EFFECT OF TUMOR CELL IMMUNOSUPPRESSIVE FACTORS ON LYMPHOCYTIC AND MACROPHAGAL CYTOKINE PRODUCTION AND ON MITOGENIC ACTIVITY OF INTERLEUKIN-2

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Tumor growth is accompanied by suppression of functional activity of macrophages, natural killer cells, and T and B lymphocytes, which correlates with disturbance of production and reception of monokines and lymphokines [10]. The mechanisms of action of the tumor on cytokine production have not yet been explained. In this connection research workers are increasingly turning their attention to humoral products of neoplastic cells: immunosuppressive factors (ISF) inhibiting many of the functions of lymphocytes and macrophages [6, 7]. The possible participation of these substances in the disturbance of cell-cytokine interactions during tumor growth is being vigorously studied.

The aim of this investigation was to study the effect of ISF of mastocytoma P-815, leukemia EL-4, and melanoma B16 cells on production of interleukin-1 (IL-1) by immunocytes (macrophages) and of interleukin-2 (IL-2) by splenocytes, and also on the mitogenic activity of IL-2.

EXPERIMENTAL METHOD

Cells of mastocytoma P-815, Leukemia EL-4, and melanoma B16 were cultured in vitro. The sources of ISF were 24-h cultural supernatants of the tumor cells (initial seeding density $1 \cdot 10^6$ cells/ml). Experiments were carried out on male BALB/c mice from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. The mice were killed by cervical dislocation, the organs were removed aseptically, and cells were obtained in glass homogenizers and washed three times in medium 199 with 10% inactivated bovine serum (washing medium). Macrophages were isolated from peritoneal exudate cells (PEC) three days after intraperitoneal injection of 5 ml of 3% peptone solution into the mice. The PEC were introduced into wells of a 24-well plate ("Linbro") in a dose of $5 \cdot 10^6$ cells per well, they were adsorbed on plastic for 2 h at 37°C, and nonadherent cells were removed by washing the monolayer three times with warm washing medium. The cells were cultured in medium RPMI-1640 "Flow Laboratories"), with the addition of 5% fetal calf serum ("Flow"), L-glutamine (2 mM), HEPES-buffer ("Flow," 25 mM), 2-mercaptoethanol ("Serva," $5 \cdot 10^{-5}$ M), and gentamicin (50 μg/ml). The cells producing IL-1 were peritoneal macrophages, and those producing IL-2 were splenocytes of BALB/c mice. They were exposed beforehand to ISF (culture medium in the control) for 24 h at 37°C in an atmosphere of air with 5% CO_2. At the end of incubation the cells were washed three times, their concentration was adjusted to $5 \cdot 10^6$/ml, and inducers of production of IL-1 (lipopolysaccharide — LPS, "Difco," 20 μg/ml) or of IL-2 (concanavalin A — con A, "Pharmacia," 5 μg/ml) were added. After culture for 24 h the cell-free supernatants, which were to be the sources of cytokines, were collected. The con A contained in the samples was inactivated by the addition of 0.2 M α-methyl-D-mannoside ("Sigma"). Activity of IL-2 in the samples was estimated by induction of proliferation of 96-h con A-blast cells of BALB/c mice [3]. IL-1 was tested by the mitogenic test on thymocytes of BALB/c mice [15]. The mitogenic activity of IL-2 was determined by

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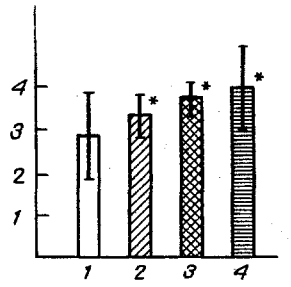

Fig. 1

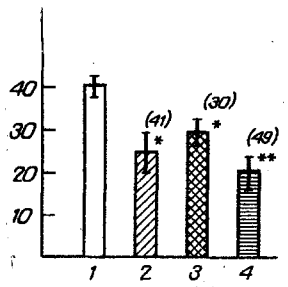

Fig. 2

Fig. 1. Effect of ISF of tumor cells on IL-1 production by peritoneal macrophages of BALB/c mice. Ordinate, index of proliferation of thymocytes in response to IL-1; macrophage pretreatment options: 1) culture medium; 2) ISF of P-815; 3) ISF of EL-4; 4) ISF of B16. *$p > 0.05$. Results of 5 experiments given (M ± m).

