

## Chapter 2

# What is the Origin of Antiphospholipid Antibodies?

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### Introduction

Antiphospholipid syndrome (APS) is an autoimmune multisystemic disorder characterized clinically by recurrent thrombosis and pregnancy morbidity, and serologically by the presence of antiphospholipid antibodies (aPL) including anticardiolipin antibodies (aCL), anti- $\beta_2$ glycoprotein-I antibodies (a $\beta_2$ GPI), and lupus anticoagulant (LA) [1–3]. It is now widely accepted that aPLs are a heterogeneous group of antibodies that react with a myriad of phospholipids (PLs), PL–protein complexes, and PL-binding proteins. The main antigenic target of these antibodies is recognized to be  $\beta_2$ GPI, which along with prothrombin accounts for more than 90% of the antibody-binding activity in APS patients [4–10].

Thus far, little is known about the origin of pathogenic aPL. Several mechanisms have been postulated including infections that were identified to contribute to the production of aPL through *molecular mimicry* and epitope spreading [11]. Additionally, there is also evidence that endogenous  $\beta_2$ GPI may get exposed to the immune system and recognized as an antigen during apoptotic cells' clearance.

This chapter reviews the most up-to-date scientific evidence regarding proposed genetic and environmental factors contributing to the development of pathogenic aPL.

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## What Is Known?

### *Genes and the Environment in Antiphospholipid Syndrome*

Various animal models and family and population studies have been used to highlight HLA associations with the occurrence of aPL and the development of thrombosis in aPL-positive patients. Thus, Major Histocompatibility Complex (MHC) genes may influence not only autoantibody production but also disease expression itself [12]. In addition, the coexistence of other inherited thrombophilia risk factors, i.e., Factor V Leiden (FVL), prothrombin 20210 mutations, may further increase thrombogenic risk in APS. [13]. These pathogenic aPL are thought to be produced after exposure to certain viral or bacterial products with sequence similarity to host antigens inducing a break in tolerance (molecular mimicry) [14]. Antiphospholipid Antibodies represent a heterogeneous group of antibodies with many different antigenic targets; the clinical experience is that not all aPL are pathogenic, making it likely that only a certain group of aPL induced by certain viral or bacterial products are important in disease development [14, 15].

### **Animal Genetic Studies in Antiphospholipid Syndrome**

There are relatively few animal studies that have assessed the genetic basis for the development of APS. The spontaneous production of IgG aCL, which exhibits cofactor ( $\beta_2$ GPI)-dependent binding to cardiolipin, has been detected in NZW  $\times$  BXSB F1 (W/B F1) male mice [16]. W/B F1 mice are SLE-prone mice, which develop several autoantibodies, circulating immune complexes, and nephritis in addition to a high incidence of degenerative coronary vascular disease with myocardial infarction and thrombocytopenia. Thus, W/B F1 mice represent a model of lupus-associated APS [16–18]. Interestingly, analysis of the genes utilized in the production of pathogenic aCL in these mice showed preferential usage of certain  $V_H$  and  $V_K$  genes, whereas other nonpathogenic aCL utilize random V gene combinations [19]. This possibly indicates that pathogenic aCL production in these mice is antigen driven rather than germline encoded.

In 1998, Ida et al. analyzed APS disease features in BXSB and NZW mice and their progeny [20]. Although male BXSB parental mice showed similar disease features to their male NZW  $\times$  BXSB F1 progeny, these features were of decreased frequency and intensity, and the disease was not apparent in female parental NZW or female NZW  $\times$  BXSB F1 progeny. These findings suggest that genes from the BXSB strain determines, while NZW genes serve to upregulate or modify, APS disease characteristics in their progeny and that modifying alleles such as BXSB Y-linked autoimmune accelerator gene (Yaa) also play a role [20–22] in disease manifestations. In the same study, genome-wide analysis using microsatellite markers was used to map BXSB alleles affecting the development of aCL, antiplatelet antibodies, thrombocytopenia, and myocardial infarction in NZW  $\times$  (NZW  $\times$  BXSB) F1 backcross male progeny [20]. This analysis showed that the generation of each disease manifestation was controlled

by two independently segregating major dominant alleles producing full expression as a complementary gene action. Although there was complete genetic concordance between antiplatelet antibodies and thrombocytopenia, other disease characteristics were independently controlled by different combinations of two dominant alleles suggesting that no single genetic factor can explain the pathogenesis of APS [20].

