CHEMILUMINESCENCE REACTIONS OF BLOOD NEUTROPHILS AND PERITONEAL EXUDATE CELLS OF SYRIAN HAMSTERS TO INTRAPERITONEAL INJECTION OF CELLULAR AND BACTERIAL MATERIALS

M. O. Prilutskaya and L. M. Kashkina

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It has become evident in the last ten years that the system of natural resistance of the body plays an important, if not determinative role in antitumor protection, whereas tumor cells possess a marked immunodepressive action [1, 6, 8]. Intraperitoneal injection of heated, irradiated, malignant tumor cells has been shown to suppress the natural resistance of the body to tumors, as shown by a marked increase in the ability of tumor cells to undergo successful inoculation and metastasization [8]. It has also been shown that the migrating capacity of peritoneal exudate cells is sharply reduced in such animals [2].

The investigation described below was undertaken to study possible changes in the activity of peritoneal exudate cells (PEC) and blood neutrophils (NP) in response to injection of preparations of these cells, compared with native irradiated tumor cells, heated irradiated normal cells, and also with preparations of heated bacterial cells of the species *Candida albicans*.

EXPERIMENTAL METHOD

The following materials, made up in medium RPMI-1640, were used to inject into the animals: 1) a microbial suspension of *C. albicans*, heated to 90°C, in a dose of $8.1 \cdot 10^8$/ml; 2) cells of a primary embryonic hamster culture (HE), inactivated by heating to 56°C and irradiation (10,000 rads), in a dose of $4.7 \cdot 10^7$/ml; 3) cells of a highly malignant strain HET-SR-MLU-1, either native or heated to 56°C, and then irradiated (10,000 rads), in a dose of $4.5 \cdot 10^7$/ml. The cells were injected intraperitoneally into Syrian hamsters aged 2-3 months, reared at the "Stolbovaya" Nursery, Russian Academy of Medical Sciences, in dose of 1 ml of suspension per hamster. The number of animals in the group varied from 5 to 7. Samples of blood and PEC were taken at various times beginning with the 2nd-3rd day and continuing until the 21st-25th day after intraperitoneal injection of the test preparations. Blood samples were taken from the orbital sinus of the animals in a volume of 0.5 ml into a test tube containing heparin solution (10 U/ml blood). The blood was diluted 1:5 with medium RPMI-1640 containing 10% of heated calf fetal serum, HEPES, and 100 U/ml of monomycin. The number of leukocytes in the sample and the blood leukocytic formula were counted. The hamsters' blood contained on average 20.8 ± 2.2% of NP, 1.0 ± 0.2% of monocytes

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TABLE 1. Cell Composition of Peritoneal Exudate and Parameters of Chemiluminescence Activity of PES of Intact Syrian Hamsters (M ± m)

Number of animals	Content in PES, %			CL: number of counts per minute per 10^5 (MP + NP)	
	MP	NP	LP	SCL	PCL
15	57,1±10,1	4,5±2,2	39,1±10,2	1117,8±272,0	4181,3±831,0

Legend. LP) lymphocytes, NP) neutrophils, MP) macrophages, SCL) spontaneous chemiluminescence, PCL) phagocytosis-dependent chemiluminescence.

(MON), and 78.2 ± 2.1% of lymphocytes (LP). It was shown previously that blood NP and MON, unlike lymphocytes, possess chemiluminescence (CL) activity [10-12]. The intensity of CL of MON and of macrophages (MP) is known to be 3-10 times lower than CL of NP taken in the same number [5], and for that reason, when counting CL of the blood NP we disregarded the presence of 1% of monocytes in the blood. PEC samples were taken by washing out the peritoneal cavity of the hamsters with 20.0 ml of culture medium containing lactalbumin hydrolysate, 5% bovine serum, 100 U/ml monomycin, and 4-10 U/ml heparin. The PEC were then washed twice by centrifugation at 1000 rpm in medium without heparin in the course of 7 min. The residue of PEC was diluted in the same medium as the blood samples to a concentration of $(5\text{-}7)\cdot10^6$/ml. The absolute numbers and relative proportions of MP, NP, and LP in 1 ml medium were then calculated. Secretin of active forms of oxygen by blood NP and PES was determined on the basis of luminol-dependent CL. A 0.01 M solution of luminol (from "Serva," Germany) in phosphate buffer not containing calcium and magnesium (pH 7.0) was used. CL was recorded at 37°C in a thermostated "Biolumat" apparatus (model 9500, from "Bertold," Germany). The reaction was stopped in darkness, in red light. CL of blood NP and PES was determined as spontaneous (SCL) and phagocyte-dependent (PCL) forms. PCL was determined with the aid of a heat-killed (90°C, 60 min) microbial suspension of *Candida albicans,* opsonized with calf serum ($1\cdot10^8$ particles/ml) [9]. To set up the PCL reaction, three ingredients were mixed in equal volumes of 100 μl each: the blood for testing (PES), *C. albicans,* and luminol. To measure SCL, 100 μl of medium was added instead of *C. albicans.* Each sample was prepared in two tubes and the mean value of two measurements was determined. Preliminary experiments showed that the optimal time for determination of SCL of NP and PES is 10-15 min after the addition of luminol to the test sample, and PCL reaches its peak values 15-25 min after addition of *C. albicans* and PES. It was during these time intervals and PCL and SCL of the blood NP and PES were measured. The intensity of CL was expressed as the number of counts per minute, per $1\cdot10^3$ NP and the number of counts per minute per $1\cdot10^5$ PES.

