Clin Exp Immunol 2004; **136**:393–401 doi:10.1111/j.1365-2249.2004.02497.x

**REVIEW**

# Antiphospholipid syndrome: multiple mechanisms

C. G. MACKWORTH-YOUNG *Kennedy Institute of Rheumatology, Imperial College, London, UK*

*(Accepted for publication 2 April 2004)*

**Keywords** antiphospholipid antibody beta 2 glycoprotein I lupus anticoagulant haemostasis thrombosis

## INTRODUCTION

It is 20 years since the use of a radioimmunoassay enabled an association to be described between antibodies to the anionic phospholipid cardiolipin and a set of clinical features [1]. These features, which included thrombosis – both venous and arterial – and recurrent foetal loss, subsequently became known as the antiphospholipid syndrome (APS) [2–4]. They had previously been shown to be associated with positive Venereal Disease Reference laboratory (VDRL) and lupus anticoagulant (LA) tests, both of which detect antiphospholipid antibodies [5–8]. However, the radioimmunoassay, and the enzyme-linked assays (ELISA) derived from it [9], are considerably more sensitive, and their use enabled the clinical syndrome to be clearly defined. Many additional clinical features have been described, such as neurological disorders, migraine, livedo reticularis and thrombocytopenia [10–12]. Some of these remain controversial, and may be features of associated connective tissue diseases such as systemic lupus erythematosus (SLE), with which APS frequently coexists. They are not all directly related to thrombosis. APS can occur as an isolated, primary condition [13–15]. It has emerged as the most common cause of acquired thrombophilia, and is a major cause of pregnancy morbidity. Because thrombosis in APS can occur at almost any site, it is a condition that is seen in almost all medical specialities.

Central to our understanding of antiphospholipid syndrome has been the demonstration that the autoantibodies in the condition probably play a direct role in pathogenesis. Strong evidence for this has come from murine models. Passive transfer of antiphospholipid antibodies (aPL) to normal mice can generally [16–19], though not always [20], produce features resembling human APS, notably impairment of foetal development. Similar results have been obtained by immunizing mice with aPL [21]. In a murine model of venous injury the addition of aPL results in increased local clot formation [22].

Another critical discovery was that antibodies to phospholipid appear to represent part of a large family of autoantibodies, many of which recognize phospholipid-binding plasma proteins either alone, or in combination with phospholipid. The first of these proteins to be recognized was beta-2-glycoprotein I ($\beta_2$GPI) [23,24]. This molecule has number of physiological functions, which include a regulatory role within coagulation pathways. Prothrombin is another important protein recognized by sera of patients with APS. Others include annexin V, high and low molecular weight kininogens, protein C and protein S (see Table 1).

In general, aPL found in patients with antiphospholipid syndrome require the presence of $\beta_2$GPI for binding to cardiolipin. Indeed, most of these antibodies appear to bind to $\beta_2$GPI itself (reviewed in [25]). This may be through an epitope on native, unbound $\beta_2$GPI. However, antibodies to $\beta_2$GPI are generally of low affinity [25], and it has been proposed that the binding of anionic phospholipid, such as cardiolipin, to $\beta_2$GPI enhances this affinity: this enhancement may occur through the revealing of a cryptic epitope on the $\beta_2$GPI molecule [26]. Furthermore, there is evidence for the binding of aPL to different domains of $\beta_2$GPI, although binding to domain I may predominate (reviewed in [26]). These conflicting results may simply reflect the existence of different subpopulations of aPL within the sera of individuals with APS. What is clear is that, for the majority of so-called antiphospholipid antibodies that are detected by ELISA in the serum of patients with APS, anionic phospholipid such as cardiolipin is not the antigen to which those antibodies are actually binding. By contrast, anticardiolipin antibodies that arise as a consequence of infection – and are not normally associated with the syndrome – do not tend to be dependent on $\beta_2$GPI for binding to phospholipid [25,27–29].

The picture that has emerged is of an autoimmune condition in which antibodies are expressed in the serum against a variety of phospholipid and protein antigens. Not only are different specificities found in different patients [30], but there may be great heterogeneity within an individual [31,32]. How is it that some of these antibodies appear to cause clinical disease?

## HAEMOSTASIS

The central feature of APS is thrombosis. Several theories have emerged to explain the association between raised serum anticardiolipin levels and vascular pathology. Many of these have built on the intimate association between phospholipid-binding proteins and antibodies found in the syndrome. The regulation of haemostasis is complex, and occurs at a number of levels. These include platelet and endothelial cell (EC) activity, as well as the

Correspondence: Dr C. G. Mackworth-Young, Department of Rheumatology, Charing Cross Hospital, London W6 8RF, UK.

**Table 1.** Antigen specificities of antibodies that have been detected in the serum of patients with antiphospholipid syndrome.

| Antigen/antigen complex | References |
|---|---|
| Phospholipids | |
| Anionic phospholipids (e.g. cardiolipin, phosphatidyl serine) | [1] |
| Zwitterionic phospholipids (e.g. phosphatidyl ethanolamine) | [73] |
| Components and regulators of coagulation and fibrinolysis | |
| Beta 2 glycoprotein I | [23,24] |
| Prothrombin | [44,46–52] |
| Thrombin | [45,54] |
| Anti-thrombin III/thrombin complex | [55] |
| Tissue factor/factor VIIa complex | [36,37] |
| High and low molecular weight kininogen | [73] |
| Prekallikrein | [55] |
| Sulphatides | [74] |
| Protein Z/protein Z protease inhibitor system | [75] |
| Protein C | [30,57] |
| Protein S | [30] |
| Thrombomodulin | [61,62] |
| Tissue plasminogen activator | [67] |
| Annexin V | [70] |
| Lipoproteins | |
| Oxidized low density lipoprotein | [106,107] |
| High density lipoprotein | [111] |
| Apolipoprotein A-I | [111] |

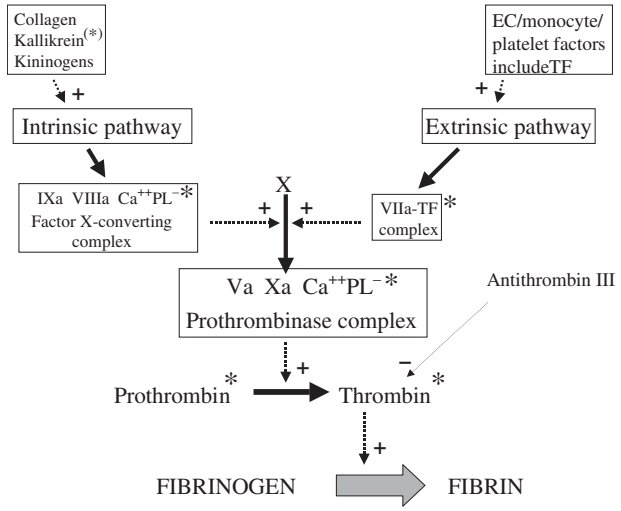


**Fig. 1.** A summary of the coagulation pathways. Only a few important stages are shown. Fibrinogen is converted to fibrin by the action of thrombin, which is generated by the action of the prothrombinase complex (containing factor Xa) on prothrombin. Two cascades can lead to the activation of factor X: the intrinsic pathway, promoted by a variety of factors, including collagen (exposed in the vessel wall), kallikrein (derived from prekallikrein*), and kininogens; and the extrinsic pathway, initiated by factors derived from endothelial cells, monocytes or platelets, including tissue factor (TF). The whole process should be envisaged as taking place at the surface of one of these cells, which, when activated, is a source of anionic phospholipid. Solid arrows indicate pathways. Dashed arrows indicate promotion (+) or inhibition (–) of a pathway. Asterisks indicate potential sites of action of antibodies in APS.

coagulation and fibrinolytic cascades and their regulatory proteins. Intriguingly, there is now considerable evidence for the ability of phospholipid-associated antibodies to interfere with all of these. This may be a reflection of the different populations of such antibodies; or it may be due to the fact that the molecules that these antibodies recognize – such as $\beta_2$GPI – play important regulatory roles at different levels.

