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Immunodeficiency thymus lymphocytes

Ultrastructure of Circulating Lymphocytes in Thymus Disorders

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Extract

Peripheral blood lymphocytes of normal children, children with viral infections, and children with congenital thymic disorders were examined by electron microscopy. Lymphoid cells were classified as small or medium lymphocytes, lymphoblasts, plasmablasts, or plasma cells by certain morphological criteria. Enumeration and classification of cells in normal children revealed that 73.4% were small lymphocytes and 26.6% were medium lymphocytes. No lymphoblasts, plasmablasts, or plasma cells were found in peripheral blood of normal children. In children with infections or congenital thymic disorders, small but significant numbers of lymphoblasts, plasmablasts, and plasma cells were found. Medium lymphocytes were twice as numerous as small lymphocytes. In congenital thymic disorders, there was an absolute depletion of small lymphocytes, whereas medium lymphocytes and more immature forms were present in normal numbers in peripheral blood.

Speculation

The absence of thymus-dependent immunological function is correlated with a deficit in small lymphocytes. This may be due to an absence of the bone marrow precursors of these cells or to a lack of thymic influence on a cell type which remains unidentified.

Introduction

Thymus disorders (TD) constitute a heterogeneous group of immunodeficiencies with variable degrees of lymphopenia and defective cellular immunity. Some of these diseases evolve with abnormal humoral immunity and immunoglobulin defects; other TD are associated with normal antibody production and immunoglobulin levels [7, 12, 23].

The most severe type is combined immune deficiency (CID), of which an autosomal recessive and an X-linked recessive type have been described. In both, lymphopenia is usually severe, and cellular immunity, including the *in vitro* blastogenic response to mitogens, is absent; these patients also exhibit agammaglobulinemia [10, 14].

A second type is the recently described congenital hypoplasia of the thymus (CHT), or DiGeorge's syndrome, with moderate lymphopenia, defective cellular immunity, and a poor *in vitro* blastogenic transformation to specific and nonspecific stimuli. These patients have normal immunoglobulin levels, and their humoral antibody production appears adequate. Such children invariably have defective parathyroid function, the first clinical manifestation of the disease being neonatal tetany due to hypocalcemia. The signs and symptoms of this syndrome have been attributed to abnormal embryogenesis of the 3rd and 4th pharyngeal pouches [6, 15].

The most striking feature of TD is the absence of cellular immunity, a response which largely depends

on the function of small lymphocytes [2]. It was thus of interest to compare the ultrastructure of lymphocytes from patients with these diseases with ultrastructure of lymphocytes of immunocompetent children. Because of their accessibility, circulating blood lymphocytes were chosen for study with an awareness of the limitation such a choice implies, since the different compartments of the lymphoid system, thoracic duct, lymphatics, blood, lymphoid organs, bone marrow, etc., cannot be considered structurally or functionally homogeneous [8, 9, 22].

Materials and Methods

Thymus Disorders

This group included four patients, two with severe combined immune deficiency of the X-linked and autosomal recessive types (these patients were 9 and 10 months of age at the time of study) and two cases of DiGeorge's syndrome (CHT) (both 18 months of age at the time of study). The immunological status of these patients is summarized in Table I.

Table I. Immunological status of patients with thymus disorders

Patient	Sex	Diagnosis[1]	Blood, lymphocytes/mm³	Delayed hypersensitivity, skin test[2]	Phytohemagglutinin response *in vitro*	Development of paracortical areas in lymph nodes
RR	M	CID	Low (900)	–	–	–
DC	F	CID	Low (850)	–	–	–
EM	M	CHT	Low normal (1200)	–	Poor	Poor
CP	M	CHT	Normal (2500)	–	Poor	Poor

Patient	Sex	Diagnosis	Immunoglobulins	Humoral antibodies	Plasma cells in bone marrow	Development of germinal centers in lymph nodes
RR	M	CID	Less than 5%	–	–	–
DC	F	CID	Less than 5%	–	–	–
EM	M	CHT	Normal	+	+	+
CP	M	CHT	Normal	+	+	+

[1] CID: combined immune deficiency; CHT: congenital hypoplasia of the thymus.
[2] Streptokinase-streptodornase, Monilia, dinitrochlorbenzene or dinitrofluorbenzene, and purified protein derivative.

