Pathogenic role of anti-β2-glycoprotein I antibodies on human placenta: functional effects related to implantation and roles of heparin

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Most of the clinical manifestations of the antiphospholipid syndrome (APS) can be related to thrombotic events; however, placental thrombosis cannot explain all of the pregnancy complications that occur in women with this syndrome. In this regard, it has been hypothesized that antiphospholipid (aPL) antibodies can directly attack trophoblasts, but it is still unclear what pathogenetic mechanisms play a role and which aPL antibodies subpopulations are involved. Although it has been assumed that aPL antibodies are directed against anionic phospholipids (PLs), current advances in the field suggest that antibodies to PL-binding plasma protein such as β2-glycoprotein-I (β2-GPI) are the clinically relevant aPL antibodies. It appears that following the attachment of β2-GPI to PLs, both molecules undergo conformational changes that result in the exposure of cryptic epitopes within the structure of β2-GPI allowing the subsequent binding of antibodies. aPL antibodies detected by anti-β2-GPI assays are associated with fetal loss. However, there is still debate on how the antibodies might induce the obstetrical manifestations. The significantly improved outcome of pregnancies treated with heparin has stimulated interest in the drug's mechanisms of action. Several mechanisms could explain its beneficial effects, because in addition to a direct effect of heparin on the coagulation cascade, it might protect pregnancies by reducing the binding of aPL antibodies, reducing inflammation, facilitating implantation and/or inhibiting complement activation. Further investigations are needed to better understand how aPL antibodies induce obstetric complications and to better clarify the functional role of heparin in the human placenta leading to more successful therapeutic options.

Key words: placenta/antiphospholipid syndrome/β2-glycoprotein I/heparin/antiphospholipid antibodies

Introduction

The antiphospholipid syndrome (APS) is a systemic autoimmune disorder, characterized by elevated levels of antiphospholipid (aPL) antibodies, recurrent fetal loss, repeated thromboembolic phenomena and thrombocytopenia (Lockshin, 1997; Levine *et al.*, 2002; Tincani *et al.*, 2003). Different pathogenic mechanisms have been suggested. Among them, attention has been given to the interaction between the aPL antibodies and the surface membranes of cells involved in the coagulation cascade (platelets, monocytes and endothelial cells). Such an interaction might be responsible for the thrombophilic diathesis in addition to the originally reported interference of aPL antibodies with coagulation factors (Levine *et al.*, 2002; Tincani *et al.*, 2003). Whole immunoglobulin G (IgG) fractions from APS patient sera or xenogenic murine anti-phosphatidylserine (PS) monoclonal antibody have been shown to displace annexin V from trophoblasts, thus creating conditions favourable to procoagulant state *in vitro* (Rand *et al.*, 1997). IgG

fractions isolated from APS patients reduce the binding of annexin V to PL-coated microtitre plates; the reduction of annexin V binding is dependent upon anti-β2-glycoprotein I (β2-GPI) antibodies and correlates with clinical thrombosis (Hanly and Smith, 2000). Recently, Rand has extended the findings with IgG fractions to experiments with monoclonal aPL antibodies and has found that monoclonal murine and human (Rand *et al.*, 1999a, b) aPL antibodies also displace annexin V and accelerate coagulation reactions.

The mechanism of fetal loss in women with APS is still unknown, although several investigators believe that placental thrombosis causes infarction and eventual fetal death (De Wolf *et al.*, 1982; Out *et al.*, 1991). This hypothesis was based on observations of extensive placental infarction and thrombosis in failed pregnancies in women with APS (De Wolf *et al.*, 1982; Sebire *et al.*, 2002a), as well as the dramatic association of aPL antibodies with systemic thrombosis. In addition to coagulation, a decidual

Table I. Trophoblast injuries linked to the presence of antiphospholipid (aPL) antibodies

Authors	Effects	
Peaceman *et al*. (1993)	Increase of placental thromboxane A2	300% versus controls
Fishmann *et al*. (1996)	Reduction of trophoblast interleukin-3	71% versus controls
Di Simone *et al*. (1997)	Reduction of β-HCG secretion	40% versus controls
Di Simone *et al*. (2000)	Reduction of trophoblast cells invasiveness	25% versus controls
Rote *et al*. (1998)	Inhibition of syncytiotrophoblast formation	82% versus controls

