Impaired expression of endometrial differentiation markers and complement regulatory proteins in patients with recurrent pregnancy loss associated with antiphospholipid syndrome

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Antiphospholipid syndrome (APS), characterized by circulating antiphospholipid (aPL) antibodies, is a major cause of early pregnancy failure and placental insufficiency. In this study, we examined whether impaired endometrial differentiation before conception contributes to the high incidence of pregnancy complications in APS. Timed secretory endometrial biopsies were obtained from a cohort of women with recurrent pregnancy loss (RPL). Real-time quantitative (RTQ)-PCR was used to determine the expression levels of transcripts that encode for decidual markers, proinflammatory cytokines and complement regulatory proteins. Expression of decidual markers such as prolactin (PRL), tissue factor (TF) and signal transducer and activator of transcription 5 (Stat5), but not insulin-like growth factor-binding protein 1 (IGFBP-1), was significantly lower in samples obtained from aPL+ patients (n = 24) when compared with aPL– group (n = 58) (P = 0.005). The abundance of transcripts encoding for interferon γ (IFNγ), tumour necrosis factor α (TNFα) or Stat1 did not differ significantly between both groups (P ≥ 0.05). However, analysis of transcripts that encode for complement regulatory proteins showed a marked decrease in decay-accelerating factor (DAF/CD55) levels in aPL+ patients (P = 0.005), which was mimicked at protein level as demonstrated by immunohistochemistry. In summary, patients with RPL have distinct endometrial gene expression profiles depending on the presence or absence of circulating aPL antibodies. In APS, impaired endometrial differentiation and lower DAF/CD55 expression before conception may compromise implantation and predispose to complement-mediated pregnancy failure.

Key words: antiphospholipid/complement/decidualization/endometrium/gene expression/miscarriage/pregnancy

Introduction

Antiphospholipid syndrome (APS) is a condition in which clinical features such as vascular thromboses, thrombocytopenia and recurrent pregnancy loss (RPL) occur in the presence of circulating antiphospholipid (aPL) antibodies (Hughes et al., 1986). APS is now recognized as the commonest cause of acquired hypercoagulability in the general population and the single most important identifiable cause of non-chromosomal RPL (Regan and Rai, 2000; Cervera et al., 2002). Moreover, some studies have linked APS to obstetric problems associated with placental insufficiency such as pre-eclampsia, fetal growth restriction, placental abruption and preterm labour (Backos et al., 1995a). Although treatment with aspirin and heparin from early pregnancy has been shown to significantly increase the live birth rate in APS (Rai et al., 1997), the incidence of severe late obstetric complications remains high (Backos et al., 1999; Stone et al., 2006). Pregnancy failure associated with APS is often attributed to thrombotic events in the fetomaternal circulation, a view seemingly reinforced by effectiveness of anticoagulant therapy in preventing early miscarriages. However, intravascular or intervillous blood clots are rarely found on histological examination of miscarriage samples from patients with APS (Sebire et al., 2002). Furthermore, recent studies have provided compelling evidence for an alternative pathological mechanism by demonstrating that aPL antibodies can target the maternal decidua and invading trophoblast directly. In vitro, aPL antibodies have been shown to bind to human trophoblast cell membranes, in both a cofactor-dependent and cofactor-independent manner and to
induce apoptosis, to decrease trophoblast fusion and to inhibit trophoblast invasiveness (Chamley et al., 1998; Bose et al., 2004; Di Simone et al., 2005; Quenby et al., 2005). Injection of human aPL antibodies in pregnant mice triggers an inflammatory cascade, characterized first by complement activation in the maternal decidua, followed by recruitment of neutrophils, expression of proinflammatory cytokines and tissue destruction (Holers et al., 2002; Girardi et al., 2003). As a consequence of these observations, the therapeutic mechanism of anticoagulant treatment in APS has also been reassessed. Several studies have now shown that heparin acts as a survival factor capable of negating the detrimental effects of aPL antibodies on human trophoblast, at least in culture (Di Simone et al., 1999; Bose et al., 2004; Bose et al., 2005). Heparin is also known to suppress natural killer cell cytotoxicity, to prevent leukocyte adhesion/influx and to antagonize interferon γ (IFNγ) signalling, a proinflammatory T-helper (Th) 1 cytokine (Yamamoto et al., 1985; Johann et al., 1995; Fritchley et al., 2000; Christopherson et al., 2002; Wan et al., 2002). Furthermore, heparin, but not other anticoagulants such as hirudin and fondaparinux, is highly effective in preventing aPL antibody-mediated fetal loss in mice by inhibiting complement activation in the maternal decidua (Girardi et al., 2004). Together, these observations suggest that heparin may prevent early pregnancy loss independently of its anticoagulant activity.