*Coagulation*

Before the description of the solid-phase assay for anticardiolipin antibodies, the most sensitive way to detect such antibodies was the LA test [33,34]. This is now understood to be an *in vitro* phenomenon, in which there is a prolongation of the kaolin clotting time (or similar measures of the intrinsic coagulation pathway) that cannot be corrected by the addition of normal plasma (i.e. a source of clotting factors). It probably arises because of an effect of aPL on the prothrombinase complex or on prothrombin itself [34,35]. This does not reflect the overall effect *in vivo*, where the balance of effects on the clotting cascade is in the direction of increased coagulation.

Many points in the coagulation cascade are phospholipid-dependent, and it was postulated early on that these might be potential sites of action of aPL. Inhibition of action at some of these would be expected to have an anticoagulant effect, while at others it would be predicted to promote clotting. An example of the latter is the complex of factors IXa and VIIIa (the so-called 'tenase' complex; see Fig. 1). While it is possible that such mechanisms contribute to the actions of aPL, it now seems more likely that the disturbances of coagulation seen in APS are primarily due to antibodies that recognize phospholipid-binding proteins. These include components of the extrinsic and intrinsic coagulation pathways, respectively the complex of tissue factor and factor VIIa [33–38], and prekallikrein [39].

Tissue factor acts as a major initiator of the extrinsic coagulation pathway, being a cofactor for the activation of factor VII. Tissue factor activity is up-regulated in patients with APS, probably due to increased expression by endothelial cells and monocytes (see below) [40–43].

The end result of both the extrinsic and intrinsic pathways is the conversion of prothrombin to thrombin (Fig. 1). This has received increasing attention in the context of APS, with antibodies to both molecules being well described, in particular prothrombin [44]. While anti-prothrombin antibodies may be responsible for the *in vitro* phenomenon of the lupus anticoagulant, *in vivo* their presence may be associated with a tendency to increased coagulation. This is an area of controversy, with some groups reporting an association between the presence of anti-prothrombin antibodies and clinical or haematological features of APS [45–47], and other groups finding no association [48,49,44]. This may be explained by different detection methods, and by the presence of antibodies with different epitope specificities [50], resulting in different functional properties [51,52]. One group has shown that antibodies that recognize the complex of prothrombin and phosphatidyl serine (an anionic phospholipid) are distinct from those that bind prothrombin alone, and are well correlated with features of APS [53].

Most patients with APS who have anti-prothrombin antibodies also have antibodies to thrombin [45,54]. These may have a procoagulant effect by protecting thrombin from inactivation by

the regulatory protein anti-thrombin III. Similarly, antibodies have been described to the complex of anti-thrombin III and thrombin [55].

*Protein C pathway*

Another area that has received considerable attention is the protein C pathway (Fig. 2). This is an important feedback mechanism for controlling thrombin formation, and thus has an anti-thrombotic effect. Protein C is a vitamin-K-dependent serine proteinase, a heterozygous deficiency of which results in recurrent thrombotic disease [56]. Activated protein C combines with another cofactor, protein S, in the presence of phospholipid to catalyse the degradation of factors Va and VIIIa of the coagulation pathway. For this to take place, protein C is first converted to its active form by thrombin in the presence of thrombomodulin, an EC-derived cofactor.

Protein C is a potential target for antibodies in APS. aPL derived from patients' serum have been shown *in vitro* to impair the degradation of factor V by protein C [30,57,58]. This effect has been shown to be phospholipid dependent [59], and may be due to an inhibitory effect on the protein C/protein S complex [60].

The activation of protein C by thrombomodulin could be another target for antibodies in APS. IgG from patients with the lupus anticoagulant have been shown to inhibit the activity of thrombomodulin [61]. Its ability to activate protein C is enhanced by phospholipid; this enhancement was found to be neutralized by an IgM antibody with lupus anticoagulant activity [62].

*Fibrinolysis*

Reduced activitation of protein C could also have an effect on the fibrinolytic system. Fibrin, which is the end product of the coagulation cascade, is degraded by plasmin, which itself is generated as a result of a complex cascade (Fig. 3). It is derived from plasminogen through the action of tissue plasminogen activator (tPA). An important modulator of this process is plasminogen activator inhibitor (PAI), which is another endothelial-derived protein. Activated protein C has been shown to decrease the PAI activity of cultured EC, and may therefore act indirectly as a promoter of fibrinolysis [63,64]. Thus the binding of antibodies to protein C could impair clot degradation.

The data on the role of tPA and PAI in APS are conflicting. Some groups have shown a raised level of PAI antigen or activity in APS compared with control patients [65], while others have failed to show any difference [66]. One group has demonstrated the presence of antibodies to tPA in patients with APS [67]. They showed in two cases that these antibodies bind to the catalytic domain of the molecule, suggesting that they could reduce tPA activity, and thus reduce fibrinolysis.

Our own data showed no difference between patients with APS and SLE controls in respect of tPA levels and PAI activity; nor was there any significant correlation between these and levels of anticardiolipin antibodies as measured by ELISA or the lupus anticoagulant [68]. However, we did find a strong positive correlation between levels of von Willebrand factor and IgG anticardiolipin levels, and a strong negative correlation between von Willebrand factor levels and the platelet count. These findings may be explained by an increase in the release of von Willebrand factor from EC. This could lead to enhanced platelet adhesion to vessel walls, resulting in an increased tendency to thrombosis, and a reduction in circulating platelet number.

Kallikrein is another promoter of the conversion of plasminogen to plasmin. Reduced prekallikrein activity has been shown in a group of patients with the lupus anticoagulant, suggesting a further mechanism for impaired fibrinolysis [39].

*Annexin V*

Another protein that regulates the clotting cascade is annexin V. It has anticoagulant activity, interfering with the binding of procoagulant factors to procoagulant membranes [69]. Notably, it is expressed by endothelial cells in the placenta and the placental precursor, the trophoblast, where it is thought to function as a natural anticoagulant. It probably does so by crystallizing over anionic phospholipids, thus inhibiting them from participating in coagulation reactions. Sera from about half of patients with serum aPL contain antibodies that bind to annexin V [70]. It has been shown that these antibodies can disrupt the annexin shield, allowing increased generation of thrombin [71]. Another group found that IgG anti-annexin antibodies only bind to free annexin, and not when it is associated with phospholipid [72]. Either way, anti-annexin V activity could represent part of the mechanism of increased foetal loss in APS (see below).

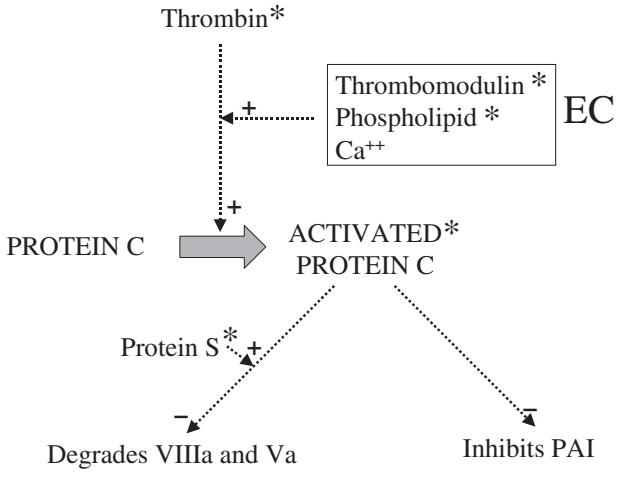


**Fig. 2.** A summary of the protein C pathway. Endothelial cells (EC) are a source of thrombomodulin, and anionic phospholipid. Solid arrows indicate pathways. Dashed arrows indicate promotion (+) or inhibition (–) of a pathway. Asterisks indicate potential sites of action of antibodies in APS.

