BASAL MEMBRANES OF THE BLOOD–THYMUS BARRIER OF THE CORTICAL ZONE OF THE HUMAN THYMUS, REVEALED BY MEANS OF ANTIBODIES TO TYPE IV COLLAGEN

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Intensive study of the physiology of the thymus has revived interest in the study of its structural organization [1, 12, 13, 15]. In particular, elucidation of the structural features of the vascular bed of the thymus and the degree of its permeability for macromolecules and cellular constituents is continuing [8, 9, 15]. Although active 2-way communication exists between the blood stream and the internal medium of the thymus [8, 11, 15], some investigators have stated that there exists a blood-thymus barrier [3, 14]. It has been postulated that this barrier is limited solely to the cortical zone and that it participates mainly in the selection of mature T cells during their emigration from the thymus into the blood stream [3, 13, 15]. Data relating to the study of the anatomical components of the blood-thymus barrier have been obtained with the electron microscope [11, 13, 15], which does not permit the simultaneous elective discovery and demonstration of all its structures.

The aim of this investigation was to study the basal membranes of the epithelial tissue of the thymus and blood vessels — the main structures of the blood-thymus barrier, by demonstration of type IV collagen, one of the principal components of membranes, in it [6, 10].

EXPERIMENTAL METHOD

The test material consisted of the thymus from adults (4 cases) and children (aged 7 and 10 years) dying from acute trauma, and from human fetuses (16 and 26 weeks). Other organs (skin, heart, kidneys, lung) served as the control. Tissue fragments were frozen at −20°C or −96°C in a mixture of dry ice and acetone. Sections 5 μ thick were cut in a cryostat, and used either unfixed or fixed for 3 min in acetone or ethanol. Type IV collagen was revealed with the aid of an antiserum, prepared in rabbits by the method in [6]. Antibodies to rabbit immunoglobulins, prepared by the method in [2] and labeled with fluorescein isothiocyanate, were used for the immunofluorescence tests. For preliminary treatment sections were moistened with physiological saline made up with phosphate buffer pH 7.0–7.3 (PBS), serum against type IV collagen was layered on them, and they were incubated in a humid chamber for 45 min at room temperature. After rinsing in PBS for 10 min the sections were treated with labeled antibodies to rabbit immunoglobulins for 30 min under the same conditions, washed again, and mounted under a coverslip in 60% neutral glycerin. The sections were studied in ML-2 and LYUMAM-2 luminescence microscopes. They were photographed on RF-3 film with a 40x objective (water immersion) and homal 3 ocular. For the histological control sections were fixed in ethanol and stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Components of basal membranes with eosinophilia, like components of most connective-tissue structures, could not be demonstrated in the thymus or other organs selectively by staining the sections with hematoxylin and eosin. A similar difficulty also arises when reticular fibers are impregnated with silver, when components of argyrophilic fibers appear not only in the basal membranes, but also in other structures of the stroma of the fibers [4].

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Fig. 1. Section through human skin. Reaction in region of basal membrane of vessels (more intense) and of epithelium of skin (less intense). Indirect immunofluorescence method. 120×.

