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Research Article

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Defective Expression of T Cell-associated Glycoprotein in Severe Combined Immunodeficiency

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Abstract

A T cell surface membrane-associated glycoprotein, Tp40 (40,000 mol wt), also designated as CD-7, was not expressed by the T cells of a patient with severe combined immunodeficiency. In addition to this abnormality, T cell proliferative responses to mitogens were defective and the IL-2 receptor expression was deficient on the patient's T lymphocytes. However, his T cells were found to provide help for the differentiation of normal B cells to Ig-secreting cells. Abundant circulating B cells were detected. These B cells proliferated normally in the presence of anti-μ antibodies and B cell growth factors, but did not differentiate into antibody-secreting cells when provided with the help of normal T cells. In addition, his activated B cells did not proliferate to IL-2 even though IL-2 receptors were expressed. A successful allogeneic histocompatible bone marrow transplantation resulting in T cell engraftment corrected both the T and B cell immunodeficiencies. These findings support the hypothesis that the Tp40 deficiency present in this patient is related to a defect of the T cell precursors, and that Tp40 plays important roles not only essential to T cell interactions but also to certain aspects of T-B cell interaction during the early lymphoid development.

Introduction

Severe combined immunodeficiency (SCID)[1] is a heterogenous group of diseases (1, 2). Certain variants of SCID, such as those associated with adenosine deaminase deficiency (3, 4) or the bare lymphocyte syndrome (5-7), have been shown to have identifiable underlying pathogenetic mechanisms. However, the pathologic basis for most cases of SCID are obscure and have been variously attributed to stem cell defects (8) or deficiencies of the thymic influence (9, 10). Recent development of monoclonal reagents has led to identification of deficiencies of helper T cells in certain cases (11-13). However, selective deficiency of a major T cell-associated marker has not yet been reported. In this report we present a case of SCID in which the T cells have been shown to be selectively deficient in a T cell-associated glycoprotein, Tp40 (14). The abnormality was associated with defective T cell proliferation and with defects in B cell differentiation. After a successful bone marrow transplantation (BMT) from a histocompatible sibling, T cell reconstitution was accompanied by the appearance of Tp40$^+$ lymphocytes and correction of both T and B cell defects. These findings suggest that Tp40 plays an important role in the ontogeny of T and B cell functions and that its absence may have been responsible for the SCID in this patient.

Methods

Patient. A 5-mo-old Mexican male was admitted to Oklahoma Children's Memorial Hospital with a history of failure to thrive, candidiasis, protracted diarrhea, and upper respiratory tract infection since the age of 2 mo. Immunological workup revealed the absolute lymphocyte count to be 10,000/mm^3. His T cell numbers were greatly diminished. 5.2% of the lymphoid cells expressed OKT11, 2.7% OKT3$^+$, 6.1% OKT4$^+$, and 0.5% OKT8$^+$ surface markers. These data were obtained with OKT Series antibodies in the Clinical Laboratory at the Oklahoma Children's Memorial Hospital with a Spectrum III instrument (Ortho Diagnostic Systems, Inc., Westwood, MA). As shown in Fig. 1, the majority of the T cells isolated by a sheep erythrocyte rosette method in our laboratory were stained with our own anti-T3 antibody, 235(15). The difference between our results and those obtained in the clinical laboratory might be due to different antibodies and instrumentation. Because of limited blood samples available, the difference was not pursued further. The majority of circulating lymphocytes had surface IgD (79.1%) and/or IgM (77.5%).

The child had low levels of circulating immunoglobulin: IgA 15.1 mg/dl, IgG 55.9 mg/dl, and IgM 59.2 mg/dl. Serum thymulin (FTS) was undetected when his serum was diluted to 1:4. Mitogenic responses to phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) were 1,435 cpm (normal > 75,000 cpm), 529 cpm (normal > 40,000 cpm), and 1,381 cpm (normal > 10,000 cpm), respectively. Because there was a functional deficiency of both T and B lymphocytes, a diagnosis of SCID was made. Adenosine deaminase levels in erythrocytes (RBCs) were normal. There was no family history of early infant deaths or immunodeficiency.

