KERATIN PHENOTYPE OF HUMAN THYMIC EPITHELIUM DURING PRENATAL AND POSTNATAL DEVELOPMENT

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UDC 612.438.015.3:547.962.9]612.647].648

KEY WORDS: thymus, keratins, monoclonal antibodies.

Intermediate filament proteins are differential markers of tissue specificity. Intermediate filaments of epithelial tissues consist of proteins known as keratins. The keratins are a large family of 19 (numbered 1 to 19 according to Moll's classification [9]) closely similar but not identical proteins. Adult human epithelia with different types of structure have been shown to possess different sets of these proteins [5]. For instance, in simple epithelia the keratins with lowest molecular weight (Nos. 7, 8, 18, 19) are expressed, but those with the highest molecular weight (Nos. 1-6, 9-16) in stratified epithelia. The structural and functional organization of the epithelial tissue of the thymus is unique. The epithelial tissue of this organ is distinguished, on the one hand, by a reticular structure and, on the other hand, by polarity in the distribution and differentiation of the cells within the lobules into cortical, intermediate, and medullary layers [6, 10]. Thymic epithelial cells are known to express different sets of keratins of simple and stratified epithelia [7, 9]. However, the spatial expression of keratin proteins in the three-dimensional epithelial tissue of the thymus has not yet been mapped.

The aim of this investigation was a comparative immunomorphological study of the principles governing the distribution of keratin-expressing cells in the human thymus during prenatal and postnatal development, by the use of monoclonal antibodies (MCAB) to individual keratins, differing in their histological specificity.

EXPERIMENTAL METHOD

The thymus from human fetuses aged 15-30 weeks of intrauterine development, obtained from clinically healthy women during premature labor, and the thymus of a 2-year-old child dying from trauma were studied. The immunomorphological investigations were conducted on serial frozen sections [1, 2]. For application of the second layer of "developing" antibodies FITC and TRITC and labeled sheep's antibodies to mouse and rabbit immunoglobulins were used. The following mouse MCAB were used: H_1 — against human keratin No. 8, E_3 against human keratin No. 17, H_3 and C_{12} — against epitopes of human keratins obtained in the Laboratory of Mechanisms of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and L001 to keratin No. 14, and an antiserum (AS) to human keratin No. 5.

EXPERIMENTAL RESULTS

The thymus of human fetuses at early (15-16 weeks) and later (30 weeks) times of development has a similar spatial organization. The epithelial tissue of the thymus consists of a heterogeneous population of branching cells, jointed together to form reticular structures around the lymphoid elements. Within the limits of the lobules, the cortical and medullary layers can be distinguished, together with a more or less marked subcapsular epithelial layer. Immunomorphological mapping of expression of keratin proteins with the aid of the MCAB and AS mentioned above demonstrated a similar pattern in the distribution of these proteins in the epithelial tissue of the human thymus in prenatal and postnatal periods of development. Similar reticular structures in the epithelial tissue of the cortex and medulla were brightly stained by MCAB to keratin No. 8 and to keratin-

Central Laboratory of Neuroanatomy, and Laboratory of Embryonic Histogenesis, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 1, pp. 87-90, January, 1991. Original article submitted February 14, 1990.

