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EFFECT OF NEUTROPHILOKINES ON THE IMMUNE RESPONSE OF MICE

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KEY WORDS: neutrophilokines, neutrophils, macrophages, immunocompetent cells, sheep's red blood cells, splenocytes.

An urgent problem in modern immunology is the study of interaction between cells of the immune system. The least studied aspect of this problem is the role of neutrophils in immune reactions and their connection with other immunocompetent cells. The writers showed previously that neutrophils, on incubation in medium 199 without activators or with activators secrete products capable of altering the functional activity of cells of the mononuclear phagocytic system [3, 4]. The nature of these products and their effect on the immune response remain unexplained.

The aim of the present investigation was to study the nature of products of neutrophils and their effect on the immune response of mice.

TABLE 1. Effect of Neutrophilokine Fractions on Induction of AFC-SRBC and AGR Function of (CBA × C57BL)F_1 Mouse Macrophage (M ± m)

Experimental conditions	Immunization with SRBC		Immunization with macrophages	
	number of AFC per spleen (× 10^2)	number of AFC per 10^6 spleen cells	number of AFC per spleen (× 10^2)	number of AFC per 10^6 spleen cells
Control (medium 199)	228,1±24,2	206,7±22,3	70,0±5,67	48,96±3,77
Injection of U_1	138,1±16,8	90,0±9,09	37,5±3,61	30,31±3,38
p	<0,02	<0,01	<0,001	<0,01
A_1	231,3±18,0	238,8±13,6	107,0±11,34	98,09±8,59
p	—	—	<0,02	<0,001
U_2	187,5±17,4	156,9±14,4	53,5±10,3	42,8±10,24
A_2	180,6±13,39	161,9±19,12	77,0±5,15	55,96±6,35
U_5	225,0±25,4	165,8±12,1	71,0±9,79	53,27±11,34
A_5	386,9±19,3	382,0±21,3	116,0±6,19	104,09±10,33
p	<0,001	<0,001	<0,001	<0,001

Legend. Here and in Table 2: p — compared with control.

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TABLE 2. Effect of Neutrophilokine Fractions on Ability of Splenocytes of CBA Mice to Induce GVHR in (CBA × C57BL)F_1 Hybrids

Experimental conditions	GVHR index	p
Control (medium 199)	3,67±0,288	—
Injection of U_1	3,87±0,238	—
A_1	4,34±0,204	—
U_2	2,74±0,124	<0,02
A_2	2,66±0,117	<0,02
U_5	3,34±0,261	—
A_5	4,71±0,257	<0,05

EXPERIMENTAL METHOD

CBA mice and (CBA × C57BL)F_1 hybrids, obtained from the "Rappolovo" Nursery of Laboratory Animals, Academy of Medical Sciences of the USSR, were used. Neutrophils were obtained from peripheral blood by centrifugation at 1500 rpm on a double Ficoll—Verografin gradient (d 1.077 and 1.093); the Ficoll was obtained from Pharmacia (Sweden), the Verografin from Spofa (Czechoslovaia) [8]. Neutrohils obtained from 25 first-time blood donors, were adjusted to a concentration of $5 \cdot 10^6$ cells in 1 ml and incubated in medium 199 with particles of monodispersed polystyrene latex 1.7 μ in diameter (activated neutrophils) or without latex (unactivated neutrophils) for 1 h at 37°C. The ratio of neutrophils to latex was 1:100 (the latex was obtained from the All-Union Synthetic Rubber Research Institute, Leningrad). After incubation the cells and latex particles were removed by centrifugation at 1500 rpm and filtered through filters with pores 0.24 μ in diameter ("Millipor," USA). Later the pools of supernatants were subjected to gel chromatography on Sephadex G-15 ("Pharmacia") [1]. The fractions thus obtained were concentrated and the content of peptides of average molecular weight in them was determined by the microbiuret method, using Benedict's reagent [1]. Fractions eluted immediately after the void volume were conventionally called A_1, A_2 ...A_n for supernatants of activated neutrophils and U_1, U_2 ... U_n for unactivated neutrophils. The isolated fractions were injected intravenously in a dose of 0.0004 mg peptides in a volume of 0.2 ml per mouse 4 times at intervals of 24 h (the concentration was calculated as peptide products secreted by 10^6 neutrophils). Sheep's red blood cells (SRBC) were injected intravenously in a dose of $2 \cdot 10^8$ into animals of group 1, 24 h after the last injection, in order to determine antibody-forming cells (AFC-SRBC) by the method in [6]. SRBC were injected intraperitoneally into mice of group 2 in a dose of $5 \cdot 10^8$ in order to determine the antigen representing function (AGR function) of the macrophages [2]. In the animals of group 3, splenocytes were taken at the same times after injection of the preparations to set up the local graft versus host reaction (GVHR) in the popliteal lymph node, by semisyngeneic transfer [7]. The GVHR was set up in the genetic combination CBA → (CBA × C57BL)F_1. Intact mice, receiving an injection of medium 199, served as the control. The results were subjected to statistical analysis of variance by Student's test.

