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Heterogeneity of stem cells in severe combined immunodeficiency

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SUMMARY

Two patients with severe combined immunodeficiency disease (SCID) having variable B-cell development have been shown to have marrow precursors of lymphoid cells which can be induced *in vitro* by thymic factors to express certain T-cell surface characteristics (HTLA$^+$ phenotypes). Their marrow cells could not, however, be induced by these same factors to develop the E-rosette marker or functional activities of T lymphocytes. The marrow of these children also showed, when compared to that of normal adults, a different distribution of cellular elements on density gradient fractionation. The findings support the view that the disorder under study has a different pathogenesis from other forms of SCID previously analysed.

INTRODUCTION

Precursor cells isolated from the marrow of healthy volunteers can be induced to express T-lymphocyte markers and complement receptors upon exposure *in vitro* to various human or bovine thymic extracts (Incefy, Touraine & Good, 1974a) or polypeptides purified from bovine thymus (Goldstein, 1974; Goldstein *et al.*, 1975). Both human thymic lymphocyte antigenicity (HTLA) and spontaneous E-rosette formation with sheep erythrocytes (SRBC) can regularly be so induced (Touraine *et al.*, 1974a; Incefy, L'Espérance & Good, 1975a). By contrast, it was found that marrow cells of three patients with severe combined immunodeficiency (SCID) could not be induced in this way, whereas marrow cells of patients with several other forms of primary immunodeficiency including DiGeorge syndrome, infantile X-linked agammaglobulinaemia and common variable immunodeficiency are inducible by the same influences (Incefy *et al.*, 1974b, 1975c). These findings were interpreted to show that primary immunodeficiencies may be dissected in a new way which could improve understanding of the immunodeficiency diseases.

Much evidence suggests that SCID comprises a heterogeneous group of disorders (Fudenberg *et al.*, 1971; Seligman *et al.*, 1975; Rosen, 1975). Some patients with SCID apparently lack adenosine deaminase, others do not (Giblett *et al.*, 1972). A deficiency of nucleoside phosphorylase has been reported in one child with only T-cell deficiency (Giblett *et al.*, 1975). Pyke and Gelfand recently described a patient with SCID who had B-cell markers and whose marrow cells could be induced to bear T-lymphocyte markers as well as to develop B-cell function by incubation with foetal thymus in tissue culture (Pyke *et al.*, 1975). In pursuing the lead afforded by the inducibility of marrow cells with thymus factors to cells bearing T-lymphocyte markers, we have found two patients with SCID whose marrow cells can be induced by thymic factors to express surface antigenicity characteristic of T lymphocytes but which could not be induced to form spontaneous E-rosettes with SRBC. These findings indicate in still another way that SCID is heterogeneous and can be further dissected by analysis of the responses of marrow cells to the inducing influence of thymic polypeptides and thymic extracts.

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MATERIALS AND METHODS

Two patients were studied and treated at the Memorial Sloan-Kettering Cancer Center (MSKCC), New York. The diagnosis for each was SCID, characterized by marked deficiency of both humoral and cellular immunity.

Case report No. 1. K.M., a 22-month-old white male, was admitted to MSKCC for evaluation at age 3 days because two previous siblings had died of immunodeficiency disease. The first a male, died with disseminated vaccinia following smallpox vaccination (Fulginiti *et al.*, 1968). The second, a female, died in 1967 at 3 months of age with disseminated cytomegalovirus infection. A third female sibling is normal.

Radiologic examination on admission revealed no thymic shadow; absolute lymphocyte counts ranged from 128–2100/mm^3. Immunoglobulins in mg/100 ml were IgG (maternal) of 1000, IgM of 15 and IgA was not detectable. Subsequently, IgG has ranged from 200–700, IgM from 0–165 and IgA from 10–225. No specific antibody was detected following tetanus or typhoid immunization and no isohaemagglutinins (A negative) were present. Of the circulating lymphocytes, 5% formed E rosettes and 80% had surface immunoglobulins. Mitogen responses were repeatedly absent but a minimal response in mixed lymphocyte culture was observed. Red blood cell and lymphocyte levels of adenosine deaminase were normal.

