IMMUNOCHEMICAL TEST FOR LTAG FOR FUNCTIONAL EVALUATION OF HUMAN LEUKOCYTES

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The normal functioning and composition of neutrophilic granulocytes were studied initially by the investigation of Intracellular processes, in which microorganisms were killed and digested in phagocytic vacuoles, where granule-associated hydrolases, secreted in them, act effectively during phagocytosis [7].

Contrary to these old ideas, the emphasis now is laid on active secretory release of effector molecules [8]. By their secretion, phagocytes act on other mediator systems and destroy extracellular objects, which are too large to be ingested. Such is evidently the case with emphysema of the lungs [9], reactions to immune complexes [6], and acute and chronic inflammation [10]. It has also been shown that spontaneous secretion of secondary granules also exists in vitro in the presence of nonspecific adhesion to the surface [11]. All these data are evidence that exocytosis of specific granules constitutes the secretory response of neutrophils and their normal function as cells of inflammation.

The aim of the present investigation was to study the possibility of using the level of secretion of one component of the specific granules of neutrophils, namely leukocytic thermostable alpha-glycoprotein (LTAG), to evaluate the functional state of leukocytes in inflammatory and autoimmune diseases. This substance is a cationic protein of granules of neutrophils, which is found in trace amounts in healthy human blood serum, and in increased concentrations in the serum of patients with immunodependent diseases: systemic lupus erythematosus, psoriasis, rheumatoid arthritis [2, 5].

EXPERIMENTAL METHOD

Blood (3 ml) was collected from 20 healthy blood donors and 23 patients from the cubital vein, and the heparinized blood (5 U) and leukocytic preparation, isolated from heparinized blood by separating the erythrocytes by sedimentation with phytohemagglutinin [3] and transferring the leukocytes into buffered physiological saline (pH 7.2) containing 1 mM $CaCl_2$, were incubated in glass and polypropylene flasks at 4, 20, and 37°C, with periodic gentle mixing. Samples of plasma and incubation medium were analyzed for their LTAG content before and 3, 6, 24, and 48 h after incubation. The cells were separated by centrifugation at 150*g* for 5 min. The hemolysate was obtained by freezing and thawing the blood cells once. LTAG was determined by double immunodiffusion in agar, using a monospecific standard test system with sensitivity of 1 mg/liter [4] (in the modification of Khramkova and Abelev). The method of obtaining the monospecific antiserum to LTAG was described previously [1]. The fraction of dead cells was determined by staining with 0.2% trypan blue solution.

The percentage of secreted LTAG was determined as:

$$\% \text{ of secreted protein} =$$

$$\text{LTAG concentration in plasma/LTAG conc. in hemolysate + LTAG conc. in plasma} \times 100\%.$$

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TABLE 1. Extracellular Secretion of LTAG (in 2 of total LTAG concentration) from Neutrophils of Healthy Blood Donors ($M \pm m$)

Conditions of incubation	Incubation time, h				
	0	3	6	24	48
Glass flasks					
4 °C	1,0±0,2	1,0±0,2	1,0±0,2	1,0±0,2	1,0±0,2
20 °C	1,0±0,2	1,0±0,2	1,0±0,2	1,0±0,2	4,0±0,5
37 °C	1,0±0,2	1,3±0,2	1,5±0,3*	15,0±3*	88,0±15
Polypropylene flasks					
4 °C	1,0±0,2	1,0±0,2	1,0±0,2	1,0±0,2	1,0±0,2
20 °C	1,0±0,2	1,0±0,2	1,0±0,2	1,0±0,2	1,3±0,2
37 °C	1,0±0,2	1,0±0,2	1,2±0,2	1,5±0,3*	2,4±0,4

Legend. $*p < 0.05$ compared with initial level.

