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SUCCESSFUL BONE MARROW TRANSPLANTATION IN A PATIENT WITH HUMORAL AND CELLULAR IMMUNITY DEFICIENCY

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SUMMARY

An immunity deficiency disease occurring in three siblings is described. Two siblings, a boy and a girl, died at ages $1\frac{1}{2}$ years and $4\frac{1}{2}$ years respectively with overwhelming varicella, varicella pneumonia and sepsis. Their disease included thymic hypoplasia, lymphopenia, deficient humoral and cellular immunity, absent serum IgA, neutropenia and eosinophilia.

Transplantation of bone marrow, identical by cytotoxic and mixed leucocyte assay, red cell antigens and Gm and Inv factors was given to an affected girl from a normal sibling on two occasions. The first transplant given at 6 months of age resulted in clinical improvement of the patient and some evidence of immunologic reconstitution. Complete correction of the immunity defect was achieved following a second bone marrow transplant at 11 months of age. A delayed onset and prolonged course of GVH reaction was observed following the second transplant. The patient survived the GVH without specific therapy. Evidence for complete immunologic reconstitution continued to be present 1 year following the second transplantation.

INTRODUCTION

Transplantation of allogeneic bone marrow to immunity deficient subjects has rarely been accomplished. To date, evidence for reconstitution has been presented in two cases of lymphopenic hypogammaglobulinaemia (Gatti *et al.*, 1968; de Konig *et al.*, 1969), and a patient with Wiskott-Aldrich syndrome (Bach *et al.*, 1968). We report successful bone marrow transplantation in a girl with an autosomal form of thymic hypoplasia associated with lymphopenia, neutropenia, eosinophilia, absent cellular immunity and deficient antibody formation. Two siblings, a male age $1\frac{1}{2}$ years and a female age $4\frac{1}{2}$ years, died from overwhelming varicella infection and sepsis and were shown to have IgA deficiency (Hoyer *et al.*, 1967).

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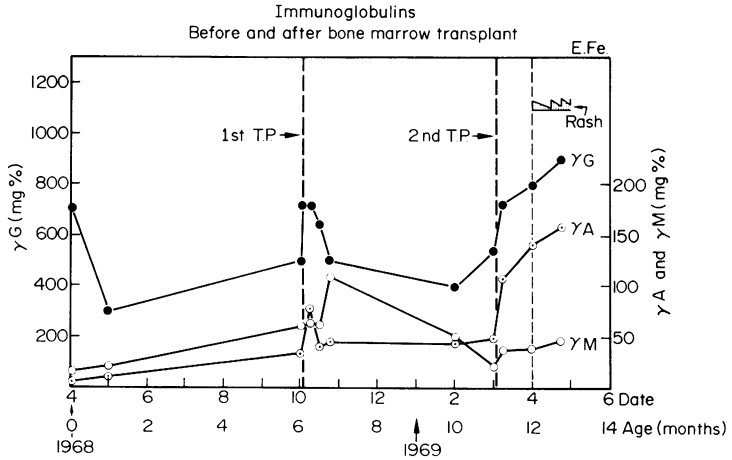

