Ultrastructural Characteristics of Type A Epithelioid Cells during BCG-Granulomatosis and Treatment with Lysosomotropic Isoniazid

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We studied BCG-granulomas, their cellular composition, and ultrastructure of type A epithelioid cells in the liver of male BALB/c mice with spontaneous granulomatous inflammation. The animals received free isoniazid or isoniazid conjugated with lysosomotropic intracellularly prolonged matrix (dialdehyde dextran, molecular weight 65-75 kDa). Lysosomotropic isoniazid was accumulated in the vacuolar apparatus of epithelioid cells and produced a stimulatory effect on plastic processes in these cells.

Key Words: *tuberculous granulomas; morphometry; ultrastructure of type A epithelioid cells; lysosomotropic isoniazid-dialdehyde dextran complex*

Tuberculosis by the incidence, life threat, and social importance ranks high among a variety of diseases morphologically characterized by granulomatosis [4,8].

Tuberculous granulomas consist of epithelioid cells (EC). EC exhibit low endocytosis capacity, but have a well-developed vacuolar and lysosomal apparatus. Taking into account the prevalence of EC in granulomas and ability to undergo spontaneous death, these characteristics are associated with high risk of destructive complications. Granulomas do not have microcirculatory structures. Mycobacteria persist in the vacuolar-and-lysosomal apparatus of phagocytizing cells in granulomas. Therefore, it is difficult to supply medicinal drugs to these structures.

Here we studied the number and size of granulomas, ultrastructural organization of EC, and manifestations of endocytosis capacity in these cells. This is one of the approaches to affect EC and pathogenic agent by new medicinal preparations that can be used in the therapy of infectious granulomatous diseases with intravacuolar pathogens.

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MATERIALS AND METHODS

During tuberculous inflammation induced by virulent tuberculous mycobacteria, destruction zones are localized in the central part of granulomas. This specific feature makes difficult to study EC in this zone. Destructive processes in granulomas are rare under conditions of BCG-granulomatosis. Disseminated tuberculous granulomatous inflammation was induced in male BALB/c mice aging 2 months, weighing 20-22 g, and obtained from the nursery of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences). The BCG vaccine was dissolved in aqueous solution of 0.9% NaCl and injected intraperitoneally in a single dose of 0.5 mg [6,7].

The mice were divided into 3 groups of 10 specimens each. Group 1 included untreated animals. Group 2 consisted of mice receiving isoniazid (isonicotinic acid hydrazide). Isoniazid was dissolved in aqueous solution of 0.9% NaCl and injec-

ted intraperitoneally (14 mg/kg, 2 times a week) for 5 months. This treatment started 1 month after infection of mice with the BCG vaccine. Group 3 animals intraperitoneally received the same dose of isoniazid conjugated with modified dextran (dialdehyde dextran, molecular weight 65-75 kDa). The scheme and duration of treatment in group 3 mice were similar to those in group 2 animals. Dialdehyde dextran exhibits tropic activity for the vacuolar apparatus of phagocytizing cells and is characterized by intravacuolar (lysosomal) prolongation [5]. Treatment of group 2 and 3 mice started 1 month after infection and lasted 5 months.

Liver samples were obtained from mice of various groups 6 months after BCG infection. The number of mononuclear phagocytes in the liver is much higher compared to other organs [3,4,7]. Besides this, the liver is most sensitive to polychemotherapy of tuberculosis. The animals were killed by cervical dislocation under ether anesthesia.

Liver samples were prepared by standard methods [1,2]. The number of granulomas was estimated under a light microscope using a multipurpose test system (squares, 1.72×10^5 μ^2). The diameter of granulomas was estimated with an ocular micrometer. The relative number of EC (%) was calculated. The number of all cells in granulomas was taken as 100%. Type A and B epithelioid cells were differentiated by means of transmission electron microscopy. Ultrastructures of type A EC were examined using a multipurpose test system. A morphological study was performed with projections of ultrathin sections from 45-55 type A EC obtained during transmission electron microscopy.

The results were analyzed by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

The ratio between the numbers of EC of types A and B in group 1, 2, and 3 mice was 32:1, 19:1, and 13:1, respectively.

