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Antiphospholipid antibodies in obstetrics: new complexities and sites of action

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The antiphospholipid syndrome, the cause of which remains unknown, is characterized by severe pregnancy complications. Fetal losses have been attributed to thrombosis of the uteroplacental vasculature and placental infarction. Polyclonal and monoclonal antiphospholipid antibodies seem able to recognize a 'plasma cofactor' on the endothelial and trophoblast cell surfaces and to affect cell function, inducing a procoagulant state. Although thrombosis is observed frequently in the decidua and placentas of patients with antiphospholipid antibodies, this observation was not universal, nor present in a sufficient degree to account for the pregnancy loss associated with this syndrome. Recent observations have suggested that antiphospholipid antibodies decreased placental hormone production and trophoblast intercellular fusion and invasion, suggesting that many of the obstetric complications observed in the syndrome may be due to antiphospholipid antibody-induced trophoblast dysfunction. However, the complex antigens on the trophoblast surfaces are still to be characterized and correlated with clinical manifestation. It is clear that successful pregnancies with the syndrome are more likely to occur after maternal treatment. Although prednisone may still be needed to treat manifestations associated with autoimmune disorders, the use of heparin, together with low-dose aspirin, has replaced prednisone for treatment of pregnant women. Maternal treatment and careful monitoring of fetal well-being are mandatory in the management of these high-risk pregnancies.

Key words: antiphospholipid syndrome/fetal loss/phospholipid antibodies/placental thrombosis/trophoblast invasiveness

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Historical notes

The occurrence of thromboses in patients with systemic lupus erythematosus (SLE) was first reported in 1963 (Bowie *et al*., 1963). Later, Hughes developed sensitive immunoassays for the detection of antibodies to cardiolipin (aCL) and recognized that the peripheral vascular manifestations of SLE were associated with these antibodies: the author proposed that a syndrome could occur in SLE, and it was initially referred to as anticardiolipin syndrome (Hughes *et al*., 1986).

Since some patients had clinical manifestations (recurrent fetal loss, arterial occlusions, repeated venous thrombosis, haemolytic anaemia and thrombocytopenia) associated with high titres of antiphospholipid antibodies (aPL), but had no clinical nor serological evidence of SLE, some authors (Alarcon-Segovia and Sanchez-Guerrero, 1989) reported these patients as having primary antiphospholipid syndrome (APS).

In the literature, different studies were performed to clarify the role of aPL in women with a history of poor pregnancy outcome. Investigators presumed an association between first-trimester pregnancy losses and aPL, because these antibodies were observed in the serum of women who had previously experienced pregnancy losses (Maier and Parke, 1989). Recently, APS was found to occur in one-third of patients with recurrent pregnancy loss (Drakeley *et al*., 1998), and a prevalence rate of 42% has previously been reported (Unander *et al*., 1987).

The reason that some individuals form aPL is unknown, but the relationship between HLA class II alleles and other autoantibodies makes it probable that class II alleles also predispose to aPL formation (Christiansen *et al*., 1998). Although the results are not in complete agreement, the bulk of the evidence points at HLA-DR4, DR7 and DQ7 as primary candidates for being HLA risk factors for the development of aPL.

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Whether aPL cause miscarriage in recurrent miscarriage patients is still unclear. Published data (Christiansen *et al*., 1998) showed no correlation between the severity of the disease (the number of previous miscarriages) and the prevalence of aPL. However, it became clear that these antibodies were also present in women who had a normal term delivery. For example, among 552 French blood donors, IgG aCL were found in 6.5% and IgM aCL in 9.4% (Vila *et al*., 1994). aPL can also occur in certain infectious diseases (McNeil *et al*., 1991) and drug reactions (Zarrabi *et al*., 1979; Canoso and de Oliveira, 1988), but only those that occur in patients with autoimmune disease seem related to the clinical syndrome.

Recently, it has been stressed that in addition to recurrent pregnancy loss, other reproductive processes such as endometriosis, unexplained infertility and in-vitro fertilization (IVF) failure may be affected by aPL abnormalities (Gleicher *et al*., 1993). The possible role for aPL in failure of nidation after IVF/embryo transfer is matter of controversy in recent literature. Some authors support that circulating aPL may be responsible for implantation failure in IVF (Geva *et al*., 1994). However, other opinion cast doubt on these observations (Bronson, 1995; Balash *et al*., 1996) and interestingly Gleicher has recently contradicted his own preliminary hypothesis that autoantibody abnormalities are predictive of IVF success (Gleicher *et al*., 1994).

While unable to find a correlation with IVF failure, a surprising statistical association was noted between the presence of abnormal aPL and intrauterine growth retardation (IUGR) (Birdsall *et al*., 1996). Antiphospholipid antibodies are highly predictive of IUGR in normal pregnancies, women with chronic hypertension and in pre-eclampsia (El-Roeiy *et al*., 1991; Gleicher, 1997). This should not be surprising because placental antibody deposits have been suggested as a cause of IUGR (Katsuragawa *et al*., 1997). The association between aPL and IUGR carries over independently into an association between aPL and perinatal risk due to hypertensive conditions of pregnancy (Gleicher, 1997). The risk for perinatal morbidity increases with increasing amounts of aPL (El-Roeiy *et al*., 1991; Gleicher *et al*., 1993).

A picture seems to emerge which suggests that the immune system can cause infertility (Gleicher, 1997). Maybe more importantly, however, if we succeed in establishing a pregnancy, this pregnancy is at considerable risk. This risk involves an increased chance of pregnancy loss, IUGR and increased perinatal morbidity as well as mortality (El-Roeiy *et al*., 1991; Geva *et al*., 1994; Lockwood and Rand, 1994; Nurat *et al*., 1994; Oliveness *et al*., 1996).

The best way to monitor the fetus in such pregnancies has not yet been established. The predictive value of abnormal uterine artery waveforms between 20–30 weeks gestation for fetal distress, pre-eclampsia, fetal growth retardation and prematurity has been demonstrated (Benifla *et al*., 1992), while a progressive increase of arcuate artery resistance index with gestational age before the clinically apparent signs of growth retardation has also been noted (Meizner *et al*., 1988). More recently, we observed (Caruso *et al*., 1993) that a colour Doppler evaluation of the uterine artery resistance index (18–20 weeks) was a good tool for early identification of pregnancies at major risk of having earlier delivery and lower birth weight and birth percentile.

In our opinion, colour Doppler can allow identification of at-risk pregnancies, allowing antepartum intensive care and optimal timing of delivery. On the other hand, the good negative predictive value of the normal resistance index could confirm early a successful pregnancy outcome and reduce the cost of prenatal care.

Pregnancy in patients with APS is more likely to be successful with maternal treatment, even though complications related to placental insufficiency are still seen (Cowchock *et al*., 1992; Hasegawa *et al*., 1992; Lima *et al*., 1996; Caruso *et al*., 1997). Treatments for these women have included moderate-to-high doses of prednisone and low-dose aspirin (Lubbe *et al*., 1983; Foraouzan *et al*., 1993) on the rationale that these patients have a subtle autoimmune disorder and an altered prostaglandin/thromboxane production. The use of heparin has been advocated based on its anticoagulant activity, and more recently for its ability to cause a dose-dependent decrease in IgG binding to phospholipids in an enzyme-linked immunosorbent assay (ELISA) (McIntyre *et al*., 1993; Di Simone *et al*., 1997). The role of intravenous gamma globulins (IVG) for the treatment of pregnant patients with aPL is still doubtful and this is certainly not routine treatment. Generally, case reports or series have combined IVG with anticoagulant therapy (low-dose aspirin and/or heparin) when routine treatment has resulted in another fetal death or a serious obstetric complication such as early-onset pre-eclampsia (Cowchock, 1996). However, there have been no placebo-controlled trials available to assess its true efficacy.