Controls, untreated trophoblast cells.

vasculopathy and placental inflammation (Abramowsky *et al*., 1980; Salafia and Cowchock, 1997; Magid *et al*., 1998) have been proposed as contributing mechanisms of fetal death. However, studies in humans have shown that thrombotic events as well as decidual inflammation cannot account for all of the histopathologic findings in placentae from women with the APS (Out *et al*., 1991; Salafia *et al*., 1996).

The possibility of direct trophoblast damage by aPL antibodies through the recognition of PS exposed during syncytium formation has been suggested (Rote *et al*., 1998). Reported direct effects of aPL antibodies on trophoblasts have included inhibition of the intercytotrophoblast fusion process (Adler *et al*., 1995; Quenby *et al*., 2005), of HCG secretion (Di Simone *et al*., 1995) and of trophoblast invasiveness (Katsuragawa *et al*., 1997; Di Simone *et al*., 1999; Table I).

Direct activity of anti-β2-GPI antibodies in reproductive failure

Although it has been assumed that aPL antibodies are directed against anionic phospholipids (PLs), current advances in the field suggest that antibodies to PL-binding plasma protein such as β2-GPI can be detected in standard aPL antibody assays (Roubey, 1994). Only aPL antibodies with affinity for β2-GPI are thought to be clinically relevant (de Laat *et al*., 2004). Although the 3-D structure of β2-GPI was known for >5 years, the mechanism by which the anti-β2-GPI antibodies recognize β2-GPI is unclear (Bouma *et al*., 1999; Schwarzenbacher *et al*., 1999). Two main theories have been proposed to explain the binding of aPL antibodies to β2-GPI. The first is known as the 'dimerization theory'; one antibody must bind two β2-GPI molecules to obtain considerable avidity (Arnout *et al*., 1998; Lutters *et al*., 2001). To achieve this, a high density of β2-GPI is essential. The studies in favour of the dimerization theory seem rather convincing, but several observations remain unexplained and are in favour of a second hypothesis. The second hypothesis is based on the recognition of a cryptic epitope by aPL antibodies. This epitope is only exposed after binding of β2-GPI to a negatively charged surface (Wang *et al*., 2000; Merrill, 2001). Supporting this latter hypothesis is the fact that the structure of β2-GPI in solution differs from that of crystallized β2-GPI.

Blank *et al*. (1999) induced experimental APS in mice after immunization with β2-GPI and showed that the clinical effects of the induced anti-β2-GPI could be prevented by injecting three synthetic peptides that mimic different epitopes on β2-GPI. This suggested that the sequences covered by these peptides were pathological epitopes on β2-GPI. The three identified epitopes are located on completely different domains of the molecule. Such diversity of antibody specificity may form the basis of the well-recognized heterogeneity of APS.

In fact, anti-β2-GPI antibodies are a heterogeneous group, with subpopulations of antibodies recognizing different domains of β2-GPI (Arvieux *et al*., 1998; Iverson *et al*., 2002). de Laat *et al*. (2004) recently published a study in which the population of anti-β2-GPI antibodies recognizing epitope G40–R43 cause lupus anticoagulant (LAC) and strongly correlate with thrombosis. Another group of anti-β2-GPI antibodies recognized other parts of β2-GPI and did not correlate with thrombosis.

In vitro studies showed that β2-GPI plays a role in the coagulation system as a natural procoagulant/anticoagulant regulator. β2-GPI inhibits prothrombinase activity on platelets or PLs on vesicles, inhibits activation of factor X and XII and modulates ADP-dependent activation of platelets. On the contrary, β2-GPI exerts procoagulant activities by the reduction of activated protein C and inhibition of the protein Z anticoagulant pathway (Shi *et al*., 1993; Forastiero *et al*., 2003). Apart from specific haemostatic functions, β2-GPI is a multifunctional plasma protein that regulates many physiological reactions. β2-GPI activates lipoprotein lipase (Nakaya *et al*., 1980), lowers triglyceride level (Whurm *et al*., 1982), binds to oxidized low-density lipoprotein to prevent the progression of atherosclerosis (Hasunuma *et al*., 1997) and binds to non-self particles or apoptotic bodies to allow their clearance (Chonn *et al*., 1995; Sheng *et al*., 2001). However, despite the regulatory functions of β2-GPI in the coagulation cascade, homozygous β2-GPI null mice appear anatomically and histologically normal (Sheng *et al*., 2001), and genetic deficiency of β2-GPI does not represent a major risk of either thrombosis or bleeding in humans.

