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Thymic activity in severe combined immunodeficiency diseases

(thymic hormone/human marrow cells/differentiation)

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ABSTRACT **Thymic function was evaluated by quantitation of circulating thymic factor in patients with several forms of severe infantile immunodeficiency diseases. Direct quantitation of thymic factor in serum of patients with severe combined immunodeficiency revealed heterogeneity of this syndrome by this parameter, as was also shown by study of susceptibility of the marrow cells to differentiation *in vitro*. Thymic factor was not detectable in one patient with severe combined immunodeficiency, but was present in normal or near-normal concentrations in three others. Circulating levels of this hormonal activity were also not detectable in a patient with DiGeorge athymic syndrome. Following marrow or fetal liver transplantation, which corrected the severe combined immunodeficiency, thymic factor levels either increased slightly or did not change appreciably. Fetal thymic transplantation, which together with fetal liver transplantation corrected the immunodeficiency in one patient with severe combined immunodeficiency, was associated with increase of thymic factor to normal levels. Fetal thymus transplantation alone, which was employed to correct the immunodeficiency of DiGeorge athymic syndrome, caused an increase in thymic factor activity to normal or near normal levels in this patient.**

Presence of a functional thymus in children with severe combined immunodeficiency (SCID) has not been extensively investigated. The defect in humoral and cell-mediated immunity in these patients has been attributed to either a lack or an abnormality of lymphoid stem cells in their marrow. Normal human marrow cells can be differentiated *in vitro* into cells bearing thymus-derived (T)-lymphocyte characteristics by thymic extracts or purified peptides (1–3). In contrast, narrow cells from patients with variants of SCID are either refractory to such differentiative influences or capable of only partial differentiation, limited to appearance of human T-lymphocyte antigenicity (HTLA) marker (4). Prior studies have not, however, permitted evaluation of the thymic function *in vivo* in this apparently heterogenous group of patients. Lack of full differentiation to normal T-cell function, which is sometimes observed following transplantation of stem cells from bone marrow or fetal liver to correct the immunodeficiency, might reflect the absence of a normally functioning thymus (5). It is especially important, then, to study thymic function in patients with these diseases, because the thymus is often small and poorly developed, and the small thymus shows considerable morphologic heterogeneity (6).

We have now extended our studies of patients with various forms of immunodeficiency, using direct determination of circulating thymic factor (TF) in serum (7). This parameter was investigated in five patients with SCID and one with thymic hypoplasia or DiGeorge syndrome. Serum TF was measured at different times before and after bone marrow, fetal liver, fetal liver plus thymus transplantation, or thymus transplantation only. The direct analysis of serum TF activity could provide yet another means to evaluate the presence of a functional thymus before transplantation. In certain patients with immunodeficiency, either SCID or DiGeorge syndrome, recognition of a functional thymic deficiency could establish a need for transplantation of a thymus either alone or along with fetal hematopoietic stem cells. Further, measurement of serum TF activity after thymic transplantation should permit evaluation of the influence of the thymus graft.

Abbreviations: B lymphocytes, bone-marrow-derived lymphocytes; BMT, bone marrow transplantation; FLT, fetal liver transplantation; FTT, fetal thymus transplantation; FL+TT, fetal liver plus thymus transplantation; HLA, human leukocyte antigens; HTLA, human T-lymphocyte antigenicity; MLC, mixed leukocyte culture; PT, parathyroid transplantation; SCID, severe combined immunodeficiency diseases; T lymphocytes, thymus-derived lymphocytes; TF, circulating thymic factor.

MATERIAL AND METHODS

Marrow Cell Differentiation. Differentiation studies on human marrow precursor cells were performed *in vitro* by the procedure of Incefy *et al.* (3, 8). After incubation with human or bovine thymic extracts (9) and purified thymic peptides (10, 11), appearance of T-lymphocyte characteristics on precursor cells was detected by two T-cell surface markers: human T-lymphocyte antigenicity (HTLA phenotypes) (2) recognized by an anti-human T-cell serum in a microcytotoxicity test, and receptors for sheep erythrocytes as detected by the spontaneous E-rosette formation technique with sheep erythrocytes (3).

TF Evaluation. Rosette-forming cells from the spleen of thymectomized mice are less sensitive to azathioprine than are those from normal mice (70 μg/ml required for rosette inhibition instead of 1 μg/ml). Thymic extracts or human and animal sera with TF activity restore normal sensitivity to azathioprine of rosette-forming cells from adult thymectomized mice after a short incubation at 37°. This change is the basis for the biological assay used for evaluation of serum TF activity.

