Experimental Cytomorphological Studies of the Reaction of Mononuclear Phagocyte System in Granulomatosis of Mixed (Silicotic and Tuberculous) Etiology

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Silicon dioxide in combination with *Mycobacterium tuberculosis* in BCG vaccine is characterized by a significantly higher granuloma-inducing activity than BCG or silicon dioxide alone. Cell "dissociation" from granulomas is not characteristic of granulomas induced by silicon dioxide or its combination with BCG (in contrast to BCG-induced granulomas). A steady increase in the counts and size, particularly on days 120-180, mainly at the expense of fibroblast accumulation and subtotal fibrosis, are intrinsic to these granulomas. Monocyte retention in the bone marrow is characteristic starting from day 56 until day 180 after injection of both granulomatous factors alone or in combination, particularly so in BCG granulomatosis.

Key Words: *granulomatosis; silicotuberculosis; mononuclear phagocyte system; liver*

Granulomas consisting mainly from phagocytic cells and their derivatives are morphological manifestations of tuberculosis and silicosis [3,4]. Exogenous BCG *Mycobacterium tuberculosis* and silicon dioxide, irrespective of the order of their injection, persist in the macrophage vacuolar system and hence can mutually aggravate the pathological process at all stages of its development [3,5,7,9], which was confirmed in clinical studies [11]. On the other hand, clinical studies do not provide the possibility of studying the processes in the main compartments of the mononuclear phagocyte system and specific features of the pathogenesis aggravating these diseases and their complications.

We studied the reaction of the mononuclear phagocyte system (central and peripheral compartments) under conditions of combined exposure to granulomatous factors, BCG *Mycobacterium tuberculosis* and silicon dioxide (SiO_2).

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MATERIALS AND METHODS

The study was carried out on 2-month-old male CBA mice (n=210; 20-22 g) from Breeding Center of Institute of Cytology and Genetics. The animals were divided into 4 groups, 3 groups of 60 per group and group 4 consisted of 30 animals, 10 mice per term of experiment. In group 1 mice, BCG granulomatosis was induced by intraperitoneal injection of 0.5 mg BCG vaccine (Allergen) in 0.2 ml 0.9% NaCl. In group 2, granulomatosis was induced by a single intravenous injection (into the caudal vein) of SiO_2 particles (S-563, 1-5 μ, Sigma) in a dose of 100 μg/kg in 0.5 ml sterile 0.9% NaCl solution. More than 90% SiO_2 particles used for injection were 0.9-1.5 μ in size. Group 3 mice were intravenously injected with SiO_2 suspension in the same dose as in group 2 and after 10 days intraperitoneally injected with 0.5 mg BCG vaccine in the same dose as in group 1. Group 4 mice (controls) were intravenously injected with 0.5 ml sterile 0.9% NaCl. The liver was examined, because it is the organ in which the most representative compartment of the mononuclear phagocyte system is located [4]. Liver specimens were collected on days 3, 10, 28, 56, 120,

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180 after injection of BCG vaccine (in groups 1 and 3) and SiO_2 suspension (in group 2), fixed in 10% neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin. Histological sections were stained with Meyer's hematoxylin and eosin [12]. Numerical density (N_{ai}) of granulomas in the test area (1600 μ^2) was evaluated and their diameters were measured [4]. The bone marrow and peripheral blood were collected in all mice for cytological studies; the smears were stained after Romanowskii–Giemsa. Blood and bone marrow monocytes were counted [1]. The significance of differences between the means was estimated by Student's *t* test. The differences were considered significant at $p<0.05$.

RESULTS

Histological study of liver specimens from animals of 3 groups revealed hepatocytes in a state of vacuolar and balloon degeneration, necrosis, and epithelioid cell granulomas with macrophage, monocyte, and lymphocyte apoptosis. No foreign bodies were detected in the granulomas of animals in groups 2 and 3. This was presumably due to small size of SiO_2 particles [5]. Silicon dioxide injected in combination with BCG and alone is characterized by higher granulomatous activity (Fig. 1), this activity manifested later than after injection of BCG alone. No "dissociation" of cells from granulomas (due to spontaneous elimination of the granulomogenic factor, BCG) was observed in these cases [4], due to SiO_2 persistence in the macrophage vacuolar system. This was seen from the increase in the concentration and size of granulomas on days 120 and 180 of the experiment in the livers of mice in groups 2 and 3, whereas in group 1 mice the granulomas became smaller and less numerous (Figs. 1 and 2). Presumably (judging from enlargement of granulomas), SiO_2 is characterized by higher phlogogenic potential than the BCG vaccine mycobacteria. However, it remains unclear, why the number of granulomas was still increasing (Fig. 1) in the livers of mice in groups 2 and 3 on days 120 and 180, while in group 1 the number of granulomas decreased 4-fold, presumably due to elimination of mycobacteria. Presumably, the injected SiO_2 dose was sufficient for its capture by resident macrophages and macrophages, which differentiated into organ-specific ones from monocytes newly recruited from the bone marrow. These latter cells then "acquired" the status of resident macrophages and granuloma-forming centers, which seemed to take some time. Changes in the quantity and size of granulomas suggest that animals injected with SiO_2 had the highest concentration of macrophageal cells. However, it is true only for granulomas on days 3-28. On days 56-180, the sizes of granulomas in groups 2 and 3 were determined by fibrous connective tissue.