Effects of anti-β2-GPI antibodies on trophoblast tissues

The *in vivo* immunohistologic demonstration of β2-GPI on trophoblast surfaces (McIntyre, 1992; La Rosa *et al*., 1994) and the induction of fetal loss by anti-β2-GPI antibodies in experimental animal models (Blank *et al*., 1994; George *et al*., 1998) suggested a role of anti-β2-GPI antibodies on placenta.

Recently, we found that β2-GPI can adhere to human trophoblast cells *in vitro* (Di Simone *et al*., 2000). Our results are consistent with the hypothesis that the visibility of anionic PLs on the external cell surface during intertrophoblastic fusion might offer a useful substrate for the cation PL-binding site (Katsuragawa *et al*., 1997; Rote *et al*., 1998). The binding to anionic structures induces the expression of new cryptic epitopes and/or increases the antigenic density, two events that are apparently pivotal for the antibody

binding (Wang *et al.*, 2000; Figure 1). *In vitro* studies with both murine and human monoclonal antibodies as well as with polyclonal IgG antibodies from APS patients clearly demonstrated a binding to trophoblast monolayers (Lyden *et al.*, 1992; Vogt *et al.*, 1996; Di Simone *et al.*, 2000).

Interestingly, once bound these antibodies can affect the trophoblast functions. Adler (Adler *et al.*, 1995) provided direct evidence that aPL antibodies were able to react with syncytiotrophoblast and to prevent intertrophoblast fusion, while Katsuragawa (Katsuragawa *et al.*, 1997) showed that an anti-PS monoclonal antibody bound trophoblast cells and prevented their *in vitro* invasiveness and HCG secretion. We have reported comparable results with spontaneously occurring polyclonal IgG fractions from APS patients as well as human IgM monoclonal antibodies with anti-β2-GPI activity (Di Simone *et al.*, 2000). The fact that human anti-β2-GPI monoclonal antibody inhibits trophoblast invasiveness provided new information on the role of this autoantibody subpopulation in APS-associated fetal loss.

To better characterize the pivotal role of β2-GPI adhesion to trophoblast cell membranes in mediating the aPL antibody effects on human placenta, we investigated whether specific mutations in the PL-binding site of β2-GPI might affect its binding to trophoblast and, in turn, the anti-β2-GPI antibody-induced functional effects (Figure 2). It has been suggested that the highly positively charged amino acid sequence, Cys 281-Lys-Asn-Lys-Glu-Lys-Lys-Cys 288, in the fifth domain of the molecule is the putative PL-binding site responsible for the β2-GPI binding to cardiolipin-coated. Single or multiple amino acid substitutions of Lys with Glu progressively decrease the ability of the molecule to bind to anionic structures (Hunt and Krilis, 1994; Sheng *et al.*, 1996). Interestingly, the same PL-binding site is involved in the adhesion of β2-GPI to human endothelial cell monolayers, because Lys substitution with Glu significantly decreases the presence of β2-GPI on endothelial monolayers, as shown by the lack of anti-β2-GPI antibody binding (Del Papa *et al.*, 1998). When trophoblast cells were incubated with serial protein concentrations of mutant 1K (single amino acid substitution from Lys 286 to Glu 286), there was approximately a 50% reduction in anti-β2-GPI antibody binding to the cells in comparison with trophoblasts cultured with

comparable protein concentrations of purified β2-GPI. The lowest antibody binding was detected with the mutant 3K (substitution from Lys 284, 286, 287 to Glu 284, 286, 287). Once bound to trophoblast-adhered β2-GPI, anti-β2-GPI antibodies significantly inhibited GnRH-induced HCG secretion from trophoblast cell cultures. Experiments carried out with trophoblast cells incubated with anti-β2-GPI antibodies and 3 K mutant show HCG secretion comparable with that found in control cultures. These observations indicated that a large alteration to the PL-binding site on the fifth domain of the molecule does not allow efficient β2-GPI adhesion, antibody binding and, in turn, antibody-mediated cell function modulation.

