Chlorpropamide-Induced Pure White Cell Aplasia

By Lee J. Levitt

We investigated the mechanism for isolated agranulocytosis and marrow pure white cell aplasia in an elderly man receiving 0.5 to 1.0 g per day of chlorpropamide (Chl) without other toxic drug exposure or overt systemic illness. Patient marrow revealed an absence of recognizable granulocytic precursors; megakaryocytes and erythroid precursors were normal. The WBC count was 1800/mm³ on admission with only 2% neutrophils; the absolute neutrophil count first exceeded 500/mm³ on the 17th day following cessation of Chl. A serum Chl level on admission was 100µg/mL (acute phase, AP); no Chl was detected in serum (convalescent phase, CP) assessed on the 22nd hospital day. Antineutrophil antibodies were not detected, and T cell depletion failed to augment patient in vitro granulopoiesis. Patient AP serum produced potent complement-mediated inhibition (87% ± 7%) of autologous granulocyte progenitors (CFU-GM) with minimal inhibition of erythroid (11% ± 5%) or multipotent (5% ± 4%) progenitor cells. Selective inhibition by patient AP serum of CFU-GM (74% ± 11%) was also seen against two allogeneic marrows. Patient CP serum no longer inhibited (6% ± 4%) autologous CFU-GM. Addition of Chl (5 to 120 µg/mL) to CP serum but not to control serum resulted in potent drug concentration-dependent complement-mediated inhibition of autologous and allogeneic CFU-GM. Inhibition of CFU-GM in the presence of Chl was no longer demonstrable following immunosorbent removal of IgG from patient serum. Patient serum in the presence of Chl had limited activity against morphologically recognizable marrow granulocytic precursors in a microimmunofluorescence assay. These results are most consistent with the development of Chl-dependent, selective antibody-mediated immune inhibition of granulopoiesis.

CASE REPORT

An 81-year-old man was admitted to the hospital in May 1985 for fever and severe neutropenia. A routine hemogram performed in March 1985 had demonstrated a normal WBC count. The patient was subsequently admitted to Stanford Hospital with fever, otalgia, and neutropenia. He had a prior history of adult onset (Type II) diabetes mellitus treated with dietary restriction and for the last three months had taken 500 to 1,000mg PO daily of Chl. The patient was also taking occasional acetaminophen and oxycodone, and there was no family history of blood dyscrasias. On physical examination he was febrile with a left otitis externa. Phimosis and balanitis were present. The nodes, spleen, and liver were not palpable, and the remainder of the examination was unremarkable. Routine serum chemistries were normal except for a creatinine of 2.7mg/dL. The hematocrit was 35%, the platelets were 486,000/µL, and the WBC count was 1,800/µL with 2% neutrophils, 93% lymphocytes, 3% monocytes, and 2% eosinophils. Direct and indirect (with and without 100µg/mL Chl) Coombs’ tests were negative. The reticulocyte count was 1.0%. A bone marrow aspirate and biopsy revealed a mild decrease in overall cellularity (35%). There was virtually a complete absence of granulocytes and all morphologically recognizable myeloid precursors. Erythroid maturation was normal, and megakaryocytes appeared increased in number. No evidence for a thymoma was detected on chest roentgenograms. The patient had a normal serum B₁₂, and folate level and an unremarkable serum immunoelectrophoresis. A serum Chl level obtained on the second hospital day was 100µg/mL (average plasma Chl concentrations following ingestion of 250 mg; range from 19-114 µg/mL, with the variation most dependent upon differences in drug half-life and variations in the volume of distribution). Chlorpropamide was not detectable when the level was again assessed on the 22nd hospital day. The patient was begun on tobramycin and cefoperazone, and his Chl was discontinued. His fever gradually defervesced over the ensuing seven days with a gradual improvement in his balanitis and otitis. The patient’s absolute neutrophil count slowly increased and first exceeded 500µL on the 17th hospital day. A repeat bone

From the Department of Medicine, Stanford University Medical Center, Stanford, California, and the Veterans Administration Hospital, Palo Alto, California.

Submitted April 25, 1986; accepted August 19, 1986.

Supported in part by a grant (1 ROI HL-35774-01) from the National Heart, Lung and Blood Institute.


