Reactivity against phospholipids during pregnancy

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Antibodies against phospholipids (PLa) are often thought to be associated with the development of activated protein C (APC) resistance. In the present study, PLa were followed throughout 29 healthy pregnancies and compared to APC resistance. The level of PLa did not change during pregnancy [6.9 ± 3.7 GPL week 12 versus 6.3 ± 2.8 GPL week 37; 3.3 ± 1.8 MPL versus 3.2 ± 1.5 MPL; and lupus anticoagulant (LA) coefficient 0.99 ± 0.11 versus 0.94 ± 0.09], in contrast to the APC resistance, which was suppressed (0.96 ± 0.22 versus 0.78 ± 0.13, P < 0.05), but both parameters elevated after delivery (up to 8.7 ± 4.2 GPL; 1.13 ± 0.1 LA coefficient; 1.11 ± 0.22 nAPC ratio; P < 0.05). Three women possessed PLa (1 CLa IgG + IgM; 1 IgG CLa + PSa + PEa; 1 PSa), no LA activity was registered. In the PLa-positive women, dynamics of the nAPC ratio during pregnancy was not related to PLa.

Key words: antiphospholipid antibodies/APC resistance/pregnancy

Introduction

Antibodies against phospholipids (PLa) are clinically associated with thromboembolic events, pregnancy complications (e.g. repeated fetal loss, placental insufficiency and fetal growth retardation, pre-eclampsia) and thrombocytopenia (McNail et al., 1991; Gleicher et al., 1993). The pathogenic effect of PLa is often related to disturbances of the protein C anticoagulant pathway (reviewed by Cines and McGrae, 1995). It has been observed that some PLa bind directly to activated protein C (APC), to complexes between phospholipids and APC, 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. However, the ability of PLa to diminish phospholipid-dependent inactivation of factor Va by APC seems to be the most probable mechanism contributing to thrombosis. A low anticoagulant effect of APC was termed APC resistance (Dahlibick et al., 1993; Bertina et al., 1994) and was frequently found in PLa-positive individuals (Bokarewa et al., 1995). It has recently been shown that the response to APC decreases gradually in the course of pregnancy, with the development of transitory APC resistance in about a half of cases (Cumming et al., 1995; Schilt et al., 1996; Bokarewa et al., 1997). The effect was related neither to thrombosis history nor to the presence of the Arg506-Gln mutation in factor V (Bokarewa et al., 1996).

In the present study, we addressed the question of whether a change in the reactivity against phospholipids might give rise to APC resistance during pregnancy.

Material and methods

Twenty-nine pregnant women aged 21–37 years (mean 28.8) visiting the maternal care unit affiliated with Karolinska Hospital, having no history of miscarriages or thromboembolic events, were included in the study. None of the women carried the Arg506-Gln mutation in factor V. Samples were taken consecutively at weeks 12, 20, 28, 32, and 37 of gestation and after delivery (range 7–21 weeks).

The study was approved by the Ethics Committee of Karolinska Hospital.

PLa were determined as antibodies to cardiolipin (CLa), phosphatidylserine (PSa), phosphatidylethanolamine (PEa) and phosphatidylcholine (PCa) by an ELISA procedure following the recommendations of the Standardisation Workshop (Harris, 1990). The amount of CLa was expressed as standard units of IgG or IgM (1 IU/ml = 1 µg/ml of antibodies). The absence of an international standard for PSa, PEa and PCa permitted the calculation of the results by a semi-quantitative method. The cut-off level for PLa was determined as 95% confidence interval, and as 3 SD above the mean value of 100 controls (aged 18–45 years). In both cases, values >17 IU/ml for IgG CLa and >11 IU/ml for IgM CLa were estimated as positive. For PSa, PEa and PCa, the values of serial dilution of a highly positive sample were used as standard.

Lupus anticoagulant (LA) was investigated by dilute Russell’s Viper Venom Time (dRVVT; Biopool, Umeå, Sweden) in the 1 + 1 mixture of test sample and pooled normal plasma. A ratio of clotting times between the test mixture and the pooled normal plasma (LA coefficient) >1.3 was taken as a positive result.

