Granulocyte-Associated IgG in Neutropenic Disorders

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We applied a radiolabeled antiglobulin test to a study of patients with a variety of neutropenic disorders. After defining the nature of the interaction of radiolabeled anti-IgG with the neutrophil, we studied 16 patients with neutropenia of uncertain etiology and adequate bone marrow granulocyte precursors. Twelve of these 16 patients had increased neutrophil-associated IgG (PMN-IgG). Patients with the highest levels of PMN-IgG had the lowest neutrophil counts. The majority of patients with neutropenia and increased PMN-IgG had an underlying immunologic disorder that included immune thrombocytopenic purpura in 5 patients and autoimmune hemolytic anemia in 1 patient. In some patients, elevated PMN-IgG preceded other evidence for immunologic disease. The direct antiglobulin test helped to distinguish neutropenic patients with increased PMN-IgG both from patients with neutropenia due to a known nonimmune disorder and from nonneutropenic patients with rheumatoid arthritis or systemic lupus erythematosis. Each of four patients with increased neutrophil-associated IgG treated with systemic corticosteroids responded clinically with an associated fall in neutrophil IgG and a rise in the circulating neutrophil count. The radiolabeled antiglobulin test appears useful in defining a subpopulation of patients with neutropenia due to an underlying immunologic disorder.

THE PATHOGENESIS of chronic neutropenia is difficult to establish. Neutropenia may occur as an isolated finding or in association with an underlying immunologic disorder. In some patients antineutrophil antibodies are thought to contribute to the development of granulocytopenia. A variety of tests have been developed to detect antibodies in plasma directed at granulocytes in neutropenic patients with cellular bone marrows. These tests have demonstrated the presence of antineutrophil antibodies in several conditions. However, antineutrophil activity has also been detected in the plasmas of patients with underlying immune disorders who are not neutropenic. Furthermore, studies in autoimmune hemolytic anemia and immune thrombocytopenic purpura, in which antienzyme and antiplatelet antibodies are present, suggest that antineutrophil antibodies would be most easily detected on the neutrophil surface. Attempts to assess antibody on the granulocyte surface by antiglobulin consumption assays have been complicated by nonspecific interactions between the antiglobulin reagent and the neutrophile. Recently, such assays have been refined and increased granulocyte-associated IgG has been observed on the granulocytes of neutropenic patients with Felty's syndrome and systemic lupus erythematosis (SLE). In addition, increased binding of staphylococcal protein A to the granulocytes of some patients with suspected immune neutropenia has been noted. However, a systematic, quantitative study of the nature of the interaction of IgG with the granulocyte surface both in normals and in patients with unexplained neutropenia has not been reported.

In the present study, we employed a radiolabeled antiglobulin test to measure granulocyte-associated IgG (PMN-IgG) in patients with a variety of neutropenic disorders. In doing so, we analyzed in detail the interaction of the radiolabeled antiglobulin reagent with the granulocyte surface. We observed that many patients with unexplained neutropenia have increased PMN-IgG and that the level of PMN-IgG correlates with the patients' clinical course. Furthermore, we found that corticosteroids can be efficacious in raising granulocyte counts and in helping to resolve infections in selected neutropenic patients with increased PMN-IgG.

MATERIALS AND METHODS

Rabbit antiserum monospecific for human IgG was prepared, and the IgG fraction isolated and radiolabeled as previously described. Rabbit anti-IgG was prepared by pepsin digestion of this IgG. Normal rabbit IgG and normal human IgG were isolated by affinity chromatography (Miles Laboratories, Inc., Elkhart, Ind.) and similarly radiolabeled, as was human serum albumin (Sigma Chemical Corp., St. Louis, Mo.).

