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Antibodies to Serine Proteases in the Antiphospholipid Syndrome

Pojen P. Chen · Ian Giles

Abstract It is generally accepted that the major autoantigen for antiphospholipid antibodies (aPL) in the antiphospholipid syndrome (APS) is β_2-glycoprotein I (β_2GPI). However, a recent study has revealed that some aPL bind to certain conformational epitope(s) on β_2GPI shared by the homologous enzymatic domains of several serine proteases involved in hemostasis and fibrinolysis. Importantly, some serine protease–reactive aPL correspondingly hinder anticoagulant regulation and resolution of clots. These results extend several early findings of aPL binding to other coagulation factors and provide a new perspective about some aPL in terms of binding specificities and related functional properties in promoting thrombosis. Moreover, a recent immunological and pathological study of a panel of human IgG monoclonal aPL showed that aPL with strong binding to thrombin promote in vivo venous thrombosis and leukocyte adherence, suggesting that aPL reactivity with thrombin may be a good predictor for pathogenic potentials of aPL.

Keywords Thrombosis · Thrombin · Activated protein C · Plasmin · Hemostasis

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Introduction

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the persistent presence of antiphospholipid antibodies (aPL) and clinical features of vascular thrombosis and pregnancy morbidity [1–4]. The aPL include lupus anticoagulants (LA), as detected by their abilities to prolong certain in vitro phospholipid (PL)-restricted blood clotting tests, and anticardiolipin antibodies (aCL) [5, 6]. Accumulated studies show that aPL in patients with APS represent a heterogeneous group of antibodies that recognize various PL in the presence of protein cofactors [3]. The involved proteins include prothrombin (PT), β_2-glycoprotein I (β_2GPI), and activated protein C (APC) [7–9]. Antibodies directed against β_2GPI and its complexes with cardiolipin probably account for most of the aCL activity found in patients with APS [3], whereas anti-PT antibodies and anti-β_2GPI antibodies are responsible for most LA activity [10].

Importantly, in vivo studies have shown that aPL from patients promote fetal loss and thrombosis in animals, indicating that circulating aPL are directly involved in the clinical features of APS [11, 12]. To explain the pathogenic properties of aPL in APS, numerous different mechanisms have been proposed [3]. First, aPL may bind to protein C and APC, and functionally inhibit activation of protein C and the anticoagulant function of APC [9, 13–15]. Second, aPL may bind to platelets and functionally promote platelet activation [16, 17]. Third, aCL may interact with endothelial cells and functionally induce expression of adhesion molecules and monocyte adhesion, resulting in expression of tissue factor and a procoagulant state [18–21]. Fourth, aPL may activate the complement pathway, generating split products that lead to fetal loss and thrombosis [22, 23]. In addition, direct effects of aPL upon

monocytes, endometrial cells, and trophoblast cells have also been demonstrated [3].

The aPL-induced expression of tissue factor on endothelial cells and monocytes is particularly important because tissue factor is a major initiator of the coagulation cascade. Tissue factor binds and accelerates the activation of factor VII, and the activated factors VII (FVIIa) form complexes with tissue factor (TF-FVIIa), which, in turn, activate factors IX and, to a lesser degree, factor X (generating factors FIXa and FXa, respectively).

Subsequently, FIXa works with activated factor VIII to generate more FXa, and FXa with activated factor V (FVa) to convert prothrombin to thrombin, which then converts fibrinogen to a fibrin clot [24]. The coagulation cascade is subjected to three major feedback regulation mechanisms: 1) the tissue factor–pathway inhibitor inhibits TF-FVIIa complexes from activating FIX and FX; 2) antithrombin binds to thrombin, FIXa, FXa, and TF-FVIIa, and inactivates their enzyme activity; and 3) protein C is activated by the thrombin–thrombomodulin complex on endothelial cells surface, and then APC forms a complex with protein S on PL surfaces and proteolytically inactivates factors FVa and FVIIIa [24]. Congenital, heterozygous deficiency in either antithrombin or protein C increase the risk of thrombosis about five- to tenfold [24–26].

