Antibodies to Myeloid Precursor Cells in Autoimmune Neutropenia


Antibodies to mature blood neutrophils and to bone marrow myeloid cells have been described in the sera of some patients with apparent autoimmune neutropenia. To further explore the prevalence and specificities of antibodies to myeloid precursor cells, we evaluated sera from 148 patients with suspected autoimmune neutropenia for the presence of antibodies to neutrophils, to cultured myeloid cell lines, and to highly purified bone marrow myeloid progenitor cells. Using an immunofluorescence flow cytometric assay, we identified IgG antibodies in 42 (28%) of these sera that bound specifically to K562 cells, a multilineage cell line originally derived from a patient with chronic myelogenous leukemia. Twenty-two (15%) of the sera also contained IgG antibodies that bound specifically to the primitive myelomonocytic leukemia cell line KG1a. Twenty-five (17%) of the sera had IgG antibodies to myeloid cell lines in the absence of antibodies to mature neutrophils. There was a trend toward more severe neutropenia in patients with antibodies to K562 cells, without antineutrophil antibodies. In further studies, antibodies from 12 sera bound to mononuclear CD34+ cells that had been purified from normal human bone marrow by an immunomagnetic separation procedure. Moreover, two of these sera suppressed the growth of granulocyte-macrophage colony-forming units (CFU-GM) in methylcellulose cultures. The presence of antibodies to primitive hematopoietic cells in the sera of some patients with suspected immune neutropenia suggests that these antibodies may have a role in the pathogenesis of the neutropenia observed. This is a US government work. There are no restrictions on its use.

Autoimmune Neutropenia may occur as an isolated clinical entity, or it may be associated with other clinical conditions such as collagen vascular disorders, drug ingestion, viral infections, or malignancy. Antibodies directed against mature blood neutrophils have been identified in the sera of patients with neutropenia of suspected immune etiology. In some cases of immune neutropenia, the neutrophil surface membrane molecules that bear antigenic binding sites for these autoantibodies have been identified. Antigenic target molecules on mature blood neutrophils include the adhesion glycoprotein complex CD11b/CD18, and the Fc receptor type III. Other molecular targets include thrytropin receptor-like molecules on neutrophil surfaces, and cell surface membrane actin-like molecules. In addition to antibodies to mature neutrophils, some patients with neutropenia have IgG antibodies apparently directed toward less mature bone marrow myeloid elements. Furthermore, cultured myeloid cell lines have been used as target cells for the study of antibody reactivity in patients with autoimmune neutropenia. Myeloid cell lines contain antigenic target molecules that are present on primitive hematopoietic precursor cells, but that are lost during the process of myeloid maturation and are absent on mature blood neutrophils. The relative contribution of antibodies directed against maturational antigens in the pathogenesis of autoimmune neutropenia is not known.

Both humoral and cellular immune mechanisms are probably involved in suppression of hematopoiesis. Antibodies from the sera of rare patients with pure white blood cell aplasia, autoimmune panleukopenia, and acquired neutropenia have been reported to inhibit the in vitro growth of myeloid colonies from human bone marrow. Furthermore, many patients with suspected autoimmune neutropenia have a bone marrow picture of myeloid arrest, with an absence of mature neutrophils in the marrow, suggesting that myeloid cells fail to mature, or that they are destroyed in the bone marrow rather than in the blood. Igs from a significant proportion of patients with autoimmune neutropenia may be involved in the suppression of myeloid cell growth and proliferation, and may contribute to the pathogenesis of neutropenia in these patients.

To further explore the prevalence and specificities of antibodies to myeloid precursor cells, we studied sera from 148 patients with suspected autoimmune neutropenia for the presence of antibodies to neutrophils and to the cultured myeloid leukemia cell lines K562 and KG1a. In addition, sera were studied for antibodies against highly purified progenitor cells isolated from normal human bone marrow, and for suppression of clonal growth in culture.

