genetic information and, in particular, these cells may develop tumorigenic properties as a result of introduction of oncogenes. This cell clone can accordingly be recommended for use as target cells with which to study the properties of an introduced foreign DNA

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DYNAMICS OF CHEMILUMINESCENCE REACTION OF SYRIAN HAMSTER BLOOD NEUTROPHILS TO GROWTH OF TEN DIFFERENT TUMOR CELL STRAINS

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UDC 616-006-018.1-02:615.155.34]-092.9-07

KEY WORDS: chemiluminescence; neutrophils; tumor cells

One of the most important characteristic features of the blood neutrophils, responsible through phagocytosis for their bactericidal and cytotoxic properties, is their ability to generate an oxygen burst, i.e., to release active forms of oxygen [4, 12, 14].

Products of the oxygen burst of neutrophils (O_2^-, OH, and, especially, H_2O_2) have been shown to be highly toxic for tumor cells [12]. Since tumor cells can secrete factors inhibiting activity of neutrophils (prostaglandins of the E_2 type, for example) [1], it is not yet clear how that cytotoxic activity of neutrophils is modified and, in particular, how active forms of oxygen are secreted during growth of different types of malignant tumors.

The aim of this investigation was to use the luminol-dependent chemiluminescence test to discover what effect growth of experimental subcutaneous tumors, differing in their origin and level of malignancy, has on the process of secretion of hydrogen peroxide (H_2O_2) by peripheral blood neutrophils of tumor-bearing hamsters.

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(Presented by Academician of the Russian Academy of Medical Sciences N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 9, pp. 315-318, September, 1992. Original article submitted February 19, 1992.

0007-4888/92/0009-1358$12.50

TABLE 1. Characteristics of 10 Cell Strains used in the Work

Strain of cells	Selection in vivo	TGA log ID_{50}	EMA, min dose	SMA
STHE	—	2,7—3,5	$<10^{-6}$	—
STHE-75/18	+	0,7—0,9	10^{-5}	—
STHE-LM^8	+	1,4—1,7	10^{-4}	+++
THE-SR	—	0,5—1,4	10^{-4}	—
THE-SR-MLU^4	+	0,8—1,4	10^{-4}	—
THE-SR-MLU^1	+	0,8—1,3	10^{-4}	—
THE-SR^2 PK-MLU	+	0,5—1,1	10^{-4}	+++
OPH-SR	+	0,9—1,2	10^{-4}	+
THE-SR^1	—	0,8—1,2	10^{-4}	+++
THE-SR^{10}	—	1,1—1,3	10^{-5}	++

Legend. TGA) Tumorigenic activity, expressed as log 50% of inoculated dose (log ID_{50}) of the test tumor cells, EMA) experimental metastatic activity (the minimal dose of the test cells giving growth on average of more than 10 experimental metastases in the lungs following intravenous injection of the cells). SMA) Spontaneous metastatic activity (expressed as the discovery of 10 or more metastases in the lungs of animals with subcutaneously inoculated test cells).

EXPERIMENTAL METHOD

The 10 cell strains used in the work differed in a number of characteristics: transforming agents (spontaneous transformation in vitro or transformation by Rous sarcoma virus (RSV) (Schmidt–Ruppin strain), tumorigenic and metastatic activity (spontaneous and experimental), and history of selection in vivo [5, 6]. They included: hamster epithelial cells transformed spontaneously in vitro (STHE strain), variants of STHE cells selected in vivo (strains STHE-LM^8 and STHE-75/18), cells of strains THE-SR, THE-SR^1, and THE-SR^{10}, independently transformed in vitro by RSV, variants of the parental strain THE-SR selected in vivo (THE-SR-MLU^1, THE-SR-MLU^4, and THE-SR^2 PK-MLU), selected in vivo, and also a hamster tumor line, induced in vivo by RSV (strain OPH-SR). Characteristics of the strains are illustrated in Table 1. They include determination of tumorigenic activity (TGA), experimental metastatic activity (EMA), and spontaneous metastatic activity (SMA). The STHE cell strains were maintained in tissue culture in Eagle's medium and lactalbumin hydrolysate with 10% cow serum with the addition of antibiotics (100 U/ml) For strains induced by RSV, medium F-12, containing 10% cow serum and antibiotics, was used. The 2-3-month old Syrian hamsters used in the experiments were obtained from the "Stolbovaya" nursery, Russian Academy of Medical Sciences. Tumor cells were injected subcutaneously in a dose of $2.0\text{-}5.0 \cdot 10^5$ cells/1.0 ml, into groups of animals (8-34 animals in a group) with individual markers. Intact animals of the same age, and also animals with individual markers, tested before inoculation of the tumor, served as the control. Blood was first taken 24 h before subcutaneous inoculation of the tumor cells, and each successive 7-10 days thereafter. Blood samples were taken from the animals' orbital sinus in a volume of 0.5 ml into a test tube containing heparin solution (10 U/ml blood). The blood was diluted 5 times with medium RPMI-1640 with 10% heated fetal serum, with HEPES buffer, and with 100 U/ml of monomycin. The number of leukocytes in the sample and the leukocytic formula of the blood were determined. Secretion of active forms of oxygen by blood neutrophils (Nph) was determined on the basis of luminol-dependent chemiluminescence (Chl) by the method in [8, 9]. A 0.01 M solution of luminol ("Serva," Germany) in phosphate buffer without calcium and magnesium (pH 7.0) was used Chl was recorded at 37°C [7] in a thermostated "Biolumat" instrument (model 9500, from "Bertold," Germany). The reaction was carried out in darkness and in red light. Chl of Nph was determined as spontaneous (SChl) and phagocytosis-dependent (PChl). To

