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Fetal cells and cell-free fetal DNA in maternal blood: new insights into pre-eclampsia

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The examination of fetal cells, specifically erythroblasts, and cell-free fetal DNA from the blood of pregnant women is currently the subject of intense research with the aim of developing new risk-free methods for prenatal diagnosis. An unexpected finding made during these studies was that the traffic of fetal erythroblasts into the maternal peripheral circulation was enhanced in pre-eclampsia. Independent prospective studies examining samples collected in the second trimester indicated that this perturbation in fetal cell trafficking occurs early in pregnancy, well before the onset of pre-eclampsia symptoms. The quantitative analysis of cell-free fetal and maternal DNA levels indicated that these concentrations were elevated in a co-ordinate manner in manifest pre-eclampsia, and that these elevations corresponded to disease severity. On the other hand, analysis of prospectively collected samples indicated that only cell-free fetal but not maternal DNA levels were elevated before onset of symptoms in pregnancies which subsequently developed pre-eclampsia. These data support hypotheses suggesting that pre-eclampsia is a multi-step disorder, initiated by a placental lesion that occurs early in pregnancy and which subsequently leads to a systemic maternal inflammatory response and associated endothelial cell damage.

Keywords: cell-free DNA/fetal cells/hypothesis/maternal blood/pre-eclampsia

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Fetal cell traffic in pre-eclampsia

The first cited evidence linking pre-eclampsia with the trafficking of fetal cells into the maternal periphery was made during the late 19th century by the German pathologist Schmorl, who detected multinucleate trophoblast-like cells in the lungs of 14 out of 17 women who had died from eclampsia, but not in four other pregnant women who had died from other causes (Schmorl, 1893).

Sixty-seven years later, a much more extensive study was performed when post-mortem material from 220 pregnant women who had died either during pregnancy, at delivery or shortly post partum was examined (Attwood and Park, 1960). In this landmark study it was determined that large trophoblast cells could be detected in the lungs of almost half the women examined, with the highest numbers of cells being recorded in cases of eclampsia.

Further studies regarding trophoblast deportation (Douglas *et al*., 1959) indicated that this phenomenon was probably common to all pregnancies. A conundrum of this study was that trophoblast cells were detected in the uterine vessels but not in the peripheral circulation. This led to the conclusion that the large trophoblast cells were rapidly filtered from the maternal circulation by the lungs. A deficit of the study was that no cases with pre-eclampsia were included, and so it was impossible to determine whether trophoblast deportation was increased under such conditions.

More recent studies regarding trophoblast deportation have included those of Redman's group in Oxford where, as in previous reports, trophoblast cells could be detected in blood samples taken from the uterine vein, but not the antecubital vein (Sargent *et al*., 1994). In the latter study, trophoblasts were found in uterine vein samples from 16 of 18 women with pre-eclampsia,

but only in two of 11 controls. Although this study suggested that trophoblast deportation was enhanced in pre-eclampsia, no correlation could be made between the number of cells recovered and the degree of disease severity.

In 1905, it was suggested that feto-maternal bleeding might be associated with pre-eclampsia, the proposal being that incompatible fetal bleeding caused eclampsia (Dienst, 1905). Indeed, later epidemiological studies supported such an idea in that Rh isoimmunization was observed to be more frequent in pregnancies affected by pre-eclampsia than in those with normal healthy deliveries (Knox, 1965, 1968; Pilkington *et al*., 1966). Although this suggested that pre-eclampsia was associated with a feto-maternal haemorrhaging of red cells, the tools needed to address this issue systematically were only developed much later.

A first step in the direction of being able to quantify the extent of feto-maternal bleeding was the development of a staining technique which permitted a distinction to be made between fetal and maternal erythrocytes (Kleihauer *et al*., 1957). Using this technique, it could be shown that the occurrence of fetal erythrocytes was indeed more frequent in pregnancies affected by pre-eclampsia than in healthy controls (61 versus 51%) (Jones *et al*., 1969). However, this effect was most dramatic in samples taken before 36 weeks pregnancy, where significant differences in the numbers of fetal erythrocytes were noted in those pregnancies subsequently affected by pre-eclampsia, in contrast to those with normal deliveries.

