# COMPARATIVE IMMUNOCHEMICAL AND IMMUNOCYTOCHEMICAL STUDY OF THREE SOLUBLE GRANULOCYTE PROTEINS IN BLOOD CELLS AND SERUM

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UDC 616.155.34-008.939.6-092.18-078.73

KEY WORDS: thermostable leukocytic α-glycoprotein; leukocytic $\beta_1$-globulin; lactoferrin; polymorphonuclear leukocytes.

Immunochemical detection of "pathological" antigenic components, or of those specific for certain tissues or states [3, 5, 6] is particularly important for the diagnosis and study of molecular mechanisms of physiological and pathological processes. Thermostable leukocytic α-glycoprotein (TLAG), with mol. wt. of 90,000 ± 7000 daltons , has been found in the blood serum in some immunodependent diseases [3]. In systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) a leukocytic $\beta_1$-globulin (LBG) with mol. wt. of 35,000 ± 3000 also has been found in the blood serum [4]. It is not yet clear whether the appearance of intracellular blood cell components in the serum is caused by their specific secretion, due to the pathological process, or whether it takes place as a result of destruction of the cells and subsequent discharge of their soluble components into the plasma. It has likewise not been settled to what concrete blood cell populations these proteins belong.

This paper describes the immunocytologicl study of TLAG, LBG, and also of lactoferrin (LF) in the blood cells and their comparative analysis in the serum in different forms of pathology.

## EXPERIMENTAL METHOD

To obtain monospecific antiserum against LF and TLAG, rabbits were immunized with eluate obtained after elution with $Na_2HPO_4$ from previously washed charcoal after hemoperfusion of patients with SLE and systemic vasculitis, and to obtain a similar antiserum against LBG, a preparation obtained from human colostrum after centrifugation, precipitation with acetic acid, and adsorption chromatography on silica-gel was used [8]. The antisera were exhausted with dried donors' plasma under control of immunodiffusion analysis. Antiserum against LF was compared with the standard (Behringwerke, West Germany) against TLAG, and antiserum against LBG with antisera obtained by Petrunin [3, 4]. Antigens were determined semiquantitatively by double immunodiffusion with standard test systems [7], whose sensitivity was 1 mg % for LF and 0.5 mg % for TLAG and LBG. If necessary, to increase the sensitivity of immunodiffusion, the concentration of test samples or their volume was increased by widening the reservoir for the antigen.

For the immunocytologic investigation films of donors' blood were fixed in cold absolute ethanol for 20 min, dried in air, and used immediately or after keeping at 4°C for 1-3 days. The films were incubated in a moist chamber at room temperature with antiserum against LF, TLAG, or LBG for 45 min, and then treated as described previously [1]. The masks were rinsed for 10 min in physiological saline made up in phosphate buffer, pH 7.0-7.4, incubated for 30 min with the preparation of antibodies against rabbit immunoglobulins labeled with florescein isothiocyanate, then washed for 10 min, and dried in air. The films were studied in ML-2 and Opton (West Germany) luminescence microscopes.

To abolish nonspecific luminescence the intermediate antisera and labeled antibodies were reabsorbed with mouse liver powder. Films treated with labeled antibodies but without the use of intermediate antiserum, and also treated by the other methods described previously [1], were used as the control. The films were examined with a phase-contrast system.

Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 7, pp. 53-56, July, 1986. Original article submitted June 28, 1985.

 0007-4888/86/0007-0934$12.50 

TABLE 1. Immunodiffusion Determination of Leukocytic Proteins in Blood Serum (M ± m)

| Diagnosis | Number of samples | Protein concentration, mg% | | | |
|---|---|---|---|---|---|
| | | LF | TLAG | LBG | CRP |
| Healthy blood donors (control) | 27 | 0,18±0,02 | 0,15±0,02 | 0,3±0,08 | — |
| IHD (complicated forms) | 11 | 0,22±0,04 | 0,11±0,01 | 0,19±0,01 | 1,9±0,2 |
| Rheumatic heart disease | 17 | 0,12±0,01 | 0,21±0,02 | 0,6±0,1 | 1,6±0,2 |
| SLE | 23 | 0,4±0,1 | 0,9±0,1* | 0,6±0,1 | 1,3±0,2 |
| RA | 19 | 0,7±0,1* | 3,2±0,3* | 0,7±0,1 | 8±1 |
| Systemic scleroderma | 5 | 0,4±0,1 | 1,0±0,2* | 0,5±0,1 | 2,1±0,3 |
| Diabetes | 11 | 0,28±0,03 | 0,32±0,04 | 1,6±0,3* | 0,5±0,1 |

Legend. *P < 0.05 compared with control.