The response to activated protein C was investigated using the APC resistance kit (Chromogenix AB, Möln达尔, Sweden) and represented as a ratio of the activated partial thromboplastin time (aPTT; a surface induced coagulation procedure) obtained with the addition of APC and without it. A normalized APC ratio (nAPC ratio) ≤0.75 indicated APC resistance.

For statistical evaluation of the results, the paired Student’s t-test and Spearman’s correlation coefficient were computed. P values ≤0.05 were considered significant.

Results

The results of PLa determination in the six consecutive samples are given in Table I. They revealed only minimal
M.Bokarewa, M.Wramsby and K.Bremme

Figure 1. Changes of reactivity against cardiolipin (CLA) and nAPC ratio during pregnancy in PLa-positive women. X-axis: sampling points with respect to gestation age (week of pregnancy). Y-axis (to the left): response to activated protein C expressed as a normalized APC ratio (nAPC ratio/aPTT with APC/aPTT). APC resistance is registered if nAPC ratio < 0.75. YY-axis (to the right): reactivity against cardiolipin (CLA), expressed as standard PL units (GPL for IgG CLa and MPL for IgM CLa). Levels exceeding 19 GPL and 11 MPL are considered positive for CLa.

Table I. Level of PLa and response to activated protein C in healthy pregnant women. Results were considered positive if IgG CLa > 17 IU/ml, IgM CLa > 11 IU/ml, LA coefficient > 1.30

<table>
<thead>
<tr>
<th>PLa type</th>
<th>IgG CLa (IU/ml)</th>
<th>IgM CLa (IU/ml)</th>
<th>LA coefficient</th>
<th>APC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women (n = 29)</td>
<td></td>
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<tr>
<td>Week 12</td>
<td>6.4 ± 3.1</td>
<td>3.2 ± 1.8</td>
<td>0.97 ± 0.11</td>
<td>0.96 ± 0.22</td>
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<tr>
<td>Week 20</td>
<td>6.0 ± 3.2</td>
<td>2.8 ± 1.3</td>
<td>0.98 ± 0.13</td>
<td>0.89 ± 0.18</td>
</tr>
<tr>
<td>Week 28</td>
<td>6.2 ± 2.8</td>
<td>3.0 ± 2.0</td>
<td>1.02 ± 0.19</td>
<td>0.80 ± 0.18</td>
</tr>
<tr>
<td>Week 32</td>
<td>6.5 ± 3.0</td>
<td>3.4 ± 2.1</td>
<td>1.05 ± 0.18</td>
<td>0.78 ± 0.13</td>
</tr>
<tr>
<td>Week 37</td>
<td>6.3 ± 3.2</td>
<td>2.9 ± 2.0</td>
<td>0.94 ± 0.09</td>
<td>0.78 ± 0.13</td>
</tr>
<tr>
<td>Post partum</td>
<td>8.7 ± 4.2</td>
<td>3.4 ± 1.7</td>
<td>1.13 ± 0.10</td>
<td>1.11 ± 0.22</td>
</tr>
</tbody>
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CLA = antibodies to cardiolipin; LA = lupus anticoagulant.

fluctuation of the mean values during pregnancy (IgG CLa 6.4 ± 3.1 at week 12 versus 6.3 ± 3.2 at week 37; IgM CLa 3.2 ± 1.8 versus 2.9 ± 2.0; and LA coefficient 0.97 ± 0.11 versus 0.94 ± 0.09), followed by an increase in IgG CLa and LA coefficient in the post-partum sample (8.7 ± 4.2 GPL and 1.13 ± 0.10 respectively; \( P < 0.05 \)). One woman turned positive for IgG CLa (from 15 to 21 GPL), but no LA activity (LA coefficient > 1.3) was registered. PSc, PEa and PCa were determined as the IgG isotype. Optical density in most of the samples correlated to IgG CLa (Spearman test, \( 0.54 < r^2 < 0.71; \) \( P < 0.05 \)).

Discussion

Pregnancy is a natural model for transitory APC resistance. As we have observed previously, a decrease in APC ratio during pregnancy was not related to the mutation in the factor V gene, and occurred in healthy women as well as in those with a history of thrombosis (Bokarewa et al., 1996, 1997).

