

HUMAN PROTHYMOCYTES  
Membrane Properties, Differentiation Patterns,  
Glucocorticoid Sensitivity, and Ultrastructural Features

By U. GALILI, A. POLLIACK, E. OKON, R. LEIZEROVITZ, H. GAMLIEL,  
A. KORKESH, J. G. SCHENKAR, AND G. IZAK\*

*From the Department of Hematology, the Department of Pathology, and the Department of Obstetrics and Gynecology, Hadassah University Hospital, Jerusalem, Israel*

It is now evident that thymocytes originate from a subpopulation of bone marrow precursor cells that migrate into the thymus (1, 2). Most of the thymic lymphocytes reside in the cortex, which contains a subcapsular band of large lymphoid cells and a major population of smaller, immunoincompetent midcortical and juxtamedullary thymocytes, whereas the remaining 5–10% of the thymic cells are immunocompetent medullary thymocytes (3, 4). Pulse-label experiments have shown that the cortical and medullary thymocytes are derived from the large subcapsular thymocytes (5, 6). In the postnatal thymus only small numbers of these large cells are found, but they do compose a large proportion of the fetal thymocyte population (7, 8). In the mouse, the large subcapsular thymocytes, unlike the cortical cells, lack specific T cell antigens such as  $\theta$ , TL, and G<sub>IX</sub>, which are expressed after 2–5 d of *in vitro* incubation (7, 9, 10). Accordingly, this subset of thymocytes is regarded as thymic precursor cells and has been designated prothymocytes (11).

In man, one of the most reliable markers for recognizing thymic-derived lymphocytes is their spontaneous ability to bind sheep erythrocytes and form E rosettes (12, 13). Whereas most postnatal thymocytes form E rosettes (14), varying proportions of fetal thymocytes lack this capacity, depending upon the age of the fetus (15, 16). Thus, it is assumed that these nonrosetting cells represent the preceding differentiation stage of the thymocytes. The purpose of the present study was to characterize membrane properties, *in vitro* differentiation patterns, glucocorticoid sensitivity, and ultrastructural features of human prothymocytes isolated from human fetal thymic tissue. A lymphoid cell population, similar to the prothymocytes described in mice and rats, was obtained by depletion of the E-rosetting T cells from fetal thymic cell suspensions.

The results obtained in this study show that prothymocytes also differ from thymocytes in relation to cell size, peanut agglutinin (PNA)<sup>1</sup> binding (17), expression of the natural-attachment (NA) phenomenon (18), and susceptibility to *in vitro* cytolysis by hydrocortisone (19). After an *in vitro* incubation, the prothymocytes express thymocyte-like characteristics. Furthermore, the data presented in this study

\* Deceased.

<sup>1</sup> *Abbreviations used in this paper:* NA, natural attachment, PAS, periodic acid Schiff; PBL, peripheral blood lymphocytes; PNA, peanut agglutinin; SEM, scanning electron microscopy; SRBC, sheep erythrocytes.

suggest that the rate of prothymocyte proliferation is regulated by a feedback mechanism mediated by the thymocytes themselves.

## Materials and Methods

### *Thymic Cell Suspension*

Pieces of fetal human thymus were obtained in the course of routine pathological examination from cases of induced abortion in the second trimester of pregnancy after administration of prostaglandin  $F_{2\alpha}$  into the amniotic sac, and from cases of spontaneous abortions as a result of incompetence of os internum. Postnatal thymic tissues were obtained from individuals undergoing open heart surgery. The thymuses were gently teased in sterile saline and pressed through a stainless-steel mesh. Dead cells were removed by gradient centrifugation with Ficoll-Hypaque. The cells were washed and resuspended in RPMI-1640 medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% fetal calf serum. These suspensions contained <3% dead cells as indicated by the trypan blue exclusion test. With similar methods, mononuclear cells were isolated from fetal spleens and livers.

### *Isolation of Prothymocytes*

The prothymocytes were functionally defined as thymic lymphocytes that lacked the ability to form E rosettes. Thus fetal thymocytes were allowed to form E rosettes according to the procedure described below. The mixture of cells and rosettes were layered on top of a Ficoll-Hypaque barrier and centrifuged at 400 *g* for 20 min. The cells that failed to form E rosettes (prothymocytes) remained at the saline-barrier interphase. 98% of the Ficoll-Hypaque-sedimented cells were in rosette form (i.e., thymocytes). Rosetting sheep erythrocytes were lysed with 0.85% ammonium chloride. The isolated subsets of thymic cells were then washed and resuspended in RPMI-1640 medium supplemented with 10% fetal calf serum. It should be noted that in this study we do not discriminate between cortical and medullary thymocytes.

### *Surface Markers*

The rosetting techniques have been described previously (20). Briefly, E rosettes were performed by mixing 0.25 ml of 1% washed sheep erythrocytes with an equal volume of lymphocytes ( $2-5 \times 10^6$  cells/ml). The mixture was spun at 200 *g* for 5 min and incubated for 1 h at room temperature (22–24°C). Thereafter the pellet was gently resuspended, and the percentage of rosette-forming cells was scored in a hemocytometer.

