probably present in his serum.

Evaluation of the oxygen consumption of polymorphonuclear leukocytes engaged in phagocytosis of inactivated granulocytes opsonized by patient 1’s serum showed that this activity was also affected (Table 5). Despite the possible trace DPH content of patient 1’s serum, which could inhibit polymorphonuclear leukocyte oxygen consumption, the effector leukocytes showed a substantial increase in oxygen consumption when exposed to granulocytes opsonized by patient 1’s serum. In contrast, the mean change in oxygen consumption after opsonization with normal serum is only 2 ± 0.22% (mean ± SEM), indicating that the changes observed with patient 1’s serum are significant. These two assays taken together
indicate the presence of a serum factor affecting and binding to mature polymorphonuclear leukocytes that may serve as an opsonin, leading to possible phagocytosis of mature polymorphonuclear leukocytes by reticuloendothelial cells.

The effect of patient 1's acute serum on the $^3$H-thymidine incorporation of autologous and allogeneic bone marrow and mitogen-stimulated peripheral blood mononuclear cells is shown in Table 5. Results in this table have been expressed as percent of incorporation in the presence of patient 1's convalescent serum (for autologous cells) or normal serum (for allogeneic cells). When the effect of patient 1's acute serum on short-term bone marrow cultures was measured, inhibition of $^3$H-thymidine incorporation was observed with patient 1's marrow (5 mo after recovery) but not normal marrow (Table 5). This observation suggests antigenic differences between the patient's bone marrow and the normal control marrow. This observation was corroborated by the finding that the patient's acute serum suppressed $^3$H-thymidine incorporation into his own PHA-stimulated mononuclear cells but not into normal donor mononuclear cells (Table 6). This effect was not drug-dependent. It is possible this inhibition was due to a metabolite contained in the patient's serum to which only his cells were sensitive. However, this would be in marked contrast to the effects of his acute serum on both his own and allogeneic granulocyte/macrophage precursors in the absence of drug, where an actual increase in cell growth was noted (Tables 1 and 2). It seems probable that suppression of thymidine incorporation in the presence of acute serum was mediated by the same antibody that caused drug-dependent suppression of his granulocyte/macrophage precursors. The effects of the serum on his bone marrow cells but not control cells suggest the existence of antigenic differences between the patient's cells and control cells. The serum inhibition of lymphocyte blastogenesis observed also appeared to be limited to the patient's lymphocytes and supports this hypothesis.

Table 7 presents the results of attempts to remove the bone marrow colony-inhibiting activity from patient 1's serum by absorption with single-donor and pooled normal donor peripheral blood cells. Absorption of patient 1's serum with autologous mononuclear cells removed the colony-inhibiting activity, whereas absorption with normal single-donor or pooled mononuclear cells did not. In contrast, absorption with either his own or pooled allogeneic polymorphonuclear leukocytes could remove this activity. This indicates the probable presence of an antigen on patient 1's mononuclear cells not shared by the majority of normal mononuclear cells, but apparently universally present on polymorphonuclear leuko-
Table 7. Summary of Absorption of Colony-Inhibitory Activity From Patient 1 Acute Serum by Autologous and Allogeneic Peripheral Blood Cells

<table>
<thead>
<tr>
<th>Drug-Dependent Inhibiting Activity Removed by Absorption With</th>
<th>No cells</th>
<th>Mononuclear cells</th>
<th>Polymorphonuclear leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cells</td>
<td>No</td>
<td>Patient 1</td>
<td>Yes</td>
</tr>
<tr>
<td>Single donor</td>
<td>No</td>
<td>Single donor</td>
<td>Yes</td>
</tr>
<tr>
<td>Pooled donor</td>
<td>No</td>
<td>Pooled donor</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Absorption of the bone marrow suppressor activity by mature polymorphonuclear leukocytes also strongly suggests a common identity for the bone marrow suppressor and the leukocyte opsonizing activity noted above.

DISCUSSION

Despite widespread use since 1938, reports of severe granulocytopenia associated with DPH have been extremely uncommon. Six cases have been reported in detail previously (Table 8). Bone marrow morphology, when described, has been characterized as showing "maturation arrest,"21,22 with normal erythroid precursors and megakaryocytes, but with "degeneration" of myeloid cells.21 Only one fatality secondary to DPH-associated agranulocytosis has been previously reported, in a patient with concomitant severe alcoholic liver disease.18

Few studies regarding the mechanism of induction of this particular drug reaction have appeared. Pisciotta3 noted suppression of 3H-5-uridine uptake in marrows from three patients with anemia and leukopenia associated with DPH. Reid and Chanarin23 have been able to demonstrate suppression of 3H-thymidine uptake by normal human marrow in the presence of DPH, but no effect was seen with incubation periods shorter than 24 hr. Others have noted abnormal deoxyuridine suppression tests in marrows of patients treated with DPH, but at DPH concentrations 5--10 times higher than therapeutic levels.24 Recently, Menitove et al.9 have reported the presence of antibody cytotoxic to polymorphonuclear leukocytes and able to suppress in vitro marrow CFU-C growth in a 17-yr-old girl with a

