EFFECT OF BLOOD SERUM FRACTIONS FROM CANCER PATIENTS ON LEUKOCYTE MIGRATION FROM CAPILLARY TUBES

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In more than half of the cases studied the blood serum from patients with carcinoma of the stomach and urinary bladder inhibited migration *in vitro* of autologous leukocytes, leukocytes from blood donors and control patients, and also guinea pig macrophages. Chromatography of these sera on Sephadex G-100 showed that activity inhibiting leukocyte migration was present in fraction I (mol. wt. over 100,000 daltons) and in fractions IV and V (mol. wt. under 30,000 daltons). The blood serum of cancer patients and its fractions did not abolish inhibition of leukocyte migration induced by tumor antigens, by contrast with leukocyte migration in medium with control serum without antigens. It is suggested that activity of fraction I inhibiting leukocyte migration is due to an antigen–antibody complex, whereas in IV and V it is due to a factor similar in its properties to the factor secreted *in vitro* by lymphocytes stimulated by antigens or mitogens.

KEY WORDS: *carcinoma of the stomach; carcinoma of the urinary bladder; blood serum factor inhibiting leukocyte migration in vitro.*

The blood serum of patients with cancer can inhibit cellular reactions of immunity *in vitro*. It blocks the cytotoxic action of lymphocytes on tumor target cells [9-11] and can abolish the inhibition of migration of leukocytes of cancer patients by antigens prepared from the corresponding tumors [1, 4, 12].

The object of the present investigation was to study the effect of blood serum fractions from patients with carcinoma of the stomach and urinary bladder on migration of the leukocytes from these patients and control subjects *in vitro* in medium without antigens and in the presence of tumor antigens.

EXPERIMENTAL METHOD

The blood plasma and serum from 40 patients with carcinoma of the stomach in stages III IV and three patients in stage II and from 15 patients with carcinoma of the urinary bladder in stages II-IV and from 12 blood donors were tested for their effect on migration of leukocytes from blood donors, autologous leukocytes, and peritoneal exudate macrophages of guinea pigs from glass capillary tubes. The leukocytes were isolated from heparinized blood [5] and the macrophages were obtained on the fourth day after intraperitoneal injection of 15 ml sterile mineral oil into guinea pigs; the cells were suspended in medium No. 199 in concentrations of 5-10 million cells/ml. The test and control sera were added to equal portions of these leukocytes up to concentration of 20 and 10%. Five capillary tubes were filled with each portion of leukocytes [5] and dipped into wells containing medium with the same serum as was used for addition to the leukocyte suspension. The plates were incubated for 24 h at 37°C. The number of cells leaving the capillary tubes into the wells with the medium was then counted [5] and the migration inhibition index (MII) calculated:

$$MII = 100\% - \frac{\text{Mean number of cells in wells from test serum}}{\text{Mean number of cells in wells with control serum}} \times 100\%$$

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TABLE 1. Effect of Blood Serum from Cancer Patients on Migration of Autologous and Donors' Blood Leukocytes and Guinea Pig Macrophages

Blood serum (from whom obtained)	Number of sera	Indicator leukocytes	Effect on migration of leukocytes		
			inhibition	stimulation	no effect
Patients with carcinoma of the stomach	43	Autologous	25	5	13
	38	Allogeneic	22	4	12
Patients with carcinoma of the urinary bladder	15	Autologous	7	2	6
	14	Allogeneic	6	2	7
	12	Guinea pig macrophages	5	—	7
Healthy donors	12	Allogeneic	—	—	12
		Guinea pig macrophages	—	—	10