Certain aPL Bind to Thrombin and Hinder Inactivation of Thrombin by Antithrombin

Increasingly, evidence has emerged that identifies direct interactions between aPL and components of the coagulation cascade that may promote thrombosis. Around 1997, increasing attention was paid to anti-PT antibodies (aPT) in APS. In this context, Rao et al. [10] had previously shown that affinity purified IgG aPT bound to immobilized phosphatidylserine in the presence of Ca^{++} and prothrombin, suggesting that aPT crosslinks prothrombin molecules and thus increases the valence of interactions between prothrombin and phosphatidylserine. Subsequently, the same investigators showed that IgG purified from an LA-positive plasma sample (designated LA IgG; from a patient with hypoprothrombinemia) enhanced the binding of prothrombin to human umbilical vein endothelial cells (HUVEC) and increased thrombin generation on the surface of HUVEC [27].

Unfortunately, the latter studies used LA IgG (which might contain IgG against $β_2$GPI, prothrombin, PL, and complexes of these antigens) instead of purified aPT IgG. Thus, to test the hypothesis that aPT may concentrate prothrombin on cell surface PL and thus lead to a hypercoagulable state, a monoclonal IgG aPT (designated IS6) was derived from a patient with APS. Indeed, IS6 enhanced prothrombin binding to HUVEC and shortened plasma clotting time measured on HUVEC [28]. In addition, IS6 also induced tissue factor expression on endothelial cells, and promoted thrombosis in mice [29]. Therefore, it was concluded that IS6 was likely to promote thrombosis in the host patient by increasing prothrombin concentration on endothelial cells and inducing tissue factor expression on endothelial cells.

An additional way in which aPT may induce thrombosis is through crossreactivity with thrombin (a key effector enzyme in the coagulation cascade) derived from the zymogen prothrombin. Thrombin converts fibrinogen to fibrin, leading to the formation of fibrin clots. It also feedback-amplifies the coagulation cascade by activating factors V and VIII, which, in turn, enhance conversion of prothrombin to thrombin [24]. Once thrombin is generated in vivo, it is tightly regulated by antithrombin that binds to thrombin in the presence of heparin-like glycosaminoglycans on endothelial cell surface and inactivates the enzyme irreversibly [24, 30]. Therefore, it is conceivable that some aPT may bind to thrombin at a site where thrombin interacts with antithrombin and interfere with antithrombin inactivation of thrombin. Indeed, IS6 aPT/aPL was found to react with thrombin, and antibodies against thrombin were found in 10 of 13 patients with APS [31]. Moreover, on screening an additional panel of seven monoclonal IgG aCL/aPL derived from two patients with the APS, five of these antibodies (IS3, IS4, CL1, CL15, and CL24) were also found to bind to thrombin (Table 1) [31]. Importantly, CL24 at 11 μg/mL (equivalent to about 0.1% of plasma IgG) could reduce antithrombin inactivation of thrombin by about 30% (Table 1) [31]. These findings defined a novel anti-thrombin autoantibody in patients with APS and showed that such antibodies may interfere with negative feedback regulation of circulating thrombin and thus contribute to thrombosis. Of note, antithrombin binds to the active site of thrombin. Therefore, some antithrombin–interfering antithrombin antibodies may also hinder thrombin interaction with fibrinogen, resulting in anticoagulant effect. Further studies to address this issue are warranted.

Notably, CL24 promotes thrombosis in a pinch-induced thrombosis model in mice [32] and binds to thrombin with a relative K_d value of $1.7×10^{-6}$ (Table 1), which is at least tenfold better than relative K_d value of CL24 binding to prothrombin [31]. Therefore, CL24 is more specific for thrombin than prothrombin. Interestingly, thrombin converts fibrinogen to fibrin in formation of a stable clot during secondary hemostasis and also binds to and activates platelets in primary hemostasis. Consequently, CL24 may also activate platelets via unchecked thrombin, and thus account for some of the aforementioned effects on aPL on platelet activation [17].

