ULTRASTRUCTURE AND FUNCTION OF THYMUS CELLS DURING THE FORMATION OF IMMUNITY TO TUBERCULOSIS

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UDC 616-002.5-097.3-092:[611.438-018.1+612.438

An electron-microscopic and histochemical study of the thymus of growing guinea pigs was made under normal conditions and during development of the immune response to BCG vaccination. In the postvaccination period, especially from the 3rd to the 7th days, the ultrastructure of cells of the blood-thymus barrier was found to be changed. The greatest changes were found in the reticulum and endothelial cells. Evidence was obtained of increased morphological and functional activity of these cells of the thymus during the formation of immunity to tuberculosis.

KEY WORDS: *Thymus*; *BCG vaccine*; *cells of the blood–thymus barrier*.

The thymus performs several functions, the most important of which is correlation of the immunobiological state of the organism. This accounts for the increased attention being paid to the study of the ultrastructure of the thymus [1-4, 9, 15].

On the basis of electron-microscopic investigations the basic structural unit of the thymus — Clark's follicle [7] — has been described. However, there is as yet no clear idea about the structure of the blood–thymus barrier (BTB), of the morphofunctional significance of the individual cells, and of the character of relations between the cells.

The object of this investigation was to study, at the submicroscopic level, the state of the BTB and lymphocytes under normal conditions and during the formation of the immune response, when morphological and functional activity of the thymus is intensified [4].

EXPERIMENTAL METHOD

BCG vaccine was injected subcutaneously in a dose of 0.1 mg into growing guinea pigs. The animals were killed 3, 7, and 14 days and 1, 2, 3, and 6 months after vaccination. The material was examined in the electron microscope. Histological sections were stained with hematoxylin–eosin and histochemical investigations were undertaken for acid phosphatase (Gormori's method).

EXPERIMENTAL RESULTS

The BTB comprises the endothelial lining, the subendothelial space, the basement membrane, the ground substance of the connective tissue, and the collagen structures found in it (Fig. 1a). By contrast with the tissue–blood barrier of other immunocompetent organs, the BTB also incorporates reticulum cells. They occupy the intermediate region between the capillary wall and thymocytes, forming a continuous network the spaces of which are filled with thymocytes.

Two types of reticulum cells were distinguished electron-microscopically in the thymus of the unvaccinated animals. Reticulum cells of the first type (Fig. 1b) were located beneath the basement membrane in contact with each other through a large number of desmosomal structures. Frequently the desmosomes were found directly in the cytoplasm of the cells. A distinguishing feature of reticulum cells of this type was the presence of microfibrils, which lay freely in the cytoplasm or penetrated through the cell membrane and projected into contact with the desmosomes. The cytoplasm of these cells contained round mitochondria with

Pathomorphological Laboratory, Central Research Institute of Tuberculosis, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. K. Bogush.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 10, pp. 447-451, October, 1977. Original article submitted April 28, 1977.

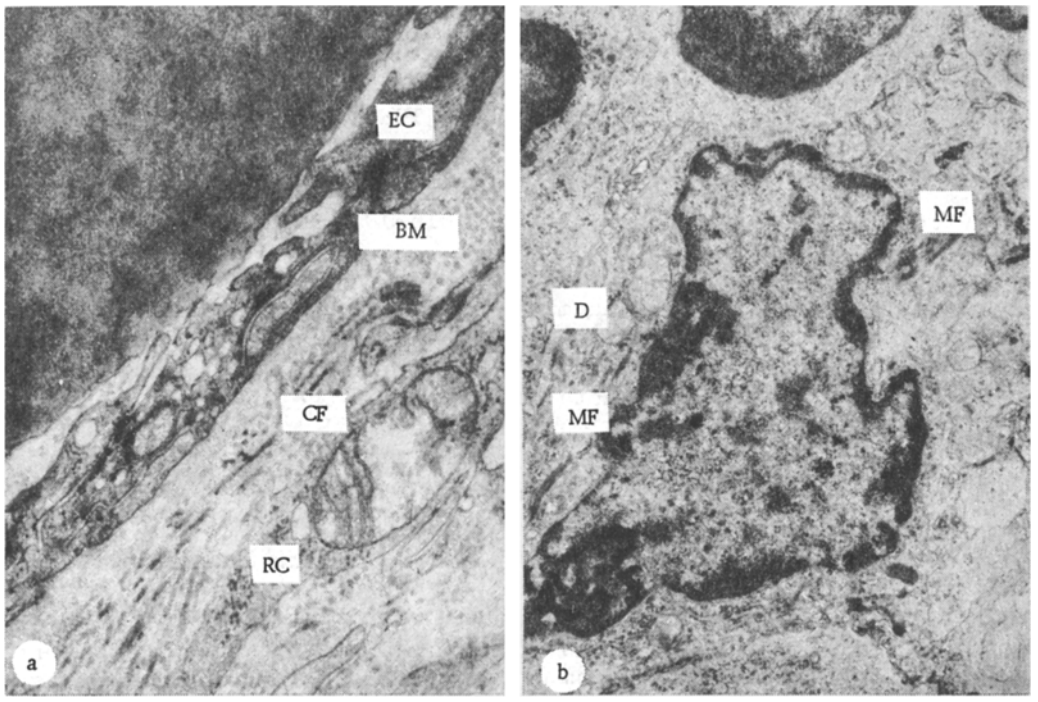

Fig. 1. Thymus of control guinea pig: a) region of BTB: endothelial cells (EC), basement membrane (BM), collagen fibers (CF), area of reticulum cell (RC) (54,000×); b) reticulum cell of first type: desmosomes (D), microfibrils (MF) (12,500×).

sparsely arranged cristae, numerous ribosomes and, in some places, fragments of the granular endoplasmic reticulum.