As clinicians have become aware of the APS, screening patients with recurrent pregnancy loss for aPL has increased. The laboratory measures of aPL may indicate risk at the beginning of a pregnancy. The reproductive performance for women with APS can be improved by treatment during pregnancy and the uterine artery velocimetry may estimate the impaired trophoblastic invasion, reflected by an earlier delivery, lower birth weight and lower birth percentile (Caruso *et al*., 1993).

Antiphospholipid antibodies: new complexities

Studies of aPL have included lupus anticoagulant (LAC), aCL, antiphosphatidylserine antibody (aPS) and antibodies against other phospholipids (phosphatidylglycerol, phosphtidylinositol). Although the specific antibodies most commonly detected are against phospholipids, current advances in the field suggested that phospholipid-binding proteins, such as annexin V, protein C

and S and β2-glycoprotein I (β2-GPI) are involved in the binding of sera from patients with APS to anionic phospholipids (Roubey, 1994). It has also been shown that annexin V and aPL compete for phospholipid both in an in-vitro clotting assay and in ELISA systems (Sammaritano *et al*., 1992). Actually, whole IgG fractions from APS sera or xenogenic murine aPS monoclonal antibody were shown to displace annexin V from trophoblast and endothelial cell surfaces favouring a procoagulant state *in vitro* (Rand *et al*., 1997; Rote *et al*., 1998).

It has been demonstrated (Oosting *et al*., 1993) that IgG from some patients with aPL and/or a history of thrombosis interfere with the anticoagulant activity of protein C and S, and that that some aPL bind directly to activated protein C, to complexes between phospholipids and protein C or to protein S. They are reported to inhibit thrombomodulin-mediated activation of protein C, prevent binding of protein S to cell surfaces and interfere in its interaction with C4b-binding protein (Bokarewa *et al*., 1998). However, the ability of aPL to diminish phospholipid-dependent inactivation of factor Va by activated protein C seems to be the most probable mechanism contributing to thrombosis. A low anticoagulant effect of activated protein C was termed 'activated protein C resistance' (Bertina *et al*., 1994) and was frequently found in aPL-positive patients (Bokarewa *et al*., 1995). However in aPL-positive women, the dynamics of the activated protein C during pregnancy is not related to aPL (Bokarewa *et al*., 1998), which implies that autoimmune reactions against phospholipid and activated protein C response have different regulation mechanisms.

In 1990, investigators reported that binding of aPL to phospholipid was dependent on a serum protein co-factor, identified as β2-GPI (Matsuura *et al*., 1990). It is now apparent that a significant proportion of antibodies detected in the conventional aPL ELISA may be directed against serum phospholipid-binding proteins such as β2-GPI rather than against phospholipid itself. These antibodies are detected in the aPL ELISA by virtue of the fact that phospholipid-binding proteins in the patient's serum or in the bovine serum used to block the ELISA plate, interact with the immobilized phospholipid and thus become available for antibody binding (Emlen, 1996). The aPL measured in the conventional ELISA may therefore include at least several subsets of aPL: the β2-GPI-independent and the β2-GPI-dependent aPL. The nature of the aPL–β2-GPI–phospholipid interaction is important, because β2-GPI is believed to be a physiological inhibitor of clotting (Schousboe, 1985) and interruption of its inhibitory activity by aPL might predispose to thrombosis (Hunt and Krillis, 1994).

A relevant observation with respect to β2-GPI–phospholipid interaction was the report by Gharavi: the immunization of mice with β2-GPI induced aCL and anti-β2-GPI antibodies (Gharavi *et al*., 1992). However, the associations of β2-GPI-induced aPL with clinical complications in normal mice are controversial. One group did not find fetal death associated with β2-GPI-induced aPL in Balb/c mice (Branch *et al*., 1990), while others reported low fecundity and higher rate of resorption following induction of aPL by β2-GPI immunization (Blank *et al*., 1991). More recently, a clear pathogenic role was demonstrated for β2-GPI-induced aPL in the development of an experimental model of APS in mice carrying genes that may predispose it for autoimmunity (Garcia *et al*., 1997).

The recognition that some aPL are β2-GPI-dependent has led to the development of assays to measure directly antibodies to β2-GPI, allowing a clear distinction between these antibodies and those that bind phospholipid alone. Actually, antibodies specific for β2-GPI have been identified and found to be associated with all the clinical manifestation of APS, including fetal losses (Arvieux *et al*., 1991) and thrombosis (Martinuzzo *et al*., 1995). It was also suggested (Aoki *et al*., 1995) that in women with reproductive autoimmune failure, the use of an ELISA to detect anti-β2-GPI antibodies is superior to other assays in the prediction of autoantibody-associated reproductive failure. In a recent report (McNally *et al*., 1995), sera from 114 patients with infections were assayed, and 11.4% of these patients were found to have aCL antibodies, though none had anti-β2-GPI antibodies, confirming that these are found more specifically in autoimmune disease.

In contrast, aPL that are independent of β2-GPI have been shown also in patients with primary APS and SLE (Pierangeli *et al*., 1992); and, conversely, naturally occurring anti-PL antibodies, that are β2-GPI-dependent, have been detected in healthy subjects and infectious patients (Hojnik *et al*., 1994).

Even monoclonal antibodies (mAbs) reacting specifically with anionic phospholipid in the absence of any plasma co-factor have been shown to reproduce fetal loss, growth retardation and placental deposition with necrosis in mice (Ikematsu *et al*., 1998), and a growing number of observations have suggested that thrombotic tissue injury may occur in patients with infections, in association with a high titre of aPL (Yamazaki *et al*., 1991; Rubbert *et al*., 1994; Levin *et al*., 1995; Manco-Johnson *et al*., 1996).

These findings suggest that at least some β2-GPI independent aPL may be pathogenic, and the activities of these antibodies may therefore potentiate the pathogenicity of the β2-GPI-dependent human aPL autoantibodies.

Phospholipids location

An interesting aspect of aPL is their potential reactivity with cell membranes. Whereas cardiolipin (CL) is confined to mitochondrial membranes, phosphatidylserine (PS) and phosphatidylethanolamine are important constituents of the cell membranes (Dale and Robinson, 1988). PS is found almost exclusively in the inner monolayer, while phosphatidylethanolamine is distributed more evenly, although it displays a preference for the inner monolayer of the cell membranes (Schick *et al*., 1976).

The current dogma is that antibodies do not freely or normally cross the membranes of living cells, therefore the relevant antigen must be expressed on the surface of the affected cells. CL does

not meet that criterion, whereas PS, phosphatidylethanolamine and phosphatidylinositol are more likely antigenic targets (Op den Kamp, 1979). Because of the normal inaccessibility of PS on the cytoplasmic surface of membranes, the key to understanding the pathophysiology associated with aPL is in understanding the physiological conditions under which antigenic phospholipid-dependent conformations are accessible to circulating aPL (Rote *et al*., 1998).

