Structural Changes in the Liver of Fetuses from Female Mice Infected with BCG Vaccine

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Hypotrophy was found in fetuses of female C57Bl/6 mice infected with BCG vaccine. Light microscopy of liver samples revealed destructive processes, impaired reparative regeneration, and fibrosis.

Key Words: *BCG granulomatosis; mouse fetuses; liver; morphometry*

Disseminated tuberculosis of pregnant women is often accompanied by transplacental infection of the fetus [4]. Morphofunctional activity of fetal liver partly determines the possibility for prevention of congenital disseminated tuberculosis, since the liver includes the majority of cells of the mononuclear phagocyte system [3]. Fetal health in disseminated tuberculosis is determined by not only morphofunctional activity of the placenta, but also function of maternal liver. Destructive processes in maternal liver occur due to intoxication with *M. tuberculosis* metabolites. The type and mechanisms of pathological changes in fetal liver remain unknown [6].

Here we studied morphofunctional changes in the liver of fetuses from female mice infected with BCG vaccine.

MATERIALS AND METHODS

Experiments were performed on pregnant female C57Bl/6 mice (2 month-old animals) and fetuses. The control group (group 1) included 10 intact C57Bl/6 females. Group 2 consisted of 10 C57Bl/6 females infected with BCG vaccine 30 days before pregnancy. Ten C57Bl/6 females of group 3 were infected with BCG vaccine on day 13 of gestation. Differentiated Kupffer cells appear in fetal liver during this period. These cells constitute 70% of the macrophage population in the mononuclear phagocyte system [7,8]. The animals fed a standard diet and had free access to water and food. Disseminated tuberculous inflammation was induced by intraperitoneal injection of BCG vaccine (single dose 0.5 mg, Allergen) in 0.2 ml 0.9% aqueous solution of NaCl.

The samples were obtained on day 21 of pregnancy. The fetuses were removed under ether anesthesia and weighted. Adult females were killed by cervical dislocation under ether anesthesia.

The liver from fetuses and female mice was subjected to histological and morphometric examination. For light microscopy, liver samples were fixed in 10% aqueous solution of neutral formalin, dehydrated with alcohols of increasing concentrations, and embedded into paraffin. Histological sections were stained with Mayer hematoxylin and eosin [5]. Morphometry of the liver from fetuses and female mice was performed using a closed test system (1.16×10^5 μ^2) [1]. The volume density of hepatocyte degeneration and necrosis was calculated. The volume density of extramedullary hematopoiesis and numerical density of megakaryocytes were additionally evaluated in fetal liver. The de-

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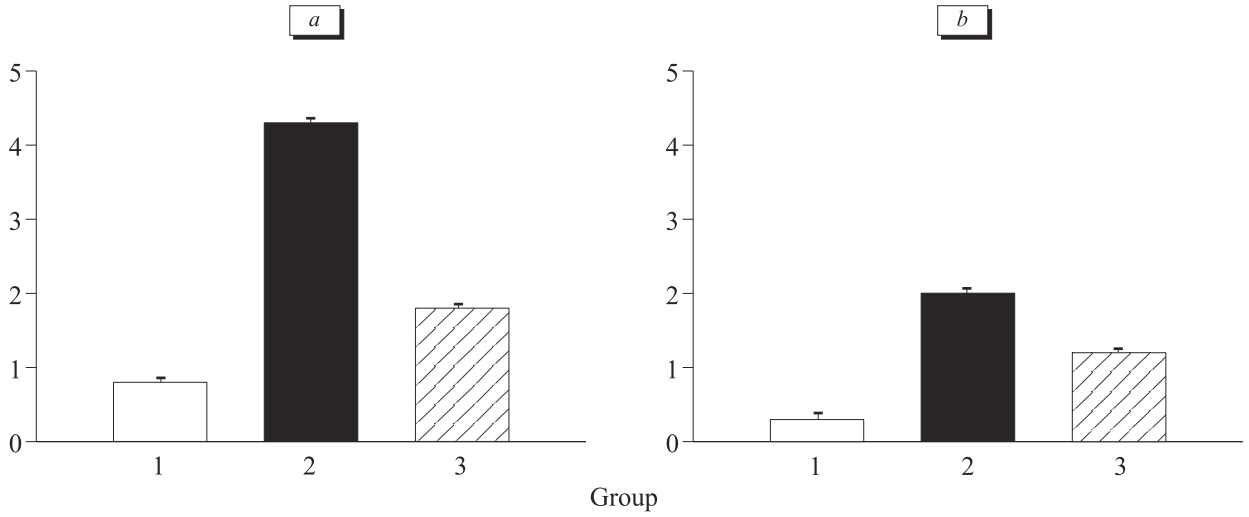

