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Circulating thymic hormone levels in severe combined immunodeficiency

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SUMMARY

Twenty-three patients with severe combined immunodeficiency disease were studied for circulating thymic hormone levels (facteur thymique serique, FTS), 21 prior to treatment by transplantation of bone marrow, thymus or fetal liver. Thirteen showed undetectable FTS activity. Only two had normal levels of this hormone. In serial determinations of FTS activity prior to and after transplantation, patients given bone marrow transplants developed sustained increments of serum FTS activity early in the course of their immunological reconstitution. However, patients given transplants of fetal liver alone or fetal liver plus thymus from fetuses of less than 12 weeks gestation generally did not show an increment of FTS activity during the period of observation. Transplantation of irradiated thymus derived from fetuses of more than 14 weeks gestation produced sustained increases of thymic hormone activity. These observations suggest that a cell of haematopoietic origin provides a stimulus necessary for differentiation or maturation of thymic secretory activity and that this cell(s) is present in post-natal marrow, but is either undeveloped or immature in the early fetal liver or fails to migrate to the thymus of an allogeneic host.

Keywords serum thymic factor FTS SCID transplantation

INTRODUCTION

Severe combined immunodeficiency disease (SCID) derives from a heterogeneous group of disorders characterized by profound deficiencies of both humoral and cell-mediated immune functions (WHO Scientific Group on Immunodeficiency, 1979). The primary deficiency of several types of SCID has been attributed to abnormalities intrinsic to lymphoid precursors (Pahwa *et al.*, 1978a), but an abnormality of the thymus has also been suggested as the basic defect in some patients (Pyke *et al.*, 1975). Whatever the primary defect, the morphology of the thymus is regularly abnormal (Hoyer *et al.*, 1968). Therefore, it seemed of particular importance to assess thymic function *in vivo* by evaluation of levels of circulating thymic hormones in sera of patients with SCID.

The serum thymic factor (FTS) is a thymic epithelium derived nonapeptide (Bach *et al.*, 1977; Monier *et al.*, 1980; Savino *et al.*, 1982) that is capable of inducing various T cell markers and functions (Bach *et al.*, 1978; Incefy *et al.*, 1980). FTS can be quantitated in serum or thymic extracts by a bioassay, the rosette inhibition assay (Dardenne & Bach, 1975). FTS activity may be considered to be one measure of the secretory function of the thymic epithelium.

In preliminary studies, we reported FTS deficiencies in the sera of two of four patients with SCID (Incefy *et al.*, 1977). In this report, we present analyses of FTS activity in the sera of 23

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patients with SCID, prior to and following transplants of bone marrow, fetal liver in combination with fetal thymus, irradiated fetal thymus or cultured thymic epithelium, and examine the relationship between engraftment of haematopoietic cells and regeneration of both FTS activity and T cell functions.

MATERIALS AND METHODS

FTS activity was determined in the sera of SCID patients by the rosette inhibition assay of Dardenne & Bach (1975) with minor modifications (Iwata *et al.*, 1981). The assay is based on the ability of FTS to render θ-negative rosette forming cells from the spleen of adult thymectomized mice θ-positive and sensitive to azathioprine (AZ).

To ascertain that the serum factor producing rosette inhibition was indeed FTS, aliquots of the serum filtrates were incubated overnight at 4°C with either specific anti-FTS or a control antibody (anti-α_2-macroglobulin) cross-linked to cyanogen bromide activated Sepharose 4B beads (Dardenne, Pléau & Bach, 1980). After incubation, the suspensions were centrifuged and the activity of the supernatant evaluated by the rosette inhibition assay. Under the conditions of the assay, FTS is completely and specifically absorbed by the anti-FTS immunosorbent, whereas other factors active in the rosette inhibition assay, particularly T lymphocyte derived allogeneic factor, remain unabsorbed in the supernatant.

Lymphocyte transformation responses to PHA or allogeneic cells in MLC were analysed by standard methods (Cunningham-Rundles, Hansen & Dupont, 1976). The number of T cells was evaluated by establishing the number and proportion of lymphocytes that form rosettes with sheep erythrocytes (E rosette) (Bentwich *et al.*, 1973). B cells were enumerated by standard methods for detecting lymphocytes bearing surface immunoglobulin using fluorescent $F(ab')_2$ antiglobulin reagents (Preud'homme & Seligman, 1972; Aiuiti *et al.*, 1974).