The first marrow study was part of the initial evaluation; the second was done at 12 months of age. The patient had been maintained in sterile laminar flow isolation while a search was made for a matched marrow donor. As this was unsuccessful, he was transplanted with liver and thymus tissues from an 11–12 week foetus at 14 months of age, after institution of germ-free decontamination. The liver cells were given i.v. and the thymus was implanted into the rectus muscle. The third marrow study was performed 2½ months following transplantation of the foetal tissues. Although lymphopenia persisted, 50% E-rosetting lymphocytes were now present. However, neither reconstitution of cellular or humoral immune functions nor development of a chimeric state could be demonstrated at this time.

Case report No. 2. M.W. is a 10-month-old white male born after a normal pregnancy. There are no siblings and no family history of immunodeficiency disease. The child developed oral moniliasis at 2½ months and was admitted to the Henrietta Egleston Hospital for Children in Atlanta, Georgia, at 5½ months suffering from oral and cutaneous moniliasis and pneumonia. Radiological examination demonstrated the absence of thymic and adenoidal shadows. On clinical examination, no lymph nodes were detectable. The lymphocyte count ranged from 1000–2200/mm^3; marrow aspiration showed no plasma cells.

Immunological evaluation revealed E-rosetting cells to be 2·5% and surface immunoglobulin-bearing cells 85% of circulating lymphocytes. Quantitative immunoglobulins in milligrams per 100 ml were: IgG 71, IgM 21 and IgA not detectable. The blood type was O Rh+ and isohaemagglutinins were: anti-A 1:1 and anti-B 1:2. Delayed hypersensitivity skin tests to candida and tuberculin-purified protein derivative were non-reactive. No proliferative response to mitogens or allogeneic cells was demonstrated. Red cell levels of adenosine deaminase were normal. The child was treated for moniliasis, pneumonia and intermittent bacterial sepsis, and for suspected *Pneumocystis carinii* infection. He received monthly injections of gammaglobulin.

At 7 months of age he was transferred to MSKCC where immunological analysis showed 22% E rosettes and 83% immunoglobulin-bearing cells, all of which had IgD specificity and approximately 50% of which had surface IgM. Lymphocytes were repeatedly unresponsive to mitogens, antigens or allogeneic cells. Complement analysis showed the C1q values to be less than two standard deviations below normal while C3 was slightly elevated. It was at this time that the bone marrow study was performed.

Inducers of differentiation. Tissue for extracts was obtained from young children undergoing cardiac surgery who routinely have part of the thymus removed to permit maximum operative exposure. Partially purified human or calf thymic extracts were prepared according to the procedure of A. Goldstein (Hooper *et al.*, 1975) and the protein content of these preparations, fractions 3 or 5 (F3 or F5) was determined using bovine serum albumin as a standard (Lowry *et al.*, 1951). The purified polypeptides, thymopoietin and ubiquitin, were isolated from bovine thymus by methods previously described (Goldstein, 1974; Goldstein *et al.*, 1975).

Precursor cell isolation. Bone marrow from normal human volunteers (aged 21–41 years) was aspirated from several sites on the iliac crest. It was defibrinated with glass beads and precursor cells were isolated by discontinuous density gradient centrifugation according to published procedures (Incefy *et al.*, 1975a).

Induction of markers. Cells from the original suspension prior to fractionation on discontinuous density gradient and those obtained from each gradient layer were resuspended to a concentration of $2{\cdot}5 \times 10^6$/ml in RPMI-1640 medium (Gibco, Grand Island, New York) containing penicillin, 50 u/ml and streptomycin, 50 μg/ml. This preparation of cells was incubated with equal volumes of the putative inducers for 15–17 hr in a humidified 5% CO_2–95% air incubator. After incubation, cells were washed twice in the same medium. The cell concentration was adjusted to 2×10^6/ml and the HTLA$^+$ phenotypes were determined by a microcytotoxicity test in the presence of a specific anti-human-T-cell serum (ATCS) and complement (Touraine *et al.*, 1974a). E-rosette formation with SRBC (Incefy *et al.*, 1975a) and EAC-rosette formation (Ross *et al.*, 1973) were also assayed.

