Etiology of Preeclampsia: An Update

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Preeclampsia still ranks as one of obstetrics major problems. Clinicians typically encounter preeclampsia as maternal disease with variable degrees of fetal involvement. More and more the unique immunogenetic maternal - paternal relationship is appreciated, and as such also the specific 'genetic conflict' that is characteristic of haemochorial placentation. Factors influencing the unique maternal-fetal (paternal) interaction probably include the length and type of sexual relationship, the maternal (decidual natural killer cells) acceptation of the invading cytotrophoblast (paternal HLA-C), and seminal levels of transforming growth factor-b and probably other cytokines. The magnitude of the maternal response would be determined by factors including a maternal set of genes determining her characteristic inflammatory responsiveness, age, quality of her endothelium, obesity/ insulin resistance and probably a whole series of susceptibility genes amongst which the thrombophilias received a lot of attention in recent years.

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Shallow, endovascular cytotrophoblast invasion in the spiral arteries, inappropriate endothelial cell activation and an exaggerated inflammatory response are key features in the pathogenesis of preeclampsia. In the late 1980s, generalized endothelial dysfunction was considered to be one the triggering steps in its pathogenesis[1]. More recently evidence has been gathered that inappropriate endothelial activation is part of more generalized intravascular inflammatory reaction involving intravascular leucocytes as well as the clotting and complement systems[2].

Preeclampsia occurs in 3-5% of pregnancies and is a major cause (15-20%) of maternal mortality in developed countries and a leading cause of (iatrogenic) preterm birth and intra-uterine growth restriction (IUGR). Understanding the etiology and pathogenesis of preeclampsia would be the major breakthrough in clinical obstetrics. This review aims to provide un update on our current level of understanding of preeclampsia, still standing as the **NUMBER ONE** major obstetrical syndrome.

A number of hypotheses on the etiology and early pathogenesis of preeclampsia are currently popular[3]. It should be stressed that these hypotheses are certainly not mutually exclusive, but most likely interactive to some extend:

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1. The placental ischemia hypothesis

Increased trophoblast deportation, as a consequence of placental ischemia, may inflict endothelial cell dysfunction. In more recent publications, the Oxford investigators[2] explain that poor placentation should be considered to be a separate pathologic mechanism, not the cause of preeclampsia but rather a powerful predisposing factor. In the adapted form this hypothesis dictates that poor placentation is a separate disorder that once established usually but not always leads to the maternal syndrome, depending on the extent to which it causes inflammatory signals (which may depend on fetal genes) and the nature of the maternal response to those signals (which would depend on maternal genes).

2. The very low-density lipoprotein (VLDL) versus toxicity-preventing activity (TxPA) hypothesis

In preeclampsia, circulating free fatty acids (FFA) are increased already 15-20 weeks before the

onset of disease. These FFA have a variety of adverse effects on endothelial physiology. Plasma albumin exists as several isoelectric species, which range from isoelectric point (pI) 4.8 to pI 5.6. The more FFA are bound to albumin the lower the pI. Plasma albumin exerts a toxicity-preventing activity (TxPA) if it is in the pI 5.6 form. Since higher ratios of FFA to albumin cause a shift from the pI 5.6 to the pI 4.8 form of albumin, preeclamptic patients will have lower amounts of protective TxPA (pI 5.6) than normotensive pregnant women. A low ratio of TxPA to VLDL would result in cytotoxicity and triglyceride accumulation in endothelial cells. In some pregnant women (low albumin concentrations?), the burden of transporting extra FFA from adipose tissue to the liver as a response to the increase energy demands, is likely to reduce the concentration of TxPA to a point where VLDL toxicity is expressed, leading to endothelial injury[4,5].

3. The hyperdynamic disease model

According to the hyperdynamic disease model, early in pregnancy, preeclamptic patients have an elevated cardiac output with compensatory vasodilatation. The dilated systemic terminal arterioles and renal afferent arterioles may expose capillary beds to syste-mic pressures and increased flow, eventually leading to endothelial cell injury characteristic of the 'injury' seen in preeclampsia[6].

