GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Formation of SiO_2-Induced Granulomas in Mice of Various Strains

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 1, pp. 20-24, January, 2000
Original article submitted May 28, 1999

Peculiarities of SiO_2-induced granulomas in the liver and subcutaneous fat were studied in C57Bl/6, CBA/Lac, BALB/c, C57BR, and C3H/F mice. Infiltrative and fibrogenic responses were interrelated and strain-specific primarily due to various intensities of myelopoiesis.

Key Words: *mouse strains; granulomas; macrophages; SiO_2*

Granulomatous process underlies many inflammatory diseases and is directed to sequestration and further degradation of foreign materials in mononuclear infiltration focus. The most pronounced sequestration is observed in granulomas undergoing fibrous transformation. Relatively inert and hardly degraded particles, silicon dioxide (SiO_2), carrageenan, asbestos, polyacrylamide, and suture material exhibit extremely low immunogenicity and induce the formation of nonimmune fibrous granulomas consisting of macrophages (MP) and fibroblasts but containing little T and B cells. The latter are typical of immune granulomas formed during delayed-type hypersensitivity (DTH).

There are considerable interstrain difference in the production of inflammatory mediators and cytokines by MP involved in granuloma formation. Therefore, mice of various strains probably have genetically determined differences in granuloma formation. The differences were demonstrated for immune granulomas but strain-specific peculiarities of nonimmune granulomas are little studied, while convenient experimental models are very limited.

Here we elaborated a new model of nonimmune granulomatous response, confirmed interstrain differences, revealed the mechanisms associated with strain-specific reactivity of MP, and evaluated the interrelation between the intensity of granulomatous reactions and the degree of fibrous changes. Taking into account high prevalence of silicoses, in our experiments granumatous process was induced by intravenous and subcutaneous injections of SiO_2 particles. Dissemination of these particles often causes extrapulmonary manifestations of lung silicoses (including liver silicosis). SiO_2 was applied in various concentrations, because the size of infiltration foci in lung silicoses depends on the dose of SiO_2.

MATERIALS AND METHODS

Granulomas in C57Bl/6, CBA/Lac, BALB/c, C57BR, and C3H/F mice were induced by intravenous or subcutaneous injections of Silica S-563 (1-5 μ) in physiological saline; control animals received isotonic NaCl. For evaluation of the number of phagocytic Kupffer cells (KC) and their clustering the animals were intravenously injected with 0.1 ml 20% colloidal carbon 1 h before killing or 1 h before SiO_2 administration, respectively. The number of KC and the formation of granulomas were analyzed morphometrically [12], the content of collagen was measured morphometrically and colorimetrically [6]. Blood leukocyte count and

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0007-4888/00/0001-14$25.00

total cellularity of the bone marrow from the femur were estimated on a Specol cell counter. The number of myeloid precursors was evaluated by the growth of granulocyte-macrophage colony-forming units (GM-CFU) in cultured bone marrow cells on a semisolid medium [14]. The data were analyzed by Student's *t* test.

RESULTS

Intravenous injection of SiO_2 caused the formation of granulomas consisting of dozens and hundreds of cells (primarily mononuclear cells) in liver parenchyma and in the lungs, spleen, and bone marrow. After injection of 100 mg/kg body weight SiO_2, the number of granulomas (Nv) in the liver parenchyma was similar in BALB/c and CBA/Lac mice, while in C57Bl/6 mice this parameter was 1.5-1.8-fold higher. One month postinjection, the volume density (Vv) of silicosis foci in C57Bl/6 mice 1.8-1.9-fold surpassed that in BALB/c and CBA/Lac mice (9.0 and 8.6% slice area, respectively). At the late stages of observations, CBA/Lac mice exhibit intermediate values of these parameters between BALB/c and C57Bl/6 mice. Interstrain differences in Vv of granulomas were primarily due to their various numbers, since granuloma volumes (V) were similar in studied mouse strains (Fig. 1). After injection of 200 mg/kg body weight SiO_2, Vv of granulomas increased primarily due to their enlargement; however, interstrain differences in this parameter were retained. Due to progressive enlargement and fusion of granulomas their mean size increased while their number decreased (Fig. 1).