Although the murine model of APS has been highly informative, it differs from the human situation in several aspects. For example, in this model, pregnant animals are exposed to a bolus of aPL antibodies, whereas women with RPL associated with APS have chronically elevated aPL antibody levels before conception. Unlike the murine model, the human endometrium may express both TNFα and IFNγ, and it is also capable of secreting aPL antibodies before conception. The human endometrium is also capable of secreting aPL antibodies in the presence or absence of an implantation blastocyst (Brosens et al., 2002). These observations led us to speculate that the human endometrium could be a target for aPL antibodies before conception and that impaired decidualization may predispose to early pregnancy failure in women with APS. This conjecture was further supported by our previous study demonstrating that expression of decidual markers, such as prolactin (PRL) and insulin-like growth factor-binding protein 1 (IGFBP-1), in primary endometrial cultures is inhibited in the presence of recombinant human interferon γ (IFNγ) and activated protein C resistance (APCR), IgG and IgM anticardiolipin antibodies and lupus anticoagulant. aPL antibodies were assayed on at least two occasions, more than 6 weeks apart. Only persistently positive tests for either lupus anticoagulant or anticardiolipin antibodies were considered to be diagnostic for APS. The aPL patients included in this study also tested negative for all other investigations.

**Subjects and methods**

**Patient selection**

Endometrial biopsies were obtained from patients attending the St Mary’s Hospital Recurrent Miscarriage Clinic (London, UK). The study was approved by St Mary’s Hospital NHS Trust Ethics Committee, and informed written consent for the use of the tissue was obtained from each patient. All women recruited for this study had normal uterine anatomy as demonstrated by transvaginal ultrasonography, a regular menstrual cycle, normal early follicular phase gonadotrophin levels, normal androgen screen and normal peripheral blood karyotype. They were also screened for the Factor V Leiden mutation (1691G→A), activated protein C resistance (APCR), IgG and IgM anticardiolipin antibodies and lupus anticoagulant. aPL antibodies were assayed on at least two occasions, more than 6 weeks apart. Only persistently positive tests for either lupus anticoagulant or anticardiolipin antibodies were considered to be diagnostic for APS. The aPL patients included in this study also tested negative for all other investigations.

**Endometrial sampling and dating**

Participants were instructed to monitor their urinary LH peak, using a home LH ovulation predictor kit, and an endometrial biopsy was scheduled 10–12 days after the putative LH surge. This interval was chosen to minimize cycle-dependent variation in gene expression and to coincide with decidual transformation of the endometrial stroma. Endometrial tissue was fixed in formaldehyde for routine processing, paraffin embedding and histological dating, using established morphological criteria, and an additional sample was immersed in RNAlater (Ambion, Austin, TX, USA) and stored at −80°C until further analysis.

**Real-time quantitative-PCR**

Total RNA was isolated using STAT-60 (Tel-Test, Friendswood, TX, USA). DNase I treated, reversed transcribed using the Superscript First-Strand Synthesis System for RT–PCR (Invitrogen, Grand Island, NY, USA) and the resulting first-strand cDNA used as template in the real-time quantitative (RTQ)-PCR analysis. Detection of L19 and target gene expression was performed with SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 7700 Sequence Detection System (Applied Biosystems), using the relative standard curve method. L19 represents a non-regulated ribosomal gene, and its expression served as internal control and was used to normalize for variance in input cDNA. All measurements were performed in triplicate. Gene-specific primer pairs were designed using the ABI Primer Express software (Table I).

