Sideroblastic Anemia Treated With Immunosuppressive Therapy

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A case of apparently primary sideroblastic anemia, in an elderly woman, refractory to hematonic therapy and requiring many blood transfusions, was treated with azathioprine. Over a period of 6 mo, the hemoglobin level gradually rose to normal, no further transfusions have been required in the 30 mo since then, and the marrow picture shows a considerable decrease in the degree of mitochondrial iron overload. There was a simultaneous fall in the titer of HL-A cytotoxic antibodies. The possible role of immunologic factors in this sequence of events is discussed.

Sideroblastic anemia is a dyshemopoietic anemia characterized by defective heme synthesis and the presence of ring sideroblasts in the bone marrow. Cases are classified as congenital and acquired, the latter being further subdivided into a primary form, in which no underlying systemic or hematologic disorder can be identified, and secondary disease, in which the sideroblastosis may disappear if the underlying disorder can be effectively treated. We describe here a case of apparently primary acquired sideroblastic anemia, which led a chronic course over several years, had shown no response to the usual hematinics, but showed a dramatic and sustained improvement with immunosuppressive therapy.

CASE HISTORY

In September 1968, a woman of 70 was admitted to the Manchester Royal Infirmary with a history of anemia for at least 18 mo. Physical examination showed conjunctival pallor, slight splenomegaly (1 cm) and hepatomegaly (1 cm). The blood count on admission was Hb, 5.2 g/100 ml; PCV, 15%; MCHC, 34%. The red cells were predominantly normochromic and normocytic but showed increased anisocytosis and poikilocytosis, with some macrocytosis. Reticulocytes were 1.2%, ESR 43 mm/hr, WBC 4900/μl (neutrophils 70%, lymphocytes 24%, monocytes 6%), platelets 213,000/μl. Serum iron was 120 μg/100 ml, serum folate was 3.87 μg/ml, and serum B₁₂ was 161 pg/ml. Direct Coombs test was negative, and liver-function tests showed serum albumen, 4.1 g/100 ml; serum globulin, 2.1 g/100 ml; serum bilirubin, 0.6 mg/100 ml. Serum alkaline phosphatase was 6 U, and blood urea was 30 mg/100 ml. Bone marrow aspirate was hypercellular with an M:E ratio of 1:1. The erythroid series showed evidence of “maturation arrest” with an increase in proerythroblasts and early normoblasts, megaloblastoid and occasional diploid cells; granulopoiesis also showed a left “shift,” but myeloblasts accounted for less than 5% of the total cells; thrombopoiesis appeared orderly. There was gross iron overload, with many ringed sideroblasts (40% of the total erythroblasts). Many appeared to be early and intermediate normoblasts. Plasma cells accounted for 3%. Erythroblasts gave a negative reaction in the PAS stain.

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A diagnosis of refractory sideroblastic anemia was made; no underlying systemic disorder was recognized, and treatment with pyridoxine (100 mg twice daily by mouth) started on September 19, 1968. There was no significant response either to this or subsequent folic acid therapy (10 mg/day). The patient’s subsequent progress is shown in Fig. 1. Blood transfusions at gradually diminishing intervals were required to maintain the hemoglobin at a mean level of 7.0 g/100 ml, the patient’s general clinical condition otherwise remaining unchanged. During the second half of 1970, occasional transfusion reactions were noted; HL-A cytotoxic antibodies, of multiple specificity, and at high titer, were demonstrated in the patient’s serum in December 1970. Physical examination in February 1971 showed no significant change except that the spleen was slightly larger (2 cm). The blood count on February 23, 1971 was: Hb, 6.3 g/100 ml; PCV, 17.4%; MCV 106 µ; MCHC, 33%. Hypochromic red cells were now more obvious; reticulocytes, 2.6%; WBC, 5000/µl (P 60%, L 33%, M 5%, E 1%, M 1%). Platelets were 220,000/µl; serum iron was 115 µg/100 ml, and TIBC was 260 µg/100 ml. Marrow examination showed true megaloblastic features, and serum folate was 2.0 ng/ml, but a further course of folic acid was given without any decisive response. Transfusion reactions were now severe, necessitating the use of leukocyte-poor blood; in the hope of reducing their severity, and in view of the immunologic studies described below, azathioprine, at a dosage of 150 mg daily, was started on March 22, 1971. On May 6, 1971, the hemoglobin was 7.8 g/100 ml. No transfusion was given at this stage, and subsequently the hemoglobin slowly rose. On June 10, it was 8.5 g/100 ml, on July 8, 11.7 g/100 ml, on September 9, 13.7 g/100 ml, and on October 21, 15.5 g/100 ml. The lowest white count recorded was 4400/µl, on June 10. The patient has since required no transfusions. The red cells now show some macrocytosis and considerable anisocytosis; a few deformed hypochromic cells persist. A bone marrow aspirate in September 1971 showed erythroid hyperplasia, and, although there was still considerable iron overload, a substantial reduction in the number of ring sideroblasts (5% of the total erythroblasts). However, the serum iron had risen at 215 µg/100 ml,