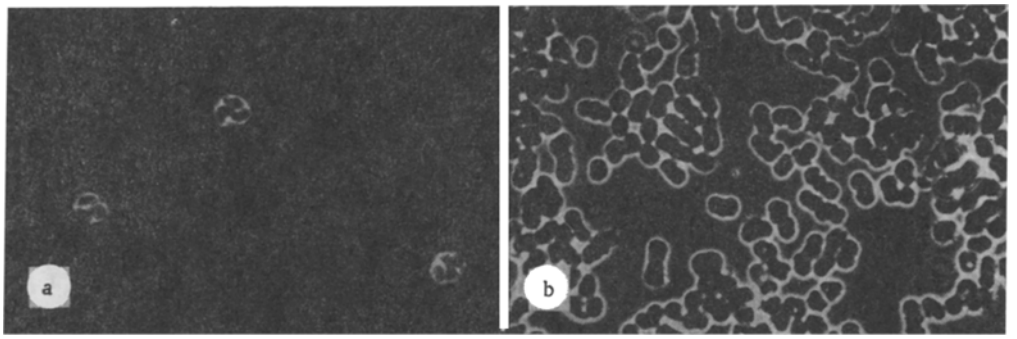


Fig. 1. Blood film from clinically healthy person: a) Treatment with rabbit antiserum against LF; reaction present in cytoplasm of PNL. Indirect immunofluorescence method. Objective 90× (oil immersion), ocular, homal 3×; b) the same film, control. Phase-contrast photograph.

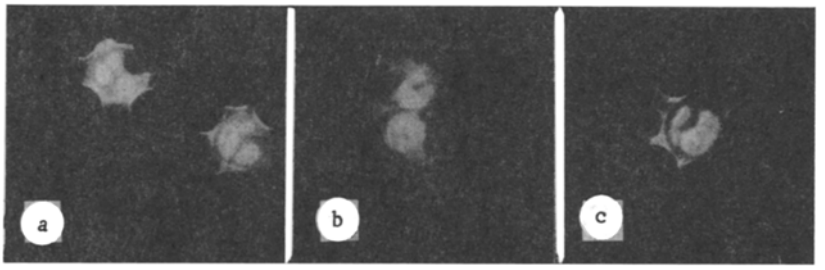


Fig. 2. Blood film from clinically healthy person: a) Treatment with antiserum to LF. Reaction in perinuclear zone of leukocytes, in zone of cytoplasmic membrane and around cell; b) treatment with antiserum to TLAG. The same immunomorphologic picture; c) treatment with antiserum to LBG. Reaction in same location. Indirect immunofluorescence test. Objective 90× (oil immersion), ocular homal 3×.

The preparations were photographed on RF-3 film with 90× objective (oil immersion) and homal 3× ocular.

## EXPERIMENTAL RESULTS

The results of immunochemical determination of the LF, TLAG, and LBG concentrations in the blood serum are given in Table 1. These proteins can be found in trace quantities in normal human blood serum, in complicated forms of ischemic heart disease (IHD), and in patients for cardiac surgery with rheumatic diseases of one or two heart valves.

On treatment of blood films from a clinically healthy person with rabbit antiserum against LF the immunofluorescence test was positive in polymorphonuclear leukocytes (PNL) (Fig. 1). Treatment of the films with antisera against TLAG and LBG also was accompanied by a reaction

with components of PNL. In the other blood cells no such reaction was found. On treatment of the films with a preparation of pure antibodies to rabbit immunoglobulins, labeled with fluorochrome, as the control, no reaction was obtained with leukocytes. The immunofluorescence test with LF, TLAG, and LBG was positive in the perinuclear zone of the leukocyte, the cytoplasmic cell membrane, and extracellularly. The zone of the extracellular reaction was limited to erythrocytes surrounding a PNL (Fig.2). As Fig. 2 shows, the immunomorphological picture was similar in character for all three proteins.

The results indicate that TLAG and LBG, like LF, are soluble components of PNL, mainly of neutrophils, although they may also be linked with other cells of the granulocyte series. Like LF, TLAG and LBG evidently escape from neutrophils by exocytosis into the blood plasma, which determines their "background" level observed in serum from clinically healthy persons and patients with certain diseases.

As Table 1 shows, the serum TLAG level was considerably raised in immunoinflammatory diseases, whereas the LF and LBG concentrations were normal or only a little raised. A high serum TLAG level has often been observed in the presence of high levels of C-reactive proteins (CRP). Elevation of the LBG concentration (to 5 mg %) accompanied by normal LF, TLAG, and CRP levels has been found in severe forms of diabetes mellitus, and also in glomerulonephritis (up to 10 mg %). Immunochemical determination of LF in the blood serum is important for the diagnosis of septic complications in surgery [12]. Selective secretive secretion of TLAG and LBG into the blood serum, which is an established fact, is interesting for subsequent clinical study, with a view to assessing the possibility of using these proteins for diagnosis, and for investigation of the pathogenesis of diseases and their complications. To study increased TLAG and LBG secretion in pathology, the level of sensitivity of immunodiffusion analysis is evidently optimal, whereas for the more accurate study of their "background" level in the blood serum, more sensitive methods of determination must be developed.

Thus a selectively increased secretion of soluble components of PNL into the blood serum occurs: of TLAG in active immunoinflammatory conditions, of LBG in severe forms of diabetes. Increased production of these proteins and their release into the serum are probably associated with intact granulocytes, but considering the presence of TLAG and LBG in other organs and tissues [3, 4, 9], the presence of another source of the raised concentrations of these antigens in the blood serum in pathology cannot be ruled out. The intracellular localization and the biological function of TLAG and LBG demand special study.

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