

The Role of Decidual Natural Killer Cells in Normal Placentation and in the Pathogenesis of Preeclampsia

Genevieve Eastabrook, BSc(Hons), MD,^{1,2} Yuxiang Hu, MSc,²
Peter von Dadelszen, MB, ChB, DPhil, FRCSC^{1,2}

¹Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver BC

²Child and Family Research Institute, Vancouver BC

Abstract

Adequate invasion of the human placenta during the first weeks of pregnancy is a critical step in ensuring both fetal and maternal health. A rapidly expanding body of evidence suggests that decidual natural killer (dNK) cells, a distinct population of CD56^{bright}CD16⁻ lymphocytes, are key regulators of this complex process. Experiments using murine models and *in vitro* evidence using human tissue cultures suggest that dNK cells modulate extravillous trophoblast (EVT) invasion and remodelling of maternal spiral arteries via both contact-dependent and contact-independent mechanisms. In addition, the differential expression of surface receptors by dNK cells may have a role in determining reproductive success through modulation of the maternal immune system at the time of implantation and placentation. The roles of cytokines, chemokines, and growth factors secreted by dNK cells and their influence on EVT migration, invasion, and pseudovasculogenesis are of particular interest. We reviewed the available experimental evidence related to the functional relationships between dNK cells and trophoblasts at the time of placentation to elucidate potential clinical correlations with human pathologies, including preeclampsia, recurrent pregnancy loss, IVF failure, and placenta accreta.

Résumé

L'implantation adéquate du placenta humain au cours des premières semaines de la grossesse constitue une étape cruciale pour ce qui est d'assurer la santé tant fœtale que maternelle. Un ensemble de données de plus en plus imposant laisse entendre que les cellules déciduales tueuses naturelles (dNK), soit une population distincte de lymphocytes CD56^{clair}CD16⁻, constituent des régulateurs clés de ce processus complexe. Les expériences menées sur des modèles murins et les données *in vitro* issues de cultures de tissus humains semblent indiquer que les cellules dNK

modulent l'implantation du trophoblaste extravilloux (TEV) et le remodelage des rameaux flexueux de l'artère utérine maternelle tant par l'intermédiaire de mécanismes qui dépendent du contact que par des mécanismes qui n'en dépendent pas. De plus, il est possible que l'expression différentielle des récepteurs de surface par les cellules dNK joue un rôle dans la détermination de la réussite génésique par l'intermédiaire de la modulation du système immunitaire maternel au moment de l'implantation et de la placentation. Les rôles des cytokines, des chimiokines et des facteurs de croissance sécrétés par les cellules dNK et leur influence sur la migration, l'implantation et la pseudovasculogenèse du TEV revêtent un intérêt particulier. Nous avons analysé les données expérimentales disponibles en ce qui a trait aux relations fonctionnelles entre les cellules dNK et les trophoblastes au moment de la placentation, et ce, afin d'éclaircir les corrélations cliniques potentielles avec les pathologies humaines, dont la prééclampsie, l'interruption de grossesse récurrente, l'échec de la FIV et le placenta accreta.

J Obstet Gynaecol Can 2008;30(6):467-476

INTRODUCTION

The process of human placentation is complex and still poorly understood, despite being central to reproductive success. Inadequate placental invasion has been associated with such reproductive complications as preeclampsia, IUGR, and recurrent pregnancy loss,¹⁻³ while uncontrolled invasion is implicated in the spectrum of placenta accreta, percreta, and increta, gestational trophoblastic disease, and choriocarcinoma.⁴⁻⁶

The burden of disease associated with placental dysfunction is great. Ischemic placental disease, including preeclampsia, placental abruption, and growth restriction, was implicated in more than half of the induced preterm births in one large population-based study.⁷ The effect of preeclampsia is particularly significant: complicating approximately 5% of pregnancies, it remains one of the two most common causes of maternal death, both in developed⁸⁻¹³ and in developing¹⁴⁻¹⁷ nations. Pulmonary embolism and

Key Words: Assisted reproductive technology, chemokines, decidua, interferon type II, natural killer cells, placenta, preeclampsia, recurrent pregnancy loss, trophoblasts

Competing Interests: None declared.

Received on August 29, 2007

Accepted on January 17, 2008

preeclampsia are the most common direct causes of maternal mortality in Canada; preeclampsia contributed to 20% of the maternal deaths reported between 1997 and 2000.¹⁸ Preeclampsia also leads to increased risk of perinatal morbidity and mortality, particularly due to prematurity.^{19,20} In addition to preterm delivery for fetal indications, many otherwise healthy fetuses are delivered before term because of deteriorating maternal health associated with preeclampsia.⁷

Our knowledge of the cellular and molecular processes of human trophoblast invasion is based mainly on *in vitro* studies and animal models; there is considerable evidence that dNK cells are crucial in successful placentation.^{21–25} They are key mediators of maternal immune system interactions with fetal cells. They are also involved in modulating EVT invasion and the remodelling of maternal spiral arteries.^{22,25,26} They express a wide range of surface receptors and signalling molecules, including cytokines, chemokines, and growth factors,^{27–29} and their functions in modulating EVT migration, invasion, and alteration from epithelial to endothelial phenotype are only beginning to be revealed.^{27,30}

METHODS

Medline and Google Scholar searches were carried out using the terms assisted reproductive technology, chemokines, cytokines, decidua, interferon gamma, natural killer cell, placenta, placenta accreta, preeclampsia,

trophoblast, and uterus. Wherever possible, primary research papers were cited in place of topic reviews. Articles were selected on the basis of the quality of their scientific method and relevance to human placentation.

DISCUSSION

Decidual Natural Killer Cells

Decidual natural killer cells are a unique population of CD56^{bright}CD16⁻ cells, phenotypically different from the main population of CD56^{dim}CD16^{bright} pbNK cells.^{31,32} Decidual natural killer cells are functionally distinct from pbNK (they have lower cytolytic activity), and they express a different cytokine repertoire. Their cytotoxicity, proliferation, and cytokine production are enhanced *in vitro* by interleukin-2 (IL-2) and IL-15.³³