![Fig. 1. The hematologic progress of the patient before and after azathioprine therapy; no transfusions have been given since March 1971.](image-url)
with TIBC, 250 µg/100 ml. This improvement in the patient’s blood count was accompanied by a feeling of well-being and corresponding increase in exercise tolerance: the spleen is now im-palpable, but the liver (March 1973) is enlarged 5 cm. A recent bone marrow aspiration (October 1972) still showed a considerable increase in reticulum cell iron, but true ring sideroblasts are rare, though some cells show conspicuous siderotic granules. Azathioprine has now been reduced to 50 mg/day (February 1973).

MATERIALS AND METHODS

Standard hematological methods were used. With the patient’s informed consent, red cell survival studies were carried out using 51Cr-labeled cells from an isologous compatible donor who had sickle cell disease and a persistent reticulocytosis of 15%. The cells were separated into reticulocyte-rich and reticulocyte-poor fractions and survival values determined for each fraction in patient and donor.

Serum immunoglobulins were estimated quantitatively by the Hyland plate technique, while their qualitative characteristics were determined by the Mancini immunoelectrophoretic technique.

HL-A cytotoxic antibodies were sought in the patient’s serum, using the lymphocytotoxic microtest technique of Troup and Walford, and a panel of lymphocytes obtained from five unrelated individuals.

Cyto-immunofluorescent studies were performed using the patient’s serum and bone marrow; the patient’s IgG globulins were extracted from her plasma by fractionation with cold ethanol at a temperature of −5°C. The IgG fraction was conjugated with fluorescin isothiocyanate according to the method of Wood. The conjugated globulin was purified on DEAE cellulose.

Bone marrow cells were washed three times in 5% albumin in an isotonic phosphate-saline buffer at pH 7.4, and the smears were air dried. The slides were fixed in absolute methanol for 5 min, washed in the buffered saline solution for 15 min, and flooded with IgG conjugated with fluorescin for a further 30 min. The slides were examined with a Zeiss microscope fitted with a dark-ground condenser, and a mercury arc lamp. The slides were then stained for further identification of cells by Jenner-Giemsa.

Controls consisted of (1) patient’s marrow, pretreated with unlabeled IgG fraction obtained from the patient, washed three times, and then treated in the same way as the test marrow; (2) a labeled IgG fraction obtained in the same way from a normal individual and tested against the patient’s marrow; (3) normal marrow prepared in the same way, treated with normal labeled IgG fraction; (4) normal marrow prepared in the same way and treated with patient’s labeled IgG fraction.

RESULTS

There was a marked reduction in the survival of reticulocyte-rich blood (51Cr $\tau_1 = 3$ days) as compared with “old” cells (51Cr $\tau_2 = 10$ days); survival times in the donor were, respectively, 19 and 21 days.

Immunoelectrophoresis using antisera specific for IgG, IgA, IgM, kappa and lambda chains revealed an abnormal kappa chain in the serum. The only precipitation lines detectable in a 1:200 concentration of the urine on immunoelectrophoresis were due to albumin and another fast-moving protein which, electrophoretically, resembled kappa chain. Estimations of individual immunoglobulins showed values of IgG, 895 mg/100 ml; IgA, 240 mg/100 ml; and IgM, 235 mg/100 ml before treatment. After azathioprine therapy for 15 mo, the values were IgG, 980 mg/100 ml; IgA, 250 mg/100 ml; IgM, 130 mg/100 ml. Free kappa chains were still demonstrable in serum and urine.

