Cytokine-Secreting Activity of Blood Eosinophils in Pulmonary Tuberculosis

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Modern immunological studies showed that eosinophilic granulocytes producing the key mediators of cellular and humoral immune response contribute to the common cytokine imbalance developing in tuberculous infection. A significant increase in BCG-induced secretion of IL-2, IL-5, and TNF-α by eosinophils in patients with pulmonary tuberculosis indicated high reserve reactivity of eosinophilic cells realizing their functional potential in regulation of the specific resistance reactions of the microorganism under conditions of *M. tuberculosis* infection.

Key Words: *tuberculosis; eosinophils; cytokines*

Cell composition of granuloma forming in the lung tissue in response to *M. tuberculosis* infection includes eosinophilic granulocytes (in addition to macrophages and lymphocytes). The peripheral blood of patients with pulmonary tuberculosis often contains excessive amounts of eosinophils [2,7,10,11]. New data on functional potentialities of eosinophils suggest regarding these unique cells as full-value participants in the effector mechanisms of antibacterial defense. On the other hand, possible role of eosinophilic granulocytes in the regulation of congenital and adaptive immunity processes is actively discussed. It is known that eosinophilic leukocytes present a variety of receptor structures on their surface: Toll-like receptors (TLR), γδT-cellular receptor (γδTCR), HLA-DR, receptors for cytokines and chemokines, adhesion molecules, *etc*. They also secrete more than 30 cytokines, including mediators with proinflammatory (IL-2, IL-12, IFN-γ, TNF-α) and anti-inflammatory (IL-4, IL-5, IL-10, IL-13, transforming growth factor β (TGF-β) activities, involved in the realization of Th1- and Th2-mediated immune response, respectively [7,9,11,12,15]. As a result, mutually directed effects form between the immune cells and eosinophils due to immunomodulatory effects of immunocompetent cells and the capacity of eosinophilic leukocytes to stimulate lymphocytes and monocytes and cause polarization of the immune response, directing it by either cellular or humoral pathway.

High secretory and receptor-expressing activities of eosinophilic granulocytes suggest their active involvement in the immunopathogenesis of inflammation in pulmonary tuberculosis and in regulation of specific and nonspecific resistance of the host in response to *M. tuberculosis* infection.

We studied the secretion of immunoregulatory cytokines (IL-2, IL-5, TNF-α) by eosinophilic granulocytes *in vitro* in patients with pulmonary tuberculosis.

MATERIALS AND METHODS

Thirty-five patients at the age of 18-55 years with newly diagnosed disseminated infiltrative destructive pulmonary tuberculosis were observed. The diagnosis was based on the clinical picture, x-ray picture of the lungs, and results of microscopic and bacteriological analysis of the sputum. Two groups were formed,

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depending on the absolute counts and percentage of eosinophils in the peripheral blood: 1) 16 patients with pulmonary tuberculosis with eosinophilia (absolute count of eosinophils 0.85±0.01 G/liter and relative content 8.00±0.46%) and 2) 19 patients with pulmonary tuberculosis without eosinophilia (absolute count 0.29±0.01 G/liter and relative content 3.0±0.3%).

Control group consisted of 12 age- and sex-matched donors (absolute count of eosinophils 0.07±0.01 G/liter and relative content 1.23±0.30%).

All studies in tuberculous patients were carried out before specific antituberculous therapy. Venous blood served as the object of studies.

The peripheral blood eosinophilic leukocytes were isolated by density gradient centrifugation on Percoll intermittent density gradient (ρ=1.133 g/liter; Sigma Life Science). In order to prepare supernatants, the isolated cells were resuspended in complete nutrient medium (90% RPMI-1640, 10% inactivated FCS (Sigma), 0.3 mg/ml L-glutamine, and 100 μg/ml gentamicin). Cell concentration in the suspension was brought to 2×10^6/ml. The cytokine-producing activity of eosinophils was stimulated by adding BCG vaccine strain (50 μg/ml; Microgen Firm) into the samples. Cell suspensions were incubated at 37°C and 5% CO_2 for 24 h. The supernatants were collected and the cytokines in them were measured by sandwich enzyme immunoassay (ELISA) according to instructions from manufacturers (Biosource, Protein Contour). The results were processed by Statistica 6.0 software.