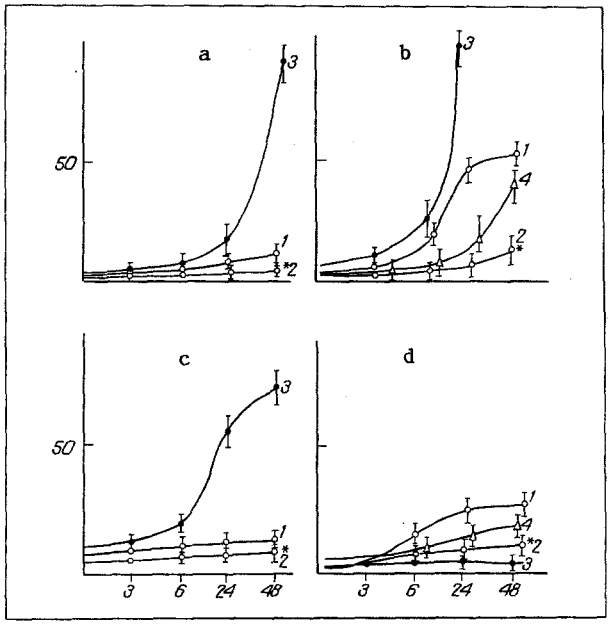

Fig. 1. Extracellular LTAG secretion from human neutrophils from 20 healthy blood donors (a), from six patients with acute psoriatic polyarthritis (b), from 10 patients with acute bronchitis (c), and from seven patients with psoriatic erythroderma, under treatment with corticosteroids (d). Abscissa, incubation time (in h); ordinate, percentage of total content of LTAG ($M \pm m$). Incubation of washed leukocytes ($3 \cdot 10^6$/ml) in buffered physiological saline with 1 mM $CaCl_2$ polypropylene (2) flasks at 37°C. 3) Incubation of whole heparinized blood (3 ml with 5 U heparin), 4) incubation of washed patients' leukocytes in plasma from healthy blood donors.

EXPERIMENTAL RESULTS

Data on the dynamics of extracellular secretion of LTAG by neutrophils are given in Table 1. During incubation of heparinized blood from clinically healthy individuals (20 blood donors aged from 18 to 45 years) at 37°C in glass flasks, a significant increase in the percentage of secreted LTAG was observed: by 0.5% after 6 h, by 14% after 24 h, and by 87% after 48 h, compared with the initial level. Incubation of the samples at a lower temperature (4 and 20°C) and also in polypropylene flasks, had hardly any effect on the LTAG level. Consequently, mainly glass flasks were used in this test and incubation was carried out at 37°C, as the optimal value for secretion. Data on the time course of LTAG secretion by neutrophils of patients and healthy donors are given in Fig. 1. The initial LTAG level in patients with acute psoriatic polyarthzitis was 2% higher than that of the healthy subjects; moreover, during incubation for 6 and 24 h this difference increased by 11.2 and 85% respectively

(Fig. 1a, b). The level of LTAG secretion was increased compared with that by leukocytes of healthy individuals and patients with acute bronchitis (Fig. 1c). The depression of secretory activity of the patients' leukocytes after separation of plasma from cells and their incubation under new conditions is noteworthy: the curve of the level of LTAG secretion with time in healthy human plasma approximated to that of the healthy blood donor although it did not correspond exactly to it (Fig. 1b).

During the study of secretory activity of washed leukocytes from patients with acute psoriatic polyarthritis a small fall of the level of LTAG secretion was observed compared with its level by washed leukocytes from healthy blood donors, evidence that the patients' own cells were responsible, and not plasma with its numerous activators of secretory activity of neutrophilic granulocytes, for maintaining a high LTAG level during incubation (Fig. 1b). Parallel with measurement of the level of protein secretion, the viability of the leukocytes also was determined, by intravital staining with 0.2% trypan blue. Throughout the incubation time (0-24 h) the number of cells stained by trypan blue did not increase, and was 2-3% just as in the control. Thus the contribution of the dying cells to the LTAG level during incubation from 0 to 24 h was minimal. After incubation for 48 h the percentage of stained leukocytes increased to 5-8%, but even this number of dead cells did not significantly affect the LTAG level.

Some interesting data were obtained on incubation of blood from patients with psoriatic erythroderma under treatment with corticosteroids. The level of secretion by leukocytes throughout the incubation period (0-48 h) remained absolutely unchanged. However, after separation of the cells from the patients' plasma and incubation in buffered physiological saline with 1 mM $CaCl_2$ in plasma from a healthy blood donor, the level of secretion rose appreciably by 20 and 15% respectively. The change in secretory activity of the neutrophils in this case can be explained by termination of the action of glucocorticoids on the membranes, their destabilization, and an increase in the outflow of lysosomal proteinases, and also by a change in the adhesive properties of the neutrophils and monocytes and, consequently, spontaneous secretion of secondary granules in vitro.

The incubation test which we propose can thus be used to evaluate the functional state of leukocytes both in vivo and in vitro: of impairment of leukocyte function in patients, and also the possibility of correcting these disturbances, and in the second case, to create artificial or natural factors capable of acting on cells and to determine the degree of their reactivity to a change of medium.

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