Although CL would only be accessible after cell death and fragmentation, there are several physiological circumstances under which plasma membrane phospholipids are externalized. Erythrocytes and lymphocytes undergoing senescence or apoptosis, express surface PS that may be part of the recognition by which aged cells are removed from the circulation by macrophages (Fadok *et al*., 1992; Koopman *et al*., 1994). Externalization of membrane phospholipids has also been related to both spontaneous and differentiation-related intercellular membrane fusion events. Myoblasts during the formation of myotubes enrich the amount of surface PS as a prelude to intercellular fusion.

With use of monoclonal antibodies Vogt and colleagues showed that placental trophoblasts also externalize PS during differentiation (Vogt *et al*., 1996). The differentiation process culminates in intertrophoblastic fusion, which can be completely blocked by the presence of antibody against PS-dependent antigen (Rote *et al*., 1995). Because the trophoblastic fusion process continues throughout most of pregnancy for the syncytial portion of the placenta, the trophoblast is perhaps the only cell that expresses PS on its surface for a long duration.

Platelets also undergo a localized and inducible fusion reaction as cytoplasmic granules fuse to the plasma membrane, resulting in the mixing of inner and outer monolayer lipids, and thereby exposing PS to the extracellular fluid. Thus, physiological membrane fusion events result in phospholipid redistribution and transient local changes in conformation from bilaminar to non-bilayer and potentially antigenic structures.

Sites and mechanisms of action

The presence of circulating aPL is associated with a considerable increase in thrombotic and vascular disease. Polyclonal as well as monoclonal aPL and anti-β2-GPI antibodies derived from APS patients are able to recognize plasma co-factors on the endothelial cell surfaces and to affect cell function, inducing a procoagulant state (Del Papa *et al*., 1997). The upregulation of endothelial adhesion molecules can favour leukocyte–endothelial adherence and the cytokine-mediated effects may then activate leukocytes, enhancing their own procoagulant effect (Korneberg *et al*., 1994).

Furthermore, the capacity of aPL to affect the generation of eicosanoids such as thromboxane (TX) or prostacyclin (PGI_2), deriving from platelets, vascular and placental cells, has been claimed as a reasonable basis to explain some of the clinical manifestations of the syndrome. Endothelial monolayers incubated with aPL or anti-β2-GPI mAb displayed a dose-dependent increase in the production of 6-keto-$PGF_{1\alpha}$ (Del Papa *et al*., 1997). This finding suggests that aPL activate the cells and consequently upregulate PGI_2 metabolism: an increase of the inducible cyclooxygenase has been reported in HUVEC incubated in the presence of human aPL IgG, explaining the enhanced metabolic degradation of PGI_2 (Habib *et al*., 1993). These findings are in accordance with those of a recent study showing an increase in urinary excretion of TX A-platelet-derived metabolites and a smaller increase in 6-keto-$PGF_{1\alpha}$, the PGI_2 vascular cell metabolite in APS patients (Lellouche *et al*., 1991).

Moreover, it has been demonstrated (Peaceman and Rehnenberg, 1993) that IgG from patients with APS increase TX production by normal placental tissue, but do not inhibit PGI_2 generation. An important role of TX formation in the development of APS was also demonstrated (Shoenfeld and Blank, 1994) by showing that treatment with an antagonist of TX receptors was able to suppress different manifestations of APS in an experimental mouse model. This drug produced reduction of fetal resorption, increased the embryo weights and platelet count, and decreased the activated partial thromboplastin time. The capacity of aPL to affect the generation of eicosanoids such as TX or PGI_2, derived from platelets, vascular and placental cells, has been claimed as a reasonable basis to explain some of the clinical manifestations of the syndrome.

Recently, whole IgG fractions from APS sera were shown to displace annexin V from trophoblast and endothelial cell surfaces *in vitro*, so favouring a procoagulant state (Rand *et al*., 1997). This protein, whose physiological function has not yet been established, has potent anticoagulant properties that are based on its high affinity for anionic phospholipids and its capacity to displace coagulation factors from phospholipid surfaces (Andree *et al*., 1992; Krikun *et al*., 1994).

Annexin V was found on the apical surface of the placental syncytiotrophoblasts, and the concentrations of this protein were markedly reduced on placental villi in patients with APS. The decrease in concentrations of annexin V induced by aPL was accompanied by a shortening of the coagulation time of plasma (Rand *et al*., 1997). In addition, incubating umbilical vein endothelial cells with polyclonal rabbit anti-annexin V resulted in faster coagulation of plasma than that induced by the control polyclonal IgG. These findings (Rand *et al*., 1997) are consistent with the concept that annexin V has an antithrombotic function on the vascular and trophoblast surface, and the aPL-induced reduction in the level of annexin V at these sites may account for the thrombosis that occurs in the APS. All these events might occur simultaneously *in vivo*, contributing to thrombus formation. Nevertheless, decidual/placental thrombosis is not specific to aPL-positive patients. Thrombosis has also been reported in cases of IUGR, pre-eclampsia, SLE and fetal death unrelated to aPL.

Table I. Summary of antiphospholipid antibody effects on trophoblast function

Antibody	Cell type	Binding	Trophoblast proliferation	HCG and HPLsecretion	Trophoblast invasiveness	Trophoblast fusion	Reference
Polyclonal aPL (IgG)	Primary trophoblast cells	++		Reduced by 40%	Completely blocks	Completely blocks	Di Simone *et al.* (1999)
Anti-β2-glycoprotein I	Choriocarcinoma cells	++	Completely blocks				Chamley *et al.* (1998)
Antiphosphatidylserine	Choriocarcinoma cells	++		Reduced by 40%	Completely blocks	Completely blocks	Rote *et al.* (1995, 1998)
Antiphosphatidylserine	Primary trophoblast cells	++		Reduced by 50%	Completely blocks		Katsuragawa *et al.* (1997)

HCG = human chorionic gonadotrophin; HPL = human placental lactogen.

Decreased levels of interleukin (IL)-3 production have been demonstrated in humans with APS (Fishman *et al*., 1993) and in mice with experimental APS (Fishman *et al*., 1996). As IL-3 plays an active role in modulating placental growth, it is conceivable that its downregulation in APS could explain the clinical features of the syndrome (i.e. fetal loss). It was suggested previously (Francis *et al*., 1988) that one of the mechanisms for the pathogenicity of the aPL is the ability of these autoantibodies to inhibit plasminogen activity. It is reasonable to assume that, by its ability to stimulate this enzyme activity, IL-3 can support implantation and trophoblast invasion, thus preventing early fetal resorption (Fishman *et al*., 1993) Accordingly, exogenous administration of recombinant IL-3, or a potentiator of IL-3 production such as low-dose aspirin, in mice with APS prevented fetal loss (Fishman *et al*., 1993; Blank *et al*., 1998).

In line with the hypothesis that several pathogenic mechanisms can be present at the same time and even in the same patient, an alternative hypothesis proposed that aPL could have a detrimental effect on the trophoblastic layer of the human placenta (Lyden *et al*., 1992). The trophoblastic differentiation process is characterized by the externalization of PS, and this physiological event may explain why the placenta is sensitive to the effects of aPL (Rote *et al*., 1995). Monoclonal aPL reacted directly with the trophoblast region of the human placenta and we have demonstrated recently that IgG fractions obtained from patients with APS displayed a binding to trophoblast cells *in vitro*. The highest trophoblast binding was found after 72 h of culture, when the cells displayed the largest syncytial groups (Di Simone *et al*., 1999). Our results are in line with the demonstration that PS is exposed on the external surface during intertrophoblastic fusion with a maximal exposure after 72 h of culture (Rote *et al*., 1998). As we used aPL IgG fractions displaying a clear binding to CL-coated as well as to PS-coated plates, we can assume that the trophoblast binding might be, at least in part, related to antibodies cross-reacting with negatively charged phospholipid or to antibodies specific for PS.