Histologically, dNK are identifiable as small granular cells found in proliferative endometrium. They increase abundantly during the secretory phase of the menstrual cycle, indicating that they may have a role in implantation.³⁴ Furthermore, dNK may have a role in endometrial differentiation and breakdown and may aid the initiation of menstruation via apoptosis.³⁵ The appearance of dNK is one of the histological hallmarks of decidualization.³⁶ There is evidence that whenever or wherever decidual tissue is formed, dNK are invariably present, even in foci of endometriosis remote from the uterus.³⁵ Interestingly, dNK are found in higher numbers in the decidual tissue of women experiencing breakthrough bleeding while using the levonorgestrel-releasing intrauterine system (Mirena) than in amenorrhic users.³⁷

dNK and EVT Interactions at the Maternal-Fetal Interface

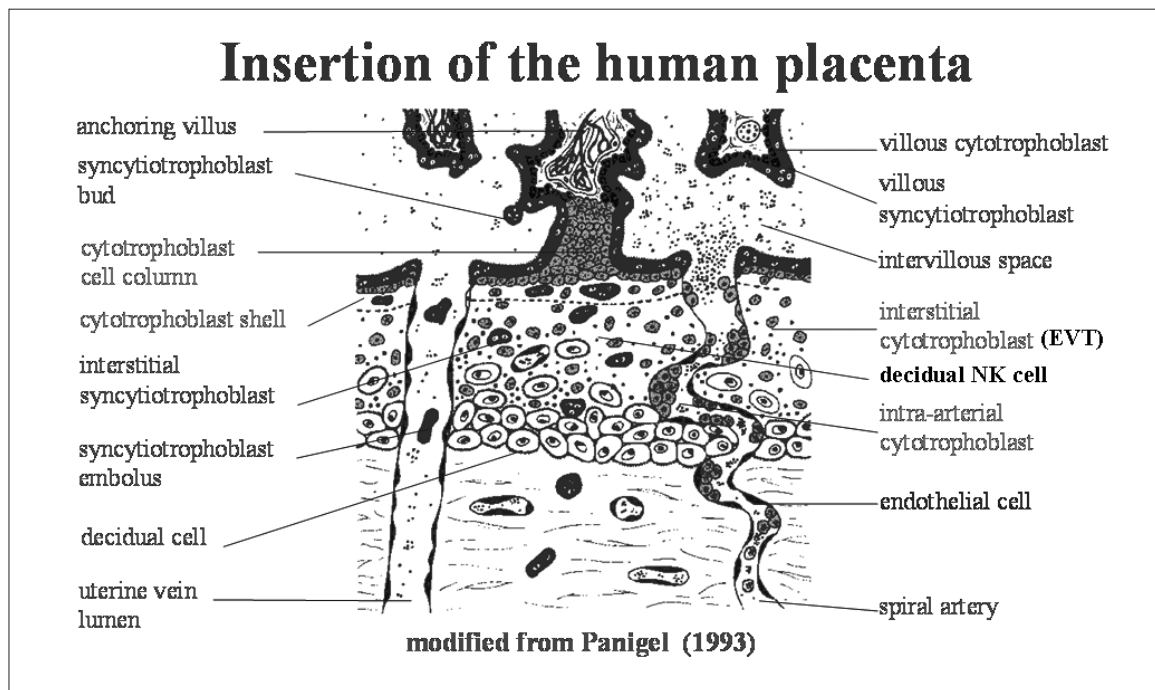
The close temporal-spatial relationship between dNK and EVT suggests that dNK regulate implantation and uterine artery remodelling through receptor-ligand interactions. Numerous dNK are found close to infiltrating EVT during implantation^{34,38} (Figure 1).³⁹ EVT lack human leukocyte antigen-A (HLA-A) and HLA-B expression, which may prevent interaction with maternal cytotoxic T-cells.⁴⁰ EVT express HLA-C which, through interaction with members of the KIR gene family expressed on NK cells,^{33,41,42} influences placental development.⁴¹ This process may be mediated through the action of the dNK-produced cytokine, IFN- γ .⁴⁰

EVT also express two non-classical MHC antigens, HLA-E and HLA-G.^{43,44} dNK express KIRs which bind to HLA-E and HLA-G, thus inhibiting NK cytotoxicity and increasing expression of pro-inflammatory and pro-angiogenic cytokines, respectively.^{43,45,46} Expression of HLA-G may protect EVT against dNK lysis.⁴⁷ Usually, EVT invading

ABBREVIATIONS

dNK	decidual natural killer cells
EVT	extravillous trophoblast
HLA	human leukocyte antigen
IFN- γ	interferon-gamma
IL-8	interleukin-8
IP-10	interferon-inducible protein-10
IUGR	intrauterine growth restriction
IVF	<i>in vitro</i> fertilization
KIR	killer immunoglobulin-like receptor
LAK	lymphokine activated killer cells
MIG	monokine inducible by gamma interferon
pbNK	peripheral blood NK cells
PIGF	placental growth factor
RANTES	regulated on activation, normal T-cell expressed and secreted
RPL	recurrent pregnancy loss
sEng	soluble endoglin
sFlt-1	soluble fms-like tyrosine kinase-1
VEGF	vascular-endothelial growth factor

Figure 1. Insertion of the Human Placenta. The close temporal-spatial relationship between dNK and EVT suggests that dNK regulate implantation and uterine artery remodelling through receptor-ligand interactions. Numerous dNK are found close to infiltrating EVT during implantation.



the decidua express and upregulate HLA-G,⁴⁸ but a lack of HLA-G expression has been noted in EVT from the placentas of pre-eclamptic women. This suggests that failed EVT invasion may precede the abnormal placentation of preeclampsia.^{49–51} Recent *in vitro* and *in vivo* studies demonstrate the anti-angiogenic and pro-apoptotic actions of the soluble form of HLA-G, secreted by EVT.^{52,53} Thus, in addition to its immunological functions, HLA-G may directly regulate spiral artery remodelling.⁵³

The actions of activating and inhibitory receptors expressed by dNK may influence human reproductive success. Varla-Leftherioti et al.⁵⁴ found that women with recurrent spontaneous abortions have a limited range of KIRs (compared with normal controls), and that the majority of these women lacked KIRs expressed by their partners. The authors concluded that in this population, miscarriages may occur as dNK lacking the appropriate inhibitory KIRs recognize trophoblastic HLA class I molecules.⁵⁴ In addition, specific combinations of maternal dNK, KIR, and fetal HLA-C genes appear to alter the balance between risk of preeclampsia and reproductive success.⁴¹ An imbalance in KIR expression may also have a role in implantation failure following IVF.^{55,56}

The differentiation of trophoblasts to the invasive extravillous phenotype is seen as a crucial part of placental angiogenesis.⁵⁷ This process involves the switching of $\alpha\beta$

