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ORIGINAL ARTICLE

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Disturbances in placental immunology: ready for therapeutic interventions?

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Abstract Recent studies have provided new insight into aberrations in the immunological interplay between mother and fetus and their potential role in the development of recurrent fetal loss and preeclampsia. The action of anti-phospholipid antibodies in recurrent fetal loss is now proposed to involve the complement system, neutrophil activation and the production of TNFα by immune bystander cells. A clear involvement of the immune system is emerging in preeclampsia, involving mainly the innate arm, especially neutrophils. The activation of peripheral neutrophils by placentally released inflammatory debris triggers the induction of neutrophil extracellular traps (NETs), which may lead to an occlusion of the intervillous space, thereby further promoting a condition of placental hypoxia. It has, hence, been suggested that new therapeutic strategies be developed, including the possible use of TNFα antagonists in cases of recurrent miscarriage. These strategies need to be addressed with caution due to the possible induction of fetal congenital abnormalities.

Introduction

Even though pregnancy presents an immunological riddle in that the mother does not reject her semi-allogeneic fetus [1], few alterations of this critical balance appear to occur during pregnancy, as most pregnancies proceed to term without major complications. Disturbances in this delicate balance may, however, play an important role in serious pregnancy-related disorders, most prominently in cases with recurrent fetal loss (RFL) or preeclampsia. In this review, we will focus on a few select mechanisms and attempt to elucidate how these are implicated in the underlying aetiology of RFL or preeclampsia.

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Recurrent fetal loss and preeclampsia: separate disorders or a continuum?

Recurrent fetal loss, also termed recurrent spontaneous abortion (RSA) or recurrent miscarriage, is defined by the loss of three or more consecutive pregnancies of non-viable fetuses [2, 3]. This may occur before 12 weeks of pregnancy (early loss) or post 12 weeks of gestation (late loss). Although a small number of these cases (3–5%) are caused by gross chromosomal anomalies, the causal aetiology in the vast proportion of cases is not readily evident. RFL has been shown to be associated in some cases with thrombophilia, anti-phospholipid antibodies or aberrant NK cell activity [2]. These may serve to indicate that the underlying aetiology may involve some form of immune dysfunction.

Preeclampsia, a severe disorder of late pregnancy, is a major cause of maternal and fetal morbidity worldwide [4, 5]. The disorder is characterised by hypertension and oedema in a previously normotensive individual, which can also progress to eclampsia characterised by fits and convulsions, leading to neurological damage or death. Preeclampsia may frequently be complicated by the associated hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome affecting the liver. No treatment exists for this enigmatic disorder other than the delivery of the fetus [4, 5]. The underlying aetiology clearly involves the placenta as most preeclampsias resolve after delivery. Placentation in preeclampsia is frequently defective in that the maternal spiral arteries fail to be effectively transformed by invasive extravillous trophoblast (EVT) from a system of pulsatile high pressure to one of constant flow low pressure. Consequently, the placenta does not receive an optimal blood supply. The maternal syndrome is characterised by systemic damage of the endovasculature, especially of the kidney, liver and brain. Several lines of evidence indicate that the placental alterations precede the clinical manifestation by a period of weeks or even months [4–6].

Akin to RFL, it has recently been suggested that preeclampsia be divided into those cases with early onset (<34 weeks of gestation) and those with late onset (>34 weeks of gestation) [7]. In general, cases with early-onset preeclampsia are clinically more severe and usually result in the delivery of a very immature, growth-retarded fetus. Of interest is that late-onset preeclampsia frequently does not exhibit the placental changes, such an unmodified spiral arteries, characteristic of this disorder [8].

Although RFL and preeclampsia are regarded as separate disorders, it is possible that they may be linked. The first line of evidence in support of this notion is from clinical studies in which women with a history of RFL and associated anti-phospholipid syndrome were treated with low molecular weight heparin and aspirin [2]. These drugs have been shown to ameliorate the condition, permitting a large number of such affected pregnancies to proceed to term. Of interest, however, is that a considerable proportion of those pregnancies which did proceed to term were affected by preeclampsia [2]. The second line of evidence stems from research into the possible role of oxidative stress in the development of RFL and preeclampsia [9]. These data indicate that fetal loss can occur as a result of conditions of extreme oxidative stress early in pregnancy. If these conditions of oxidative stress are not as high, then the pregnancy may progress to term, but preeclampsia will most likely develop. These two sets of data, hence, suggest that a causal link may exist between the development of RFL and preeclampsia and that preeclampsia may arise from conditions which are not severe enough to lead to outright fetal loss.

Preeclampsia: an immune disorder?

Due to the severity of the symptoms, most research on preeclampsia has focussed on factors related to hypertension and recent evidence has emerged here that an imbalance in placentally derived angiogenic factors may play a significant role in the onset of maternal symptoms [10].

Although exciting these angiogenic factors alone cannot account for the multi-factorial nature of events which contribute to the development of this complex disorder. This is because the key placental events, such as inadequate modification of the maternal spiral arteries, which are thought to play a major role in the development of preeclampsia, precede the alterations in angiogenic factors by a period of weeks or months [4, 5]. This facet is further underscored by genetic studies which indicate that hereditary traits do exist which can predispose a pregnancy to an increased risk for developing preeclampsia. However, even though both partners or children born from preeclamptic pregnancies can play a role in the transmission of such risk factors, they appear to be mediated by a genetic component which cannot be accounted for by a single or a few genes but appear rather to be attributable to a host of genes [4, 5].

On the other hand, several lines of evidence seem to indicate that an altered immune response may play a key role in the development of preeclampsia [11, 12], as listed below:

1. *Primiparity or primipaternity* Preeclampsia frequently occurs in first pregnancies, especially in teenage pregnancies. It is, however, also seen when the woman has a new partner. Preeclampsia can be consequently viewed as either a disorder of primiparity or of primipaternity.
2. *Period of cohabitation* The period of cohabitation, especially the exposure to the partner's semen, even orally, appears to have a protective effect. Although it had been recorded that prolonged sexual intercourse using condoms does not provide such protection, this was most clearly illustrated in an analysis of pregnancies conceived by in vitro fertilisation in couples when the male partner was aspermic. In these cases, where the wife had never been exposed to the partner's semen, the incidence of preeclampsia was particularly high.
3. *Protective effect of previous pregnancy* Previous pregnancies, even fetal losses, can reduce the risk of having a preeclampsia; however, the time lapse between the previous and the subsequent pregnancy cannot be too long.
4. *Change of partner* Even though previous pregnancies do protect against the possibility of preeclampsia, this effect is lost with a change in partner. Under these conditions, the risk for preeclampsia is the same as for any first pregnancy.
5. *Ooocyte donation* Conflicting reports exist on whether pregnancies resulting from oocyte donations are more frequently affected by preeclampsia. Of note is that, in these cases, the embryo represents a completely allogeneic graft, having no genetic component from the mother.
6. *Requirement of an intact T-cell-mediated immune system* A study on pregnant HIV+ women has shown that HIV-1-related immune deficiency is associated with a low rate of preeclampsia. This is elevated in those cases receiving anti-retroviral therapy.

