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Placental Deficiency of Interleukin-10 (IL-10) in Preeclampsia and its Relationship to an *IL10* Promoter Polymorphism

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The placenta is pivotal in the acceptance of the feto-placental unit by the maternal immune system. Imbalance at the maternal–fetal interface of tissue pro- and anti-inflammatory cytokines may be partly involved in disease causation. Previous work has shown conflicting levels of IL-10. IL-10 levels have been shown to increase, decrease, or remain unchanged in women with preeclampsia. This study examines the difference in serum and placental IL-10 expression in women with preeclampsia and investigates if the *IL10* (−1082) A promoter polymorphism contributes to lower concentrations. In a prospective case–control study of 12 women with preeclampsia and 31 controls we assessed serum IL-10 by ELISA, placental mRNA by quantitative PCR and protein by immunohistochemistry as well as placental *IL10* promoter genotype. Comparisons were made with non-parametric tests where necessary and chi-square. We found a significant reduction in placental IL-10 mRNA and protein expression in women with preeclampsia compared to controls. Women with the AA IL-10 promoter genotype expressed less placental IL-10 mRNA compared to women with AG or GG genotype. There was no difference in serum IL-10 concentrations between different genotypes. Preeclampsia is associated with a deficiency of placental IL-10. Placental AA genotype in the promoter region results in significantly less placental IL-10.
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INTRODUCTION

The aetiology of preeclampsia is multi-factorial and abnormalities of oxidation, endothelial function, immunological response and gene expression have been implicated. Although it is generally accepted that the clinical syndrome of preeclampsia (hypertension and proteinuria) is predominantly contributed to by vascular dysfunction and inflammation, we still lack a clear understanding of the changes of the inflammatory markers in preeclampsia. If involvement is demonstrated, we further need to elucidate if such changes occur in early pregnancy and if they are involved in the pathogenesis of preeclampsia.

IL-10 is a key regulator of the inflammatory process and has been a cytokine of interest because of its pleiotropic activities. It is an anti-inflammatory (Th2) cytokine which inhibits cytokines, chemokines and antigen presentation [1]. There is already some evidence for the importance of the role of IL-10 at the feto-maternal interface as an important immune regulator. The trophoblasts [2], decidual macrophages [3], natural killer (NK) cells [4] and lymphocytes found within the placenta produce IL-10. Placental IL-10 is expressed in a gestation-dependent manner; it is up-regulated in the first trimester and down-regulated at term [5]. In early gestation it is most heavily expressed at the feto-maternal interface by the extra-villous cytotrophoblasts and in late gestation mostly expressed by villous cytotrophoblasts [6]. Human leukocyte antigen-G (HLA-G) expression is induced by IL-10, which contributes to fetal allograft tolerance by inhibiting lysis by maternal NK cells [7]. IL-10 also increases the resistance of trophoblasts to Fas-mediated apoptosis [8] and is an autocrine inhibitor of cytotrophoblast matrix metalloproteinase-9 (MMP-9) activity and consequently invasiveness [9]. Thus, abnormalities of IL-10 production may alter trophoblast invasion into the uterus.

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There are several lines of evidence from women with gestational disorders and animal studies to support the role of IL-10 in accepting the fetal allograft and contributing to trophoblast invasion. Women with first trimester missed abortions have a deficiency of decidual IL-10 [10]. Fetal loss can be prevented in abortion prone CBA × DBA/J mice by the administration of IL-10 [11]. Furthermore, IL-10 null mutant mice have structurally different placentas, demonstrating a greater cross-sectional area and reduced density of trophoblasts compared to wild type mice [12].

The pathogenesis of preeclampsia is related to shallow placental development, however; the clinical syndrome is effected by molecules of vascular dysfunction (such as vascular endothelial growth factor receptor-1) and inflammation. The studies performed to date investigating the changes in plasma and placental IL-10 in women with preeclampsia have resulted in conflicting evidence. It has been shown that circulating IL-10 is lower [13], not different [14] and higher [15,16] in women with preeclampsia compared with normal controls. Studies examining the change in placental IL-10 expression in women with preeclampsia have also resulted in inconsistent findings. Placental IL-10 has been shown to be both increased [16,17] and decreased [13,18].