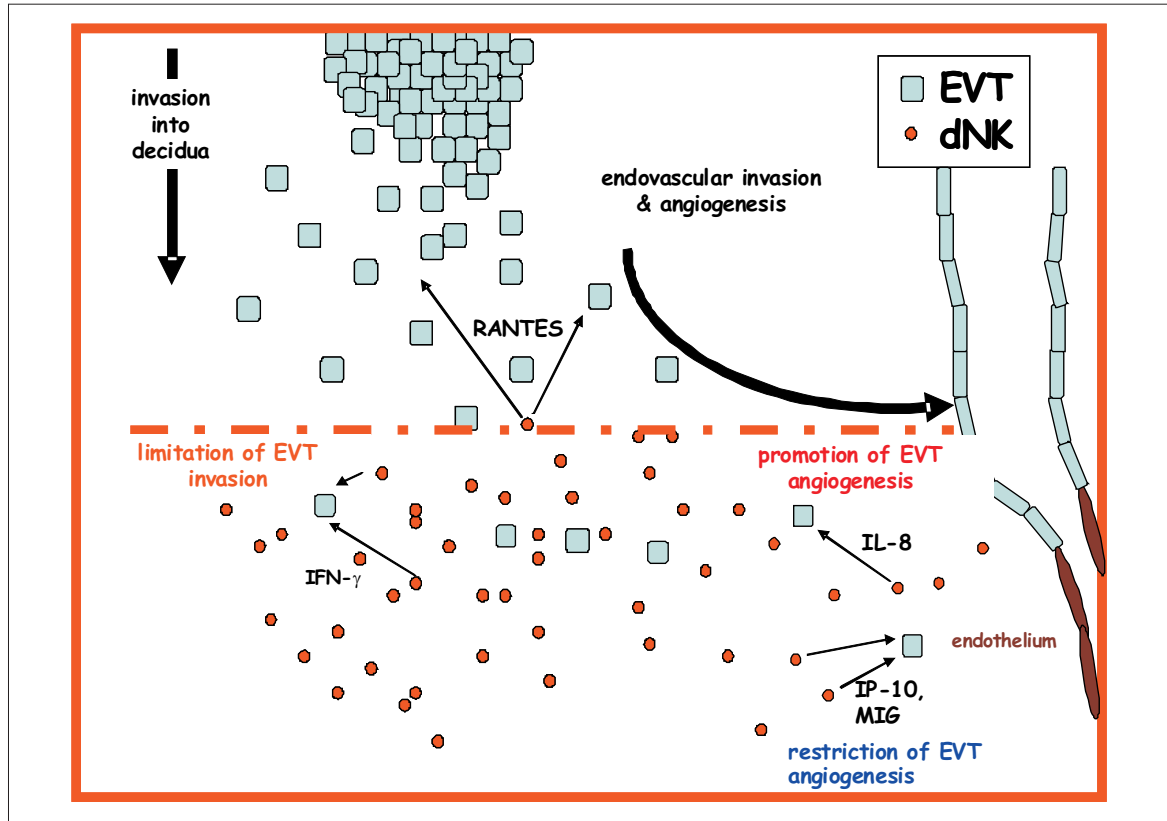
to $\alpha V\beta 3$ integrins (adhesion molecules involved in cell-ECM interactions) and the downregulation of E-cadherin (adhesion molecule involved in cell-cell interactions).^{58–60} As a result, trophoblasts evolve from an epithelial to an endothelial phenotype, a process described as pseudovasculogenesis.^{58,61–63} Pseudovasculogenesis is one of the key processes that becomes impaired in the placentas of women with preeclampsia.⁶²

Decidual NK cell-derived cytokines, growth factors, and other soluble products modulate EVT cell adhesion molecule expression during EVT invasion.³⁰ For example, in *in vitro* experiments, E-cadherin mRNA and protein expression levels increase in EVTs following exposure to both dNK contained within hollow fibres and to dNK conditioned medium³⁰; both are contact-independent mechanisms. E-cadherin expression may^{61,62} or may not⁵⁹ be maintained in EVT that exhibit the shallow invasion described in placental bed biopsies from women with preeclampsia.

The Role of Cytokines in dNK-EVT Interactions

Decidual natural killer cells produce numerous cytokines implicated in regulating trophoblast invasion, including granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage CSF (GM-CSF), macrophage CSF (M-CSF), leukaemia inhibitory factor (LIF), tumour necrosis factor alpha (TNF- α), and IFN- γ .²⁸

Figure 2. dNK-EVT Interactions at the Maternal-Fetal Interface. The complex interplay between dNK cells and EVT at the maternal-fetal interface is mediated by cytokines, chemokines, and growth factors. dNK-produced IFN- γ may limit EVT migration alone or in concert with IFN-inducible chemokines such as MIG and IP-10. IL-8 and IP-10 may both promote EVT migration while enhancing (IL-8) and restricting (IP-10) angiogenesis. RANTES may contribute to EVT migration and differentiation to an invasive phenotype.



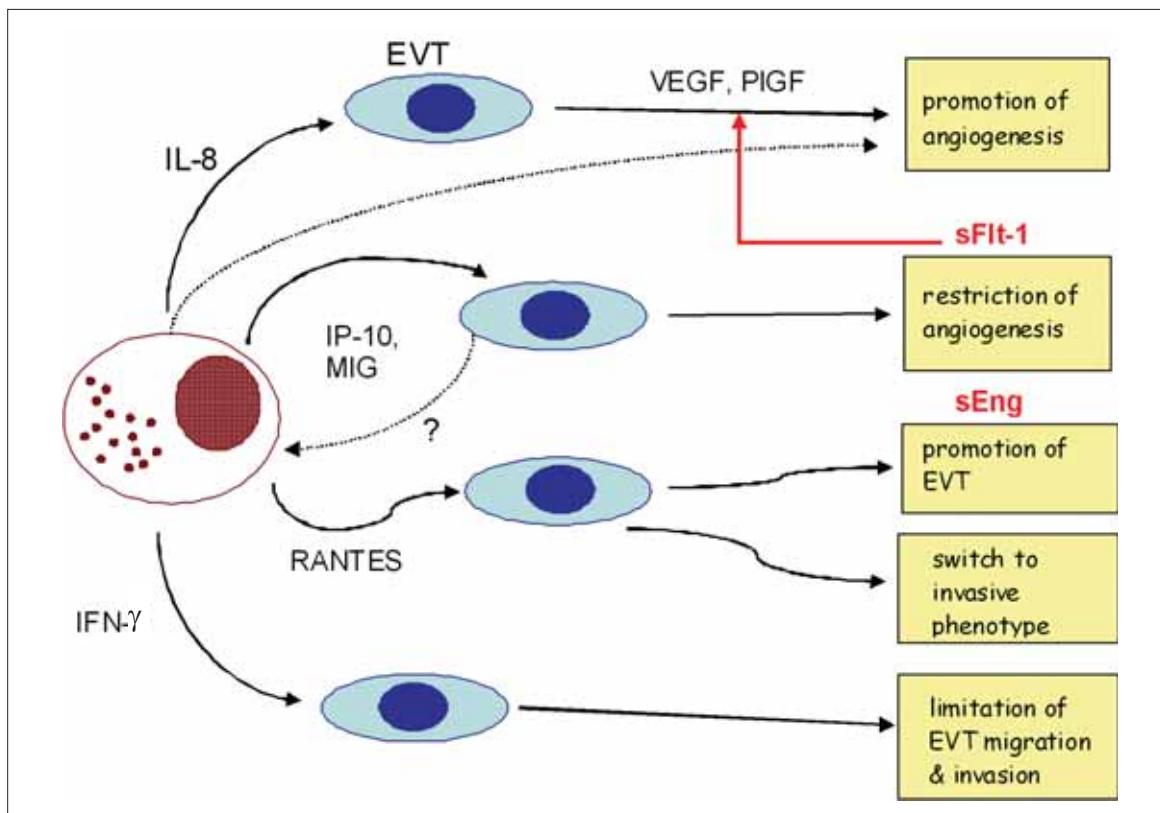
IFN- γ is of particular interest because knock-out mice lacking genes for IFN- γ production or for IFN- γ receptors exhibit abnormal placentation, and consequently have adverse pregnancy outcomes.^{26,64} Conversely, mice lacking cells of the NK lineage have histologically abnormal pregnancies, but the outcomes of their pregnancies are more variable.^{65,66} In mouse models, IFN- γ modulates uterine vascular modification, decidual integrity,²⁶ and dNK maturation.^{22,26} In a novel collagen (two-dimensional) model of placentation developed in our laboratory, the presence of dNK resulted in contact-independent inhibition of normal cytotrophoblast migration.³⁰ This was associated with changes in the cytotrophoblast expression of metalloproteases-2 and 9 (proteolytic enzymes associated with ECM degradation), and plasminogen activator inhibitor-1 (an inhibitor of fibrinolysis). In contrast, dNK did not affect EVT proliferation, apoptosis, or cell column formation. dNK effects were partially reversed by neutralizing antibodies against IFN- γ . Thus, this model provides

