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The Thymus as Haematopoietic Tissue of Non-Lymphoid Cells

N. Bourgeois, G. Bergmans, and N. Buyssens
Department of Pathology, University of Antwerp, U.I.A., Belgium

Summary. Non-lymphoid haematopoiesis in the thymus was studied in 33 cases ranging from 14 weeks gestation up to 15 months postnatally.

All thymuses displayed focally granulopoiesis in the connective tissue septa and in the lymphoid tissue, where it was localized in the peripheral cortex and at the cortico-medullary junction. Within the lymphoid tissue the granulocytic series showed many precursor cells with large rounded nuclei. Their origin could only be identified by the naphtol AS D chloroacetate esterase stain. In cases with thymic atrophy these precursor cells were present in relatively large numbers. Within the connective tissue septa many granulocytes with nuclear segmentation were intermingled with precursor cells.

The erythroid series were detected with an immunohistochemical method for demonstration of haemoglobin. The positively stained nucleated cells occurred mainly in nests mostly localized in the peripheral cortex. These precursor cells were usually found in different places from the granulopoietic cells. No megakaryocytes were found.

With appropriate techniques non-lymphoid haematopoiesis can be demonstrated in the developing thymus and must be considered as an expression of normal growth.

Key words: Thymus – Non-lymphoid haematopoiesis

Introduction

For many years neutrophils and eosinophils have been observed in the thymus (Bargmann 1956; Leeson et al. 1970; Haar 1974; Potter 1976; Valdés-Dapena 1979). Recent studies on the central role of the thymus in lymphopoiesis have diverted the attention from the possible role of this organ in non-lymphoid haematopoiesis.

Offprint requests to: Dr. N. Bourgeois, Department of Pathology, Akademisch Ziekenhuis Antwerpen, Wilrijkstraat 10, B-2520 Edegem-Antwerpen, Belgium

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Our study was done in order to establish the extent, nature and localization of non-lymphoid haematopoiesis in the thymus.

Material and Methods

33 thymuses of human fetuses, neonates and infants were obtained at autopsy. Fetal, neonatal and postnatal age, the pathological findings in the thymus and in other organs are mentioned in Table 1.

The tissues were fixed in 4% Formalin or in B_5 fixative (a mixture of formalin with mercury chloride 6%).

Sections were stained with haematoxylin eosin, Giemsa, P.A.S., trichrome Masson and malachite green acridine red (Hitchcock and Ehrich 1930), controlled by ribonuclease digestion according to the method of Brachet (1953). The naphtol AS D chloroacetate esterase stain (Leder and Stutte 1975) was performed for demonstration of the myeloid series. The unlabeled antibody enzyme

Table 1

Case	Fetal age in weeks	Neonatal Age	Thymus weight in g	General findings
1	14	Stillborn		Immaturity
2	16	Stillborn		Immaturity
3	18	Stillborn		Immaturity
4	23	Stillborn		Immaturity
5	23	Stillborn		Immaturity
6	26	9 days	1, atrophy	Hyaline membrane disease
7	29	1 day	7	Hyaline membrane disease
8	30	9 h	9	Congenital malformations
9	30	Unknown	3	Hyaline membrane disease
10	30	$1^1/_2$ days		Hyaline membrane disease
11	30	1 day	7	Anoxia
12	30	Unknown	8	Anoxia
13		Stillborn	2	Dysmaturity
14	32	Unknown		Congenital malformations
15	32	17 h	7	Hyaline membrane disease
16	32	1 day	10,5	Hyaline membrane disease
17	32	Unknown	9,5	Dysmaturity, anoxia
18	32	2 days	17	Congenital malformations
19	34	2 days	atrophy	Dysmaturity
20	34	5 h	9	Listeriosis
21	35	$2^3/_4$ h	11,5	Congenital malformations
22		$5^3/_4$ h	7	Hyaline membrane disease
23	38	$1^1/_3$ days	5	Dysmaturity
24	38	Stillborn	4	Dysmaturity, anoxia
25	40	$^1/_4$ h	8	Congenital malformations
26	40	Stillborn	25	Anoxia
27		2 weeks	atrophy	Sepsis
28		4 weeks	7	Trisomy 18
29		$2^1/_2$ months	35	Respiratory infection
30		6 weeks		Sepsis
31		7 months	14 atrophy	Pneumonia
32		6 months	25	Pulmonary oedema
33	34	15 months	5 atrophy	Hydropyocephalus

method of Sternberger (1970) was performed with antihaemoglobin serum to detect erythropoiesis in B_5 fixed specimens.

