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The Placental Bed in Pregnancies Complicated by Primary Antiphospholipid Syndrome

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Pregnancy in women with primary antiphospholipid syndrome (APS) is frequently associated with placental insufficiency leading to intrauterine growth restriction (IUGR) ± fetal death, pre-eclampsia, placental abruption, premature delivery or thrombosis. The aim of this study was to investigate the placental bed in APS pregnancies for evidence of impaired trophoblast invasion, endothelial cell activation (ECA) and macrophage infiltration.

Methods: Biopsies from the presumed site of the placental bed were obtained from 12 women with treated primary APS and 16 controls. Immunohistochemical methods were employed to investigate expression of cytokeratin (trophoblasts), α-actin (smooth muscle), CD68 (macrophages) and VCAM-1 (as marker of ECA). Fibrinoid and elastin distribution and expression were determined by periodic acid/Schiff and orcein stain, respectively.

Results: Three APS pregnancies developed IUGR, one with concurrent pre-eclampsia. Eight of 12 APS biopsies were confirmed to be from the placental bed; one patient failed to meet APS criteria and was excluded from analysis; six included spiral arteries in the biopsy; 11 of 16 controls' biopsies were from the placental bed. APS biopsies had a higher concentration of inflammatory cells ($p = 0.0001$), particularly macrophages ($p = 0.014$). Three APS biopsies showed necrosis with hyperplastic vessels; one demonstrated arterial thromboses, but none had profound vasculopathy/atherosis or ECA.

Conclusion: Inflammatory mechanisms in the placental bed may contribute to APS pregnancy complications.
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INTRODUCTION

Pregnancies complicated by the antiphospholipid syndrome (APS) are predisposed to fetal compromise through uteroplacental insufficiency manifested by miscarriage, intrauterine growth restriction (IUGR) or fetal death [1]. Pre-eclampsia, placental abruption and premature delivery have also been associated with this condition [2]. Treatment with low dose aspirin with or without low molecular weight (LMW) heparin, close obstetric surveillance and timely intervention have improved fetal survival among women with primary APS. However, complications still frequently occur.

Studies of the placenta from APS pregnancies with poor outcome have shown extensive infarction and thrombosis together with other non-specific features accredited to hypoxia [3]. In addition, isolated case reports have proposed that impaired trophoblast invasion of the spiral arteries may be a feature of the placental bed of APS/systemic lupus erythematosus (SLE) pregnancies but this has not been verified in larger series of pregnancies complicated by primary APS, i.e. APS alone in the absence of SLE or other autoimmune disorders [4–6].

In this study, placental bed biopsies were obtained from women with primary APS in order to establish if impaired trophoblast invasion is prevalent amongst a larger cohort than previously studied. The endothelial cell layer in the uteroplacental bed vasculature was also investigated as recent studies have suggested that antiphospholipid antibodies (aPLs) may contribute to endothelial cell activation [7]. Evidence for any abnormality amongst the leucocyte population in placental bed biopsies from APS women and, in particular, the presence of

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macrophages was also examined. Placental bed biopsies from women with normal pregnancy outcome were studied for comparison.

MATERIALS AND METHODS

Local Ethics Committee approval was obtained for the removal of placental bed biopsies at caesarean section. Pregnant women with primary APS were recruited and compared with a cohort of healthy pregnant women recruited on the labour ward immediately prior to elective caesarean section or in early labour. Twelve women with primary APS and 16 control women provided biopsies and placental samples at the time of caesarean section. Placental samples, but not biopsies were obtained after vaginal delivery from a further eight APS patients. Control women were matched for parity and ethnicity with no past history of adverse pregnancy events, hypertension, diabetes or SLE. Pregnancies which were normotensive, non-proteinuric, not complicated by IUGR and delivered between 37 and 42 weeks were considered normal.