Materials and Methods

Sera and Igs. Sera screened for antibodies to neutrophils and to myeloid precursor cells were collected from 148 patients with suspected immune neutropenia, referred from the adult and pediatric services of the Walter Reed Army Medical Center, Washington, DC, and elsewhere. Patients eligible for study included those with isolated chronic neutropenia, as well as those with neutropenia (defined as an absolute neutrophil count ≤1,500) and associated clinical conditions, including immune cytophenias such as autoimmune hemolytic anemia or immune thrombocytopenic purpura (ITP); collagen vascular disorders such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), mixed connective tissue disease, Sjögren's syndrome, Hashimoto's thyroiditis, and Grave's disease; viral infections such as Epstein Barr virus, cytomegalovirus, and human immunodeficiency virus (HIV); lymphoproliferative disorders such as Hodgkin's disease, lymphoma, thymoma, or lymphoproliferative disorder of granular lymphocytes (LDGL); drug ingestions, including penicillins, phenothiazines, antihypothyroid drugs, hydantoins, antiarrhythmics; and associated clinical conditions.

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(eg, procainamide), sulfa drugs, nonsteroidal antiinflammatory agents, and levamisole. Sera from patients with drug ingestions were collected during the period of neutropenia; however, they were not studied in the presence of added drugs. Sera were also collected from 20 normal healthy individuals for use as controls. Additional controls included sera from 81 nonneutropenic patients with collagen vascular diseases, including seropositive RA and SLE. All sera were collected according to a protocol approved by the Walter Reed Army Medical Center Human Use Committee. Purified IgG was prepared from the sera of selected patients and controls using protein A-Sepharose affinity chromatography.

Cell lines. Cultured myeloid cell lines were maintained using standard techniques. KG1a cells were maintained in Iscove's modified Dulbecco's medium (IMDM) with 10% fetal bovine serum (FBS). K562 cells were maintained in RPMI with 10% FBS. Cultures were incubated at 37°C with 5% CO₂ and 100% humidity. Cultures were restarted from cryopreserved stocks of early passaged cells on a periodic basis to avoid changes in the phenotypic character of the cell lines during the course of maintenance culture.

Neutrophils. Neutrophils were purified from the venous blood of healthy donors. Briefly, heparinized blood was separated by density gradient centrifugation and the mononuclear cell layer discarded. Neutrophils were separated from red blood cells (RBCs) by sedimentation in 3% dextran, and further purified by hypotonic lysis of residual RBCs. Neutrophils prepared in this manner were shown to be more than 95% pure and approximately 98% viable by trypan blue exclusion.

Immunofluorescence flow cytometry. Immunofluorescence flow cytometry was performed using mature neutrophils as described. In addition, cultured myeloid cell lines (K562 and KG1a), and normal human bone marrow mononuclear cells (see below), were used to detect antibodies to primitive myeloid surface membrane proteins. Cells for immunofluorescence assays were suspended in phosphate-buffered saline with 2% FBS and 0.1% sodium azide (PBS/FBS) at 10⁶/mL (except for purified bone marrow progenitor cells, which were studied at 5 × 10⁶/mL), and incubated with patient or control serum, diluted 1:10, for 30 minutes at 4°C. The cells were then washed twice with PBS/FBS, incubated with fluorescein isothiocyanate (FITC)-labeled goat antihuman IgG (Fab' fragments, heavy and light chain–specific; Sigma Chemical Co, St Louis, MO), diluted 1:100 in PBS/FBS. Samples were analyzed on a FACSCAN instrument (Becton Dickinson, Mountain View, CA), and results expressed as computer-generated histograms of cell number versus fluorescence. Histograms obtained using the control sera from 20 normal healthy individuals, when studied with K562 or KG1a cells, all fell within a narrow range. Neutrophenic patients' sera and control sera from patients with collagen vascular disorders were compared with individual normal sera for evaluation of enhanced antibody binding. Results were graded as positive if more than 50% of the patient's fluorescent events were greater than control.

