MORPHOLOGY AND PATHOMORPHOLOGY

Pathomorphological Characteristics of Experimental Toxemia Induced by Thermostable *Yersinia pseudotuberculosis* Toxin

L. M. Isachkova, N. F. Timchenko, E. P. Nedashkovskaya, and S. D. Raznik

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Pathogenic properties of thermostable toxin responsible for pathogenicity of *Yersinia pseudotuberculosis* were experimentally studied. The toxin exerted a pronounced polyorgan cytopathogenic effect with predominating degenerative destructive changes and membranolytic effect on cell ultrastructure of parenchymatous organs. The toxin is believed to be directly involved in the development of typical pathomorphological picture of pseudotuberculosis, which confirms its pathogenetic role.

Key Words: *Yersinia pseudotuberculosis; thermostable toxin; pathomorphology*

Microbial toxins play an important role in the pathogenesis of infectious diseases [1,2]. *Y. pseudotuberculosis* produces various toxins [8,10], the best studied are thermolabile [6,9] and thermostable toxins [4]. The pathomorphology of toxemia caused by thermostable (TS) toxin of *Y. pseudotuberculosis* is little studied. It is known to cause mouse death 12-16 h after parenteral administration. We studied the pathological changes in experimental animals injected with *Y. pseudotuberculosis* TS toxin, a protein with molecular weight of 45 kD [4].

MATERIALS AND METHODS

Thermostable toxin was isolated from *Y. pseudotuberculosis* strain 512 by an original method. Random-bred mice (16-18 g) were intraperitoneally injected with the toxin in a dose of 20 μg protein in 100 μl. Controls were injected with the same volume of normal saline. Thirty mice were used. The animals were decapitated after 3, 6, 12, and 24 h. Liver, lung, heart, spleen, brain, and fragments of the small intestine with mesenteric lymph nodes were taken for pathomorphologic analysis. Paraffin sections were stained with hematoxylin and eosin and semithin sections with methylene blue-Azur II-eosin. Ultrathin sections prepared from blocks embedded in epon-araldite mixture were contrasted with uranyl acetate and lead citrate and examined under a JEM-100S electron microscope.

RESULTS

The clinical picture developed during 3 h after injection of toxin, rapidly progressed, and the majority of mice died in 12-22 h with tremor, convulsions, diarrhea, and prostration.

Macroscopic examination 3 h postinjection showed plethoric liver and spleen, solitary punctate point hemorrhages in the lungs, and in some animals bloody contents of the gallbladder. After 6 h manifestations of enteroadsorption became universal: sharp uneven swelling of the gut loops and sometimes of the stomach, accumulation of yellow-straw liquid in the gut ,

Institute of Epidemiology and Microbiology, Siberian Division, Russian Academy of Medical Sciences, Vladivostok

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2-4-fold enlargement of the spleen in comparison with the control. These changes were pronounced 12-22 h after injection of TS toxin, visceral plethora increased, some animals developed shock kidney and shock lung. Mesenteric lymph nodes were swollen.

Pathohistological study showed changes in the liver, heart, lungs, kidneys, and lymph tissue. The pathological process was characterized by total capillary reaction with degenerative and necrotic changes in the viscera. Erythrocyte aggregations and fibrin threads were seen in capillary lumen during early period of toxemia (3-6 h postinjection), indicating thrombus formation. These changes were most clearly seen in the liver primarily involved in detoxication after intraperitoneal injection of TS toxin and in the lungs. Vascular changes were not associated with essential increase in vascular permeability and hemorrhages, in contrast to the pathomorphological picture of toxemia induced by *Y. pseudotuberculosis* lipopolysaccharide [3]. In parallel, structural changes in parenchymatous organs progressed by 12-22 h postinjection, with augmenting necrosis. The most pronounced changes were

