Defective endovascular trophoblast invasion in primary antiphospholipid antibody syndrome-associated early pregnancy failure

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BACKGROUND: Primary antiphospholipid antibody syndrome (PAPS) is an established cause of recurrent pregnancy loss, traditionally presumed to be due to ‘intraplacental thromboses’. This study examines products of conception (POC) from early pregnancy failures to investigate the mechanism of pregnancy loss. METHODS: POC from patients attending a recurrent miscarriage clinic and from terminations of pregnancy for non-medical reasons were examined histologically with particular regard to the presence or absence of vascular or intervillous thromboses and decidual endovascular trophoblast invasion. RESULTS: There were 31 PAPS-positive, 50 PAPS-negative, 34 aneuploid and 20 control cases at 6–14 weeks gestation. Villous morphology and frequency of intervillous thrombosis were not different among groups. Normal intradecidual endovascular trophoblast invasion was identified significantly less frequently in PAPS cases (24%), compared with controls (75%), aneuploid (53%), or PAPS cases (61%; Z = -3.0, P < 0.01). In all cases there was apparently normal interstitial extravillous trophoblast invasion. CONCLUSIONS: Defective decidual endovascular trophoblast invasion, rather than excessive intervillous thrombosis, is the most frequent histological abnormality in PAPS-associated early pregnancy loss. Furthermore, endovascular trophoblast invasion is not significantly reduced in the majority of fetal aneuploidy-associated pregnancy failures.

Key words: antiphospholipid antibodies/antiphospholipid antibody syndrome/endovascular trophoblast/pregnancy loss/recurrent miscarriage

Introduction

Primary antiphospholipid antibody syndrome (PAPS) is an established cause of recurrent pregnancy loss (Clifford et al., 1994; Rai et al., 1995; Rai and Regan, 1998). Women with PAPS may present with a history of recurrent early pregnancy loss and/or pregnancies affected by late complications including pre-eclampsia and severe intrauterine growth restriction (Rai and Regan, 1997; Backos et al., 1999). The mechanism of the pregnancy loss and other complications has traditionally been presumed a consequence of ‘intraplacental thromboses’, since antiphospholipid antibodies (aPLs) have been associated with intravascular thrombosis in other organs (Voland and Main, 1995). The aim of this study was to examine histologically the products of conception (POC) obtained from women with and without PAPS suffering first trimester miscarriage attending a recurrent miscarriage clinic, in order to identify a potential mechanism underlying the early pregnancy failures.

Materials and methods

A computer-based search was conducted to identify all first trimester POC examined histologically in a single centre from first trimester pregnancy losses in patients attending a dedicated recurrent miscarriage clinic. All patients from the recurrent miscarriage clinic had been investigated according to the clinic’s published protocol (Regan, 1992; Clifford et al., 1994; Rai et al., 1996). The phospholipid antibody status of all women was known and the fetal karyotype of the failed pregnancies was available in all cases. In addition to the cases from the recurrent miscarriage clinic, POC were additionally obtained from 20 women with no history of recurrent pregnancy loss, who underwent termination of pregnancy for social reasons, these acting as a control group. The study was approved by the local ethics committee and written informed consent was obtained from each woman. Histological sections stained with haematoxylin and eosin were reviewed by a pathologist, blinded to the clinical details, with particular regard to the presence or absence of vascular or intervillous thromboses and decidual endovascular trophoblast invasion in cases in which implantation site fragments were present (Figure 1). An implantation site was identified by the presence of interstitial extravillous trophoblast invasion surrounding decidual vessels. In each case, all decidual vessels present were examined and cases were classified as endovascular trophoblast invasion present or absent, according to whether any intradecidual vessels demonstrated definite endovascular trophoblast invasion/‘plugging’ (Figure 1). The pregnancy loss cases were divided into groups for analysis: those with a fetal chromosomal abnormality (aneuploid), those with PAPS (PAPS+) and normal karyotype, and
those without PAPS (PAPS−) and normal karyotype. Comparison of differences in frequency of the histological findings between the groups was carried out using comparison of proportions test.

**Results**

A total of 135 cases was included in the study: 31 PAPS+, 50 PAPS− and 34 aneuploid recurrent miscarriage cases (including one each of trisomies 7, 8, 9, 10, 14, one XO, one complex mosaicism, two trisomy 15, two trisomy 18, three trisomy 13, six trisomy 16, two trisomy 20, two trisomy 21, three trisomy 22 and seven triploidy), and 20 terminations of pregnancy in women with no history of recurrent pregnancy loss. The median gestational age at diagnosis of pregnancy failure was 8 weeks (range 7–10), 9 weeks (range 6–12), 9 weeks (range 6–14) and 9 weeks (range 6–14) for each of the groups respectively. In all cases, scattered small amounts of perivillous fibrinoid material could be identified irrespective of gestational age, but there was no subjective increase in perivillous or intervillous fibrin deposition in the PAPS+ cases. In no case from any of the study groups was significant intervillous or intravascular thrombosis identified. There were no major differences in gross villous morphology among any of the groups.

