Received Date : 19-Jan-2016

Revised Date : 22-Feb-2016

Accepted Date : 10-Mar-2016

Article type : Forum

Pathogenesis of the Antiphospholipid Syndrome Revisited – Time to Challenge the Dogma

Karl J. Lackner, Nadine Müller-Calleja

Institute of Clinical Chemistry and Laboratory Medicine, University Medical Centre Mainz, Germany

Address for correspondence:

Karl J. Lackner, MD
Institute of Clinical Chemistry and Laboratory Medicine
University Medical Centre Mainz
D-55131 Mainz
Germany

Ph: +49 6131 177190
Fax: +49 6131 176627
e-mail: karl.lackner@unimedizin-mainz.de

Abstract

Since more than a decade the antiphospholipid syndrome (APS) is reported to be caused mainly by antiphospholipid antibodies (aPL) which are not directed against phospholipids but against a complex of phospholipids and phospholipid binding proteins so called cofactors, e.g. β2-glycoprotein I (β2GPI). In fact, many researchers propose that the only relevant antigens in the APS are the cofactors themselves, with β2GPI being the most important. Antibodies which bind to phospholipids in a cofactor independent manner are considered insignificant for the pathogenesis of the APS. We review the evidence for this current pathophysiologic concept and argue that it has never been proven and is now clearly no longer tenable. First, there is undisputable evidence that cofactor independent aPL are pathogenic and present in the blood of APS patients. Second, available epidemiologic and clinical studies do not support a dominant pathogenic role for anti-β2GPI.

The antiphospholipid syndrome (APS) is characterized by recurrent thromboembolic events or severe pregnancy morbidity in the presence of pathogenic antiphospholipid antibodies (aPL) which are highly heterogeneous [1]. The prevailing view is that pathogenic aPL bind anionic phospholipids like cardiolipin only in the presence of a protein cofactor or bind directly to the protein cofactors of which β2-glycoprotein I (β2GPI) is considered the most important. Cofactor dependence or direct cofactor binding is believed to distinguish pathogenic from non-pathogenic aPL. Cofactor independent aPL are generally regarded as irrelevant for the APS. This concept is presented in almost all recent reviews [e.g. 2-5]. For instance, Meroni [2] states "β2glycoprotein I-dependent autoantibodies seem to be the main pathogenic subpopulation of aPL". Brandt [3] postulates "the main antigenic target of aPLA is the plasma phospholipid binding protein β2-glycoprotein 1". Du et al. [5] state "It is now well accepted that, although the name antiphospholipid syndrome implies direct binding to phospholipids, aPLs bind phospholipid-binding plasma proteins, of which beta2-glycoprotein I is regarded as most relevant".

While this view is shared by most authorities in the field and is allegedly backed by a wealth of literature, we propose that the postulated almost exclusive pathogenic role of anti-β2GPI or β2GPI-dependent anticardiolipin has never been proven and is today clearly untenable. We do not negate a role for anti-β2GPI, but challenge the view that cofactor independent aPL are irrelevant for the pathogenesis of the APS. We further demonstrate that the evidence that anti-β2GPI or β2GPI-dependent aPL are the main pathogenic subpopulation of aPL is sketchy.

We will address two major questions:

(1) Is there evidence that cofactor independent aPL contribute to the pathogenesis of the APS?

(2) Is there evidence that the contribution of cofactor independent aPL to the pathogenesis of the APS is negligible or at least minor compared to anti-β2GPI or β2GPI-dependent aPL?

Table 1 lists four criteria that must be fulfilled to consider an aPL pathogenic. The answer to the second question can only come from appropriate clinical studies. We will analyze these issues in the light of the available literature.