4. The immune maladaptation hypothesis

The interaction between decidual leukocytes and invading cytotrophoblast cells is essential for normal trophoblast invasion and development. Immune maladaptation may cause the shallow invasion of spiral arteries by endovascular cytotrophoblast cells and endothelial cell dysfunction mediated by an increased decidual release of Th1 cytokines, proteolytic enzymes, and free radical species[3].

5. The genetic hypothesis

The development of preeclampsia-eclampsia may be based on a single recessive gene or a dominant gene with incomplete penetrance. Penetrance may be dependent on the fetal genotype.

6. The genetic conflict hypothesis

The maternal and fetal genomes perform different roles during development. Inheritable paternal, rather than maternal, imprinting of the genome is necessary for normal trophoblast development. Preeclampsia may relate to a 'hefty' genetic conflict, or a mother unable to cope with a 'physiologic' genetic conflict.

Risk Factors

The etiology of preeclampsia is still unknown. Many risk factors have been identified[7] (Table 1). Knowing these risks factors is useful for the clinician to target groups of patients at a higher risk of developing preeclampsia.

Sperm Exposure, Primipaternity and the Paternal Factor

Generally, preeclampsia occurs in first pregnancies[1]. A previous normal pregnancy is associated with a markedly lowered incidence of preeclampsia, even a previous abortion provides some protection in this respect[8]. The protective effect of being multiparous, is however lost with change of partner. The term primipaternity was introduced by Robillard et al

Table 1. Risk factors for preeclampsia

PRECONCEPTIONAL AND/OR CHRONIC RISK FACTORS
Partner-related risk factors
nulliparity/primipaternity/teenage pregnancy
limited sperm exposure, donor insemination, oocyte donation
oral sex *(risk reduction)*
partner who fathered a preeclamptic pregnancy in another woman
Non-partner related risk factors
history of previous preeclampsia
age, interval between pregnancies
family history
Presence of specific underlying disorders
chronic hypertension and renal disease
obesity, insulin resistance, low birth weight
gestational diabetes, type I diabetes mellitus
activated protein C resistance (factor V mutation), protein S deficiency
antiphospholipid antibodies
hyperhomocysteinemia
sickle cell disease, sickle cell trait (?)
Exogenous factors
smoking *(risk reduction)*
stress, work related psycho-social strain
in utero DES exposure
PREGNANCY-ASSOCIATED RISK FACTORS
multiple pregnancy
structural congenital anomalies
hydrops fetalis
chromosomal anomalies (trisomy 13, triploidy)
hydatidiform moles
urinary tract infection

[9,10] exploring the relationship between severe preeclampsia and changes in pater-nity patterns among multig-ravidae.

Change of partner in a subsequent pregnancy [10,11] or oocyte donation[12] increase a woman's risk of preeclampsia, suggesting that prior exposure to fetal (paternal) antigens is protective[13]. Robillard postulated that preeclampsia may be a problem of primipaternity rather than primigravidity[10]. Previous sperm exposure also conveys protection. The risk of preeclampsia was tripled in women pregnant by intracytoplasmic sperm injection (ICSI) done with surgically obtained sperm (i.e. these women were never exposed to their partner's sperm) compared to women pregnant after in vitro fertilization (IVF) or ICSI with ejaculated sperm[14]. Oral tolerization to paternal antigens by oral sex and swallowing sperm is associated with a lower incidence of preeclampsia[15].

