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Short communication

Altered expression of immune-associated genes in first-trimester human decidua of pregnancies later complicated with hypertension or foetal growth restriction

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ABSTRACT

During pregnancy the maternal immune system has to coordinate uterine spiral-artery remodelling, trophoblast invasion, and acceptance of the semi-allogenic fetus simultaneously. As dysregulation of the immune system is associated with adverse pregnancy outcomes, we analysed first-trimester deciduas of pregnancies for immune parameters in later complicated pregnancies. Higher IL6 and macrophage mRNA expression, and lower ratios of regulatory macrophages were found in first-trimester deciduas of pregnancies later complicated with pregnancy-induced hypertension. Lower Gata3 (Th2) mRNA expression was found in deciduas of pregnancies with later foetal growth restriction. Our results suggest that adverse pregnancy outcomes are associated with immunological disturbances in first-trimester deciduas.

1. Introduction

Complications of human pregnancy like pregnancy-induced hypertension (PIH) and intrauterine foetal growth restriction (IUGR) are associated with dysfunctional adaptation of the maternal immune system towards the fetus [1–3]. Apart from the necessary adaptations of the maternal immune response to accommodate the semi-allogeneic fetus, the maternal immune system has a role in the regulation of uterine spiral artery remodelling and trophoblast invasion [4–6]. T cells, Natural-Killer (NK) cells, monocytes and macrophages are regarded as being important in these processes [4,6,7]. Altered immune balances, such as changes in macrophages and T cell subsets, also influence spiral artery remodelling and trophoblast invasion, and could lead to adverse pregnancy outcomes via this mechanism [6,8–14]. The aim of this study was to analyse immune parameters in first-trimester deciduas of pregnancies with and without later complications.

2. Materials and methods

First-trimester decidual tissue was obtained from surplus tissue at chorionic villus sampling (CVS), which was performed vaginally between 10 and 12 weeks of gestation for maternal age or serum screening related risk for aneuploidy [15,16]. Immediately after sampling, decidual tissue was mechanically separated from villi by a qualified, experienced laboratory technician to minimize trophoblast contamination. Earlier studies showed only very sporadically trophoblast cells in decidual samples [15,16]. Patients were informed that otherwise discarded material could be used for research. Follow-up of pregnancies was available by a questionnaire returned by the patient postpartum. Decidual tissue from pregnancies later complicated by PIH (without proteinuria) or IUGR (without hypertension) were selected from our database, and controls were selected matched for maternal age, parity and gestational age at time of sampling. In total, decidual tissue of 38 pregnancies was selected, as RNA quality of 10 was too low, only decidual tissue of 14 control and 7 IUGR and 7 PIH complicated pregnancies was used (Table 1). PIH was defined as blood pressure >140/90 after 20 weeks of gestation on 2 occasions at least 6 h apart [17], and IUGR as a birth weight below the 10th percentile [18].

RNA was isolated and purified as previously described [19]. cDNA was reverse transcribed using a Superscript-II Reverse Transcriptase kit (Invitrogen, USA). HPRT was used as housekeeping gene [19]. We analysed mRNA expression of Interleukin-6 (IL6), IL10, Tbet21 (Th1 response), Gata 3 (Th2 response), Rorγt (Th17 response), Foxp3 (Treg), CD56 (NK cells), CD68 (pan macrophage marker), NOS2 (iNOS, M1 macrophages), and CD206 (M2 macrophages), using On-Demand-Gene-Expression Assays (Applied Biosystems, USA). As recent data show macrophage differentiation into subsets with different roles in inflammation or tissue remodelling and vascularisation, respectively M1 and M2 macrophages, specific macrophage mRNA expression was tested in this study [9,20,21].

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Table 1
Characteristics of the IUGR and PIH study groups and matched controls.

