Effect of Natural Antiphospholipid Antibodies on Antithrombotic Activity of Vascular Wall

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Natural latent human antibodies cross-reacting with DNA and cardiolipin, interact with human endothelial cells, decrease antiaggregation activity of rat aortic wall, and increase fibrinolytic activity of the wall of the inferior vena cava. It is assumed that natural antiphospholipid antibodies present in immunoglobulin preparations in a latent state modify antithrombogenic activity of the vascular wall and are a potential cause of antiphospholipid syndrome.

Key Words: *natural latent antibodies; endothelium; vascular antiaggregation and fibrinolytic activity*

Human and animal serum and immunoglobulin preparations contain natural polyspecific antibodies. These antibodies can be latent and free [4]. These subpopulations of antibodies cross-react with DNA and phospholipids [3], but free antibodies react mainly with phosphatidylcholine, while latent ones react with cardiolipin. Natural latent antibodies possess the properties of lupus anticoagulant and can be a potential cause of the antiphospholipid syndrome [2].

We investigated the effects of natural free and latent antiphospholipid antibodies on antiaggregation and fibrinolytic activities of the vascular wall.

MATERIALS AND METHODS

Natural antibodies were isolated from immunoglobulin preparations (G. I. Gabrichevskii Institute of Epidemiology and Bacteriology) or Sandoglobulin (Sandoz) [4]. Free antibodies were isolated by affinity chromatography on DNA-cellulose in a buffer containing 0.15 M NaCl, 0.02 M Tris-HCl (pH 8.0), and 0.005 M EDTA, followed by elution with 2 M NaCl. Exhausted immunoglobulin fraction was dialyzed against 0.01 M potassium phosphate buffer (pH 7.3), and ion exchange chromatography on QAE Sephadex A-50 was performed. Natural antibodies present in the initial preparation in a latent state were isolated from the resultant fractions 1 (passed through ion exchanger) and 2 (adsorbed and eluted with 0.05 M NaCl) by subsequent chromatography on DNA cellulose.

The interactions of antibodies with endothelial cells were studied *in vitro*. Endothelial cell were obtained from human umbilical vein. Enzyme immunoassay was carried out on live cells in Flow Labs. plates. Antibodies in different concentrations fixed in wells with 4% formaldehyde were incubated with 1% BSA and then with peroxidase-labeled second antibodies. o-Phenylene diamine was used as the substrate. The results were expressed in optical density units. Blood vessels, platelet-rich and platelet-free plasma were obtained from intact Wistar rats of both sexes (200-250 g) narcotized with sodium ethaminal (45 mg/kg intraperitoneally).

Antiaggregation activity of the vascular wall were studied according to McInture's method [8]. Two segments of the abdominal aorta (5 mm) were taken from each animal; one segment was incubated with free or latent antibodies (40 μg/sample) for 10 min at 37°C and 80 min at 18-20°C and the other under the same conditions in 0.05 M Tris-HCl buffer (pH 8.0). Then

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the vascular segment was put into platelet-rich plasma and aggregation intensity was evaluated according to Born [5] with a final ADP concentration of 10^{-5} M. Platelet aggregation in the absence of vascular segment was used as a reference value in estimations of antiaggregation activity by the formula: $AW=[(I_{PP}-I_{VW})\times100]/I_{PP}$, where I_{PP} and I_{VW} are intensity of platelet aggregation in the presence and absence of vessel segment.

Fibrinolytic activity of the wall of the inferior vena cava and effects of antibodies on this parameter were evaluated by the method proposed by V. N. Romanovskaya. Two 5-mm segments were taken from the inferior vena cava between the liver and right atrium, one was incubated with antibodies (40 μg protein in 200 μl 0.05 M Tris-HCl) for 30 min at 37°C or for 10 min at 37°C and 80 min at 18-20°C. Control segment from the same vein was incubated without antibodies. After incubation, both segments were transferred to citrate platelet-free plasma (500 μl) and incubated for 3 min at 18-20°C. Vascular segments were then removed, and fibrinolytic activity in plasma samples was measured [1].

The results were statistically processed using Student's *t* test for independent and paired samples.

