IMMUNOCHEMICAL DETECTION OF HETEROGENEITY OF THERMOSTABLE LEUKOCYTIC α-GLYCOPROTEIN

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Thermostable leukocytic α-glucoprotein (TLG), identified immunochemically in 1982 [2], is a specific protein of human granulocytes [3, 8]. In some diseases and pathological states TLG appears in the blood plasma [1, 2, 8]. The TLG concentration is high in the seminal fluid during disturbances of spermatogenesis [7]. The biological function of this protein has not been finally settled, but it is known to differ from lactoferrin, lysozyme, and transcobalamin. In connection with the discovery of elastase activity in TLD preparations the view has been expressed that this protein is identical with the elastase of human granulocytes. In diseases of an inflammatory character and in immunodependent diseases the concentration of granulocytic enzymes, including elastase, in the blood plasma is increased [9, 10]. Considering the possible enzyme activity of TLG and also the presence of this protein in different biological materials, it is reasonable to suggest the existence of molecular forms of TLG which differ in their functional activity or their physicochemical properties. Discovery of heterogeneous forms of TLG may be of great importance for the elucidation of the biological role of this protein under normal and pathological conditions.

To detect heterogeneity of TLG, a comparative immunochemical and physicochemical study was made of it in pus, leukocytes, and blood plasma.

EXPERIMENTAL METHOD

Monospecific antiserum against TLG was obtained as a result of immunization of rabbits and subsequent absorption. An extract of pus was mixed with Sephadex SP-C-50 (Pharmacia, Sweden) in the ratio of 1:1 by volume, washed with 0.9% NaCl solution, and eluted with 0.5 M Na_2HPO_4 solution. The eluate was dialyzed and used to immunize rabbits. Antiserum was absorbed with the free fraction obtained by chromatography of the extract of pus on Sephadex

TABLE 1. Comparative Analysis of Some Physicochemical Properties of TLG in Pus, Hemolysate, and Blood Plasma (M ± m)

Property	Extract of pus (n = 10)	Hemolysate (n = 11)	Blood plasma (n = 3)
Relative electrophoretic mobility	0,73±0,1	0,49±0,03	0,65; 0,71; 0,77
Change in electrophoretic mobility under the influence of neuraminidase	Reduced	Unchanged	Not tested
Thermostability, % of thermostable fraction	6,25—12,5	12,5—25	50—75

Legend. Number of determinations given in parentheses.

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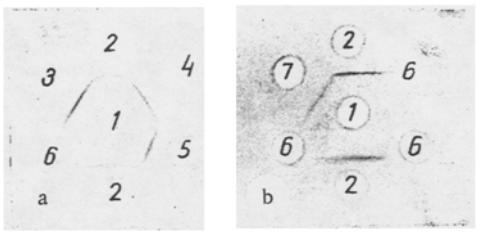

Fig. 1

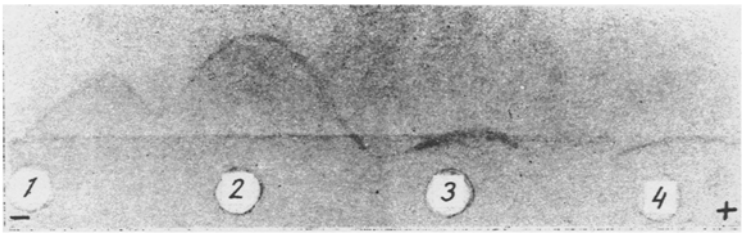

Fig. 2

Fig. 1. Comparative immunochemical analysis of TLG in human blood plasma, hemolysate, and pus (a) and in lyzed human leukocytes (b). 1) Monospecific antiserum against TLG; 2) solution of standard antigen (extract of pus in dilution of 1:8); 3) extract of pus; 4) blood serum from patient for heart surgery; 5) hemolysate; 6) 0.9% NaCl solution; 7) lyzed leukocytes.

Fig. 2. Crossed immunoelectrophoresis of TLG. 1) Blood serum from patient for heart surgery; 2) extract of pus; 3, 4) preparations of TLG obtained from extract of pus by ion-exchange chromatography. Electrophoresis in agarose gel containing 15% monospecific antiserum against TLG.

DEAE A-50, not containing TLG. Completeness of exhaustion was verified by immunodiffusion analysis with blood plasma proteins and lyzed leukocytes from normal blood donors, and the specificity of the antiserum was tested by comparison with antiserum against TLG [2]. Immunodiffusion analysis was carried out [6] with a standard test system, and extract of pus in a dilution of 1:8 was used as the standard antigen. Immunoelectrophoresis was carried out in 1% agarose or agar gel (Sigma, USA) in 0.05 M Veronal-Medinal buffer (pH 8.6).

