Correlation of Lymphocyte Transformation and Morphology in the Human Fetal Thymus

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A study was made of PHA-stimulated thymic lymphocyte transformation in 10 human fetuses ranging from 12 to 22 weeks of gestation. The results were compared with those obtained using circulating blood lymphocytes of the corresponding fetuses in seven cases. The degree of transformation of blood lymphocytes is independent of fetal age. On the other hand, thymic lymphocyte transformation is age-related. Until the 18th week of gestation, the per cent of transformed thymic lymphocytes is comparable to that of blood lymphocytes. After the 18th week of gestation, the per cent of transformed thymic lymphocytes diminishes to the level of adult thymic lymphocytes. The significance of these findings is discussed.

There is controversy concerning the capacity of thymic cells to respond to phytohemagglutinin (PHA). Studies of adult human thymic lymphocytes have brought a better understanding of the problem.[1-4] It appears that adult thymic lymphocytes are composed of two distinct populations. The smaller of these populations is composed of cells which are transformed into blast cells in the presence of PHA. The cells belonging to the larger population do not respond to PHA. For blood lymphocytes, on the contrary, the PHA reacting pool is the larger.

Thymic lymphocytes of the human fetus are also capable of responding to PHA stimulation.[5-8] This capacity can be detected as early as 12 weeks, at a time when the lymphocytes in circulation are rare or totally absent. The relationship of fetal age to degree of thymic lymphocyte transformation is still obscure. The object of the present work is to study this relationship at fetal ages ranging from 12 to 22 weeks.

Two techniques were used to measure the degree of PHA-induced transformation after 72 hours of culture: (1) the percentage of blast cells found; (2) the precentage of labeled cells on autoradiography after tritiated thymidine incorporation. The transformation of blood cells was compared to the transformation of thymic cells for the same fetus under the same technical conditions. A histological study of the thymic maturation was carried on, parallel to the functional study of the thymic lymphocyte population. Particular attention was paid to the partition of cortical and medullary lymphocytes. A relationship was found between changes in this partition and changes in thymic cell response to PHA.

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Materials and Methods

Twenty-two fetuses, ranging in age from 10 to 22 weeks, were studied. Fetal age was estimated on the basis of vertex to coccyx length, using Hamilton standard embryological tables.[9] Histological sections of the thymus were made on all fetuses. Due to contamination or autolysis, lymphocytes suitable for culture were obtained from only 10 fetuses. The thymus was removed under sterile conditions. One fragment was set aside for histological sections. The remainder was utilized for cell cultures. Blood was obtained from the heart.

Histological Study

The thymic fragment was immediately fixed in 0.5 per cent picric acid–alcohol solution. After imbedding in paraffin, numerous sections were cut at 4μ and stained with hematin-eosin-red safranin.

Cell Cultures

The thymus was placed in a sterile Petri dish containing 2 ml. of 199 nutrient medium (Institut Pasteur). It was finely divided with Patchef scissors. Further cell separation was carried out by agitating the fragments for 10 minutes with a magnetic agitator.

The thymic cells were then resuspended in 199 medium containing 10 per cent human AB serum. The final cell concentration was approximately 1000 cells/cu. mm. one ml. portions of this cellular suspension were divided into Leighton tubes. Antibiotics were added (100 U. of penicillin, 50 γ streptomycin/ml.). PHA, 0.05 ml., (Difco) was placed in half of the tubes.

Fetal blood cultures were prepared in the same way by adding several drops of blood to the medium. The final cell concentration was about 700 cells/cu. mm.

All the tubes were incubated at 37° C for 72 hours.

Morphologic and Autoradiographic Study

One μCi. ml. of tritiated thymidine was added to all culture tubes 5 hours prior to the end of incubation (after 67 hours). After 5 hours of incubation with this marker, the cultures were centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded.

Each cell button was used to make smears stained with May-Grünwald-Giemsa and smears for use in autoradiography. Those prepared for autoradiography were fixed in methyl alcohol for 3 minutes, rinsed with tap water and air dried. The slides thus prepared were dipped into Ilford K5, and after 6 days of exposure in the dark were developed with Kodak D19 and fixed with Kodak AL4. The slides were rinsed with tap water for 1 hour and stained with May-Grünwald for 15 minutes, followed by 15 minutes in water.