RESULTS

Enzyme immunoassay showed that only latent natural antibodies, but not free antibodies interacted with endothelial cells (Fig. 1). It has been demonstrated that

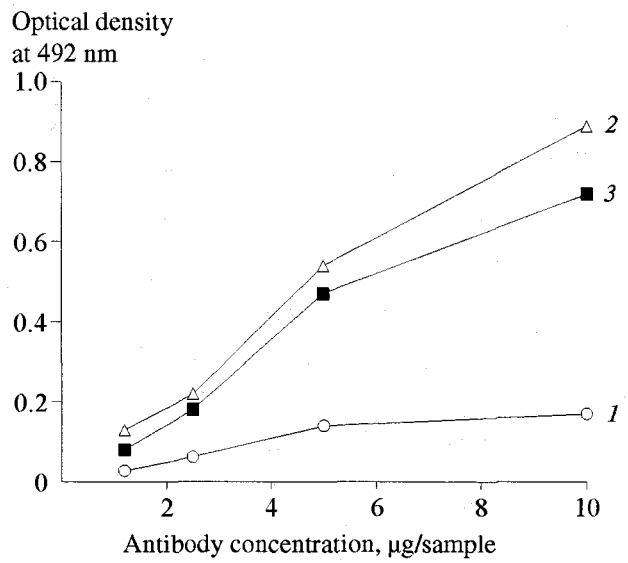

Fig. 1. Reaction of free (*1*) and latent (*2, 3*) antibodies with endothelial cells in enzyme immunoassay. *2*) fraction 1; *3*) fraction 2.

natural and immune antibodies to DNA and phospholipids can react with endothelial cell membrane and modify their functional activity [6,11].

Study of biological role of natural antiphospholipid antibodies by analogy with immune antiphospholipid antibodies includes analysis of their involvement in hemostasis and blood clotting. Aortic wall possessed high antiaggregation activity and decreased the intensity of aggregation by 50-67% (Table 1). Preincubation of the vascular wall with immunoglobulins

TABLE 1. Effect of Latent Anticardiolipin Antibodies on *In Vitro* Antiaggregation Activity of Rat Aortic Wall (*M±m*)

Concentration of factors, μg/sample		Antiaggregation activity, %		Difference between paired variants, %
		control	experiment	
Natural anticardiolipin antibodies	40 (*n*=8)	67±6.5	48±6.0	18±2.0*
	20 (*n*=5)	55±8.4	57±7.8	2.0±2.5
Immunoglobulin	40 (*n*=5)	50±5.9	48±5.3	2.0±1.8

Note. Mean intensity of platelet aggregation in the absence of aortic wall in 5 observations was 100±9%; *$p<0.05$ *vs.* the control.

TABLE 2. Effect of Latent Anticardiolipin Antibodies on *In Vitro* Fibrinolytic Activity of the Wall of Rat Inferior Vena Cava (*M±m*)

Parameter	Control plasma (*n*=13)	Plasma activated by vascular wall incubated with antibodies for			
		30 min (*n*=8)		90 min (*n*=7)	
		control	experiment	control	experiment
Time of clot lysis, min	191±4.7	150±5.0*	141±3.8*	171±4.1*	149±3.8*

Note. *$p<0.05$ *vs.* control plasma, **$p<0.01$ *vs.* the control.

and natural antibodies (free and latent) in a concentration of 20 μg/sample) did not change its antiaggregation activity. Increasing the concentration of latent antibodies in the incubation medium to 40 μg/sample considerably decreased antiaggregation activity of the vascular wall, which apparently reflected the dependence of the effect on the concentration of antibodies. The decrease in antiaggregation activity of the vascular wall suggests that natural latent antiphospholipid antibodies can induce a prethrombotic state, which is in line with published reports [10].

A different picture was observed in studies of the effect of natural antiphospholipid antibodies on fibrinolytic activity of the vascular wall. Vascular segment incubated with plasma activated fibrinolysis and accelerated the lysis of fibrin clot by 10-20% (Table 2). Preincubation of the vessel segment with antiphospholipid antibodies resulted in additional statistically significant ($p<0.01$) activation of fibrinolysis.

It has been demonstrated that activation of fibrinolysis caused by venous occlusion does not correlate with the incidence of thrombotic complications in systemic lupus erythematosus [7]. However the reactions in the blood flow differ from the events on the surface of the epithelium [8]. The interaction between antiphospholipid antibodies and endothelium promotes accumulation of C_3 complement component and loss of heparan sulfate on its surface and increases monocyte adhesiveness to the endothelium, which contributes to a prethrombotic state [9,11]. The increase in fibrinolytic activity of the vascular wall caused by latent antiphospholipid antibodies can be due to the participation of C_3 complement component in activation of fibrinolysis. In general, our findings indicate that natural antiphospholipid antibodies can be involved in the pathogenesis of the antiphospholipid syndrome.

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