MORPHOLOGY AND PATHOMORPHOLOGY

Immunohistochemical Characteristics of the Liver in Patients with Peritonitis (Early Autopsy)

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Immunomorphological study of the liver in patients died from diffuse peritonitis showed specific changes in the number and morphology of Kupffer cells and endotheliocytes in various zones of the acinus in peritonitis of different origin.

Key Words: *immunohistochemistry; Kupffer cells; liver; peritonitis*

Acute hepatic failure developing in 40-60% patients with peritonitis is the leading cause of death. The pathogenesis of liver dysfunction in peritonitis is complex. The key pathogenetic factor is intoxication caused by massive release of bacterial toxins from the abdominal cavity into the blood. This leads to generalized endogenous intoxication and absorption of hepatotoxic products from the intestine into the blood [1, 15]. Our studies demonstrated that microcirculatory disorders and hepatocyte damage underlie the development of acute hepatic failure.

The system of resident liver macrophages is a true barrier for the development of generalized endotoxemia, including that in patients with peritonitis [10]. Experimental rats showed that 5 min after injection of endotoxin in the portal vein it is accumulated in Kupffer cells (KC), where it undergoes partial degradation and deactivation [13]. In addition, sinusoidal cells of the liver as a component of the blood-liver barrier participate in the maintenance of many functions.

Immunomorphological method with mono- and polyclonal antibodies to some intracellular proteins, *i.e.* specific cell markers, is a very reliable method for investigation of individual cell populations, including sinusoidal cells.

Our aim was immunomorphological study of liver tissue in order to elucidate the significance of disorders in cell interactions in liver acini (LA) in diffuse peritonitis.

MATERIALS AND METHODS

Material collected at 35 early autopies was examined. Clinical material was selected and classified on the basis of case histories and autopsy protocols in order to identify the underlying disease, its complications, and direct causes of death. Clinical criteria of hepatic failure were hypoproteinemia, dysproteinemia, hypoglycemia, hyperbilirubinemia, and increased transaminase activities. The underlying disease and direct cause of death were diagnosed on the basis of early autopsy (45-90 min after death) findings, clinical data, and urgent histological and histoenzymatic analysis of tissues and organs using common pathological diagnostic criteria [2-4,7].

All cases of early autopsy were divided into 3 groups: 1) 17 cases (9 men and 8 women aged 42-68 years) with peritonitis complicating the course of small intestinal diseases: gangrene resultant from acute thrombosis of mesenteric arteries (n=6), comissural ileus

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(*n*=4), and incarcerated hernias (*n*=7). Group 2 included 12 cases with peritonitis (5 men and 7 women aged 48-79 years) developing in diseases of the large intestine: perforation of the large intestine (*n*=8) and gangrene (*n*=4). Control group included 6 forensic medical autopsies (4 men and 2 women aged 42-71 years): sudden cardiac death (*n*=4) and trauma incompatible with life (*n*=2).

According to case histories and results of autopsies, none of the patients previously had liver diseases or hepatotropic intoxications. Paraffin sections of liver tissue were stained with hematoxylin and eosin. The preparations were examined under a Hitachi-200 electron microscope.

Immunomorphological study of the liver was carried out using a panel of mono- and polyclonal antibodies (Dako). Markers CD-68 (clone KP1) to KC, CD-31 (clone JC/70A) to endothelial cells (EC), and LCA (clone 2B11+PD 7/26) to leukocytes were used.

RESULTS

Histological analysis of the liver in the control group showed a slightly uneven sinusoidal plethora, solitary monocellular necroses in the perivenular zones of the acini.

Pronounced disorders of circulation and damaged LA structure were seen in all liver samples from patients died from intoxication caused by diffuse peritonitis. Portal vessels were plethoric, in some cases with plasma sedimentation. Sinusoidal vessels were dilated and plethoric, with aggregations of erythrocytes, leukostasis, and fibrin clots. Pronounced leukostasis and hemorrhagic foci, including those along the portal tracts, were seen mainly in peritonitis caused by perforation of the large intestine.

Vacuolation of hepatocyte cytoplasm was observed mainly in the perivenular and intermediate zones of LA. Small- and large-droplet fatty degeneration and signs of hepatocyte lipophanerosis were seen in the same zones. Sites of necrotic cells formed monocellular, perivenular, triangular, periacinar, and bridge necroses, whose type was determined by the degree of intoxication and determined the hepatocellular insufficiency syndrome. Disseminated necroses were seen mainly in the liver of patients died from peritonitis caused by small intestinal gangrene due to acute thrombosis of mesenteric vessels.