Blocking studies. Blocking studies were performed using murine monoclonal antibodies (MoAbs) to myeloid precursor cell antigens. The MoAbs My-9 (anti-CD33, Coulter Immunology, Hialeah, FL) and My-10 (anti-CD34, Becton Dickinson, Mountain View, CA) were studied with KG1a cells. KG1a cells were first incubated with MoAb or murine control MoAb, 1 µg/10⁶ cells, for 30 minutes at 25°C. The cells were then added to individual tubes containing sera (final dilution 1:10), and incubated for an additional 30 minutes. The cells were washed twice, incubated with antihuman IgG-FITC, washed, and studied on the flow cytometer as above. Evidence for competition for a specific antigenic epitope would be seen as a decrease in human IgG binding, as reflected by a decrease in antihuman IgG-FITC fluorescence.

Immunoblotting studies. Cell lysates were prepared by nitrogen cavitation of KG1a cells or neutrophils. Nuclei and cellular debris were removed by centrifugation at 10,000 g for 30 minutes. Five percent to 15% gradient polyacrylamide gel electrophoresis was performed using the lysate preparation (~50 µg protein per lane) and the proteins were then electrophoretically transferred to nitrocellulose paper. The paper was cut into strips, incubated with study or control sera (1:100), and developed using an enzyme-linked (horseradish peroxidase) method.

Suppression studies of myeloid progenitor cell growth in vitro. Studies of the effect of immunoglobulins on the in vitro proliferation of myeloid progenitor cells were performed in methylcellulose cultures. Bone marrow was collected from normal healthy volunteers following a protocol approved by the Walter Reed Army Medical Center Human Use Committee. For these studies, marrow mononuclear cells were separated by density gradient centrifugation and adherent cell depletion, and progenitor cells were enriched by positive immunomagnetic selection. Cells purified using the immunomagnetic technique were kindly provided by Dr Stephen Kessler (Naval Medical Research Institute, Bethesda, MD) and were used for both flow cytometry studies as above and clonal inhibition studies described below. Briefly, light density bone marrow mononuclear cells were rosetted with the anti-CD34 MoAb K6,22 linked to modified magnetic Dynabeads (Dynal Inc, Great Neck, NY) and separated by one or four cycles of attraction to a rare earth magnet. The cells were nonenzymatically detached from the beads, and the beads subsequently removed from the cells magnetically.23 The enriched progenitor cells were plated at 10⁴ cells/mL in 35-mm diameter, girded tissue culture plates (Nunc Inc, Naperville, IL) in IMDM containing 1.0% methylcellulose (4,000 CP, Tridom/Fluka, Buchs, Switzerland), 10% human serum, 10% phytohemagglutinin lymphocyte-conditioned media (PHA-LCM), 20% FBS (HyClone, Logan, UT). Human serum and FBS were heat-inactivated before use, and there was no other source of added complement. Cultures studied with purified immunoglobulin (1 mg/mL, final concentration) also had no added complement. Cultures were incubated at 37°C with 5% CO₂ and 100% humidity, and hematopoietic colonies were counted with an inverted-phase microscope at 40X magnification at 14 to 21 days. Granulocyte-macrophage colony-forming units (CFU-GM), hemoglobinized erythroid burst-forming units (BFU-E), and mixed erythroid-erythroid colonies (CFU-Mix) were recognized using standard criteria of clonal morphology.

RESULTS

Antineutrophil antibodies. Sera from 148 patients with suspected immune neutropenia were studied using immunofluorescence flow cytometry with mature blood neutrophils as target cells. Thirty-six percent (53/148) of sera had evidence of enhanced binding of IgG to mature blood neutrophils, interpreted as positive for antineutrophil antibodies. Sera were also studied from nonneutropenic patients with seropositive RA, SLE, and other collagen vascular disorders collected from patients seen in a rheumatology clinic. Seventeen percent (14/81) of sera from these patients with collagen vascular disorders also had evidence for enhanced IgG binding to neutrophils, compared with normal control sera.

