EFFECT OF THE $C1_q$ SUBCOMPONENT OF COMPLEMENT ON PLATELET ADHESION AND SPREADING

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UDC 612.111.7.017.1-063

KEY WORDS: adhesion of platelets; subcomponent of complement.

When a leading role is ascribed to platelets in hemostatic reactions *in vivo*, the ever-increasing interest in the study of the role of platelets in immune processes must be noted. Platelets are effector cells responsible for interaction of an antigen with the lymphocyte receptor and for immune complexes (IC), and they participate in clearance of microorganisms, tumor cells, and other particles. F_c receptors and receptors for components of complement ($C3_a/C4_a$, $C5_a$, and $C1_q$) have been found on the membrane of human platelets. Anaphylatoxins ($C3_a$, $C4_a$, and $C5_a$), during binding, induce dose-dependent release of serotonin; $C1_q$ participates in clearance of IC and it modulates the functional properties of platelets: it inhibits collagen-induced adhesion and aggregation [5]. However, the biological role of the $C1_q$ receptors on platelets has not yet been explained.

The aim of this ivestigation was to study the effect of the first subcomponent of complement on platelet activation.

EXPERIMENTAL METHOD

$C1_q$ was isolated by affinity chromatography [8] on macroporous glass from the euglobulin fraction of serum. The preparation of $C1_q$ was freed from preservation by dialysis against physiological saline and was added to platelet-enriched plasma (1000 rpm, 7 min) in concentrations of 240, 120, 60, 30, 16, 8, 4, and 2 μg/ml. Spreading of the platelets was studied by the method in [3]: 0.1 ml of a solution of $C1_q$ and 0.2 ml of platelet-enriched plasma, diluted 200 times, were applied to a slide. Adhesion of platelets to the glass was determined by the method in [2]: 0.05 ml of $C1_q$ and 0.2 ml of whole blood. Platelet aggregation was recorded graphically on an aggregometer.

EXPERIMENTAL RESULTS

$C1_q$ greatly stimulated adhesion of the platelets to glass. In a dose of 8-250 μg/ml the percentage of adherent platelets was increased by 50-100%. The most marked changes ($p < 0.001$) were found when $C1_q$ was used in a concentration of 16-30 μg/ml. Low doses (4-60 μg/ml) also stimulated spreading of the platelets. The percentage of completely spread forms of platelets was increased two-threefold (Table 1). Incidently, while interacting with platelets and activating their spreading and adhesion, $C1_q$ did not cause aggregation of platelets and did not affect ADP-, adrenalin-, or thrombin-induced aggregation. Thus $C1_q$, on binding with the homonymous receptor of the platelets, promotes their fixation and transfer into the marginal pool.

Free $C1_q$, when entering the plasma, binds with fibronectin [7] and quickly forms the macromolecular complex Clq ($C1r_2$ $C1s_2$), which circulates in the blood stream. Activation of this complex on interaction between antigen and antibody leads to interaction with C1-esterase inhibitor, which removes both $C1_r$ and $C1_s$ from the $C1_q$ subcomponent. IC which remains bound with $C1_q$ can react with the $C1_q$ receptor [12].

These IC formed in the blood stream evidently interact initially with platelets, as the most numerous blood cells with $C1_q$ and F_c receptors. Binding with the $C1_q$ receptor strengthens adhesion of the platelets to the capillary endothelium, were local interaction between antigen and immunocompetent cells evidently take place [4].

Department of Normal Physiology, Chita Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 12, pp. 694-696, December, 1985. Original article submitted December 10, 1984.

 0007-4888/85/0012-1676$09.50

TABLE 1. Effect of Cl_q Subcomponent of Complement on Functional Properties of Human Platelets (M ± m)

Parameter	Concentration of Cl_q, μg/ml								
	0	240	120	60	30	16	8	4	2
Adhesion of platelets to glass, %:									
After 5 min	10±2	17±3	19±2*	17±3	18±2*	25±4*	20±3*	12±2	10±2
After 10 min	15±2	22±3*	25±3*	25±3*	28±3**	32±3**	29±4*	20±2	18±2
Spreading of platelets on glass, %:									
Completely spread out forms	5±1	7±1	15±5	15±4*	11±3*	17±3**	18±4**	14±2**	11±4
Transitional forms	55±2	56±2	53±2	51±2	61±3	52±1	55±2	55±3	58±3
Normal platelets	39±2	37±2	32±4	35±4	28±3*	30±3*	27±4*	32±4	30±4*
Aggregation index of platelets (in %), induced by									
ADP ($5 \cdot 10^{-7}$ M)	21±2	22±2	19±3	20±2	18±3	21±2	20±2	20±2	20±3
Thrombin (2.5 units/ml)	17±2	21±2	18±3	21±1	17±2	15±2	19±2	18±2	18±2
Thrombin (2.5 units/ml)									
Adrenalin (10^{-5} M)	22±2	24±4	20±2	24±4	22±4	22±4	19±2	18±2	20±2

Legend. *P < 0.05, **P < 0.001.

