Low-molecular weight heparin restores in-vitro trophoblast invasiveness and differentiation in presence of immunoglobulin G fractions obtained from patients with antiphospholipid syndrome

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The present study was designed to investigate the effects of immunoglobulin G obtained from patients with antiphospholipid syndrome (APS) on in-vitro models of trophoblast invasiveness and differentiation. We tested the binding of affinity-purified immunoglobulin G to human primary trophoblast cells. These antibodies affected the invasiveness and differentiation of cytotrophoblast cells after binding to the cell surface. In addition, we determined whether the drugs used to treat APS might be able to restore the trophoblast functions. Low-molecular weight heparin, in a dose-dependent manner, significantly reduced the immunoglobulin G binding to trophoblast cells and restored in-vitro placental invasiveness and differentiation. No effect was observed in the presence of acetylsalicylic acid. These observations may help in understanding the role of these treatments in women with APS.

Key words: antiphospholipid antibodies/heparin/invasiveness/trophoblast

Introduction

Antiphospholipid antibodies (aPL) are associated clinically with thrombocytopenia, recurrent thrombosis, repeated pregnancy losses or a combination of these events (Branch et al., 1992). Patients whose pregnancies last beyond the middle of the second trimester may have a variety of collateral obstetric complications, such as early and severe pregnancy-induced hypertension and intrauterine growth retardation (Harris, 1990). Although the specific antibodies most commonly detected in these patients are against cardiolipin- and phosphatidylserine-dependent antigens, current advances in the field suggest that phospholipid-binding plasma proteins, such as beta-2-glycoprotein I (β2-GPI), human prothrombin, proteins C and S and annexin V are involved in the binding of sera from patients with the antiphospholipid syndrome (APS) to anionic phospholipids (Roubey, 1994).

However, the mechanism by which aPL might cause recurrent miscarriages remains the subject of research. Fetal losses have been attributed to thrombosis of the uteroplacental vasculature and placental infarction (De Wolf et al., 1982; Out et al., 1991). Although thrombosis is observed frequently in the decidua and placentas of aPL-positive patients, this observation is not universal, nor present in a sufficient degree to account for the pregnancy loss associated with this syndrome. An alternative hypothesis proposed that aPL have a detrimental effect on the trophoblastic layer of the human placenta (Lyden et al., 1992).

In the present study, we demonstrated that immunoglobulin (Ig) G fractions, obtained from patients with APS, displayed a binding to trophoblast cells, and also investigated the effects of IgG on in-vitro models of trophoblast invasiveness and differentiation.

In line with the recent idea that several pathogenic mechanisms can be present simultaneously in the same patient, monoclonal aPL have been shown to prevent placental human chorionic gonadotrophin (HCG) and human placental lactogen (HPL) secretion (Katsuragawa et al., 1997). We demonstrated that sera from patients with APS could interfere with gonadotrophin-releasing hormone (GnRH)-induced signal transduction (Di Simone et al., 1995) and that in-vitro heparin treatment of cytotrophoblast cells was able to restore the placental HCG secretion (Di Simone et al., 1997).

Several regimens have been proposed for the treatment of APS, and recent studies have suggested that aspirin plus heparin might be superior to prednisone or aspirin taken alone, for the treatment of aPL-associated recurrent pregnancy losses (Sher et al., 1994; Kutteh, 1996).

The second purpose of the present study was to investigate whether some drugs used for conventional treatment of pregnant women with APS would be able to interfere with the ability of IgG, obtained from sera containing high concentrations of aPL antibodies, to bind trophoblast cells, thus restoring the in-vitro placental differentiation and invasiveness.

Materials and methods

Sera

The clinical presentations and aPL specificities of the four women who provided sera for the study are summarized in Table I. The two aPL-positive patients (N3, N4) satisfied the criteria for a diagnosis of APS (Alargon-Segovia and Sanchez-Guerrero, 1989; Harris, 1990). Two normal women with no history of pregnancy complications and with negativity for aPL antibodies were used as controls (N1, N2).