A separate count was made of the blast cells and the labeled cells found among the whole population. The results are given in percentages. The labeled cells are those involved in DNA synthesis during the incubation period (Fig 3). Since 5 hours of incubation with ^{3}H-thymidine was employed, some cells were near the end of S phase when the marker was added and were also labeled.

Results

Blood Lymphocyte Cultures

Blood lymphocytes of seven fetuses, aged from 15 to 22 weeks, were cultured (Table 1, Fig 1). Three fetuses, aged 12–15 weeks, had an absence or paucity of blood lymphocytes and could not be cultured.

The PHA had induced a lymphoblastic transformation in all the blood lymphocyte cultures from the 15th to the 22nd week of gestation. The percentage of blast cells ranged between 62 and 98 per cent; the per cent of thymidine-labeled cells between 30 and 60 per cent. These results are independent of fetal age.

Table 1.—Per Cent of Blast Cells and Labeled Cells in Cultures of Human Fetal Circulating and Thymic Lymphocytes Stimulated with PHA

No.	E_1		E_{34}		E_{32}		E_{30}		E_{15}		E_{27}		E_{21}		E_{18}		E_{23}		E_{12}	
Age (Weeks)	12		14		15		15		17		17		18		19		20		21	
Cell	B	L	B	L	B	L	B	L	B	L	B	L	B	L	B	L	B	L	B	L
Blood	No Lymphocyte				Rare lymphocytes		69	37	98	60	70	37	89	53	84	50	62	30	92	45
Thymus	70		52	38	85	43	70	41	75	58	56	37	30	24	30	25	12	2	33	21

Percentages are those after 72 hours of incubation. Starting with the 18th week of gestation, the counts for thymic lymphocytes are significantly lower than the counts for circulating lymphocytes.

B, blast cells; L, labeled cells (found among the whole population of cells).

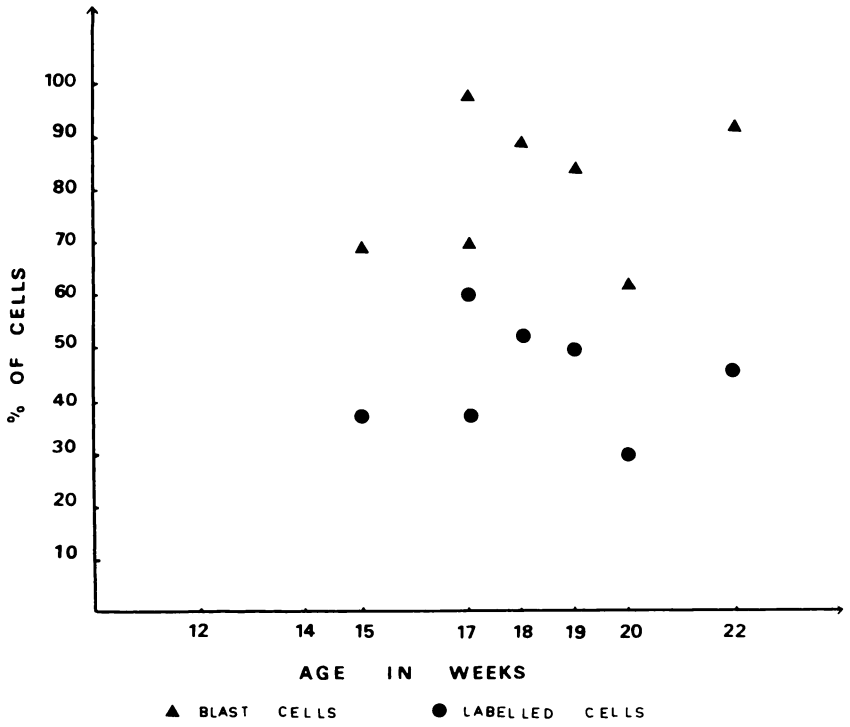

Fig. 1.—Cultures of human fetal blood lymphocytes. Percentage of blast and isotope-labeled cells as a function of fetal age.