EXPERIMENTAL RESULTS

It was first necessary to determine the normal levels of CL of the blood NP and PES in intact animals. For this purpose, individual blood samples of 120, and PES of 15 intact animals were tested. It was found previously that values of SCL and PCL for blood NP vary widely in different animals; the mean values are 40.7 ± 17.2 for SCL of NP and 414 ± 170.3 for PCL of NP [3].

Data on the cell composition of the peritoneal exudate of 15 intact Syrian hamsters and values of their CL activity are given in Table 1. The results show the dominant cells in the composition of PES were MP and LP, whereas the number of NP was 4.5 ± 2%. However, since lymphocytes, as has already been stated, do not possess CL activity, and since NP activity is very considerable, we considered it impossible to disregard even the small number of NP in the composition of PES, and we calculated total CL activity of PES, to include both MP and NP.

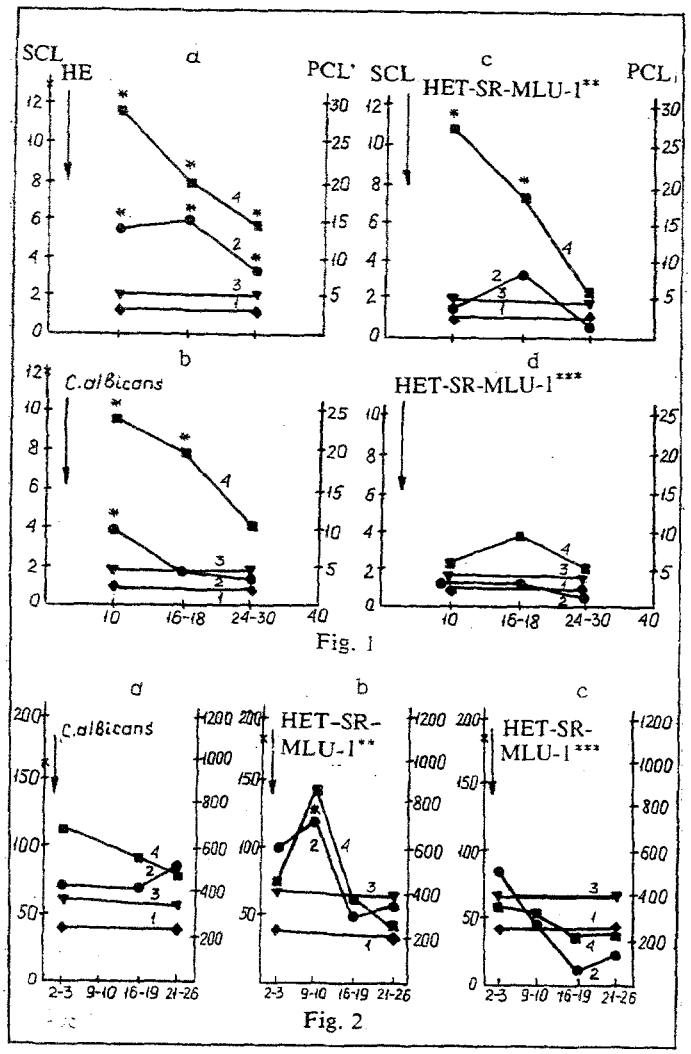

Fig. 1

Fig. 2

Fig. 1. Values of SCL and PCL of PES in animals inoculated with cellular and bacterial materials (a-d). Abscissa, days on which animals were tested; ordinate, values of CL expressed as number of counts $\cdot 10^3$ per minute/10^5 PES. *) Significant difference from control ($p < 0.01$), **) native irradiated tumor cells HET-SR-MLU-1, ***) heated irradiated tumor cells HET-SR-MLU-1. 1) SCL of control; 2) SCL of experiment; 3) PCL of control, 4) PCL of experiment.

Fig. 2. Values of SCL and PCL of NP in blood of animals inoculated with cellular and bacterial materials (a-c). Abscissa, days on which animals were investigated; ordinate, values of CL expressed as number of counts per minute/10^3 NP. *) Significant difference from control ($p < 0.05$). Remainder of legend as to Fig. 1.

Investigation of the dynamics of changes in CL of PES of the inoculated animals showed that intraperitoneal injection of heated, irradiated HE cells and particles of *C. albicans,* as will be clear from Figs. 1a, b, caused a significant increase in the intensity of both SCL and PCL of PES as early as on the 10th day, followed by a gradual fall of these parameters to the 16th-24th day. Figure 1 shows points whose values differ significantly from those of the same parameters in the control. It will be evident that during the first days after injection, *C. albicans* and heated normal HE cells caused marked local activation of SCL of PES, which lasted 18-20 days.

