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Autotransplantation lymph node
immunoglobulins thymic
leukocytes dysplasia

Hereditary Thymic Dysplasia: A Graft-Versus-Host Reaction Induced by Bone Marrow Cells with a Partial 4a Series Histoincompatibility

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Extract

A nine-month-old male with recurrent pulmonary and gastrointestinal infections and persistent lymphopenia was presumed to have hereditary thymic dysplasia. He was unresponsive to intradermal injections of Monilia antigen, Varidase®, and diphtheria toxoid, and could not be sensitized to dinitro fluorbenzene. He failed to reject a skin allograft. Peripheral blood lymphocytes were unresponsive to phytohemagglutinin stimulation. The serum contained markedly diminished concentrations of γA, γM, and γG globulins, and he could not form specific antibodies to a variety of injected antigens.

A female sibling of the child was presumed to have leukocytes that shared the major histocompatibility antigens with the leukocytes of her brother. Consequently, 100×10^6 bone marrow cells were transplanted from the sister into the affected infant. Five days later, what appeared to be a graft-versus-host reaction was noted with hepatosplenomegaly, a maculopapular rash, edema, diarrhea, and, terminally, profuse hemorrhage. At autopsy the presumptive diagnosis was confirmed: a rudimentary thymus gland weighing 1.5 g was found. Lymph nodes and spleen lacked lymphoid follicles. Plasmacytoid cells, presumably of donor origin, were found in great abundance in the spleen, blood, and bone marrow.

Speculation

Further attempts to reconstitute immunocompetence in infants with thymic dysplasia should be pursued with transplants of histocompatible tissues. The limitations of current methodology in assessing minor differences in histocompatibility suggest, however, that immunosuppressive therapy is required to prevent the lethal effects of seemingly histocompatible cells from an immunocompetent donor in an immunologically unresponsive host. Ideally, such transplants should be performed only when a donor identical with the recipient at the HL-A locus is available.

Introduction

The clinical syndrome of hereditary thymic dysplasia has been well defined. Infants affected with this syndrome have undue susceptibility to viral, bacterial, and fungal infections, an inability to manifest delayed hypersensitivity reactions or to reject allografts. Although such infants may or may not be profoundly lymphopenic, peripheral blood lymphocytes are unresponsive to phytohemagglutinin stimulation. Serum

usually lacks all immunoglobulins, but several variant forms in which serum contains one or more of the immunoglobulins in normal concentration have been described [7, 14].

The intramuscular injection of gamma globulin does not alter the inexorably fatal outcome of this immune defect; therefore, various attempts have been made to establish immunologic competence with transplants of thymus [12], bone marrow cells [11, 13], or fetal hemapoietic cells [6, 7]. These attempts have either failed to achieve the aim of such therapy or have resulted in a graft-versus-host reaction by the donor cells.

In view of the risk of transplanting immunocompetent cells into infants affected with this syndrome [5, 11], it appeared warranted to select donors by testing for histocompatibility between the leukocytes of the donor and the recipient. The present report concerns an attempt to restore immunological competence in an infant with hereditary thymic dysplasia by transplanting cells from a normal female sibling who was presumed to be reasonably histocompatible with the patient.

Case Report

The patient was born on August 16, 1966. Antenatal and perinatal histories were normal. At the age of three months the infant developed chronic upper respiratory infection; at four months of age he had pneumonia, which slowly responded to antibiotic therapy. At six months of age he sustained a prolonged bout of gastroenteritis. Examination by paper electrophoresis revealed a low concentration of gamma globulin in serum and the patient was given 8 ml of immune serum globulin by intramuscular injection. At nine months of age he was admitted to the hospital for evaluation.

Physical examination revealed a chronically ill-appearing infant, in the 50th percentile for height and below the 3rd percentile for weight. Tonsils and adenoid tissue were absent. No lymph nodes were palpable. Inspiratory rales were heard at both lung bases and a chest x-ray revealed generalized peribronchiolar thickening with irregular aeration. No thymus shadow was evident. Cultures of tracheal aspirates repeatedly yielded *Klebisiella aerobacter* and *Candida albicans*. Levels of calcium and phosphorus in serum and electrolytes in sweat were normal.