Control Groups

The first control group was comprised of four immunologically normal children, whose ages varied between 6 and 8 months, admitted for noninfectious problems to the Orthopedic or Ophthalmology Services at Children's Hospital Medical Center in Boston.

A second control group was comprised of six immunologically normal children, aged 6–21 months, admitted to the Pediatric Hospital, Centro Medico Nacional, I.M.S.S., in Mexico City for acute viral infections (Table II).

Table II. Immunologically normal children with acute viral infections (infected controls)

Patient	Age, months	Disease
MR	8	Measles
JS	8	Measles with interstitial pneumonia
FS	6	Enterovirus
SC	16	Laryngotracheitis, herpes simplex
FG	15	Measles
LG	21	Viral hepatitis

From each child, 10 ml heparinized venous blood were obtained and centrifuged for 20 min at 1,500 rpm. The buffy coat and supernatant plasma were separated and recentrifuged to obtain a cell pellet which was fixed for 5 min in 2.5% phosphate-buffered glutaraldehyde (pH 7.4) and then trimmed to 1-mm blocks and fixed for another 60–90 min. Tissues were postfixed in 1% osmium-tetroxide, dehydrated, and embedded in Epon-812. Ultrathin sections (600–1000 Å) were stained with uranylacetate and lead citrate, and examined in an electron microscope [28]. Lymphoid cells were photographed and classified according to characteristics shown in Table III. Cell morphology was evaluated according to recent electron microscopic studies of the lymphoid system [1, 3, 13, 17, 20, 27]. Examples of each cell type are shown in Figures 1–7. Cells were classified as blood monocytes when they contained more than five dense, membrane-bound bodies per cell, and a large number of vesicles in the cytoplasm [4]. Such cells were not included in the final count.

Results

The number of cells photographed, the absolute numbers of each cell type, as well as the corresponding percentages for each patient, are shown in Table IV.

Thymus Disorders

In the children with TD, the pooled percentages of the five cell types were, respectively, 31.4, 57.1, 5.6, 5.6, and 0.3. The counts obtained for patients with severe CID were not significantly different from those found in patients with CHT, which permitted pooling of the two results. It is possible, however, that the analysis of

Table III. Characteristics of lymphoid cells

Nomenclature	Figure	Nucleus	Cytoplasm	Endoplasmic reticulum	Polyribosomes	Membrane-bound dense bodies	Approx. size, μ
Small lymphocyte	1, 2, 5, 7	Central, indented, coarse chromatin pattern	Scarce	Minimal	Few or none	Few or none	5–10
Medium lymphocyte	2, 3		Moderate	Occasional	Few	Occasional	10–15
Lymphoblast	4, 7	Central, round, finely dispersed chromatin and heterochromatin; prominent nucleolus	Abundant	Moderate	Prominent	None	10–15
Plasmablast	5, 7		Abundant	Prominent, occasionally laminated	Prominent	None	*10–15
Plasma cell	6, 7	Eccentric round, large blocks of dense chromatin in the periphery	Abundant	Prominent, laminated	Occasional	None	10–15