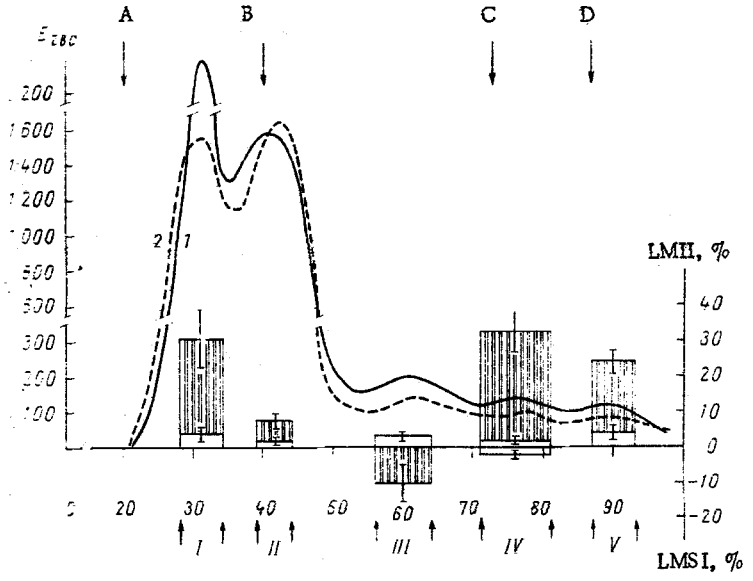

Fig. 1. Effect of blood serum fractions from patients with carcinoma of the stomach on migration of donors' leukocytes. 1) Elution curve of serum protein of cancer patients; 2) the same for serum of healthy donors. I-V) Blood serum fractions collected from tubes of corresponding numbers (abscissa). LMII and LMSI indices of inhibition and stimulation respectively of leukocyte migration by blood serum fraction from 12 cancer patients (shaded columns) and 4 healthy donors (unshaded columns) with confidence intervals at p = 0.05. Elution peaks of: A) Blue dextran (mol. wt. 2 million daltons); B) bovine serum albumin (mol. wt. 67,000 daltons); C) trypsin (mol. wt. 24,000 daltons); D) ribonuclease (mol. wt. 13,600 daltons).

The significance of differences between the means was determined by Student's method.

Sera inhibiting migration and control sera were chromatographed on Sephadex G-100 in phosphate buffer, pH 7.0. The columns were calibrated with blue dextran and proteins with known molecular weight. The fractions were dialyzed against distilled water and concentrated so that the volume of each fraction was not less than half the volume of serum applied to the column. Dry Eagle's medium was added to the fractions, which were sterilized by filtration through a 0.3 μ filter, to the physiological concentration, after which they were tested

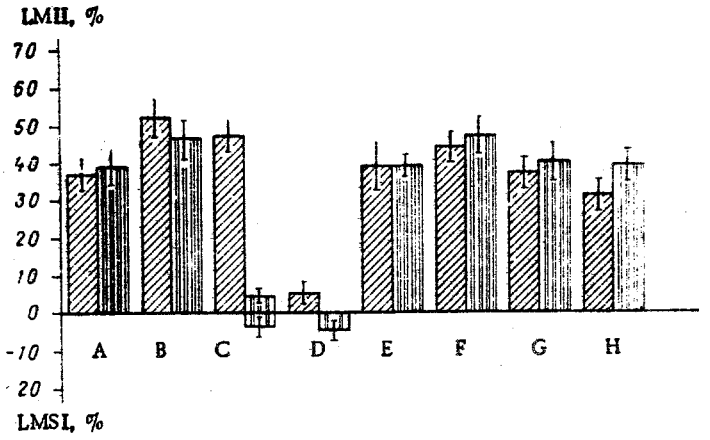

Fig. 2. Effect of active fractions of blood serum from cancer patients and of gastric carcinoma antigens on leukocyte migration. Obliquely shaded columns represent leukocytes of six cancer patients; vertically shaded columns leukocytes of six healthy donors. SMII and SMSI) indices of inhibition and stimulation respectively of leukocyte migration. A) Fraction I, B) fraction IV of blood serum of 6 cancer patients; C) gastric carcinoma antigens; D) antigens of normal gastric mucosa adjacent to tumor; E) fraction I of blood serum of cancer patients and antigens.*

in various dilutions (from 9.0 to 0.002 mg/ml protein) for their effect on migration of the indicator leukocytes, in the same way as the blood serum.

The effect of blood serum fractions from cancer patients on inhibition of leukocyte migration (ILM) induced by the antigens of the same tumor also were studied. Suspensions of leukocytes from cancer patients and control subjects were made up in medium containing different fractions of blood serum, as indicated above. Saline extracts prepared from tumors and adjacent areas of normal gastric mucosa were added in a dose of 0.1-0.5 mg/ml protein to the leukocytes, capillary tubes were filled with the samples, and the ILM test carried out [5] in the same way as with the leukocytes of other cancer patients [4, 6].