Apart from these phenomenological observations implying a role for an immune aberration [11,12], several lines of more concrete evidence have recently emerged. These range from observations documenting an overt activation of the maternal innate immune

response in preeclampsia [13] to reduced expression of key regulatory molecules directly at the placental interface [14].

Antiphospholipid syndrome and recurrent fetal loss

High titres of antiphospholipid (aPL) antibodies occur in 30–40% of patients with a history of recurrent miscarriage [2, 3]. Although these antibodies have been shown to bind to a wide variety of membrane phospholipids (e.g. cardiolipin and phosphatidyl serine), they do not appear to bind directly to phospholipid but rather to phospholipid-binding proteins or to phospholipid/phospholipid-binding protein complexes [3, 15]. One of these proteins is beta 2 glycoprotein (β2GPI), which is localised on extravillous cytotrophoblast and syncytiotrophoblast [15, 16]. It is possible, therefore, that the function of aPL antibodies may be to disrupt the function of β2GPI. Although such antibodies have been implicated in coagulation and thrombosis, a number of studies indicated that antiphospholipid antibodies can affect trophoblast behaviour in vitro [2, 3]. These effects include inhibition of trophoblast proliferation and the diminished invasion of maternal spiral arteries [17]. The production of trophoblast-specific proteins (human chorionic gonadotropin) and adhesion molecules (α1 and α5 integrins, as well as E and VE cadherins) was also reduced [18, 19]. Certain antibodies, such as those against annexin V, were also shown to induce trophoblast apoptosis [3]. These effects have recently been confirmed by the use of placental explant cultures, where it was shown that aPL antibodies increased placental apoptosis, decreased villous trophoblast proliferation and reduced extra villous trophoblast invasion through matrigel [20]. These features were also observed in the analysis of placental sections from pregnancies which were miscarried [21]. Therefore, these reports extend previous observations that defective trophoblast proliferation and invasion is a key pathological feature in placenta of patients with antiphospholipid syndrome (APS) [2, 3]. Of considerable interest is that these effects could be attenuated in vitro by heparin and aspirin, drugs commonly used for the treatment of APS in pregnancy [22].

The action of aPL antibodies may not be restricted to trophoblast but may also affect the endovasculature, as recent evidence indicates that these antibodies bind to activated, but not resting, endothelium [23].

An exciting alternative to the direct action of aPL antibodies on trophoblast cells is that these actions are mediated via complement [24]. These data, obtained by injecting pregnant mice with human aPL antibodies, indicated that inhibition of complement activation prevented fetal loss and growth retardation [25]. This effect appeared to be mediated by the murine complement receptor 1-related protein y and complement component C5 [25, 26]. It also appears to require the participation of neutrophils [25, 26]. Akin to what has been observed in other systems discussed above, heparin was shown to prevent the activation of complement by aPL antibodies, thereby preventing fetal loss in a murine model system [27].

Of considerable interest is the very recent finding that aPL antibodies appear to induce the expression of TNFα in decidual tissues [28]. This activity appears to occur downstream of complement activation. As TNFα has been shown to be very deleterious to fetal and placental tissues [29] and leads to fetal loss in pregnant mice, this could be a further explanation for the pathology of aPL antibodies in RFL. On the basis of these data, it has been suggested that anti-TNFα therapy may be useful for treating patients with RFL due to aPL antibodies [28].

Although we do propose a link between RFL and preeclampsia, aPL may not play a role in the latter. In our own analysis, we could not find any association between aPL and incidence of preeclampsia [30], a feature also observed by others [31].

HLA-G and its interaction with uNK cells

HLA-G was discovered in 1986 as a novel HLA class I molecule expressed on extravillous trophoblast [32]. Its distinct expression pattern in very select placental trophoblast cells led the authors to raise the question of whether this molecule could provide new insight into the immune relationship between mother and fetus [32]. In the interim, it has been shown that HLA-G differs largely from other HLA class I molecules in that it is largely monomorphic and that very few differences exist between population groups [33–35]. The structural features of HLA-G have been determined to be quite complex in that five distinct forms exist, three membrane-anchored forms and two soluble forms. While the expression of the full-length HLA-G1 molecule seems to be restricted to invasive cytotrophoblast and thymic epithelium, the expression of the other variants, especially the soluble HLA-G5 variant, is more widespread, occurring throughout the placenta, chorionic membrane and decidua [34].

HLA-G has been shown to possess a number of immune-suppressive or immune-modulatory activities, especially in reducing the lytic activity of NK cells [34, 35]. The activation of NK cells differs largely from that of T cells in that these cells of the innate arm of the immune system are not triggered by a specific antigen presented on either class I or class II HLA molecules [36]. In contrast, NK cells are activated when they sense the absence of HLA class I molecules. This “missing self” phenomenon is used by NK cells to recognise and kill cells which no longer correctly express their HLA repertoire, such as tumour cells or cells which have become infected by viruses. This activity is mediated via the interaction of HLA molecules with specific receptors on NK cells termed killer immunoglobulin receptors (KIRs) or immunoglobulin-like transcripts (ILTs) [36]. In general, the binding of a particular HLA class I molecule to its KIR would, therefore, down-modulate the lytic activity of the NK cell. Some KIRs, however, have been shown to lead to NK cell activation.

In this context, the expression of HLA-G by trophoblast cells is particularly interesting as, although the lack of HLA expression by the trophoblast would render these fetal cells “invisible” to maternal cytotoxic CD8 T cells, the failure to express classical class I molecules would render them susceptible to lysis by maternal NK cells [37]. As uterine NK (uNK) cells are the predominant group of leucocytes in the decidua, it was therefore proposed that HLA-G could prevent the inadvertent activation of these cells and, hence, trophoblast lysis [37].

This scenario has turned out to be more complex than that proposed above in that uNK cells do not appear to possess any cytolytic activity, unlike their circulatory counterparts [38]. The main function of uNK indeed now seems to be the promotion of implantation and successful placentation by ensuring optimal vascularisation and modification of the maternal spiral arteries. This is proposed to occur largely via the production of cytokines such as IFN-γ [39]. Therefore, these maternal immune cells do not appear to be cytotoxic guardians ready to act against the migration of abnormal fetal cells.