In 1990 two groups described cofactor dependency of aPL [6,7]. McNeil [6] purified aPL from two patients. The purified immunoglobulins did not bind in a cardiolipin ELISA blocked with milk powder rather than bovine serum, whereas the blocking procedure had no effect on the usual 1:50 dilution of patient serum. Thus, apparently a serum factor not present in the purified aPL supported binding which they identified as β2GPI. Galli [7] also purified aPL from two APS patients. They blocked ELISA plates with bovine serum albumin (BSA). Again purified aPL did not bind under these conditions. Addition of human serum enabled binding of aPL.

Two aspects of these publications are noteworthy: (1) both used affinity purified aPL rather than whole IgG. Accordingly, the purified aPL may not be representative for the whole spectrum of aPL of the patients. (2) These two highly cited papers and their far reaching conclusions are based on only 4 patients.

Matsuura [8] partly confirmed these data. A very remarkable result of this study is the fact that two out of three APS patient IgG-fractions bound either in a completely or partially cofactor independent fashion. Another 15 affinity purified aPL fractions were all cofactor dependent. Pierangeli [9] reported only moderate enhancement of binding of 5 APS-IgG to cardiolipin by the addition of β2GPI. This early paper provides convincing evidence, that many if not the majority of APS patients harbor cofactor independent aPL. In 1996 Forastiero [10] presented a larger series of 35 APS and SLE patients. In 14 out of 35 aPL positive serum samples binding was not enhanced by addition of cofactor. Furthermore, 2 out of 10 IgG fractions isolated from these patients were cofactor independent. We observed that 10 out of 15 APS-IgG fractions bound in a fully cofactor independent manner to cardiolipin while the remaining 5 showed reduced binding in the absence of cofactor [11].

Today, it is recommended that anticardiolipin should be measured by enzyme-linked immunosorbent assays for β2GPI–dependent anticardiolipin antibodies. Unfortunately, this suggests that such assays are specific for β2GPI–dependent aPL. However, this assay format only ensures binding of β2GPI-dependent aPL. Since cofactor independent aPL will also bind, it is impossible to distinguish between cofactor dependent and independent aPL. Assay formats designed to distinguish between β2GPI-dependent and independent aPL have been devised but never gained wider acceptance [12].

In summary, the available data show that patients with the APS have quite heterogeneous antibody profiles. Contrary to common belief, there is ample evidence in the literature that a significant proportion if not the majority of APS patients harbor cofactor independent antibodies against cardiolipin (criterion 1).

Common to all experimental approaches to show pathogenic effects of aPL in vitro is the need for a source of aPL. This source is obviously of major importance for the interpretation of results. Patient immunoglobulins are highly heterogeneous so that it is impossible to assign effects to a specific antibody if serum or purified immunoglobulins are used. Affinity purification of aPL has been notoriously ineffective to provide pure aPL subfractions which are devoid of other aPL species [e.g. 13]. Human monoclonal aPL are another option. A relevant limitation here is the difficulty to prove that a particular monoclonal aPL is relevant for the APS. This can be solved by comparing the effects of monoclonal aPL with effects of IgG-fractions from APS patients. If they induce the same effects this is strong evidence that the effects of the monoclonal aPL are relevant for the APS.

We have previously shown that two human monoclonal cofactor independent aPL which have been obtained from patients with the APS can induce proinflammatory and procoagulant responses in monocytes and endothelial cells [11,14,15]. The aPL enter the endosomal route where they activate NADPH-oxidase 2 (NOX2) followed by induction of cytokines, inflammasome components, and tissue factor. All effects could be reproduced by IgG fractions obtained from APS patients. Analysis of the aPL spectrum of these patients showed that all of them had cofactor independent aPL only or in combination with cofactor dependent aPL [11]. These combined data prove that cofactor independent aPL have proinflammatory and procoagulant cellular effects (criterion 2).