EA and EAC' rosettes were used for the detection of surface Fc and complement receptors, respectively. Ox erythrocytes coated with subagglutinating titers of rabbit anti-ox erythrocyte IgG were used for the formation of EA rosettes, whereas ox erythrocytes coated with rabbit anti-ox IgM and complement were used for EAC' rosettes. The procedures for rosetting were similar to those performed for E rosettes.

**SURFACE IMMUNOGLOBULINS.** Direct immunofluorescence tests were performed with fluorescein-conjugated goat anti-human immunoglobulin (Behring-Werke AG, Marburg/Lahn, Federal Republic of Germany).  $1 \times 10^6$  cells in pellet were resuspended in 0.1 ml of diluted (1:10) anti-Ig solution and incubated for 30 min at 4°C. Thereafter the cells were washed three times in cold saline, and the proportion of fluorescent cells was determined in a Zeiss UV fluorescence microscope (Carl Zeiss, Inc., New York).

**PNA RECEPTORS.** PNA is a lectin that binds specifically to cortical thymocytes (17), whereas PNA receptors on medullary thymocytes and circulating T lymphocytes are masked by sialic acid. The cells that bind PNA were identified by fluorescein-conjugated PNA (Miles-Yeda, Israel). The cells were resuspended in a solution of 250  $\mu$ g/ml PNA. The staining procedure was identical to that used in immunofluorescence technique.

**SURFACE T ANTIGEN.** Lymphocyte suspensions in saline ( $10^6$  cells/ml) were mixed with an equal volume of rabbit anti-T cell antiserum (Institut Mérieux, Lyon, France) and rabbit complement. The mixture was incubated at 37°C for 45 min, and the percentage of dead cells was assessed by viable staining with trypan blue.

**NA CAPACITY.** The NA phenomenon has been described previously and reflects the ability of lymphocytes to attach to a variety of normal and malignant cells in a species restricted manner (18, 21). Cultured erythromyeloid K562 cells (22) were used as target cells. Lymphocytes

and K562 cells were adjusted to a concentration of  $10^6$  cells/ml. 0.5 ml of lymphocytes was mixed with 0.1 ml of K562 cell suspension in Falcon 2058 tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). The tubes were spun for 5 min at 200 *g* and incubated at 37°C for 30 min. Thereafter the pellets were gently resuspended, and the number of lymphocytes attached to 100 K562 cells was scored in a hemocytometer. Lymphocytes were easily distinguished from the K562 target cells by their size (K562 cells are three times larger than thymic lymphocytes).

#### *In Vitro Cytolysis by Hydrocortisone*

This test has been described in earlier studies (19, 23) and has shown that glucocorticoids cause specific *in vitro* cytolysis of activated human T cells but do not affect resting peripheral blood lymphocytes (PBL) or postnatal thymocytes. Aliquots of 0.2-ml cell suspensions ( $10^6$  cells/ml) were incubated with varying concentrations of hydrocortisone succinate (Solu-Cortef, The Upjohn Co., Kalamazoo, Mich.), or cortisol (Ikapharm, Ramat-Gan, Israel), in flat-bottom microwells (Cooke Engineering Co., Alexandria, Va.) for 20 h at 37°C in a 5% CO<sub>2</sub> humidified atmosphere. Because a proportion of the cells incubated with the glucocorticoids was completely lysed within 20 h of incubation, the number of permanently damaged cells was assessed by determining the concentration of the remaining viable cells (trypan blue exclusion test) in a hemocytometer. The percentage of cytolysis was calculated by the formula  $(a - b)/a \times 100$ , where *a* is the concentration of viable cells in wells that contain medium without steroids, and *b* is the concentration of viable cells in wells that contain the drug.

#### *Long-Term Cultures*

The various lymphoid suspensions ( $10^6$  cells/ml) were incubated in RPMI-1640 medium plus 10% fetal calf serum (100 U/ml penicillin and 100 µg/ml streptomycin), at 37°C, in a 5% CO<sub>2</sub> humidified atmosphere, for up to 7 d. Aliquots of these cultures were examined at 24-h intervals. The following parameters were studied: (a) proliferation rate as determined by the percentage of cells with mitotic figures, after staining with May Grunwald-Giemsa; (b) the proportion of cells that formed E rosettes; and (c) the capacity of NA to K562 cells.

#### *Morphological Studies*

**CYTOCHEMISTRY.** Routine staining for the following enzymes was performed on the different thymic lymphoid populations: myeloperoxidase, acid phosphatase, β-glucuronidase, and non-specific esterase with and without fluoride inhibition; Sudan black and periodic acid Schiff (PAS) stains were also performed. References for the above staining procedures may be found in an earlier publication (24).

**TRANSMISSION ELECTRON MICROSCOPY.** Cell pellets ( $6-8 \times 10^6$  cells) were fixed with phosphate-buffered 1.25% glutaraldehyde (pH 7.3, 4°C) for at least 1 h, rinsed with 0.2 M phosphate buffer, postfixed in osmium tetroxide for 1 h at 4°C, dehydrated through a graded series of ethanols, embedded in low-viscosity epoxy resin embedding medium according to Spürr (25), and sectioned with an MT-2 Porter-Blum microtome equipped with a diamond knife. Thin sections were mounted on uncoated copper grids, stained with uranyl acetate and lead citrate, and viewed with a Philips EM-300 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N. J.).