evidence for the role of dNK in directly modulating EVT differentiation during column formation and migration from anchoring villi.³⁰

Placental Angiogenesis: A Role for dNK?

Although it is known that dNK affect trophoblast migration and invasion both *in vivo* and *in vitro*, the mechanisms behind this effect are incompletely understood. In particular, the role of dNK in mediating angiogenesis has not been explored to any great extent. Decidual NK express angiogenic growth factors throughout the menstrual cycle, which suggests a role in endometrial angiogenesis and regeneration.²⁹ Decidual NK express high levels of VEGF-C, PIGF, and angiotensin 2 during the secretory phase of the menstrual cycle.²⁹ VEGF-C acts via VEGF-R2 and R3 receptors, which are expressed solely on dNK within the endometrium.²⁹ Although abnormal expression of VEGF and its receptors has been reported in cases of placenta accreta,⁶⁷ the role of dNK cells in the development

Figure 3. The Putative Roles of dNK Cells in EVT Migration, Invasion, and Placental Angiogenesis. Experimental evidence suggests that dNK-produced chemokines, cytokines, and growth factors may contribute to EVT migration, invasion, phenotypic switch, and ultimately, angiogenesis.



of this disorder has not been investigated in any studies published to date.

Recently it has been demonstrated that dNK, but not pbNK subsets, regulate trophoblast invasion both in vitro and in vivo through the chemokines IL-8 and IP-10.²⁷ These studies provide evidence that dNK can secrete an array of angiogenic factors and that EVT express the corresponding receptors. This suggests that dNK may induce vascular growth during placentation (Figure 2). In the development of malignancies, IL-8 is pro-angiogenic, while IP-10 is potently angiostatic.^{68,69} An appropriate balance of pro- and anti-angiogenic chemokines produced by dNK may help to regulate maternal spiral artery remodelling and placental angiogenesis (Figure 3); this warrants further investigation.

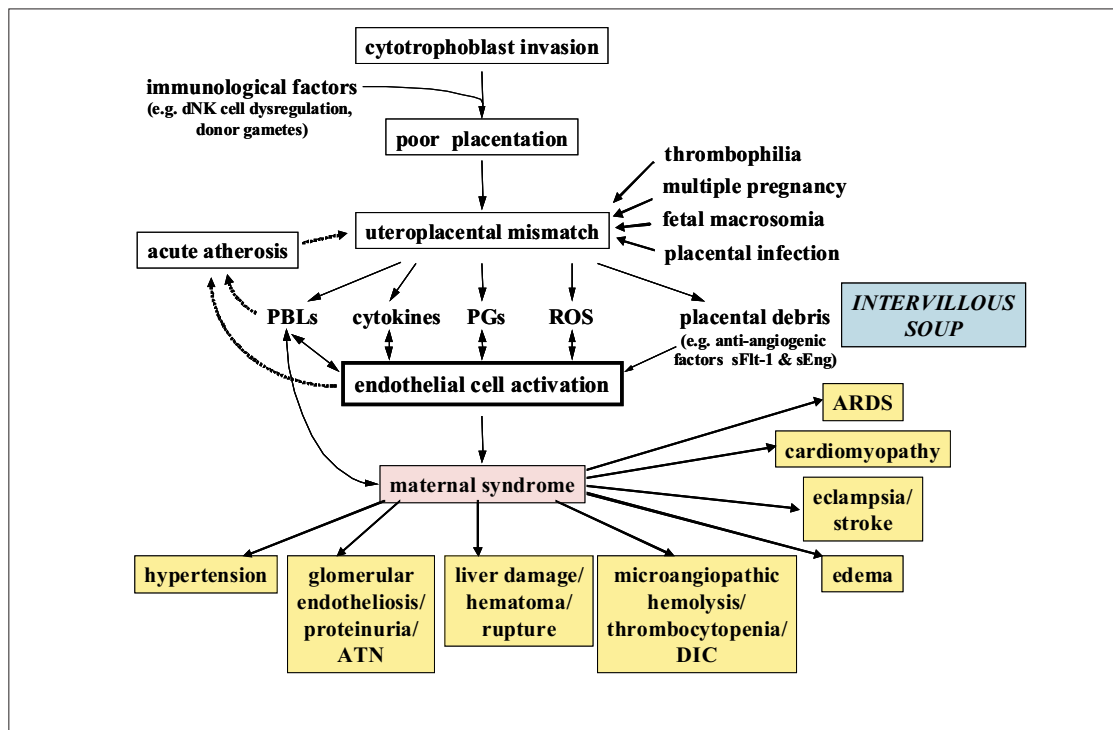
LAK cells mediate angiogenesis in vitro.⁷⁰ This subset of peripheral blood mononuclear cells (which include NK cells) is activated using IL-2, a cytokine elevated in the sera of women with preeclampsia.⁷¹ EVT angiogenesis is reduced by LAK cells in vitro.⁷⁰ This effect is modulated through EVT-derived sFlt-1, a truncated and inactive receptor for both VEGF and PlGF.⁷² Serum levels of sFlt-1

are elevated in women with preeclampsia.⁷³ Shedding of this anti-angiogenic protein from the placenta into the maternal circulation may contribute to the systemic endothelial dysfunction^{73–75} which is a hallmark of preeclampsia (Figure 4).⁷⁶ Another anti-angiogenic protein of interest in women with preeclampsia is sEng, thought to impair TGF-β1 binding to cell surface receptors and to decrease endothelial nitric oxide signalling.⁷⁷ Recent studies indicate that levels of sEng (produced by the placenta) are elevated in the serum of women with preeclampsia, increasing with disease severity and falling after delivery of the placenta^{78,79}; it has also been implicated in the pathophysiology of HELLP syndrome.⁵⁷

dNK-Derived Chemokines in EVT Migration and Differentiation

Chemokines, which were originally identified as critical regulators of leukocyte migration,⁶⁸ are abundant in endometrial epithelial and decidual cells at the time of implantation and trophoblast invasion.^{80–83} The chemokine repertoire of dNK in particular is diverse. For example, dNK express IL-8 mRNA,^{27,28} and produce large amounts of this chemokine without stimulation.²⁸ dNK also produce

Figure 4. The Pathogenesis of Preeclampsia. The maternal syndrome of preeclampsia (multiple organ dysfunction) is the result of an interplay between numerous maternal and fetal factors, beginning with impaired placental invasion.