Pierangeli showed in an injury dependent mouse model that thrombus size in mice with aPL induced by immunization was independent of the presence of anti-β2GPI [16]. Unfortunately, cofactor dependence of the induced anticardiolipin is not known. Later on this group also used human monoclonal aPL to induce thrombosis in the same model [17]. The aPL with the most pronounced thrombus induction (IS2) was not dependent on β2GPI nor did it bind to this protein. IS2 appears to be dependent on a so far unidentified cofactor present in mouse but not in human serum. These data strongly suggest that dependence on or specificity for β2GPI is not a prerequisite of pathogenicity. We have recently shown that the two monoclonal human aPL used in our in vitro experiments induce thrombus formation in an in vivo mouse model [18]. Induction is rapid within three hours after infusion of the aPL. NOX2 deficient mice are protected from the prothrombotic effects underscoring that the signaling cascade observed *in vitro* is also activated *in vivo*. Furthermore, we showed that thrombus formation depends largely on tissue factor induction in monocytes. This is the first evidence that cofactor independent aPL can be pathogenic in vivo (criterion 3) and that the signal transduction underlying this process can be induced by IgG fractions from APS patients.

Having shown conclusive evidence for the first three criteria listed in table 1, finally, the results of clinical studies shall be discussed. Fortunately, this has been reviewed extensively in the past. The first concise reviews of clinical studies were published in 2003 [19,20]. They summarize that LA shows the strongest association to thromboembolic events. Anticardiolipin antibodies show a significant association in many but not all studies [19] which is similar to anti-β2GPI [20]. In 2014 a further systematic review and meta-analysis of clinical studies on patients with aPL in the absence of systemic lupus was published [21]. LA and cardiolipin antibodies again were significantly associated with an increased risk of venous and arterial thrombosis. Anti-β2GPI was only associated to arterial thrombosis. Basically, these authors come to similar conclusions as Galli [19,20] 10 years earlier even though their methodological approach was not the same.

Thus, the results from clinical studies can be summarized as follows: (1) LA is the aPL test most consistently and strongly associated with thromboembolic events. (2) Anticardiolipin is still the immunoassay with the strongest and best documented association with thromboembolic events. (3) As outlined above, none of the clinical studies was designed to differentiate between cofactor dependent and independent aPL. Therefore, clinical data cannot support a pathophysiologic role of one or the other.

The surprising conclusion is that the commonly favored view that only cofactor dependent aPL or aPL against cofactor proteins are clinically relevant has never been substantiated by appropriate clinical studies. The assumption that persistent cofactor independent aPL are irrelevant has not been addressed at all.

The major conclusions that derive from these reflections are as follows:

- Anticardiolipin are clearly pathogenic. This is independent of cofactor requirement.
- The differentiation of pathogenic and non-pathogenic aPL must be based on other not yet defined criteria.

- The statement that β2GPI is the major antigen in the APS and anti-β2GPI are the dominant pathogenic autoantibodies lacks clinical proof.

Authors state no conflicts of interests.

References

1. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, DE Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006; 4: 295-306
2. Meroni PL, Borghi MO, Raschi E, Tedesco F. Pathogenesis of the antiphospholipid syndrome: understanding the antibodies. Nat Rev Rheumatol 2011; 7: 330-9
3. Brandt KJ, Kruithof EKO, de Moerloose P. Receptors involved in cell activation by antiphospholipid antibodies. Thromb Res 2013; 132: 408-13
4. Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. N Engl J Med 2013; 368: 1033-44
5. Du VX, Kelchtermans H, de Groot PG, de Laat B. From antibody to clinical phenotype, the black box of the antiphospholipid syndrome: pathogenic mechanisms of the antiphospholipid syndrome. Thromb Res 2013; 132: 319-26
6. McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta 2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci U S A 1990; 87: 4120-4
7. Galli M, Comfurius P, Maassen C, Hemker HC, de Baets MH, van Breda-Vriesman PJ, Barbui T, Zwaal RF, Bevers EM. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. Lancet 1990; 335: 1544-7
8. Matsuura E, Igarashi Y, Fujimoto M, Ichikawa K, Suzuki T, Sumida T, Yasuda T, Koike T. Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. J Immunol 1992; 148: 3885-91
9. Pierangeli SS, Harris EN, Davis SA, DeLorenzo G.Beta 2-glycoprotein 1 (beta 2GP1) enhances cardiolipin binding activity but is not the antigen for antiphospholipid antibodies. Br J Haematol 1992; 82: 565-70
10. Forastiero RR, Martinuzzo ME, Kordich LC, Carreras LO. Reactivity to beta2 glycoprotein I clearly differentiates anticardiolipin antibodies from antiphospholipid syndrome and syphilis. Thromb Haemost 1996; 75: 717-20
11. Müller-Calleja N, Köhler A, Siebald B, Canisius A, Orning C, Radsak M, Stein P, Mönnikes R, Lackner KJ. Cofactor-independent antiphospholipid antibodies activate the NLRP3-inflammasome via endosomal NADPH-oxidase: implications for the antiphospholipid syndrome. Thromb Haemost 2015; 113: 1071-83