Antibodies to cultured myeloid cell lines. Sera from neutropenic patients were studied for evidence of antibody binding to the primitive myeloid cell lines KG1a and K562. The KG1 cell line was originally derived from a child with myelomonocytic leukemia, and the KG1a cell line arose from it as a spontaneous tissue culture variant that retains features of myeloid blast cells.20-32 This cell line expresses the hematopoietic cell molecule CD34, as well as the early
myeloid marker CD33. The K562 cell line was derived from a patient with chronic myelogenous leukemia in blast crisis, and retains features of both myeloid and primitive erythroid cells. It may also be differentiated into cells with some megakaryocytic characteristics in maintenance culture, and does not express membrane proteins present on mature neutrophils.23 This cell line retains promyelocytic characteristics in maintenance culture, and does not express membrane proteins present on mature neutrophils.23

Twenty-eight percent (42/148) of sera from neutropenic patients contained IgG antibodies that bound specifically to K562 cells, and 15% (22/148) of sera contained IgG antibodies that bound to KG1a cells. Further studies were also performed using sera from nonneutrophic patients with various autoimmune disorders including RA and SLE. Six percent (5/81) of these sera had enhanced IgG binding to K562 cells and 4% (3/81) had enhanced IgG binding to KG1a cells. Some of these patients (2/5 and 1/3) also had apparent antibodies to neutrophils.

There did not appear to be any consistent pattern of IgG binding from patients' sera compared with IgG binding from normal control sera for each cell type studied.

Clinical characteristics of patients with antibodies to neutrophils and/or to myeloid cell lines. Sera with evidence for enhanced IgG binding to neutrophils, or to one of the myeloid cell lines, had been collected from patients with a variety of clinical diagnoses (see Methods). This heterogeneous group of patients all had neutropenia, without another reasonable explanation for its presence, such as cytotoxic chemotherapy or malignancy involving the bone marrow. The diagnostic categories of neutropenic patients with IgG antibodies reactive with mature blood neutrophils and/or with myeloid cell lines are presented in Table 2. Sera from patients who carried hematologic diagnoses, such as RA and SLE, appeared to be more likely to have antibodies to one or more of the cell types than were sera from patients in other diagnostic categories.

Some of these patients with enhanced antibody binding to the KG1a cell line did not have a more profound neutropenia than did the patients who had antibodies to mature blood neutrophils (data not shown). However, when binding to the K562 cell line was considered, there was a statistically significant trend for patients to have more severe neutropenia in the presence of antibodies to K562 cells, without antineutrophil antibodies, as shown in Table 3. The clinical characteristics of the 17 patients with antibodies to K562 cells, in the absence of antibodies to mature blood neutrophils, are shown in Table 4. There did not appear to be a predominance of patients from any single diagnostic category in this group, which contains patients with mild and moderate, as well as severe neutropenia.

Bone marrow biopsies from study patients were examined when available. Some patients had evidence for decreases in bone marrow cellularity. However, the majority of these patients had adequate cellularity in the bone marrow, with
Table 3. Correlation of Degree of Neutropenia With Antibody Specificity for Patients With Autoantibodies Reactive With Mature Blood Neutrophils and/or With the K562 Cell Line

<table>
<thead>
<tr>
<th>Antibody Reactivity</th>
<th>Mild/Moderate Neutropenia (ANC = 500-1,500)*</th>
<th>Severe Neutropenia (ANC &lt;500)*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil-positive/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K562-negative</td>
<td>11</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>Neutrophil-positive/</td>
<td>10</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>K562-positive</td>
<td>4</td>
<td>8†</td>
<td>12</td>
</tr>
<tr>
<td>Neutrophil-negative/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K562-positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>24†</td>
<td>49</td>
</tr>
</tbody>
</table>

* ANC, absolute neutrophil count at the time of study × 10⁹ L⁻¹.
† Statistically significant by χ² analysis, χ² = 2.010, df = 2.

Evidence of decreased mature myeloid cells. Hypoplasia of myeloid precursor cells in the marrow was documented in bone marrow biopsies from three of 21 patients who had IgG antibodies to myeloid cell lines.