Thymic Lymphocyte Cultures

The thymic lymphocytes of the 10 fetuses studied, ranging in age from 12 to 22 weeks, responded to PHA stimulation with lymphoblastic transformation and thymidine uptake (Table 1, Fig. 2). In contrast to blood lymphocytes, the degree of response to PHA stimulation for thymic lymphocytes is related to fetal age and is different before and after the 18th week of gestation.

Six fetuses aged less than 18 weeks were studied. The per cent of blast cells was 52–85 per cent and of labeled cells 37–58 per cent. In the three cases

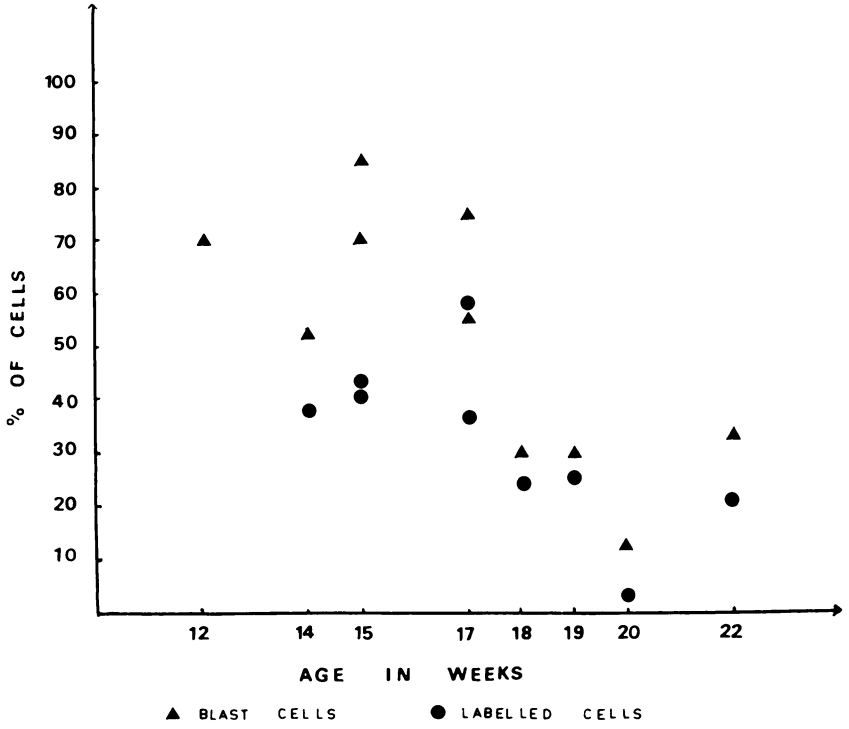

Fig. 2.—Cultures of human fetal thymic lymphocytes. Percentage of blast and isotope-marked cells as a function of fetal age.

(E_{30}, E_{15}, E_{27}) where both blood and thymic lymphocytes could be studied (Table 1), the results for the two types of cell cultures are not significantly different.

Four fetuses, ranging in age between 18 and 22 weeks, were studied. In each case, when degree of transformation of the blood culture is compared to the thymic culture of the same fetus, the latter is found to be significantly lower. The percentage of cells undergoing blastic transformation in thymic cell cultures is between 12 and 33 per cent and the percentage of labeled cells is between 2 and 25 per cent.

Control results are summarized in Table 2. Some degree of spontaneous

Table 2.—Percentages of Blast Cells and Labeled Cells in Control Thymic and Blood Lymphocyte Cultures

No.	E_1		E_{34}		E_{32}		E_{30}		E_{15}		E_{27}		E_{21}		E_{18}		E_{23}		E_{12}	
Age (Weeks)	12		14		15		15		17		17		18		19		20		22	
Cell	B	L	B	L	B	L	B	L	B	L	B	L	B	L	B	L	B	L	B	L
Blood	No Lymphocyte				Rare lymphocytes		5	0	31	11	5	1	12	7	12	12	10	2	16	1
Thymus	19		5	0	12	10	9	2	23	15	5	3	5	1	9	10	4	2	7	4

Percentages are those after 72 hours of incubation without PHA stimulation.
B, Blast cells; L, labeled cells (found among the whole population).