Address reprint requests to Dr Lee J. Levitt, Hematology Division, Stanford University Medical Center, Stanford, CA 94305.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1987 by Grune & Stratton, Inc.

0006-4971/87/6902-0003$3.00/0

Blood, Vol 69, No 2 (February), 1987: pp 394-400
marrow aspirate performed on the 25th hospital day demonstrated reconstitution of marrow myeloid precursors with normal maturation and a myeloid/erythroid (M:E) ratio of approximately 2:1.

METHODS

Assay systems for hematopoietic progenitors. Two to four milliliters of marrow aspirate from normal adult bone donors were drawn into heparinized syringes, layered over Ficoll-Hypaque (Pharmacia, Piscataway, NJ) gradients, and interface mononuclear cells collected. All donors gave fully informed consent prior to marrow aspiration, and all studies were approved by the Stanford University Human Experimentation Committee. Marrow mononuclear cells (5 to 15 x 10^5/mL) were cultured in the presence or absence of 0.5 IU of purified human urinary erythropoietin (1,140 U/mg of protein, National Institutes of Health) in triplicate 0.25-

ml aliquots in methylcellulose microwell cultures containing 1% Mo-conditioned medium (MoCM, a medium conditioned by a T lymphoblast cell line containing both myeloid colony-stimulating activity and erythroid burst-promoting activity). Committed erythroid progenitors (burst-forming units-erythroid [BFU-E]) and granulocyte-macrophage progenitors (colony-forming units-granulocyte-macrophage [CFU-GM]), as well as multipotent hematopoietic stem cells (colony-forming units giving rise to mixed colonies containing granulocytic, erythroid, macrophage, and megakaryocytic cells [CFU-GEMM]), were enumerated by in situ observation containing (CFU-GEMM) and committed granulocyte-macrophage (CFU-GM) and erythroid (BFU-E) progenitor cells. Patient colony yield per 2 x 10^5 buoyant marrow cells included 158 ± 26 (mean ± SEM) BFU-E, 67 ± 9 CFU-GM, and 4.0 ± 1.8 CFU-GEMM. These results are comparable to those obtained from prior and/or concurrent culture of ten normal human marrows in our laboratory (Table 1). Colony cytologic composition, examined after micropipette selection and histochemical staining, did not differ between patient and control marrows after assessment of individual CFU-GM and CFU-GEMM from each experiment. Independent assessment of patient CFU-GM, using placental-conditioned medium rather than Mo-conditioned medium as a source of myeloid colony-stimulating activity, yielded colony numbers comparable to those obtained after plating of normal human marrow. No increment in patient marrow CFU-GM was seen following marrow T cell depletion by immunoadsorption using a pan-T Leu-1 monoclonal antibody (data not shown). These data together indicate that neither a deficiency of marrow granulocyte-macrophage or multipotent progenitors nor qualitative changes in patient colony cytotologic composition could explain the patient’s agranulocytosis and marrow white cell aplasia. These results also provide no evidence for autologous T cell inhibition of granulopoiesis.
Patient AP serum was next examined for antineutrophil activity. Both the direct and the indirect Cowan I staphylococcal slide tests performed to detect antineutrophil antibodies were negative. Serum granulocyte cytotoxicity and granulocyte agglutinating activity were assessed against a panel of 10 normal donors. Neither the cytotoxicity nor the microagglutination assay detected antineutrophil activity in patient serum. The patient's acute and chronic-phase sera were cytotoxic for lymphocytes derived from only three of 19 normal blood donors.

We next assessed patient AP serum (obtained on the second hospital day) for its effect on autologous marrow hematopoietic progenitors. In two separate experiments using MoCM as a source of myeloid colony-stimulating activity, patient heat-inactivated serum in the presence of complement produced potent (mean 87%) inhibition of autologous marrow CFU-GM with minimal inhibition of either BFU-E or CFU-GEMM (Table 1). Similarly, patient AP serum produced potent complement-mediated inhibition of autologous marrow CFU-GM when patient granulocyte macrophage progenitors were cultured in the presence of placental conditioned medium (Fig 1). The further addition of as much as 120 μg/mL of Chl to patient AP serum was not associated with any further significant decrement in autologous marrow CFU-GM. Similar selective inhibition of CFU-GM was noted against two separate allogeneic human marrows after treatment with patient serum plus complement (Fig 1). Patient serum did not demonstrate cytotoxicity for lymphocytes derived from either of the two allogeneic marrow donors. Minimal inhibition (12% to 19%) of allogeneic BFU-E or CFU-GEMM was noted after treatment of marrow with patient serum plus complement. Patient serum in the absence of complement had little inhibitory effect on allogeneic hematopoietic progenitors.

