STUDY OF OPSONIC FACTORS BY THE NITROBLUE TETRAZOLIUM REDUCTION REACTION BY HUMAN NEUTROPHILS

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A method of determining nonspecific opsonins in the system of human neutrophilic phagocytosis is suggested. The method is based on assessment of the intensity of metabolic stimulation of neutrophils by killed Serratia marcescens cells in the presence of opsonizing substrate. Serum opsonic activity levels were determined in 44 healthy blood donors.

KEY WORDS: nonspecific opsonins; human neutrophils; method.

Realization of the biological potential of the neutrophil depends on its interaction with many internal environmental factors [2, 3]. Analysis of functional contacts is essential for a correct understanding of the role of neutrophils in physiological processes and pathological situations, many of which are formed with their participation [6, 13]. In the modern view, an important place in the manifestation of the primary function of the neutrophil (phagocytosis) belongs to a combination of humoral factors with properties of nonspecific opsonins. They include various classes of immunoglobulins [7, 8], certain components of the complement [5] and properdin [10] systems, and the α_2-fraction of blood serum [11].

The discovery of "extracellular" defects is an important step in the investigation of patients with disturbances in the phagocytosis system. The undertaking of such tasks is restricted by the absence of objective methods of testing [14]. During the last decade the reaction of stimulation of intracellular reduction of nitroblue tetrazolium (nitro-BT) by objects of phagocytosis [4, 9] has been widely used to study the bactericidal potential of human neutrophils.

This paper gives the results obtained by the use of this reaction to assess the functional dependence of neutrophils on nonspecific serum factors.

EXPERIMENTAL METHOD

Leukocytes were isolated from the venous blood of a healthy blood donor. Erythrocytes were sedimented by the addition of a 6% solution of dextran T-500 in a final concentration of 1.5% for 60 min at 4°C. The dextran solution was made up in isotonic 0.1 M phosphate buffer, pH 7.2. The leukocytes were washed three times with Hanks' solution with 0.1% gelatin and 5 units/ml* (200g, 5-10 min). The washed leukocytes were suspended in Hank's solution with 4.4% human serum albumin (from Reanal, Hungary) in a concentration of 10^7 cells/ml. All manipulation with blood and cells were carried out in glassware treated twice with silicone.

The neutrophils were stimulated by a cell suspension of Serratia marcescens (2×10^9 bacterial cells), killed by heating at 100°C for 60 min. Test sera were obtained from venous blood of 44 healthy donors aged 20-40 years and were used in the course of 24 h. Specific reactions between S. marcescens vaccine and the test sera were excluded by a negative developed agglutination reaction.

The incubation medium (pH 7.2) consisted of a mixture of 0.1 ml of leukocyte suspension, 0.05 ml of the test serum (final concentration 15%), 0.1 ml of stimulator (the object of phagocytosis), and 0.1 ml of 0.2% nitro-BT solution (Lachema-Chemapol, Czechoslovakia). After incubation at 37°C for 30 min the reaction was stopped by the addition of 3 ml 0.1 N HCl and the sample was centrifuged for 5 min at 200g. Films were prepared from the residue and fixed with methanol. The nuclei were counterstained with 2% methyl green solution and the percentage of activated neutrophils with diformazan inclusions was calculated as described by the writers previously [1].

*Substance not given in Russian original – Publisher.

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TABLE 1. Individual Indices of Activation of Neutrophils of Healthy Donors in Positive and Negative Controls

Positive control	Negative control	Ratio of positive to negative controls
72	21	3,4
55	39	1,4
18	7	2,6
66	13	5,1
35	6	5,8
31	2	15,5
40	31	1,3
45	7	6,4
42	11	3,8
28	1	28,0
32	8	4,0
28	1	28,0
41	10	4,1
50	26	1,9
41	5	8,2
41,6±3,7	12,5±3,0	8,0±2,3
	$P < 0,001$	

The viability of the cells at the beginning of the experiment and immediately after incubation was determined by the Trypan blue test.

The reactions were standardized against positive and negative controls. In the positive control, standard serum (a pool from 6 healthy donors), kept at −20°C, was added instead of the test sera. In the negative control the serum was replaced by an equal volume of Hanks' solution. The results were expressed as an index of opsonic cooperation (IOC) between serum and neutrophils, by the following equation:

$$10C = \frac{A - B}{C - B},$$

where A is the percentage of activated neutrophils in the reaction with the test serum, B in the negative control, and C in the positive control.

EXPERIMENTAL RESULTS

Preliminary experiments with neutrophils from 15 healthy donors showed that differences between activation of neutrophils in the positive and negative controls were significant ($P < 0.001$; Table 1). The ratio between them averaged 8.0 ± 2.3, and significant values of this index ($P < 0.05$) lay within the interval 3.2-12.8. Test systems with a ratio of the positive control to the negative of over 3 were used in these experiments.