The assay procedure and the mode of preparation of sera for this evaluation have been described in detail by Dardenne and Bach (12). Sera from patients with various immunodeficiency diseases were tested before and at different times after bone marrow, fetal liver, fetal liver plus thymus, or thymus transplantation. Determination of TF activity was performed on coded sera and carried out as a blindfold analysis.

PATIENTS

Sera from five male infants, 1–39 months old, with severe combined immunodeficiency (M.R., S.C., K.M., M.W., and J.J.) were studied for circulating TF activity. Marrow cells from these patients were investigated for their ability to be differentiated *in vitro* by thymic inducers before and after successful reconstitution with bone marrow or fetal liver plus thymus transplantation. Patient M.R. was given seven bone marrow transplants from an unrelated, female donor matched in mixed

Table 1. *In vitro* differentiation of marrow T-lymphocyte precursor cells by thymic extracts, and thymic factor levels in severe combined immunodeficiency and thymic hypoplasia before and after transplantation

Subject	No. and type of transplants*	Age, months	Before transplantation: Inducibility *in vitro* of T-cell markers by thymic inducers: HTLA+ cells	Before transplantation: Inducibility *in vitro* of T-cell markers by thymic inducers: E-rosettes	Before transplantation: Circulating serum thymic factor activity	After transplantation: Inducibility *in vitro* of T-cell markers by thymic inducers: HTLA+ cells	After transplantation: Inducibility *in vitro* of T-cell markers by thymic inducers: E-rosettes	After transplantation: Circulating serum thymic factor activity
Normal								
0–20 years					1/32, 1/64			
20–30 years			+++	+++	1/8, 1/16			
30–40 years			+++	+++	1/4, 1/8			
Severe combined immunodeficiency								
M.R.		2	−	−				
		3.5			1/16, 1/32			
	3 BMT	7						1/16, 1/32
		10.5				+++	+++	
	4 BMT	18.5				+++	+++	
	5 BMT	24						1/16, 1/32
S.C.		1.5	−	−	1/8, 1/16			
	1 BMT	3						1/8, 1/16
	2 BMT	5				+++	+++	1/32, 1/32
	3 BMT	12						1/32, 1/32
K.M.		1	−	−				
		12	+	−	1/16, 1/16			
	1 FL+TT	16.5				+++	+++	
	1 FL + 2 TT	20						1/16, 1/16
	2 FL + 3 TT	28.8						1/32, 1/32
M.W.		7.5	+	−	<1/2, <1/2			
	2 FLT	11				+	−	<1/2, <1/2
	3 FL + 1 TT	16.5				+	±	1/16, 1/16
J.J.								
	FLT	14				+	±	1/12
		14.5						1/12
		15.5						1/16
Thymic hypoplasia								
O.T.		1.5	++	++	<1/2, 1/2			
1 day after	1 FT+PT	2.8						1/8, 1/16
13 days after	1 FT+PT	3.2						1/16, 1/32
19 days after	1 FT+PT	3.7						1/8, 1/16
1 day after	2 FTT	4				+	+	1/8, 1/16
5 months after	2 FTT	9						1/16, 1/16

* Abbreviations for transplantation procedures are: bone marrow (BMT), fetal liver (FLT), fetal thymus (FTT), fetal liver plus fetal thymus (FL+TT), and parathyroid (PT).

leukocyte culture (MLC) and mismatched in human leukocyte antigens (HLA) (13). Patient S.C., who had an apparent adenosine deaminase deficiency in lymphocytes and erythrocytes, received three bone marrow transplants from a MLC- and HLA-matched male sibling donor (14). Patients K.M. and M.W. differed from M.R. and S.C. in that 80% of their circulating lymphocytes had surface immunoglobulins. No specific antibody, however, could be detected in their blood following tetanus or typhoid immunization, and immunoglobulin levels were low (4). Each was given both fetal liver and thymus transplants to correct their immunodeficiency. Patient J.J. was admitted to Memorial Sloan-Kettering Cancer Center after having received a fetal liver transplantation for SCID. He was 11 months old and was not studied prior to transplantation.

Another patient (O.T.) with thymic hypoplasia (DiGeorge syndrome) was studied before and after fetal thymus transplantation.

Serum TF levels were established previously in normal subjects of various ages (7); differentiation studies were conducted on marrow cells of 31 healthy adult volunteers as controls.

