MORPHOLOGY AND PATHOMORPHOLOGY

Dynamics of Mononuclear Phagocytes in Lymph Nodes and Granulomas in Chronic Tuberculous Inflammation

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Quantitative changes in mononuclear phagocytes in lymph nodes and tuberculous granulomas in patients with generalized tuberculosis suggest that accumulation of these cells in granulomas occurs due to their recruiting from bone marrow precursors, rather than from lymph nodes. Antimycobacterial therapy leads to dissociation of granulomas accompanied by accumulation of macrophages in lymph nodes. The number of granulomas decreases, but not their size and relative content of epithelioid cells remain unchanged. With due regard of the microanatomy of granulomas, epithelioid cells are regarded as the main site of mycobacterium persistence and the object of targeted drug delivery.

Key Words: *lymph node; phagocytes; granuloma; tuberculosis*

The population of mononuclear phagocytes is maintained due to bone marrow precursors [13], dynamically reacting to functional changes in the organism and organs. Subdivision of differentiated cells of the mononuclear phagocyte system into mobile and resident is arbitrary [8,14], and their counts in organs and anatomical regions of organs are apparently determined by different concentrations of chemattractants, which is explained by physiological characteristics of organs, infection, inflammation, *etc.* This is proven by the data on differences in macrophage (MP) concentrations in tissues of different organs and redistribution of MP by the chemotaxic gradient [8,14].

Presumably, the number of typical mononuclear phagocytes in lymph nodes (LN) is also determined by dynamically changing gradients of chemattractants (metabolites, high-molecular-weight proteins, microorganisms, *etc.*). In case of infection with *M. tuberculosis* these microorganisms always involve LN in primary tuberculosis and, persisting there, become the source of infection in secondary tuberculosis [13]. On the other hand, it is unknown how the population of mononuclear phagocytes in LN changes in "spontaneous" chronic granulomatous tuberculous inflammation and during antimycobacterial therapy modifying the concentration of chemattractants (mycobacteria and cell necrosis products) in them. Investigation of these aspects was the aim of our study.

MATERIALS AND METHODS

The study was carried out on 90 BALB/c male mice (Breeding Center of the Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences) aged 2 months (20-22 g). The animals were kept on standard laboratory diets with free access to water and food. Before the experiment the animals were adapted to vivarium conditions for 2 weeks.

Generalized tuberculous process was induced by a single injection of BCG vaccine (N. F. Gamaleya Institute of Epidemiology and Microbiology) in a dose of 0.5 ml vaccine in 0.9% NaCl/mouse [7]. After 1 month, all animals developed generalized tuberculous inflammation [9,11]. In group 1, isoniazide ther-

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apy was started 1 month postinfection (14 mg/kg intraperitoneally twice a week) [5,11]. Untreated mice 1 and 6 months postinfection comprised groups 2 and 3, respectively.

The animals were sacrificed by cervical dislocation under ether narcosis. Axillary LN were collected from 8-10 mice of each group and from intact mice, fixed in Tellesnetskii fixature (100 ml ethanol, 5 ml concentrated acetic acid, 5 ml 40% formaldehyde) for 24 h, dehydrated in alcohols, and embedded in paraffin-wax mixture. Sections were made through the entire LN along the maximal length. The sections (5-6 μ) were stained with hematoxylin and eosin, azur-2-eosin, by Van-Gieson method, and embedded in Canadian balm.

Morphometry of LN (×990) and granuloma cells (×110 and ×440) was carried out using square test systems and object micrometer. The number of granulomas per unit of section area was counted and cell numbers in granuloma were expressed in percent of total number of granuloma cells.

The data were statistically processed using Student's *t* test, the differences between mean values were considered significant at $p<0.05$.

