ACTION OF TUMOR NECROSIS FACTOR ON THE MICROVASCULAR ENDOTHELIUM AND ITS ROLE IN MORPHOLOGICAL CHANGES IN THE INTERNAL ORGANS

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Despite definite progress in the study of the causes of serious complications of bacterial infections, the mechanisms of development of these threatening states still remain insufficiently clear. Previously the development of pathological processes was linked with the presence of products of bacterial cells circulating in the blood stream, but nowadays more and more evidence is being obtained to show that most lesions in organs during sepsis are due, not to the direct action of these substances, but to the action of various substances, including the so-called cytokines, whose production in macrophages and other cells is induced by bacterial toxins [11]. Recent studies have shown that one of the cytokines, namely tumor necrosis factor (TNF), is synthesized in large amounts after injection of the above-mentioned bacterial toxins [4, 5], and also in patients with severe bacterial meningoencephalitis [12], and in children with sepsis [3]. Despite the active study of the biological properties of TNF in the last 5 years, the target cells for the action of this multitarget factor in vivo have not yet been discovered [5], although there are indications that interaction of TNF in vitro with endothelial cells (EC) and polymorphonuclear leukocytes (polymorphs) can induce lesions similar to changes produced by endotoxin [5, 7, 10]. A conceptual achievement of the last decade has been proof of the active role of EC in inflammation and immunity and their involvement in systemic reactions during the development of sepsis [8]. The considerations expressed above led us to analyze the reaction of EC of the microvessels in different regions in vivo in the course of time and its correlation with changes in the internal organs in response to injection of TNF.

EXPERIMENTAL METHOD

A single injection of TNF (recombinant human with specific activity of $5 \cdot 10^7$ U/mg protein) was given into the caudal vein of C57BL/6 mice weighing 19-20 g, in a dose of $5 \cdot 10^5$ U per mouse, which corresponds approximately to LD_{50}. The TNF was generously provided by V. G. Korobko (M. M. Shemyakina Institute of Bioorganic Chemistry, Academy of Sciences of the USSR). Fragments from various parts of the internal organs were taken 1, 4, and 24 h after injection of the TNF. The method of taking the material and of preparing it for examination in scanning and transmission electron microscopes was described previously [1]. The material was analyzed in the JXCA-733 scanning electron microscope ("Jeol," Japan) and the Jem-100CX transmission electron microscope from the same firm. Mice receiving heat-inactivated TNF served as the control.

EXPERIMENTAL RESULTS

Transmission electron microscopy (TEM) 1 h after injection of TNF revealed structural changes in EC in the lungs, in the form of slight thickening of the nucleus-containing zone in EC of the capillaries of the alveolar septa and an increase in the number of filaments in the cytoplasm. Parallel with these changes, signs of commencing edema were seen in the interstitial tissue. In the liver some degree of thickening of the nucleus-containing zone of some EC in the sinusoids was found, with an increase in volume of the Kupffer cells. There were no changes in the kidneys.

