ASSESSMENT OF ACTIVITY OF TUMOR NECROSIS FACTOR UNDER NORMAL CONDITIONS AND IN OVARIAN TUMORS

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Activated cells of the monocytic-macrophagal series, which possess direct cytotoxicity, are one of the factors of antitumor protection and cause lysis of tumor cells, and also act indirectly through the production of monokines, including tumor necrosis factor (TNF) [2, 3, 5]. The functions of TNF are not limited to cytolysis, but it also activates certain immunocompetent cells and stimulates the production of many cytokines and its own synthesis [6, 11, 12]. The biological effects of TNF and mechanisms of its production have been studied mainly in experimental models [4, 9, 10]. Data on the cytotoxic activity of TNF, obtained by the study of patients with neoplastic conditions, are extremely contradictory [4, 6-8], and the role of TNF in the immunopathogenesis of tumors is not clear; the mechanisms regulating its activity have not been adequately studied and the criteria which would allow activity of TNF to be used as a diagnostic test in clinical immunology have not yet been determined.

The aim of this investigation was to compare the cytotoxic activity of TNF of normal individuals and of patients with benign and malignant ovarian tumors.

EXPERIMENTAL METHOD

The subjects tested comprised 12 healthy blood donors (seven men and five women) aged from 26 to 60 years, 13 women aged from 38 to 80 years with malignant ovarian tumors (adenocarcinoma), and five women aged from 52 to 72 years with benign ovarian tumors (serous papillary cystadenoma).

Mononuclear cells (MNC) were isolated from peripheral blood by the method in [1].

Activity of TNF in supernatants of MNC was determined by the method in [5]. To obtain supernatant containing TNF activity a suspension of isolated MNC was adjusted to a concentration of 10^6/ml with medium 199, containing 10% embryonic calf serum ("Gibco," England) and gentamicin in a final concentration of 50 μg/ml. Before incubation the cell suspension was treated with lipopolysaccharide (LPS) from *Escherichia coli* (0111:B4, from "Sigma" USA) in a final concentration of 10 μg/ml. Supernatants from unstimulated cell cultures were used as the control. The cell suspensions were incubated for 24 h at 37°C in an atmosphere with 5% CO_2. At the end of the period of culture the supernatants were collected, purified by centrifugation (60 min at 400*g*), sterilized by filtration through a filter with pore diameter of 0.22 μ, and kept at −25°C until required for testing. As target cells we used transformed mouse fibroblasts of the L-929 line. The target cells were introduced into 96-well flat-bottomed plastic microplates ("Nunc", Denmark) in a concentration of $5 \cdot 10^5$/ml in a volume of 0.1 ml per well in order to obtain a firm monolayer. After 24 h a tenfold dilution of supernatants of the effector cells in a volume of 0.1 ml and actinomycin D ("Serva," West Germany) in a final concentration of 24 μg/ml in a volume of 0.1 ml were added to the monolayer. After culture for 18 h at 37°C the supernatant was removed and the plates were stained for 10 min with a 0.2% solution of crystal violet in 2% ethanol, after which the plates were washed with water and dried.

The optical density of the dye in the surviving monolayer was measured on a "Multiscan" instrument ("Labsystems," Finland) at a wavelength of 540 nm. The index of cytotoxicity was calculated by the equation: $IC = (1 - E/C) \cdot 100\%$, where IC

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TABLE 1. Cytotoxic Activity of TNF Determined by Ratio to Spontaneous Lysis of Target Cells

Groups tested, %	IC, %	
	spontaneous	induced
Blood donors (n = 12)	15,2±5,5	33,4±4,9
Malignant ovarian tumors (n=13)	38,4±4,7*	28,3±5,7
Benign ovarian tumors (n = 13)	20,7±10,5	33,7±9,8

Legend. *$p < 0.05$ indicates significant difference from control.

stands for the index of cytotoxicity, E the optical density (relative units) in the experimental well, and C the optical density in the well containing the control supernatant. To calculate IC the mean optical density of two or three measurements was calculated.

EXPERIMENTAL RESULTS

The cytotoxic activity of TNF from healthy blood donors was 28.4 ± 3.5%, in women IC was 26.5 ± 5.2%, and in men it was 29.9 ± 4.8% ($p > 0.05$), and for that reason, when the results were analyzed, we used one common value of IC for the whole group of healthy blood donors.

