CHANGES IN MONOCYTE CYTOTOXIC MEDIATOR PROFILE IN PATIENTS WITH CANCER AND PRECANCEROUS CONDITIONS OF THE STOMACH

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UDC 616.33-006.6-076.5

KEY WORDS: monocytes, tumor necrosis factor α, active forms of oxygen, carcinoma of the stomach

The study of the antitumor activity of monocytes from cancer patients has shown that it may be inhibited, may remain within normal limits, or activated [5, 13, 15]. This diversity of action of monocytes on tumor cells may be the result of a change in the profile of cytotoxic factors produced by them, such as active forms of oxygen (AFO) [6], tumor necrosis factor α (TNF-α), interleukin I, interferon (α/β), as well as unidentified cytotoxic mediators [7, 9]. Accordingly, the study of the profile of cytotoxic mediators of monocytes from patients with cancer and precancerous states may be of definite practical importance.

This paper describes a comparative study of spontaneous and stimulated production of cytotoxic mediators, cytotoxic factors with TNF-like activity [11], and AFO by peripheral blood monocytes from healthy persons and patients with cancer and precancerous conditions of the stomach.

EXPERIMENTAL METHOD

The investigation was carried out on monocytes obtained from venous blood of 28 individuals of both sexes aged from 30 to 60 years: seven healthy blood donors, nine patients with precancerous conditions of the stomach (chronic gastric ulcer, gastric polyps, chronic atrophic gastritis), and 13 patients with carcinoma of the stomach. Mononuclear cells were isolated from heparinized blood (10 U/ml) on a Ficoll density gradient (1.077 g/ml, 800g, 20 min). To obtain a cell population rich in monocytes (60-70%), centrifugation was repeated on a Percoll density gradient ("Pharmacia," Sweden; 1.067 g/ml, 400g, 30 min) [2]. The cells thus obtained ($200 \cdot 10^3$ cells/well) were cultured in 96-well flat-bottomed planchets ("Costar," USA) in medium RPMI-1640 ("Serva," West Germany) with the addition of 10% fetal calf serum ("Flow Laboratories," England) for 24 h (37°C, 5% CO_2) in the presence of 10 mg/ml of *E. coli* lipopolysaccharide ("Sigma," USA). TNF-like activity in the supernatant was determined in the cytotoxic test with fibroblastlike L929 cells, compared with recombinant human TNF-α ("Ferment" Research and Production Combine, Vilnius) [1]. The dilution of supernatant corresponding to 50% lysis of the cells was taken as one unit.

Production of AFO was recorded by a chemiluminescence method on the 1251 luminometer (LKB, Sweden) in the presence of luminol ("Sigma," USA) [10]. Luminol (100 μl) was added to a measuring vial containing 800 μl of a suspension of monocytes in culture medium (50% RPMI-1640 with 10% fetal calf serum and 50% Hanks' solution). After recording of the spontaneous chemiluminescence (average background value during 15 min, I_b, mV) 100 μl of opsonized zymosan (1 mg/ml) was added to the vial and recording was continued for 60 min. The data were collected and analyzed by IBM PC computer (USA), using the LUMOGRAF program (Tomsk). The maximal response (I_{max}, mV) and the light sum during 30 min from the time of addition of the stimulator (mV · min) were used as parameters of reactive chemiluminescence.

Research Institute of Oncology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 2, pp. 175-177, February, 1991. Original article submitted November 21, 1989.

 0007-4888/91/0002-0210$12.50

TABLE 1. TNF-Like Activity (U/ml of supernatant) and Parameters of Chemiluminescence (mV, mV·min·10^5) of Monocytes from Patients of Groups Tested

Parameters of chemiluminescence	Healthy subjects	Precancerous diseases of the stomach	Carcinoma of the stomach
Minus LPS	14,9±3,3	224,1±41,3	5,9±2,8
	n=5	n=9	n=11
	p 1,2<0,001	p 2,3<0,001	p 3,1<0,01
Plus LPS	30,2±4,4	276,9±51,1	10,0±3,3
	n=6	n=9	n=13
	p 1,2<0,001	p 2,3<0,001	p 3,1<0,001
I_b	0,8±0,1	1,3±0,3	1,9±0,3
(mV)	n=6	n=7	n=6
	p 1,2<0,05	p 2,3<0,05	p 3,1<0,05
I_{max}	143,3±7,7	111,3±21,4	383,0±162,5
	n=6	n=7	n=6
		p 2,3<0,05	p 3,1<0,05
Light sum in 30 min (mV·min·10^5)	1,6±0,2	1,5±0,3	5,3±2,3
	n=6	n=7	n=6
		p 2,3<0,05	p 3,1<0,05