The presence of IgG aCL has also been demonstrated in other lupus-prone mice, including the MRL/MP/lpr/lpr (MRL/lpr) and MRL<sup>lpr/lpr</sup> mice [23]. Similar to aCL produced in W/B F1 mice, those produced in MRL/lpr mice showed nonrandom V<sub>H</sub> and V<sub>K</sub> gene usage and also evidence of somatic mutation indicating a role for antigen-driven affinity maturation [24]. Anticardiolipin antibodies are also produced in normal C57BL/6J mice with estrogen treatment increasing the incidence and levels of these antibodies, underscoring the role that environmental factors such as hormones modifying genetic susceptibility in APS patients [25]. However, aCL produced in these estrogen-treated C57BL/6J mice and those in MRL/lpr mice are not  $\beta_2$ GPI dependent but rather show decreased binding to cardiolipin in the presence of human  $\beta_2$ GPI [26]. Interestingly, NZW  $\times$  NZB F1 mice, another classic murine model of SLE, fail to produce aCL despite the production of other autoantibodies, such as anti-dsDNA [23].

### **Family and Population Studies: Human Leukocyte Antigen (HLA) and Non-HLA Associations**

Multiple HLA-DR and DQ associations with the occurrence of aPL have been described, but small patient sample sizes and difficulties regarding obtaining appropriately as well as ethnically matched control populations make interpretation problematic [12, 13]. A familial clustering of individuals with persistently false-positive tests for syphilis in whom overt autoimmune disease developed years later was perhaps the first indication of familial APS [27]. Since 1980, several studies have described families with high incidences of primary APS associated with LA, aCL, and other autoantibodies [28, 29]. The increased incidence of aCL in first-degree relatives of APS patients with or without SLE has also been demonstrated [30, 31]. A 1998 study which assessed 7 families with a high incidence of primary APS, 30 of 101 family members meeting diagnostic criteria, suggested either a dominant or codominant model for inheritance of the disease by segregation analysis but failed to find linkage to HLA and other candidate genes, including  $\beta_2$ GPI and Fas [32]. Other family studies, however, have reported several HLA associations. The paternal haplotype A30; Cw3; B60; DR4; DRw53; DQw3 has been shown to be associated with aCL in an English Canadian family, both in asymptomatic individuals and those with APS associated with SLE and autoimmune thyroid disease [33]. The occurrence of LA in families with haplotypes containing either DR4 or DR7 has also been demonstrated [34, 35]. In a family study in which all members had SLE and presented with various APS manifestations, a mother and her twins shared a haplotype that included DR4, DRw53, and DQw7 [36].

Nonfamilial population studies also highlight several HLA associations of APS. A 1991 study of 20 patients with SLE and LA demonstrated an association with HLA-DQw7 (HLA-DQB1\*0301) linked to HLA-DR4 and/or -DR5 [37]. In 13

English patients with primary APS, DR4 and DRw53 were found with increased frequency [38]. Other HLA loci associated with primary APS include DRB1\*04, DR7, DQB1\*0301/4, DQB1\*0604/5/6/7/9, DQA1\*0102, and DQA1\*0301/2 [39–41]. In a large Italian study of SLE patients, aCL was positively associated with HLA-DRB1\*04, -DRB1\*07, -DQA1\*0201, -DQA1\*0301, -DQB1\*0302, and -DRB3\*0301, and  $\alpha\beta_2$ GPI was positively associated with DQB1\*0302 [42]. The association of aCL with DRB1\*09 has been reported in Japanese patients with APS associated with SLE [43]. Anti- $\beta_2$ glycoprotein-I in Caucasian and Mexican Americans is strongly associated with HLA-DR4 haplotypes, especially those carrying HLA-DQ8 (DQB1\*0302), while in African-American and white British patients with primary APS,  $\alpha\beta_2$ GPI is strongly associated with the HLA-DRB1\*1302 and DQB1\*0604/0605 haplotypes [39, 44]. The association of C4A or C4B null alleles with the presence of aCL has been reported in black American populations; however, patients in the Hopkins Lupus Cohort who were homozygous for C4A deficiency had a lower frequency of aCL and LA than patients without this deficiency [45–47].