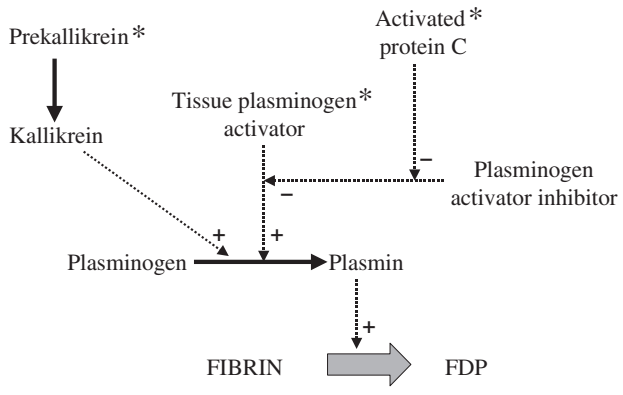


**Fig. 3.** A summary of the fibrinolytic pathway. Solid arrows indicate pathways. Dashed arrows indicate promotion (+) or inhibition (–) of a pathway. Asterisks indicate potential sites of action of antibodies in APS. FDP: fibrin degradation products.

*Other regulatory proteins*

In the wealth of literature on antibody specificity in APS, a number of other antigens have been described that are recognized by sera of patients with the condition, binding of which could alter haemostasis. For instance, antibodies have been identified that bind to phospholipid in association with high or low molecular weight kininogens [73]. Antibodies have also been described that bind to sulphatides [74]. These are sulphated glycosphingolipids that are expressed on the surface of erythrocytes, leucocytes and platelets, and that interact with several adhesion molecules involved in haemostasis. Another group has shown an impairment in patients with APS of the protein Z/protein Z protease inhibitor system, another regulatory mechanism that inhibits factor Xa. They also showed that aPL from these patients inhibit this mechanism *in vitro* [75].

*Platelets*

Platelets play a central role in coagulation: they provide intrinsic coagulation proteins, and they also form procoagulant membrane surfaces, characterized by the exposure of anionic phospholipids. There is now a growing body of evidence to suggest that some of the pathogenic activity of antibodies in APS may occur through effects on platelets.

Increased platelet activation can be been demonstrated in patients with APS [76], and there is evidence that aPL can directly promote this. Activation of platelets has been shown in an *in vitro* model, using polyclonal and monoclonal aPL from patients with APS [77]. It has also been shown in a different model that anti$\beta_2$GPI antibodies can promote platelet binding to vascular subendothelium [78]. Another group has found that complexes of aPL and $\beta_2$GPI can increase the production from platelets of thromboxane A2, an eicosanoid that promotes vasoconstriction and clotting [79]. This appears to occur through an increase in the activity of platelet cyclic AMP [80].

Recently there has been more direct evidence for the role of anti$\beta_2$GPI antibodies in promoting platelet adhesion and aggregation. Using an *in vitro* flow system, de Groot and colleagues [81] have shown that dimerized $\beta_2$GPI (which mimics the effects of $\beta_2$GPI–anti$\beta_2$GPI complexes) can increase adhesion of platelets to collagen, and their aggregation. They have further demonstrated, by coimmunoprecipitation, that this activity is probably mediated by the apolipoprotein E receptor 2′, which is a member of the low density lipoprotein (LDL) receptor family [81]. Since many types of cell express members of this receptor family on their surface, such a mechanism could mediate the activation of other cells in APS.

However, not all groups have confirmed the ability of aPL or anti$\beta_2$GPI antibodies to activate platelets [82]. The picture is complicated by the presence of specific antiplatelet antibodies in the serum of patients with APS and associated connective tissue diseases.

There has been much controversy as to whether thrombocytopenia is a manifestation of APS. If it occurs it is generally mild, and does not usually lead to problems with bleeding. What is clear is that the administration of aPL to experimental animals generally results in a lowering of the platelet count [17,19]. The mechanism is uncertain. It may be due to platelet consumption, or may result from the presence of antibodies to platelet glycoproteins [83,84].

*Endothelial cells*

In the study of APS pathogenesis, the area that has received perhaps the greatest attention in recent years has been the endothelial cell. In its normal state, the endothelial lining of blood vessels plays a central part in homeostasis, helping to maintain blood fluidity via a number of mediators that inhibit coagulation. However, certain stimuli can alter the phenotype of EC, allowing them to act as a surface that promotes coagulation. There has been accumulating evidence that aPL may have a direct effect on these cells, helping to promote the switch to the pro-coagulant phenotype. This state parallels the 'pro-adhesive' or pro-inflammatory phenotype.

A relatively early observation was that aPL may interfere with the release from endothelial cells of prostacyclin [85]. This is an eicosanoid that has actions broadly opposed to those of thromboxane. It was suggested at the time that this action of aPL might occur through an effect on cell surface phospholipid. Although the finding was controversial [86,87], it was soon recognized that the sera of patients with APS frequently contain antibodies that bind to the surface of endothelial cells [88]. However, there did not appear to be a close relationship between antiendothelial cell and antiphospholipid binding. For instance, antiendothelial activity could only be poorly absorbed by preincubation with phospholipid micelles [88–91]. This is consistent with the resting state of the endothelial cell membrane, in which anionic phospholipids are not exposed on the outside. However, even when endothelial cells are activated, the binding of aPL-positive sera is not necessarily enhanced.

These findings can be explained by the observation that $\beta_2$GPI may be the chief molecule involved in the binding of aPL-positive sera to endothelial surfaces [92,93]. Sera that contained anticardiolipin and anti$\beta_2$GPI antibodies were found to have reduced antiendothelial cell activity when the EC had been cultured in serum-free medium. The antiendothelial cell activity was restored when purified human $\beta_2$GPI was added. It was postulated that $\beta_2$GPI in the culture medium adhered to EC, and was recognized by anti$\beta_2$GPI antibodies in the test sera; when serum-free medium was used, this source of $\beta_2$GPI was not available.

These observations are supported by the finding that $\beta_2$GPI can bind to EC *in vitro*, and can be demonstrated on trophoblast EC *in vivo* (reviewed in [88]). The binding of $\beta_2$GPI to EC appears to occur through the cationic, phospholipid-binding site in the fifth domain of the molecule.

If $\beta_2$GPI is indeed present on the surface of EC *in vivo*, this could suggest a potential pathway for the action of antibodies in APS. The pro-inflammatory, procoagulant phenotype can be induced in EC *in vitro* by incubation with anti$\beta_2$GPI antibodies [42,92–97]. This has been shown with both monoclonal and polyclonal antibodies. Characteristics of this change in phenotype include the up-regulation of adhesion molecules such as E-selectin, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM –1), and the increased secretion of pro-inflammatory cytokines, including interleukin 1 (IL-1) and interleukin 6 (IL-6). It can also result in the expression of tissue factor. This activator of the extrinsic coagulation pathway can be expressed by a variety of cells, including EC, in response to inflammatory cytokines (e.g. IL-1$\beta$ and tumour necrosis factor $\alpha$) or endotoxin. Tissue factor production by EC can be up-regulated by anti$\beta_2$GPI antibodies *in vitro* [42]; and raised levels of tissue factor have been demonstrated in patients with APS [41]. Anti-$\beta_2$GPI antibodies can induce the production of other

procoagulant proteins by EC [97]. In this way, antibodies that bind to $\beta_2$GPI – and are generally present in the sera of patients with APS – could promote a hypercoagulable state.