Patients. Experimental observations were made in 23 patients with SCID. The diagnosis of SCID was based on the absence of organized lymphoid tissue, failure of lymphocytes to transform in response to mitogens, antigens or allogeneic cells, and absence of delayed type hypersensitivity reactions or antibody responses following *in vivo* antigenic stimulation. Five patients had a classical form of SCID characterized by a profound deficiency of both T (E rosette positive) and B (sIg positive) lymphocytes, and agammaglobulinaemia. Three patients had SCID with adenosine deaminase (ADA) deficiency; one patient had SCID with the cartilage hair hypoplasia. The remaining 14 patients had SCID without ADA deficiency but with variable numbers of B lymphocytes.

Therapeutic measures. Of the 21 patients evaluated prior to transplantation, nine were given transplants of bone marrow (BM) from an histocompatible donor.

Seven patients, for whom no histocompatible marrow donor could be identified, were transplanted with a cell suspension from the liver (FL) of a fetus which differed from the recipient at all serologically detectable loci of the major histocompatibility complex HLA A,B,C (fully allogeneic). The transplanted livers were obtained from fetuses of less than 12 weeks gestation following elective hysterotomy or prostaglandin-induced abortions. At this stage of embryogenesis, the fetal liver represents a rich source of normal lymphoid precursors, but is essentially free of thymus differentiated lymphocytes capable of inducing lethal GvHD (O'Reilly *et al.*, 1980.) Five of the seven patients were given multiple sequential transplants of fetal liver before engraftment was achieved. Sustained chimerism with lymphoid elements bearing the HLA phenotype of one of these transplants was eventually achieved in five of the seven patients. Details regarding these transplants will be presented in a separate report to be presented soon.

Twelve patients received one or more transplants of thymic tissue to provide a milieu for lymphoid differentiation and to correct observed deficiencies of thymic secretory function. Five of these patients received no additional transplant during the time of observation. The other seven patients also received fetal liver grafts.

Three types of thymic graft were used. Three patients received transplants of thymus derived from the same fetus used as the source of fetal liver cells (FLT). These thymuses were epithelial in

histology and of less than 12 weeks gestation. Nine patients were given one or more transplants of lymphoid thymus (FTx) derived from fetuses of more than 14 weeks gestation and irradiated with 800–1,000 rad to eliminate the capacity of the transplanted thymic lymphocytes to proliferate and induce GvHD. Five patients were transplanted with cultured thymic epithelium (CT) prepared according to the method of Pyke *et al.* (1975). Fetal thymus, cut into fragments, was administered as an intramuscular implant. Cultured thymic epithelial transplants were injected intraperitoneally.

Immunological reconstitution achieved following transplantation was characterized according to the degree of immunological functions. Reconstruction of humoral immune functions (B lymphocytes) and cell-mediated immune functions (T lymphocytes) were graded according to criteria previously reported (Incefy *et al.*, 1981) and summarized below:

Humoral immunity:	4^+	3^+	2^+	1^+	*0*
Concentrations of immunoglobulins (IgG, M, A)	normal	IgG, IgM, normal	increased but low for age	increased but low for age	no change
Antibody responses to immunizations	normal	subnormal	weak	none	none
Cell-mediated immunity:					
E rosette+ lymphocytes	normal	normal	normal	increased but low for age	deficient no change
Transformations to mitogens, antigens	normal	>30% of normal	<30% of normal	none	none
Delayed type hypersensitivity (DNCB, DT, Candida)	present	present	none	none	none

RESULTS

The serum titres of FTS detected in each of the 21 patients studied prior to transplantation are depicted in Fig. 1. Patients are segregated according to variant of SCID, and compared with an age matched group of normal children (less than 10 years of age). As can be seen, 13 of 21 patients had no detectable FTS activity (< 1/4). Six had low but detectable activity, including each of the patients