Other genes outside the MHC region also contribute to both autoantibody production and disease expression in APS. A polymorphism in domain 5 of  $\beta_2$ GPI, valine instead of leucine at position 247, is found more frequently in patients with APS than matched controls and is associated with  $\alpha\beta_2$ GPI production in these patients [48–50]. One study found an increased frequency of this polymorphism in patients with arterial thrombosis than those without [50]. There are other prothrombotic genetic factors that can modify disease expression in APS patients. Those genetic factors clearly related to thrombophilia that have been seen in APS patients include factor V Leiden (FVL) and prothrombin mutations and antithrombin III, protein C, and protein S deficiencies [51]. The gain-of-function FVL G1691A mutation is highly prevalent in Caucasian populations with population frequencies ranging from 1% to 15% [52, 53].

Several reports have demonstrated an increased incidence of thrombosis in APS patients with FVL mutation when compared to those without FVL mutation. However, this mutation seems to have a more moderate effect on the development of thrombosis in APS than in the general population [54–56]. The G20210A prothrombin mutation (F2 G20210A) is associated with venous thromboembolism in the general population, but there have been conflicting reports of the increased risk of thrombosis related to this gene mutation in APS patients. Initial reports indicated no increased risk, but some of the subsequent studies have demonstrated the association between the mutation and thrombosis in APS patients: the first case was in a young female with SLE-associated APS homozygous for the G20210A mutation [57–60]. Protein C, S, and antithrombin III deficiencies are uncommon diseases, making it difficult for an accurate assessment of the relative contributions of these mutations to thrombus generation in aPL-positive patients. However, there have been reports of increased thrombosis rates in patients with protein C and protein S deficiency [61, 62]. Other polymorphisms that potentially impact the risk of thrombosis in APS patients include platelet glycoproteins GP Ia/IIa and GP IIb/IIIa, platelet Fc $\gamma$  receptor IIa, tissue factor pathway inhibitor, thermolabile variant of methylenetetrahydrofolate reductase, type-I plasminogen activator inhibitor, tumor necrosis factor  $\alpha$ , thrombomodulin, annexin A5, P-selectin, P-selectin glycoprotein ligand-1, toll-like receptor 4, factor XIII, and CD40 [63–73].

**Fig. 2.1** Amino acid similarities of peptides from viral and bacterial origin with GDKV (a 15 amino acid peptide) from the fifth region of  $\beta_2$ GPI. K=lysine residues. In red: sequence similarities among peptides

Peptides	Sequence	Origin
GDKV	GD <b>K</b> VSFFC <b>K</b> N <b>K</b> E <b>K</b> K <b>C</b>	$\beta_2$ GPI
TADL	TADLAIAS <b>K</b> <b>K</b> <b>K</b> <b>K</b> KRSP <b>K</b> PE	HAdV-2
TIFI	<b>T</b> IFILFCC <b>S</b> <b>K</b> E <b>K</b> R <b>K</b> <b>K</b> KQAAT	CMV
VITT	VITTILYYRR <b>K</b> <b>K</b> KSPSDT	CMV
SGDF	SGDFEYTY <b>K</b> G <b>K</b> <b>K</b> <b>K</b> KMAFATS	<i>B. sub</i>

Environmental Factors and the Origin of Antiphospholipid Antibodies

The processes underlying the production of aPL in APS patients remain undetermined. When these antibodies were first described, aPLs were defined as antibodies reacting to cardiolipin; however, it is now well accepted that these antibodies recognize various PL and protein antigenic complexes [4–7]. Indeed, as stated previously, the main antigenic target for these antibodies is  $\beta_2$ GPI, an abundant serum protein that is a necessary cofactor for aPL’s binding to phospholipid. In fact, efforts to induce high titer production of pathogenic aPL in animal models succeeded only after immunization with heterologous  $\beta_2$ GPI rather than pure phospholipids [4, 74]. This led researchers to believe that perhaps in vivo binding of foreign PL-binding proteins resembling  $\beta_2$ GPI to self-phospholipids in APS patients may lead to the formation of immunogenic complexes against which aPL are produced.