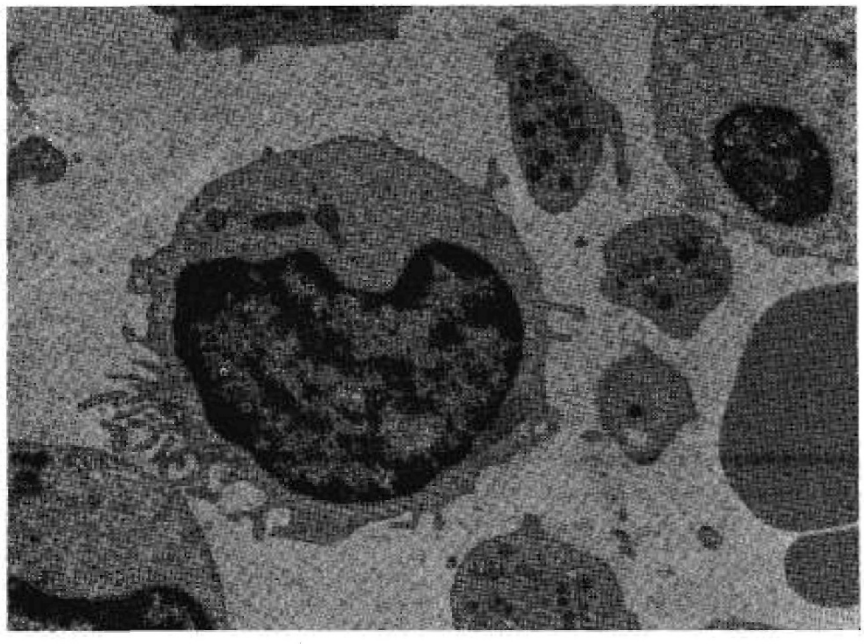

Fig. 1. Typical small lymphocytes from a normal child. The cytoplasm is scarce and contains few organelles. The nucleus is indented and contains dense clumps of chromatin, and the nucleolus is not visible. × 8,500.

a large number of patients will yield significant differences between them. When average percentages of each cell type in the three groups were compared statistically, the differences were highly significant for all cell types ($P = 0.002$) (Table IV). It was considered necessary, however, to compare the absolute numbers of each cell type calculated by extrapolating the percentages observed in the electron microscope with the number of circulating lymphocytes at the time of sampling. These values are shown in Table V. The differences between total circulating lymphocytes were significant when the group with TD was compared with each control group ($P = 0.01$), whereas comparison of the two control groups yielded no significant difference. When absolute numbers of each cell type were compared in control groups, the decreased numbers of small lymphocytes in infected, as compared with normal controls, was found to be significant ($P < 0.01$). The difference in amount of medium lymphocytes was not significant, and it is evident that the presence of lymphoblasts, plasmablasts, and plasma cells in infected immunocompetent children must be considered significant, since they were not found in normal controls.

The comparison of absolute numbers of the five cell types in patients with TD and in normal controls

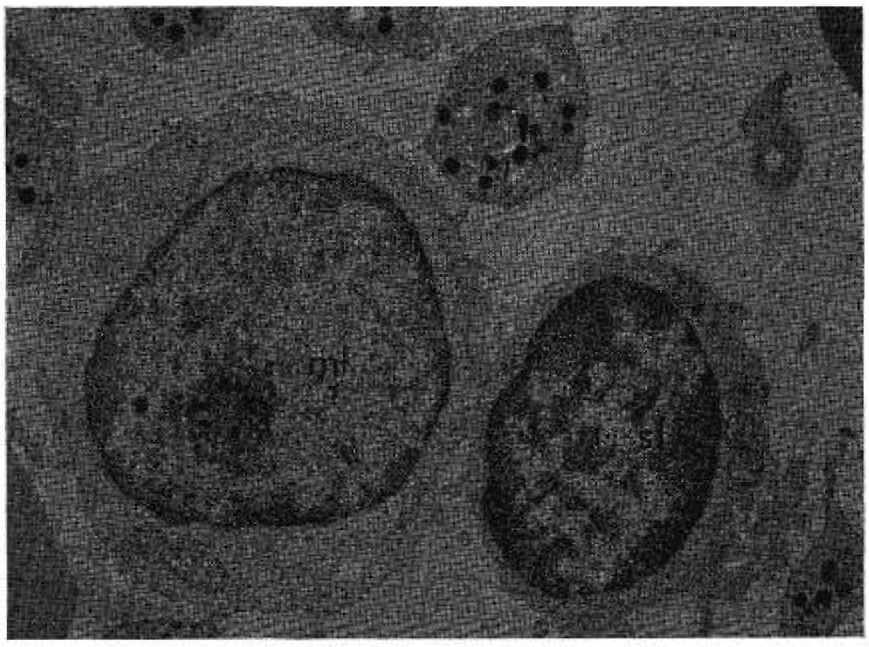

Fig. 2. Buffy-coat section from a child (*RR*) with X-linked severe combined immune deficiency. A small lymphocyte (sl) is shown next to a medium lymphocyte (ml) with a large nucleus and prominent nucleolus. The cytoplasm of both cells is almost devoid of organelles. × 10,000.