RESULTS

Despite the key role of alveolar macrophages, dendritic cells, and various T lymphocyte subpopulations in the formation of immunity to tuberculous infection, it was found that eosinophilic granulocytes possessed a significant armory of factors due to which they made an important contribution to the mechanisms of antiinfectious immunity. It was shown that eosinophils presented TLR-2 on their surface, through which these cells reacted with *M. tuberculosis*, this resulting in release of α-defensins and eosinophilic peroxidase, initiating cell wall injury and lysis of mycobacteria [7]. It was also found that mycobacteria stimulated eosinophilic granulocytes through γδTCR with subsequent cell degranulation and secretion of active oxygen species, NO, cytotoxic proteins and cytokines. According to some data, eosinophilic cells suppress the growth of mycobacteria by phagocytosing them and participate in numerous reactions of antituberculosis immunity through presentation of antigen and production of the key mediators mediating the realization of Th1- and Th2-immune response [6,9,11,14].

The key factor of T lymphocyte growth, IL-2, directing differentiation of null T-helpers (Th0) into Th1 and regulating delayed hypersensitivity reactions plays an important role in the pathogenesis of tuberculous infection [2,4,12]. The main producers of IL-2 are stimulated Th1 lymphocytes, but eosinophilic granulocytes also secrete this mediator and express an appropriate receptor [9,15]. Binding of CD28 costimulatory molecules on the surface of eosinophilic granulocytes led to release of IL-2 and IFN-γ (cytokines responsible for protective cell-mediated immune response) [15].

Our study detected basal hyperproduction of IL-2 by eosinophilic granulocytes *in vitro* in patients with pulmonary tuberculosis irrespective of their blood eosinophilic reaction (Table 1).

It is noteworthy that the data on IL-2 production by blood cells in *M. tuberculosis* infection are contradictory. The majority of authors indicate deficiency of IL-2 production in destructive pulmonary tuberculosis and a correlation between inhibition of the production of this cytokine and severity of the infectious process [3,4]. Our previous studies also demonstrated low secretion of IL-2 by mononuclear leukocytes *in vitro* during the acute period of pulmonary tuberculosis as a result of toxic effect of *M. tuberculosis* on the cytokine synthesis and release [2]. It seems that this mechanism is also responsible for disorders in the production of IL-2 by eosinophils actively reacting with *M. tuberculosis* and absorbing it.

One more mediator secreted by eosinophils and involved in regulation of the host antibacterial resistance mechanisms is TNF-α. Local release of this cytokine stimulates leukocyte chemotaxis, the absorption and digestion functions of phagocytic cells, production of inflammation mediators, promotes re-expression of HLAI/II and a shift of the cytokine balance towards Th1 [2,8,13]. According to modern data, the role of TNF-α in tuberculous infection is determined by its level: the deficiency of its production mediates a severe course of infectious process with disorders in granuloma formation and hence, dissemination of mycobacteria in the body; extremely high level of TNF-α production mediates destructive changes in the lungs [2,3].

Tuberculous infection is usually characterized by hyperproduction of TNF-α by blood mononuclear leukocytes, alveolar macrophages, pleural exudation macrophages, and other cells, this in the majority of cases promoting an unfavorable time course of the infectious process [3,8]. We studied TNF-α secretion by blood eosinophilic leukocytes in patients with pulmonary tuberculosis. Irrespective of hemic eosinophilia, the basal production of this cytokine in our patients was significantly higher than in the controls. The most pronounced changes were found in patients with tuberculous infection associated with eosinophilia (Table

1). This was in line with published data on TNF-α capacity to prolong the eosinophil persistence in the peripheral blood by intense secretion of GM-CSF through translocation of NF-κB nuclear factor [13].