The Infectious Origin of Antiphospholipid Antibodies

Many infections may be accompanied by aPL elevations and, in some, these elevations may be accompanied by clinical manifestations of the APS. Several reviews on this important topic have been deeply detailed in [75–77]. Skin infections (18%), human immunodeficiency virus infection (HIV) (17%), pneumonia (14%), hepatitis C virus (HCV) (13%), and urinary tract infections constituted the most common infections found as “triggering” factors. Viral, bacterial, and parasitic infections have been implicated in aPL production.

Peptides from microorganisms with functional and sequence similarity to that of the PL-binding site of  $\beta_2$ GPI have been used to induce pathogenic aPL and anti- $\beta_2$ GPI production. Pathogenic aPL production in mice was achieved by immunization with a synthesized 15 amino acid peptide, GDKV, which spanned an area of the fifth domain of  $\beta_2$ GPI known to be a major PL-binding site of the molecule [78–80] (Fig. 2.1). The peptides TIFI and VITT from cytomegalovirus (CMV), TADL from adenovirus (AdV), and SGDF from *Bacillus subtilis* all had greater degrees of PL binding compared to GDKV and induced high-titer aPL and  $\beta_2$ GPI production in mice. Subsequent in vivo and in vitro experiments confirmed the pathogenicity of antibodies induced in TIFI-immunized mice [11, 80, 81].

Further supporting evidence for molecular mimicry as a possible mechanism for APS development was provided by a study evaluating the APS-related pathogenic

potential of microorganisms carrying sequences related to a hexapeptide, TLRVYK, known to be specifically recognized by a pathogenic monoclonal  $\alpha\beta_2$ GPI [82]. Following immunization with *Haemophilus influenzae*, *Neisseria gonorrhoeae*, or tetanus toxoid, high-affinity anti-peptide (TLRVYK) and  $\alpha\beta_2$ GPI were observed in BALB/c-immunized mice. TLRVYK affinity-purified antibodies were then infused into naive mice at day 0 of pregnancy. At day 15, these mice had significant thrombocytopenia, prolonged activated partial thromboplastin times (aPTT), and increased frequency of fetal loss compared to controls. An additional example was provided by a synthetic peptide (named peptide A, NTLKTPRVGGC) that shares similarity with common bacterial antigens, which reverse aPL-mediated thrombosis in mice in vivo [83].

Infections are thought perhaps to be the most prominent environmental trigger for aPL production and APS development. Syphilis was the first infectious disease recognized to be linked to aPL production and these infectious-type aPL were initially thought to be nonpathogenic [84]. However, several subsequent reports have shown that many infections not only trigger aPL production but are associated with the development of APS manifestations as well [85]. This is perhaps best exemplified by catastrophic APS, a rare presentation of APS characterized by multiple small vessel occlusions affecting multiple organ systems with a high mortality rate, which is strongly linked to preceding infections and/or trauma [86]. CMV, parvovirus B19, HIV, hepatitis B and C viruses, human T-cell lymphoma/leukemia virus (HTLV), and Varicella Zoster Virus (VZV) are just a few of the infectious agents that have been reported to have associations with aPL production and APS manifestations [87]. In addition to molecular mimicry, infectious agents can potentially induce autoimmune responses by selectively activating or destroying unique lymphocyte subsets, directing cytokine/chemokine release, or exposing cryptic autoantigens during cell necrosis and/or apoptosis causing aPL production by bystander activation [88–90].

## Other Environmental Factors

Other potential environmental triggers of APS development include vaccination, drugs, and certain malignancies. However, to date, there is no conclusive evidence linking vaccination to the development of APS [91, 92]. Only case reports have been published addressing the use of diverse adjuvants, such as silicone, vaccination, and others as a trigger for generation of aPL. In a case report, a 38-year-old patient with previous silicone breast implants was reported to develop APS 3 years after the operation, manifested by recurrent fetal loss, venous and arterial thromboses, and high titers of aCL and  $\alpha\beta_2$ GPI [93]. In addition, several case reports addressed correlation between exposure to acrylamide and elevated aCL,  $\alpha\beta_2$ GPI, and antiphosphatidylserine antibodies [94]. However, the direct association of APS with implants has not been proven.