The effect of patient acute (day 2, containing 100 μg/mL Chl) and convalescent (day 25, containing no detectable Chl)-phase serum with and without exogenous Chl was next assessed on growth of autologous marrow CFU-GM. Patient marrow was obtained for this set of experiments upon recovery of the WBC count to 6,800 per μL, four weeks following cessation of Chl. Previously frozen patient AP serum in the presence of complement again selectively inhibited autologous marrow CFU-GM (Fig 2). However, patient convalescent-phase serum in the presence of complement failed to inhibit either autologous or allogeneic marrow granulocyte-macrophage progenitors. Patient convalescent-phase serum in the presence of Chl (60 μg/mL final concentration) did inhibit (79% ± 21%) autologous CFU-GM (Fig 2). Inhibition of autologous CFU-GM by patient convalescent-phase serum was seen when the previously prepared drug-serum mixture was incubated with marrow plus complement. Inhibition was also noted when marrow, serum, drug suspension, and complement were incubated together simultaneously (88% ± 26%). Autologous CFU-GM inhibition was not seen, however, when the drug suspension was preincubated with marrow, after which marrow cells were washed and then incubated with serum plus complement prior to plating (11% ± 7%). No inhibition was noted after marrow cell incubation with AB serum plus 60 μg/mL Chl plus complement (Fig 2).

Inhibition of patient CFU-GM colony growth by convalescent-phase serum was drug-concentration dependent (Fig 3)
CHLORPROPAMIDE-INDUCED PURE WHITE CELL APLASIA

Fig 2. Effects of patient AP (containing 110 μg/mL) and convalescent-phase (containing no detectable levels of Chl) serum with or without exogenous Chl (60 μg/mL final concentration) on growth of autologous marrow granulocyte-macrophage (CFU-GM) progenitor cells. C', complement; D, chlorpropamide. Results expressed as means ± SEM from two separate experiments. Patient convalescent-phase serum inhibited growth of autologous CFU-GM only in the presence of exogenous drug. Marrow was obtained from the patient following recovery of neutropenia.

with demonstrable progenitor inhibition occurring at Chl concentrations of 30 to 120 μg/mL (the patient's AP serum Chl level on the second hospital day was 100 μg/mL). Patient serum plus Chl in the absence of complement had little demonstrable inhibitory activity against autologous CFU-GM (Fig 3). Patient convalescent-phase serum plus Chl with or without complement had little inhibitory activity against autologous BFU-E.

Absorption studies of patient serum were next performed utilizing protein A-sepharose affinity chromatography to deplete patient serum of IgG immunoglobulin. As demonstrated in Fig 4, inhibition of allogeneic CFU-GM in the presence of 100μg/mL Chl could no longer be demonstrated following immunoabsorbent removal of IgG from patient convalescent-phase serum. Absorption of patient's serum with pooled platelets from 14 random donors to remove possible anti-HLA antibody activity did not affect the inhibitory activity of patient convalescent-phase serum plus Chl against allogeneic CFU-GM.

Patient serum was next assessed for activity against morphologically recognizable marrow myeloid precursors utilizing an indirect immunofluorescent assay. Results were compared with similar assays on the same allogeneic target marrow population using an antihuman IgM monoclonal antibody termed 80H.5. This antibody recognizes an antigen common to mature granulocytes and immature marrow granulocytic cells and was positive by immunofluorescence for 54% ± 9% of nonadherent allogeneic marrow mononuclear cells. This percentage corresponded to the number of morphologically recognizable granulocytic precursors as assessed by Wright-Giemsa staining of marrow target cell cytocentrifuged preparations. Patient convalescent-phase serum in the presence or absence of 100μg/mL Chl was positive for 9% ± 3% of allogeneic marrow cells (n = 3). These results were not different from those obtained after incubation with allogeneic marrow and AB serum with or without Chl (11% ± 4%). The results suggest that patient serum in the presence or absence of Chl had limited immunofluorescent activity against morphologically recognizable allogeneic marrow granulocytic precursors.

