Antibody-Mediated Acquired Sideroblastic Anemia: Response to Cytotoxic Therapy

By A. Kim Ritchey, Ronald Hoffman, Nicholas Dainiak, Sue McIntosh, Richard Weininger, and Howard A. Pearson

A 5'/2-yr-old child developed severe anemia with erythroid hypoplasia and 50% ringed sideroblasts in his bone marrow. A serum inhibitor of erythropoiesis was demonstrated, utilizing syngeneic and autologous bone marrow in a plasma-clot culture system. The IgG fraction of the patient’s serum effected similar suppression of erythroid colony formation. Prednisone therapy was ineffective, but following treatment with cyclophosphamide, normal erythropoiesis was established, ringed sideroblasts disappeared, and his serum no longer inhibited erythropoiesis in vitro. Cyclophosphamide was discontinued, and the patient has remained hematologically normal. This patient is an example of antibody-mediated sideroblastic anemia successfully treated with a cytotoxic drug.

Sideroblastic anemia is a distinctly unusual occurrence in childhood. Lead intoxication and abnormalities of heme synthetic enzymes are known causes of sideroblastic anemia in children, but in most instances, the pathogenesis is unclear. Treatment for idiopathic sideroblastic anemia in the majority of cases is nonspecific and supportive.

We report the case of a 5'/2-yr-old boy who developed severe anemia and ringed sideroblasts in his erythroblastopenic bone marrow. After treatment with cyclophosphamide, the anemia remitted and ringed sideroblasts disappeared from the bone marrow. Prior to treatment, an inhibitor of erythropoiesis in an IgG serum fraction was demonstrated, utilizing the plasma-clot culture technique. This inhibitor was no longer present following recovery.

CASE REPORT

A 5'/2-yr-old black boy has been followed since birth because of an unusual, and as yet unclassified, constellation of abnormalities. The more prominent defects include hypopigmentation of the skin, bilateral cataracts, atrial septal defect and pulmonic stenosis, peripheral cutaneous hypalgesia, and growth retardation. Throughout multiple hospitalizations in the past, many hematologic studies, including bone marrow examination at the age of 11 mo to rule out a storage disease, had been normal.

In August 1977 he presented to the hospital in congestive heart failure secondary to a hemoglobin level of 2.2 g/dl. He had experienced the gradual onset of fatigue, but there was no recent drug ingestion, toxin exposure, unusual dietary pattern, or hematologic disease in family members. Review of previous laboratory values from his hospital record revealed a falling hemoglobin during the preceding 3 mo (Fig. 1).

Other laboratory studies were as follows: hematocrit (Hct) 7%, mean cell volume (MCV) 54 fl, MCH 15.6 pg/RBC, MCHC 28.5 g/dl, hypochromic microcytic red cells and normal platelets on smear.

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Fig. 1. Course of patient.

reticulocytes 0.0%, white blood cell count (WBC) 13,800/cu mm, serum iron 240 µg/dl, total iron binding capacity 360 µg/dl, free erythrocyte protoporphyrin 109 and 24.8 µg/dl (normal 5–40 µg/dl) on two separate occasions, blood lead 10 µg/dl (normal <40), haptoglobin 31.5 mg/dl, direct Coomb's negative, and negative stool guaiac. Antinuclear antibody was negative prior to cyclophosphamide therapy during the period of steroid treatment. No mediastinal mass was seen on chest x-ray. Bone marrow examination revealed a cellular marrow with normal megakaryocytic and granulocytic maturation, but hypoplasia of the erythroid series with a myeloid to erythroid ratio (M/E) of 8:1. Megaloblastic changes and prominent vacuolization of proerythroblasts were observed (Fig. 2). With Prussian blue staining, 50% of nucleated red cells were ringed sideroblasts (Fig. 3). The majority of these were acidophilic normoblasts.

The patient was slowly transfused with packed red cells. Therapeutic trials of pyridoxine, 75 mg/day for 6 wk, and prednisone, 2 mg/kg/day for 3.5 wk, yielded no response, and he remained transfusion dependent (Fig. 1). Treatment with oral cyclophosphamide, 2 mg/kg/day, was begun, and after 3 wk the dose was increased to 3 mg/kg/day. Ten days after the higher dose of cyclophosphamide was initiated, a reticulocytosis was noted and bone marrow revealed an intense erythroid hyperplasia (M/E = 1.2). Prussian blue stain showed only a rare ringed sideroblast. The patient's hemoglobin stabilized, no further transfusions were necessary, and cyclophosphamide was discontinued after 2 mo. No further hematologic problems have occurred during a follow-up period of 9 mo, and hemoglobin level and reticulocyte counts remain normal.

