little at this period. The view is held [3] that the appearance of immunoglobulins on T-lymphocytes may reflect a certain stage of their maturation and activation.

Formation of the B-lymphocyte population in man thus begins in early prenatal ontogeny; Ig-positive cells with surface IgG-receptors are present both in the thymus and in the spleen, but the density of immunoglobulin receptors on their surface and the ability of the lymphocytes to form "caps" of fluorescence in the presence of antigens are evidence of the degree of differentiation of the lymphocyte.

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COOPERATION OF ANTIGENS, IMMUNOGLOBULINS, COMPLEMENT, AND ANTIMICROBIAL ENZYMES IN REGULATION OF MOBILITY OF BLOOD GRANULOCYTES

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Cooperation between specific and nonspecific factors of humoral immunity in the regulation of granulocyte mobility was studied. Bacterial antigens of dental plaque, immunoglobulins, lysozyme, peroxidase, ribonuclease, and trypsin, each separately, were shown to produce moderate stimulation of chemotaxis and chemokinesis of granulocytes. The strongest chemotaxic effect was given by ribonuclease and the strongest chemokinetic effect by lysozyme. The strongest chemotaxic stimulus was generated on activation by complement in the classical way. Lysozyme sharply enhanced whereas ribonuclease and trypsin depressed the formation of the chemotaxis factor of complement in the classical way. Treatment of granulocytes with antimicrobial enzymes lowered their sensitivity to this factor.

KEY WORDS: chemotaxis and chemokinesis of granulocytes; bacterial antigens; immunoglobulins; complement; antimicrobial enzymes.

In the intact organism complement activation takes place parallel with an increase in the activity of antimicrobial enzymes in a focus of inflammation [1, 2, 6]. Because of the absence of data in the literature on relations between these two manifestations of humoral immunity in the generation of the chemotaxic stimulus, their separate and combined effect on granulocyte chemotaxis and chemokinesis was studied.

EXPERIMENTAL METHOD

Mobility of granulocytes isolated from 30 blood samples from clinically healthy donors was studied. The granulocytes were isolated from whole blood, diluted 1:2, by centrifugation in a two-step density gradient of

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TABLE 1. Effect of Antimicrobial Enzymes on Chemotaxis of Human Blood Granulocytes and on Generation of Chemotaxic Stimulus

Contents of open compartment	Statistical index	Granulocytes in Eagle's medium									
Density of granulocyte front	M	22	451	130	163	235	123	574	387	121	206
	$\pm m$	1,8	32	47	78	52	41	35	30	8	9
	$P<$		0,01	0,03	0,08	0,01	0,02	0,01	0,04	0,01	0,01
	$P_1<$		0,01	0,01	0,01	0,01	0,01	0,01	0,14	0,01	0,01
Contents of closed compartment		PS	DP Ig BP	L	P	R	T	L, DP BP Ig	P, DP BP Ig	R, DP BP Ig	T, DP BP Ig

Legend. Here and in Table 2: 1) index of significance P calculated relative to negative control, $\overline{P_1}$ relative to positive control.
2) PS) Physiological saline; DP) dental plaque; Ig) immunoglobulins; BP) blood plasma; L) lysozymes; P) peroxidase; R) ribonuclease; T) trypsin.

TABLE 2. Effect of Antimicrobial Enzymes on Chemokinesis and Sensitivity of Granulocytes to Standard Chemotaxic Stimulus

Contents of open compartment	Statistical index	Granulocytes in Eagle's medium							
		L	P	R	T	L	P	R	T
Density of granulocyte front	M	434	337	231	168	255	167	201	323
	$\pm m$	73	54	57	22	88	62	68	43
	P	0,01	0,01	0,01	0,01	0,01	0,03	0,01	0,01
	P_1	0,84	0,08	0,01	0,01	0,01	0,01	0,01	0,02
Contents of closed compartment		PS	PS	PS	PS	DP Ig BP	DP Ig BP	DP Ig BP	DP Ig BP