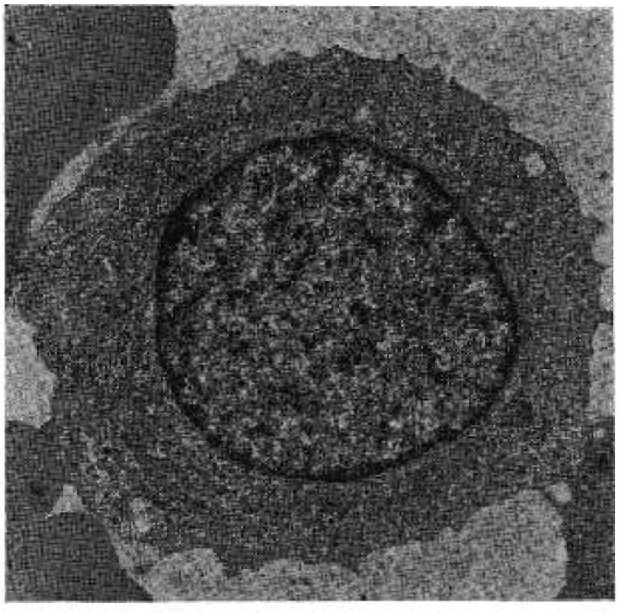

Fig. 3. Medium lymphocyte from a patient with DiGeorge's syndrome showing some polyribosome clusters and several strands of endoplasmic reticulum. × 10,000.

yielded a highly significant ($P = 0.001$) decrease of small lymphocytes, and this was the main reason for lymphopenia in these patients. When absolute numbers of medium lymphocytes were compared between patients with TD and normal controls, no significant difference was noted. Finally, comparison of absolute numbers of small lymphocytes in patients with TD and in infected controls did not yield a significant difference, probably due to the wide range of values

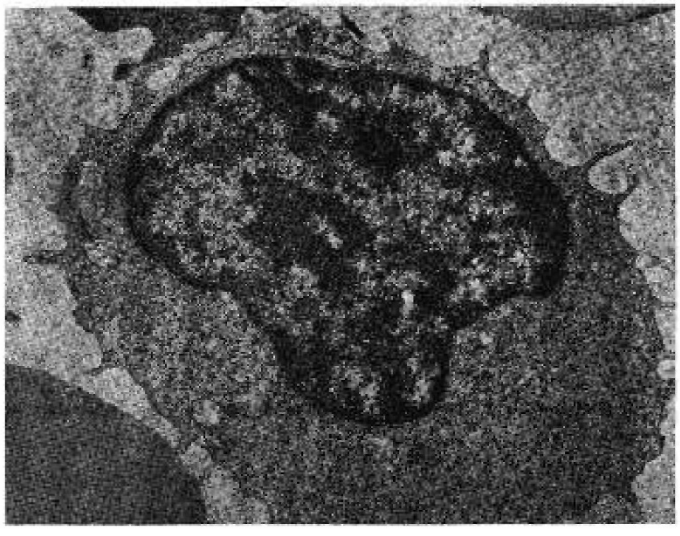

Fig. 4. Lymphoblast from an immunocompetent patient with measles. Cytoplasm contains a large number of polyribosomes. × 10,000.