12. Levy RA, de Meis E, Pierangeli S. An adapted ELISA method for differentiating pathogenic from nonpathogenic aPL by a beta 2 glycoprotein I dependency anticardiolipin assay. Thromb Res 2004; 114: 573-7
13. Arad A, Proulle V, Furie RA, Furie BC, Furie B. β2-glycoprotein-1 autoantibodies from patients with antiphospholipid syndrome are sufficient to potentiate arterial thrombus formation in a mouse model. Blood 2011; 117: 3453-9
14. von Landenberg C, Lackner KJ, von Landenberg P, Lang B, Schmitz G. Isolation and characterization of two human monoclonal antiphospholipid IgG from patients with autoimmune disease. J Autoimmun 1999; 13: 215-223
15. Prinz N, Clemens N, Strand D, Pütz I, Lorenz M, Daiber A, Stein P, Degreif A, Radsak M, Schild H, Bauer S, von Landenberg P, Lackner KJ. Antiphospholipid-antibodies induce translocation of TLR7 and TLR8 to the endosome in human monocytes and plasmacytoid dendritic cells. Blood 2011; 118: 2322-32
16. Pierangeli SS, Liu XW, Anderson G, Barker JH, Harris EN. Thrombogenic properties of murine anti-cardiolipin antibodies induced by b2 glycoprotein 1 and human immunoglobulin G antiphospholipid antibodies. Circulation 1996; 94: 1746-51
17. Pierangeli SS, Liu X, Espinola R, Olee T, Zhu M, Harris NE, Chen PP. Functional analyses of patient-derived IgG monoclonal anticardiolipin antibodies using in vivo thrombosis and in vivo microcirculation models. Thromb Haemost 2000; 84: 388-95
18. Manukyan D, Müller-Calleja N, Jäckel S, Luchmann K, Mönnikes R, Kiouptsi K, Reinhardt C, Jurk K, Walter U, Lackner KJ. Cofactor independent human antiphospholipid antibodies induce venous thrombosis in mice J Thromb Haemost 2016; doi: 10.1111/jth.13263
19. Galli M, Luciani D, Bertolini G, Barbui T. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. Blood 2003; 101: 1827-32
20. Galli M, Luciani D, Bertolini G, Barbui T. Anti-beta 2-glycoprotein I, antiprothrombin antibodies, and the risk of thrombosis in the antiphospholipid syndrome. Blood 2003; 102: 2717-23
21. Reynaud Q, Lega J-C, Mismetti P, Chapelle C, Wahl D, Cathébras P, Laporte S. Risk of venous and arterial thrombosis according to type of antiphospholipid antibodies in adults without systemic lupus erythematosus: A systematic review and meta-analysis. Autoimmun Rev 2014; 13: 595-608

Table 1: Criteria for pathogenicity of aPL

1.	Presence in many or most APS patients
2.	Induction of humoral or cellular responses compatible with the clinical manifestation of the APS
3.	Induction of clinical manifestations of the APS *in vivo*
4.	Association with clinical manifestations of the APS in humans