The family history was negative for evidence of possible immunologic deficiency disease. The parents were healthy and two female siblings, aged 10 and 11 years, were both normal.

Immunologic Studies

Immunoelectrophoretic examination of serum using an equine antihuman serum revealed trace amounts of γG and γA and an absence of γM globulin (fig. 1). Estimation of levels of immunoglobulin in serum by radial diffusion techniques yielded the following results per 100 ml: γG 260 mg, γA 23 mg, and γM 18 mg. Persistent lymphopenia was observed throughout the hospital course (fig. 2). An aspirate of tibial bone marrow contained 9 % lymphocytes (normal for age, 20 %) and no plasma cells were found. The infant was unresponsive to a booster dose of tetanus and diphtheria toxoids. Although his red blood cells were type O Rh positive, isohemagglutinins were not detected in the serum. Total hemolytic complement and levels of C'1 and C'3 were normal. No delayed hypersensitivity response was obtained with intradermal injections of diphtheria toxoid, Monilia antigen, streptokinase-streptodornase, or intermediate strength PPD. Following sensitization with a vesicant dose of 5 % dinitrofluorbenzene in acetone, the infant was unresponsive to challenge with 1:1000, 1:100, and 1:40 dilutions of the sensitizing solution in corn oil. A full thickness skin allograft, 25 × 5 mm, from an unrelated donor, was

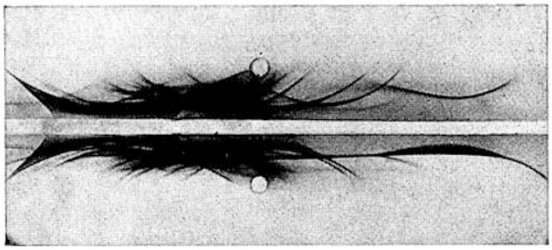


Fig. 1. Immunoelectrophoresis of patient's serum (top), of normal serum (bottom), and of a lymph node to the left. Pattern was developed with equine antihuman serum.

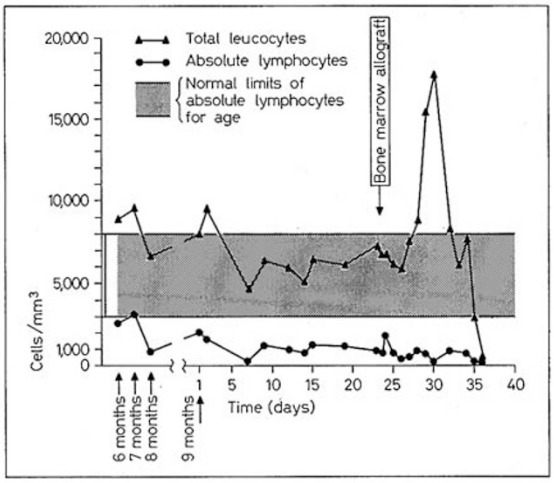


Fig. 2. Sequential leukocyte and lymphocyte counts of peripheral blood.

placed on the anterior surface of the left thigh. There was no microscopic evidence of rejection 8 and 26 days later. Attempts to induce mitoses in lymphocytes from peripheral blood by stimulation with phytohemagglutinin failed on three occasions.

Examination of cells from the buffy coat of peripheral blood by electron microscopy revealed no mature small lymphocytes. A few immature lymphocytes with large, pale nuclei and relatively large amounts of cytoplasm with an unusual amount of ergastoplasm and vesicles were found (fig. 3).

The parents and two sisters of the infant responded normally to intradermal injections of Monilia antigen and streptokinase-streptodornase. Levels of immunoglobulin of their serum were normal.

