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Expression of TGF beta in the placental bed is not altered in sporadic miscarriage

E. Ball [a,b,*], S.C. Robson [a], S. Ayis [c], F. Lyall [d], J.N. Bulmer [b]

[a] School of Surgical and Reproductive Sciences (Obstetrics and Gynaecology), Newcastle University, UK
[b] School of Clinical and Laboratory Sciences (Pathology), Newcastle University, UK
[c] Department of Social Medicine, University of Bristol, UK
[d] Maternal and Fetal Medicine Section, Institute of Medical Genetics, University of Glasgow, Yorkhill, Glasgow, UK

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Abstract

Extravillous trophoblast invasion of uterine stroma and spiral arteries (SA) is essential for normal pregnancy and is reduced in preeclampsia and late miscarriage. The control mechanisms are not understood, but transforming growth factor-beta (TGF-β) may be a candidate. Placental and placental bed biopsies were obtained from early (8^{+0}–12^{+6} weeks) euploid miscarriages ($n = 10$), early aneuploid miscarriages ($n = 10$), late (13^{+0}–19^{+6} weeks) euploid miscarriages ($n = 10$) and controls of the same gestation ($n = 20$). Frozen sections were immunostained for TGF-β1, 2 and 3. Immunoreactivity of trophoblast and uterine cell populations was assessed semi-quantitatively. TGF-β1 immunolocalization was limited to extracellular matrix in cytotrophoblast islands and cytotrophoblast shell, perivascular fibrinoid and interstitial trophoblast and did not differ in miscarriage compared with controls. TGF-β2 was expressed additionally in endovascular trophoblast and multinucleate trophoblast giant cells. There was no aberrant TGF-β2 immunolocalization in late miscarriage, but TGF-β2 immunoreactivity was increased in extracellular matrix in cytotrophoblast islands in early miscarriage. TGF-β3 was absent from all cell populations. Stromal and extravillous trophoblast TGF-β2 immunolocalization suggests a more important role in trophoblast invasion than TGF-β1, but neither isoform was altered in miscarriage. Altered TGF-β localization is therefore unlikely to play a role in abnormal trophoblast invasion and SA transformation in miscarriage.

Keywords: TGF beta 1, 2 and 3; Miscarriage; Early pregnancy; Trophoblast; Placental bed

1. Introduction

In the UK miscarriages represent the commonest gynecological emergency (70,000–90,000 per year) [1], complicating up to 30% of pregnancies before 20 weeks of gestation. Around 85% of miscarriages occur at or before 12 weeks of gestation and half of these are due to aneuploidy [2]; the cause of the remainder is unclear. In contrast, later miscarriages are more often associated with underlying maternal causes such as antiphospholipid syndrome, cervical incompetence, uterine anomalies or genital infection [3].

The orderly invasion by extravillous trophoblast into the uterine placental bed and spiral arteries, leading to the loss of the arterial musculo-elastic media and replacement by fibrinoid [4], is a requirement for successful pregnancy. Deficient trophoblast invasion, particularly into myometrial segments of spiral arteries, has been associated with a variety of pregnancy complications including preeclampsia [5] and pre-term birth [6]. Recently we demonstrated a similar deficiency of trophoblast invasion in second trimester miscarriage [7]. Interestingly, although trophoblast invasion has been reported to be deficient at the level of the cytotrophoblast shell in first trimester miscarriage [8], we recently demonstrated normal trophoblast invasion into deeper decidua, myometrium and spiral arteries [9]. Normal endovascular trophoblast invasion has also been reported in recurrent antiphospholipid-negative miscarriage [10].

* Corresponding author. St Bartholomew's Hospital, Obstetrics and Gynaecology, West Smithfield, London EC1A 7BE, UK. Tel.: +44 01727 867287.

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Trophoblast invasion appears to be orchestrated by transcription factors expressed by trophoblast cells [11] and a complex network of local autocrine and paracrine factors, including cytokines and proteinases [12]. Several lines of evidence suggest that transforming growth factor-beta (TGF-β) may play a role in the control of trophoblast invasion; TGF-β isoforms have been localized to the feto-maternal interface [13–16] and TGF-β1 has both anti-proliferative and anti-invasive effects on cultured cytotrophoblast cells [17, 18]. Indeed elevated serum TGF-β1 levels have been reported during and after pregnancy failure [19]. Caniggia et al. [14] reported an anti-invasive effect of TGF-β3 as well as temporal regulation of villous trophoblast expression during normal pregnancy, with dysregulation in pregnancies complicated by preeclampsia. In contrast, using a range of techniques, others have failed to detect evidence of temporal regulation of TGF-β3 or altered expression in preeclampsia [16, 20]. TGF-β effects may be mediated by downregulation of inducible nitric oxide synthase (iNOS) in trophoblast cells [21] and enhanced plasminogen activator inhibitors (PAI) [22].

In order to address the hypothesis that TGF-β immunolocalization is altered at the feto-maternal interface of women with miscarriage we compared immunolocalization of TGF-β1, TGF-β2 and TGF-β3 in placental and placental bed samples from women suffering early and late miscarriage with a group of gestationally matched normal pregnancies.

