Common Regularities in Changes of the Neutrophil Functional Activity during *In Vivo* Growth of Tumors of Different Immunogenic Activity

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Studies on BALB/c mice with tumors of different immunogenic activity (nonimmunogenic J774, WEHI 164 and immunogenic NS0) have showed that the development of a tumor is associated with changes in the neutrophil morphology and functions: the counts and size of the cells migrating to the focus increase and their capacity to produce active oxygen species is changed.

Key Words: *neutrophil; active oxygen species; tumor growth model*

The interactions between the immune system and tumor developing in the body are an important and intricate problem of oncoimmunology. The immune system cells realize the immunological surveillance, due to which transformed cells are recognized and destroyed [9]. Neutrophils, whose functional activity is mediated by proteolytic enzymes present in granules and reactive oxygen species (ROS), are actively involved in the formation of antitumor immune response [6].

The impact of the tumor and its products for functional activity of neutrophils peripheral for the tumor is little studied by the present time. Studies of the neutrophil function modification during tumor growth are difficult because the changes are determined by tumor type, location, and stage [2]. Studies on experimental cancer models help to evaluate the role of these factors [3,5]. In order to evaluate the interactions between the immune system and the tumor, it is essential to select the most adequate of the numerous models, so that the results were correlating with the processes associated with tumor development in human body.

The aim of our study was to detect specific features and regularities in changes of functional activity of neutrophils on the models of growth of tumors with different immunogenic activity *in vivo*.

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MATERIALS AND METHODS

The study was carried out on the peripheral blood and peritoneal induced neutrophils of adult male BALB/c mice using J774 (sarcoma), WEHI 164 (fibrosarcoma), and NS0 (melanoma) cell cultures. Solid tumors were induced by intramuscular injection of 10^6 cells in 150 μl into the hip. Controls were injected with the same volume of saline. The neutrophils of controls and animals with tumors were analyzed at different stages of tumor growth in parallel experiments. Blood specimens and peritoneal neutrophils were processed as described previously [1]. The production of ROS was evaluated by luminol-dependent chemiluminescence (CL) on a Chemilum-12 chemiluminometer. Respiratory burst was stimulated with opsonized zymosan (0.25 mg/ml) or chemotaxic peptide N-formyl-Met-Leu-Phen (FMLP). The data were presented as mean±mean square deviation for 10-15 independent experiments, each performed on cells of the same animal. The differences between the groups were evaluated by Student's *t* test.

RESULTS

The levels of spontaneous and opsonized zymosan-stimulated production of ROS in nonfractionated blood increased during the growth of the studied tumors

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TABLE 1. Changes in Spontaneous and Opsonized Zymosan-Stimulated (0.25 mg/ml) Production of ROS by Blood Cells in the Control (day 0) and on Days 7, 12, and 26 after Transplantation of Tumor Cells Evaluated by CL (*M*±*m*)

Tumor	Spontaneous production, arb. units×10^{-3} (*n*=10)				Stimulated production of ROS, arb. units×10^{-3} (*n*=10)			
	day after tumor transplantation				day after tumor transplantation			
	0	7	12	26	0	7	12	26
J774	40±3	45±4	48±12*	54±7*	175±8	180±21	190±20	250±40*
WEHI 164	40±3	40±3	51±4*	270±23*	175±18	625±35*	750±90*	675±70*
NS0	40±3	109±32*	178±69*	167±52*	175±18	1400±90*	1010±73*	840±90*

Note. Here and in Table 2: *p<0.001 compared to the control (day 0). Neutrophils of controls and animals with tumors were studied in parallel experiments at different stages of tumor growth.