A recent Norwegian study[16] claimed that it is prolonged birth interval and not primipaternity as risk factor for preeclampsia in multiparous women. After adjustment for the interval between births in a Norwegian nationwide study, change of partner was no longer associated with an increased risk of preeclampsia. However, the latter study was based on data from the Norwegian Birth Registry. It is known that birth registries do not contain the details necessary to adequately investigate paternity in humans. There is a significant rate (5-20%) of false claimed paternities in stable couples in developed countries[17]. Those nevertheless fall into the 'same father' group in the latter study. Conclusions concerning the association between paternity and preeclampsia based on birth registry alone should be avoided, since they are a very crude indication of the real paternities and do not contain the necessary information on sexual cohabitation. In this study, when the interbirth interval in multiparous women was 10 years or more, the risk for preeclampsia approximated that among nulliparous women. This may actually have revealed genetic vascular/thrombophillic predisposition for preeclampsia which are well-known risk factors for preeclampsia with advancing age, or may have included women with one or more miscarriages between the births. They fail to discuss the findings of the previous study showing that the effect of partner change was influenced by the outcome of the preceding pregnancy. Partner change was found to convey protection if the first pregnancy was complicated by preeclampsia[18]. Interestingly, the risk to develop preeclampsia for mothers who had preeclampsia in the first pregnancy was 13.1% if she had her second pregnancy with the same partner. This risk dropped to 11.8%, if she changed the partner[19]. If anything one would assume a longer birth interval after experiencing a traumatic life event, such as a pregnancy complicated by preeclampsia. The results from these studies contradict the 'birth interval hypothesis'[18,19].

As mentioned earlier, the duration of sexual cohabitation is also an important determining risk factor. The number of sexual cohabitation's preceding pregnancy is about three times higher in normal pregnant women than in preeclamptic women and this finding provides an explanation for the high incidence of preeclampsia in teenage pregnant girls[20]. In a case-control study comparing the contraceptive histories of primiparous women with and without preeclampsia, the risk of preeclampsia for users of contraceptives that prevent exposure to sperm increases 2.4 fold[21]. In a prospective study on the relationship between sperm exposure and preeclampsia,the incidence of pregnancy-induced hypertension was 11.9% among primigravidae, 4.7% among samepaternity multigravidae, and 24.0% among new-paternity multigravidae. For both primigravidae and multigravidae, length of sexual cohabitation before conception was inversely related to the incidence of pregnancy-induced hypertension ($p < 0.0001$). Taking women cohabitating for more than 12 months as reference, a cohabitation period of 0-4 months was shown to be associated with a typical odds ratio (OR) of 11.6, a period of 5-8 months with a typical OR of 5.9, and a period of 9-12 months with a typical OR of 4.2. The very high incidence (24.0%) of pregnancy-induced hypertension among new-paternity multiparous women was found to be related to a remarkable short period of sperm exposure preceding conception[10].

The exact way by which the female organism is exposed to the paternal HLA message is uncertain. Sperm exposure does provide protection against developing preeclampsia. Actual exposure to the sperm cells appears to be important for that matter. Deposition of semen in the female genital tract provokes a cascade of cellular and molecular events that resemble a classic inflammatory response. The critical seminal factor appears to be seminal vesicle derived transforming growth factor 1 (TGF1). Seminal vesicle-derived TGF1 is secreted predominantly in a latent form. Seminal plasmin and uterine factors transform the latent form into bioactive TGF1[22]. Intra-uterine insemination of TGF1 in-vivo results in an increase in granulocyte-monocyte colony stimulating