Fig. 1

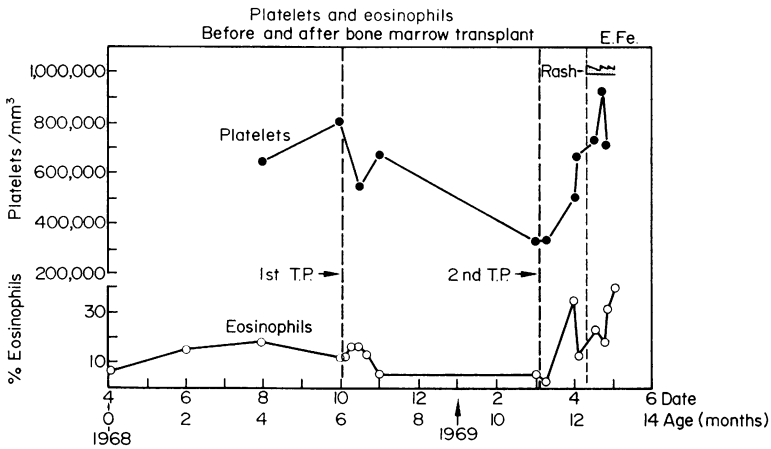

Fig. 2

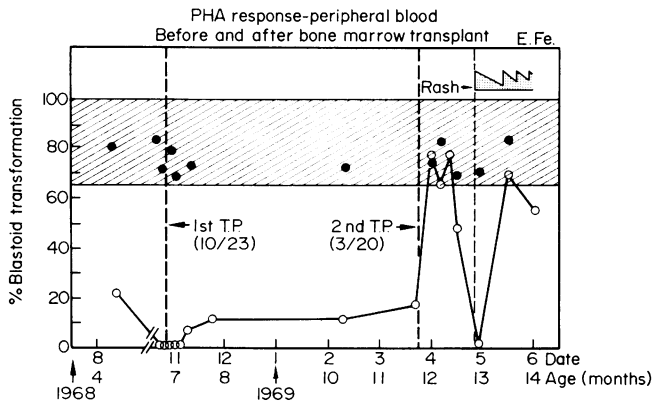

Fig. 3

FIG. 1. Immunoglobulin production in patient before and after bone marrow transplantations.
FIG. 2. Platelet and eosinophil counts before and after bone marrow transplantations.
FIG. 3. PHA response of peripheral lymphocytes before and after bone marrow transplantations.

Case Report

E.F., a female infant, was first evaluated at 2 weeks of age. The only positive physical finding was a mild seborrheic rash. Laboratory studies revealed a haemoglobin of 18 g/100 ml, white blood cell count (WBC) 6850/mm^3 with 33% polymorphonuclears, 49%

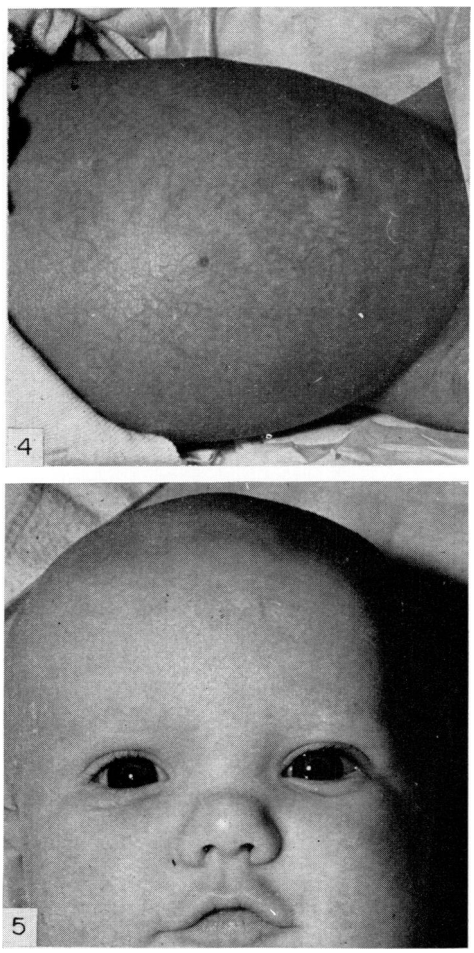

FIG. 4. Rash on patient at 6 months of age. Note maculopapular appearance and fine desquamation.

FIG. 5. Patient at 6 months of age. Note absence of hair and eyebrows.

lymphocytes, 11% monocytes and 7% eosinophils. Immunoglobulins were (mg/100 ml), IgG 700, IgM<2, IgA<2 (Fig. 1). The lymphocytes responded subnormally to phytohaemagglutinin (PHA). Subsequently, the lymphocytes became completely unresponsive to PHA (Fig. 3). Peripheral eosinophilia and thrombocytosis appeared (Fig. 2). No thymus shadow was seen on tomograms of the mediastinum. Over the next 6 months a maculopapular erythematous, desquamating eruption appeared, initially involving only the scalp

and then spreading to the entire body (Fig. 4). Hair on the scalp and eyebrows became scant (Fig. 5).

At 6 months of age the patient was admitted to the University of Minnesota Hospitals for detailed immunologic evaluation and possible bone marrow transplantation. The physical examination revealed a chronically ill infant. She was in the 10th percentile for height and weight. The skin was as previously described.

Studies (summarized in Table 1) showed deficient humoral and cellular immunity. A bone marrow biopsy revealed megakaryocytosis, eosinophilia and the presence of plasma cells. Biopsy of a stimulated node showed generalized depletion of lymphocytes and poor follicular formation (Fig. 6). Rectal biopsy showed increased numbers of plasma cells and inflammatory cells. The skin showed slight lymphoid infiltration of the epidermis and vacuolization in the basal cell layer.

