Analysis of Fibrotic Depositions in Granulomas in Chronic Silicotuberculosis in Mice

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The stimulating effect of silicon dioxide on fibroblast proliferation in granulomas of male CBA mice surpasses that of BCG vaccine mycobacteria. The number of fibroblasts in granulomas after combined treatment with BCG and SiO_2 increased by more than 3 times compared to individual treatment with BCG and by 2 times compared to treatment with SiO_2 alone. In silicosis and silicotuberculosis, collagen and argyrophilic fibers in granulomas during the period from 4 to 6 months after administration of granulomogenic factors occupied more than 90% granuloma volume, which 3-fold surpassed the corresponding parameter in mice infected with BCG vaccine alone. In silicosis, pronounced fibrosis was determined by relatively high proliferative and synthetic activities of fibroblasts, while in silicotuberculosis it was achieved due to significantly higher proliferative activity against the background of lower synthetic activity.

Key Words: *granulomatosis; silicotuberculosis; fibroblasts; fibrotic depositions; liver*

The term granulomatoses unites about 70 nosologies; morphological manifestations of these pathologies are granulomas consisting primarily of mononuclear phagocyte system cells and fibroblasts [2]. Exposure to granulomogenic factors, *M. tuberculosis* and silicon dioxide (SiO_2) particles, often induces the development of silicotuberculosis [12]. Apart from destructive processes in the granulomas and surrounding tissues, this pathology is characterized by extensive fibrotic complications. The mechanisms underlying the development of these complications are poorly studied. Morphological manifestations of silicotuberculosis in the lungs are best studies [8,12]. At the same time, damage to the liver in tuberculosis and after inhalation of SiO_2 nanoparticles are described [2-7]. Moreover, the liver is the largest compartment of the mononuclear phagocyte system and the target organ for glucocorticoid hormones regulating proliferative processes in the bone marrow, and therefore it is an appropriate organ for modeling granulomatoses and studies of changes in developing in granulomas, *e.g*. fibrotic complications in silicotuberculosis [10,12].

Here we studied fibrotic complications in liver granulomas in mice with chronic silicotuberculosis.

MATERIALS AND METHODS

The study was performed on 180 male CBA mice weighing 20-22 g obtained from Nursery of Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences. The animals were divided into 3 groups, 60 mice per group (10 mice per point). In group 1 animals, BCG granulomatosis was modeled by intraperitoneal injection of 0.5 mg BCG vaccine (Allergen) in 0.2 ml 0.9% NaCl aqueous solution. Spontaneous necroses extremely rare develop in granulomas induced by BCG vaccine mycobacteria. This provides the possibility of studying changes in cellular composition of granulomas not related to destructive processes during the entire period of chronic experi-

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ment [2]. In group 2 mice, granulomatosis was induced by single injection of SiO_2 suspension (S-563 grade, 1-5-μ particles, Sigma) in a dose of 100 μg/kg body weight in 0.5 ml 0.9% NaCl sterile aqueous solution into the caudal vein. It was found that more than 90% particles in the suspension had a diameter of 0.9-1.5 μ. Group 3 mice received SiO_2 suspension (intravenous) in the same dose as group 2 mice and after 10 days they received 0.5 ml BCG vaccine (intraperitoneal) in the same dose as group 1 mice. Liver samples were taken on days 3, 10, 28, 56, 120, and 180 after administration of BCG vaccine (groups 1 and 3) and SiO_2 suspension (group 2). The samples were fixed in 10% neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin. Histological sections were stained with Mayer's hematoxylin and eosin and after van Gieson. For detection of argyrophilic fibers, the sections were stained after Sweet and Gordon [1]. The sections were examined under an Axiostar light microscope (Carl Zeiss). Volume densities of collagen and argyrophilic fibers in granulomas and their sum in the granuloma were determined; fibroplastic activity of fibroblasts was evaluated as the ratio of volume densities of all collagen and all argyrophilic fibers in the granuloma to the number of fibroblasts in it and expressed in arbitrary units. The relative content of fibroblast in the granuloma was calculated (% of all cells in the granuloma) [2]. The significance of differences between the means was evaluated using Student *t* test, the differences were significant at $p<0.05$.

RESULTS

Microscopic examination of the mouse liver revealed granulomas starting from day 3 after administration of the granulomogenic factors; their number and size and visual cytological signs of apoptosis in granuloma cells increased throughout the experiment, especially in groups 2 and 3. On days 120 and 180, van Gieson staining revealed bulbous sclerosis of granulomas typical of silicotic inflammation in group 3 mice (Fig. 1).

As soon as on day 3 of the experiment, more rapid increase in the number of fibroblasts in liver granulomas was observed in groups 1 and 3 (Fig. 2). This parameter gradually increased from the 28th to the 180th day of observation. In mice of groups 2 and 3 receiving SiO_2, the number of fibroblasts most drastically increased starting from day 120; this increase was most pronounced in mice with silicotuberculosis (Fig. 2).

