Heterogeneity of Lymphocyte Subpopulations

in Severe Combined Immunodeficiency

EVIDENCE AGAINST A STEM CELL DEFECT

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A B S T R A C T Surface markers typical of T and B
lymphocytes were present on varying proportions of
peripheral blood lymphocytes from three infants with
severe combined immunodeficiency disease. Despite this,
functions mediated by T and B cells were either absent
or very minimal in all three, including cell-mediated re-
sponses in vivo; the in vitro proliferative response to
mitogens, allogeneic cells, or antigens; effector cell func-
tion in lymphocyte-antibody lymphocytolytic interaction
assays; and in vitro synthesis of IgG, IgA, and IgM.
In contrast, mononuclear cells from one of the infants
were tested and found capable of lysing both human and
chicken antibody-coated erythrocyte targets normally.
Co-cultivation experiments with unrelated normal con-
trol lymphocytes failed to demonstrate suppressor cell
activity for immunoglobulin synthesis in these infants.
Augmentations of immunoglobulin production from 310
to 560% over that expected on the basis of individual
culture data were noted in co-cultures of one of the in-
fants' cells with two different unrelated normal control
cells. These findings suggest that that infant may have
had a T helper cell defect or that his T cells were unable
to produce soluble factors necessary for B cell differen-
tiation. The finding of cells with differentiation markers
characteristic of T and B lymphocytes in each of these
patients, though in variable quantities, is further evi-
dence for heterogeneity among patients with the clinical
syndrome of severe combined immunodeficiency and
argues against the concept that their immunodeficiency
was due to a stem cell defect.

INTRODUCTION

Infants with severe combined immunodeficiency (SCID)[1]
usually have no evidence of functions mediated by T
or B cells (1) except for occasional low-grade mixed
leukocyte or mitogen responses in vitro (2, 3). Since
these patients do have thymus glands and because im-
mune function has been conferred on a number of them
by grafts of histocompatible bone marrow, it has been
hypothesized that the primary defect in this condition is
an abnormal stem cell (1). This hypothesis was put
forth before methods for detecting and enumerating
human peripheral blood T and B lymphocytes were de-
veloped in the early 1970's (4, 5). Due to the rarity of
SCID, only sporadic reports have appeared describing
results of studies of lymphocyte subpopulations in such
infants by these techniques (3, 6–10). The present re-
port describes results of studies of the membrane charac-
teristics and functional capacities of peripheral blood
lymphocytes from three male infants with the adenosine
deaminase (ADA)-positive form of SCID (11). Despite
absences or very minimal evidence of T and B cell func-
tions, all three patients had both T and B cells, as
defined by surface markers, and, in case 1, the percent-
ages of each were near normal throughout a 4½-mo
study. The lymphocytes from two of the patients were
studied for suppressor activity by co-cultivation with
normal B cells and none was found.

[1] *Abbreviations used in this paper:* ADA, adenosine de-
aminase; LALI, lymphocyte-antibody lymphocytolytic inter-
action; SCID, severe combined immunodeficiency.

METHODS

Cases. Patient 1, a 7-mo-old white male, was admitted to the Duke University Medical Center in 1973 with interstitial pneumonia and a history of weight loss, diarrhea, and oral moniliasis of 1–2-mo duration. Two brothers and four maternal uncles had died in early infancy with severe infections. Serum immunoglobulin concentrations were: IgG 240 (after gamma globulin therapy), IgA 4, IgM 5, and IgD < 1 mg/dl, and IgE 1 IU/ml. No antibodies were detected to type A or B erythrocytes (blood type O) or to diphtheria or tetanus toxoids, despite adequate immunization. Erythrocyte ADA levels were normal. Skin tests for cell-mediated immunity to *Candida* and streptokinase-streptodornase were negative. He was treated with pyrimethamine and sulfa and improved sufficiently to be given a fetal liver transplant when he was 11 mo of age; the details of this are reported elsewhere (12).