PBLs: peripheral blood lymphocytes; PGs: prostaglandins; ROS: reactive oxygen species; ARDS: acute respiratory distress syndrome; ATN: acute tubular necrosis; DIC: disseminated intravascular coagulation.

a large quantity of RANTES, interferon-inducible protein-10 (IP-10), and monokine inducible by gamma interferon (MIG).²⁷ CC-chemokine receptor 1 (CCR1) ligands (chemokines) including RANTES and macrophage inflammatory protein-1 α (MIP-1 α) have been identified in decidual tissues. These chemokines promote migration of EVT isolated from explant cultures in vitro⁸⁴ (Figure 2).

The expression of chemokine receptors appears to differ temporally, spatially, and by trophoblast cell type. For example, the chemokine receptors CX3CR1 and CCR1 have been immunolocalised to endovascular EVT but not to invading interstitial EVT (iEVT). Conversely, CCR3 has been localised to invading iEVT and to syncytial microvilli.⁸⁵ In addition, trophoblasts acquire CCR1 as they differentiate to an invasive phenotype at villus-anchoring sites.⁸⁶ This suggests a role for the chemokine-CCR1 system in the initial step of trophoblastic invasion toward maternal tissue.

dNK Cells in Recurrent Pregnancy Loss and IVF Failure

Several studies of women experiencing idiopathic recurrent pregnancy loss have found abnormalities in the dNK cell populations in the peri-implantation endometrium of these

patients. Decreased populations of CD56^{bright}CD16⁻ dNK cells and increased numbers of CD56^{dim}CD16⁺ dNK cells have been reported.^{87–89} However, the results of functional studies have been less consistent. For example, while one study reported decreased dNK cytotoxicity in vitro during normal pregnancies in comparison with anembryonic gestations or in women with RPL,⁹⁰ another study reported decreased dNK cytotoxicity in cells isolated from women with RPL.⁹¹ At present, there does not appear to be any prognostic value in predicting the outcomes of future pregnancies by measuring dNK from endometrial biopsies of RPL patients. Although the numbers of dNK cells are higher in women with a history of RPL than in normal controls, the significance of this finding is uncertain.⁹²

In patients experiencing repeated implantation failure following IVF, abnormalities in dNK cell CD56 and CD16 expression have been correlated with uterine artery Doppler abnormalities and altered expression of the cytokines IL-12 and IL-18.^{93,94} However, this finding has not been consistent in all studies reported to date.⁹⁵

NK Cell Dysregulation in Preeclampsia

It has been proposed previously that an imbalance of TH1/TH2 immunity may be involved in poor placentation

and consequently in preeclampsia.^{96,97} However, this putative TH1/TH2 imbalance is likely an oversimplification of more complex immune processes that involve not only these subsets of T cells but also NK cells (Figure 4). NK cells may mediate changes in systemic type 1 and type 2 immunities, both in normal pregnancy and in women with preeclampsia.⁹⁸ Greater numbers of CD56^{dim} and CD94⁺ cells have been found in the decidua of women with preeclampsia than in normal controls at term,⁹⁹ indicating a role for altered expression of dNK cell receptors in the development of this disorder. In addition, villous trophoblasts in the placentas of women with preeclampsia express significantly less IL-12 than normal controls.⁹⁹ In contrast, women with preeclampsia have been found to have significantly elevated serum levels of IL-12 and IL-15.⁹⁹ Together, these findings point to a role for NK dysregulation in the pathogenesis of preeclampsia. However, because the decidua and trophoblasts were examined at term, it is still unknown whether NK dysregulation is a cause or an effect of preeclampsia in these cases.

Future Directions

Continued investigation of the role of dNK in trophoblast invasion and spiral artery remodelling will provide insights into the mechanisms of both normal and pathological placentation. Within general obstetrics and maternal-fetal medicine, a more thorough understanding of placentation is critical in understanding preeclampsia, IUGR, intrauterine fetal demise, and other disorders associated with abnormal placentation. Evidence of altered dNK KIR repertoires⁵⁴ and reduced expression of CD56^{bright}CD16⁻ dNK cells in women with recurrent pregnancy loss⁸⁷⁻⁸⁹ and recurrent post-embryo transfer IVF failure^{93,94} has implications within the disciplines of reproductive endocrinology, infertility, and assisted reproductive technologies. As the rates of Caesarean section continue to rise,^{100,101} there is likely to be a concomitant increase in the incidence of placenta accreta, increta, and percreta¹⁰²; examination of the cellular and immunological mechanisms underlying uncontrolled invasion of the trophoblast are crucial in better understanding these disorders. The presence of altered dNK receptor repertoires within the placentas of preeclamptic women, and elevated levels of pro-inflammatory cytokines in the serum of preeclamptic women^{96,98,99} demonstrate the roles of both the basic and clinical sciences in developing diagnostic tests and eventually, in the creation of targeted therapies for this disorder.

Further work is necessary in order to characterize the actions of dNK-derived cytokines (particularly IFN- γ), chemokines (IL-8, IP-10, and RANTES), and growth factors (VEGF, PlGF) at the human maternal-fetal interface (Figure 2). Our ongoing investigation of dNK-trophoblast

interactions will provide crucial insights into the relationship between the maternal immune system and placental angiogenesis, a link which has ramifications within all facets of reproductive health.

ACKNOWLEDGEMENTS

Special thanks to Dr Roger Pierson for editorial assistance and to the Strategic Training Initiative in Research in Reproductive Health Sciences (STIRRHs) for research support.