Table 1 Summary of binding and functional properties of eight monoclonal IgG antiphospholipid antibodies derived from two patients with antiphospholipid syndrome

Antibodies	IS1	IS2	IS3	IS4	IS6	CL1	CL15	CL24
Antigens[a]								
CL/BS	+	+	+	+	+	+	+	+
Human β_2GPI	–	–	10^{-6}	+	+	+	–	+
Human thrombin	–	–	7×10^{-6}	+	+	+	8×10^{-6}	2×10^{-6}
Human APC	–	–	4×10^{-6}	+	+	+	2×10^{-6}	+
Human plasmin	–	–	3×10^{-7}	5×10^{-7}	2×10^{-7}	6×10^{-8}	1×10^{-7}	1×10^{-6}
Human tPA	–	–	3×10^{-7}	+	5×10^{-7}	4×10^{-7}	3×10^{-7} +	
Human FIXa	–	–	+	+	8×10^{-7}	+	2×10^{-6}	5×10^{-6}
Prothrombotic activities								
Thrombus[b]								
Size	–	3+	+	3+	2+	–	2+	2+
Duration	–	2+	2+	+	3+	–	+	3+
Functional activities[c]								
Inhibit thrombin inactivation	–	–	–	–	–	–	–	+
Inhibit APC activity	–	–	–	–	–	–	+	–
Inhibit plasmin activity	–	–	–	–	–	–	+	–
Inhibit tPA activity	–	–	–	–	–	+	+	–
Inhibit FIXa inactivation	–	–	–	–	+	–	+	+

[a] Binding to cardiolipin (CL) in the presence of bovine serum (BS) and β_2-glycoprotein I (β_2GPI) is compiled from Zhao et al. [28] and Zhu et al. [37]. Binding to thrombin, activated protein C (APC), plasmin, tissue plasminogen activator (tPA), and factor IXa (FIXa) are from Hwang et al. [31, 33], Yang et al. [34], Lu et al. [38], and Yang et al. [50••], and are given in +, –, or K_d (if known).

[b] For prothrombotic activities, the relative activities of all aCL within each category are given. Original and more quantitative data are in Vega-Ostertag et al. [29] and Pierangeli et al. [32].

[c] Functional activities are from Hwang et al. [31, 33], Yang et al. [34], Lu et al. [38], and Yang et al. [50••].

Certain aPL Bind to APC and Inhibit the Anticoagulant Function of APC

Thrombin is a serine protease, and the serine protease family contains several members involved in hemostasis and fibrinolysis; these include the anticoagulant APC, several activated coagulation factors (FVIIa, FIXa, and FXa), plus plasmin and tissue plasminogen activator (tPA) of the fibrinolytic system. The discovery of thrombin-reactive aPL raised a possibility that such antibodies may also react with other serine proteases in hemostasis and fibrinolysis. Because the enzymatic domain of APC is most homologous to that of thrombin (sharing a similarity of 50.5% at the protein level), investigators first analyzed six thrombin-reactive monoclonal aPL against APC, and found that all six bind to APC (Table 1) [33]. Importantly, when the APC-reactive aPL were examined for their effects on APC anticoagulant activity in plasma coagulation, CL15 at 25 µg/mL (equivalent to about 0.25% of plasma IgG) significantly inhibited the anticoagulant function of APC (Table 1) [33]. These data provide further support to the possible structural basis for the aforementioned inhibition of APC by aPL [14, 15]. Of note, CL15 also promotes thrombosis in the pinch-induced thrombosis model (Table 1) [32].