EXPERIMENTAL RESULTS

Autologous blood plasma from patients with carcinoma of the stomach and urinary bladder inhibited leukocyte migration compared with migration in medium containing blood plasma or serum from group IV blood donors or bovine blood serum. Blood plasma and serum from cancer patients had a similar action on leukocytes of donors with noncancerous diseases and on guinea pig macrophages (Table 1). In only 5 of 43 cases did plasma from cancer patients selectively inhibit migration of autologous leukocytes, and in 4 of 38 cases this selectivity was observed in relation to allogeneic leukocytes. Usually MII was 15-40%, less frequently 60%. Occasionally blood serum from cancer patients stimulated migration of the indicator leukocytes (Table 1). There was no difference in the degree of ILM when sera from patients with cancer in stages II and IV were tested.

The blood serum from 12 patients with carcinoma of the stomach, 2 patients with carcinoma of the urinary bladder, and 4 blood donors was chromatographed on columns with Sephadex G-100. The chromatographic spectrum of the blood sera from the cancer patients was heterogeneous. Usually five protein fractions with different molecular weights could be obtained (Fig. 1). However, in 4 cases fraction III (mol. wt. 50,000-40,000 daltons) was not detected in the serum of the cancer patients. The blood serum of healthy donors had less protein in fractions IV and V than the serum of cancer patients.

*Descriptions of F), G), and H) ommitted in Russian original — Consultants Bureau.

Fraction I (mol. wt. over 100,000 daltons) of the blood serum of cancer patients induced ILM in a protein concentration of not less than 1 mg/ml (in 6 cases, 3-9 mg/ml. Fraction II inhibited migration of indicator leukocytes in only 2 of 12 experiments in protein concentrations of 2.7 and 5.2 mg/ml. In 3 cases a tendency was observed for leukocyte migration to be stimulated by the proteins of this fraction. Fraction III (mol. wt. 50,000-40,000 daltons) in 2 of 8 cases stimulated, but did not inhibit leukocyte migration. Fractions IV and V (mol. wt. 10,000-30,000 daltons) had the strongest inhibitory effect on leukocyte migration. This inhibitory activity was observed in a much lower protein concentration (0.6-.03 mg/ml) than in fraction I. No fraction had a cytotoxic action on the leukocytes, for the cells remained viable in the trypan blue test.

Activity inhibiting migration of test leukocytes was thus present in fractions I, II and V of the blood serum of cancer patients. In fraction I this activity was evidently due to an antigen–antibody complex, which can inhibit migration of guinea pig macrophages [13] and blood leukocytes [7, 15]. As regards fractions IV and V, their ability to inhibit leukocytemigration could be due to substances resembling the factors inhibiting migration of macrophages and leukocytes (MIF, LIF) and isolated from the supernatant of lymphocytes stimulation *in vitro* [3, 16]. Activity similar to these factors has been found in the blood sera of patients with lymphoproliferative [8] and chronic inflammatory diseases. After injection of a specific antigen into patients allergic to it, similar factors also appear in their blood serum [2]. However, in cancer this factor was absent in fractions with mol. wt. of 40,000-50,000 daltons and was detected only in fractions of low molecular weight (under 30,000 daltons).

The effect of blood serum fractions from patients with carcinoma of the stomach on ILM of these patients induced by the antigen of the same tumor also was studied. Both the original serum of the cancer patients and its active fractions induce the same ILM as the tumor antigen. Addition of the serum and its fractions with antigens did not potentiate and did not abolish ILM by comparison with leukocyte migration in medium with control serum and without antigen (Fig. 2). Consequently, only when migration of leukocytes of cancer patients in medium with their plasma (serum) and without antigens is compared with their migration in medium with the same plasma and with tumor antigens was this false effect of abolition of ILM observed. This effect was due to the ability of the blood plasma (serum) of cancer patients without antigens to inhibit leukocyte migration. This ability of plasma may be due to the presence of an antigen–antibody complex in it (ILM of fraction I) or the presence of activity similar to the migration-inhibiting factors.

The latter could appear in the blood as a result of prolonged stimulation of the patients' lymphocytes *in vivo* by specific tumor antigens or (and) other antigens accompanying growth of the tumor. Leukocytes of cancer patients, after adsorbing such a factor from the blood, evidently sometimes become unable to react by inhibition of migration to tumor antigens *in vitro*, for their migration is already inhibited *in vivo*. This phenomenon can explain the negative results of the ILM test in some cancer patients [1, 4, 6]. Factors inhibiting the cytotoxic effect of lymphocytes, their blast transformation, and their ability to form antibodies have also been found in the blood serum of cancer patients. These factors may be antigen–antibody complexes [9, 11], α globulin, or substances present in its fraction [14].

The blood serum of cancer patients thus contains qualtitatively different factors which affect the reactivity of lymphocytes and other leukocytes.