Reticulum cells of the second type were not connected with each other by desmosomes and did not make direct contact with other cells, despite the fact that the cytoplasmic processes, which penetrated for a considerable distance into the intercellular space, reached other cells. Their cytoplasm was large in volume and, unlike that of the reticulum cells of the first type, it did not contain microfibrillary structures. Together with mitochondria, microvacuoles, a well-developed Golgi complex, polysomes, and elements of the granular endoplasmic reticulum were detected in the cytoplasm.

In the postvaccination period the reticulum cells of the first type were transformed into cells performing a secretory function. This was expressed by the formation of rosettes of polysomes, which accumulated in large numbers in vacuoles surrounded by a single membrane. The vacuoles could open freely into the intercellular space, where lymphocytes migrating from Clark's follicles were found. Presumably it was these vacuoles that were the location of proteins synthesized by the polysomes. These changes are evidence of increased functional activity of these cells during the period of formation of immunity to tuberculosis. Many investigators have linked the manifestation of the protective reaction of the body with the state of this reticulum-cell component of the thymus [5, 12].

Reticulum cells of the second type are transformed during the immune response into cells capable of functioning as macrophages. Results obtained by several workers also indicate that cells of this type are mesenchymal in origin and, under certain conditions, may be transformed into macrophages [10, 12].

Some increase in the volume of the cytoplasm was observed in the reticulum cells of the second type 3-7 days after vaccination and many ribosomes and polysomes, fairly uniformly distributed throughout the cell, appeared in it. The number of mitochondria also increased. They became elongated in shape. Their matrix was condensed and the number of internal cristae increased. Meanwhile some degree of hypertrophy of the Golgi zone took place, with the appearance in it of vacuoles filled with finely granular osmiophilic material. The contents of these vacuoles became denser and they come to look like lysosomes, varying in size and in electron-optical density (Fig. 2a). The results of an investigation of the tissue enzymes at this period showed an increase in acid phosphatase activity in the cytoplasm of

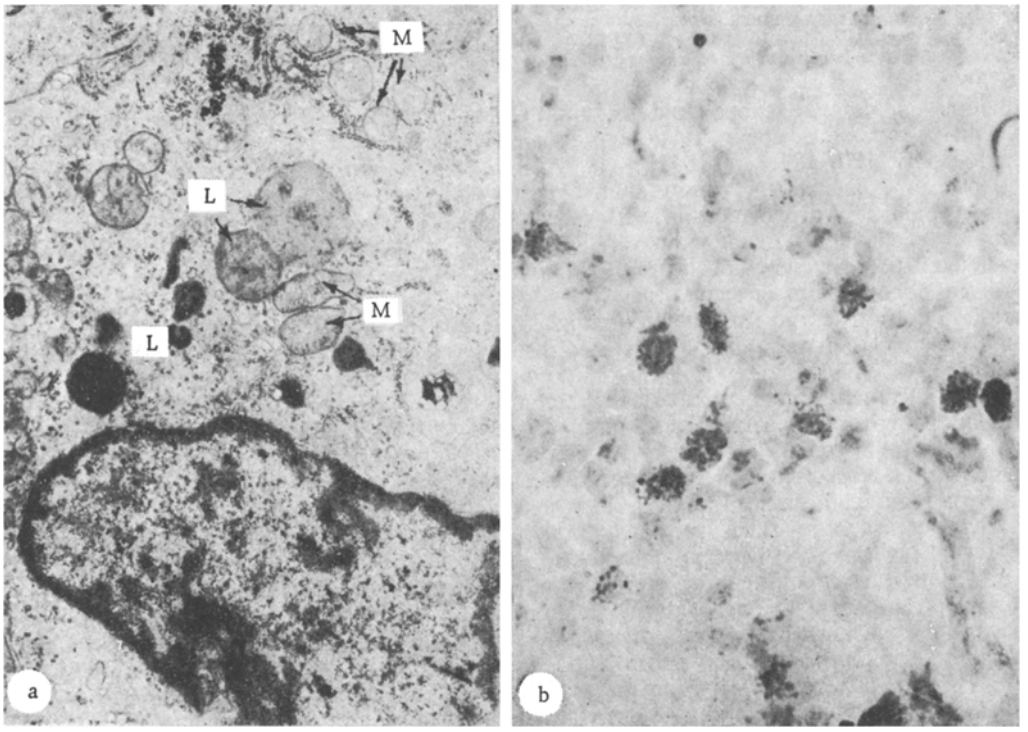

Fig. 2. Thymus of guinea pig 3 days after BCG vaccination: a) Activated reticulum cell of second type: lysosomes (L), mitochondrion (M) (11,600×); b) high acid phosphatase activity in activated reticulum cell of second type (Gormori's reaction; 460×).

the cells of this type (Fig. 2b). At this stage the morphology of the activated reticulum cells was identical with that of macrophages of the spleen and of lymph nodes, but phagosomes and phagolysosomes, typical of phagocytes, were rarely found in their cytoplasm. Activated reticulum cells of the second type are evidently a reserve component of the BTB, performing a phagocytic function only when this barrier is disturbed.