Blocking studies with monoclonal antibodies. In an attempt to identify the antigenic target molecules present on primitive myeloid cells that were bound by IgG antibodies from patients’ sera, blocking studies were performed using the MoAbs My-9 (anti-CD33) and My-10 (anti-CD34). Cultured KG1a cells were found to be positive for these primitive myeloid antigens by immunofluorescence flow cytometry, as previously described. Because the K562 cells did not express CD34, the KG1a cells were studied with 22 sera that had been shown to be positive for anti-KG1a antibodies. There was no evidence for antibodies to the My-9 or My-10 antigenic epitopes, present on the CD33 or CD34 molecules, in any of these sera using the blocking assay (data not shown).

Immunoblot studies. Further studies were undertaken to evaluate the specificity of immunoglobulin binding to myeloid precursor cell antigens, using an immunoblot technique with cell lysates. Again KG1a cells were chosen, because they express both CD33 (molecular weight 40 kD) and CD34 (molecular weight 114 kD) antigens. Sera from patients with neutropenia and IgG antibodies reactive with KG1a cells were studied, and IgG from four of these sera identified a protein band of approximately 55 kD. Two of these four sera reacted only weakly with this protein, and two produced a more prominent band, as illustrated in Fig 1. The four sera did not bind to any similar protein band in immunoblot studies using lysates from mature neutrophils. Immunoglobulin binding to KG1a cell proteins of various other molecular

Table 4. Clinical Characteristics of 17 Patients With IgG Antibodies to K562 Cells in the Absence of Antibodies to Mature Neutrophils

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yrs)/Sex</th>
<th>Diagnosis*</th>
<th>WBC Count (per μL)</th>
<th>ANC (x10⁹/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11/F</td>
<td>AHA</td>
<td>—†</td>
<td>—†</td>
</tr>
<tr>
<td>2</td>
<td>1/F</td>
<td>Childhood</td>
<td>11.2</td>
<td>336</td>
</tr>
<tr>
<td>3</td>
<td>1/M</td>
<td>Childhood</td>
<td>5.0</td>
<td>325</td>
</tr>
<tr>
<td>4</td>
<td>44/M</td>
<td>Chronic</td>
<td>1.3</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>39/F</td>
<td>Chronic</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>36/M</td>
<td>Chronic</td>
<td>3.5</td>
<td>1,500</td>
</tr>
<tr>
<td>7</td>
<td>36/M</td>
<td>Chronic</td>
<td>1.1</td>
<td>88</td>
</tr>
<tr>
<td>8</td>
<td>—/F</td>
<td>Chronic</td>
<td>—†</td>
<td>—†</td>
</tr>
<tr>
<td>9</td>
<td>52/M</td>
<td>Chronic</td>
<td>0.9</td>
<td>200</td>
</tr>
<tr>
<td>10</td>
<td>43/F</td>
<td>Drug</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>27/F</td>
<td>Drug</td>
<td>—†</td>
<td>—†</td>
</tr>
<tr>
<td>12</td>
<td>39/M</td>
<td>HIV</td>
<td>2.6</td>
<td>—†</td>
</tr>
<tr>
<td>13</td>
<td>40/F</td>
<td>ITP</td>
<td>1.7</td>
<td>850</td>
</tr>
<tr>
<td>14</td>
<td>39/F</td>
<td>LDGL</td>
<td>2.2</td>
<td>164</td>
</tr>
<tr>
<td>15</td>
<td>—/F</td>
<td>Other</td>
<td>4.0</td>
<td>—†</td>
</tr>
<tr>
<td>16</td>
<td>—/M</td>
<td>RA</td>
<td>3.3</td>
<td>693</td>
</tr>
<tr>
<td>17</td>
<td>16/F</td>
<td>SLE</td>
<td>2.8</td>
<td>1,276</td>
</tr>
</tbody>
</table>

* Diagnostic categories include autoimmune hemolytic anemia (AHA), idiopathic neutropenia of childhood, adult chronic neutropenia, drug-related neutropenia, human immunodeficiency virus disease (HIV), lymphoproliferative disorder of granular lymphocytes (LDGL), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and ITP.
† Data not available. All patients had neutropenia (ANC ≤1,500). However, precise neutrophil counts, at the time of serum collection, were not available for some patients.