The ability of drugs to bind and perhaps alter the processing and presentation of self-antigens such that cryptic antigens are presented makes the development of an autoimmune response possible [95]. Indeed, agents, such as chlorpromazine,

amoxicillin, phenytoin, chlorothiazide, propranolol, oral contraceptives, antibiotics, antiarrhythmic drugs, antihypertensive medications, quinine, alpha-interferon, or infliximab, have been associated with aPL, but data regarding the prevalence of drug-induced aPL in APS patients are still lacking [96–100].

The presence of aPL has been reported in patients with both solid and hematological malignancies and the significance of this finding lies in the increased risk for thrombosis and the potential for precipitating CAPS in these patients. The mechanisms leading to aPL production remain unclarified but may result from an immune response directed against tumor antigens or perhaps against neoantigens formed due to immunomodulatory drug therapy, such as interferon- $\alpha$  (IFN $\alpha$ ) [101].

### *Presentation of Neoepitopes of $\beta_2$ GPI on Apoptotic Cells*

The normal immune system is exposed to millions of apoptotic cells per day because of the fact that cells continuously die as a result of tissue turnover and as a response to different homeostatic stimuli. An efficient clearance of apoptotic cells occurs mainly in the thymus and bone marrow. Due to the lack of costimulatory signals in these central lymphoid organs, no induction of autoantibodies can occur.

One of the clues linking cell death to the onset of autoimmunity is provided by autoantibodies that bind apoptotic cells and recognize surface epitopes that include complexes of anionic phospholipids, such as phosphatidylserine (PS) and  $\beta_2$ GPI [102–104]. Phosphatidylserine, a negatively charged phospholipid that normally is located almost exclusively on the inner cytoplasmic leaflet, flips to the outside of the cell membrane when the cell undergoes apoptosis [105, 106]. The target of many antiphospholipid autoantibodies has been shown to be a complex between anionic phospholipid and  $\beta_2$ GPI, or  $\beta_2$ GPI alone [107]. In the context of apoptotic cells, the sequestered phospholipid, PS, triggers specific recognition and removal by macrophages [108], and the PS/ $\beta_2$ GPI complex recruits a $\beta_2$ GPI autoantibodies to enhance clearance of apoptotic cells to preserve tissue homeostasis [109].

Piroux et al. [104] coined the concept that cell damage may be an origin of aPL. Since then, accumulating evidence paved the way for apoptotic cells/ $\beta_2$ GPI complexes as sources of a $\beta_2$ GPI. Levine et al. [102] demonstrated that  $\beta_2$ GPI binds selectively to the surface of apoptotic cells, but not viable cells, and that binding of  $\beta_2$ GPI to the surface of apoptotic cells generates an epitope recognized by aPL from patients with primary APS and SLE. Mice immunized with apoptotic cells or apoptotic cells in complex with  $\beta_2$ GPI developed enhanced production of aPL autoantibodies [110–113]. A recent study identified that  $\beta_2$ GPI precipitates in apoptotic bodies through the Ro60 receptor [114].

Due to a defect in the clearance of apoptotic cells, specifically in lupus, the quantity of apoptotic cells may have overwhelmed the normal clearance [109–113, 115]. Therefore, long-lasting circulating apoptotic cells presenting PS on the apoptotic blebs may lead to presentation of hidden epitopes or neoepitopes of  $\beta_2$ GPI on the PS/ $\beta_2$ GPI complex. Furthermore, novel membrane cluster rearrangements due to



posttranslational modifications, phosphorylations, and glycosylations may establish  $\beta_2$ GPI complexes with cellular particles or clusters, as adjuvants, presenting  $\beta_2$ GPI or phospholipids as neoantigen or novel autoantigen, such as cardiolipin as an immunogen, thus triggering an immune response to self, resulting in elevated circulating autoantibodies, including aPL (cardiolipin, PS, phosphatidylethanolamine and  $\alpha\beta_2$ GPI).