IOC of healthy donors' sera are given in Table 2. The mean index was 1.05 ± 0.097 ($P < 0.05$). Most sera (77.3%) had values of IOC of between 0.8 and 1.6, but in two cases lower values were observed (under 0.4), and in one case the index exceeded 1.6. The viability of the cells in all experiments was not less than 93%.

The study of the reactivity of neutrophils in the test system devised by the writers thus enabled levels of the potentiating effect of nonspecific humoral factors on metabolic stimulation during phagocytosis to be differentiated. This index can be regarded as the biochemical criterion of opsonization. The choice of vaccines from S. marcescens – a species of the Enterobacteriaceae – rarely in contact with man [12], as the object of phagocytosis rules out the effect of specific reaction on the process of neutrophil stimulation. The suggested method can also be used to assess other manifestations of cooperation in the system of neutrophilic phagocy-

TABLE 2. Values of IOC for Sera from Different Donors

Values of IOC	Number of sera
0,4	2
0,4—0,8	7
0,8—1,2	19
1,2—1,6	15
1,6	1

tosis. The accessibility of the test and the possibility of rapid and objective evaluation make its introduction into scientific research and practical medicine well worth while.

LITERATURE CITED

1. M. E. Viksman and A. N. Mayanskii, Kazan. Med. Zh., No. 5, 99 (1977).
2. A. N. Mayanskii, M. E. Viksman, L. G. Popova, et al., Byull. Éksp. Biol. Med., No. 2, 187 (1978).
3. G. Mowat, in: Information, Immunity, and Hypersensitivity [Russian translation], Moscow (1975), pp. 9-128.
4. R. L. Baehner and D. G. Nathan, New Engl. J. Med., 278, 971 (1968).
5. I. M. Goldstein, H. B. Kaplan, A. Radin, et al., J. Immunol., 117, 1282 (1976).
6. P. M. Henson, Immunol. Commun., 5, 757 (1976).
7. J. Menzel, H. Jungfer and D. Gemsa, Infect. Immun., 19, 659 (1978).
8. I. Miler, Faktory Prirozene Rezistence Organismu, Prague (1976).
9. D. G. Nathan, R. L. Baehner, and D. K. Weaver, J. Clin. Invest., 48, 1885 (1969).
10. G. Nathenson, M. E. Miller, K. A. Myers, et al., Clin. Immunol. Immunopath., 9, 269 (1978).
11. T. M. Saba, in: Immune System and Infectious Diseases, Basel (1975), pp. 489-504.
12. J. Sedlak and H. Rische, (Editors), Enterobacteriaceae – Infektionen, Leipzig (1968).
13. P. C. Wilkinson, Clin. Exp. Immunol., 25, 355 (1976).
14. M. Yamamura and H. Valdimarsson, Immunology, 34, 689 (1978).

SEASONAL VARIATIONS IN THE CATECHOLAMINE CONCENTRATION IN THE ALBINO RAT BRAIN

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Seasonal changes in the concentrations of dopamine (DA), noradrenalin (NA), and homovanillic acid (HVA) in the forebrain and diencaphalon were studied in 258 Wistar albino rats. Experiments were carried out monthly for six years. The concentrations of DA, NA, and HVA in the brain changed significantly in the course of the year, and in both parts of the brain the changes were in the same direction. The DA concentration in the winter and spring months was higher than the average for the year, whereas in summer it was lower. The NA concentration was much higher in spring (by one-third in the diencephalon) than the mean values. In the summer months (June-August) the average NA concentration corresponded to more rapid metabolism of DA (a decrease in DA and an increase in HVA).

KEY WORDS: catecholamines; seasonal variation; rat brain.

Experiments to study the concentrations of indolamines and catecholamines in the tissues and fluids of laboratory animals were carried out previously (during 1972-1976). The experimental animals were kept under standard conditions of temperature and lighting, and the conditions of their food and fluid intake were identical. Material for investigation was always taken at the same time of day (at 10 a.m.) and the monoamines were determined by the same method. Despite this fact, the results varied appreciably in the course of the year. Seasonal variations were particularly marked in the concentration of serotonin and its metabolites [5]. The serotonin concentration, in turn, is known to be connected with catecholamine metabolism. The decisive role in the changes in indole metabolism is played by activity of the serotonin coenzyme A, N-acetyltransferase, whose activity is regulated by noradrenalin through β-adrenoreceptors [8, 9, 14]. The diurnal rhythm of the serotonin concentration in the rat brain also depends on fluctuations in the activity of serotonin decarboxylase, which is under the control of the competitive effect of catecholamines and serotonin on the enzyme [8]. In

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