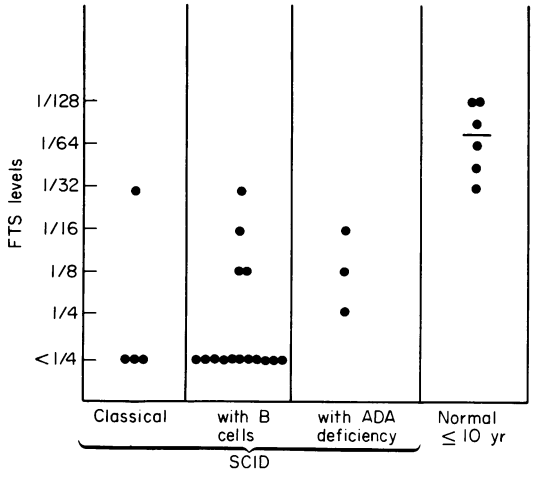

Fig. 1. Serum titres of FTS activity in 21 patients with SCID studied prior to transplantation. Patients are segregated according to variant of SCID and compared to normal children ≤10 years of age.

Table 1. Patients with FTS deficiency transplanted with bone marrow. Levels of FTS activity in the sera pre- and post-transplantation

Patients (≤3 years old)	Before transplants: SCID ADA	SCID B cell	SCID Ig	FTS levels	After transplants: No. and type of transplants*	Immunological reconstitution† T cells	Immunological reconstitution† B cells	FTS levels
UPN 33‡	+	+	−	<1/4	1BM	4+	4+	1/128
UPN 51	+	−	−	<1/4	1BM	4+	3+	1/128
UPN 1	−	−	−	1/8–1/16	3BM	4+	4+	1/32
UPN 14	−	+	+	1/4–1/8	1BM	4+	4+	1/64
UPN 21§	+	+	+	≤1/4	1BM	4+	4+	1/32–1/64
UPN 85	+	+	+	<1/4	1BM	4+	4+	1/32
Normals (≤10 years old)				1/32–1/128				

* BM = bone marrow transplantation.

† Immunological reconstitution is graded as described in Methods and in more details elsewhere (Incefy *et al.*, 1981).

‡ UPN = unique patient numbers.

§ Patient with neutropoenia and cartilage hair hypoplasia anomaly.

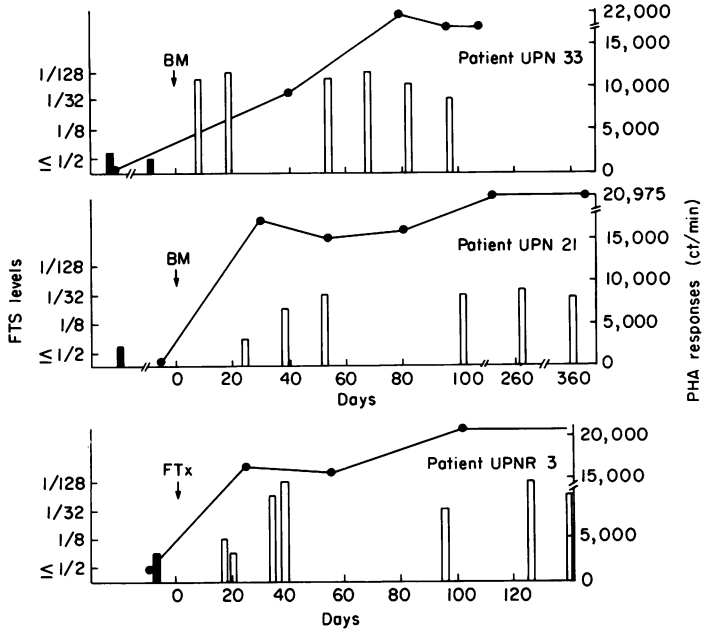

Fig. 2. Serial determinations of FTS activity in the sera of three SCID patients; two were studied before and after bone marrow transplantation (BM) and the other before and after irradiated fetal thymus transplantation (FTx). The patients' UPN numbers are the same as those in Tables 1 and 2. PHA responses (●—●).

with ADA deficiency (1/4–1/16). Only two patients had normal titres of FTS prior to transplantation (1/32).

