A Single Endotoxin Aggression Causes Dose-Dependent Reversible Activation of Rat Liver Ito Cells without Their Transdifferentiation into Myofibroblasts

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The effect of Gram-negative bacterial lipopolysaccharide on rat hepatocytes and sinusoidal cells was studied. The damage and regeneration potential of the liver were evaluated by activation of perisinusoidal Ito cells and proliferative activity of liver cells. Compensatory and repair reactions in the liver induced by lipopolysaccharide manifested by proliferation of liver cells and reversible activation of Ito cells without their transdifferentiation into myofibroblasts.

Key Words: *endotoxin; Ito cells; hepatocytes; compensatory adaptive processes*

Gram-negative enteric microflora is the main source of endotoxin in the organism. It is now known that the liver is the main organ responsible for endotoxin clearance [3,4]. Endotoxin binds primarily to Kupffer cells (KC) via CD14 membrane receptor. The receptor binds both lipopolysaccharide (LPS) and its complex with plasma lipid A-binding protein [2]. Interaction of LPS with liver macrophages triggers cascade reactions based on production and release of cytokines and bioactive mediators [2,7].

The role of liver macrophages (KC) in the clearance of bacterial LPS has been extensively investigated, but the interaction of endothelium with other mesenchymal cells, *e. g.* perisinusoidal Ito cells, has virtually never been studied.

MATERIALS AND METHODS

Highly purified lyophilized *E. coli* (strain 0111) LPS in doses of 0.5, 2.5, 10, 25, and 50 mg/kg dissolved in 1 ml sterile normal saline was intraperitoneally injected to male albino rats (200 g). After 0.5, 1, 3, 6, 12, 24, 72 h and 1 week the viscera were removed under narcosis and placed in 10% buffered formalin. The material was embedded in paraffin. The sections (5 μ) were stained by immunohistochemical streptavidin-biotin method with antibodies to desmin, α-smooth muscle actin (A-SMA), and proliferating cells nuclear antigen (PCNA, Dako). Desmin was used as a marker of perisinusoidal Ito cells, A-SMA as myofibroblast marker, and PCNA as proliferating cell marker. Endotoxin was detected in liver cells using anti-Re-glycolipid antibodies (Institute of General and Clinical Pathology, Moscow).

RESULTS

In a dose of 25 mg/kg and higher LPS induced shock with a lethal outcome 6 h postinjection. Acute exposure of the liver tissue to LPS activated Ito cells and increased their count. The count of desmin-positive cells increased starting from 6 h postinjection and reached the maximum by 48-72 h (Fig. 1. *a, b*). After 1 week, the number of desmin-positive cells decreased but remained above the control. No A-SMA-positive cells appeared in liver sinusoids. Detection of smooth-muscle cells containing A-SMA in portal blood ves-