In conclusion, the presence of β2-GPI on the trophoblast cell membranes could be one of the main targets for β2-GPI-dependent aPL antibodies in the placental circulation (McIntyre, 1992). Such a finding is a prerequisite for a pathogenic role for these antibodies, as suggested by the clinical association between recurrent fetal loss and β2-GPI-dependent aPL or anti-human β2-GPI antibodies themselves. At the same time, it also might explain the aPL tropism that has been described in experimental animal models of aPL antibody-associated fetal loss. In fact, when exogenous human aPL antibodies are passively infused in pregnancy-naïve mice, they undergo rapid plasma clearance (Ikematsu *et al.*, 1998). It has been suggested that the rapid clearance could be related to aPL antibody binding to placental structures, as the same antibodies can be eluted from the placenta (Blank *et al.*, 1991). Moreover, there is also evidence from immunohistochemical studies that β2-GPI is expressed in higher quantity on the trophoblastic villi of placentas from women with APS who have had fetal loss than in control placentas, and an Ig deposition with a comparable immunohistochemical pattern is also detectable (La Rosa *et al.*, 1994). These findings suggest that most of the circulating β2-GPI-dependent aPL antibodies (or even the anti-β2-GPI antibodies themselves) might be bound to placental β2-GPI *in vivo*. The fact that aPL antibodies can be absorbed by placental structures has been thought to be pivotal for allowing the potential pathogenic effect of the antibodies on the placenta and for explaining, at least in part, why maternal IgG aPL antibodies do not often cause thrombotic events in fetuses or neonates (Avcin *et al.*, 2002).
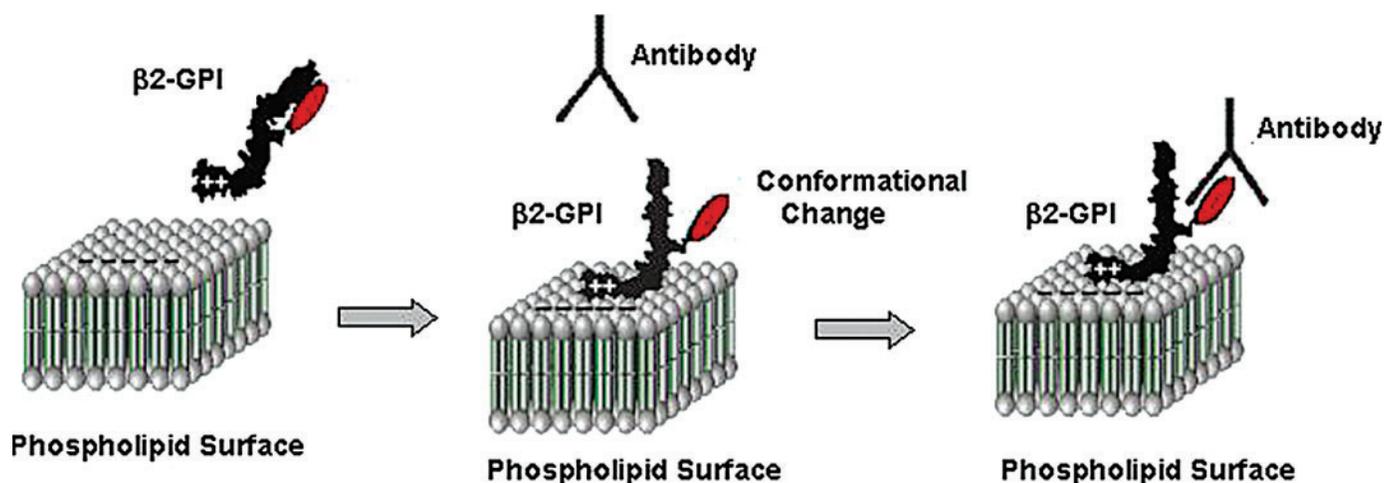
Figure 1. Mechanism describing the binding of antiphospholipid (aPL) antibodies to β2-glycoprotein I (β2-GPI). aPL antibodies cannot bind β2-GPI in solution, because the epitope is covered by one of the carbohydrate chains. Binding to a phospholipids membrane induces a conformational change in β2-GPI. As result, the carbohydrate chain is no longer able to cover the epitope and is now able to bind aPL antibodies. Modified from de Laat *et al.* (2004).
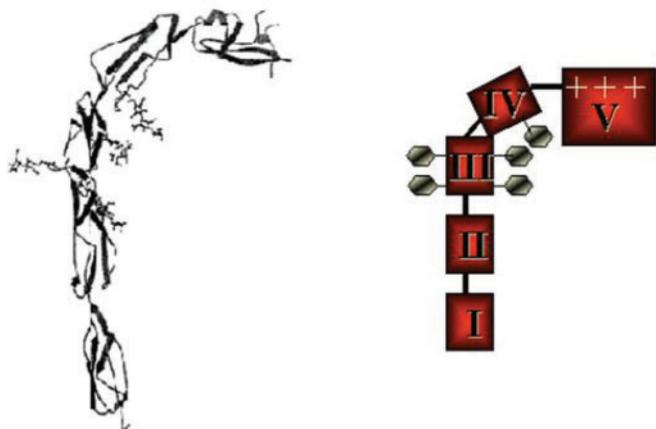
Figure 2. β2-glycoprotein I (β2-GPI) structure. It is composed of five contiguous domains, four of which are highly homologous to one another. The fifth domain, containing 80 amino acids, a long C-terminal tail and an extra disulfide bond, has high affinity for negatively charged macromolecules.