MATERIALS AND METHODS

The patient and 14 randomly selected normal controls were used as donors of peripheral blood. Bone marrow cells were obtained by aspiration from the posterior iliac crest of the patient and 16 controls. Informed consent was obtained from both the patient and controls.

IgG fractions were extracted and purified by the standard techniques of ammonium sulfate precipitation and DEAE cellulose column chromatography and dialyzed against NCTC 109.2 Purity of the initial protein peak as IgG was established by immunoelectrophoresis. The concentration of the IgG fraction was measured by its refractive index. All sera were Millipore filtered prior to use.

The in vitro plasma-clot culture technique was employed for measuring the growth of erythroid colonies from human bone marrow, as described by Tepperman et al.2 Dispersed bone marrow cells in a final concentration of 6–8 x 10^5 cells/1.1 ml were cultured in quadruplicate in the presence of 2 IU of

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PYRIDOXINE PREDNISON CYTOXAN
Fig. 2. Patient’s bone marrow prior to treatment, demonstrating vacuolization of proerythroblast (Wright-Giemsa stain, ×630).

Fig. 3. Ringed sideroblast in patient’s marrow (Prussian blue technique ×1000).
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human urinary erythropoietin (Lot M-12 TaLSL, U.S. National Insitutes of Health). In studies of humoral inhibitors, 0.05-0.1 ml of serum was added. When the IgG fractions were used, the concentration was adjusted so that the final cultures contained the same concentration of IgG as in the patient’s plasma. Cultures were maintained in a humidified atmosphere of 5% carbon dioxide at 37°C. At 6 and 12 days of incubation, the clots were removed and transferred to glass slides, fixed in glutaraldehyde, and stained with benzidine and hematoxylin. Under 100 times magnification, each clot was examined, and erythroid colonies of between 8 and 49 benzidine-positive cells, appearing on day 6, were counted as CFU-E (colony forming unit-erythroid) derived colonies. Colonies consisting of between 50 and 500 benzidine-positive cells or clusters of 3 or more CFU-E-derived colonies, appearing on day 12, were considered to be BFU-E (burst-forming unit-erythroid) derived colonies. Statistical analysis was performed with the two-sample rank test of Wilcoxon-White.

RESULTS

Effect of Serum on the Patient’s Marrow

A sample of the patient’s marrow was placed in culture prior to initiation of prednisone and incubated with normal human serum and the patient’s serum. In the presence of erythropoietin alone, 165 ± 28 CFU-E were formed (Fig. 4). This represents more than double the number of CFU-E found in normal marrows (77 ± 7.2/8 x 10⁴ cells). The addition of 0.1 cc of normal serum effected no change (p > 0.10), while the autologous serum reduced the colony count by over one-half (73 ± 7.67/8 x 10⁴ cells; p < 0.05). Counts of 10.25 ± 3.9 BFU-E (normal 20.6 ± 4.0/8 x 10⁴ cells) were enumerated on day 12 of incubation. BFU-E formation was not influenced by the addition of autologous serum to cultures.

Fig. 4. Effect of serum on patient’s marrow. CFU-E formed per 8 x 10⁴ bone marrow cells cultured. Result is mean of quadruplicate determination with brackets representing 2 standard errors of the mean.
Effect of Serum on Control Marrow

Figure 5 shows the effect of normal human serum and the patient’s serum on CFU-E formation by control allogeneic bone marrow. Again, no inhibitory effect is seen with the addition of 0.1 cc of normal serum, while a similar aliquot of patient’s serum inhibited CFU-E formation by approximately 50% (p < 0.05).

Effect of IgG Fraction on Control Marrow

When the IgG fraction of normal human serum was added to the culture (with a source of complement), no inhibition of CFU-E formation was seen (Fig. 6). A second control had comparable results. Addition of the patient’s IgG fraction at a similar concentration markedly inhibited CFU-E formation (13.5 ± 3.9 colonies/8 x 10^4 cells; p < 0.05) compared to control (37 ± 3.9 colonies/8 x 10^4 cells; p > 0.10).