A further puzzle which needs to be solved is what the receptor for HLA-G on uNK cells is exactly. The receptor for HLA-G on monocytes appears to be ILT4, while on lymphocytes

ILT2 appears to be the predominant form [36, 37]. Uterine NK cells, however, express negligible levels of ILT2 or ILT4 [36, 37] (Bürk MR and Hahn S, unpublished observations).

HLA-G, however, also appears to affect the activity of other leucocytes, and in vitro experiments have indicated that highly purified HLA-G1 can induce Fas-mediated apoptosis in activated but not resting CD8+ T cells [40]. In CD4+ T helper cells, HLA-G has been noted to suppress proliferation and reduce activation by dendritic cells [41, 42].

Although the true function of HLA-G is not clear, its highly specific expression in certain placental tissues implies that it may play a significant role in human pregnancy [34]. It is consequently no great surprise that alterations in HLA-G expression have been proposed to be involved in RFL and preeclampsia [33, 35]. In the latter, several reports now indicate that HLA-G expression is reduced in preeclamptic placentae [43], a feature we have also observed in both the placental basal plate and septum (Fig. 1). As these observations were made in placentae obtained at delivery, it is not certain whether this alteration in expression is a consequence of other placental changes occurring during the course of this disorder or whether it is a key initiating lesion. In addition, a number of HLA-G polymorphisms, many of which appear to affect expression levels, have been associated with the incidence of RFL [33, 44–47].

HLA-G itself may be important in implantation, as the presence of soluble HLA-G in the culture supernatant of embryos generated by IVF generally indicates that these embryos implant successfully [48]. On the other hand, in those cultures where it is missing and, hence, probably is not expressed, the embryos frequently fail to implant successfully [48].

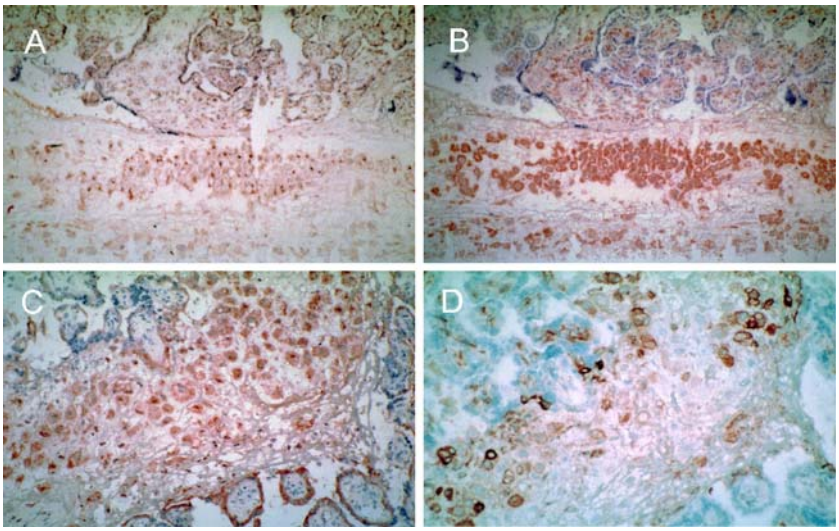

Fig. 1 Decreased expression of HLA-G in preeclamptic placentae. Cytokeratin expression in the placental septum of normal pregnancies. HLA-G expression in the placental septum of normal pregnancies. Cytokeratin expression in the placental septum of normal pregnancies. HLA-G expression in the placental septum of normal pregnancies

Other interactions between trophoblast and uNK

In addition to HLA-G, extravillous trophoblast (EVT) also expresses the ubiquitous HLA-C and HLA-E molecules [37]. The monomorphic HLA-E molecule functions by presenting the leader peptide of other HLA molecules. HLA-E consequently serves to indicate which other HLA molecules are being expressed by the cell. The receptor for HLA-E is the dimer CD94/NKG2A, and this molecule is highly expressed on uNK cells [36].

Of the three class I antigens expressed by EVT, HLA-C is the only one that exhibits a considerable degree of polymorphism [14]. As the paternal allele is expressed, this implies that a truly foreign antigen is present on EVT. As the various HLA-C forms are recognised by different members of the KIR family, it could be that preeclampsia involves such ligand–receptor interactions. The KIR members that interact with HLA-C exist as two haplotypes, A and B. The A haplotype has seven KIR loci, only one of which, KIR2DS4, is an activating receptor. In individuals who are homozygous for this haplotype (AA), this activating receptor is frequently deleted. These individuals consequently express only inhibitory receptors. The B haplotype has an additional locus which contains several activating receptors (KIR2DS1, 2, 3 and 5 and KIR3DS1). On the other hand, HLA-C allotypes consist of two groups:

1. HLA-C1, which interacts with inhibitory receptors KIR2DL2/3 and the activatory receptor KIR2DS2
2. HLA-C2, which interacts with inhibitory receptor KIR2DL1 and the activatory receptor KIR2DS1

In an analysis of KIR and HLA-C genotypes in pregnancies affected by preeclampsia and those which had uncomplicated deliveries, it was noted that preeclampsia was frequently associated with a maternal KIR AA genotype and a fetal (placental) HLA-C2 genotype [14]. This suggests that the lack of activatory KIRs on maternal uNK cells is deleterious, implying that the activation of these cells is required for successful placentation. These findings extend and confirm the hypothesis described above that uNK activity may be required for efficient vascularisation and modification of the maternal spiral arteries, features which are deficient in preeclampsia [38].

IDO

One of the most stunning observations in the field of reproductive immunology was that made by Andrew Mellor and colleagues [49] and begged the question of whether the entire question of maternal tolerance to fetal antigens could be attributable to the rudimentary biochemical requirement for an essential amino acid. Their initial intriguing observation was made with macrophages, which were shown to induce T cell tolerance by an indirect biochemical mechanism rather than by a T-cell-receptor mediated pathway. The process was shown to be caused by the enzyme indoleamine 2,3-dioxygenase (IDO), which depleted all tryptophan in the micro-environment around the T cells, thereby preventing their activation [50]. As IDO is also expressed in human syncytiotrophoblast cells, they tested for IDO activity in murine pregnancy. In this case, they made the astounding observation that the inhibition of this enzyme by a pharmacological agent (1-methyl-DL-tryptophan) had an

exceptionally strong abortive effect, leading to the loss of the entire litter when applied early during gestation [49].

The influence of IDO in human pregnancy currently, especially in pregnancy-related complications is not clear, with a solitary report indicating reduced levels in preeclampsia [51]. It has recently been suggested that indeed an alteration in the Th1/Th2 phenotype may have a greater consequence for the maintenance of pregnancy than a reduction in IDO activity [52].