	Controls ($n = 14$)	IUGR ($n = 7$)	PIH ($n = 7$)
At CVS			
Age (years)	37.4 ± 2.5	39.0 ± 2.5	36.6 ± 2.2
GA (days)	78.4 ± 3.7	80.1 ± 1.9	81.0 ± 5.9
Parity	1.5 ± 0.8	1.9 ± 0.7	0.7 ± 1.1
At delivery			
GA (days)	281.9 ± 4.6	279.9 ± 12.9	274.4 ± 6.9*
Birth weight (g)	3766.4 ± 388.6	2672.1 ± 624.4***	3134.3 ± 642.4

(mean ± SD), * = $p < 0.05$, *** = $p < 0.001$, compared to controls, using one way ANOVA with Dunnett post-hoc testing).

PCR reactions were performed as described previously, in triplicates in a total volume of 10 μL [19]. Runs were performed by a 7900HT Fast Real-Time PCR System (Applied Biosystems, USA), mRNA data were normalised to HPRT mRNA expression using $2^{-\Delta Ct}$. Undetectable Ct values (>40) were analysed as the maximum Ct value (40). For statistical analysis SPSS was used. For each gene statistical comparisons were performed on log-transformed data, to assure normal distribution, using an ANOVA followed by a Dunnett post-hoc test, in which both the PIH and IUGR group were compared to the total control group.

3. Results and discussion

In this study we used unique decidual material, collected during routine CVS between 10 and 12 weeks of pregnancy, which allowed us to study immunological parameters in early pregnancy deciduas with a known pregnancy outcome. A significantly higher mRNA expression of IL6 ($p < 0.01$), a trend towards higher CD68 ($p < 0.10$) (pan macrophage marker), and significantly lower ($p < 0.05$) CD206 (regulatory macrophage subset)/CD68 mRNA expression ratio was found in PIH complicated pregnancies compared to controls (Fig. 1ab).

The higher expression of IL6, probably produced by immune cells being active in the decidua, especially by higher numbers of macrophages [22], might indicate altered angiogenesis and decidualisation in first-trimester decidua [6], requiring higher blood pressure in the third-trimester to guarantee adequate placental flow, and with that ultimately leading to systemic complications of pregnancy such as PIH. It could also skew the local immune response in the decidua away from a tolerating environment into an (Th17) inflammatory environment, leading to altered balances of macrophage and T cell subsets. Indeed, we observed a relative decrease of the tissue repairing, regulatory macrophage subset (CD206-expressing macrophages). Our results, therefore, suggest a role for macrophages in the success of pregnancy and support recent data showing lower numbers of regulatory macrophages in decidua of pre-eclamptic patients compared to deciduas of control pregnant women [11]. Interestingly, levels of Foxp3 mRNA (Treg cells) were low in first-trimester decidual tissue, and we did not find differences in levels of Foxp3 mRNA between PIH complicated and control pregnancies. This could indicate that at the moment of CVS (between 10 and 12 weeks of pregnancy), macrophages are more important for placental development than T cells. It could also indicate, as animal data suggest [23], that Treg cells are especially important prior to tissue sampling, i.e. before 10 weeks.

A trend towards lower expression of Gata3 (Th2 response) mRNA ($p = 0.06$) was found in pregnancies complicated by IUGR (Fig. 1a) as compared with control pregnancies. The lower Gata3 mRNA expression in complicated pregnancies is in line with earlier