factor (GM-CSF) production that is sufficient to initiate an endometrial leukocytosis comparable with that seen following mating[22]. The introduction of TGF1 into the uterus in combination with paternal ejaculate antigens favours the growth and survival of the semi-allogenic fetus, as evidenced by a significant increase in fetal and placental weight in animal studies, in two ways. Firstly, by initiating a post-mating inflammatory reaction, TGF1 increases the ability to sample and process paternal antigens contained within the ejaculate. Another important role of TGF1 and the subsequent post-coital inflammatory response is the initiation of a strong Type 2 immune deviation. The processing of an antigen by antigen-presenting-cells in an environment containing TGF1 is likely to initiate a Th2 phenotype within these responding T-cells[23]. By initiating a Type 2 immune response towards paternal ejaculate antigens, seminal TGF1 may inhibit the induction of Type 1 responses against the semi-allogenic conceptus that are thought to be associated with poor placental development and fetal. Decidual macrophages, present in an immune-suppressive phenotype from the moment of implantation, may inhibit NK cell lytic activity through their release of molecules such as TGF, Interleukin-10 (IL-10) and prostaglandin-E2 (PGE2). Under the influence of the local cytokine environment, antigen-presenting-cells such as macrophages and dendritic cells may take up, process and present ejaculate antigens (sperm, somatic cells, and soluble antigens) to T cells in the draining lymph nodes loss[22,23]. In mice, uptake of sperm mRNA encoding for paternal HLA by decidual antigen presenting cells has been shown to occur, with subsequent translation of sperm mRNA encoding paternal MHC class I within these maternal antigen presenting cells. These antigen-presenting cells traffic from the uterus to the draining lymph nodes during the post-coital inflammatory response. It is unknown whether or not this fascinating mechanism is operative in humankind. HLA-G is certainly not involved here, since human sperm cells do not have mRNA for HLA-G[24,25]. Since HLA-G is monomorphic it would also be a very unlikely candidate to represent the paternal HLA specificity.

In summary, the primipaternity hypothesis continues to stand strong. However, there might be an additional effect associated with prolonged birth intervals. Structural changes of the spiral arteries needed for the pregnancy, do not completely resolve following parturition. The degree of anatomical changes are related to the number of previous pregnancies; duplication and fragmentation of the internal elastic lamina and the proportion of non-muscular tissue increased with increasing parity[26]. It would be very important to know whether or not these changes regress with prolonged birth interval.

To reduce the risk of preeclampsia/eclampsia, it is better for the human female to avoid conception soon after initiating sexual relations with a new partner, regardless of her gravidity. The human female has to tolerate the semi-allogeneic graft through the father's alloantigens, and this is accomplished after an immunological stimulation following an extended duration of sexual exposure to the father's sperm. In the case of a first pregnancy with a particular father, she is able to tolerate it given a long constant exposure to the father's antigens through his sperm[11,12,27]. All these findings on the effects of partner change, the protective effect of sperm exposure and the 'dangerous' partner are consistent with the 'Immune maladaptation hypothesis'.

Investigating the immunogenetic pathways potentially involved in preeclampsia, the human leukocyte antigen (HLA) system has been implicated. Many of the reported findings have been inconsistent or contradictory[28]. HLA-G, a major histocommpatibility tissue specific antigen expressed in extravillous trophoblast (EVT) cells (fetal derived), may protect trophoblasts from maternal-fetal immune intolerance and allow these cells to invade the uterus to ensure proper placentation[29]. Extravillous trophoblast cells express an unusual combination of HLA class I molecules: HLA-C, HLA-E and HLA-G. The maternal decidua is infiltrated by a population of natural killer (NK) cells with a distinctive phenotype. NK cells typically function by cell killing or by cytokine production, which is enhanced by cytokines such as IFN a, IFN b, IL-2, IL-12 and IL-15[30]. These cells are particularly numerous in the decidua basalis at the implantation site where they come into close contact with invading EVT cells. These NK cells express a variety of receptors (CD94/NKG2, KIR and ILT) which are known to recognize HLA class I molecules. Interaction between these NK cells and EVT cells might provide the controlling influence for implantation[31].