After subcutaneous injection of SiO_2, interstrain differences in the size of infiltrates were similar to those observed after intravenous injection. Maximum infiltrative response was observed in C57Bl/6 mice. In BALB/c mice, the infiltrative reaction was more pronounced than in CBA/Lac mice, but these changes were insignificant except for day 3 postinjection. MP

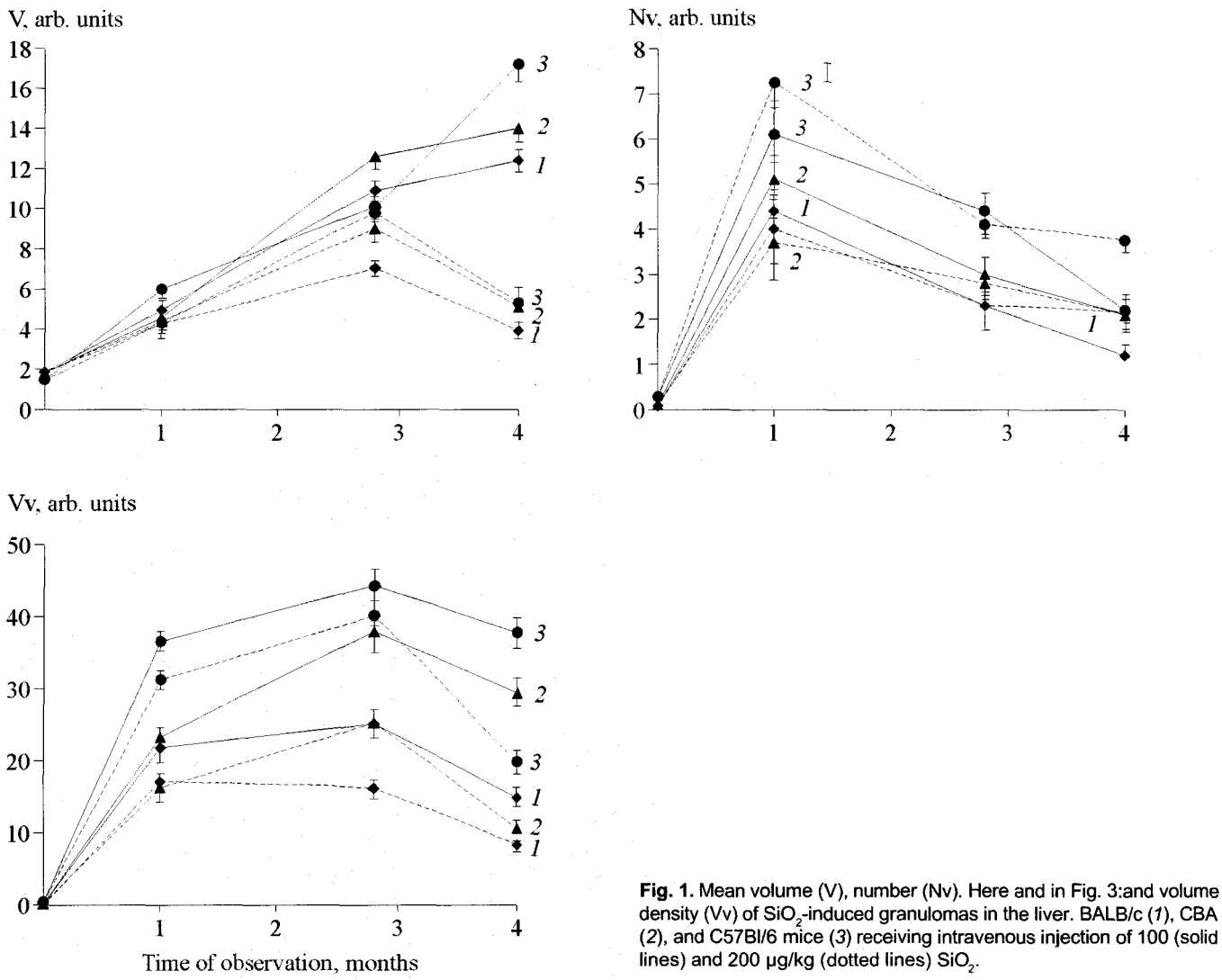

Fig. 1. Mean volume (V), number (Nv). Here and in Fig. 3:and volume density (Vv) of SiO_2-induced granulomas in the liver. BALB/c (*1*), CBA (*2*), and C57Bl/6 mice (*3*) receiving intravenous injection of 100 (solid lines) and 200 μg/kg (dotted lines) SiO_2.

reactivity in C3H/F mice estimated by their infiltrative response was minimum (Fig. 2).

On control liver slices, collagen formed delicate structures along vascular regions. Marked collagen deposits were seen in granulomas 4 weeks after intravenous injection of 100 mg/kg SiO_2 and, therefore, Vv of infiltrates strictly correlated with collagen content in the liver and interstrain differences in these parameters were similar (Fig. 3). As granulomas grow, the content of collagen increased, and interstrain differences were observed throughout the observation period. At later stages, granulomas became smaller, and Vv of collagen deposits surpassed that of granulomas due to stability of these deposits and formation of fibrous septa containing collagen. SiO_2 in a dose of 200 mg/kg body weight increased the amount of collagen, but did not change interstrain differences (Fig. 3).