**SCANNING ELECTRON MICROSCOPY (SEM).** Six million cells were mixed in suspension in 1% phosphate-buffered glutaraldehyde (pH 7.3, 310 mosmol) for at least 1 h, at room temperature and then collected onto glass coverslips covered by poly-L-lysine as described by Sanders et al. (26). Coverslips with monolayers of cells were fixed for another hour at room temperature and then prepared for SEM by further dehydration in a graded series of ethanol and Freon 113 and critical point dried with Freon 113 as described in earlier studies (27). The specimens were then coated with a thin layer of gold-palladium and examined with a Jeol SM-35X scanning electron microscope, at an accelerating voltage of 35–39 KV.

## Results

**Characteristics of the Human Prothymocyte.** Prothymocytes were isolated from the fetal thymic cell suspensions after depletion of the E-rosetting cells. The prothymocytes

were identified as precursors of thymocytes by their spontaneous expression of thymocyte characteristics upon prolonged *in vitro* incubation (Fig. 4). Prothymocytes differed from fetal or postnatal thymocytes in the following ways: Thymocytes are small cells (5–7  $\mu\text{m}$ ) with a dense nuclear chromatin pattern, whereas prothymocytes are larger cells (>7  $\mu\text{m}$ ) with a more delicate nuclear chromatin pattern and more ample basophilic cytoplasm containing organelles (Figs. 1 and 2; Table I). Thymocytes consistently show NA capacity (19, 21) in that they readily adhere to K562 cells, whereas prothymocytes fail to interact with these target cells (Table I). Thymocytes have the ability to bind the lectin PNA via galactosyl residues of certain surface glycoproteins (28). Fetal thymocytes bind PNA to the same degree as postnatal

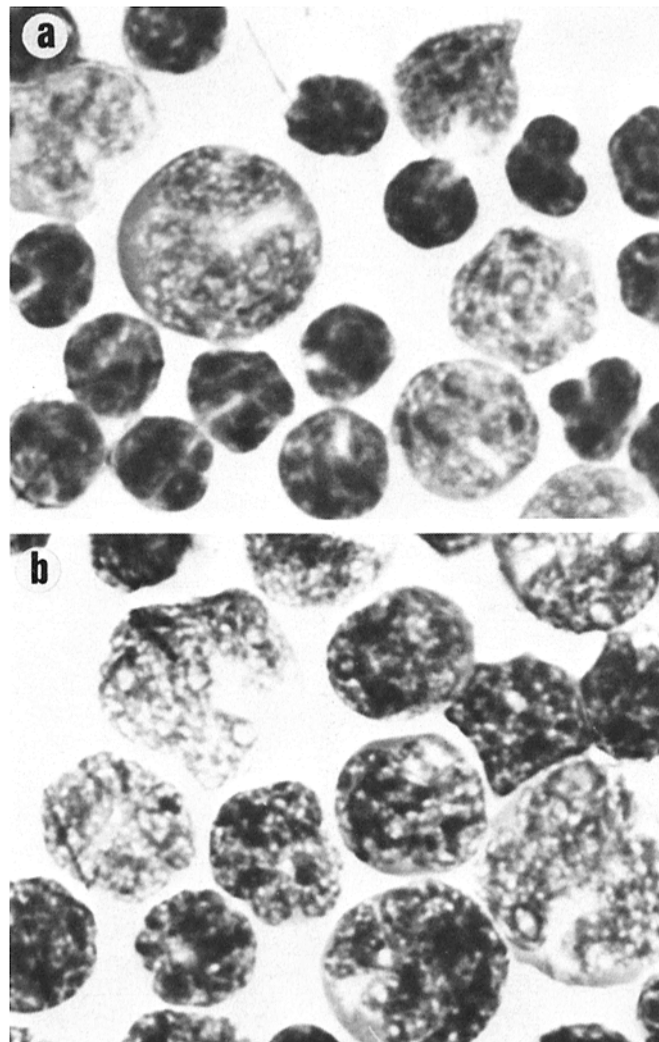


FIG. 1. (a) A suspension of human fetal thymic cells that shows the difference in size and nuclear chromatin patterns. Large prothymocytes have more delicate chromatin and ample cytoplasm.  $\times 1,000$ . (b) A suspension of human prothymocytes that was enriched after depletion of the smaller E-rosetting thymocytes with Ficoll-Hypaque gradient centrifugation.  $\times 1,000$ .