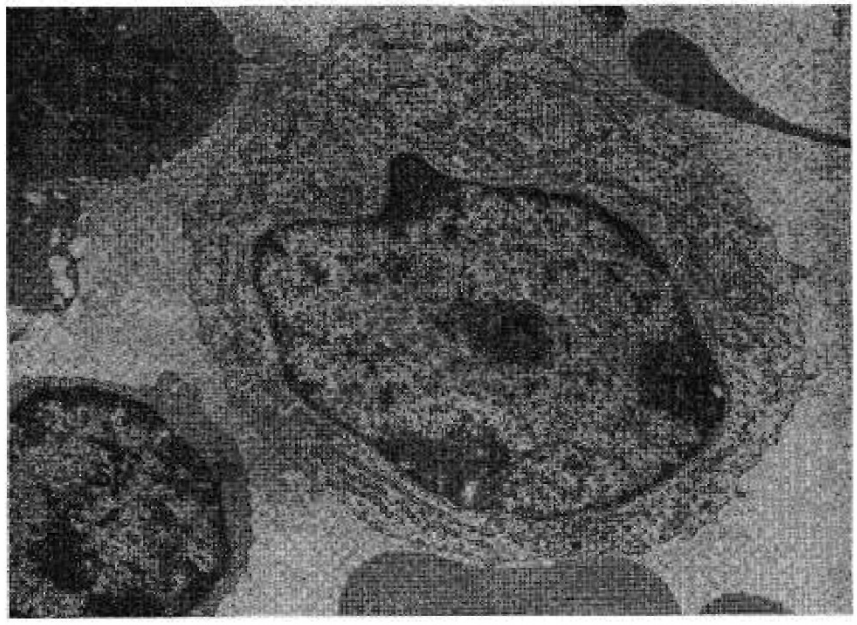

Fig. 5. Plasmablast from a patient with severe combined immune deficiency showing a large nucleus with finely dispersed chromatin and at least two nucleoli (nc). The cytoplasm contains abundant polyribosomes and some strands of endoplasmic reticulum. A small lymphocyte (sl) may be seen in the upper and lower left corners. × 7,200.

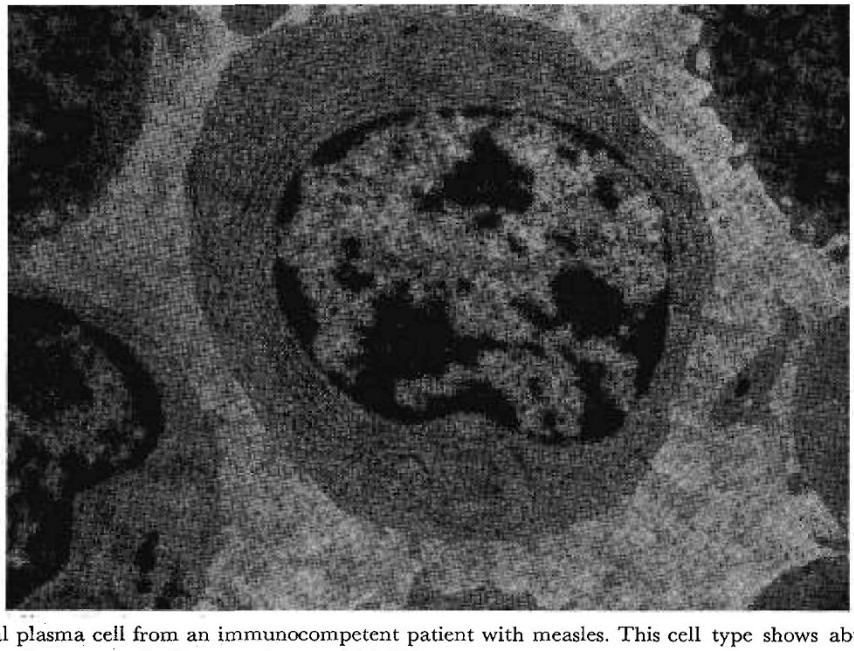

Fig. 6. Section of a typical plasma cell from an immunocompetent patient with measles. This cell type shows abundant laminated endoplasmic reticulum and very few or no polyribosome clusters. × 9,000.

found in these controls (Table V). A significantly lower number of medium lymphocytes, lymphoblasts, and plasmablasts was found in patients with TD when they were compared with infected controls ($P = 0.02$, 0.01, and 0.02, respectively). The differences in plasma cells were not significant.