**Immunohistochemistry**

Four-micron endometrial sections, placed on 1% w/v poly-lysine slides, were treated with xylene and graded alcohol and equilibrated in PBS. Antigen retrieval was performed by pressure cooking for 4 min in sodium citrate buffer, and endogeneous peroxidase was quenched by incubating the slides in methanol containing 3% H2O2. Immunostaining was carried out using anti-human

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**Table I. Sequence of primers used for real-time quantitative (RTQ)-PCR**

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Forward (5'-3')</th>
<th>Reverse (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>L19</td>
<td>GCGGAAGGGTGACGCAAT</td>
<td>GCAGCCGGCGCAA</td>
</tr>
<tr>
<td>PRL</td>
<td>AACGCTGAGGATTTGAGGAGCAAC</td>
<td>TCAGGTGAACTCTGGTACTA</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>CGAAGGCTCTCCTATGCACCA</td>
<td>TTCTCTCGTGCTGGAAGTAAAC</td>
</tr>
<tr>
<td>TF</td>
<td>TTTTAAGAGGATAGAATCATTAGGAAACGC</td>
<td>TCCAGGTCAATCAAGGTTTTTTAAGAC</td>
</tr>
<tr>
<td>Stat5</td>
<td>CATACATCCCGCTCAGGAGTC</td>
<td>CCGCGGTTGAATGTTT</td>
</tr>
<tr>
<td>TNFα</td>
<td>TTCTCTGCCACCATCGGGTGA</td>
<td>GGAGCTGCCCCTACAGCTT</td>
</tr>
<tr>
<td>IFNγ</td>
<td>TGGTACCGCTGATAATGGAACCTTTC</td>
<td>AATTTGCTCTGATTATTTTCTTG</td>
</tr>
<tr>
<td>Stat1</td>
<td>TCCATCCCTTGAGCACTACGCT</td>
<td>GCCCATGTTGAGTCAAAG</td>
</tr>
<tr>
<td>DAF/CD55</td>
<td>TTGGCCCTTCCCCCAGAT</td>
<td>TCCCTCGGAAAATCTGACG</td>
</tr>
<tr>
<td>MCP/CD46</td>
<td>TTTCTTCCCTGGCGGCTTTC</td>
<td>AAATGTGTTGGTCCTCACA</td>
</tr>
<tr>
<td>Proteind/CD59</td>
<td>TCACATGGAACCTTTCTTACAAC</td>
<td>ACCACATATGGAACATTGCG</td>
</tr>
</tbody>
</table>

DAF, decay-accelerating factor; IFNγ, interferon γ; IGFBP-1, insulin-like growth factor-binding protein 1; MCP, membrane cofactor protein; PRL, prolactin; Stat, signal transducer and activator of transcription; TF, tissue factor; TNFα, tumour necrosis factor α.
CD55/decay-accelerating factor (DAF) antibody (R&D Systems, Minneapolis, MN, USA) (diluted at 1:50), biotinylated anti-goat IgG (diluted at 1:100) and peroxidase-labelled streptavidin (ABC Kit, Vector Laboratories, Burlingame, CA, USA). In control slides, the primary antibody was omitted. Counterstaining was performed with haematoxylin (Sigma, St. Louis, MO, USA).

Immunostaining was assessed, by a histopathologist blinded to the clinical history and results of other assays, using a semiquantitative histological scoring system (HSCORE) as described previously (Young et al., 2002). Briefly, HSCORE was calculated using the equation $HSCORE = \sum P_i \times (I + 1)$, where $I$ represents staining intensity (1 = weak, 2 = moderate and 3 = strong) and $P_i$ is the percentage of stained epithelial cells for each intensity.

**Statistical analysis**

Data were analysed using the Statistical Package for Social Sciences software, version 12.0 (SPSS, Chicago, IL, USA). Because normality and/or homoscedasticity were not observed, statistical analysis was performed using the non-parametric Mann–Whitney U-test to compare the levels of different transcripts between different patient populations. The Mann–Whitney U-test was also used to compare the CD55/DAF HSCOREs between patients with or without the APS in different endometrial compartments. A P-value <0.05 was considered statistically significant.

**Results**

**Expression of decidual markers**

Endometrial biopsies were obtained from 112 cycling patients with RPL, but six samples were discarded because of insufficient tissue. Of the remaining 106 samples, 82 were histologically confirmed to be between cycle days 22 and 26 and used for further analysis. Twenty-four samples were from aPL+ patients and 58 from women with no evidence of APS (aPL−). The mean age, number of previous miscarriages and number of previous live births did not differ significantly between both groups ($P > 0.05$; Table II).