On the other hand, trophoblast expression of anionic phospholipid could offer a substrate for circulating cationic β2-GPI. The immunohistological demonstration of the presence of β2-GPI on trophoblast surfaces (McIntyre, 1992; La Rosa *et al*., 1994) and the fetal loss induced by anti-β2-GPI in experimental animal models (Blank *et al*., 1994; George *et al*., 1998), suggested a pathogenic role for these antibodies. Trophoblast differentiation is characterized by the development of extravillous trophoblast that migrates into maternal myometrium. Matrigel cultures of human trophoblast cells can differentiate into villous structures comparable with those observed *in vivo*, making it a useful in-vitro assay to evaluate trophoblast invasiveness. Incubation with polyclonal aPL IgG significantly reduced trophoblast cell invasiveness (Di Simone *et al*., 1999). Murine allo-antibodies have also been shown to prevent in-vitro trophoblast invasion (Katsuragawa *et al*., 1997). We made a similar observation with autoantibodies which should recognize different epitopes and occur spontaneously during the syndrome. Findings by other researchers (Chamley *et al*., 1998) are in line with the pathogenetic activity of anti-β2GPI in an aPL-mediated fetal loss.

These proposed aetiopathogenic events would allow the development of the villous placenta, with inadequate implantation limiting the success of the pregnancy in aPL-positive women, leading to the obstetric complications associated with aPL. However, the mechanism by which aPL can affect trophoblast invasiveness is not clear, but antibody binding to anionic PL or to adhered β2-GPI on trophoblast cell membranes seems to be a necessary pre-requisite. In-vitro invasion is dependent upon several factors, including adherence to the extracellular matrix, response to external cytokine signals, and expression and alteration of adhesion proteins. The effects of aPL on the individual steps of this process have not yet been studied.

Recent observations have suggested that syncytium formation and hormonal differentiation are separate, but parallel events: the adequate production of trophoblast hormones is also necessary for a successful pregnancy. In primary trophoblast cultures, monoclonal aPLs reactive with phospholipid antigens decreased placental human chorionic gonadotrophin (HCG) and human placental lactogen (HPL) secretion to approximately 40% of control levels (Katsuragawa *et al*., 1997). The process by which this occurs is yet unknown.

Hormone production seems responsive to signal transduction through membrane-bound phospholipase C and protein kinase C: sera containing aPL can block the induction of HCG production by exogenous phospholipase C (Gleicher *et al*., 1992), dependent on membrane phospholipid, or by gonadotrophin-releasing hormone (Di Simone *et al*., 1995). It is possible that aPL can interfere with signal transduction in trophoblast cells and prevent the induction of hormone production by the placenta.

Table II. Pregnancy outcome and obstetric complications in women with prednisone/aspirin-treated pregnancies (*n* = 53) with antiphospholipid syndrome (*n* = 47)

Outcome/complication	n	%
Obstetric history (untreated pregnancies, *n* =116)		
Fetal loss	100	86
Live birth	16	14
Neonatal death	11/16	69
Pregnancy outcome (treated pregnancies, *n* = 53)		
Fetal loss	7/54[a]	13
Live birth	47/54[a]	87
Neonatal death	1/47	2
Obstetric complications		
Preterm delivery (≤36 weeks)	25/47	53
IUGR	6/47	13
Birth weight <2500 g	25/47	53
Pre-eclampsia[b]	10/50	20
Gestational diabetes mellitus	21/53	40

[a]One twin pregnancy
[b]Excluding spontaneous abortions
IUGR = intrauterine growth retardation.

In conclusion, the inhibitory effects of aPL on trophoblast intercellular fusion, hormone production and invasion (Table I) suggest that many of the obstetric complications observed in the APS may be due to aPL-induced trophoblast dysfunction.

Therapeutic implications

Successful pregnancies with APS are more likely to occur after maternal treatment, even if complications related to placental insufficiency are observed (Cowchock *et al*., 1992; Hasegawa *et al*., 1992; Caruso *et al*., 1993, 1997; Lima *et al*., 1996). These women can have a high rate of fetal loss when no specific treatment is given during pregnancy. However, the best treatment of aPL-associated complications is not well defined (Rai *et al*., 1995). Early therapeutic efforts for recurrent fetal loss included high-dose prednisone and aspirin (Lubbe *et al*., 1983; Branch *et al*., 1985). The suppression of lupus anticoagulant (LAC) with prednisone/aspirin in five of six patients treated, all resulting in live birth has been described (Lubbe *et al*., 1983). Our experience with prednisone/aspirin-treated pregnancies in APS is summarized in Table II (unpublished data). The rate of fetal loss decreased from 86% to 13% after treatment, even if the incidence of obstetric complications was high. Gestational diabetes mellitus and hypertension are important side effects of this therapy, and a significantly higher incidence of preterm delivery is described in treated pregnancies (Caruso *et al*., 1997; Laskin *et al*., 1997).

Low-dose aspirin or heparin are currently the favoured treatments (Cowchock, 1996). Several studies have shown that the output of TXA metabolites was higher during the entire pregnancy in patients with APS (Kaaja *et al*., 1993), while observations *in vitro* showed that plasma containing LAC inhibits the production of PGI_2 in vascular tissues (Carreras *et al*., 1981; Elias and Eldor, 1984); the increase of TXA metabolites associated with the decrease in PGI_2 production could then lead to the observed clinical symptoms such as thrombosis.

The basis for the use of low-dose aspirin in the prevention of these pregnancy complications is that such a regimen inhibits almost exclusively platelet cyclooxygenase activity and thereby blocks the synthesis of the vasoconstrictory and pro-aggregatory TXA_2, without affecting the synthesis of PGI_2, which exerts vasodilatation and anti-aggregation. Recently (Tulppala *et al*., 1997), showed that 50 mg of aspirin during early pregnancy in women with recurrent spontaneous abortions, without aPL, significantly reduced TXA_2 production but left PGI_2 unchanged. However, the treatment did not improve pregnancy outcome. Even Balash's data (Balash *et al*., 1993) advocated treatment with low-dose aspirin for prevention of pregnancy losses in patients with APS, though no prospective placebo-controlled trial has been carried out to compare aspirin with placebo. It might seem that modulation of arachidonic acid metabolism by aspirin had important implications in APS, but to have a clear idea about the effect of aspirin in aPL-positive women with recurrent miscarriage, low-dose aspirin treatment should be tested against placebos in prospective trials.

Two recent publications (Kutteh, 1996; Rai *et al*., 1997) looked at treated pregnant patients with APS, comparing low-dose aspirin alone versus low-dose aspirin and heparin. Both studies showed a statistically significant improvement in live birth rate for the patients treated with low-dose aspirin and heparin. Both reports were of prospective trials, but were not comparable in terms of study design or inclusion criteria. The trial reported by Kutteh did not use prospective random assignment to treatment and excluded patients with lupus antibodies (LA); in the report by Rai *et al*., over half of the patients were positive for LA. A randomized controlled trial is needed (Drakeley *et al*., 1998).

