

Pathogenesis of the Antiphospholipid Syndrome

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Abstract

Antiphospholipid syndrome (APS) is characterized by the presence of pathogenic autoantibodies against β 2-glycoprotein-I (β 2GPI). Studies of experimental APS models emphasized that molecular mimicry between β 2GPI related synthetic peptides and structures within bacteria, viruses (cytomegalovirus) and tetanus toxoid could explain APS. In this review we discuss the association of antiphospholipid antibodies with infectious agents.

Autoimmune diseases have a multifactorial etiology influenced by both genetic and environmental factors. Infectious agents can induce autoimmune diseases by a variety of mechanisms. Antiphospholipid syndrome (APS) is a multisystem autoimmune disease, (1-2) mechanisms characterized by vascular thrombosis, recurrent fetal loss, thrombocytopenia and other clinical manifestations in the presence of persistent circulating antiphospholipid antibodies (aPL), such as lupus anticoagulant and anticardiolipin antibodies. aPL antibodies target phospholipid molecules, mainly via β 2-glycoprotein-I (β 2GPI) (3-5).

Human β 2GPI molecule is a heavily glycosylated membrane-adhesion glycoprotein, present in blood plasma at a concentration of ~150-300 μ g/ml (6). β 2GPI exhibits several anticoagulant properties in vitro (7-8) and was found to be immunogenic in vivo: immunization of BALB/c, PL/J mice, or New Zealand white rabbits with β 2GPI resulted in generation of anti- β 2GPI Abs (9-12). β 2GPI-immunized mice developed high titers of β 2GPI-dependent anticardiolipin antibodies (aCL) associated with increased fetal resorption (the equivalent of fetal loss in human APS), thrombocytopenia, and prolonged activated partial thromboplastin time (aPTT) indicating the presence of lupus anticoagulant (11). In addition, tolerance was induced in β 2GPI orally fed mice, and APS was prevented (13). Anti- β 2GPI antibodies exert direct pathogenic effect by interfering with

homeostatic reactions occurring on the surface of monocytes, platelets and vascular endothelial cells (14-16). They activate monocytes leading to tissue factor release (15-16) and activate endothelial cells via induction of adhesion molecule expression including E-selectin, ICAM-I, and VCAM-I, NFkB expression (17-19). In an ex-vivo model of thrombosis these antibodies induce thrombus formation (20-21).

APL antibodies are found in 5% - 8% of healthy control subjects, and the level increases with age and coexistent chronic diseases (22). Most antibodies do not have any clinical significance and disappear within 1.9 years. In a study from Europe, low titer anticardiolipin and anti- β 2GPI antibodies were found in 11% and 7% of healthy children respectively (23). Most antibodies are transient and non pathogenic. The levels of IgA anticardiolipins are lower in children than in adults, while IgG anti- β 2GPI levels are highest in preschool children (23). Similar findings were also observed in a large Mexican study, where higher levels of anti- β 2GPI were found in a group of 360 healthy Mexican children aged from 1 month through 8 years compared to Mexican adults (24). Correlation between the prevalence of aPL antibodies and history of previous infections and vaccinations was documented.

Several infectious states may cause aPL titer elevation, but only rarely cause APS. In a study of infection-related APS, the main "triggering" factors were found to be skin infections (18%), Human immunodeficiency virus infection (HIV) (17%), pneumonia (14%), Hepatitis C Virus (HCV) (13%) and urinary tract infections.. Other infections less frequently associated with APS are pulmonary tuberculosis, mycoplasma, malaria, *P. carinii* and leptospirosis.