Patient 2, a 12-mo-old white male, was admitted to the Duke University Medical Center in 1974 for immunologic evaluation after a history of weight loss and oral and cutaneous moniliasis beginning at 8 mo and biopsy-proven *Pneumocystis carinii* pneumonia at age 10 mo. Family history was negative for immunodeficiency or early death. Serum immunoglobulin concentrations were: IgG 20, IgA 0, IgM 0, and IgD < 1 mg/dl and IgE 6.3 IU/ml. No antibodies were detected to type A or B erythrocytes (blood type 0) or to diphtheria or tetanus toxoids, despite adequate immunization. Erythrocyte ADA levels were normal. Skin tests to *Candida* and streptokinase-streptodornase were negative. He, like case 1, had no histocompatible potential donors of bone marrow and was also given a fetal liver transplant at age 13 mo, the details of which are reported elsewhere (12).

Patient 3, a 5½-mo-old white male, was admitted to the Duke University Medical Center in 1974 with a history of recurrent otitis since 2 mo, of intermittent diarrhea since 3 mo, and of chronic thrush and recurrent furunculosis since 4 mo of age. Family history was negative for immunodeficiency or early death. Serum immunoglobulin concentrations were: IgG 19, IgA 0, IgM 0, and IgD < 1 mg/dl, and IgE 0.8 IU/ml. No antibodies were detected to type A or B erythrocytes (blood type A) or to diphtheria or tetanus toxoids, despite adequate immunization. Erythrocyte ADA levels were normal. Skin tests to *Candida* and streptokinase-streptodornase were negative. HLA typing by lymphocyte cytotoxicity revealed no HLA-identical family members. Repeated mixed leukocyte culture studies over 4½ mo demonstrated the mother to be mixed leukocyte reaction-compatible with the infant. Maternal bone marrow cells were given to the patient at age 10 mo; 8 days later he underwent a transient febrile episode lasting 4 days, development of a graft-versus-host rash, and enlargement of the liver with elevated liver enzymes and bilirubin in the serum. Splenomegaly did not occur, chimerism was established, the graft-versus-host reaction was subsiding, and his course appeared stable when he developed aspiration pneumonia and died 28 days after the transplant. Post-mortem examination revealed *Candida* pneumonia, an epithelial thymus weighing only 0.4 g and containing no Hassall's corpuscles, very few lymphoid elements and no follicles in the spleen, and no detectable lymph nodes or gut-associated lymphoid tissue.

Methods. Rosette formation, membrane immunofluorescence, and lymphocyte stimulation studies were carried out as described earlier (13), except that spontaneous sheep erythrocyte (E or T) rosette studies were performed with neuraminidase-treated as well as untreated sheep erythrocytes (14), human type A erythrocytes coated with rabbit IgG antibody (HEA) were used to test for the Fc receptor, and only human erythrocytes coated with rabbit antibody and mouse complement (HEAC) were used to test for complement receptor B cells. Ficoll-Hypaque-purified peripheral blood lymphocytes were used for all of these studies and the cells were also preincubated with carbonyl iron to deplete monocytes for the fluorescence and rosette studies. The purity of such lymphocyte suspensions was > 99% and the yield averaged 60–70% of the cells available in the whole blood. Studies were carried out on from two to six occasions in each patient.

Lymphocyte-antibody lymphocytolytic interaction (LALI) studies were carried out as described by Trinchieri et al. (15), using macrophage-depleted, Ficoll-purified peripheral blood lymphocytes as effectors, and ^{51}Cr-labeled Ficoll-purified human lymphocytes coated with alloantibodies as targets. The effector lymphocyte suspensions were adjusted to 10^6/ml and the effector to target cell ratio was 100:1. A result of more than 10% ^{51}Cr release was considered positive. All alloantisera used had been shown effective in preparing their respective target lymphocytes for killing by a variety of normal effector cells. The experiments were done in triplicate and the percent specific release of chromium was calculated by the formula E-$S/TI \times 100$, where E is the experimental release (mean value), S is the spontaneous release (target + effector + media) and TI is the total incorporation of chromium. Antibody-dependent cell-mediated cytotoxicity was also tested with similarly prepared macrophage-depleted or undepleted mononuclear effector cells and either chicken erythrocytes coated with rabbit IgG antibody or human O⁺ erythrocytes coated with Ripley IgG anti-D labeled with ^{51}Cr as targets.

