Treatment of Autoimmune Hemolytic Anemia in Children with Azathioprine (Imuran)

By W. H. HITZIG AND L. MASSIMO

Many cytostatic agents have been found to impair immunologic responses. The search for new drugs with this property led to the development of azathioprine (Imuran), a derivative of 6-mercaptopurine. The efficacy of purine antagonists has been proved in clinical trials by suppression of homograft rejection and autoimmune diseases, particularly autoimmune hemolytic anemia. Several publications deal with the application of this treatment to adults, but to the best of our knowledge this is the first communication describing its use in infants and small children with autoimmune hemolytic anemia. The inherent difficulties in treating this age group and the success achieved by Imuran justify a brief communication on 3 small children with acquired hemolytic anemia associated with autoantibodies.

Case 1 (Fig. 1)

II. II., born October 10, 1961, is the sixth child of healthy, nonrelated parents. His birth and early development were unremarkable. After an undiagnosed febrile illness at the age of 3 months, the first clinical symptoms appeared, with vomiting and rapidly progressive anemia. At the age of 4 months he was admitted to a local hospital, where the diagnosis of acquired hemolytic anemia with a strongly positive direct antiglobulin test was made. The antibodies were shown by the Central Laboratory of the Swiss Red Cross to be "incomplete" autoantibodies which reacted with all the test erythrocytes available and were of nongamma type (=gamma, or 19S antibody. Treatment with prednisone and ACTH improved the child's condition only temporarily, and as a result the patient was transferred to our hospital at the age of 7 months.

On admission, the boy was very pale and apathetic, but of normal physical development. The skin was clean and no petechiae were seen. There were no skeletal deformities. The tone of muscles was normal. The head, thorax and abdomen were normal. The lymph nodes were not enlarged and the spleen was not palpable. The liver was felt ¼ fingerbreadth below the costal margin.

Laboratory Findings: Hb was 3.5 Gm. per cent, reticulocytes 93 per cent, leucocytes 16,800 per cu.mm. with essentially normal differential count. There were 16 nucleated red cells per 100 white blood cells. The sternal marrow

From the Department of Pediatrics (Director: Prof. A. Prader), University of Zurich, Switzerland, and the Department of Pediatrics (Director: Prof. G. de Toni), University of Genova, Italy.

This work was supported by a grant from the Swiss National Foundation.

First submitted Oct. 25, 1965; accepted for publication May 1, 1966.

W. H. HITZIG, M.D.: Professor of Pediatrics, University of Zurich, Zurich, Switzerland.

L. MASSIMO, M.D.: Professor of Pediatrics, University of Genova, Genova, Italy.

840

BLOOD, Vol. 28, No. 6 (DECEMBER), 1966
showed intense erythroid hyperplasia with an otherwise normal distribution of
the marrow cells. Total protein was 5.5 Gm. per cent, serum iron 221 gamma
per cent, bilirubin 3.2 mg. per cent, osmotic resistance slightly diminished.
Direct and indirect antiglobulin test showed +++. The antibody was char-
acterized as an "incomplete" non-gamma globulin antibody with great thermic
amplitude.

A diagnosis of severe acute acquired hemolytic anemia with warm anti-
bodies of nongamma type was made.

Treatment with relatively high doses of prednisone (40 mg./day) was insti-
tuted. On the second day a transfusion of packed erythrocytes was given.
The hemoglobin rose to above 8 Gm. per cent and the reticulocyte count
dropped to 10-25 per cent. The child recovered rapidly and within two weeks
was in quite good condition.

During the following months several attempts to decrease the prednisone
therapy were made.* Each time, however, new signs of active hemolysis be-
came apparent as soon as the dose was reduced to below 10 mg./day. These,
in most instances, seemed to be precipitated by intercurrent infections. The
direct antiglobulin test was positive throughout this time. However, a gradual
change in the characteristics of the antibodies from nongamma to gamma-type
was noted. As a side effect of the longstanding prednisone therapy, a marked
rounding of the face developed and growth of bone and soft tissue was grossly
impaired. It was therefore decided to institute azathioprine therapy. Because
of the young age of the patient, it seemed advisable to test his antibody-
forming capacity to standard antigens while still on steroid therapy.