Special attention was given to the location of non-lymphoid precursor cells.

Results

Our studies revealed that in all thymuses non-lymphoid haematopoiesis was found. This consisted mainly of cells of the myeloid series and to a much lesser degree also of the red series. Megakaryocytes and their precursors were always absent; only one megakaryocyte, in one single section, has been found.

The myeloid series were localized (1) focally in the connective tissue septa and (2) focally in the lymphoid tissue:

(1) Septal Location

The foci of myelopoiesis in the connective tissue septa were readily recognized in HE stained sections because of the regularity of the growth pattern. The groups of myeloid cells were present in the deepest expansions of the connective tissue septa penetrating into the lobules. At this site the septa contained large veins and smaller arteries which lay close to the corticomedullary zone of the lymphoid tissue. Focally the connective tissue space between the vessels and the adjacent lymphoid tissue was filled with cells of the myeloid series. Occasionally these cells were so numerous that they expanded into the neighbouring lymphoid tissue (Fig. 1).

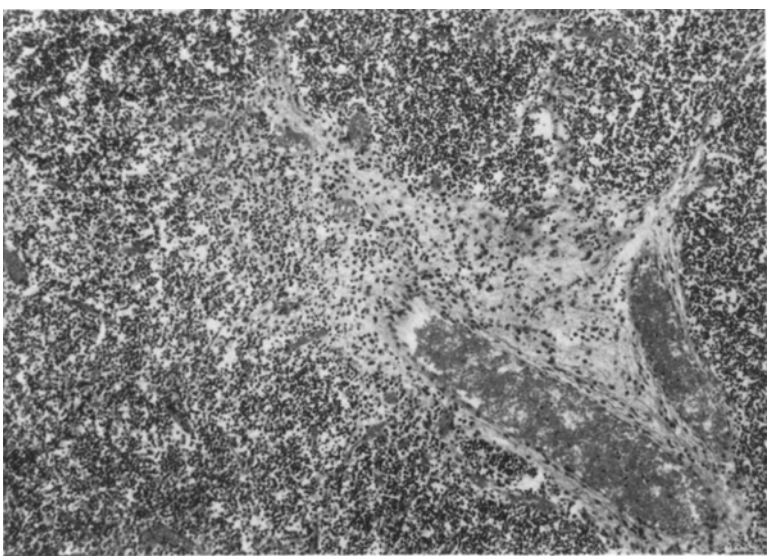


Fig. 1. Case 12. Connective tissue septum penetrating deep into the thymus in which many immature granulocytes are present. HE × 75

The morphology of the myeloid cells was pleiomorphic. Large rounded cells were intermingled with medium sized rounded and indented cells and with smaller polymorphous nuclear segmented cells. The density of the chromatin increased with the decrease of nuclear size. The cytoplasm was always wide, irrespective of cell size and contained numerous granules. The outline of individual eosinophilic granules was clearly seen in HE and Giemsa stains. The more numerous but smaller neutrophilic granules showed less staining and were recognizable by the inhomogenous appearance of the cytoplasm. Under oil-immersion, the fine granules became more clearly defined. In the Giemsa stain the importance of the neutrophilic granulation was overwhelming so that intense basophilia of the cytoplasm was not observed, even not in precursor cells. The large cells with rounded nuclei and a delicate chromatin pattern had numerous granules which influenced the tone of the cytoplasm which varied from faint pink to grey, but never reached the deep blue colour of plasma cell cytoplasm.

These observations were confirmed by the findings with the naphtol AS D chloroacetate esterase stain for the neutrophilic series. The large non-basophilic cells were densily packed with brightly staining red coarse granules filling the cytoplasm. The red stained granules were slightly larger than in HE and Giemsa stains but never spilled outside the cytoplasmic boundaries. The clear demonstration of the granularity was due to the prominence of the red colour and the slight enlargement of the granular size. This last phenomenon was dependent on the deposition of the enzymatic reaction product. In the eosinophilic series the enzyme naphtol AS D chloroacetate esterase was not present. The cells containing unstained granules could still be recognized because of the coarse granularity of the cytoplasm.