Leukocytes were isolated from whole blood [5], the preparation of lyzed leukocytes was obtained by freezing and thawing a leukocyte suspension, and the hemolysate was obtained by freezing and thawing a blood clot. Pus was obtained after opening an abscess. Blood from healthy donors, from patients after operations for cardiac surgery, and patients with rheumatoid arthritis was used. To investigate TLG, blood serum or plasma from patients from heart surgery, with a titer of this protein of not less than 1:4, was used.

The relative electrophoretic mobility was determined against albumin after immunoelectrophoresis in agar gel [4]. To determine the molecular weight of the TLG, gel-filtration through Sephadex G-100 was used [12]; a set of standard proteins was obtained from Serva, West Germany. For comparative analysis of the thermostability of TLG, the test samples were boiled at 100°C for 1 min, cooled, the residue was removed, and the TLG concentration determined in the supernatant. The TLG concentration in the thermostable fraction was expressed as a percentage of the concentration of this protein in the original sample before boiling. Protease (trypsin-like) activity in the test samples was detected as hydrolysis of fibrinogen, conjugated with peroxidase (Sigma), after incubation with these samples, and also with trypsin (Sigma), used as the positive control.

EXPERIMENTAL RESULTS

The results of comparative immunochemical analysis of TLG in an aqueous extract of pus, the hemolysate, and blood plasma are shown in Fig. 1a. Within the limits of the resolving power of immunodiffusion analysis using the test system, complete immunochemical identity of the TLG present in different biological materials was found. Meanwhile, in two of 10 samples of lyzed leukocytes, prepared from peripheral blood leukocytes, TLG was found but it was partly changed from the antigenic standpoint. In one case this change took the form of the appearance of a classical "spur", in the other, by a characteristic change in the precipitation arc (Fig. 2b), also indicating absence of some antigenic determinants in the test sample compared with antigen of the test system.

Immunoelectrophoretic analysis of TLG in different samples of extract of pus, hemolysate, and blood plasma showed that the electrophoretic mobility of this antigen may vary from that of α_2- to β_1-globulins. The results of comparative analysis of the relative electrophoretic mobility of TLG are given in Table 1. Discovery of TLG in blood plasma or serum by immunoelectrophoresis is difficult because the concentration of this protein is not high enough, and it proved to be possible only in individual cases when its titer reached 1:16-1:32. These

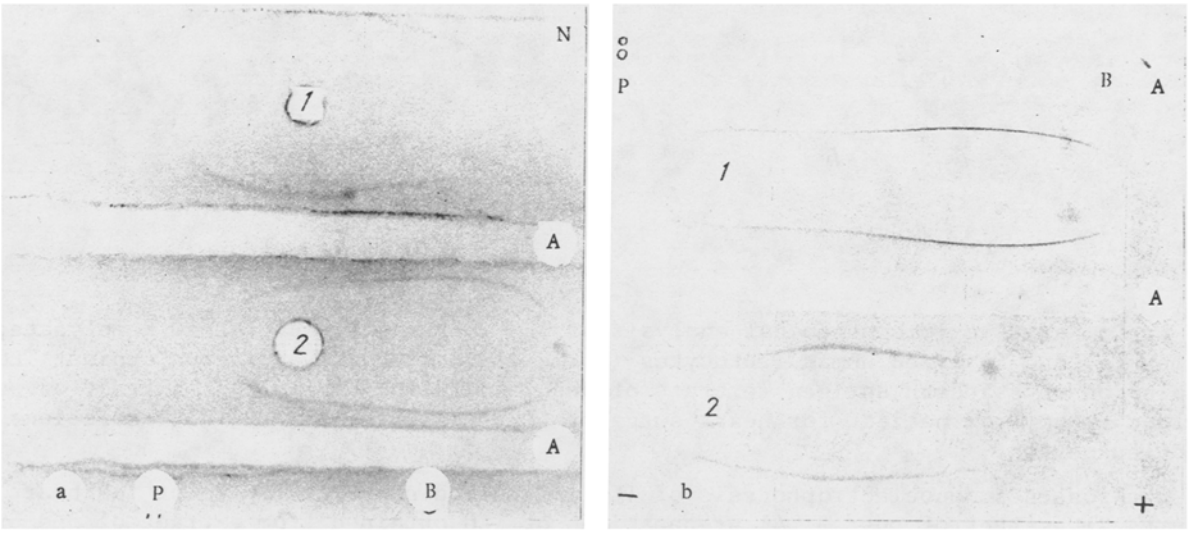

Fig. 3. Immunoelectrophoretic analysis of TLG in hemolysate and blood serum (a), and effect of action of neuroaminidase on electrophoretic mobility of TLG (b). 1) Hemolysate; 2) blood serum from patient for heart surgery; 3) extract of pus; 4) extract of pus incubated with neuroaminidase (100 U of enzyme to 1 ml of extract). A) Monospecific antiserum against TLG. Reference substances for immunoelectrophoresis: P) Pyronine; B) Evans' blue.