The Th1–Th2 paradigm

The possibility of a shift in T helper subsets during pregnancy continues to remain a highly controversial hypothesis [53]. CD4 T helper 1 (Th1) cells were initially characterised by the production of interleukin 2 (IL-2), interferon γ (IFN-γ) and tumour necrosis factor α (TNFα), which would activate a cell-mediated immune response [54]. On the other hand, CD4 T helper 2 (Th2) would promote a humoral immune response by the synthesis of interleukins 4, -5 and -10 [54]. We have previously shown that Th1 T cells possess FasL-mediated cytolytic activity, which is lacking in their Th2 counterparts [55, 56]. These traits are not restricted to CD4+ T cells and are also evident in CD8+ T cells, which tend to be mainly of a Th1 phenotype [57].

As pregnancy is characterised by diminished cell-mediated immune response and a vigorous humoral response, this can be seen as evidence of a shift towards a Th2 type immune response [58]. Such a change in phenotype could be mediated by the prodigious production of Th2-promoting cytokines (IL-4, -5 and -10) by placental tissues [59].

Furthermore, successful pregnancy outcome has been shown to be incompatible with a Th1 phenotype, as the presence of Th1 cytokines (IL-2, IFN-γ or TNFα? results in destruction of trophoblast in vitro and fetal loss in pregnant mice [29]. In humans, the presence of IFN-γ or TNFα in the placenta in the case of malarial infection during pregnancy is strongly associated with poor pregnancy outcome [60]. Recent studies with a murine model have indicated that the adoptive transfer of activated Th1 lymphocytes leads to preeclampsia-like symptoms exclusively in pregnant mice [61]. In our own analyses, we observed that there was a reduction in the activation levels, as measured by the expression of CD69, in both CD8+ and CD4+ T cells in late pregnancy (Schatt et al., in preparation). In a flow cytometrical analysis, we observed that the production of both IFNγ and IL-4 by these two T cell populations was reduced in late pregnancy. Even though the production of both IFNγ and IL-4 was reduced, in these instances, there appeared to be a slight shift towards Th2 phenotype (Schatt et al., in preparation). On the other hand, the capacity to produce IFNγ was the same in NKT cells and was enhanced in circulatory NK cells (Schatt et al., in preparation).

These various data, therefore, support the proposal that the aberrant presence of inflammatory Th1 cytokines could be deleterious for pregnancy, negatively influencing implantation and fetal development. It is also possible that a Th1 phenotype may promote the development of preeclampsia or may even, in extreme circumstances, be pro-abortive.

Innate inflammatory response in pregnancy

Pregnancy has been observed to be associated with an enhanced inflammatory response, particularly of the innate arm of the immune system, which is overt in preeclampsia [13, 62]. In their original hypothesis, Sargent et al. [63] proposed that this may be brought about by the deportation of inflammatory debris by the placenta. Due to the replenishment of the syncytiotrophoboblast, the continuous monolayer covering the entire villous tree with a surface area of several m^2, a massive amount of material is released into the maternal circulation (in the order of several grams per day in pregnancies close to term) [64]. Although this material may be released in the form of syncytial knots, a large proportion may exist as microparticles, frequently termed syncytiotrophoblast microparticles (STBM). The release of this material in normal healthy pregnancies occurs largely by apoptosis, but in preeclampsia, it has been suggested to involve necrotic or apo-necrotic pathways [64]. In this case, the material released in preeclampsia would be more inflammatory than that deported in normal pregnancy [6, 63].

In vitro studies have suggested that STBM prepared by mechanical dissection of placental material can activate human umbilical vein endothelial cells and can inhibit the proliferation of circulatory lymphocytes or even induce apoptosis [65, 66] in the Jurkat T cell lymphoma cell line [67]. In recent tests, we observed that the in vitro activity of STBM depends on the mode of preparation, in that those prepared largely by necrotic means exhibit the greatest effect on endothelial cell cultures, inhibiting their proliferation and inducing apoptosis [68].

Our analysis of their activity on circulatory T lymphocytes again indicated that those produced by necrotic means (crude dissection) significantly inhibited T-cell activation, proliferation and cytokine release by activated T lymphocytes [69]. On the other hand, those produced by close to physiological conditions (placental perfusion) significantly induced T cell proliferation and even caused slight increase in IFNγ release [69].

Our experiments also indicated that the effect of placentally released mediators is not restricted to particulate matter but also includes soluble particle-free factors. In this context, we observed that placental explant cultures release prodigious quantities of the highly inflammatory IL-1, which can contribute to endothelial cell dysfunction in preeclampsia [70]. We also observed that these supernatants can significantly inhibit T lymphocyte responsiveness, indicating that other, as yet uncharacterised, immune-modulatory molecules are produced by the placenta [71].

Neutrophil extracellular traps

The activation of circulatory polymorphonuclear neutrophils has been proposed to be a major contributor to the enhanced inflammatory response observed in pregnancy, and their overt activation, resulting in the release of superoxide radicals, has been suggested to contribute to endothelial damage in preeclampsia [72]. The activation of these neutrophils has been proposed to be mediated via the presence of STBM in normal pregnancy and their elevated release in preeclampsia. For this reason, we were very interested in a recent report indicating that inflammatory signals, such as that mediated by IL-8 or bacteria, can trigger neutrophils to generate extracellular DNA-containing fibrous lattices, termed neutrophil extracellular traps (NETs) [73]. This report indicated that these NETs may have bactericidal activity in that they can ensnare and kill bacteria [73].

We were also intrigued by this observation because a large portion of our previous research focused on the increased presence of cell-free DNA in preeclampsia [6, 74]. We consequently set out to determine whether a link existed between these two phenomena. For these studies, we used in vitro generated STBM as well as placentally derived IL-8. We observed that these agents efficiently induced NETs in isolated neutrophils (Fig. 2) and that, furthermore, STBM could be seen clearly trapped in these extracellular DNA lattices.

