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NATURAL KILLER CELLS IN PATIENTS WITH PULMONARY TUBERCULOSIS

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KEY WORDS: natural killer cells; lymphocytes; tuberculosis; immune phenotyping.

The close study of immune reactivity in patients with tuberculosis has led to the creation of a sufficiently sound system of ideas on the connection between the time course of the disease and the level of T-lymphocytes ($CD3^+$-cells) and their principal subpopulations, namely helper and suppressor cells ($CD4^+$- and $CD8^+$-cells) [1-3, 7, 9-11]. Meanwhile, few studies of yet another lymphocyte subpopulation, namely natural killer (NK) cells, in tuberculosis have been published. According to data in the literature NK cells are polypotent: they play an important role in antiviral protection, they carry out antibody-independent lysis of tumor and virus transformed cells; they have also an Fc-receptor, which enables them to perform cytolytic functions with the involvement of antibodies, and they can directly kill certain bacteria [4-6, 13, 14].

Considering the mainly intracellular parasitism of *Mycobacterium tuberculosis,* involvement of NK cells in the mechanisms of resistance to mycobacterial infection cannot be ruled out. According to data in the literature, in some cases an increase in the number of NK cells is observed in the blood and pleural exudate of patients with tuberculosis compared with healthy individuals [8]. Activity of NK cells in the blood and exudate, according to these same workers, does not extend beyond normal limits. Onwubalili and co-workers [9] also noted the absence of changes in the corresponding parameters of NK cells in tuberculosis, which they analyzed in relation to the form of the disease or treatment given. They point out that the NK cell system evidently does not play an active role in protective mechanisms in tuberculosis. In other cases [12], a certain tendency was nevertheless found toward an increase in the activity of these cells, associated more with the duration of the disease. Similar views are held by Yoneda and co-workers [15], who observed correlation between a low level of NK cell activity and worsening of the roentgenologic data and progression of the disease.

The aim of the investigation described below was to determine the number of NK cells in the blood of patients with pulmonary tuberculosis, to establish correlation between their number and various other clinically important parameters, studied previously, relating to the mean level of $CD3^+$-, $CD4^+$-, and $CD8^+$-lymphocytes, and determination of the intensity of fluorescence of NK cells and of $CD3^+$-, $CD4^+$-, and $CD8^+$-lymphocytes.

EXPERIMENTAL METHOD

In order to carry out this task we undertook immunologic testing of the blood of patients with pulmonary tuberculosis and healthy volunteers. For immune phenotyping of the lymphocytes we used monoclonal antibodies from the firm Behringwerke (West Germany) ($CD3^+$ — BW264/56, $CD4^+$ — BW264/123, and $CD8^+$ — BW135/80, NK cells BMA070) in

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TABLE 1. Mean Number of $CD3^+$ and NK Cells in Blood of Patients with Pulmonary Tuberculosis and Healthy Blood Donors (M ± m)

Method of assessment	Group of subjects tested	Number of subjects tested	Mean level of lymphocytes	
			$CD3^+$	NK
Luminescence microscopy	Healthy	21	65,2±1,9	14,2±1,16
	p		<0,05	<0,05
	With pulmonary tuberculosis	20	58,92±2,34	22,9±2,69
Laser flow cytofluorometry	Healthy	9	61,5±2,7	12,77±2,56
	p		<0,05	<0,05
	With pulmonary tuberculosis	21	49,35±4,09	20,37±1,74

TABLE 2. Intensity of Fluorescence of $CD3^+$-, $CD4^+$-, and $CD8^+$-Lymphocytes and NK Cells in Healthy Individuals and Patients with Pulmonary Tuberculosis

Group of subjects tested	Number of subjects tested	Average fluorescence channel (1-256)			
		$CD3^+$	$CD4^+$	$CD8^+$	NK cells
Healthy	9	63,27±9,18	64,99±7,17	111,89±4,39	82,04±7,69
p				<0,05	<0,05
NK cells	21	81,19±4,66	67,12±3,16	90,38±4,3	56,33±2,28