high titers of TLG were found by immunodiffusion in three of 22 patients for heart surgery, undergoing operations requiring an assisted circulation for a long time. Results of crossed immunoelectrophoresis of TLG in blood serum, extract of pus, and semipurified preparations obtained from pus by ion-exchange chromatography, are given in Fig. 2, and the results of immunoelectrophoretic analysis of TLG in pus and blood serum in Fig. 3a. It will be clear from Figs. 2 and 3 and from Table 1 that TLG in the hemolysate as a rule possessed the mobility of β-globulins, that in pus the mobility of α_2- and β-globulins, and that in the plasma or serum mobility of α_2-globulins; in other words, mobility of TLG was greater in serum or plasma than in pus or hemolysate. Preliminary incubation of samples of pus or hemolysate with healthy human blood plasma not containing TLG likewise led to a greater or lesser increase in anodal electrophoretic mobility of this protein. TLG in plasma may perhaps be bound with a carrier protein or, for example, with an inhibitor of enzyme activity, which would be responsible for the higher mobility of this antigen.

Besides the electrophoretic heterogeneity of TLG found in individual samples or in different biological materials, heterogeneity of this protein also was observed in the same sample, and was manifested during electrophoresis by the presence of two fractions with mobility of α- and β-globulins (Fig. 3b). Treatment with neuroaminidase led to a decrease in the anodal electrophoretic mobility of TLG, indicating the presence of sialic acids in the composition of the molecule. However, as will be clear from Fig. 3b, sialic acids evidently were present in only one TLG fraction, that with the mobility of α-globulins. In the hemolysate, the change in mobility of TLG under the influence of neuraminidase was hardly perceptible or could not be found at all, and as regards mobility this protein belonged to the β-globulins. Thus sialic acids are components of TLG, but their content may vary. There is possibly an antigenically active TLG which does not contain sialic acids [2].

Comparative analysis of the thermostability of TLG in pus, hemolysate, and blood plasma showed that only a small part of the antigenic activity of TLG remained in the extract of pus after boiling, whereas there was not less than half in plasma (Table 1). The thermostability of TLG is evidently increased when it is bound with plasma components, or there exists a form of this antigen which is resistant to the action of temperature, and its content in plasma is relatively high. This is a most interesting fact that deserves further study, for evaluation of the properties of TLG in the blood plasma may determine its biological role in pathology.

Determination of the molecular weight of TLG contained in extract of pus also showed the presence of heterogeneity: most of the antigen was eluted from the column in the fraction

with mol. wt. of 100 kilodaltons (kD), but TLG also was found to be present in fractions with mol. wt. of between 20 and 70 kD.

All samples of lyzed leukocytes, of hemolysate, and extract of pus which were investigated possessed fibrinogenolytic activity, but no relationship could be found between its value and the TLG content in these samples. Since the test material contained a large quantity of proteinases and their inhibitors [11], it is evidently advisable that enzyme activity be tested only in purified preparations of TLG.

The results of this investigation showed that immunochemically detectable TLG is heterogeneous with respect to the charge on the molecules, the composition of the carbohydrate component, resistance to the action of temperature, and also (in some cases, possibly) molecular weight and individual antigenic determinants. It can be tentatively suggested that complexes of the enzyme with a carrier protein, inhibitor, or substrate, and also forms of the active enzyme and its inactive precursor (proenzyme) may possess antigenic activity. Probably at least two forms of antigen designated as TLG can be distinguished: an α-globulin, found in plasma under pathological conditions, and a β-TLG, evidently present in leukocytes. Although the observed heterogeneity of TLG is evidence that the initial name of this antigen is conventional, it would not seem that any useful purpose may be served by changing the name before the biological function of TLG is discovered, in order to avoid confusion. When the functional activity of TLG is studied the existence of different, possibly inactive, forms of it must be taken into account. Probably determination not only of the total concentration of all TLG molecules, but also the concentration of its individual forms or the normal ratio between them may be of practical importance.

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