Granulocytes

Granulocytes were isolated by a modification of established methods. Whole blood in heparin (10 U/ml) was diluted 2:1 with 6% Dextran-70 in normal saline (Cutler Laboratories, Inc., Berkeley, Calif.). From 20 ml to 150 ml of blood was obtained from each patient, depending on the level of neutropenia. The erythrocytes were sedimented by gravity for 45 min at room temperature, and 20 ml of the leukocyte-enriched supernatant was layered onto 10 ml of Hypaque-Ficoll (Biometric Laboratory Products, Kensington, Md.).
and centrifuged at room temperature for 20 min at 400 g. Erythrocytes in the granulocyte pellet were lysed by a brief exposure to hypotonic saline. The granulocytes were harvested, suspended in modified Tyrodes-albumin-EDTA (10 mM) buffer,30 washed twice, and resuspended to 5 x 10⁶ leukocytes/ml in this buffer. The yield of granulocytes from whole blood for all experiments was 68% ± 6% (± 2 SD), with <1% platelet and erythrocyte contamination. Wrights-Giemsa staining revealed that granulocyte preparations from normals contained 95% ± 6% polymorphonuclear leukocytes, 3% ± 6% eosinophiles, and 2% ± 3% lymphocytes and monocytes. Granulocyte preparations from neutropenic patients and neutropenic controls contained 92% ± 10% polymorphonuclear leukocytes, 5% ± 6% eosinophiles, and 3% ± 7% lymphocytes and monocytes. Simultaneous mononuclear cell controls were prepared from most patients using defibrinated or heparinized whole blood, which was sedimented on a Hypaque-Ficoll density gradient. Mononuclear cell preparations (containing <1% erythrocytes and granulocytes) were harvested, washed twice, and resuspended to 5 x 10⁶ leukocytes/ml with Tyrodes-EDTA buffer.

**Granulocyte-Radiolabeled Antiglobulin Test**

This assay is a modification of that previously reported for platelets.30 In the direct antiglobulin test, 1 ml of 5 x 10⁶ granulocytes in Tyrodes-EDTA buffer was incubated with a constant aliquot of 125I-lgG anti-IgG for 45 min at 37°C in a shaking water bath. In the indirect antiglobulin test, 1 ml of 5 x 10⁶ normal granulocytes in Tyrodes-EDTA buffer was first incubated with 1 ml of patient plasma for 45 min at 37°C; the granulocytes were washed 3 times with 5 ml of Tyrodes-EDTA buffer, sedimented at 160 g, and resuspended to 1 ml in Tyrodes-EDTA buffer. The sensitized granulocytes were then incubated with 125I-lgG anti-lgG as in the direct test. In certain experiments, 125I-F(ab';) anti-lgG was substituted for 125I-lgG. In both the direct and indirect tests, after incubation with 125I-lgG, granulocytes or similarly studied control mononuclear cells were washed 4 times with 5 ml of Tyrodes-EDTA buffer, sedimented at 160 g, resuspended to 1 ml, and the leukocyte-associated radioactivity determined in a gamma scintillation counter. Loss of leukocytes due to washing was <5%.

**Expression of Granulocyte-Associated Radioactivity**

The radiolabeled antiglobulin test provides a quantitative measure of the amount of anti-lgG bound to leukocytes. This can be directly expressed as percent radioactivity bound per 5 x 10⁶ cells. In addition, we used two methods to estimate the amount of lgG per granulocyte detected with the radiolabeled antigen lgG reagent. Radioactivity bound to granulocytes was compared to the radioactivity bound to an equal number of erythrocytes coated with a known amount of lgG antibody, as previously described.30 Granulocyte-associated lgG (PMN-IgG) was expressed as antiglobulin combining sites. One antiglobulin combining site per granulocyte adsorbs an amount of 125I-anti-lgG equivalent to that adsorbed by 1 molecule of lgG antibody per erythrocyte. A second method was also employed to quantitate PMN-IgG based on a modification of the method of Ohanian and Borsos.31,32 Quantitation of PMN-IgG was determined by the extent of inhibition by PMN of the binding of 125I-anti-lgG to antibody-coated erythrocytes. One milliliter of 125I-anti-lgG was incubated with 0.1 ml of granulocytes (5 x 10⁷/ml) or purified human lgG (2-2000 ng in solution) for 45 min at 37°C, and each mixture was sedimented at 1500 g for 10 min. The supernatant fluid containing unbound 125I-anti-lgG was removed and incubated with 10⁶ unsensitized erythrocytes (E) or erythrocytes sensitized with lgG anti-D (EA) in Tyrodes-albumin-EDTA buffer for 45 min at 37°C. These erythrocytes were then washed 4 times, and the binding of residual 125I-anti-lgG to EA as compared to E determined. A standard curve was constructed relating the amount of soluble lgG to inhibition of 125I-anti-lgG binding to EA and E. A biphasic inhibition curve was obtained (Fig. 1) with an initial slope approaching 1.0. The amount of PMN-IgG was determined by comparing soluble IgG and granulocytes in their ability to inhibit 125I-anti-lgG binding to EA and E. The amount of IgG associated with granulocytes was similar, using as a standard curve either erythrocytes coated with a known amount of IgG30 or the inhibition curve using IgG in solution. Mononuclear cell controls from normals and patients were studied. In no case did the binding of 125I-anti-lgG to mononuclear cells contribute significantly to total leukocyte-bound radioactivity.