RTQ-PCR analysis was used to determine the relative expression levels of transcripts that encode for three major endometrial differentiation markers: PRL, IGFBP-1 and tissue factor (TF) (Gellersen and Brosens, 2003). As shown in Figure 1, endometrial PRL mRNA expression was approximately 2.5-fold lower in the aPL+ group [median 2.0 arbitrary units (a.u.), SD 4.0] when compared with aPL− patients [median 5.4 a.u., SD 11.9; $P = 0.026$]. TF transcripts were also less abundant in endometrial samples obtained from aPL+ women (median 5.9 a.u., SD 8.2) compared with aPL− women (median 10.0 a.u., SD 22.7) ($P = 0.039$). Although there was a trend towards lower IGFBP-1 levels in the aPL+ group (median 0.15 a.u., SD 0.9 versus median 0.35 a.u., SD 63.2, respectively; $P = 0.12$), this did not reach statistical significance because of the very large sample-to-sample variation in expression levels. Notably, none of the patients in the APS group had IGFBP-1 mRNA levels above 3.5 a.u. compared with 11 (19%) aPL− patients. Activation of Stat5, a member of the Stat signal transducers and activators of transcription) family of latent cytoplasmic transcription factors, is essential for the expression of decidual marker genes in human endometrial stromal cells (Mak et al., 2002; Gellersen and Brosens, 2003). As shown in Figure 1, Stat5 mRNA levels mimicked those of PRL and TF in that they were significantly lower in the aPL+ group (median 0.66 a.u., SD 1.0 versus median 1.1 a.u., SD 1.6; $P = 0.013$).

**Expression of inflammatory mediators**

Injection of human aPL antibodies in the murine APS model elicits a profound inflammatory response at the fetal–maternal interface (Girardi et al., 2006). Furthermore, proinflammatory Th1 cytokines such as tumour necrosis factor $\alpha$ (TNF$\alpha$) and IFN$\gamma$ are known potent inhibitors of the decidual response (Gellersen and Brosens, 2003). This prompted us to examine the abundance of TNF$\alpha$ and IFN$\gamma$ transcript levels in endometrial samples from aPL+ and aPL− patients. As shown in Figure 2, neither the levels of IFN$\gamma$ mRNA (median 1.9 a.u., SD 4.7 versus median 1.7 a.u., SD 8.9; $P = 0.7$) nor the levels of TNF$\alpha$ mRNA (median 0.3 a.u., SD 0.5 versus median 0.5 a.u., SD 2.5; $P = 0.05$) differed significantly between both groups. IFN$\gamma$ has been shown to antagonize progesterone responses in endometrial stromal cells through induction and activation of Stat1, another member of the Stat family of transcription factors (Zoumpoulidou et al., 2004). However, Stat1 mRNA levels in aPL+ patients were comparable with those of aPL− women (median 1.6 a.u., SD 2.8 versus median 2.5 a.u., SD 3.9; $P = 0.1$).

**Expression of transcripts that encode for complement regulatory proteins**

Next, we examined the expression of transcripts that encode for three complement regulatory proteins: membrane cofactor protein (MCP/CD46), DAF/CD55 and protectin (protectin/CD59). No significant differences were found in the endometrial expression levels of either MCP/CD46 or protectin/CD59 transcripts between aPL+ and aPL− patients as shown in Figure 3. In contrast, transcripts encoding DAF/CD55 were found to be markedly lower in women with APS (median 0.37 a.u., SD 3.0 versus median 0.9 a.u., SD 2.6; $P = 0.005$).

Amongst all the transcripts examined, DAF/CD55 mRNA levels discriminated best between the two patient groups. To further validate this finding, we examined DAF/CD55 protein expression by immunohistochemistry in 30 tissue samples. As shown in Figure 4A, DAF/CD55 immunoreactivity was largely confined to the endometrial epithelial cells with little or no staining in the stromal compartment. A semiquantitative analysis of 17 aPL− and 13 aPL+ endometrial samples using the HSCORE method is shown in Figure 4B. In agreement with the mRNA analysis, DAF/CD55 protein expression was lower in luminal as well as in glandular endometrial epithelial cells in aPL+ samples ($P = 0.008$ and $P = 0.04$, respectively).