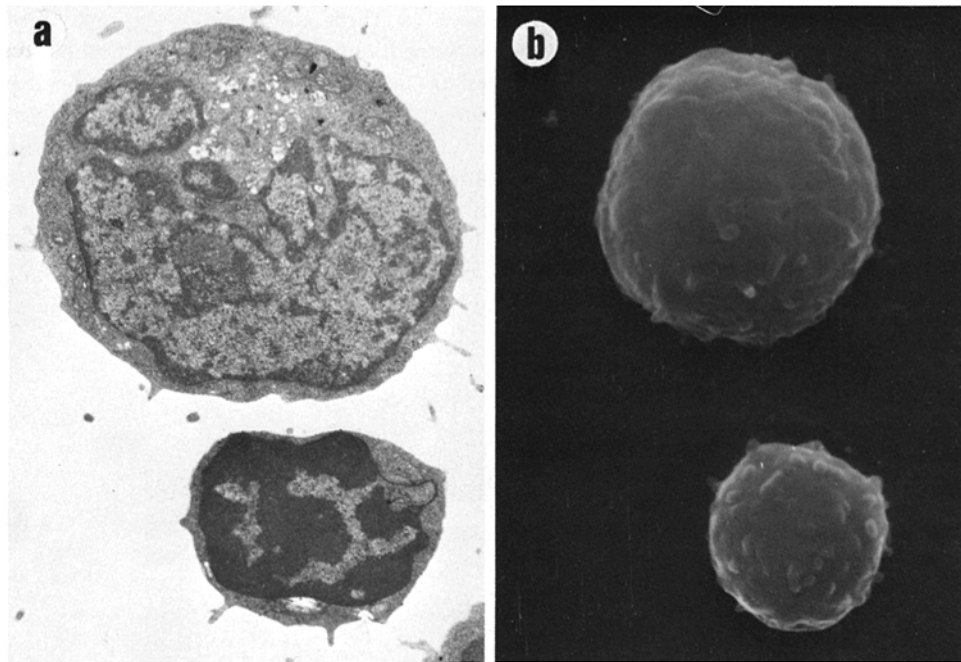


FIG. 2. (a) A transmission electron micrograph of thymic cells that shows a larger prothymocyte alongside a smaller thymocyte. Note the difference in nuclear chromatin pattern and cytoplasmic features.  $\times 3,600$ . (b) A scanning electron microscopic photograph that shows the thymic cells' size difference. The upper cell is a prothymocyte, and the lower cell is a thymocyte.  $\times 2,700$ .

TABLE I  
*Surface and Enzymatic Characteristics of Prothymocytes in Comparison with Thymocytes\**

Characteristic	Fractionated fetal thymic lymphocytes							
	Fetal thymic lymphocytes (nonfractionated)		Fractionated fetal thymic lymphocytes				Postnatal thymocytes	
	Mean	Range	Prothymocytes (non-E-rosetting cells)		Thymocytes (E-rosetting cells)		Mean	Range
E rosettes, %	67	58-80	7	4-10	95	93-98	98	97-99
EA rosettes, %	<1		2	1-4	<1		<1	
EAC rosettes, %	<1		1.3	1-3	<1		<1	
Surface immunoglobulins, %	<1		<1		<1		<1	
Large cells ( $>7\mu\text{m}$ ), %	35	14-55	83	69-92	3	2-4	<1	
NA	90	80-97	4	3-4	132	120-140	192	150-230
PNA receptors, %	81	67-90	10	7-15	>98		>95	
T antigen, %	78	73-86	60	39-69	95	94-97	98	97-100
Acid phosphatase	Positive		Positive		Positive		Positive	
Nonspecific esterase	Negative		Negative		Negative		Negative	
$\beta$ -glucuronidase	Positive		Positive		Positive		Positive	
PAS	Negative		Negative		Negative		Negative	
Sudan black	Negative		Negative		Negative		Negative	

\* The data are expressed as the mean and range of the results obtained of at least three different thymic tissues.

thymocytes, whereas most of the prothymocytes seem to lack receptors for this lectin (Table I). Both thymocytes and prothymocytes lacked surface immunoglobulins, whereas the few EA rosettes found were formed by monocytes within the thymic

suspensions and were detected by positive esterase and peroxidase staining (Table I). In contrast to thymocytes, only a proportion of prothymocytes exhibited the membrane T antigen, as demonstrated by the cytotoxicity assay (Table I); however, the focal paranuclear staining for acid phosphatase and  $\beta$ -glucuronidase, usually exhibited by T cells and thymocytes, was also found in the prothymocytes. All types of thymic cells lacked positive staining for Sudan black myeloperoxidase and esterases (characteristic for myelo-monocyte cells) as expected, and the PAS staining was consistently negative (Table I).

SEM studies revealed cells with relatively smooth undulating surfaces with a few microvilli on the surface of prothymocytes. Thymocytes showed a similar topography. SEM also illustrated size differences in these two subsets of cells (Fig. 2). Incubation of various thymic subsets ( $10^6$  cells/ml) with [ $^3$ H]thymidine (2  $\mu$ Ci/ml) for 20 h showed that prothymocytes had highly active DNA synthesis when compared with the thymocytes (Table II).

*In Vitro Sensitivity of Fetal Lymphoid Subsets to Glucocorticoid-mediated Cytolysis.* The viability of fetal thymocytes incubated in vitro with 1 mg/ml hydrocortisone succinate or with  $10^{-4}$  M cortisol remained unaffected, whereas prothymocytes were found to be highly sensitive to cytolysis by these drugs (Fig. 3). Cytolysis of prothymocytes was also demonstrated with pharmacological concentrations of cortisol ( $10^{-5}$  M). Thus the partial sensitivity of the whole fetal thymic suspension seems to be a result of the specific lysis of the prothymocyte subset. The fetal spleen lymphocyte suspension, composed mainly of B cells (>1% E rosettes), and the hemopoietic cells isolated from the fetal liver were unaffected by hydrocortisone (Fig. 3).