Red blood cell antigens and gamma globulin and haptoglobin types were determined on blood samples obtained from the propositus, his mother, father, and two siblings (table I). Peripheral blood white cells were obtained for histocompatibility typing [8]. The results are given in table II. Because sister C. was thought to be histocompatible with the propositus in the 4b and 8a series of the HL-A locus, she was chosen to donate bone marrow cells to the patient. The possibility of a partial incompatibility in the 4a series was not appreciated at the time of the transplant.

Heparinized bone marrow was obtained from the iliac crest of the sister under general anesthesia; 100×10^6 bone marrow cells were present in a volume of 40 ml. Eleven percent of the cells appeared to be

Table I.

	Father	Mother	Sister R.	Sister C.	Patient
			Blood antigens		
A	—	—	—	—	—
B	—	—	—	—	—
D	—	—	—	—	—
C	—	—	—	—	—
E	—	—	—	—	—
c	+	+	+	+	+
e	+	+	+	+	+
C^W	—	—	—	—	—
G	—	—	—	—	—
P	+	+	+	—	±
K	—	—	—	—	—
Kp^a	—	—	—	—	—
Kp^b	+	+	+	+	+
Le^a	—	—	—	—	—
Le^b	+	+	+	+	+
M	—	+	+	+	+
N	+	—	+	+	+
S	—	+	—	—	—
s	+	+	+	+	+
Lu^a	+	—	—	+	+
Lu^b	+	+	+	+	+
Fy^a	—	—	—	—	—
Jk^a	+	+	+	+	+
Wr	—	—	—	—	—
VeL	+	+	+	+	+
Yt^a	+	+	+	+	+
			S. proteins		
Gm^a	+	+	—	+	+
Gm^b	+	+	Ab	+	+
Gm^x	—	+	—	—	+
Inv	—	—	—	—	—
Hp	2–1	2–1	2–1	1–1	1–1