RESULTS

Fractionation of bone marrow cells

Marrow cells were fractionated by discontinuous density gradient centrifugation for each study. Their

relative distribution in each of the five Ficoll gradient layers is shown in Fig. 1 for patient K.M. at age 4 weeks and 12 months (prior to transplantation) and at age 16 months after foetal thymus and liver transplantation. When the patterns of cell distribution in the patient's marrow are compared to the

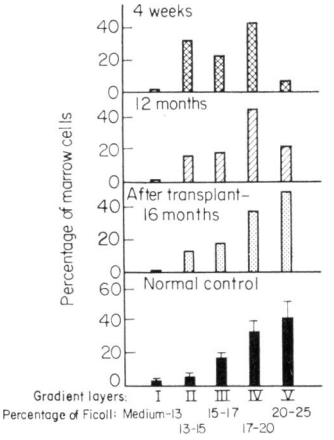

FIG. 1. Relative distribution of marrow cells from patient K.M. and from normal controls obtained after fractionation by discontinuous density gradient centrifugation. The different patterns with cell of K.M. at age 4 weeks and 12 months, and at age 16 months after liver and thymus transplantation from an 11–12-week-old foetus are compared to the mean cell distribution (±s.d.) from eight healthy volunteers (range 21–41 years).

pattern obtained with marrow from eight healthy volunteers (range 21–41 years), irregularity in cell distribution was observed especially at age 4 weeks. This may be an abnormal pattern, but one cannot be certain since normal infants of the same age have not yet been studied; nonetheless, the pattern seemed gradually to change toward that of the adult when the marrow was studied at 12 months. After the transplant, the marrow exhibited the adult cell distribution. Most noticeable in these patterns is the low number of cells found in layer V, particularly at the time of the first study. The missing cell population(s) in this layer increased with age and after transplantation reached the proportion found in adults.

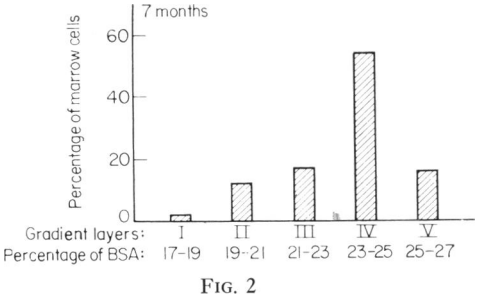

FIG. 2

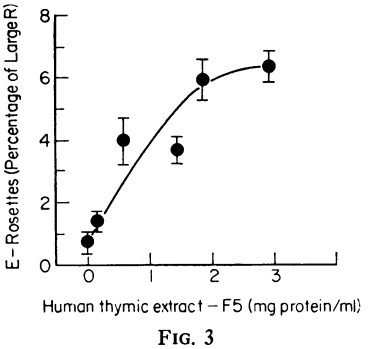

FIG. 3

FIG. 2. Relative distribution of marrow cells from patient M.W. at 7 months of age before transplantation. Cells were fractionated on a BSA discontinuous density gradient centrifugation as described previously (Incefy *et al.* 1975a).

FIG. 3. Response of normal human marrow cells to varying concentrations of human thymic extract. Representative dose–response curve obtained with normal human marrow cells treated with our human thymic extract, fraction 5 (HT-F5) prepared according to Hooper *et al.* (1975). Marrow cells from a 23-year-old healthy volunteer were isolated on a Ficoll discontinuous density gradient by centrifugation and cell concentration was adjusted to $2{\cdot}5\times10^6$/ml. Equal volumes of cell suspension from layers II and III were combined and 100 μl ($2{\cdot}5\times10^5$ cells) incubated 15 hr in a 5% CO_2–95% air humidified incubator with varying amounts of HT-F5. Cells were washed with RPMI-1640 by slow centrifugation at the end of the incubation and induction of T-cell markers was determined by spontaneous E-rosette formation with SRBC.

In a similar manner, the distribution obtained with marrow cells from our second child, M.W., at age 7 months is shown in Fig. 2. This was the only time his marrow was studied by this procedure and the cell distribution obtained in the five BSA gradient layers closely resembled that observed with the marrow of patient K.M. at 12 months.

Induction of surface markers

We have demonstrated previously that certain populations of cells isolated from marrow of healthy volunteers can be induced *in vitro* to express T-cell markers after incubation in the presence of thymic extracts (Incefy *et al.*, 1974a, 1975a; Touraine *et al.*, 1974a). Fig. 3 represents the degree of induction obtained with varying concentrations of human thymic extract (F5) as demonstrated by spontaneous E-rosette formation with SRBC. Induction of surface markers with these extracts or purified peptides has also been investigated in various immunodeficiency diseases (Incefy *et al.*, 1975c). The findings are summarized in Table 1.