Lymphocytes were tested for their ability to synthesize immunoglobulins in vitro as described elsewhere (16). To determine whether defective immunoglobulin synthesis in vivo was associated with the presence of suppressor cells, the lymphocytes of the patients were co-cultured with an equal number of lymphocytes from normal individuals in the presence of pokeweed mitogen (16). The synthesis of immunoglobulin (Ig) by cells of two subjects in co-culture was related to the sum of the expected contribution by each cell population as follows:

Synthesis of Ig by cells in co-culture as percentage of Ig synthesized individually = $100 \times$ (synthesis of Ig by 10^6 cells from both subjects in co-culture/($\frac{1}{2} \times$ sum of Ig synthesized by 2×10^6 cells of each subject cultured separately).

RESULTS

The absolute lymphocyte counts and percentages of T and B cells in the peripheral blood of each of the three patients at the time of their initial evaluations are presented in Table I. Cases 1 and 2 were lymphopenic, but the lymphocyte count of patient 3 was well above the normal mean for age. Cells having surface characteristics of T or B lymphocytes were found in all three infants. In patients 1 and 2 the percentages of cells forming spontaneous sheep erythrocyte (E) rosettes were very low, whereas the percentages of cells bearing the complement receptor were elevated in both, particularly in case 2 where 91% of the cells formed HEAC rosettes. In addition, the percentages of patient 2's cells bearing IgM and IgD on their surfaces were

TABLE I

T and B Cells in the Peripheral Blood of Three Infants with Severe Combined Immunodeficiency

Case	Age	Absolute lymphocyte count	Ig-bearing B cells		HEAC(B) rosettes	HEA(Fc) rosettes	E(T) rosettes	En(T) rosettes
			M	D				
	mo	per mm³	%		%	%	%	%
1	7	2,500	18.6	4.6	38.0	0	3.5	8.5
2	12	2,376	54	69	91.0	4.0	1.0	6.0
3	5½	10,175	7	12	9.0	3.0	61.0	76.0
Normals*		7,500	7.6	4.5	15.7	6.23	55.4	74.9
		(4,000–10,500)	±3.0	±3.6	±5.6	±4.07	±8.9	±6.2

* See legend to Fig. 1 for source of normal data for rosette studies. Normal data for membrane immunofluorescence studies have been reported previously (13). The mean and range of absolute lymphocyte counts in normal infants from 5 to 10 mo of age are taken from Altman, P. L.: Blood and Other Body Fluids. FASEB publication, Table 46, p. 125, Washington, D. C., 1961.

markedly elevated. The sum of the percentages of IgD and IgM-bearing cells exceeded 100%, suggesting that immunoglobulins of both of these classes were present on many cells, as in the case of the normal co-existence of IgM and IgD (17). In addition, by combining the immunofluorescence and E-rosetting techniques, we looked in case 2 for cells similar to those reported by Dickler et al. (18), which bear both T and B lymphocyte markers. Only 2% such cells were found; this is within the range of percentages (1.0–6.0%) found in normals by the latter workers. Membrane immunofluorescence studies yielded essentially normal results in cases 1 and
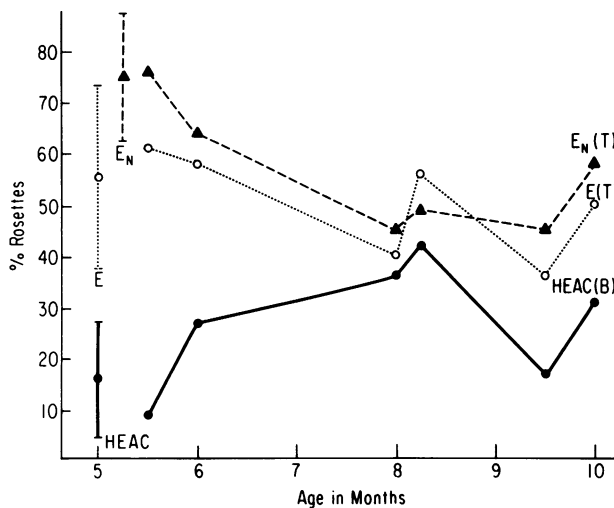

FIGURE 1 Serial determinations of percentages of peripheral blood lymphocytes from case 3 forming rosettes with sheep erythrocytes (E or T), neuraminidase-treated sheep erythrocytes (En or T), and human erythrocytes coated with rabbit antibody and mouse complement (HEAC or B) over 4½ mo. The means±2 SD of the data from 21 En, 62 E, and 39 HEAC evaluations of normal subjects are indicated by the vertical bars in the upper left.