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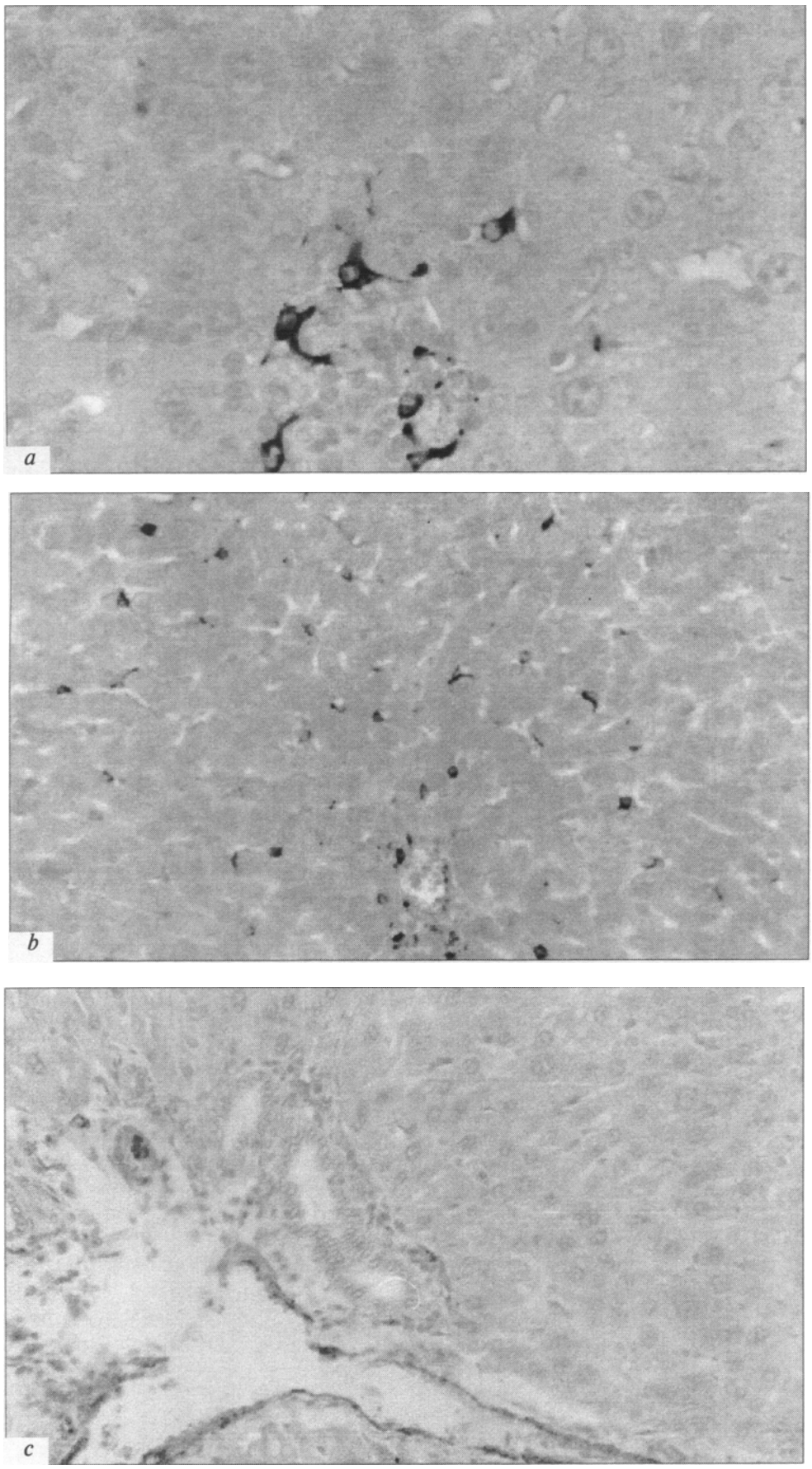

Fig. 1. Rat liver slices treated with LSAB-labeled antibodies to desmin (*a, b*) and α-smooth muscle actin (*c*), ×400 (*a, b*), ×200 (*c*). *a*) before injection of endotoxin: solitary desmin-positive Ito cells in the periportal zone; *b*) 72 h after injection of endotoxin: numerous desmin-positive Ito cells; *c*) 120 h after injection of endotoxin: α-smooth muscle actin is present only in vascular smooth-muscle cells.

sels served as the positive control in staining with anti-A-SMA antibodies (Fig. 1, *c*). Therefore, despite increased count of Ito cells, single injection of LPS did not lead to their transformation (transdifferentiation) into myofibroblasts.

The number of desmin-positive cells in the portal zone increased. From the 6th to 24th h after injection of LPS, perisinusoidal cells were detected only around the portal tracts, *i. e.* in the first acinus zone. From the 48th to 72nd h, when the number of desmin-positive cells reached the maximum, they appeared in other acinus zones, but the greater part of Ito cells was located periportally.

Presumably this is due to the fact that periportal KC are the first to capture endotoxin entering from the