RESULTS

In vitro differentiation of human marrow cells by thymic inducers

Summarized in Table 1 are findings obtained with studies of marrow cell differentiation induced *in vitro* by thymic inducers in five children with SCID and one child with DiGeorge syndrome. As shown, cells from these patients were analyzed before and after successful bone marrow transplantation (BMT), fetal liver plus thymus transplantation (FL+TT), or fetal thymus transplantation (FTT) performed to correct their immunodeficiency. Inducibility of marrow cells was evaluated by the appearance of two T-cell surface markers after incubation of cells with thymic extracts or purified peptides (4, 8, 14). The presence or absence of circulating serum thymic factor activities is also indicated in relation to these investigations.

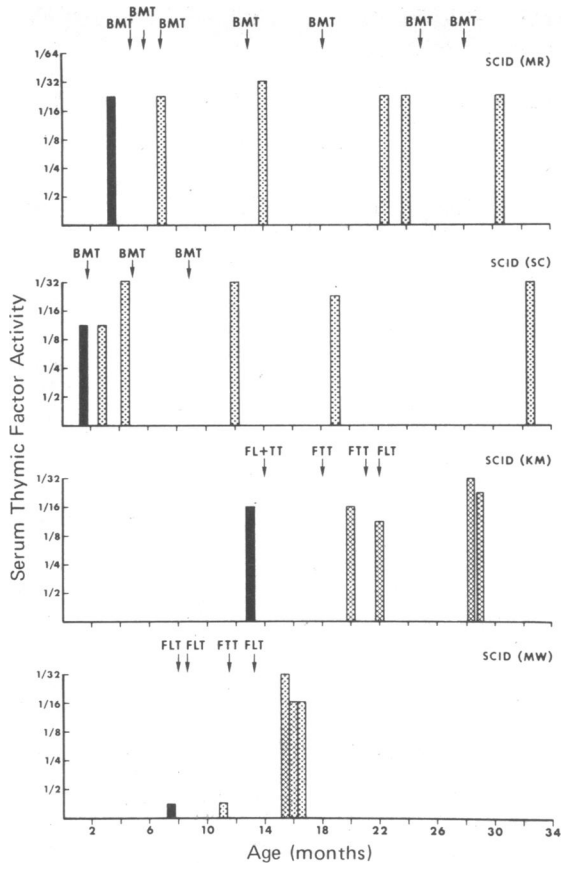

FIG. 1. Circulating thymic factor activity in severe combined immunodeficiency before (solid bars) and after (shaded bars) cellular engineering.

Marrow cells from patients M.R. and S.C. with SCID, the latter with an adenosine deaminase deficiency, could not be induced *in vitro* by thymic extracts known to be active on marrow cells from normal volunteers (14). However, these two children possessed almost normal levels of TF in their blood. After BMT, induction of T-lymphocyte characteristics *in vitro* was observed in cells from their marrows, and TF levels did not change appreciably in patient M.R., but the levels were slightly increased in serum of patient S.C. In contrast, two additional patients with SCID, K.M. and M.W., who had circulating bone-marrow-derived (B) lymphocytes with immunoglobulins at their surface but lacked T-lymphocytes, had marrow cells that could be induced to bear only $HTLA^+$ phenotypes but not sheep erythrocyte receptors (4). After the initial fetal liver plus thymus transplantation, which failed to produce either chimerism or functional immunity, marrow cells from patient K.M. could be induced to develop both T-cell markers. Immunologic reconstitution with sustained chimerism developed following engraftment of a second fetal liver. The latter patient (M.W.), who did not possess detectable levels of TF in his serum before transplant or after two fetal liver grafts, developed almost normal levels of this hormone after having been given a thymus transplant and another fetal liver. This child showed clinical and laboratory evidence of successful reconstitution, but the bone marrow abnormality was not corrected. Marrow cells from another patient (J.J.) were studied for susceptibility to inducers of differentiation only following fetal liver transplantation and showed a weak response. J.J.'s serum TF level was low on three occasions, but activity was not completely absent. Patient O.T., with DiGeorge syndrome, had cells in his marrow that could be induced to differentiate to cells with T-cell markers both before and after fetal thymus transplantation given to correct his immunodeficiency. No serum TF could be detected prior to a thymus graft; however, this hormone was detectable one day following the graft and has been present in the same or greater concentrations ever since.