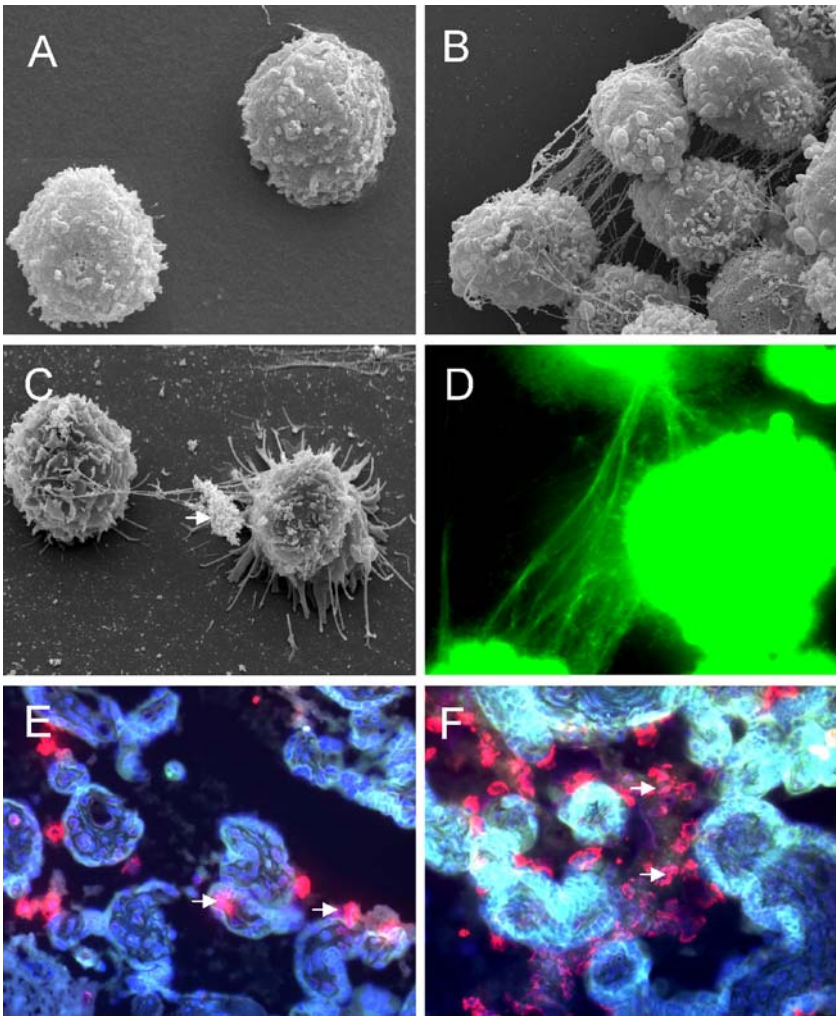

Fig. 2 Induction of NETs by STBM and presence in preeclamptic placentae. Untreated neutrophils showing a typical round structure. Induction of NETs in neutrophils treated with placentally derived IL-8. Entrapment of STBM in induced NETs. Staining of NETs with the DNA binding dye cytox green. Minimal presence of NETs in the intervillous space of normal placentae. Massive presence of NETs in the intervillous space of preeclamptic placentae

An examination of placental cryosections indicated that large numbers of NETs were present directly in the intervillous space of preeclamptic placentae, in stark contrast to the few neutrophil NETs observed in normal placentae (Fig. 2).

As these NETs are capable of trapping large bacteria (*S. aureus*) [73], it is possible that, when they are present in large numbers, they will hinder the flow of blood through the intervillous space. In this manner, NETs could contribute to the condition of oxidative stress thought to occur in preeclampsia [9].

MIF: another immune-modulator important in pregnancy?

Macrophage migration inhibition factor (MIF) was described almost 40 years ago as a T cell soluble factor which inhibited macrophage migration [75]. Since then, MIF has been shown to be expressed by several other cell types, including macrophages, neutrophils, anterior pituitary cells and bronchial alveolar cells [76]. Of particular interest was a report on the presence of MIF in the anterior chamber of the eye, where it was shown to hinder the

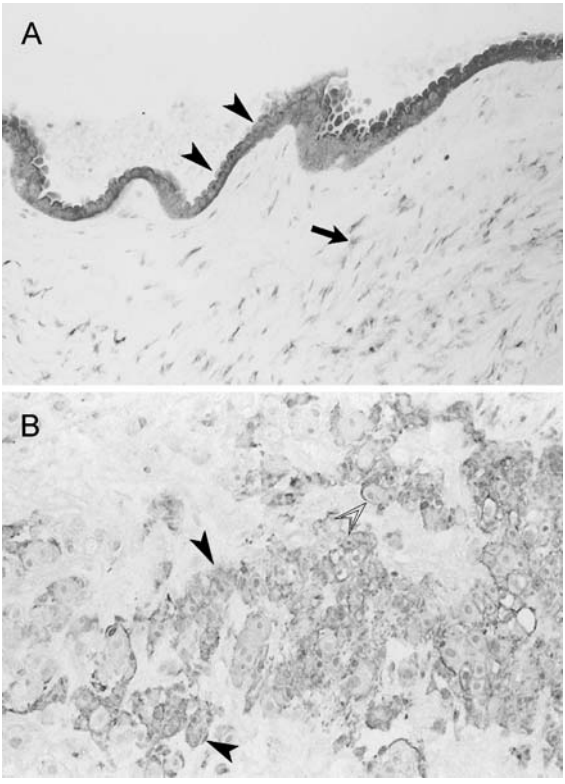

Fig. 3 Expression of MIF in amnion and placenta MIF expression in amnion epithelium. MIF expression in multinucleate trophoblast giant cells in the chorionic plate

chemotaxis of neutrophils and macrophages, to suppress T- and B-cell proliferation in vitro and also to prevent the activation and lytic activity of CD8+ T cells and NK cells [77].

As both the anterior chamber of the eye and placenta are examples of immune privileged tissues, in which inflammatory immune reactions are generally suppressed [78], we were interested to examine whether MIF may also play a similar role in the placenta. Our studies indicated that MIF is expressed in placental tissues, being produced in both the fetal and maternal compartments of the placenta, as shown by the intense staining observed in the amnion epithelium, extravillous cytotrophoblast giant cells in the chorionic plate, and in the invasive extravillous trophoblast located in the decidua (Fig. 3). This implies that MIF may play an important role in these tissues. Although it has recently been reported that circulatory MIF concentrations are elevated under conditions of preeclampsia [79], we could not confirm this finding in a study with a larger cohort [80]. Although MIF clearly influences the behaviour of T and NK cells in the eye, its role in the placenta may be different and, in this case, may rely more on its ability to promote neovascularisation, which is certainly of importance for efficient placentation.

Therapeutic possibilities

Therapeutic interventions are particularly difficult in pregnancy as there is a constant danger of congenital birth defects. Therapeutic efforts are further hampered by the lack of screening tools to reliably identify pregnant women at risk for certain pregnancy-related disorders such as preeclampsia, which develops many weeks after the occurrence of the initiating placental lesion [4, 5, 81]. Clinicians are only consequently presented with these cases once the maternal symptoms have become manifest and are left with few alternatives other than delivering a premature baby in the severe cases. Another problem is the multi-factorial nature of pregnancy-related disorders, such as RFL or preeclampsia, which makes it difficult to develop a single therapeutic strategy.

Current therapies for RFL in association with aPL antibodies rely on treatment with heparin and aspirin, which can have a success rate of up to 70% [2]. It is interesting that recent in vitro or murine studies are starting to elucidate the pathways by which heparin acts under these conditions [22, 27]. Although the use of other anti-inflammatory agents and anti-TNFα strategies is being discussed [28], no clinical studies reporting on the efficacy of such planned approaches exist. The potential effect of such therapies on fetal development need careful appraisal.