By contrast with *C. albicans* and HE cells, intraperitoneal injection of tumor cells (native and, in particular, heated) into the animals did not induce activation of SCL of PES (Fig. 1c, d). Investigation of PCL of PES of these same animals showed that toward the 10th day after injection, native tumor cells induced significant activation of PCL, whereas heated tumor cells did not induce such activation.

To study the dynamics of changes in CL of blood NP, the animals were inoculated intraperitoneally with three types of materials: 1) microbial particles of *C. albicans*; 2) and 3) irradiated tumor cells of strain HET-SR-MLU-1 (native and heated). The results showed (Fig. 2a) that no significant changes took place in SCL and PCL of the blood NP in animals inoculated with microbial particles of *C. albicans* either on the 3rd-9th day or at later times after injection.

Intraperitoneal injection of native tumor cells led, after an initial (on the 9th-10th day), significant increase in the intensity of SCL and, to a lesser degree, of PCL of the blood NP, to a subsequent fall of these parameters by the 16th-19th day to normal.

Injected of heated irradiated tumor cells caused a decrease in the values of SCL and PCL of the blood NP as early as on the 10th day, but later, on the 16th-21st day after inoculation, this decrease was even more marked.

Thus intraperitoneal injection of heated irradiated microbial particles and normal HE cells causes marked local activation of CL of PES, but has no significant effect on the value of CL of the blood NP of these animals, whereas intraperitoneal injected of heated irradiated tumor cells not only does not activate PES, but also exhibits a marked tendency toward suppression of the CL activity of the blood NP, possible evidence of the systemic character of the response of the body to injection of this material.

Since it was shown previously [7] that tumor cells of the HET-SR-MLU-1 strain are able to secrete prostaglandins of the E_2 type (PGE_2) it can be postulated that suppression of CL of NP following injection of these cells may be linked with the immunodepressive activity of PGE_2. According to our data, blood NP are highly sensitive to the immunodepressive activity of PGE_2. It has also been shown that a single intraperitoneal injection of a commercial preparation of PGE_2, known for its instability, into Syrian hamsters, was able to induce long-term (until the 14th day) suppression of the cytostatic activity of PES [4]. It is thus clear from our data given above that the response of the animal to intraperitoneal injection of different materials was a differential one and remained local for microbial bodies and normal cells, but was more general in the case of injection of malignant tumor cells.

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EFFECT OF ASCITES FLUID GLOBULINS ON GROWTH OF LEUKEMIA P388/DOX AND EHRLICH'S CARCINOMA IN MICE

F. V. Donenko, A. O. Kabieva, and L. V. Moroz

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The writers showed previously that ascites fluid (AF) of Ehrlich's carcinoma affects the growth of this tumor in mice [1]. This effect can be explained by the presence in AF of what have been called potentiating antibodies. However, they were described previously only for the blood serum of tumor-bearing animals, including those with Ehrlich's carcinoma. It has been shown in the latter case that antibodies isolated from the blood serum of tumor-bearing animals, can interact directly with tumor cells and cause their death [2].

The question of the ability of the globulin fraction of AF to accelerate tumor growth has not been studied The aim of the present investigation was accordingly to study the effect of the globulin fraction of AF of Ehrlich's carcinoma and of leukemia P388/DOX on the rate of tumor growth in vivo.

EXPERIMENTAL METHOD

In the experiments with Ehrlich's carcinoma male (CBA × C57BL/6)F_1 hybrid mice were used, in experiments with leukemia P388/DOX, male BDF_1 hybrid mice (DBA/2 × C57BL/6) weighing 24-26 g were used. AF was isolated from mice on the 10th day after intraperitoneal transplantation of Ehrlich's ascites carcinoma (from the Tumor Strains Bank of the All-Union Oncologic Scientific Center, Russian Academy of Medical Sciences, Moscow), and on the 8th day after intraperitoneal transplantation of leukemia P388/DOX (this strain was described previously) [4]. To remove cells and fragments of cell membranes the AF was centrifuged at 3000*g* for 15 min, then at 20,000*g* for 30 min.

The blood serum globulin fraction of animals with an intramuscularly inoculated tumor and globulin fraction of AF were obtained by precipitation of proteins with ammonium sulfate. The protein precipitate was dissolved in a volume equal to the original volume of blood serum or AF, and the ammonium sulfate was removed by dialysis. Dialysis was carried out and the specimens dissolved in 0.15 M NaCl, 0.02 M phosphate buffer, pH 7.4.

To exhaust 10 ml of the globulin fraction tumor cells were isolated from 100 ml of AF. The cells were fixed in 5% formalin (pH 2.8), then washed in physiological saline and incubated with the globulin fraction for 1 h at 37°C (pH 7.4).

To record the effects the animals received an intramuscular transplantation of $1 \cdot 10^4$ tumor cells per mouse in 0.1 ml of Hanks' solution 15 min after intraperitoneal injection of AF or globulins (Gl) of AF and blood serum Gl. Animals of the control groups were given the corresponding volumes of physiological saline. The action of AF Gl

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