*Long-Term Culture of Prothymocytes.* The incubation of prothymocytes ( $0.5 \times 10^6$  cells/ml) in RPMI-1640 plus 10% fetal calf serum resulted in the spontaneous expression of cortical thymocyte characteristics. Within 72 h 40% of the prothymocytes formed E rosettes with sheep erythrocytes (SRBC) and expressed NA capacity (Fig. 4B). These two characteristics reached optimal levels of expression by the 5th d. When the whole fetal thymic cell suspension was incubated for 3 d the E-rosetting cells increased from 58–80 to >90% (not shown).

In vitro incubation of the prothymocytes resulted in a striking proliferation of these cells, as indicated by the proportion of mitotic figures (Fig. 4A). The peak proliferation was detected on the 3rd d of incubation when ~5% of the cells showed mitotic figures, and >80% of the entire population were large cells with basophilic cytoplasm. Upon further incubation, the mitotic rate decreased. By the 6th d, only a few mitoses were

TABLE II  
*Thymidine Incorporation of Various Thymic\* Lymphoid Populations*

Cell population	[ $^3$ H]thymidine incorporation <i>cpm</i>
Fetal thymus	
Whole thymic suspension	41,950 $\pm$ 7,420
Prothymocytes	103,970 $\pm$ 13,700
Thymocytes	36,640 $\pm$ 6,100
Postnatal thymus	
Whole thymic suspension	27,600 $\pm$ 6,810

\* The results are the mean  $\pm$  SE of three different experiments.

## HUMAN PROTHYMOCYTES

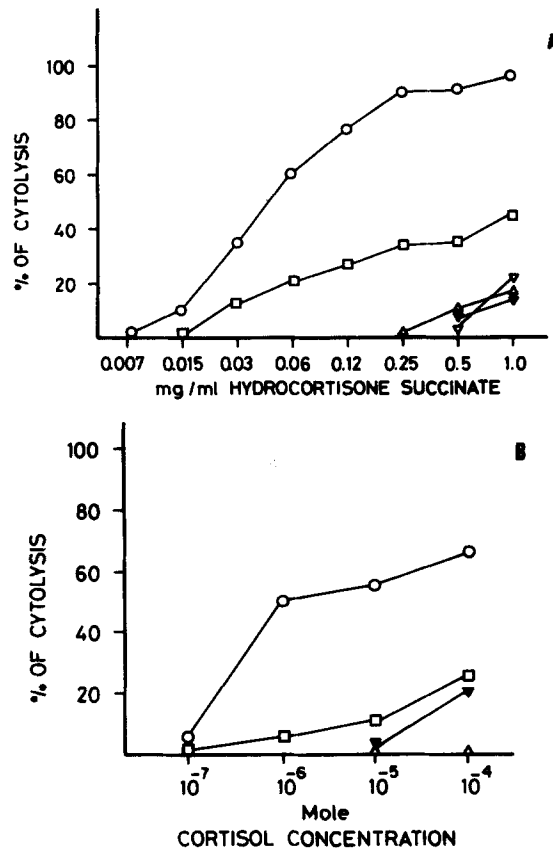


FIG. 3. A representative experiment (similar results were obtained in three different experiments) that shows *in vitro* sensitivity of fetal lymphoid cells to glucocorticoid-induced cytolysis. A. Hydrocortisone succinate. B. Cortisol. Whole thymic cell suspension (□); prothymocytes (○); E-rosetting thymocytes (Δ); splenocytes (▽); and liver hematopoietic cells (▼).

seen, and the majority of cells were small lymphocytes with dense nuclear chromatin. Additional incubation resulted in varying degrees of cell death.

When the unseparated fetal thymic cell suspension was tested for spontaneous *in vitro* proliferation, very few mitotic figures were detected during the various stages of incubation despite the fact that prothymocytes comprised 20–30% of the entire population. The E-rosetting thymocytes also failed to show any spontaneous proliferation after prolonged incubation *in vitro* (Fig. 4 A) and died after 4 d of incubation. These findings suggested that the rate of prothymocyte proliferation may be regulated by the more-mature E-rosetting thymocytes. Accordingly,  $0.5 \times 10^6$  prothymocytes/ml were incubated for 3 d with varying numbers of E-rosetting thymocytes, and the percentage of cells in mitosis was scored in each culture. As seen in Fig. 5, incubation of  $0.1 \times 10^6$  thymocytes with  $0.5 \times 10^6$  prothymocytes decreased the amount of mitotic figures by ~50%. Incubation of equal number of thymocytes and prothymocytes ( $0.5 \times 10^6$  cells) resulted in a 90% reduction of mitotic figures. These results imply that the concentration of thymocytes present in the cell suspension regulate the rate of prothymocyte proliferation.

