Morphological Changes in the Liver of Pregnant C57Bl/6 Mice with BCG Granulomatosis

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Morphological changes in the liver of pregnant C57Bl/6 mice infected with BCG vaccine before pregnancy and on day 13 of gestation were studied by means of light microscopy and morphometry. The formation of BCG granulomas in mice of both groups was followed by a decrease in the numerical density and diameter of granulomas, increase in the volume density of hepatocyte degeneration and necrosis, and rise in the numerical density of binucleate hepatocytes (compared to nonpregnant mice infected with BCG vaccine).

Key Words: *BCG granulomatosis; pregnancy; liver*

Significant increase in the morbidity and mortality rate from tuberculosis in all population groups, including pregnant women, is an urgent medical and social problem [2]. The combination of tuberculous inflammation and pregnancy is a risk factor for the negative reciprocal influence of these processes. The development of pregnancy complications is determined by the period of infection and type of tuberculosis [3,5]. Infection in the early period of pregnancy is associated with severe tuberculous inflammation and high risk for complications of pregnancy and delivery [2,3].

Pregnancy is accompanied by hormonal changes and increased production of pregnancy hormones (progesterone, estradiol, *etc*.), placental hormones, and glucocorticoids [7]. The liver is the target organ for these hormones. Hence, functional load in the liver increases during pregnancy. Moreover, the liver is a major compartment for cells of the mononuclear phagocyte system. They play a major role in granulomatous inflammation during tuberculosis, which is associated with severe morphofunctional changes in the liver [4].

Here we studied morphological changes in the liver of mice infected with BCG vaccine before and during pregnancy.

MATERIALS AND METHODS

Experiments were obtained on pregnant C57Bl/6 mice aging 2 months and obtained from the nursery of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences, Novosibirsk).

In series I, the animals were divided into 3 groups of 10 specimens each. Group 1 included intact pregnant C57Bl/6 mice. Control group 2 consisted of nonpregnant C57Bl/6 mice with BCG-induced granulomatous inflammation (8 days). Group 3 included pregnant C57Bl/6 mice infected with BCG vaccine on day 13 of pregnancy.

In series II, the animals were divided into 3 groups. Group 4 included intact pregnant C57Bl/6 mice. Control group 5 consisted of nonpregnant C57Bl/6 mice with BCG-induced granulomatous inflammation (51 days). Group 6 was composed of

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pregnant C57Bl/6 mice infected with BCG vaccine 30 days before pregnancy. Pregnancy was confirmed by the presence of vaginal plugs.

Disseminated tuberculous inflammation was induced by intraperitoneal injection of BCG vaccine (single dose 0.5 mg, Allergen, Stavropol) in 0.2 ml 0.9% aqueous solution of NaCl.

The liver was examined. In series I, the samples were obtained from nonpregnant and pregnant animals on day 8 after infection with BCG vaccine and on day 21 of pregnancy (day 8 of BCG infection), respectively. In series II, the samples were obtained from nonpregnant and pregnant mice on day 51 after infection with BCG vaccine and on day 21 of pregnancy (day 51 of BCG infection), respectively.

The mice were killed by cervical dislocation under ether anesthesia. Liver samples were fixed in 10% aqueous solution of neutral formalin, dehydrated in alcohols of increasing concentrations, and embedded into paraffin. Histological sections were stained with Mayer hematoxylin and eosin [6].

Morphometry of the liver was performed using a closed test system (1.16×10^5 μ^2) [1]. We calculated the numerical density and diameter of hepatic granulomas, as well as the volume density of hepatocyte degeneration and necrosis. The degree 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

Series I showed that destructive changes in the liver parenchyma of group 2 and 3 animals develop on day 8 after infection with BCG vaccine and include vacuolar degeneration and diffuse focal micronecrosis. Macrophage granulomas were mainly localized in the periportal and perivascular area. The numerical density of granulomas in group 3 mice was 1.7-fold lower than in group 2 animals (Table 1). The diameter of granulomas in the liver of group 2 mice was 10% higher than in group 3 animals.