During a second hospital admission, from September 13 to Novemeber 11,
1963, immunoelectrophoresis and quantitative determination of immunoglobul-

*We wish to thank Dr. A. Fischer, Hitzkirch, for his most valuable help in controlling
the patient's course at home.
lins (\(\gamma_a, \gamma_A, \gamma_M\)) showed normal values. Subsequently, he was immunized with 3 injections of tetanus/diphtheria/pertussis-antigens at 4 weekly intervals. Fourteen days after the end of this course, the following antibody titers were determined: tetanus, 3 I. U. per ml.; diphtheria 1 I. U. per ml.; pertussis, positive in a titer of 1:1024.* These results are in the normal range for children of this age.

On December 17, 1963, treatment with azathioprine (Imuran) was started with a dose of 18 mg./day (2.0 mg./Kg./day). On February 5, 1964, this dose was increased to 25 mg./day (3.0 mg./Kg./day). At the same time, the dose of prednisone was very slowly reduced and finally on April 23, 1964, it was discontinued. During this period the hemoglobin values remained above 13 Gm. per cent and the reticulocyte count slowly dropped from 2–5 per cent to 0.2–2.0 per cent. The child did not suffer from intercurrent infections, nor did he develop whooping cough, even after close contact with his sister, who had a typical attack of the disease. No drugs have been given since July 9, 1964. No exacerbation of the hemolytic process has occurred and the antiglobulin test became, and has remained, completely negative.

Once prednisone treatment was stopped, a rapid physical and mental development took place. Height and weight, which were grossly below normal, increased rapidly, and at the same time the bone age made rapid progress. By July 1965, the child had reached normal values for height, weight, and skeletal development. This was considered to be a typical example of “catch-up” growth12 (Fig. 2).

Summary: An apparently normal 3-month-old boy developed a severe acquired hemolytic anemia with incomplete warm autoantibodies. The hemolytic process could barely be controlled, even with doses of prednisone that grossly impaired growth and skeletal development. Treatment with azathioprine (Imuran) was therefore considered fully justified. The hyperhemolysis was controlled by this agent and subsequently both prednisone and Imuran were successfully withdrawn. Once prednisone therapy was stopped, rapid “catch-up” growth took place, even though Imuran was being given. After one year of observation without treatment, the child is normal.

Case 2 (Fig. 3)

G. M., born August 13, 1962, is a boy of a healthy family and from a normal pregnancy. Precocious and intense jaundice of the newborn was noted, but bilirubinemia was not measured and no exchange transfusion was done. At the age of 4 months the child was admitted to a local hospital, where a diagnosis of acute otitis and bronchitis with edema of the hands and hepatosplenomegaly was made. The hemoglobin was 11 Gm. per cent. On February 26, 1963, at the age of 6 months, shortly after being discharged from hospital, he was admitted to this hospital for further study because of high fever and pallor.

On admission, he was very ill with high fever, prostration and marked ane-
Hemolytic anemia. H Herbert, 9560/63

Fig. 2.—Case 1. Growth and development are grossly impaired by long-term steroid medication; after suppression of prednisone, growth is resumed and its velocity is even greater than normal ("catch-up growth"). Imuran was given up to the age of 2 10/12; it has no inhibiting effect.

A: Height curve in terms of percentiles.

miae. Purulent otitis was still present; marked hepatosplenomegaly (liver 4 cm., spleen 5 cm. below the costal edge) was noted.

Laboratory Investigations: Hb was 5.4 Gm. per cent, red cells 1,800,000/cu. mm., reticulocytes 32–50 per cent. Marked microspherocytosis and normoblastemia were seen in the blood smear. There was intense erythroid hyperplasia in the bone marrow. Blood groups: O, Rh pos., probable genotype CDE/cDE, MN. Direct antiglobulin test was strongly positive (1:160), indirect negative; incomplete warm antibodies with a specificity anti-Rh, were demonstrated (with trypsin at a titer of 1:32, with bromeline of 1:32).