In the non-pregnant population, circulating IL-10 concentrations and tissue IL-10 expression have been shown to be influenced by polymorphisms in the IL-10 promoter region [19]. Suarez et al. showed that healthy volunteers with GG at the −1082 position produced significantly more IL-10 mRNA compared to AG or AA. Conversely, certain promoter polymorphisms have predisposed to the development of disease [20]. Daher et al. [21] have investigated the relationship between recurrent pregnancy loss and the IL-10 promoter polymorphism which is G → A at the −1082 position. This meta-analysis has revealed that the GG promoter genotype has a significant association with recurrent pregnancy loss ($p = 0.03$, OR 1.75). To date, the relationships between IL-10 G → A (−1082) promoter polymorphisms, preeclampsia and tissue or circulating levels of IL-10 have not been examined.

Thus, this study aimed to investigate differences in circulating or placental immunosuppressive IL-10 at term in pregnant women with preeclampsia, compared with women who had normal pregnancy outcomes. Furthermore, we investigated the contribution of the *IL10* G/A promoter polymorphism to these differences and to the development of the clinical syndrome of preeclampsia.

METHODS

Subjects

The study was approved by the local institutional human ethics research committee. Women were consecutively recruited and all women gave written informed consent. There were a total of 43 women who underwent caesarean section and were enrolled in the study comprising 31 control subjects who had a normal pregnancy (control group) and 12 women who developed preeclampsia (preeclampsia group). Patients were excluded from the study if they had commenced labour, had labour induced or had any evidence of an intercurrent maternal or fetal infection. Women were considered to have preeclampsia as defined by the Australasian Society for the Study of Hypertension in Pregnancy (ASSHP) [22]. Briefly, women had a blood pressure greater than or equal to 140 mmHg systolic and or 90 mmHg diastolic after 20 weeks gestation on two occasions at least 4 h apart. This was associated with evidence of end organ dysfunction, which was proteinuria (greater than or equal to 2+ on dipstick or 300 mg/24 h) or renal insufficiency (serum creatinine greater than 0.09 mmol/L), liver disease (raised serum transaminases and severe epigastric pain), neurological problems (excluding convulsions) and haematological disturbances, all of which resolved within three months post partum.

Enzyme-linked immunosorbent assay (ELISA)

Venous blood (9 mL) was taken prior to caesarean section using a Vacuette® system (Greiner Bio-One, Kremsmuenster, Austria). The samples were centrifuged within 2 h of venipuncture for 10 min at 3000 rpm and the serum was aliquoted and stored at −80 °C until required. The concentration of IL-10 in the serum was assayed using a commercially available ELISA kit (Becton Dickinson, North Ryde, Australia). Briefly, a 96 well plate was incubated at 4 °C overnight with capture antibody. The wells were blocked to reduce any non-specific binding. The samples and standards were then added and incubated. Detection antibody and horseradish peroxidase were added to each well. A colour producing substrate 3′,3′,5′,5′-tetramethylbenzidine (TMB: Becton Dickinson, North Ryde, Australia) was added and the reaction was stopped after 30 min using 2 mol/L sulphuric acid. The results were read using a spectrophotometer (Sunrise, Tecan, Germany) at 450 nm with 570 nm as the reference wavelength. The intra-assay variability was 4.5% and the inter-assay variability was 13% in these studies. Further validations of the IL-10 ELISA were performed in our laboratory. The IL-10 assay had a lower limit of detection of 0.96 pg/mL calculated by assaying 30 zero specimens in duplicate and calculating the concentration of the mean plus two standard deviations of the mean. The recovery of IL-10 spiked to human serum was 99.9% (+SD 11.1%) at physiologic concentrations.

Tissue collection

The placentas were collected within 30 min of delivery. Five small (2 × 2 cm) separate samples were taken systematically from different areas of the placenta. The samples were taken from the centre and one from each quadrant to avoid sampler bias. The periphery, areas of calcification and necrosis were not sampled. The samples were rinsed in Dulbecco's phosphate-buffered saline (PBS; Sigma-Aldrich, St Louis, USA). Tissue samples required for immunohistochemistry were fixed in 10% formalin. Subsequently, tissue samples for

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