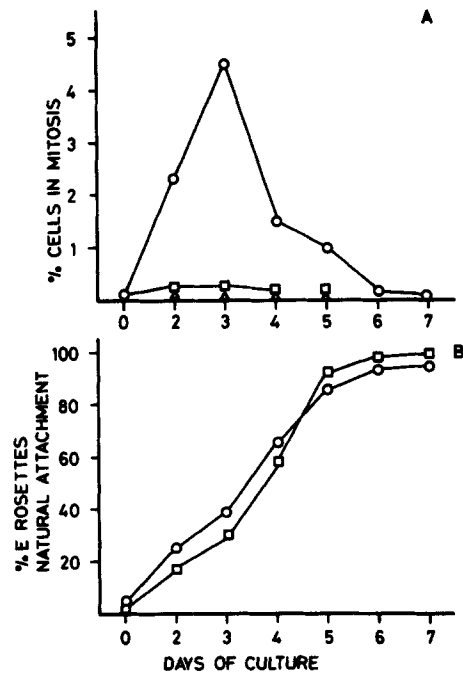


FIG. 4. A representative experiment (similar results were obtained in three different experiments) that shows *in vitro* long-term culture of prothymocytes. A. Cell proliferation rate as indicated by the percentage of cells with mitotic figures. Whole thymic cell suspension (□); prothymocytes (○); and E-rosetting thymocytes (△). B. Expression of thymocyte characteristics by cultured prothymocytes. E rosettes (○); and NA (□).

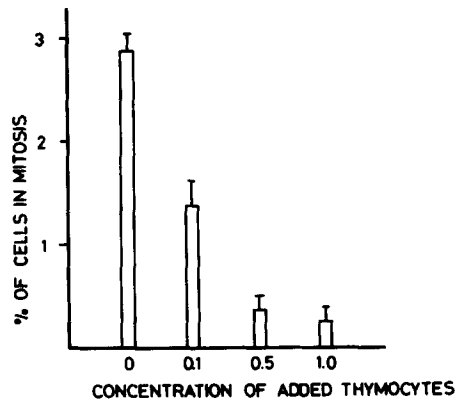


FIG. 5. The effect of coincubation with thymocytes on the proliferation rate of prothymocytes. The cultures were incubated for 3 d before examining the proliferation rate. Mean  $\pm$  SE of three different experiments.

### Discussion

In man, the formation of E rosettes was found to be a useful method for distinguishing between the stem cells recently migrated to the thymus (prothymocytes) and the more-mature, differentiating thymocytes. Most of the current knowledge on prothymocytes has been obtained from experimental animals such as the mouse and the rat



(7, 9–11, 29). Up to the 14th d of gestation, most of the murine thymic lymphoid cells are large cells that lack the T membrane antigens such as TL, Thy-1, Ly-1,2,3, and G<sub>IX</sub> (7, 9). After *in vitro* incubation of these prothymocytes, thymic antigens are expressed within 4–6 d of culture (10, 11). The results of this study show that human prothymocytes are also able to differentiate *in vitro* into cells expressing the SRBC receptor and NA capacity independently of the thymic environment. *In vitro* differentiation of non-E-rosetting fetal prothymocytes, designated as “precursor cells,” was first observed in 1975 by Gaten et al. (16), who separated these cells on the basis of their buoyant density on a bovine serum albumin gradient.

The stimulus for the differentiation and maturation of the prothymocytes to thymocytes is presumed to originate from the thymic epithelial cells, and previous studies have shown that thymic epithelial products can induce differentiation of bone marrow stem cells into lymphocytes that bear recognizable T cell markers in mouse (30, 31) and in man (32, 33). Thus, it seems that the *in vitro* expression of thymocyte characteristics after prolonged culture of prothymocytes is a manifestation of the differentiation stimuli delivered to the prothymocyte *in situ*.

Further similarities between prothymocytes of human and murine origin include the absence of Fc receptors and the lack of readily detectable cell-surface immunoglobulins (11). As in the mouse (34), there is also a difference in the PNA binding capacity of human prothymocytes and thymocytes. Studies on T cell differentiation during murine ontogeny have indicated that prothymocytes lack the ability to bind this lectin; but during fetal development, the cortical thymocytes acquire PNA binding ability. The results of this study also demonstrate that human prothymocytes lack PNA binding capacity as opposed to the strong binding displayed by small E-rosetting thymocytes. PNA interacts with galactosyl residues of a glycoprotein in the membrane of the cortical thymocytes (17, 27), and, through differentiation to medullary thymocytes and mature circulating T cell, a sialic acid group is attached to the galactosyl residue, thus preventing the interaction with PNA in these cells. Treatment of PBL with neuraminidase eliminates the sialic acid and restores the PNA binding capacity of the mature T cells.

In this study, the NA phenomenon was expressed by thymocytes, but not by prothymocytes. Earlier studies (35) have shown that the circulating T lymphocytes do not express NA because of the high negative surface charge, attributed mainly to the presence of sialic acid. Once this negative electrical charge is reduced either by immunoactivation or by neuraminidase, the T cells express the NA ability and interact nonspecifically with normal and malignant cells in a species-restricted manner (18, 21).

In view of the above findings, it is possible that the PNA receptor that coincides with the thymocyte NA site serves as the base for the mounting of sialic acid in the course of T cell differentiation. This, in turn, would inhibit the expression of the NA capacity of the T cell until activation occurs. In activated T cells, the NA phenomenon seems to play an important role in the cellular immune response (36).