Therapy was instituted with antibiotics, prednisone (25–30 mg./day) and 6-mercaptopurine. Only after mastoidotomy under general anesthesia and two blood transfusions was a remission of the hemolytic process achieved. However, immediately after discontinuation of the prednisone, a new hemolytic crisis necessitated reinstitution of the steroid treatment. During the following year continuous therapy, with not less than 5 mg./day of prednisone, was given. In spite of this, five hemolytic crises occurred during
Hemolytic anemia.

Fig. 2B.—Development of height, weight and bone maturation in relation to normal mean.

which the dose had to be increased to 15 mg./day; each crisis was initiated by infection, and a second paracentesis was needed. Despite this, no satisfactory control of the hemolytic process could be obtained and the antiglobulin test remained strongly positive. The spleen was therefore removed on May 9, 1964. This resulted in a moderate and unsustained improvement.

On July 17, 1964, therapy with azathioprine (2.5 mg./Kg. daily) was begun. ACTH (10 mg./day) was given for the first 15 days as the course of treatment. General and hematologic conditions were satisfactory until the end of November 1964. Because of a new hemolytic episode with drop of the Hb to 6.8 Gm. per cent and rise of the reticulocytes to 10 per cent, the dosage of azathioprine was increased to 33, then 50 mg./day (=3 and 4 mg./Kg./day). Under this treatment a moderate, although not completely satisfactory, subsidence of the hemolytic activity was noted.

In the beginning of January 1965 the child had a bout of bronchopneumonia during which prednisone (2 mg./Kg./day) was given and the azathioprine was discontinued. After this period, azathioprine (2.5 mg./Kg./day) was given together with a low dose of prednisone (5 mg./day), with a satisfactory result. At the beginning of June the general condition is good—height 82 cm., weight 11 Kg., liver at 2 cm. from the costal edge. The blood picture was essentially
IMURAN IN TREATMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA

normal (Hb 12.5 Gm. per cent, red cells 4,100,000 per cu.mm., reticulocytes 4 per cent, white cells 10,600 per cu.mm. with the following differential: N 70 per cent, E/, B/, L 27 per cent, M 3 per cent; platelets 200,000/cu.mm.). The antiglobulin test was weakly positive (titer 1:5). Growth and development, which were grossly retarded during the long-lasting prednisone therapy, improved gradually when this drug was stopped or decreased.

Summary: In this boy the first signs of autoimmune hemolytic anemia started around the age of 4 months. Prednisone treatment controlled the hemolysis only partially, but despite its administration new episodes of hyperhemolysis occurred, some of them after infections. Splenectomy produced only moderate and transient benefit. The institution of azathioprine therapy resulted in a definite reduction in the abnormal hemolysis, but the best results were achieved when azathioprine and prednisone was given concurrently.

CASE 3 (SEE REFS. 1, 2, 9) (FIG. 4)

M. L. was born August 29, 1960. The parents of this boy are first cousins. The first child was stillborn. This pregnancy was complicated by toxemia; birth weight was 3.3 Kg. His early development was normal, but from the age of 4 months he had many infections (bronchopneumonia, tonsillitis, gastroenteritis, pertussis, and three attacks of meningoencephalitis). At the age of 2 years, after an attack of acute bronchitis, a hemolytic anemia appeared, which was treated in another hospital with numerous blood transfusions. A second bout of hemolytic anemia some months later could not be improved by transfusions, which even seemed to aggravate the anemia. The child was therefore transferred to our department.
On admission, the boy was seriously ill with very marked anemia and mild jaundice. He had a mongoloid appearance, with macrocephaly, prominent frontal and maxillary bones and a wide, open fontanelle (4 × 4 cm.). There was a soft systolic murmur of the heart. Liver and spleen were palpable 4 cm. below the costal edge.

Laboratory Findings: Hb was 3.5 Gm. per cent, red cells 1,650,000 per cu. mm., reticulocytes 32 per cent. On the smear: aniso-, poikilocytosis, polychromasia and anisochromia; marked erythroblastosis (basophils 2, polychromatics 2, orthochromatics 28 per 100 white cells). Leucocytosis was 16,200–22,000 per cu. mm. with preponderant lymphocytosis. Bilirubin was 2.0 mg. per cent (indirect 1.2 mg. per cent; direct 0.8 mg. per cent). Marked erythroid hyperplasia in the bone marrow (210.7 per cent). Blood groups: B, Rh+, MN. Antiglobulin test: direct strongly positive, indirect negative. Incomplete warm agglutinins were found, and in addition incomplete cold agglutinins in a low titer. From the patient's red cells cold antibodies active on erythrocytes O Rh+ were eluted.