In the cyto-immunofluorescent studies, dense, uniform cytoplasmic fluorescence was seen in approximately 10% of cells identified as erythroblasts when the patient’s marrow was treated with fluorescein-labeled IgG obtained from the patient’s serum. Some erythroblasts also showed much weaker, patchy,
nuclear fluorescence. Granulocytes showed much less intense, and an occasional lymphocyte weak, diffuse, cytoplasmic fluorescence, but no nuclear fluorescence. Erythoblast fluorescence was present in control system No. 4, in which occasional positive cells were seen; weak leukocyte fluorescence was also seen in this system.

The patient's serum, when tested against a "panel" of normal lymphocytes obtained from five normal donors, with varying HL-A specificities, was found to be cytotoxic to all of these at titers of greater than 1:128. Six months after continuous treatment with azathioprine, the patient's serum showed no cytotoxicity against the lymphocytes of three members of the same panel, and reduced cytotoxicity, at a titer of less than 1:32, against the remainder. Twelve months after therapy, tests for cytotoxicity against a larger panel (50 members) showed only HL-A1 and HL-A8 antibodies to a titer of 1:4.

DISCUSSION

Sideroblastic anemia includes a heterogeneous group of disorders, both hereditary and acquired, having in common defective heme synthesis, disturbed iron metabolism, and the morphologic hallmark of the disease, the "ring sideroblast." In many cases, the abnormal accumulation of iron in the developing erythroblast is believed to be due to a mitochondrial defect, but the manner in which this damage is achieved is not clear.

This elderly patient developed sideroblastic anemia which, in the absence of any family history and of any demonstrable underlying disorder, was originally diagnosed as an example of the primary acquired type. The subsequent failure to respond to hematins, distribution of sideroblasts among early as well as late erythroid precursors, and the chronic course are all features of this type of disease. The presence of slight splenomegaly has been noted as an occasional feature of both hereditary and acquired disease. The patient's transfusion requirements gradually increased over a period of 2 1/2 yr, although no red cell antibodies were demonstrated, and the DCT remained negative. The survival of reticulocyte-rich isologous blood was decreased, as compared with that in the donor, and this might have been related to the presence of powerful leukocyte antibodies evoked by numerous transfusions. Immunosuppressive therapy was given in the hope of reducing the titer of leukocyte antibodies and therefore preventing transfusion reactions, but there was also evidence, from the in vitro immunologic studies, that an antibody directed against some subcellular component of the developing erythroblast might be present. Since HL-A antibodies may destroy young red cells, a slight reduction in transfusion requirements might be expected, but it is doubtful whether this would be noticeable clinically. The improvement in hemoglobin, which was unexpected, did not become obvious until 2 mo later; although not associated with a brisk reticulocytosis, the steady and sustained rise in the hemoglobin suggests that this was an effect of azathioprine and not a spontaneous fluctuation in the severity of the underlying marrow disorder.

Although the marrow showed gross reticuloendothelial cell iron overload, the presence of ring sideroblasts suggests that the block in iron utilization lay at the erythroblast level rather than in its release from the R-E cell. It is possi-
ble that this patient had an inhibitor of heme synthesis, and that immunosuppressive therapy reduced its avidity. The cyto-immunofluorescent studies might be interpreted in favor of this hypothesis. The patient’s serum showed the presence of powerful HL-A antibodies; since they were presumably evoked by numerous blood transfusions, it is likely that they were isoantibodies. Therefore, although it is known that erythroblasts do possess HL-A antigens, it is unlikely that the cyto-immunofluorescence observed in these cells was due to a reaction within this system. It is tempting to speculate that the intense cytoplasmic fluorescence seen in some of the erythroblasts was due to an autoantibody directed against some cytoplasmic component(s) in the cell. Antibodies to erythroblast nuclei have been reported in pure red cell aplasia, however, our patient’s marrow showed erythroid hyperplasia, and the inhibitor may have been directed against the cell mitochondria. Alternatively, damage at the stem cell level might have produced a clone of erythroblasts characterized by a defect in heme synthesis. Even though the hemoglobin rose to normal after immunosuppressive therapy, the red cells remained macrocytic, and abnormally large siderotic granules are still present in the marrow. A similar state of affairs obtains in pyridoxine-responsive sideroblastic anemia, in which an underlying metabolic defect persists, and in which deformed red cells persist even if the hemoglobin rises to normal.