Administration of hydrocortisone *in vivo* to either mouse or man results in the involution of the thymus and disappearance of the cortical thymocytes (6, 14). Microscopical examination of the thymus has revealed that murine cortical thymocytes die after such treatment (6). *In vitro* experiments have shown that incubation of rat or murine thymocytes with hydrocortisone also resulted in cytolysis (37), whereas

human thymocytes are unaffected by the same treatment (19). The results of the present study suggest that the thymic involution after hydrocortisone administration in a glucocorticoid-resistant species such as man (37), may be a result of the selective elimination of the prothymocyte population. Subsequent depletion of the thymic cortex would then be a result of normal cell maturation or cell death. At present it is unclear why prothymocytes are sensitive to hydrocortisone while thymocytes are resistant. However, similar differences in glucocorticoid sensitivity have been detected in other stages of T cell maturation. Thus, circulating T lymphocytes are glucocorticoid resistant but become sensitive to these hormones after immune activation (19, 23). The possibility that these differences may be a result of variations in the cytoplasmic or nuclear level of steroid receptors or to the expression of certain genes is currently under study.

The *in vitro* studies of the rate of DNA synthesis, as measured by [<sup>3</sup>H]thymidine incorporation, indicated that human prothymocytes display active DNA synthesis. Thymocytes, however, also synthesize substantial amounts of DNA, which suggests that cell proliferation is not terminated by the expression of T cell-surface markers. High rates of DNA synthesis by human prothymocytes have also been demonstrated previously by Parkman and Merler (8), who isolated cells from fetal thymuses with the buoyant density technique. In the present study long-term *in vitro* incubation of prothymocytes for >24 h resulted in marked proliferation that peaked on the 3rd d, and it seems that the suppression of prothymocyte proliferation after coinubation with thymocytes, may reflect a physiological regulatory mechanism in this system. Studies of murine bone marrow-derived prothymocytes have also shown that the production and proliferation of cells that precede intrathymic prothymocytes are controlled by the homeostatic regulation of the thymus (38). The possibility that such regulation involves thymocyte-secreted humoral factors is, at present, under study.

### Summary

Thymic precursor cells (prothymocytes) comprise a large proportion of the fetal thymic cell population, but are less frequently encountered in the postnatal thymus, where they compose <1% of the entire population. In the present study we attempted to characterize a number of properties of the prothymocytes obtained from human fetal thymic tissues after depletion of the E-rosetting thymocytes on a Ficoll-Hypaque gradient. The prothymocytes are larger than the thymocytes and show a different nuclear chromatin pattern. This subset of cells lacks the E-rosetting and natural-attachment capacities and, unlike thymocytes, does not bind the lectin peanut agglutinin. Human prothymocytes are highly sensitive to the *in vitro* cytolytic effect of hydrocortisone, whereas the thymocytes are resistant. Long-term *in vitro* culture of prothymocytes resulted in the expression of thymocyte characteristics together with a burst of mitotic activity. Results of this study indicate that the rate of the prothymocyte proliferation is regulated by the small thymocytes present in the same suspension.

*Received for publication 19 May 1980.*

### References

1. Ford, C. E. 1966. Traffic of lymphoid cells in the body. In thymus. Experimental and clinical studies. *Ciba Found. Symp.* 131.

2. Moore, M. A. S., and J. J. T. Owen. 1967. Experimental studies on the development of the thymus. *J. Exp. Med.* **126**:715.
3. Bloom, W., and D. W. Fawcett. Textbook of Histology. W. B. Saunders Co., Philadelphia, Pa. 10th edition. 458.
4. Cantor, H., and I. Weissman. 1976. Development and function of subpopulations of thymocytes and T lymphocytes. *Prog. Allergy.* **20**:1.
5. Yoffey, J. M., W. O. Reinhard, and N. B. Everett. 1961. The uptake of tritiated thymidine by lymphoid tissue. *J. Anat.* **95**:293.
6. Weissman, I. L. 1973. Thymus cell maturation. Studies on the origin of cortisone-resistant thymic lymphocytes. *J. Exp. Med.* **137**:504.
7. Owen, J. J. T., and M. C. Raff. 1970. Studies on the differentiation of thymus-derived lymphocytes. *J. Exp. Med.* **132**:1216.
8. Parkman, R., and E. Merler. 1973. Discontinuous density gradient analysis of the developing human thymus. *Cell. Immunol.* **8**:328.
9. Kamarck, M. E., and P. D. Gottlieb. 1977. Expression of thymocyte surface alloantigens in the fetal mouse thymus in vivo and in organ culture. *J. Immunol.* **119**:407.
10. Ritter, M. A. 1978. Embryonic mouse thymus development: stem cell entry and differentiation. *Immunology.* **34**:69.
11. Basch, R. S., and J. L. Kadish. 1977. Hematopoietic thymocyte precursors. II. Properties of the precursors. *J. Exp. Med.* **145**:405.
12. Jondal, M., G. Holm, and H. Wigzell. 1972. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. *J. Exp. Med.* **136**:307.
13. Wybran, J., M. C. Carr, and H. H. Fudenberg. 1972. The human rosette forming cell as a marker for a population of thymus derived cells. *J. Clin. Invest.* **51**:2537.
14. Galili, U., and M. Schlesinger. 1975. Subpopulation of human thymus cells differing in their capacity to form stable E-rosettes and in their immunologic reactivity. *J. Immunol.* **115**:827.
15. Hayward, A. R., and G. Ezer. 1977. Development of lymphocyte populations in the human foetal thymus and spleen. *Clin. Exp. Immunol.* **17**:169.
16. Gatien, J. G., E. E. Schneeberger, and E. Merler. 1975. Analysis of human thymocyte subpopulations using discontinuous gradient of albumin: precursor lymphocytes in human thymus. *Eur. J. Immunol.* **5**:312.
17. Reisner, Y., M. Linker-Israeli, and N. Sharon. 1966. Separation of mouse thymocytes into two subpopulations by use of peanut agglutinin. *Cell. Immunol.* **25**:129.
18. Galili, U., N. Galili, F. Vanky, and E. Klein. 1978. Natural species restricted attachment of human and murine T lymphocytes to various cells. *Proc. Natl. Acad. Sci. U. S. A.* **75**:2396.
19. Galili, N., U. Galili, E. Klein, L. Rosenthal, and B. Nordenskjold. 1980. Human T lymphocytes become glucocorticoid sensitive upon immune activation. *Cell. Immunol.* **50**:440.
20. Galili, U., E. Klein, and M. Schlesinger. 1977. Human T lymphocyte receptors for sheep red blood cells and specific antigens: are they identical sites on the cell membrane? *J. Immunol.* **119**:104.
21. Galili, U., E. Klein, G. Klein, and P. Biberfeld. The natural attachment of thymocytes and activated T lymphocytes to normal and malignant cells: an interspecies study. *Dev. Comp. Immunol.* In press.
22. Lozzio, C. B., and B. B. Lozzio. 1975. Human chronic myelogenous cell line with positive Philadelphia chromosome. *Blood.* **45**:321.
23. Galili, U., E. Klein, G. Klein, and I. S. Bal. 1980. Activated T lymphocytes in infiltrates and draining lymph nodes of nasopharyngeal carcinoma. *Int. J. Cancer.* **25**:85.
24. Ben-Bassat, H., S. Mitrani-Rosenbaum, H. Gamliel, E. Naparstek, R. Leizerowitz, A.