These effects of anti$\beta_2$GPI antibodies on EC appear to be mediated by nuclear factor kappa B [98]. Annexin II may also play a role [99].

*Other cells*

Monocytes can contribute directly to coagulation under physiological conditions, and this also appears to be the case in APS. Serum from patients with APS can induce a procoagulant, tissue factor-like activity in monocytes *in vitro* [40,42]; and monocytes taken from such patients also show an enhanced production of tissue factor, which correlates with a history of thrombosis [100]. This may be due to binding of $\beta_2$GPI at the cell surface [77]: there is a correlation between the expression on monocytes of $\beta_2$GPI and of tissue factor in patients with APS [43].

Although serum antibodies in APS can be found to bind to surface molecules on a variety of cells, it is unlikely that this involves anionic phospholipid, which is not exposed on the surface of intact cell membranes. aPL do, however, bind to apoptotic cells, where the membrane is disrupted [101]. This may provide a stimulus for sustaining or enhancing the autoimmune process, but it is uncertain whether it contributes directly to the pathogenic action of the antibodies.

## VESSEL TONE

Another way in which endothelial cells could mediate the effects of aPL is through their control of vessel tone. EC release a number of factors that can alter the tone of the vessel wall, including vasoconstrictors such as endothelin and platelet activating factor, and vasodilators such as nitric oxide and prostacyclin. As noted above, aPL may reduce the release of prostacyclin from EC [85]. It has also been shown that levels of the vasoconstrictor endothelin I peptide are raised in patients with thrombosis due to APS [102]. A perturbation of the balance of vessel wall regulation towards vasoconstriction could explain the increased frequency of Raynaud's phenomenon and livedo reticularis seen in APS, and could also contribute to thrombosis.

## ATHEROSCLEROSIS

Over the last few years it has become increasingly recognized that many autoimmune conditions carry with them an increased risk for the development or progression of atherosclerosis. In the case of APS there are no prospectve studies to confirm this; and the picture is confused by the close association with SLE, in which there may be many risk factors for the enhancement of atheroma formation, including the inflammatory disease and corticosteroid treatment [103]. Nevertheless, it does appear that APS is an independent risk factor for an increased development or progression of atherosclerosis [104].

There are a number of potential mechanisms through which antibodies found in APS could promote atherogenesis (reviewed in [105]). Perhaps the most compelling is through binding to $\beta_2$GPI. This molecule (also known as apolipoprotein H) is present in various lipoprotein fractions, including oxidized LDL. It is the uptake of oxidized LDL by macrophages in the blood vessel wall to form foam cells that is thought to initiate atheroma plaque formation. Antibodies to oxidized LDL are well described in APS, although there has been some controversy as to whether they cross-react with $\beta_2$GPI [106,107]. One group has isolated the ligand on oxidized LDL to which $\beta_2$GPI binds. They have shown in an *in vitro* model that liposomes containing this ligand are taken up by macrophages, and that this process is enhanced by $\beta_2$GPI and anti-$\beta_2$GPI antibodies [108]. Furthermore, LDL-receptor-deficient mice immunized with $\beta_2$GPI show accelerated atherosclerosis [109]. If macrophages are activated by the uptake of oxidized LDL, this could result in damage to endothelial cells, and subsequent promotion of thrombosis [110].

Another possible mechanism is interference with the protective effect of high-density lipoprotein (HDL) and apolipoprotein A-I (apo A-I). HDL helps to prevent the oxidation of LDL, while apo A-I stabilizes paraoxonase, an antioxidant enzyme within the HDL particle. Patients with APS have a high frequency of antibodies to HDL and apo A-I, a large percentage of which cross-react with cardiolipin [111].

At this stage the link between specific antibodies and atherogenesis in APS is less strong than for thrombosis, although clearly the two processes are related [112].

## FOETAL LOSS

It was long thought that miscarriage in APS could largely be explained by impaired foetal blood supply caused by placental thrombosis and infarction. Placental infarcts have been described in cases of foetal loss due to APS (reviewed in [113]). Any of the potential mechanisms for increased coagulation outlined above could play a role, notably antiannexin V activity. However, placental infarction is not always present, and it is thought likely that other mechanisms are equally or even more important [114,115].

It is known that the spiral arteries of the placenta show abnormal development in APS [116]. This could be due to an effect on endothelial function, as outlined above. However, it has also been shown that purified aPL can bind specifically to placental antigens [117], providing a potential mechanism for nonthrombotic placental damage and impaired foetal blood supply. aPL and antibodies to $\beta_2$GPI have also been found to modify trophoblast proliferation and differentiation [118,119]. aPL may bind directly to trophoblast cell membranes through exposed anionic phospholipid and adhered $\beta_2$GPI: this may result in altered gonadotrophin secretion [119]. One group has shown a direct effect of aPL on embryonic implantation in a murine model [18]. Using elegant embryo transfer experiments, they have demonstrated that defects in both the embryo and the mother contribute to pregnancy failure [120].

There is emerging evidence that the complement pathway may also mediate foetal damage in APS. Salmon and colleagues have shown in a mouse model of APS that activation of the C3 component of complement is needed for foetal loss to occur [121]; in the same model they have also demonstrated a requirement for complement C5 as a mediator of foetal injury [122]. It has been suggested that local complement activation could be a mechanism for damage to tissues such as vascular endothelium and the trophoblast [121]. This would fit with the observation that local complement inhibition appears to be a requirement for normal murine pregnancy [123]. A drawback to such animal experiments is the uncertainty that remains about how relevant murine models of APS are to the human disease, particularly those that involve the transfer of heterologous antibodies, which could result in immune complex formation and complement activation.

However, a number of findings in humans do support these initial conclusions: inflammatory changes have been described in placentae from women with APS [124,125]; elevated levels of complement split products have been demonstrated in the serum of patients with cerebral thrombotic events due to APS [126]; and the complement-fixing ability of aPL has been shown to be associated with foetal loss (and indeed thrombosis) [127].

## NEUROLOGICAL DAMAGE

A wide variety of neurological disorders have been reported in APS [3,10]. Many of these, such as stroke and mononeuritis, can be explained by thromboembolism. Even here there remains some controversy about the precise relationship between aPL and such events. For instance, a large American study has recently found that the presence of aPL in patients with ischaemic stroke does not predict an increased risk for subsequent vascular occlusive events [128]. Unfortunately animal models are unhelpful here: among the many that have been reported, thrombosis outside the placenta is not a characteristic feature, and nor are specific neurological abnormalities.

There are other neurological features seen in human APS that are less readily explained by thrombosis: examples include cerebral dysfunction (for instance poor concentration or forgetfulness) and multiple sclerosis-like lesions. Although such features could be due to microthrombi, there is increasing evidence that aPL can in fact cause direct damage to neurones. Antibodies to the anionic phospholipid phosphatidylserine have been shown to bind directly to neuronal tissue [129], as have antibodies to $\beta_2$GPI [130]. Subsequent experiments have indicated that there may be functional effects of such antibodies on neuronal cells. For instance, it has been demostrated that aPL can cause depolarization of synaptoneurosomes in an *in vitro* preparation, suggesting that these antibodies could disrupt neuronal function by a direct action on nerve terminals [131]. Shoenfeld and colleagues performed *in vivo* experiments, administering purified IgG from patients with APS into the cerebral ventricles of normal mice: they found impairment of learning and memory, again suggesting a direct antineuronal effect [132].