Cells of the vascular endothelium in culture can activate lymphocytes [4]. Additonally, the Cl_q molecules, which possess lectin-like and mitogenic properties [9, 14], probably also facilitate stimulation of lymphocytes. When fixed to the membrane of these cells, Cl_q interacts with immunoglobulins and acts as receptor IgM or IgC complexes [13]. It can be postulated that IC are fixed to the endothelium by platelets, and thus facilitate contact of B lymphocytes through the receptor for antigen and Cl_q, thus leading to activation and capping of the complex. Cl_q, with six binding points, can play the role of ligand, cross-reacting with the receptors, thus ensuring an adequate density of hapten on the cell surface to activate it.

The presence of Cl_q has been demostrated in the central organs of immunity: the spleen lymph nodes, and Peyer's patches in mice [10]. The epithelium synthesizes 400-3700 times more Cl than monocytes and fibroblasts [11], thus promoting the formation of "local" immunity.

Meanwhile lymphocytes facilitate degradation of C3 [6], and activation of complement by the alternative pathway causes increased spreading of the cells [1]. During spreading of the platelets, straightening of the crinkled membranes evidently leads to exposure of new receptors of platelets participating in the immune response. For instance, gel-filtration of platelets leads to unavoidable modification of the membrane and its receptor apparatus, for Cl_q changes the aggregation properties of such platelets [15].

As a result of cooperation between spread-out platelets, lymphocytes, and endothelium the cells become sensitized to the antigen, and macrophages are subsequently involved in the process, in particular through isolation of chemotaxic and anaphylactic peptides $C3_a$ and $C5_a$ in the course of activation of complement by IC, and secretion of macrophage migration inhibiting factor by endothelial cells. Macrophages and fibroblasts, when activated, synthesize an increased quantity of Cl_q and take part in clearance of IC.

The platelet receptor for Cl_q can thus perhaps coordinate mobile cells in blood vessels for the effective realization of the body's response.

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INACTIVATION OF GLUTAMIN-(ASPARAGIN)-ASE FROM *Pseudomonas aurantiaca* 548 BY AZASERINE AND 6-DIAZO-5-OXO-L-NORLEUCINE

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UDC 615.277:[579.222:577.152].012.8

KEY WORDS: inhibition, glutamin-(asparagin)-ase, azaserine, methotrexate, 6-diazo-5-oxo-L-norleucine.

The bifunctional enzyme glutamin-(asparagin)-ase (GA-ase) was isolated comparatively recently in a pure form from microorganisms [1, 2, 5, 6, 9]. By now experimental and clinical evidence that GA-ase has an inhibitory effect on growth of cells from different types of human and animal tumors has been collected [4, 7, 8, 10]. Enhancement of the therapeutic effect of the enzyme has been observed when used in conjunction with other antitumor therapeutic preparations widely used in clinical oncology, for example, with chemical analogos of L-glutamine [8].

However, on direct contact with the enzyme, substrate analogs may induce its reversible or irreversible inhibition, and this usually leads to reduction or total loss of the catalytic activity of the enzyme.

The aim of this investigation was to make a preliminary assessment of the inhibitory action of glutamine analogs on GA-ase activity *in vitro*.

EXPERIMENTAL METHOD

Homogeneous GA-ase isolated from a biomass of *Pseudomonas aurantiaca* VKMV-548 by the method devised by the writers previously. Enzyme activity was measured the quantity of ammonia formed during hydrolysis of L-glutamine, by a continuous method, in the coupled glutamate dehydrogenase reaction at 25°C [3]. The reaction mixture (1 ml) contained 0.05M Tris-HCl (pH 7.4), 5 mM sodium α-ketoglutarate, 0.25 mM NADH, 0.5-10 mM L-glutamine, 0.5 mg of glutamate dehydrogenase, and 0.5 mg of bovine serum albumin.

To measure the degree of inactivation by the action of diazo coupling the enzyme (0.05-0.25 U/ml) was incubated with 0.05-8 mM of 6-diazo-5-oxo-L-norleucine (DON) or with 0.1-20 mM azaserine at 25°C in 0.1M Tris-HCl (pH 7.4) and aliquots were taken after known time intervals to measure enzyme activity in a medium of the above-mentioned composition. To investigate the effect of the reaction products and substrate analogs on the degree of inhibition of GA-ase by the action of DON the enzyme was incubated in the presence of the compounds indicated in Table 1 for 10 min at 25°C.

EXPERIMENTAL RESULTS

The effect of analogs of L-glutamine, most of them used in the treatment of cancer patients in conjunction with enzymes during combination treatment of malignant neoplasms, on activity of GA-ase from *Pseudomonas aurantiaca* 548 was investigated in the experiments of series I. These compounds were added directly to the system for measuring enzyme activity without preincubation with enzyme preparations. Table 2 shows that none of the substances tested had any inhibitory action, in a concentration of 0.5 mM, on enzyme activity in the presence of 0.5 mM L-glutamin

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 12, pp. 696-698, December, 1985. Original article submitted November 21, 1984.

 0007-4888/85/0012-1678$09.50