MORPHOLOGY AND PATHOMORPHOLOGY

Dynamics of Morphofunctional Changes in Immune Organs of BALB/c Mice with Experimental Hepatitis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 4, pp. 451-454, April, 2006
Original article submitted October 6, 2005

The time course of morphofunctional changes in the liver, thymus, and spleen was observed in BALB/c mice with Con A-induced experimental hepatitis. The progress of alterative changes in the liver was paralleled by intensification of accidental involution of the thymus. On day 1 of hepatitis development high proliferative and cytostatic activity of splenocytes was paralleled by hemorrhagic necroses and depletion of the periarteriolar lymphoid sheaths in the spleen (T-zones), presumably due to migration of activated lymphocytes to the liver and barrier tissues. Later normalization of lymphocyte proliferative activity was paralleled by recovery and hyperplasia of the splenic T-cell zones.

Key Words: *experimental hepatitis; concanavalin A; liver; thymus; spleen*

The number of patients with hepatitis of different etiology is increasing [2]. Study of the pathogenesis of hepatic diseases requires available models for detection of cellular and molecular mechanisms of hepatocyte injury. It is difficult to evaluate the development of liver injuries on simple cell systems *in vitro* because of highly specialized morphological organization of the liver, intricate cell-cell cooperation of different cell types in this organ, and functional heterogeneity of hepatocytes; therefore, the most adequate method is simulation of these processes in experimental animals. Various models of *in vivo* hepatitis are known, induced by leukotriene D_4, TNF, hepatotoxins of fungal origin, gram-negative bacterial lipopolysaccharide [4]. Hepatitis developing in response to systemic treatment with Con A (lectin actively used in immunology as a T-cell mitogen) is a widely used model. The choice of this model is explained by the fact that, according to published data, the mechanism of liver injury in viral, alcoholic, and autoimmune hepatitis is common (activation of cellular immunity with subsequent production of cytokines inducing hepatocyte apoptosis and necrosis) [3,10,11]. This model of hepatitis attracts the attention of immunologists investigating cellular and humoral factors of inflammatory process. Morphological studies of the liver and immune organs during the development of Con A-induced hepatitis are extremely scanty and give no idea on the time course of structural and functional changes in these organs [5-8].

We studied the morphology and function of the thymus and spleen of BALB/c mice with Con A-induced hepatitis.

MATERIALS AND METHODS

Experiments were carried out on male BALB/c mice (18-20 g) from Stolbovaya Breeding Center. In order to induce hepatitis, 20 experimental animals

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0007-4888/06/1414-0465

were intravenously injected with Con A (10 mg/kg in 200 μl saline) under ether narcosis. Controls (n=5) were injected with 200 μl saline. The animals were sacrificed on days 1, 2, 3, and 7 after injection of Con A by cervical dislocation under ether narcosis.

Liver function was evaluated by serum levels of AST and ALT (Pliva-Lachema a. s.). In order to characterize splenocyte function, the levels of spontaneous and IL-2 and Con A-induced proliferation were evaluated *in vitro* by routine methods in 4-h test with ^{3}H-thymidine and the cytotoxic activity was evaluated using ^{51}Cr-labeled T-cell tumors Yac-1 (target for natural killers - NK) and EL-4 (target for cytotoxic T-lymphocytes — CTL) in the 100:1, 50:1, and 10:1 effector:target ratios. For morphological studies the liver, spleen, and thymus were fixed in 10% neutral formalin and after standard histological processing the sections were stained by hematoxylin and eosin. The significance of differences was evaluated by Student's t test.

RESULTS

Hepatocytes with diffuse small droplet degeneration, numerous small necrotic foci consisted of 3-5 hepatocytes, and solitary larger foci consisted of several tens of cells, with solitary neutrophils and histiocytes around necrotic zones, were detected in the liver 24 h after injection of Con A. On day 2 the progress of degenerative changes in hepatocytes was associated with more diffuse and manifest necrotic foci, with perifocal inflammatory reaction; the morphological picture corresponded to acute necrotic hepatitis. On days 3 and 7 the manifestations of acute hepatitis persisted; inflammatory changes combined with fibroblastic reaction augmented in the demarcation zone around large necrotic foci (Fig. 1). Serum levels of ALT and AST on days 1-3 of experimental hepatitis were significantly higher than in the control, this confirming morphological signs of hepatocyte alteration. On day 7 ALT and AST tended to decrease, but did not reach the basal level.