Using different staining methods septal myelopoiesis was shown to consist of many neutrophils and a moderate number of eosinophils. Mitotic figures were frequent. In the cell maturation sequence precursor cells were generally more numerous than the more mature segmented neutrophils. Only in the eosinophilic series did the segmented forms occasionally outnumber the precursor cells.

(2) Lymphoid Location

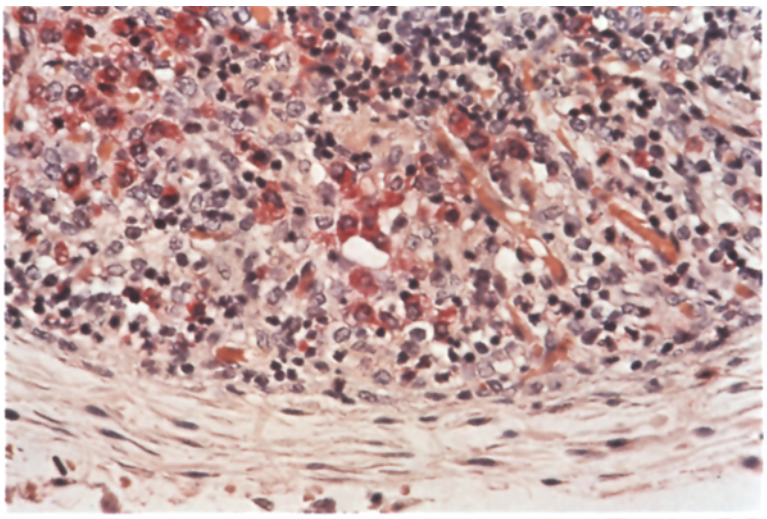
Myelopoiesis within the lymphoid tissue occured at two different sites: at the outer peripheral cortical rim of the lobules and at the cortico-medullary zone.

The first location was less frequent. Precursor cells with large or medium sized rounded nuclei and granular cytoplasm formed an unbroken layer. Rather

Fig. 2. Case 27. Atrophic thymus: peripheral cortex depleted from thymocytes. Many cells of the granulocytic series have red cytoplasm stained for naphtol AS D chloroacetate esterase. HE × 250

Fig. 3. Case 27. Atrophic thymus: the large cells with red granular cytoplasm have rounded nuclei with moderate clear chromatin. The naphtol AS D chloroacetate esterase stain reveals these cells as precursor cells in the granulocytic series. HE × 400

Fig. 4. Case 24. Thymus with nests of cells positively stained for haemoglobin with the unlabeled antibody enzymemethod. HE × 400



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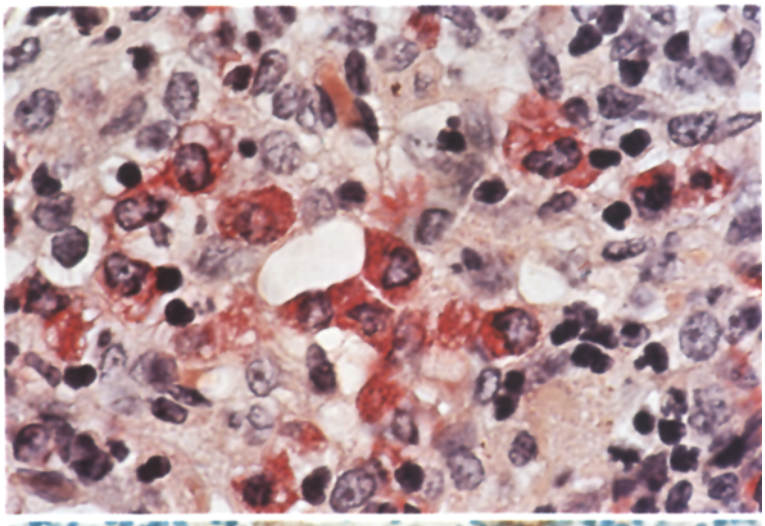