3, except for an elevated percentage of IgM-bearing cells in patient 1. The E rosette percentages for patients 1 and 2 remained below 5% when evaluated for 17 and 7 wk, respectively. Unexpectedly, in case 3 the percentages of cells forming spontaneous E rosettes and rosettes with neuraminidase-treated sheep erythrocytes (En) were initially entirely normal.

Due to the long period of follow-up of patient 3 before definitive therapy was attempted, we had an opportunity to monitor peripheral blood lymphocyte characteristics in this infant for 4½ mo. The results of serial studies of patient 3's cells, as defined by rosette tests, are presented in Fig. 1. The percentages of E(T) rosetting cells remained within 2 SD of the normal mean throughout the follow-up, although the percentages of rosettes with neuraminadase-treated sheep erythrocytes (En) usually fell somewhat under 2 SD below the normal mean. The percentages of cells in patient 3 bearing the complement receptor were above 2 SD of the normal mean on most determinations, but were within the normal range at the initial and 9½-mo evaluations, and never exceeded 45% of the peripheral blood lymphocyte population. Serial absolute lymphocyte counts for patient 3 were variable but were well within the normal range at the initial and 7½-mo evaluations and were very close to the lower range of normal (4,500/mm³) on all but four of the remaining evaluations, when values ranged from 2,300 to 3,300/mm³.

Despite the presence of cells in each of these infants with surface markers characteristic of T or B lymphocytes, functions normally attributed to T or B cells were either lacking or only feebly manifested in all three. Each was markedly deficient in serum immunoglobulins and none had demonstrable antibody production. Moreover, as shown in Table II (lymphocyte stimulation studies in all three revealed either no significant DNA synthesis or an extremely low level in response to the

TABLE II
Lymphocyte Responses to Mitogens and Allogeneic Cells

Case and age	Exp.		PHA Unstim	PHA Stim	ConA Unstim	ConA Stim	PWM Unstim	PWM Stim	MLR Unstim	MLR Stim
mo										
1	1	Pt.	2,838	5,870*	2,948	4,926	2,725	3,876	1,900	1,948
7		Cont.	2,506	13,360*	2,659	33,422*	3,056	34,884*	1,488	40,248*
9	2	Pt.	1,406	4,687*	1,794	2,286	210	359	—	—
		Cont.	5,838	92,092*	821	71,228*	1,275	74,349*	—	—
2	1	Pt.	1,639	3,316	2,165	4,030	—	—	433	1,795
12		Cont.	1,544	73,660*	1,594	81,169*	—	—	613	53,546*
13	2	Pt.	430	1,477*	539	568	581	749	431	447
		Cont.	479	70,968*	438	46,594*	740	65,510*	851	63,742*
3	1	Pt.	296	1,491*	465	717	329	1,485*	726	2,233*
5½		Cont.	195	87,128*	307	61,058*	543	71,001*	455	14,030*
6½	2	Pt.	857	2,508*	1,543	5,140*	1,543	4,614*	691	1,380
		Cont.	513	76,598*	1,482	46,706*	820	56,915*	579	15,736*

PHA, Phytohemagglutinin; Con A, concanavalin A; PWM, pokeweed mitogen; MLR, mixed leukocyte reaction.
* Statistically significant stimulation ($P = < 0.0001$) as determined by Student t tests on the logarithmically transformed data. See legend to Fig. 2 for description of derivation of data.

mitogens or to allogeneic cells. In addition, no significant response occurred to the antigens diphtheria and tetanus toxoids or *Candida*. Results of serial lymphocyte
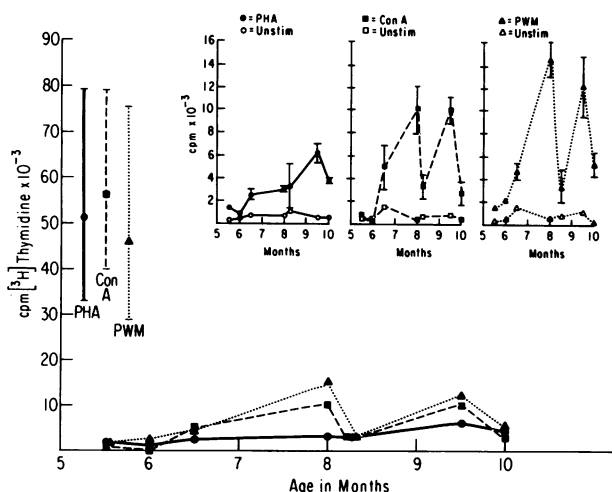