## SECOND HIT PHENOMENON

There seem, therefore, to be multiple ways in which aPL and related antibodies could cause pathology. Yet many individuals with high IgG aPL levels do not develop features of APS. This may be due to the particular pattern of antibody specificities in their serum. However, it appears that for many people other factors may be needed for the expression of APS, i.e. a 'second hit' is required. Thus pregnancy (a hypercoagulable state) can lead to the development of thrombosis in patients with raised aPL levels [133,134]. Other promoters of thrombosis in APS include the presence of factor V Leiden [135], vascular injury and infection [136].

## CONCLUSIONS

In the last 20 years a wealth of information has emerged about the potential action of autoantibodies in APS. It seems very likely that at least some of these antibodies are directly pathogenic. A large number of mechanisms have been proposed, most of which involve disturbance of coagulation pathways, their regulatory systems, and the cells that control them. It is improbable that they all have a significant role *in vivo*: much of the evidence comes from *in vitro* experiments; and in some areas it depends on single reports. Although many of these putative mechanisms are closely related, it may well be that their multiplicity reflects the wide heterogeneity of antibody specificities within individuals and between different people with the condition. It may indeed be that thrombosis represents the final common pathway of many disease processes, each of which is dependent on its own particular autoantibody profile. The same could apply to foetal loss and neuronal disease. One of the chief aims of research over the next few years will be to establish which of these many mechanisms are truly central to the disease process, so that specific therapies can be designed for this unusual and often devastating condition.

## REFERENCES


1 Harris EN, Gharavi AE, Boey ML *et al.* Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet 1983; **ii**:1211–4.

2 Alarcon-Segovia D, Deleze M, Oria CV *et al.* Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. Medicine (Baltimore) 1989; **68**:353–65.

3 Cuadrado MJ, Hughes GRV Hughes (antiphospholipid) syndrome. clinical features. Rheum Dis Clin North Am 2001; **27**:507–24.

4 Lockshin MD, Druzin ML, Goei S *et al.* Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. N Eng J Med 1985; **313**:152–6.

5 Laurell B-B, Nilsson IM. Hypergammaglobulinaemia, circulating anticoagulant, and biologic false positive Wassermann reaction. J Laboratory Clin Med 1957; **49**:694–707.

6 Bowie EJW, Thompson JH Jr, Pascuzzi CA, Owen CA Jr. Thrombosis in systemic lupus erythematosus despite circulating anticoagulants. J Laboratory Clin Med 1963; **62**:416–30.

7 Firkin BG, Howard MA, Radford N. Possible relationship between lupus inhibitor and recurrent abortion in young women. Lancet 1980; **ii**:366.

8 Gastineau DA, Kazmier FJ, Nichols WL, Bowie EJW. Lupus anticoagulants. an analysis of the clinical and laboratory features of 219 cases. Am J Hematol 1985; **19**:265–75.

9 Loizou S, McCrea JD, Rudge AC *et al.* Measurement of anticardiolipin antibodies by enzyme linked immunosorbent assay: standardisation and quantitation of results. Clin Exp Immunol 1985; **62**:738–44.

10 Navarrete MG, Brey RL, Levine SR. Cerebral disease in the antiphospholipid syndrome. In: Khamashta MA, ed. Hughes Syndrome, Antiphospholipid Syndrome. London: Springer Verlag 2000:43–58.

11 Gibson GE, Su WP, Pittelkow MR. Antiphospholipid syndrome and the skin. J Am Acad Dermatol 1997; **36**:970–82.

12 Cuadrado MJ, Mujic F, Munoz E *et al.* Thrombocytopenia in the antiphospholipid syndrome. Ann Rheum Dis 1997; **56**:194–6.

13 Mackworth-Young CG, David J, Loizou S, Walport MJ. Primary antiphospholipid syndrome: features of patients with raised anticardiolipin antibodies and no other disorder. Ann Rheum Dis 1987; **26**:94.

14 Alarcon-Segovia D, Sanchez-Guerrero J. Primary antiphospholipid syndrome. J Rheumatol 1989; **42**:1309–11.

15 Asherson RA, Khamashta MA, Ordi-Ros J *et al.* The 'primary' antiphospholipid syndrome: major clinical and serological features. Medicine (Baltimore) 1989; **68**:366–74.

16 Branch DW, Dudley DJ, Mitchell MD *et al.* Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss. Am J Obstet Gynecol 1990; **163**:210–6.

17 Blank M, Cohen J, Toder V, Shoenfeld Y. Induction of antiphospholipid syndrome by passive transfer of anti-cardiolipin antibodies. Proc Natl Acad Sci USA 1991; **88**:3069–73.

18 Sthoeger ZM, Mozes E, Tartakovsky B. Anti-cardiolipin antibodies induce pregnancy failure by impairing embryonic implantation. Proc Natl Acad Sci USA 1993; **90**:6464–7.

19 Mason AN, Mageed RA, Mackworth-Young CG. The effects of a human IgM monoclonal anticardiolipin antibody on pregnancy in a transgenic mouse model. Lupus 2001; **10**:289–94.

20 Silver RM, Smith LA, Edwin SS *e al.* Variable effects on murine pregnancy of immunoglobulin G fractions from women with antiphospholipid antibodies. Am J Obstet Gynecol 1997; **177**:229–33.

21 Bakimer R, Fishman P, Blank M *et al.* Induction of primary antiphospholipid syndrome in mice by immunisation with a human monoclonal anticardiolipin antibody (H-3). J Clin Invest 1992; **89**:1558–63.

22 Pierangeli SS, Harris EN. Antiphospholipid antibodies in an *in vivo* thrombosis model in mice. Lupus 1994; **3**:247–51.

23 Galli M, Comfurius P, Maassen C *et al.* Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. Lancet 1990; **335**:1544–7.

24 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 USA 1990; **87**:4120–4.

25 Roubey RAS. Immunology of the antiphospholipid syndrome. Arthritis Rheum 1996; **39**:1444–54.

26 Giles IP, Isenberg DA, Latchman DS, Rahman A. How do antiphospholipid antibodies bind $\beta$2-glycoprotein I? Arthritis Rheum 2003; **48**:2111–21.

27 Hunt JE, McNeil HP, Morgan GJ *et al.* A phospholipid-beta 2-glycoprotein I complex is an antigen for anticardiolipin antibodies occurring in autoimmue disease but not with infection. Lupus 1992; **1**:75–81.

28 Forastiero RR, Martinuzzo ME, Kordich LC *et al.* Reactivity to beta 2 glycoprotein I clearly differentiates anticardiolipin antibodies from antiphospholipid syndrome and syphilis. Thrombosis Haemostasis 1996; **75**:717–20.

29 Gharavi AE, Sammaritano LR, Wen J *et al.* Characteristics of HIV and chlorpromazine induced antiphospholipid antibodies: effect of $\beta$2-glycoprotein I on binding to phospholipid. J Rheumatol 1984; **21**:94–9.

30 Oosting JD, Derksen RH, Bobbink IW *et al.* Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C, or protein S. an explanation for their pathogenic mechanism? Blood 1993; **81**:2618–25.

31 Lieby P, Soley A, Levallois H *et al.* The clonal analysis of anticardiolipin antibodies in a single patient with primary antiphospholipid syndrome reveals an extreme antibody heterogeneity. Blood 2001; **97**:3820–8.

32 Shoenfeld Y, Krause I, Kvapil F *et al.* Prevalence and clinical correlations of antibodies against six beta2-glycoprotein-I-related peptides in the antiphospholipid sydrome. J Clin Immunol 2003; **23**:377–83.

33 Lee SL, Saunders M, Kahny HMA. A disorder of blood coagulation in systemic lupus erythematosus. J Clin Invest 1955; **34**:1814–22.