It is noteworthy that the formation of the blood eosinophilic reaction in disease is mainly attributed to hyperproduction of IL-5, the key cytokine regulating the eosinophilic leukocyte homeostasis. In addition to the eosinophil-stimulating effects, IL-5 (the main mediator of humoral immunity) stimulates B cell proliferation and differentiation by stimulating the expression of IL-2 receptor on B cells and induces cytokine production by B cells (in their transformation into plasma cells) and production of immunoglobulins of various classes [9,15]. It is known (and shown in our work) that tuberculous infection is associated with increase of B lymphocyte count in the blood and stimulation of these cells, hyperproduction of IgG, IgM, IgA, and increase of the circulating immune complexes concentration [2]. Some authors noticed high serum levels of IgE in tuberculosis, correlating with the disease severity; this indicated that certain mycobacterial proteins, immunodominant by IgE response, stimulated Th2 cells and simultaneously inhibited the production of Th1 cytokines [1]. These data are in line with the findings of other studies, demonstrating hyperproduction of IL-4, IL-5, IL-6, and other humoral immunity factors in tuberculous infection [3]. Our findings indicated that spontaneous secretion of IL-5 by peripheral blood eosinophilic granulocytes was significantly higher in patients with pulmonary tuberculosis associated with eosinophilia in comparison with the parameter in donors and tuberculous patients without eosinophilia (Table 1).

Hence, our data indicate that eosinophilic cells stimulate cellular (by producing IL-2 and TNF-α) and humoral immunity reactions (by producing IL-5) and act as autocrine regulators of their own differentiation and/or stimulation, mediating further persistence of eosinophils in the peripheral blood in pulmonary tuberculosis.

Modifying the experiment, it is possible to evaluate not only basal secretion of the mediators (reflecting total cellular functional activity), but also mitogen-induced production, indicating the reserve reactivity of the cells. We evaluated the cytokine secretion by eosinophilic granulocytes in response to addition of vaccine BCG strain (*M. bovis* live attenuated strain) into cell culture suspension. This inductor was selected because eosinophilic leukocytes directly reacted with various mycobacterial species with subsequent release of a wide spectrum of factors. Our study showed a significant elevation of BCG-induced secretion of IL-5 and TNF-α *in vitro* in tuberculous patients with eosinophilia; the production of IL-2 increased in tuberculous patients with normal blood levels of eosinophils (Table 1). These changes indicate high reserve reactivity of eosinophilic cells, realizing their functional potential in regulation of adaptive antituberculosis immunity in response to extra antigen challenge.

TABLE 1. Cytokine Levels in Eosinophilic Granulocyte Culture Suspension Supernatants (pg/ml) in Patients with Pulmonary Tuberculosis ($Me(Q_1\text{-}Q_3)$)

	Parameter	Donors	Patients with pulmonary tuberculosis with eosinophilia	Patients with pulmonary tuberculosis without eosinophilia
IL-2	intact culture	512.95	208.30*	307.50*+
		(204.5-605.0)	(177.50-290.80)	(247.50-525.00)
	BCG-induced culture	573.35	246.05*	575.20+o
		(307.00-657.60)	(214.50-569.85)	(485.10-676.80)
TNF-α	intact culture	615.25	883.30*	717.40*+
		(553.50-1014.20)	(819.40-1533.00)	(604.80-992.40)
	BCG-induced culture	755.4o	1629.50*o	732.0+
		(622.90-1352.00)	(669.0-2141.0)	(627.2-1583.0)
IL-5	intact culture	3.03	5.41*	2.67+
		(2.13-4.75)	(4.97-6.17)	(1.34-2.91)
	BCG-induced culture	4.46o	7.03*o	3.73+
		(3.51-6.62)	(4.75-8.33)	(2.51-4.46)

Note. $p<0.05$ in comparison with: *donors, +tuberculous patients with eosinophilia, otuberculous patients without eosinophilia.

Hence, eosinophilic granulocytes secreting mediators of different profiles seem to make their contribution to total cytokine imbalance forming in tuberculous infection. Further studies of a wide spectrum of cytokines produced by eosinophils and playing the key role in the immunopathogenesis of pulmonary tuberculosis will give new data on the immunoregulatory activity of eosinophilic cells polarizing the immune response by the cellular or humoral pathway and hence, promoting or preventing effective response of the host to *M. tuberculosis*.

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