ERYTHROCYTE MEMBRANES IN CANCER

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Many investigators have observed a reduction in the intensity of chemiluminescence of the blood plasma and serum in cancer [4, 7]. These results have been subjected to detailed analysis [5, 11]. The suggestion accordingly arises: If the quencher (antioxidant) is present in the blood, its action may be expected to be exerted on the blood cell membranes. However, it has been postulated [3] that the quencher enters the plasma from the blood cells after their destruction, and that the quencher appears in the blood cells several days before it appears in the plasma. Changes in the intensity of chemiluminescence during the primary immune response of lymphocytes and the participation of hydrogen peroxide (H_2O_2) in this process have recently been described [13]. The immune response of lymphocytes, as we know, is of great importance in carcinogenesis.

The aim of this investigation was to study chemiluminescence of the blood cells induced by addition of hydrogen peroxide to the system (PCHL) in normal subjects and cancer patients.

EXPERIMENTAL METHOD

PCHL was measured on the apparatus described in [6].

Altogether 61 cancer patients were investigated at the No. 1 City Hospital: nine with carcinoma of the stomach in stage II, 12 with carcinoma of the stomach in stage III, eight with carcinoma of the stomach in stage IV, and 32 with carcinoma of the rectum in stages II and III.

The presence of cancer was confirmed in all patients histologically. In addition, nine healthy blood donors were studied.

Blood was taken from the cubital vein of all subjects by the usual method. Sodium citrate in a concentration of 10 mg to 1 ml of blood was used as anticoagulant. All manipulations with the blood were carried out at 20°C except in cases when the temperature is indicated specially.

Blood from the tube in a volume of 0.02 ml was diluted in 4.0 ml of physiological saline. Since most blood cells are erythrocytes, it can be taken conventionally that what was measured was PCHL of erythrocyte membranes. The number of erythrocytes in the suspension was counted in a Goryaev's chamber.

To measure PCHL of the erythrocytes 0.5 ml of suspension was introduced into a cuvette and the solution made up to 4.0 ml by the addition of a solution of 134 mM phosphate buffer, pH 7.4. Next, 2.0 ml of a 9 mM solution of H_2O_2 was added and a flash of PCHL consisting of two parts (fast and slow) was recorded (Fig. 1).

The following parameters of PCHL were determined [12]: the light sum of the slow flash (during 120 sec after addition of H_2O_2, in pulses), reflecting the resistance of the cell membranes to the action of H_2O_2 [9, 10], and the time taken for PCHL to reach maximal intensity (T), reflecting the state of antioxidant activity [8]: the higher the value of T, the higher the antioxidant activity.

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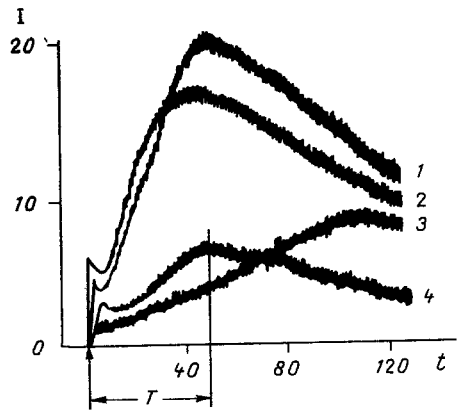

Fig. 1

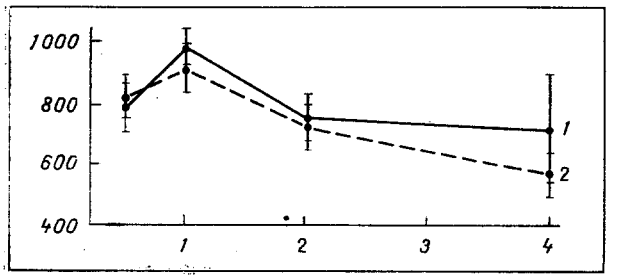

Fig. 2

Fig. 1. Kinetic curves of PCHL of cancer patients (2, 3) and healthy blood donors (1, 4), recorded at 20°C (1, 2) and 5°C (3, 4). Abscissa: t) time after addition of H_2O_2 (in sec); ordinate: I) intensity of PCHL (in pulses/sec), T) time taken for PCHL to reach maximal intensity (in sec). Arrow indicates time of addition of H_2O_2.