TABLE 1. Immunologic function before and following first and second bone marrow transplantation

Time	Age (months)	Small lymphs (%)	PHA	DNFB†	Isohaemagglutinins titre
Before transplant	6	2	0*	negative	0
After 1st transplant	7–11	5	15	positive	0
After 2nd transplant	12	14	100	positive	1:4

* Per cent transformation. † Positive skin response to challenge dose following sensitization.

A normal 6-year-old sister (the only surviving sibling) was found to be histocompatible with the patient as determined by cytotoxic assay and mixed leucocyte culture.

The patient received an intraperitoneal infusion of 45 ml of bone marrow from the normal sibling consisting of approximately $1{\cdot}25 \times 10^3$ nucleated cells. The marrow was collected as described previously (Gatti *et al.*, 1968).

Over the next 5 months the patient improved. Some evidence of engraftment was provided by the following observations: 1—increased responsiveness of the lymphocytes to PHA stimulation although this response still did not reach normal intensity (Fig. 3), 2—development of cutaneous reactivity to DNFB (Table 1), 3—the amelioration of the skin rash (see below). A decrease in the peripheral eosinophilia (Fig. 2) and generalized improvement in the appetite and disposition occurred. Nevertheless, no specific humoral antibody responses could be demonstrated (Table 1). These findings were interpreted as an indication that only partial immunologic reconstitution had been accomplished. Accordingly a second bone marrow transplantation from the same donor was performed at 11 months of age. Marrow from the normal HL-A matched sibling was again utilized. At this time 153 ml of bone marrow containing approximately 9×10^9 nucleated cells were given. In order to minimize dilution of the bone marrow by peripheral blood no more than 3 ml was taken from any one site. No adverse reactions were observed in the donor or host as a consequence of the transplant. One week following the second transplant the eosinophils decreased to 2%, the platelets decreased to 326,000, the lymphocytes now transformed normally when stimulated by PHA, and the immunoglobulins were (mg/100 ml) IgG720 IgM 43, IgA 110

(Figs. 1, 2, 3). Evidence for further immunologic reconstitution following this marrow transplant was thus obtained.

The patient continued to do well until the 40th day following the second transplant.

