Relative Content of Cytokines in Different Tissues in Mycosis Fungoides

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The concentrations of tumor necrosis factor-α, interleukin-1β, interleukin-2, interleukin-4, and the content of antibodies to H1 histone fraction in peripheral blood lymphocytes, epidermis, and serum were evaluated in patients with mycosis fungoides. It was found that as the disease progresses, cytokine synthesis is switched from Th1 to Th2 class and production of proinflammatory cytokines increases, which indirectly promotes the development of the autoimmune reaction.

Key Words: *mycosis fungoides; cytokines*

Impaired cytokine production in tumors originating from immunocompetent cells disturbs cytokine regulation of the immune system in cancer patients. Cytokine genes are activated together with oncogenes in chromosome aberrations and other abnormalities of the gene apparatus in cells, so that tumor cells produce cytokines stimulating proliferation of neoplastic immunocompetent cells [6]. Malignant skin lymphomas are a special case of systemic tumor process in the lymphoid tissue. Mycosis fungoides is one of the most extensive morphological groups of skin T-cell lymphomas [4]. The imbalance of Th1 and Th2 cytokine production at different stages of the disease was detected *in vitro* and became the object of discussion, specifically the imbalance of interleukin-2 (IL-2) and interleukin-4 (IL-4) production [5]. Enhanced production of Th2 cytokines is associated with more aggressive forms of the disease and increased production of proinflammatory cytokines by tumor microenvironment. The possibility of autoimmune reaction in tumor tissue is discussed. We compared the contents of proinflammatory cytokines IL-2 and IL-4 in different tissues and the content of antibodies to H1 histone fraction as the marker of autoimmune process in mycosis fungoides.

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MATERIALS AND METHODS

The concentrations of IL-2, IL-4, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and content of antibodies to H1 histone fraction were evaluated in 46 patients with mycosis fungoides at different stages of the disease. Control group consisted of 20 donors.

The stage of the disease was determined by clinical and histological data. Lymphocytes were routinely isolated from the peripheral blood in a Ficoll-Verograffin density gradient [1]. The cells were destroyed by three successive freezings-thawings and centrifuged at 2000*g* for 15 min.

The supernatant was collected for the analysis. Biopsy specimens of the epidermis (7-15 mg) were homogenized on the cold and centrifuged in 0.1 M K-phosphate buffer at 2000*g* for 10 min; the supernatant was collected for the analysis [2]. The concentrations of IL-2, IL-4, and TNF-α in the serum, lymphocytes, and epidermis were measured by ELISA with commercial kits (Protein Contour).

The concentration of antibodies to H1 histone fraction was assayed with IMMCO kits in accordance with the manufacturer's protocol. The results were recorded on an EFOS 9305 photometer at 450 and 492 nm.

The results were statistically processed using Student's *t* test.

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TABLE 1. Concentrations of IL-2 and IL-4 in the Serum and Lymphocytes of Patients with Mycosis Fungoides ($M \pm m$)

Parameter		Control (n=20)	Mycosis fungoides, stage		
			I (n=14)	II (n=12)	II (n=20)
IL-2, IU	per 10^6 lymphocytes	7.9±0.5	2.8±0.3*	7.5±0.2	2.4±0.9*
	per 1 ml serum	24.7±5.2	23.8±3.2	26.3±3.7	23.8±2.6
IL-4, pg	per 10^6 lymphocytes	3.9±0.6	21.0±1.3*	2.4±0.3	12.2±1.5*
	per 1 ml serum	50.9±11.0	8.9±2.3*	87.4±13.0	16.8±3.0*

Note. Here and in Table 2: *p<0.05 compared to the control.

TABLE 2. Concentrations of TNF-α, Antibodies to H1 Histone Fraction, and IL-1β in the Serum and Lymphocytes of Patients with Mycosis Fungoides ($M \pm m$)

Parameter		Control (n=20)	Mycosis fungoides, stage		
			I (n=14)	II (n=12)	II (n=20)
TNF-α, pg	per 1 ml serum	57.1±5.8	58.6±7.5	122.8±15.0*	142.5±15.0*
	per 1 mg skin tissue	4.7±0.3	3.9±0.3	8.7±1.8*	6.8±0.9*
IL-1β, pg/ml serum		56.4±8.0	68.7±5.1	26.9±3.2*	86.8±9.0*
Antibodies to H1 histones, %					
	serum	3.7±0.2	3.8±1.4	2.1±0.5	12.2±3.0*
	skin	0.18±0.02	9.3±1.0*	0.4±0.1	4.3±0.5*

RESULTS

The concentrations of IL-2 and IL-4 in peripheral blood lymphocytes of patients with mycosis fungoides underwent opposite changes at different stages of the disease and differed significantly from the normal. The content of IL-2 during stages I and III 2.5-3-fold surpassed the normal, while the concentration of IL-4 decreased 5-fold during stage III and 3-fold during stage I (Table 1). This is in line with published reports about switching of the cytokine synthesis from Th1 to Th2 class with disease transformation into a more severe form [5]. A trend to normalization of both cytokine levels during stage II probably reflects a transitional form of activation of the pathological process.

Serum concentration of IL-2 virtually did not differ from normal at all stages of the disease (Table 1), which could result from increased content of soluble IL-2 receptor [3]. Serum concentration of IL-4 at the initial and final stages of the disease was far below the normal (Table 1). The difference in serum and lymphocyte concentrations of cytokines can reflect changes in the intensity of their synthesis by tumor microenvironment in regions more distant from the focus and the imbalance between free and bound cytokine forms.

The mean serum concentrations of proinflammatory cytokines TNF-α and IL-1β in patients with mycosis fungoides little differed from the normal during stage I and 2-3-fold surpassed the normal during stage III (Table 2). The concentration of TNF-α in the epidermis increased during stages II and III and did not differ from the normal during the initial stage (Table 2).

Serum content of antibodies to H1 histone fraction increased significantly in patients with mycosis fungoides only during the terminal stages of the disease (Table 2). The content of these antibodies in the epidermis increased significantly during stages I and III.

These data confirm switching of the cytokine synthesis from Th1 to Th2 class with the progress of mycosis fungoides. Increased production of proinflammatory cytokines deserves special attention, because they indirectly promote the development of autoimmune reaction in the body.

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