Hume et al. have described a group of elderly women with normochromic normocytic anemia, very high erythrocyte sedimentation rates, and a dysproteinemia, who responded hematologically to prednisolone; however, these patients did not show conspicuous iron overload, and ring sideroblasts were not present.

The anemia of rheumatoid arthritis, and that of polymyalgia rheumatica, may be associated with marrow iron overload, and also respond to prednisolone, but this patient showed no clinical stigmata of either disease.

Although there was no other evidence of an autoimmune disorder, such as has been demonstrated in certain patients with thymoma and red cell aplasia, there was a “marker” of an immunologic disturbance, as evidenced by the presence of free K-chains in the serum and urine. The marrow aspirate showed no evidence of myeloma, and the response to azathioprine was quite unlike that of a patient with myeloma. Moreover, free K-chains at low concentration have been reported in a few individuals in this age group who do not have other evidence of an immunoproliferative disorder. The concentration of free K-chains in our patient’s serum has not been affected by therapy.

Dameshek has suggested a close relationship between refractory sideroblastic anemia and erythroleukemia, but Eastman and his colleagues have recently proposed a division of those cases of chronic refractory anemia characterized by morphologic and functional evidence of ineffective erythropoiesis into a “di Guglielmo” group, in which the risk of clinical leukemia is relatively high, and a nonleukemic group in which leukemia does not occur and the clinical course is benign. Catovsky et al. have described cases in which leukemia and myeloma have supervened in cases originally diagnosed as examples of primary sideroblastic anemia; they postulate that this represents a clonal progression of the disease. Red cell dimorphism in RSA might be inter-
interpreted in favor of a clonal origin of the disease, as is the evidence that one population of cells may have decreased heme synthetase activity. However, our patient has not shown any evidence of leukemia clinically, although the earlier marrow showed a striking increase in proerythroblasts with some megaloblastoid forms.

We have treated a number of other cases of primary sideroblastic anemia with azathioprine without noteworthy success; however, these patients did not have “markers” of immunologic abnormality. While the improvement on immunosuppressive therapy in this unusual case cannot be explained with certainty, and the possibility that the patient has an underlying myeloproliferative or chronic leukemic disorder cannot be entirely excluded, it seems more likely that the sideroblastic features of the marrow resulted from immunologic damage mediated by a humoral factor(s) which was partly eliminated by therapy, for she is now in good health 5 yr after the original diagnosis and 30 mo after starting immunosuppressive therapy.

**ADDENDUM**

A decision to withdraw azathioprine was made in June 1973, since it seemed of importance to both patient and physician to establish that long-term immunosuppressive therapy was essential to maintain hematologic remission.

On June 8, 1973 when treatment was stopped, the hemoglobin was 15.2 g/100 ml. On September 13 it had fallen to 11.7 g/100 ml. Azathioprine was restarted at a dosage of 100 mg per day on September 16; bone marrow aspiration in October once again showed the presence of ring sideroblasts, accounting for about 10% of the total erythroblasts; early as well as late forms were present. On October 29, the hemoglobin had fallen to 7.8 g/100 ml, when azathioprine was increased to 150 mg daily. On November 26, the hemoglobin reached a nadir of 6.9 g/100 ml; thereafter it steadily rose and on December 6 was 7.9 g/100 ml, on December 21 10.0 g/100 ml, on January 11, 1974 11.9 g/100 ml, and on February 8, 13.1 g/100 ml.

This supports our impression that azathioprine therapy was essential for maintenance of remission of this patient’s sideroblastic anemia.

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