Lupus anticoagulant was detected with a standard activated partial thromboplastin time (aPTT), a diluted phospholipid aPTT (Alving et al., 1985), a kaolin clotting time (Exner et al., 1978) and tissue thromboplastin inhibition test (Schleider et al., 1976). Lupus anticoagulant was defined by prolongation of coagulation times (patient:control ratio of ≥:1.5) which persisted after the addition of control plasma (patient ratio 1:1) in at least two tests.
Table I. Clinical characteristics and serum antiphospholipid antibodies profile in normal patients (N1, N2) and patients with antiphospholipid syndrome (N3, N4)

<table>
<thead>
<tr>
<th></th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30</td>
<td>27</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>Spontaneous first-trimester abortion (n)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Spontaneous second- and third-trimester fetal loss (n)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Live births (n)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>History of thrombosis</th>
<th>No</th>
<th>No</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Anticardiolipin IgG antibodies (OD values)</td>
<td>109</td>
<td>110</td>
<td>1845</td>
<td>1650</td>
</tr>
<tr>
<td>Antiphosphatidylserine IgG antibodies (OD values)</td>
<td>98</td>
<td>89</td>
<td>1927</td>
<td>1558</td>
</tr>
</tbody>
</table>

| Lupus anticoagulant   | No  | No  | Yes | No  |
| Veneral disease research laboratory | No  | No  | No  | No  |

Anticardiolipin and antiphosphatidylserine antibodies are expressed as optical density values ($\times 10^{-3}$).

Antiphospholipid antibodies assay

Anticardiolipin and antiphosphatidylserine antibodies were detected by a solid-phase enzyme-linked immunosorbent assay (ELISA) as described previously (Di Simone et al., 1997). Results were expressed as the mean (± SD) optical density (OD) values of duplicate determinations. Sera were considered positive for OD values $>0.150$ or 0.165, respectively (mean ± 3 SD of 50 normal healthy controls).

Immunoglobulin (IgG) purification

The IgG fractions were affinity-purified on protein G–Sepharose columns (MabTrap-GII; Pharmacia-Biotec, Uppsala, Sweden). The concentration of IgG was determined using a spectrophotometric analysis.

Dispersion and culture of trophoblast cells

Placentas were obtained immediately after uncomplicated vaginal delivery at 36 weeks of gestation from normal women delivering at the Obstetrics and Gynecology Department of the Catholic University, Rome, Italy. Trophoblast cell cultures were established as detailed elsewhere (Di Simone et al., 1995), except for the addition of a Percoll gradient centrifugation step (Kliman et al., 1986). Briefly, placental tissues were rinsed two to three times in cold Dulbecco’s modified Eagle’s medium (DMEM; Gibco BRL, Grand Island, NY, USA). After mincing, the tissues were submitted to repeated enzymatic digestion in Ringer-bicarbonate buffer containing 0.25% trypsin (Gibco BRL) and DNase I (0.2 mg/ml; Sigma-Aldrich S.r.l., Milan, Italy) at 37°C in a shaking water bath for 2 h. The supernatants were filtered through a 42 µm mesh filter and centrifuged; the suspension was layered over a preformed Percoll gradient in Hanks’ balanced salt solution (HBSS; Gibco BRL). The gradient was prepared from 70% to 5% Percoll (v/v) by dilutions of 90% Percoll (9 parts Percoll:1 part 10 X Hanks’) and layered in a 50-ml conical polystyrene centrifuge tube. After centrifugation of the gradient for 20 min at 1200 g, the middle layer containing a relatively uniform population of mononuclear cells was removed, washed once with DMEM, and then resuspended in medium.

Cell viability evaluated by Trypan blue exclusion was $>90\%$. Cell purity was determined by immunostaining for markers of fibroblasts (2%, antivimentin; Labsystems Helsinki, Finland), macrophages (3%, anti-61-chymotrypsin; Dako, Santa Barbara, CA, USA) and syncytiotrophoblast (2%, monoclonal antibody against low-molecular weight cytoeratin; Labsystems, Chicago, IL, USA; Kliman et al., 1986). The enriched cytotrophoblast cells (95%) were counted and the volumes adjusted so that the cell concentration was $5 \times 10^5$ cells/ml. Cells were cultured at 37°C under 5% CO$_2$ and 95% air with DMEM and 10% fetal bovine serum (FBS). The medium was changed daily.

Binding assay

For the binding assay the cell suspension was transferred to the wells of an ELISA plate. On day 3 (72 h) of culture the medium was removed and the cells incubated at room temperature for 1 h with serial protein concentrations of the IgG preparation (ranging from 0 to 400 µg/ml) in complete medium at the final volume of 100 µl. The plates were then extensively washed to remove unbound antibodies and proteins. A secondary alkaline phosphatase-conjugated anti-human IgG antibody (Sigma Chemical Co. Ltd, St Louis, MO, USA) was added to the plates. After incubation and washing, p-nitrophenylphosphate substrate (Sigma Diagnostics, St Louis, MO, USA) was used to measure the IgG binding on trophoblast cells. The OD values of the samples were read at 405 nm by a microplate photometer (Bio-Rad Platereaders Model, Bio-Rad Laboratories S.r.l., Milan, Italy).