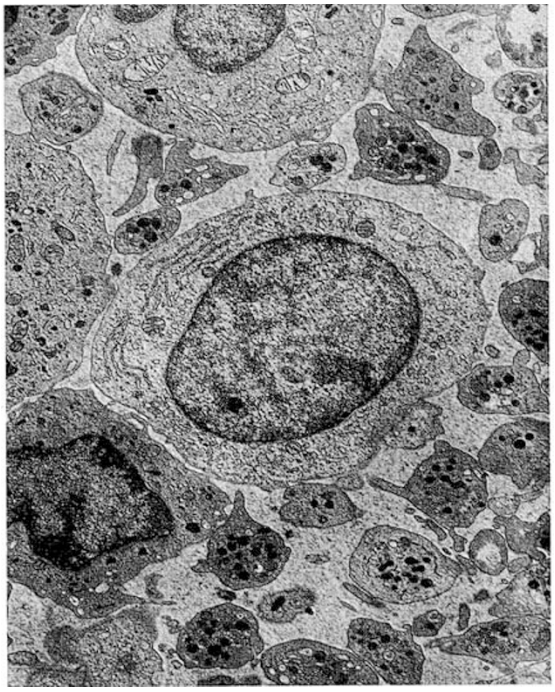


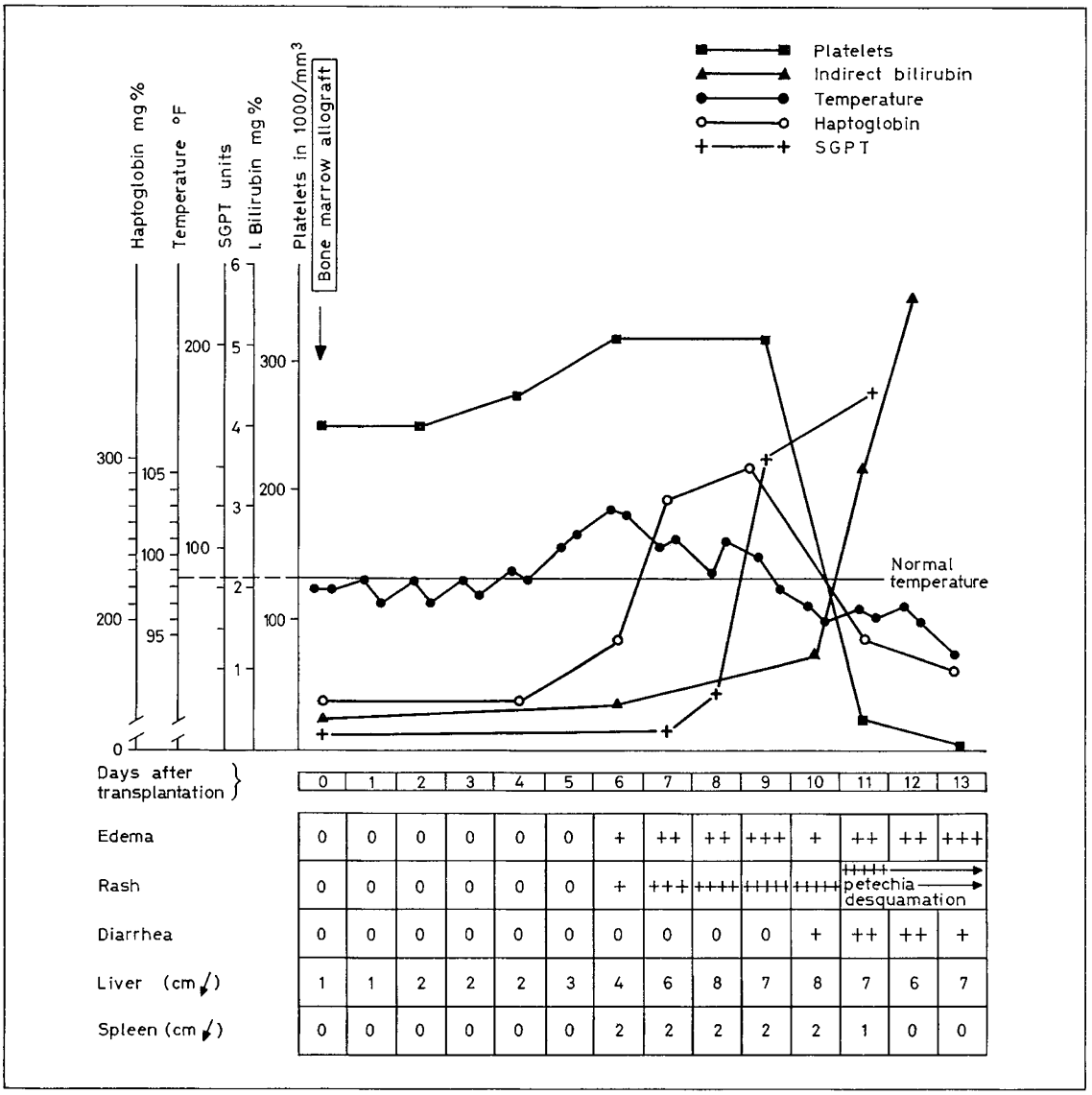
Fig. 3. Electromicrograph of cells from the buffy coat. In the center is shown a lymphoblast of the type most frequently found in this patient, with a large immature nucleus, abundant cytoplasm, and an unusual amount of endoplasmic reticulum with small vesicles. 2.5 % glutaraldehyde fixation, stained with Uranyl acetate and Reynolds' lead citrate. Mag. × 5800.

Fig. 4. Clinical course of patient following bone marrow transplantation. ▶

Table II. Results of histocompatibility typing

Previously recognized antigenic series	Serums used for typing in this study[1]	Family R				
		Father	Mother	Sister R	Sister C	Patient
4b	AHN/TRO	—	+	+	+	+
	PLG/BRE/COF	—	+	—	—	—
	LOO/DA	+	+	+	+	+
4a	R/HAM/COH	+	—	+	—	+
8a	J	+	+	—	+	+
	LIS/PAL	+	+	—	+	+

[1] Only serums exhibiting differences among family members are reported. Thirty-seven other serums that revealed no differences in family members are not cited.