Control Groups

Out of 214 classified cells in the noninfected immunocompetent children, 73.4% were small lymphocytes and 26.6% medium lymphocytes. No other cell types were found in this group. The group of infected im-

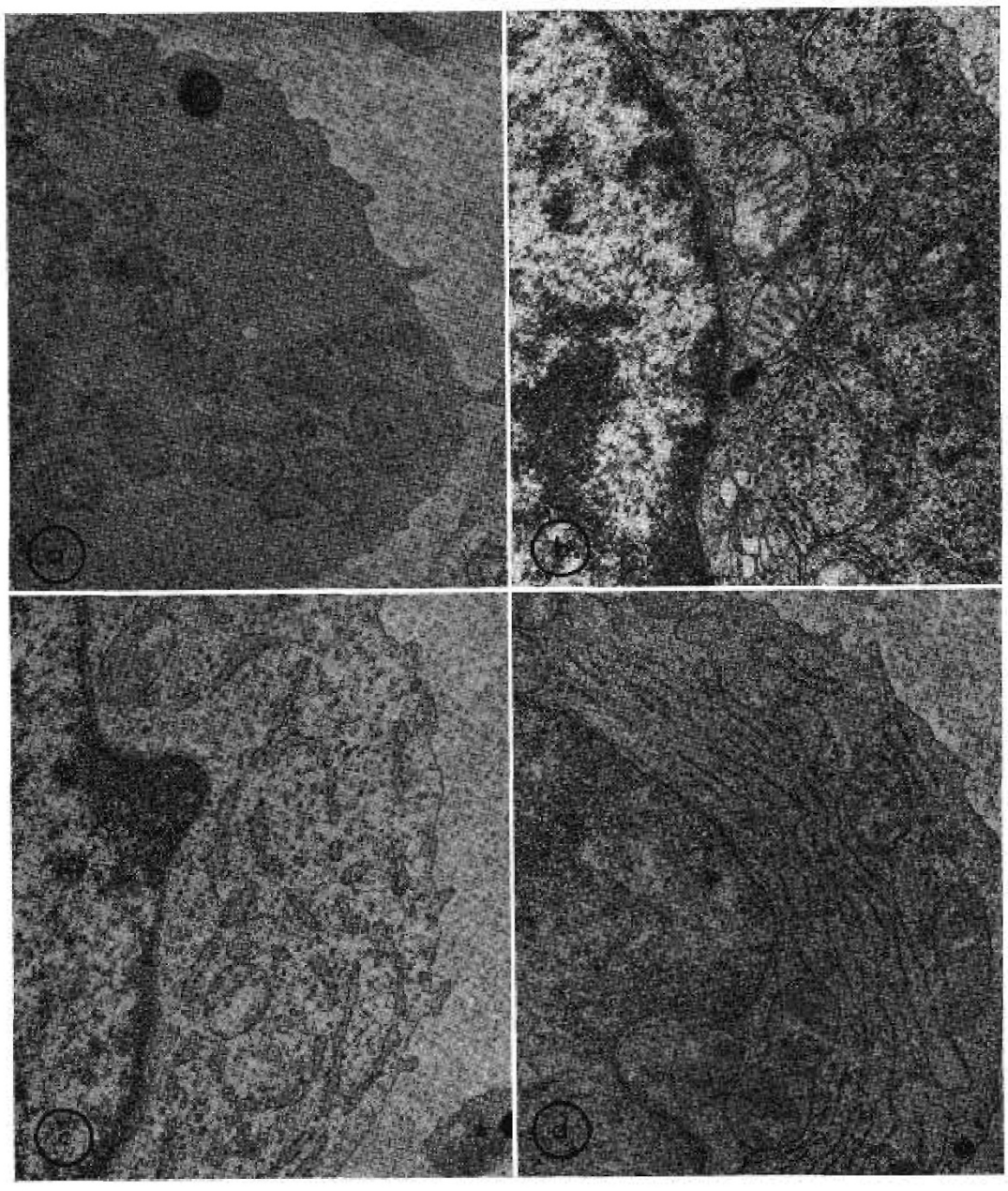

Fig. 7. Detail of lymphoid cells to illustrate differences in cytoplasmic structure. (*a*) Small lymphocyte with single ribosomes, a strand of endoplasmic reticulum and one dense body. × 17,000. (*b*) Detail of a lymphoblast, with abundant polyribosomes and some strands of rough endoplasmic reticulum. × 17,000. (*c*) Detail from plasmablast in Figure 5, illustrating large number of polyribosomes and prominent endoplasmic reticulum. × 17,000. (*d*) Plasma cell from a patient with DiGeorge's syndrome. Cytoplasm contains abundant distended rough endoplasmic reticulum and almost no polyribosomes. × 20,000.