**Specificity of Anti-lgG Binding**

The interaction of 125I-lgG anti-lgG with the granulocyte may be influenced by a number of factors other than the presence of lgG on the granulocyte surface. These include: (1) the presence of radiolabeled protein in trapped buffer sedimenting with the granulocytes, (2) nonspecific binding of 125I-anti-lgG, due to alteration of the lgG molecule during radiolabeling,(3) binding of 125I-anti-lgG to the granulocyte Fc receptor, and (4) endocytosis of 125I-anti-lgG by

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**Fig. 1. Standard curve for PMN-IgG.** PMN-IgG was determined by the percentage inhibition by PMN of increasing concentrations of soluble human lgG of the binding of 125I-anti-lgG to antibody-coated erythrocytes. Here the percent inhibition of binding by soluble human lgG is shown.
granulocytes. A series of experiments were performed to address these possibilities.

The distribution of radiolabeled protein in trapped buffer was determined by assessing the interaction of $^{14}$C-inulin or $^{125}$I-albumin with granulocytes. Trapped buffer refers to radioactivity associated with granulocytes. A series of experiments were performed to address these possibilities. Trapped buffer refers to radioactivity associated with the test-tube–PMN mixture. A quantity of granulocytes (5 x $10^9$) in 0.3 ml of Tyrodes-EDTA buffer were preincubated with 0.1 ml of a 1:5 mixture of Hi-phenyl silicone oil (DC-550) (William Nyc, Inc., New Bedford, Mass.) and methyl silicone oil (DC-200) in a 1.5 ml conical tube. Cell-associated radioactivity was assessed following sedimentation of the granulocytes at 15,600 g for 2 min (Eppendorf centrifuge, model 5412, Brinkman Instruments, Westbury, NY). The tip of the centrifuge tube containing the cell pellet was sliced and placed in a gamma scintillation counter for determination of $^{125}$I radioactivity. To determine $^{14}$C activity, the tip was incubated overnight in a liquid scintillation vial with 0.5 ml of Protosol (New England Nuclear, Boston, Mass.). The ($^{14}$C) radioactivity of this dissolved cell pellet was then determined in a liquid scintillation counter. Incubation of granulocytes with $^{125}$I-albumin or $^{14}$C-inulin (radioactivity equal to that of the $^{125}$I-anti-IgG) resulted in 16% ± 7% of the radioactivity becoming associated with granulocytes. Qualitatively similar results were obtained in studies where leukocytes were washed in Tyrodes-EDTA buffer or isolated through silicone oil. In the studies assessing specific binding of $^{125}$I-anti-IgG, correction was made for percent radioiodel in the trapped space.

The specificity of binding of $^{125}$I-anti-IgG to the granulocyte surface was first examined by comparing the ability of unlabelled anti-IgG and other plasma proteins to inhibit the subsequent binding of radiolabeled anti-IgG to granulocytes. Granulocytes (5 x $10^9$) in 0.3 ml of Tyrodes-EDTA buffer were preincubated with 0.1 ml of increasing concentrations of unlabeled human albumin, normal rabbit IgG, or rabbit IgG anti-IgG for 45 min at 37°C. One-tenth milliliter of $^{125}$I-anti-IgG was then added for 45 min at 37°C. The mixture was layered over the combination of silicone oils, and the cell-associated radioactivity determined as described earlier. The percent inhibition of $^{125}$I-anti-IgG binding to each cell was determined using the formula:

$$\text{% cpm of cells incubated with } ^{125}\text{I-anti-IgG} - \text{cpm of cells preincubated with buffer} \times 100$$

$$\text{cpm of cells preincubated with buffer}$$

Qualitatively similar results were obtained with cells washed in Tyrodes-EDTA buffer and with cells isolated through silicone oil. A 100-fold excess of unlabeled IgG to $^{125}$I-IgG inhibited the net binding of nonantibody $^{125}$I-IgG to a level accounted for by trapped buffer. Therefore, specific binding was defined in inhibition studies as that binding of $^{125}$I-anti-IgG inhibitable by a 100-fold excess of unlabeled anti-IgG, but not by an equal amount of nonantibody IgG. In all studies, granulocytes were compared to control erythrocytes and antibody-coated erythrocytes. In the studies employing erythrocytes sensitized with IgG anti-D, 85% ± 3% of the binding of $^{125}$I-anti-IgG was specific for IgG.