REFERENCES

1. Brosens I, Dixon HG, Robertson WB. Fetal growth retardation and the arteries of the placental bed. *Br J Obstet Gynaecol* 1977;84(9):656-63.
2. Khong TY, De WF, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by preeclampsia and by small-for-gestational age infants. *Br J Obstet Gynaecol* 1986;93(10):1049-59.
3. Norwitz ER. Defective implantation and placentation: laying the blueprint for pregnancy complications. *Reprod Biomed Online* 2006;13(4):591-9.
4. Bulmer JN. Immune aspects of pathology of the placental bed contributing to pregnancy pathology. *Baillieres Clin Obstet Gynaecol* 1992;6(3):461-88.
5. Labarrere CA, Althabe OH. Primary chronic abortion, preeclampsia, idiopathic intrauterine growth retardation, hydatidiform mole, and choriocarcinoma: a unifying concept. *Am J Reprod Immunol Microbiol* 1986;10(4):156-7.
6. Harma M, Harma M. Defective placentation and resultant oxidative stress play a similar role in complete hydatidiform mole to that in preeclampsia and early pregnancy loss. *Med Hypotheses* 2006;66(1):100-2.
7. Ananth CV, Vintzileos AM. Maternal-fetal conditions necessitating a medical intervention resulting in preterm birth. *Am J Obstet Gynecol* 2006;195(6):1557-63.
8. Liston RM. The path to prevention. *J Obstet Gynaecol Can* 2005;27(2):117-21.
9. MacKay AP, Berg CJ, Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. *Obstet Gynecol* 2001;97(4):533-8.
10. von DP, Magee LA, Devarakonda RM, Hamilton T, Ainsworth LM, Yin R, et al. The prediction of adverse maternal outcomes in preeclampsia. *J Obstet Gynaecol Can* 2004;26(10):871-9.
11. Why mothers die 2000-2002—The sixth report of confidential enquiries into maternal deaths in the United Kingdom. Lewis G, ed. London: Royal College of Obstetricians and Gynaecologists; 2004.
12. Wen SW, Huang L, Liston R, Heaman M, Baskett T, Rusen ID et al. Severe maternal morbidity in Canada, 1991-2001. *CMAJ* 2005;173(7):759-64.
13. Zhang J, Meikle S, Trumble A. Severe maternal morbidity associated with hypertensive disorders in pregnancy in the United States. *Hypertens Pregnancy* 2003; 22(2):203-12.
14. Duley L. Maternal mortality associated with hypertensive disorders of pregnancy in Africa, Asia, Latin America and the Caribbean. *Br J Obstet Gynaecol* 1992;99(7):547-53.
15. Ngoc NT, Merialdi M, bdel-Aleem H, Carroli G, Purwar M, Zavaleta N, et al. Causes of stillbirths and early neonatal deaths: data from 7993 pregnancies in six developing countries. *Bull World Health Organ* 2006;84(9):699-705.
16. Romero-Gutierrez G, Espitia-Vera A, Ponce-Ponce de Leon AL, Huerta-Vargas LF. Risk factors of maternal death in Mexico. *Birth* 2007;34(1):21-5.
17. World Health Organization. Revised 1990 estimates of maternal mortality: a new approach by WHO and UNICEF. Geneva: WHO; 1996.

18. Rusen ID, Liston RM. Special report on maternal mortality and severe morbidity in Canada: enhanced surveillance: the path to prevention. Public Health Agency of Canada; 2008.
19. Xiong X, Buekens P, Pridjian G, Fraser WD. Pregnancy-induced hypertension and perinatal mortality. *J Reprod Med* 2007;52(5):402-6.
20. Hayter MA, Anderson L, Claydon J, Magee LA, Liston RM, Lee SK, et al. Variations in early and intermediate neonatal outcomes for inborn infants admitted to a Canadian NICU and born of hypertensive pregnancies. *J Obstet Gynaecol Can* 2005;27(1):25-32.
21. Ashkar AA, Black GP, Wei Q, He H, Liang L, Head JR, et al. Assessment of requirements for IL-15 and IFN regulatory factors in uterine NK cell differentiation and function during pregnancy. *J Immunol* 2003;171(6):2937-44.
22. Ashkar AA, Croy BA. Functions of uterine natural killer cells are mediated by interferon gamma production during murine pregnancy. *Semin Immunol* 2001;13(4):235-41.
23. Croy BA, He H, Esadeg S, Wei Q, McCartney D, Zhang J, et al. Uterine natural killer cells: insights into their cellular and molecular biology from mouse modelling. *Reproduction* 2003;126(2):149-60.
24. Croy BA, Chantakru S, Esadeg S, Ashkar AA, Wei Q. Decidual natural killer cells: key regulators of placental development (a review). *J Reprod Immunol* 2002;57(1-2):151-68.
25. Parham P. NK cells and trophoblasts: partners in pregnancy. *J Exp Med* 2004;200(8):951-5.
26. Ashkar AA, Di Santo JP, Croy BA. Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J Exp Med* 2000;192(2):259-70.
27. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, et al. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* 2006;12(9):1065-74.
28. Saito S, Kasahara T, Sakakura S, Enomoto M, Umekage H, Harada N, et al. Interleukin-8 production by CD16-CD56bright natural killer cells in the human early pregnancy decidua. *Biochem Biophys Res Commun* 1994;200(1):378-83.
29. Li XF, Charnock-Jones DS, Zhang E, Hiby S, Malik S, Day K, et al. Angiogenic growth factor messenger ribonucleic acids in uterine natural killer cells. *J Clin Endocrinol Metab* 2001;86(4):1823-34.
30. Hu Y, Dutz JP, MacCalman CD, Yong P, Tan R, von DP. Decidual NK cells alter in vitro first trimester extravillous cytotrophoblast migration: a role for IFN-gamma. *J Immunol* 2006;177(12):8522-30.
31. Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, et al. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J Exp Med* 2003;198(8):1201-12.
32. Lopez-Botet M, Moretta L, Strominger J. NK-cell receptors and recognition of MHC class I molecules. *Immunol Today* 1996;17(5):212-4.
33. Verma S, Hiby SE, Loke YW, King A. Human decidual natural killer cells express the receptor for and respond to the cytokine interleukin 15. *Biol Reprod* 2000;62(4):959-68.
34. Starkey PM, Sargent IL, Redman CW. Cell populations in human early pregnancy decidua: characterization and isolation of large granular lymphocytes by flow cytometry. *Immunology* 1988;65(1):129-34.
35. King A. Uterine leukocytes and decidualization. *Hum Reprod Update* 2000;6(1):28-36.
36. Moffett-King A, Entrican G, Ellis S, Hutchinson J, Bainbridge D. Natural killer cells and reproduction. *Trends Immunol* 2002;23(7):332-3.
37. Peloggia A, Petta CA, Bahamondes L, Oliveira-Ribeiro M, Zhang J, Salamonsen LA. Endometrial chemokines, uterine natural killer cells and mast cells in long-term users of the levonorgestrel-releasing intrauterine system. *Hum Reprod* 2006;21(5):1129-34.
38. Moffett-King A. Natural killer cells and pregnancy. *Nat Rev Immunol* 2002;2(9):656-63.
39. Panigel M. The origin and structure of the extraembryonic tissues. In: Redman CWG, Sargent IL, Starkey PM, eds. *The Human Placenta*. Oxford: Blackwell Scientific; 1993:3-32.
40. King A, Burrows TD, Hiby SE, Bowen JM, Joseph S, Verma S, et al. Surface expression of HLA-C antigen by human extravillous trophoblast. *Placenta* 2000;21(4):376-87.
41. Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CW, Carrington M, Trowsdale J, et al. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J Exp Med* 2004;200(8):957-65.
42. Jacobs R, Hintzen G, Kemper A, Beul K, Kempf S, Behrens G, et al. CD56bright cells differ in their KIR repertoire and cytotoxic features from CD56dim NK cells. *Eur J Immunol* 2001;31(10):3121-7.
43. King A, Allan DS, Bowen M, Powis SJ, Joseph S, Verma S, et al. HLA-E is expressed on trophoblast and interacts with CD94/ NKG2 receptors on decidual NK cells. *Eur J Immunol* 2000;30(6):1623-31.
44. Yelavarthi KK, Fishback JL, Hunt JS. Analysis of HLA-G mRNA in human placental and extraplacental membrane cells by in situ hybridization. *J Immunol* 1991;146(8):2847-54.
45. Rajagopalan S, Bryceson YT, Kuppusamy SP, Geraghty DE, van der MA, Joosten I, et al. Activation of NK cells by an endocytosed receptor for soluble HLA-G. *PLoS Biol* 2006;4(1):e9.
46. Rajagopalan S, Long EO. A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. *J Exp Med* 1999;189(7):1093-100.
47. Dietl J, Honig A, Kammerer U, Rieger L. Natural killer cells and dendritic cells at the human feto-maternal interface: an effective cooperation? *Placenta* 2006;27(4-5):341-7.
48. McMaster MT, Librach CL, Zhou Y, Lim KH, Janatpour MJ, DeMars R, et al. Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. *J Immunol* 1995;154(8):3771-8.
49. Goldman-Wohl DS, Ariel I, Greenfield C, Hochner-Celnikier D, Cross J, Fisher S, et al. Lack of human leukocyte antigen-G expression in extravillous trophoblasts is associated with pre-eclampsia. *Mol Hum Reprod* 2000;6(1):88-95.
50. Lyall F. Mechanisms regulating cytotrophoblast invasion in normal pregnancy and pre-eclampsia. *Aust N Z J Obstet Gynaecol* 2006;46(4):266-73.
51. Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH, et al. Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. *Am J Pathol* 1997;151(6):1809-18.
52. Fons P, Chabot S, Cartwright JE, Lenfant F, L'Faqih F, Giustiniani J, et al. Soluble HLA-G1 inhibits angiogenesis through an apoptotic pathway and by direct binding to CD160 receptor expressed by endothelial cells. *Blood* 2006;108(8):2608-15.
53. Le BP, Fons P, Hérault JP, Bono F, Chabot S, Cartwright JE, et al. Soluble HLA-G and control of angiogenesis. *J Reprod Immunol* 2007;76(1-2):17-22.
54. Varla-Leftherioti M, Spyropoulou-Vlachou M, Niokou D, Keramitsoglou T, Darlamitsou A, Tsekoura C, et al. Natural killer (NK) cell receptors' repertoire in couples with recurrent spontaneous abortions. *Am J Reprod Immunol* 2003;49(3):183-91.
55. Coulam CB, Roussev RG. Correlation of NK cell activation and inhibition markers with NK cytotoxicity among women experiencing immunologic implantation failure after in vitro fertilization and embryo transfer. *J Assist Reprod Genet* 2003;20(2):58-62.