Treatment with intravenous immunoglobulin, although promising in certain studies [82], needs to be confirmed in large-scale studies. The putative desensitisation of the maternal immune system with paternal antigens appears to be entering a renaissance after having lost much credibility previously [83, 84].

For cases of RFL without aPL antibodies, the treatment is more problematic, and a Cochrane review does not recommend conventional heparin/aspirin treatment [85]. For those cases where a chromosomal anomaly is suspected, the use of preimplantation genetic diagnosis of IVF-generated embryos is recommended [86].

The treatment of preeclampsia remains more problematic due to the lack of an effective tool to identify at-risk pregnancies [87]. Although several small-scale studies have indicated that the use of anti-inflammatory agents (aspirin) or anti-oxidants (vitamin C and E) may be

beneficial if given early in pregnancy before the onset of symptoms, this remains to be confirmed in large-scale analyses [88].

In those cases where preeclampsia symptoms have become manifest, the use of conservative treatment strategies with anti-hypertensive drugs such as ketanserin, although beneficial under certain conditions [89], remains contentious with some indications existing that these are not useful in cases with severe early-onset preeclampsia [90].

Effective treatment awaits a better understanding of the underlying chain of events leading to the development of either RFL or preeclampsia and, perhaps, of the steps linking these two disorders.

Conclusions

This review has attempted to describe the complex immune interactions occurring during pregnancy and how aberrations in these networks can contribute to severe pregnancy-related disorders such as preeclampsia or even to fetal loss. We have also tried to underscore that these two separate disorders may have common underlying aetiologies and that there may even be a continuum between RLF and preeclampsia. Investigations in this field have yielded several surprises, ranging from aPL antibodies acting via the complement system and TNFα to the new proposal that uNK cells may not act in a cytolytic manner but rather may serve to promote efficient vascularisation, spiral artery modification and placentation. The fact that T cells can be incapacitated by simple biochemical deprivation of an essential amino acid was also certainly surprising. The role of IDO in pregnancy-related disorders, however, needs to be clearly demonstrated. Our own observation concerning the presence of NETs in preeclampsia, which awaits independent verification, may provide new insights into inflammation and placental oxidative stress. It seems appropriate to close with a quotation by Sir Peter Medawar, the first to address the immune riddle of pregnancy, who so clearly stated: “The human mind treats a new idea the way the body treats a strange protein; it rejects it.”

References

1. Medawar PB (1961) Immunological tolerance. Nature 189:14–17
2. Carrington B, Sacks G, Regan L (2005) Recurrent miscarriage: pathophysiology and outcome. Curr Opin Obstet Gynecol 17:591–597
3. Meroni PL, di Simone N, Testoni C, D’Asta M, Acaia B, Caruso A (2004) Antiphospholipid antibodies as cause of pregnancy loss. Lupus 13:649–652
4. Redman CW, Sargent IL (2005) Latest advances in understanding preeclampsia. Science 308:1592–1594
5. Sibai B, Dekker G, Kupferminc M (2005) Pre-eclampsia. Lancet 365:785–799
6. Hahn S, Huppertz B, Holzgreve W (2005) Fetal cells and cell free fetal nucleic acids in maternal blood: new tools to study abnormal placentation? Placenta 26:515–526
7. von Dadelszen P, Magee LA, Roberts JM (2003) Subclassification of preeclampsia. Hypertens Pregnancy 22:143–148
8. Sebire NJ, Goldin RD, Regan L (2005) Term preeclampsia is associated with minimal histopathological placental features regardless of clinical severity. J Obstet Gynaecol 25:117–118
9. Burton GJ, Jauniaux E (2004) Placental oxidative stress: from miscarriage to preeclampsia. J Soc Gynecol Investig 11:342–352

10. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA (2004) Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 350:672–683
11. Robillard PY, Dekker GA, Hulsey TC (1999) Revisiting the epidemiological standard of preeclampsia: primigravidity or primipaternity? Eur J Obstet Gynecol Reprod Biol 84:37–41
12. Dekker GA, Robillard PY, Hulsey TC (1998) Immune maladaptation in the etiology of preeclampsia: a review of corroborative epidemiologic studies. Obstet Gynecol Surv 53:377–382
13. Redman CW, Sargent IL (2000) Placental debris, oxidative stress and pre-eclampsia. Placenta 21:597–602
14. Hiby SE, Walker JJ, O'shaughnessy KM, Redman CW, Carrington M, Trowsdale J, Moffett A (2004) Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. J Exp Med 200:957–965
15. Chamley LW (1997) Antiphospholipid antibodies or not? The role of beta 2 glycoprotein 1 in autoantibody-mediated pregnancy loss. J Reprod Immunol 36:123–142
16. Chamley LW, Allen JL, Johnson PM (1997) Synthesis of beta2 glycoprotein 1 by the human placenta. Placenta 18:403–410
17. Chamley LW, Duncalf AM, Mitchell MD, Johnson PM (1998) Action of anticardiolipin and antibodies to beta2-glycoprotein-I on trophoblast proliferation as a mechanism for fetal death. Lancet 352:1037–1038
18. di Simone N, Castellani R, Caliandro D, Caruso A (2002) Antiphospholid antibodies regulate the expression of trophoblast cell adhesion molecules. Fertil Steril 77:805–811
19. di Simone N, Castellani R, Caliandro D, Caruso A (2001) Monoclonal anti-annexin V antibody inhibits trophoblast gonadotropin secretion and induces syncytiotrophoblast apoptosis. Biol Reprod 65:1766–1770
20. Bose P, Black S, Kadyrov M, Bartz C, Shlebak A, Regan L, Huppertz B (2004) Adverse effects of lupus anticoagulant positive blood sera on placental viability can be prevented by heparin in vitro. Am J Obstet Gynecol 191:2125–2131
21. Bose P, Kadyrov M, Goldin R, Hahn S, Backos M, Regan L, Huppertz B (2006) Aberrations of early trophoblast differentiation predispose to pregnancy failure: lessons from the anti-phospholipid syndrome. Placenta (In press) PMID 16289730
22. Bose P, Black S, Kadyrov M, Weissenborn U, Neulen J, Regan L, Huppertz B (2005) Heparin and aspirin attenuate placental apoptosis in vitro: implications for early pregnancy failure. Am J Obstet Gynecol 192:23–30
23. Chen Q, Stone PR, Woon ST, Ching LM, Hung S, McCowan LM, Chamley LW (2004) Antiphospholipid antibodies bind to activated but not resting endothelial cells: is an independent triggering event required to induce antiphospholipid antibody-mediated disease? Thromb Res 114:101–111
24. Salmon JE, Girardi G (2004) The role of complement in the antiphospholipid syndrome. Curr Dir Autoimmun 7:133–148
25. Holers VM, Girardi G, Mo L, Guthridge JM, Molina H, Pierangeli SS, Espinola R, Xiaowei LE, Mao D, Vialpando CG, Salmon JE (2002) Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. J Exp Med 195:211–220
26. Girardi G, Berman J, Redecha P, Spruce L, Thurman JM, Kraus D, Hollmann TJ, Casali P, Caroll MC, Wetsel RA, Lambris JD, Holers VM, Salmon JE (2003) Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. J Clin Invest 112:1644–1654
27. Girardi G, Redecha P, Salmon JE (2004) Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. Nat Med 10:1222–1226
28. Berman J, Girardi G, Salmon JE (2005) TNF-alpha is a critical effector and a target for therapy in antiphospholipid antibody-induced pregnancy loss. J Immunol 174:485–490
29. Yui J, Garcia-Lloret M, Wegmann TG, Guilbert LJ (1994) Cytotoxicity of tumour necrosis factor-alpha and gamma-interferon against primary human placental trophoblasts. Placenta 15:819–835
30. Hristoskova S, Holzgreve W, Hahn S (2004) Anti-phospholipid and anti-DNA antibodies are not associated with the elevated release of circulatory fetal DNA in pregnancies affected by preeclampsia. Hypertens Pregnancy 23:257–268
31. Bowen RS, Moodley J, Dutton MF, Fickl H (2002) Antibodies to oxidised low-density lipoproteins and cardiolipin in pre-eclampsia and eclampsia. J Obstet Gynaecol 22:123–126
32. Ellis SA, Sargent IL, Redman CW, McMichael AJ (1986) Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line. Immunology 59:595–601
33. Hviid TV (2006) HLA-G in human reproduction: aspects of genetics, function and pregnancy complications. Hum Reprod Update (In press)