Certain aPL React with Plasmin and Reduce Fibrinolysis

Subsequently, these investigators studied IgG antibodies against plasmin. Again, all six thrombin-reactive aPL bind to plasmin (Table 1) [34]. In addition, a small study of the plasma samples from 25 patients with APS showed that seven (28%) of these patients had IgG antiplasmin antibodies, using the mean optical density plus three standard deviations (SD) of 20 normal controls as the cutoff [34]. Furthermore, functional analyses of the plasmin-reactive aPL revealed that CL15 could inhibit plasmin-mediated fibrinolysis (Table 1) [34]. This finding was consistent with a report that IgG from patients with APS impaired the fibrin dissolution with plasmin, and provided the potential structural basis for the observation [35].

Intriguingly, the aforementioned aPL bound to plasmin with the relative K_d values ranging from 1×10^{-6} to 6×10^{-8} M (Table 1) [34]. Of note, affinity-purified IgG anti-β_2GPI antibodies (from 5 patients with APS) bound to β_2GPI with the relative K_d values ranging from 3.4 to 7.2×10^{-6} M [36]. Taken together, these data showed that some IgG aPL in patients with APS bind to plasmin with higher affinities than those to β_2GPI, and suggested that

plasmin may be the primary autoantigen that drives such aPL in patients with APS. In support of this hypothesis, recent studies have shown that plasmin immunization in mice induces pathogenic aPL.

Certain aPL Bind to tPA and Decrease Plasmin Activation

Based on the high affinity of aPL with plasmin, these investigators switched their attention from thrombin to plasmin and searched for the relevant serine protease that was most homologous to plasmin, which led them to focus on tPA. As predicted, all six plasmin-reactive aPL were found to bind to tPA (Table 1) [37, 38]. Importantly, two tPA-reactive aCL (CL1 and CL15) could inhibit tPA activity in converting plasminogen to plasmin [38]. These findings were consistent with reports that anti-tPA antibodies were found in 15% of patients with APS and were inversely correlated with the plasma tPA activity in patients, and that anti-tPA IgG from two positive patients bound to the enzymatic domain of tPA [39].

Crossreactive aPL Bind to the Enzymatic Domains of Serine Proteases

Of the four serine protease that react with aPL, thrombin contains only an enzymatic domain, whereas the other three serine proteases contain additional domains, including the epidermal growth factor (EGF) domain (in APC and tPA), the kringle domain (in plasmin and tPA), and the fibronectin domain (in tPA). Therefore, it is most likely that the serine protease–reactive aPL bind to the enzymatic domains common to all these target serine protease. To prove this hypothesis experimentally, the CL15 monoclonal antibody (mAb) was used to perform a crossinhibition experiment with α-thrombin and tPA. It was shown that α-thrombin could inhibit CL15 from binding to tPA (that contains a fibronectin domain, an EGF domain, two kringle domains, and an enzymatic domain) [38]. Therefore, it was concluded that CL15 (and most likely other serine protease–reactive aPL) bind to the enzymatic domains of the target serine protease [38].

Certain aPL Recognize Conformational Epitopes Shared by the Homologous Enzymatic Domains of Several Serine Proteases and β_2GPI

It is intriguing to note that, of the six serine protease–reactive patient-derived IgG monoclonal aPL, five bind to human β_2GPI (Table 1), suggesting that some aPL in

patients with APS recognize certain antigenic epitopes shared by the homologous enzymatic domains of the aforementioned serine protease and β_2GPI. To test this hypothesis, four new IgG monoclonal aPL (including two screened against human β_2GPI, one against thrombin and one against protein C) were generated from two other patients with APS [40••]. Analyses of these monoclonal aPL showed that both IgG anti-β_2GPI mAbs (designated B1 and B2) bound to thrombin, APC, and plasmin [40••]. On the other hand, one anti-thrombin mAb (T1) and one anti-APC mAb (P1) also bound to β_2GPI [40••]. Moreover, the binding of the P1 mAb to human β_2GPI was inhibited by α-thrombin (the structurally simplest serine protease with only an enzymatic domain) [40••]. Furthermore, all four new monoclonal aPL displayed aCL activity, as assayed for binding to cardiolipin in the presence bovine serum that contained bovine β_2GPI [40••]. Taken together, these data demonstrated that certain aPL in patients with APS recognize conformational epitopes shared by β_2GPI and the enzymatic domain of several regulatory serine protease in hemostasis and fibrinolysis.