The success of heparin on pregnancy outcome in women with phospholipid syndrome stimulated interest on its mechanism of action. Investigators studied the possibility that heparin binds to and interferes with recognition of either the aPL–protein complex or directly with the aPL. It was suggested (McIntyre *et al*., 1993) that there was direct binding of heparin to aPL, but in the ELISA a decrease in aPL concentration was

shown with increasing doses of heparin. This was not thought to be due to an electrostatic interaction because chondroitin sulphate, a molecule with a negative charge similar to heparin, had no effect on aPL concentrations in the ELISA. In addition, low-molecular weight heparin seemed to be more effective at pharmacological and lower concentrations than regular heparin (Di Simone *et al*., 1997), suggesting that steric hindrance was not a significant problem. The mechanism by which heparin could bind to aPL has still to be ascertained. Findings indicate that β2-GPI binds to heparin (McIntyre, 1992), and this might interfere with the aPL binding, depleting the necessary co-factor for the binding.

We recently reported that IgG fractions obtained from patients with APS, displaying both CL and PS, do bind to trophoblast cells displaying an inhibitory effect on the in-vitro invasiveness and differentiation of human trophoblast cells. Furthermore, we demonstrated a direct interference of heparin in the IgG binding to primary trophoblast cells and that heparin treatment is able to restore the normal trophoblast and HCG secretion (Di Simone *et al*., 1997; Figure 1B), in-vitro invasiveness and differentiation (Di Simone *et al*., 1999; Figure 1A).

These observations suggest that aPL may cause pregnancy loss by binding to phospholipids expressed on the invading trophoblast, thereby inhibiting successful embryonic implantation into the endometrium. Once placentation is established, their thrombogenic action leads to decreased placental perfusion and subsequent infarction. Low-dose aspirin might improve pregnancy outcome in women with aPL by irreversibly blocking the action of cyclooxygenase in platelets, thereby inhibiting platelet thromboxane synthesis and preventing thrombosis of the placental vasculature. Heparin may act to reduce fetal loss protecting the trophoblast phospholipids from attack of aPL and promoting successful implantation in early pregnancy, in addition to its anticoagulant action.

Nonetheless, it is clear that these are in-vitro models and in patients with APS several pathogenetic mechanisms can be present at the same time. Even if the heparin concentrations used in our in-vitro studies were higher than the therapeutic plasma concentrations, we have to consider that in this model we tested the short-term effect of the drug, that is different from the in-vivo condition in which the trophoblast tissue is exposed to a longer drug action.

Future clinical studies should be able to determine the benefits of preconceptual administration of heparin and whether it can stopped after 13 weeks gestation without adversely affecting the rate of live births (Rai *et al*., 1997).

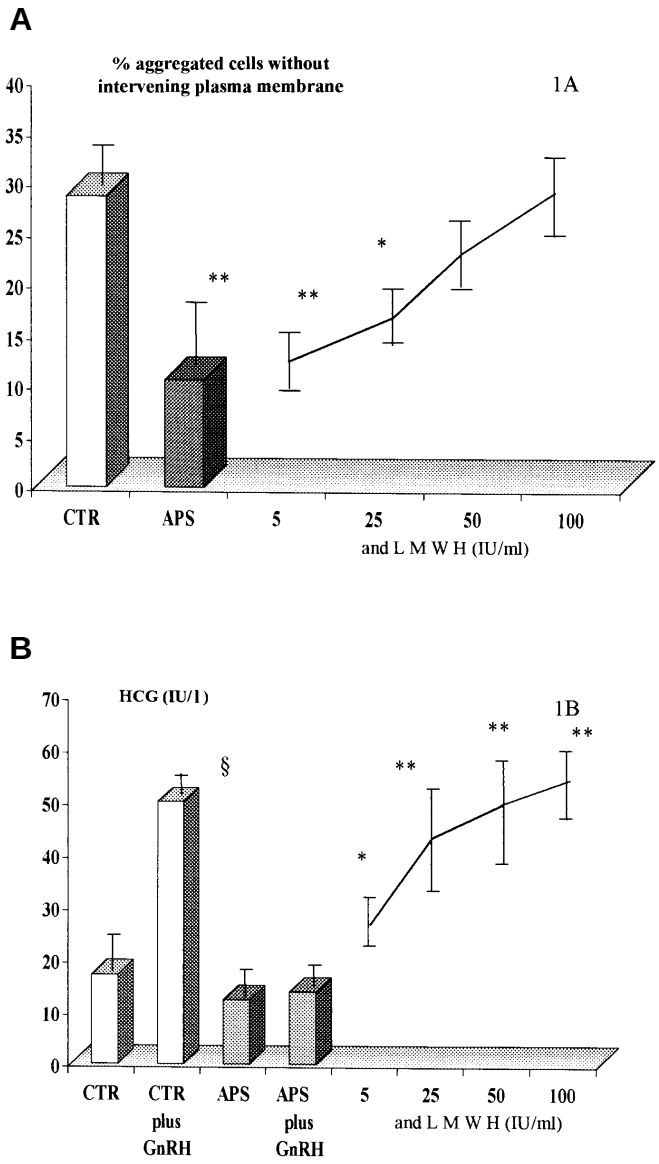

Figure 1. (**A**) Effects of low-molecular weight heparin (LMWH) on trophoblast cellular aggregation (36 h of culture). CTR: IgG (100 μg/ml) obtained from normal patients. APS: IgG (100 μg/ml) obtained from patients with antiphospholipid syndrome. Significant differences compared with the corresponding IgG fractions obtained from normal patients (CTR): **P* < 0.05; **P < 0.001. (**B**) Effects of LMWH on gonadotrophin-releasing hormone (GnRH)-induced human chorionic gonadotrophin (HCG) production by human trophoblast cells. CTR: IgG (100 μg/ml) obtained from normal patients. APS: IgG (100 μg/ml) obtained from patients with antiphospholipid syndrome. GnRH (10^{-7} M) treatment plus CTR versus CTR or versus GnRH plus APS: §*P* < 0.01. LMWH significantly increases GnRH-induced HCG secretion in presence of IgG obtained from patients with antiphospholipid syndrome: **P* < 0.05; ***P* < 0.01.

Conclusions

Although clinical complications associated with aPL are now well established, questions about both pathogenesis and optimal therapy remain. The mechanis4ms of aPL interactions with anionic phospholipids is slowly being understood, and such interaction, mediated by phospholipid-binding coagulation proteins, has now been demonstrated in the laboratory. However, the complex antigens on the trophoblast surfaces are still to be characterized and eventually correlated with clinical manifestation. The heterogeneity of these

antibodies thus far has precluded the design of predictive tests able to identify antibody subsets responsible for a defined clinical risk. Assays need to be developed which detect the different subpopulations of antibodies in the plasma of patients at risk and which could predict the type of clinical problems likely to be encountered.

References

Alarcon-Segovia, D. and Sanchez-Guerrero, J. (1989) Primary antiphospholipid syndrome. *J. Rheumatol*., **16**, 482–489.

Andree, H.A.M., Hermens, W.T., Hemker, H.C. *et al*. (1992) Displacement of factor Va by annexin V. In Andree, H.A.M. (ed.), *Phospholipid Binding and Anticoagulant Action of Annexin V.* Universitaire Pers Maastricht, Maastricht, The Netherlands, pp. 73–85.

