Anticardiolipin Antibodies Dissociate from Protein in Thermal Shock

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Anticardiolipin antibodies are revealed in rat serum by solid-phase immunoenzyme assay 3 h after thermal burn of 30% of the body surface. An increase in serum concentration of the antibody after thermal burn may be due to dissociation of the antibody-protein complex.

Key Words: *masked antibodies; cardiolipin; thermal shock; immunoenzyme assay*

It was demonstrated that an increase in serum concentration of anticardiolipin antibodies is associated with higher probability of thrombosis, spontaneous abortions, thrombocytopenia [4], stroke, and myocardial infarction [6]. Clinical manifestations associated with increased concentration of anticardiolipin antibodies were termed the antiphospholipid syndrome [5].

We showed that a considerable amount of anticardiolipin antibodies in serum of healthy persons is inactivated, i.e., these antibodies are bound to serum proteins (masked). Anticardiolipin antibodies react with antigen only after gel-filtration on Sephadex G-200 (pH 4.5) or ion-exchange chromatography [2], which was confirmed by others [3].

A question arises whether anticardiolipin antibodies dissociate from protein and exert their effects *in vivo*?

We hypothesized that these antibodies dissociate under conditions of shock. While studying the properties of masked antibodies which react with bacterial endotoxins, we found that the activity of these antibodies in the serum markedly increases at the early stages of traumatic shock [1].

In the present study the activity of antibodies reacting with cardiolipin was studied in rats with burn shock.

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180-200 g. A IIIB degree thermal burn of 30% body surface was produced by exposure of sheared back to light from an electrical bulb. Serum was prepared 3 h after the exposure. Serum from intact rats served as the control.

Solid-phase immunoassay was performed as follows. Ethanol solution of cardiolipin (50 μl, Sigma) was dried in immunological plates (Nunc) at 4°C overnight. Nonspecific binding sites were blocked by incubation with 0.3% gelatin (100 μl) in phosphate-buffered saline (PBS, pH 7.4) for 2 h at 37°C followed by washing with PBS. Incubation with rat antisera (50 μl, 1:500) was carried out for 1 h at room temperature. After washing with PBS, anticardiolipin antibodies were identified by incubation for 1 h at room temperature with biotinylated sheep anti-anticardiolipin antibodies (50 μl, Amersham). Streptavidin-peroxidase conjugate (Amersham) was then added in the corresponding dilution and incubated for 1 h at room temperature. After thorough washing with PBS, incubation with o-phenylenediamine was carried out for 20 min at 37°C. The reaction was terminated by the addition of 1 M H_2SO_4, and light absorbance was measured at 492 nm. Three series of experiments were performed; the results were statistically processed.

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TABLE 1. Interaction of Antibodies with Cardiolipin in Immunoenzyme Assay (*M±m*)

Experimental series	Biological control	Burn
I	0.306±0.005 (*n*=3)	0.599±0.050 (*n*=5)**
II	0.330±0.064 (*n*=5)	0.446±0.059 (*n*=3)
III	0.247±0.070 (*n*=5)	0.512±0.027 (*n*=4)*
Mean value	0.292±0.035 (*n*=13)	0.531±0.032 (*n*=12)***

Note. *p=0.01, **p=0.001, ***p=0.0002 in comparison with the biological control.

RESULTS

As seen from Table 1, serum content of anticardiolipin antibodies significantly increases 3 h after thermal burn. Bearing in mind that *de novo* synthesis of these antibodies is impossible for such a short time period, it can be hypothesized that this increase is due to their demasking, i.e., dissociation from serum proteins.

Thus, *in vivo* anticardiolipin antibodies are masked. It is likely that free antibodies play an important role in the pathogenesis of burn disease and cause disseminated intravascular coagulation. This suggestion is confirmed by the finding that naturally occurring cardiolipin antibodies dissociated from proteins during isolation from serum of healthy subjects exhibit an anticoagulant activity. A similar anticoagulant activity was demonstrated for lupus erythematosus anticoagulants that determine the development of the antiphospholipid syndrome [5].

So far, the mechanism of binding and dissociation of anticardiolipin antibodies has not been elucidated. The problem is complicated by the fact that sometimes the binding of anticardiolipin antibodies to epitope requires the presence of a cofactor (apolipoprotein H).

The present study shows that anticardiolipin antibodies are recovered in burn shock. Special investigation is required to elucidate the mechanism of this process.

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