The numbers of colloidal carbon-containing KC in control C57Bl/6, CBA/Lac, and BALB/c mice were 32.4±2.5, 27.2±2.4, and 32.1±2.8 cells/view field, while 12 weeks after injection of 100 mg/kg SiO_2 it increased by 56, 84, and 46%, respectively. KC localized in granulomas were not labeled with colloidal carbon and, therefore, their actual accumulation was considerably higher. The number of KC decreased and did not differ from the control 16 weeks after SiO_2 administration. After injection of 200 mg/kg body weight SiO_2 this parameter only slightly increased or even decreased probably due to accelerated recruitment and elimination of MP in large infiltrates. In parallel with accumulation of KC induced by SiO_2 (100 mg/kg) Vv of colloidal carbon in these cells increased and 12-16 weeks postinjection it 1.2-1.4-fold surpassed the control (2.6, 2.7, and 2.5% of slice area for control C57Bl/6, CBA/Lac, and BALB/c mice, respectively). Increasing the dose of SiO_2 to 200 mg/kg produced no considerable changes in colloidal carbon uptake. Interstrain differences in the number of KC and colloidal carbon uptake were insignificant in control and treated animals.

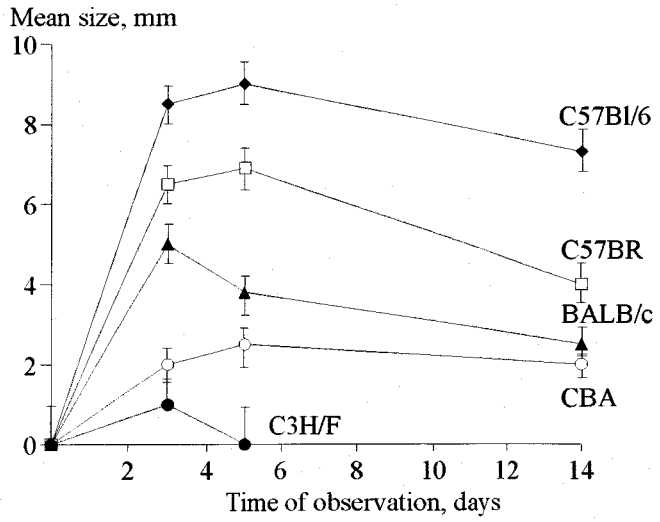

Fig. 2. Local granulomatous infiltration induced by subcutaneous injection of SiO_2 particles to mice of various strains.