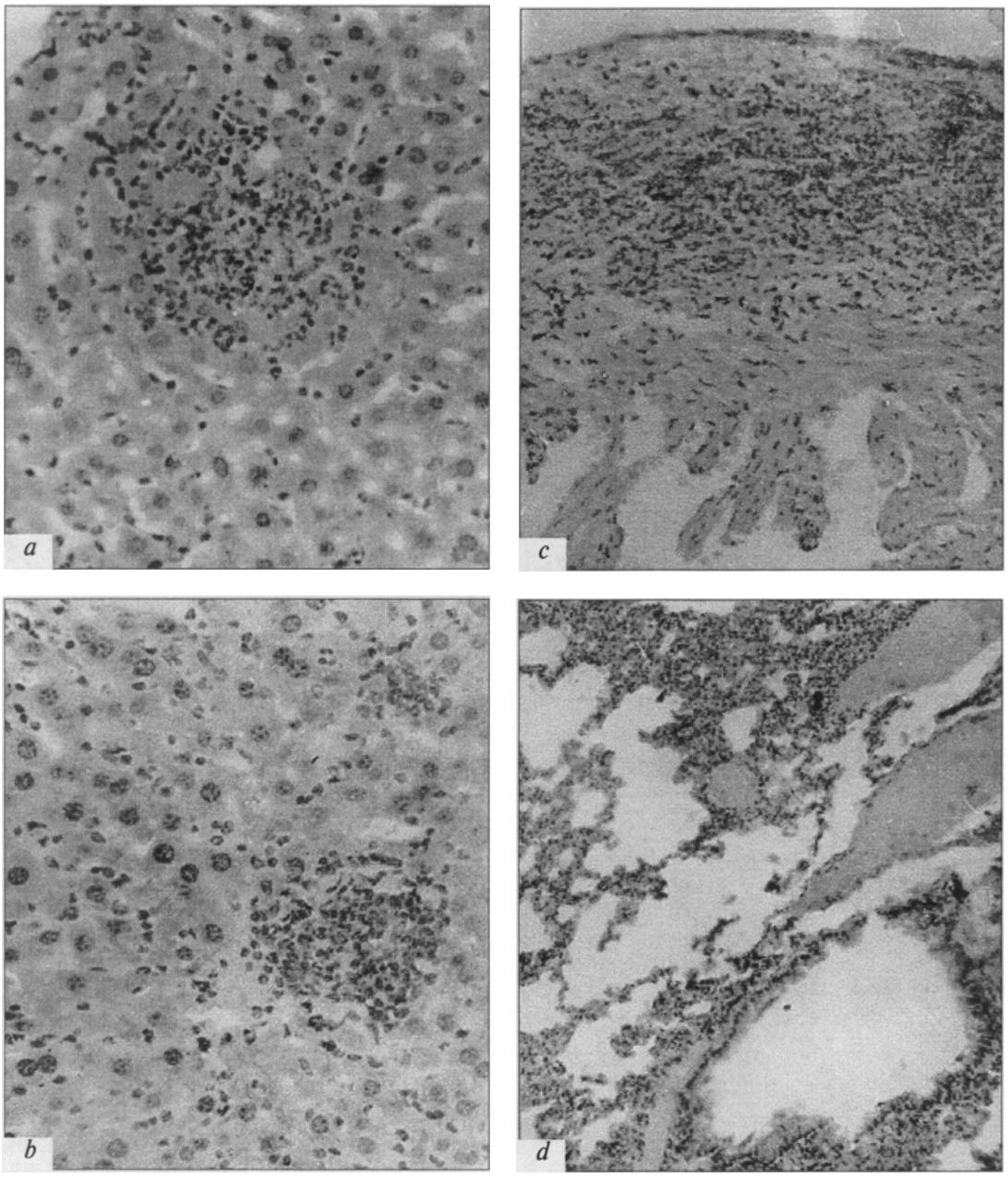

Fig. 1. Histopathology of experimental toxemia caused by *Y. pseudotuberculosis* thermostable toxin. Hematoxylin-eosin staining. *a*) necrotic focus in the liver with polymorpho-cellular reaction, ×200; *b*) diffuse and nodular proliferation of Kupffer cells, ×200; *c*) focus of destruction in the right auricula atrii with lymphoid neutrophilic reaction, ×125; *d*) lung edema, sites of atelectasis and emphysema, ×125.