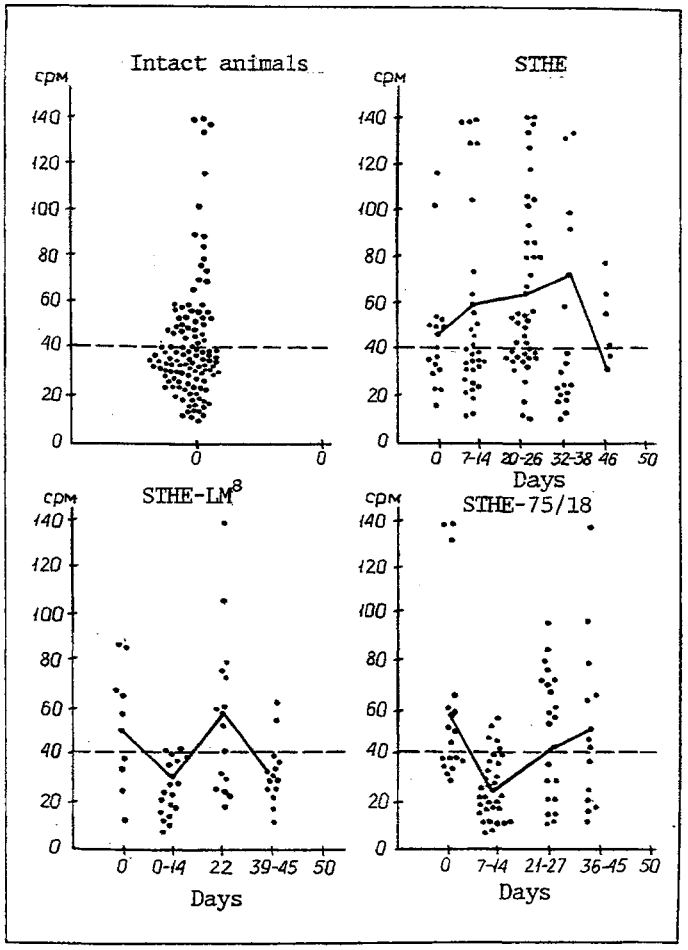

Fig. 1. SChl of blood Nph of intact animals and of animals bearing tumors of the STHE strain and its variants selected in vivo. Values of SChl of individual animals at different times after inoculation of the tumor is shown in Figs. 1-3. Dots indicate values of SChl for each animal in the course of time. Abscissa, time (in days) after inoculation of tumor cells into animals; ordinate, number of counts per minute per 10^3 Nph. Continuous line indicates mean values for animals of the given group during tumor growth.

determine PChl, a microbial suspension of *Candida albicans* (CA, $1 \cdot 10^8$ particles/ml), killed by heating to 90°C for 60 min and opsonized with cow serum [7], was used. To set up the PChl reaction, three ingredients were mixed together in equal volumes of 100 μl: the blood for testing, CA, and luminol. To measure SChl, 100 μl of medium was added instead of CA. Each sample was prepared in two tubes and the mean value of two measurements were calculated. In preliminary experiments the optimal time for determination of SChl was established, namely 10-15 min after addition of luminal to the sample. PChl of blood neutrophils rises to a maximum 15-25 min [11, 13] after addition of CA. It was in these time intervals that SChl and PChl of the blood Nph were measured. The intensity of Chl was expressed as the number of counts per minute per 10^3 Nph.