56. Ntrivalas EI, Bowser CR, Kwak-Kim J, Beaman KD, Gilman-Sachs A. Expression of killer immunoglobulin-like receptors on peripheral blood NK cell subsets of women with recurrent spontaneous abortions or implantation failures. *Am J Reprod Immunol* 2005;53(5):215–21.
57. Zhou Y, McMaster M, Woo K, Janatpour M, Perry J, Karpanen T, et al. Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. *Am J Pathol* 2002;160(4):1405–23.
58. Damsky CH, Librach C, Lim KH, Fitzgerald ML, McMaster MT, Janatpour M, et al. Integrin switching regulates normal trophoblast invasion. *Development* 1994;120(12):3657–66.
59. Floridon C, Nielsen O, Holund B, Sunde L, Westergaard JG, Thomsen SG, et al. Localization of E-cadherin in villous, extravillous and vascular trophoblasts during intrauterine, ectopic and molar pregnancy. *Mol Hum Reprod* 2000;6(10):943–50.
60. Aplin JD. Expression of integrin alpha 6 beta 4 in human trophoblast and its loss from extravillous cells. *Placenta* 1993;14(2):203–15.
61. Zhou Y, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M, et al. Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? *J Clin Invest* 1997;99(9):2139–51.
62. Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? *J Clin Invest* 1997;99(9):2152–64.
63. Aplin JD. Expression of integrin alpha 6 beta 4 in human trophoblast and its loss from extravillous cells. *Placenta* 1993;14(2):203–15.
64. Ashkar AA, Croy BA. Interferon-gamma contributes to the normalcy of murine pregnancy. *Biol Reprod* 1999;61(2):493–502.
65. Croy BA, Ashkar AA, Minhas K, Greenwood JD. Can murine uterine natural killer cells give insights into the pathogenesis of preeclampsia? *J Soc Gynecol Investig* 2000;7(1):12–20.
66. Croy BA, Ashkar AA, Foster RA, DiSanto JP, Magram J, Carson D, et al. Histological studies of gene-ablated mice support important functional roles for natural killer cells in the uterus during pregnancy. *J Reprod Immunol* 1997;35(2):111–33.
67. Tseng JJ, Chou MM, Hsieh YT, Wen MC, Ho ES, Hsu SL. Differential expression of vascular endothelial growth factor, placenta growth factor and their receptors in placentae from pregnancies complicated by placenta accreta. *Placenta* 2006;27(1):70–8.
68. Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* 2000;18:217–42.
69. Strieter RM, Polverini PJ, Kunkel SL, Arenberg DA, Burdick MD, Kasper J, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem* 1995;270(45):27348–57.
70. Matsubara K, Nagamatsu T, Fujii T, Kozuma S, Taketani Y. Lymphokine-activated killer cells induced from decidual lymphocytes reduce the angiogenic activity of trophoblasts by enhancing the release of soluble fms-like tyrosine kinase-1 from trophoblasts: an implication for the pathophysiology of preeclampsia. *J Reprod Immunol* 2005;68(1–2):27–37.
71. Hamai Y, Fujii T, Yamashita T, Nishina H, Kozuma S, Mikami Y et al. Evidence for an elevation in serum interleukin-2 and tumor necrosis factor-alpha levels before the clinical manifestations of preeclampsia. *Am J Reprod Immunol* 1997;38(2):89–93.
72. Kendall RL, Wang G, Thomas KA. Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. *Biochem Biophys Res Commun* 1996;226(2):324–8.
73. Dimitrakova ED, Dimitrakov JD, Karumanchi SA, Pehlivanov BK, Milchev NP, Dimitrakov DI. Placental soluble fms-like tyrosine-kinase-1 (sFlt-1) in pregnant women with preeclampsia. *Folia Med (Plovdiv)* 2004;46(1):19–21.
74. Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN et al. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol Reprod* 1998;59(6):1540–8.
75. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;111(5):649–58.
76. Magee LA, Helewa M, Moutquin J-M, Von Dadelszen P. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy. SOGC Clinical Practice Guideline No. 206, March 2008. *J Obstet Gynaecol Can* 2008;30(Suppl 1).
77. Toporsian M, Gros R, Kabir MG, Vera S, Govindaraju K, Eidelman DH, et al. A role for endoglin in coupling eNOS activity and regulating vascular tone revealed in hereditary hemorrhagic telangiectasia. *Circ Res* 2005;96(6):684–92.
78. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006;355(10):992–1005.
79. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006;12(6):642–9.
80. Drake PM, Red-Horse K, Fisher SJ. Reciprocal chemokine receptor and ligand expression in the human placenta: implications for cytotrophoblast differentiation. *Dev Dyn* 2004;229(4):877–85.
81. Drake PM, Red-Horse K, Fisher SJ. Chemokine expression and function at the human maternal-fetal interface. *Rev Endocr Metab Disord* 2002;3(2):159–65.
82. Red-Horse K, Drake PM, Fisher SJ. Human pregnancy: the role of chemokine networks at the fetalmaternal interface. *Expert Rev Mol Med* 2004;2004:1–14.
83. Red-Horse K, Drake PM, Gunn MD, Fisher SJ. Chemokine ligand and receptor expression in the pregnant uterus: reciprocal patterns in complementary cell subsets suggest functional roles. *Am J Pathol* 2001;159(6):2199–213.
84. Thirkill TL, Lowe K, Vedagiri H, Blankenship TN, Barakat AI, Douglas GC. Macaque trophoblast migration is regulated by RANTES. *Exp Cell Res* 2005;305(2):355–64.
85. Hannan NJ, Jones RL, White CA, Salamonsen LA. The chemokines, CX3CL1, CCL14, and CCL4, promote human trophoblast migration at the feto-maternal interface. *Biol Reprod* 2006;74(5):896–904.
86. Sato Y, Higuchi T, Yoshioka S, Tatsumi K, Fujiwara H, Fujii S. Trophoblasts acquire a chemokine receptor, CCR1, as they differentiate towards invasive phenotype. *Development* 2003;130(22):5519–32.
87. Lachapelle MH, Miron P, Hemmings R, Roy DC. Endometrial T, B, and NK cells in patients with recurrent spontaneous abortion. Altered profile and pregnancy outcome. *J Immunol* 1996;156(10):4027–34.
88. Quenby S, Farquharson R. Uterine natural killer cells, implantation failure and recurrent miscarriage. *Reprod Biomed Online* 2006;13(1):24–8.
89. Yamamoto T, Takahashi Y, Kase N, Mori H. Decidual natural killer cells in recurrent spontaneous abortion with normal chromosomal content. *Am J Reprod Immunol* 1999;41(5):337–42.
90. Chao KH, Yang YS, Ho HN, Chen SU, Chen HF, Dai HJ, et al. Decidual natural killer cytotoxicity decreased in normal pregnancy but not in anembryonic pregnancy and recurrent spontaneous abortion. *Am J Reprod Immunol* 1995;34(5):274–80.