LITERATURE CITED

1. V. I. Govallo, M. P. Grigor'eva, and G. A. Kosmiadi, Vopr. Onkol., No. 11, 18 (1973).
2. A. D. Gorbach, D. K. Novikov, and I. N. Gorbach, Zh. Mikrobiol., No. 4, 106 (1977).
3. A. A. Mikhailova and R. V. Petrov, in: Progress in Science and Technology. Series: General Problems in Pathology [in Russian], Vol. 5, Moscow (1977), pp. 7-34.
4. D. K. Novikov and G. P. Adamenko, Dokl. Akad. Nauk SSSR, 207, 715 (1974).
5. D. K. Novikov, G. P. Adamenko, and V. I. Novikova, Byull. Éksp. Biol. Med., No. 6, 707 (1976).
6. D. K. Novikov, G. P. Adamenko, and N. P. Dainyak, Zh. Ushn. Nos. Gorl. Bol., No. 1, 31 (1977).
7. V. I. Novikova and D. K. Novikov, Sov. Med., No. 8, 31 (1975).
8. S. Cohen et al., New Engl. J. Med., 290, 882 (1974).

9. J. Hellström et al., Proc. Nat. Acad. Sci. USA, 62, 362 (1969).
10. J. Hellström et al., Int. J. Cancer, 11, 280 (1973).
11. S. M. Jagarlamoody, et al., Proc. Nat. Acad. Sci. USA, 68, 1346 (1971).
12. P. Khaer, Acta Path. Microbiol. Scand., 382, 294 (1974).
13. P. Kotkes and E. Pick, Clin. Exp. Immunol., 35, 1489 (1975).
14. B. R. Nimberg et al., Cancer Res., 35, 1489 (1975).
15. T. Raskalen and J. Wasseman, Int. Arch. Allergy, 42, 790 (1971).
16. R. E. Rocklin, J. Immunol., 112, 1461 (1954).

CHANGES IN RESISTANCE OF MICE OF VARIOUS STRAINS TO TUMORS UNDER THE INFLUENCE OF IMMUNOLOGIC FACTORS

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In experiments on mice with administration of a carcinogen in the early period after immunization (BCG vaccine, Freund's complete adjuvant) it was shown that an increase or decrease in the resistance of the animal to tumors may be determined by hereditary features. Antitumor resistance was reduced under these circumstances only in mice of strains in which a known predisposition to the formation of a state of allergy to tuberculin has been observed. In the late stages after immunization, when the intensity of the allergic component of reactivity was reduced, antitumor resistance was substantially higher than initially.
KEY WORDS: *heredity; immunologic factors; allergy; antitumor resistance.*

The nature of changes in the functional state of an animal after exposure to various conditions is largely determined by genetic factors [6, 7, 11, 12], and this must be reflected in the character of changes in antitumor resistance. The object of the present investigation was to study this problem after immunologic procedures.

EXPERIMENTAL METHOD

Experiments were carried out on mice of various lines (C3HA, C3H/He, BALB/c, C57BL/6) and noninbred mice, 519 animals altogether. The experimental animals were immunized at the age of 3-4 months. The immunizing agent used was BCG, either alone or as a constituent of Freund's complete adjuvant (FCA). The concentration of mycobacteria in the FCA was 10 mg/ml. BCG vaccine was injected intraperitoneally in doses of 0.01 or 1 mg in 0.2 ml physiological saline. The FCA was injected into the footpad of one hind limb in a dose of 0.02 ml. The carcinogen was administered 13 days after FCA or 30 days after vaccination. These times of administration of the carcinogen were based on the fact that they correspond to the state of the strongest response of the animal to the immunologic stimulus [1, 3, 9, 10].

Changes in resistance to the development of tumors also were studied in C57BL/6 mice in experiments in which the carcinogen was administered 70 days after FCA, when the immunologic response was mainly at an end and, in particular, antibody formation was on a reduced scale [3, 10].

In all series of experiments the carcinogen (20-methylcholanthrene) was injected into the soft tissues of the thigh in a dose of 1 mg in 0.1 ml purified vegetable oil.

Tuberculin tests were used as the control of changes in reactivity. Tuberculin was injected into a footpad of a hind limb in a dose of 0.02 ml of a solution containing 50,000 tuberculin units/ml. The reactions to tuberculin were recorded in points, and two degrees of intensity were distinguished: hyperemia of the footpad and part of the medial surface of the

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