Table 8. Characteristics of Reported Cases of Agranulocytosis Associated With DPH

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>M/F</th>
<th>Duration of Therapy (Days)</th>
<th>Total Dose (g)</th>
<th>Outcome</th>
<th>Other Drugs at the Time of Diagnosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>F</td>
<td>24</td>
<td>8.0</td>
<td>Recovered</td>
<td>None</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>M</td>
<td>10</td>
<td>3.0?</td>
<td>Died</td>
<td>Ethanol chlorothiazide</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>M</td>
<td>38</td>
<td>15.2</td>
<td>Recovered</td>
<td>Phenobarbital</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>F</td>
<td>14</td>
<td>4.2</td>
<td>Recovered</td>
<td>Prior DPH therapy</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>M</td>
<td>30</td>
<td>Unknown</td>
<td>Recovered</td>
<td>Prior DPH therapy</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>F</td>
<td>11</td>
<td>3.3</td>
<td>Recovered</td>
<td>None</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>M</td>
<td>19</td>
<td>7.0</td>
<td>Recovered</td>
<td>None</td>
<td>Present study</td>
</tr>
<tr>
<td>8</td>
<td>75</td>
<td>M</td>
<td>22</td>
<td>4.3</td>
<td>Died</td>
<td>Aminophylline</td>
<td>Present study</td>
</tr>
</tbody>
</table>
serum sickness-like illness associated with DPH. Min-Fu et al. noted phagocytosis of polymorphonuclear leukocytes by histiocytes in a marrow specimen from a 15-yr-old girl with DPH-associated agranulocytosis. Parker and Gumnit have also presented clinical evidence for a dose-related leukopenia in a single patient on DPH.

In a period of 6 mo, we had occasion to see 2 patients with DPH-associated agranulocytosis. Neither showed signs or symptoms suggestive of a hypersensitivity reaction. Sufficient data were available in patient 1 to suggest that metabolism of the drug in this patient was not abnormal. In both patients, bone marrow examination strongly suggested selective suppression of granulopoiesis as a component of their drug reaction. One patient recovered uneventfully, but the second succumbed to multiple medical complications.

The concept of immune suppression of hemopoiesis as a mechanism for induction of blood dyscrasias has been recently reviewed. Kelton et al. have recently reported drug-dependent suppression of CFU-C and CFU-E production in a patient with pancytopenia associated with quinidine therapy. Barrett et al. noted suppression of marrow colony growth in the presence of serum and drug from a patient with aminopyrine-induced agranulocytosis. Both patients studied here also demonstrated drug-dependent suppression of granulopoiesis in culture by a serum factor. The in vitro assay for granulocyte/macrophage precursors using allogeneic bone marrow demonstrated the presence of a drug-dependent serum factor capable of suppressing granulopoiesis. Extensive studies in patient 1 showed the serum factor to be active against autologous as well as allogeneic bone marrow colony formation. The factor was found to be heat stable, nondependent on complement, and nondialyzable. Passage of the serum through an antiimmunoglobulin column resulted in removal of the drug-dependent inhibitor, strongly suggesting that it is an antibody.

In our study, cultures were conducted in the continuous presence of serum. This situation may more closely approximate the situation in vivo, but makes the site of action of the antibody somewhat more uncertain. Inhibition of proliferation could have occurred at any point in the mitotic compartment (cells less mature than a late myelocyte). The profound colony suppression seen in some cultured bone marrow specimens in the presence of patient 1’s serum indicates probable suppressive activity at a very early stage of proliferation.

When patient 1’s acute phase serum was tested against his own bone marrow in short-term culture, suppression of 3H-thymidine incorporation was observed. This serum also demonstrated evidence suggesting inhibition of phagocytosis by mature polymorphonuclear leukocytes and definite evidence of ability to opsonize these cells. Absorption with polymorphonuclear leukocytes could remove the drug-dependent colony-inhibiting activity from the serum. These data demonstrate that the antibody affects mature, well differentiated granulocytes, as well as immature, dividing granulocyte precursors. Opsonization of mature polymorphonuclear leukocytes could lead to peripheral destruction of these cells in the reticuloendothelial system, as observed by Min Fu et al., whereas coating of immature cells could lead to the leukopenic bone marrow picture by injuring these cells.

These findings suggest a multifactorial etiology for induction of this drug-induced agranulocytosis. The antibody-mediated agranulocytosis in patient 1 was
capable of both suppressing granulopoiesis and of mediating peripheral destruction of mature polymorphonuclear leukocytes. Variable clinical manifestations of drug-induced antigranulocyte antibodies may therefore also be present in other patients, depending on whether leukocyte destruction, decreased production, or both predominate. Aminopyrine, a drug long thought to induce agranulocytosis primarily by antibody-mediated peripheral leukocyte destruction, can induce a serum factor that also suppresses hemopoiesis in culture. DPH, on the other hand, may induce an antibody whose major mode of action is suppression of granulopoiesis, although the antibody may also effect the destruction of mature polymorphonuclear leukocytes.