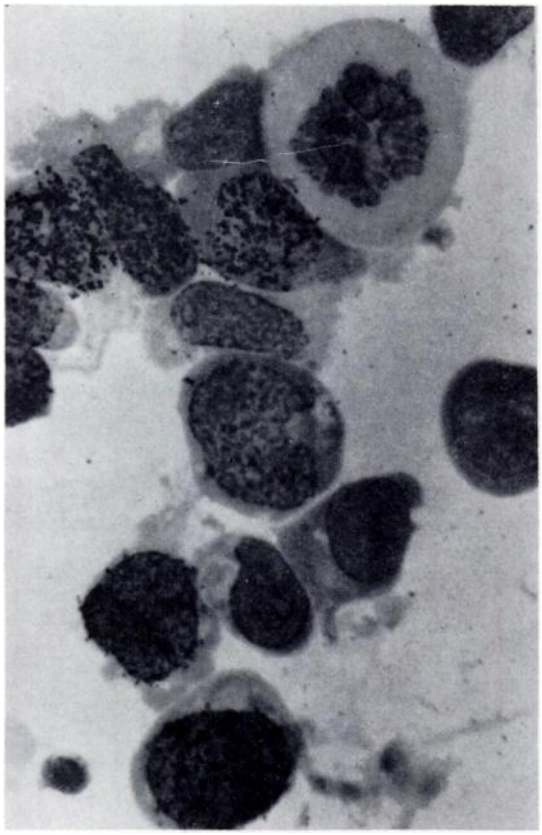

Fig. 3.—Autoradiographic slide of human fetal lymphocytes (fetus E_{15}, 17 weeks gestation). Note large transformed cells with abundant granules.

transformation was observed in the control cultures, which were prepared for both morphologic and autoradiographic studies and incubated for 72 hours without addition of PHA. The degree of spontaneous transformation found in controls is considerably below that of PHA-stimulated cultures, does not differ significantly between blood and thymic cultures and is apparently not related to fetal age.

Histologic Study of Thymic Sections

Twenty-two fetal thymuses, aged between 10 and 24 weeks, were studied. Only 10 of these thymuses were used for tissue culture, as described above.

The thymus is the first lymphoid organ to appear in fetal development. Lymphocytes are first seen in thymic tissue at about the 9th week. At first, the lymphocytes are evenly distributed throughout the thymic tissue, leaving a lightly stained, lymphocyte-free subcapsular zone of varying thickness. This light zone consists of large clear cells, which are scattered throughout the organ, but which are more prominent at the periphery where they have a pseudoepithelial arrangement. In later stages of development, a distinct cortex appears, consisting of small lymphocytes which are more and more densely packed at the periphery. The light subcapsular zone, seen in early stages of development, gradually disappears as the organ matures.

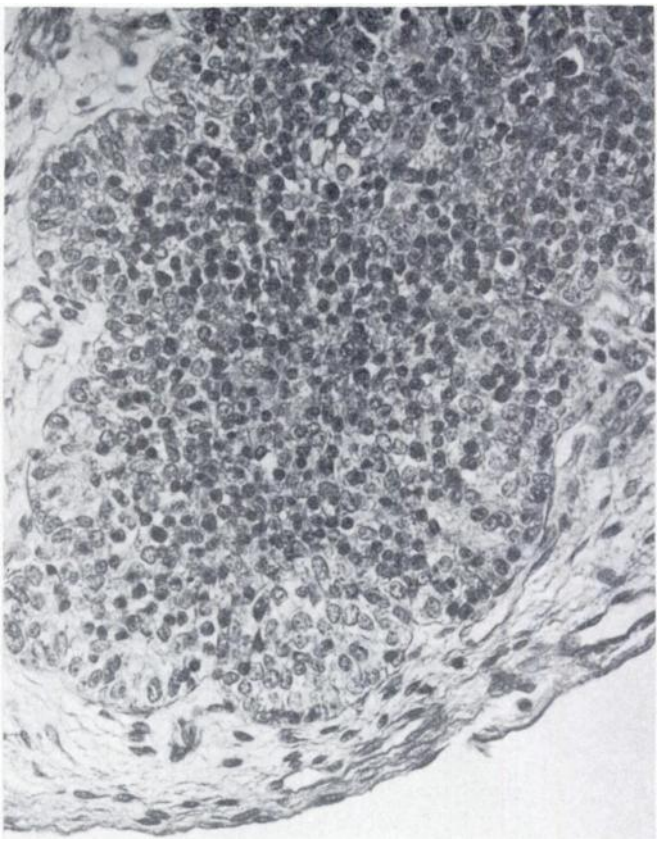

Fig. 4.—Human fetal thymus at 12 weeks of gestation (E_{41}). Medullary region is absent and clear subcapsular zone is distinct. (Hematin - eosin - safranin red.