Catastrophic APS, a rare form of APS, is an acute widespread small vessel coagulopathy resulting in almost simultaneous multiorgan disease (most common renal pulmonary, CNS, cardiac) with mortality in a half of cases. In this disease, "triggering" factors have become increasingly apparent and were present in 51% of cases in the latest analysis (25). These triggering factors include trauma, anticoagulant withdrawal, malignancy and most commonly infections, which were identified in 24% of catastrophic APS patients. These infections included urinary tract infections (4%), respiratory (10%), cutaneous (including infected leg ulcers) (4%), gastrointestinal (2%), sepsis (1%) and other infections (3%). Molecular "mimicry" has been proposed as one of the major mechanisms responsible for the development of catastrophic APS following infections (26) but there may be an interplay of other mechanisms.

Passive transfer of anti- β 2GPI related synthetic peptides with homology to common bacteria to naive mice resulted in an induction of APS (27-28). Exchanging heavy and light chains between pathogenic and non-pathogenic anti- β 2GPI single chain Fv, demonstrated that the pathogenic part of the anti- β 2GPI molecule is located on the CDR3 of the heavy chain of the immunoglobulin (29). Molecular mimicry between common pathogen and anti- β 2GPI peptide epitopes is a possible origin for anti- β 2GPI antibodies. This assumption is based on the fact that there is a correlation between APS clinical manifestations and infectious agents in human, also supported by the homology found between β 2GPI related peptides (target epitopes for anti- β 2GPI Abs) and different common pathogens, as demonstrated in the protein data bases.

We have previously identified several synthetic peptides as target epitopes for anti- β 2GPI Abs.

These β 2GPI related peptides were found to be located on domain I-II (mimotope), domain-III and domain-IV (both linear sequences) of β 2GPI molecule. All 3 synthetic peptides inhibited activation of endothelial cell in-vitro and induce APS in naïve mice via neutralizing of the pathogenic anti- β 2GPI Abs (18). Moreover, the prevalence of circulating autoantibodies against these peptides in a sera of 295 APS patients ranged between 18% to 47.5% (30). In addition, homology exists between these peptides and common infectious agents. Following immunization of naive mice with microbial pathogens (which share structural homology with the TLRVYK hexapeptide), mouse anti-TLRVYK were affinity purified from the immunized mice on a TLRVYK-column and then passively infused into naive mice at day 0 of pregnancy. Following this latter immunization, various levels of mouse anti- β 2GPI Abs were observed. The highest was detected in those mice immunized with *Haemophilus influenzae*, *Neisseria gonorrhoeae* or Tetanus toxoid. Mice infused with these anti- β 2GPI Abs had significant thrombocytopenia, prolonged aPTT and increased fetal loss, similarly to a control group of mice given pathogenic anti- β 2GPI monoclonal antibody (31). Hence, this established a mechanism of molecular mimicry in experimental APS, demonstrating that β 2GPI-structure homologous bacteria are able to induce the generation of pathogenic anti- β 2GPI Abs along with APS manifestations (31).

There is structural similarity between various common pathogens, including *Helicobacter pylori*, *Streptococcus pyogenus*, *Borrelia burgdorferi*, *Saccharomyces cerevisiae*, *Vaccinia virus*, Epstein-Barr virus and β 2GPI and β 2GPI related peptides. One theory is that pathogen particles are phagocytized and digested by antigen presenting cells (macrophages, dendritic cells or B cells). After presentation to T cells with appropriate HLA molecules and cytokine milieu, plasma cells are generated and secrete anti- β 2GPI Abs which are directed against the pathogen particles with structural homology (molecular mimicry) with the β 2GPI molecule. Whether an individual will develop APS will depend mainly on his genetic predisposition. Therefore, a mimicking antigen, similar in only one epitope, may initiate a primary cross-reactive response to that epitope that subsequently results in recognition of numerous epitopes on the host β 2GPI. Mimicry may be one of the mechanisms for breaking the tolerance and triggering the autoimmune response. Yet, the mere presence of a self-determinants on a virus or bacteria, will not necessarily result in disease. The full-blown APS will emerge only if appropriate genetic predisposition exists.