1.077/1.097 g/ml, made up from a mixture of 9% Ficoll-400 solution (from Pharmacia, Sweden) and 60% verografin solution (from Spofa, Czechoslovakia). A suspension of 250,000 granulocytes in 0.5 ml Eagle's medium was used. Granulocyte mobility was studied in blind chambers of brand 200-312 (from Neuro Probe, USA), which are an up-to-date modification of Boyden's chambers [5]. The intensity of chemotaxis and chemokinesis was estimated from the degree of filling pores, 3 μ in diameter, in Mark 11302 filters (from Sartorius Membranfilter, West Germany). A suspension of dental plaque, sterilized by ultrasound (44 kHz, 25 W/cm^2, 30 min), in a final concentration of 5 mg/ml was used as bacterial antigen. Immunoglobulins isolated from mixed human saliva by gel-filtration on Sephadex G-200 were used in a final concentration of 0.17 mg/ml, of which 74% consisted of secretory IgA and 26% of IgG, fixing the complement contained in the immune complex most actively [3]. Autogenous blood plasma, after removal of cells, was used as the source of complement. Other reagents were used in the following final concentrations: egg albumin lysozyme (from Reakhim, USSR) 20 units/ml; peroxidase (from Reanal, Hungary) 50 units/ml; alkaline ribonuclease, isolated by the writers themselves from rabbit saliva [4] 0.5 unit/ml; trypsin (from Spofa, Czechoslovakia) 0.5 unit ml.

EXPERIMENTAL RESULTS

Activation of complement on the immune complex was shown to stimulate oriented movement of leukocytes against the concentration gradient of the chemotaxic factor sharply, so that it was 20 times greater than the index of spontaneous migration of the cells through a millipore filter (Table 1). Mixed human saliva, in which activation of complement on the complex bacterial antigens of dental plaque with salivary immunoglobulins is continuously maintained, had a rather weaker chemotaxic action (279 ± 68; $P < 0.001$). Bacterial antigens and immunoglobulins themselves gave even weaker stimulation of chemotaxis, namely 147 ± 27 ($P < 0.001$) and 45 ± 9.6 ($P < 0.03$) respectively.

The study of the effect of antibacterial enzymes on chemotaxis of granulocytes showed for the first time that each one has a marked positive chemotaxic effect, which is especially strong in the case of ribonuclease (Table 1). The chemotaxic action of trypsin increased sharply during incubation with blood plasma (409 ± 79; $P < 0.01$) through the formation of chemotaxic factors following the splitting of complement and kininogen by

this enzyme [7, 8]. The study of the effect of antimicrobial enzymes on formation of the chemotaxic factors in the immune complex–complement system yielded important facts (Table 1). Lysozymes sharply stimulated this process, peroxidase had virtually no effect on its activity, and ribonuclease and trypsin significantly inhibited it. The reason for this phenomenon will evidently be explained by a study of the modification of the surface receptors of the granulocytes and of the antigen, antibody, and complement molecules under the influence of these enzymes.

The addition of the enzymes directly to the granulocyte suspensions sharply increased their chaotic mobility (Table 2). This property was particularly marked in the case of lysozyme, possible evidence of the important role of polysaccharide structures on the surface of the granulocytes in the regulation of chemokinesis. However, the response of the granulocytes to an additional chemotaxic stimulus arising from the lower compartment of the chamber in response to activation of complement of the immune complex, was sharply reduced under these conditions. This phenomenon may be due either to hydrolysis of the chemotaxic factor in the upper compartment of the chamber or to preliminary modification of the reactivity of the granulocytes by the enzymes. The results of this last experiment, it should be noted, agree with data in the literature on the inhibitory effect of trypsin on chemotaxic reactivity of leukocytes, although they do not confirm data showing that egg lysozyme does not affect this process [9].

The results of the present experiment thus points to close interaction between antigens, immunoglobulins, and antimicrobial enzymes both in generation of the chemotaxic stimulus and in modification of cellular reactivity of granulocytes.

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