A MORPHOLOGICAL AND HISTOCHEMICAL STUDY OF THE THYMUS DURING PROGRESSIVE TUBERCULOSIS

V. I. Puzik and M. P. El'shanskaya

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A combined morphological and histochemical study was made of the thymus and changes in the gland compared with changes in other organs during progressive tuberculosis. In the first stages of development of experimental tuberculosis evidence of increased morphological and functional activity of the thymus appeared: the cells of the epithelial part of the gland swelled and their enzyme activity increased. Thymus-dependent zones of the lympth glands and spleen enlarged and numerous antibody-forming cells appeared in the sinuses. In the period of development of severe generalized tuberculosis the state of the thymus changed and activity of the epithelial reticulum was reduced and the cortex became delymphotized. These changes were accompanied by disappearance of the cells from the thymus-dependent zones and a decrease in the number of antibody-forming cells in immunogenetic organs. The parallel described between the histological and functional state of the thymus and the immunogenetic organs suggests that the thymus participates in the reorganization of tissue immunity in tuberculosis.

The thymus is the central organ controlling immunobiological processes in the body [4, 6]. Its state in tuberculosis has not been studied. The view is now held that changes in tissue immunity against tuberculosis take place not only after vaccination but also in manifest forms of the disease and also during recovery [2, 5].

The object of this investigation was to study morphological and histolochemical responses in the thymus in progressive experimental tuberculosis and also during healing of the lesions in animals as a result of treatment and then to compare changes in the thymus with immunomorphological reactions in the immunogenetic organs.

EXPERIMENTAL METHOD

Experiments were carried out on 40 growing guinea pigs weighing 200-250 g infected subcutaneously with Mycobacterium tuberculosis strain Bovinus 8 in a dose of 0.0001 mg. Some of the animals 1 month after infection were treated for 3 months with isoniazid in a daily dose of 0.15 mg. The animals were killed 1, 7, and 14 days and 1, 2, 3, and 4 months after infection. Uninfected control guinea pigs were killed parallel with the experimental animals. Sections were stained with hematoxylin-eosin and studied histochemically for RNA (Brachet), DNA (Feulgen), and neutral muco- and glycoproteins (Hotchkiss). The following enzymes also were determined: succinate dehydrogenase (SDH; Nachlas), acid and alkaline phosphatases (Gomori), adenosinetriphosphatase (ATPase; Moisel and Wachstein), esterase (Nachlas–Seligman), and lipase (Gomori, in Mark's modification). Antibody-forming cells were detected by the direct immunofluorescence method of Coons. For this purpose the nonspecific immunoglobulins were determined in sec-

Central Scientific-Research Institute of Tuberculosis, Ministry of Health of the USSR, Moscow.
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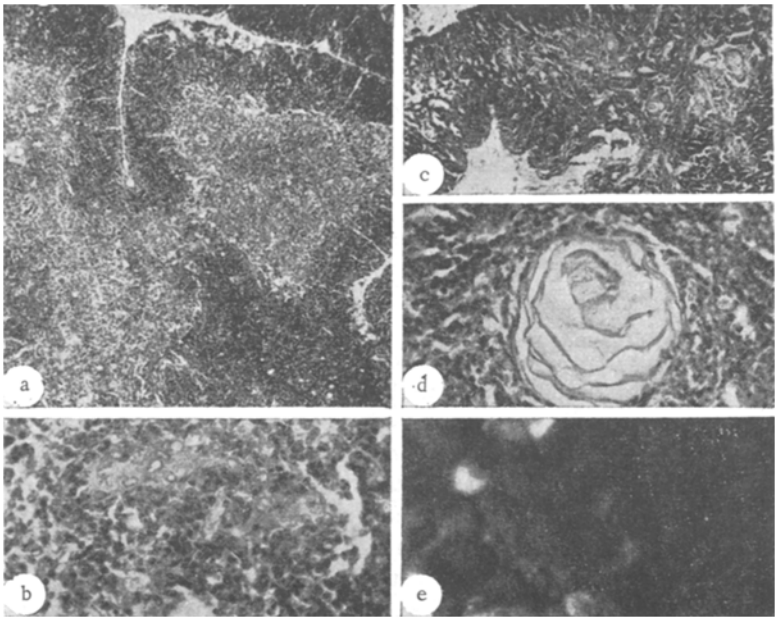

Fig. 1. Thymus of infected guinea pigs (hematoxylin-eosin): a) enlargement of lobules 7 days after infection (72×); b) epithelial islets in medulla of lobules (detail from preceding preparation; 400×); c) decrease in size of lobules of thymus 1 month after infection (72×); d) keratinizing, degenerating Hassall's corpuscle (detail of preceding preparation; 400×); e) antibody-forming cells in sinus of lymph gland 7 days after infection (direct Coon's method, 350×).

tions prepared by Sainte-Marie's method by the use of a standard luminescence anti-species serum prepared by the N. F. Gamaleya Institute of Epidemiology and Microbiology.