34 Derksen RH, Hasselar P, Blokzijl L *et al.* Coagulation screen is more specific than the anticardiolipin aantibody ELISA in defining a thrombotic subset of lupus patients. Ann Rheum Dis 1998; **47**:364–71.

35 Bevers EM, Galli M, Barbui T, Comfurius P, Zwaal RF. Lupus anticoagulant IgGs (LA) are not directed to phosholipids only, but to a complex of lipid-bound human prothrombin. Thrombosis Haemostasis 1991; **66**:629–32.

36 Skjonsberg OH, Wisloff F, Godal HC. Inhibition of the tissue factor-factor VII mediated activation of coagulation factor X by a monoclonal antibody expressing lupus anticoagulant activity. Thrombosis Res 1990; **58**:349–52.

37 Bidot CJ, Horstman LL, Huisheng H *et al.* Factor VII/VIIa: a new antigen in the antiphospholipid antibody syndrome. Br J Haematol 2003; **120**:618–26.

38 Angles-Cano E, Guillin M-C. Antiphospholipid antibodies and the coagulation cascade. Rheum Dis Clin North Am 2001; **27**:573–86.

39 Sanfelippo MJ, Drayna CJ. Prekallikrein inhibition assocoated with the lupus anticoagulant. Am J Clin Pathol 1982; **77**:275–9.

40 Kornberg A, Blank M, Kaufman S, Shoenfeld Y. Induction of tissue factor-like activity in monocytes by anti-cardiolipin antibodies. J Immunol 1994; **153**:1328–32.

41 Atsumi T, Khamashta MA, Amengual O, Hughes GRV. Up.-regulated tissue factor expression in antiphospholipid syndrome. Thrombosis Haemostasis 1997; **77**:222–3.

42 Amengual O, Atsuni T, Khamashta MA, Hughes GRV. The role of the tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid syndrome. Thrombosis Haemostasis 1998; **79**:276–81.

43 Conti F, Sorice M, Circella A *et al.* Beta-2-glycoprotein I expression on monocytes is increased in antiphospholipid antibody syndrome and correlates with tissue factor expression. Clin Exp Immunol 2003; **132**:509–16.

44 Amengual O, Atsumi T, Koike T. Specificities, properties and clinical significance of antiprothrombin antibodies Arthritis Rheum. 2003; **48**:886–95.

45 Sorice M, Pittone V, Circella A *et al.* Anti-thrombin but not 'pure' anti-cardiolipin antibodies are associated with the clinical features of the antiphospholipid antibody syndrome. Thrombosis Haemostasis 1998; **80**:16–22.

46 Nojima J, Kuratsune H, Suehisa E *et al.* Anti-prothrombin antibodies combined with lupus anti-coagulant activity is an essential risk factor for venous thromboembolism in patients with systemic lupus erythematosus. Br J Heamatol 2001; **114**:647–54.

47 Nojima J, Kuratsune H, Suehisa E *et al.* Acquired protein C resistance is associated with the co-existence of anti-prothrombin antibodies and lupus anticoagulant activity in patients with systemic lupus erythematosus. Br J Haematol 2002; **118**:577–83.

48 Pengo V, Biasiolo A, Brocco T *et al.* Autoantibodies to phospholipid-binding proteins in patients with thrombosis and phospholipid-reactive antibodies. Thrombosis Haemostasis 1996; **75**:721–4.

49 Forastiero R, Martinuzzo M, Cerrato G *et al.* Relationship of anti-beta2-glycoprotein I and anti prothrombin antibodies to thrombosis and pregnancy loss in patients with antiphospholipid antibodies. Thrombosis Haemostasis 1997; **78**:1008–114.

50 Horbach D, van Oort E, Derksen R, de Groot P. The contribution of anti-prothrombin antibodies to lupus anticoagulant activity – discrimination between functional and non-functional anti-prothrombin antibodies. Thrombosis Haemostasis 1998; **79**:790–5.

51 Galli M, Beretta G, Daldossi M *et al.* Different anticoagulant and immunological properties of anti-prothrombin antibodies in patients with antiphospholipid antibodies. Thrombosis Haemostasis 1997; **77**:486–91.

52 Horbach D, van Oort E, Donders R *et al.* Lupus anticoagulant is the strongest risk factor for both venous and arterial thrombosis in patients with systemic lupus erythematosus: comparison between different assays for the detection of antiphospholipid antibodies. Thrombosis Haemostasis 1996; **76**:916–24.

53 Atsumi T, Ieko M, Bertolaccini ML *et al.* Association of autoantibodies against the phosphatidylserine-prothrombin complex with manifestations of the antiphospholipid syndrome and with the presence of lupus anticoagulant. Arthritis Rheum 2000; **43**:1982–93.

54 Hwang K-K, Grossman JM, Visvanathan S *et al.* Identification of antithrombin antibodies in the antiphospholipid syndrome that interfere with the inactivation of thrombin by antithrombin. J Immunol 2001; **167**:7192–8.

55 Shibata S, Harpel PC, Gharavi AE *et al.* Autoantibodies to heparin from patients with antiphospholipid antibody syndrome inhibit formation of antithrombin III-thrombin complexes. Blood 1994; **83**:2532–40.

56 Gonias SI, Pizzo SV. The biochemistry of haemostasis. Clin Laboratory Haematol 1986; **8**:281–305.

57 Marciniak E, Romond EH. Impaired catalytic function of activated protein C. a new manifestation of lupus anticoagulant. Blood 1989; **74**:2426–32.

58 Borrell EM, Sala N, de Castellarnau C *et al.* Immunoglobulin fractions isolated from patients with antiphospholipid antibodies prevent the inactivation of factor Va by activated protein C on human endothelial cells. Thrombosis Haemostasis 1992; **68**:268–72.

59 Smirnov MD, Triplett DT, Comp PC *et al.* On the role of phosphtidylethanolamine in the inhibition of activated protein C activity by antiphospholipid antibodies. J Clin Invest 1995; **95**:309–16.

60 Malia RG, Kirchens S, Greaves M, Preston FE. Inhibition of activated protein C and its cofactor protein S by antiphospholipid antibodies. Br J Haematol 1990; **76**:101–7.

61 Comp PC, DeBault LE, Esmon NL, Esmon CT. Human thrombomodulin is inhibited by IgG from two patients with non-specific anticoagulants. Blood 1983; **62**:299a.

62 Freyssinet JM, Wiesel M-L, Gauchy J *et al.* An IgM lupus anticoagulant that neutralizes the enhancing effect of phospholipid on purified endothelial thrombomodulin activity. A mechanism for thrombosis. Thrombosis Haemostasis 1986; **55**:309–13.

63 Sakata Y, Curriden S, Lawrence D *et al.* Activated protein C stimulates the fibrinolytic activity of cultured endothelial cells and decreases anti-activator activity. Proc Natl Acad Sci USA 1985; **82**:1121–5.

64 Van Hinsberg VWM, Bertina RM, van Wijngaarden A *et al.* Activated protein C decreases plasminogen activator-inhibitor activity in endothelial cell-conditioned medium. Blood 1986; **65**:444–51.

65 Violi F, Ferro D, Valesini G *et al.* Tissue plasminogen activator inhibitor in patients with systemic lupus erythematosus and thrombosis. Br Med J 1990; **300**:1099–102.

66 Keeling DM, Campbell SJ, Mackie IJ *et al.* The fibrinolytic response to venous occlusion and the natural anticoagulants in patients with antiphospholipid antibodies both with and without systemic lupus erythematosus. Br J Haematol 1991; **77**:354–9.