To investigate possible interactions of low-molecular weight heparin (LMWH, Enoxaparina, Cleaxone; Rhone-Poulenc Rorer, France) or acetylsalicylic acid (AA; Sigma Immunocochemicals, St Louis, MO, USA), we studied the effects of these drugs on the binding assay. Various dilutions of LMWH (1–100 IU/ml) or AA (0.03–1.6 M) were made in 1 ml of DMEM/10% FBS. DMEM/10% FBS (100 µl) as a control or DMEM/10% FBS with different concentrations of LMWH or AA was added to the cells immediately before IgG (200 µg/ml). All assays were carried out in triplicate.

In-vitro invasion assay

To investigate the possible interactions of LMWH and AA with aPL, we studied the effects of these drugs on trophoblast cell invasiveness using a Matrigel invasion assay (Biocoat Matrigel Chamber; Sigma Immunocochemicals). The protein concentration of the IgG in the present study was 200 µg/ml. The trophoblast cells were labelled for 36 h with 10 µCi/ml $[^3]$H]TdR (Amersham Life Science, Milan, Italy) in DMEM/10% FBS. The cells were then trypsinized, washed and resuspended in complete medium at a concentration of $10^5$ cells/ml (Graham et al., 1994).

A 500-µl aliquot of the cell suspension with or without different concentrations of LMWH (5–100 IU/ml) or AA (0.03–1.6 M) was added in duplicate to the upper well of transwell chambers; 700 µl of 3T3 (American Type Culture Collection, Rockville, MD, USA) medium was then added to the lower wells. After a 72 h incubation period, the media of the upper and lower wells were removed and placed in separate tubes. The upper wells were washed once with phosphate-buffered saline (PBS) and the washings pooled with the media from the upper wells.

A 500-µl portion of a 0.01% trypsin solution in PBS was added to each lower well with the transwell in place in order to remove the cells adhering to the plastic surface. The trypsin solutions of the lower wells were pooled with the incubation media from the lower wells. The membranes were removed with a small scalpel and placed in separate tubes for the determination of radioactivity.

The fractions from each compartment of the invasion chambers as well as the Matrigel-coated filters were counted in a Beckman scintillation counter to determine associated radioactivity. The invasion index was calculated from the amount of radioactivity in the lower wells expressed as a percentage of the sum of the radioactivity in all compartments plus the membranes. Each experiment was carried out in the presence of: (i) medium alone; (ii) normal IgG (200 µg/ml); and (iii) aPL IgG (200 µg/ml).
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Figure 1. Immunoglobulin G binding to primary trophoblast cells (72 h of culture). Control patients: N1, ■; N2, ▲. Patients with antiphospholipid syndrome: N3, ▼; N4, ▼. Data are means ± SD of five experiments. Significance versus untreated cells (control IgG 0 μg/ml; optical density (OD) value = 250 ± 38): *, P < 0.05; **, P < 0.02; ***, P < 0.01.

Cell counting

The highly purified cytotrophoblasts were cultured in eight-well Nunc multidishes with coverslips (Nalge Nunc International, Naderville, IL, USA) in humidified 5% CO₂/95% air at 37°C with or without LMWH (5–100 IU/ml) or AA (0.03–1.6 M). The protein concentration of the IgG in the present study was 200 μg/ml.

At the end of culture, the media were removed, the cells on coverslips were washed twice with PBS, and then transferred to a −80°C freezer for later haematoxylin and eosin staining.

Cell counting (Shi et al., 1993) began at a random point near the middle of the coverslips where the differentiation was most obvious. The haematoxylin and eosin-stained cells were used for counting aggregated cells, which do not have intervening membranes. Cell counting was performed under a light microscope at a magnification of ×300. Aggregated cells without apparent intervening plasma membranes represent cells that are either completely, or close to being, fused.

All experiments were repeated three times on different placentas, with triplicates within each experiment. The results were expressed as the percentage of 1000 cells that aggregated with no visible intervening plasma membranes. The media of control (untreated) cells were stored at −20°C until assayed for HCG. The hormone assay was performed using a commercial radioimmunoassay kit (generously provided by Radim, Rome, Italy).

Statistical analysis

Statistical analyses were performed using a two-way analysis of variance for multiple comparisons.