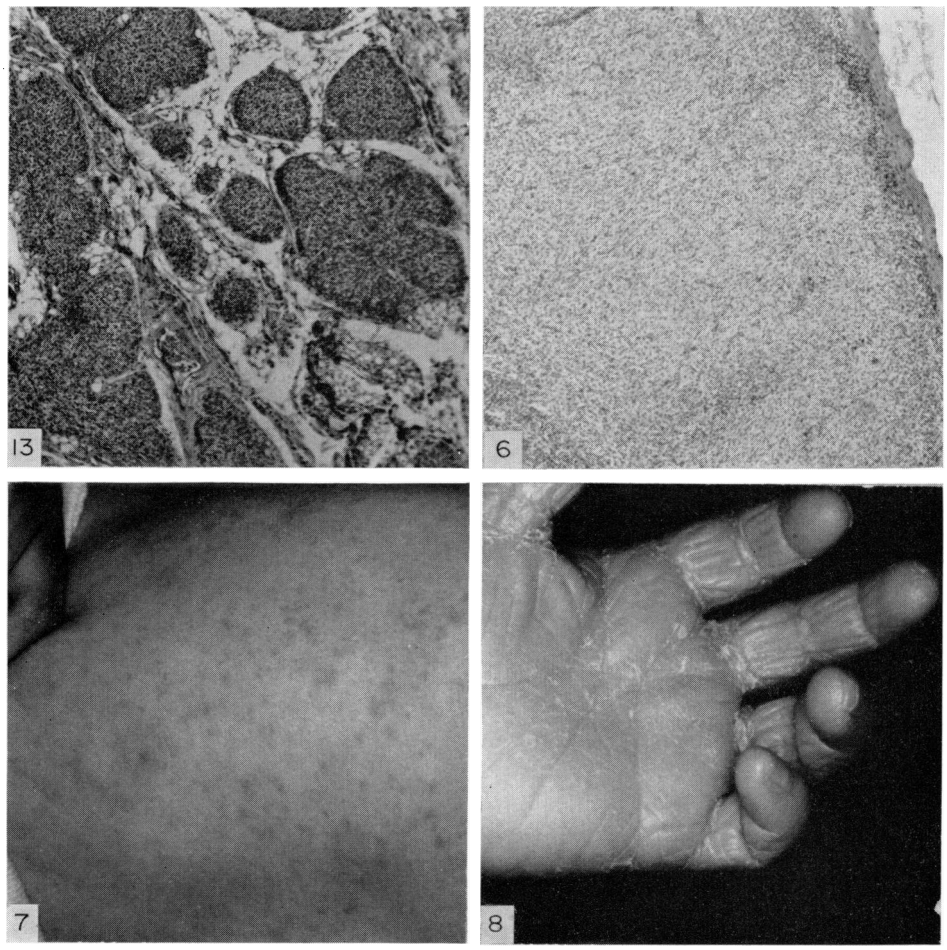

FIG. 13. Thymus (× 32 magnification, H and E stain) of Je.F., brother of the patient, showing absence of corticomedullary differentiation and Hassel's corpuscles.

FIG. 6. Stimulated lymph node (× 32 magnification, H and E stain), showing generalized lymphoid depletion and lack of follicle formation.

FIG. 7. Generalized maculopapular rash 40 days following second transplant.

FIG. 8. Hand of patient 8 weeks following second transplant showing waxy appearance and desquamation.

At this time a faint erythematous, maculopapular rash was observed primarily on the trunk. During the following weeks the rash increased in severity and extent (Fig. 7) and was associated with eosinophilia and thrombocytosis (Fig. 2). It was felt at this time that the patient was experiencing a mild graft versus host (GVH) reaction. Over the next 4 weeks

an episode of otitis media, upper respiratory illness, and DPT immunization, each occurring as separate events, resulted in an exacerbation of the rash, eosinophilia and thrombocytosis.

Eight weeks following the second transplant the patient was admitted to the hospital with fever and cough. Physical examination revealed erythematous, waxy skin with marked

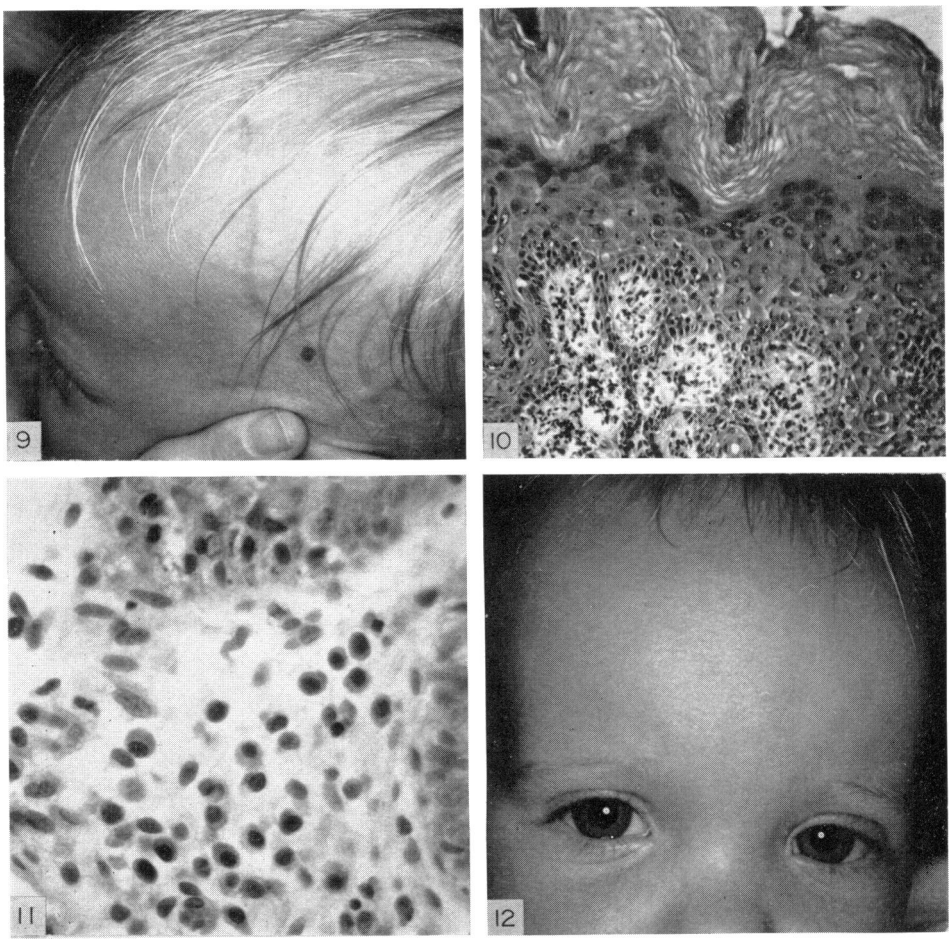

FIG. 9. Patient 8 weeks following second transplant showing loss of hair.