With prolongation of hepatitis development, involutive changes progressed in the thymus: diffuse focal depletion of the cortical layer, blurred interface between the medullary and cortical layers, increased number of Hassal's bodies and their appearance in the cortical layer on day 7 of experiment. Accidental involution of the thymus (nonspecific reaction developing against the background of general adaptation syndrome, in which glucocorticoids play the main role) increased in the course of Con A-induced hepatitis. Depletion of the thymic cortical layer seemed to be caused by progressive death of hydrocortisone-sensitive lymphocytes, including subpopulations of phenotypically and functionally immature minor T-lymphocytes located mainly in the subcapsular zone. Increased content of Hassal's bodies and their atypical distribution in the thymus could result from disordered communications between epithelial cells and thymocytes or from toxic effect of Con A on the thymic epithelium (direct and mediated through the cytokine cascade elements).

Depletion of the white pulp with reduction of periarteriolar lymphoid sheaths (PALS) was seen in the spleen 24 h after injection of Con A. Functional tests *in vitro* showed sharply increased indexes of spontaneous splenocyte proliferation and increased proliferative response of these cells to IL-2 in experimental animals. High level of spontaneous proliferation persisted for 3 days of hepatitis development and normalized only by the end of the period of observation (Fig. 2), while induced proliferation decreased to the control level on day 3. This discrepancy between the morphological picture of depletion of T-cell zones in the spleen and increased proliferative activity of splenocytes can be due to intensive migration of activated lymphocytes into the liver and barrier tissues. On day 2 vast solitary large foci of hemorrhagic necrosis were detected in splenic subcapsular zone; these foci were separated from intact tissue by a cell bank consisting of histiocytes and lymphocytes; the number of cells in PALS increased. On days 3-7 diffuse lymphocyte, histiocyte, and neutrophil infiltration with initial manifestations of fibroblastic reaction was observed around condensed subcapsular necrotic foci. The area of the white pulp increased; PALS hyperplasia was noted starting from day 3.

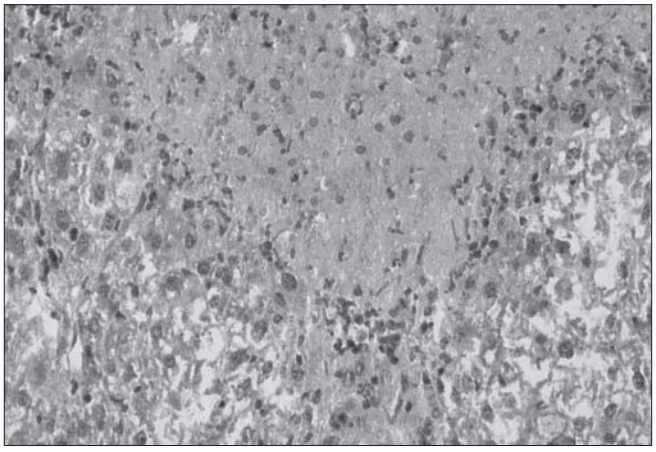

Fig. 1. Morphological changes in the liver of BALB/c mice on day 7 after injection of Con A. Large necrotic focus with perifocal inflammatory infiltration. Hydropic degeneration of hepatocytes. Hematoxylin and eosin staining; ×400.