A 15-yr-old female sibling was shown to be HLA compatible, nonreactive in mixed leukocyte cultures (MLC), and ABO matched with the patient. A marrow transplantation was performed without any preparation of the patient. Bone marrow cells from the donor in a dose of 4.79×10^8 nucleated cells/kg were infused. Within 2 wk after the transplant, the T cell numbers increased, as revealed by surface marker analysis. During this period, the child developed a skin rash, had profuse diarrhea, and a diagnosis of graft-vs.-host disease (GVHD) was made. The patient was treated with 1 mg/kg of methylprednisolone for 3 wk and the GVHD completely resolved. The child was discharged from the Bone Marrow

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1. *Abbreviations used in this paper:* BCGF, B cell growth factor; BMT, bone marrow transplantation; Con A, concanavalin A; FITC, fluorescein isothiocyanate; GVHD, graft-vs.-host disease; IL-2, interleukin 2; mAb, monoclonal antibody; MLC, mixed leukocyte cultures; MϕS, macrophage factor; PFC, plaque-forming cell; PHA, phytohemagglutinin; PWM, pokeweed mitogen; RBC(s), erythrocyte; rIL-2, recombinant IL-2; SCID, severe combined immunodeficiency; TdR, thymidine.

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Transplantation Unit 2 mo after the marrow transplantation and has remained in good clinical condition since discharge.

7 mo after BMT, B cell function was demonstrated. Isoagglutinins were present. Serum immunoglobulin determination, 2 mo after the discontinuation of intravenous gammaglobulin therapy, revealed IgA, 8.5 mg/dl; IgM, 67 mg/dl; and IgG, 860 mg/dl. T cell reconstitution was demonstrated by the lymphoproliferative responses to PHA (90,000 cpm), Con A (42,000 cpm), and PWM (13,000 cpm). Karyotypic analyses of bone marrow cells also confirmed the establishment of a donor marrow graft. Circulating T cells were of donor origin while B cells were of host type.

Cell preparation. Peripheral blood mononuclear cells from the patient or control subjects were isolated on Ficoll-Hypaque density gradient centrifugation. After monocyte depletion by adherence to plastic, the mononuclear cells were separated into T and non-T cells by rosetting techniques using neuraminidase-treated sheep erythrocytes as described (16).

Monoclonal antibody (mAb) production. mAb 69.3.4 was produced in a fusion between SP2/0 tumor cells and splenocytes of a BC_3F_1 female mouse previously immunized with PHA-activated human T cells. Hybridoma supernatants were screened for their specific binding on T cells. This hybridoma was cloned twice on soft agar. Details of these procedures have been described previously (17). mAb 3A1 was kindly provided by Dr. B. Haynes, Duke University School of Medicine, Durham, NC.

Lymphocyte proliferation assay. 2×10^4 or 10^5 lymphocytes in 0.2 ml of medium were stimulated in microtiter wells with PHA-P (Difco Laboratories Inc., Detroit, MI), PWM, or Con A (Gibco, Grand Island, NY), as described previously (18). B cell proliferation assays were performed as described (19).

Reverse plaque assay. Reverse plaque assays for Ig secretion were performed as described (19).

Immunofluorescence studies. The presence of Tp40 on T cells was analyzed using fluorescent microscopy or an Epics V flow cytometer (Coulter Electronics Inc., Hialeah, FL). Two-color fluorescence microscopy was carried out by staining T cells with 69.3.4 mAb conjugated with rhodamine and counterstained with biotinated AT-1 (anti-IL-2 receptor) and fluoresceinated avidin. Details of these methods have been described (18, 19).

Immune rosette. Human RBCs collected in Alsever's solution were washed thoroughly in saline. Equal volumes of packed red cells and goat anti-mouse Ig (1 mg/ml) were mixed and then suspended in saline at red cell concentration of 20% vol/vol. Equal volumes of a 0.006% (wt/vol) $CrCl_3$ solution were added dropwise to the RBC/goat anti-mouse Ig suspension, which was gently vortexed. After incubation for 1 h at 30°C, the conjugated red cells were washed thoroughly with RPMI-1640 and resuspended at 5% vol/vol for use.