Fig 1. Immunoblot detection of IgG binding to KG1a cell proteins. Lane A: Immunoblot of serum from a healthy, normal volunteer. Lanes B and C: Immunoblots of sera from two patients with neutropenia and IgG antibodies to KG1a cells, detected by immunofluorescence, illustrate IgG binding to a prominent KG1a cell protein of approximately 55 kD. Enhanced IgG binding to KG1a cell proteins of various other molecular weights is also visible.
weights was also detected (Fig 1), although binding to proteins of 40 kD or 114 kD was not detected.

Studies of autoantibody binding to human bone marrow mononuclear cells. To confirm the presence of antibodies to primitive myeloid antigens, we evaluated binding of IgG from patients’ sera to highly purified CD34+ cells that had been isolated from normal human bone marrow by an immunofluorescence purification technique (see Methods). The presence of cell-surface CD34 was confirmed using the anti-CD34 MoAb My-10. Twelve of 25 sera, which contained antibodies to the primitive myeloid cell lines KG1a or K562, were also positive for IgG antibodies to these human bone marrow progenitor cells using the immunofluorescence assay.

Inhibition of clonal growth of human bone marrow myeloid progenitor cells in culture. Normal CD34+ marrow cells are known to be highly enriched for clonogenic hematopoietic progenitors. Therefore, we examined the possibility that antibodies reactive with these cells might interfere with their clonal proliferation in vitro. CD34+ marrow cells were isolated from the marrow of healthy normal donors, and growth of myeloid progenitors (CFU-GM) was measured in methylcellulose cultures.

Sera from 15 patients, from whom adequate quantities of serum were available, were studied in the clonal assay system. Cultures incubated with normal control sera had approximately 46 CFU-GM colonies per 107 highly purified CD34+ marrow cells plated (Fig 2, closed circles). This high clonogenic efficiency is similar to that obtained in the absence of human serum in this culture system. Sera from two patients with neutropenia without antibodies to myeloid cell lines did not suppress CFU-GM growth (not shown). Sera from eight patients with neutropenia and enhanced antibody binding to K562, KG1a, or purified CD34+ marrow cells, detected by immunofluorescence, also failed to suppress CFU-GM (data from five of these sera are shown in Fig 2). Partial inhibition of CFU-GM growth was observed with sera from two neutropenic patients who had IgG antibodies to K562 cells, but not to CD34+ marrow cells (Fig 2, patients A and B, open circles), as well as with serum from a neutropenic patient who had IgG antibodies to mature neutrophils only (Fig 2, patient C). Moreover, sera from two patients with strongly positive antibody binding to CD34+ marrow progenitor cells suppressed the clonal growth of these cells in culture, as shown in Fig 2, patients D and E. These findings were confirmed with the purified IgG from one of these patients.

DISCUSSION

We studied the sera from 148 patients with neutropenia of suspected immune etiology for antibodies to myeloid precursor cells, and in a large proportion of these sera we demonstrated the presence of IgG antibodies to antigens present on cultured myeloid cell lines and on highly purified bone marrow progenitor cells. The prevalence of antibodies to myeloid precursor cells in a significant proportion of patients with suspected autoimmune neutropenia has not been widely appreciated. Although some antigens may be shared by mature blood neutrophils and the myeloid cell lines that were studied, many of the sera appeared to contain IgG antibodies directed against primitive myeloid antigens that are not present on normal neutrophils. It is likely that many of these sera contain multiple autoantibodies, some reactive with antigens on mature neutrophils, and others reactive with antigens on bone marrow myeloid precursor cells.