TABLE 3. Mean Number of $CD3^+$-, $CD4^+$-, and $CD8^+$-Lymphocytes and NK Cells in Blood of Patients with Tuberculosis with More than 15% and with Not More than 15% of NK Cells (M ± m)

Group of patients	Number of patients	Mean level of lymphocytes				
		$CD3^+$	$CD4^+$	$CD8^+$	NK	$CD4^+/CD8^+$
With more than 15% of NK cells	13	55,5±2,97	36,24±3,15	23,68±3,21	25,65±1,63	1,65±0,12
p				<0,05	<0,05	<0,05
With not more than 15% NK cells	8	44,38±9,64	34,16±4,9	12,91±4,1	11,72±1,6	2,8±0,56

the indirect immunofluorescence test in a microlunar modification. Cells isolated on Ficoll and washed, were incubated in the cold with 1st order antibodies (normal IgG), and after washing were incubated once again with FITC-labeled antimouse second order antibodies (IgG — for $CD3^+$, $CD4^+$, $CD8^+$, and IgM — for NK cells).

The results of the reaction were assessed by luminescence microscopy and by laser flow cytofluorometry on the EPICS (Coulter, USA) apparatus. The patients were tested at the 1st and 2nd months of treatment.

Altogether 41 patients with pulmonary tuberculosis and 30 healthy volunteers were investigated. The patients with tuberculosis included 30 men and 11 women, 27 of whom had been found to have the specific disease in the lungs for the first time, whereas in 14 patients the disease followed a chronic course. All patients were HIV negative. While in the clinic, all patients received combination chemotherapy against tuberculosis.

EXPERIMENTAL RESULTS

The results of immune phenotyping of the blood cells in patients with pulmonary tuberculosis and healthy volunteers are given in Table 1.

Analysis of the results indicates that against the background of a significant decline in the number of $CD3^+$-lymphocytes, in patients with pulmonary tuberculosis there was a significant increase in the number of NK cells ($p < 0.05$). It will be noted that when different methods were used to analyze the results of the immune phenotyping tests (luminescence microscopy or laser flow cytofluorometry), similar values were obtained for the levels of $CD3^+$-lymphocytes and NK cells, i.e., the results of assessment by the two methods are comparable.

By laser flow cytofluorometry it is possible to determine not only the average number of cells carrying a particular antigenic marker on their surface, but also the average number of molecules of this antigen located on the cells, on the basis of the intensity of fluorescence (Table 2). Intensity of fluorescence is a morphological and functional parameter and gives some idea about the potential functional activity of the cells.

Analysis of the data indicates that the average number of antigen molecules on the surface of $CD3^+$- and $CD4^+$-lymphocytes was identical in the two groups studied – patients with pulmonary tuberculosis and healthy individuals. The situation was different when the intensity of fluorescence of the $CD8^+$ and NK cells was compared. A significant decrease in the number of antigen molecules was found on the cell surface of the lymphocytes of these subpopulations in patients with pulmonary tuberculosis. Changes of this kind may be reflected in the functional activity of the cells and, in particular, may lead to inhibition of antibody-dependent cytotoxicity.

Further analysis showed that the average number of NK cells in the blood was independent of the duration of the disease and of its clinical form.

To detect the role of NK cells in the immunopathogenesis of tuberculosis and to establish correlation between this parameter and the numbers of the cell populations studied previously, we compared the results obtained by laser flow cytofluorometry in two groups of patients. The first group consisted of patients with tuberculosis with an average number of NK cells of over 15%, the second group consisted of subjects with a mean number of more than 15% of NK cells (Table 3).

It will be evident that a low level of NKC (11.72 ± 1.6) corresponds to a significantly lower level of $CD8^+$-lymphocytes and, conversely, a higher level of NK cells in the blood (25.65 ± 1.63) corresponds to a significantly higher content of $CD8^+$-cells. Correspondingly, the $CD4^+/CD8^+$ ratio correlated negatively with the number of NK cells.

