Effect of Leukocyte Transfusion in a Child with Type II Mucopolysaccharidosis
(Hunter's syndrome/glycosaminoglycans degradation)

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ABSTRACT Treatment of a child affected by type II mucopolysaccharidosis (Hunter's syndrome) with leukocyte transfusions produced dramatic biochemical and clinical changes. The biochemical changes, consisting of greatly increased urinary excretion of glycosaminoglycans and their products of degradation, were transient. The clinical changes, on the other hand, were protracted, which suggests that the mobilization and degradation of stored glycosaminoglycans was not followed by their reaccumulation to initial levels. The effects obtained with this treatment are superior to those produced by plasma infusion, and have implications for therapeutic attempts in the treatment of other hereditary diseases.

The available therapeutic armamentarium for the large majority of inborn errors of metabolism in man is so meager that any promising new approach is welcome. This paper presents observations made on a child affected by mucopolysaccharidosis type II which suggest that transfusion of normal human leukocytes may constitute a useful therapeutic approach in this syndrome.

The experimental plan was based on several fundamental observations. (a) Di Ferrante et al. (1) have observed clinical and biochemical improvement after the administration of normal human plasma to various patients affected by mucopolysaccharidoses I or II. Their study was based upon the report of Hors-Cayla, Maroteaux, and de Grouchy (2) that normal human serum inhibits the intracellular accumulation of glycosaminoglycans (GAG) in cultured fibroblasts derived from patients affected by one of the mucopolysaccharidoses. (b) Muir, Mittwoch, and Bitter (3) have described the occurrence of metachromatic inclusions in the lymphocytes of various patients affected by mucopolysaccharidoses, a finding that may indicate the presence in normal lymphocytes of those enzymes normally involved in the degradation of GAG. (c) Fratantoni, Hall, and Neufeld (4) have demonstrated that intracellular accumulation of GAG does not occur in mixed cultures of fibroblasts derived from patients with type I and type II mucopolysaccharidoses. In their experiments, even the culture medium from one type of cell could "correct" defective cells of the other type, suggesting that the factor(s) missing in one type of cell may be liberated by cells of the other type and transferred to the deficient cells. A similar corrective mechanism in vivo could account for the lack of any observable intracellular accumulation of GAG in females heterozygous for the sex-linked type II mucopolysaccharidosis. The presence in such carrier females of two populations of cells, as predicted by the Lyon hypothesis, has been demonstrated by Danes and Bearn (5).

These observations suggest that normal lymphocytes might be a source of the "corrective factors" present in normal human plasma and that their transfusion might allow a more efficient correction of the abnormal cells of the recipient. Moreover, the known passive transfer of a specific immune response by transfused lymphocytes may provide a precedent for transfer of ability to synthesize a specific protein such as an enzyme. On this theoretical basis, a plan was developed to transfuse leukocytes from an unaffected donor to a patient affected by mucopolysaccharidosis II.

The patient was a 6-year-old white male who was admitted to the Texas Research Institute of Mental Sciences in Houston in June 1970, with the diagnosis of Hunter's syndrome, or mucopolysaccharidosis type II. Clinical and radiological findings were typical for this disorder. The family history revealed that two brothers had died at ages 7 and 12 years with the same condition, that a 13-year-old sister was quite normal, and that no other members of the family had been affected by the disease. An electroencephalogram revealed extremely slow activity, with paroxysms of generalized very slow activity, indicative of severe diffuse encephalopathy probably involving both cortical and subcortical structures. There was, in addition, an active focus of spike and slow activity in the sleeping record. Laboratory data revealed no abnormalities, except for metachromatic granulation of peripheral lymphocytes and a marked elevation of plasma and urine GAG. Biopsy of a typical skin nodule revealed intracellular accumulation of metachromatic granules in distended dermal fibroblasts and extracellular metachromatic material between collagen bundles. Dr. Elizabeth Neufeld has demonstrated that the GAG accumulation in the patient's fibroblasts could be markedly decreased in the presence of factors obtained from mucopolysaccharidosis I (Hurler) cells and media but not from mucopolysaccharidosis II (Hunter) cells and media (6).

METHODS
Urine was collected daily, without preservative, starting 2 days before the leukocyte infusions and continuing until the biochemical changes had stabilized. The daily excretion of "large molecular weight GAG" and of "GAG fragments" was measured as reported by Di Ferrante et al. (1) and expressed as milligrams of hexuronic acid per 24 hr. The ratio "(large molecular weight GAG)/(GAG fragments)" reflects the ex-

Abbreviations: GAG, glycosaminoglycans; KLH, Keyhole limpet hemocyanin.
tent of GAG degradation; its value is 0.2–0.4 in normal children and 0.75–3.0, or even higher, in patients with mucopolysaccharidosis I, II, or III (7). Both “large molecular weight GAG” and “GAG fragments” were analyzed for hexuronic acid (1), iduronic acid (8), and 2-deoxy-2-sulfamino-hexose (9).