TF levels in severe combined immunodeficiency diseases

Fig. 1 shows three different patterns of circulating serum TF activity in patients with SCID. Patients M.R. and S.C. had normal or near normal levels of TF before bone marrow transplantation and normal levels of TF following marrow transplantation. Similarly, patient K.M. had near normal levels before transplantation and reached normal levels following fetal liver plus thymus transplantation. In contrast, patient M.W. lacked serum TF activity before transplantation and did not exhibit TF activity in his serum after two fetal liver transplants, the second of which established a chimeric state. This patient, however, acquired normal levels of TF after he was given a fetal thymus transplant which, with the fetal liver transplants, corrected his SCID.

TF levels in DiGeorge syndrome

Fig. 2 represents the marked changes in serum TF activity observed in an infant with thymic hypoplasia. Absence of cir-

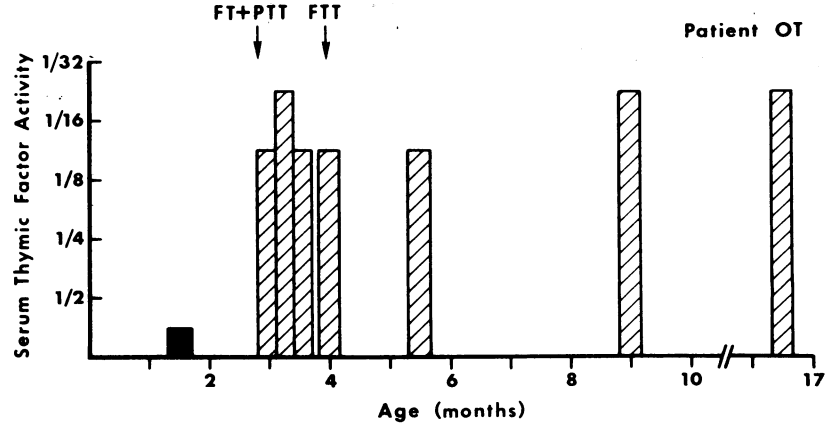

FIG. 2. Circulating thymic factor activity in thymic hypoplasia (DiGeorge syndrome) before (solid bar) and after (hatched bars) thymus transplantation.

culating TF before fetal thymus transplantation and levels increasing to normal after reconstitution are clearly apparent.

DISCUSSION

Our investigations and those of others indicate that the term *severe combined immunodeficiency* encompasses a heterogenous group of disorders. In our prior studies, we showed heterogeneity in the response of marrow of patients with this syndrome *vis à vis* susceptibility of marrow cells to differentiation (4, 8, 14). In one group we found that marrow cells which can respond to induction with thymic extracts or thymic peptides were apparently lacking, and in the other group, in which some B-cell development was evident, we found the marrow cells could be induced to form certain markers but not others (4).

In the present study, by applying a new method for assay of circulating TF (7, 12, 15), we have also obtained additional evidence of heterogeneity in patients with SCID. Three different patterns were observed. One patient (M.R.) had nearly normal amounts of TF, which did not change significantly following immunologic reconstitution with marrow cells from a non-related donor matched at the HLA-B and -D loci. Two other patients (S.C. and K.M.) had somewhat lower levels of TF, which reached normal range in one instance following successful immunologic reconstitution with marrow from a histocompatible sibling donor. The other reached normal range following correction by fetal liver plus thymus transplantation. The third form, in patient M.W., showed the TF to be undetectable before and after engraftment of fetal liver cells. Immunologic reconstitution and establishment of normal TF levels occurred only after transplantation of a fetal thymus followed by engraftment of a second fetal liver.

In the patient with thymic hypoplasia, syndrome of DiGeorge, TF was undetectable, as might have been expected. Following thymic transplantation to correct the immunologic function, TF became detectable 1 day after transplantation and achieved levels slightly lower than normal thereafter. These studies show clear influence of thymic transplantation to correct deficiencies of TF levels. The findings in patient S.C. suggest also that bone marrow transplantation can influence thymic function as assayed in this way. Such an influence could be due to trophic effect of T-cell precursors on thymic development and function, a possible influence that needs further study.

This method of analysis of SCID may well be clinically useful in defining patients with SCID whose thymic function is so defective as to necessitate transplantation of a thymus along with fetal hematopoietic precursors. Coupled with our previously reported assessment of precursor cell abnormalities or potentialities using induction of marrow cell differentiation, variants of SCID potentially correctable by engraftment of a fully differentiated, lymphocyte-depleted thymus might be delineated by this technique (16). These findings establish the clinical validity of the assay used, because the studies carried out in blindfold fashion readily detected the thymic deficiency in a patient with DiGeorge syndrome and revealed the thymic influence reflected in clinical evidence of immunologic reconstitution following thymic transplantation.

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