Fig. 2. Dependence of PCHL on number of erythrocytes in cuvette. Abscissa, number of erythrocytes in cuvette ($\times 10^6$); ordinate, light sum of PCHL (in pulses). 1) Healthy blood donors, 2) cancer patients.

EXPERIMENTAL RESULTS

With an increase in the number of erythrocytes in the cuvette the light sum of PCHL rose initially to reach a peak when the number of erythrocytes in the cuvette was 10^6, after which it flattened out on a plateau, and this was followed by a decrease in the light sum of PCHL: The decrease was greater in the patients than in the blood donors (Fig. 2).

In the next experiments the number of erythrocytes in the cuvette was $1.5 \cdot 10^6$, and 2.0 ml of H_2O_2 was added in different concentrations.

With an increase in the H_2O_2 concentration to 500 mM the light sum of PCHL of the erythrocytes from both blood donors and patients increased, but a further increase in the H_2O_2 concentration was not accompanied by any increase in the light sum (Fig. 3A). The light sum of PCHL of the blood donors' erythrocytes in the presence of H_2O_2 in a concentration of 0.5-1.0 M was greater than that of the patients ($p < 0.05$). The kinetics of PCHL differed in low and high H_2O_2 concentrations (Fig. 3B). In low concentrations of added H_2O_2 (3.75, 7.5, and 9.0 mM) the PCHL flash had fast and slow components, but in high concentrations (0.25, 0.5, and 1.0 M) the flash was characterized by a sharp rise and slow decay, with transition to steady-state fluorescence.

The results thus show that Gurvich's view [3] on transfer of the quencher by the blood cells in cancer may be quite correct. Changes obtained in T indicate that this quencher of chemiluminescence in all probability possesses antioxidant properties in the erythrocyte membrane.

Heating the erythrocytes to 40°C led to inversion of the ratio between the light sums of PCHL of the blood donors and cancer patients. For instance, whereas at 20°C the light sum of PCHL of the donors was higher than that of the cancer patients, at 40°C, on the contrary, the light sum of PCHL of the cancer patients' erythrocytes was higher than that of the blood donors (Table 1). These results agree with data in the literature [2], showing that at 40°C PCHL of cancer cells is higher than that of normal cells.

A distinct difference was observed in the kinetics of PCHL of donors and patients at 5°C (Fig. 1). The PCHL curve of the patients at this temperature had no fast flash, and the slow flash was more sloping than that of the donors. T for the patients was greater ($p < 0.05$) than for the donors. Thus whereas the PCHL curve of the donors at 5°C did not differ significantly in its kinetics from the curves at 20 and 40°C, there were clear differences in the cancer patients. The effect of temperature on the kinetics of PCHL was evidently due to a change in the microviscosity of the lipid layer of the erythrocyte membranes in the patients and to the state of the proteins on their surface. Since the light sum of PCHL of the patients at 5 and 40°C was higher than that of the donors, it can be tentatively suggested that the

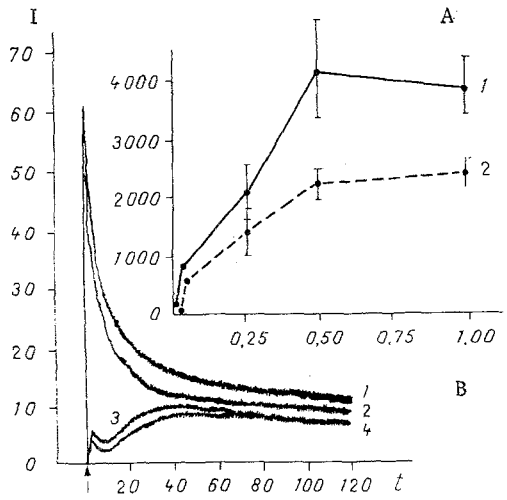

Fig. 3. Dependence of PCHL on H_2O_2 concentration. A: abscissa, concentration of H_2O_2 added to cuvette (in M); ordinate, light sum of PCHL flash (in pulses). 1) Donors, 2) cancer patients.