The detailed analysis of rare nucleated fetal cells from the maternal circulation was, however, only made possible by the development of new technologies which permitted the efficacious enrichment of these cells. This was achieved by the use of flow cytometry (FACS) (Herzenberg *et al*., 1979) and magnetic cells sorting (MACS) (Ganshirt-Ahlert *et al*., 1992; Holzgreve *et al*., 1992). Indeed, while exploring the use of the MACS system for the enrichment of fetal erythroblasts, a fortuitous observation was made which indicated that the number of erythroblasts was elevated in the circulation of 43 pregnant women with pre-eclampsia when compared with 222 controls (Ganshirt *et al*., 1994). This finding suggested that the trafficking of fetal erythroblasts into the maternal circulation might be perturbed in pre-eclampsia, though as it had not been discerned whether these erythroblasts were of fetal or maternal origin it was also possible that this was simply an increase in the number of maternal erythroblasts. At this time, considerable controversy existed as to whether any fetal erythroblasts occurred in the maternal circulation at all (Slunga-Tallberg *et al*., 1995, 1996). Consequently, this phenomenon was examined more closely by means of a case-control study in which only pregnancies with a singleton male fetus were included (Holzgreve *et al*., 1998). This study again strongly suggested that the median number of erythroblasts was significantly elevated in the maternal periphery of pregnancies with manifest pre-eclampsia when compared with a control cohort. Moreover, as only pregnancies with male fetuses were examined, it was a relatively simple task to determine the origin of these cells by use of fluorescence in-situ hybridization (FISH) for the X and Y chromosomes. This analysis showed that a large proportion of the erythroblasts were of fetal origin, and confirmed that the trafficking of fetal cells into the maternal circulation was elevated in pre-eclampsia.

It is noteworthy that analogous observations regarding enhanced numbers of fetal erythroblasts in pre-eclampsia have subsequently been reported independently by other groups, thereby emphasizing the validity of the original observation (Al Mufti *et al*., 2000b; Jansen *et al*., 2001; Simchen *et al*., 2001). A feature observed in all reports made to date was that the numbers of fetal erythroblasts was not increased in all cases of pre-eclampsia examined, but that a degree of overlap was seen to exist between the study group and the controls. However, it is possible that this might reflect the heterogeneous nature of this disorder.

Taken together, these disparate data—which have been collated over a period of more than a century—suggest that pre-eclampsia is associated with an increased influx of trophoblasts, fetal erythrocytes and erythroblasts into the maternal circulation. It is very likely that this traffic of fetal cells across the placental barrier is not restricted to these few cell types, but may possibly also involve fetal immune effector cells and well as a variety of fetal progenitor cells. It is currently unclear what the effect of the increased presence of such a diverse variety of semi-allogeneic cells might be on the mother, and what possible role this event might play in the manifestation of pre-eclampsia. Nonetheless, this feature may have important connotations for a recently proposed hypothesis (Dekker *et al.*, 1998) which suggests that pre-eclampsia is the result of an immune maladaptation of the mother to paternal antigens. This aspect is discussed below.

Is fetal cell traffic enhanced before onset of pre-eclampsia symptoms?

A consensus among many researchers studying pre-eclampsia is that the underlying aetiology involves events that occur early during pregnancy, perhaps even at implantation (Roberts and Redman, 1993; Dekker and Sibai, 1998). In this way, pre-eclampsia has been shown to involve alterations in placentation in that the maternal arteries are not modified from a high pressure system to one of low pressure with increased blood flow by the invasive cytotrophoblast (Brosens *et al*., 1972; Starzyk *et al*., 1997). This failure leads to placental hypoxia as the spiral arteries are unable to provide an adequate blood supply to the placental villi—a feature which has severe consequences for the fetus.

Prompted by these speculations, an investigation was made to determine whether the increase in the number of fetal erythroblasts observed in manifest pre-eclampsia was associated with early defects in placentation, or whether it was a consequence of later clinically manifest stages of the disorder. For this purpose, a prospective study was performed in almost 100 pregnant women from whom maternal blood samples were collected at approximately 20 weeks gestation (Holzgreve *et al*., 2001). In order to increase the cohort size of pregnant women at risk of pre-eclampsia, 43 cases were selected with a Doppler ultrasound-identified anomaly of the uterine arteries; this phenomenon has predictive value in determining those pregnant women who are at risk of pre-eclampsia or fetal growth retardation (Harrington *et al*., 1991). A group of 54 women with no such anomaly was studied as controls.

Numbers of erythroblasts present in the blood samples were then determined and compared with the subsequent pregnancy outcome. On many previous occasions it had been shown—by single-cell PCR (Troeger *et al.*, 1999; Di Naro *et al*., 2000; Zhong

et al., 2000) or by FISH (Hahn *et al.*, 1999)—that a large proportion (up to 50%) of the erythroblasts in the maternal circulation were of fetal origin. Thus, for expediency the total number of erythroblasts was counted, with no attempt being made to distinguish between fetal and maternal cells. This strategy also had the advantage that all of the cases recruited could be evaluated rather than only those with male fetuses, as current methods only permit reliable distinction to be made between single male fetal and maternal erythroblasts on the basis of Y chromosome-specific assays.