Treatment with free isoniazid (FI) decreased numerical density of granulomas by 47% (Fig. 1, *a*). In mice receiving lysosomotropic isoniazid (LI; Fig. 1, *a*) this parameter changed by 3 times. Our findings confirm high therapeutic effectiveness of the test drugs. The formation and size of granulomas are determined by the gradient of chemoattractants, mainly those produced and induced by live

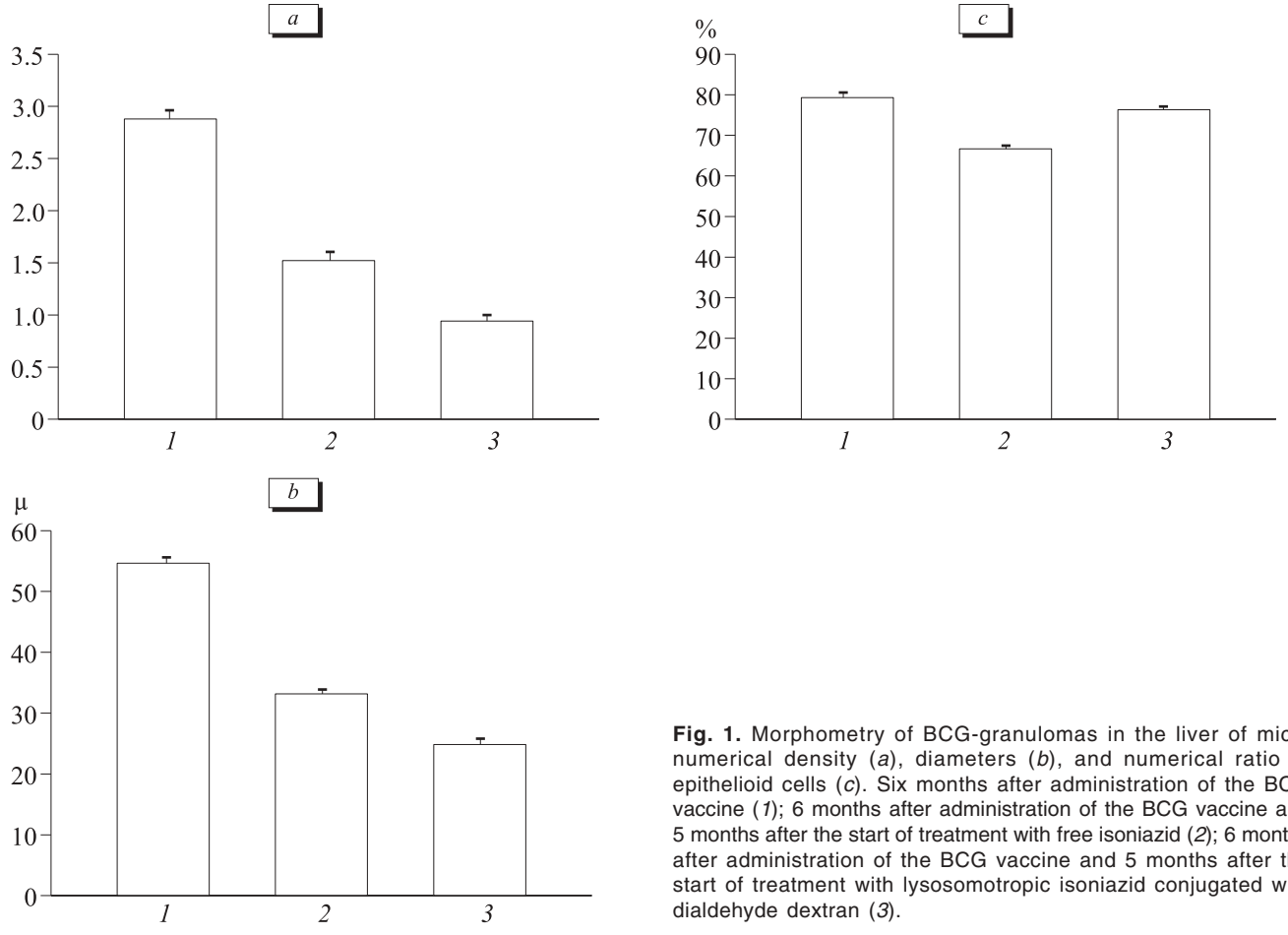

Fig. 1. Morphometry of BCG-granulomas in the liver of mice: numerical density (*a*), diameters (*b*), and numerical ratio of epithelioid cells (*c*). Six months after administration of the BCG vaccine (*1*); 6 months after administration of the BCG vaccine and 5 months after the start of treatment with free isoniazid (*2*); 6 months after administration of the BCG vaccine and 5 months after the start of treatment with lysosomotropic isoniazid conjugated with dialdehyde dextran (*3*).

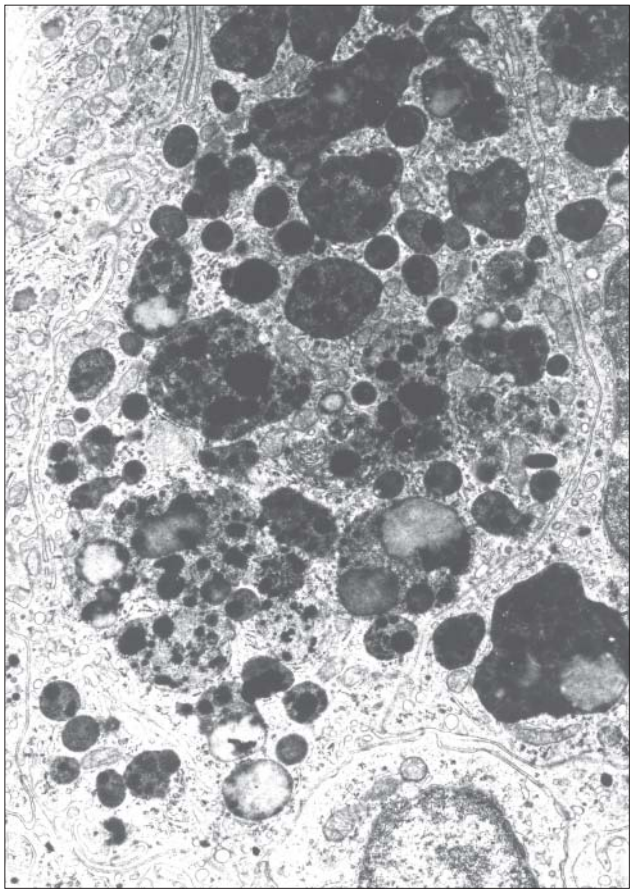

Fig. 2. Ultrastructural organization of type A epithelioid cells 6 months after infection with the BCG vaccine and 5 months after the start of treatment with lysosomotropic isoniazid conjugated with dialdehyde dextran. Numerous primary and secondary lysosomes (×8750).