FIG. 10. Skin biopsy (× 80 magnification, H and E stain) taken during GVH showing lymphocyte infiltration, basal cell vacuolization, dyskeratosis and epidermal thickening.

FIG. 11. Rectal biopsy (× 340 magnification, H and E stain) during GVH showing increased plasma cells and inflammatory cells.

FIG. 12. Patient 12 weeks following second transplant showing growth of scalp hair, eyebrows and eyelashes.

desquamation (Fig. 8), loss of hair (Fig. 9), eyebrows, and eyelashes and splenomegaly. Laboratory studies revealed a 40% eosinophilia, 926,000 platelets/ml^3, increased orinithine carbamyl transferase (OCT) of 40 deca units, white cells which reduced nitroblue tetrazolium (NBT) and a normal electrocardiogram. Perihilar infiltrate was present in the chest

X-ray. A lung biopsy demonstrated increased number of alveolar macrophages and a mononuclear cell infiltrate. Silver and Giemsa stains did not reveal Pneumocystis carinii organisms. A skin biopsy revealed marked epidermal infiltration with lymphocytes, basal cell layer vacuolization, dyskeratosis and epidermal thickening, all characteristic of a graft versus host reaction (Fig. 10).

Thirteen weeks following the second transplant the patient continued to show desquamation of the skin and had several febrile episodes with temperatures up to 104–105°F. Prednisone (5 mg/day) was given in an attempt to decrease the GVH reaction. However, because of hypertension (160/110), the steroid was discontinued after 3 days. Nevertheless, gradual improvement occurred with a cessation of desquamation of the skin, no further hair loss, return of temperature to normal and decrease of platelets, eosinophil counts and OCT to normal. Hypertension persisted to a mild degree but the etiology remained obscure despite extensive studies. Urinalyses were always normal and renal function tests, intravenous pyelogram, urinary catecholamines and metanephrines were all normal. The patient did not exhibit any gastrointestinal tract symptoms during the entire GVH reaction. A rectal biopsy showed increased numbers of inflammatory and plasma cells (Fig. 11). The only indication of liver involvement was the transitory elevation of the OCT.

Gradual improvement in the patient continued. Twelve weeks following the second transplant no evidence of skin rash was present and scalp hair, eyebrows, eyelashes and new nail growth was present (Fig. 12). Diffuse skin pigmentation was observed. Hypertension gradually decreased and was normal 20 weeks following the second transplant. The patient continues to do well 1 year following the second transplant.

Siblings of patient

Je.F., the sister of the patient, was evaluated at age 2½ years for recurrent otitis media. Initial studies revealed lymphopenia, neutropenia and eosinophilia. Between the ages of 2 and 4 years she had numerous episodes of fever, otitis media and pneumonia. Death occurred at 4½ years following severe varicella complicated by varicella, pneumonia and staphylococcal sepsis. Absence of serum IgA was demonstrated at this time.

An autopsy showed a small thymus with absence of Hassall's corpuscles, lymphoid depletion, and absence of corticomedullary differentiation (Fig. 13). The lymph nodes showed marked depletion of lymphocytes in the thymic dependent area (paracortical). Plasma cells were abundant and normal primary and secondary follicular formation was seen. Ischemic necrosis with type-A intranuclear inclusions were observed throughout the viscera.

Ed.F., the brother of the patient, contracted varicella at the same time as his sister and died from an overwhelming infection with varicella pneumonia and pseudomonas sepsis. The patient was shown to have lymphopenia, neutropenia, eosinophilia and absent serum IgA. An autopsy revealed essentially similar findings to Je.F. In addition, inclusions characteristic of cytomegalic inclusion cell disease were observed. These two patients were reported in detail in a previous communication (Hoyer *et al.*, 1967).

DISCUSSION

Immunologic reconstitution of our patient was achieved utilizing two separate allogeneic bone marrow transplants from a normal sibling shown to be identical to the patient at the

HL-A locus by cytotoxic assay (Terasaki, Vredevoe & Mickey, 1967), and mixed leucocyte culture (Bach & Voynow, 1966). In addition the patient and sibling were determined to be identical in the major and minor blood groups and Gm and Inv factors tested.