FIGURE 2 Results of serial in vitro lymphocyte stimulation studies in case 3 over 4½ mo. The data presented are the means of results of quadruplicate cultures expressed in counts per minute per 10^5 lymphocytes and represent the maximum responses obtained for patient or normal control lymphocytes. The geometric means and standard deviations of maximal responses in 129 phytohemagglutinin, 114 Concanavalin A, and 109 pokeweed mitogen determinations on a normal population are indicated by the vertical bars in the upper left. The insets in the upper right portion of the figure represent the same data presented on an expanded scale ±1 SD along with results from the unstimulated cultures. Very low but statistically significant ($P = < 0.04–$ < 0.0001) stimulation was noted on all determinations from 6½ to 10 mo of age.

stimulation studies carried out in case 3 over 4½ mo are presented in Fig. 2. Despite some slight fluctuation and statistically significant responses in several of the studies, it can be seen that the maximum responses were still far below normal with each of the mitogens.

The ability of peripheral blood leukocytes to act as effector cells in LALI was tested in patients 2 and 3. As seen in Fig. 3, despite the presence of normal percentages of Fc receptor-bearing cells in case 2, no significant specific release of ^{51}Cr was effected by his cells when they were tested against five different targets. These tests were conducted when the patient was 14 mo old (target 1),
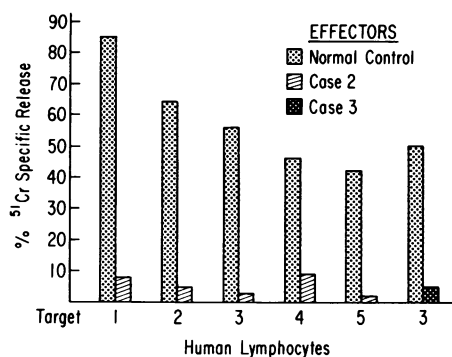

FIGURE 3 Results of LALI studies in cases 2 and 3. Target 1: HLA 2,5, 30/31, 12, antiserum to HLA 5, W5, W18,2. Target 2: HLA 1, 8, antiserum to HLA 8 (long). Target 3: HLA 1, 8, antiserum to HLA 8 (long). Target 4: HLA 1,7,2,5, antiserum to HLA1, W10. Target 5: HLA 1, 8, W28,W17, antiserum to HLA 8 (long). Case 2 was tested at ages 14 mo (target 1), 17½ mo (targets 2 and 3), 21 mo (target 4), and 24 mo (target 5). Case 3 was tested at 10 mo. The results presented represent the means of triplicate determinations.

17½ mo old (targets 2 and 3), 21 mo old (target 4), and 24 mo old (target 5). Similarly, cells from patient 3, who also had a normal percentage of cells bearing the Fc receptor, failed to effect significant specific ⁵¹Cr release from target 3 when he was 10 mo old. In studies by McConnachie et al. (19), leukocytes from all normal infants and children from 1 to 6 yr of age tested were able to serve as effectors in this type of antibody-dependent lymphocyte cytotoxicity test and the cells from 7 of 11 infants ranging from 3 to 12 mo were found to have this ability. In other (unpublished) studies, we found a mean specific release of 32% in LALI assays with cord blood lymphocyte effectors from 10 normal infants. In contrast to the negative results with case 2 and 3's cells in LALI, case 2's cells, whether macrophage-depleted or not, were able to induce lysis of antibody-coated chicken and human erythrocyte targets (Fig. 4).