Results

Antiphospholipid activity of the IgG fractions

The anticardiolipin and antiphosphatidylserine binding activities of the IgG fractions at serial protein concentrations are listed in Table II. The IgG of patients (N3, N4) retained significant levels of anticardiolipin and antiphosphatidylserine binding activity, while the two normal IgG fractions (patients N1, N2) displayed background binding.

IgG binding to trophoblast cells: effect of heparin and acetylsalicylic acid

Time-dependent trophoblast binding was identified for IgG fractions from aPL-positive sera (data not shown). The highest binding was observed at 72 h of culture, when the differentiation of the cytotrophoblast in syncytiotrophoblast is supposed to be complete. Trophoblast binding of IgG is illustrated in Figure 1. IgG from patients with APS (N3, N4) displayed clear binding at protein concentrations as low as 15 μg/ml. In contrast, IgG from controls (N1, N2) showed negligible background values.

Experiments were performed to evaluate the potential role of LMWH or AA on the trophoblast binding of IgG. IgG (200 μg/ml) was assayed in the absence and with increasing amounts of LMWH (1–100 IU/ml) or AA (0.03–1.6 M), and binding was determined using ELISA. A total of four experiments was performed.

As shown in Table III, LMWH did not reduce the IgG (patients N3, N4) binding at concentrations between 1 and 10 IU/ml, but at concentrations >25 IU/ml the binding was significantly decreased. AA did not cause any inhibition of IgG binding.

In-vitro invasion assay

Figure 2 illustrates the results of a 3-day in-vitro Matrigel invasion assay. Normal control IgG fractions (200 μg/ml; N1, N2) did not show any effect on trophoblast cell invasiveness, whereas this was significantly reduced by IgG from aPL-positive sera (N3, N4) (P < 0.01). Treatment with LMWH, from 25 IU/ml, restored placental invasiveness. In these experiments invasiveness was not affected by exogenous AA.
Table II. Binding of serial protein concentrations\(^a\) of immunoglobulin G fractions from normal patients (N1, N2) and patients with antiphospholipid syndrome (N3, N4)

<table>
<thead>
<tr>
<th>IgG (µg/ml)</th>
<th>Cardiolipin-coated plates</th>
<th>Phosphatidylserine-coated plates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N1</td>
<td>N2</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>34</td>
</tr>
<tr>
<td>90</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>130</td>
<td>70</td>
<td>81</td>
</tr>
<tr>
<td>260</td>
<td>100</td>
<td>110</td>
</tr>
</tbody>
</table>

\(^a\)Optical density values (\(×\)10\(^{-3}\)).

Table III. Low-molecular weight heparin effect on immunoglobulin G binding to primary trophoblast cells (72 h of culture)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>IgG (200 µg/ml)</th>
<th>Low-molecular weight heparin (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>N1</td>
<td>330 ± 38(^a)</td>
<td>308 ± 42</td>
</tr>
<tr>
<td>N2</td>
<td>308 ± 42</td>
<td>306 ± 32</td>
</tr>
<tr>
<td>N3</td>
<td>1380 ± 180</td>
<td>1300 ± 119</td>
</tr>
<tr>
<td>N4</td>
<td>1050 ± 98</td>
<td>1028 ± 101</td>
</tr>
</tbody>
</table>

N1, N2 = control patients.
N3, N4 = patients with antiphospholipid syndrome.

\(^a\)Optical density (OD) values (\(×\)10\(^{-3}\)). Data are mean ± SD of four experiments. Untreated cells: IgG 0 µg/ml; OD = 270 ± 35.

Low-molecular-weight heparin (IU/ml) in presence of IgG from patients with antiphospholipid syndrome versus IgG alone: \(b\), \(P < 0.05\); \(c\), \(P < 0.02\); \(d\), \(P < 0.01\).

Figure 2. Matrigel invasion by trophoblast cells. Results were calculated as means ± SD of five experiments and expressed as % of the invasion index of control (untreated) trophoblast cells. Immunoglobulin G (\(\square\), 200 µg/ml) from patients with antiphospholipid syndrome (N3, N4) significantly inhibited trophoblast invasiveness: *, \(P < 0.01\). No effect of immunoglobulin G from controls (N1, N2) on the invasion index was observed. Low-molecular-weight heparin (\(\square\), 5 IU/ml; \(\square\), 25 IU/ml; \(\square\), 100 IU/ml) starting from 25 IU/ml restored trophoblast invasiveness: §, \(P < 0.05\); §§, \(P < 0.01\).