DISCUSSION

This patient's severe neutropenia and marrow pure white cell aplasia completely resolved following discontinuation of Chl and in the absence of immunosuppressive therapy. Patient AP serum (containing 100μg/mL Chl) produced potent complement-mediated inhibition of both autologous and allogeneic granulocyte-macrophage (CFU-GM) hematopoietic progenitors with little demonstrable cytotoxic activity against autologous or allogeneic erythroid (BFU-E) and multipotent (CFU-GEMM) progenitors. Patient serum also possessed little activity against autologous and allogeneic lymphocytes, granulocytes, or morphologically identifiable marrow granulocytic precursors. Patient convalescent-phase serum (obtained after recovery of marrow and peripheral

Fig 3. Effects of patient convalescent-phase serum plus varying amounts of exogenous Chl on growth of autologous marrow granulocyte-macrophage (CFU-GM, A) and early erythroid (BFU-E, B) hematopoietic progenitor cells. Marrow was obtained following recovery of neutropenia. C', complement. Results expressed as means ± SEM from two separate experiments. Patient serum plus drug produced selective drug concentration-dependent inhibition of allogeneic CFU-GM.

Fig 4. Effects of Chl (100 μg/mL final concentration) plus immunoabsorbed convalescent-phase patient serum on growth of allogeneic granulocyte-macrophage (CFU-GM) progenitor cells. C', complement; D, chlorpropamide. CFU-GM inhibition was no longer demonstrable following protein A immunoabsorbent removal of IgG from patient serum. Pooled platelet immunoabsorption of patient serum to remove possible anti-HLA alloantibodies had no effect on CFU-GM inhibition. Results expressed as means ± SEM from two to three separate experiments. ▼, Patient serum; □, patient serum after protein A immunoabsorption; ◊, patient serum after pooled platelet immunoabsorption.
blood granulocytopenia and four weeks following cessation of Chl) no longer inhibited autologous or allogeneic CFU-GM. However, the addition of Chl to patient convalescent-phase serum resulted in potent drug concentration-dependent, complement-mediated selective inhibition of autologous and allogeneic CFU-GM. Inhibition of allogeneic CFU-GM was no longer demonstrable following protein A-sepharose immunoadsorbent removal of IgG from patient serum. These results are most consistent with the development of Chl-dependent selective antibody-mediated immune inhibition of granulopoiesis.

The etiology and pathogenesis of marrow pure white cell aplasia appear in many ways analogous to the previously described syndrome of acquired pure red cell aplasia. Primary chronic pure red cell aplasia has been associated in about 50% of cases with IgG autoantibodies causing either complement-dependent erythroblast lysis, complement-independent inhibition of erythroid progenitor colony growth, or, rarely, inhibition of endogenous circulating erythrocytins. We have previously described a patient with pure white cell aplasia associated with a spontaneously arising (non-drug-dependent) complement-fixing IgG autoantibody that selectively inhibited autologous and allogeneic CFU-GM proliferation. Thymomas have also been reported in association with both acquired pure red cell aplasia and with acquired marrow granulocytic hypoplasia. At least four cases of Chl-associated pure red cell aplasia have been previously reported. Agranulocytosis has also been described as a rare complication of Chl administration; marrow smears in these patients have revealed varying degrees of hypocellularity and only partial preservation of erythroid elements and megakaryocytes. Stein et al have described a female patient with fatal agranulocytosis associated with Chl therapy and the presence of serum leukoagglutinins. The marrow in this case was described as hypocellular with "profound depression of myeloid cells" but with normal erythroid cells and megakaryocytes. No further studies were undertaken to evaluate the mechanism of the selective marrow myeloid hypoplasia.

A number of mechanisms have been postulated to explain the pathogenesis of drug-induced immune cytolysis. In penicillin-induced hemolytic anemia, the drug is tightly bound to erythrocytes in vitro and in vivo; inhibition of autologous CFU-GM was not seen in our patient, however, when Chl was preincubated with target marrow mononuclear cells prior to washing and incubation with patient serum plus complement, thus making a drug-adsorption mechanism unlikely. An "innocent bystander" mechanism has been described in a case of Chl-induced immune hemolytic anemia in which the causal antibody was found to be a complement-requiring IgG immunoglobulin. Alternatively, drugs may in some patients lead to the production of "autoantibodies" directed against intrinsic red cell antigens. In this instance, however, antibodies are usually demonstrable in the absence of drug, and cell cytolysis usually persists for several weeks to months following cessation of drug therapy.