10^7/ml of lymphoid cells were incubated with an equal volume of hybridoma supernatant for 30 min at 4°C. After washing, the lymphoid cells were resuspended to twice the original volume. Antibody-coated RBCs were then added to the lymphoid cell suspension at a final concentration of 2%. The cell mixture was centrifuged at 1,000 rpm for 5 min and the pellet incubated at 4°C for 30 min. The cell pellet was then resuspended gently and rosetting cells were separated from nonrosetting cells by Ficoll-Hypaque density gradient sedimentation. Residual red cells were lysed with buffered $(NH_4)Cl$ solution.

Cytokine preparations. Recombinant interleukin 2 (rIL-2) was purchased from Genzyme (Boston, MA). Macrophage factor (MϕS) was produced in our laboratory according to the procedures of Finelt and Hoffmann (20) with minor modifications. 5×10^6 mononuclear cells in 1 ml RPMI-1640 containing 20% fetal calf serum and 2 mM glutamine were incubated at 37°C in tissue culture dishes (Falcon 3001, Becton-Dickinson & Co., Oxnard, CA) for 1 h. Nonadherent cells were then washed off and the remaining cells incubated in serum-free Mischell Dutton medium with 10 μg/ml lipopolysaccharide (*Escherichia coli* type 055:B5, Difco Laboratories, Inc.) for 24 h at 37°C in 5% CO_2. The supernatant was harvested, filtered through a 0.45-μm filter, and stored at −20°C. Conditioned media containing B cell stimulatory factors were produced as described (19). Partially purified B cell growth factor (BCGF) was a kind gift of Dr. A. Maizel (M.D. Anderson Cancer Center, Houston, TX).

Results

Characterization of mAb 69.3.4. mAb 69.3.4, an IgG_1 antibody, stained 60–80% of isolated T cells. It also stained thymocytes and leukemic T cell lines. It did not stain B cells, granulocytes, monocytes, erythrocytes or platelets. By immunoprecipitation, a 40,000-mol-wt polypeptide was identified as its reactive antigen. mAb 69.3.4 was found to react with a protein similar to that precipitated by mAb 3A1 (21). Sequential immunoprecipitations were then carried out. mAb 69.3.4 showed no precipitate with a T cell lysate that had been first reacted with mAb 3A1 plus Sepharose 4B-linked goat anti-mouse Ig antibodies. Thus, mAb 69.3.4 was reactive with the same molecule identified by mAb 3A1. This molecule, which has been shown to be a glycoprotein (22), has been designated as CD-7 by the First International Workshop on Human Leucocyte Differentiation Antigens (14).

Absence of Tp40 on SCID T cells. Although only a few T cells were present in the blood of our patient with SCID, the T cells present stained for T3, T4, T8 and T11. Despite the presence of these mature T cell markers, the isolated T cells from the patient failed to proliferate when stimulated with T cell mitogens and alloantigens. Additional mAb were used to further study these T cells. The patient's T cells expressed HLA antigens and the LFA-1 antigen (23). However, his isolated T cells did not stain with mAb 69.3.4 (Table I and Fig. 1 *B*) in three separate experiments. By contrast, activated T cells from normal individuals regularly stained strongly and numerously with the 69.3.4 antibody (Table I). Further efforts to activate the patient's T cells did not significantly increase the numbers of $Tp40^+$ cells. As an additional control, T cells from five cord blood samples were studied, and each was shown to contain $Tp40^+$ T lymphocytes, which showed staining intensity, and numbers similar to those present in the blood of adults.

After BMT, the patient's T cell numbers gradually increased.

