A NEW INFORMATIVE INDEX OF POLYMORPHONUCLEAR NEUTROPHIL FUNCTION

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An important role in the formation of defensive reactions of the organism is played by neutrophils, the level of whose function can be judged from morphologic, cytochemical, biochemical, biophysical, and immunobiological indices.

This paper describes an attempt to assess the state of function of polymorphonuclear neutrophils (polymorphs) on the basis of changes in the biophysical properties of their surface in a hypertonic medium [2, 6]. By this approach, additional information on the reactivity of these blood cells can be obtained under conditions of loading

To investigate this problem experiments were carried out in which changes were produced in polymorph function *in vivo* and *in vitro*. According to data in the literature, phagocytic activity of polymorphs can be simulated by activity aimed at the sympathetic nervous system [1, 9]. It has also been shown that a pathological process such as malignant growth is accompanied by depression of polymorph function [5, 8]. These two experimental models were chosen for the present investigation.

EXPERIMENTAL METHOD

Lowering of the tone of the sympathetic nervous system, accompanied by depression of polymorph function, was produced by chemical desympathization of rats with isobarin (guanethidine) (20 mg/kg), injected subcutaneously into animals for 4 weeks starting on the first day after birth. Intact rats of the same litter, reared together with the experimental rats, served as the control. Experiments were carried out on animals aged 1.5 months. To obtain the stimulating effect of the neurotransmitter on polymorphs, experiments were carried out *in vitro*, for it is only under those conditions that a concentration of the preparation in the microenvironment corresponding to that arising locally in response to excitation of the sympathetic nervous system can be created [7].

Tumors were induced in rats by intramuscular injection of an oily solution of 20-methylcholanthrene (2 mg of carcinogen per animal). Investigation of the peripheral blood was undertaken at the time of mass appearance of tumors (4.5-6 months after induction). Two series of experiments were carried out with an interval of 1 month to confirm the reproducibility of the results.

Halo-forming ability of the polymorphs was studied by the method in [4], modified somewhat. In animals with altered polymorph function due to desympathization or tumor growth one capillary tube of blood was taken (from the tail) and added to a mixture of three capillary tubes of 0.9% NaCl solution + 0.5 capillary tube of a 5% solution of sodium citrate, and a "crushed drop" preparation was made at once on a slide, by mixing equal volumes (0.03 ml of each) of the resulting blood cell suspension and of 15% NaCl solution. Alongside it on the same slide a preparation was made for counting leukocytes, by mixing the cell suspension in the above proportions with a 3% solution of acetic acid. In experiments with addition of the neurotransmitter to the incubation medium blood from healthy animals was mixed in the proportions indicated above with physiological saline and a solution of noradrenalin in physiological saline (1 mg/ml). Together with parallel control samples (i.e., in physiological

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TABLE 1. Changes in Halo Formation Following Action Directed toward the Sympathetic Nervous System (M ± m)

Procedure	Control	Expt.	P
Desympathization	2,39±0,51 (12)	14,67±2,62 (17)	<0,001
Noradrenalin	7,05±1,13 (10)	2,9±0,57 (10)	<0,01

Legend. Here and in Table 2, number of experiments given in parentheses.

TABLE 2. Changes in Halo Formation during Growth of Chemically Induced Rat Sarcomas (M ± m)

Group of animals	Series I (4.5-5 months after induction)	Series II (5.5-6 months after induction)
Age control	2,75±0,30 (44)	2,35±0,32 (26)
Growth of tumor (volume): up to 20 cm^3	4,08±0,80 (46)	3,30±0,61 (21)
20-40 cm^3	5,72±1,28 (47)*	7,93±2,16 (20)*

Legend. *P < 0.05.

saline only) the cell suspension was incubated for 1 h at 37°C. The subsequent course of preparation of specimens followed the method described previously.

Halos were counted after 1-2 h in 10 fields of vision under magnification of the microscope: ocular 15, objective 9. The results were expressed as a percentage of the total number of leukocytes in 10 analogous fields of vision in the corresponding preparation.

EXPERIMENTAL RESULTS

As Table 1 shows, after desympathization of the animals the relative percentage of halo-forming cells in the peripheral blood increased considerably, whereas addition of noradrenalin to the incubation medium of the blood cells led to a decrease in this parameter.

Development of a tumor (Table 2) was accompanied by a progressive increase in the number of halo-forming cells in the peripheral blood of the experimental animals. The results of the two series of experiments were identical.

Desympathization of the animals and tumor growth, leading to depression of polymorph function, were thus characterized by increased halo formation, but the stimulating action of noradrenalin on the blood cells weakened halo formation.

This phenomenon of halo formation (vesiculocytosis) around living cells placed in a hypertonic medium has been described previously [2, 6]. For instance, vesicle formation in hypertonic solution takes place on account of separation of erythrocytes by a substance secreted from a leukocyte as a result of destructive processes [6]. It is considered [3, 4] that halo formation is the result of the action of electrical and mechanial factors.

The mechanisms of halo formation still remain a topic for study. Nevertheless, the results of the present investigation indicate its biological importance. It follows from the data described above that a change in the ability of polymorphs to form halos can be used as an indicator of the state of their function and to assess the defensive reactions of the organism when exposed to the action of various pathogenic agents.

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