34. Hunt JS, Petroff MG, McIntire RH, Ober C (2005) HLA-G and immune tolerance in pregnancy. FASEB J 19:681–693
35. Le Bouteiller P, Pizzato N, Barakonyi A, Solier C (2003) HLA-G, pre-eclampsia, immunity and vascular events. J Reprod Immunol 59:219–234
36. Lanier LL (2005) NK cell recognition. Annu Rev Immunol 23:225–274
37. Parham P (2004) NK cells and trophoblasts: partners in pregnancy. J Exp Med 200:951–955
38. van den Heuvel MJ, Xie X, Tayade C, Peralta C, Fang Y, Leonard S, Paffaro VA Jr, Sheikhi AK, Murrant C, Croy BA (2005) A review of trafficking and activation of uterine natural killer cells. Am J Reprod Immunol 54:322–331
39. Monk JM, Leonard S, McBey BA, Croy BA (2005) Induction of murine spiral artery modification by recombinant human interferon-gamma. Placenta 26:835–838
40. Contini P, Ghio M, Poggi A, Filaci G, Indiveri F, Ferrone S, Puppo F (2003) Soluble HLA-A, -B, -C and -G molecules induce apoptosis in T and NK CD8+ cells and inhibit cytotoxic T cell activity through CD8 ligation. Eur J Immunol 33:125–134
41. Le Friec G, Laupeze B, Fardel O, Sebti Y, Pangault C, Guilloux V, Beauplet A, Fauchet R, Amiot L (2003) Soluble HLA-G inhibits human dendritic cell-triggered allogeneic T-cell proliferation without altering dendritic differentiation and maturation processes. Hum Immunol 64:752–761
42. Bahri R, Hirsch F, Josse A, Rouas-Freiss N, Bidere N, Vasquez A, Carosella ED, Charpentier B, Durrbach A (2006) Soluble HLA-G inhibits cell cycle progression in human alloreactive T lymphocytes. J Immunol 176:1331–1339
43. Goldman-Wohl DS, Ariel I, Greenfield C, Hochner-Celnikier D, Cross J, Fisher S, Yagel S (2000) Lack of human leukocyte antigen-G expression in extravillous trophoblasts is associated with pre-eclampsia. Mol Hum Reprod 6:88–95
44. Christiansen OB, Mohapeloa HP, Steffensen R, Jersild C (1997) HLA-C and -Bw typing of couples with unexplained recurrent miscarriages. J Reprod Immunol 37:63–77
45. Karhukorpi J, Laitinen T, Tiilikainen AS (1997) HLA-G polymorphism in Finnish couples with recurrent spontaneous miscarriage. Br J Obstet Gynaecol 104:1212–1214
46. Aldrich CL, Stephenson MD, Karrison T, Odem RR, Branch DW, Scott JR, Schreiber JR, Ober C (2001) HLA-G genotypes and pregnancy outcome in couples with unexplained recurrent miscarriage. Mol Hum Reprod 7:1167–1172
47. Pfeiffer KA, Fimmers R, Engels G, d van V, d van V (2001) The HLA-G genotype is potentially associated with idiopathic recurrent spontaneous abortion. Mol Hum Reprod 7:373–378
48. Sher G, Keskintepe L, Fisch JD, Acacio BA, Ahlering P, Batzofin J, Ginsburg M (2005) Soluble human leukocyte antigen G expression in phase I culture media at 46 hours after fertilization predicts pregnancy and implantation from day 3 embryo transfer. Fertil Steril 83:1410–1413
49. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL (1998) Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 281:1191–1193
50. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL (1999) Inhibition of T cell proliferation by macrophage tryptophan catabolism. J Exp Med 189:1363–1372
51. Santoso DI, Rogers P, Wallace EM, Manuelpillai U, Walker D, Subakir SB (2002) Localization of indoleamine 2,3-dioxygenase and 4-hydroxynonenal in normal and pre-eclamptic placentae. Placenta 23:373–379
52. Clark DA, Blois S, Kandil J, Handjiski B, Manuel J, Arck PC (2005) Reduced uterine indoleamine 2, 3-dioxygenase versus increased TH1/TH2 cytokine ratios as a basis for occult and clinical pregnancy failure in mice and humans. Am J Reprod Immunol 54:203–216
53. Chaouat G, Ledee-Bataille N, Dubanchet S, Zourbas S, Sandra O, Martal J (2004) TH1/TH2 paradigm in pregnancy: paradigm lost? Cytokines in pregnancy/early abortion: reexamining the TH1/TH2 paradigm. Int Arch Allergy Immunol 134:93–119
54. Mosmann TR, Coffman RL (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 7:145–173
55. Hahn S, Gehri R, Erb P (1995) Mechanism and biological significance of CD4-mediated cytotoxicity. Immunol Rev 146:57–79
56. Stalder T, Hahn S, Erb P (1994) Fas antigen is the major target molecule for CD4+ T cell-mediated cytotoxicity. J Immunol 152:1127–1133
57. Hahn S, Erb P (1999) The immunomodulatory role of CD4-positive cytotoxic T-lymphocytes in health and disease. Int Rev Immunol 18:449–464