mycobacteria and granuloma cells [5,8]. Studying the size of granulomas provided evidence for these data (Fig. 1, *b*). The size of granulomas in animals treated with LI and FI decreased by 2.2 times and 35%, respectively, compared to untreated mice. Therefore, LI was more effective than FI. Probably, dialdehyde dextran increases the incidence of phagolysosomal fusion [4]. Granulomas were not characterized by progression of destructive processes that could affect their number and size. "Dissociation" of granulomas is probably related to migration of cells with a decrease in the gradient of chemoattractants, which results from destruction of several mycobacteria [3,4,6].

These changes probably determine the therapeutic effect and preservation of a greater number of EC in granulomas [3,4]. EC have complex intercellular contacts, which contributes to strong fixation of cells in granulomas (Fig. 2). The number of EC was highest in granulomas (Fig. 1, *c*).

The total density of primary and secondary lysosomes in EC of untreated animals was more than 7% of the cytoplasmic volume (Table 1). Under normal physiological conditions, this index in Kupffer cells varies from 6 to 24% (taking into account erythrophagosomes) [5]. Hence, EC are close to phagocytizing cells by the development of vacuoles and lysosomes (Fig. 2). However, the area of plasmalemma free of intercellular contacts and capable of endocytosis is 12.00±0.54% of the total area. This structure was most often directed toward narrow intercellular spaces (Fig. 2).