From these observations, one could speculate that the clustering effect of anti-β2-GPI antibodies on trophoblast-adhered β2-GPI might be the HCG down-regulation as well as the impaired invasiveness, contributing to the defective placentation in APS. In other studies, the *in vitro* exposure of cultured trophoblast to β2-GPI-dependent aPL antibodies caused regulatory changes to occur in the transcription and translation of certain adhesion molecules (Di Simone *et al*., 2002): the coordination and phenotypic expression of certain trophoblast integrins (α1 to α5) and cadherins (VE, E) necessary to achieve successful implantation and vascularization were modified. These aPL antibody-induced changes in trophoblast cell surface molecules resulted in altered expression and may result in marginal, incomplete or total failure of blastocyst implantation.

Heparin and aPL antibodies

Different regimens have been proposed for the treatment of APS, including aspirin, monotherapy, prednisone and aspirin, or heparin and aspirin (Table II). In 1992, Cowchock (Cowchock *et al*., 1992) compared the use of low-dose heparin with a standard dose of 40 mg prednisone daily for treatment of pregnant women with APS. The frequency of live birth after treatment was 75% in each group. In contrast to the similar live birth rates in the two treatment groups, women randomly assigned to prednisone were significantly more likely to be delivered preterm (6/6 versus 2/8, P = 0.006). Preterm delivery was associated with premature rupture of the membranes (3/6 versus 0.9, P = 0.004) or pre-eclampsia. Recently, the combination of low molecular weight heparin (LMWH) and low-dose aspirin seemed to have the highest success rate (Noble *et al*., 2005). Three trials of aspirin alone (Cowchock *et al*., 1997; Pattison *et al*., 2000; Tulppala *et al*., 1997) showed no significant reduction in pregnancy loss (relative risk[RR] 1.05, 95% confidence interval[CI] 0.66, 1.68), while heparin combined with aspirin (Kutteh *et al*., 1996; Rai *et al*., 1997) provides a significantly better pregnancy outcome. The success of heparin treatment on pregnancy outcome in women with APS stimulated interest on the drug's mechanism of action. The use of heparin to prevent pregnancy loss in this syndrome was based on the premise that some pregnancy losses were caused by a placental thrombosis and that thromboprophylaxis with heparin could prevent this process (Rai *et al*., 1997). However, the examination of placentas and first-trimester decidua from APS-complicated pregnancies has found little evidence of specific thrombotic placental pathology (Salafia and Cowchock, 1997; Sebire *et al*., 2003). Defective decidual endovascular trophoblast invasion, rather than excessive intervillous thrombosis, was the most frequent histological abnormality in APS-associated early pregnancy loss (Di Simone *et al*., 2000; Sebire *et al*., 2002b).

