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Induction of antiphospholipid antibodies by peptide immunization and proof of their pathogenicity in an animal model

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Keywords

Antiphospholipid syndrome, autoantibodies, autoimmunity, ?2GPI, endothelial cells, systemic lupus erythematosus

Context

Antiphospholipid (aPL) antibodies (Abs) represent autoantibodies associated with thrombosis, pregnancy loss, and thrombocytopenia in patients with SLE or primary aPL syndrome. The induction of these autoantibodies remains enigmatic. Phospholipid (PL) alone is not able to induce autoantibodies, whereas a variety of studies could demonstrate that cardiolipin complexed with *Staphylococcus aureus, Salmonella minnesota* particles have the capacity to induce aPL. However, generation of these antibodies did not result in pathogenic effects when studied in applied experimental settings. Notably, ?2glycoprotein I (?2GPI), also known as apolipoprotein H, is a cofactor for binding of aPL to cardiolipin. It has been previously shown that aPL can be generated in mice by immunization with ?2GPI and these Abs were thrombogenic and caused pregnancy loss in mice. To test whether aPL induced by immunization with a 15 amino acid polypeptide, including the phospholipid-binding site of ?2GPI is thrombogenic and activates endothelial cells (EC) *in vivo* and *in vitro*.

Significant findings

The binding capacities of GKDV and GKDV2 to cardiolipin (CL) were analyzed in comparison to ?2GPI with GDKV having a 43% and GKDV2 a 56% reduced binding to CL than the whole ?2GPI protein. In addition, the authors demonstrate the specificity of GKDV and GKDV2 to bind to CL based on competetive ELISA experiments. Animals immunized with free GKDV only (without carrier proteins) did not induce antibodies to PL, ?2GPI or GKDV. Altogether, this study demonstrated that the ?2GPI protein is the strongest immunogen, followed by the two peptides coupled to bovine serum albumin (BSA). Most notably, GDKV or keyhole limpet hemocyanin (KLH) did not induce such a strong immune response as it could been observed with GDKV-KLH. Free GKDV and KLH alone were not able to induce any antibodies against PL, ?2GPI or against GDKV itself. In the animal experiments,

mice perfused with GKDV-induced aPL had larger thrombi and persisted longer than controls. Moreover, it could be shown that animals perfused with the monoclonal anti-GKDV Ab exhibited a greater number of sticking WBC to the ECs in comparison to controls. This enhanced adhesion of WBC to ECs in animals infused with the monoclonal Ab was consistent with EC activation in *in vivo* micromanipulation experiments. This was confirmed in part by the detection of elevated expression of ICAM-1, VCAM-1 and E-selectin after 4 h exposure of ECs to GDKV-induced aPL Ab versus RPMI culture medium and lipopolysaccharide (LPS) incubation. Anti-GKDV Ab were able to activate significantly HUVEC monolayers as it was detected for LPS.

Comments

This report demonstrates a number of interesting points. First, immunization of animals with an amino acid sequence of the functionally active site of the relevant autoantigen is able to generate autoantibodies with a pathogenic impact. This completes the picture already obtained by a number of previous reports about autoantigens, such as DNA-histone, RNA-Ro/La/RNP complexes providing evidence that the functionally active site is highly immunogenic. Second, the results are consistent with observations of the pathogenic role of antibody interaction with these functionally active sites. However, the study emphasizes that peptides alone do not have the capacity to induce (auto)antibodies. Therefore, additional factors, such as time-dependent exposure to other (exogenous) antigens or transient protein complexes, appear to be essential for establishing autoimmunity. In any case, these factors appear to be of outstanding importance for the breakdown of tolerance.

Methods

A 15 amino acid peptide spanning Gly274-Cys288 called GKDV and a mutant called GKDV2, with a stronger binding capacity to phopsholipids, were used for the immunization of NIH/Swiss mice to generate monoclonal antibodies from spleen cells of a GKDV-BSA-immunized mouse. After two booster injections with a 2 week interval, the sera from the mice were tested in enzyme-linked immunosobant assay (ELISA) (competitive PL-binding ELISA using a monoclonal Ab for testing ?GPI) for their binding to PL. Normal CD-1 male mice were then infused with the generated monoclonal aPL. Endothelial cell activation was analyzed *in vivo* by direct visualization and quantitation of adhering white blood cells (WBC) to ECs of the exposed right femoral vein that was pinched with a standard pressure to introduce an injury leading to clot formation.

Further, *in vitro* exposure of endothelial cells (human umbilical vein ECs) to GDKV-induced aPL Abs was analyzed by the surface expression of E-selectin, intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) by using colorimetric ELISA.

References

1. Gharavi AE, Pierangeli SS, Colden-Stanfield M, Liu XW, Espinola RG, Harris EN: GDKV-induced antiphospholipid antibodies enhance thrombosis and activate endothelial cells *in vivo* and *in vitro*. J Immunol. 1999, 163: 2922-2927.