Morphological Changes in the Placenta of Pregnant Mice in Experimental Tuberculosis

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Changes in the placentas of pregnant C57Bl/6 mice infected with BCG vaccine were studied by light microscopy and morphometry. The decrease in the numbers of maternal and fetal vessels was paralleled by destructive changes and disorders in the compensatory reactions, which led to fetal hypotrophy after infection with BCG vaccine.

Key Words: *tuberculosis; placenta; morphometry*

For women of reproductive age tuberculosis is a high risk factor of pregnancy and labor complications, such as gestosis, miscarriage, preterm amniorrhea, chronic placental insufficiency, *etc.* [2]. Intrauterine development of the fetus is largely determined by the morphology and function of the mother-placenta-fetus system, and the risk of the development of small-for-date fetus, congenital developmental defects, perinatal death, and stillbirth is high [4,5,9]. However, the mechanisms of development of these complications as risk factors of pathological development of pregnancy in tuberculosis in the mother and fetus are insufficiently studied [4].

We studied morphological changes in the placenta of pregnant C57Bl/6 mice and the status of their fetuses in experimental tuberculosis.

MATERIALS AND METHODS

The study was carried out on pregnant 2-month-old C57Bl/6 females and their fetuses. The following groups were formed, 10 animals in each: 1) intact; 2) infected with BCG vaccine before pregnancy; and 3) infected with BCG vaccine on day 13 of gestation. Generalized tuberculous inflammation was induced by single intraperitoneal injection of BCG vaccine (Allergen Company) in a dose of 0.5 mg in 0.2 ml 0.9% isotonic NaCl.

The animals were sacrificed by cervical dislocation under ether narcosis and the material for the study was collected. The placentas of pregnant females and their fetuses were examined. Material for light microscopy was collected on day 21 of gestation, fixed in 10% neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin. Histological sections (5-6 μ) were stained with Mayer's hematoxylin and eosin and after van Gieson [5]. Morphometry of placental structures was carried out using a closed square test system of 100 points. Numerical density (N_{ai}) of vessels was studied in a test area of 1.16×10^5 μ^2 [1,5]. The maternal and fetal vessel N_{ai}, volume density (V_v) of degenerative sites in the chorionic epithelium of the placental labyrinthine compartment, trophoblast and collagen fibers V_v in the placenta were evaluated. Newborn mice were weighed directly after birth.

The probability of significant differences between the compared means of the studied parameters was evaluated using Student's *t* test. The differences were considered significant at $p<0.05$.

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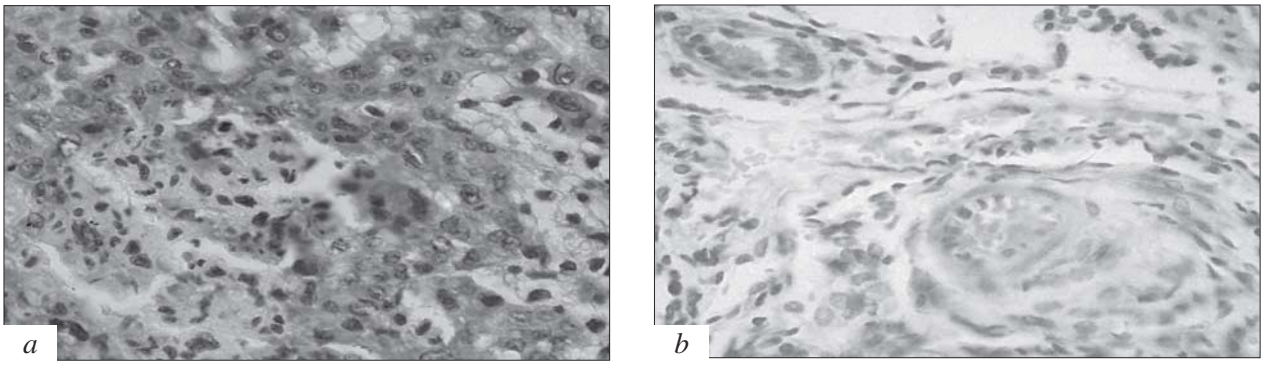

Fig. 1. The placentas of C57Bl/6 mice infected with BCG vaccine before pregnancy. Mayer's hematoxylin and eosin staining, ×200. *a*) vacuolar degeneration of islets of glycogen-containing cells, interstitial edema; *b*) vasculites, mainly in maternal vessels: plethora, stasis, plasma imbibition of vascular walls, interstitial edema. Focal perivascular mononuclear infiltration.

RESULTS

Weighing of mouse fetuses showed significant and equal reduction of their body weights in the group of females infected with BCG vaccine before pregnancy (1243.1±2.0) and during pregnancy (1256.3± 1.8) in comparison with the group of intact females (1739.6±2.0).