- Korbesh, M. Sagi, R. Voss, G. Kohn, and A. Polliack. 1980. Establishment in continuous culture of a T-lymphoid cell line (HD-MAR) from a patient with Hodgkin's lymphoma. *Int. J. Cancer*. **25**:583.
25. Spürr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**:31.
  26. Sanders, S. K., E. L. Alexander, and R. C. Braylan. 1975. A high yield technique for preparing cells fixed in suspension for scanning electron microscopy. *J. Cell Biol.* **67**:476.
  27. Polliack, A. 1977. Normal, transformed and leukemic leukocytes. In *A Scanning Electron Microscopy Atlas*. Springer-Verlag, Berlin. 5.
  28. Pereira, M. E., F. A. Kabat, R. Lotan, and N. Sharon. 1976. Immunological studies on the specificity of peanut (*Arachis hypogea*) agglutinin. *Carbohydr. Res.* **51**:107.
  29. Order, S. E., and B. H. Waksman. 1969. Cellular differentiation in thymus. Changes in size, antigenic characteristics, and stem cell function of thymocytes during thymus repopulation following irradiation. *Transplantation (Baltimore)*. **8**:783.
  30. Bach, J. F., M. Dardenne, A. L. Goldstein, A. Guha, and A. White. 1971. Appearance of T-cell markers in bone-marrow rosette forming cells after incubation with thymosin a thymic hormone. *Proc. Natl. Acad. Sci. U.S.A.* **68**:2734.
  31. Komuro, K., and E. A. Boyse. 1973. In vitro demonstration of thymic hormone in the mouse by conversion of the precursor cells into lymphocytes. *Lancet*. **I**:740.
  32. Touraine, J. L., J. W. Hadden, and R. A. Good. 1977. Sequential stages of human T lymphocyte differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **74**:3414.
  33. Touraine, J. L., G. S. Incefy, F. Touraine, Y. M. Rho, and R. A. Good. 1977. Differentiation of human bone marrow cells into T lymphocytes by in vitro incubation with thymic extracts. *Clin. Exp. Immunol.* **17**:151.
  34. London, J., S. Berrih, and M. Papiernik. 1979. Peanut agglutinin. III. Study of T lymphocyte differentiation during murine antigeny. *Dev. Comp. Immunol.* **3**:343.
  35. Galili, U., P. Häyry, and E. Klein. 1979. Loss of net negative surface charge during MLC stimulation of human T lymphocytes. Correlation to stable E rosette formation and natural attachment to normal and malignant cells. *Cell. Immunol.* **48**:91.
  36. Galili, U., L. Rosenthal, N. Galili, and E. Klein. 1979. Activated T cells in the synovial fluid of arthritic patients: characterization and comparison with in vitro activated human and murine T cells in cooperation with monocytes in cytotoxicity. *J. Immunol.* **122**:878.
  37. Claman, H. N. 1972. Corticosteroids and lymphoid cells. *N. Engl. J. Med.* **287**:388.
  38. Roelants, G. E., and K. S. Mayor-Witley. 1977. The regulation of the T lymphocyte precursor pool by a humoral factor released by the thymus. *Cell. Immunol.* **34**:420.