The specificity of $^{125}$I-anti-IgG binding was also assessed by comparing its binding to granulocytes with the binding of $^{125}$I-IgG (rabbit) not having anti-IgG activity. In these experiments, equal amounts of IgG anti-IgG and IgG not having anti-IgG activity were employed. These preparations had comparable specific activity. Bound radioactivity was determined both by washing in Tyrodes-EDTA buffer as well as by centrifugation through silicone oil as described above with similar results. Percent specific binding was defined as the difference in binding to the granulocyte surface between $^{125}$I-IgG anti-IgG and $^{125}$I-IgG not having anti-IgG activity using the formula:

$$\text{% cpm of cells incubated with } ^{125}\text{I-anti-IgG} - \text{cpm of cells incubated with } ^{125}\text{I-IgG} - \text{cpm of cells incubated with } ^{125}\text{I-anti-IgG} \times 100$$

$^{125}$I-anti-IgG could bind to granulocyte IgG and undergo endocytosis with subsequent release from the granulocyte. To examine this possibility, the effect of inhibitors of endocytosis was studied. Granulocytes were preincubated for 45 min at 37°C with either cycloheximide (0.5 μg/ml), 2-deoxyglucose (0.02 M), antimycin A (2 μg/ml), or cytochalasin B (10 μg/ml), concentrations previously demonstrated to inhibit endocytosis. Following this preincubation, granulocytes were washed twice, resuspended to 1 ml, and the direct antiglobulin test performed as above.

**Plasma Antineutrophil Activity**

The binding of heat-aggregated IgG to normal PMN was compared with the binding to Raji cells as previously described. The addition of 30–50 μg/ml of heat-aggregated normal human IgG to normal autologous sera increased the binding of $^{125}$I-anti-IgG to autologous granulocytes. To assay for complement-fixing anti-PMN activity, normal sera, or sera from patient S.G. (diluted 1:4 with phosphate-buffered saline containing 0.15 M NaCl and 0.5 mM Mg++) was incubated with 5 x 10^6 PMN at room temperature for 30 min. Lysis of PMN was determined by light microscopy. Normal plasma did not cause visual lysis of PMN. Following this interaction, the sera were assayed for residual C4 hemolytic activity by effective molecule titration.

**Patients**

Sixteen consecutive patients with neutropenia (<1800 PMN/cu mm) of uncertain etiology were studied (Table 1). Two additional patients with severe neutropenia could not be studied because of the inability to obtain a PMN population of greater than 85% purity. All patients had cellular bone marrow biopsies with a relative increase in the number of granulocyte precursors. Except for one patient (AS) who had non-Hodgkin's lymphoma, there was no evidence of any disorder that might limit or alter granulocyte production. Three patients had palpable spleens (A.S., S.G., J.P.), but had marked neutropenia in the absence of, or out of proportion to, thrombocytopenia or anemia. Ten patients had neutropenia in association with an underlying immunologic disorder (patients 1–10). Two patients had active systemic lupus erythematosus (K.M., F.S.) and 5 patients had a clinical diagnosis of ITP with increased levels of platelet-associated IgG. Of these 5, 2 developed neutropenia in association with a viral syndrome and HAA-negative hepatitis ML, RM). The granulocyte count of these two patients returned to normal following resolution of the infection. Three patients developed positive serum latex fixation titers 1–6 for development of severe neutropenia (S.G., G.D., P.H.); one of these (P.H.) developed frank arthritis 8 yr after presentation with neutropenia. Six patients presented with isolated neutropenia (patients 11–16). A history of recurrent infection in these 16 patients was variable. Five patients have had aphthous ulcers or pharyngitis, and 5 have had recurrent urinary tract infections. Only one patient developed bacterial sepsis (PH) and one developed a pyogenic lung abscess (S.G.). The case reports of two patients are summarized below.