TABLE 2. Time Course of Counts and Size of Neutrophils from Acute Inflammation Focus during Tumor Growth (*M*±*m*)

Tumor	Cell count, ×10^6 (*n*=10)				Cell diameter, μ (*n*=10)			
	day after tumor transplantation				day after tumor transplantation			
	0	7	12	26	0	7	12	26
J774	6.8±0.8	9.8±1.2*	10.5±2.3*	10.4±1.4*	6.2±0.7	6.8±0.8	6.9±0.9	11.8±1.2*
WEHI 164	6.8±0.8	7.3±0.8	9.0±1.2*	8.10±0.06*	6.2±0.7	8.7±0.7*	9.6±0.9*	9.1±1.0*
NS0	6.8±0.8	8.5±0.9*	8.1±0.7	7.8±0.7	6.2±0.7	7.2±1.1	8.1±1.1*	9.4±0.8*

(Table 1). The time course of ROS production was peculiar in each of the studied tumors. In the mice with J774 and WEHI 164 tumors, the levels of spontaneous and stimulated production of ROS gradually increased and significantly differed from the control on day 12 after tumor cell transplantation. In the animals with immunogenic tumors formed by NS0 cells, ROS production drastically increased as early as on day 7.

The increase of ROS production in the blood is a common regularity for all the studied tumors. A possible explanations of this fact is the primed status of the cells. Cell priming is caused by various factors, including increased cytokine level [10] observed in cancer [4,11].

Neutrophils make the main contribution to ROS production in the blood. We studied changes in their functional activity over the course of tumor growth. The counts of neutrophils attracted to the peritoneal cavity increased in all three tumor types (Table 2). This could be a result of the following processes: increase in blood neutrophil count, increase in adhesion, and lifespan prolongation. Cancer is associated with elevated relative neutrophil content in the blood [8]. In addition, chronic exposure of neutrophils to cytokines and growth factors released by tumor cells and immune cells infiltrating the tumor results in inhibition of apoptosis and prolongation of the neutrophil lifespan [7].

We observed changes in neutrophil morphology: the cells in animals with tumors were larger (Table 2), with larger nuclei and more pronounced granularity of the cytoplasm (data not presented).

The time course of ROS production by isolated neutrophils is determined by the tumor type (Fig. 1). The level of spontaneous ROS production increased in all tumor growth models (Fig. 1, *b*). However, the level of spontaneous ROS production in animals with NS0 tumor increased sharply as early as on day 7 of tumor growth and continued to increase significantly with tumor growth. Thus, we observed a trend to an increase of ROS production in comparison with that in controls (Fig. 1, *b*).

The most potent stimulator of neutrophil function, including ROS production, is chemotactic peptide FMLP. The intensity of ROS production stimulated with 1 μM FMLP in neutrophils of animals with J774 and WEHI 164 tumors markedly decreased and significantly differed from the control on day 12 of tumor

