RESTORATION OF ANTITUMOR REACTIVITY OF LEUKOCYTES OF CANCER PATIENTS AFTER PREINCUBATION

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Specific antitumor sensitization in patients with gastric cancer was detected by the leukocyte migration inhibition test. After preincubation for 24 h at 4°C in serum-free medium, areactive leukocytes from patients with gastric cancer in stages III-IV reacquired the ability to react specifically to allogenic antigens of a tumor in the same location. Preincubation did not affect inhibition by cancer antigens of migration of leukocytes from patients with noncancerous diseases. The supernatants of preincubated leukocytes from cancer patients contained substances inhibiting migration of the indicator leukocytes.

KEY WORDS: leukocytes; cancer antigens; leukocyte migration inhibition.

A progressively growing tumor causes inhibition of the patient's immune reactions [2, 9] and this is an obstacle to successful immunotherapy and immunodiagnosis of cancer. An important role in the depression of cellular reactions of immunity is played by blood serum factors of cancer patients absorbed on leukocytes [4, 6, 10]. The reactivity of the patients' leukocytes can thus be enhanced or restored after procedures deblocking their receptors [8, 17].

The object of this investigation was to study the possibility of deblocking receptors of leukocytes of cancer patients and restoring their reactivity.

EXPERIMENTAL METHOD

Leukocytes were isolated from 7-10 ml of heparinized blood taken the day before operation from patients with cancer of the stomach, patients with noncancerous diseases, and healthy blood donors [4, 5]. The leukocytes (5-10 million/ml) were used for the leukocyte migration test (LMIT) immediately after being washed twice with medium 199 or they were incubated for 24 h at 4°C in medium 199 either without serum or in the presence of 1% bovine serum (BS). Another portion of the leukocyte suspension was incubated in autologous plasma. After incubation, the supernatant was discarded and the leukocytes were washed twice with medium 199 and diluted in the same medium with 20% BS to a concentration of 5-10 million cells/ml. Antigens of cancer or normal mucosa of the stomach (0.05-0.2 mg/ml protein) were added to different portions of this leukocyte suspension. No antigen was added to the control. In some experiments the leukocytes were incubated with an equal volume of the supernatants after preincubation of the leukocytes for 30 min at 37°C, washed once with medium 199, and used in the LMIT with the antigens [4, 5]. The antigens consisted of saline extracts of allogeneic tumors and adjacent areas of mucosa, obtained during operation. Glass capillary tubes were filled with leukocyte suspensions, dipped in wells in plates containing the same medium as the capillary tubes, and incubated for 24 h at 37°C. The number of cells migrating from the capillary tubes was then counted and values of the leukocyte migration inhibition index calculated [4, 5].

EXPERIMENTAL RESULTS

Altogether, 95 patients with gastric cancer in stages I-IV were studied (7 in stage I, 10 in stage II, 43 in stage III, 35 patients in stage IV). The control group consisted of 110 patients (61 with gastric and duodenal ulcer, 7 with chronic gastritis, 6 with benign tumors of the stomach, 36 with other diseases or healthy subjects).

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TABLE 1. Effect of Preincubation of Leukocytes from Gastric Cancer Patients in Medium without Autologous Plasma on Inhibition of their Migration by Gastric Cancer Antigens

Antigen	Number of patients	Before preincubation			After preincubation		
		ILM	SLM	no effect	ILM	SLM	no effect
Gastric cancer	35/30	10/2	5/4	20/24	29/3	1/1	5/26
Gastric mucosa	35/30	6/0	7/4	22/26	8/3	3/3	24/24

Legend. Numerator gives number of patients with gastric cancer, denominator number of control patients. ILM) Inhibition of leukocyte migration, SLM) stimulation of leukocyte migration.

TABLE 2. Effect of Supernatant of Leukocytes from Cancer Patients on Migration of Test Leukocytes

Supernatant of leukocytes from	Leukocyte migration inhibition (stimulation) index*	
	of cancer patients	of control subjects
Gastric cancer patients		
1	0,58	1,0
2	0,62	1,0
3	0,73	0,94†
4	0,56	0,64
5	0,62	0,63
Healthy subjects		
1	1,08 †	1,04†
2	0,94 †	0,78†
3	1,13 †	0,99†

* Leukocyte migration inhibition index = mean level of leukocyte migration in medium with supernatant. Mean level of leukocyte migration in medium without supernatant.
†Differences from control not significant ($P > 0.05$); elsewhere $P < 0.05$-0.01; when index is 1.0 there is no difference from the control.