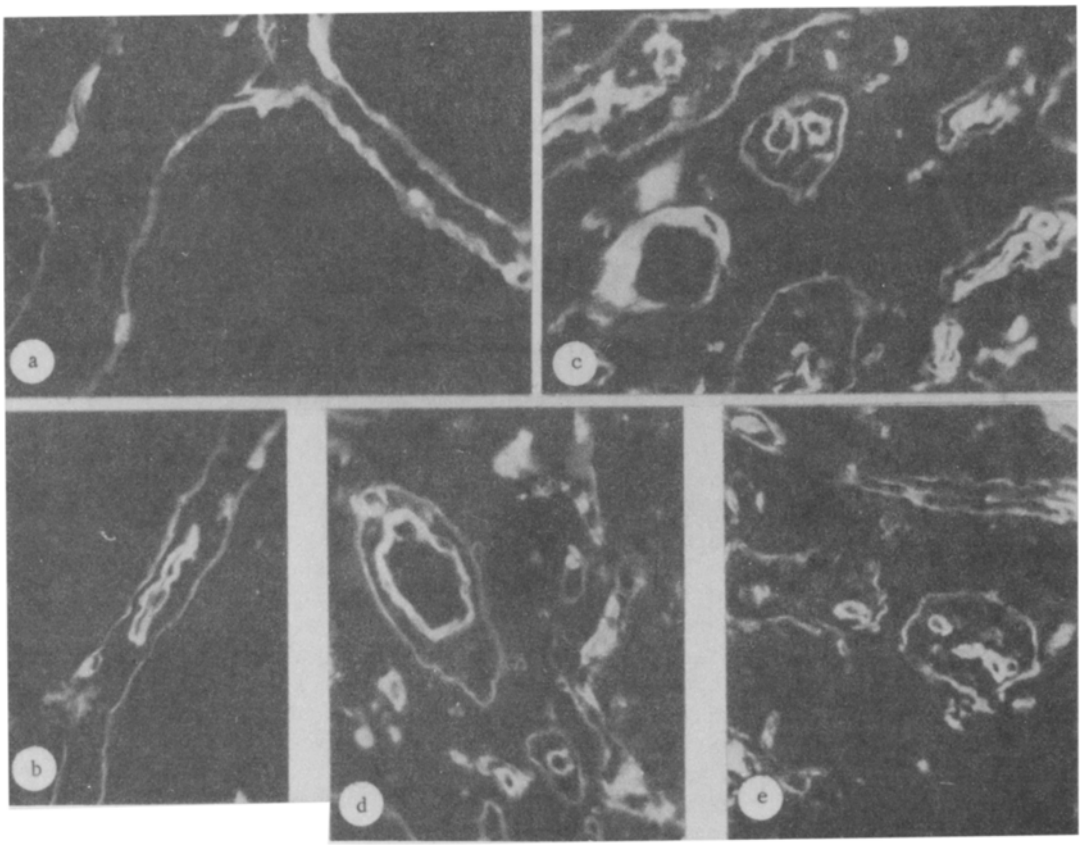

Fig. 2. Sections through human thymus. a) type IV collagen in basement membrane at periphery of lobule. No type IV collagen present in connective tissue of interlobular septa; b) collagen localized in basal membrane of a radially oriented vessel and basal membrane of epithelial tissue of thymus, bordering on vessel; c, d, e) most vessels of cortical zone separated from parenchyma of thymus by basal membrane of epithelium. Indirect immunofluorescence method. 120×.

On treatment of sections through the tissues of all the organs studied (thymus, skin, heart, kidneys, lungs), whether fixed in alcohol and acetone or used unfixed, with serum against type IV collagen in the indirect immunofluorescence test, only structures containing type IV collagen were revealed: the basal membranes of epithelial tissues, blood vessels, and the sarcolemma of the muscle fibers. The strongest reaction in all cases was observed in the blood vessel walls, rather weaker in the zone of the sarcolemma, and weaker still in the basal membrane of epithelial tissues (Fig. 1). The basal membrane in the thymus containing type IV

collagen surrounds each lobule of the gland (Fig. 2a) and forms evaginations protruding into the cortical zone and accompanying blood vessels which penetrate into the lobule from the interlobular connective tissue (Fig. 2b). When the walls of such vessels are impregnated with silver, usually many collagen (reticular) fibers are observed [4], and for this reason, evidently, when type IV collagen was demonstrated by the immunofluorescence test, the strongest reaction was observed (Fig. 2c, d, e). Collagen fibers located elsewhere, outside basal membranes, were not revealed by serum against type IV collagen. Investigation of the cortical zone at different levels showed that the basal membrane of the epithelial tissue of the thymus separates all or the great majority of vessels from the parenchyma of the organ. Connective tissue accompanying the vessels, in which no type IV collagen is discovered, lies between the vessel wall and the basal membrane of the epithelium. This can be seen particularly clearly on the transverse section of the vessel (Fig. 2d). Often the basal membrane of the epithelium surrounds a group of vessels, which evidently includes an artery and two veins or an arteriole and venule (Fig. 2e).