Transitory reduction of APC response correlated inversely to the level of factor VIII. The increased level of factor VIII was not considered a cause of APC resistance. The low APC ratio preceded an increase in factor VIII, which suggested that high levels of factor VIII during pregnancy could have been a result of its protection from inactivation and its accumulation in the circulation (Bokarewa et al., 1997).

The determination of PLa levels throughout pregnancy showed that the decrease in the response to APC was not dependent on PLa. Lack of correlation between PLa level and APC ratio was found either in women negative or in women positive for PLa. The dynamics of PLa and nAPC ratio in the women who revealed positive results for CLa at pregnancy week 12 is shown in Figure 1. One woman (case A) had PLa of IgG type reactive with CL, PS and PE. In this woman, titres of PLa showed only a slight variation during pregnancy (15–19 IU/ml) with a significant increase after delivery (to 26 IU/ml). The nAPC ratio in this woman was low throughout pregnancy (0.47–0.68) but increased to a normal level after delivery (0.91). The other woman (case B) was positive for IgG (29 GPL) and IgM (20 MPL) CLa. The level of antibodies fell already in the next sample, taken at week 20, to 9 IU/ml of IgG, and to 14 MPL IU/ml of IgM. The achieved level sustained during pregnancy, but elevated again in the post-partum sample (17 IU/ml of IgG). The third woman was positive for PSc and had low levels of CLa in all the samples (data not shown). The response to APC in this woman followed the pattern of the main group (see Table I). It was above the level of APC resistance in all the samples (nAPC ratio 0.99, 0.82, 0.80, 0.76, 0.82 and 1.04 respectively). The response to APC showed no correlation to PLa level even in the PLa-positive individuals, which implies that autoimmune reaction against phospholipids and APC response
have different regulation mechanisms in healthy women. PLa do not contribute to the suppression of the response to APC observed during pregnancy. Similar conclusions were drawn after the comparison of APC response in thrombophilic women (Bokarewa et al., 1995; Bokarewa and Bremme, 1998). APC ratio showed no decrease in the presence of CLa or PSA, and was not correlated with the level of PLa. PLa possessing LA activity could be, however, the only group of PLa contributing to the development of acquired APC resistance.

The relationship between PLa and pregnancy loss has been addressed in many studies (reviewed by McNail et al., 1991; Gleicher et al., 1993). PLa is a prevalent finding in women with repeated fetal loss as compared to healthy pregnant women. Attempts to predict the pregnancy outcome following natural changes in the level of PLa gave inconsistent results, however (Rix et al., 1992; Ober et al., 1993; Pattison et al., 1993; Lynch et al., 1995). The verification of PLa seemed to be more important for women who had already experienced fetal loss than in healthy or primary pregnant women. Together with this, the suppression of PLa during the treatment was not always a reliable indicator of a successful outcome in women with repeated fetal loss (Kwak et al., 1994; Melk et al., 1995; Branch et al., 1997). PLa in healthy pregnant women correlated seldom to an adverse pregnancy outcome. The observations presented in our study are in concordance with this postulate.

All three women positive for PLa had a successful pregnancy outcome. This was true even for woman A, with a combination of unfavourable findings such as polyreactivity of PLa, antibodies against prothrombin (not shown) and a strong resistance to APC, comparable to homozygous carriers of the Arg506-Gln mutation in factor V.

Reviews of literature analysing the immunological aspects of recurrent miscarriage have concluded that autoantibodies probably do not cause the miscarriage, but merely accompany the event (Bulletti et al., 1996; Christiansen, 1996). To understand the reasons and mechanisms driving the production of autoantibodies (PLa, in particular) in healthy subjects is one of the ways to explain their role in the human organism. It has been suggested that the increased reactivity with phospholipids may be a non-specific autoimmune reaction of the woman to the exposure of self-antigens during pregnancy (Gleicher et al., 1993). In favour of the suggestion is a relatively high incidence of CLa in women with successful pregnancies compared to those who have never been pregnant (Parke et al., 1991), and a difference in the isotype pattern of PLa production between these groups (Ober et al., 1993). The role of PLa as indicators of non-functioning cells enhancing their elimination from the bloodstream is also possible (Carsiola-Rosen et al., 1996). The increase of PLa, following recurrent miscarriage and associated with the excessive exposure of distracted placental or fetal tissue, may well reflect the nature of PLa.

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References

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PLa in healthy women