CYTOKINES AND ITS ROLE IN UTEROPLACENTAL DYSFUNCTION

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Pre-eclampsia is a multisystem disorder of unknown etiology that is unique to human pregnancy. It is characterized by abnormal vascular response to placentation that is associated with increased systemic vascular resistance, enhanced platelet aggregation, activation of the coagulation system, and endothelial-cell dysfunction. The clinical findings of pre-eclampsia can manifest as either a maternal syndrome (hypertension and proteinuria with or without other multisystem abnormalities) or fetal syndrome (fetal growth restriction, reduced amniotic fluid, and abnormal oxygenation). In clinical practice, the maternal syndrome is probably more than one disease with major differences between near-term pre-eclampsia without demonstrable fetal involvement and pre-eclampsia that is associated with low birthweight and preterm delivery. The disorder is heterogeneous for which pathogenesis can differ in women with various risk factors. Pathogenesis of pre-eclampsia in nulliparous women may differ to that in women with pre-existing vascular disease, multi fetal gestation, diabetes mellitus, or previous pre-eclampsia. Additionally, the pathophysiology of the disorder leading to onset before 34 weeks' gestation could differ to that developing at term, during labour, or postpartum.

Despite advances in perinatal care, frequency of pre-eclampsia has not changed. Research addressing this disorder has been extensive during the past decade, but has not resulted in substantial improvement in methods of prediction or prevention of the disorder. A major impediment in the development of such methods is our poor understanding of the various pathological mechanisms that lead to pre-eclampsia as well as the inconsistent criteria used to define it. Indeed, many studies have suggested that women who develop pre-eclampsia are at increased risk of cardiovascular complications later in life.

Recent attention has focused on elucidating the immuno-biological roles of cytokines in normal human pregnancy following the accumulated reports of complex cytokine activity within uteroplacental tissues. T helper (Th) cells can differentiate into subsets with distinctive patterns of cytokine release. It has been proposed that Th1-type responses (e.g. the production of IL-2 and IFN-γ) are systemically suppressed in murine pregnancy and that local expression of Th2-type cytokines (e.g. IL-4, IL-6, IL-10) in placental tissue might be beneficial for fetal survival. Whether an analogous situation exists in human pregnancy is unclear as yet, although partial systemic impairment of Th1 responses is compatible with clinical evidence that a number of infectious diseases caused by intracellular pathogens can sometimes be exacerbated in pregnancy, e.g. cytomegalovirus and malaria.

Abnormally shallow cytotrophoblast invasion of the maternal spiral arteries, defective placentation, poor trophoblastic perfusion, elaboration of endothelial cell toxins and subsequent endothelial cell injury resulting in activation of the coagulation system, have all been implicated as important events associated with the pathophysiology of pre-eclampsia.

Based on the fact that placental tissue is both essential and sufficient to generate the end stages of the clinical syndrome of pre-eclampsia, placental factors have been suggested to be

involved in causing the endothelial activation in pre-eclamptic patients. The placental ischaemia that ensues secondary to the initial defective placentation results in the release of placental factors into the maternal circulation, which perturb endothelial function followed by the clinical sequelae associated with this condition. The identity of the deleterious factors that link placental ischaemia and generalized endothelial dysfunction during pre-eclampsia are however unknown at present.

The role of cytokines in acceptance of the fetal-allograft and success of human pregnancy has been a subject of debate. Aberrations in the cytokines network could possibly be implicated in pregnancy-associated disorders including pre-eclampsia. We hypothesized that inadequate placentation in pre-eclampsia which results in foci of placental ischaemia/hypoxia leads to overproduction of proinflammatory cytokines.

Although, studies have demonstrated alterations in the expression of cytokines in blood of women presenting with pre-eclampsia when compared with those undergoing normal pregnancy, the data is scarce and inconsistent, and hence more extensive investigations are needed to understand the role of Th1/Th2 cytokine balance in the pathology of pre-eclampsia.

We present here the first comprehensive investigation to examine the expression profiles of a range of Th1 and Th2 cytokines at protein and mRNA level in the placental tissues obtained from pre-eclamptic patients compared to the normotensive control group. The results of the present study are in accordance with the view that aberrations in the cytokine network within the feto-placental unit are associated with pre-eclampsia and demonstrate for the first time that there is an imbalance in both type 1 and type 2 cytokines in pre-eclampsia compared to normal term human placenta.

Previous reports on changes in the levels of IL-1 in pre-eclampsia patients are highly conflicting. While decreased amniotic fluid concentrations of IL-1 in pre-eclampsia has reported in one study, no differences in the plasma levels were observed by another study. Results of the present study, demonstrate an enhanced secretion of immunoreactive IL-1α and IL-1β by the pre-eclampsia placenta.