In nine other experiments, IgG fractions from normal individuals were incubated with control marrow. In no instance was there statistically significant suppression of CFU-E growth. Furthermore, IgG fractions were obtained from six multiply transfused patients with the following diagnoses: idiopathic sideroblastic anemia, aplastic anemia, congenital hypoplastic anemia, chronic lymphocytic leukemia, hemophilia, and thalassemia. In no instance was there evidence of suppression of CFU-E when these fractions were added to control marrow.

Effect of Posttreatment Serum on Control Marrow

Figure 7 illustrates the effect on CFU-E growth of the patient’s serum taken before and after a cyclophosphamide-induced remission. As in previous experi-
ments, 0.1 cc of pretreatment serum markedly decreased CFU-E-derived colony formation (p < 0.05), while posttreatment serum did not possess this inhibitory activity (p > 0.10).

DISCUSSION

The sideroblastic anemias are a group of disorders that may be hereditary or acquired. Common to both types are anemia, a low percentage of reticulocytes, erythroid hyperplasia of the bone marrow often with dyserythropoiesis, and ringed sideroblasts in the marrow.4 Because of the lack of similarly affected family members, his unresponsiveness to pyridoxine, and normal hematologic values prior
and subsequent to the episode of anemia described, this patient’s sideroblastosis is presumed to be an acquired disease. No evidence of toxin exposure or underlying disease known to be associated with a secondary sideroblastic process was found. The demonstration of an inhibitor of erythropoiesis in the IgG fraction of the patient’s serum and his response to an immunosuppressive drug would strongly suggest antibody mediation of his anemia.

Antibody mediation of sideroblastic anemia appears to be an exceedingly rare phenomenon. No evidence of antibody suppression of erythroid colony formation in adults with sideroblastic anemia has previously been found in our laboratory (unpublished data). The only case we have found in the literature was described by Zervas et al., who reported a 70-yr-old woman with acquired sideroblastic anemia who responded to azathioprine therapy with a rising hemoglobin and a decrease in the number of ringed sideroblasts.

Krantz has described the presence of an IgG inhibitor of erythropoiesis in some individuals with acquired pure red cell aplasia. A similar inhibitor of erythropoiesis may be present in some children with transient erythroblastopenia of childhood. Ringed sideroblasts have not been described in children with acquired hypoplastic anemia, although there has been one case report of a child with congenital hypoplastic anemia who developed sideroblastic anemia at the time of relapse. Our patient’s prolonged course is atypical for transient erythroblastopenia of childhood, and conclusive responses to cytotoxic therapy in this disorder have not been reported.

The mechanism of ringed sideroblast production in this child is unclear. That his marrow findings are related to the immunologic nature of his anemia seems likely from the in vitro findings and clinical course. Recent studies describing refractory anemia and ineffective erythropoiesis prior and subsequent to the development of pure red cell anemia would lend support to this relationship. It is conceivable that the production of ringed sideroblasts by this patient was a nonspecific response to the immunologic attack of his erythroid-committed stem cells and the subsequent alterations of erythropoiesis.

Since the patient’s bone marrow and serum used in these studies were not obtained until after transfusion had been given, it is possible that his antibody against red cell precursors was not a true autoantibody, but rather the result of sensitization to transfused blood products. Although we cannot absolutely state that this is not the case without testing pretransfusion serum (which was unavailable), certain factors weigh against this possibility. IgG fractions from multiply transfused patients have not caused suppression of CFU-E growth in our laboratory, as reported above. In addition, this child’s hematologic illness was present long before transfusions were administered, and the dramatic clinical response to cyclophosphamide, with the disappearance of the serum inhibitor, substantiate an antibody as a causative agent.

It is of interest that the patient’s serum inhibitor was capable of inhibiting the in vitro differentiation and proliferation of CFU-E but not BFU-E. Because the BFU-E is a more primitive erythroid progenitor cell than the CFU-E, it might have antigenic components that differ from those of its more differentiated progeny. Apparently, the antigenic determinants against which the patient’s serum inhibitor was directed are present in CFU-E but are either absent in BFU-E or lacking the quantity sufficient for inhibition of in vitro differentiation.
REFERENCES

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