Maternal uteroplacental blood flow increases during pregnancy. Altered uteroplacental blood flow is a core predictor of abnormal pregnancy. Normally, the uteroplacental arteries are invaded by endovascular trophoblast and remodeled into dilated, inelastic tubes without maternal vasomotor control. Disturbed remodeling is associated with maintenance

of high uteroplacental vascular resistance and intrauterine growth restriction (IUGR) and preeclampsia. Recent studies have shown that endovascular trophoblast invasion involves a side route of interstitial invasion[32]. Only humans have such extensive placental invasion, possibly because of the long intrauterine period that is required for the development of the fetal brain[30,33].The invasion of trophoblast cells into the decidua and the subsequent arterial transformation requires and results in close tissue contact between allogeneic cells. The mucosal lining of the uterus is transformed from endometrium in the non-pregnant state to the decidua of pregnancy. Progesterone plays a major role in this transformation. A massive leukocyte infiltration is the major cellular characteristic of this change[30]. A true decidua is only formed in species that an invasive form of placentation. The process begins in the luteal phase before (potential) implantation. The uterine NK cells are probably derived from blood NK cells. During early pregnancy the uterine NK cells accumulate as a dense infiltrate around the invading cytotrophoblast cells. From mid-gestation onwards they progressively disappear. Therefore, their presence coincides with the period of cytotrophoblast invasion, since placentation in humans is complete by about 20 weeks gestation[30]. NK cells influence both trophoblast invasion and the actual maternal placental bed vascular changes[30]. The uterine NK cells produce a series of cytokines that are involved n angiogenesis and vascular stability, including vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and angiopoietin 2 (ANG2).

The major ligands for the NK cell receptors are the HLA-A, C and E. Both the invading trophoblast cells and surrounding maternal cells express HLA-E. This HLA-E may be of importance in preventing lysis by NK cells by having preferential binding to NK cell inhibitory receptors[30]. Interestingly, the sequence of the monomer peptide derived from other HLA proteins that is bound to HLA-E also influences binding. Only the HLA-G leader sequence complexed with HLA-E binds preferentially to activating NK cell receptors triggering an NK cell response[30]. So, uterine NK cells could respond differently to trophoblast HLA-E complexed with HLA-G compared with the surrounding HLA-E positive but HLA-G negative maternal cells. In this way, HLA-G, which is expressed only by extravillous trophoblast, could influence the maternal immune response indirectly through presentation by HLA-E. This mechanism would still occur if the fetus is homozygous for the HLA-G null allele, because the leader peptide will continue to be translated 30.

As mentioned earlier, this activation of the NK cells is probably of major importance in mediating the major vascular adaptations.

In summary, the major function of appropriate interaction between trophoblast HLA and uterine NK cells may not be simply to prevent lysis, but instead an active positive recognition resulting in major changes in decidual leukocyte cytokine production stimulating trophoblast growth, invasion and vascular remodeling. The term immunotrophism has been used to describe this active process. The great importance of these new insights is that we start to understand how pregnancy is based on an unique couple specific immune interaction not involving T-cells. It also provides an explanation how previous sperm exposure could induce partner specific tolerance (paternal HLA-C) translating in a more optimal placentation proving protection against preeclampsia. Future studies are required to confirm or refute this exciting hypothesis.

The Mother

The immunogenetic interaction between two partners is important to determine the degree of endovascular trophoblast invasion and as such the successfulness of placentation. There is however a clear and important maternal contribution that is probably of pivotal importance in determining the magnitude of the maternal response syndrome. There is a well described inherited maternal predisposition to preeclampsia[34].

The other side of the equation, besides the genes controlling the maternal inflammatory response, is the way the maternal cardiovascular system, and more specific the endothelium is coping with the exaggerated inflammatory response and the degree of increased shear stress. Factors having a significant negative impact on the coping capacity of the endothelium include: (1) age, (2) chronic hypertension, (3) thrombophilic disorders, (4) the homocysteine level, (5) obesity, (6) insulin resistance/hyperinsulinemia, (7) type I diabetes.