Immunomorphological study of liver samples (Fig. 1, *a*, fig. 2, *a*) showed intracinar differences in the zonal distribution of stellate reticuloendotheliocytes: their ratio in the 1st and 3rd zones of the acinus was 1.6:1. Immunohistochemical study of the liver of patients died from diffuse peritonitis showed decreased count of KC. In peritonitis caused by small intestinal disease their counts were decreased by 30.4 and 35.7% in the 1st and 3rd LA zones, respectively, in comparison with the control (both $p<0.05$; Fig. 1, *b*). In patients died from peritonitis complicating diseases of the large intestine KC counts were decreased *vs.* the control still more: by 60.7 and 71.4%, respectively ($p<0.05$; Fig. 1, *c*).

Endothelial injuries in the periportal sinusoids were seen in patients died from peritonitis caused by gangrene of the small intestine (Fig. 2, *b*). Immunomorphological study confirmed the presence of leukocytes in necrotic foci and more pronounced leukostasis in patients with peritonitis caused by diseases of the small intestine (Fig. 3). Leukostases predominated in the 1st LA zone.

Electron microscopy showed sinusoidal cell damage in all liver samples from patients died from intoxication. More pronounced signs of damage to stellate reticuloendotheliocytes and EC (edema and decreased number of ultrastructures) were seen in liver samples from patients with peritonitis caused by perforation of the sigmoid colon. The detected signs of sinusoidal cell destruction indicate impaired cell-to-cell cooperation in the hepatocyte-KC system and essentially decreased detoxifying function of the liver in peritonitis.

Summing up our findings [5,6] and published data [8,9,12,14], we suggest the following order of events. Increased concentration of toxins from the abdominal cavity entering through the portal vein activates KC and release of chemoattractants (interleukins, leukotrienes, C5 complement component) by them, which, in turn, promote the neutriophil migration: activated neutrophils with adhesion molecule receptors adhere to EC, while adhesion molecules promote the leukocyte migration into the liver parenchyma. Activated neutrophils produce cytokins and free-radical oxygen forms, which damage liver cells. Liver macrophages produce toxic mediators and included platelet aggregation, which leads to circulatory disorders aggravation local hypoxia and leading to cell damage and liver dysfunction.

It is noteworthy that sinusoidal cell of the liver as a component of the blood-tissue barrier are the first to react to endotoxemia. Many cytokines along with the direct toxic action of endotoxin provoke the formation of “breaches” in the sinusoidal lining, which drastically increases endothelial penetrability for toxic substances. On the one hand, these EC lesions indicate the presence of endotoxin receptors on their membranes and on the other, confirm the role of specialized endothelium of liver capillaries in the realization of many effects of endotoxin [11].

It is noteworthy that in peritonitis of different origin endotoxemia is maintained by “vicious circle”