TABLE 1. *In vitro* differentiation of bone marrow T-lymphocyte precursors by thymic extracts in various immunodeficiency diseases

Group	T-cell markers (No. positive/No. in group)	
	HTLA$^+$ phenotypes	E rosettes
Normal	11/11	22/22
Common variable immunodeficiency	1/1	1/1
Thymic hypoplasia (DiGeorge syndrome)	3/3	3/3
X-linked agammaglobulinemia	2/2	2/2
Ataxia telangectasia	1/1	1/1
SCID autosomal recessive	0/3	0/3
SCID with immunoglobulin synthesis no antibody synthesis	1/1	0/1
SCID without immunoglobulin synthesis no antibody synthesis	1/1	0/1
SCID with ADA deficiency	0/2	0/2

These patients were under study by the 'Combined Study Group of Immunodeficiency Diseases' at Memorial Sloan-Kettering Cancer Center, except two of them, one partial DiGeorge syndrome and one SCID with ADA deficiency were studied in France (Touraine *et al.*, 1975a, b).

Patient K.M. was 4 weeks old when cells from this marrow were first studied. At that time, after fractionation into five gradient layers, no cell population could be induced *in vitro* to bear HTLA$^+$ phenotypes or receptors for SRBC, the latter demonstrated by formation of spontaneous E rosettes. These findings were similar to those previously obtained with two other SCID patients of the autosomal recessive type who had been studied before marrow transplantation by this procedure using thymic extracts regularly capable of inducing marrow cells of healthy volunteers to develop both T-cell markers (Touraine *et al.*, 1974b; Incefy *et al.*, 1975b). At the time of the second study, when he was 12 months old (Table 2), the fractionated marrow cells of the Ficoll gradient layer II could now be induced to

TABLE 2. *In vitro* differentiation of marrow cells by thymic hormones in SCID patient (K.M.) before transplant

	Cytotoxic index (Percentage of HTLA$^+$ cells) Gradient layers					E rosettes (Percentage of Large R) Gradient layers					EAC rosettes (%) Gradient layers				
Additions*	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
None	—	25	12	18	0	—	0	0·5	0	0	—	0	0	0·5	0·5
CT-F3	—	56	25	10	6	—	0	0	0	0	—	0	0	0	0
Thymopoietin	—	51	29	4	7	—	0	0	0	0	—	0	0	0	0
Ubiquitin	—	42	15	20	16	—	0	0	0	0	—	0	0	0	0
						Percentage of cells induced									
CT-F3	—	41	15	0	6	—	0	0	0	0	—	0	0	0	0
Thymopoietin	—	35	19	0	7	—	0	0	0	0	—	0	0	0	0
Ubiquitin	—	23	3	2	16	—	0	0	0	0	—	0	0	0	0

Marrow cells were fractionated on a Ficoll discontinuous density gradient and incubated 17 hr in a 5% CO_2–95% air-humidified incubator in presence of inducers.

* CT-F3 is calf thymic extract, fraction 3 (Hooper *et al.*, 1975) which contains 290 μg protein/ml; thymopoietin I and ubiquitin (Goldstein 1974, 1975) are purified-peptide preparations used at 0·50 and 0·25 μg/ml respectively. Percentage of cells induced = [(b−a)/(100−a)]100, where a = percentage of cells bearing markers in controls after incubation with RPMI medium, b = percentage of cells bearing markers after incubation with inducers.

express HTLA$^+$ phenotypes after a 17-hr incubation with either partially purified calf thymic extract (F3) or the purified peptides thymopoietin or ubiquitin. A smaller number of cells in layer III could also respond to the influence of thymopoietin and calf thymic extract (F3) but not to ubiquitin. Cells of layer IV and V did not express this marker after exposure to thymic extract or thymopoietin, but in layer V a small number responded to the influence of ubiquitin. By contrast, the same fractionated marrow cells could not be induced to bear receptors for sheep erythrocytes or for the third component of complement.