The cellular mechanisms by which heparin exerts its beneficial effects still have to be ascertained. Several authors (McIntyre *et al*., 1993; Ermel *et al*., 1995; Franklin and Kutteh, 2003) suggested direct binding of heparin to aPL antibodies showing a decrease in aPL antibody binding with increasing dose of heparin. This was not thought to be due to an electrostatic interaction, as chondroitin sulphate which has a negative charge similar to that of heparin had no effect on aPL concentrations in the enzyme-linked immunosorbent assay.

Heparin's mechanisms of action

In previous studies, we demonstrated that LMWH was able to reduce the aPL antibody binding to trophoblast cells and to restore *in vitro* placental invasiveness and differentiation (Di Simone *et al*., 1999). Our observations suggested that the rationale for the clinical use of heparin could be that it inhibits the binding of aPL antibodies, thus protecting the trophoblast PL and promoting implantation in early pregnancy (Di Simone *et al*., 1999).

Table II. Treatments for women with antiphospholipid syndrome (APS)

Authors	Treatments	Results
Cowchock *et al*. (1992)	Corticosteroids versus heparin	Not statistically significant (difference 75%)*
Kutteh (1996)	Heparin + low-dose aspirin versus aspirin alone	80% viable infants versus 44%
Rai *et al*. (1997)	Heparin + aspirin versus aspirin alone	71% live births versus 42%
Backos *et al*. (1999)	Aspirin and LMWH	71 % live births
Branch *et al*. (2000)	Intravenous immune globulin + heparin + low-dose aspirin versus heparin + low-dose aspirin	Not statistically significant (difference 15%)[†]
Noble *et al*. (2005)	LMWH + low-dose aspirin versus unfractionated heparin + low-dose aspirin	Not statistically significant (difference 82%)*

LMWH, low molecular weight heparin.
*Live births.
[†]Birthweights.

Recently Guerin, using an expression/site-directed mutagenesis approach, demonstrated that the primary heparin-binding site of β2-GPI is the positively charged site located within the fifth domain of the protein, which also binds to PL (Guerin *et al*., 2002). Then heparin seems to prevent the binding of β2-GPI to negatively charged PL, which in turn prevented the deposition of the anti-β2-GPI antibodies in tissues. Furthermore, heparin at concentrations that are reached therapeutically *in vivo* greatly enhanced the plasmin-mediated cleavage of β2-GPI. Considering that the cleaved forms of β2-GPI cannot bind to PL and may be cleared more rapidly from the circulation than native β2-GPI (Horbach *et al*., 1999), interaction with heparin should greatly reduce the prothrombotic effects of anti-β2-GPI antibodies. Then, the rationale for the clinical use of heparin could be that, in addition to its anticoagulant action, it inhibits the binding of β2-GPI to PL, thus protecting the trophoblast from injury. In the second mechanism, heparin potentiates the generation of an inactive form of β2-GPI by plasmin.

Bose *et al*. (2005) successfully tested the hypothesis that heparin is able to prevent trophoblast apoptosis: Bewo cells cultured in media supplemented with sera obtained from nonpregnant donors (IVF failure) were associated with increased apoptosis, and addition of heparin to those cultures attenuated Bewo apoptosis. The potential mechanism as to how heparin inhibits execution of trophoblast apoptosis may involve augmentation of cellular-protective mechanisms. In fact, levels of Bcl-2, a known inhibitor of apoptosis, were increased by heparin treatment of cultured placental explants, whereas Bcl-2 levels are depleted in syncytiotrophoblast of failing pregnancies (Lea *et al*., 1997). Moreover, heparin has been shown to regulate apoptosis caused by toxic glycoproteins (Twu *et al*., 2002) as well as oxidants (Ishikawa and Kitamura, 1999).