The impression was obtained of balance between the effectors of different types of immune response in pulmonary tuberculosis. For instance, corresponding to a disturbance of the principal regulators of T-lymphocyte subpopulations and, thus, to a disturbance of the classical pathway of protection against *Mycobacterium tuberculosis,* there was a significant increase in the NK cell level. This may be linked with activation of another pathway of resistance to infection, namely antibody-independent cytotoxicity, of which NK cells are the effectors.

Thus a significant increase in the number of NK cells compared with that observed in healthy individuals was revealed by laser flow cytofluorometry in the blood of patients with pulmonary tuberculosis. This is evidence of possible activation, not only of the classical mechanisms of resistance to *M. tuberculosis,* but also of the phylogenetically oldest mechanism of antibody-independent cytotoxicity, mediated through NK cells.

The intensity of fluorescence of NK cells, determined by the intensity of expression of the FC-receptor on the surface of these cells, was significantly lower than the corresponding parameter in normal individuals. This parameter is a combined morphological and functional characteristic and can provide de evidence of a disturbance of antibody-dependent cytotoxicity of NK cells.

The blood NK cell level in patients with pulmonary tuberculosis correlates with the number of $CD3^+$- and $CD8^+$-lymphocytes and also with the $CD4^+/CD8^+$ ratio, which are closely interlinked with the time course of pulmonary tuberculosis. This indicates a definite role of NK cells in the immunopathogenesis of tuberculosis.

The results of immune phenotyping of the blood cells, estimated by luminescence microscopy and by laser flow cytofluorometry, can be considered to be comparable.

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INCREASED PRODUCTION OF TUMOR NECROSIS FACTOR DURING ENDOTOXIN SHOCK IN MICE PRESENSITIZED WITH SERUM OF MICE WITH TUMORS OR WITH TUMOR CELL FACTORS

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KEY WORDS: endotoxin shock; tumor necrosis factor; sensitization; tumor.

Experimental treatment of tumors based on the use of components of bacterial cells and, in particular, bacterial glycoconjugates, is currently regarded as highly effective [2, 6, 11, 12]. The main obstacle to the use of components of bacterial cell walls in clinical oncology is their toxicity. We know that animals with tumors are most sensitive to the toxic action of immunomodulators of bacterial origin [3, 4].

An increase in the sensitivity of animals with growing tumors to the toxic action of endotoxin correlates with the granulocytosis [3] and enhancement of the bactericidal activity of macrophages [4], and the monocytes of cancer patients can produce an increased quantity of tumor necrosis factor (TNF) and prostaglandin E_2 in response to stimulation by lipopolysaccharide [10]. The authors cited consider that the increase in sensitivity of tumor-bearing animals to the toxic action of lipopolysaccharide (LPS) is linked with activation of macrophages and is effected through increased production of cytokines (TNF etc.) by cells of the reticuloendothelial system.

The aim of this investigation was to study what factors are responsible for sensitizing an animal with tumor cells to the toxic action of a combination of LPS and glucosaminylmuramyldipeptide (GMDP), and to shed light on the causes of the increased mortality of tumor-bearing animals following administration of bacterial immunomodulators.

EXPERIMENTAL METHOD

Mice of strains C57BL/6 (H-2^b), DBA/2 (H-2*), BALB/c (H-2*), A/Snell (H-2*), and (CBA × C57BL/6)F_1 hybrids (H-2*) of both sexes, aged 2-3 months, were obtained from the "Stolbovaya" and "Svetlye Gory" nurseries Syngeneic tumor cells were injected into the mice: leukemia EL-4, melanoma B16, mastocytoma P815, and plasmacytoma MOPC 315, subcutaneously (10^6 cells).

*Haplotypes omitted in original – Translator.

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