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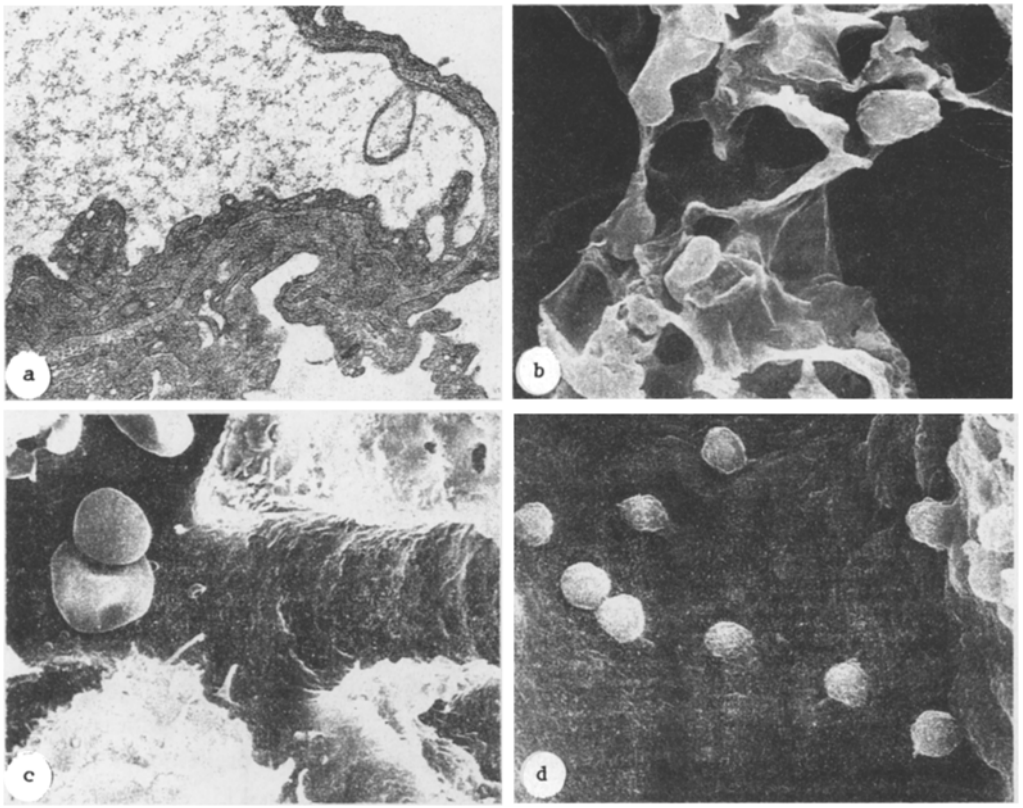

Fig. 1. Morphological changes in internal organs of mice 4 h after injection of TNF. a) detachment of layer of peripheral zone of cytoplasm in capillary EC in alveolar septum of the lung. 28,000×; b) unevenness of endothelial lining in capillary in alveolar septum of the lung. Nucleus-containing zones of EC projecting into capillary lumen. 3100×; c) reduction in number of large pores in EC of liver sinusoids. Erythrocytes in lumen of microvessel. 3600×; d) unevenness of endothelial lining of hepatic vein. Adhesion of polymorph to surface of EC. 2400×.

TEM 4 h after injection of the TNF revealed accumulation of polymorphs in the microvessels of the alveolar septa. In some EC the shape of the nucleus-containing zone was changed, with, an increase in the number of filaments, of structures of the lamellar/complex, and of the endoplasmic reticulum in the cytoplasm. Occasionally microvilli, vesicles, and regions of detachment of a layer of EC from the vessel wall were observed in the peripheral zone (Fig. 1a). Signs of interstitial edema were intensified. Scanning electron microscopy (SEM) revealed unevenness and individual defects of the endothelial lining in the capillaries of the alveolar septa, with the formation of folds and microvilli (Fig. 1b). Against the background of an increase in volume of nucleus-containing zones of EC, adhesion of some polymorphs to the inner surface of veins of different caliber could be seen. TEM in this zone showed signs of polarization of the polymorphs, and a tight junction between the cytolemma of the leukocytes and EC; at junctions between the latter cells, separation of the margins of neighboring cells could be seen.

In the liver, unevenness of width of the lumen of the sinusoids with an increase in the number of polymorphs present in them compared with the control could be observed. The lumen of the sinusoids was partly blocked by Kupffer cells with signs of membrane activity and with a large number of vacuoles, lysosomes, and phagosomes.