References

1. Oldstone MB. Molecular mimicry and immune-mediated diseases. *FASEB J* 1998; 12: 1255-1265.
2. Karlsen AE, and Dyrberg T. Molecular mimicry between non-self, modified self and self in autoimmunity. *Semin Immunol* 1998;10:25-34.
3. Harris EN, Gaharavi AE, Boey ML, Patel BM, Mackworth-Young CG, Loizou S, Hughes GRV: Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet* 1983;2:1211-1214.
4. Hughes GRV, Harris EN, AE Gharavi. The anti-cardiolipin syndrome. *J Rheumatol* 1986; 13:486-489.
5. Asherson RA, Cervera R, Piette JC, Shoenfeld Y. Milestones in the antiphospholipid syndrome. In:

- Asherson RA, Cervera R, Piette JC, Shoenfeld Y, (eds). The antiphospholipid syndrome II - Autoimmune thrombosis. Elsevier, Amsterdam, 2002
6. Schwarzenbacher R, Zeth K, Diederichs K, Gries A, Kostner GM, Laggner P, Prassl R. Crystal structure of human beta2-glycoprotein I: Implications for phospholipid binding and the antiphospholipid syndrome. *EMBO J* 1999;18:6228-6239.
 7. Brighton TA, Hogg PJ, Dai YP, Murray BH, Chong BH and Chesterman CN. Beta 2 glycoprotein I in thrombosis: evidence for a role as a natural anticoagulant. *Br J Haematol* 1996;93: 185-194.
 8. Koike T, Ichikawa K, Atsumi T, Kasahara H, Matsuura E. Beta 2-glycoprotein I-anti-beta 2-glycoprotein I interaction. *J Autoimmun* 2000;15:97-100.
 9. Gharavi AE, Summaritano LR, Wen J, Elkouf EB. Induction of antiphospholipid antibodies by immunization with β 2-glycoprotein I (apolipoprotein H). *J Clin Invest.* 1992;90:1105-1111.
 10. Pierangeli SS, Harris EN. Induction of phospholipid-binding antibodies in mice and rabbits by immunization with human β 2-glycoprotein I or anticardiolipin antibodies alone. *Clin Exp Immunol.* 1993;93:269-273.
 11. Blank M, Faden D, Tincani A, Kopolovic J, Goldberg I, Gilburd B, Balestrieri G, Valesini G, Shoenfeld Y. Immunization with anticardiolipin cofactor (β 2-glycoprotein-I) induces experimental APS in naive mice. *J Autoimmun* 1994;7:441-447.
 12. Garcia C, Kanbour-Shakir A, Tang H, Espinoza LR, Gharavi AE. Induction of experimental antiphospholipid syndrome in PL/J mice following immunization with β 2-glycoprotein I. *J Invest Med* 1996; 44:69A.
 13. Blank M, George J, Barak V, Tincani A, Koike T and Shoenfeld Y. Oral Tolerance to Low Dose β 2-Glycoprotein I: Immunomodulation of experimental antiphospholipid syndrome. *J Immunol* 1998;161: 5303-5312.
 14. Shi W, Chong BH, and Chesterman CN. Beta 2-glycoprotein I is a requirement for anticardiolipin antibodies binding to activated platelets: differences with lupus anticoagulants. *Blood* 1993;81:1255-1262.
 15. Kornberg A, Blank M, Kaufman S, and Shoenfeld Y. Induction of tissue factor-like activity in monocytes by anti-cardiolipin antibodies. *J Immunol* 1994;153:1328-1332.
 16. Amengual O, Atsumi T, Khamashta MA, and Hughes GR. The role of the tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid syndrome. *Thromb Haemost* 1998;79:276-281.
 17. 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.
 18. Blank M, Shoenfeld Y, Cabilli S, Heldman Y, Fridkin M and E Katchalski-Katzir. Prevention of experimental antiphospholipid syndrome and endothelial cell activation by synthetic peptides. *Proc Natl Acad Sci* 1999;96:5164-5168.
 19. Meroni PL, Raschi E, Testoni C, Tincani A, Balestrieri G, Molteni R, Khamashta MA, Tremoli E, Camera M. Statins prevent endothelial cell activation induced by antiphospholipid (anti-beta2-glycoprotein I) antibodies: effect on the proadhesive and proinflammatory phenotype. *Arthritis Rheum*

2001;44:2870-2878.