The patients with gastric cancer included 54 men and 42 women. The ages of the patients ranged from 38 to 74 years. The diagnosis in the patients with gastric cancer was confirmed histologically.

Gastric cancer antigens inhibited leukocyte migration in four of seven patients with stage I gastric cancer in medium containing plasma from a cancer patient and in six of seven patients in medium containing BS or serum from donors with blood group AB_0 (IV). In stage II gastric cancer, inhibition of leukocyte migration was observed in five of ten cases when the leukocytes were cultured in medium with autologous plasma and in eight of ten cases in medium with BS or donors' serum. In patients with stage III gastric cancer, migration of leukocytes from 12 of 39 patients in medium with autologous plasma and in 24 of 43 patients in medium with a control serum was inhibited, whereas in stage IV gastric cancer it was inhibited in 9 of 31 and in 10 of 35 patients respectively. Consequently, the frequency of a positive LMIT in patients with gastric cancer to antigens of cancer in this situation in other patients depended on the stage of the tumor process.

Antigens of allogeneic gastric mucosa adjacent to the tumor inhibited migration of leukocytes of gastric cancer patients in 21 of 95 cases (22%) in medium with BS or with serum from blood donors with group AB_0(IV) and in nine of 81 cases (11%) in medium with plasma of a cancer patient.

Leukocytes from 25 patients with gastric cancer in stages III and IV, migration of which was not inhibited by allogeneic tumor antigens, and also leukocytes from ten patients which did respond to these antigens, were

studied in preincubation experiments (Table 1). When leukocytes of gastric cancer patients which did not react to gastric cancer antigens were preincubated for 24 h in medium without serum or in the presence of 1% BS at 4°C, they acquired the ability to respond by inhibition of migration to tumor antigens in 19 of 25 cases. Meanwhile the number of cases of stimulation of leukocyte migration fell from 5 to 1 (Table 1). If these leukocytes from gastric cancer patients were preincubated in autologous plasma, no restoration of their reactivity to tumor antigens was observed. Leukocytes of ten gastric cancer patients, which responded to tumor antigens by inhibition of migration before preincubation at 4°C, preserved their ability after incubation also.

The specificity of action of allogeneic gastric cancer antigens on migration of leukocytes from gastric cancer patients was proved by simultaneous tests with leukocytes from patients of the control group. Gastric cancer antigens caused inhibition of migration of leukocytes from only 13 (11.8%) of 110 control patients in medium with BS. After preincubation of leukocytes from 30 of these patients, inhibition of leukocyte migration which had not been observed before preincubation was found in only two cases. Consequently, only preincubation of leukocytes from cancer patients can facilitate inhibition of their migration by gastric cancer antigens.

After preincubation of leukocytes from gastric cancer patients, inhibition of their migration by anitgens from the mucosa adjacent to the tumor was observed in a further two of the 35 cases, and the number of cases of stimulation of migration fell from seven to three.

The effect of the supernatant of preincubated leukocytes, clarified by centrifugation at 2000 rpm for 10 min, on migration of other leukocytes was tested. In the presence of this fluid, gastric cancer antigens did not inhibit migration of leukocytes from cancer patients which responded to them previously, compared with migration of leukocyte migration was due to the fact that supernatant without antigens could inhibit migration of leukocytes from gastric cancer patients, and it could do so more often than leukocytes from the control group (Table 2). It can be tentatively suggested that during preincubation the leukocytes from cancer patients secrete an antigen-antibody complex into the supernatant which can inhibit their migration [7, 9, 11], or substances resembling the migration-inhibiting factor [1, 3, 6]. Leukocytes from healthy subjects did not secrete any such substances into the supernatant, for they were unable to inhibit migration of the test leukocytes (Table 2).

Restoration of the reactivity of leukocytes from gastric cancer patients to antigens of this tumor after preincubation in serum-free medium was thus due to the deblocking of their receptors as a result of secretion of immunologically active factors by them. It can be tentatively suggested that after preincubation of lymphocytes from cancer patients they regained their ability to synthesize a factor inhibiting migration under the influence of tumor antigens, or after preincubation the polymorphs regained their sensitivity to this factor. Binding the blocking factors by receptors of the leukocytes evidently depends on temperature, and a fall in temperature is accompanied by deblocking of the receptors. This evidently takes place because in the cold the receptors lose their ability to move and to form caps, and they are distributed diffusely on the surface of the cells [12].

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