The leukocytes administered to the patient were collected with the IBM–NCI Blood Cell Separator (International Business Machines, Inc., Endicott, N.Y.) as described by Freireich et al. (10). Fractionation was performed under conditions where 70–90% of the leukocytes collected were lymphocytes. The cells were collected in 100–200 ml of autologous plasma and administered to the patient shortly thereafter by intravenous drip over a period of 30–60 min (11).

After completion of baseline urine collection, a series of 10 leukocyte transfusions was begun on September 14, 1970 (day 2 of study, see Fig. 1). On each of days 2–6 and days 9–13 the patient received a single transfusion of peripheral blood leukocytes collected from his father. 3 months later a second series of four leukocyte transfusions was given to the patient on successive days. On this occasion the leukocyte donor was the patient’s sister. The father, sister, and patient were ABO-compatible. HL-A typing (Histocompatibility Testing Laboratory, Baylor College of Medicine, Houston) revealed that the father’s lymphocytes had three antigens not shared by the patient and that the patient had no antigens not shared by his father. The lymphocytes of the sister had one antigen not shared by the patient. These results were supported by studies of mixed-leukocyte cultures (12).

The immunocompetence of the patient was assessed by delayed-hypersensitivity skin testing and by the in vitro lymphocyte blastogenic response to phytohemagglutinin and several antigens. 14 days before the start of a series of leukaphereses the patient’s father was sensitized to the antigen Keyhole limpet hemocyanin (KLH, 11). The transfer of the father’s immunity to KLH to the patient was assessed by delayed-hypersensitivity skin testing, measurement of KLH hemagglutinins, and the development of increased in vitro responsiveness of the patient’s lymphocytes to this antigen.

Chromosome analysis of the patient’s peripheral blood was done daily during the leukocyte transfusions from the patient’s sister and twice weekly for 4 weeks after completion of this series of infusions.

RESULTS

Before the infusion, the urinary excretion of “large molecular weight GAG” and “GAG fragments” was typical for a patient with mucopolysaccharidosis type I or II; the excretion of “large molecular weight GAG” was approximately 40 mg/24 hr (normal 5–8 mg/24 hr) and that of “GAG fragments” about 5–10 mg/24 hr (normal 10–50 mg/24 hr), with a ratio of former to latter of 4.8. Immediately after the initial administration of leukocytes from the patient’s father the excretion of “large molecular weight GAG” increased and continued to do so (maximum 112 mg/24 hr) for 4 days, and then declined (see Fig. 1, left panel, A). During the same period the “GAG fragments” increased even more strikingly (to a maximum of 150 mg/24 hr) and then declined. The ratio fell to a minimum of 0.5 and then rose again. During days 9–13 the changes were somewhat different. The “large molecular weight GAG” actually decreased to a minimum of 28 mg/24 hr while the “GAG fragments” rose to a maximum of 54 mg/24 hr, and the

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**Figure 1.** Left panel, excretion of urinary GAG, as influenced by the first series of leukocyte transfusions. A, total urinary GAG; B, iduronic acid-containing GAG; C, sulfamino-hexose-containing GAG; D, ratio (large molecular weight GAG)/(GAG fragments)” for total urinary GAG. The arrows indicate days in which leukocytes were transfused.

Right panel, excretion of urinary GAG, as influenced by the second series of leukocyte transfusions.
ratio fell to 0.6. On day 16 all values had returned to control levels. Fig. 1 (left panel, B and C) indicates that as a result of lymphocyte infusion, the excretion of dermatan sulfate and heparan sulfate (both as “large molecular weight GAG” and as “GAG fragments”) underwent modifications qualitatively similar to those demonstrated for the total GAG. It is evident, however, that the degradation products of dermatan sulfate and heparan sulfate account for a modest part of the total GAG fragments, a conspicuous portion of which is represented by degradation products of other GAG. Similar results have been obtained after administration of normal human plasma to patients affected by mucopolysaccharidosis I and II (1).

Other laboratory data during this period included transiently elevated serum glutamate-oxalacetate and glutamate-pyruvate transaminases (EC 2.6.1.1 and 2.6.1.2). The peripheral leukocyte count rose to 11,700/mm³ (50% granulocytes), fell to 3900/mm³ (43% granulocytes), and then returned to normal. The leukaphereses were well tolerated by the donors and the transfusions by the patient. The major effect on the patient was an elevation of temperature to 38–39°C about 1 hr after each leukocyte transfusion.

Several clinical changes were noted, beginning with day 8. There was a marked softening of the skin and diminution in the heaviness of the face. The nodular skin lesions became smaller and less elevated and a biopsy revealed a diminution of the extracellular metachromasia. There was a considerable increase in joint mobility, particularly in the hands. The abdomen became much less protuberant and the liver smaller. The patient’s hyperactivity decreased noticeably.

During the next 3 months in the hospital and at home the patient’s improvement was maintained, despite the fact that the excretion of mucopolysaccharides reverted to pretreatment levels. It was felt that although the metabolic effects had been temporary, the protracted clinical benefits may have resulted from the extensive degradation and excretion of stored GAG. If this were the case, a second administration of leukocytes might not have so profound an effect. In order to test this possibility the patient was readmitted in January 1971, 3.5 months after the first course of treatment was completed.