## What Is Controversial and/or Unknown?

The topic of aPL and APS has attracted considerable interest in the scientific community and significant knowledge has been gathered in the last 20 years with respect to pathogenesis of the disease as well as development and standardization of diagnostic tools and treatments. However, the question of how “pathogenic” aPL are generated has not been completely examined and many questions still remain unanswered. For example, the extent of the involvement of central and/or peripheral tolerance mechanisms is not completely understood. In addition, the role of dendritic cells, B cells, and/or T cells has not been properly addressed and requires further attention. Furthermore, studies have focused mainly on  $\beta_2$ GPI as the main target antigen, but insufficient attention has been placed in addressing other protein antigenic targets in APS, such as prothrombin and/or complexed antigens of those proteins with various negatively charged phospholipids. Finally, it is not known whether different subtypes of aPL are potentially generated via different pathways.

## Current Research

Several ongoing research studies were recently presented at the 13th International Congress on Antiphospholipid Antibodies (Galveston, April 2010) or “APLA 2010.” In a study presented by Papalardo et al., the authors presented the first evidence of MHC class II involvement in vivo on the production of pathogenic aPL. The investigators showed—in an animal model of thrombosis and APS—that thrombogenic aPL are not produced after immunization with human  $\beta_2$ GPI and tissue factor is not upregulated in MHC II-deficient mice. However, significant titers of pathogenic aPL were produced after immunization with  $\beta_2$ GPI in MHC class II-deficient mice transgenic for the human DR4, DQ6, and DQ8 genes, hence confirming the involvement of certain class II haplotypes in the production of aPL antibodies [116]. In another presentation, van Os et al. showed that  $\alpha\beta_2$ GPI can be generated by immunization with surface protein H of *Streptococcus pyogenes*, underscoring once again the importance of infections and molecular mimicry in APS [117]. Similarly, a report presented by Vista et al. indicated that aCL can be produced following influenza vaccination [118]. On the other hand, Wen et al. identified three loci of genetic susceptibility to APS in 89 patients [119], while Kato et al. showed a higher prevalence of CD36 single-nucleotide polymorphism (SNP) in APS patients, suggesting that



scavenger receptor function correlates with resistance to developing APS [120]. Interestingly, two independent groups reported at the same congress that oxidation plays a role as a trigger of the autoimmune response in APS after oxidative modification of immunoglobulins from normal individuals [121, 122].

Dr. Joyce Rauch proposed—in a plenary session at APLA 2010—that innate immunity plays a dual role in APS [123]. She hypothesized that innate immunity contributes to the pathogenesis of APS in two distinct phases: (1) an “initiation phase,” where the role of innate immunity would be to amplify the adaptive immune response (e.g., to phospholipid-binding proteins, such as  $\beta_2$ GPI), resulting in the long-lived production of aPL and other SLE autoantibodies; and (2) an “effector” (or pathologic) “phase,” where the role of innate immunity would be to enhance the prothrombotic effects of aPL via priming the vascular endothelium (e.g., cellular activation and/or disruption) at the site of thrombosis. During both phases, innate immunity may be triggered by events, such as injury, infection, inflammatory processes, infarction, or ischemia, factors known to be associated with the onset of aPL-related clinical manifestations (“second hit” hypothesis). Dr. Rauch presented compelling evidence to support her hypothesis from the work of her own group that also confirmed previous publications from others who have shown involvement of TLR4 on aPL-mediated thrombosis and endothelial cell activation (“effector phase”) [73, 124]. Dr. Rauch also underscored a possible role of apoptosis in APS [123].

Other groups are currently working on the involvement of innate immunity and the role of dendritic cells, antigen presentation, and breaking tolerance that would lead to the production of autoimmunity mediated by aPL. To that extent, Brogгинi et al. showed—at APLA 2010—that SNPs of proinflammatory genes are associated with thrombosis in APS, including Nox3, MAPK8, and IRAK 2, all of which play a role in TLR-mediated signaling pathway [125], underscoring once more the link between the innate immunity responses and APS. Recent observations from Dr. Pierangeli’s laboratory indicate a role of TLR7 and TLR9 in the production of thrombogenic aPL in mice [126].