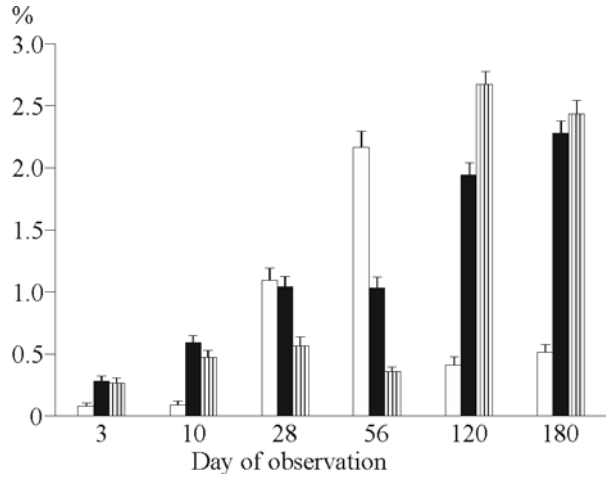

Fig. 1. Numerical density (N_{ai}) of granulomas in the livers of male CBA mice with chronic granulomatous inflammation of mixed (silicotic and tuberculous) etiology. Here and in Fig. 2: light bars: BCG granulomatosis; dark bars: SiO_2 granulomatosis; vertically hatched bars: BCG+SiO_2 granulomatosis.

Under conditions of a generalized process, the granuloma monocytes and macrophages together with epithelioid cells can be regarded, to a certain measure, as a provisional and the largest peripheral compartment of the mononuclear phagocyte system. The epithelioid-cell "cores" of granulomas started to form from day 3 after injection of granulomogenic factors; this process was more active in group 3. Later their percentage in the granulomas changed little in animals of all groups (Table 1). The percentage of monocytes/macrophages in granulomas decreased as the counts of epithelioid cells increased, but not in the proportions which would allow us to explain these processes by their differentiation into epithelioid cells [4]. The percent of cells

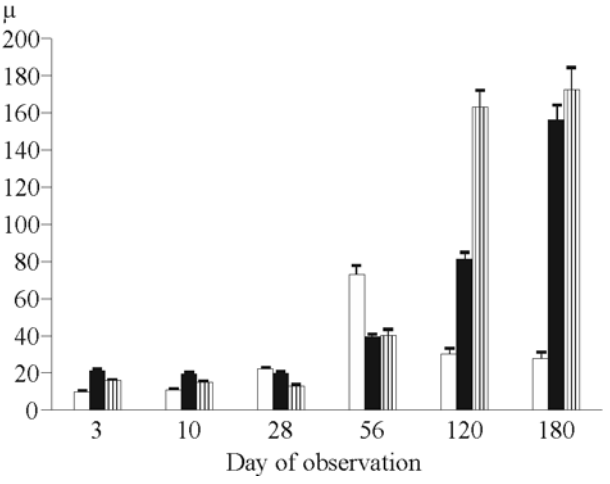

Fig. 2. Diameters of granulomas in the livers of male CBA mice with chronic granulomatous inflammation of mixed (silicotic and tuberculous) etiology.