Girardi *et al*. (2004) hypothesized that aPL antibodies activate complement in the placenta, generating split products that mediate placenta injury and lead to fetal loss and growth restriction. To test this hypothesis, Girardi used a murine model of APS in which pregnant mice were injected with human IgG containing aPL antibodies. Mice were injected on days 8 and 12 of pregnancy with IgG isolated from patients with high titres of aPL antibodies, and approximately 40% of the embryos were resorbed, the ones that survived were growth restricted. When the mice were injected with F(ab)′2 fragments of IgG aPL antibody, aPL antibody was required to induce damage (Girardi *et al*., 2004), suggesting that the Fc portion might activate the complement system. Then, Girardi's group proposed that aPL antibodies, preferentially targeted at deciduas and placenta, activate complement through the classical pathway, leading to generation of potent anaphylatoxins and mediators of effector-cell activation. The recruitment of inflammatory cells accelerates local alternative pathway activation and creates a proinflammatory amplification loop that enhances complement component 3 (C3) activation and deposition, generates additional C3a and C5a and results in further influx of inflammatory cells into the placenta (Girardi *et al*., 2003). Interestingly, treatment with heparin prevented complement activation *in vivo* and protected mice from pregnancy complications induced by aPL antibodies (Figure 3). Even mice treated with subanticoagulant doses of heparin were protected from aPL antibody-induced pregnancy complications (Girardi *et al*., 2004). Such low doses of heparin, lacking anticoagulant effects, inhibited inflammatory responses at the level of leukocyte adhesion and influx and limited tissue injury (Friedrichs *et al*., 1994; Koenig *et al*., 1998; Wang
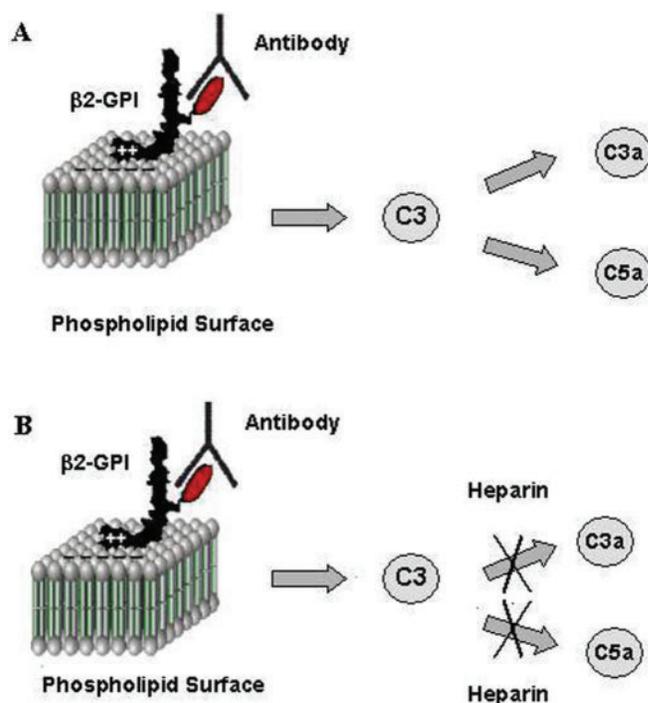
Figure 3. Anticomplement activity of heparin. Antiphospholipid (aPL) antibodies activate complement through the classical pathway enhancing complement component 3 (C3) activation and deposition, and generating additional C3a and C5a with consequent influx of inflammatory cells into the tissues (**A**). Treatment with heparin (unfractionated or low molecular weight) is able to prevent complement activation and to protect from complications induced by antiphosphoplipid antibodies (**B**). Modified from Girardi *et al*. (2004).

et al., 2002; Rops *et al*., 2004). Neither fondaparinux nor hirudin, other anticoagulants without known effects on complement (Mollnes *et al*., 2002), prevented pregnancy loss, demonstrating that anticoagulant therapy is insufficient protection against APS-associated miscarriage (Girardi *et al*., 2004).