It is also possible that some of the antibody reactivities that we found represent alloantibodies, rather than autoantibodies. Alloantibodies are generally found in patients after multiple transfusions, and in some women after pregnancy. HLA antibodies are expressed on neutrophils, although the antigenic density has been reported to be variable and lower than that of lymphocytes. KG1a cells express the HLA A30, A31, B35, and CW4 antigens, but HLA antigens have not been detected on K562 cells. The majority of the CD34+ bone marrow mononuclear cells do express HLA antigens. However, in our studies, those sera with positive IgG binding as detected by immunofluorescence flow cytometry have remained consistently positive between neutrophil and bone marrow donors. Preincubation of patients’ sera with pooled platelets has been used by neutrophil serotyping laboratories as a method to eliminate anti–HLA reactivity, although we did not use this procedure in our.
studies. Certain antibody binding reactivities reported here may be due to alloantibodies directed against HLA antigens.

The presence of antibodies reactive with myeloid cell lines may define a subgroup of immune neutropenia patients with distinct clinical features. Currie et al. studied antibody binding to HL60 cells, a cell line that in maintenance culture has the phenotype of immature myeloid progenitor cells. They found that neutropenic patients with IgG antibodies that bound to HL60 cells, in the absence of binding to mature neutrophils, generally had selective myeloid hypoplasia of the bone marrow, defined as a decreased percentage of myeloid lineage cells found on bone marrow specimens from these patients. These findings, as well as those of others, suggest that patients with antibodies that bind to primitive myeloid maturational antigens may have a decrease in myeloid elements in the bone marrow, when these marrows are histologically evaluated. In studies presented here, the presence of IgG antibodies to the myeloid cell lines K562 and KG1a did not appear to be clearly associated with myeloid hypoplasia. Nonetheless, in selected cases, there was an associated hypoplasia of marrow myeloid elements.

The use of cell lines may serve as a basis for developing studies designed to identify specific, immature myeloid antigens that may be involved in the pathogenesis of myelosuppression in these patients. Although varying patterns of cell line reactivity have been described, little is currently known about the identity of the myeloid antigens that are recognized by autoantibodies detected in patients’ sera. The 55-kD protein identified in our studies may be the same as a 54-kD antigen, with an isoelectric point of 6.2 on isoelectric focusing gels, that has been previously identified on immunoblots of HL60 cell membranes. The identity of this protein remains to be determined.

Antibody inhibition of hematopoietic cell production may be as important as increased peripheral blood cell destruction in the pathogenesis of autoimmune neutropenia, and inhibition of the clonal growth of myeloid progenitor cells in vitro has been studied by previous investigators. Fitchen and Cline found immunoglobulin inhibitors of CFU in culture of 19 of 104 patients with various disorders, including three patients with acquired neutropenia. Antibodies from the sera of rare patients with pure white blood cell aplasia or autoimmune panleukopenia have also been found to inhibit the in vitro growth of myeloid colonies from human bone marrow. We found that the sera that showed the most significant suppression of clonal growth each had strongly positive IgG antibody reactivity with primitive CD34+ marrow mononuclear cells, and that this suppression was not complement-dependent. Partial suppression of CFU-GM was also seen in sera that did not have antibody binding to the CD34+ bone marrow mononuclear cells. Antibodies to maturational antigens present on proliferating cells in bone marrow, but not present on the most primitive progenitor cells, may have accounted for this partial suppression of clonal growth.

In summary, we find that antibodies to primitive hematopoietic cells are present in the sera of a significant proportion of patients with autoimmune neutropenia, and that these antibodies may occur in the presence or in the absence of antibodies to mature neutrophils. Myeloid cell lines can be used to detect antibodies that recognize myeloid antigens that are not present on mature neutrophils. We found that the presence of antibodies to the K562 cell line, in the absence of antibodies to mature neutrophils, correlated with the presence of more severe neutropenia. Further studies are needed to identify the antigenic targets on primitive myeloid cells that are bound by these antibodies. Several sera suppressed the growth of CFU-GM in clonal assays, raising the possibility that these antibodies may have a role in the pathogenesis of the myelosuppression observed in these neutropenic patients.

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Antibodies to myeloid precursor cells in autoimmune neutropenia

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