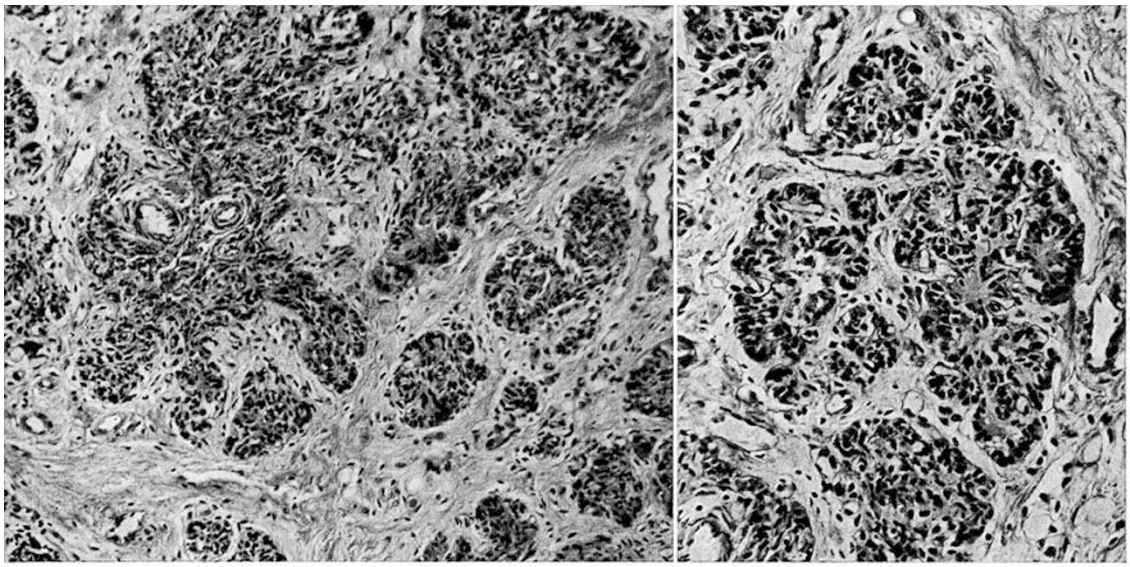
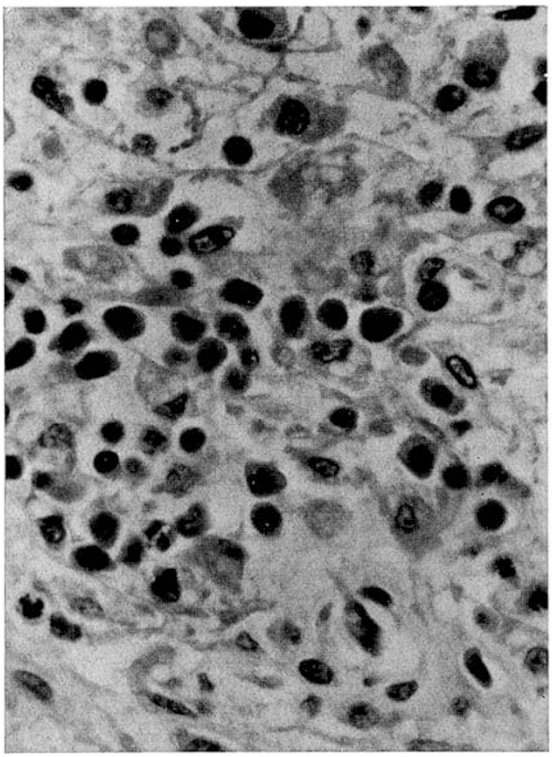


Fig. 5A. Photomicrograph of thymus, ×250.
Fig. 5B. Photomicrograph of thymus, ×280.