Individual signs of "contraction" were found in EC of the sinusoids; their cytoplasm contained numerous filaments, and some elements of the endoplasmic reticulum were vacuolated. Besides individual projecting nucleus-containing zones of EC, SEM of the surface of the sinusoids also revealed the almost complete disappearance of large pores in the cytoplasm in different zones of the lobule (Fig. 1c). Solitary polymorphs were adherent to the endothelial lining. In hepatocytes at this time individual lipid inclusions were seen, with dilatation of the tubules of the endoplasmic reticulum. In EC of the central veins an increase in volume of the nucleus-containing zones was observed, accompanied by an increased number of elements of the cytoskeleton and endoplasmic reticulum. Many polymorphs were fixed to the endothelial lining (Fig. 1d).

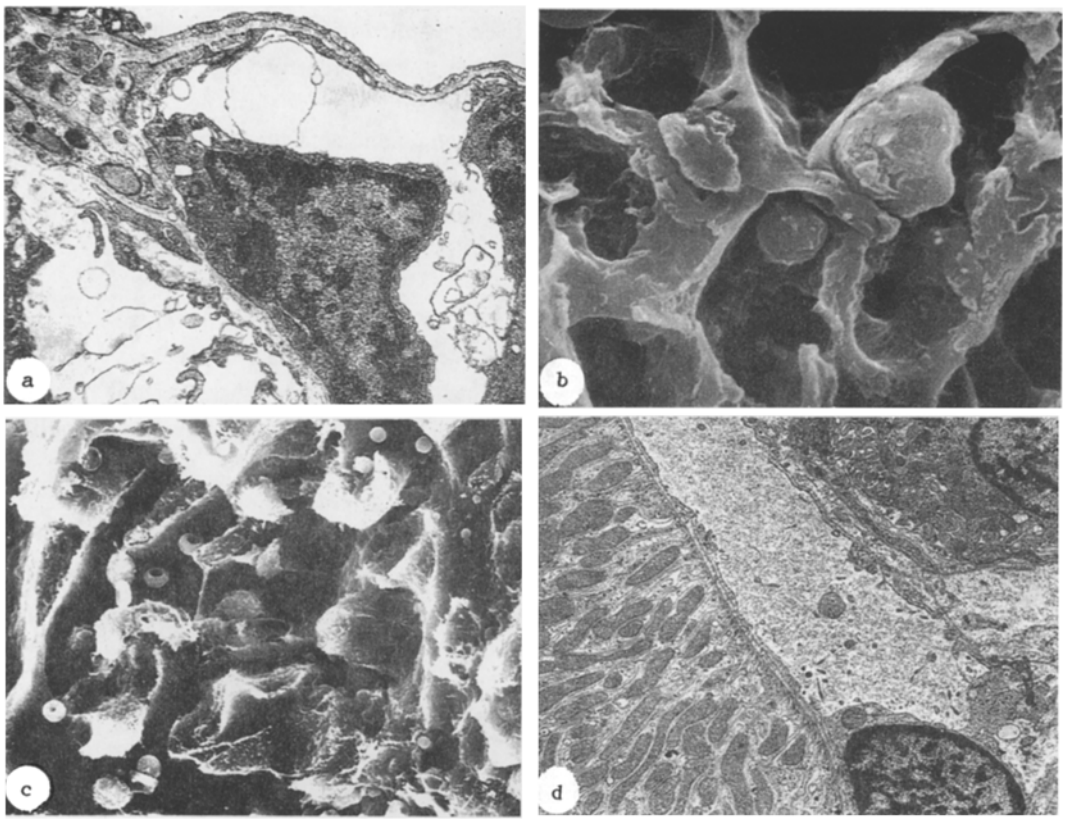

Fig. 2. Morphological features of mouse internal organs 24 h after injection of TNF. a) Dilatation of structures of endoplasmic reticulum and thickening of nucleus-containing zone in EC of capillary of alveolar septum of lung. 25,000×; b) defects and unevenness of endothelial lining in capillaries in alveolar septum of lung. Adhesion of polymorphs to EC. 4400×; c) adhesion of numerous polymorphs to EC from basement membrane. Peritubular capillary in kidney. 7200×.