67 Cugno M, Cabibbe M, Galli M *et al.* Antibodies to tissue-type plasminogen activator (tPA) in patients with antiphospholipid syndrome: evidence of interaction between the antibodies and the catalytic domain of tPA in two patients. Blood 2004; **00**:000–000. in press

68 Mackworth-Young CG, Andreotti F, Harmer IJ *et al.* Endothelium-derived haemostatic factors and the antiphospholipid syndrome. Br J Rheumatol 1995; **34**:201–6.

69 Andree HA, Stuart MC, Hermens WT *et al.* Clustering of lipid-bound annexin V may explain its anticoagulant effect. J Biol Chem 1992; **267**:17907–12.

70 Matsuda J, Saitoh N, Gohchi K *et al.* Anti-annexin V antibody in systemic lupus erythematosus patients with lupus anticoagulant and/or anticardiolipin antibody. Am J Hematol 1994; **47**:56–8.

71 Rand JH, Wu XX, Quinn AS *et al.* Human monoclonal antiphospholipid antibodies disrupt the annexin A5 anticoagulant crystal shield on phospholipid bilayers: evidence from atomic force microscopy and functional assay. Am J Pathol 2003; **163**:1193–200.

72 Arai T, Matsubayashi H, Sugi T *et al.* Anti-annexin A5 antibodies in reproductive failures in relation to antiphospholipid antibodies and phosphatidylserine. Am J Reprod Immunol 2003; **50**:202–8.

73 Sugi T, McIntyre JA. Autoantibodies to phosphatidylethanolamine (PE) recognize a kininogen-PE complex Blood. 1995; 86:3083–9.

74 Merten M, Motamedy S, Ramamurthy S *et al.* Sulfatides: targets for anti-phospholipid antibodies. Circulation 2003; **108**:2082–7.

75 Forastiero RR, Martinuzzo ME, Lu L, Broze GJ. Autoimmune antiphospholipid antibodies impair the inhibition of activated factor X by protein Z/protein Z-dependent protease inhibitor. J Thrombosis Haemostasis 2003; **1**:1764–70.

76 Joseph JE, Harrison P, Mackie IJ *et al.* Increased circulating platelet-leucocyte complexes and platelet activation in patients with antiphospholipid syndrome, systemic lupus erythematosus and rheumatoid arthritis. Br J Haematol 2001; **115**:451–9.

77 Reverter JC, Tassies D, Font J *et al.* Effects of human monoclonal anticardiolipin antibodies on platelet function and on tissue factor expression on monocytes. Arthritis Rheum 1998; **41**:1420–7.

78 Font J, Esinosa G, Tassies D *et al.* Effects of beta2-glycoprotein I and monoclonal anticardiolipin antibodies in platelet interaction with subendothelium under flow conditions. Arthritis Rheum 2002; **46**:3283–9.

79 Robbins DL, Leung S, Miller-Blair DJ *et al.* Effect of anticardiolipin/beta2-glycoprotein I complexes on production of thromboxane A2 by platelets from patients with antiphospholipid syndrome. J Rheumatol 1998; **25**:51–6.

80 Opara R, Robbins DL, Ziboh VA, Cyclic AMP agonists inhibit antiphospholipid/beta2-glycoprotein I induced synthesis of human platelet thromboxane A2 in vitro. J Rheumatol 2003; **30**:55–9.

81 Lutters BCH, Derksen RHWM, Tekelenburg WL *et al.* Dimers of $\beta$2-glycoprotein I increase platelet deposition to collagen via interaction with phospholipids and the apolipoprotein E receptor 2′. J Biol Chem 2003; **278**:33831–8.

82 Reverter JC, Tassies D. Mechanisms of thrombosis in the antiphospholipid syndrome: binding to platelets. In: Hughes Syndrome, Antiphospholipid Syndrome. Khamashta, MA, ed. Springer Verlag, 2000:pp 290–8.

83 Galli M, Daldossi M, Barbui T. Anti-glycoprotein IIb/IX and IIb/IIIa antibodies in patients with antiphospholipid antibodies. Thrombosis Haemostasis 1994; **71**:571–5.

84 Godeau B, Piette J-C, Fromont P *et al.* Specific antiplatelet glycoprotein autoantibodies are associated with the thrombocytopenia of primary antiphospholipid syndrome. Br J Haematol 1997; **98**:873–9.

85 Carreras LO, Defreyn G, Machin SJ *et al.* Arterial thrombosis, intrauterine death and 'lupus' anticoagulant: detection of immunoglobulin interfering with prostacyclin formation. Lancet 1981; **i**:244–6.

86 Rustin MHA, Bull HA, Machin SJ *et al.* Effects of the lupus anticoagulant in patients with systemic lupus erythematosus in endothelial cell prostacyclin release and procoagulant activity. J Invest Dermatol 1988; **90**:744–8.

87 Coade SB, van Haaren E, Loizou S *et al.* Endothelial prostacyclin relaese in systemic lupus erythematosus. Thrombosis Haemostasis 1989; **61**:97–100.

88 Riboldi P, Gerosa M, Raschi E *et al.* Endothelium as a target for antiphospholipid antibodies. Immunobiology 2003; **207**:29–36.

89 Rosenbaum J, Pottinger BE, Woo P *et al.* Measurement and characterisation of circulating anti-endothelial cell IgG in connective tissue diseases. Clin Exp Immunol 1988; **72**:450–6.

90 Vismara A, Meroni PL, Tincani A *et al.* Relationship between anticardiolipin and anti-endothelial cell antibodies in systemic lupus erythematosus. Clin Exp Immunol 1988; **74**:247–53.

91 Hassalaar P, Derksen RHWM, Blokzijl L, de Groot PG. Cross reactivity of antibodies directed against cardiolipin, DNA, endothelial cells and blood platelets. Thrombosis Haemostasis 1990; **63**:169–73.

92 Del Papa N, Guidali L, Spatola L *et al.* Relationship between antiphospholipid and anti-endothelial cell antibodies III. $\beta$2 glycoprotein I mediates the antibody binding to endothelial membranes and induces the expression of adhesion molecules. Clin Exp Rheumatol 1995; **13**:179–85.

93 Simantov R, LaSala JM, Lo SK *et al.* Activation of cultured vascular endothelial cells by antiphospholipid antibodies. J Clin Invest 1995; **96**:2211–9.

94 Gharavi AE, Pierangeli SS, Colden-Stanfield M *et al.* GDKV-induced antiphospholipid antibodies enhance thrombosis and activate endothelial cells in vivo and in vitro. J. Immunol 1999; **163**:2922–7.

95 Pierangeli SS, Colden-Stanfield M, Liu JH *et al.* Antiphospholipid antibodies from antiphospholipid syndrome patients activate endothelial cells in vitro and in vivo. Circulation 1999; **99**:1997–2002.

96 George J, Blank Y, Levy P *et al.* Differential effects of anti-beta 2 glycoprotein I antibodies on endothelial cells and on the manifestations of experimental antiphospholipid syndrome. Circulation 1998; **97**:900–6.

97 Meroni PL, Raschi E, Camera M *et al.* Endothelial activation by aPL. a potential pathogenetic mechanism for the clinical manifestations of the syndrome. J Autoimmun 2000; **15**:237–40.

98 Dunoyer-Geindre S, de Moerloose P. Galve-de Rochemonteix B *et al.* NFκB is an essential intermediate in the activation of endothelial cells by anti-$\beta$2-glycoprotein I antibodies. Thrombosis Haemostasis 2002; **88**:851–7.

99 Ma K, Simantov R, Jing-Chuan Z *et al.* High affinity binding of $\beta$2-glycoprotein I to human endothelial cells is mediated by annexin II. J Biol Chem 2000; **275**:15541–8.