EXPERIMENTAL RESULTS

From 1 to 7 days after infection, before the appearance of tuberculous changes in the internal organs, the weight and size of the lobules of the thymus were greater than in uninfected animals. Congestion of the vascular network and widening of the medulla of the thymus lobules (Fig. 1a) on account of swelling and proliferation of the cells of the epithelial reticulum and endothelium of the blood vessels, with an increase in their ATPase content, were observed histologically. The swollen cells of the epithelial reticulum showed high SDH, lipase, and nonspecific esterase activity. The number of Hassall's corpuscles also was increased, so that epithelial islets consisting of epithelial cells accumulating granules of PAS-positive material in their cytoplasm became predominant. Accumulation of many phagocytic macrophages with high acid phosphatase activity was observed in some Hassall's corpuscles. These morphological and histochemical changes indicated increased functional activity of the thymus and, in particular, of its epithelial component, the state of which is considered by many investigators to reflect the manifestation of the protective response of the body [1, 3], at this period of the experiment.

From 14 days to 1 month after infection the animals developed tuberculosis with the initial phase of generalization. The lobules of the thymus gradually diminished in size (Fig. 1c). Epithelial islets and newly formed Hassall's corpuscles were found much less frequently than at the preceding time of the experiment. The number of degenerating Hassall's corpuscles showing keratinization (Fig. 1d) and necrosis increased. A reduction in the morphological and functional activity of the epithelial part of the thymus was accompanied by its delymphotization. Chromatin was redistributed in the cortical lymphocytes which became hyperchromic and pycnomorphic, "adhered" to the reticular cells, and underwent phagocytosis and lysis. Their enzyme activity diminished. The number of lymphocytes in the cortex fell. Side by side with accumulation of lymphocytes, in some areas they were completely absent.

In the period of development of severe generalized tuberculosis the degree of alteration of the thymus increased. The cortical lymphocytes died, with consequent delymphotization, deformation, and collapse of the lobules and fibrosis of the connective-tissue stroma of the gland, the capsule, and the blood vessels. However, complete delymphotization of the thymus did not develop.

Restoration of the structure of the thymus was incomplete in the animals infected and treated with isoniazid. The lobules were enlarged. The density of packing of the lymphocytes in the cortex was less than in the uninfected animals. Some lymphocytes had pycnomorphic, hyperchromic nuclei, affected by lysis in some places.

The morphological study of the immunogenetic organs (lymph glands and spleen) revealed changes in the thymus-dependent zones of these organs at certain periods of development and treatment of experimental tuberculosis.

In the first stage of the disease (1-7 days after infection), before specific changes took place in the internal organs, the thymus-dependent (paracortical) zone of the lymph glands was widened and contained an increased number of blast cells with a high level of RNA, SDH, acid and alkaline phosphatases, and nonspecific esterase. The periarteriolar thymus-dependent zones of the spleen also were rich in blast cells. The study of the spleen and lymph glands by the immunofluorescence method of Coons showed an increase in the number of antibody-forming cells in these organs at this stage of the experiment (Fig. 1e): these were chiefly immature plasma cells located in the medulla of the lymph glands along the course of the sinuses of the spleen, and also in the peribronchial lymphoid concentrations in the lungs. In addition, activation of the macrophagal reaction with an increase in acid phosphatase activity was observed in the lung tissue. All these facts are evidence of the intensification of tissue immunity in the late stage of the disease.

Later during the development and progression of the tuberculosis (2-4 months after infection) a gradual decrease in the number of blast cells in these thymus-dependent zones was observed, with their total disappearance in some animals with severe generalized tuberculosis. The number of antibody-forming cells in the sinuses of the spleen and lymph glands fell. Meanwhile the macrophagal reaction in the lungs and spleen decreased in intensity, with a decrease in acid phosphatase activity. These facts indicate the depression of the tissue defensive reactions and of the resistance of the animals during progressive tuberculosis.

In the treated animals not only did repair processes develop in the thymus, but the thymus-dependent zones of the lymph glands and spleen also were restored.

In the first stages of development of tuberculosis the morphological and functional activity of the epithelial part of the thymus was thus increased. During progression of the tuberculosis the state of the thymus gradually altered, with a decrease in the activity of the epithelial reticulum and with delymphotization of the cortex.

The parallel observed during progressive tuberculosis and its treatment between the histological and functional state of the thymus and the intensity of tissue immunity suggests that the thymus participates in the reorganization of tissue immunity against tuberculosis.

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