When the marrow cells from this same child, who was now 16 months old, were studied a third time 2 months after foetal liver and thymus transplantation (Table 3), marrow cells of layer I were

TABLE 3. *In vitro* differentiation of marrow cells by thymic hormones in SCID patient (K.M.) after foetal liver and thymus transplants

	Cytotoxic index (Percentage of HTLA$^+$ cells) Gradient layers					E rosettes (Percentage of Large R) Gradient layers					EAC rosettes (%) Gradient layers			
Additions*	I	II	III	IV	V	I	II	III	IV	V	II	III	IV	V
None	8	20	39	60	48	3	4	7	2	1	8	6	11	11
HT-F5	16	18	24	51	—	2	7	23	1	2	0·5	8	24	12
Thymopoietin	15	25	12	24	—	2	5	20	2	1	1	10	23	6
Ubiquitin	—	14	11	43	—	—	3	14	0·8	1	6	7	12	9
						Percentage of cells induced								
HT-F5	9	0	0	0	—	0	3	18	0	1	0	2	15	1
Thymopoietin	8	6	0	0	—	0	1	14	0	0	0	4	13	0
Ubiquitin	—	0	0	0	—	0	0	8	0	0	0	1	1	0

Marrow cells were fractionated on a Ficoll gradient and incubated 16 hr in presence of inducers.

* HT-F5 is human thymic extract, fraction 5 (Hooper *et al.*, 1975) which contains 140 μg protein/ml; thymopoietin II and ubiquitin were used at 0·50 and 0·25 μg/ml respectively.

induced to develop HTLA$^+$ phenotypes by a human thymic extract (F5) and by thymopoietin. A few cells of layer II were also induced by thymopoietin to express this phenotype. However receptors for SRBC, as recognized by the E-rosette technique, could now be induced significantly in cells of layer III by all three inducers. In addition, complement receptors were expressed by cells of layer IV after incubation with human thymic extract (F5) and thymopoietin.

The second child, M.W., was 7 months old when his marrow cells were studied before transplant to determine their ability to be differentiated *in vitro* by thymic inducers. As shown in Table 4, cells from BSA gradient layer I and IV could be induced to bear HTLA$^+$ phenotypes by another human thymic extract (F5) and by thymopoietin. Ubiquitin appeared to induce this marker only in cells of layer III. However, as with our other child before transplantation (Table 2), no receptors for SRBC (E rosettes) could be induced in these same marrow cells after 15 hr incubation with these inducing agents. This child's marrow had been studied earlier, however, by another procedure in which his marrow cells were fractionated by velocity sedimentation (Kagan *et al.*, 1976). Receptors for SRBC and complement receptors, recognized respectively by E-rosette and EAC-rosette formation techniques, were the markers studied. After an 8-hr incubation with thymopoietin or ubiquitin, these T- and B-cell markers were not expressed in the lymphocyte fraction. Thus, in quite a different cell isolation system, the essential cellular abnormality of this patient appears to be confirmed.

Functional studies

In an attempt to determine whether marrow cells before thymus transplantation could aquire any functional activity after exposure to thymic inducers, mitogen studies were conducted on cells of layers

TABLE 4. *In vitro* differentiation of marrow cells by thymic hormones in SCID patient (M.W.) before transplant

	Cytotoxic index (Percentage of HTLA$^+$ cells)						E rosettes (Percentage of Large R)					
		Gradient layers						Gradient layers				
Additions*	Original B.M.	I	II	III	IV	V	Original B.M.	I	II	III	IV	V
None	16	7	59	27	1	2	0·7	2	0·3	1	0	0
HT-F5	37	43	44	21	19	10	2	1	2	0	0	0
Thymopoietin	15	13	6	28	9	1	0	3	1	0	0	0
Ubiquitin	—	—	17	37	—	1	—	—	0·3	0	0	0
						Percentage of cells induced						
HT-F5	25	39	0	0	19	8	1	0	2	0	0	0
Thymopoietin	0	6	0	0	9	0	0	1	0	0	0	0
Ubiquitin	—	—	0	14	0	0	—	—	0	0	0	0

Marrow cells were fractionated on a BSA discontinuous density gradient and incubated 15 hr with inducers.

* HT-F5 is human thymic extract, fraction 5, which contains 390 μg protein/ml; thymopoietin II and ubiquitin were used at 0·50 μg/ml each.

II, III, IV and V that had been used for the studies shown in Tables 2 and 4. No significant response to PHA or Con A was induced in marrow cells of either patient.

Foetal thymus biopsy

One lobe of the thymus implanted in K.M. was biopsied 102 days following the foetal liver and thymus transplant. The formalin-fixed tissue was embedded in paraffin and sections stained by standard techniques. Histologically, the thymus was clearly viable and possessed the stromal pattern, architecture and vascularity of an early embryonal thymus (Fig. 4). Neither lymphoid infiltration nor Hassall's corpuscles

were evident. Thus, no development of thymic cellularity or differentiation of architecture had occurred in the implant.