In the group of women with benign ovarian tumors IC was only half as high as in healthy blood donors, namely 15.0 ± 5.2% ($p < 0.05$). In patients with ovarian cancer IC was negative, its value being −10.9 ± 4.5% ($p < 0.05$). It will be seen from the formula used to calculate IC that a negative value of this parameter occurs when the ratio E/C exceeds 1, i.e., when the survival rate of the target cells in the control is lower than in the experiment. This means that the cytotoxic activity of the supernatant of the unstimulated MNC is higher than that of the stimulated MNC or, in other words, spontaneous production of TNF is higher than induced production. To obtain some idea of the level of spontaneous TNF activity we estimated IC of the supernatant of unstimulated MNC relative to spontaneous lysis of target cells in the medium. To do this, we calculated: IC (spontaneous) = $(1 - S/M) \cdot 100\%$, where S denotes the optical density in the well with the supernatant of unstimulated cells and M denotes optical density in the well with medium (spontaneous lysis of target cells).

In healthy blood donors and patients with benign ovarian tumors IC of spontaneous TNF activity was 15.2 ± 5.5 and 20.7 ± 10.5% respectively ($p > 0.05$) (Table 1). In patients with malignant tumors the same parameter was significantly higher than in the control, namely 38.4 ± 4.7% ($p < 0.05$), evidence of increased spontaneous production of TNF in this group.

For comparative analysis with the level of spontaneous TNF activity we estimated the induced cytotoxicity of the supernatant relative to spontaneous lysis of target cells in the medium also, using the following formula: $IC = (1 - IM) \cdot 100\%$, where I denotes the optical density in the well with supernatant of stimulated MNC, M the optical density in the well with medium (spontaneous lysis of target cells) (values of IC are given in Table 1). Supernatants of stimulated MNC had cytotoxicity twice as high as the supernatant of unstimulated cells in healthy donors. The value of IC was increased from 15.2 ± 5.5 to 33.4 ± 4.9% ($p < 0.05$). Conversely, in patients with neoplastic processes of the ovaries levels of spontaneous and induced cytotoxicity did not differ significantly; in patients with ovarian carcinoma, moreover, a tendency, paradoxically as it may seem, was observed for cytotoxicity of the supernatant of the stimulated cells to decrease. This decrease in the responsiveness (benign tumors) and nonresponsiveness (malignant tumors) of MNC to stimulation by LPS with increased production of TNF was evidence of disturbance of the functioning of these cells. On the other hand, stimulation of mononuclears by LPS could evidently lead to the production not only of cytokine, but also of suppressor factors, reducing the cytotoxic action of the supernatant on target cells. Prostaglandins may be one such factor [3]. To study the role of prostaglandins, indomethacin was added together with LPS to MNC from patients with ovarian cancer. The presence of indomethacin in the culture medium of the MNC did not affect spontaneous cytotoxicity of the cell supernatants. In the absence of indomethacin IC was 25.7 ± 2.5%, but after the addition of indomethacin it was −13.7 ± 7.1% ($p > 0.05$). Conversely, the cyeotoxic activity of supernatants of stimulated cells was increased in the pzesence of indomethacin from −11.0 ± 2.0% to −0.37 ± 2.0% ($p < 0.05$). The results are evidence that in ovarian cancer there is an imbalance in TNF and P_9E production by the monocytes, with predominance of the latter.

Determination of TNF activity can thus be used as a test of function of the immune system in patients with neoplastic conditions, but the mechanisms of regulation of TNF activity require further study.

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DETERMINATION OF TROPHOBLASTIC β_1-GLYCOPROTEIN IN TUMOR TISSUE AND BLOOD SERUM IN OVARIAN CANCER

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The specific protein of pregnancy, trophoblastic β_1-glycoprotein (TBG), is known as a biological marker of trophoblastic tumors [7]. In some cases, however, TBG can be found in the blood of patients with nontrophoblastic tumors [12, 13]. There is also evidence of detection of TBG by the immunoperoxidase method in the tissue of an ovarian adenocarcinoma [11]. There is no general agreement on the serum TBG concentration in ovarian cancer. An increase in the seruin TBG concentration to 3-10 μg/liter in ovarian cancer has been found by radioimmunoassay in 16.7% [12] of cases, and up to 12-100 μg/liter in 75% [13].

This paper gives the results of immunochemical identification of TBG in tumor tissue and blood serum in ovarian cancer.

EXPERIMENTAL METHOD

Extracts from tumor and normal tissues were prepared under standard conditions: Tris-glycine buffer (pH 8.3) was added to a weighed sample of tissue in the ratio (*v/w*) of 2:1. Various fractions were obtained from tumor extracts by precipitation with

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