An increase in the functional activity of the endothelial cells of the blood capillaries contained in BTB also was observed after vaccination. The number of ribosomes in the cytoplasm of the endothelial cells increased. Hypertrophy of the Golgi zone and the formation of lysosomal structures were observed (Fig. 3a). In some cases vacuoles with phagocytosed particles appeared. The phagolysosomes had the characteristic ultrastructure of these organoids. Acid phosphatase activity was intensified (Fig. 3b). Consequently, the endothelial cells of the thymus, the initial component of BTB making contact with antigens circulating in the blood stream, can perform a phagocytic function in the postvaccination period, preventing antigen from penetrating into the parenchyma of the organ.

The electron-microscopic study of the lymphocytes of the thymus showed that in unvaccinated animals they contained few intracellular organoids. Besides free ribosomes and polysomes, the cytoplasm contained solitary small mitochondria. As a rule the chromatin was uniformly distributed throughout the nucleus. Vaccination was not followed by profound intracellular structural changes in the thymocytes. No transformation of thymocytes into plasma cells was observed.

The slight morphological changes in the thymocytes during development of the immune response were expressed primarily as a very small increase in the volume of the cell and the appearance of many free ribosomes in the cytoplasm. The number of mitochondria also increased. In a few cases fragments of the granular endoplasmic reticulum were found. In addition, lysosomes were formed in the cytoplasm of the activated thymocytes; these were equal in size to the mitochondria and had well-marked electron-optical density.

The study of the ultrastructure of the thymocytes in the various stages of the immune response showed that activated cells were most numerous in the initial stages of the postvaccination period (3-7 days). Later the number of activated thymocytes fell, so that by 6 months after vaccination only solitary cells with the ultrastructure described above could be found.

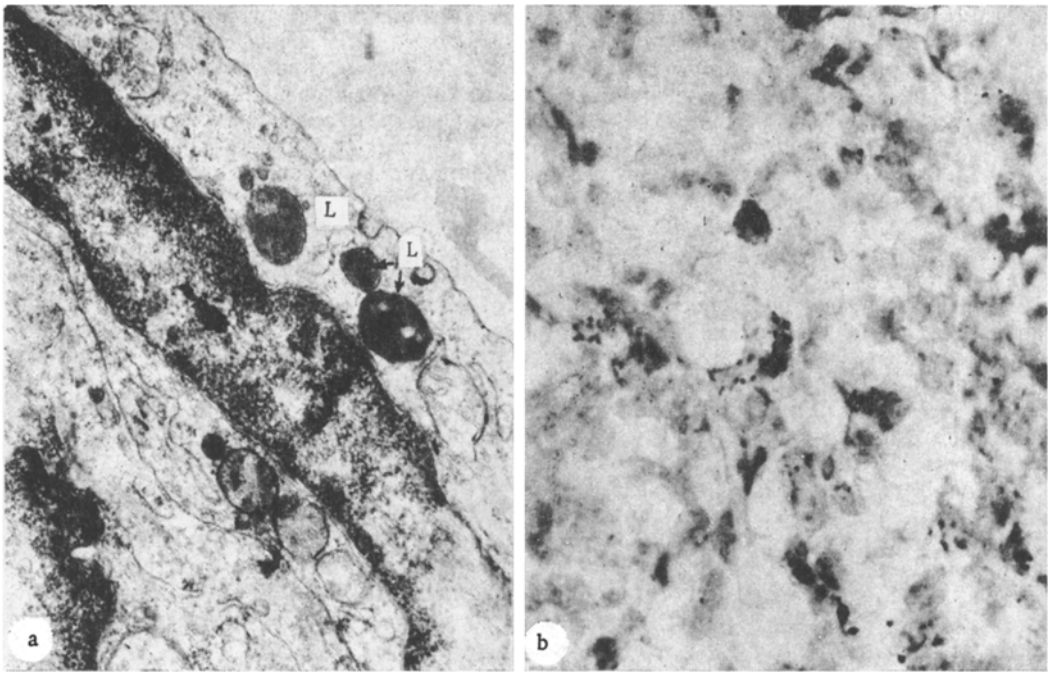

Fig. 3. Thymus of guinea pig 3 days after BCG vaccination: a) area of BTB; numerous lysosomes (L) in cytoplasm of endothelial cells (18,000×); b) acid phosphatase activity in endothelial cells (Gomori's reaction; 460×).

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