BCG Granulomatosis in Adult Mice of Different Strains with a History of Intrauterine Hypoxia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Suppl. 1, pp. 93-96, 2008
Original article submitted July 29, 2008

Specific features of the formation and spontaneous regression of BCG granulomas, depending on mouse genotype and history of intrauterine hypoxia, were detected in 2-month-old male CBA and C57Bl/6 mice. The numerical density of granulomas, their size and (partially) cell composition varied. There are good grounds to assume that the number of BCG granulomas in the liver, irrespective of the experimental conditions and animal strains, decreases after elimination of *M. tuberculosis* persisting in granuloma phagocytes and subsequent migration of cells constituting the granuloma.

Key Words: *BCG granulomatosis; intrauterine hypoxia; liver; genotype; postnatal period*

Tuberculous granulomas are formed mainly by cells of the mononuclear phagocyte system (MPS) [9]. Exposure to destructive factors, *e.g.* hypoxia, often occurring *in utero* seems to nonspecifically modulate the formation of MPS in the fetuses. This can modify the structural and functional parameters of phagocytic cells during the late postnatal period, determining its reactions to factors of bacterial nature, *e.g. M. tuberculosis* [5]. Individual sensitivity or resistance to both factors (hypoxia and infection agents) in the same species can be largely determined by its genotype [1]. A natural model for the study of this phenomenon are animals of different strains with genetically determined structural and functional characteristics of the systems determining the reaction to external factors [1,2,7].

We studied morphological manifestations of generalized BCG granulomatosis in the liver during the late postnatal period in male mice of opposite strains (CBA and C57Bl/6) with a history of intrauterine hypoxia (IUH).

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MATERIALS AND METHODS

The study was carried out on CBA and C57Bl/6 mice (20-22 g), differing by genetically determined structural and functional parameters of organ and systems, determining, among other things, the type of reaction to pathogenic bacteria, for example, *M. tuberculosis* [5,7,10]. The animals were obtained from Breeding Center of Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences (Novosibirsk).

Animals of each strain were divided into 2 groups. Groups 1 of both strains were intact pregnant females. Females of groups 2 of both strains on day 13 of gestation were placed into a pressure chamber and "elevated" to a height of 9 km (4 h daily for 7 days) [6].

The progeny (males) of females from both groups and strains at the age of 2 months received intraperitoneal injections of 0.5 ml BCG vaccine in 0.2 ml 0.9% saline. The animals received standard diet.

Material for the analysis (liver samples) was collected 1, 2, and 3 months after infection. The animals were sacrificed by cervical dislocation under ether narcosis. Liver samples were fixed in 10% neutral formalin, dehydrated in ascending alcohols,

0007-4888/08/1466-0842

and embedded in paraffin. Histological sections (5-7 μ) were stained with hematoxylin and eosin [4]. The numerical density of granulomas in a test area of 1.56×10^5 μ^2 and the diameters of granulomas were evaluated. The percentage of cells in granulomas was estimated [8].

The significance of differences between the means was evaluated by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

Histological study showed areas of degenerative and necrotic hepatocytes in the liver parenchyma in mice of both strains during all periods after injection of BCG vaccine. Mainly epithelioid-cell and macrophage granulomas were detected in the portal tracts and inside the liver lobules of animals of both groups.

In spontaneous BCG granulomatosis, the reactions of MPS in animals of the two strains to *M. tuberculosis* infection manifested by opposite time course of granuloma formation during inflammation development, the densities of granulomas 1 month after infection being the same (Fig. 1, *a*). Later, the number of granulomas decreased and stabilized in CBA mice and increased almost 2-fold by month 3 in C57Bl/6 mice (Fig. 1, *a*). Exposure of CBA mice to IUH led to a delay in the increase in the number of granulomas, while by month 3 this parameter returned to values observed in controls during the same period. Activation of granuloma formation in group 2 C57Bl/6 mice was realized 1 month earlier than in CBA mice, after which the process stabilized and the count of granulomas in the liver did not surpass that in mice of the same strain one month postinfection (Fig. 1, *a*).

During all periods of the study under the studied experimental conditions (injection of the same doses of mycobacteria incapable of active multiplication) the granulomas were larger in C57Bl/6 mice, which could indicate either higher phagocytic activity and hence, higher chemoattractant potential formed by their resident macrophages and granuloma cells, or higher potentialities of the bone marrow producing macrophage precursors, despite the fact that the production of glucocorticoid hormones by their adrenals was much higher (3.5 times) than in CBA mice normally and in response to a stimulus equally strong for animals of both groups [7]. However, these functional peculiarities of the adrenals in C57Bl/6 mice were not realized in the studied situation, which could be regarded as stress, because of appearance of the so-called bone marrow "locking" effect for monocytes. Comparison of the dynamics of granuloma size and number during the same periods in mice of the two strains suggested that the number of granulomas in the liver decreased because of rapid migration of cells from some granulomas, because no cell death (a possible mechanism of reduction of the number and size of granulomas) was observed in them. This is particularly obvious, if we compare the changes in the numbers and diameters of granulomas in CBA mice not exposed to IUH, in which the numbers of granulomas by months 2 and 3 after infection decreased more than 3-fold in comparison with the parameter one month after infection (Fig. 1, *a*). The diameters of granulomas remained the same, and the variability of this parameter was negligible, indicating that there were no transitional (by size) granulomas resulting from their gradual shrinkage. Quantitative evaluation of cell composition of gra-