Transplantation of bone marrow alone led to normalization of serum levels of FTS activity in six of six patients found to be deficient in FTS activity prior to transplantation (Table 1). As shown in Fig. 2, deficiencies of circulating FTS activity were corrected 8–52 days following bone marrow transplantation. In each case, FTS levels have remained normal through the post-transplant period, that is, for intervals ranging from 6 to 27 months. Full immunological reconstitution has been achieved in each of these patients.

As summarized in Table 2, four patients (UPN4, 30, R2, 43) with FTS deficiency were initially transplanted with liver alone (UPN4, 30, R2) or liver plus thymus (UPN43) from fetuses of less than 12 weeks gestation. Three patients (UPN4, R2, 43) were engrafted and evaluable, of whom two (UPN4, 30) failed to achieve sustained recovery of FTS activity despite engraftment of fetal lymphoid elements. The third patient (UPNR2) was found to have normal FTS levels on a single sample obtained 124 days after fetal liver transplantation (75 days after engraftment had been documented.)

Two patients with a deficiency of FTS (UPN43, 32) received unirradiated thymus transplants (FT) derived from fetuses of 12 weeks or less gestation, either alone (UPN32) or in combination with liver of the same fetus (UPN43). These transplants failed to alter the deficiency of serum FTS. In contrast, six of the nine evaluable irradiated lymphoid thymus grafts derived from fetuses of ≥ 14 weeks gestation administered to six patients (UPN30, 43, 32, R3, 52, 75) with pre-existing deficiencies of circulating FTS activity, induced rapid and sustained normalization of FTS levels. As

Table 2. Patients with FTS deficiency transplanted with fetal or cultured tissues. Levels of FTS activity in the sera pre- and post-transplantation

		Before transplants			After transplants					
								Immunological reconstitution†		
Patients	ADA	SCID B cells	Ig	FTS levels	Patient's age at transplant (months)	Type of transplants*	Gestational age (weeks)	T cells	B cells	Peak FTS levels
UPN 4	+	+	–	≤1/4	8	FL	8	–	–	1/4
					8·5	FL	12·5	–	–	1/4
					11·5	FTx	18	–	–	ND‡
					13	FL	9·5	4+	–	1/32
UPN 30§	+	+	–	<1/4	13·5	2FL	11	–	–	ND
					14·0	FTx	16	–	–	1/128
					14·5	FL	11	–	–	1/64
					18·5	FLT	11·5	–	–	1/64
UPNR 2¶	+	+	–	<1/4	1	1FL	11	–	–	ND
					5	1FL	11	–	–	1/32
UPN 43	+	–	–	≤1/4	10	FLT	11·5	–	–	1/4
					13	FTx	16	–	–	1/16
					15	FTx	20	3+	–	1/64—1/128
UPN 32	+	+	+	1/4–1/8	11	FT	16	–	–	1/4
					12·5	FT	12	–	–	1/4
					16·5	CT	adult	–	–	1/32→1/4
				1/4	25	FLT+FTx	10·5,20	–	–	1/4
				1/4	27	FL	13	–	–	ND
				1/4	31	FTx	14	–	–	1/128
UPNR 3	–	+	–	1/4	13	FTx	14·5	2+	1+	1/128
UPN 52	+	+	+	<1/4	10·5	FTx	16·5	–	–	1/64
UPN 75	+	+	–	<1/4	12	FTx	16·5	–	–	1/128
UPNR 4	+	+	+	<1/4	18	CT	adult	3+	2+	1/128

* Type of transplants (donor of engrafted lymphocytes): FLT = fetal liver plus thymus from the same fetus; FL = fetal liver; FT = fetal thymus; FTx = irradiated fetal thymus; CT = cultured thymic epithelium.

† Immunological reconstitution is graded as described in Methods and elsewhere (Incefy *et al.*, 1981).

‡ N.D. = not done.

§ Patient with T cells (E rosetting lymphocytes) of maternal origin.

¶ UPNR = unique patient number referral.

shown in Fig. 2 (i.e. UPNR3), FTS activity reached normal levels between 8 and 33 days after transplantation. Normal titres of FTS activity have been maintained for periods from 4 to 32 months post-transplantation. Two patients (UPN32, R4) with deficient thymic function received cultured thymic epithelial grafts (Table 2). A sustained increment in FTS activity was observed in one patient (UPNR4). However, this patient was also shown to be engrafted with lymphocytes derived from the cultured thymus.