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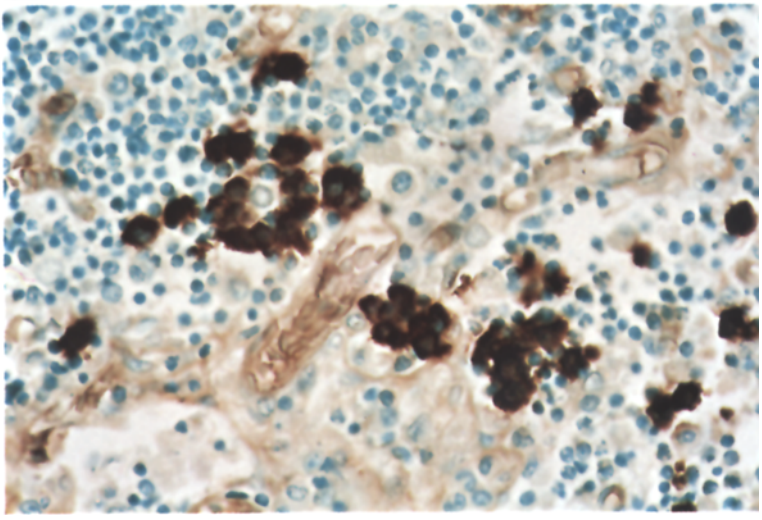

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fewer cells had smaller lobulated nuclei. In cases with thymic atrophy several of such granulocytic cell layers could be present in the narrowed cortex. In such conditions lymphocytes were scarce. The granulocytic precursors were intermingled with many large pale non-basophilic cells (Figs. 2, 3). Using the naphtol AS D chloroacetate esterase reaction the red stained granulocytic precursors could be easily separated from the unstained cells which probably belonged to the collapsed epithelial framework. The most impressive location of the myelopoiesis was observed at the transitional zone between cortex and medulla. Here the naphtol AS D chloroacetate esterase stain revealed loose nests of large and medium sized rounded cells with a pronounced granulation of the cytoplasm. More advanced cell maturation was generally not observed in the neighbourhood of these nests. On the contrary in the central part of the medulla where numerous Hassall bodies were found, isolated or small groups of segmented granulocytes were present. In this location groups with 6–10 segmented granulocytes contained cells with eosinophilic granules and the isolated cells had neutrophilic granules: only the latter cells could be seen within the Hassall corpuscules.

In the corticomedullary zone some mitotic figures occurred in the cytoplasm of cells heavily stained with the naphtol AS D chloroacetate esterase reaction. Such mitoses were not seen in the central part of the medulla.

Erythroid haematopoiesis could hardly be detected with the usual staining methods in a few thymuses. The nucleated precursor cells were localized in small groups of 6–10 cells outside the lymphoid tissue. Their regular shape and regular nuclear contour and the dense evenly distributed chromatin recalled the erythroid islets observed in the bone marrow. However large nucleated precursor cells were discovered in B_5 fixed specimens after application of the unlabeled antibody enzyme method using an antihaemoglobin serum. The positive staining of the cytoplasm increased slightly after a previous incubation in a diluted trypsin solution, and at the same time the non-specific staining diminished except for the collagen which remained brown (Fig. 4).

The location of the haemoglobin containing precursor cells was entirely unpredictable. A few larger cells were intermingled with the myelocytic series in the intralobular septa. At the outer cortical rim of the lobules some scattered large haemoglobin containing nucleated cells were found. In the same section a few lobules displayed many of these large cells but most lobules were devoid of them.

Smaller haemoglobin containing nucleated cells were only recognized by means of the immunohistochemical method. The positive stained cells formed nests in the densily packed cortical tissue. The nuclei of the red cell precursors and the thymocytes resembled each other so closely that only the application of the haemoglobin marker allowed the separation of these two cell types.

In the medulla no erythroid precursors were observed.

Megakaryocytes and their precursors were also looked for. We expected them to be easily found because of the large size of the nuclei and their peculiar lobulation. As already mentioned, only one section out of many showed one megakaryocyte. In this section, which showed a moderate thymic involution, the one and only mature megakaryocyte was found close to a widened capillary in the periphery of the cortex.

Discussion

Thymuses at all fetal ages are involved in haematopoiesis. The extent of myelopoiesis and erythropoiesis is underestimated when no special techniques are applied. The use of tissue sections seems more appropriate (Taylor et al. 1976; Schaefer et al. 1971) than the examination of cytologic smears of thymus tissue (Kelemen et al.1979).