Since the family comes from a region with a high incidence of thalassemia and because of the mongol-like features of the child, investigations were extended. They revealed in the patient and in his father the existence of a new hemoglobin with electrophoretic mobility similar to Hb L. This has since received the designation of Hb L δενομα. By fingerprinting and peptide analysis the formula \( \alpha^A \gamma^{G Y} \delta^A_2 \) could be identified.\(^1,2,10\)

Therapy with prednisone (40 mg./day) was immediately successful (rise in Hb, return of reticulocytes to normal and reversion of the antihemoglobin test to negative). Five weeks later, however, recurrence of the hyperhemolysis
made continuous prednisone therapy necessary. During the next 15 months, four more hemolytic crises necessitated drastic increases of the maintenance dose of 5–10 mg. to 30–40 mg./day.

On July 7, 1964, azathioprine, 33 mg./day ( =2.5 mg./Kg./day), was added with the hope that the prednisone dosage could be reduced. After a transient rise of the reticulocyte count, a slow and steady improvement was noted with continuous rise in hemoglobin and a drop of reticulocytes.

In April, 1965, prednisone was stopped altogether, and in May azathioprine was reduced to the dose of 1 mg./Kg./day. General and hematologic conditions are presently satisfactory and the antiglobulin test is slightly positive. The physical development is within the normal range.

**Summary:** This son of consanguineous parents is an example of a new hemoglobinopathy with acute acquired hemolytic anemia due to incomplete warm autoantibodies. The hemolytic process became evident at the age of 2 years and was first treated with blood transfusions, then with high doses of prednisone. Despite maintenance therapy of corticosteroids for 1 1/2 years, five episodes of severe hyperhemolysis occurred. Therefore, treatment with azathioprine was started which, within a year, apparently checked the autoantibody formation.

**Discussion**

Autoimmune hemolytic anemia is a rare disease in small children and infants. Corticosteroids are standard treatment in this potentially fatal condition and their beneficial effect is usually attributed to “immunosuppression,” although it is difficult to define this effect more exactly. In the cases recorded in this paper, certain facts have to be considered.

1. The “immunosuppressive” effect of corticosteroids was temporary and lasted only as long as they were given.
2. The effect was enhanced by giving azathioprine and the benefit so obtained continued, even though steroid therapy was stopped.
3. The Coombs’ Tests remained positive, despite corticosteroid and azathioprine therapy, until a remission occurred.
4. A normal antibody response to bacterial antigens occurred in one case, despite corticosteroid and azathioprine therapy.

Most of the clinical data on the “immunosuppressive” effect of Imuran and the closely related drug, 6-mercaptopurine, are based on the prevention of kidney graft rejection. Presumably inhibition of transplantation immunity is via the effects on “cellular immunity.” One possible explanation of the beneficial effect of steroid and Imuran therapy in the cases reported here is that “cellular,” not “humoral,” immunity is depressed and that, as a result, phagocytosis of red blood cells did not occur. This hypothesis can be studied in further cases, using tagged red blood cells.

A major problem in the treatment of children with corticosteroids is their profound inhibitory effects on normal growth. It is of some importance that with the relatively small doses of Imuran in our patients, interference with growth was not clinically detectable and, at the same time, “immunosuppres-
sion” was sufficient to control the hemolytic process. In addition, Imuran obviously did not have the physiologic actions of cortisone and therefore did not produce osteoporosis, diabetes mellitus, gastrointestinal ulcers, psychoses, and other side effects of corticosteroids.

Further work is needed to determine the best way of combining the use of steroids and Imuran in order to obtain maximal “immunosuppressive” action with minimal side effects. When the nature of the beneficial “immunosuppressive” action of these drugs can be defined in exact terms, it may be possible to find drugs which act specifically on this mechanism with a better therapeutic index than those currently available.