RESULTS

In "spontaneous" inflammation the number and size of granulomas essentially increased (Table 1). It was previously shown that the number and size of tuberculous granulomas reflect activity of the inflammatory process, which depends on the amount of bacteria in macrophages of granuloma both during spontaneous development of the process and during antimycobacterial therapy [6,10,11]. We assumed that MP for the formation of granulomas are primarily recruited from LN. One month after infection, the number of monocytes in LN decreased 2.6 times, and after 6 months much more. The number of MP after 1 month increased 2.8-fold and then did not change (Fig. 1). One can assume that monocyte count decreased due to their differentiation into MP and recovery of monocyte population proceeded more slowly the increase in MP number (Fig. 1). It is also possible that the decrease in monocyte count in LN was due to their death, but the intensity of these processes was negligible in comparison with the rate of the decrease in monocyte count (Fig. 1). Dead cells cannot be infected, and therefore they were presumably not only monocytes. Removal of monocytes from LN could be caused by the formation of granulomas, *i.e.* differentiation of monocytes into MP and epithelioid cells (EC) (Table 1), the most abundant cells in granulomas. However, all these changes did not cause the decrease in MP count in LN (this parameter increased almost 3-fold); this can be regarded as a peculiar reserve in cases when higher intensity of limiting reaction is needed, particularly for blocking monocyte entry from the bone marrow. Such potentialities are limited in non-lymphoid tissues.

At the beginning of isoniazide therapy, mycobacteria were partially suppressed, including those in granuloma macrophages, which can be regarded as decrease of the chemattractant concentration. This manifested by an essential decrease in the number of granulomas in LN during the fifth month of therapy (sixth from the moment of infection, Table 1). Necrosis of granuloma cells is not characteristic of the model of tuberculous inflammation used in our study, and therefore decrease in the number of granulomas seems to be caused by cell "dissociation" [6,9]. Interestingly, the number of MP after the start of therapy decreased in granulomas (Table 1), and increased in LN (Fig. 2). However during the first 3 months of therapy these MP and other cells in granulomas presented as "transitory" forms, because 6 months postinfection LN in untreated animals were enlarged by 76% compared to intact animals. After the first month of therapy LN returned to normal (in untreated animals this parameter returned to normal only 6 months postinfection, *i.e.* after 5 months of therapy). The enlargement of LN was not caused by edema or congestion, which was confirmed by cell concentrations.

TABLE 1. Parameters of Tuberculous Granulomas in LN (per 1.049×10^6 μ² Section Area) and MP and EC Counts in Granulomas (*M*±*m*)

Parameter	Time postinfection, months					
	untreated controls		isoniazide therapy			
	1	6	2	3	4	6
Number of granulomas	0.76±0.06	2.57±0.06	0.87±0.05	1.75±0.35	1.16±0.08	0.95±0.07
Diameter of granulomas, μ	16.33±0.42	49.13±0.64	20.58±0.30	25.13±0.78	28.92±0.54	41.60±0.55
Percentage of cells						
MP	22.82±0.40	15.49±0.25	14.10±0.56	6.85±0.20	4.77±0.17	3.53±0.17
EC	73.80±0.51	81.30±0.27	82.89±0.67	84.89±0.48	83.81±0.45	81.42±0.23

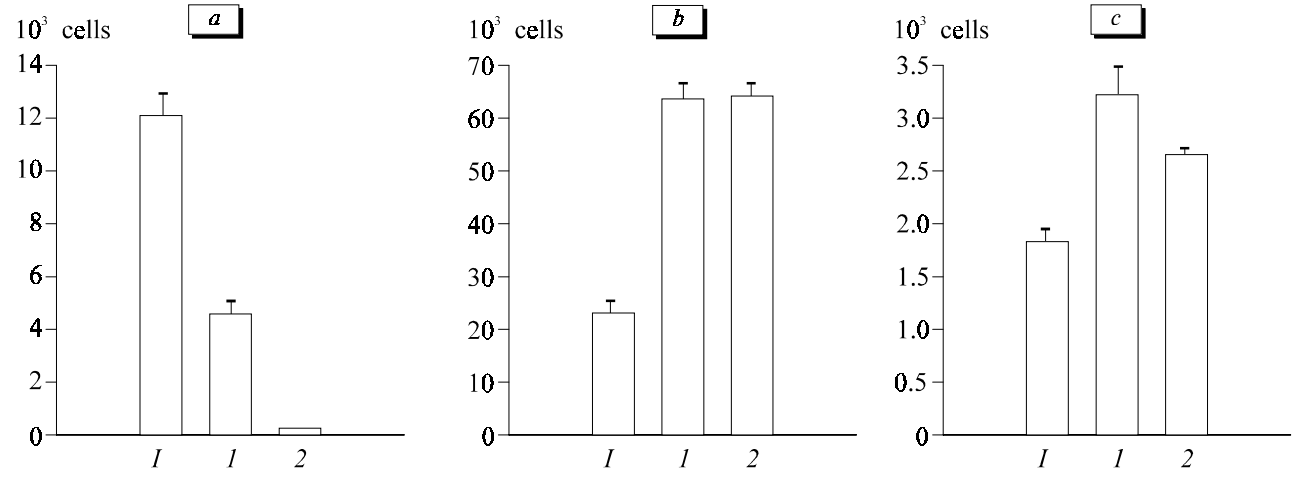

Fig. 1. Number of monocytes (*a*), macrophages (*b*), and dead cells (*c*) in lymph node (per 1.098×10^4 μ² section area). *I*: intact animals; *1* and *2*: untreated animals 1 and 6 months after infection with BCG, respectively.