Aoki, K., Dudkiewicz, A.B., Matsuura, E. *et al*. (1995) Clinical significance of β2-glycoprotein I-dependent anticardiolipin antibodies in the reproductive autoimmune failure syndrome: correlation with conventional antiphospholipid antibody detection systems. *Am. J. Obstet. Gynecol.*, **172**, 926–931.

Arvieux, J., Roussel, B., Jacob, M.C. *et al*. (1991) Measurement of antiphospholipid antibodies by Elisa using β2-Glycoprotein I as an antigen. *J. Immunol. Methods*, **143**, 223–230.

Balash, J., Carmona, F., Lopez-Soto, A. *et al*. (1993) Low-dose aspirin for prevention of pregnancy losses in women with primary antiphospholipid syndrome. *Hum. Reprod*., **8**, 2234–2239.

Balash, J., Creus, M., Fabregues, F. *et al*. (1996) Antiphospholipid antibodies and human reproductive failure. *Hum. Reprod*., **11**, 2310–2315.

Benifla, J.L., Tchobroutsky, C., Uzan, M. *et al*. (1992) Predictive value of uterine artery velocity waveforms in pregnancies complicated by systemic lupus erythematosus and the antiphospholipid syndrome. *Fetal Diag. Ther*., **7**, 195–202.

Bertina, R.M., Koeleman, B.P.C., Koster, F. *et al*. (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature*, **369**, 64–67.

Birdsall, M.A., Lockwood, G.M., Ledger, W.L. *et al.* (1996) Antiphospholipid antibodies in women having in-vitro fertilization. *Hum. Reprod*., **11**, 1185–1189.

Blank, M., Cohen, J., Toder, V. *et al*. (1991) Induction of antiphospholipid syndrome by positive transfer of anticardiolipin antibodies. *Proc. Natl Acad. Sci. USA*, **88**, 3069–3073.

Blank, M., Faden, D. and Tincani, A. (1994) Immunization with anticardiolipin cofactor (β2-glycoprotein I) induces experimental antiphospholipid syndrome in naive mice. *J. Autoimmun.*, **7**, 441–455.

Blank, M., George, J., Fishman, P. *et al*. (1998) Ciprofloxacin immunomodulation of experimental antiphospholipid syndrome associated with elevation of interleukin-3 and granulocyte-macrophage colony-stimulating factor expression. *Arthritis Rheum*., **41**, 224–232.

Bokarewa, M.I., Bremme, K., Falk, G. *et al*. (1995) Studies on phospholipid antibodies, APC resistance and associated mutation in the coagulation factor V gene. *Thromb. Res*., **78**, 193–200.

Bokarewa, M.I., Wramsby, M. and Bremme, K. (1998) Reactivity against antiphospholipids during pregnancy. *Hum. Reprod*., **13**, 2633–2635.

Bowie, W.E.J., Thompson, J.H., Pascuzzi, C.A. *et al*. (1963) Thrombosis in Systemic Lupus Erythematosus despite circulating anticoagulants. *J. Clin. Invest*., **62**, 416–430.

Branch, D.W., Scott, J.R., Kochenour, N.K. *et al*. (1985) Obstetrics complications associated with the lupus anticoagulant. *N. Engl. J. Med*., **313**, 1322–1326.

Branch, D.W., Dudley, D.J., Mitchell, M.D. *et al*. (1990) 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*., **163**, 210–216.

Bronson, R. (1995) Immunology and reproductive medicine. *Hum. Reprod*., **10**, 755–757.

Canoso, R.T. and de Oliveira, R.M. (1988) Chlorpromazine-induced anticardiolipin antibodies and lupus anticoagulant: absence of thrombosis. *Am. J. Hematol*., **27**, 272–275.

Carreras, L.O., Defreyn, G., Machin, S.J. *et al* (1981) Recurrent arterial thrombosis, repeated intrauterine death and 'lupus' anticoagulant: detection of immunoglobulin interfering with prostacyclin formation. *Lancet*, **i**, 244–246.

Caruso, A., De Carolis, S., Ferrazzani, S. *et al*. (1993) Pregnancy outcome in relation to uterine artery flow velocity waveforms and clinical characteristics in women with antiphospholipid syndrome. *Obstet. Gynecol*., **82**, 970–977.

Caruso, A., De Carolis, S., Ferrazzani, S. *et al*. (1997) Antiphospholipid antibodies in pregnant patients. *Int. J. Immun. Pharmacol*., **10**, 137–138.

Chamley, L.W., Duncalf, A.M., Mitchell, M.D. *et al*. (1998) Action of anticardiolipin and antibodies to β2-glycoprotein-I on trophoblast proliferation as a mechanism for fetal death. *Lancet*, **352**, 1037–1038.

Christiansen, O.B., Ulcova-Gallova, Z., Mohapeloa, H. and Krauz, V. (1998) Studies on associations between human leukocyte antigen (HLA) class II alleles and antiphospholipid antibodies in Danish and Czech women with recurrent miscarriages. *Hum. Reprod*., **13**, 3326–3331.

Cowchock, S. (1996) Prevention of fetal death in the antiphospholipid antibody syndrome. *Lupus*, **5**, 467–472.

Cowchock, S., Reece, E.A., Balaban, D. *et al*. (1992) Repeated fetal losses associated with antiphospholipid antibodies: a collaborative randomized trial comparing prednisone with low-dose heparin treatment. *Am. J. Obstet. Gynecol*., **166**, 1318–1324.

Dale, M.P. and Robinson, N.C. (1988) Synthesis of cardiolipin derivatives with protection of the free hydroxyl: its application to the study of cardiolipin stimulation of cytochrome C oxidase. *Biochemistry*, **27**, 8270–8275.

Del Papa, N., Guidali, L., Sala, A. *et al*. (1997) Endothelial cells as a target for antiphospholipid antibodies. *Arthritis Rheum*., **40**, 551–561.

Di Simone, N., De Carolis, S., Lanzone, A. *et al.* (1995) *In vitro* effect of antiphospholipid antibody-containing sera on basal and gonadotrophin releasing hormone-dependent human chorionic gonadotrophin release by cultured trophoblast cells. *Placenta*, **16**, 75–83.

Di Simone, N., Ferrazzani, S., Castellani, R. *et al*. (1997) Heparin and low-dose aspirin restore placental human chorionic gonadotrophin secretion abolished by antiphospholipid antibody containing sera. *Hum. Reprod*., **12**, 2061–2065.

Di Simone, N., Caliandro, D., Castellani, R. *et al*. (1999) Low-molecular weight heparin restores *in vitro* trophoblast invasiveness and differentiation in presence of immunoglobulin G from patients with antiphospholipid syndrome. *Hum. Reprod*. **14,** 489–495.

Drakeley, A.J., Quenby, S. and Farquharson, R.G. (1998) Mid-trimester loss-appraisal of a screening protocol. *Hum. Reprod*., **13**, 1975–1980.

Elias, M. and Eldor, A. (1984) Thromboembolism in patients with the lupus-type circulating anticoagulant. *Arch. Intern Med*., **144**, 510–515.

El-Roeiy, A., Myers, S.A. and Gleicher, N. (1991) The relationship between autoantibodies and intrauterine growth retardation in hypertensive disorders of pregnancy. *Am. J. Obstet. Gynecol*., **164**, 1253–1261.

