Mononuclear Phagocyte System in Mice with Intrauterine *Candida albicans* Infection and Postnatal Experimental Tuberculosis

A. P. Nadeev, V. A. Shkurupii, S. V. Pozdnyakova, and M. A. Travin

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Intrauterine *Candida albicans* infection in mouse fetuses affected the type of granulomatous inflammation induced by BCG vaccine during the postnatal period. It manifested in increased formation of granulomas and variations in their cellular composition.

Key Words: *tuberculous granulomatous inflammation; intrauterine C. albicans infection; macrophages*

Intracellular destruction of microorganisms by tissue macrophages in fetuses is impaired due to reduced production of reactive oxygen species [9]. It results in persistence of pathogens in the vacuolar apparatus of macrophages and, probably, replication and dissemination of the pathological agents. Intrauterine *Candida albicans* infection (ICI) is followed by modification of the mononuclear phagocyte system (MPS) and development of granulomatous inflammation induced by another infectious agent during the postnatal period.

Here we studied the effect of ICI of mouse fetuses on the reaction of MPS and severity of hepatic granulomatous inflammation in mice induced by BCG vaccine during the postnatal period.

MATERIALS AND METHODS

Experiments were performed on 2-month-old pregnant C57Bl/6 mice (nursery of the Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences), C57Bl/6 males, and their offspring. On days 13-14 of pregnancy, mouse fetuses received a 1-day-old culture of *C. albicans* (2.5×10^6 microbial bodies) in 0.2 ml 0.9% isotonic NaCl. The offspring of intact pregnant mice served as controls. Two-month-old male offspring was divided into 4 groups. Group 1 included intact males. Group 2 consisted of animals with ICI. The offspring of intact pregnant mice receiving intraperitoneal injections of 2.5 mg/kg BCG vaccine (Allergen) in 0.2 ml 0.9% isotonic NaCl entered group 3 [2]. Group 4 consisted of mice with ICI receiving BCG vaccine in the same dose. The animals fed a standard laboratory diet.

A morphological study was performed with 8-10 mice of each group. The animals were decapitated under ether anesthesia. Liver samples were obtained from 2-month-old mice 1, 2, and 3 months after infection with BCG vaccine. These samples were fixed in 10% neutral formalin, dehydrated with alcohols in increasing concentrations, and embedded into paraffin. Histological sections (5-6 μ) were stained with hematoxylin and eosin. Cellular composition, numerical density (test area 5.63×10^5 μ^2), and diameter of hepatic granulomas (μ) were evaluated [6]. The numerical density of Kupffer cells (KC; staining with Lysozyme monoclonal antibodies, DAKO), binucleated hepatocytes, and sinu-

Research Center of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences; Novosibirsk State Medical Academy. ***Address for correspondence:*** nadeevngma@mail.ru. A. P. Nadeev

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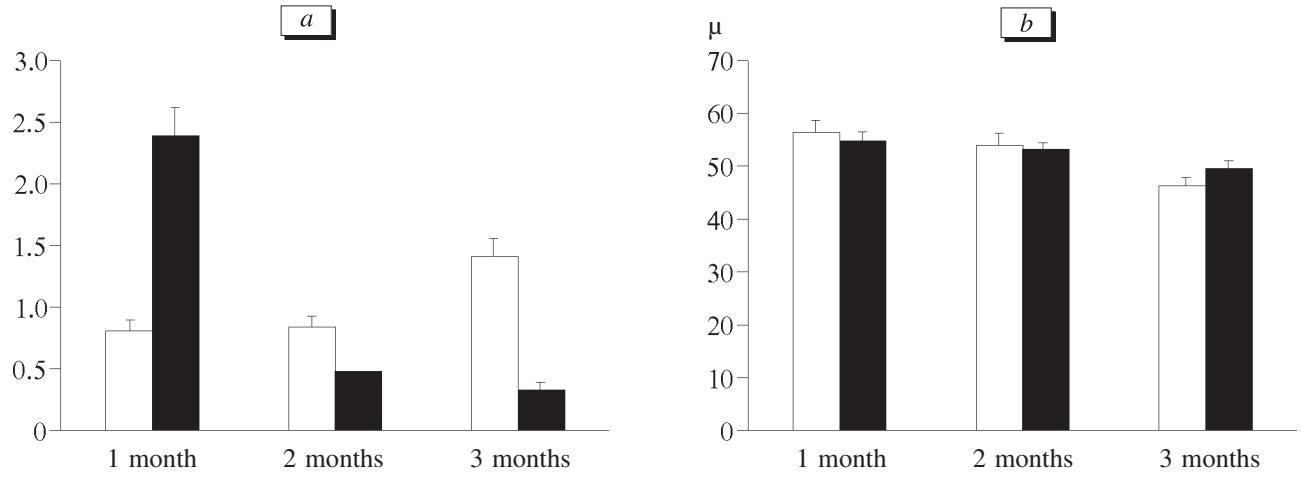

Fig. 1. BCG vaccine granulomas in the liver of male C57Bl/6 mice with intrauterine *Candida albicans* infection: (*a*) numerical density of BCG vaccine granulomas; (*b*) diameter of granulomas. Light bars, group 3; dark bars, group 4.

soidal cells and volume density of dystrophic and necrotic hepatocytes were measured in group 1 and 2 mice. The relative number of monocytes was evaluated in the bone marrow and peripheral blood [2]. The significance of differences between the mean values was estimated by Student's *t* test ($p<0.05$).