Patient S.G. is a 55-yr-old white male who presented with a 1-yr history of neutropenia, malaise, and oral ulcerations. Four years earlier, several subcutaneous nodules compatible with rheumatoid nodules were removed from his elbows. The patient had no history of arthritis and was taking no medications. Physical examination was unremarkable except for several deep oral ulcers and a palpable spleen tip. The absolute neutrophil count was 60 PMN/cu mm. The
LE-cell preparation was positive on one occasion, latex fixation titer was 1:20, and the antinuclear antibody (ANA) was 1:640. The latex fixation titer subsequently rose to 1:5120 in the absence of clinical arthritis and with serial negative LE-cell preparations. The patient's bone marrow was hypercellular with a M:E ratio of 4:1, but with a relative decrease in bands and polys. The hemoglobin and platelet counts were normal. Other laboratory and x-ray studies revealed no abnormalities. Peripheral blood smear was unremarkable except for the absence of neutrophiles. Over the next several weeks he developed worsening oral and cutaneous ulcers with cellulitis unresponsive to antibiotics. PMN-IgG was elevated, and he was then begun on prednisone, 80 mg a day.

Patient GD. is a 47-yr-old white male who presented with a 4-mo history of progressive fatigue and malaise that caused him to cease working. The patient had a normal physical examination. Hemoglobin and platelet counts were normal. The absolute granulocyte count was 180 PMN/cu mm. Peripheral blood smear was normal except for decreased neutrophiles. His bone marrow biopsy was hypercellular with a M:E ratio of 4:1, but with a relative decrease in bands and polys. Laboratory and x-ray studies were normal, including rheumatoid factor and ANA. The latex fixation test subsequently became positive with a titer of 1:160-1:1280 by day 140 of his course. Because of profound malaise in the presence of increased PMN-IgG, he was begun on prednisone, 80 mg a day.

Other patients studied included 12 patients with active systemic lupus and 10 patients with seropositive rheumatoid arthritis who were not neutropenic. Other neutropenic patients studied included 8 patients with Felty's syndrome (neutropenia with rheumatoid arthritis), 2 patients with hypersplenism, 4 patients with neutropenia secondary to marrow infiltration with tumor, and 4 patients neutropenic secondary to myelosuppressive chemotherapy. Most patients were also studied when there was no clinical evidence for infection. In addition, in most patients studied, the level of PMN-IgG was determined on more than one occasion so as to document that a change in the level of PMN-IgG in a given patient did not represent methodological variation.

**Association of ^125I-Anti-IgG With Normal Granulocytes**

**Total Binding**

Using the direct radiolabeled antiglobulin test, the granulocytes from 43 normal volunteers bound an amount of ^125I-anti-IgG equivalent to 3–23 x 10^5 total anti-IgG binding sites per cell. This binding represented approximately 0.5% of the total radioactivity incubated with the granulocytes. Mononuclear cell controls prepared from defibrinated blood bound a similar amount of ^125I-anti-IgG as did normal granulocytes and, therefore, did not contribute significantly to total leukocyte-associated radioactivity. Using the same donor, there was little variation in the total ^125I-anti-IgG binding sites on sequential determina-
tions: 8300 ± 2500 anti-IgG sites/cell (± 2 SD; n = 17).

Specific Binding

We employed two experimental designs to assess the specific binding of \( ^{125}\)I-anti-IgG to normal granulocytes (Table 2). First, we compared the capacity of unlabeled anti-IgG and IgG to inhibit the subsequent binding of \( ^{125}\)I-anti-IgG. Preincubation of an 100-fold excess of unlabeled anti-IgG or IgG inhibited the binding of \( ^{125}\)I-anti-IgG 85% and 22%, respectively. Thus, 63% of the binding of \( ^{125}\)I-anti-IgG was specifically inhibited by unlabeled anti-IgG. Second, when the binding of \( ^{125}\)I-anti-IgG and \( ^{125}\)I-IgG to normal granulocytes was compared, 0.41% and 0.14% of the radioactivity became associated with the granulocytes, respectively. Thus, 66% of the \( ^{125}\)I-anti-IgG binding was specific for IgG, a result similar to that observed with the studies of the inhibition of \( ^{125}\)I-anti-IgG binding.