munocompetent children exhibited the five cell types included in Table III.

Discussion

Circulating lymphocytes do not constitute a morphologically or functionally homogeneous cell population. Functionally, two populations have been described: one, thymus-dependent, with a long half-life and fundamentally involved in cellular immunity; and another, thymus-independent population, with a short half-life and apparently not related to these phenomena [11].

Table IV. Percentage of lymphocytes in thymus disorders and control groups[1]

Group[2]	Patient	Blood, lymphocytes/mm³	Cells counted	Cell type				
				Small lymphocyte	Medium lymphocyte	Lymphoblasts	Plasmablast	Plasma cell
Normal controls	*AK*	4,500	35	23/65.7	12/34.3	0	0	0
	JM	5,000	39	23/59.0	16/41.0	0	0	0
	LH	5,500	68	59/86.8	9/13.2	0	0	0
	MD	6,400	72	52/72.2	20/27.8	0	0	0
	Average	5,350	214	157/73.4	57/26.6	0	0	0
Infected (viral) controls	*MR*	10,700	68	18/26.5	14/20.6	7/10.3	13/19.1	16/23.5
	JS	8,400	74	13/17.6	46/62.1	14/18.0	1/ 1.4	0/ 0
	FS	3,100	58	2/ 3.4	31/53.6	10/17.2	10/17.2	5/ 8.6
	SC	8,000	44	1/ 2.3	22/50.0	9/20.4	11/25.0	1/ 2.3
	FG	12,700	22	1/ 4.5	12/59.1	4/18.2	4/18.2	0/ 0
	LG	6,200	57	23/40.4	19/33.3	4/ 7.0	10/17.5	1/ 1.8
	Average	8,200	323	58/18.0	145/44.8	48/14.9	49/15.2	23/ 7.1
Thymic disorders								
CHT	*EM*	1,200	86	33/38.4	42/48.8	2/ 2.3	9/10.5	0
	CP	2,500	85	26/30.6	47/55.3	8/ 9.4	3/ 3.5	1/ 1.2
	Average	1,850	171	59/34.5	89/52.0	10/ 6.2	12/ 7.0	1/ 0.6
CID	*RR*	900	56	8/14.3	43/76.8	3/ 5.4	2/ 3.6	0
	DC	850	60	23/38.3	32/53.3	3/ 5.0	2/ 3.3	0
	Average	875	116	31/26.7	75/64.7	6/ 5.2	4/ 3.4	0
CHT + CID		1,362	287	90/31.4	164/57.1	16/ 5.6	16/ 5.6	1/ 0.3

[1] All ratios expressed as absolute numbers/percent of total cells.
[2] CID: combined immune deficiency; CHT: congenital hypoplasia of the thymus.

Table V. Absolute number of lymphocytes

Group[1]	Patient	Small lymphocyte	Medium lymphocyte	Lymphoblast	Plasmablast	Plasma Cell
Normal controls	*AK*	2,957	1,543	0	0	0
	JM	2,950	2,050	0	0	0
	LH	4,774	726	0	0	0
	MD	4,621	1,779	0	0	0
	Averages	3,826	1,524	0	0	0
Infected controls (virus)	*MR*	2,840	2,207	1,104	2,047	2,518
	JS	1,481	5,226	1,590	118	0
	FS	106	1,677	538	538	269
	SC	186	4,033	1,645	2,016	185
	FG	572	7,509	2,312	2,312	0
	LJ	2,489	2,051	431	1,078	111
	Averages	1,279	3,784	1,270	1,352	514
CHT	*EM*	460	586	28	126	0
	CP	765	1,383	235	88	30
	Averages	613	984	131	107	15
CID	*RR*	128	691	49	33	0
	DC	325	453	43	28	0
	Averages	227	572	46	30	0
Pool TD		420	778	88	69	8

[1] CHT: congenital hypoplasia of the thymus; CID: combined immune deficiency; TD: thymus disorders.