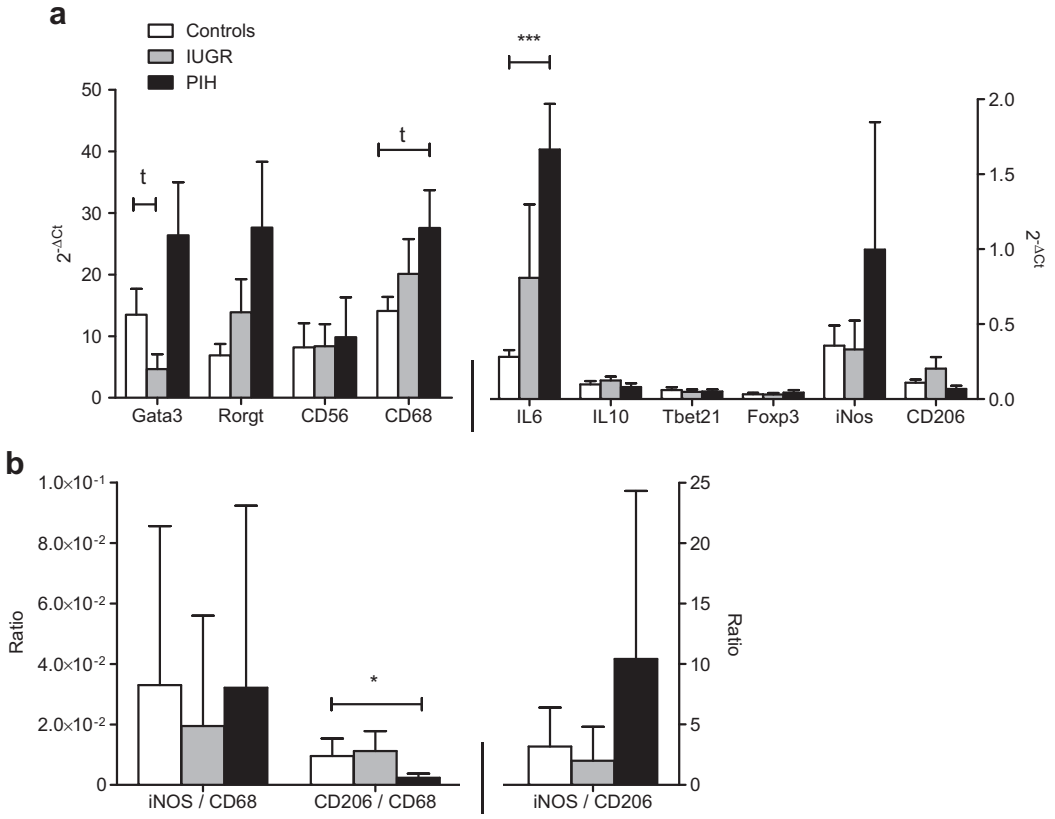

Fig. 1. mRNA expression in first-trimester decidual tissue of cases and controls. mRNA expression (a) and macrophage subset ratios (b) in first-trimester decidual tissue of control pregnancies (open bars), and pregnancies complicated by IUGR (grey bars) or PIH (black bars). Higher mRNA expression of IL6 and CD68 in pregnancies complicated by PIH, and lower mRNA expression of Gata3 (Th2) in decidual tissue of pregnancies complicated by IUGR (a) were observed. Lower CD206/CD68 (regulatory macrophage subset) mRNA ratios were observed in pregnancies complicated by PIH (b). mRNA target gene expression is shown as relative expression to household gene. ($t = p < 0.10$, * = $p < 0.05$, *** = $p < 0.001$, compared to controls, using one way ANOVA with Dunnett post-hoc testing).

research showing that complicated pregnancies are associated with a shift away from the Th2 environment as seen in normal pregnancies [1]. This shift away from a Th2 environment might indicate an inflammatory reaction towards the fetus and placenta, causing placental dysfunction and with that foetal growth restriction [24].

In summary, we show that adverse pregnancy outcomes are associated with altered mRNA expression of immune parameters, especially mRNA of macrophages or their cytokines, in first-trimester human deciduas. We found higher IL6 and CD68 mRNA expression, as well as lower CD206/CD68 mRNA ratios in deciduas of pregnancies complicated by PIH, and lower Gata3 mRNA expression in IUGR complicated pregnancies. Although we studied decidual tissue of women with PIH and not of women with preeclampsia, our findings do indicate immunological changes in early pregnancy with later hypertensive complications, including preeclampsia. These differences not only imply immunological mechanisms in the pathophysiology of adverse pregnancy outcomes, but also suggest different aetiologies between PIH and IUGR, and hold potential for the prevention and treatment of immune based pathologies of pregnancy.

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