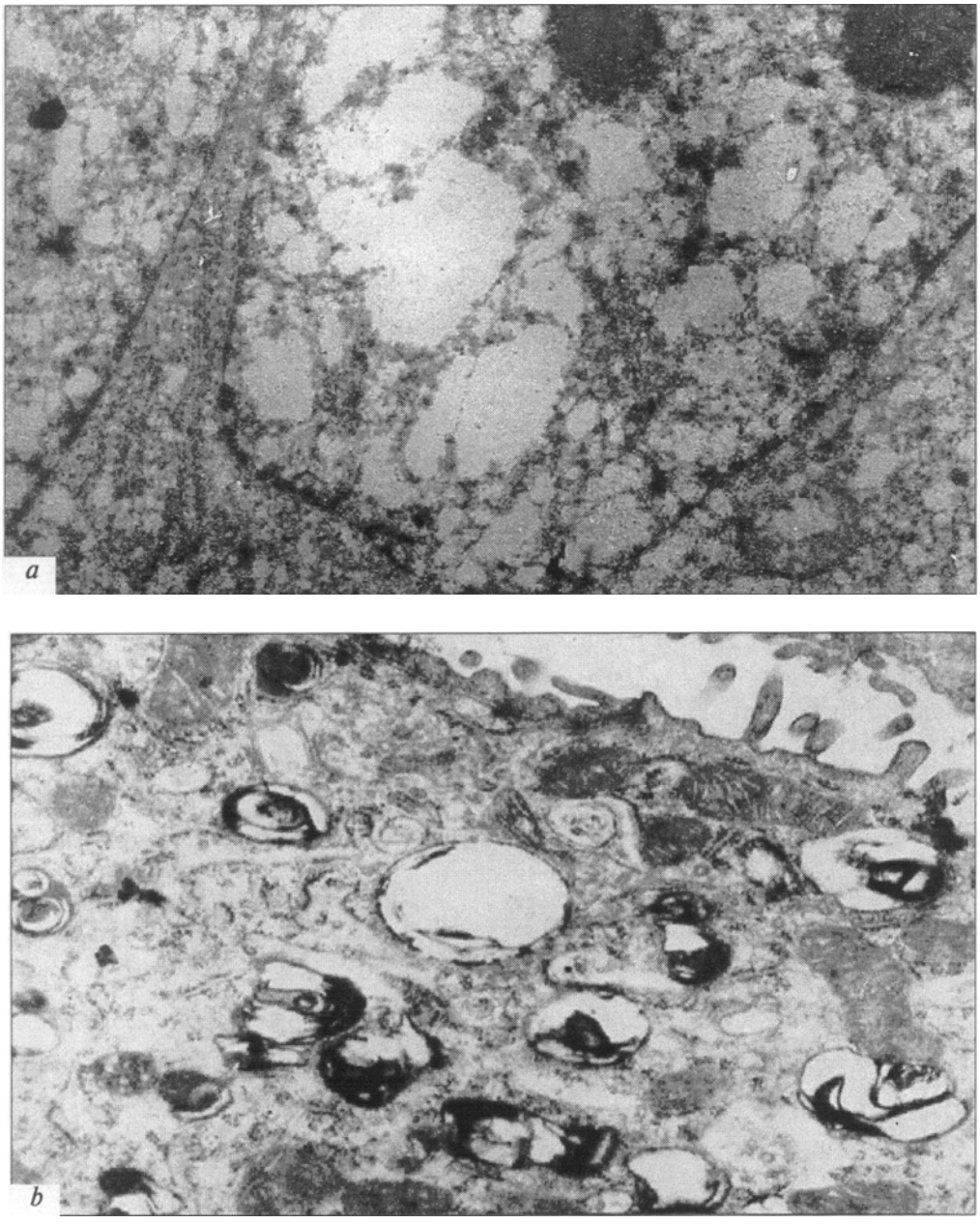

Fig. 2. Ultrastructural changes in cells treated with *Y. pseudotuberculosis* thermostable toxin. *a*) hepatocyte fragment, lysis of nuclear membrane, sharp vacuolation, ×10,000; *b*) type II alveolocyte, drastic injury to osmiophilic lamellar corpuscles, ×37,500.

found in the liver. Three hours postinjection toxic degeneration developed. It manifest by uneven staining of liver parenchyma with alteration of eosinophilic and basophilic hepatocytes with granular cytoplasm. Numerous small oval foci of necrosis were infiltrated with polymorphonuclear leukocytes and macrophages (Fig. 1, *a*). These changes were observed during all terms of the experiment. Condensation of nuclei or chromatin dispersion, fragmentation of the nucleolus were seen in hepatocytes outside necrotic foci; hyperchromatosis and nuclear hypertrophy were seen in some hepatocytes. Binucleated hepatocytes appeared. Dilatation and plethora of central veins and portal vessels were seen; sinusoidal capillaries were sometimes filled with hyaline-like mass, leukocytes, and hemosiderin grains.