RESULTS

Group 2 mice were characterized by delayed somatic development compared to group 1 animals. The body weight of these mice was 12.70±1.06 *vs.* 24.00±0.24 g in group 1.

Histological study showed that destructive changes (necroses and dystrophic hepatocytes) in the liver parenchyma of group 2 mice were more extensive than in group 1 animals (Table 1). These differences were probably associated with activated state of KC and presence of macrophageal infiltrates in group 2 mice. It should be emphasized that cells of macrophageal infiltrates synthesize biocidal substances capable of inducing liver alteration [1]. No intergroup differences were revealed in the number of binucleated hepatocytes reflecting the intensity of proliferative processes in the liver. However, a greater volume of liver destruction in group 2 mice reflects higher intensity of reparative processes [3]. Published data show that fungi suppress cell proliferation [4]. It can be hypothesized that the decrease in reparative activity of hepatocytes in group 2 mice was associated with long-term persistence of *C. albican* or fungal fragments in KC [4]. The number of sinusoidal cells in group 2 mice was lower than in group 1 animals (Table 1). These differences were probably related to a greater area of necrosis in group 2 mice. Moreover, reparative processes in these animals were suppressed not only in hepatocytes, but also in sinusoidal cells.

No differences were revealed in the number of KC and count of monocytes in the peripheral blood (Fig. 2) and bone marrow (Fig. 3) of group 1 and 2 mice. One months after administration of BCG vaccine the number of granulomas in group 4 mice was 3-fold higher than in group 3 animals (Fig. 1, *a*). Three months after infection the number of granulomas decreased by 7 times in group 4 mice, but increased by 1.6 times in group 3 animals. Previous studies showed that administration of living fungi or fungal polysaccharides activates KC. These cells serve as the site for granuloma formation in the liver [6,8]. Administration of BCG vaccine during

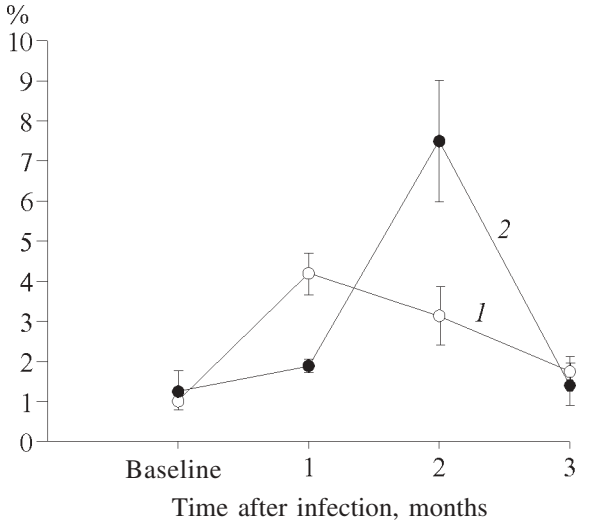

Fig. 2. Peripheral blood monocytes in male C57Bl/6 mice with experimental tuberculosis and intrauterine *Candida albicans* infection (ICI). Here and in Fig. 3: experimental tuberculosis (*1*); experimental tuberculosis and ICI (*1*).

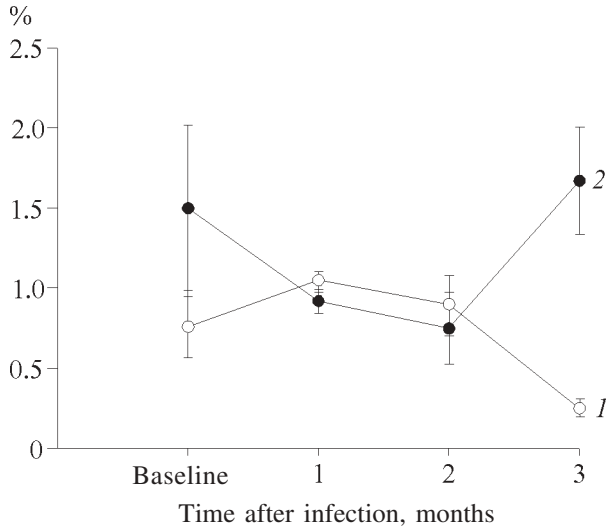

Fig. 3. Bone marrow monocytes in male C57Bl/6 mice with experimental tuberculosis and intrauterine *Candida albicans* infection.

the postnatal period probably stimulates KC to secrete substances activating MPS, inducing migration of mononuclear cells to the inflammatory focus, and contributing to the formation of a considerable number of granulomas. Our results are consistent with published data that *C. albicans* can increase the severity of mycobacterial infection [7]. The degree of mycobacterial elimination estimated by the number and size of granulomas [6] was highest in group 4 mice. It was probably associated with a greater activity of the oxygen radical system, high concentration of NO, and completeness of phagolysosomal fusion in phagocytes.