The association of rabbit IgG protein with granulocytes accounts for approximately 20% of the binding of \( ^{125}\)I-anti-IgG (Table 2). This most likely represents an association with the granulocyte IgG (Fc) receptor, as normal granulocytes bound 10%–20% fewer molecules of \( ^{125}\)I-(Fab') anti-IgG than native \( ^{125}\)I-anti-IgG. On the other hand, the endocytosis inhibitors cycloheximide, 2-deoxyglucose, antimycin A, and cytochalasin B did not alter the binding of \( ^{125}\)I-anti-IgG to granulocytes. These studies do not exclude internalization of \( ^{125}\)I-anti-IgG, but suggest that significant internalization and release from the granulocyte does not occur under these conditions. Taken together these studies indicate that approximately two-thirds of the radiolabeled anti-IgG associated with normal granulocytes is specific for granulocyte-associated IgG. The remainder appears to be due primarily to the Fc receptor and to trapped plasma.

Table 2. Determination of Specific \( ^{125}\)I-Anti-IgG Binding to Granulocytes

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<th>Percent inhibition of ( ^{125})I-anti-IgG binding</th>
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<td>Preincubation with anti-IgG</td>
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*Results represent the mean ± SEM of three experiments. 100-fold excess cold anti-IgG or IgG was employed.
†Results represent mean ± SEM percent granulocyte-associated radioactivity using granulocytes from 25 normal controls.
‡Specific binding to granulocytes determined by comparing binding of \( ^{125}\)I-anti-IgG and \( ^{125}\)I-IgG as illustrated in Materials and Methods.

Association of \( ^{125}\)I-anti-IgG With Patient Granulocytes

Total Binding

The granulocytes from 10 patients with neutropenia secondary to hypersplenism, bone marrow infiltration from tumor, or bone marrow hypoplasia secondary to chemotherapy had normal levels of PMN-IgG (4–17 × 10³ total anti-IgG sites/cell). In contrast, all 8 patients with Felty's syndrome studied had increased PMN-IgG (Fig. 2). An inverse relationship between PMN-IgG and the absolute neutrophil count was observed (Fig. 3). Patients with Felty's syndrome (4/4) with a moderately increased level of PMN-IgG (≤ 40,000 sites/cell) were found to have mild–moderate neutropenia (≤ 800 PMN/cu mm). In contrast, marked elevations of PMN-IgG (4/4 patients with Felty's syndrome) were associated with more severe neutropenia (≤ 800 PMN/cu mm). Granulocytes from 5 of 22 patients with systemic lupus or seropositive rheumatoid arthritis who were not neutropenic also had increased PMN-IgG, although generally to a lesser extent (Fig. 2).
Twelve of the 16 patients studied with neutropenia of uncertain etiology had increased PMN-IgG. Of these 12 patients, 10 had evidence of an associated immunologic disease (Table 1). Similar to the results observed in the patients with Felty's syndrome, there was an association between PMN-IgG and the absolute neutrophil count in those 12 patients with neutropenia of uncertain etiology who had increased PMN-IgG (Fig. 3). Patients (6/6) with moderate elevations of PMN-IgG (≤ 40,000 sites/cell) had moderate neutropenia (≥ 800 PMN/cu mm), while patients (5/6) with larger amounts of PMN-IgG were more severely neutropenic.

Five patients with neutropenia and increased PMN-IgG had increased platelet-associated IgG as well and a clinical diagnosis of immune thrombocytopenic purpura (ITP). One of these patients (L.A.) had concomitant autoimmune hemolytic anemia, immune thrombocytopenic purpura, and neutropenia associated with increased PMN-IgG in the absence of any evidence for systemic lupus.

Specific Binding

It is difficult to obtain sufficient granulocytes from granulocytopenic patients to perform all the studies related to specific anti-IgG binding that were performed with normal granulocytes. The results cited earlier demonstrate that the determination of specific binding of 125I-anti-IgG to normal granulocytes is similar using either comparison of the binding of 125I-anti-IgG and 125I-IgG or studies of the inhibition of 125I-anti-IgG binding with unlabeled anti-IgG.

Therefore, we assessed the specific binding of 125I-anti-IgG to patient granulocytes by comparing the binding of 125I-IgG (no anti-IgG activity) with that of 125I-anti-IgG. When the binding of these two reagents to the granulocytes of patients with increased PMN-IgG (n = 15) was compared, 79% (range 60%–85%) of the 125I-anti-IgG binding was specific for PMN-IgG.

