oxidant effect of HDL will add a new dimension to our understanding of their antiatherogenic action.

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LEUKOCYTIC THERMOSTABLE α-GLYCOPROTEIN: IMMUNOCHEMICAL STUDY AND INVESTIGATION OF ITS ENZYME ACTIVITY

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KEY WORDS: leukocytic α-glycoprotein; granulocytic elastase; amidolytic activity; granulocytic antigens

Leukocytic thermostable α-glucoproetin (LTαG), with mol. wt. of 90 ± 7 kilodaltons, identified in 1982 [4], is a specific protein of human granulocytes [7]. A particularly important fact is that in certain diseases [1-4, 7], and also after operations involving the use of an assisted circulation [6], LTαG is found in raised concentrations in the patients' blood serum. It is impossible to assess the importance of this factor and the possibility of using it for diagnostic purposes and for studying the pathogenesis of diseases and their complications, without a study of the biological function of LTαG, but this has not hitherto been undertaken.

The investigation described below was devoted to the systematic immunochemical identification and characterization of the protein components of peripheral blood leukocytes, namely soluble leukocytic antigens (SLA) [5], LTαG is one of the proteins included in this system (SLA-2). The approach chosen to assess the functional state of the leukocytes was by determining concentrations of individual SLA in hemolysates from patients, for it has been shown

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Fig. 1. Comparative immunodiffusion analysis of LTαG with lysozyme and lactoferrin. 1) Standard antiserum against human lysozyme; 2) colostrum in a dilution of 1:4; 3) monospecific antiserum against LTαG; 4) monospecific antiserum against leukocytic β_1-microglobulin; 5) pus extract in dilution of 1:10; 6) standard antiserum against human lactoferrin; 7) seminal plasma in solution of 1:2; 8) monospecific antiserum against leukocytic α_2-macroglycoprotein.

[1, 3, 6] that in certain diseases the normal relations between these proteins may be altered.

In the investigation described below, a comparative immunochemical analysis was undertaken of LTαG with known components of granulocytes, and their enzyme activity was studied.

EXPERIMENTAL METHOD

Monospecific antisera against LTαG were obtained as described previously [4, 8]. For immunochemical determination of the antigens the double gel diffusion test was used. Antisera against human lactoferrin, lysozyme, and fibronectin were obtained from Boehringwerke (West Germany); polyspecific antisera against normal human blood plasma proteins and white cell lysate, and monospecific antisera against leukocytic α_2-macroglycoprotein (SLA-1) and β_1-microglobulin (SLA-3), obtained as described previously [5, 7], also were used.

Immune percipitates were stained for enzymes after washing and drying of the agar plates. For the reaction for nonspecific esterases [8], α-naphthyl acetate (Serva, West Germany) and Fast blue B (Chemapol, Czechoslovakia) were used. To detect peroxidase activity of the precipitates, the histochemical method [11] was used.

Elastase activity was determined by the method in [15]. The reaction mixture consisted of 100 µl of the test solution and 50 µl of substrate. The substrate for elastase, namely Suc-[Ala]$_3$ Nan (Serva), was diluted in 10 mM diemthyl sulfoxide. Substrates for thrombin (chromozym TH), plasminogen (chromozyme PL; Boehringer, West Germany), and kallikrein (S-2302, Kabi, Sweden), were used in aqueous solutions of the same concentration. Changes in optical density of the reaction mixture taking place during hydrolysis of the substrate and liberation of the p-nitroaniline residue were recorded at 405 nm on an FP-901 biochemical analyzer (Labsystems, Finland).

To test protease activity with chromogenic substrates LTαG was isolated on a bioaffinity sorbent with a fraction of pus proteins, obtained by ion-exchange chromatography on DEAD Sephadexes A-50 and SP C-50, immobilized on sepharose. Cyanogen bromide-activated sepharose (Pharmacia, Sweden) was used. Semipurified fractions of an aqueous extract of pus and samples of blood serum from patients with an LTαG content of up to 2 mg % were used as the original material for affinity isolation of LT/G. Unbound proteins were washed off with 0.15M NaCl and 0.1M phosphate buffer, pH 7.4. Elution was carried out with 0.1M glycine-HCl buffer with 0.1M NaCl (pH 2.4). The eluates were assayed for LTαG and impurities by immunodiffusion analysis with monospecific antiserum against LTαG and polyspecific antisera against blood plasma proteins and against proteins from normal human white cell lysate.

EXPERIMENTAL RESULTS

Comparative immunochemical analysis, using standard commercial antisera (Fig. 1), showed that LTαG is not identical with other known proteins of human granulocytes — lactoferrin and lysozyme. A reaction of complete identity was found in this case between lactoferrin and SLA-1 and SLA-3 [5]. LTαG also was not identical with fibronectin, the concentration of which in polymorphonuclear leukocytes and plasma may vary significantly in pathology.