Nevertheless, in treating children with a potent drug such as azathioprine, the following precautions seem advisable: It should be evident that in a given case the disease is chronic and no spontaneous cure can be expected; that is, several attempts to taper steroid therapy should result in clear-cut increases of the hemolytic process, or the hemolysis should be uninfluenced by sufficiently high cortisone doses. Furthermore, before starting immunosuppressive treatment, a course of primary immunization (in infants) or a booster injection (in older children) with the normal antigens should be given to take advantage of the child's own antibody forming capacity as much as possible (see Case 1). During therapy the exposure to infection should be controlled as far as this is possible. Reduction of the steroid dose seems advisable only after 4 to 8 weeks of azathioprine treatment; sufficient time should be allowed to taper it off. Evidence of recurrence of signs of hyperhemolysis should be sought and, if they appear, the dose of the steroid increased. If the corticoids can be stopped, treatment with azathioprine should be continued for a period of 3 to 4 months, followed by gradual reduction of the dose to nothing.

The recommended dose of azathioprine is 2.5 mg./Kg./day or 50 mg./m²/day. This can be doubled if the response seems to be sufficient (Case 2). Prolongation of the treatment for more than 8 to 12 months without success is not justifiable. Because of the steroid-sparing treatment with azathioprine resulting in growth and development (“catch-up” growth) in Case 1, data on height, weight, and skeletal and intellectual maturation should be collected throughout the whole treatment period.

The prognosis of autoimmune hemolytic anemia, which formerly was quite gloomy, has greatly improved since the introduction of steroids into the therapeutic armamentarium. About 80 per cent of affected children survive, either attaining complete remission after a differing period of time or establishing a more or less tolerable new equilibrium. According to the present data, formation of tolerance can be greatly enhanced and accelerated by immunosuppressive chemicals.

Taking into account and watching carefully all the possibilities of complications, immunosuppressive treatment is therefore a very valuable means to break the vicious circle of autoimmune hemolytic anemia also in children. It should certainly be considered seriously before more dramatic measures, such as thymectomy, are carried out.
IMURAN IN TREATMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA 849

SUMMARY

Three cases of autoimmune hemolytic anemia in infants are described. This disease is very rare in the first few months of life. One case (No. 3) was complicated with a rare hemoglobinopathy, which may have been coincidental. Corticosteroid treatment in large doses was partially and temporarily effective in controlling the disease. Immunosuppressive therapy (azathioprine = Imuran, 2–5 mg./Kg./day) produced a complete cure in Case 1 and allowed a marked decrease in steroid doses in the other two cases. Most probably, a vicious circle of autoimmunity was broken by this drug. Failure to gain weight on steroids was more than compensated for by the clear-cut "catch-up growth" in Case 1, even though Imuran was being given. Indications regarding precautions and dosage of azathioprine are stated. The exact mode of action of these drugs is discussed in an attempt to explain the nature of immunosuppression.

SUMMARIO IN INTERLINGUA

Es describite tres casos de autoimmun anemia hemolytic in infantes. Iste morbo es rar durante le prime menses del vita. Un del casos (le tertie) esseva complicate per un rar hemoglobinopathia, sed isto esseva possibilemente un coincidentia. Le therapia a corticosteroide in grande doses esseva partialmente e transientemente efficace in maestrar le morbo. Un therapia immunosuppressive (con azathioprina, i.e., Imuran, in un dosage de inter 2 e 5 mg per kg de peso corporee per die) produceva un curation complete in le prime caso e permetteva un marcate reduction del dosage de steroides in le altere duo. Il pare probabilissime que un circulo vitiouse de autoimmunitate esseva rumpite per iste pharmaco. Le non-augmento in peso durante le uso de steroides esseva plus que cm per le nette crescentia resaliente in le prime caso in despecto del facto que Imuran esseva continue. Es notate le desiderato de proceder cautamente in le uso de azathioprina. Le dosage del pharmaco es commentate. Le modo de action de iste pharmacos es analysate con le objectivo de explicar le natura de immunosuppression.

ACKNOWLEDGMENT

We are grateful to the Wellcome Foundation for advice and for the Imuran used in these studies.

REFERENCES


4. Hitchings, G. H., and Elion, G. B.: Chemical suppression of the immune...
Treatment of Autoimmune Hemolytic Anemia in Children with Azathioprine (Imuran)

W. H. Hitzig and L. Massimo