All the thymuses studied were distinctly lymphoid. Three histologic criteria of maturation were noted: presence and extent of light subcapsular zone (Fig. 4); presence and extent of defined cortex, consisting primarily of small lymphocytes (Fig. 5); presence of Hassal's corpuscles.

The light subcapsular zone disappears about the 17th week (Table 3). The cortical zone begins to appear as early as the 14th week, continues to enlarge up to the 17th week, and reaches its maximum thickness and lymphocyte concentration at about the 20th week. At the 20th week, the relative extent of the medulla and cortex is similar to that of a neonate thymus. Hassal's corpuscles are first seen at 14 weeks.

Discussion

Thymic lymphocytes of human fetuses aged between 12 and 22 weeks are capable of transformation in the presence of PHA. This transformation is accompanied by incorporation of tritiated thymidine by the cells. The lymphocytes that undergo transformation are the thymus cells and not cells that have come from blood in thymic vessels; for it was shown that rich cultures and many transformed lymphocytes were obtained from fetal thymuses of 12, 14 and 15 weeks when their blood lymphocytes were rare or absent.

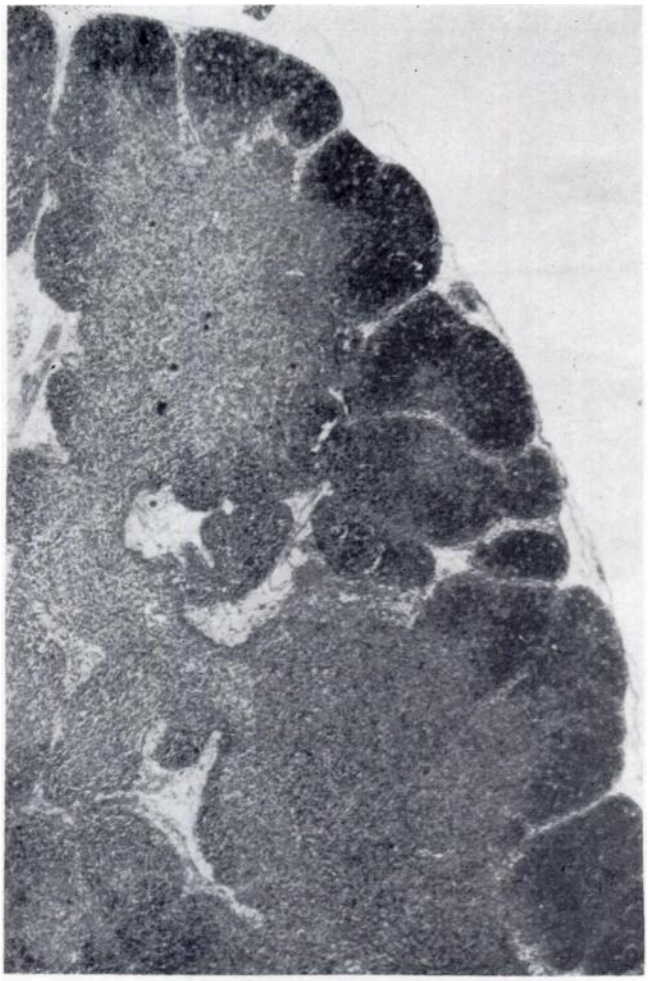

Fig. 5.—Human fetal thymus at 22 weeks of gestation (E_{12}). Distinct medullary and cortical regions are evident. Hematin - eosin - safranin red.

The blood lymphocytes of fetuses aged 15 to 22 weeks undergo transformation to an extent comparable to what has been observed in postnatal studies. The transformation of blood cells is independent of fetal age. On the contrary, our results show that the degree of transformation of thymic cells is related to the age of the fetus.