In the kidneys, 4 h after injection of TNF, sludging of erythrocytes, the formation of outgrowths on the luminal cytolemma of EC, and partial detachment of the peripheral area of cytoplasm could be seen in the peritubular capillaries.

Changes in the lungs 24 h after injection of TNF were mosaic in character, ranging from moderately severe interstitial edema to signs of intensive intraalveolar edema. Clotted plasma, polymorphs without glycogen granules, a few lymphocytes, and fibrin fibrils were found in the capillary lumen. Darkly stained fibrin deposits could be seen in the interstitial tissue.

The capillary EC were distinguished by condensation of their cytoplasm, vacuolation of the mitochondria, and dilatation of structures of the endoplasmic reticulum. The cytoplasmic outgrowth diverged, to leave exposed the connective-tissue base of the air-blood barrier (Fig. 2a). Areas of cytoplasm surrounded by membrane were found in the lumen of the microvessels. Some capillary EC were characterized by an increase in the number of organelles in the perinuclear zone against the background of vacuolation of peripheral areas of the cytoplasm. Edema of the cytoplasm was observed in the type 1 alveolocytes, and they formed cytoplasmic outgrowths. The degree of damage to EC and type 1 alveolocytes affected the development of intraalveolar edema. Polymorphs in various stages of flattening, adherent to the luminal surface, could be seen in the venules and capillaries of the alveolar septa on SEM. Here also defects of the endothelial layer and deposition of fibrin were observed (Fig. 2b).

After 24 h, against the background of the changes discovered previously and, in particular, the absence of pores, vesicle formation could be seen in the liver in EC of the sinusoids on the luminal surface. In the lumen of some sinusoids polymorphs formed tight junctions with the cytolemma of EC (Fig. 2c). In some narrowed sinusoids sludging of erythrocytes and individual fibrin clots were observed. Glycogen was absent in the hepatocytes, but the content of free lipids, in the form of large vacuoles, was sharply increased. In the central veins, SEM clearly revealed enlarged nucleus-containing zones of EC, with individual defects (stomata and stigmata) between EC. Polymorphs were adherent to the endothelial lining. In the central vein and larger blood vessels fibrin deposits were found.

Various degrees of dystrophic changes in the epithelium of the convoluted proximal and distal tubules with vacuolation of elements of the endoplasmic reticulum and partial destruction of the brush border, were seen in the kidneys 24 h after injection of TNF. Changes of this kind were focal in character and they evidently arose against the background of the damaged EC of the peritubular capillaries, with vacuolation of the endoplasmic reticulum and detachment of areas of cytoplasm (Fig. 2d). Fibrin fibrils, deformed erythrocytes, and solitary polymorphs were seen in the lumen of such vessels. Edema of parts of the cytoplasm and its detachment in some places from the basement membrane, with dilatation of tubules of the endoplasmic reticulum were noted in EC of the glomerular capillaries. Individual fibrin filaments were discovered in the lumen of the microvessels.

The results are evidence of a systemic effect of the doses of TNF used on EC; structural changes in the endotheliocytes, moreover, lead to lesions that are characteristic of each organ. Interstitial, followed by intraalveolar edema develops in the lungs, in the liver activation of Kupffer cells is observed, with changes in porosity of the blood-parenchymatous barrier, and signs of fatty degeneration of the hepatocytes, while in the kidneys there is damage to the glomerular and interstitial capillaries combined with initial manifestations of degenerative changes in the epithelium of the convoluted tubules.

The changes as a whole revealed by the investigation resemble the series of lesions developing in response to injection of endotoxin [1, 6], and during the development of Gram-negative infection [2]. This points to a key role of TNF in the damage done to organs in Gram-negative infection. The results confirm data on the action of TNF on EC in culture, with structural changes in the cytoskeleton and modification of some of their functional properties [5, 7, 9, 10]. Accumulation of polymorphs followed by their adhesion to EC is evidently connected with induction of adhesion of leukocytes on the membranes of EC [8].

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