To our surprise, patient 1's serum also showed suppression of autologous but not allogeneic lymphocyte PHA transformation. This activity was non-drug-dependent in our studies, although this may merely reflect differences in the assay systems used to test effects on CFU-Cs and lymphocytes, or trace contamination of the patient's serum by DPH. It is possible that the serum's effects on lymphocytes were due to a separate factor or antibody. However, absorption studies demonstrated that autologous but not allogeneic lymphocytes were capable of removing the bone marrow colony suppressing factor, indicating a cross-reaction between his lymphocytes and bone marrow granulocyte/macrophage precursors from all donors studied. Of additional interest are recent reports noting augmentation of colony growth by anti-T-cell sera, since patient 1's serum augmented colony growth in this study.

Considerable data exist to support the existence of cross-reacting antigens between lymphocytes and committed granulocyte precursors. Human bone marrow CFU-C production is suppressed by antilymphocyte globulin and anti-B-cell-specific serum. B-lymphocyte antigens have also been identified on myeloid leukemic cells. Cross-reaction of the serum factor suppressing the patient's lymphocytes and that suppressing granulocyte/macrophage colony formation is strongly suggested by absorption of the latter activity by the patient's mononuclear cells. Others have also noted antilymphocyte activity in antibody-mediated suppression of granulopoiesis. Cline et al. noted intermittent lymphocytotoxic activity in serum from a patient with antibody-mediated autoimmune panleukopenia. A review of our two patients' lymphocyte counts showed that they too declined during the period of agranulocytosis (Fig. 1). This observation suggests antilymphocyte as well as antigranulocyte activity in vivo as well as in vitro, although other explanations are possible. The possibility that the original target of the DPH-induced antibody may be a lymphocyte antigen, cross-reacting with granulocyte precursors, but occurring with a very restricted population distribution, may provide an explanation for the infrequency with which this particular drug complication is observed.

The means by which an antilymphocyte antibody might induce agranulocytosis are at present speculative. Antigens shared by early myeloid precursors and B cells have already been mentioned. Nathan et al. have demonstrated that proliferation of peripheral blood erythroid burst-forming units is dependent on the presence of T cells. Similarly, Richman et al. have shown that proliferation of peripheral blood CFU-Cs seems to require a cell–cell interaction with a mononuclear cell subpopulation. It is therefore possible that an antibody directed against a T-
lymphocyte cell population might significantly impair granulocyte production. Mitogen-stimulated lymphocytes are known to produce colony-stimulating activity. An antibody that inhibited colony-stimulating activity production by these cells might also significantly impair granulocyte production. Further studies in other patients will be necessary to delineate the possible sites of action of such antibodies.

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REFERENCES

24. Taguchi H, Laudy M, Reid C, Reynolds EH, Chanarin I: The effect of anticonvulsant...
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drugs on thymidine and deoxyribose nucleic acid
synthesis by human marrow cells. Br J Haematol
36:181, 1977

25. Parker WA, Gummit RJ: Diphenylhydanto-
toin toxicity: Dose-dependent blood dyscrasia.
Neurology 24:1178, 1974


27. Netzel B, Radt H, Hoffmann-Fezer G,
Thiel E, Thierfelder S: The effect of crude and
differently absorbed anti-human T-cell globulin
on granulocytic and erythropoietic colony forma-

marrow suppression by antilymphocyte globulin.
Br Med J 2:541, 1975

29. Kaplan J, Inoue S, Ottenbreit MJ: Myeloid
colony-forming cells express human B lymphocyte

G, Gale R, Terasaki P: Human B-lymphocyte
antigens expressed by lymphocytic and myelocytic

31. Cline M, Billing R: Antigen expressed by
human B lymphocytes and myeloid stem cells. J
Exp Med 146:1143, 1977

32. Cline MJ, Opelz G, Saxon A, Fahey JL,
Golde DW: Autoimmune panleukopenia. N. Engl

33. Dale DC, Fauci AS, Guerry D, Wolff SM:
Comparison of agents producing a neutrophilic

34. Nathan DG, Chess L, Hillman DG, Clarke
B, Breard J, Merier E, Housman D: Human
erythroid burst-forming unit: T-cell requirement
1978

35. Richman CM, Chess L, Yankee RA: Purifi-
cation and characterization of granulocytic
progenitor cells (CFU-C) from human peripheral
blood using immunologic surface markers. Blood
51:1, 1978

36. Cline MJ, Golde DW: Production of colony
stimulating activity by human lymphocytes.
Nature 248:703, 1974
Drug-induced agranulocytosis: in vitro evidence for immune suppression of granulopoiesis and a cross-reacting lymphocyte antibody

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