91. Vassiliadou N, Bulmer JN. Functional studies of human decidua in spontaneous early pregnancy loss: effect of soluble factors and purified CD56+ lymphocytes on killing of natural killer- and lymphokine-activated killer-sensitive targets. *Biol Reprod* 1998;58(4):982–7.
92. Tuckerman E, Laird SM, Prakash A, Li TC. Prognostic value of the measurement of uterine natural killer cells in the endometrium of women with recurrent miscarriage. *Hum Reprod* 2007;22(8):2208–13.
93. Ledee-Bataille N, Bonnet-Chea K, Hosny G, Dubanchet S, Frydman R, Chaouat G. Role of the endometrial tripod interleukin-18, -15, and -12 in inadequate uterine receptivity in patients with a history of repeated in vitro fertilization-embryo transfer failure. *Fertil Steril* 2005;83(3):598–605.
94. Ledee-Bataille N, Dubanchet S, Coulomb-L'hermine A, Durand-Gasselini I, Frydman R, Chaouat G. A new role for natural killer cells, interleukin (IL)-12, and IL-18 in repeated implantation failure after in vitro fertilization. *Fertil Steril* 2004;81(1):59–65.
95. Matteo MG, Greco P, Rosenberg P, Mestice A, Baldini D, Falagario T, et al. Normal percentage of CD56bright natural killer cells in young patients with a history of repeated unexplained implantation failure after in vitro fertilization cycles. *Fertil Steril* 2007;88(4):990–3.
96. Dong M, He J, Wang Z, Xie X, Wang H. Placental imbalance of Th1- and Th2-type cytokines in preeclampsia. *Acta Obstet Gynecol Scand* 2005;84(8):788–93.
97. Wilczynski JR, Tchorzewski H, Banasik M, Glowacka E, Wiczorek A, Lewkowicz P, et al. Lymphocyte subset distribution and cytokine secretion in third trimester decidua in normal pregnancy and preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2003;109(1):8–15.
98. Borzychowski AM, Croy BA, Chan WL, Redman CW, Sargent IL. Changes in systemic type 1 and type 2 immunity in normal pregnancy and pre-eclampsia may be mediated by natural killer cells. *Eur J Immunol* 2005;35(10):3054–63.
99. Bachmayer N, Rafik HR, Liszka L, Bremme K, Sverremark-Ekstrom E. Aberrant uterine natural killer (NK)-cell expression and altered placental and serum levels of the NK-cell promoting cytokine interleukin-12 in pre-eclampsia. *Am J Reprod Immunol* 2006;56(5–6):292–301.
100. Chaillet N, Dumont A. Evidence-based strategies for reducing cesarean section rates: a meta-analysis. *Birth* 2007;34(1):53–64.
101. Liu S, Rusen ID, Joseph KS, Liston R, Kramer MS, Wen SW, et al. Recent trends in caesarean delivery rates and indications for caesarean delivery in Canada. *J Obstet Gynaecol Can* 2004;26(8):735–42.
102. Gielchinsky Y, Rojansky N, Fasouliotis SJ, Ezra Y. Placenta accreta—summary of 10 years: a survey of 310 cases. *Placenta* 2002;23(2–3):210–4.