2. Materials and methods

2.1. Subjects and sample collection

Placental bed biopsies were obtained from pregnant women at the Royal Victoria Infirmary, Newcastle upon Tyne with ethical approval and written patient consent. For the miscarriage group, only women with a fetal pole on ultrasound scan, regular menstrual cycles (28 ± 2 days), a definite record of last menstrual period (LMP), no cervical weakness or sepsis and no more than one previous early or late miscarriage were included in the study. Both early (gestational age 8^{+0}–12^{+6} weeks, $n = 20$) and late miscarriages (gestational age 13^{+0}–19^{+6} weeks, $n = 10$) were included. Gestational age was calculated from the date of the LMP. Fetal crown–rump length or biparietal diameter was also measured using ultrasound. All women underwent surgical evacuation of retained products of conception.

The control group consisted of women who underwent surgical termination of apparently normal pregnancies under clause C of the 1969 Abortion Act by suction curettage (gestational age 8^{+0}–12^{+6} weeks, $n = 10$) or dilatation and evacuation (gestational age 13^{+0}–19^{+6} weeks, $n = 10$), as determined by ultrasound measurement of crown–rump length or biparietal diameter immediately prior to the procedure. Prior to surgery cervical priming was achieved with 400 μg of vaginal Misoprostol (Cytotec®) for women up to 14 weeks of gestation and with 2 mg of vaginal prostaglandin E1 (Cervagem®) for later gestational ages.

The technique of ultrasound guided placental bed biopsy has been described previously [23]. Placental and placental bed biopsies were snap frozen in liquid nitrogen-cooled methyl-2-butane and cut at 7 μm. In order to confirm placental bed by the presence of extravillous trophoblast and to facilitate histological orientation, serial sections of placental bed biopsies were immunostained for cytokeratin (LP34; 1:800, Novocastra Laboratories, Newcastle upon Tyne, UK) to demonstrate trophoblast cells, desmin (1:100 Novocastra Laboratories) for medial smooth muscle and myometrium, and von Willebrand factor (1:800, Dako, Ely, UK) to detect endothelium, as previously described [7, 9].

Placental chorionic villi were cultured and karyotyped [24]. Details of the subjects included in the study are given in Table 1. Ten early (8^{+0}–12^{+6} weeks) euploid miscarriages and 10 early aneuploid miscarriages were matched for gestational age with 10 controls (presumed to be euploid). Ten late (13^{+0}–19^{+6}) euploid miscarriages were similarly matched for gestational age with 10 controls.

2.2. Immunohistochemistry

Sections were immunostained using a streptavidin–biotin peroxidase technique (Vectastain Elite rabbit kit, Vector Laboratories, Burlingame, CA, USA) as described previously [23]. Optimal concentrations of rabbit anti-TGF-β1 (1:200, SC146), TGF-β2 (1:250, SC90), and TGF-β3 (1: 200, SC82; all from Santa Cruz Biotechnology, CA, USA) were those that resulted in optimal staining on positive control sections of normal adult skin with minimal background staining [16, 20]. Phenylhydrazine was applied for 45 min to block endogenous peroxidase activity. The reaction was developed with 1 mg/ml diaminobenzidine. Sections were counterstained with Mayer's hematoxylin and mounted in DPX synthetic resin. For each of the TGF-β isoforms, all miscarriage and control sections were immunostained in one run in order to avoid day-to-day variation between staining runs. Normal adult human skin was used as a positive control in each staining run. Negative controls were performed for each tissue sample by substitution of primary antibody with normal rabbit serum diluted 1:200 in TBS.

2.3. Histological assessment

The histological structures assessed in placenta included the chorionic villous stroma, vessels, Hofbauer cells, cytotrophoblast and syncytiotrophoblast, as well as trophoblast cells and extracellular matrix within cytotrophoblast columns and islands. In the placental bed interstitial, endovascular and multinucleate extravillous trophoblast populations were assessed separately, as well as spiral artery endothelium, perivascular fibrinoid deposits, decidual stromal cells, myometrial cells and endometrial glands. The intensity of staining was graded as absent, weak, moderate, strong or variable (some cells positive, some cells negative; Fig. 1a–c). Scoring was performed independently by two observers (EB, JNB) who were blinded to both pregnancy outcome and gestational age. There was a high level of agreement (range 78–100%) between observers and thus the results of one observer (EB) were used for subsequent analysis.

2.4. Statistical analysis

Fisher's exact test was used to compare TGF-β1, TGF-β2 and TGF-β3 immunolocalization between different gestational age groups and pregnancy

Table 1
Subjects

	Early euploid miscarriage $n = 10$	Early aneuploid miscarriage $n = 10$	Early normal pregnancy[a] $n = 10$	Late euploid miscarriage $n = 10$	Late normal pregnancy $n = 10$
Maternal age	30.5 [19–39]	29 [21–34]	25 [18–40]	28 [23–42]	25 [18–35]
Median parity	1 [0–3]	1 [0–3]	0 [0–2]	1 [0–2]	0 [0–4]
Gestational age (weeks)	10 [8–11]	11 [8–12]	10.5 [8–12]	15.5 [13-19]	15.5 [13–19]

Figures are median [range].
Mann–Whitney test showed no significant differences between groups.
[a] Presumed euploid.