Table I. Absence of Tp40 on T Cells of Our SCID Patient and Its Expression after BMT as Analyzed by Immunofluorescence

	Resting T cells	PHA-activated T cells
	%	%
Patient		
Pre BMT	0	<1
Post BMT		
2 wk	Not done	27.9
7 wk	18.7	32.0
16 wk	41.7	Not done
22 wk	60.0	Not done
Family members		
Father	76.5	>95
Mother	70.6	>95
Sister (donor)	81.1	>95
Controls (average of five)	77.7 (range 69.4–95.8)	88.3 (range 79.3–99.8)

Lymphocytes isolated by an E rosette separation technique were first incubated with mAb 69.3.4 and then stained with fluorescein isothiocyanate (FITC) goat anti-mouse Ig. After thorough washings, cells were analyzed on an Epics V flow fluorocytometer; 2,000–5,000 cells were analyzed.

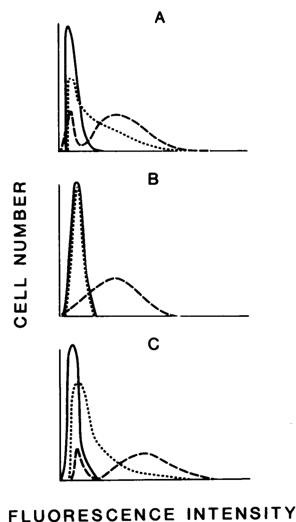

Figure 1. Cytofluorometric analysis of T cell-associated antigens on sheep erythrocyte rosettes forming cells from (*A*) control, (*B*) SCID patient before BMT, and (*C*) SCID patient after BMT: (——), control; (- - -), T3; and (-----), Tp40. The fluorescence intensity is expressed on a linear scale.

Table II. Effect of Cytokines on T Cell Proliferation in SCID Patient

	^{3}H-Thymidine (TdR) incorporation (*cpm*)	
	Patient	Control
Medium	95±40	327±70
MφS	185±100	207±50
rIL-2	150±14	443±80
PHA	1,026±420	2,650±260
PHA + MφS	695±160	11,997±380
PHA + rIL-2	1,135±200	9,144±70
PHA + rIL-2 + MφS	658±30	17,750±750

20,000 T cells were cultured for 3 d in 0.2 ml medium in the presence of 25% MφS, rIL-2 (250 U/ml), and PHA (10 μg/ml) either singly or in combination. 0.5 μCi of tritiated thymidine (^{3}H-TdR) was added for the last 6 h of culture.

22 wk posttransplantation, 60% of the T cells stained for Tp40 (Table I and Fig. 1 *C*) and were shown to be of donor origin with XX chromosomes.

Both parents and the bone marrow donor (sister) were studied with respect to the expression of Tp40. Normal numbers of Tp40$^+$ cells were detected in the blood preparations of each of these family members. The staining intensities of the T cells of these family members were indistinguishable from those of normals.

A brief survey of various immunodeficiency states showed that Tp40 deficiency was not present in lymphocytes of patients with X-linked agammaglobulinemia (three cases), Wiskott-Aldrich syndrome (three cases), or common variable hypogammaglobulinemia (six cases). T cells from two other cases of SCID were examined and Tp40 was detected in an expected proportion of isolated T cells from these patients.

Defective T cell proliferative responses and lack of response to cytokines. The functional capacities of the Tp40-deficient T cells were studied in our patient. His isolated peripheral blood mononuclear cells did not respond with significant proliferations to any of the T cell mitogens or to alloantigens (see case history). In view of the recent finding that T cell from patient with Nezelof's syndrome respond to mitogens in the presence of exogenous cytokines (24), the influence of exogenous cytokines on the patient's isolated T cells were determined. Normal T cells responded well to PHA in the presence of macrophage factors (Table II). The addition of interleukin 2 (IL-2) enhanced the PHA-induced T cell proliferation. By contrast, the patient's T cells responded minimally to these cytokines in the presence of PHA.

Deficient IL-2 receptor expression by the SCID patient's Tp40$^-$ T cells. Isolated T cells from our patient or from normal controls were activated with PHA-P for 3 d. The T cells were evaluated for the expression of IL-2 receptors using our mAb AT-1 (19). Only 16% of patient T cells expressed IL-2 receptors, while 81.5% of control T cells expressed IL-2 receptors (Table III). 5 mo after the successful bone marrow transplant, the patient's T cells, which now were Tp40$^+$ and of donor origin, expressed IL-2 receptors in a normal fashion.