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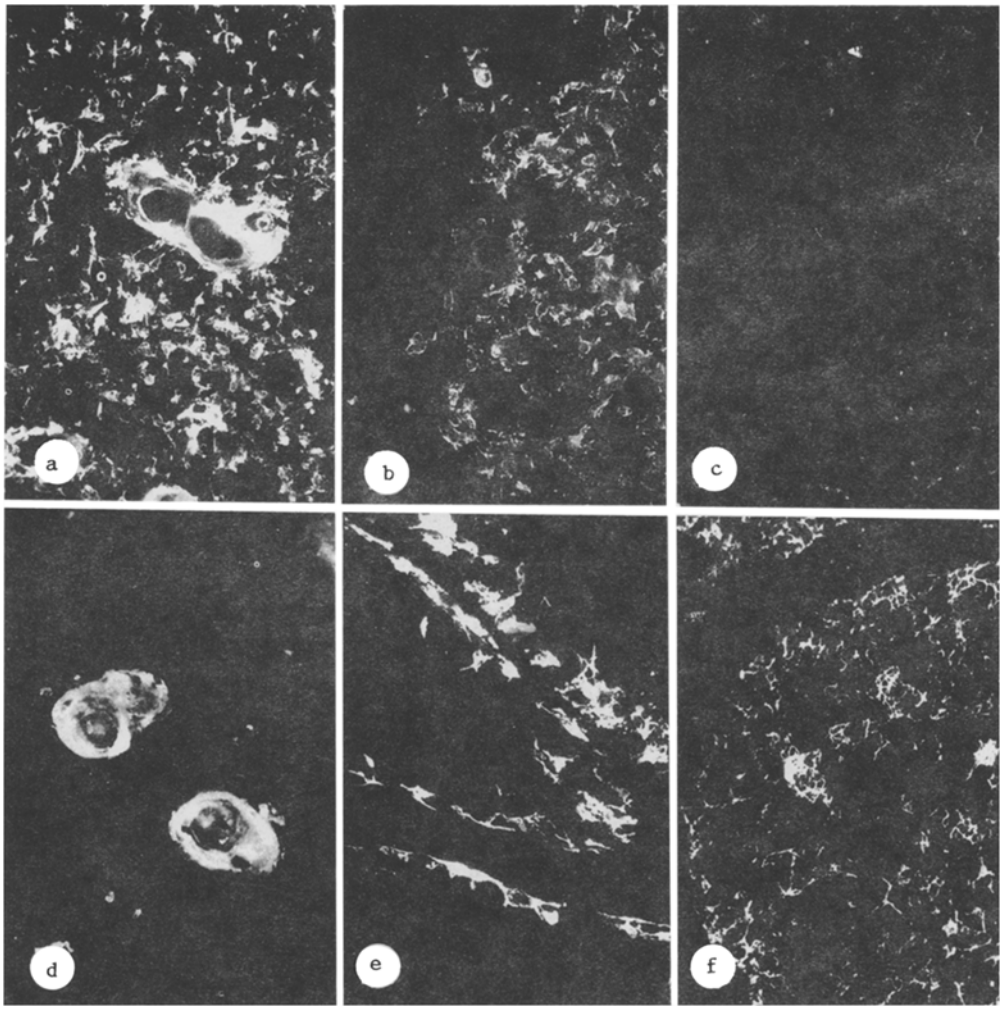

Fig. 1. Epithelial network and Hassall's corpuscles. 400×. a) Staining of cells of medullary layer and cells adjacent to Hassall's corpuscles with MCAB to keratin No. 8; b) staining of cells of medullary layer with MCAB to epitope H_3. Absence of specific staining of Hassall's corpuscles; c, d) absence of specific staining by MCAB to C_{12} epitope of medullary epithelium, bright fluorescence of cells forming Hassall's corpuscles by these antibodies; e) selective staining of cells of subcapsular zone by MCAB to keratin No. 14; f) uniform staining of reticular net of epithelium by AS to keratin No. 5.

associated epitope H_3 (Fig. 1a, b, f). Cells adjacent to the Hassall's corpuscles, either developed or in the process of development, expressed this same set of keratins. However, cells expressing keratin No. 5 and epitope H_3 were not found in the composition of the Hassall's corpuscles themselves (Fig. 1b; Fig. 2a, c), but cells expressing keratin No. 8 were found (Fig. 1a). When MCAB to keratins Nos. 14 and 17 were used, selective staining of solitary cells of the subcapsular zone, located on the basement membrane, and diffuse, bright staining of a network of epithelial cells in the medullary layer were observed (Fig. 1e; Fig. 2d). Cells in the composition of the Hassall's corpuscles in this case stained brightly with MCAB to keratin No. 17 but were not stained by MCAB to keratin No. 14 (Fig. 2b, d). Solitary cells expressing the C_{12} epitope were found in the medullary layer of the thymus. As a rule, this epitope was found only in cells of the outer (in fetuses aged 15-25 weeks) and inner (in fetuses at 25-30 weeks and in the 2-year-old child) layers of fully formed Hassall's corpuscles (Fig. 1c, d). In the immunomorphological investigation using FITC and TRITC labeled antibodies as developing antibodies, only solitary cells of the subcapsular zone expressed keratins Nos. 5, 14, and 17, whereas most cells of the subcapsular zone expressed keratins Nos. 5, 14, and 17, whereas most cells of this zone did not express keratins Nos. 14 and 17, but preserved their reactivity toward MCAB to keratins Nos. 5 and 8 and to epitope H_3. An even greater diversity of the keratin phenotype was found in the epithelium of the medullary layer of the thymus. Unlike in the subcapsular zone, where the distribution of cells expressing keratin No. 14 in serial sections