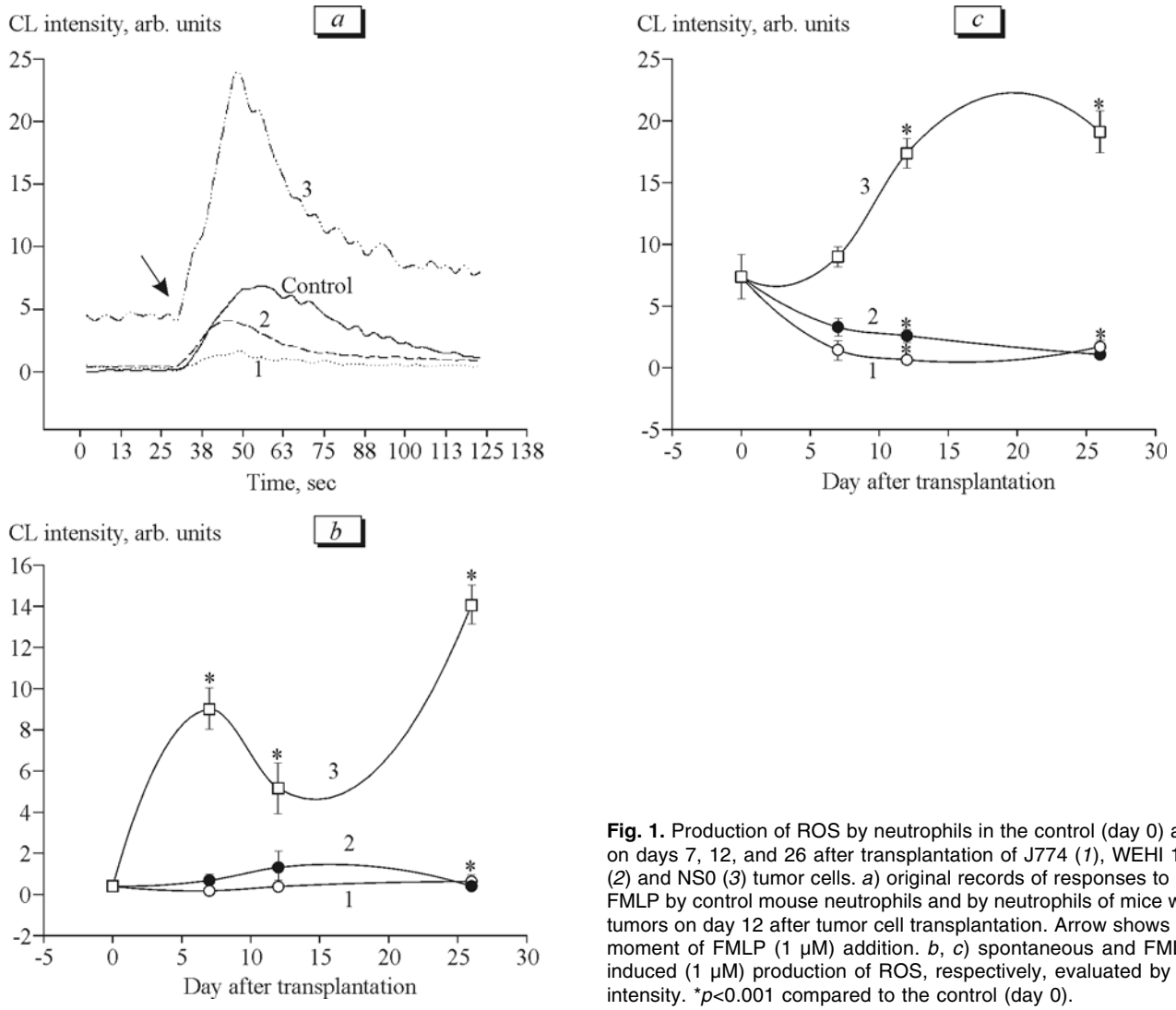

Fig. 1. Production of ROS by neutrophils in the control (day 0) and on days 7, 12, and 26 after transplantation of J774 (*1*), WEHI 164 (*2*) and NS0 (*3*) tumor cells. *a*) original records of responses to 1 μ FMLP by control mouse neutrophils and by neutrophils of mice with tumors on day 12 after tumor cell transplantation. Arrow shows the moment of FMLP (1 μM) addition. *b*, *c*) spontaneous and FMLP-induced (1 μM) production of ROS, respectively, evaluated by CL intensity. *$p<0.001$ compared to the control (day 0).

growth (Fig. 1, *a*, *b*). A different trend was observed in animals with NS0 tumor. The amplitude of neutrophil response to 1 μM FMLP gradually increased in this group of animals starting from day 7 and on day 12 differed significantly from the control (Fig. 1, *a*, *c*). A significant increase of spontaneous and FMLP-stimulated production of ROS by neutrophils from mice with NS0 tumor was in line with the results obtained on whole blood and confirmed the development of immune response.

Hence, the time course of spontaneous and opsonized zymosan-stimulated production of ROS by blood cells, of inflammatory reaction parameters (neutrophil count and size) is characterized by common regularities during the studied tumors growth. A common trend of changes in ROS production by isolated neutrophils from mice with tumors is a higher level of spontaneous production and a lower intensity of response to stimulation, which is a characteristic sign of weaker cytotoxicity. The time course of ROS production was determined by the tumor immunogenic activity. The growth of an immunogenic tumor (NS0 in our study) was paralleled by the development of immune response. This model is not fit for studies of the modulatory effect of the tumor on immune system cells.

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