Activated normal B cells have recently been shown to express IL-2 receptors (19). Before BMT, the patient's B cells were activated with anti-μ antibodies and BCGF. His activated B cells expressed IL-2 receptors normally (Table III) as compared with control B cells.

The possibility that Tp40$^-$ T cells from a normal individual also did not proliferate and failed to express IL-2 receptors was considered. Tp40$^+$ cells were depleted from T cell preparations of normal donors using an immune rosette technique. This T cell preparation was found to be 75–95% Tp40$^-$ by immunofluorescent microscopy. Remaining Tp40$^+$ cells were weakly stained and could not be further depleted by repeated rosetting. These cell preparations were stimulated with PHA, Con A, or PWM. No differences were observed in thymidine uptake of the Tp40$^-$ T cell preparations as compared with the unseparated T cell preparation that contained >80% Tp40$^+$ cells (data not shown).

The expression of IL-2 receptors by mitogen-activated Tp40$^+$ and Tp40$^-$ T cells was determined by two-color immunofluorescence microscopy using anti IL-2 receptor mAb AT-1. In three separate experiments, both Tp40$^+$ and Tp40$^-$ T cells activated by mitogens such as PHA and Con A expressed IL-2 receptors (Table IV). These data indicated that Tp40$^-$ normal T cells were capable of proliferation in response to T cell mitogen and of IL-2 receptors expression.

Defective B cell functions of the Tp40-deficient SCID patient before BMT. B cells were isolated from peripheral blood of con-

Table III. Expression of IL-2 Receptors on Activated Lymphocytes

	Lymphocytes expressing IL-2 receptors (%)		
	Activated T cells		Activated B cells
	Pre-BMT	Post	Pre-BMT
Patient	16.1	84.6	62.2
Control	81.5	76.1	57.2

10^6/ml T cells were activated with PHA-P (10 μg/ml) for 3 d. 10^6/ml non-T cells were activated with rabbit anti-IgM (10 μg/ml) and BCGF for 3 d. Lymphocytes were first incubated with mAb AT-1 (anti-IL-2 receptor) and then counterstained with FITC-goat anti-mouse Ig. After thorough washing, the cells were analyzed with fluorescence microscopy. In the case of non-T cells, rhodamine goat anti-IgM was used to identify B cells. Results were expressed as percentage of cells stained by AT-1.

Table IV. Expression of IL-2 Receptors by Activated Normal T Cell Subsets

Mitogen	T cell subsets	Activated T cells expressing IL-2 receptors (%)		
		Exp. 1	Exp. 2	Exp. 3
PHA	Tp40$^+$	92.7	92.6	85.5
(10 μg/ml)	Tp40$^-$	90.7	87.5	79.9
Con A	Tp40$^+$	—	79.8	62.4
(5 μg/ml)	Tp40$^-$	—	95.2	55.6
Medium	Tp40$^+$	—	20.2	26.9
	Tp40$^-$	—	10.1	5.0

10^6/ml E$^+$ cells were activated with various mitogens for 3 d. After activation, the cells were stained with rhodamine-conjugated 69.3.4. and counterstained with biotinated AT-1 (anti-IL-2 receptor) and FITC-avidin. Numbers of cells expressing IL-2 receptors were counted as percentage of either Tp40$^+$ or Tp40$^-$ cells.

trols and the patient by depletion of T cells and monocytes using sheep erythrocyte rosette technique and plastic adherence. Rabbit anti-human IgM was used as a first stimulant and a partially purified BCGF preparation (a kind gift of Dr. A. Maizel, M.D. Anderson Hospital and Tumor Institute) was added to stimulate the isolated B cells. B cells from normal donors proliferated vigorously in the presence of these stimulants. The patient's B cells showed proliferation similar to that of normal control B cells (Table V).

Normal B cells developed IL-2 receptors after activation in vitro and became responsive to IL-2 (19). In sharp contrast, while the patient's activated B cells acquired IL-2 receptors in a manner comparable to normal B cells (Table II), these cells did not respond with proliferation to exogenous rIL-2 (Table VI).