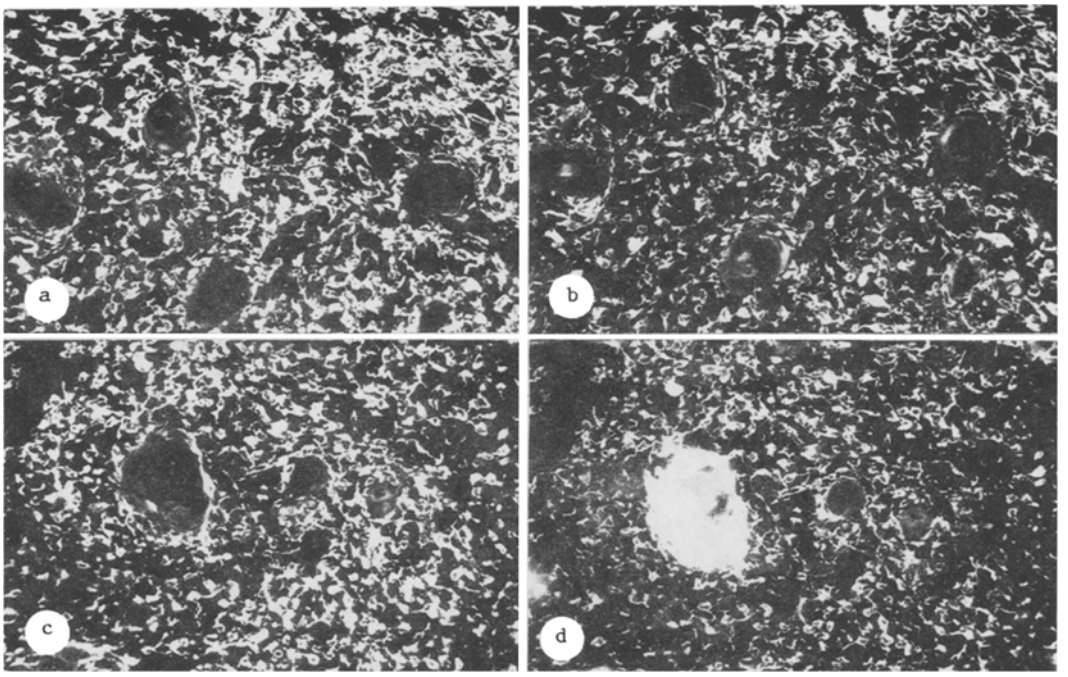

Fig. 2. Method of immunofluorescence staining using a double label to detect keratins in human thymic epithelium. 400×. a, b) Staining with MCAB to keratin No. 14 and AS to keratin No. 5; c, d) staining with AS to keratin No. 5 and MCAB to keratin No. 17.

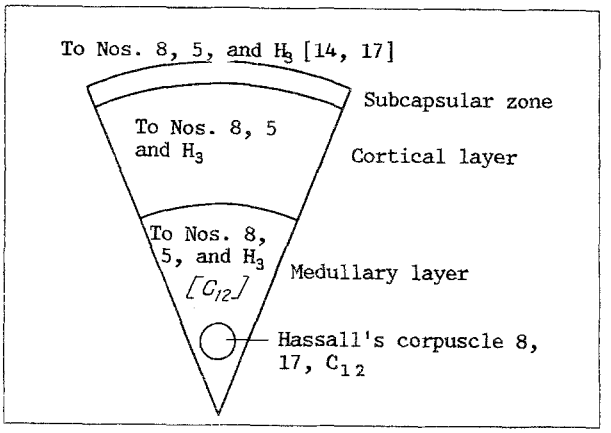

Fig. 3. Keratin phenotype of epithelial cells of human thymus.