The explanation regarding cellular specificity of drug-induced immune cytolysis remains to be elucidated. Recent evidence suggests, however, that target cells may have receptors for some drugs or drug-antibody complexes. Chlorpropamide-induced immune hemolytic anemia has been described in at least one instance to be associated with an antibody directed against Jk\(^+\) antigenic determinants. Human red blood cell surface antigenic determinants have not, however, been found on hematopoietic progenitor cells. Claas et al have provided evidence that certain drugs may induce autoantibody formation by reacting with restricted non-HLA polymorphic determinants present on the surface of either platelets or granulocytes. Cellular specificity in drug-antibody reactions may also be conferred as a consequence of alternative mechanisms of drug-antibody binding to target cell determinants: binding of antidrug antibody to target cells, which occurs only in the presence of drug, has been shown in preliminary experiments utilizing quinidine-dependent antiplatelet antibodies to be mediated by the Fab domain of the antibody rather than the Fc portion of the immunoglobulin. Our data seem most consistent with the hypothesis that binding of patient drug-antibody complex occurs via an antigenic determinant or receptor that is relatively restricted in expression to the surface of human CFU-GM and which appears to be lost during the early stages of progenitor cell differentiation.

Granulocyte-macrophage progenitors, obtained from the patient during a time of profound neutropenia, grew normally in the absence of patient serum or drug. These observations suggest that the assayed colonies may have arisen from primitive precursors that did not bind the patient's drug-antibody complex. Erythroid colonies are often similarly present in normal or increased numbers in the marrow of patients with antibody-mediated inhibition of erythropoiesis and pure red cell aplasia. Marrow colony growth in patients with humoral inhibitors of autologous hematopoiesis has also been described in spontaneously arising pure white cell aplasia and in patients with antibody-mediated aplastic anemia. Normal growth of patient CFU-GM may, alternatively, reflect differences in vivo and in vitro in the kinetics of drug-antibody receptor binding and cytolysis.

Drug-associated inhibition of granulocyte-macrophage progenitor growth has been described infrequently in patients with agranulocytosis. Bone marrow smears, however, in a number of these patients have demonstrated coexistent suppression of erythroid and megakaryocytic activity or, alternatively, considerable preservation of myeloid activity with a relative paucity of only the more mature granulocytic elements. In some cases of purported drug-induced suppression of marrow colony formation, the drug inhibited growth of normal allogeneic myeloid colonies but did not suppress growth of patient recovery marrow colonies. Taetle et al have described two patients with diphenylhydantoin-associated antibody-mediated inhibition of granulopoiesis; sera from these patients, however, also appeared capable of mediating destruction of polymorphonuclear leukocytes. Kelton et al have reported a patient with quinidine-induced pancytopenia and marrow hypoplasia; quinidine plus patient serum inhibited growth of both autologous and allogeneic erythroid and granulocytic progenitor...
cells. Very recently, Mamus et al. have described the development of reversible marrow granulocytic aplasia in association with ingestion of ibuprofen. Inhibition of in vitro granulopoiesis in this instance was observed only in the presence of patient serum or IgG plus ibuprofen plus complement.

Further investigations of patients with drug-induced immune suppression of hematopoiesis should provide a better understanding of the mechanisms underlying drug-induced myelosuppression and may identify either surface antigenic determinants or antibodies which would prove useful in the recognition and purification of early hematopoietic progenitor cells.

ACKNOWLEDGMENT

We are indebted to Lorela Fajardo for her expert technical assistance and to Robin Kizer and Janet Scrimger for their expertise in the preparation of this manuscript.

REFERENCES

36. Habibi B, Bretagne Y: Blood group antigens may be the receptors for specific drug-antibody complexes reacting with red blood cells. C R Acad Sci Paris (III) 396:693, 1983
40. Smith ME, Jordan JV, Reid DM, Jones CE, Shulman NR: Drug-antibody binding to platelets is mediated by the Fab domain and is not Fe-dependent. Blood 64:91a, 1984 (abstr).


Chlorpropamide-induced pure white cell aplasia

LJ Levitt