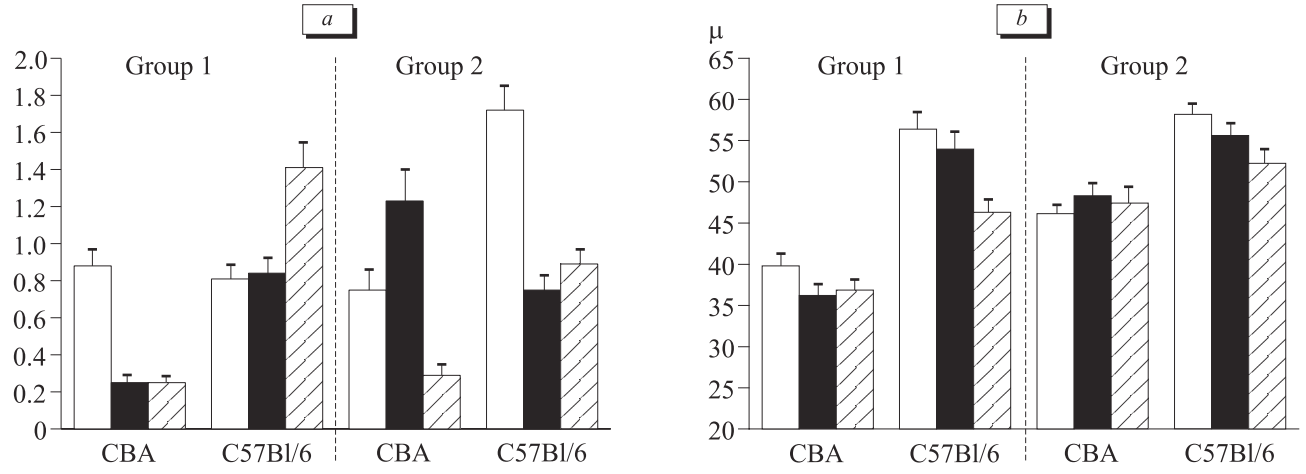

Fig. 1. Time course of granuloma formation in the livers of male CBA and C57Bl/6 mice after IUH and infection by BCG vaccine. *a*) numerical density of BCG granulomas; *b*) diameter of BCG granulomas. Light bars: 1 month; dark bars: 2 months; cross-hatched bars: 3 months after infection.

TABLE 1. Cell Composition (%) of BCG Granulomas in the Liver of Male CBA and C57Bl/6 Mice with a History of IUH (*M*±*m*)

Time after infection, month	Cell composition of granulomas	Group			
		1		2	
		CBA	C57Bl/6	CBA	C57Bl/6
1	Monocytes/macrophages	16.56±1.01	22.69±0.99^{o}	24.04±1.18^{+}	25.78±1.00
	Epithelioid cells	79.10±1.10	74.30±1.08^{o}	71.40±1.31^{+}	65.25±1.20^{+o}
2	Monocytes/macrophages	19.53±1.48	23.37±1.23	23.27±1.09^{+}	20.58±1.31*
	Epithelioid cells	76.93±1.51	73.68±1.23	74.78±1.94	72.98±1.22*
3	Monocytes/macrophages	20.12±1.17	21.18±1.01	24.00±1.44	24.31±0.95*
	Epithelioid cells	76.87±1.36	74.52±1.15	72.51±1.65	70.46±1.24

Note. All granuloma cells are taken for 100%. $p<0.05$ compared to: *previous period, $^{+}$control, ^{o}CBA.

nulomas showed that epithelioid cells predominated in mouse of both strains in all experiments during all periods of BCG granulomatosis development (Table 1); normally these cells are absent in mice. This parameter was the same in animals of both strains, exposed or not to IUH (Table 1).

Monocytes and macrophages ranked second, their number remaining virtually constant. These data also suggest rapid migration of cells from certain granulomas under condition of effective elimination of *M. tuberculosis* persisting in them by macrophages constituting these granulomas.

Hence, the number of granulomas in the liver of mice with spontaneous BCG granulomatosis gradually decreased after infection, irrespective of animal appurtenance to one of the opposite strains. In some granulomas this process is rapid presumably after elimination of *M. tuberculosis* persisting in the vacuolar system of their phagocytes and the resultant reduction of the chemoattractant gradient, which allows migration of cells from the granuloma. The appurtenance to one of the opposite mouse strains and a history of IUH determine the dynamics of the numbers of granulomas in the organ and less so the size of granulomas. Epithelioid cells predominated in BCG granulomas irrespective of the experimental conditions during all periods of observation, their percentage being in fact constant.

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