Plasmin Immunization in Mice Induce Pathogenic IgG aPL

Given that serine protease–reactive aPL bind plasmin more strongly (relative K_d values ranging from 1×10^{-6} to 6×10^{-8} M [34]) than the reported affinities of aPL to β_2GPI, it was suggested that plasmin may be the autoantigen that drives such aPL. To determine whether plasmin may drive some IgG aCL in patients with APS, mice were immunized with human plasmin in complete Freund's adjuvant and examined for the presence of IgG antiplasmin antibodies and IgG aCL. These experiments showed that plasmin immunization induced high titers of IgG antiplasmin antibodies in all mice, and that five of 10 (50%) immune sera also displayed aCL activity [41•].

Subsequently, eight mAb were isolated from these plasmin-immunized mice and studied. The results showed that three of eight antiplasmin mAb (designated B12, E9, and F10) displayed aCL activity [41•]. In addition, one of these mAb (E9) bound to thrombin, and one other mAb (C5) reacted with APC [41•]. These data showed that plasmin could drive some IgG aCL, and that some plasmin-driven antibodies could react with thrombin and APC, which are homologous to plasmin in their enzymatic domains.

To determine the LA activity of the plasmin-induced antibodies, all mAb were analyzed by the dilute Russell's viper venom time (dRVVT) test and the dRVVT-confirm test according to Exner et al. [42]. The results showed that the E9 mAb (with the strongest aCL activity) displayed LA activity, prolonging the clotting time in the in vitro PL-

Table 2 Summary of binding properties and biological effects of five monoclonal IgG derived from native IS4 monoclonal antiphospholipid antibody

Heavy chain	Light chain	CL binding[a]	β₂GPI binding[a]	Thrombin binding[a]	Thrombus size[b]	Leukocyte adherence[b]
IS4VH	IS4VL	Strong	Weak	Strong	16[c]	8[c]
IS4VHi&ii	IS4VL	None	None	None	2.6	1.7
IS4VHi&ii	B3VL	Strong	Weak	Strong	22[c]	6[c]
IS4VH	B3VL	Strong	Medium	None	2.7	3
IS4VH	UK4VL	Weak	None	None	6.5	3.5

[a] The relative binding of each heavy-chain variable region (VH)/light-chain variable region (VL) sequence combination to cardiolipin (CL), β₂-glycoprotein I (β₂GPI), and thrombin, and their biological effects on thrombus size and leukocyte adherence in vivo are from Giles et al. [45, 46••]. The identity of native heavy and light chains is clearly indicated. IS4VHi&ii contains two Arg to Ser replacements at positions 96 and 97

[b] Fold increase was calculated by dividing the median value of each group of animals/cells treated with monoclonal IgG by the median value of corresponding animals/cells treated with monoclonal control IgG, which lacks cardiolipin and thrombin binding

[c] Statistically significant differences are indicated

restricted coagulation test by 20%. When this mAb was subjected to the dRVVT confirmatory test, it remained positive, with a dRVVT/dRVVT-confirm ratio of 1.26 [41•].

Thereafter, the pathogenic effects of two antiplasmin mAb (E9 and C5) on pregnancy morbidity in mice were studied. These mAb were chosen because the E9 mAb displays both diagnostic aCL and LAC activity, whereas the C5 mAb binds to APC, suggesting that it may mimic the CL15 mAb in reducing activated protein C activity and promoting thrombosis [32]. The results showed that the fetal resorption rates in mice treated with E9 and C5 were 25.4 and 18.5%, respectively, compared with 11.2% in the control mice treated with normal mouse IgG [41•]. Combined, these data provide further evidence that plasmin may serve as a driving antigen for some pathogenic aPL.