Genetic Conflict Hypothesis

According to the genetic conflict theory[35,36], fetal genes will be selected to increase the transfer of nutrients to the fetus, and maternal genes will be selected to limit transfers in excess of some maternal

optimum. The phenomenon of genomic imprinting means that a similar conflict exists within fetal cells between genes that are maternally derived and genes that are paternally derived. Endovascular trophobast invasion has three consequences: (1) the fetus gains direct access to its mother's arterial blood. Therefore, a mother cannot reduce the nutrient content of blood reaching the placenta without reducing the nutrient supply to her own tissues, (2) the volume of blood reaching the placenta becomes largely independent of control by the local maternal vasculature, and (3) the placenta is able to release hormones and other substances directly into the maternal circulation. The conflict hypothesis predicts that placental factors (fetal genes) will act to increase maternal blood pressure, whereas maternal factors will act to reduce blood pressure. The conflict hypothesis suggests that the mother reduces vascular resistance early in pregnancy to ration fetal nutrients and that the subsequent physiologic increase in vascular resistance represents the changing "balance of power" as the fetus grows larger. A corollary is that placental factors contribute to the increase in maternal cardiac output. Placental factors have an opportunity to preferentially increase nonplacental resistance because the uteroplacental arteries are highly modified and unresponsive to vasoconstrictors. The intrinsic effects of a high maternal systemic blood pressure are ultimately beneficial to the fetus. Thus, genetic conflict hypothesis predicts that fetal genes will enhance the flow of maternal blood through the intervillous space by increasing maternal blood pressure (perfusion pressure). Maternal blood pressures form a continuum so that the dividing line between normotensive and hypertensive pregnancies is arbitrary. The conflict hypothesis predicts that a mother's position on this continuum is determined by the balance between fetal factors increasing blood pressure and maternal factors decreasing blood pressure. This mechanism may be operative in gestational hypertension, where fetal prognosis is known to be good. In contrast, in preeclampsia the hypertension results from vasoconstriction rather than increased cardiac output. An hypoxic placenta may release cytotoxic factors that damage maternal endothelial cells, and thus cause vasoconstriction and an increase in maternal blood pressure. In this situation, small increments in the birthweight of semistarved fetuses may often have caused major increases in subsequent survival despite substantial costs to the mother. Thus, endothelial cell dysfunction may have evolved as a high risk fetal strategy to increase nonplacental resistance when a fetus's uteroplacental blood supply is inadequate.

The recent studies on soluble Flt provide the molecular pathways predicted by Haig[37,38]. Placental soluble fms-like tyrosine kinase 1 (sFlt1), an antagonist of decidual (maternal) VEGF and placental growth factor (PlGF), is upregulated in preeclampsia, leading to increased systemic levels of sFlt1 that fall after delivery [37]. Increased circulating sFlt1 in patients with preeclampsia is associated with decreased circulating levels of free VEGF and PlGF, resulting in endothelial dysfunction in vitro that can be rescued by exogenous VEGF and PlGF. In a rat model VEGF and PlGF cause microvascular relaxation of rat renal arterioles in vitro that is blocked by sFlt1, while administration of sFlt1 to pregnant rats induces hypertension, proteinuria, and glomerular endotheliosis, the classic lesion of preeclampsia. Excess circulating sFlt1 may contribute to the pathogenesis of preeclampsia, and may represent an important fetal 'rescue' strategy by increasing maternal blood pressure and as such uteroplacental perfusion pressure.

The conclusions derived from the studies reviewed in this paper may have practical consequences for practicing physicians, even if the exact etiology of preeclampsia remains unre-sol-ved:

1. According to the primipaternity concept, a multiparous women with a new partner should be approached as being a primigravid women.

2. Artificial donor insemination, oocyte donation and especially embryo donation are associated with an increased risk of developing pregnancy-induced hypertensive disor-ders.

3. A more or less prolonged period of sperm exposure provides a partial protection against pregnancy-induced hypertensive disorders. Nowadays all women with changing partners are strongly advised to use condoms in order to prevent sexually transmitted diseases. However, a certain period of sperm exposure within a stable relation, when pregnancy is aimed for, is associated with a partial protection against preeclampsia.

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