We sought to determine whether the cells bearing surface markers characteristic of B lymphocytes in the peripheral blood of cases 2 and 3 were capable of synthesizing immunoglobulin when stimulated by a nonspecific B cell stimulant in vitro. As shown in Table III, no IgG, IgA, or IgM were detected in supernates taken at 7 and 21 days from cultures of case 3's washed peripheral blood lymphocytes stimulated with pokeweed mitogen. A small quantity of IgM was detected in 7- and 21-day supernates from case 2 7 mo after his fetal liver transplant, but no IgG or IgA were present. To determine whether certain of their lymphocytes might be functioning as suppressor cells, causing suppression like that caused by T lymphocytes in some patients with common variable hypogammaglobulinemia (16), lymphocytes from cases 2 and 3 were studied in co-cultivation experiments. As seen in Table III, no inhibition of poke-week mitogen-stimulated immunoglobulin synthesis by the normal cells was observed when they were co-cultured with cells from cases 2 and 3. Indeed, there appeared to be considerable augmentation of the quan-
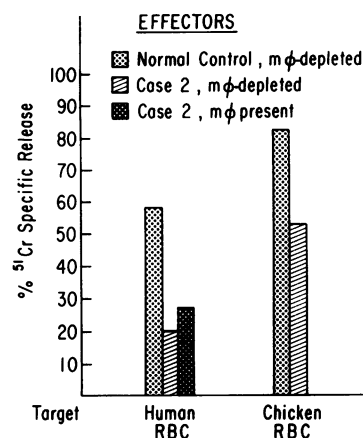

FIGURE 4 Results of antibody-dependent cell-mediated cytotoxicity studies using macrophage (mø)-depleted or undepleted lymphocyte suspensions from case 2 as effector cells and antibody-coated human or chicken erythrocytes as targets. Significant specific release is seen with all effector cell preparations.

tity of IgM, IgA, and IgG produced with two different sets of normal control cells when co-cultured with cells from case 2. The mean quantities of immunoglobulin synthesized over that expected were 560% for IgM, 440% for IgG, and 310% for IgA (Table III). Although this augmentation could be due to donor B cells derived from the fetal liver, the patient had a high percentage of cells bearing B cell differentiation markers before transplantation, and this percentage declined after transplantation. Moreover, when these studies were done, he had no evidence of immunoglobulin production in vivo (12) and, as noted above, his cells produced only a small amount of IgM when cultured alone with pokeweed.

DISCUSSION

The finding of variable, even normal, percentages of cells having surface characteristics of T and B lympho-

TABLE III
Immunoglobulin Biosynthetic Studies with Lymphocytes From Two Infants with Severe Combined Immunodeficiency

Subjects	Immunoglobulin biosynthesis						Synthesis in co-cultures with lymphocytes of unrelated normal individuals§		
	IgM		IgG		IgA				
	7 days	21 days	7 days	21 days	7 days	21 days	IgM	IgG	IgA
	ng/2 × 10⁶ cells/ml culture						% of expected		
Case 2	76	182	0	0	0	0	560	440	310
Case 3	0	0	0	0	0	0	95	93	88
Normal controls (20)‡	4,910	12,134	1,625	5,260	1,270	5,320	106	93	93

* Amount biosynthesized in RPMI 1640 with 10% fetal calf serum. Pokeweed mitogen was used in all cultures as a trigger for B cell maturation and immunoglobulin synthesis.
‡ Geometric mean values for 20 normal controls.
§ Values represent mean values from co-culture experiments with two normal individuals for case 2 and with three normal individuals for case 3.

cytes in these three infants with SCID fails to support the concept of a stem cell defect as their primary problem. Moreover, the heterogeneity observed in their lymphocyte subpopulations adds to that already apparent in SCID from the findings that some of these infants are extremely deficient in ADA (11) or nucleoside phosphorylase (20), key enzymes in the catabolic pathway of purine metabolism. Thus, the syndrome of SCID may result from several different primary biologic errors. The severe immunodeficiency in the patients in the present report appears to be due to functional abnormalities in cells already influenced during development to acquire certain characteristics of T or B lymphocytes. The observations also illustrate the hazard of relying solely on tests to detect and enumerate T and B cells in evaluating the immunologic competence of patients. Despite a normal absolute lymphocyte count and normal percentages of T and B cells on the initial evaluation of case 3, the functional impairment of his cells was severe.