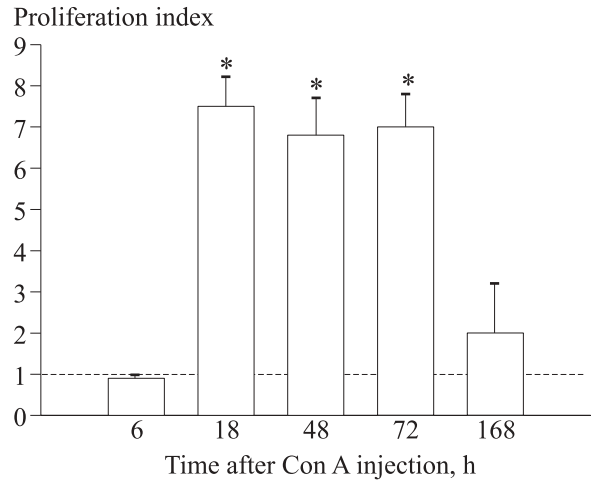

Fig. 2. Spontaneous proliferation of splenic cells of BALB/c mice after intravenous injection of Con A (200 μg). Interrupted line: control. *p<0.001 compared to the control.

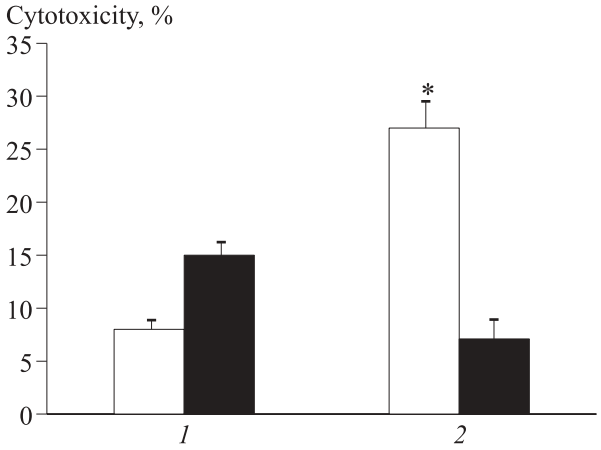

Fig. 3. Cytotoxic activity of splenocytes in adherent (light bars) and common (dark bars) population of splenocytes from intact mice (*1*) and animals with Con A-induced hepatitis, 24 h after injection (*2*). *p<0.001 compared to intact mice.

Recovery and hyperplasia of the splenic functional T-cell zone cannot be due to migration of forming T-lymphocytes from the thymus subjected to accidental involution, when the export of maturing T-cells into peripheral lymphoid organs is blocked. Presumably, the hyperplastic process develops as a result of disorders in homeostatic proliferation of T-lymphocytes. It is triggered by low-affinity recognition by T-lymphocytes of their own peptides in the context of the main histocompatibility complex molecules, and the level of proliferation is determined by the area of devastated lymphoid space [9].

Splenocytes from mice with experimental hepatitis are characterized by significantly higher (*vs.* controls) cytotoxic activity towards Yac-1 target, while lysis of EL-4 target did not differ in the two groups of animals. Adhesive interactions play an important role in the development of Con A-induced hepatitis, and therefore we carried out a comparative analysis of cytotoxic splenic cells fractionated by adhesion characteristics. The fraction of highly adherent splenic cells from animals of both groups exhibited higher cytolytic activity than total splenocyte population. Highly adherent splenic cells from intact mice, similarly as nonfractionated splenocytes, showed a pronounced cytotoxic activity towards Yac-1 target (Fig. 3). In mice with Con A-induced hepatitis highly adherent population of splenic cells showed a significantly higher (*vs.* control) cytotoxicity towards EL-4 target, but the lysis of Yac-1 was low in comparison with that in intact animals and in the common splenocyte population in this group. This indicates the generation of NK and CTL in the spleen of mice 24 h after induction of hepatitis, the latter cells being present mainly in the adherent splenocyte fraction. Previously detected deficient production of IL-2 during the same period [1] can also promote hyperplasia of the functional T-cell zones, because this cytokine stimulates the expression of CTLA-1 (negative regulator of T-cell activation) and promotes the generation of regulatory $CD4^+CD25^+$ T-cells [10].

Hence, the time course of structural changes in the liver, thymus, and spleen in experimental Con A-induced hepatitis is characterized. The data of morphological analysis of the liver, central and peripheral organ of the immune system together with the results of functional tests extend the potentialities of using this model for preclinical evaluation of the immunomodulating and hepatoprotective drugs.

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