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Anticoagulant Activity of Naturally Occurring Anticardiolipin Antibodies

V. M. Zyablitskii, A. N. Starosel'skaya, I. V. Lekakh, V. I. Kiseleva, and A. M. Poverennyi

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Natural antibodies isolated from a commercial immunoglobulin preparation react with cardiolipin *in vitro* and exhibit anticoagulant activity in the thromboplastin suppression and kaolin time tests performed with the use of Wistar rat plasma.

Key Words: *antiphospholipid syndrome; antibodies; cardiolipin; anticoagulant activity*

The antiphospholipid syndrome (APS), a thrombotic state associated with hereditary or acquired deficiency of blood coagulation inhibitors and fibrinolytic disorders, has been recently discovered. Two APS types have been defined: anticoagulant-thrombotic lupoid and anticardiolipin-thrombotic, manifesting themselves as certain differences in the thrombosis of cardiac, cerebral, and renal arteries and veins, repeated spontaneous abortions, and thrombocytopenia. APS is developed against the background of lupus erythematosus, in other connective tissue diseases, autoimmune and malignant disorders, in response to some drugs for syphilis and some infectious diseases in persons below 50 as a secondary syndrome, and as a primary syndrome in patients healthy in all other respects [7]. The APS has been associated with the emergence of antibodies against IgG, IgM, and IgA. Previously, we showed that latent anticardiolipin antibodies (IgG and IgM) are present in the serum of healthy subjects [4].

In this study a possible role of these antibodies in the development of APS is evaluated.

MATERIALS AND METHODS

Experiments were performed *in vitro* on citrated platelet-free plasma of intact Wistar rats.

Commercial Ig preparations for intramuscular administration were used as a source of antibodies. These preparations were fractionated by ion exchange chromatography on QAE A-50 Sephadex in 0.01 M potassium phosphate buffer (pH 7.3) as described elsewhere [5]. The fraction of positively

Department of Radiation Biochemistry, Laboratory of Experimental Radiation Hematology, Medical Radiology Research Center, Russian Academy of Medical Sciences, Obninsk

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TABLE 1. Kaolin Time (sec) for Rat Plasma Clotting after the Addition of Fractions A1 and A2 of Normal Antibodies and Serum of Patient with Lupus Erythematosus (*M±m*)

Component of reaction mixture	Component ratio	Incubation time, min	
		5	60
Intact plasma	-	72±2.4 (18)	
Plasma+0.85% NaCl	1:1	99±2.9 (19)	98±5.2 (12)
Plasma+antibodies A1	4:1	115±17.6 (2)	158±18.2 (4)
	3:1	127±5.0* (9)	162±16.0* (9)
	1:1	143±16.9* (8)	148±16.0* (8)
Plasma+antibodies A2	4:1	119±15.8 (2)	135±10.0 (4)
	3:1	128±4.6* (9)	134±6.6* (9)
	1:1	145±18.9* (8)	146±20.0* (8)
Plasma+patient's serum diluted 3-fold	4:1	208±19.0 (4)	200±25.0 (9)
	2:1	223±19.9* (8)	266±17.0* (8)
	1:1	Coagulation blockade	Coagulation blockade
Plasma+γ-globulin	3:1	106±6.1 (9)	117±9.4 (6)
	2:1	94±6.8 (8)	94±7.3 (8)
	1:1	106±4.6 (8)	108±4.6 (8)

Note. The initial concentration of A1 and A2 antibodies and γ-globulin was 3.5 mg/ml; *$p<0.05$ compared with the control. Here and in Table 2: the number of patients is given in parentheses.

charged Ig, which had passed through the column, was designated as A1, the fraction eluted from the column with 0.5 M NaCl as A2. Both fractions were dialyzed against phosphate buffered saline. The protein concentration was determined spectrophotometrically at 280 nm. The ability of Ig preparations and A1 and A2 fractions to react with cardiolipin was assessed in an innumoenzyme assay [4]. The results were expressed in units of light absorbance.

The anticoagulant activity (ACA) of normal antibodies was determined in the kaolin time test and in the thromboplastin inhibition test [1] at a final antibody concentration in the reaction mixture ranging from 1.75 to 0.7 mg protein/ml.

Prior to the ACA measurements, serum from a patient with lupus erythematosus was diluted 3-fold.

Equal volumes of 0.85% NaCl or commercial γ-globulin in the corresponding protein concentration were added to the control samples.

The results were analyzed using Student' s *t* test [6].

RESULTS

The presence of antibodies against the contact phase phospholipids and thromboplastin in the plasma is one of the diagnostic criteria of anticoagulant-thrombotic APS. They are detected *in vitro* by prolongation of the prothrombin and kaolin times required for the clotting of healthy donors' plasma diluted with patients' plasma or serum.

In the present study, naturally occurring anticardiolipin antibodies were detected in A1 and A2 fractions of commercial Ig preparation after chromatography on QAE A-50 Sephadex. The preparation weakly reacted with cardiolipin, while A1 and A2 fractions exhibited marked activity towards this antigen.

Both fractions prolonged the kaolin time or inhibited prothrombinase formation by the intrinsic pathway (Table 1).

Anticoagulant activity of the antibodies tended to increase with the increase in their concentration in the reaction mixture. There was no relationship between ACA and the incubation time. Anticoagulant activity of serum from patient with lupus erythematosus was higher than that of normal antibodies and did not increase with prolongation of the incubation period. Commercial Ig preparation had no ACA.

Similar results were obtained in the thromboplastin inhibition test. Both fractions of normal antibodies and patient's serum showed high ACA, which did not increase with the incubation time.

These findings indicate that the fraction of naturally occurring antibodies contains antibodies against blood coagulation phospholipids, which may be potential inducers of APS.

There is controversy over the specificity of APS-inducing antibodies. Some researchers believe that antiphospholipid syndrome is induced by the lupoid type antibodies displaying ACA *in vitro* [7-9]. A question arises whether APS-inducing antibodies are cross-reactive or they are represented by several po-

TABLE 2. Prothrombin Time (sec) after the Addition of Antibody Fractions and Serum of Patient with Lupus Erythematosus (*M±m*)

Component of reaction mixture	Thromboplastin incubation time, min	
	5	10
Control	27±0.3 (21)	
Dilution control (0.85% NaCl)	28±0.7 (16)	33±1.4 (4)
Antibody fraction A1	62±2.4** (9)	58±1.4** (4)
Antibody fraction A2	50±0.9** (21)	54±0.8** (4)
Patient's serum diluted 3-fold	62±2.6** (9)	43±1.4** (4)
γ-globulin	29±0.6 (21)	32±1.4 (4)

Note. Concentration of antibodies and γ-globulin in the reaction mixture was 1.75 mg/ml; $p<0.05$: *compared with the dilution control, *compared with the γ-globulin data.

pulations. Previously, we detected anti-DNA antibodies in fractions of naturally occurring antibodies [2,3], which are known to possess polyspecificity and cross-reactivity. Therefore, it can be assumed that antibodies reacting with cardiolipin, cross-reacting with DNA, and participating in the inactivation of the prothrombin complex are present among the antibodies exhibiting ACA after ion-exchange chromatography.

Thus, the Ig fraction of healthy subjects contains latent antibodies against cardiolipin. They have the properties of the antibodies detected in patients with phospholipid syndrome and exhibit ACA. The conditions under which this activity can be exhibited will be elucidated in further investigations.

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