In addition to studies of B cell proliferation, differentiation of B cells was studied. Normal B cells differentiated into antibody-secreting cells when stimulated by PWM plus either autologous or allogeneic T cells (Table VII). In an allogeneic system, the patient's T cells, despite their apparent functional deficiency, provided helper activity to normal B cells in the PWM system.

Table V. B Cell Proliferation Induced by Anti-IgM and BCGF in SCID

Rabbit anti-IgM (10 μg/ml)	BCGF (final dilution)	^{3}H-TdR incorporation (*cpm*)	
		Control	Patient
−	—	325±70	198±45
+	—	3,799±320	4,847±635
−	1:8	28,881±200	868±120
+	1:8	68,693±1,300	54,729±2,800
+	1:16	42,626±3,600	44,797±1,150
+	1:32	16,827±1,800	35,123±280
+	1:64	19,217±3,500	26,085±1,400
+	1:128	16,315±4,300	18,827±900

Nonadherent non-T cells were used as B cells at 10^5 cells/microtiter well and cultured for 3 d. Anti-IgM and BCGF were added in appropriate doses at initiation of culture. 0.5 μCi ^{3}H-TdR was added to culture wells for the last 6 h of culture.

Table VI. IL-2 Response of Activated B Cells from SCID Patient

	^{3}H-TdR incorporation (*cpm*)		
		Patient	
	Control	Pre-BMT	Post-BMT
Med	4,533±260	1,622±180	1,150±80
rIL-2 (250 U/ml)	10,651±250	1,872±180	3,576±110

Isolated B cells were activated with anti-IgM (10 μg/ml) and BCGF (10%) for 3 d. After washing, the activated B cells were cultured for an additional 2 d with either media or 250 U/ml rIL-2. 0.5 μCi ^{3}H-TdR was added for the last 6 h of culture. rIL-2 significantly ($P < 0.05$) augmented proliferation of the control B cells and the patient's B cells post BMT.

Although his T cells were nonreactive in MLC, they were able to provide helper cell activity to his parents' isolated B cells in this system (data not shown). In marked contrast, the patient's B lymphocytes did not respond to PWM in the presence of either autologous or allogeneic T cells.

After normal B cells had been stimulated to proliferate by *Staphylococcus aureus* or anti-IgM together with BCGF, the activated cells could be driven to differentiate into antibody-secreting cells by addition of conditioned media that contained B cell differentiation factors (19). In this system, our patient's B cells were not inducible to differentiate into antibody-secreting cells (Table VIII).

Restoration of B cell function after BMT. B cell function in this patient was followed serially for several months after BMT. He made specific antibodies and his serum immunoglobulin levels rose to the normal range 6–8 mo after BMT. Cytogenetic

Table VII. Generation of PWM-dependent Plaque-forming Cells (PFC) by T and Non-T Cells in Patient with SCID before and after BMT

Lymphoid cells added		PFC/10^5 cells cultured			
		Pre-BMT		Post-BMT	
T	Non-T	Exp. 1	Exp. 2	5 mo	9 mo
Control	—	90	14	42	1
—	Control	100	52	8	11
Control	Control	303	360	362	180
Patient	—	0	7	19	4
—	Patient	Not done	6	12	2
Patient	Patient	10	Not done	33	97
Control	Patient	84	25	56	20*
Patient	Control	374	355	159	46*

Peripheral blood mononuclear cells were depleted of macrophage by plastic adherence and then separated into T and non-T cells by sheep erythrocyte rosettes and Ficoll Hypaque density gradient sedimentation. 5 × 10^5 cells of each lymphoid cell preparation were cultured in 1 ml of medium in 30 × 100-mm culture tubes in the presence of 1% PWM for 7 d. Reverse plaque assay was performed as described in Methods.

* These low numbers are likely due to allogeneic suppression in the MLC reaction in this experiment. When the control T cells were irradiated with 1,500 rad, 185 PFC were detected. This reversal of suppression was not seen when the patient's T cells were irradiated, suggesting that a defect of T cells was still present.