The results of this study demonstrating a significant increase in the secretion of immunoreactive TNFα by pre-eclamptic placenta confirms earlier findings and also suggests that placenta could be one of the source of increased TNFα levels in circulation and in amniotic fluid. However, TNFα mRNA was not detectable in the placental tissues from both the groups. Earlier studies have also reported low to negligible levels of TNFα mRNA in pre-eclamptic placenta and could not be detected in normal tissues.

In contrast to the increased production of the proinflammatory cytokines IL-1α, IL-1β and TNFα by the pre-eclamptic placenta, the results of this study demonstrated a significant reduction in expression of IL-8 and IL-5 mRNA, accompanied with a reduced secretion of the respective immunoreative proteins by the pre-eclamptic placental explants in culture when compared to the normal controls.

The observation of decreased production of IL-6 by the pre-eclamptic placenta is in agreement with the previous reports. In addition, in the present study, we also found a decrease in the IL-6 mRNA levels. Since placenta is an important source of IL-6 during pregnancy, the decreased IL-6 production by the pre-eclampsia placenta could be the reason for the observed decrease in AF IL-6 levels.

Both protein and mRNA levels of the anti-inflammatory cytokine IL-10 are increased in the pre-eclamptic placenta.

It can thus be inferred from the present study that there is an imbalance in both Th1 and Th2 cytokine expression in the placental tissues from pre-eclamptic patients compared to those from normotensive control group.

It has been demonstrated by *in vitro* experiments that reduced oxygen tension stimulates placental production of inflammatory cytokines like TNFα, IL-1α and IL-1β. This suggests that placental ischaemia, a characteristic of pre-eclampsia may be the causative factor for the aberrations in the placental cytokine production.

An abnormal activation of the immune system has been postulated to have a role in the aetiology of pre-eclampsia and it has been suggested that this syndrome is the result of an exaggerated inflammatory reaction of the mother towards the fetal-allograft. There are substantial published evidences supporting a systemic activation of maternal inflammatory cell responses in pre-eclampsia, accompanied with an increase in the levels of proinflammatory cytokines like IL-1α and TNFα into the maternal circulation. Increased expression and release of these pivotal proinflammatory cytokines by the pre-eclamptic placenta as demonstrated in the present study supports this hypothesis.

Cytokines have been demonstrated as one of the important causative factors for endothelium activation in pre-eclampsia. It has been suggested that increased circulating levels of proinflammatory cytokines in pre-eclamptic patients induces a variety of structural and functional alterations in the endothelial cells. Inflammatory cytokines are known to regulate the gene expression of signaling molecules involved in endothelial activation.

IL-1β has been shown to induce expression of cell adhesion molecules in endothelial cells, which in turn may contribute to the loss of endothelial function through increased neutrophil adhesion. In this regards, serum levels of ICAM-1 and VCAM-1 have been shown to be enhanced in pre-eclamptic patients.

TNFα, which has emerged as an important factor involved in the pathogenesis of pre-eclampsia has been shown to be involved in transcriptional regulation of PDGF and ET-1 genes. In addition, TNFα also induces the expression of plasminogen activator inhibitor-1 (PAI-1) in human endothelial cells. Levels of PAI-1 and its mRNA have been shown to be increased in the placenta of pre-eclampsia and IUGR patients. Increase TNF levels in pre-eclamptic placenta may also contribute to increased placental vasoconstriction by stimulating cyclooxygenase activity leading to increased thromboxane synthesis.

Thus, cytokines-induced changes in endothelial cell markers suggest a link between cytokine dysregulation and endothelial dysfunction in pre-eclampsia.

In addition to their detrimental effects on the endothelium, the alterations in the placental levels of these cytokines in pre-eclampsia could have other important implications within the feto-placental unit. Therefore, cytokine network in human pregnancy plays a role not only in immune protection of the fetal-allograft, but also in regulating other complex events of pregnancy including implantation, trophoblast cell invasion and placental growth and development.

A highly regulated invasion of the trophoblast cells into the uterine wall and the remodeling of the uteroplacental vessels occur during the development of human placenta. Abnormally shallow cytotrophoblast invasion resulting in failure of physiological remodeling of decidual vessels, with concomitant structural defects in the spiral arterioles contributes to the pathogenesis of pre-eclampsia. Cytokines have an important role in regulating trophoblast invasion. The disturbances in the cytokine network in pre-eclampsia may give rise to inadequate trophoblast invasion and defective placentation. Therefore, aberrations in cytokine network within the fetoplacental unit could be implicated in the aetiology of pre-eclampsia.