Effect of Preliminary Load of Macrophages with Silicium Dioxide on Phagocytosis of BCG Strain Micobacteria by Macrophages and Antimicrobial Activity

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We studied the effect of preliminary loading of peritoneal macrophages with silicium dioxide on *in vitro* viability, phagocytosis of BCG strain mycobacteria, and the capability to destroy the phagocytosed mycobacterium tuberculosis. It was shown that preliminary loading of macrophages with silicium dioxide did not reduce their viability and stimulated phagocytosis of BCG strain mycobacteria, but reduced their antibacterial activity.

Key Words: *macrophages; silicium dioxide; BCG mycobacteria*

Tuberculosis infection can complicate various types of pneumoconioses, but it is most frequently caused by inhaling quartz-containing dust (tuberculosis is diagnosed in more that 35% miners [13]). Among employers engaged in plants with occupational silicosis hazard, silicosis was diagnosed in 22.7%, tuberculosis in 65.4%, and silicotuberculosis in 11.9% workers [10]. However, pathogenetic regularities determining high incidence of tuberculous infection against the background of silicosis inflammation and the severity of complications are poorly understood. In the development of silicotubercuosis, the leading role is played by phagocytizing cells, because they are the key effectors in both silicosis and tuberculosis [3,4]. There are data that capture of silicium dioxide (SiO_2) by macrophages (MP) can modify their metabolic activity [7,9].

The aim of the study was to analyze the effect of SiO_2 particles on viability, phagocytosis, and potential antibacterial activity against strain BCG mycobacteria.

MATERIALS AND METHODS

In vitro experiments were carried out on MP of peritoneal exudate from BALB/c mice (2-month-old males weighing 21-22 g, obtained from Nursery of Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk). Peritoneal cells were isolated after animal sacrifice via routine cervical dislocation (under ether anesthesia) [1]. The mice received an intraperitoneal injection of SiO_2 particles (S-563 mark, 500 μg/0.3 ml) with a diameter of 1-5 μ (>90% particles with a diameter of 0.9-1.5 μ) in 0.9% NaCl aqueous solution. After 24 h, the peritoneal transudate cells were explanted into culture and cultured in glass flasks (37°C) on coverslips (10^6 cells in 2 ml medium 199 containing 10% FCS) for 24 h. For evaluation of SiO_2 phagocytosis by MP, the total number of MP and the number of MP containing SiO_2 in the cytoplasm were determined. Parameters of phagocytosis activity were evaluated by the percent (in %) of phagocytitizing MP (phagocytosis index, PI) and by the mean number of SiO_2 particles or BCG strain mycobacteria captured by one phagocytizing MP (phagocytosis number). PI was calculated by the formula: $PI=(N_1/N_2)\times100\%$, where N_1 is the number of MP phagocytozing SiO_2 particles and N_2 is the total number of MP. The cultures containing MP phagocytizing SiO_2 were assigned to "SiO_2" group. The cultures containing MP phagocytizing SiO_2 to which BCG strain mycobacteria were added after 24-h culturing (the medium of peritoneal transudate was

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replaced with similar medium containing BCG mycobacteria in a concentration of 0.1 mg/ml) and cultured for additional 5 h were denoted as "SiO_2+BCG" cultures. The cultures containing peritoneal cells from intact mice to which BCG strain mycobacteria were added after 24-h culturing (the medium of peritoneal transudate used for cell explantation into culture was replaced with medium of the same composition containing BCG mycobacteria in a concentration of 0.1 mg/ml) and cultured for additional 5 h were denoted as "BCG" cultures. Culturing in all groups of cultures was stopped 29 h after explantation of cells into cultures.

Viability of MP in culture was evaluated by the method of vital trypan blue staining [1]. BCG mycobacteria in MP were visualized by fluorescent microscopy after acryidine orange staining (10 μg/ml for 15 min at 37°C) [11]. The study was performed using an AxioImager Z1 microscope (Zeiss). In the fluorescence mode, living mycobacteria had green color and damaged and dead mycobacteria had orange-yellow and red color [11,12]. The cells were photographed using an AxioImager Z1 microscope (Zeiss) and an AxioCamHr camera (×100 objective magnification) in two modes for each field of view: phase contrast for visualization of SiO_2 particles in MP and fluorescence for visualization of BCG mycobacteria and evaluation of their viability. The production of reactive oxygen species in MP was indirectly evaluated by the reaction nitroblue tetrazolium reduction (NBT test) [8] followed by computer morphometry using Video Test-Morpho 3.2 software [5]. Digital cell images were binarized by the color of formazan formed in MP, while the total area of all binary images of formazan granules was taken as an arbitrary parameter of their total content in MP and a measure of production of reactive oxygen species (ROS) in MP. The significance of differences of the studied parameters between experimental groups of cultures was evaluated using nonparametric White test. Correlation analysis was performed using Spearman test The data were processed using Statistica software and presented as $M \pm m$, the differences were significant at $p<0.05$.