Table 1 shows that the volume density of hepatocyte degeneration in group 3 mice is higher than in group 1 and 2 animals (by 3.2 and 1.4 times, respectively). The volume density of focal micronecrosis in hepatocytes of the liver parenchyma in group 3 mice was higher than in group 1 and 2 animals (by 7.5 and 1.5 times, respectively).

TABLE 1. Morphometric Study of BCG Granulomas and Liver Structure in Pregnant C57Bl/7 Mice Infected with BCG Vaccine (Series I, $M\pm m$)

Parameter	Group		
	1	2	3
Numerical density of granulomas	—	0.46±0.12	0.26±0.12$^+$
Diameter of granulomas, μ	—	28.90±0.68	16.40±0.38$^+$
Volume density of hepatocyte degeneration, %	10.32±0.20	21.50±0.18*	26.40±0.20*$^+$
Volume density of zones with hepatocyte necrosis, %	2.61±0.08	16.80±0.16*	19.70±0.12*$^+$
Numerical density of binucleate hepatocytes	7.82±0.20	9.80±0.20*	12.20±0.16*$^+$

Note. $p<0.05$: *compared to group 1; $^+$compared to group 2.

TABLE 2. Morphometric Study of BCG Granulomas and Liver Structure in Pregnant C57Bl/7 Mice Infected with BCG Vaccine (Series II, $M\pm m$)

Parameter	Group		
	1	2	3
Numerical density of granulomas	—	0.84±0.15	0.46±0.18$^+$
Diameter of granulomas, μ	—	46.90±0.84	38.10±0.46$^+$
Volume density of hepatocyte degeneration, %	12.82±0.16	22.60±0.83*	32.40±0.21*$^+$
Volume density of zones with hepatocyte necrosis, %	2.90±0.08	18.51±0.83*	20.61±0.14*$^+$
Numerical density of binucleate hepatocytes	12.41±0.20	14.45±0.21*	16.98±0.18*$^+$

Note. $p<0.05$: *compared to group 4; $^+$compared to group 5.

The numerical density of binucleated hepatocytes in the liver of group 3 mice was 1.3-fold higher than in group 1 and 2 animals (Table 1).

Table 2 shows that the numerical density and diameter of granulomas in the liver of group 6 mice are lower than in group 5 animals (by 1.8 and 1.2, times, respectively).

The volume density of hepatocyte degeneration in group 6 mice was lower than in group 4 and 5 animals (by 2.5 and 1.4 times, respectively; Table 2). The volume density of focal micronecrosis in hepatocytes of the liver parenchyma in group 6 mice was higher than in group 4 and 5 animals (by 7.1 times and 10%, respectively).

Table 2 shows that activation of reparative processes in group 6 mice is manifested in an increase in the numerical density of binucleated hepatocytes (by 1.3 and 2.3 times compared to group 4 and 5 animals, respectively).

Hence, infection with BCG vaccine before and during pregnancy is accompanied by similar changes in the liver parenchyma of C57Bl/6 mice. The decrease in the numerical density and diameter of BCG granulomas is probably associated with the increase in the concentration of pregnancy hormones and placental hormones in the blood during pregnancy [7]. These hormones induce the formation of monocytes in the bone marrow and their differentiation into macrophages. These changes result in increased elimination of mycobacteria that persist in granuloma macrophages. The increase in the severity of destructive changes in the liver parenchyma of pregnant mice infected with BCG vaccine before and during pregnancy is probably related to the cytopathic effect of *M. tuberculosis* metabolites and induction of free radical generation by activated macrophages. It should be emphasized that granuloma formation is followed by an increase in the number of liver macrophages [5]. Destructive changes in the liver parenchyma of pregnant C57Bl/6 mice infected with BCG vaccine before and during pregnancy are accompanied by the corresponding increase in reparative processes in the liver parenchyma. These features are probably determined by the anabolic effect of pregnancy hormones (progesterone, estradiol, *etc*.) and placental hormones [7].

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