This expression of thymic haematopoiesis is entirely different from the harmonic trilinear haematopoiesis in the normal bone marrow. In the thymus non-lymphoid hematopoiesis is not trilinear and is not harmonic. As a rule megakaryocytes or their precursors are absent: the incidental finding of one megakaryocyte in only one tissue section could result from circulation. Disharmony exists between myeloid and erythroid series, the former predominating over the latter. Moreover within a given pathway of cell differentiation a gradual maturation sequence may be present or absent: in intralobular septa myelopoiesis shows a gradual maturation, whereas at the corticomedullary junction mainly large rounded cells are found.

Without the use of the naphtol AS D chloroacetate esterase stain these cells would be wrongly interpreted as belonging to the lymphoid group. This disharmonic ripening is also present in the erythropoiesis were large cells are recognized separately from smaller nucleated erythroid precursors.

The sensitivity of the technique applied is of the utmost importance. For the granulocytic series the naphtol AS D chloroacetate esterase stain is the most suitable to detect precursors. Cells with mitotic figures can now be identified as granulocytic because of the positive cytoplasmic stain.

In some sections very small lymph nodes were found between the fat lobules outside the thymic tissue. Because of their small size these nodes could not be recognized in the wet fixed tissue by naked eye examination. At microscopic examination of these lymph nodes many granulocytic precursor cells were discovered by the napthol AS D chloroacetate esterase stain in fetuses of 35 and 40 weeks, again indicating disharmonic extramedullary haematopoieis.

For erythropoiesis the immunohistochemical method detecting haemoglobin reveals unexpected positive cells at unpredictable sites. The smaller nucleated cells are grouped in nests irregulary distributed in the cortex and their nuclei can hardly be distinguished from those of cortical thymocytes. The larger precursor cells have a delicate fine granular chromatin and a basophilic cytoplasm. They are usually isolated. Taylor et al. (1976) mentioned the presence of basophilic cells mainly located in the connective stroma of the thymus and concluded that these cells represented non-lymphoid haematopoiesis, granulocytic as well as erythroid.

With the very sensitive malachitegreen-acridine red stain on B_5 fixed material and with the Giemsa stain the basophilia can be clearly demonstrated. The application of these stains reveals the large basophilic cells which are localized in connective tissue septa or in the lymphoid cortex. In few cells the basophilia is pronounced and present over the entire cytoplasm. In many large cells with moderate basophilia neutrophil granules are grouped focally in the cytoplasm. The basophilic cells never show the chromatin pattern of transformed lymphocytes as encountered in stimulated lymph nodes within and outside the germinal

centers. The conspicuous nucleoli characteristic of transformed lymphocytes or the so-called immunoblast, are absent in the basophilic cells observed in the thymus.

In thymuses of young infants with or without thymic involution the myelopoiesis persists in the connective tissue septa as well as in the lymphoid tissue. This observation has already been made by Schaefer et al. (1971) who mentioned the persistance of myelopoiesis in the thymus until puberal involution took place.

Myeloid, and to a lesser degree, erythroid haematopoiesis in the thymus may be considered to be a natural feature of development and not restricted to early fetuses where bone marrow activity has not been developed. The quantitative contribution to the total cell pool may be small or insignificant. The simultaneous development of thymocytes, granulocytes and erythrocytes in close vicinity to each other raises the question of the ubiquitous presence of pluripotent stem cells. The presence of these cells in the thymus is not demonstrable by morphological criteria, but the development of their descendants in a suitable micro-environment like the thymus cannot be denied.

Finally studies on terminal deoxynucleotidyl transferase activity (Stein 1978) have shown that prethymocytes and thymocytes contain this enzyme. In haematopoietic malignancies the presence of this enzyme does not indicate the lymphoid origin of the neoplasia specifically, as immature blast cells in the acute phase of chronic myeloid leukemia (Sarin and Gallo 1974) or the blast cells of acute myeloid leukemia (Marcus et al. 1976) may contain this marker-enzyme. The fact that in normal growth lymphoid and non-lymphoid cell lines are present simultaneously may account for the finding of common marker enzymes in neoplastic conditions.

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