Indices of myelopoiesis did not differ between control CBA/Lac and BALB/c mice and were lower than in C57Bl/6 mice (Table 1). SiO_2 considerably increased leukocyte count in the blood and total cellularity and content of GM precursors in the bone marrow. The basal content of GM precursors in C57Bl/6 mice 2-fold surpassed this parameter in CBA/Lac and BALB/c mice. After administration of SiO_2, the contents of GM precursors in C57Bl/6, CBA/Lac, and BALB/c mice increased by 3, 2.26, and 1.84 times, respectively.

Thus, SiO_2-induced nonimmune granulomatous reactions differed between various mouse strains. In C57Bl/6 mice this reaction was maximum, and the infiltrate persisted for a long time. CBA/Lac and BALB/c mice displayed similar infiltrative reactions, but the granulomatous response in BALB/c mice was less pronounced. These interstrain differences in granuloma formation were stable and practically did not depend on the localization and size of infiltrates and the type of administered substance. Our previous experiments showed that the same interstrain differences are typical of zymosan-induced nonimmune granulomas.

The differences in nonimmune granulomas observed by us are consistent with the data on strain-specific peculiarities of the formation of types Th1 and Th2 immune mononuclear infiltrates. In contrast to BALB/c mice, C57Bl/6 and CBA mice are resistant to *Leishmania major* infection due to intense granuloma formation [9]. Granulomatous infiltration in the liver induced by *Schistosoma japonicum* eggs is more pronounced in C57Bl/6 mice compared to CBA/H and BALB/c mice [3] probably due to different intensity of DTH reactions and inverse correlation between the size of granulomas and production of antibodies against shistosomal antigens. Differences in granulomatous reactions to *Mycobacterium lepraemurium* inoculation in C57Bl/6J and C3H mice were also reported [7].

Taking into account that nonimmune and immune DTH granulomas primarily contain MP [11] and are strain-specific, these differences cannot be explained only by peculiarities of T cell sensitization and antibody production. It can be assumed that interstrain differences are associated with different reactivity of the mononuclear phagocyte system.

The role of MP and their mediators in the formation of strain-specific immune granulomas was reported [15], but studies of nonimmune granulomogenesis received little attention [11]. Our findings of granulomatous reactions in mice of various strains agree with