One more characteristic feature was diffuse and focal hyperplasia of stellate endotheliocytes (Kupffer cells) functioning as tissue macrophages (Fig 1, *b*). Active reaction of Kupffer cells indicates their involvement in the response to *Y. pseudotuberculosis* TS toxin injected intraperitoneally and spreading through the portal system. Accumulations of macrophages and lymphoid cells presenting as nodules were seen in the

liver parenchyma. Such changes, varying in expression in different animals, were observed at all terms of experiment.

Severe heart injuries were detected: plethoric vessels, toxic myocardiodystrophy, cardiomyocytolysis, lymphoid infiltration in the interstitium. Alterative changes manifested by nuclear vacuolation, presence of ribbon and hyperchromatic cardiomyocytes, cleavage and clumpy degradation of muscle fibers. These changes were sometimes paralleled by focal lymphoid-neutrophilic inflammatory infiltration 12 h after injection of TS toxin (Fig. 1, *c*).

In the lungs pathological changes manifested by thinning and decrease in the cell count of alveolar septae, sites of atelectasia, and focal emphysema. In cases with shock lung, microscopic analysis showed serous hemorrhagic or hemorrhagic pulmonary edema; no bronchus-associated lymphoid tissue was detected (Fig. 1, *d*).

In the kidneys the vessels between the tubules were plethoric, sharp granular degeneration and necrotic changes were seen in the epithelium of the proximal and distal convoluted tubules. Focal lymphoid infiltration in the renal interstitium was observed 12-22 h postinjection. There were no severe destructive changes in the gut, but the epithelium was edematous and mucosa vessels were plethoric.

Toxic changes involved the lymphoid tissue. Small lymphoid follicles without signs of antigenic stimulation and necrotic changes in the follicles and red pulp were seen in the spleen. The most pronounced changes were pulp delymphatization and active erythrophagocytosis.

Electron microscopy of mouse liver and lung samples at the peak of toxemia (12 h after injection of TS toxin) showed pronounced ultrastructural changes in cells, involving both the nucleus and organelles. In hepatocytes, nuclear pores were open, the nuclear membrane were sometimes lyzed, and perinuclear space was unevenly dilated. Lumps of condensed chromatin under nuclear membrane were seen in some hepatocytes. Rarefaction and vacuolation of dispersed chromatin was observed in other cells (Fig. 2, *a*). Abnormal nuclei (prolapses and protrusions) were seen in many hepatocytes. Presumably, this nuclear pathology can be due to nuclear membrane lysis, but it can also be due to nuclear hyperfunction caused by regeneratory processes. Tubules of the granular endoplasmatic reticulum (GER) were unevenly dilated, the number of ribosomes on their membrane, polysomes, and glycogen granules decreased. GER damage was more pronounced at the periphery of hepatocyte cytoplasm. Mitochondria were poorly seen. Hepatocyte cytoplasm was vacuolated because of severe injury of endoplasmatic (including agranular) reticulum membranes and mitochondria. Hyaline-like formations and groups of lysosome were seen. In the lungs, in type II alveolocytes (AC-1) the membranolytic effect of TS toxin manifested by total destruction of osmiophilic lamellar corpuscles (phospholipid-rich producers of surfactant) (Fig. 2, *c*). Microvilli on free surface of AC-2 were leveled, lyzed and fragmented at some sites, which indicated involvement of the external cytoplasmatic membrane.

These data indicate a pronounced polyorgan cytopathic effect of *Y. pseudotuberculosis* TS toxin, with predominance of degenerative destructive component of the pathological process. According to A. I. Strukov *et al.* [5], numerous micronecroses in the organs with nodular inflammatory, mainly polymorpho-cellular infiltration, are characteristic of bacterial endotoxicosis caused by protein toxins. The pattern of ultrastructural changes in parenchymatous cells indicates membranolytic effect of *Y. pseudotuberculosis* TS toxin, which is in line with disorders in protein-producing function and redox processes in the cells [7]. Cytotoxic changes in the cells after injection of TS toxin are similar by many signs to morphological changes observed in pseudotuberculosis induced in humans and animals by virulent and toxigenic *Y. pseudotuberculosis* strains [3]. This gives us grounds to suggest that development of typical granulomas with central karyorhexis, a morphological differential diagnostic sign of pseudotuberculosis, is caused by direct effect of TS toxin, one of toxic substances produced by *Y. pseudotuberculosis*.

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