Response of Mononuclear Phagocyte System to Experimental Tuberculosis in Mice of Opposite Strains

A. P. Nadeev, V. A. Shkurupii, T. A. Uvarova, and S. V. Pozdnyakova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 8, pp. 227-230, August, 2005
Original article submitted April 28, 2005

Genetically determined peculiarities of the reaction of the mononuclear phagocyte system to BCG vaccine manifested in CBA and C57Bl/6 mice by differences in the number and size of granulomas and time course of changes in the cellular composition of granulomas, peripheral blood, and bone marrow.

Key Words: *mice of opposite strains; tuberculosis; granulomogenesis; macrophages*

Differences in the realization of response to environmental factors in individuals are genetically determined peculiarities of organs and systems, including hormonal and mononuclear phagocyte systems [8]. This suggests the existence of appreciable differences in the realization of response to infectious factors, *e.g.*, to *Mycobacterium tuberculosis* [11].

We studied the role of phenotypical determination of the mononuclear phagocyte system reactions in inbred CBA and C57Bl/6 mice with chronic granulomatous inflammation induced by injection of BCG vaccine.

MATERIALS AND METHODS

The study was carried out on 2-month-old male CBA and C57Bl/6 mice (20-22 g) from Breeding Center of Institute of Cytology and Genetics. These mouse strains were selected because they are opposite by sensitivity to tuberculosis [2,3]. Electron microscopy showed differences in the ultrastructural organization of hepatocytes and adrenals in these two strains. C57Bl/6 mice are characterized by significantly higher basal and ACTH-stimulated levels of 11-hydroxycorticosteroids compared to CBA mice [8].

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

Tuberculous granulomatous inflammation was induced by intraperitoneal injection of 0.5 mg BCG vaccine (Allergen Firm) in 0.2 ml 0.9% isotonic NaCl [7,9]. Control group consisted of intact 2-month-old mice of both strains. Ten mice from each group were taken for morphological studies. The animals were kept on a standard working diet. The mice were decapitated under ether narcosis. Liver samples were collected 1, 2, and 3 months after infection of BCG vaccine and fixed in 10% neutral formalin solution. Histological sections (5-6 μ) were stained with hematoxylin and eosin [6]. The number of BCG granulomas in the test area, their diameters (μ), and cell composition of BCG granulomas in the liver were evaluated [7,9,10]. Numerical density of Kupffer cells in the liver stained using Lysozyme Monoclonal Antibodies (DAKO) was evaluated by the streptavidin-biotin method. The proportion of monocytes (Mn) in the bone marrow (central compartment of the mononuclear phagocyte system; MPS) and peripheral blood was evaluated [6]. The results were processed using Student's *t* test. The differences between the means were considered significant at $p<0.05$.

RESULTS

The pattern of infectious granulomatous inflammation is determined by several factors: type and dose of the agent, immune status, specifically MPS, morphology and function of macrophages (Mp), their count, *etc.*