Analyses of Recombinant Monoclonal aPL Reveal that Thrombin Binding Best Predicts Prothrombotic Potential of aPL

APS may occur either alone (ie, primary APS) or with other autoimmune diseases, such as systemic lupus erythematosus (SLE). About 30% to 40% of SLE patients have aPL, but only about one third of these patients with aPL and SLE will experience clinical manifestations of APS [3]. In addition, the prevalence of aPL among young, healthy control subjects has been shown to be 1.5% to 5% for both aCL and LA, which increases with age [43]. The question of whether individuals in both the SLE and general population with aPL but no APS require primary prophylaxis with aspirin remains controversial [44]. Unfortunately, current routine clinical aPL (aCL, anti-β₂GPI, and LA) assays do not allow accurate prediction of which patients with aPL will develop APS. Therefore, it is critically important to study whether other binding reactivities of aPL better predict the pathogenic potentials of the detected aPL.

To address this question, Giles et al. [45] used an in vitro expression system to produce a panel of human monoclonal IgG aPL that were all based on IS4 (Table 1). These recombinant mAb were engineered to have small differences in sequence between their antigen-binding sites (known as the variable regions), which led to large changes in their binding properties. Variant forms of whole IgG were produced by site-directed mutagenesis in IS4 variable region heavy-chain (VH) and variable region light-chain sequence (VL) exchange. IS4VL was exchanged with closely related human 2a2-derived VL sequences, from B3 (a human antinucleosome antibody) and UK4 (a β₂GPI-independent aPL). In particular, altering one or more of four arginine residues in IS4 VH and/or the paired VL had dramatic effects on binding different antigens [45]. Recently, they selected five of these mAbs for further study on the basis of their different patterns of strength and selectivity of binding to different PL (including cardiolipin) and β₂GPI and correlated in vivo and in vitro biological properties
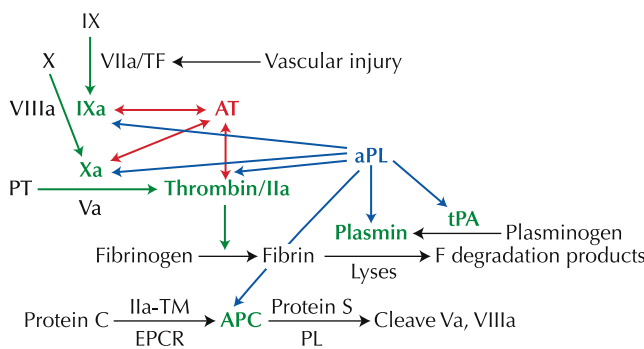
Fig. 1 The target serine proteases in coagulation that are recognized and affected by antiphospholipid antibodies (aPL). *Green* indicates proteases; *red* indicates antithrombin (*AT*) and its irreversible interaction; and *blue* indicates aPL and its target identification. *APC* activated protein C, *EPCR* endothelial protein C receptor, *F* fibrin, *PL* phospholipid, *PT* prothrombin, *TF* tissue factor, *TM* thrombomodulin, *tPA* tissue plasminogen activator

with their binding to cardiolipin, β2GPI, and thrombin (Table 2). Intraperitoneal injection of these IgG into mice subjected to a femoral vein pinch stimulus showed that only those IgG (native IS4 and IS4VHi&ii/B3VL) which showed strong binding to thrombin promoted in vivo venous thrombosis and leukocyte adherence compared with control IgG [46••]. In contrast, recombinant IS4VH/B3VL (which differs from IS4VHi&ii/B3VL by only two arginine to serine mutations) displayed strong cardiolipin and moderate β2GPI binding with negligible binding to thrombin did not significantly increase thrombus size in treated mice compared with control human IgG. Therefore, it is not just the strength of binding to cardiolipin that controls the ability to enhance thrombus formation in this panel of monoclonal aPL but selectivity of binding is also important. In particular, binding to thrombin was most closely associated with the ability of the monoclonal aPL to cause thrombosis.