In-vitro cytotrophoblast differentiation

Initially, the isolated cytotrophoblasts were mononuclear single cells with two or three aggregates, and complete morphological syncytial formation occurred by 72 h of culture (data not shown). Therefore, we first investigated the time-course of differentiation in order to decide when to terminate the cultures such that LMWH or AA responses could be quantified reliably.

Differentiation was shown to progress in trophoblast cultures from 24–48 h of culture (Table IV). Recent observations have suggested that syncytium formation and hormonal differentiation are separate, though parallel, events (Katsuragawa et al., 1997). To confirm the reliability of the present method to study the in-vitro trophoblast differentiation, we analysed the time-dependent HCG concentration (IU/ml) in the medium of untreated (control) cells (24 h of culture, 5 ± 1 mIU/ml; 36 h, 15 ± 2 mIU/ml; and 48 h, 40 ± 5 mIU/ml) (Table IV). Consequently, a 36-h culture period was used in subsequent experiments. When compared with control (untreated) cells, the presence of IgG (200 µg/ml) from patients with APS (N3, N4; Table IV) reduced the aggregation of cells without intervening plasma membranes by 36 h, and this continued until 48 h of culture.

LMWH (at concentrations >25 IU/ml) restored the aggregation of cells without intervening plasma membranes (Table V). However, no effect on aggregate cells without intervening plasma membranes was observed in the presence of aspirin.

Discussion

Many investigators have identified a relationship between increased concentrations of aPL and the presence of lupus anticoagulant with increased obstetrical complications, including intrauterine growth retardation, recurrent pregnancy loss, pregnancy-induced hypertension and stillbirth (Branch et al.,
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Table IV. Time dependency of the effects of immunoglobulin G fractions on trophoblast aggregation

<table>
<thead>
<tr>
<th>Duration of culture (h)</th>
<th>% Aggregated cells without intervening plasma membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTR N1 N2 N3 N4</td>
</tr>
<tr>
<td>24</td>
<td>12 ± 4a</td>
</tr>
<tr>
<td>36</td>
<td>13 ± 4a</td>
</tr>
<tr>
<td>48</td>
<td>11 ± 3a</td>
</tr>
</tbody>
</table>

CTR = control, untreated cells.
N1, N2 = IgG (200 µg/ml) obtained from normal patients.
N3, N4 = IgG (200 µg/ml) obtained from patients with antiphospholipid syndrome.
The values in each column with different letters (a and b–c) are significantly different: b, P <0.05; c, P <0.01.
aSignificant differences compared with the corresponding control and with IgG fractions obtained from normal patients (N1, N2), P <0.02.

Table V. Effects of low-molecular weight heparin (LMWH) on cellular aggregation (36 h of culture)

<table>
<thead>
<tr>
<th>LMWH (IU/ml)</th>
<th>% Aggregated cells without intervening plasma membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTR N1 N2 N3 N4</td>
</tr>
<tr>
<td>0</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>5</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>25</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>100</td>
<td>31 ± 3</td>
</tr>
</tbody>
</table>

CTR = control, untreated cells.
N1, N2 = IgG (200 µg/ml) obtained from normal patients.
N3, N4 = IgG (200 µg/ml) obtained from patients with antiphospholipid syndrome.
The values in each column with different letter (a and b–c) are significantly different: b, P <0.05; c, P <0.02.
aSignificant differences compared with the corresponding control and with IgG fractions obtained from normal patients (N1, N2), P <0.01.

Figure 3. In-vitro differentiation of primary cytotrophoblast cells (36 h of culture). Percoll gradient-purified cytotrophoblast cells were cultured, fixed and stained with haematoxylin and eosin. The micrograph shows that some of the aggregated cells do not have intervening plasma membranes, indicating that fusion was either complete or close to being complete. Scale bar = 30 µm.

1992). Although many different phospholipids have been studied, cardiolipin and phosphatidylserine have received the most attention as the phospholipids of primary importance in APS. Nonetheless, the exact pathogenic mechanism of APS-associated pregnancy loss has still to be clarified, with several reports having suggested different mechanisms, in addition to the observation that APS-associated recurrent fetal losses may be the result of placental thrombotic phenomena.

In the present study we have demonstrated that IgG fractions obtained from patients with APS, displaying both anticardio-
lipin and antiphosphatidylserine antibodies, bind to human trophoblast cells and bear an inhibitory effect on the cells’ invasiveness. Recently, Katsuragawa et al. (1997) showed that murine alloantibodies prevent in-vitro trophoblast invasion. We have now made a similar observation with autoantibodies that should recognize different epitopes and which occur spontaneously during this syndrome.