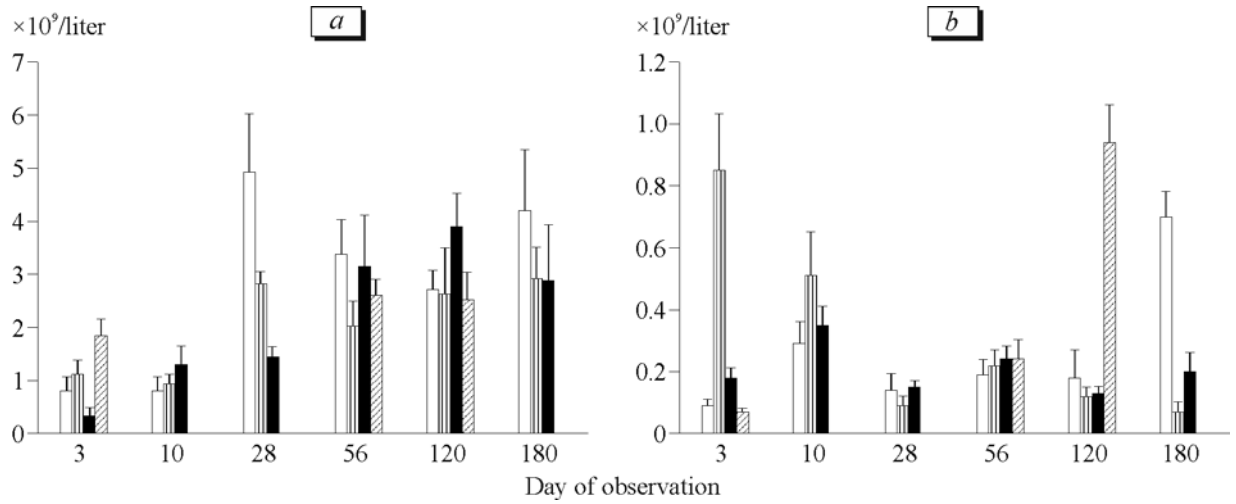

Fig. 3. Absolute counts of monocytes in chronic granulomatous inflammation of mixed (silicotic and tuberculous) etiology in male CBA mice. *a*) bone marrow; *b*) peripheral blood. Light bars: group 1; vertically hatched bars: group 2; dark bars: group 3; obliquely hatched bars: group 4.

of both types in groups 1 and 2 was about 90%, in group 3 it was 10% less on days 120 and 180 (Table 1). However, the percent of these cells in granulomas in later periods of observation was significantly lower than during earlier periods because of massive fibrosis of granulomas, which was shown in other studies as well [3]. It is possible that this was caused by the toxic effect of SiO_2 on macrophages [10].

Changes in the counts of monocytes in the peripheral blood and bone marrow were in line with the dynamics of granuloma formation (Fig. 3, *a*, *b*). On days 3 and 10 of the experiment, capture of granulomogenic factors by macrophage was associated with monocyte release from the bone marrow to the peripheral blood. Monocyte elimination from the blood was more rapid in animals injected with BCG. Later (days 28, 56, 120) the reduction of blood monocyte counts corresponded to the dynamics of reduction of the number of granulomas in the liver. On day 180, the reduction in the number of granulomas in group

TABLE 1. Content of Epithelioid Cells and Macrophages (%) in Liver Granulomas of CBA Mice with Chronic Granulomatosis of Mixed (Silicotic and Tuberculous) Etiology (*M*±*m*)

Cell type	Day postinfection	Experimental conditions		
		group 1 (BCG granulomatosis)	group 2 (SiO_2 granulomatosis)	group 3 (SiO_2+BCG granulomatosis)
Epithelioid	3	47.54±9.77	40.54±8.14	77.66±7.28*+
	10	50.69±9.70	44.65±5.78	72.35±5.91*+
	28	59.10±11.05	57.06±5.26	72.29±6.45*+
	56	75.61±6.78	65.93±5.56	68.71±5.96*+
	120	76.85±6.14	66.45±5.54	70.02±5.69
	180	75.79±5.33	72.92±7.47	65.86±6.08
Monocytes/ macrophages	3	38.88±9.18	42.95±7.67	13.67±5.49*+
	10	37.34±8.81	45.83±5.48	17.51±4.57*+
	28	33.71±8.87	35.85±5.90	18.25±4.83*+
	56	15.17±6.07	27.42±5.89*	17.99±4.61*+
	120	13.67±4.46	21.72±5.86*	10.37±3.27
	180	12.61±4.54	16.43±5.79	8.70±3.34

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

1, which started on day 120, was characterized by an increase of the blood monocyte count (presumably partially "dissociated" from granulomas) and of the bone marrow monocytes, not "needed" in the granulomas (Table 1, Fig. 3, *a*, *b*). On the other hand, the increase in the number of granulomas in the liver of mice in groups 2 and 3 was paralleled by monocyte elimination from the blood (Figs. 1, 3, *b*). At the same time, cells counts in fibrosed granulomas on days 56-180 (presumably because of hypoxia and apoptosis, morphological signs of which were observed in the liver granulomas in groups 2 and 3) were significantly lower than during the previous 3 periods. Presumably, phagocytosis of SiO_2 particles was also involved in this phenomenon, leading to toxic injury of macrophages by blocking their surface receptors [10], to increase of active oxygen forms production [4,14], and to apoptosis induction [8,13,14]. These events were paralleled by the release of proinflammatory mediators and chemoattractants, causing migration of monocytes to the focus of inflammation and the formation of numerous granulomas in the liver in groups 2 (silicosis) and 3 (silicotuberculosis).

Starting from day 28, obvious signs of delayed release of monocytes from the bone marrow were seen in group 1 mice and later in mice of the rest groups, this process being more pronounced in mice injected with BCG (Fig. 3, *a*). This was presumably caused by lesser summary counts of macrophages/monocytes and epithelioid cells in granulomas at the latest stages of the experiment in animals injected with SiO_2 (Table 1).

Hence, granulomatosis induced by BCG, SiO_2, or their combination is characterized by high elimination of monocytes from the bone marrow, their incorporation in granulomas during the early periods after injection of granulomogenic factors, and their subsequent retention in the bone marrow on days 28-180. This is presumably explained by the stimulatory effect of SiO_2 injection on the production of glucocorticoid hormones [12].

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