Fig. 2. Inhibition of IL-2 production by spleen cells of BALB/c mice after preliminary treatment with ISF of tumor cells. Ordinate, index of proliferation of con A — blast cells in response to IL-2. Splenocyte pretreatment options: 1) culture medium; 2) ISF of P-815; 3) ISF of EL-4; 4) ISF of B16. *$p < 0.05$; **$p < 0.01$. Index of inhibition (in per cent) shown in parentheses. Results of 3 experiments given (M ± m).

adding 96-h con A-blast cells to wells of a 96-well flat-bottomed plate ("Linbro," $5 \cdot 10^4$ cells per well) together with recombinant human IL-2 ("Biogen" Interdepartmental Scientific Technical Combine, Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, 100 U/well). After incubation of the plate at 37°C for 24 h in an atmosphere of air with 5% CO_2 the contents of the wells were transferred to glass-fiber filters ("Flow") by means of a harvester and the samples were counted on a beta-spectrometer (LKB). The effect of ISF on cytokine production and mitogenic activity of IL-2 was estimate with the aid of an inhibition index (II), calculated by the equation: $II = [(A - B)/A] \cdot 100$, where A and B denote incorporation of ^3H-thymidine (in cpm) in the control (pretreatment with culture medium) and experimental (pretreatment with ISF) samples.

EXPERIMENTAL RESULTS

The present writers [5] and others [6, 12] showed previously that tumor cell ISF inhibits most strongly the proliferative response of mouse splenocytes to mitogens and alloantigens on the addition of these substances in the early stage (0-48 h) of culture. Products of P-815 cells suppressed the formation of cytotoxic T lymphocytes in mixed lymphocyte culture but did not affect the lytic activity of mature allogeneic killer cells [2]. The inhibitory action of ISF was exhibited even during short-term incubation with splenocytes followed by intensive washing of the cells [5]. It has been suggested that tumor products may interfere with the monokine — lymphokine cascade of lymphocyte activation.

To confirm this hypothesis we studied the effect of ISF on IL-1 and IL-2 production by macrophages and splenocytes respectively, and also on the mitogenic activity of IL-2. The method of preliminary treatment of the ISF-producing cells for 24 h followed by washing off and the addition of inducers of IL-1 (LPS) or IL-2 (con A) synthesis, was used. The data in Fig. 1 show that tumor products did not depress the ability of macrophages to produce IL-1. Meanwhile these substances strongly inhibited IL-2 production (Fig. 2). The ISF of B16 and P-815 cells exhibited maximal suppressor action (II 49 and 41%); the inhibitory effect of humoral factors of EL 4 cells was weaker.

Proliferation and differentiation of lymphocytes depends not only on the level of key cytokine production, but also on the ability of the cells to respond to them [1]. It was accordingly important to discover whether ISF can also be involved in the cell response to particular cytokines. We studied the effect of the tumor products on mitogenic activity of IL-2. As the model we used the test of induction of lymphoblast proliferation under the influence of exogenous IL-2. We know that

TABLE 1. Effect of ISF of Tumor Cells on Mitogenic Activity of Human Recombinant IL-2

ISF (1:40)	IL-2 (100 units per well)	Proliferation of con A-blasts (incorporation of ^{3}H-thymidine, cpm), M ± m	IP	II,%
—	—	763±123	—	—
—	+	34 729±2033	46*	—
ISF P-815	+	23 650±1599	30*	35
ISF EL-4	+	697±105	0,9**	98
ISF B16	+	1 171±343	1,5**	97

Legend. IP) Index of proliferation. *$p < 0.05$, **$p < 0.001$. Results of one typical experiment are shown.

96-h con A-blasts leave their proliferative cycle, but remain on the surface of the receptor for IL-2, and on the addition of this cytokine they proliferate intensively [3]. Combined culture of lymphoblasts with exogenous recombinant IL-2 was carried out in the presence of ISF (in the presence of culture medium in the control). The inhibitory effect of the tumor products was judged by the decrease in IL-2-dependent proliferation. Products of all three tumors were found to inhibit the mitogenic activity of IL-2 (Table 1). Judging by data in the literature, the tumor products did not disturb binding of IL-2 with receptors, but prevented activation of the lymphocyte at other levels [14]. The writers showed previously that the action of ISF can be recorded by phorbol-myristate acetate, ionomycin, or a combination of both [11]. This indicates an affect of ISF on phosphoinositol phosphate metabolism [5]. The action of tumor products on internalization of the IL-2 — receptor for IL-2 complex, on protein kinase C activity, and so on, likewise cannot be ruled out. These hypotheses will be topics for further research.

Thus ISF from tumor cells inhibited IL-2 production by splenocytes, inhibited the mitogenic activity of IL-2, but did not affect IL-1 production by macrophages. The writers showed previously that tumor products suppress secretion of tumor necrosis factor (TNF) by macrophages [9]. TNF is known to be a growth factor for thymocytes, T lymphocytes, and T-cell lines [13], and it is also involved in the regulation of expression of the α-chain of the IL-2 receptor [8]. Accordingly, despite the fact that IL-1 can still be produced, the action of ISF on macrophages may disturb the monokine lymphokine cascade of lymphocyte activation and may lead to depression of IL-2 production and of the response to it. Another possible indirect mechanism of inhibition of IL-2 production is inhibition of the producers of IL-2 by suppressor cells activated by ISF [5]. The possibility of direct inhibition of cells producing IL-2 by tumor products likewise cannot be ruled out.