Furthermore, heparin is increasingly recognized as a modulator of the inflammatory responses. It possesses the ability to inhibit lipopolysaccharide-induced proinflammatory cytokines [tumour necrosis factor α, interleukin (IL)-6, IL-8 and IL-1β; Hochart *et al*., 2006], involved in recurrent fetal loss of a murine model of APS (Berman *et al*., 2005).

It is still unknown if heparin can directly affect placental functions. In a recent study, we demonstrated that heparin plays a role in extravillous trophoblast cell (EVCT) invasion with an enhancement of the activity of specific proteases, such as metalloproteinases (MMPs) involved in trophoblast invasion into endometrial tissues (Librach *et al*., 1991). We isolated trophoblast cells from first trimester spontaneous abortions, investigated for genetic defects, infections, autoimmune diseases, endocrine profile and glucose intolerance. A different secretion profile of MMP-2 and MMP-9 was found between EVCT and VCT, and LMWH, at concentrations that are reached therapeutically *in vivo* (0.1–1 IU/ml), greatly enhanced both total and active MMPs. Furthermore, we demonstrated that the production of tissue inhibitors of MMPs (TIMPs) was inhibited at both the mRNA and protein levels by a higher dose of LMWH (10 IU/ml). The decline in TIMPs expression seems able to remove the inhibitory influence on MMPs activity. Even if it is possible that EVCTs obtained from spontaneous abortions have
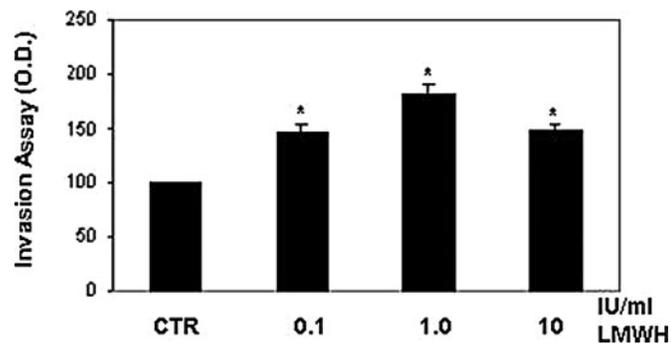
Figure 4. Extracellular matrix invasion by human extravillous cytotrophoblast cells. Incubation with low molecular weight heparin (LMWH) significantly increased cytotrophoblast cells invasiveness. Results were calculated as means ± SE of four experiments and expressed as % of control cells [untreated cells; control (CTR)]. The stimulation percentage of 0.1 IU/ml heparin (LMWH) at 24 h was 40%; at 1 IU/ml heparin greatly enhanced (80%) cytotrophoblast cells invasion. Significance versus CTR: $^*P < 0.05$. Modified from Di Simone *et al.* (2006).

different features, these results (Di Simone *et al.*, 2006) led us to consider heparin as a potent regulator of MMP production, trophoblast invasion (Figure 4) and synthesis of specific TIMPs.

Conclusions

Over the last years, our understanding of APS has dramatically changed. Although initial studies focused their attention on decidual vasculopathy and placental thrombosis, a growing body of evidence suggested a direct role of aPL antibodies on trophoblast cell placental biology. aPL antibodies are a heterogeneous class of antibodies with differing clinical significance. The diagnosis of APS is based on clinical and laboratory Sidney criteria (Miyakis *et al.*, 2006). Laboratory criteria included the presence of LAC, anticardiolipin IgG and IgM, and IgG and IgM anti-β2-GPI assays are added in the revised criteria.

The success of heparin treatment on pregnancy outcome in women with APS stimulated investigator's interest on the drug's action. Several mechanisms could explain the beneficial effects of heparin, because, in addition to its anticoagulant action, it inhibits the binding of aPL antibodies and the activation of complement, it modulates trophoblast apoptosis, and it directly promotes trophoblast cell invasiveness. Understanding the regulation of intracellular trophoblast-signalling mechanisms and placental function by extracellular heparin and the subsequent application of this knowledge *in vivo* might provide an exciting avenue of future research.

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