Table VIII. Effect of Allogeneic T Cell-conditioned Media on Differentiation of S. aureus-activated B Cells

Activated B cells	Conditioned media	PFC/10^5 cells cultured	
		Pre-BMT	Post-BMT
Control	−	45	8
	+	329	137
Patient	−	<1	2
	+	<1	83

B cells were isolated after two cycles of depletion of sheep RBC rosettes and cultured with 0.01% (vol/vol) formalinized *S. aureus* at 10^6 cells/ml. After 3 d, the cells were thoroughly washed and cultured at 10^6 cells/ml for an additional 3 d in the presence of 20% conditioned media. Reverse PFC assay was performed as described in Methods.

analysis revealed that B cells in the blood were of host origin 6 mo after BMT. Karyotypes were analyzed from a culture of the patient's B cells stimulated with formalinized *S. aureus* and BCGF, and all were shown to be of male type. Despite the chimeric nature of the lymphoid population, the patient's B cells proliferated to IL-2 after activation (Table VI) 5 mo after transplantation. In addition, host B cells collaborated with both donor T cells and allogeneic T cells and differentiated to Ig-secreting PFC under the influence of PWM (Table VII). Activated B cells were also able to respond to maturation factors to secrete immunoglobulins (Table VIII).

Discussion

In this report, we have presented evidence that a membrane glycoprotein (Tp40) was lacking in the T lymphocytes of a child with SCID syndrome. Recently, membrane defects have been identified in patients with this syndrome as well as with certain other immunodeficiencies. Bare lymphocyte syndrome in which HLA antigens are absent (5–7), and defects of the membrane cytoskeleton have been associated with SCID (25, 26). Deficiencies of leukocyte function antigens have been associated with neutrophil and lymphocyte functional defects (27–32). Deficiency of a membrane glycoprotein gp 115 has been identified in patients with Wiskott-Aldrich syndrome and in patients with certain other T cell deficiency states (33–35). These membrane abnormalities involve primarily structures present in cells of several cell lineages. The marker Tp40 appears to be a peptide specific for a major population of T lymphocytes (21), although it has been detected occasionally in some blast cells of patients with acute myelogenous leukemia and with chronic myelogenous leukemia in blastic crisis (36, 37). Thus, our patient represents a unique instance in which a major T cell marker deficiency has been identified. Despite our unsuccessful attempt thus far to identify other patients with this defect, further investigation of immunodeficient patients for this molecular deficiency seems warranted.

Although our patient had a demonstrable deficiency of T cell numbers, the circulating T cells had markers of mature T cells including T3, T11, T4 and T8. Despite the presence of these mature T cell markers, his T cells did not proliferate well in response to stimulation with mitogens or antigenic stimulation. Attempts to correct the unresponsiveness with exogenous cytokine were not successful. These T cells were also deficient in IL-2 receptor expression. The presence of mature T cell markers on the patient's T cells before transplantation would make the explanation that the absence of Tp40 is secondary to T cell immaturity unlikely.

One possible explanation for the T cell defects seen is that the major T cell subset, $Tp40^+$ T cells, was absent and that the remaining minor $Tp40^-$ T cells were normally nonfunctional. This possibility, however, was ruled out by the experiments presented herein (Table IV). The reported results of Haynes et al. (21) also indicated that normal $Tp40^-$ T cells are capable to proliferate to mitogens, though to a lesser extent than normal $Tp40^+$ T cells. Furthermore, Morishima et al. (38) observed that $Tp40^-$ T cell population proliferated to alloantigens in vitro and contained the precursors for cytotoxic T cells. In contrast, the patient's T cells, which were $Tp40^-$, were abnormal in proliferation to mitogens and in the ability to express IL-2 receptors. Thus, the coexistence of the absence of Tp40 and abnormalities of the other T cell functions in this patient suggest a cause-effect relationship.

Interestingly, the T cells from our patient were able to provide helper activities for normal allogeneic B cells to differentiate to Ig-secreting cells. These helper activities are usually provided by $Tp40^+$ cells in normal individuals (21). Thus, the acquisition of Tp40 and of the functional helper activity for later stages of B cell differentiation were independent events during T cell ontogeny.