Certain aPL Bind to FIXa and Hinder the Antithrombin Inactivation of FIXa

In addition to thrombin, antithrombin also binds to FIXa and inactivates FIXa that is a key mediator of tissue factor–induced coagulation (Fig. 1). Patients with high FIX levels are associated with increased risk of venous and arterial thromboembolism [47]. Therefore, FIXa is tightly regulated by antithrombin in normal hemostasis. Inherited heterozygous deficiency in antithrombin increases the risk of thromboembolism by about fivefold and women with the deficiency are at particularly high risk of abortion during pregnancy [24, 48]. Hence, it is conceivable that interference of antithrombin inactivation of FIXa may promote both thrombosis and pregnancy morbidity in patients with the APS.

Interestingly, FIXa also belongs to the serine protease family and its enzymatic domain is homologous to those of thrombin [49]. Specially, at the protein level, the catalytic domains of FIXa and thrombin share a similarity of 52.7%, suggesting that some aPL in APS may bind to FIXa and interpose antithrombin inactivation of FIXa. Indeed, 10 of 12 patient-derived monoclonal IgG aPL described previously with anti–serine protease reactivity were found to react with FIXa. Furthermore, IgG anti-FIXa antibodies in patients with APS were significantly higher in 11 of 38 (28.9%) APS patients tested, compared with 30 healthy controls using the mean + 3 SD of 30 normal controls as the cutoff [50••]. Importantly, four of the 10 FIXa-reactive monoclonal aPL (including the B2 mAb generated against β2GPI) significantly hindered antithrombin inactivation of FIXa [50••]. More importantly, IgG from two positive plasma samples were found

to interfere with antithrombin inactivation of FIXa [50••]. Because FIXa is an upstream procoagulant factor, impaired antithrombin regulation of FIXa may contribute more toward thrombosis than the dysregulation of the downstream thrombin.

Conclusions

It is generally accepted that heterogeneous aPL bind to cardiolipin in the presence of bovine serum and/or certain plasma proteins, including β2GPI and prothrombin. Recent accumulated studies have shown that some aPL also bind to the homologous enzymatic domains of several serine protease in hemostasis and fibrinolysis, including thrombin, APC, plasmin, tPA, and FIXa (Fig. 1). Of these aPL–serine protease interactions, some hinder inactivation of the target serine protease (eg, FIXa and thrombin) by antithrombin, whereas others directly inhibit the enzyme activities of the target proteases in fibrinolysis, plasmin activation, and inactivation of cofactors FVIIIa and FVa (Fig. 1). Furthermore, anti-thrombin antibody reactivity has been shown to most closely predict thrombogenicity of monoclonal aPL in animal models. Consequently, such aPL could promote thrombus formation at multiple points in coagulation, and from both ends—by promoting clot formation and inhibiting resolution of clots. In addition, these findings reveal a novel class of autoantibodies that recognize several members of an enzyme family instead of a single autoantigen (eg, DNA for anti-DNA antibodies). Surprisingly, certain aPL recognize conformation epitopes shared by β2GPI and the homologous enzymatic domains of the reactive serine protease. Of note, the prothrombotic IS2 mAb in Table 1 does not react with any serine protease, but has been shown to induce expression of adhesion molecules and promote in vivo leukocyte adhesion to endothelial cells in microcirculation, suggesting that IS2 may promote thrombosis by activating endothelial cells [32]. Further research, however, is required to clarify the exact diagnostic utility and pathogenic role of these anti–serine protease antibodies.

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•• Of major importance

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