No intergroup differences were found in the diameter of granulomas (Fig. 1, *b*). However, the diameter of granulomas in animals of various groups slightly decreased by the 3rd month after infection. Published data show that the diameter of candidal granulomas does not exceed 30 μ [3], while the diameter of BCG vaccine granulomas is not less than 50 μ [2]. It can be hypothesized that granulomas with a diameter of at least 46 μ consist of epithelioid cells and are induced by BCG vaccine (Fig. 1, *b*).

Granulomas in group 3 and 4 mice were mainly presented by epithelioid cells (Table 2). In group 4 mice with ICI the number of macrophages, lymphocytes, and fibroblasts increased, while the count of epithelioid cells decreased compared to group 3 animals. The number of granuloma cells remained unchanged in group 3 mice. In group 4 animals the number of macrophages increased, while the count of epithelioid cells decreased by the 3rd month after infection. Our results show that candidal granulomas mainly consist of macrophages, but not of epithelioid cells. However, group 4 mice had granulomas of both cell types.

Taking into account the fact that the content of monocytes in the bone marrow in group 3 and 4 mice did not differ from normal 1 and 2 months after infection (Fig. 3), we conclude that in group 4 animals mononuclears are recruited into granulomas from the peripheral blood (Fig. 2). One month after infection the number of peripheral blood monocytes in group 4 mice was lower than in group 3 animals. By the 3rd month after infection the 8-fold

TABLE 1. Numerical Density of KC and Structure of the Liver Parenchyma in 2-Month-Old Male C57Bl/6 Mice with ICI (*M*±*m*)

Parameter	Group 1	Group 2
KC	3.35±0.31	2.68±0.18
Degenerative changes	47.28±1.92	73.90±1.18*
Necroses	3.03±0.45	20.04±1.13*
Binucleated hepatocytes	12.97±0.47	13.70±0.69
Sinusoidal cells	43.11±0.84	26.66±1.04*

Note. *$p<0.05$ compared to group 1.

TABLE 2. Cellular Composition of BCG vaccine Granulomas in the Liver of Male C57Bl/6 Mice with ICI (*M*±*m*)

Cellular composition of granulomas	Group 3	Group 4
1 month		
Monocytes/macrophages	22.69±0.99	25.30±1.33
Epithelioid cells	74.30±1.08	59.77±1.61*
Lymphocytes	0.54±0.17	6.88±0.58*
Fibroblasts	1.68±0.30	6.42±0.62*
Neutrophils	0.79±0.22	1.62±0.29
2 months		
Monocytes/macrophages	23.37±1.23	34.88±1.37*
Epithelioid cells	73.68±1.23	53.10±1.57*
Lymphocytes	0.73±0.16	6.60±0.52*
Fibroblasts	1.16±0.26	4.61±0.42*
Neutrophils	1.06±0.27	0.81±0.20
3 months		
Monocytes/macrophages	21.18±1.01	37.60±1.52*
Epithelioid cells	74.52±1.15	52.41±1.53*
Lymphocytes	1.64±0.23	3.84±0.46*
Fibroblasts	1.60±0.24	4.20±0.35*
Neutrophils	1.06±0.34	1.95±0.50

Note. *$p<0.05$ compared to group 3.

decrease in the number of granulomas was accompanied by a significant increase in the count of peripheral blood monocytes in group 4 mice (Fig. 2). During this period the number of bone marrow monocytes in group 4 mice returned to the level observed in 2-month-old animals (Fig. 3). These changes were accompanied by an increase in the ratio of macrophages in residual granulomas. A decrease in the number of granulomas was probably associated with elimination of tuberculous mycobacteria from the vacuolar apparatus of phagocytes. It can be hypothesized that the increase in macrophage number was related to persistence and activation of *C. albicans* in residual granulomas. These changes illustrate the cyclic nature of inflammation [5]. During this period we revealed an increase in the number of granulomas and decrease in the count of bone marrow monocytes in group 3 animals. It was probably due to migration of these cells from the bone marrow to granulomas (Fig. 3).

Our results indicate that ICI of mouse fetuses accelerates the initial stage of BCG vaccine-induced granulomatous inflammation in the liver of animals during the postnatal period. Liver inflammation in these animals is rapidly reduced in the follow-up period.

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