Implantation site fragments were present in 80% (16/20) of the cases from terminations of pregnancy and normal endovascular trophoblast invasion with ‘plugging’ of decidual vessels was identified in only 24% (4/17; Figure 1). Normal endovascular trophoblast invasion was identified significantly less frequently in the PAPS+ cases compared with PAPS− cases and terminations of pregnancy (Z = −2.4, P = 0.02 and Z = −3.0, P < 0.01 respectively). There was no significant difference in the frequency of endovascular trophoblast invasion identified between terminations of pregnancy and PAPS− POC [Z = 0.96, P = not significant (0.34)]. Implantation site fragments were present in 17 of the 34 (50%) aneuploid cases and endovascular trophoblast invasion was present in nine of the 17 (53%). This was not significantly different from the chromosomally normal PAPS− group [Z = −0.51, P = not significant (0.61); Table I]. In all cases in which decidual implantation site fragments were present, there was apparently normal interstitial extravillous trophoblast invasion, the difference between the PAPS+ and other groups being specifically in endovascular extravillous trophoblast invasion. In all pregnancies in which implantation site fragments were present and endovascular trophoblast ‘plugging’ was identified, there were additional intradecidual vessels present which appeared uninvolved by endovascular trophoblast invasion.

**Discussion**

This study demonstrates that histological examination of villous, intervillous and implantation site tissue is possible in the majority of samples obtained from evacuation of retained POC in women with recurrent early pregnancy loss. Furthermore, these data suggest that there may be a specific defect in decidual endovascular trophoblast invasion in PAPS+ associated early...
pregnancy loss, as compared with PAPS− cases or terminations of pregnancy. In this study, it was not possible to identify any cases of early pregnancy loss, from women with or without PAPS, in which there was evidence of excessive intervillous thrombosis as a cause of the miscarriage. Furthermore, the frequency of normal endovascular trophoblast invasion was not significantly different in cases with fetal chromosomal abnormality. Hence the results of this study do not support the hypothesis that defective trophoblast invasion and formation of endovascular trophoblast plugs is the major cause of pregnancy failure in cases of fetal aneuploidy. It should be noted that in this study, it is defective invasion of the intradecidual portions of the spiral arteries to which we are referring, rather than defective invasion of the intramyometrial segments, which characterize pre-eclampsia, implying a more severe and fundamental failure of endovascular trophoblastic invasion in some cases of early pregnancy failure. Furthermore, since in pregnancies in which implantation site fragments were present and endovascular trophoblast ‘plugging’ was identified, there were usually other intradecidual vessels identified which did not show endovascular trophoblast ‘plugging’, it is possible that the findings in the PAPS+ group may represent a marked exaggeration of a normal physiological process.

Our findings suggest that the commonly stated assumption that intraplacental ‘thrombosis’ is the main cause of pregnancy failure in women with PAPS is unlikely to be true in first trimester pregnancy loss, and further, that abnormalities of early trophoblast invasion may be the primary pathological mechanism in such cases. In normal early pregnancy, extravillous trophoblast invasion of the decidual stroma is associated with a subpopulation of trophoblast which invades the decidual vessels. This endovascular trophoblast initially forms aggregates or ‘plugs’ within the vessels, which subsequently dissociate, and the endovascular trophoblasts spread and convert the branches of the uterine arteries to low resistance uteroplacental vessels (Pijnenborg et al., 1980, 1981, 1983). Defective trophoblast invasion of the uteroplacental arteries is now well described in association with the development of later pregnancy complications such as intrauterine growth restriction and pre-eclampsia (Brosens, 1977; Brosens et al., 1977; Pijnenborg et al., 1991; Meekins et al., 1994; Starzyk et al., 1997), and it has been previously postulated that defective trophoblast invasion may be associated with some cases of early pregnancy loss (Michel et al., 1990). Ultrasonographic studies have demonstrated that a continuous intervillous circulation is not present until the late first trimester (Jurkovic et al., 1991; Jauniaux et al., 1997). In early pregnancy, trophoblast ‘plugging’ of the maternal circulation may play an important role in limiting the extent of intervillous blood flow with the result that both pressure-related and oxidative damage to the developing placenta are limited (Jauniaux et al., 1997). Hence, defective endovascular trophoblast invasion may explain both the occurrence of early pregnancy loss and later pregnancy complications in women affected by PAPS. Severe abnormal endovascular trophoblast invasion may lead to excessive blood flow into the early intervillous space resulting in early pregnancy loss, whereas lesser degrees of abnormal trophoblast invasion may allow the pregnancy to proceed into the second or third trimester, but complications related to uteroplacental vasculopathy subsequently supervene. This hypothesis may also explain the excess of late pregnancy complications in women with PAPS and a history of early pregnancy loss treated with aspirin and heparin (Backos et al., 1999). This treatment appears to reduce the severity of the defective endovascular trophoblast invasion, effectively converting potential early pregnancy losses into continuing pregnancies. However, later pregnancy complications secondary to the underlying uteroplacental vasculopathy remain a significant consideration.