Effect of Corticosteroid Therapy

Figure 4 describes the effect of corticosteroid therapy in patient S.G. At the time of presentation the patient was infected, granulocytopenic, and had increased PMN-IgG. Incubation of S.G. serum with normal PMN caused lysis of 80% of the cells and depletion of 96% of the hemolytically available C4 from the serum (normal less than 5%). The patient was begun on corticosteroid therapy with prednisone (80 mg q.d.) with a fall in the level of PMN-IgG. Several days after the initiation of steroid therapy, his granulocyte count began to rise and his infection subsequently resolved. The patient was then begun on alternate-day prednisone (60 mg q.o.d.). Over the next several weeks, the granulocyte count fell, associated with a rise in the level of PMN-IgG. The patient developed recurrent oral ulcers and a pulmo-
nary infection (anaerobic lung abscess, day 244). He was then treated with reinstitution of corticosteroids and antibiotics (not shown). His PMN-IgG again decreased, his granulocyte count again rose, and his infections again resolved.

Patient G.D. (Fig. 5) is presented to illustrate a therapeutic response to alternate-day corticosteroids. He initially had increased PMN-IgG, severe granulocytopenia, and profound malaise. He was begun on daily prednisone therapy (80 mg q.d.). Within 1 wk his malaise began to resolve. When studied on day 40, his PMN-IgG had decreased and his granulocyte count had become normal. Cessation of steroids (day 120) resulted in recurrent malaise, increased PMN-IgG, and granulocytopenia. He responded to reinstitution of steroids. On alternate-day prednisone (20 mg q.o.d.) he continues to feel well and has maintained a normal granulocyte count, despite a mild increase in PMN-IgG (day 310), now 15 mo after diagnosis.

DISCUSSION

We have adapted a radiolabeled antiglobulin test for the measurement of granulocyte-associated IgG in suspected immune granulocyte disorders. We observed increased levels of PMN-IgG in 12 of 16 patients with neutropenia of uncertain etiology and 8 of 8 patients with Felty’s syndrome (Fig. 2). All of these patients had cellular bone marrows with a relative increase in granulocyte precursors. In contrast, patients with neutropenia secondary to intrinsic bone marrow disease, chemotherapy-induced myelosuppression, or splenomegaly had normal levels of PMN-IgG. In two of our patients with increased PMN-IgG (A.S. and J.P.), an enlarged spleen or altered bone marrow granulocyte production may have contributed in part to the neutropenia. In all other patients, however, no other clinical explanation for the neutropenia was evident.

Ten of our patients with neutropenia of uncertain etiology had evidence for an underlying immunologic disorder, such as ITP or SLE. Five of these patients were thrombocytopenic (Table 1) with increased platelet-associated IgG, and one patient had IgG-induced thrombocytopenia and hemolytic anemia. This combination of neutropenia and ITP occurred more commonly than previously recognized. Three of our patients presented with neutropenia, increased PMN-IgG, weakness, and malaise and subsequently developed positive latex fixation titers. Two of these patients later developed arthralgias and one frank arthritis. Although presenting with neutropenia, these patients may ultimately develop classical rheumatoid arthritis and thus may represent a clinical variant of Felty’s syndrome.

All of the neutropenic patients with evidence of another immunologic disorder had increased levels of PMN-IgG. However, in several of these patients, neutropenia with increased PMN-IgG preceded other evidence of an underlying immunologic disorder. Our series of neutropenic patients, therefore, differs from the patients generally reported as having chronic idiopathic neutropenia. Such patients usually do not have associated immunologic dysfunction and generally have decreased or ineffective granulocyte production. Only 2 of 6 such patients with neutropenia in the absence of associated immunologic disease had increased PMN-IgG in our series. The other four patients with isolated neutropenia and normal PMN-IgG were only distinguishable by a history of significant ingestion of major tranquilizers (Table 1).

The finding of increased PMN-IgG in neutropenic patients with SLE and Felty’s syndrome is in agreement with previous reports using antiglobulin consumption techniques and suggests a role for increased PMN-IgG in the development of neutropenia. However, increased PMN-IgG may represent only one of several causes of neutropenia in patients with these disorders. In addition, we have observed that a few patients with seropositive rheumatoid arthritis or SLE with normal granulocyte counts had levels or PMN-IgG above our normal range (Fig. 2).
In general, the levels of PMN-IgG in these nonneutropenic patients with rheumatoid arthritis or SLE are lower than similar patients who are neutropenic (Fig. 2). Similar results were observed recently by Starkebaum and Arend.8,34 Such patients might show increased granulocyte turnover if studied.