Whatever the abnormality in the cells of these patients was, its profound effects on a number of diverse functions included: (a) failure of the B lymphocytes to differentiate into B plasma cells capable of synthesizing and secreting immunoglobulins; (b) failure of both B and T lymphocytes to proliferate normally in response to mitogens, antigens, or allogeneic cells; and (c) failure of the cells to function as effector killer cells in one type of antibody-dependent lymphocyte cytotoxicity assay. The variable, extremely low but statistically significant responses to phytohemagglutinin by lymphocytes from all three infants, and to concanavalin A, pokeweed mitogen, and allogeneic cells by lymphocytes from case 3 are, however, also evidence against a stem cell defect, since one would not expect such responses if the abnormality occurred at that level.

The failure of mononuclear cells from cases 2 and 3 to function as effectors in LALI contrasts with the results seen when case 2's cells were tested against both human and chicken antibody-coated erythrocyte targets and with the observations of Gelfand (21), who found normal or increased antibody-dependent cell-mediated cytotoxicity by cells from three infants with SCID using chicken erythrocytes or Chang cells coated with antibody as targets. The differences observed could be due to the fact that results in tests of antibody-dependent cell cytotoxicity appear to be highly dependent on the target employed. The studies of Muchmore et al. (22) suggest that monocytes, lymphocytes, and even non-lymphoid cells may act as effectors in assays with antibody-coated heterologous erythrocytes. Other evidence accumulated by those workers, however, favors the view that the primary if not sole effector cells in the LALI type of assay are the Fc-bearing or K cells. Recent evidence reported by Frøland et al. (23) and Wisløff

et al. (24) indicates that these cells are neither T or B cells but a third subpopulation of lymphocytes. Since normal percentages of cells bearing Fc receptors were demonstrated on the surfaces of macrophage-depleted lymphocytes from cases 2 and 3, their failure to act as effectors in LALI appears not to be due to absence of Fc receptor or K cells. The observations are more consistent with an intrinsic defect in the cell, so that triggering of function by stimuli interacting with membrane receptors does not take place. Rachelefsky et al. (25) have also reported no effector cell activity in a LALI type of assay in one infant with SCID but low activity in two given transplants of bone marrow or thymus.

The data are rather more compatible with either a block of maturation of cell function or a metabolic defect that prevents normally differentiated cells from carrying out their functions. Supporting such concepts are the recent demonstrations by Pyke et al. (26) of the normal maturation of bone marrow cells from an infant with SCID when incubated on monolayer cultures of normal human thymic epithelium, and by Polmar et al. (27) of the restoration of in vitro responses of peripheral blood lymphocytes from an ADA-deficient SCID patient by the addition of exogenous ADA. The marked diminution in T cells in cases 1 and 2 indicates that the abnormality, at least in those two infants, affected T cells far more than B cells. The increased numbers of B cells in those two infants strikingly resembles the findings in cases reported by Seligmann et al. (3), Luckasen et al. (8), Geha et al. (9), and Mukhopadhyay et al. (10), suggesting that this pattern of subpopulation distribution is relatively common in SCID.

An alternative possibility is that the defect or defects in these infants could be due to either a deficiency or excess of a particular subpopulation of T cells that affects the maturation or function of other subpopulations of lymphocytes. There could be a deficiency of T-helper cells, for example, or an excess quantity of T-suppressor cells, or both. That such types of aberrations exist in human immunodeficiency has already been well documented in patients with common variable hypogammaglobulinemia, shown to have an excessive proportion of T-suppressor cells, capable not only of suppressing immunoglobulin synthesis by their own B cells but also that of normal B cells (16). In view of the near-normal percentages of T and B cells in case 3, we wondered whether the T cells might be of the suppressor variety since they did not exhibit normal T cell functions. The co-cultivation studies, however, failed to demonstrate suppression of terminal differentiation of B cells by T cells from either case 3 or case 2. This does not, however, rule out the presence of cells capable of suppressing differentiation at an earlier stage. Whether the augmentation of immunoglobulin synthesis observed in co-cultures of case 2's cells with normal cells repre-

sents a helper effect by the normal T cells or was brought about by soluble factors elaborated from normal T cells after pokeweed mitogen stimulation will have to be examined in future experiments.

ACKNOWLEDGMENTS

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