Emlen, W. (1996) Antiphospholipid antibodies: new complexity and new assays. *Arthritis Rheum*., **39**, 1441–1443.

Fadok, V.A., Voelker, D.R., Campbell, P.A. *et al*. (1992) Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J. Immunol*., **148**, 2207–2216.

Fishman, P., Falach-Vaknin, E., Zigelman, R. *et al*. (1993) Prevention of fetal loss in experimental antiphospholipid syndrome by *in vivo* administration of recombinant interleukin-3. *J. Clin. Invest*., **91**, 1834–1837.

Fishman, P., Falach-Vaknin, E., Sredni, B. *et al*. (1996) Aspirin-interleukin-3 interrelationships in patients with antiphospholipid syndrome. *Am. J. Reprod. Immunol*., **35**, 80–84.

Foraouzan, I., Cohen, A.W., Lindenbaum, C. *et al*. (1993) Umbilical artery and ductal blood flow velocities in patients treated with aspirin and

prednisone for presence of anticardiolipin antibody. *J. Ultrasound Med*., **12**, 135–138.

Francis, R.B., McGhee, W.G. and Feinstein, D.I. (1988) Endothelial dependent fibrinolysis in subjects with the lupus anticoagulant and thrombosis. *Thromb. Haemost.*, **39**, 412–414.

Garcia, C.O., Kanbour-Shakir, A., Tang H. *et al*. (1997) Induction of experimental antiphospholipid antibody syndrome in PL/J mice following immunization with β2GP-I. *Am. J. Reprod. Immunol*., **37**, 118–121.

George, J., Blank, M., Levy, Y. *et al*. (1998) Differential effects of anti-β2-glycoprotein-I antibodies on endothelial cells and on the manifestations of experimental antiphospholipid syndrome. *Circulation*, **97**, 900–906.

Geva, E., Yaron, Y., Lessing, J.B. *et al*. (1994) Circulating autoimmune antibodies may be responsible for implantation failure in *in vitro* fertilization. *Fertil. Steril*., **62**, 802–806.

Gharavi, A.E., Sammaritano, L.R., Wen, J. *et al*. (1992) Induction of antiphospholipid antibodies by immunization with β2-glycoprotein I (apolipoprotein H). *J. Clin. Invest.*, **90**, 1105–1109.

Gleicher, N. (1997) Antiphospholipid antibodies and reproductive failure: what they do and what they do not do; how to, and how not to treat! *Hum. Reprod*., **12**, 13–16.

Gleicher, N., Harlow, L. and Zilberstein, M. (1992) Regulatory effect of antiphospholipid antibodies on signal transduction: a possible model for auto-antibodies-induced reproductive failure. *Am. J. Obstet. Gynecol*., **167**, 637–642.

Gleicher, N., Pratt, D. and Dudkiewicz, A. (1993) What do we really know about autoantibody abnormalities and reproductive failure: a critical review. *Autoimmunity*, **16**, 115–140.

Gleicher, N., Liu, H., Dudkiewicz, A. *et al*. (1994) Autoantibodies profiles and immunoglobulin levels as predictors of *in vitro* fertilization success. *Am. J. Obstet. Gynecol*., **170**, 1145–1149.

Habib, A., Martinuzzo, M., Lebret, M. *et al*. (1993) Lupus anticoagulant IgGs do not depend on serum for the induction of cyclooxygenase-2 by human endothelial cells. In Samuelson, B., Paoletti, R., Granstrom, E., Ramwell, P.W., Folco, G. and Nicosia, S. (eds), *Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Raven Press, New York.

Hasegawa, I., Takakuwa, K., Goto, S. *et al*. (1992) Effectiveness of prednisolone/aspirin therapy for recurrent aborters with antiphospholipid antibodies. *Hum. Reprod*., **7**, 203–207.

Hojnik, M., Gilburd, B., Ziporen, L. *et al*. (1994) Anticardiolipin antibodies in infections are heterogeneous in their dependency on β2-glycoprotein I: analysis of anticardiolipin antibodies in leprosy. *Lupus*, **3**, 515–521.

Hughes, G.R.V., Harris, E.N. and Gharavi, A.E. (1986) The anticardiolipin syndrome. *J. Rheumatol*.,**13**, 486–489.

Hunt, J. and Krillis, S. (1994) The fifth domain of β2-glycoprotein I contains a phospholipid binding site (C45 281-C45 288) and a region recognized by anticardiolipin antibodies. *J. Immunol*., **152**, 653–659.

Ikematsu,W., Luan, F.L., La Rosa, L. *et al*. (1998) Human anticardiolipin monoclonal autoantibodies cause placental necrosis and fetal loss in BALB/c mice. *Arthritis Rheum*., **41**, 1026–1039.

Kaaja, R., Julkunen, H., Viinikka, L. and Ylikorkala, O. (1993) Production of prostacyclin and thromboxane in lupus pregnancies: effect of small dose of aspirin. *Obstet. Gynecol*., **81**, 327–331.

Katsuragawa, H., Kanzaki, H., Inove, T. *et al*. (1997) Monoclonal antibody against phosphatidylserine inhibits *in vitro* human trophoblastic hormone production and invasion. *Biol. Reprod*., **56**, 50–58.

Koopman, G., Reutelingsperger, C.P., Kuijten, G.A. *et al*. (1994) Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood*, **84**, 1415–1420.

Korneberg, A., Blank, M., Kaufman, S. *et al*. (1994) Induction of tissue factor-like activity in monocytes by anticardiolipin antibodies. *J. Immunol.*, **153**, 1328–1331.

Krikun, G., Lockwood, C.J., Wu, X.X. *et al*. (1994) The expression of the placental anticoagulant protein, annexin V, by villous trophoblast: immunolocalization and *in vitro* regulation. *Placenta*, **15**, 601–612.

Kutteh, W.H. (1996) Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. *Am. J. Obstet. Gynecol*., **174**, 1584–1589.

La Rosa, L., Meroni, P.L., Tincani, A. *et al*. (1994) β2-glycoprotein I and placental anticoagulant protein I in placentae from patients with antiphospholipid syndrome. *J. Rheumatol*., **21**, 1684–1693.

Laskin, C.A., Bombardier, C., Hannah, M.E. *et al*. (1997) Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. *N. Engl. J. Med*., **337**, 148–153.

Lellouche, F., Martinuzzo, M., Said, P. *et al*. (1991) Imbalance of thromboxane/prostacyclin biosynthesis in patients with lupus anticoagulant. *Blood*, **78**, 2894–2899.

Levin, M., Eley, B.S., Louis, J. *et al*. (1995) Postinfectious purpura fulminans caused by an autoantibody directed against protein S. *J. Pediatr*., **127**, 355–363.

Lima, F., Khamashta, M.A., Buchanan, N.M. *et al*. (1996) A study of sixty pregnancies in patients with the antiphospholipid syndrome. *Clin. Exp. Rheumatol*., **14**, 131–136.

Lockood, C.J. and Rand, J.H. (1994) The immunobiology and obstetrical consequences of antiphospholipid antibodies. *Obstet. Gynecol. Surv.*, **49**, 432–441.

Lubbe, W.F., Buther, W.S., Palmer, S.J. *et al*. (1983) Fetal survival after prednisone suppression of maternal lupus-anticoagulant. *Lancet*, **i**, 1361–1363.