RESULTS

Phagocytosis of SiO_2 particles (after 24-h culturing) or BCG (5 h after addition of mycobacteria of the BCG strain) by MP did not affect MP viability in culture of peritoneal cells (Fig. 1). This attests to low cytotoxicity of SiO_2 in the applied concentration against MP in the used concentration. Micobacteria of BCG strain 5 h after their addition to cultures did not affect viability of MP, while 50% MP contained more than 10 (up to 40-50) engulfed mycobacteria. Insignificant decrease in MP population viability was noted in cul-

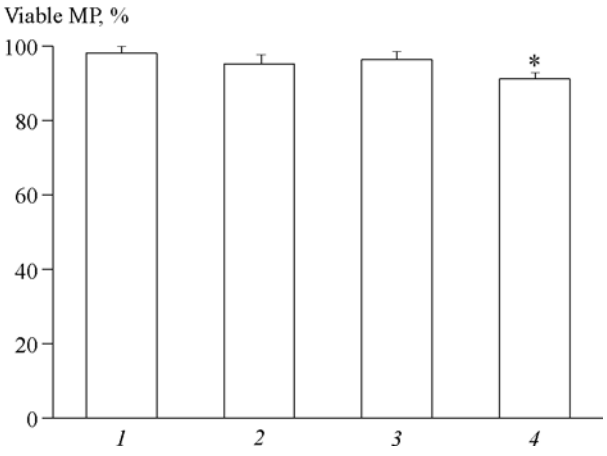

Fig. 1. Effect of SiO_2 and BCG on MP viability *in vitro*. *1*) control, *2*) SiO_2, *3*) BCG, 4) SiO_2+BCG. *$p<0.05$ compared to the control.

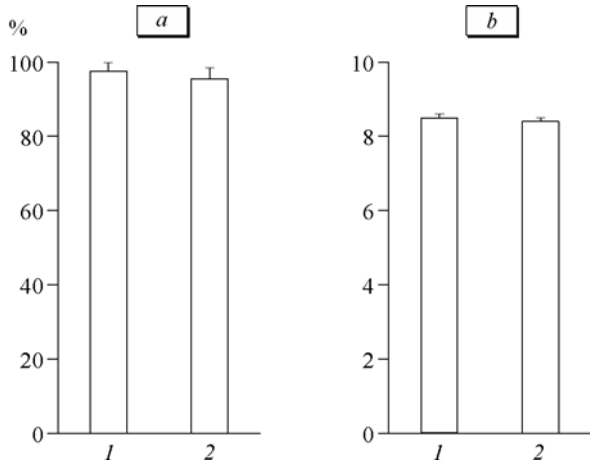

Fig. 2. Phagocytic activity of MP against SiO_2. *a*) percent of MP phagocytizing SiO_2, *b*) mean number of SiO_2 particles engulfed by one phagocytizing MP *in vitro*. *1*) SiO_2; *2*) SiO_2+BCG.

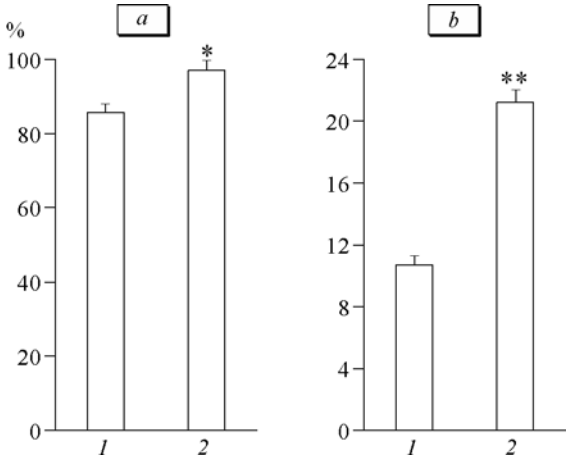

Fig. 3. Phagocytic activity of MP. *a*) percent of MP phagocytizing BCG strain mycobacteria, *b*) mean number of BCG strain mycobacteria engulfed by one phagocytizing MP. *1*) BCG, *2*) SiO_2+BCG. *$p<0.05$, **$p<0.01$ compared to the control.

tures, where MP preliminary loaded with SiO_2 phagocytized BCG strain mycobacteria.

NBT test showed that the amount of formazan characterizing the intensity of ROS production was maximum in the group "SiO_2+BCG" (1950.8±127.3 arb. unit) compared to group "SiO_2" (910.2±86.3 arb. unit, $p<0.05$). Enhanced ROS production in MP on the one hand can induce damage to cells and their lysosomal membranes, but on the other can improve their microbicidal potential *in vivo* [2,4,6,8]. PI and phagocytic number in groups "SiO_2" and "SiO_2+BCG" were similar (Fig. 2), which is determined by simultaneous formation of these groups from pooled peritoneal transudate cells from mice receiving an intraperitoneal injection of SiO_2 particles 24 h before cell explantation into culture. Evaluation of phagocytosis of BCG strain mycobacteria showed that PI and phagocytic number in group "SiO_2+BCG" were higher than in "BCG" group (Fig. 3). We also analyzed the dependence of phagocytosis of BCG strain mycobacteria by macrophages on the number of engulfed SiO_2 particles. The number of BCG mycobacteria phagocytized by MP directly correlated with the number of phagocytized SiO_2 particles. This correlation probably attests to the existence of a direct dependence of phagocytic activity of MP against BCG strain mycobacteria on the number of engulfed SiO_2 particles. This is probably related to stimulation of energy production and energy supply of the phagocytic process over a short period. At the same time, the results of fluorescent analysis showed that the percent of BCG strain mycobacteria damaged in phagocytizing MP of "BCG" group (45.2±3.1 arb. units) is higher than in MP of " SiO_2+BCG" group (26.7±3.5 arb. units, $p<0.05$).

Thus, preliminary MP loading with SiO_2 is associated with stimulation of their phagocytic activity against BCG strain mycobacteria, but simultaneously reduced their antimycobacterial activity, probably due to labilization of lysosomal membranes by ROS, which prevents completion of phagocytosis at the stage of formation of phagolysosomes and creates conditions for intracellular persistence of mycobacteria tuberculosis. *In vivo* stimulation of ROS production by SiO_2 particles can increase the destructive potential created by activated MP of tuberculous granulomas. This not only prologs mycobacteria persistence in MP, but also aggravates the destructive and fibrotic complications in organs [3].

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