Our study extends the evaluation of PMN-IgG to patients with unexplained neutropenia. In the absence of granulocyte kinetic studies, the relationship between PMN-IgG and granulocyte survival cannot be proven. However, the relationship of increased PMN-IgG to neutropenia in our patients is suggested by several findings. First, in patients with Felty's syndrome and those with unexplained neutropenia, an inverse relationship was found between the absolute neutrophil count and the level of PMN-IgG (Fig. 3). Second, in longitudinal studies performed on several individual patients, the level of PMN-IgG again varied inversely with the neutrophil count and paralleled the clinical course (Figs. 4 and 5). In addition, serum from one patient (S.G.) destroyed granulocytes in vitro.

Four of our patients with severe neutropenia and increased PMN-IgG were treated with corticosteroids. In all 4 patients (the course of 2 of whom is illustrated), a dramatic clinical response, a decrease in PMN-IgG, and an increase in granulocyte count were all noted. In patient S.G., the steroid-associated leukocytosis reversed advancing mucocutaneous ulcers that appeared in the presence of systemic antibiotics (Fig. 4). Similar results were obtained in patient L.A. (ulcerating pharyngitis) and in a patient with Felty's syndrome with an unresponsive and progressive leg ulcer. Although steroids may directly increase the level of circulating neutrophiles, the data portrayed suggest that a decrease in PMN-IgG or a decrease in clearance of IgG-coated PMN is primarily responsible for the steroid-induced clinical response. In each patient the response to either the institution or withdrawal of steroids occurred over a period of several days to weeks, arguing against an effect of steroids on neutrophil kinetics. In 2 of 4 patients (the course of one of whom is illustrated in Fig. 5), alternate-day corticosteroids were effective in maintaining a normal granulocyte count. The use of corticosteroids in the setting of infection and neutropenia presents obvious risks. However, our data suggest that a short course of steroids may be indicated in certain neutropenic patients with increased PMN-IgG.

Previous investigators have demonstrated IgG anti-
PMN activity in the plasmas of patients with unexplained neutropenia,1-6 Felty's syndrome, or SLE.5,8,18 However, similar plasma activity has also been found in these groups of patients in the absence of neutropenia.9-13 Although plasma IgG anti-PMN activity could be detected in most of our patients having increased PMN-IgG (i.e., a positive direct antiglobulin test), we also found an increased level of activity in the plasmas of nonneutropenic patients with SLE as well as in normal plasmas to which heat-aggregated IgG was added (not shown). Therefore, a positive direct antiglobulin test (increased PMN-IgG) may either be due to IgG antibody or IgG-containing immune complexes on the granulocyte surface. If circulating immune complexes are responsible for neutropenia and increased PMN-IgG in certain patients, this may represent a selective avidity of a component(s) of these immune complexes for a granulocyte antigen or the granulocyte Fc receptor. The turnover of IgG on the granulocyte surface is unknown. It is therefore possible that in some patients an increased amount of granulocyte-associated IgG may not be detected. In these patients, measurements of circulating IgG anti-granulocyte antibody (e.g., antiglobulin deposition or functional alteration of PMNs8,13) may be especially useful. Such assays may also be useful when small amounts of functionally potent antibody (e.g., certain complement-fixing antibodies) are present.

We performed extensive studies to examine whether the normal granulocyte carries IgG. In contrast to indirect antiglobulin consumption assays, the radio-labeled antiglobulin test can assess this question directly. Our studies indicate that normal granulocytes do carry significant amounts of IgG. However, a portion of the association of 125I-anti-IgG with the granulocyte surface represents interactions other than those due to the binding of anti-IgG to IgG, e.g., association caused by the granulocyte Fc receptor, nonspecific cell–protein interactions, and trapped plasma. Our data indicate that PMN-IgG levels reported with antiglobulin consumption assays probably represent cumulative interactions between granulocytes and anti-IgG. Such interactions may account for the variations in results observed among various laboratories and suggest that measurements of PMN-IgG represent only an estimate of the actual amount of IgG on the cell surface. The nature of this IgG is unknown and may represent IgG bound to granulocyte antigens, IgG bound to the Fc receptor, or IgG “nonspecifically” associated with the granulocyte surface. The radiolabeled antiglobulin test is a direct means to assess and monitor granulocyte-associated IgG in a variety of neutropenic disorders. It also provides a mechanism to differentiate specific from “nonspecific” cellular interactions with antiglobulin reagents.

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Granulocyte-associated IgG in neutropenic disorders

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