Furthermore, we have not only shown a direct interference of heparin in the IgG binding to primary trophoblast cells, but also that heparin treatment is able to restore normal trophoblast invasiveness. Even if the heparin concentrations used in this study are higher than therapeutic plasma concentrations, we should consider the present model to be an in-vitro system in which the short-term effect of this drug may be tested. This differs from the in-vivo condition, where the trophoblast tissue is exposed to a longer period of drug action.

In a previous study, we identified a failure of placental cells to respond to GnRH after 72 h of incubation in the presence of sera containing aPL. Subsequent heparin treatment of cytotorphoblast cells restored the GnRH-induced secretion of HCG (Di Simone et al., 1997). We suggested that this failure might be due to reduced syncytiotrophoblast formation and that the morphology and differentiation state of the trophoblast differed between untreated and heparin-treated cultures. In line with this hypothesis, we have now shown that heparin treatment of cytotorphoblast cells is able to restore regular differentiation. To our knowledge, this is the first demonstration of an inhibitory effect of IgG fractions from patients with APS on primary trophoblast differentiation, and of an effect by heparin on trophoblast differentiation and invasiveness.

Among the limitations of these in-vitro observations is that the situation in vivo at the feto-maternal interface is highly complex, and several pathogenic mechanisms may be present simultaneously and in the same patient. Different regimens have been proposed for the treatment of APS, including aspirin monotherapy, prednisone and aspirin, or heparin and aspirin. The success of heparin treatment on pregnancy outcome in women with APS stimulated interest on the drug’s mechanism of action. McIntyre et al. (1993) suggested direct binding of heparin to aPL and, using an ELISA, showed a decrease in aPL concentrations with increasing doses 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 ELISA. In addition, LMWH appeared more effective at pharmacological and lower concentrations than did regular heparin (Di Simone et al., 1997), suggesting that steric hindrance was not a significant problem.

The mechanism by which heparin might bind to aPL has still to be ascertained, though investigators have considered the possibility that heparin either binds to and interferes with recognition of either the anti-PL–protein complex, or binds directly with the aPL. The 54 kDa serum β2-glycoprotein (β2-GP) appears to serve as cofactor in the recognition of the putative antigens by aPL (Roubey, 1994). Either alone or in complex with anionic phospholipid, β2-GP may form an antigenic site for these antibodies (Rand et al., 1997). Findings indicate that β2-GP binds to heparin (McIntyre and Wagenknecht, 1992), which in turn might interfere with the aPL binding and thus eliminate the requirement for a cofactor in the binding reaction.

As the majority of pregnancy losses in women with APS occurred in the first trimester, attention should clearly be focused on the possible effects of aPL on embryonic implantation and placentation. Our observations might suggest that IgG from women with APS cause pregnancy loss by binding to phospholipid expressed on the invading trophoblast, thereby inhibiting placental development and, as a consequence, embryonic implantation into the endometrium. The rationale for the clinical use of heparin could be that, in addition to its anticoagulant action, it inhibits the binding of aPL, thus protecting the trophoblast phospholipids and promoting implantation in early pregnancy (Rai et al., 1997). The data of Kutteh and Ermel (1996) indicated that heparin plus low-dose aspirin provided a significantly better pregnancy outcome than low-dose aspirin alone for aPL-associated recurrent pregnancy losses. Recently, Kutteh (1996) also reported that heparin combined with aspirin is as effective as heparin alone for the treatment of pregnancy losses associated with APS. In the present study, AA caused no significant modification of in-vitro trophoblast invasiveness and differentiation.

Rai et al. (1997) suggested that low-dose aspirin might improve pregnancy outcome in women with APS by blocking the action of cyclooxygenase in platelets, thereby inhibiting platelet thromboxane synthesis and preventing thrombosis of the placental vasculature. In treating women who have autoantibodies and recurrent fetal loss with prednisone and aspirin, Laskin et al. (1997) concluded that these therapies should not be effective in preventing fetal loss, and thereby increase the risk of prematurity. However, to date, no prospective placebo-controlled trial has been carried out to compare AA treatment with placebo (Christiansen, 1995).

In conclusion, we suggest the possibility of an additional pathogenic mechanism sustained by IgG from women with APS, which could bind to human trophoblast cells and affect both in-vitro trophoblast invasiveness and differentiation. In addition, on-going studies are being performed to clarify the action of purified aPL on placental function.

References
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