Lyden, T.W., Vogt, E., Ng, A.K. *et al*. (1992) Monoclonal antiphospholipid antibody reactivity against human placental trophoblast. *J. Reprod. Immunol*., **22**, 1–14.

Maier, D.B. and Parke, A. (1989) Subclinical autoimmunity in recurrent aborters. *Fertil. Steril*., **51**, 280–285.

Manco-Johnson, M.J., Nuss, R., Key, N. *et al*. (1996) Lupus anticoagulant and protein S deficiency in children with post-varicella purpura fulminans or thrombosis. *J. Pediatr.*, **128**, 319–323.

Martinuzzo, M.E., Forastiero, R.R. and Carreras, L.O. (1995) Anti-β2-glycoprotein-I antibodies: detection and association with thrombosis. *Br. J. Haematol*., **89**, 397–402.

Matsuura, E., Igarashi, Y., Fujimoto, M. *et al*. (1990) Anticardiolipin cofactor(d) and differential diagnosis of autoimmune disease. *Lancet*, **336**, 177–178.

McIntyre, J.A. (1992) Immune recognition at the maternal-fetal interface: overview. *Am. J. Reprod. Immunol.*, **28**, 127–131.

McIntyre, J.A., Taylor, C.G., Torry, D.S. *et al*. (1993) Heparin and pregnancy in women with a history of repeated miscarriages. *Haemostasis*, **23**, 202–211.

McNally, T., Makie, I.J., Machin, S.J. *et al*. (1995) Increased levels of β2-glycoprotein I antigen and β2-glycoprotein I/binding antibodies are associated with a history of thromboembolic complications in patients with SLE and primary antiphospholipid syndrome. *Br. J. Rheumatol*., **34**, 1031–1036.

McNeil, H.P., Chesterman, C.N. and Krilis, S.A. (1991) Immunology and clinical importance of antiphospholipid antibodies. *Adv. Immunol.*, **49**, 193–289.

Meizner, I., Katz, M. and Press, F. (1988) Assessment of utero-placental blood flow velocity waveforms in a pregnant patient with systemic lupus erythematosus. *J. Clin. Ultrasound*, **6**, 55–57.

Nurat, P., Oliveness, F., de Muzon, J. *et al*. (1994) Task force report on the outcome of pregnancies and children conceived by *in vitro* fertilization. *Fertil. Steril*., **61**, 324–330.

Oliveness, F., Kadhel, P., Rufat, P. *et al*. (1996) Perinatal outcome of twin pregnancies obtained after *in vitro* fertilization: comparison with twin pregnancies obtained spontaneously after ovarian stimulation. *Fertil. Steril*., **66**, 105–109.

Oosting, J.D., Derksen, R.H., Bobbink, I.W. *et al*. (1993) Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C or protein S: an explanation for their pathogenic mechanism? *Blood*, **81**, 2618–2625.

Op den Kamp, J.A.F. (1979) Lipid asymmetry in membranes. *Annu. Rev. Biochem.*, **48**, 47–71.

Peaceman, A.M. and Rehnenberg, K.A. (1993) The effect of immunoglobulin G fractions from patients with lupus anticoagulant on placental prostacyclin and thromboxane production. *Am. J. Obstet. Gynecol.*, **169**, 1403–1406.

Pierangeli, S.S., Harris, E.N., Davis, S.A. *et al*. (1992) β2-glycoprotein-I enhances cardiolipin binding activity but is not the antigen for antiphospholipid antibodies. *Br. J. Haematol.*, **82**, 565–570.

Rai, R.S., Clifford, K., Cohen, H. *et al*. (1995) High prospective fetal loss rate in untreated pregnancies of women with recurrent miscarriage and antiphospholipid antibodies. *Hum. Reprod*., **10**, 3301–3304.

Rai, R., Cohen, H., Dave, M. *et al*. (1997) Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). *Br. Med. J*., **314**, 253–257.

Rand, J.H., Wu, X., Andree, H.A.M. *et al*. (1997) Pregnancy loss in the antiphospholipid-antibody syndrome. A possible thrombogenic mechanism. *N. Engl. J. Med*., **337**, 154–160.

Rote, N.S., Chang, J., Katsuragawa, H. *et al*. (1995) Expression of phosphatidylserine-dependent antigens on the surface of differentiating BeWo human chorioncarcinoma cells. *Am. J. Reprod. Immunol*., **33**, 114–121.

Rote, N.S., Vogt, E., De Vere, G. *et al*. (1998) The role of placental trophoblast in the pathophysiology of the antiphospholipid antibody syndrome. *Am. J. Reprod. Immunol.*, **39**, 125–136.

Roubey, R.A. (1994) Autoantibodies to phospholipid-binding plasma proteins: a new view of lupus anticoagulants and other phospholipid antibodies. *Blood*, **89**, 2854–2867.

Rubbert, A., Bock, E., Schwab, J. *et al*. (1994) Anticardiolipin antibodies in HIV infection: association with cerebral perfusion defects as detected by 99mTc-HMPAO SPECT. *Clin. Exp. Immunol.*, **98**, 361–368.

Sammaritano, L.R., Gharavi, A.E., Soberano, C. *et al*. (1992) Phospholipid binding of antiphospholipid antibodies and placental anticoagulant protein. *J. Clin. Immunol*., **12**, 27–35.

Schick, P.K., Kurica, K.B., Chacko, G.K. *et al*. (1976) Localization of phosphatidylethanolamine and phosphatidylserine in the human platelet plasma membrane. *J. Clin. Invest*., **57**, 1221–1226.

Schousboe, I. (1985) β2-glycoprotein I: a plasma inhibitor of the contact activation of the intrinsic blood coagulation pathway. *Blood*, **66**, 1086–1091.

Shoenfeld, Y. and Blank, M. (1994) Effect of long-acting thromboxane receptor antagonist (BMS 180/291) on experimental antiphospholipid syndrome. *Lupus*, **74**, 770–777.

Tulppala, M., Marttunen, M., Soderstrom, V. *et al*. (1997) Low-dose aspirin in prevention of miscarriage in women with unexplained or autoimmune related recurrent miscarriage: effect on prostacyclin and thromboxane A2 production. *Hum. Reprod*., **12**, 1567–1572.

Unander, A.M., Norberg, R., Hahn, L. and Arfors, L. (1987) Anticardiolipin antibodies and complement in ninety-nine women with habitual abortion. *Am. J. Obstet. Gynecol*., **156**, 114–119.

Vila, P., Hernandez, M.C., Lopez-Fernandez, M.F. *et al*. (1994) Prevalence, follow-up and clinical significance of the anticardiolipin antibodies in normal subjects. *Thromb. Haemost*., **72**, 209–213.

Vogt, E., Ng, A.K., Role, N.S. *et al*. (1996) A model for antiphospholipid antibody syndrome: monoclonal antiphosphatidylserine antibody induces intrauterine growth restriction in mice. *Am. J. Obstet. Gynecol*., **174**, 700–708.

Yamazaki, M., Asakura, H., Kawamura, Y. *et al*. (1991) Transient lupus anticoagulant induced by Epstein–Barr virus infection. *Blood Coag. Fibrinol*., **2**, 771–774.

Zarrabi, M.H., Zucker, S., Miller, F. *et al*. (1979) Immunologic and coagulation disorders in chlorpromazine-treated patients. *Ann. Intern. Med.*, **91**, 194–199.

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