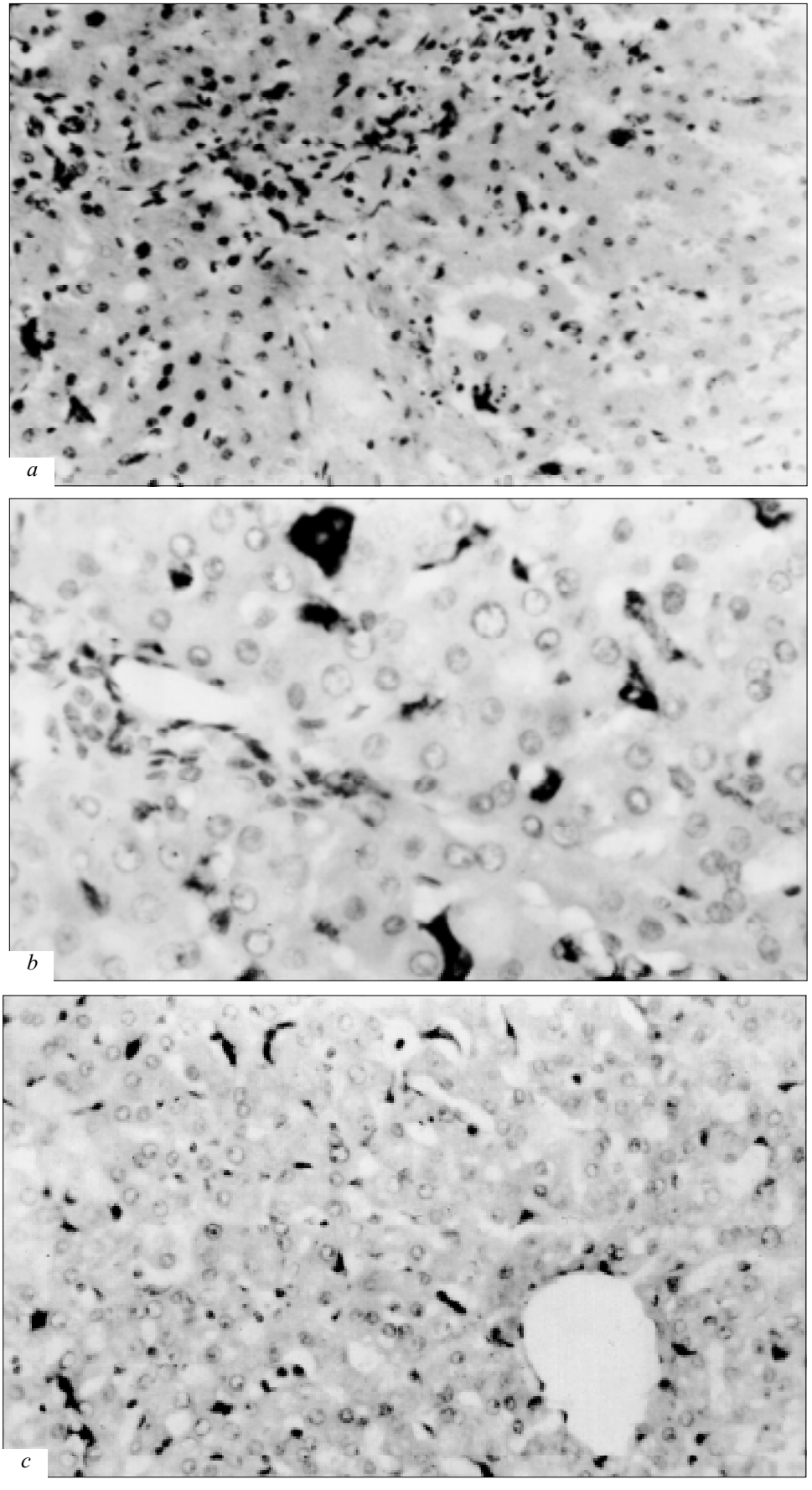

Fig. 1. Expression of CD-68 by Kupffer cells in control (*a*), in a patient with comissural ileus (small intestine) (*b*), and comissural ileus (large intestine) (*c*). CD-68 antibodies, PAP method. Poststaining with Mayer hematoxylin, ×250 (*a, c*), ×400 (*b*).