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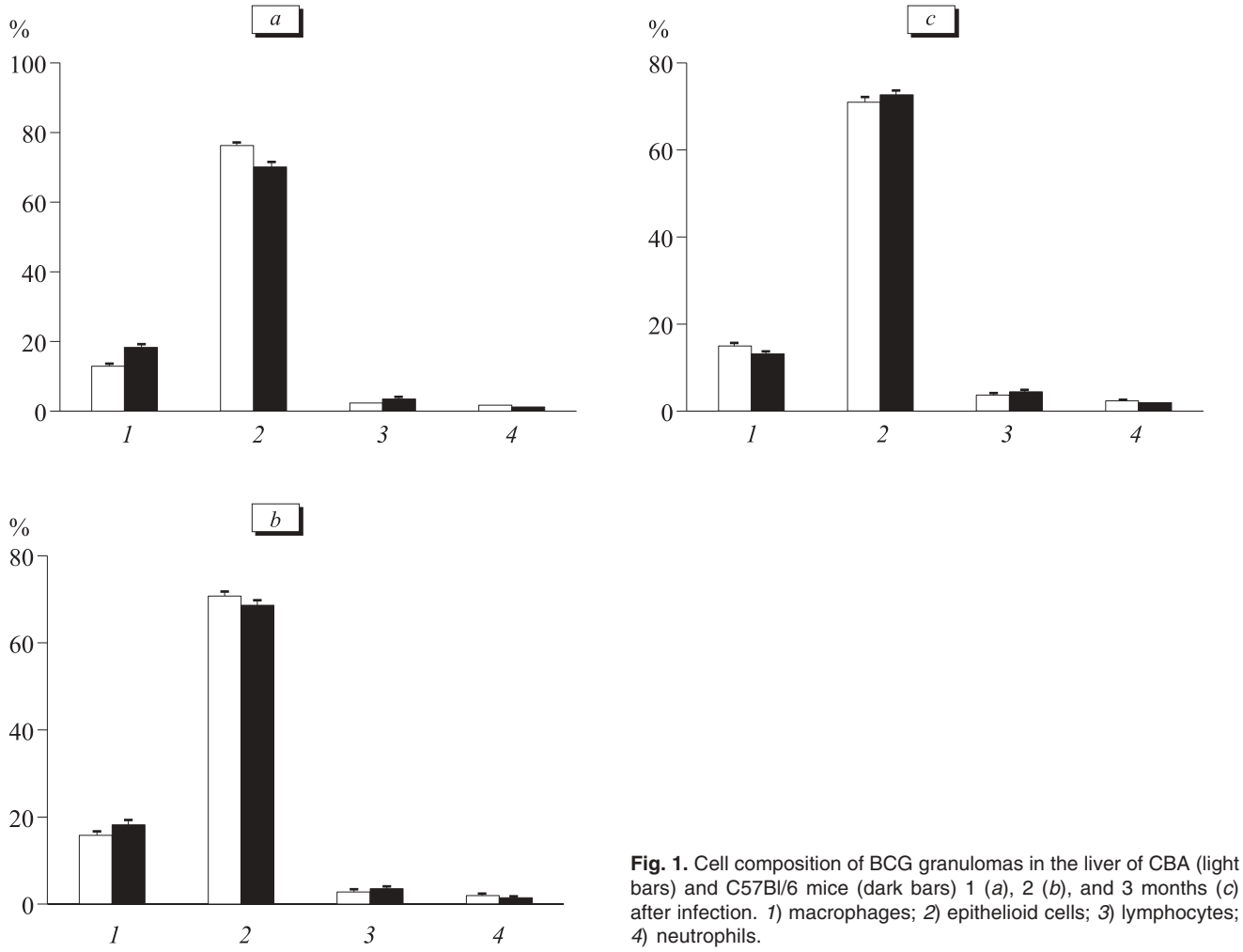

Fig. 1. Cell composition of BCG granulomas in the liver of CBA (light bars) and C57Bl/6 mice (dark bars) 1 (*a*), 2 (*b*), and 3 months (*c*) after infection. *1*) macrophages; *2*) epithelioid cells; *3*) lymphocytes; *4*) neutrophils.

[3]. Granuloma-forming centers in the liver are resident Mp (Kupffer cells). The numerical density of Kupffer cells in intact C57Bl/6 mice was 1.38 times higher than in CBA mice (3.35±0.31 *vs.* 2.43±0.20). However, 1 month after infection the number of granulomas in animals of two strains was similar (Table 1). Presumably, this was due to greater capacity of the agent to fixation in Kupffer cells of CBA mice. Two months after infection the number of granulomas in C57Bl/6 mice was 3.36 times higher than in CBA mice; 3 months after infection the number of granulomas in C57Bl/6 mice was 5.6 times higher than in CBA animals. The diameter of granulomas in C57Bl/6 mice was greater than in CBA animals at all terms of the study (by 1.4, 1.5, and 1.2 times, respectively). By month 3 the number of granulomas in CBA mice de-

TABLE 1. Results of Morphometric Studies of BCG Granulomas in the Liver of CBA and C57Bl/6 Mice ($M\pm m$; n=30)

Parameter	Period of observation, months	CBA	C57Bl/6
Numerical density of granulomas, per 5.63×10^5 μ^2	1	0.88±0.09	0.81±0.08
	2	0.25±0.04	0.840±0.085*
	3	0.25±0.04	1.41±0.14*
Diameter of granulomas, μ	1	39.8±1.48	56.4±2.12*
	2	36.2±1.36	53.96±2.16*
	3	36.84±1.32	46.28±1.64*

Note. *p<0.05 compared to CBA.