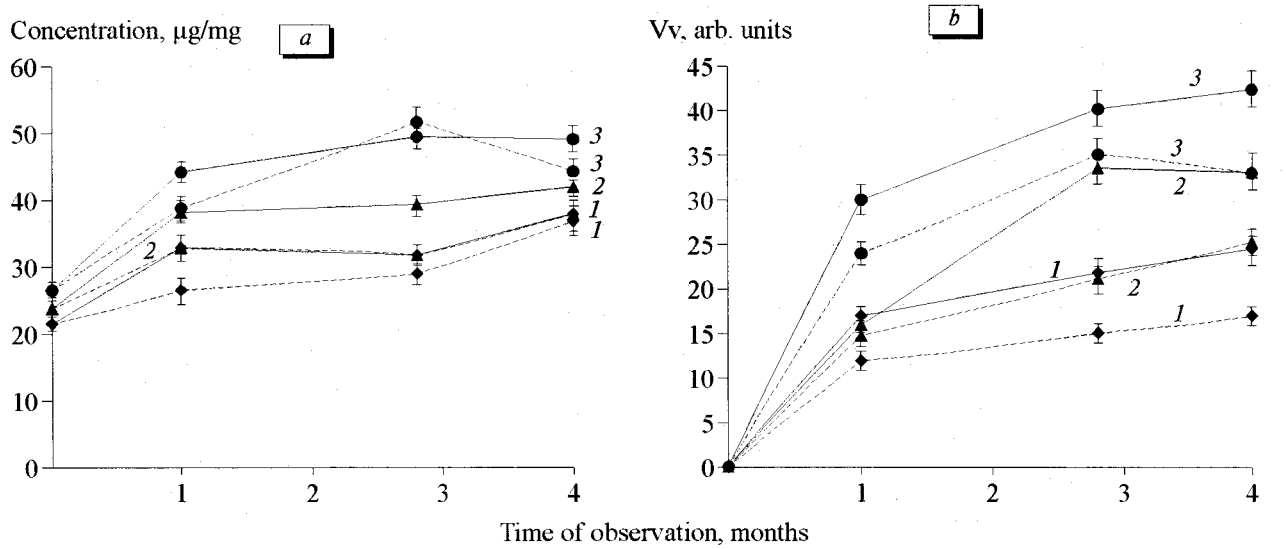

Fig. 3. Concentration (*a*) and volume density (*b*) of collagen in the liver of mice receiving intravenous injection of SiO_2.

the data on peculiarities in the production of reactive oxygen and nitrogen species, interleukins 1 and 12, and tumor necrosis factor-α [5,15,11] by MP. Since SiO_2 particles induce the production of MP mediators [8], these cells probably contribute to interstrain differences in granuloma formation.

Apart from MP mediators, the interstrain differences in the degree of infiltration can be due to differences in cluster formation and local proliferation of resident KC, dynamics of monocyte and MP recruitment from the blood, and rates of production and recruitment of monocyte precursors from myelopoiesis foci. Proliferation of resident MP contributes to the formation of infiltrates only under conditions of impaired myelopoiesis and monocytopenia [10]. In our experiments SiO_2 did not induce myelosuppression and there were no considerable differences in cell clustering. Therefore, it is unlikely that revealed interstrain differences are related to *in situ* clustering or proliferation of resident macrophages.

C. Sunderkotter *et al.* [9] showed that during immune granulomagenesis, the rate of MP maturation in C57Bl/6 mice 2-fold surpassed that in BALB/c mice, which was probably due to the production of myelocyte and granulocyte-macrophage colony-stimulating factors. Inductors of nonimmune granulomas stimulate the production of these factors, cause monocytosis, and therefore, increase the number of cells recruited into the infiltrative focus [10]. Therefore, results reported by C. Sunderkotter *et al.* [9] agree with our findings that C57Bl/6, but not CBA/Lac and BALB/c mice, intensively generate MP precursors. Strain-specific peculiarities of myelopoiesis correlate with and explain interstrain differences in the degree of granulomatous infiltration during immune or nonimmune granuloma formation.

The size of fibrous granulomas and the degree of fibrosis not always correlate [2,3]. However, in our experiments interstrain differences in the fibrogenic response and granulomatous reactions coincided, which probably reflects an interrelation between infiltration, generation of proinflammatory cytokines, and further production of antiinflammatory and profibrotic factors of the cytokine cascade, including tumor

TABLE 1. Effects of SiO_2 on Myelopoiesis in Mice of Various Strains (*M±m*)

Parameter	CBA/Lac		BALB/c		C57Bl/6	
	control	SiO_2	control	SiO_2	control	SiO_2
Leukocyte count, 10^9/liter	6.8±1.36	8.7±0.59	8.5±1.16	11.6±1.02*	12.2±1.13*+	16.5±2.45+x
Total cellularity of bone marrow, 10^6/femur	18.8±0.78	24.1±2.73	16.7±1.07	18.2±1.36	21.8±1.82+x	28.3±2.53+x
CFU-GM, 10^3/femur	2.3±0.14	5.2±0.55	2.5±0.2	4.6±0.14	4.0±0.43+x	12.0±0.6+x

Note. Significant differences between CBA and BALB/c mice (*),CBA and C57Bl/6 mice (+), and BALB/c and C57Bl/6 mice (x). All values significantly differ from the control.

necrosis factor-β, whose expression sharply increases in SiO_2-induced granulomas [13]. Moreover, expression of tumor necrosis factor-β mRNA in C57Bl/6 mice is markedly enhanced compared to BALB/c and C3H/HeJ mice [4].

Thus, SiO_2-induced granulomatous responses considerably differ in mice of various strains. Infiltrative and fibrogenic reactions induced by SiO_2 are interrelated, and the intensity of the granulomatous response is determined by strain-specific production of myeloid precursors in the bone marrow.

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