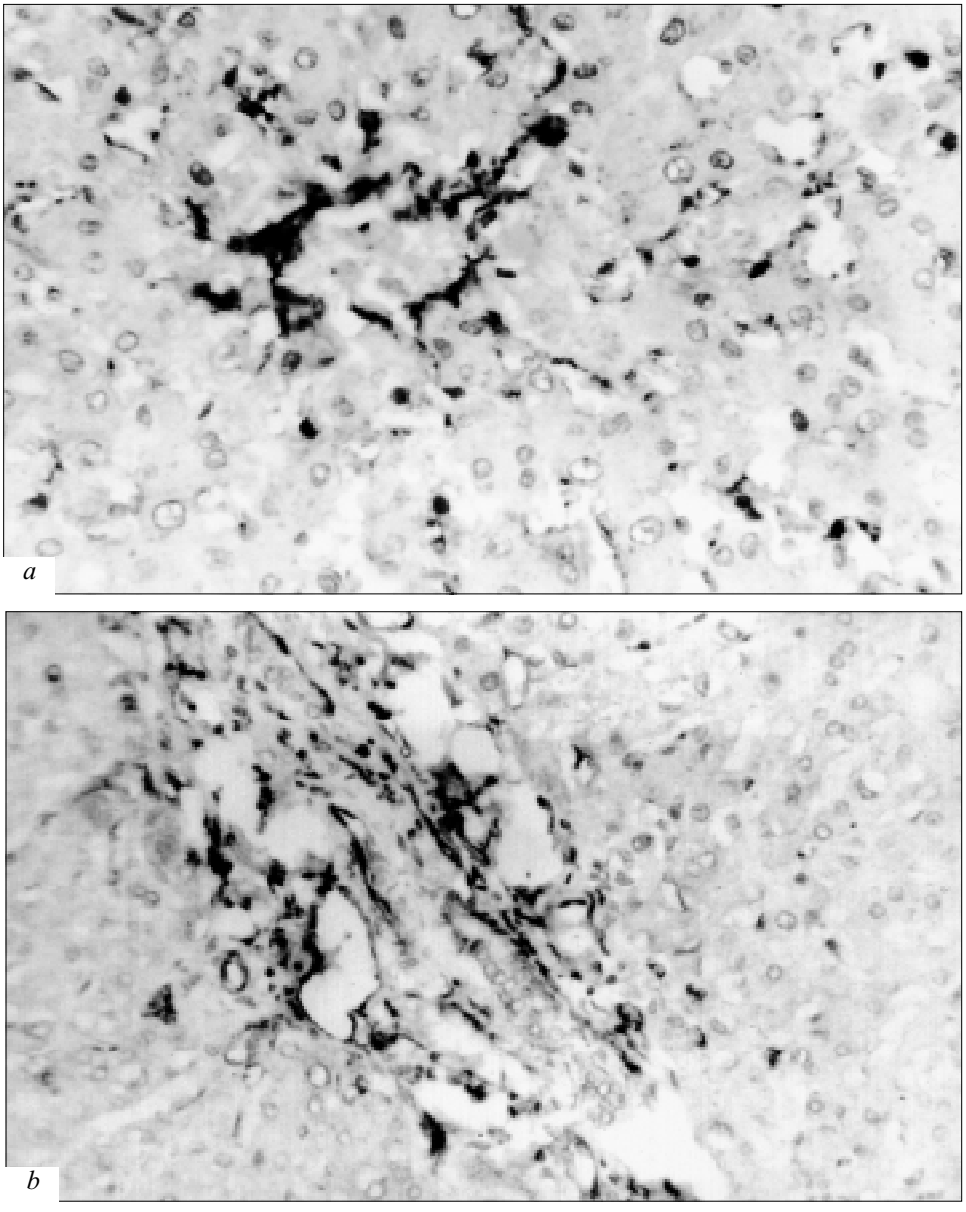

Fig. 2. Expression of CD-31 by liver endotheliocytes in control (*a*) and in ileus (small intestine, *b*), PAP immunostaining with CD-31 antibodies. Poststaining with Mayer hematoxylin, ×400 (*a, c*), ×250 (*b*).

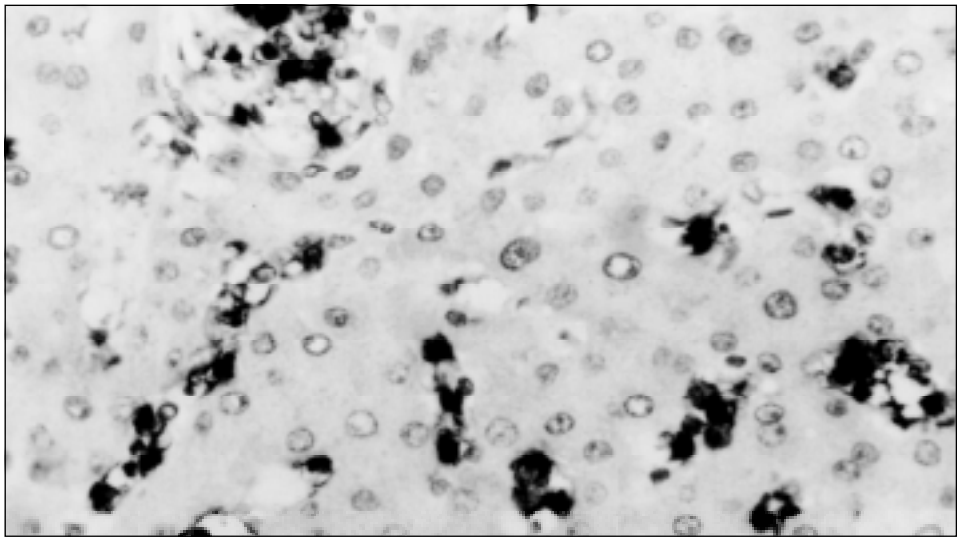

Fig. 3. Expression of LCA by liver leukocytes in a patient with colon gangrene, PAP immunostaining with anti-LCA antibodies. Poststaining with Mayer hematoxylin, ×400. Leukocyte accumulation in sinusoids.

pathogenesis at the expense of entry of bioactive metabolites from foci of visceral lesions.

Hence, hepatocytes and sinusoidal cell lesions underlie the impairment of cell cooperation and failure of detoxifying barrier of the liver in patients with peritonitis and lead to the development of hepatic and in many cases polyorgan failure.

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