100 Dobado-Berrios PM, Lopez-Pedrera C, Velasco F *et al.* Increased levels of tissue factor mRNA in mononuclear blood cells of patients with primary antiphospholipid syndrome. Thrombosis Haemostasis 1999; **82**:1578–82.

101 Price BE, Jauch J, Shia MA *et al.* Anti-phospholipid antibodies bind to apoptotic, but not viable, thymocytes in a $\beta$2-glycoprotein I-dependent manner. J Immunol 1996; **157**:2201–8.

102 Atsumi T, Khamashta MA, Haworth RS *et al.* Arterial disease and thrombosis in the antiphospholipid syndrome: a pathogenic role for endothelin I. Arthritis Rheum 1998; **41**:800–7.

103 Petri M, Perez-Gutthann S, Spence D, Hochberg MC. Risk factors for coronary artery disease in patients with systemic lupus erythematosus. Am J Med 1992; **93**:513–9.

104 MacGregor AJ, Dhillon VB, Binder A *et al.* Fasting lipids and anticardiolipin antibodies as risk factors for vascular disease in systemic lupus erythematosus. Ann Rhem Dis 1992; **38**:529–34.

105 Matsuura E, Kobayashi K, Kasahara J *et al.* Anti-$\beta$2-glycoprotein I autoantibodies and atherosclerosis. Intern Rev Immunol 2002; **21**:51–66.

106 Vaarala O, Alfthan M, Jauhiainen M. *et al.* Crossreaction between antibodies to oxidised low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. Lancet 2003; **341**:923–5.

107 Tinahones FJ, Cuadrado MJ, Khamashta MA *et al.* Lack of cross-reaction between antibodies to $\beta$2-glycoprotein I and oxidised low-density lipoprotein in patients with antiphospholipid syndrome. Br J Rheumatol 1998; **37**:746–9.

108 Kobayashi K, Matsuura E, Liu Q *et al.* A specific ligand for $\beta$2-glycoprotein I mediates autoantibody-dependent uptake of oxidised low density lipoprotein by macrophages. J Lipid Res 2001; **42**:697–709.

109 George J, Harats D, Gilburd B *et al.* Induction of early atherosclerosis in LDL-receptor-deficient mice immunised with $\beta$2-glycoprotein I. Circulation 1988; **98**:1108–15.

110 Ames PRJ. Antiphospholipid antibodies, thrombosis and atherosclerosis in systemic lupus erythematosus: a unifying 'membrane stress syndrome' hypothesis. Lupus 1994; **3**:371–7.

111 Delgado Alves J, Kumar S, Isenberg DA. Cross-reactivity between anti-cardiolipin, anti-high-density lipiprotein and anti-apolipoprotein A-I IgG antibodies in patients with systemic lupus erythematosus and primary antiphospholipid syndrome. Rheumatology 2003; **42**:893–9.

112 Sherer Y, Shoenfeld Y. Antiphospholipid antibodies: are they pro-atherogenic or an epiphenomenon of atherosclerosis? Immunobiology 2003; **207**:13–6.

113 Mackworth-Young CG, Loizou S, Walport MJ. Antiphospholipid antibodies and disease. Quart J Med 1989; **72**:767–77.

114 Stone S, Khamashta MA, Poston L. Placentation, antiphospholipid syndrome and pregnancy outcome. Lupus 2001; **10**:67–74.

115 Gharavi AE, Pierangeli SS, Levy RA, Harris EN. Mechanisms of pregnancy loss in antiphospholipid syndrome. Clin Obstet Gynecol 2001; **44**:11–9.

116 Parke AL. Placental pathology in antiphospholipid antibody syndrome. In: Khamashta MA, ed. Hughes Syndrome, Antiphospholipid Syndrome. London: Springer Verlag, 2000:281–9.

117 Donohoe S, Kingdom JCP, Mackie IJ. Affinity purified human antiphospholipid antibodies bind normal term placenta. Lupus 1999; **8**:525–31.

118 Chamley LW, Duncalf AM, Mitchell MD *et al.* Action of anticardiolipin and antibodies to beta 2 glycoprotein I on trophoblast proliferation as a mechanism for fetal death. Lancet 1998; **352**:1037–8.

119 Di Simone N, Meroni PL, Del Papa N *et al.* Antiphospholipid antibodies affect trophoblast gonadotrophin secretion and invasiveness by binding directly and through adhered beta 2 glycoprotein I. Arthritis Rheum 2000; **43**:140–53.

120 Tartakovski B, Bermas BL, Sthoeger Z *et al.* Defective maternal–fetal interaction in a murine autoimmune model. Human Reprod 1996; **11**:2408–11.

121 Holers VM, Girardi G, Mo L *et al.* Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. J Exp Med 2002; **195**:211–20.

122 Girardi G, Berman J, Redecha P *et al.* Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. J Clin Invest 2003; **112**:1644–54.

123 Xu C, Mao D, Holers VM *et al.* A critical role for the murine complement receptor Crry in fetomaternal tolerance. Science 2000; **287**:498–501.

124 Out HJ, Kooijman CD, Bruinse HW, Derksen RH. Histopathological findings in placentae from patients with intra-uterine fetal death and antiphospholipid antibodies. Eur J Obstet Gynecol Reprod Biol 1991; **41**:179–86.

125 Magid MS, Kaplan C, Sammaritano LR *et al.* Placental pathology in systemic lupus erythematosus: a prospective study. Am J Obstet Gynecol 1998; **179**:226–34.

126 Davis WD, Brey RL. Antiphospholipid antibodies and complement activation in patients with cerebral ischaemia. Clin Exp Immunol 1992; **10**:455–60.

127 Munakata Y, Saito T, Seino J *et al.* Detection of complement-fixing antiphospholipid antibodies in association with thrombosis. Thrombosis Haemostasis 2000; **83**:728–31.

128 Levine SR, Brey RL, Tilley BC *et al.* Antiphospholipid antibodies and subsequent thrombo-occlusive events in patients with ischemic stroke. J Am Med Assoc 2004; **291**:576–84.

129 Kent M, Vogt E, Rote NS. Monoclonal antiphosphatidylserine antibodies react directly with feline and murine central nervous system. J Rheumatol 1997; **24**:1725–33.

130 Caronti B, Calderaro C, Alessandri C *et al.* Serum anti-beta2-glycoprotein I antibodies from patients with antiphospholipid antibody syndrome bind central nervous system cells. J Autoimmun 1998; **11**:425–9.

131 Chapman J, Cohen-Armon M, Shoenfeld Y, Korczyn AD. Antiphospholipid antibodies permeabilize and depolarize brain synaptoneurosomes. Lupus 1999; **8**:127–33.

132 Shoenfeld Y, Nahum A, Korczyn AD *et al.* Neuronal-binding antibodies from patients with antiphospholipid syndrome induce cognitive deficits following intrathecal passive transfer. Lupus 2003; **12**:436–42.

133 Lima F, Khamashta MA, Buchanan NMM *et al.* A study of sixty pregnancies with the antiphospholipid syndrome. Clin Exp Rheumatol 1996; **14**:131–6.

134 Ringrose DK. Anaesthesia and antiphospholipid syndrome – a review of 20 obstetric patients. Int J Obstet Anaesth 1997; **6**:107–11.

135 Brenner B, Vulfons SL, Lanir N, Nahir M. Coexistence of familial antiphospholipid syndrome and factor V Leiden: impact on thrombotic diathesis. Br J Haematol 1996; **94**:166–7.

136 Rosendaal F. Thrombosis in the young: epidemiology and risk factors. A focus on venous thrombosis. Thrombosis Haemostasis 1997; **78**:1–6.