Comparison of transformation of lymphocytes from blood and from thymus shows that, at about the 18th week of gestation, a reduction in the transformation of thymic lymphocytes takes place. Before the 18th week, the thymic cell transformation is similar in degree to that of blood cells in circulation. The results obtained after 18 weeks of gestation are the same as those obtained by Claman[1,2] using adult human thymic lymphocytes.

Our study is the first to show that transformation of thymic lymphocytes in the human fetus is age-related. Lishner and Punet's[6] studies of thymic lymphocytes began at the 18th week. They also found that the thymic lymphocyte response was similar to that seen in adult thymic lymphocytes. According to

Table 3.—Morphologic Characteristic of Human Fetal Thymic Maturation Between 10th and 24th Week of Gestation

No.	Age (Weeks)	Clear Subcapsular Zone	Extent of Cortex	Hassal's Corpuscles
E_{35}	10	+	−	−
E_1	12	+	−	−
E_{41}	12	+ +	−	−
E_{24}	13	+	−	−
E_{39}	14	±	±	+
E_{34}	14	+ +	±	−
E_{17}	15	±	±	−
E_{19}	15	±	±	−
E_{32}	15	±	+	+
E_{30}	15	±	±	±
E_{14}	16	±	+	+
E_{15}	17	±	+	+
E_{27}	17	+	+	+
E_{33}	17	−	+ +	+
E_{21}	18	−	+ +	+
E_4	18	−	+ +	+
E_6	18	±	+ +	+
E_{18}	19	−	+ +	+
E_{23}	20	−	+ +	+
E_{12}	22	−	+ + +	+ +
E_{29}	22	−	+ +	+
E_{36}	24	−	+ + +	+

Jones,[8] who studied fetuses between 12 weeks and term, the results vary from one fetus to another and there is no relation between the degree of transformation and fetal age.

In the course of our work, a histological study of the fetal thymus was made along with the functional study of its lymphocytes. We found a progressive maturation of thymic cortex which becomes more and more rich in lymphocytes. This light subcapsular zone disappears. At the 20th week of gestation, the cortex and medulla reach a relative size that persists.

The reduction that we have observed in the thymic lymphocyte response to PHA stimulation seems to correspond to a change in the relative proportion of cortical and medullary lymphocytes. The young fetal thymus (before 18 weeks) has either no cortex at all or the mere rudiment of one. The lymphocytes of this thymus respond to PHA stimulation in large numbers. The older thymus (after 18 weeks) has a well developed cortex and its cellular pool contains a much smaller population which responds to PHA. We believe that the drop in the percentage of responding cells corresponds to the appearance of the cortical cellular pool. This seems to indicate that a functional distinction exists between the medullary and cortical thymic lymphocytes of the human fetus. This suggestion has previously been made for the adult thymus in animals. In the thymus of rats, stimulated by PHA in organotypic cultures, one notes an increase in the number of mitoses in the medulla.[10] Other experiments have shown that cortical lymphocytes are more sensitive to radiation[11] and to steroids[12] than are the medullary lymphocytes. Studies on chickens have indicated that the immunocompetent lymphocyte pool is located in the

medulla.[13] If one considers that PHA response indicates the immunocompetent pool of a lymphocyte population, it can also be said that, in the human fetal thymus, this pool is located in the medulla.

When lymphocytes appear in the fetal thymus at about 9 weeks, they are essentially absent from the spleen, lymph nodes and bone marrow,[14-15] as well as the blood stream.[16] At 12 weeks, when the lymphocyte concentration is still insignificant except in the thymus, we have shown that there is a rich cellular pool of immunocompetent lymphocytes in this organ. One could hypothesize that these immunocompetent cells in that early period directly populate the blood stream and the lymphoid organs elsewhere. But it could also be postulated that the cells which migrate from the thymus are nonreactive to PHA. Their paucity in the early fetal thymus may mean that they are leaving the thymus very rapidly. (the noncompetent cells might become PHA-responsive after further maturation in another setting into which they migrate). These noncompetent lymphocytes could accumulate in the cortex of the thymus when the peripheral need is less important and when the peripheralization slows.

The significance of these changes in the redistribution of thymic lymphocyte pool is far from clear. But it can be said that the fetal thymus, in the early period of the lymphoid system development has a specific and very high functional activity.

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