In all groups, synthetic activity of fibroblasts (judging from accumulation of argyrophilic fibers) was maximum starting from day 28 after administration of the granulomogenic factors (Table 1). This process was less active in mice with silicotuberculosis

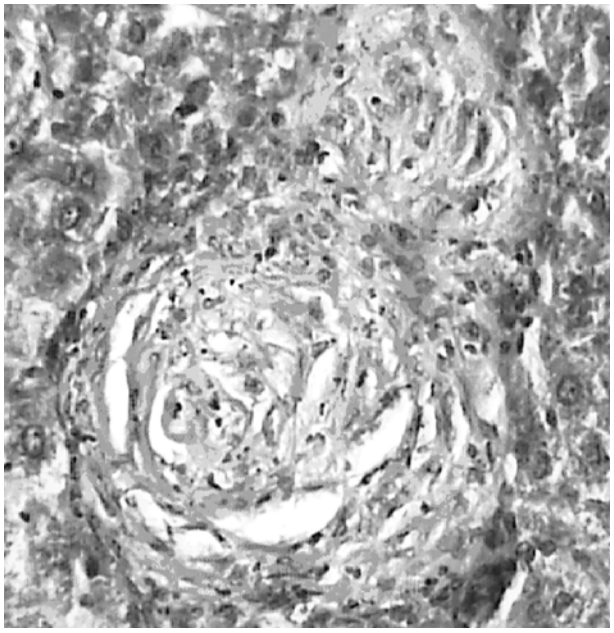

Fig. 1. Bulbous fibrosis of liver granuloma in male CBA mice with chronic silicotuberculosis on day 180. van Gieson staining, ×600.

and most active in animals of groups 1 and 2 (Table 1). During the next two periods, the content of argyrophilic fibers in granulomas of mice infected with BCG vaccine (group 1) was considerably lower, while the content of collagen fibers increased starting from day 56 and was stably high until the end of the experiment. In granulomas of groups 2 and 3 mice (both groups received SiO_2), the concentration of argyrophilic fibers continued to increase and 3-4-fold surpassed the corresponding parameter in group 1 mice. The concentration of collagen fibers in granulomas of groups 2 and 3 mice also significantly increased (Table 1) and by day 180 of the experiment 3-fold surpassed the corresponding parameter in group 1 mice infected with

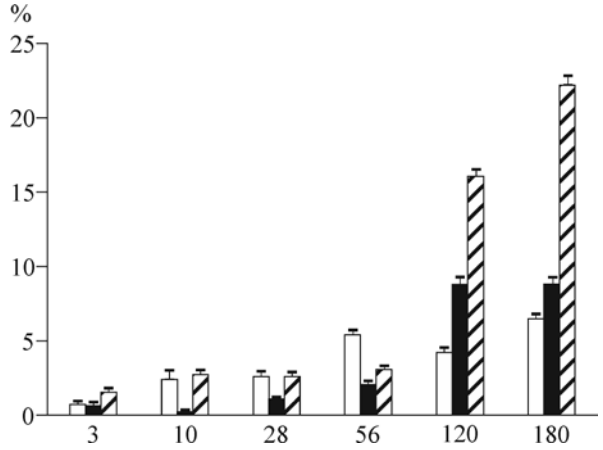

Fig. 2. Fibroblast content in liver granuloma in male CBA mice with chronic silicotuberculosis. Here and on Fig. 3: Open bars: BCG; dark bars: SiO_2; hatched bars: BCG+SiO_2.

TABLE 1. Volume Density (V_v) of Collagen and Argyrophilic Fibers in Liver Granulomas in Male CBA Mice with Chronic Silicotuberculosis (*M*±*m*)

Type of fibers	Day after infection	Experimental conditions		
		group 1	group 2	group 3
Argyrophilic fibers, %	3	1.08±0.63	2.55±0.75*	1.44±0.58
	10	1.04±0.47	1.84±0.56	2.38±0.64*
	28	15.39±1.17	18.30±1.32	6.84±0.94*+
	56	39.29±0.86	36.42±1.23	16.90±1.41*+
	120	8.74±0.75	26.11±0.78*	31.23±0.88*+
	180	7.86±0.78	22.0±0.82*	26.48±0.99*+
Collagen fibers, %	3	1.38±0.55	4.16±0.75*	3.17±0.57*
	10	2.63±0.58	4.06±0.58*	7.94±0.73*+
	28	3.47±0.65	22.08±0.97*	17.31±0.74*+
	56	22.23±1.20	19.56±0.83*	24.61±0.78+
	120	23.08±0.72	47.35±0.73*	68.92±0.81*+
	180	22.78±0.80	70.64±0.68*	70.88±0.69*
Total volume of argyrophilic and collagen fibers, %	3	2.46±0.41	6.71±0.47*	5.60±0.47*
	10	3.67±0.37	5.90±0.46*	6.44±0.43*+
	28	18.86±0.66	40.38±0.75*	28.92±0.67*+
	56	61.52±0.43	55.98±0.72	36.46±1.13*+
	120	31.82±0.51	73.46±0.56*	78.58±0.57*+
	180	30.64±0.55	92.64±0.53*	97.12±0.60*+