The results of the study indicated that fetal cell traffic, as measured by total erythroblast numbers, was already elevated at ~20 weeks gestation in those pregnancies which subsequently developed pre-eclampsia (Holzgreve *et al.*, 2001). A very similar observation was made independently in an analogous, albeit much smaller, study in which Doppler ultrasound was used to recruit 18 pregnant women at risk of pre-eclampsia, as well as 10 normal controls (Al Mufti *et al.*, 2000a).

The predictive value of these studies in detecting pregnant women at risk for pre-eclampsia, although promising, needs to be evaluated in further large-scale trials.

Cell-free fetal and maternal DNA levels correspond to disease severity

The detection of cell-free fetal DNA in the maternal circulation is a relatively recent finding (Lo *et al.*, 1997), while the advent of real-time PCR technology (Heid *et al.*, 1996), as used by Taqman® technology (Applied Biosystems, Inc., Rotkreuz, Switzerland), has permitted highly reproducible and robust quantitation of fetal genetic material. By using these techniques, the median concentration of free fetal DNA was shown to be elevated ~5-fold in samples obtained from 20 pregnant women with pre-eclampsia when compared with an equal-sized cohort of normotensive, pregnant women (Lo *et al.*, 1999).

In performing a more detailed analysis of this phenomenon in a larger, multi-centre study of 44 women with pre-eclampsia and 53 controls, the striking observation was made that not only were levels of cell-free fetal DNA elevated in pre-eclampsia, but that a similar increase in levels of maternal cell-free DNA also occurred (Zhong *et al.*, 2001b), with both increases being ~10-fold higher than in controls. Moreover, as the study group was much larger than was detailed previously (Lo *et al.*, 1999), the pre-eclamptic cases could be stratified according to severity of symptoms. This analysis, which included three cases with HELLP (haemolysis, elevated liver enzymes, low platelet count) syndrome and four with eclampsia, showed that increments in both cell-free fetal and maternal DNA corresponded to the degree of disease severity. In addition, a further 3.5-fold increase in cell-free DNA levels was observed in pregnancies complicated by HELLP syndrome or eclampsia when compared with those with manifest pre-eclampsia, or 10-fold greater than in controls. Furthermore, it could also be shown that levels of the two cell-free DNA species corresponded to each other in pregnancies affected by pre-eclampsia, but not in normal pregnancies.

Recently, a very similar observation was reported regarding cell-free fetal DNA and maternal DNA levels in pregnancies with pre-eclampsia and those further complicated with HELLP syndrome (Swinkels *et al.*, 2002). Of particular interest was that these authors also observed almost 4-fold additional increments in both cell-free fetal and maternal DNA levels among pregnancies additionally complicated by HELLP syndrome compared to those with manifest pre-eclampsia. Levels of cell-free maternal DNA were also similarly elevated. Taken together, these independent data sets strongly suggest that increased disease severity is associated with increased release of cell-free fetal and maternal DNA into the maternal circulation.

Another group, in a recent study, also examined cell-free fetal DNA levels in pregnancies affected by pre-eclampsia (Smid *et al.*, 2001). Although the study cohort was very small, these authors also observed almost 4-fold elevations in median levels of cell-free DNA in 10 women with pre-eclampsia.

As with studies reporting fetal cell numbers, investigations into cell-free fetal DNA indicated a fair degree of overlap between levels of cell-free DNA in normal pregnancies and those with manifest pre-eclampsia. In the two studies described (Zhong *et al.*, 2001b; Swinkels *et al.*, 2002), the latter of which included several cases with HELLP syndrome, the distinction was much more apparent between controls and those women with pregnancies affected by pre-eclampsia. Hence, it has been suggested that an analysis of cell-free fetal and maternal DNA levels might be of assistance in the clinical diagnosis of pregnancies affected by HELLP syndrome (Swinkels *et al.*, 2002).

Cell-free, but not maternal DNA, is elevated before onset of pre-eclampsia

Currently, two groups (Al Mufti *et al.*, 2000a; Holzgreve *et al.*, 2001) have shown that the traffic of fetal cells into the maternal periphery is enhanced before the onset of pre-eclampsia symptoms. Consequently, a natural extension of these findings was to examine whether cell-free fetal DNA levels were similarly elevated.

By examining samples collected previously and prospectively for the analysis of erythroblast numbers, median cell-free fetal DNA levels were indeed seen to be elevated ~3-fold in the 10 women who subsequently developed pre-eclampsia, compared with the 40 controls who delivered normally (Zhong *et al.*, 2001a, 2002a). Likewise, an almost 2-fold increase in median cell-free DNA level was noted in a study that compared 18 pregnant women who subsequently developed pre-eclampsia with a group of 33 women who delivered normally (Leung *et al.*, 2001a).

One very recent report verified these findings in a large group of early second-trimester (~15 weeks) samples (Cotter *et al.*, 2002). In this study, samples obtained from 88 women who subsequently developed pre-eclampsia, were compared with 176 control samples. The analysis indicated that an increase in the concentration of cell-free fetal DNA was associated with an almost 8-fold increased risk of developing pre-eclampsia.