In addition to the T cell defects demonstrated, certain B cell defects were also apparent. While the patient's B cells could proliferate in response to anti-IgM and BCGF, these activated B cells did not differentiate into antibody-secreting cells even in the presence of normal T cells and T cell factors. A related observation was that although his B cells, upon activation, expressed IL-2 receptors as detected by mAb, they remained unresponsive to IL-2 stimulation. The dissociation of receptor expression and receptor function with respect to the lymphokine, IL-2, is of considerable interest in view of the recent demonstration that IL-2 receptors express on normal activated B cells and IL-2 can promote B cell proliferation (19, 39–41).

Cytogenetic analyses showed that after bone marrow transplantation, the patient's B cells continued to be of host origin while his T cells were of donor origin. Despite this chimerism, the patient's serum immunoglobulin levels were restored and he became able to make specific antibodies. It was of additional interest that the persistent host B cells functioned normally in vitro after BMT. They differentiated to Ig-secreting cells in collaboration with normal and donor T cells after PWM stimulation. They were able to respond with proliferation to IL-2 stimulation after activation. This normal response was first observed 5 mo after T cell engraftment. Thus, the host B cells matured under the influence of normal donor T cells in vivo. It appears that Tp40 may play a significant role for early T-B cell interactions, and the absence of Tp40 does not allow these early events to occur.

In a recent communication, Tp40 was shown by Lobach et al. (42) to be present in the perithymic mesenchyme at 7 wk of embryogenesis, before the appearance of other T cell surface markers including sheep RBC receptors, T1, T3, T4 and T8. These data indicated that Tp40 may be expressed on early precursors of T cells and that passage through the epithelial thymus may not be required for its expression. The early appearance of this glycoprotein would add credence to our hypothesis that it plays an important role in the early steps of T and B cell devel-

opment and in T-B cell interactions essential to the normal development of the human immune system.

It has been shown in a murine model that T cell precursor might affect B cell development (43). Sherr and co-workers (43) found that immature murine B cells from fetal or neonatal donors, when transferred into lethally irradiated adult recipient, would acquire the capacity to reconstitute a normal heterogenous antibody response only if thymus cells were transferred together with them. This suggested that the interaction between thymus cells and fetal B cells were required for the fetal B cells to mature and supported the thesis that early T-B interaction played a crucial role in B cell ontogeny. By analogy, it is possible that the early differentiation antigen Tp40 in man might be a critical component in such interaction and absence of this protein would result in failure of such interaction and subsequent B cell function.

Tp40 deficiency seen in this patient is a unique finding to date. Studies of two other SCID patients revealed the presence of this antigen. Thus, Tp40 deficiency may only account for the pathogenesis of a small percentage of SCID cases. Despite this, it provided valuable clues to human T cell ontogeny and T-B interaction. Our finding also demonstrated that, in addition to T cell defects measured by conventional T cell function assays, other more subtle defects, such as T-B interactional defects, might be present. In addition, B cell defects observed in our case before transplantation were corrected by allogeneic T cell graft. These defects were not inherent in the B cell lineage. In the literature, B cell defects were occasionally reported in SCID. The inability to induce SCID B cells to differentiate to Ig-secreting cells in these cases led to the postulate that intrinsic B cell defects were responsible (44). Our findings would suggest extreme caution in such interpretation. Indeed, our findings would support the thesis that T cell defects are primarily responsible for the pathogenesis of SCID (45).

By immunofluorescence analysis, T cells from the patient's parents and sister expressed Tp40 on cell membranes in quantities similar to that seen in normals. Thus, the results of these analyses did not provide a basis to define the mode of inheritance of this Tp40 deficiency. The lack of family history of early infant deaths and the fact that his siblings are normal argue for the anomaly seen in our patient as a mutational event. Definitive answers as to the genetic basis of the defect must await availability of a DNA probe for the Tp40 gene and analysis of the gene by restriction mapping or sequence analysis.

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