Normal trophoblast invasion is a dynamic process which is tightly controlled via a complex series of interactions between trophoblast and decidual products (Bischof et al., 2000). The differentiation of trophoblast into an invasive phenotype is related to specific spatial and temporal alterations in the expression of cell surface adhesion and signalling molecules (Burrows et al., 1994; Damsky et al., 1994; Aboagye-Mathiesen et al., 1996; Aplin et al., 2000; Castellucci et al., 2000). Both extravascular interstitial and endovascular trophoblast invasion is required in normal pregnancy since interstitial trophoblast-mediated modification of the vessel walls precedes replacement of the endothelial cells by endovascular trophoblast (McMaster et al., 1994; Kam et al., 1999). There are three main possibilities to explain the mechanism by which the presence of aPLs may affect this process of trophoblastic invasion. Firstly, aPLs may bind to components on the cell surface of invading trophoblast, either inhibiting the function of other cell surface molecules or causing trophoblast damage by way of complement activation or other similar mechanisms. There is in-vitro evidence that aPLs can bind to receptors on trophoblast and may lead to

| Table 1. Histopathological findings in products of conception from cases of early pregnancy failure with PAPS+ or without PAPS− primary antiphospholipid antibody syndrome, fetal chromosomal abnormality (aneuploid), and from terminations of pregnancy in women with no history of recurrent pregnancy failure (TOP). Data are presented as median (range) |
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| n | Gestation (weeks) | Implantation site fragments identified | Endovascular trophoblast invasion identified | Proportion with endovascular trophoblast identified (versus PAPS+) |
| Aneuploid | 34 | 9 (6–14) | 17/34 (50%) | 9/17 (53%) |
| TOP | 20 | 9 (6–14) | 16/20 (80%) | 12/16 (75%) |
| PAPS− | 50 | 9 (6–12) | 28/50 (56%) | 17/28 (61%) |
| PAPS+ | 31 | 8 (7–10) | 17/31 (52%) | 4/17 (23%) |
alterations of cellular behaviour (Rote et al., 1998). Secondly, circulating aPLs may bind to the endothelium of maternal vessels, including those within the decidua, and either prevent the correct endothelial–trophoblast interaction via cell surface molecule modification, or lead to direct endothelial damage, thus preventing normal trophoblast invasion. Thirdly, aPLs may directly bind to the endovascular trophoblast population, leading to dissolution or abnormal formation of the endovascular trophoblast plugs.

Fetal aneuploidy is reported as the commonest ‘cause’ of early pregnancy loss, with subclassifications into various types of defect (Jauniaux et al., 1997), but the mechanism for fetal death in such cases remains undetermined. Throughout pregnancy, specific fetal chromosomal defects have different rates of intrauterine lethality (Snijders et al., 1995), with sex chromosome defects having a relatively low chance of intrauterine death and trisomies 18, 13 and triploidy having extremely high rates, whilst some chromosomal defects found in first trimester pregnancy loss specimens are never seen in later pregnancy (Jauniaux et al., 1997). The results of the present study suggest that trophoblastic invasion is not significantly altered in the presence of fetal chromosomal defects and this is also in accordance with the lack of specific or characteristic placental villous features in cases of fetal trisomy (Fox, 1997). In the majority of our cases, the karyotypic abnormality was a fetal trisomy, the vast majority of which are maternal in origin. Early placentation development appears to be predominantly controlled by paternal genes, whereas early fetal development is primarily controlled by maternally derived genes (Fisher, 1997), which may explain the lack of defective placentation development or trophoblast invasion seen in many of our cases. Nevertheless, it is possible that this mechanism may depend upon the individual chromosomal defect. In the present study, although the numbers remain too small for meaningful statistical subgroup analysis, both cases with fetal trisomy 21 and all four cases with fetal trisomy 16 had apparently normal endovascular trophoblast plugging. In contrast, the cases with fetal trisomy 7, 9, 20 and 22, all of which are recognized as lethal at an early gestation, showed no endovascular trophoblast plugging.

POC obtained from evacuation of the uterus by curettage may contain fragments of chorionic villi, gestation sac, decidua and implantation site. An implantation site is present in all pregnancies, but implantation site fragments cannot be identified in specimens of POC in all cases examined. This is related to factors associated with surgical uterine evacuation and sampling at the tissue processing stages. Furthermore, even in the specimens obtained from terminations of pregnancy, the presence of endovascular trophoblast invasion may be identified only focally within the decidual fragments examined. In this study, since specimen retrieval, handling and processing were the same in all groups, the different proportions in implantation site fragments between the groups is unlikely to be a significant factor.

This study has demonstrated that histopathological examination of POC may provide evidence for the mechanism of early pregnancy failure in women with recurrent pregnancy loss. Abnormal endovascular trophoblast invasion does not appear to be a major factor in early pregnancy loss in cases of fetal chromosomal abnormality whereas, in woman with PAPS, there may be defective early endovascular trophoblast invasion. In these cases it is possible that imaging techniques, such as colour Doppler examination of early uteroplacental blood flow, may identify abnormal intervilous flow patterns prior to pregnancy failure and hence provide useful prognostic and therapeutic information.

References


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