Several physiological differences have been described for these two cell populations; thus, thymus-dependent lymphocytes, as estimated by their ability to respond to PHA *in vitro*, are more labile to hypotonic shock [25]. Antilymphocyte serum also selectively affects the thymus-dependent lymphoid population [3]. The two populations also differ in their cytoelectrophoretic mobility [24], adherence to plastic surfaces [21], and organ distribution ("homing") upon injection in syngeneic recipients [9]. Unfortunately, it has been impossible so far to identify these two populations with a typical morphology [1, 3, 13, 17, 20].

In the present study, all cell types found in the blood of infected immunocompetent children were also found in the blood of patients with TD, and only the relative proportion of the cell types varied significantly. Thus, it was found that lymphopenia of TD was largely due to a decrease in the number of small lymphocytes. Although the percentage of medium lymphocytes was predominant in TD, their absolute numbers were not significantly different when compared with those found in normal controls, and were even significantly decreased when compared with those of infected controls. This may reflect arrested lympho- and/or blastogenesis in these patients since the medium lymphocyte does not seem to be an "end-cell" [26]. Large lymphoblasts and plasma cells were not found in the circulation of normal children, but were found in the blood of immunocompetent infected controls, as well as in patients with TD. Such cells have also been described in the course of viral infections,

collagen diseases, and in certain tumors [5], and they are thought to derive either from small lymphocyte blastogenic transformation to premitotic cells or as intermediate cells in the process of lymphogenesis [16]. The presence of these cells in TD suggests the existence of at least some degree of lympho- and/or blastogenic activity.

If they derive from blastogenic activity, then the chronic, predominantly viral infections these patients are known to sustain [12] may constitute the stimulus for this activity, notwithstanding the fact that viruses are considered poor blastogenic agents *in vitro* [19]. The fact that *in vitro* responses to PHA and antigenic stimulation are not altogether absent in lymphocytes from patients with TD seems to be in accordance with this. During the present study, only one child had a positive viral culture (adenovirus) prior to sampling, but all four showed unequivocal signs and symptoms of chronic infection.

Furthermore, it has been suggested that small lymphocytes are capable of responding to certain stimuli with an incomplete or "sterile" transformation which does not render them capable of immunological function [18]. The observation that patients with TD revealed lymphoid cell types compatible with blastogenic transformation, but lack the ultimate immunological functions associated with them, may be alternately explained in this context.

Finally, one has to be cautious in extrapolating purely morphological observations to functional aspects. It seems, though, that circulating lymphocytes in TD are undergoing some sort of reactive activity as judged by the presence of lymphoblasts, plasmablasts, and even plasma cells. Further studies are necessary to clarify these reactive potentialities.

The immunological defect in one patient, *EM*, was corrected by a thymic implant. Ultrastructural studies of his lymphocytes following this procedure revealed a distribution of cell types which was entirely normal.

Summary

Ultrastructural studies of peripheral blood lymphoid cells from infants with congenital hypoplasia of the thymus and severe combined immune deficiency disease revealed the presence of normal numbers of medium lymphocytes and the presence of immature forms (lymphoblasts, plasmablasts, and plasma cells). Such immature forms were not found in the blood of normal children but are present in age-matched controls with viral infections. Infants with congenital thymic disorders were found to have an absolute deficit in the number of small lymphocytes. Although the blood of the infected controls contained a preponderance of medium lymphocytes, there was no significant concomitant deficit in small lymphocytes.

The immunological defect in one infant with congenital hypoplasia of the thymus was corrected by an implant of fetal thymus. Subsequently, the distribution of lymphoid cells in his peripheral blood was the same as that observed in the normal controls.

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