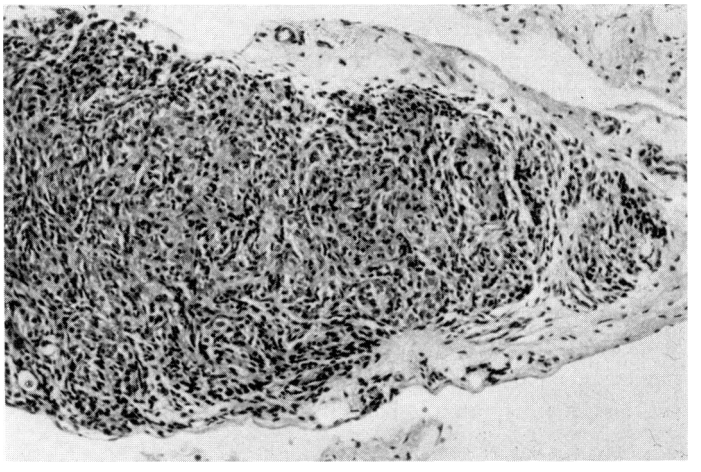

FIG. 4. Biopsy of transplanted 11–12-week foetal thymus 3 months following implantation in the rectus abdominis muscle of patient K.M. An embryonal morphology devoid of Hassall's corpuscules, lymphoid infiltration or a well-developed architecture is demonstrated in this well engrafted specimen.

DISCUSSION

Severe combined immunodeficiency disease (SCID) subsumes a spectrum of congenital disorders which have been attributed to qualitative or quantitative deficiencies in the development of lymphoid stem cells. The absence of functional immunity of both the humoral and cell-mediated types is the hallmark of this group of disorders.

Recently, it has been appreciated that a significant proportion of patients with the autosomal recessive form of SCID have an associated deficiency of adenosine deaminase (ADA) (Giblett *et al.*, 1972). We have reported two cases of SCID in which an inhibitor of adenosine deaminase was documented (Trotta, Smithwick & Balis, 1976). Patients with SCID and the associated ADA abnormality may present the same profound immunodeficiency and the same variability of lymphoid morphology (Hitzig *et al.*, 1971; Yount *et al.*, 1974) as SCID patients without associated enzymatic faults, but the pathology of the thymus in the two diseases is distinctive (Hitzig *et al.*, 1971), suggesting a different pathogenesis.

Among patients with SCID who have no currently detectable enzymopathy, the heterogeneity of disease expression has previously been reported to be largely restricted to differences in B-cell development. Thus, while in the classical autosomal form of the disease, patients usually present marked deficiencies of circulating B and T cells together with agammaglobulinemia, a number of patients have been recognized who share the functional and quantitative deficiencies of T cells, yet have normal numbers of circulating B cells and produce varying amounts of immunoglobulin despite an inability to generate antibody in response to immunization (Fireman *et al.*, 1966; Seligman *et al.*, 1975; Geha *et al.*, 1974; Pyke *et al.*, 1975). The present report documents an abnormality of *in vitro* T-cell differentiation which may separate such patients from those with the classical form of SCID. Thus, two patients with SCID possessing an expanded population of circulating B cells with variable quantities of immunoglobulin but without functional antibody are of interest in that, unlike prior patients with classical forms of SCID studied, cells in their marrow can readily be induced *in vitro* to bear antigenic markers which typify T lymphocytes ($HTLA^+$). However, these patients do not have marrow cells that can be induced to form E or EAC rosettes with SRBC by the techniques used. Further, in contrast to responsiveness of marrow cells of normals, we could not induce the stem cells of these patients to

develop functional T-cell capacities upon exposure to human thymic extract, bovine thymopoietin or ubiquitin.

The observation that marrow from the patients with SCID reported here failed to develop the capacity to rosette spontaneously with SRBC or to respond to mitogens under the influence of thymic factors also contrasts with our findings in a patient with DiGeorge syndrome whose marrow after identical stimulation produced a population of cells with both T-cell surface markers, HTLA and E-rosette (Incefy *et al.*, 1976). This finding may serve to explain in part the clinical observation that foetal thymus transplants in patients like those reported here have often failed to produce reconstitution of T cells while thymus transplants to patients with DiGeorge syndrome regularly have restored T cells and immunological vigour (Cleveland, 1975; Biggar *et al.*, 1975).