Fig. 6. Photomicrograph of spleen (×840) showing plasmacytoid cells in the red pulp.



lymphocytes. The cells were administered to the patient intravenously via an antecubital vein over a 60-minute period, after which time 1 ml of protamine sulfate was injected intravenously. The procedure was well tolerated by the donor and the recipient.

The condition of the patient remained unchanged for the four days immediately following the transfusion. On the fifth day, the child became febrile; on the following day he developed a rapidly spreading maculopapular erythematous rash which ultimately spared only the face, palms, and soles. Cultures of blood, nose, throat, spinal fluid, urine, and stool were persistently negative for bacteria and viruses. The clinical course is summarized in figure 4. On the sixth day, a bone marrow aspiration revealed the presence of large plasmacytoid cells; similar cells also constituted 3 % of the peripheral blood white cells. Up to the time of death, twelve days after bone marrow transplantation, no positive results were obtained by stimulating lymphocytes from peripheral blood with phytohemagglutinin or by intradermal injection of Monilia antigen and Varidase®.

A skin allograft showed no evidence of rejection and was not spared by the rash. On the twelfth day following the bone marrow transplant, the infant died. Death was apparently due to extensive hemorrhage into the lungs. Postmortem examination confirmed the presence of thymic dysplasia. A small, rudimentary thymus gland, weighing 1.5 g and containing no Hassell's corpuscles, was found in the anterior mediastinum (fig. 5). The lymph nodes were devoid of lymphocytic elements and plasma cells. No Peyer's patches were evident in the gastrointestinal tract. The pharyngeal and palatine tonsils could not be identified. The spleen

weighed 23 g and contained numerous pyrininophilic plasmacytoid cells in the red pulp (fig. 6). Similar cells were also found in the liver, bone marrow, and peripheral blood.

Discussion

At the present time, the immunologic incompentence of infants with hereditary thymic dysplasia, appears to result from the absence or maturational failure of a line of bone marrow cells destined to differentiate into mature lymphocytes and plasma cells [7]. The relation of the failure of normal thymic embryogenesis to the stem cell abnormality is unclear. In view of the basic abnormality in these infants, it seems likely that immunologic restoration will be achieved only when immunopotential stem cells are replaced in these infants, perhaps together with whatever influence is provided by a normal thymus. Experimental evidence has strongly pointed to bone marrow as the source of these stem cells [4, 10]. Thus far, attempts to restore immunocompetence to infants with thymic dysplasia by transplantation of stem cells from bone marrow or from fetal liver have failed, presumably because of the lethal effects of histoincompatibility.

Histocompatibility testing in man is being actively studied [1, 2]. The major and minor histocompatibility barriers in man are not well established. The infant reported in this study demonstrated compatibility with a female sibling in the 4b and 8a series of the HL-A system of antigens, which are thought to be major factors in human transplantation immunity. The partial incompatibility in the 4a series of antigens had not been established at the time transplantation was undertaken, partly because white cell typing of young infants presents considerable technical difficulties. In retrospect, sister R. would have been a more suitable donor of bone marrow. This critical difference apparently resulted in a fatal graft-versus-host reaction following bone marrow transfusion. Attempts to define further the factors in this reaction were unsuccessful. It was impossible to demonstrate antibodies in the serum of the infant, just prior to death, against white cells carrying the incompatible antigens, and no antibodies could be eluted from the red cells of the patient.

Pursuit of these attempts at immunologic restoration with histocompatible immunopotential stem cells will depend on further developments in typing techniques and presumptive genotyping of the HL-A locus in man. Meanwhile, such transplants may be rendered feasible by exposing cells prior to injection to mitotic inhibitors such as mitomycin, or by suppression and control of graft-versus-host reactions with antilymphocyte antiserum. These possibilities have been explored in experimental models [3, 9].

Summary

A nine-month-old infant with hereditary thymic dysplasia was infused with bone marrow cells taken from his sister. Histocompatibility at meaningful alleles of the HL-A locus was demonstrated, with the exception of a partial incompatibility in the 4a series. A graft-versus-host reaction ensued five days later and the child died.

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