The activity in the sera of the patients was confirmed to be exclusively FTS by specific anti-FTS immunoabsorptions in the 13 of 16 patients studied post-transplantation. Two of the remaining three patients (UPN3, R2) had both FTS and allogeneic factor in the serum. In only one patient (UPN30) was there evidence that the FTS like activity was attributable to allogeneic factor and not FTS. This patient is unique in that both prior to and following transplantation, the patient was found to be engrafted with immunoincompetent histoincompatible T lymphocytes of maternal origin (Pollack *et al.*, 1980). GvHD was not detected either before or after transplant. Since engraftment of fetal liver and thymus derived lymphocytes was never observed in this patient, the allogeneic factor produced in response to transplantation was possibly generated by T lymphocytes of maternal origin.

The development of FTS activity post-transplant was also correlated with the generation of PHA responses. For illustration (Figs 2 & 3) we have chosen patients from each transplant group. Patients UPN33, 21 (Fig. 2) were given BM transplants. In these patients, reconstitution of PHA responses was not dependent on antecedent restoration of normal levels of FTS activity. Patients given an irradiated lymphoid fetal thymus transplant without further treatment enjoyed a rapid and sustained increase of FTS activity following transplantation. However, development of responses to PHA or allogeneic cells was observed only in the ADA deficient patient UPNR3.

Patients receiving transplants of liver and prelymphoid thymus from fetuses of < 12 weeks gestation are illustrated in Fig. 3. UPN43 was engrafted with liver and thymus from the same $11\frac{1}{2}$ week old fetus (Table 2). This patient engrafted but did not achieve a sustained recovery of FTS activity or show restoration of immune function. Transplantation of an irradiated thymus from a 16 week old fetus 85 days after the initial graft did not correct the deficiency of FTS activity. However, following administration of a second irradiated thymus graft, FTS levels rose immediately, decreasing only during treatment of acute GvHD. The initial spike of FTS activity following the second irradiated thymus graft was followed by increments in the number of circulating E rosette forming lymphocytes and in proliferative responses to mitogens and allogeneic cells. These T cell functions were also depressed during GvHD, but increased steadily once this process resolved. Patient UPN4 initially received two fetal liver grafts (Table 2). Lymphoid cells bearing the HLA phenotype of the second transplant were detected in the blood 2 weeks after transplantation.

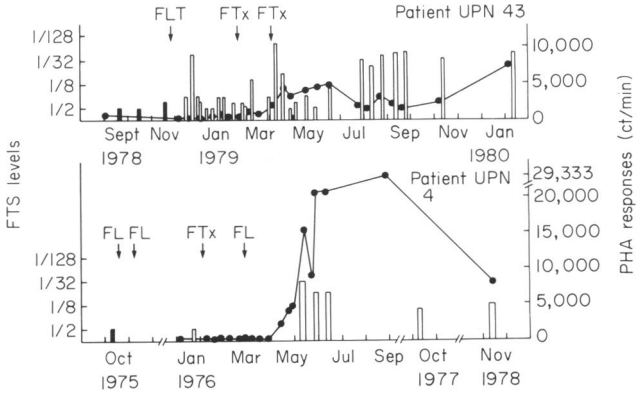

Fig. 3. Serial determinations of FTS activity in the sera of two SCID patients before and after fetal liver plus thymus (FLT) transplantation or fetal liver (FL) followed by irradiated fetal thymus transplantation (FTx). The patients' UPN numbers are the same as those in Table 2. PHA responses (●—●).

Chimerism persisted for an additional 6 weeks without evidence of immune reconstitution. FTS levels remained deficient. An irradiated fetal thymus was then administered to correct this deficiency. A subsequent fetal liver transplant engrafted and produced full reconstitution of T cell functions. Unfortunately, FTS levels were not obtained between the fetal thymus and liver grafts, but were normal thereafter. Although the correction of FTS deficits cannot be ascribed with certainty to either of these two transplants, our results with fetal liver and irradiated thymus grafts in other evaluable patients would suggest the thymus graft to be responsible for this event.