A major question regarding the origin of the observed aberration in immunologic differentiation concerns whether the lymphoid stem cell, while present is defective, or whether the thymus or its maturation, as a differentiative influence on the lymphoid system is incomplete or defective, or whether both abnormalities are present. While much of the data presented here seems to support the view that the marrow cells are abnormal, the possibility of a thymic defect must also be considered, especially in light of the findings of Pyke *et al.* (1975). These investigators observed in a case of SCID apparently very similar to M.W., that while the patient's marrow cells and peripheral blood lymphocytes failed to form E rosettes when cultured with media derived from normal thymus epithelial monolayers, incubation of both cell populations on normal thymic monolayers resulted in production of cells that formed rosettes with SRBC. More important, perhaps, was their observation that the patient's own thymus upon culture *in vitro* developed Hassall's corpuscles. Further, conditioned medium from the thymus culture of the patient showed capacity to induce E-rosetting cells when incubated with normal marrow. The latter finding suggests that *in vivo* the patient's thymus had been restricted in its maturation (pathologically, the thymus was embryonal in cell content and architecture), and that *in vitro* its functional maturation had progressed so that it became capable of inducing T-cell differentiation.

Although it is difficult to reconcile entirely the present findings with those of Pyke *et al.*, several possibilities exist which could explain the deficiencies observed in his and our patients. One possibility is that the basic abnormality responsible for the patient's disease resides at a stem cell level. This defect would be revealed in failure of the stem cells to respond normally to inductive influences as has been found in the present study. This same defect, e.g. lack of an appropriate cell surface receptor, might further interfere with traffic of the precursor cells to the thymus. If such traffic is necessary for full thymic differentiation via mesenchymal–epithelial interaction, the thymus *in vivo* might remain abnormal in morphology and function. A graft of normal stem cells might then be able to correct both the thymic abnormality and the lymphoid cell deficiencies. If on the other hand, it is the thymus which is responsible for the primary defect and which fails to provide the influence to permit lymphoid stem cell precursors to develop to the point where they can respond to other thymus factors, correction of the defect might be accomplished by appropriate thymus transplant. We have observed in one of our cases that a thymus and liver transplant from an early embryo, although producing changes in normal cells, did not correct entirely the defect of the patient and that the thymus after transplant remained embryonic. Perhaps environmental factors in the host inhibit thymic maturation and thus prevent correction of the defect by transplantation of the thymus from an early embryo. Transplantation of thymus from an older embryo could, then, be the treatment needed to correct the defect.

Ammann *et al.* (1975) have recently reported transient but significant functional T-cell reconstitution in two of three patients who apparently had another form of SCID after transplantation with foetal thymus plus treatment with transfer factor. Of interest has been their success with grafts of thymus from donors older (14–20 week gestational age) than those we have used, suggesting that more mature thymic tissue may enhance differentiation of a normal population of T cells in such patients. The differentiated T cells in these cases in each instance have been derived from the patient himself and not from the thymus donor, indicating the presence of a population of lymphoid stem cells than can be differentiated under appropriate influence.

Our observations show that patients with SCID of the type described here have a pattern of marrow

cells on density gradient fractionation that is abnormal by adult standards. Whether this difference is a component of the pathology of the disease or is attributable to age differences between patients and controls will need to be determined. It is of interest that the abnormality of cell distribution seemed to be corrected coincident with thymus and foetal liver transplantation in K.M. Again, whether this change is to be attributed to the transplants or simply to maturation with time remains unclear.

The studies presented in this report provide rudiments for a further dissection of the origins of the T-cell deficiencies observed within the spectrum of disorders collectively termed severe combined immunodeficiency disease (SCID). They also may provide a basis for assessment of the stages of T-cell differentiation and provide a means for evaluating the influences of various thymic factors on lymphoid development in man. As additional T-cell antigens such as HTLA are recognized, the ontogeny of their expression and the factors controlling T-cell morphological and functional differentiation will be increasingly understood. We believe such analyses are important because, as the several steps in T-lymphocyte differentiation are unravelled, chemical or hormonal substances which control differentiation will be developed for therapy. The findings of the present studies indicate that an understanding of each step in both thymic maturation and stem cell differentiation to T and B lymphocytes will have to be clarified if the several immunodeficiencies, already observed and those still to be defined, are to be corrected rationally by cellular and/or macromolecular engineering.

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