APS was defined according to the recent International Consensus Criteria [8]. This encompasses a history of thrombosis (venous, arterial or small vessel) and/or pregnancy morbidity i.e. three or more consecutive early miscarriages, one or more fetal death >10 weeks gestation or premature delivery (<34 weeks) because of severe pre-eclampsia or placental insufficiency, supported by laboratory evidence of the disease [lupus anticoagulant and/or anticardiolipin antibody of IgG and/or IgM isotype present in medium or high titres on ≥ 2 occasions at least 6 weeks apart]. The pregnancy outcomes of three women (two from whom placental bed biopsies were taken) were excluded from analysis on the basis of only one positive antiphospholipid antibody test result. Pre-eclampsia was defined by the International Society for the Study of Hypertension in Pregnancy guidelines [9] and small for gestational age was defined as infants born $\leq$10th centile for gestation and gender, corrected for maternal height, weight, parity and ethnicity using centile charts [10].

Women with APS were treated with 75 mg aspirin from pre-conception and low molecular weight (LMW) heparin (dalteparin 5000 IU daily) subsequent to a positive pregnancy test if there was a history of thrombosis, previous late fetal loss or recurrent miscarriages, in spite of concurrent aspirin therapy. In women with past venous thromboembolic events, heparin dosage was doubled from 20 weeks' gestation until delivery [11]. Women were reviewed monthly and fetal monitoring was instigated from 20 weeks with timely intervention. This management regime was in accordance with recently published guidelines [12].

Placental bed biopsies

Placental bed biopsies were obtained according to the method described by Robertson et al. [13] at the time of caesarean section. Two placental bed biopsies were obtained from each patient and were fixed at room temperature in phosphate buffered 4% w/v paraformaldehyde. Biopsies were cut into 1–2 mm thick sections after the first hour of fixation, in a plane perpendicular to the decidual–myometrial junction. After 24 h, specimens were transferred to 0.1 M phosphate buffer pH 7.2 containing 3% sucrose at 4 °C for a further 24 h before storage in 70% ethanol until further processing. Tissue blocks were dehydrated in a graded ethanol series and embedded in paraffin for storage. The 1–2 mm thick sections of each biopsy were embedded together in one block to allow evaluation of different depths simultaneously in each biopsy on one slide. They were positioned flat so that both decidua and myometrium could be visualised on the same slide. Sections were cut step-serially at 3 μm thickness, collected onto silane (2% 3-aminopropyltriethoxysilane 99% in acetone) coated slides and dried at 37 °C for over 12 h.

A routine panel of staining procedures was applied to serial sections in order to determine the structural characteristics of the placental bed (Table 1) and in addition slides were immunostained with antibodies to vascular cell adhesion molecule-1 (VCAM-1) as a marker of endothelial cell activation [14] and with antibodies to CD68 for detection of macrophages. After heating the slides at 55 °C for 1 h, paraffin was removed in histoclear™, and sections were rehydrated by immersion in ethanol and distilled water successively. The tissue sections were rinsed in 0.01 M Tris-buffered saline (pH 7.6, 0.9% NaCl) (TBS) before immunostaining.

Immunohistochemical techniques

Mouse monoclonal anti-human IgG antibody to cytokeratin (MNF 116) was used for immunostaining trophoblast. Tissues were treated with pepsin (Sigma, P-7012), 0.04% in 0.01 M HCl at 37 °C for 10 min. After several rinses in TBS at 4 °C, endogenous peroxidase activity was blocked using 0.5% hydrogen peroxide in absolute methanol (with 0.1% sodium

Table 1. Panel of staining procedures for the determination of structural characteristics of the placental bed

Stains applied to adjacent sections of placental bed tissue
1. Monoclonal antibody, anti-cytokeratin (MNF 116) (Dako, USA)/haematoxylin & eosin – for detection of trophoblasts
2. α-actin immunostaining (Dako, USA) – reveals all smooth muscle cells allowing distinction between decidua and myometrium. Also allows the study of smooth muscle changes in the arterial walls
3. PAS (Periodic Acid/Schi) – allows detection of fibrinoid material (stained pink) in physiologically changed spiral arteries within the placental bed
4. Orcein staining – reveals the presence or absence of elastin (stained black) in the vessel walls
5. Monoclonal mouse anti-human VCAM-1 (Dako, USA – clone 1.4C3) – for evidence of endothelial cell activation
6. Monoclonal mouse anti-human CD68 (Dako, USA – clone KP1) – for detection of macrophages