DISCUSSION

Our findings demonstrate that patients with severe combined immunodeficiency disease usually have markedly deficient thymic secretory function as revealed by levels of FTS in circulating blood. Indeed, FTS was not detectable in the serum of 13 of 20 patients studied. Deficiencies in patients with the ADA positive form of SCID are in keeping with the embyronic nature of the thymus in these patients (Cottier *et al.*, 1968; De Vries, Dooren & Cleton, 1968). The low but consistently detectable levels of FTS in ADA deficient SCID patients support morphological evidence of thymic development in this form of SCID (Meuwissen, Pollara & Pickering, 1975).

In following an individual patient's course which may involve multiple transplants, it is sometimes difficult to establish whether a change in FTS levels is a direct sequela of a particular transplant, or the end result of a series of biological events in the post-transplant course. However, in each of six evaluable patients, transplantation of marrow alone was followed by a rapid and sustained normalization of FTS activity in the serum.

Correction of deficiency of FTS production was documented in only one of three patients (UPNR2) who had been engrafted with lymphoid precursors derived from a fetal liver of 11 weeks gestation, and this correction was demonstrated only late (2–5 months) after engraftment had been established.

The functional development of the thymic epithelium initiated by thymic infiltration with lymphoid or other haematopoietic elements is further suggested by the contrast between the rapid and durable normalization of serum FTS levels which followed transplantation of irradiated lymphoid thymus from fetuses of greater than 14 week gestational age and the persistent deficiencies of FTS observed following engraftment of pre-lymphoid epithelial thymus from fetuses of less than 12 week gestational age (UPN43, 32).

Taken together, these observations suggest that a stimulus supplied by a cell or cells of haematopoietic origin is necessary for differentiation or maturation of thymic secretory functions, and that the initiator cell(s) is/are present in the marrow of normal children and adults but may not be present in the early fetal liver or pre-lymphoid thymus in an appropriate state of differentiation to exert this influence. Disruption of the fetal liver prior to transplant might abort or delay differentiation of this cell, explaining the absence or delay of FTS secretion in recipients of fetal liver grafts. Alternatively, these cells, because of their histoincompatibility with the host, might fail to traffic to or interact with the thymic epithelium, a phenomenon well documented in murine systems (Stutman & Good, 1969).

If the normal lymphoid precursor is the source of this stimulus, deficiencies of FTS observed in SCID might be another sequela of abnormalities intrinsic to these lymphoid progenitors. Alternatively, other cells of haematopoietic origin might supply this stimulus, such as the thymic dendritic or reticular cell, a cell of haematopoietic origin which has been shown by Janossy, Pizzolo & Thomas (1980) to infiltrate the embryonal thymus immediately before lymphocytes begin to be differentiated by this organ.

An abnormality of thymic maturation mediated by a deficiency of accessory inducing cells might explain the apparent paradox presented by the two exceptional patients with SCID studied by Pyke *et al.* (1975) and Pahwa *et al.* (1978b) who, on the one hand, possessed lymphoid precursors which could be induced to express T cell markers and functions when exposed *in vitro* to a normal thymic epithelium, yet also had embryonal thymuses which, when cultured *in vitro* with marrow

cells from normal individuals, would mature histologically and successfully induce the functional development of the normal T lymphocyte precursors.

Correction of FTS deficiency in transplant recipients with SCID clearly did not correlate with T cell reconstitution. For example, irradiated thymus from older fetuses restored FTS levels in six of nine cases, but was associated with partial restoration of T lymphocyte numbers and functions in only one case (Fig. 2). Furthermore, restoration of PHA responses in marrow transplant recipients in some instances antedated development of serum FTS activity (Fig. 2), probably reflecting the presence of mature T cells in the marrow graft. However, no patient who achieved immunologic reconstitution did so without correction of FTS deficiency. Furthermore, patients whose restored immune function was mediated by their own (UPNR3) or fetal liver derived cells (that is, cell populations formerly lacking mature T cells), did not develop function until FTS deficiencies were partially or fully corrected, suggesting that the functional development of these immature lymphoid cells was dependent on the thymus or its products.

Our studies thus demonstrate that most patients with SCID have a deficiency of FTS, and that this deficiency can be corrected either by supplying a stimulus to the host thymus by transplantation of normal marrow elements, or by transplantation of mature lymphoid thymus tissue.

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