Fig. 1. Volume density of destructive changes in the liver parenchyma of fetuses from female C57Bl/6 mice infected with BCG vaccine. Volume density of vacuolar hepatocyte degeneration (*a*); and volume density of focal necrosis in the liver parenchyma.

gree of reparative regeneration in the liver parenchyma was estimated from the numerical density of binucleated hepatocytes.

The significance of differences between the mean values was estimated by Student's *t* test. These differences were significant at $p<0.05$.

RESULTS

Granulomas were identified in parenchymal organs of female mice infected with BCG vaccine, which suggesed the development of disseminated granulomatous inflammation. Apart from granulomas, focal degeneration and necroses of hepatocytes were accompanied by activation of reparative processes. Tuberculous granulomas were not found in the liver of fetuses from group 3 females. However, these animals were characterized by diffuse focuses of extramedullary hematopoiesis and vacuolar degeneration and necrosis of hepatocytes. Similar, but more significant changes were revealed in the liver parenchyma of fetuses from group 2 females (Fig. 1).

The loss of hepatocytes due to destruction was not compensated by reparative regeneration of cells. This conclusion was derived from the decrease in the numerical density of binucleated hepatocytes. These changes were more pronounced in fetuses of group 2 females compared to group 1 specimens (fetuses of intact female mice, Table 1).

Destructive changes in the liver parenchyma of fetuses from female mice with BCG infection are probably related to the immaturity of barrier systems in the liver of fetuses (macrophage system and oxygenase system) and intoxication of female mice with *M. tuberculosis* metabolites. These processes can be accompanied by endotoxemia and intrauterine fetal death. Our assumption is confirmed not only by the increase in the volume density of extramedullary hematopoiesis in fetal liver, but also by the rise in the count of megakaryocytes in group 2 and 3 animals (Table 1). This parameter in group 2 animals was 2.5-fold higher than in group 1 specimens, which reflects compensatory activation of hematopoietic function in fetal liver (Table 1) [2].

We revealed a decrease in the weight of fetuses from BCG-infected female mice. These changes were most significant in fetuses from group 2 females (Table 2).

The mammalian liver serves as a major site of metabolism. Pathological changes in the liver of

TABLE 1. Morphometric Study of the Liver Parenchyma in Fetuses from Female C57Bl/6 Mice Infected with BCG Vaccine ($M\pm m$)

Group	Volume density of extramedullary hematopoiesis, %	Numerical density of megakaryocytes	Numerical density of binucleated hepatocytes
1	9.20±0.62	0.40±0.08	9.80±0.21
2	15.50±0.52*	1.80±0.06*	6.50±0.20*
3	10.20±0.68*+	1.40±0.05*+	7.80±0.21*+

Note. Here and in Table 2: $p<0.05$: *compared to group 1; +compared to group 2.

TABLE 2. Weight of Fetuses from C57Bl/6 Mice ($M \pm m$)

Group of female mice	Weight, mg
1	1739.6±2.0
2	1243.1±2.0*+
3	1256.3±1.8*

fetuses from BCG-infected female mice probably result from the fact that BCG granulomatosis in pregnant females is accompanied by intoxication with *M. tuberculosis* metabolites. These changes contribute to the progression of destructive processes and suppression of reparative regeneration in the liver parenchyma of female mice and, therefore, in the liver of fetuses (hypotrophy).

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