**Endometrial gene expression in aPL+ patients**

We further interrogated the expression data of the aPL+ group in order to determine whether endometrial transcript levels differ between patients who never had a live birth (primary RPL) and those who had one or more previous live births (secondary RPL). As summarized in Table III, none of the endometrial transcripts analysed were differentially expressed, except for TF mRNA expression which was lower in patients with secondary RPL ($P = 0.04$). Comparison of samples from patients with less than five miscarriages and those with five or more consecutive losses also yielded only one differentially expressed transcript. As summarized in Table IV, the abundance of TNF$\alpha$ mRNA was somewhat, albeit significantly, lower in women with a high number of RPL ($P = 0.04$).

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**Table II. Demographic details of study population**

<table>
<thead>
<tr>
<th></th>
<th>aPL− ($n = 58$)</th>
<th>aPL+ ($n = 24$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.95 (0.57)</td>
<td>35.08 (0.96)</td>
</tr>
<tr>
<td>Number of previous live births</td>
<td>0.36 (0.08)</td>
<td>0.62 (0.16)</td>
</tr>
<tr>
<td>Number of miscarriages</td>
<td>4.45 (0.29)</td>
<td>4.83 (0.48)</td>
</tr>
</tbody>
</table>

APL, antiphospholipid.

The data shown are expressed as mean (SEM).
Figure 1. Real-time quantitative (RTQ)-PCR analysis of prolactin (PRL), insulin-like growth factor-binding protein 1 (IGFBP-1), tissue factor (TF) and signal transducer and activator of transcription 5 (Stat5) mRNAs in timed endometrial biopsy samples (days 22–26) from patients with recurrent pregnancy loss (RPL). The abundance of each transcript was normalized to L19 and expressed in arbitrary units (a.u.). The antiphospholipid (aPL+) group comprises samples obtained from aPL+ patients, whereas aPL– denotes samples from aPL– patients.

Figure 2. Real-time quantitative (RTQ)-PCR analysis of interferon \(\gamma\) (IFN\(\gamma\)), signal transducer and activator of transcription 1 (Stat1) and tumour necrosis factor \(\alpha\) (TNF\(\alpha\)) mRNAs in timed endometrial biopsy samples (days 22–26) from patients with recurrent pregnancy loss (RPL). The abundance of each transcript was normalized to L19 and expressed in arbitrary units (a.u.). The antiphospholipid (aPL+) group comprises samples obtained from patients with antiphospholipid syndrome (APS), whereas aPL– denotes biopsies from patients with unexplained RPL.
Endometrial differentiation in APS

Discussion

The post-ovulatory rise in progesterone levels triggers a co-ordinated programme of endometrial differentiation, characterized first by secretory transformation of the glands followed by the influx of specialized uterine natural killer (uNK) cells, differentiation of endometrial stromal cells into decidual cells and remodelling of the spiral arteries (Brosens et al., 2002). Decidualization continues in pregnancy, and the extent of this process appears to correlate with the degree of trophoblast invasion in various species (Ramsey et al., 2000).
Table IV. Endometrial transcript levels in relationship to the number of miscarriages

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Number of miscarriages</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 or 4 (n = 40)</td>
<td>≥5 (n = 18)</td>
</tr>
<tr>
<td>PRL</td>
<td>5.4 (13.3)</td>
<td>5.5 (7.8)</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>0.4 (75.2)</td>
<td>0.2 (5.5)</td>
</tr>
<tr>
<td>TF</td>
<td>10.1 (26.6)</td>
<td>9.7 (6.9)</td>
</tr>
<tr>
<td>Stat5</td>
<td>1.2 (1.8)</td>
<td>0.8 (1.2)</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.5 (2.9)</td>
<td>0.4 (0.4)</td>
</tr>
<tr>
<td>IFNγ</td>
<td>1.3 (40.3)</td>
<td>1.7 (5.8)</td>
</tr>
<tr>
<td>Stat1</td>
<td>2.7 (4.4)</td>
<td>2.1 (2.3)</td>
</tr>
<tr>
<td>DAF/CD55</td>
<td>1.0 (2.9)</td>
<td>0.9 (1.6)</td>
</tr>
<tr>
<td>MCP/CD46</td>
<td>1.8 (2.9)</td>
<td>2.1 (1.2)</td>
</tr>
<tr>
<td>Proteclin/CD59</td>
<td>1.3 (1.0)</td>
<td>1.3 (0.8)</td>
</tr>
</tbody>
</table>