Rabbit antiserum against normal human white cell lysate revealed one protein in the white cell lysate whose precipitate gave a positive reaction for peroxidase: a gray-blue color which, during the next 1-6 min, changed to brown. Precipitation arcs of LTαG present in the hemolysate, pus, and patients' blood serum, and also precipitation arcs of lactoferrin, lysozyme,

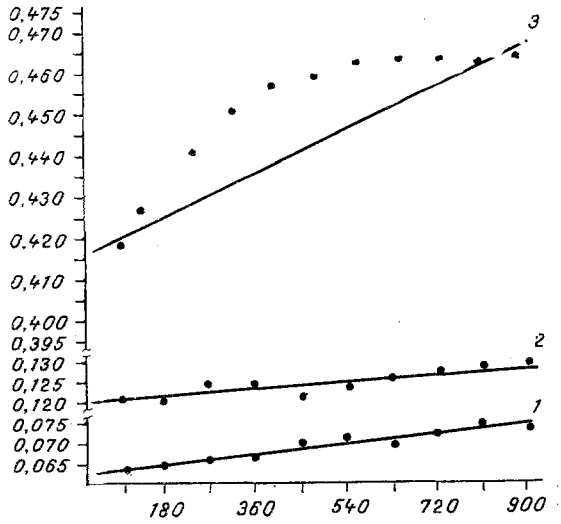

Fig. 2. Change in optical density as a result of hydrolysis of chromogenic substrate by LTαG. Abscissa, time (in sec); ordinate, optical density (in relative units). 1) Hydrolysis of substrate for plasminogen under the influence of purified LTαG; 2) hydrolysis of substrate for elastase under the influence of semipurified LTαG, 3) hydrolysis of substrate for elastase by purified LTαG.

and plasma proteins gave no such reaction. On staining for nonspecific esterases [8], the reaction of LTαG, like that of lactoferrin and lysozyme, was negative. The red-brown color of the precipitate of one blood plasma protein served as the control.

LTαG is thus not identical with lactoferrin, lysozyme, myeloperoxidase, or fibronectin. It also seems unlikely atht LTαG is identical with transcobalamine, for although the latter is a specific protein of human granulocytes, it is present in blood cells and plasma in much lower concentrations than LTαG [7]. Considering the similarity of certain physicochemical properties of LTαG and of granulocytic esterase, and also that granulocytic elastase is found in the blood serum after an assisted circulation [9] and in certain diseases [10], the protease (including elastase) activity of LTαG was studied.

Fractions from pus and white cell lysate, semipurified by ion-exchange chromatography, in which no plasma proteins were detected immunochemically, but in which, besides LTαG, from one to three leukocytic antigens were present as impurities, hydrolyzed substrates for elastase (Fig. 2). The aminolytic activity which was found may have been due to the presence of LTαG or to contamination by other serum proteases, inhibitors, and also several active and inactive forms of the enzyme. Immunochemically pure LTαG, isolated from the semipurified preparation of pus by affinity chromatography, also hydrolyzed the substrate for elastase (Fig. 2). The possibility of selective isolation of LTαG from pus on a protein ligand, also present in pus (nonspecific sorption was ruled out in control experiments using pure sepharose, purified lactoferrin immobilized on sepharose, or donkey antibodies against human immunoglobulin, immobilized on sepharose), may indicate the existence of an TLαG-binding protein in man, possibly an inhibitor of enzyme activity.

Besides the substarte for elastase isolated from pus, LTαG also hydrolyzed substrates for kallikrein, thrombin, and plasmin (Fig. 2).

The results of this investigation show that purified LTαG is an enzyme (a protease) which, in a neutral medium, can hydrolyze the bond of p-nitroanilide with the amin-acid residues of peptides in chromogenic substrates. It is very probable that LTαG is identical with granulocytic elastase [14], but the final answer to this question will be obtained only by direct immunochemical comparison. The biological role of granulocytic elastase has not yet been explained. It has been suggested that this enzyme, an activator of Hageman factor, affects processes of blood clotting during inflammation [12]. Immunochemical identification of new granulocytic antigens is currently being continued [13], and it is thus important to compare newly described leukocytic proteins not only with plasma proteins, but also with known leukocytic antigens.

Comparative analysis of the kinetics of substrate conversion under the influence of LTαG isolated from different sources will give some idea of the biological activity of this protein under normal and pathological conditions. The discovery that the LTαG preparation can hydrolyze substrates of varied specificity and, in particular, components of the hemostasis and fibrinolysis system requires further study, for it could determine both the degree of specificity of the chromogenic substrates and of the enzyme, as well as the possibility that LTαG may help to regulate the functional activity of various biological substrates *in vivo*.

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EFFECT OF PHENOTHIAZINES ON VISCOSITY AND ELECTRICAL STABILITY OF MODEL PHOSPHOLIPID MEMBRANES

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KEY WORDS: phenothiazines; membrane viscosity; breakdown voltage

Derivatives of the phenothiazine series are nowadays widely used in many different branches of medicine, but in particular in clinical psychiatry as neuroleptic drugs and antipsychotic agents. Although the history of the use of pentothiazines in medical practice goes back more than 30 years and the mechanism of their action on the CNS has been intensively studied, there are as yet no unanimously held views on the molecular mechanisms of their action. There is evidence that derivatives of the penothiazine series can affect the physical properties of the lipid bilayer of membranes [13, 15] and change the osmotic resistance of closed membrane formations [8-10, 12], and this is largely determined by the viscous properties of the phospholipid bilayer of membranes [14, 15] and also, under certain conditions, the surface charge on the membrane. However, this problem has not been systematically studied.

In the investigation described below the effect of derivatives of the phenothiazine series on the viscosity and electrical stability of model lipid membranes was studied.

EXPERIMENTAL METHOD

Monolayer liposomes for fluorescence investigations were obtained by the method in [6] from hen egg yolk phospholipids [7].

Multilayered unified liposomes were obtained by the method in [5]. The relative lateral viscosity of the lipid bilayer was determined by recording excimerization of pyrene [1]. The kinetics of excimerization of pyrene obeys the Stern–Volmer equation:

$$\frac{1}{\tau}=\frac{1}{\tau_0}+K_e\cdot C,$$

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