At the time of this second admission his appearance was similar to that on discharge. There was slight loss of joint mobility, especially in his hands. His abdomen had not reverted to its original status. Skin biopsy revealed no remarkable change from the previous (post-treatment) specimen; there was perhaps an even further decrease of the extracellular metachromasia. The urinary excretion of GAG was essentially at the pretreatment level.

On this occasion the patient received leukocytes donated by his sister on each of four successive days. The results were much less remarkable this time (see Fig. 1, right panel). The “large molecular weight GAG” fluctuated between 30 and 50 mg/24 hr while the “GAG fragments” increased transiently from 15 to 42 mg/24 hr, then declined again. The ratio was less than 1.0 on one occasion. Clinically, the patient improved still further, with diminution in the nodular skin lesions and with decrease in liver size by 1–2 cm. The active focus of spike and slow activity on the electroencephalogram was considerably reduced after the transfusions but had reappeared 3 weeks later. 3 months later (April 1971) no further changes were noticed in his condition.

The average leukocyte transfusion from the patient’s father contained 8.0 × 10⁶ leukocytes, of which 1.2 × 10⁶ were granulocytes, 5.6 × 10⁶ lymphocytes, and 1.0 × 10⁶ monocytes. The total dose of leukocytes and lymphocytes was 81 × 10⁶ and 56 × 10⁶ respectively. The total amount of plasma infused was 1600 ml. The total numbers of cells collected during the four leukapheresis procedures on the patient’s sister were 12 × 10⁶ leukocytes, 0.5 × 10⁶ granulocytes, 11 × 10⁶ lymphocytes, and 1.0 × 10⁶ monocytes in a total of 540 ml of plasma.

Immunity to KLH was successfully transferred to the patient, as indicated by delayed hypersensitivity to this antigen 7 days after the last leukocyte transfusion from the patient’s father. Immunocompetence of the patient was indicated also by delayed hypersensitivity responses to the antigens candida and streptokinase–streptodornase and by normal in vitro lymphocyte blastogenic responses to phytohemagglutinin and the antigens streptolysin O and streptokinase–streptodornase. Chromosome analyses of the patient’s peripheral blood revealed some female karyotypes only after the first leukocyte transfusion from the patient’s sister.

**DISCUSSION**

Our patient with type II mucopolysaccharidosis (Hunter’s syndrome) showed remarkable transient changes in GAG excretion and long-term clinical improvement in response to transfusion of normal human leukocytes suspended in plasma. The changes were more marked than those reported by Di Ferrante et al. (1) after infusions of plasma alone, and the difference can probably be attributed to the presence of leukocytes, since the total volume of plasma administered was approximately the same (1500 ml) in both instances. Over a 12-day period, our patient excreted a total of about 800 mg of “GAG fragments” and a nearly identical amount of “large molecular weight GAG”; thus, the average excretion of 60–70 mg per day of each component should be compared with the average excretion of 15–25 mg per day of each component in children treated with human plasma alone. It should be emphasized that most of the material mobilized might have been degraded to an extent well beyond the limit of detection of the analytical methods employed.

The clinical changes observed were similar to those found in response to plasma infusion, except that they were of longer duration. The clinical improvement in the skin was correlated with a reduction of metachromatic material in biopsy specimens. A considerable decrease in abdominal size was associated with a reduction of hepatomegaly. The increase in joint mobility was striking. A suggestive improvement in mental status was also noted, as was a transient improvement in the electroencephalogram.

Even considering the smaller number of lymphocytes infused in the second study, the biochemical changes observed were far less impressive, possibly because of the reduced pool of stored material. These results suggest that a considerable length of time may be required for an extensive reaccumulation of GAG. If this were the case, leukocyte transfusion at a rate of two or three times per year might afford sustaining benefit. Although adverse immunological reactions may occur more frequently in response to leukocytes than to plasma, the former could probably be given less frequently. Furthermore, leukocyte transfusion provides the possibility that a transfer of the missing “factors” or the machinery needed for their
synthesis might occur, either by transfer at an epigenetic level (as it may occur in the perpetuation of specific immune response) or by the establishment of a lymphocyte graft.

Other metabolic disorders besides the mucopolysaccharidoses could be amenable to treatment with leukocyte transfusion, provided that the latter contain the needed enzymes and that the storage material, once degraded, does not re-accumulate rapidly. Thus, one would expect beneficial results not only in the other mucopolysaccharidoses but also in some forms of glycogen storage diseases and sphingolipidoses. Perhaps the storage diseases involving deficiency of lysosomal enzymes would be those for which enzyme supplementation could be most readily effected. It is relevant that a biochemical improvement has been reported in Fabry’s disease after plasma infusion (13). In view of the short survival of donor leukocytes in a recipient, a further requirement for treatment with leukocyte transfusion may be the susceptibility of the abnormal cells to correction by the appropriate “factors” even in absence of contact with the normal cells. Such conditions are met in the case of various mucopolysaccharidoses (6) but not in the case of the Lesch–Nyhan syndrome, where metabolic cooperation seems to require intimate contact between normal and abnormal cells (14).

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