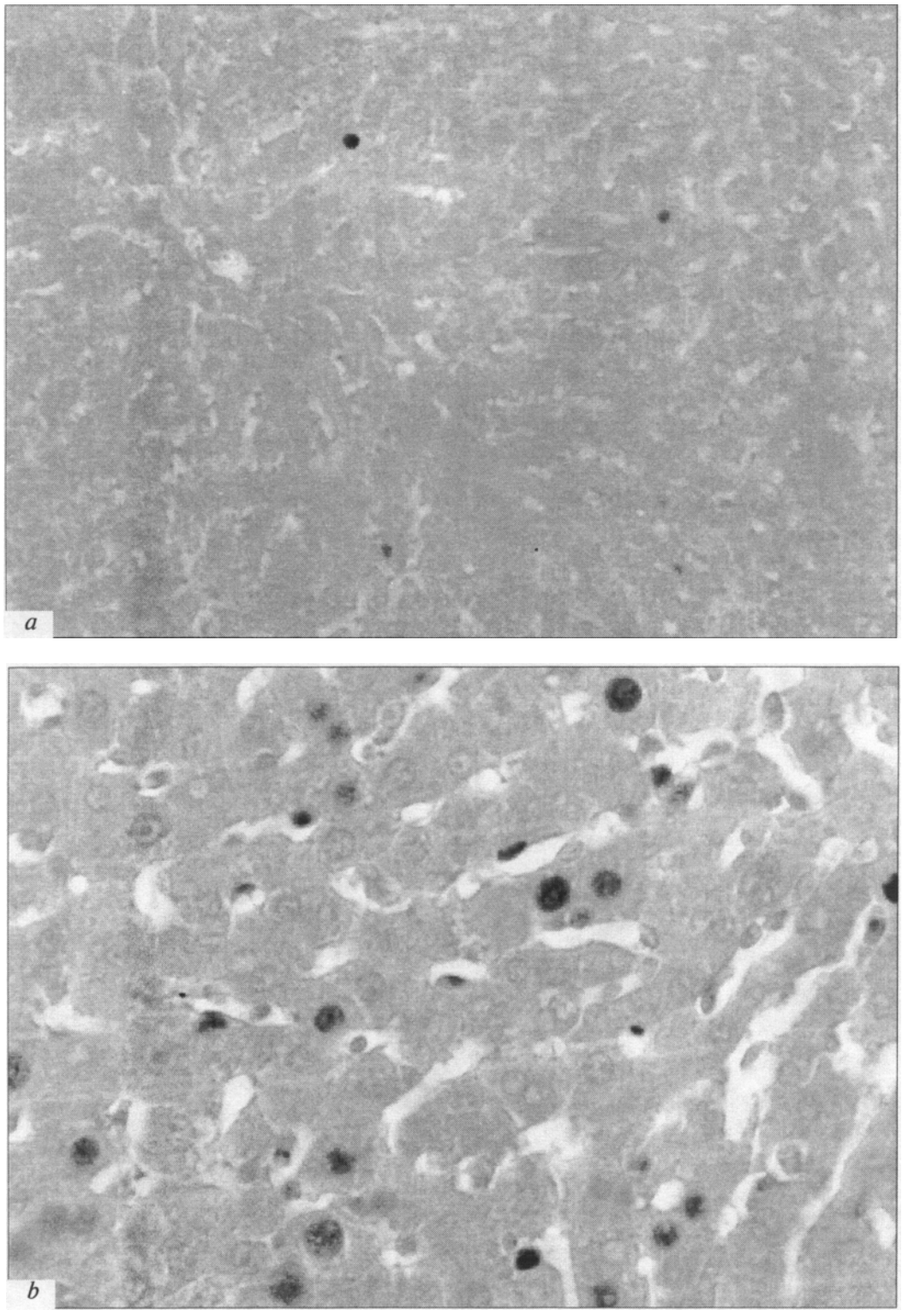

Fig. 2. Rat liver slices treated with LSAB-labeled antibodies to PCNA. *a*) before injection of endotoxin: solitary proliferating hepatocytes, ×200; *b*) 72 h after injection of endotoxin: numerous proliferating hepatocytes, ×400.

intestine via the portal vein or from systemic circulation. Activated KC produce various cytokines triggering activation of Ito cells and their transdifferentiation into myofibroblasts [5]. That is why Ito cells near activated liver macrophages (in the first acinus zone) first respond to cytokine release. However we did not observe their transdifferentiation into myofibroblasts, which suggests that cytokines produced by KC and hepatocytes after single exposure of the liver to LPS can maintain transdifferentiation but cannot trigger it.

Enhanced proliferative activity was also observed mainly in the first acinus zone, which suggests that all (or virtually all) processes aimed at the auto- and paracrine regulation of cell-cell interactions take place in the periportal zones. The number of proliferating cells increased starting from the 24th hour after injection of LPS and peaked after 72 h (maximum proliferative activity, Fig. 2, *a, b*). Both hepatocytes and sinusoidal cells proliferated. However staining for PCNA does not allow identification of proliferating sinusoidal cells. Published data suggest that LPS increases the number of KC [4] probably due to proliferation of liver macrophages and migration of monocytes from other organs [1]. Cytokines released by KC can stimulate the proliferative capacity of Ito cells. Therefore proliferating cells are most probably presented by perisinusoidal Ito cells. Increase in their number is probably needed for intensive production of growth factors and repair of damaged extracellular matrix. This can be a component of compensatory repair reactions of the liver, because Ito cells are the main source of extracellular matrix components, stem cell factor, and hepatocyte growth factor, which participate in the reparation and differentiation of epithelial liver cells [6]. The absence of Ito cell transformation into myofibroblasts indicates that single endotoxin aggression is insufficient for the development of liver fibrosis.

Hence, acute endotoxin exposure increased the number of desmin-positive Ito cells, which indirectly confirms liver damage. Apparently, the number of perisinusoidal cells increases due to their proliferation. Single endotoxin aggression caused reversible activation of perisinusoidal Ito cells without their transdifferentiation into myofibroblasts. This suggests the involvement of some other factors of cell-cell interactions, apart from endotoxin and cytokines, in the mechanisms of activation and transdifferentiation of Ito cells.

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