DAF, decay-accelerating factor; IFNγ, interferon γ; IGFBP-1, insulin-like growth factor-binding protein 1; MCP, membrane cofactor protein; PRL, prolactin; Stat, signal transducer and activator of transcription; TF, tissue factor; TNFα, tumour necrosis factor α.

The data shown are expressed as median (SD). Transcript levels are expressed in arbitrary units.

1976). It is now well established that decidual transformation of endometrial stromal cells involves extensive and sequential reprogramming of functionally related families of genes (Brar et al., 2001; Tierney et al., 2003; Talbi et al., 2006), many of which are involved in the regulation and protection of the invading semiallogenic trophoblast. For instance, decidualizing cells secrete factors such as macrophage inflammatory protein 1B, interleukin (IL)-11, IL-15 and PRL that provide the chemotactic, proliferative and differentiating signals for uNK cells (Gubbay et al., 2002; Kitaya et al., 2003; Dimitriadis et al., 2005). These innate immune cells are not only involved in conferring immunotolerance towards invading trophoblast but also play a role in the remodelling of the spiral arteries (Ashkar et al., 2000; Moffett and Loke, 2004). By producing growth factors, binding proteins such as IGFBP-1 and extracellular matrix components, decidual cells further regulate co-ordinated trophoblast invasion and differentiation (Aplin et al., 1988; Hills et al., 2004). Furthermore, differentiating stromal cells surrounding the spiral arteries highly express TF, the initiator of the extrinsic coagulation pathway, and plasminogen activator inhibitor-1, a fibrinolysis inhibitor, emphasizing their primary role in maintaining vascular stability before menstruation and during endovascular trophoblast invasion (Schatz et al., 2003).

This study demonstrates for the first time that expression of decidual markers, such as PRL and TF, is significantly lower in women with APS when compared with patients with unexplained RPL. Although the abundance of IGFBP-1 transcripts did not differ significantly between both groups, it should be noted that none of the aPL+ patients had levels above 3.5 a.u. compared with one in five in the aPL− group. Interestingly, a recent study demonstrated that IGFBP-1 expression by differentiating endometrial stromal cells is particularly sensitive to mechanical stretch (Harada et al., 2006). Asymmetrical subendometrial contraction waves are a feature of the secretory phase and may account for the large inter-patient variation in IGFBP-1 mRNA levels.

Difficulties in recruitment precluded the inclusion in this study of a matched control group of women with proven fertility. However, in our experience the prospective live birth rate in the unexplained group is high, approximately 60%. This suggests that a much larger study is needed to define differences in endometrial gene expression profile between women with unexplained RPL and fertile controls. This notion is further supported by the observation that amongst the ten transcripts examined, only TF mRNA levels differed significantly between patients with primary and secondary unexplained RPL. Similarly, only TNFα mRNA expression was significantly different between aPL+ patients with three or four miscarriages compared with those with five or more consecutive losses. The lower level of endometrial TNFα transcripts in women with a high number of consecutive pregnancy losses is rather surprising as there is a wealth of data indicating that a preponderance of proinflammatory Th1 cytokines, such as TNFα and IFNγ, predisposes to pregnancy loss and late pregnancy complications such as pre-eclampsia (Banerjee et al., 2005; Lee et al., 2005).

The mechanism whereby aPL antibodies compromise endometrial differentiation is as yet unclear. One possibility is that ovarian steroidogenesis is impaired in APS patients, resulting in either a delayed rise in post-ovulatory progesterone or lower circulating levels. This warrants further investigation, although we are not aware of any evidence in support of this hypothesis. In analogy with the murine model of APS, aPL antibodies may cause a chronic inflammatory response in the endometrium. This is most unlikely as there was no histological evidence of chronic inflammation in aPL+ endometrial samples nor did we find altered expression of TNFα, IFNγ or Stat1 transcript levels between both groups. Finally, aPL antibodies could target endometrial cells directly, thereby interfering with signal transduction pathways involved in differentiation through a mechanism yet unknown. Previously, we have shown that aPL antibodies inhibit decidualization of primary endometrial cultures by attenuating phosphorylation, activation and nuclear translocation of Stat5 (Mak et al., 2002). The observation that APS is associated with lower endometrial Stat5 mRNA expression in vivo further implicates this transcription factor in aPL antibody-mediated pathobiology.