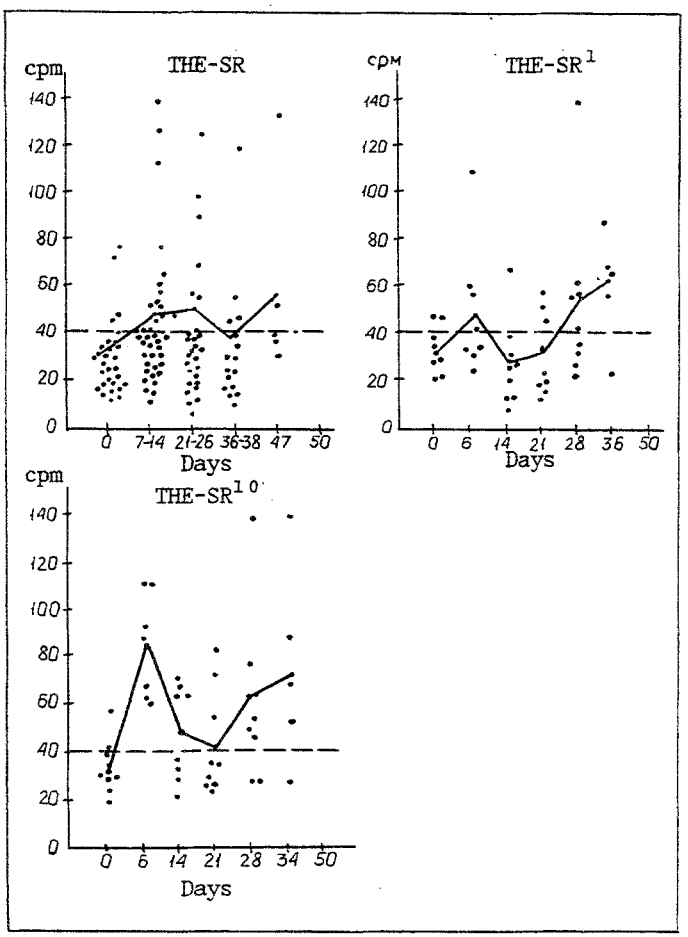

Fig. 2. SChl of blood Nph of animals during tumor growth following primary transplantation of cells not subjected to selection in vivo.

EXPERIMENTAL RESULTS

The study of Chl of Nph of a large group of control intact animals (120) showed that the values of SChl and PChl in individual animals vary significantly, being on average 40.7 ± 17.2 for SChl and 414.1 ± 170.4 for PChl (Fig. 1a). Data showing the relative levels of Chl activity of Nph and other hematologic parameters of the blood in hamsters before receiving subcutaneous injection of tumor cells, are given in Table 2. As Table 2 shows, the percentage of monocytes in the hamsters' blood averaged 1.0 ± 0.2, whereas the number of Nph reached 20.8 ± 2.2. Besides, it was shown previously that the intensity of Chl of monocytes is 3-5 times weaker than that of Chl of the same number of neutrophils, whereas Chl of whole blood is mainly accounted for by Nph [10, 13]; when the intensity of Chl of the blood Nph was calculated, we therefore disregarded the presence of monocytes in the blood.

A number of workers showed previous that as a rule a high level of Chl of Nph is observed in patients with tumors [3, 8, 9, 10]. It was therefore interesting to discover how values of Chl change during tumor formation, starting from the very earliest stages. Accordingly we studied the time course of the value of Chl of blood Nph in individual animals before and after inoculation of different strains of tumor cells into them, including the time after the appearance of a tumor nodule in the animals.