EXPERIMENTAL RESULTS

Elution profiles of products of activated and intact neutrophils were identical. Peptide products were identified only in the first, second, and fifth peaks. Later only these peaks were used for investigation. The isolated fractions of neutrophilokines differed in their activity. The U_1 fraction inhibited antibody formation and the AGR function of the macrophages statistically significantly (Table 1), the U_2 fraction inhibited activity of the splenocytes in GVHR (Table 2), and U_5 caused virtually no change in function of the immunocompetent cells.

Fractions of the supernatants of activated neutrophils caused other effects. For instance, A_1 significantly stimulated the AGR function of the macrophages (Table 1) and stimulated activity of the splenocytes in GVHR, but not significantly (Table 2); A_5 stimulated AFC-SRBC production and the AGR function of the macrophages statistically significantly (Table 2). Only fraction A_2 behaved identically with U_2 — it inhibited activity of the spleen cells in GVHR (Table 2).

The results are evidence that neutrophils can secrete peptide products regulating the function of immunocompetent cells. It is well known that neutrophils penetrate more rapidly than other cells into a focus of injury and they are the first to respond to injection of most antigens [5]. Accordingly, it is logical to postulate that activation products of neutrophils may serve as initiating factors in the development of cellular cascade reactions, involved in the development of inflammation and the immune response. Under normal conditions peptide products of intact neutrophils may act as the source of suppressor regulatory factors of immunocompetent cells.

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NATURE OF A SERUM FACTOR PRECIPITATING AUTOLOGOUS ALPHA-GLOBULINS IN RABBITS

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One of the principal phenomena in which specific interaction of serum antibodies with antigens against which they are directed is manifested is the precipitation reaction. The writers showed previously that on immunization of rabbits with human albumin, starting with the 7th day and throughout the period of the immune response precipitating activity is found in the blood serum, directed both against human albumin and against autologous alpha-globulins (AAG) of intact rabbit serum. The reaction between proteins of immune and intact blood sera was recorded by immunodiffusion and immunoelectrophoresis in agarose [2]. The appearance of precipitating activity was observed in 100% of animals immunized with human albumin in a dose of 100 mg, and was absent in intact rabbits and rabbits immunized with other antigens tested (human transferrin and IgG, bovine albumin, rabbit albumin, Vi-antigen, sheep's red blood cells).

The aim of this investigation was to study the nature of a factor in rabbit immune blood serum precipitating autologous serum alpha-globulins.

EXPERIMENTAL METHOD

Blood serum from rabbits immunized intravenously by a single injection of human albumin ("Reanal," Hungary) in a dose of 100 mg per animal was used.

Antibodies in the immune serum were determined quantitatively by the passive hemagglutination test (PHT) using sheep's red blood cells sensitized with human albumin [1]. The AAG-precipitating factor was isolated by ion-exchange and immunoaffinity chromatography.

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