The total volume and ratio between primary and secondary lysosomes in mice of the FI group did not differ from those in untreated animals (1:7, Table 1). It is beyond doubt that FI could enter EC. The molecular weight of FI is less than 300 Da. This compound diffuses into the cells and compartments. However, this characteristic is incompatible with intracellular accumulation of FI in effective concentrations.

Treatment of animals with LI was accompanied by a 53% increase in the volume of vacuoles and lysosomes in EC. The ratio between primary and secondary lysosomes decreased to 1:5.7 (Table 1). These changes were associated with a 100% increase in the number of primary lysosomes due to the ability of several dextrans to stimulate plastic processes in cells [5]. The number of free and bound ribosomes in EC from animals of this group increased by 1.7 and 1.9 times, respectively. The volume density of mitochondria increased by 61%. The total surface density of the outer and inner membrane increased by 2.2 times. The total density of organoid membranes in EC reflecting the intensity of plastic processes increased by 66%. The volume density of secondary lysosomes in these mice was 47% higher compared to untreated and FI-treated animals. These changes were associated with accumulation of slowly hydrolyzed dialdehyde dextran in lysosomes (Table 1) [5]. LI can enter EC by pinocytosis. Moreover, accumulation of LI in EC can be related to fusion of these cells with macrophages engulfing LI and mycobacteria. *In vitro* experiments with peritoneal macrophages absorbing zymosan particles and EC provide evidence for this hypothesis [4].

Our results show that LI on the dextran matrix entered EC. However, it remains unclear whether LI supply is related to pinocytosis and/or fusion of EC with macrophages engulfing LI and mycobacteria. We conclude that high-molecular-weight carriers of drugs (*e.g.*, dextrans) exhibit intrinsic biological activity and hold promise to modify function of EC, supply and concentrate medicinal drugs, and treat infectious diseases accompanied by granulomatous inflammation.

TABLE 1. Ultrastructural Morphometry of Type A EC in BCG-Granulomas from the Liver of Male BALB/c Mice (*M*±*m*)

Parameter			Conditions and stages of study		
			BCG, 6 months	FI treatment, 5 months	LI treatment, 5 months
Rough endoplasmic reticulum		Vv	8.24±0.70	9.26±0.68	10.68±0.91^{+}
		Sv	1.81±0.14	2.13±0.17	2.32±0.17^{+}
Golgi complex		Vv	2.34±0.45	2.88±0.55	3.58±0.69
		Sv	0.95±0.18	0.86±0.16	1.31±0.25
Primary lysosomes		Vv	0.91±0.18	0.85±0.16	1.80±0.35^{*+}
		Sv	0.19±0.04	0.16±0.03	0.31±0.06*
		Nai	1.16±0.22	1.31±0.24	2.77±0.54^{*+}
Secondary lysosomes		Vv	6.61±0.56	6.06±0.59	9.73±1.12^{*+}
		Sv	1.14±0.07	1.23±0.12	1.43±0.11^{+}
		Nai	2.70±0.55	3.17±0.61	3.09±0.60
Ribosomes	bound	Nai	15.46±1.30	19.51±1.65	28.87±2.37^{*+}
	free	Nai	22.44±1.81	25.82±2.13	37.66±3.11^{*+}
Mitochondria		Vv	5.96±0.69	6.13±0.71	9.63±1.12^{*+}
		Nai	2.98±0.35	3.64±0.42	3.70±0.43
Outer membrane		Sv	0.80±0.09	0.64±0.06	1.77±0.20^{*+}
Inner membrane		Sv	1.59±0.16	1.45±0.17	3.62±0.35^{*+}
Σ Sv			6.48±0.30	6.47±0.32	10.76±0.52^{*+}

Note. Vv, volume density of structures (% of the cytoplasmic volume); Sv, surface density (surface area of structures per unit cytoplasmic volume); Nai, number of structures per unit cytoplasmic volume, Σ Sv, total density of membranes in cytoplasmic organelles. $^{*}p<0.05$ compared to FI; $^{+}p<0.05$ compared to BCG.

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