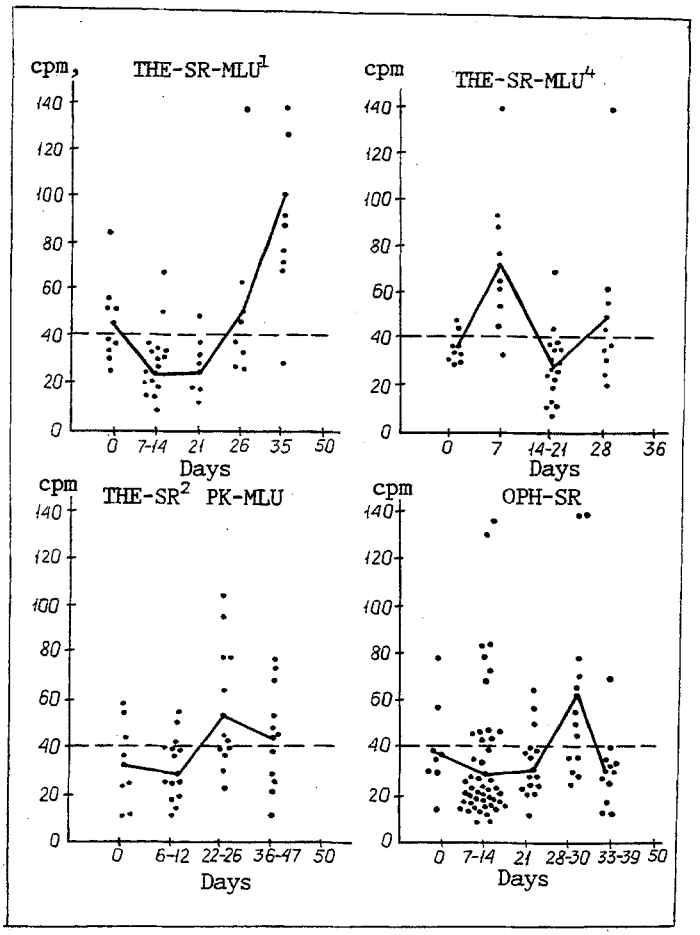

Fig. 3. SChl of blood Nph of animals during growth of tumors selected in vivo.

TABLE 2. Correlation between Chemiluminescence (Chl) of Blood Neutrophils and Other Hematologic Parameters of Intact Syrian Hamsters (M ± m)

Number of animals	Number of leukocytes, $\times 10^9$	Content in blood, in %			Chl, number of counts per minute per 10^3 Nph	
		Lph	Nph	MON	SChl	PChl
120	8,9±0,7	78,2±2,1	20,8±2,2	1,0±0,2	40,7±17,2	414,1±170,3

Legend. Abbreviations: Lph) lymphocytes, Nph) neutrophils, MON) monocytes, SChl) spontaneous, PChl) phagocytosis-dependent chemiluminescence.

Analysis of the time course of the changes in Chl of the blood Nph in the inoculated animals during growth of 10 different cell strains showed the existence of two types of development of the SChl reaction. In the first type of reaction, during the first 7-14 days after inoculation of the tumor cells, i.e., as a rule before the appearance of a palpable tumor nodule, a decrease in SChl and, to a lesser degree, in PChl is observed, and changes into an increase in Chl 20-30 days after inoculation of the tumor (the beginning of tumor growth). In animals with this type of early

reaction of Nph to late stages of tumor growth, both a decrease in SChl during growth of some tumors, and an increase in SChl during growth of others can be observed.

The second type of reaction is characterized by an increase in SChl and also of PChl sooner (by 7-10 days) after inoculation of the tumor cells, and a decrease in activity of Nph 14-21 days after inoculation of the tumor cells, and at the 30th-45th day by gradual restoration of the original level, or even reaching a higher level. The decisive factor in the present investigation, it will be noted, is SChl, for it changes, either downward or upward, frequently but not always involved changes in PChl also. As will be clear from Figs. 1 and 2, within each group of animals wide scatter of values of SChl was observed, but nevertheless, growth of each of the 10 cell strains caused a reaction of Nph of mainly one of the two types.

Comparison of the characteristics of the cell strains studied with the types of reaction of SChl of the blood Nph of these animals shows that the type of reaction did not depend on TGA, SMA, EMA, or the transforming agent. It was found, however, that the first type of reaction of Nph was characteristic of animals with tumors consisting of cells which had been selected in vivo, including a tumor induced by RSV in vivo, whereas the second type of reaction of Nph was characteristic of animals after primary inoculation of cell strains transformed in vitro, i.e., not having undergone preliminary selection in vivo. The only exception to this rule was given by the results obtained with strain THE-SR-MLU[4], selection of which in vivo was ineffective, and which in its malignant properties and its values of Chl of Nph, this cell line was indistinguishable from the parental THE-SR strain (Table 1, Fig. 3).

It is thus possible that cell strains inducing a reaction of the first type in Nph as a result of selection in vivo acquired the ability to inhibit Nph function. It can be tentatively suggested that this inhibition of the reaction of Chl of the blood Nph in the early period after inoculation of tumor cells may facilitate activation of these cells and their dissemination in vivo, and conversely, the early activation of Nph which we found during growth of transformant cells which had not undergone selection in vivo may facilitate their more efficient elimination in vivo. It must be pointed out that the reaction of blood Nph of animals bearing different tumors is evidently a systemic reaction, for growth of subcutaneous tumors is accompanied by changes in Chl activity of blood Nph.

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