A bone marrow transplant of $1{\cdot}25 \times 10^9$ nucleated cells was given at 6 months of age. Immunologic reconstitution was inadequate following the first transplant but some signs of improvement were noted: 1—improved disposition and appetite, 2—gradual fading and disappearance of rash, 3—appearance of scalp hair, eyebrows and eyelashes, 4—return to normal of platelets and eosinophils, 5—response to PHA, although suboptimal, 6—positive skin reaction to DNFB. Our interpretation of these events is that in the first transplant of immunocompetent cells and stem cells too few cells were given. In addition, the marrow was obtained from only two sites and most likely was diluted with peripheral blood.

After the second graft was given (5 months following the first transplant, age 11 months) evidence for further immunologic reconstitution was obtained. The lymphocytes transformed normally when stimulated by PHA, isohaemagglutinins were present (Table 1) and the immunoglobulins increased to (mg/100 ml) IgG 720, IgM 43, IgA 110 (Fig. 1). As the patient and the donor were identical in all antigens tested and both were females, no markers were present to permit detection of donor cells. The disposition and appearance of the patient continued to improve and weight gain was observed.

Spontaneous improvement is a consideration but we know of no cases of spontaneous conversion of a cellular immunity defect. Further, it seems unlikely that all of the tests of immunologic function could have reverted to normal in a period of 1 week as a matter of coincidence. The persistence of these responses and the continued increase of immunoglobulin levels indicates that this was not simply a passive transfer of immunocompetent cells but a true engraftment and establishment of a replicating cell line. In addition, the presence of a prolonged GVH reaction indicated persistence of the immunity defect of the host cells.

Because most GVH reactions occur 1–2 weeks following transplantation (Hathaway *et al.*, 1966; Rosen *et al.*, 1966; Miller, 1967), it was felt that our patient had passed the critical period. However, 40 days following the second transplant the first evidence of GVH appeared.

The diagnosis of GVH mediated by immunocompetent donor cells was based on the characteristic skin rash (Fig. 7) and skin biopsy showing lymphocyte infiltration of the epidermis, dyskeratosis, basal cell vacuolization and thickening of the epidermis (Fig. 10), (Kodawaki *et al.*, 1965). In addition, loss of hair, eyebrows, eyelashes, fever, thrombocytosis, eosinophilia and elevated liver enzymes occurred. Although more severe than observed in our previous case, the GVH was not fatal and spontaneously improved without significant therapy. It seems to us unlikely that the brief period of prednisone therapy had a significant influence on the course. During the entire course of the GVH a positive NBT test ($>25\%$ reduction) was observed. This test which depends on the reduction of NBT by cells which are actively phagocytizing is usually negative in viral infections ($<10\%$ reduction) but positive in bacterial infections (Park, Fikrig & Smith, 1968; Park *et al.*, 1969). It is unlikely that active bacterial infection could have been present in the patient for a period of 3 months (all cultures were normal and a lung biopsy for Pneumocystis carinii was negative). Since NBT reduction is also seen in the phagocytosis of particulate matter (Windhorst *et al.*, 1968), it is possible that the destruction of host cells occurring during a GVH reaction yields products which are eliminated by a phagocytic mechanism and could account for the

positive NBT test. The exacerbation of the GVH reaction on three separate occasions by either infection (upper respiratory infection, otitis media) or DPT antigen is of great interest. One can only speculate as to the mechanism involved, however it is possible that in all three instances antigenic stimulation of immunocompetent cells could have produced factors which exacerbated the GVH reaction non-specifically, e.g. by means of macrophage proliferation with production of factors involved in inflammatory reactions (Keller, 1968; Schild & Willoughby, 1967).

In addition to the delayed onset of the GVH (40 days) in our patient, a prolonged course was observed. The prolonged course may have been due to the large number of immunocompetent cells given in the second transplant. The delayed onset and prolonged course may also be related in some as yet unknown way.