A decrease in production of IL-2 and its mitogenic activity is a characteristic property of the ISF of many strains of tumor cells, such as murine sarcomas, melanomas, T-leukemia, and HeLa cells [7, 10]. Tumor products inhibiting IL-1 synthesis by macrophages and monocytes [5, 6] and inhibiting the mitogenic action of IL-1 [7] also are known. Thus the intervention of ISF of tumor cells in the monokine-lymphokine cascade of lymphocyte activation is an important mechanism of their inhibitory effect, and one which evidently makes an important contribution to the process of "escape" of tumors from immunologic surveillance.

LITERATURE CITED

1. B. D. Brondz, T Lymphocytes and Their Receptors in Immunologic Recognition [in Russian], Moscow (1987).
2. A. E. Medvedev, W. Dostman, and A. Flegel, Byull. Éksp. Biol. Med., No. 8, 224 (1989).
3. G. T. Sukhikh, V. V. Malaitsev, and I. M. Bogdanova, Dokl. Akad. Nauk SSSR, **278**, No. 9, 762 (1984).
4. E. Farram, M. Nelson, D. S. Nelson, and D. K. Moon, Immunology, **46**, 603 (1982).
5. B. B. Fuchs, A. L. Rakhmilevich, and A. E. Medvedev, 9th European Congress of Immunology (1988), p. 245.
6. H. Fujiwara, Z. Toossi, K. Okhnishi, et al., J. Immunol., **138**, 197 (1987).
7. P. Hersey, C. Bindon, M. Czerniecki, et al., J. Immunol., **131**, 2837 (1983).
8. J. W. Lowenthal and W. C. Green, Proc. Nat. Acad. Sci. USA, **86**, 2331 (1989).
9. A. E. Medvedev, B. B. Fuks, and A. L. Rakhmilevich, Biomed. Sci., **1**, 261 (1990).
10. M. Nelson and D. S. Nelson, Immunol. Cell Biol., **66**, 97 (1988).

11. A. L. Rakhmilevich, B. B. Fuks, A. E. Medvedev, and A. I. Shapoval, European Federation of Immunological Societies, 10th Meeting (1990), p. 34b-30.
12. Z. Ramic, M. Lazic, M. Mostarica-Stojkovic, et al., European Federation of Immunological Societies, 10th Meeting (1990), p. 34b-31.
13. G. E. Ranges, M. P. Bombara, R. A. Aiyer, et al., J. Immunol., **142**, 1203 (1989).
14. C. Siepl, S. Bodmer, K. Erei, et al., Eur. J. Immunol., **13**, 593 (1988).
15. T. Uede, H. Kohda, Y. Ibayashi, et al., J. Immunol., **135**, 3243 (1986).

DETECTION OF ANTIBODIES TO CROSS-REACTING BACTERIAL ANTIGENS IN CANCER PATIENTS

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Microorganisms belonging to different taxa contain a wide range of antigens whose epitopes cross-react with antibodies to differential antigens of normal tissues and also with antibodies to tumor-associated antigens [4]. For instance, cross reactions have been found between BCG antigens and experimental human hepatocarcinomas and melanomas [9], and between *Streptococcus pyogenes* antigens and hamster fibrosarcomas and human basal-cell carcinomas [1, 7]. Data showing a high level of homology of products of oncogenes of the ras type with proteins of microorganisms, whose synthesis is coded by analogous sequences, are particularly interesting [10]. The most extensive studies in the field of cross reactions between microorganisms and tumors have been undertaken with the saprophytic microorganism *Bacillus megaterium* H, whose cells have been shown by immunofluorescence and immunoelectron microscopy and by other methods to contain an antigen cross-reacting with monospecific sera against various human tumors [4].

The facts described above served as a basis for the present investigation, with the aim of studying the serologic activity of serum from patients with cancerous and noncancerous diseases of the gastrointestinal tract and from animals with tumors toward *B. megaterium* H antigen, using enzyme immunoassay as the test.

EXPERIMENTAL METHOD

The test object consisted of blood serum from 217 persons, of whom 30 were healthy blood donors, 160 were patients in the Gastroenterology Department of Novosibirsk No. 18 Hospital, and 27 were patients with malignant tumors under treatment at the Novosibirsk No. 1 City General Hospital. Sera from 16 A/Sn mice, into which $1 \cdot 10^5$ ascites syngeneic B-lymphoma cells had been transplanted intraperitoneally, and sera from 6 Balb/c mice in which fibrosarcoma formation had been induced by intramuscular application of methylcholanthrene (1 mg), also were tested.

A glycoprotein with mol. wt. 65-70 kD, isolated by preparative electrophoresis from growth medium of *B. megaterium* H, homogeneous in polyacrylamide gel density gradients and cross-reacting with monospecific rabbit serum against a human gastric tumor, was used as antigen.

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