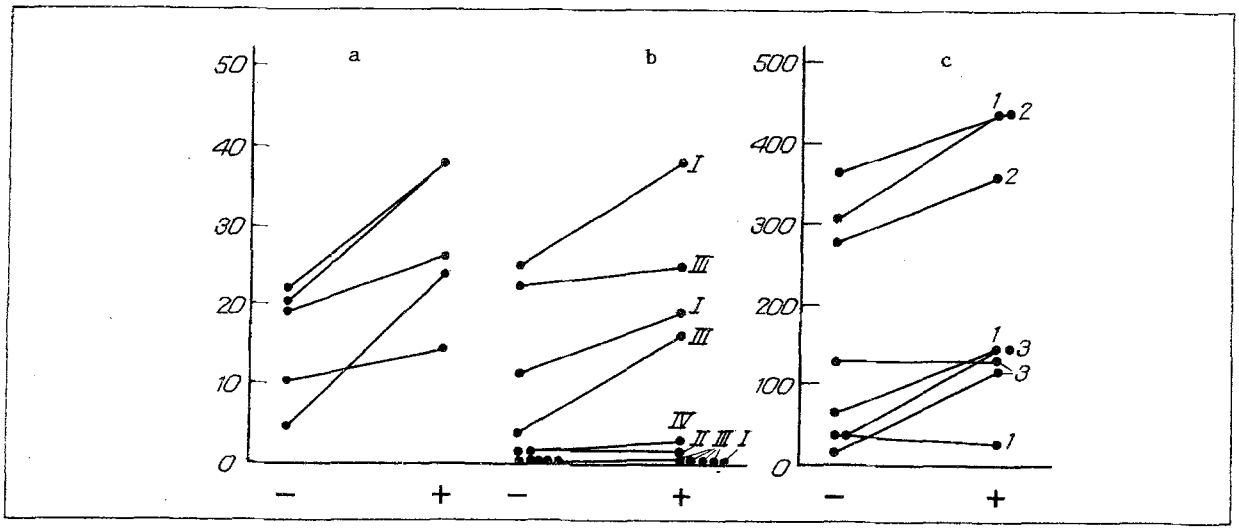

Fig. 1. Intraindividual comparison of spontaneous and LPS-stimulated production of TNF-like activity by monocytes: a) healthy individuals; b) patients with cancer (Stages I-IV); c) with precancerous states of the stomach (1 — ulcer, 2 — polyps, 3 — gastritis). Ordinate, activity in U/ml of supernatant.

EXPERIMENTAL RESULTS

The results were analyzed by Student's t-test. The experiments showed that maximal capacity for spontaneous production of TNF-like factors was possessed by monocytes from patients with precancerous conditions of the stomach. The content of cytotoxic factors in their supernatant was maximal. Even stimulation of healthy human monocytes by the lipopolysaccharide in vitro led to a level of production of TNF-like factors which was seven times lower (Table 1). The precancerous state is evidently such a powerful activating factor that additional stimulation by LPS in vitro did not lead to any further increase in the rate of production of cytotoxic factors, unlike the situation in healthy individuals and patients with carcinoma of the stomach. The increase in cytotoxic activity was perhaps the result of activation of synthesis and storage of mRNA-TNF-α in vivo in peripheral blood monocytes [14]. We know that TNF-α is a modulator of cellular immunity and a mediator of inflammatory reactions [3]. Thus an increase in spontaneous and LPS-stimulated production of TNF-α by monocytes is observed in various pathological processes, including chronic hepatitis [8] and pneumoconiosis [9]. For that reason an increase in production of TNF-α may reflect its involvement in the pathogenesis of precancerous states of the stomach.

Monocytes of patients with gastric cancer had minimal ability to produce TNF-α-like factors. The cytotoxic activity of their supernatant was three times less than that of healthy individuals. Moreover, in five of 13 patients with gastric cancer the TNF-like activity of the supernatant of their monocytes was under 1 U/ml (Fig. 1). The low level of cytotoxic activity may be the result of the presence of factors inhibiting presynthesis of mRNA-TNF-α in monocytes in vivo in the serum of these patients [12].

On the other hand, AFO production by monocytes of gastric cancer patients was increased by 2.4 and three times respectively, as reflected in the light sum of spontaneous and reactive chemiluminescence, compared with healthy individuals (Table 1). This is in agreement with the results of determination of AFO production by monocytes of gastric cancer patients in the reaction of reduction of nitro-BT [13].

Changes observed in the production of cytotoxic mediators are probably the cause of the increase in the cytostatic, but not cytolytic, activity of monocytes of gastric cancer patients [13, 15], and also of the change in their cytotoxic activity at different stages of carcinoma of the gastrointestinal tract [16]. The increase in AFO production by monocytes of gastric cancer patients together with reduced production of polypeptide cytotoxic factors may be an alternative mechanism of realization of their cytotoxic action. Changes revealed in the production of cytotoxic mediators by patients with cancer and precancerous states of the stomach provide a basis for the possible use of these parameters as additional differential diagnostic criteria.

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