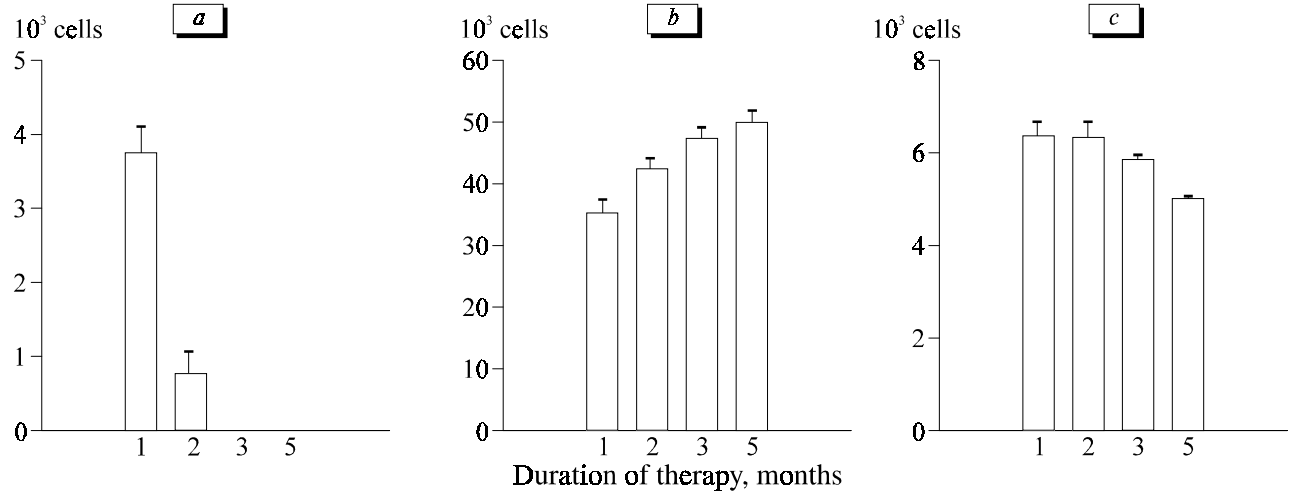

Fig. 2. Number of monocytes (*a*), macrophages (*b*), and dead cells (*c*) in lymph node (per 1.098×10^4 μ² section area) during isoniazide therapy of tuberculous process.

In granulomas persisting after therapy the relative count of EC was the same as before treatment, though at the beginning (1 month) of treatment granulomas were small, but then gradually increased and reached $^4/_5$ of the size of granulomas in untreated mice (Table 1). This probably means that mycobacterium count in granulomas increased. It was previously shown that isoniazide therapy of patients with generalized chronic tuberculosis accelerated differentiation of the granuloma monocytes into MP and led to manifestation of cytological signs of their activation [11]. Presumably, division of mycobacteria persisting in EC was stimulated by cytokines produced by MP [4].

EC possess a well-developed lysosomal system characteristic of MP. Though they have virtually no plasmalemma surface needed for phagocytosis [9], particularly in the center of granulomas, they contain many secondary lysosomes and phagosomes [12]. EC are apparently forming from MP, which captured bacteria but are unble to complete phagocytosis/ These cells are transformed into EC and then in multinuclear cells, by analogy with multinuclear cells of foreign bodies, which also could not degrade captured material. There are good grounds to suggest that EC fuse with MP [2] transporting in them both their hydrolases and phagocytosis objects, which can partially explain the presence of secondary lysosomes and phagosomes. Together with mycobacteria isolated in them, these cells presumably present as a granuloma-forming center and the site of persistence of drug-resistant mycobacteria. Granulomas have no well-developed microcirculatory structures; with certain limitations in realization of endocytosis, EC can be an object of investigation, particularly important as regards the persistence of the agent, reinfection, development of antimycobacterial drugs and methods of their “addressed” delivery.

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