TABLE 1. Effect of Temperature on Parameters of PCHL of Erythrocytes from Cancer Patients and Healthy Blood Donors (M ± m)

Temperature, °C	Erythrocytes from	Light sum, pulses	τ, sec
5	Donors	429±27,0	45±9,1
	Patients	485±10,4***	79±6,4***
20	Donors	1390±50,4**	35±3,7
	Patients	933±135,2*,***	38±3,0*
40	Donors	1164±207,8**	33±2,2**
	Patients	2220±695,1*	38±2,9*

Legend. *P < 0.05 compared with patients' erythrocytes at 5°C, **) the same compared with donors' erythrocytes at 5°C, ***) the same compared with donors' erythrocytes at corresponding temperature.

ability of the lipids of the erythrocyte membranes to undergo peroxidation was increased [9] and the resistance of the membranes to the harmful action of H_2O_2 was reduced [10]. Meanwhile antioxidant activity was increased, evidently because of the release of chemical quenchers of chemiluminescence, whose action was manifested particularly clearly at 20°C, into the blood of the cancer patients. A similar situation was mentioned in [1], in an analysis of the results of a study of lipid peroxidation in tumor membranes.

In patients with a malignant growth, an increase in antioxidant activity and activation of lipid and protein peroxidation are thus observed in the erythrocyte membranes, and are manifested as a decrease in resistance of the erythrocytes to H_2O_2. As the blood temperature falls the kinetics of PCHL of the patients' erythrocytes changes, and this phenomenon can be used as a diagnostic criterion for the detection of cancer.

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EFFECT OF ADAPTIVE TRANSFER OF SPLENOCYTES FROM TUMOR-BEARING MICE ON METASTASIS FORMATION IN THE LUNGS OF INTACT MICE

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The view that a primary tumor focus has an inhibitory action on the formation of distant metastases arose on the basis of clinical observations and experimental research [2, 5, 8, 9, 14]. In a model system developed by the writers, with transplantable mammary gland carcinoma of C3H/He mice, the inhibitory effect of the local tumor on metastasis formation in the lungs was reproduced. Surgical removal of the tumor abolished the inhibitory effect (IE) [1]. Adoptive transfer of splenocytes from tumor-bearing mice to immunodepressed recipients is known to induce changes in resistance to metastases in the recipients similar to those in the tumor-bearers [7, 10]. It appeared interesting to study the role of splenocytes in the development of IE in tumor-bearing mice using our model system.

The aim of the present investigation was to study quantitative dynamics of nucleated spleen cells in mice at different stages of growth of a syngeneic tumor and after its removal. The antimetastatic (antitumor) activity of splenocytes of tumor-bearing mice was assessed by the adoptive transfer method, by determining their ability to inhibit the formation of experimental lung metastases (ELM) in intact mice.

EXPERIMENTAL METHOD

Experiments were carried out on male C3H/f (MTV=S$^-$) mice weighing 24-26 g and on 6-8-week old mice with the nude mutation (partially inbred for the C3H/f genotype, after four back crosses to the pure line), maintained at the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR. Transplantable mammary gland carcinoma, strain MMT1, of C3H/He mice was used. A suspension of tumor cells was prepared by a method based on preliminary trypsinization of tumor tissue [5]. Growth of the tumor was induced by injection of $2 \cdot 10^6$ MMT1 cells subcutaneously in the right subscapular region. Splenocytes were counted and transferred from the tumor-bearers on the 5th, 14th, and 25th days after subcutaneous inoculation of the cells and 14 days after removal of the tumor. ELM were induced by injection of $2 \cdot 10^5$ tumor cells into the caudal vein; metastases in the lungs were counted under the MBS-2 binocular loupe with a magnification of 8. The suspension of splenocytes was prepared by the method in [12]. The cells were washed off twice in medium 199 by centrifugation at 2000 rpm for 10 min. Each experimental animal was given an intravenous injection of $3 \cdot 10^7$ splenocytes

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