The complement system plays a critical role in the innate immune responses at the fetal–maternal interface by protecting the developing conceptus against infectious agents and by removing immune complexes and apoptotic cells. Furthermore, recent studies have indicated that controlled local complement activation promotes embryonic development and is involved in the physiological remodelling of the spiral arteries (Xu et al., 2004; Girardi et al., 2006). To maintain tissue homeostasis, fetal trophoblast and maternal tissues express the complement regulatory proteins DAF/CD55, MCP/CD46 and proteclin/CD59, which function to prevent complement-mediated cell lysis. Notably, microarray studies have shown that complement regulatory proteins are amongst the most highly regulated genes in human endometrium, and in particular the expression of DAF/CD55 increases dramatically in mid-secretory endometrium (Young et al., 2002; Mirkin et al., 2005; Talbi et al., 2006). Gene ablation studies in mice have provided unequivocal evidence for the importance of local complement regulation in normal and pathological pregnancies. Mice deficient in Crry expression, a membrane-bound complement regulatory protein with functional homology to DAF/CD55 and MCP/CD46, exhibit progressive embryonic lethality. However, this phenotype is entirely rescued when heterozygote mice are inter-crossed with complement C3-deficient animals to generate C3/Crry double-knockout embryos (Xu et al., 2000). Conversely, human aPL antibodies fail to trigger embryo loss or fetal growth restriction in pregnant mice deficient in either C3 or C5, which is downstream of C3 in the complement activation cascade (Holers et al., 2002; Girardi et al., 2003).

It has been shown that the degree of cellular protection against complement-mediated lysis is proportional to the level of DAF/CD55 expression in a variety of human cells (Young et al., 2002). Our observation that APS is associated with decreased endometrial DAF/CD55 mRNA levels and immunoreactivity points towards increased vulnerability to complement-mediated cell damage during the process of trophoblast invasion and placenta formation. DAF/CD55 expression in endometrial epithelial cells during the mid- and late-secretory
phases of the cycle is not thought to be under direct progesterone control but regulated by local expression of epidermal growth factor (EGF) and heparin-binding EGF (HB-EGF) (Young et al., 2002). These growth factors are abundantly expressed by differentiating stromal cells (Chobotova et al., 2005), suggesting an intricate link between the impaired expression of decidual markers and lower DAF levels.

There is a growing consensus that aPL antibody testing in unselected women does not predict pregnancy outcome. This view is largely based on studies involving IVF patients (Practice Committee of the American Society for Reproductive Medicine, 2004; Buckingham et al., 2005). However, in addition to the supra-physiological levels of ovarian hormones, most IVF patients receive exogenous progesterone support, which potentially negates the endometrial defects associated with aPL antibodies. Alternatively, it is possible that specific somatic mutations in aPL antibodies structure are required to render them pathogenic in feto–maternal tissues. If so, analysis of endometrial gene expression before conception may be useful in identifying those patients with raised aPL antibodies levels at risk of subsequent pregnancy failure, pre-eclampsia or fetal growth restriction.

In summary, this study demonstrates that endometrial gene expression during the secretory phase of the menstrual cycle differs between patients with RPL, dependent upon the presence or absence of circulating aPL antibodies. Impaired decidualization and lower endometrial DAF/CD55 expression in non-pregnant women with APS are not associated with a local inflammatory response but may predispose to complement-mediated tissue damage during pregnancy. Our results also indicate that compounds capable of promoting endometrial differentiation, such as progesterone, hCG and phosphodiesterase inhibitors (Gellerson and Brosens, 2003; Bartsch et al., 2004), may be useful in the management of RPL associated with APS, especially if conventional anticoagulation therapy has shown to be ineffective.

Acknowledgements

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References


Endometrial differentiation in APS


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