coincided with the distribution of cells expressing keratin No. 17, both cells with coexpression of keratins Nos. 14 and 17 and cells expressing keratin No. 14, but not expressing keratin Nos. 17, and vice versa, were found in the medullary layer of the thymus. The pattern of spatial distribution of keratins was largely similar in the thymus of fetuses at the later stage of development (25-30 weeks) and in the postnatal thymus (2 years), and differed only a little from the distribution of these proteins in the thymic epithelium of fetuses at an early stage of development (15-16 weeks). The main differences were concerned, first, with the smaller number of cells in the medullary layer expressing keratin No. 17, and second, with expression of the C_{12} epitope, which was found mainly in cells of the inner layers of Hassall's corpuscles (Fig. 3). Thus, the epithelial cells of the embryonic and postnatal thymus express a quite wide range of keratin proteins. The character of expression of the keratins, from the time that the thymus becomes populated by lymphoid cells and epithelial-reticular structures begin to be formed was virtually unchanged. The predominant number of cells of the subcapsular zone of the thymus expressed keratins Nos. 8 and 5 and the H_3 epitope, but only solitary cells expressed keratins Nos. 14 and 17.

An earlier comparative study of the distribution of keratins in the prenatal human epidermis showed that only cells of the basal layer of the epidermis in early embryos and fetuses until the 28th week of development expressed such a wide range of keratins, detectable in cells of the subcapsular zone and the medullary layer of the thymus. However, by contrast with the thymus, in the epidermis cells of the basal layer ceased to express keratins Nos. 8, 17, and H_3 with the development of stratification and the formation of its appendages, namely hair follicles and sweat glands [3, 4]. Under these circumstances, the more highly differentiated subpopulations of parabasal cells in the embryonic epidermis expressed keratins Nos. 5 and 17 but did not express the range of keratins Nos. 8 and 17, and H_3, whereas cells of the surface layers of the stratified epidermis of 23-week-old fetuses stained with MCAB C_{12}. With respect to their keratin phenotype, cells of the deep layers of the thymic cortex differed from cells of the subcapsular zone and medullary layer in that they did not stain with MCAB to keratins Nos. 14 and 17. Under these circumstances, the cells of this layer preserved their reactivity toward MCAB to keratins Nos. 8, 5, and H_3. The investigations showed that with respect to the ranges of expressed keratins, the tissue of the medullary layer of the thymus consists of cell subpopulations that are most heterogeneous for their keratin phenotypes. If the method of double staining was used, cells selectively expressing only keratin No. 17 or only keratin No. 14 were found in the subpopulation of cells coexpressing keratins Nos. 14 and 17, which exceeded in number the subcapsular zone. Dissociation of the keratin phenotype of this kind was seen most clearly in the Hassall's corpuscles, the cells of which expressed only keratin No. 17 and did not express keratin No. 14. Cells expressing keratins Nos. 8 and 5 and the H_3 epitope predominated in the epithelial tissue of the medullary layer. Hassall's corpuscles, both formed and in the course of formation, did not stain with MCAB to keratin No. 5 and the H_3 epitope, but stained brightly with MCAB to keratin No. 8. Evidently Hassall's corpuscles are formed from a cell subpopulation with the keratin phenotype (8^+, 17^+). Furthermore, in the small subpopulation of cells in the medullary layer, just as in the keratinizing cells of the epidermis [3], the C_{12} epitope was expressed. Expression of this keratin-associated epitope was discovered only in those lobules of the thymus in which Hassall's corpuscles were formed. Evidently only a certain cell population is subject to embryonic keratinization, and Hassall's corpuscles are the end products of the keratinization process. In the subpopulation of epithelial cells in the medullary layer of the thymus most heterogeneous for its keratin phenotype we found no cell subpopulation that corresponded completely to the keratin phenotype of the differentiated parabasal cells of the epidermis. The keratin phenotype of the different cell subpopulations of the thymic epithelium combines expression of marker proteins of undifferentiated and differentiated subpopulations of the embryonic epidermis. Thus with respect to the set of MCAB to individual keratin proteins which we used, the keratin phenotype of the majority of cells of the epithelial reticulum of the thymus comes closest to the keratin phenotype of undifferentiated basal cells of the embryonic epidermis before processes of stratification and of appendage formation begin in it.

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