Histological study of the placentas from infected animals revealed changes in the labyrinthine compartment of the placenta and in its maternal part (Fig. 1, *a*), presenting as diffuse productive inflammation with multiple foci of mononuclear infiltration. In addition, foci of vacuole degeneration and necrosis of the chorionic epithelium in the placental labyrinthine compartments, in maternal and fetal vascular walls, and vasculites were detected (Fig. 1, *b*), mainly in maternal vessels. These changes were more pronounced in placentas of group 2 mice.

Volume density of degenerative changes in the placentas of groups 2 and 3 was 5.4 and 4.2 times higher, respectively, than in the control group. Degenerative changes were more severe in group 2, presumably because of longer specific inflammatory process in these animals and developing productive inflammation in the placenta from the beginning of gestation.

Along with destructive changes, the trophoblast V_v was 1.8 and 4.5 times reduced in the placentas of animals of groups 2 and 3, respectively, in comparison with the control (Table 1). The trophoblast V_v in group 3 mouse placentas was more markedly reduced than in group 2.

Study of fetal vessels in the placentas showed morphological signs of significant limitation of the trophic potential of this placental compartment, manifesting in reduction of the fetal vessel N_{ai} (Fig. 2, *a*), which was minimum in group 2 mice. Presumably, these changes were caused by earlier damage inflicted to the placentas in group 2 animals. This promoted early formation of inflammatory changes in vascular walls during tuberculous process in the females, which led to disorders in the formation of vessels and served as an additional factor contributing to the formation of placental insufficiency, linked with augmenting hypoxia, leading to the progress of destructive changes and development of fibrosis in the placentas [6].

The study of placental maternal vessels showed that the changes characterizing their N_{ai} were in general similar to those observed in fetal vessels, but were less pronounced (Fig. 2, *b*). These changes were concomitant with destruction of maternal vascular walls in the presence of tuberculous inflammation.

TABLE 1. Volume Density (V_v) of Morphological Structures of the Placentas in C57Bl/6 Mice (*M*±*m*)

Group	V_v (%) of		
	placental trophoblast	placental collagen fibers	foci of degenerative changes in placenta
1	2.40±0.20	0.20±0.10	1.80±0.30
2	1.00±0.80*	0.6±0.10*	9.60±0.41*
3	0.40±1.10	0.30±0.11*+	7.40±0.40*+

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

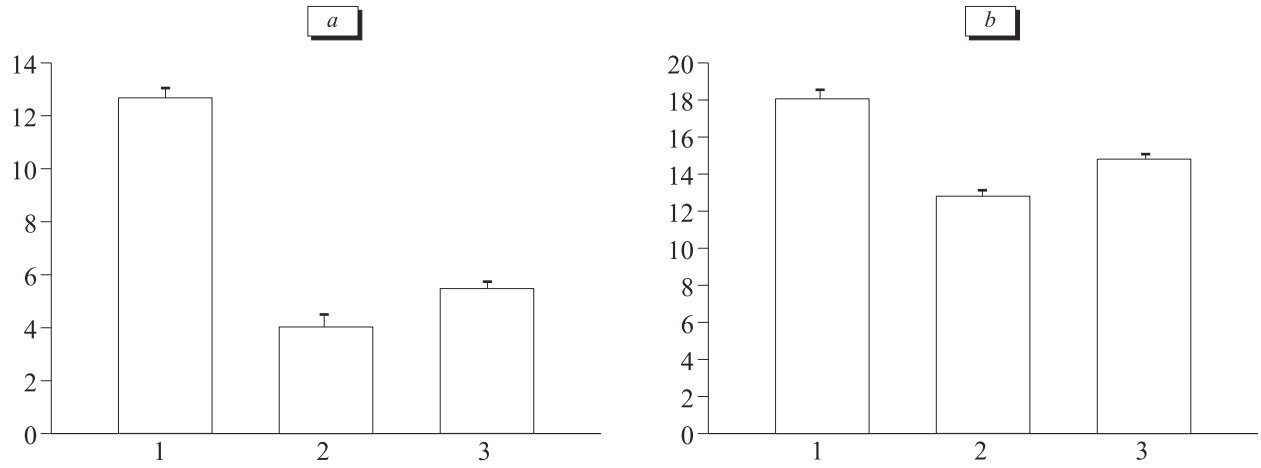

Fig. 2. Fetal (*a*) and maternal (*b*) vessel N_{ai} in the placentas of pregnant C57Bl/6 mice infected with BCG vaccine in different periods of gestation.

Fibrous tissue developed in placentas of all pregnant mice infected with BCG vaccine before and during pregnancy, the most severe fibrosis being observed in group 2 animals (Table 1), presumably because of a longer exposure of placental tissues to *Mycobacterium tuberculosis* and placental ischemia with subsequent formation of the above-mentioned structural changes.

Hence, pathological changes detected in the placental vessels suggest that *Mycobacterium tuberculosis* infection was paralleled by disorders in the growth of placental vessels, maternal and, more so, fetal ones, which on the whole led to the development of destructive and fibrotic changes in the placenta, eventuating in chronic placental insufficiency, augmented by placental fibrosis with subsequent fetal growth delay [7].

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