The barrier consisting of two basal membranes, separated by a considerable layer of loose connective tissue, can undoubtedly prevent the escape of lymphocytes from the cortical zone of the thalamus into the blood stream. In addition, collagen in the cortical zone contains a small number of narrow (from 7 to 10 μ in section) tubular formations (Fig. 2c d), resembling capillaries in size. However, since we know that the basal membrane of capillaries has no collagen (reticular) fibers [11], it must be postulated that the tubular structures are sheaths, formed by the basal membrane of the epithelium, separating blood capillaries of the cortical zone from the internal medium of the gland. Many tubular formations can be observed in the cortical zone when other compounds are revealed in the thymus, such as secretory immunoglobulin, which is bound with components of the membrane of the wall of these structures [5]. In most cases, evidently, this membrane does not contain collagen, and for that reason, in the present investigation only a few of these small tubular formations were revealed. Only substances taking part in metabolism can evidently pass through the wall of capillaries surrounded by a basal membrane of epithelium, and cells cannot pass through. Thus the discovery of two basal membranes between the blood stream and the internal medium of the thymus in its cortical area [11], confirmed by the demonstration of type IV collagen, characteristic of basal membranes, demands acceptance of the view that a blood-thymus barrier exists. However, we need to know more exactly whether the barrier is local in character and is typical of the cortical area of the thymus only [3]. The walls of the blood capillaries and of the postcapillary segments of the veins in the corticomedullary zone allow the passage of lymphoid cells and their escape into the blood stream [11, 13]. It must be postulated that the blood-thymus barrier is a component of the collector system of the thymus [3], which is responsible for selection of the most mature T cells and their release into the blood stream.

LITERATURE CITED

1. A. K. Ageev, Histopathology of the Human Thymus [in Russian], Leningrad (1973).
2. L. V. Beletskaya and T. A. Danilova, Immunoluminescence in Medicine [in Russian], Moscow (1977), p. 145.
3. L. V. Beletskaya, L. P. Katasonova, and T. S. Aliev, Byull. Éksp. Biol. Med., No. 8, 371 (1981).
4. V. K. Beletskii and L. V. Beletskaya, Byull. Éksp. Biol. Med., No. 4, 502 (1980).
5. É. V. Gnezditskaya, L. V. Beletskaya, V. P. Bukhova, and N. A. Zakharova, Byull. ÉKsp. Biol. Med., No. 7, 79 (1984).
6. S. P. Domagatskii, G. L. Idel'son, O. V. Merzlikina, et al., Immunologiya, No. 3, 78 (1985).
7. Z. Kemileva, The Thymus [Russian translation], Moscow (1984).
8. I. E. Kovalev, P. V. Sergeeva, and S. E. Imambaeva, Zh. Mikrobiol., No. 5, 85 (1971).
9. N. V. Medunitsyn, Zh. Mikrobiol., No. 4, 113 (1964).
10. L. I.Slutskii and B. Z. Simkhovich, Usp. Sovrem. Biol., 89, No. 1, 58 (1980).
11. S. L. Clark, Am. J. Anat., 112, 1 (1963).
12. K. Henry and G. Farrer-Brown (editors), A Color Atlas of Thymus and Lymph Node Histopathology, Amsterdam (1981).
13. M. D. Kendall (ed.), The Thymus Gland, London (1981).
14. A. H. E. Marshall and R. C. White, Br. J. Exp. Pathol., 42, 379 (1961).
15. J. Olah, P. Rohlich, and J. Toro, Ultrastructure of Lymphoid Organs, Budapest (1975).