## Future Research Directions

Antiphospholipid Syndrome is a multisystemic and heterogeneous disease with devastating consequences in affected individuals. New treatments are urgently needed that may hopefully provide an alternative approach to long-term anticoagulation and general immunosuppression. The current recommended anticoagulant and immunosuppressive treatments for aPL-related clinical manifestations are associated with significant side effects. A full understanding of the etiopathogenesis of the disease will most likely lead to the development of new and better alternative treatments. One of the problems that the APS field is facing is the lack of adequate multicenter and well-designed clinical studies. These would allow the recruitment of adequate numbers of subjects to conduct basic and clinical studies that would

yield meaningful results to answer critical questions. Studies should be designed to answer the following:

What is the role of central and/or peripheral tolerance in APS?

What are the relevant genetic and environmental factors predisposing individuals to APS?

What is the role of innate immunity in APS?

Can peptides that mimic relevant regions of  $\beta_2$ GPI and possible other antigenic targets inhibit the production of aPL in humans?

Group Conclusions

The relative degree to which genetic and environmental factors influence susceptibility to aPL/APS development is still uncertain. It is likely that there is a complex interplay of multiple environmental factors in a genetically susceptible patient to produce the varied autoantibodies and myriad clinical manifestations typical of this disease. The proposed mechanisms of genetic and environmental factors in aPL/APS development are summarized in Table 2.1. Improved understanding of the

**Table 2.1** Evidence for proposed mechanisms of genetic and environmental involvement in antiphospholipid syndrome development

Experimental model		Implications/findings
Genetic	Animal studies	<ul style="list-style-type: none"><li>• Evidence for antigen-driven stimulation for pathogenic aPL production</li><li>• Complementary gene action of two independently segregating major dominant alleles produces disease characteristic</li><li>• Role for modifying alleles, e.g., Yaa</li><li>• Role for hormones in modifying genetic susceptibility</li></ul>
	Human family HLA studies	<ul style="list-style-type: none"><li>• Most consistent associations: HLA-DR4 and DRw53</li></ul>
	Human population HLA studies	<ul style="list-style-type: none"><li>• Others: DR7, DQw3, DQw7, A30, Cw3, B60</li><li>• Most consistent associations: HLA-DR4, DR7, DRw53, DQB1*0302</li><li>• Others: DRB1*04, DQB1*0301/4, DQB1*0604/5/6/7/8/9, DQA1*0102, DQA1*0301/2</li><li>• DRB1*09 in Japanese patients</li><li>• C4A/C4B null alleles in African-American patients</li></ul>
	Human non-HLA studies	<ul style="list-style-type: none"><li>• Several non-HLA genes associated with increased thrombosis [G20210 A prothrombin, ATIII, F5G1691 A FVL mutations; <math>\beta_2</math>GPI val247Ieu, F13A1 Factor XIII val34, Glycoprotein Ia/IIa, polymorphisms]</li></ul>

(continued)

**Table 2.1** (continued)

Experimental model		Implications/findings
Environmental	Infectious agents	<ul style="list-style-type: none"> <li>• Molecular mimicry (CMV, Adenovirus) <i>Haemophilus influenzae</i>, <i>Neisseria gonorrhoeae</i>, <i>Clostridium tetani</i></li> <li>• Selective destruction/activation of unique lymphocyte subsets</li> <li>• Cytokine release</li> <li>• Cryptic antigen exposure (necrosis/apoptosis—PS/<math>\beta_2</math>GPI complex formation)</li> </ul>
	Drug/vaccine/adjuvant (e.g., silicone, acrylamide)	<ul style="list-style-type: none"> <li>• Neoantigen formation</li> <li>• Altered antigen processing and presentation</li> </ul>
	Malignancies	<ul style="list-style-type: none"> <li>• Neoantigen formation (tumor, immunomodulatory therapy)</li> </ul>

*aPL* antiphospholipid antibodies, *ATIII* antithrombin III,  *$\beta_2$ GPI val247leu*  $\beta_2$ glycoprotein-I valine 247 polymorphism, *CMV* cytomegalovirus, *FVL* factor V leiden mutation, *HLA* human leukocyte antigen, *Yaa* Y-linked autoimmune accelerator

relative contributions of these many factors would certainly aid in the prevention of APS and management of these patients. Future studies will certainly address those critical questions.

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