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

BCG alone. These findings suggest that SiO_2 stimulates proliferation of fibroblasts in granulomas; this effect was considerably more pronounced after combined treatment with SiO_2 and *M. tuberculosis*. Mycobacteria administered alone (without SiO_2, group 1) at later terms of the experiment less effectively stimulated fibroblast proliferation than SiO_2, probably due to their elimination by macrophages (Fig. 2). These

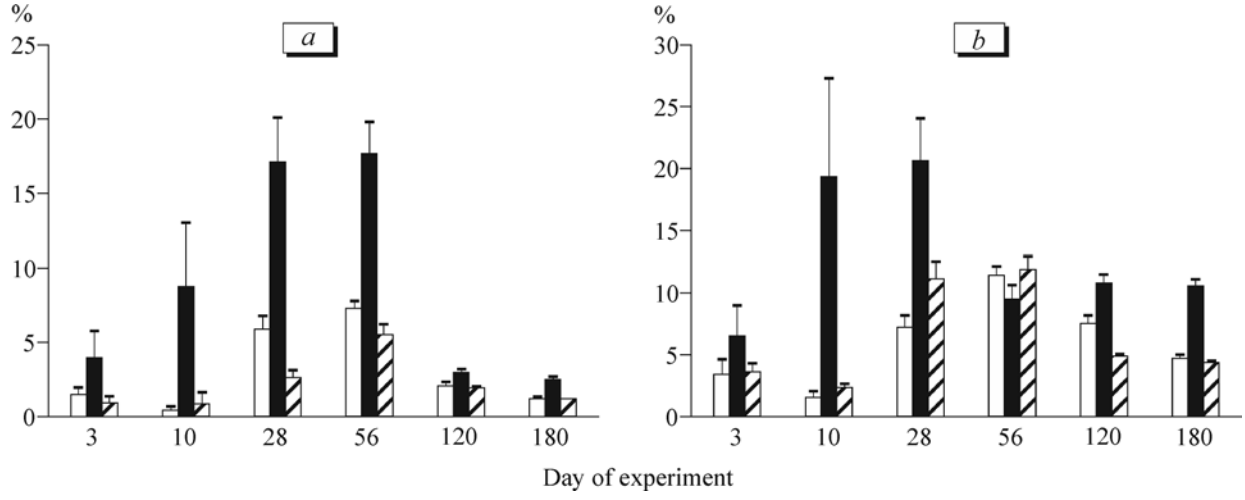

Fig. 3. Fibroplastic activity of fibroplasts in liver granuloma in male CBA mice with chronic silicotuberculosis. *a*) argyrophilic fibers; *b*) collagen fibers.

findings also suggest that SiO_2 (in contrast to *M. tuberculosis*), being a lysosomotropic non-biodegradable granulomogenic factor determining the increase in the number and size of granulomas, more effectively stimulates the synthesis and secretion of argyrophilic fibers (Fig. 3, *a*) in group 2 mice, especially during the period from the 10th to 56th day postinjection. At the same time, secretory activity of fibroblasts after combined treatment with both granulomogenic factors was significantly lower and close to the level induced by *M. tuberculosis* alone (Fig. 3, *a*). On the whole, by day 120 and 180 the volume of precursors of argyrophilic (Fig. 3, *a*) and collagen (Fig. 3, *b*) fibers secreted by granuloma fibroblasts in mice of groups 2 and 3 continued to increase and led to almost total fibrosis of granulomas (Table 1, Fig. 1). Despite sharply reduced productive capacity of fibroblasts at two late stages of the experiment (Fig. 3, *a*, *b*), the increase in the volume of the connective tissue in granulomas was achieved at the expense of increased number of fibroblasts and, probably, low rate of degradation of collagen and argyrophilic fibers synthesized during the previous periods. This was most pronounced in case of combined induction of granulomas in the liver of group 3 animals by both granulomogenic factors. Devastation of granulomas on days 120 and 180 in mice of groups 2 and 3 related to SiO_2-induced apoptotic death of phagocytes and lymphocytes (Fig. 1) probably determines impossibility of degradation of collagen and argyrophilic fibers in granulomas [11,13-15]. In light of this, possible sources of factors supporting proliferative, synthetic, and secretory activity of fibroblasts during these two periods remain unknown.

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