58. Wegmann TG, Lin H, Guilbert L, Mosmann TR (1993) Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 14:353–356
59. Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG (1993) Synthesis of T helper 2-type cytokines at the maternal–fetal interface. J Immunol 151:4562–4573
60. Fievet N, Moussa M, Tami G, Maubert B, Cot M, Deloron P, Chaouat G (2001) *Plasmodium falciparum* induces a Th1/Th2 disequilibrium, favoring the Th1-type pathway, in the human placenta. J Infect Dis 183:1530–1534
61. Zenclussen AC, Fest S, Joachim R, Klapp BF, Arck PC (2004) Introducing a mouse model for pre-eclampsia: adoptive transfer of activated Th1 cells leads to pre-eclampsia-like symptoms exclusively in pregnant mice. Eur J Immunol 34:377–387
62. Sacks G, Sargent I, Redman C (1999) An innate view of human pregnancy. Immunol Today 20:114–118
63. Sargent IL, Germain SJ, Sacks, GP, Kumar S, Redman CW (2003) Trophoblast deportation and the maternal inflammatory response in pre-eclampsia. J Reprod Immunol 59:153–160
64. Huppertz B, Kingdom J, Caniggia I, Desoye G, Black S, Korr H, Kaufmann P (2003) Hypoxia favours necrotic versus apoptotic shedding of placental syncytiotrophoblast into the maternal circulation. Placenta 24:181–190
65. Degenne D, Khalfoun B, Bardos P (1986) In vitro inhibitory effect of human syncytiotrophoblast plasma membranes on the cytolytic activities of CTL and NK cells. Am J Reprod Immunol Microbiol 12:106–110
66. Thibault G, Degenne D, Lacord M, Guillaumin JM, Girard AC, Bardos P (1992) Inhibitory effect of human syncytiotrophoblast plasma membrane vesicles on Jurkat cells activated by phorbol ester and calcium ionophore. Cell Immunol 139:259–267
67. Knight M, Redman CW, Linton EA, Sargent IL (1998) Shedding of syncytiotrophoblast microvilli into the maternal circulation in pre-eclamptic pregnancies. Br J Obstet Gynaecol 105:632–640
68. Gupta AK, Rusterholz C, Huppertz B, Malek A, Schneider H, Holzgreve W, Hahn S (2005) A comparative study of the effect of three different syncytiotrophoblast micro-particles preparations on endothelial cells. Placenta 26:59–66
69. Gupta AK, Rusterholz C, Holzgreve W, Hahn S (2005) Syncytiotrophoblast micro-particles do not induce apoptosis in peripheral T lymphocytes, but differ in their activity depending on the mode of preparation. J Reprod Immunol 68:15–26
70. Rusterholz C, Gupta AK, Huppertz B, Holzgreve W, Hahn S (2005) Soluble factors released by placental villous tissue: interleukin-1 is a potential mediator of endothelial dysfunction. Am J Obstet Gynecol 192:618–624
71. Gupta AK, Holzgreve W, Hahn S (2005) Microparticle-free placentally derived soluble factors downmodulate the response of activated T cells. Hum Immunol 66:977–984
72. Sacks GP, Studena K, Sargent IL, Redman CW (1997) CD11b expression on circulating neutrophils in pre-eclampsia. Clin Sci (Lond) 93:187—189
73. Brinkman V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A (2004) Neutrophil extracellular traps kill bacteria. Science 303:1532—1535
74. Hahn S, Holzgreve W (2002) Fetal cells and cell-free fetal DNA in maternal blood: new insights into pre-eclampsia. Hum Reprod Update 8:501–508
75. Bloom BR, Bennett B (1966) Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. Science 153:80–82
76. Mitchell RA (2004) Mechanisms and effectors of MIF-dependent promotion of tumourigenesis. Cell Signal 16:13–19
77. Apte RS, Sinha D, Mayhew E, Wistow GJ, Niederkorn JY (1998) Cutting edge: role of macrophage migration inhibitory factor in inhibiting NK cell activity and preserving immune privilege. J Immunol 160:5693–5696
78. Niederkorn JY, Wang S (2005) Immune privilege of the eye and fetus: parallel universes? Transplantation 80:1139–1144
79. Todros T, Bontempo S, Piccoli E, Ietta F, Romagnoli R, Biolcati M, Castellucci M, Paulesu L (2005) Increased levels of macrophage migration inhibitory factor (MIF) in preeclampsia. Eur J Obstet Gynecol Reprod Biol 123(2):162–166
80. Hristoskova S, Holzgreve W, Zhong XY, Hahn S (2005) Macrophage migration inhibition factor is elevated in pregnancy, but not to a greater extent in preeclampsia. Arch Gynecol Obstet 1–4:1–4
81. Holzgreve W, Hahn S (1999) Novel molecular biological approaches for the diagnosis of preeclampsia. Clin Chem 45:451–452

82. Shoenfeld Y, Katz U (2005) IVIg therapy in autoimmunity and related disorders: our experience with a large cohort of patients. Autoimmunity 38:123–137
83. Pandey MK, Rani R, Agrawal S (2005) An update in recurrent spontaneous abortion. Arch Gynecol Obstet 272:95–108
84. Pandey MK, Agrawal S (2004) Induction of MLR-Bf and protection of fetal loss: a current double blind randomized trial of paternal lymphocyte immunization for women with recurrent spontaneous abortion. Int Immunopharmacol 4:289–298
85. Di Nisio M, Peters L, Middeldorp S (2005) Anticoagulants for the treatment of recurrent pregnancy loss in women without antiphospholipid syndrome. Cochrane Database Syst Rev CD004734
86. Carp HJ, Dirnfeld M, Dor J, Grudzinskas JG (2004) ART in recurrent miscarriage: preimplantation genetic diagnosis/screening or surrogacy? Hum Reprod 19:1502–1505
87. Audibert F (2005) Screening for pre-eclampsia: the quest for the Holy Grail? Lancet 365:1367–1369
88. Rumbold A, Middleton P, Crowther CA (2005) Vitamin supplementation for preventing miscarriage. Cochrane Database Syst Rev CD004073
89. Oettle C, Hall D, Roux A, Grove D (2005) Early onset severe pre-eclampsia: expectant management at a secondary hospital in close association with a tertiary institution. BJOG 112:84–88
90. Hanff LM, Visser W, Roofthooft DW, Vermes A, Hop WC, Steegers EA, Vulto AG (2005) Insufficient efficacy of intravenous ketanserin in severe early-onset pre-eclampsia. Eur J Obstet Gynecol Reprod Biol