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EFFECT OF ENZYMES OF THE CONTACT PHASE OF BLOOD CLOTTING ON PHAGOCYTIC ACTIVITY OF THE NEUTROPHILS

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The effect of the product of the contact phase of blood clotting (CAP), i.e., factor XII and its activated form on the reaction of reduction of nitro-BT by human neutrophils was studied. In all cases CAP caused marked stimulation of the neutrophils. The response of the neutrophils to factor XII was observed irregularly. It was more regular and stronger after activation of this factor. The indirect effect of CAP on neutrophils is postulated, through activation of interconnected enzyme systems of the blood plasma.

KEY WORDS: neutrophils; phagocytosis; contact activation product.

Various humoral factors of the blood plasma participate in the regulation of metabolic processes maintaining the phagocytic function of the neutrophils. Activation products of the complement system [2, 3], the α_2-glycoprotein fraction [9, 11, 13], C-reactive protein [4], fetuin [13], and the kallikreins [5] are all stimulators of neutrophils. The functional state of the neutrophils has been observed to change during incubation of blood in glassware not treated with silicone. Accordingly, the investigation described below was undertaken to study the mechanism of this phenomenon, namely the possibility of its mediation through factors participating in the contact phase of blood clotting.

EXPERIMENTAL METHOD

Contact activation product (CAP) was obtained from human blood plasma by the method of Schoenmakers et al. [12]. The eluate from the glass was concentrated with dry Sephadex G-50. The glycine buffer was changed for isotonic 0.15 M phosphate buffer, pH 7.2, on a column with Sephadex G-25. The preparation had the property of restoring the defective clotting of plasma not containing contact factors, obtained by Nossel's method [8].

Partially purified factor XII (F-XII) – fraction IVS – also was obtained from bovine plasma [12]. During electrophoresis in polyacrylamide gel three protein zones were detected in it. The protein content in the zone capable of correcting the defective clotting of plasma deprived of contact factors was about 80% of the total pro-

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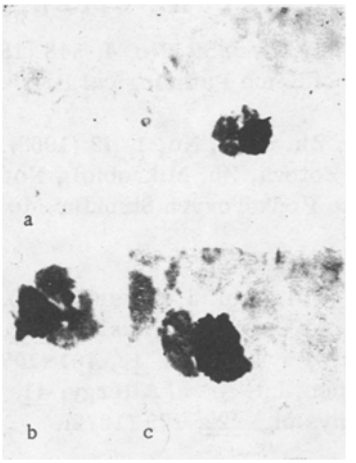

Fig. 1. Reaction of reduction of nitro-BT by human blood neutrophils. Polymorphic, coarsely dispersed black deposits (dark blue in the actual preparation) of diformazan can be seen in cytoplasm of neutrophils beside paler segments of their nuclei (green in the actual preparation). Outlines of red blood cells also visible. 900×.

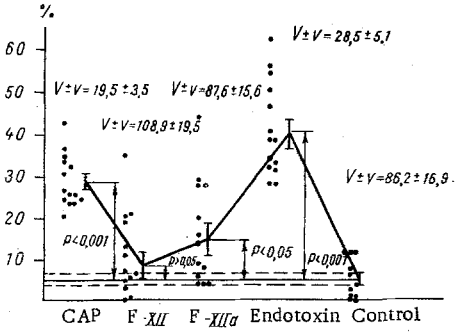

Fig. 2. Stimulation of neutrophils by CAP, F-XII, F-XIIa, and endotoxin. V ± v) Coefficient of variation and its error. Ordinate, percentage of stimulated neutrophils.

tein content. Immediately before the experiment the freeze-dried preparation was dissolved in distilled water and exchange to phosphate buffer (pH 7.2) was carried out on a column with Sephadex G-25. The F-XII was activated (converted into F-XIIa) by glass.

The reaction of the neutrophils to the test preparations was studied by intracellular substrate-free reduction of nitro-blue tetrazolium (nitro-BT) in whole blood from donors aged 20-35 years by the method of Park et al. [10] with certain modifications (Fig. 1). It is usually considered that this index reflects the ability of the neutrophils to perform phagocytosis, the result of activation of the hexose–monophosphate shunt [6, 7]. The level of stimulation was determined from the number of formazan-containing cells among 100 neutrophils. CAP, F-XII, and F-XIIa were tested in concentrations of 20-25 μg/ml. Endotoxin of S. marcescens (25 μg/ml) served as the standard stimulator, and unstimulated neutrophils were used as the control.

EXPERIMENTAL RESULTS

In the control, 0-11% of neutrophils (5.1 ± 1.3) were stimulated (Fig. 2). Of the three preparations (CAP, F-XII, F-XIIa) CAP had the strongest activity: the number of stimulated neutrophils was 21-43% (28.8 ± 1.6; $P < 0.001$). The addition of F-XII to the test system was followed by twofold stimulation or more in five of 13 cases (8.9 ± 3.0; $P > 0.05$). F-XIIa stimulated neutrophils in nine of the 13 donors (14.5 ± 3.7; $P < 0.05$). After incubation with the endotoxin the level of stimulation was 30-63% (39.8 ± 3.2; $P < 0.001$).

The individual features of the reaction of the neutrophils to the various preparations were manifested differently (Fig. 2). The lowest values of the coefficient of variation were observed in the case of stimulation by CAP and endotoxin (19.5 ± 3.5 and 28.5 ± 5.1, respectively), the maximal in the experiments with F-XII.

The results of these experiments show that the components of CAP, in a dose corresponding to their concentration in the blood and arising during the contact phase of clotting,* have a significant effect on the functional phagocytic activity of the neutrophils. The direct effect of CAP on the neutrophils cannot be ruled out, although no such information is present in the literature. The stimulating effect of the preparation could also be mediated through activation of the interconnected enzyme systems of the plasma, primarily the kallikrein–kinin and complement systems [1]. One of the probable effector components of CAP is F-XIIa, which caused significant stimulation of the neutrophils in most of the present experiments. The high variability of the individual indices observed during stimulation of the neutrophils by preparation F-XIIa and, in particular, by F-XII, is interesting. To some extent this may be associated with the character of interaction of this factor with the humoral components of the plasma responsible for its effect on the neutrophils. The possibility of individual differences in the receptor apparatus of the neutrophils, by means of which they interact directly with biologically active substances, likewise cannot be ruled out.

The results of these investigations suggest that under natural conditions stimulation of metabolic processes in the neutrophils, which is observed under the influence of various factors, may be the result of primary activation of the humoral components of the blood plasma. This must be borne in mind during analysis of clinical observations and also during the modeling of experimental systems to study the functional activity of neutrophils.

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*The final volume of the CAP preparation corresponded to the initial volume of blood taken for its preparation.