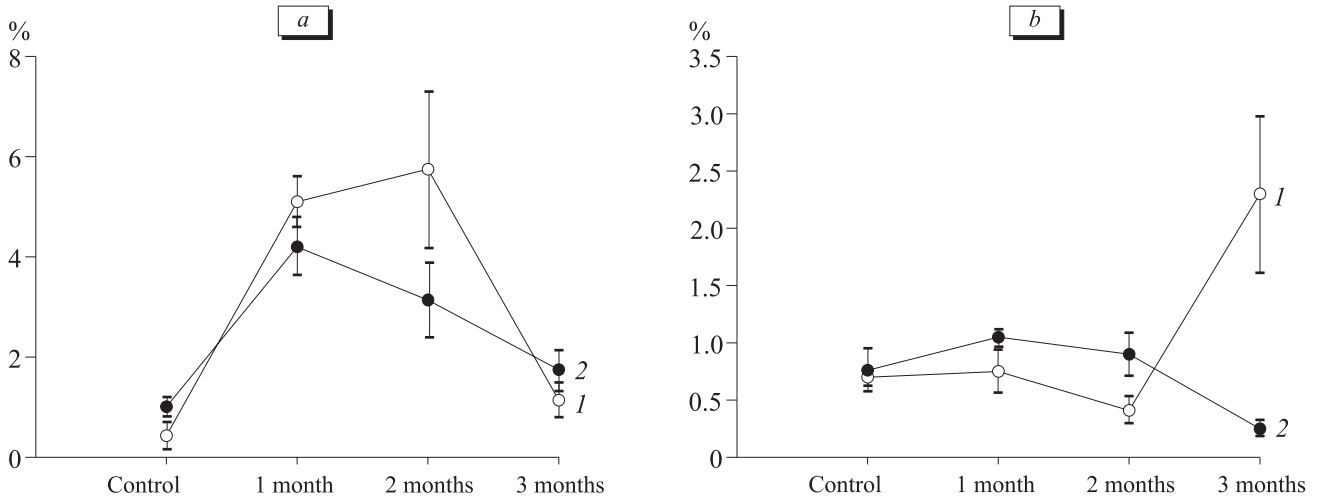

Fig. 2. Peripheral blood (*a*) and bone marrow monocytes (*b*) of CBA (*1*) and C57Bl/6 mice (*2*) in health and experimental tuberculosis.

creased and in C57Bl/6 mice increased significantly (by 60%), the diameters of granulomas in this group was greater. Presumably, MPS cells in C57Bl/6 mice are incapable of effectively eliminating the agent. This can be due to defective phagosomal-lysosomal fusion or inefficiency of oxygen-dependent mechanisms of mycobacterium suppression in Kupffer cells (in contrast to CBA mice), which is compensated by more pronounced infect localization reaction (granulomas and their size) in C57Bl/6 animals. The increase in the number of granulomas by month 3 in C57Bl/6 mice seems to indicate dissemination of the agent and, presumably, indirectly confirms lower efficiency of the mechanisms of mycobacterium suppression by phagocytes in this mouse strain.

The study of the dynamics of cell composition of granulomas revealed interstrain differences 1 month after infection (Fig. 1, *a*), involving Mp, epithelioid cells, and lymphocytes. The number of cells and size of granulomas did not change in all groups (Fig. 1). One month after infection the number of Mp in granulomas of C57Bl/6 mice was 1.4-fold higher than in CBA mice and the content of epithelioid cells was 9% higher (Fig. 1, *a*). The content of lymphocytes and neutrophils in granulomas in mice of two strains were also different (Fig. 1).

Migration of new Mn into a granulema is a condition for the maintenance of its cellular composition [4,5]. The count of Mn in intact C57Bl/6 mice was 2.3 times more than in CBA animals, indicating initial differences in this parameter in the peripheral blood of mice of different strains (Fig. 2, *a*). During the development of granulomatous inflammation, the proportion of peripheral blood Mn increased 1 and 2 months after injection in mice of both strains. By month 3 this parameter returned to the initial level, no differences between the strains were observed (Fig. 2, *a*).

Comparison of these data with the content of Mn in the bone marrow (Fig. 2, *b*) showed the same initial levels of Mn in mice of both strains, but with development of granulomatous inflammation in CBA mice the level of Mn increased 3-fold by month 3 after infection compared to the control group, while in C57Bl/6 animals this parameter was 3-fold lower. Despite high level of granuloma formation, the bone marrow Mn content in CBA mice remained at the initial level during the first 2 months after infection, while the increase in the count of these cells in the peripheral blood was presumably due to accelerated release of Mn from the bone marrow and/or recruiting of Mn from other pools. The content of Mn in the bone marrow is significantly lower than the tissue pool of Mn [4]. Increased content of Mn in the bone marrow of CBA mice by month 3 was presumably due to the decrease in functional "demand" because of decreased number of granulomas and hence, in the content of agents isolated in granulomas. On the other hand, the bone marrow pool of Mn decreased significantly in C57Bl/6 mice by month 3 in comparison with month 2, which could be due to their release into the blood and tissue, specifically, into the liver.

Changes in Mn content in the bone marrow and peripheral blood of C57Bl/6 mice seemed to be caused by the bone marrow "lock" phenomenon for Mn as a result of increased basal and ACTH-induced production of glucocorticosteroids [8] or a higher "requirement" because of increase in the number of granulomas. This effect did not manifest in CBA mice.

Hence, we showed differences in the initial counts of Kupffer cells and of Mn in the peripheral blood in the two strains, which are not related to normally different content of Mn in the bone marrow in animals of the two strains. The role of animal genophenotype in determination of the mononuclear phagocytes re-

sponse to infection by BCG vaccine at different levels and on different components of formation of defense reaction in tuberculosis is demonstrated.

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