20.Pierangeli SS, Liu X, Espinola R, Olee T, Zhu M, Harris NE, and Chen PP. Functional analyses of patient-derived IgG monoclonal anticardiolipin antibodies using in vivo thrombosis and in vivo microcirculation models. *Thromb Haemost* 2000;84:388-395.

21.Branch DW, Dudley DJ, Mitchell MD, Creighton KA, Abott TM, Hammond E, and Daynes RA. Immunoglobulin G fractions from patients with anti-phospholipid antibodies cause fetal death in BALBA/c mice: a model for autoimmune fetal loss. *Am J Obstet Gynecol* 1990;163:210-216.

22.Juby A, Davis P, Genge T, McElhaney J. Anticardiolipin antibodies in two elderly subpopulations. *Lupus* 1995;4:482-5.

23.Avcin T, Cimaz R, Meroni PL. Recent advances in antiphospholipid antibodies and antiphospholipid syndromes in pediatric populations. *Lupus*. 2002;11:4-10.

24.Cabiedes J, Trejo-Hernandez J, Loredo-Abdala A, Castilla-Serna L, Lopez-Mendoza AT, Cordero-Esperon HA, Huerta MT, Cabral AR, Alarcon-Segovia D. Anti-cardiolipin, anti-cardiolipin plus bovine, or human beta2-glycoprotein-I and anti-human beta2-glycoprotein-I antibodies in a healthy infant population. *Arch Med Res*. 2002;33:175-9.

25.Cervera R, Gómez-Puerta JA, Espinosa G, Font J, De la Red G; Gil V, Bucciarelli S, Cucho M, Ramos-Casals M, Ingelmo M, Shoenfeld Y, Piette JC, Asherson RA. "CAPS Registry". A review of 200 cases from the International Registry of patients with the Catastrophic Antiphospholipid Syndrome (CAPS). *Ann Rheum Dis* 2003; 62 (suppl. 1): 88.

26.Asherson RA, Shoenfeld Y. The role of Infection in the pathogenesis of catastrophic antiphospholipid syndrome –molecular mimicry ? *J Rheumatol* 2000;27:12 – 14.

27.Blank M, Cohen J, Toder V, Shoenfeld Y. Induction of primary anti-phospholipid syndrome in mice by passive transfer of anti-cardiolipin antibodies. *Proc Natl Acad Sci (USA)* 1991; 88:3069-3073.

28.Holers VM, Girardi G, Mo L, Guthridge JM, Molina H, Pierangeli SS, Espinola R, Xiaowei LE, Mao D, Vialpando CG, Salmon JE. Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *J Exp Med* 2002;195:211-220.

29.Blank M, Waisman A, Mozes E, Koike T, and Shoenfeld Y. Characteristics and pathogenic role of anti-beta2-glycoprotein I single-chain Fv domains: induction of experimental antiphospholipid syndrome. *Int Immunol* 1999;11:1917-1926.

30.Shoenfeld Y, Krause I, Kvapil F, et al. Prevalence and clinical correlations of antibodies against six beta2-glycoprotein-i-related peptides in the antiphospholipid syndrome. *J Clin Immunol*. 2003 (in press)

31.Blank M, Krause I, Fridkin M, Keller N, Kopolovic J, Goldberg I, Tobar A, Shoenfeld Y, Bacterial induction of autoantibodies to beta2-glycoprotein-I accounts for the infectious etiology of antiphospholipid syndrome. *J Clin Invest* 2002; 109:797-804.