Modification of the GVH has been attempted by several different means. Theoretically the transplant should contain only the desired stem cells. Separation of stem cells from other immunocompetent cells which could participate in the GVH reaction has been attempted utilizing an albumin gradient (de Konig *et al.*, 1969). Nevertheless the patient described in whom successful marrow transplantation was achieved with such a preparation also experienced a mild GVH reaction. Pretreatment with immunosuppressive agents has been advocated, based upon experience in animal experimentation (Thomas *et al.*, 1962), but limited use of these agents in man has not yet proved effective (Mathe *et al.*, 1969). Antilymphocyte serum treatment of the donor marrow was utilized in one of our previous cases but experience with this method in man is also limited. Careful antigenic matching is a necessity if GVH reaction is to be avoided or kept to a minimum in bone marrow transplantation. In our patient identity of the recipient with the donor was established utilizing a cytotoxic assay (Terasaki *et al.*, 1967), and mixed leucoyte culture (Bach & Voynow, 1966). Not all white cell antigens are detected by these techniques however, and further, the sensitivity of the mixed leucocyte culture is insufficient to indicate differences in minor (non HL-A) loci. Currently a 'mild' GVH reaction may have to be accepted as an unavoidable complication of bone marrow transplantation prior to the establishment of a tolerant state. We believe that optimal testing includes evidence of donor and host match on both cytotoxic and mixed leucocyte assays. Of the two assay systems the mixed leucocyte culture seems more discriminating since antigenic differences may be detected by this method which cannot yet be detected by cytotoxic assay.

Severe GVH reactions are associated with characteristic features such as skin rash, fever, hepatosplenomegaly, diarrhoea and jaundice. In our patient skin rash was the predominant feature. No gastrointestinal tract symptomatology nor jaundice was present and fever, splenomegaly and elevated liver enzymes were only transiently present. Hair loss, not previously emphasized in man but a predominant feature in protracted GVH in animals appeared to be an important feature of the GVH reaction in our case. Return of hair and nail growth coincided with improvement in the patient. In addition, certain laboratory studies appear to be sensitive indicators of the onset and severity of the GVH. Eosinophilia and thrombocytosis preceded clinical symptomatology of GVH, increased with the severity of the GVH, and returned to normal more slowly than the clinical findings. It is possible that thrombocytosis may be an early and sensitive indicator of GVH. The finding of thrombocytosis in autoimmune disease (Bean, 1965) indicates that intense immunologic reactivity may be associated with this phenomenon.

The long term effects and complications of chronic GVH are unknown. Although our

patient is currently doing well, low grade hypertension persisted for several months perhaps representing a complication of GVH reactions in those who survive. Renal involvement in fatal GVH has been described (Miller, 1967); however, our patient had normal renal function tests and urinalysis. A renal biopsy was not performed.

Certain features of the patient's initial presentation suggest that a GVH reaction may have been present shortly after birth. The diffuse maculopapular rash was similar to that observed following the second transplant which resulted in a GVH reaction. The patient also lacked scalp hair, eyebrows and eyelashes. A rectal biopsy showed inflamatory cell infiltrate in the mucosa and blood eosinophilia and thrombocytosis were present. Following the first transplant, hair, eyebrow and eyelash growth occurred and the skin rash resolved. Eosinophilia and thrombocytosis decreased (Fig. 2). The initial findings were interpreted as evidence of a GVH reaction, the graft being of maternal origin. The clinical picture was highly reminiscent of the case reported by Kodawaki *et al.* (1965). Also consistent with a GVH reaction in early life was the finding of low levels of all three immunoglobulins rather than the pattern of IgA deficiency seen in the two other siblings. In Kodawaki's case, it was suggested that the graft was able to produce small amounts of immunoglobulins. Unfortunately, no erythrocyte or γ-globulin antigenic markers could be employed in our case to verify this assumption.

The exact classification of the immunologic defect in the patient and her siblings is difficult. The syndrome consists of thymic hypoplasia, isolated IgA deficiency, lymphopenia, neutropenia, eosinophilia and defective humoral and cellular immunity. It is not apparent what form of inheritance exists in these patients but the involvement of both male and female siblings in a single generation would be compatible with autosomal recessive inheritance.

The ethical and legal implications of bone marrow donation from children is a subject of proper concern. Several aspects of marrow transplantation make its utilization different from that of organ transplantation. So far as is currently known, the removal of bone marrow has no detrimental effect on the donor, and further, unlike removal of organs, leaves the donor with a remaining replicating cell population. Although the use of non-sibling donors has resulted in some successful renal transplants (Barnes, 1965), bone marrow transplantations from non-siblings have resulted either in failure of engraftment or fatal GVH reactions.

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