It is important to note that in neither of the above studies was any increase in cell-free maternal DNA observed during the early stages of pregnancies which subsequently developed pre-eclampsia.

Fetal cell traffic, cell-free DNA and current hypotheses of pre-eclampsia

As discussed earlier, data obtained from a variety of sources have shown that pre-eclampsia is associated with increased trafficking

of fetal cells across the placental barrier into the maternal circulation (Ganshirt *et al*., 1994; Holzgreve *et al*., 1998; Al Mufti *et al*., 2000b; Jansen *et al*., 2001). Studies performed on samples collected prospectively during the second trimester further indicate that this perturbation occurs early in those pregnancies which will subsequently develop pre-eclampsia (Al Mufti *et al*., 2000a; Holzgreve *et al*., 2001). These data therefore suggest that the underlying placental lesions leading to the increased traffic of fetal cells into the maternal periphery occur early in those pregnancies which subsequently develop pre-eclampsia. This correlates well with previous reports which indicate that placental defects associated with pre-eclampsia most likely occur early during gestation (Brosens *et al*., 1972; Starzyk *et al*., 1997).

In a similar manner, it has been shown that cell-free fetal DNA levels are also elevated long before onset of clinical symptoms of pre-eclampsia (Leung *et al*., 2001a; Zhong *et al*., 2001a, 2002b). The data relating to cell-free DNA, however, have provided an additional unexpected insight regarding the aetiology of pre-eclampsia. Recent evidence indicates that cell-free fetal DNA is most likely of placental origin (Bianchi and Lo, 2001) and is not derived from fetal cells which have traversed the placental barrier (Zhong *et al*., 2002b). It is, furthermore, proposed that cell-free DNA is liberated by some form of cell turnover or cell death (Holdenrieder *et al*., 1999; Stroun *et al*., 2001). Therefore, the increased presence of cell-free fetal DNA indicates that pre-eclampsia is associated with damage to the placenta (Zhong *et al*., 2001b). That cell-free fetal DNA levels are elevated before disease onset also indicates that the placental lesion occurs well before the clinical symptoms are established. On the other hand, the increments in cell-free maternal DNA observed once the disorder has become manifest indicate that this stage, but not the a symptomatic preclinical stage, is associated with some form of cell damage in the mother.

In addition, it has been observed (Zhong *et al*., 2001b; Swinkels *et al*., 2002) that the degree of free DNA liberation correlates with disease severity, which implies that the more severe forms of this disorder are associated with increased levels of damage to the placental and maternal tissues

In the present authors' view, these two aspects of fetal and maternal circulatory DNA levels in pre-eclampsia can best be reconciled to a model whereby localized placental damage leads to a more systemic damage of maternal tissues. In this manner, the present data might add new insight supporting two standing hypotheses, namely that pre-eclampsia involves the systemic damage of the maternal endothelium (Roberts *et al*., 1991a,b) and that the causative agent for such damage might be the release of placental toxins, in the form of microscopic cell debris (Redman and Sargent, 2000). In this regard, a number of experiments have indicated that such placental microdebris can cause the activation and destruction of endothelial cells *in vitro* (Smarason *et al*., 1993; Cockell *et al*., 1997; Knight *et al*., 1998). It has been proposed furthermore that this placental subcellular debris could lead to the unspecific activation of the maternal immune system, leading to a maternal inflammatory response in normal pregnancy (Redman and Sargent, 2000, 2001). The elevated release of these particles in pre-eclampsia would then lead to a hyperactivation of the maternal immune system in pre-eclampsia (Johansen *et al*., 1999; Redman *et al*., 1999). The inflammatory cytokines, which would include factors such as tumour necrosis factor (TNF)-α and interferon (IFN)-γ, released by these immune effector cells could themselves have negative effects both on the placental trophoblast (Yui *et al*., 1994) as well as on the maternal endothelium (Choy *et al*., 2001). This would potentiate the cascade of inflammatory events, leading to pre-eclampsia (Redman *et al*., 1999).

It is therefore proposed that the release of free fetal DNA might be the result of placental breakdown or damage, and that this process is associated with the release of subcellular particles (Figure 1A). This proposal is supported by data indicating that the release of placental microdebris is an intrinsic feature of the differentiation of the rapidly dividing cytotrophoblast cells into the resting multinucleate syncytiotrophoblast cell layer, and that this feature is disturbed under conditions of pre-eclampsia (Huppertz *et al*., 1999, 2001). As this process involves apoptosis-like processes (Huppertz *et al*., 1999), it is highly likely that the excess redundant nuclei of the syncytiotrophoblast are released as cell-free DNA into the maternal circulation. Further support for this hypothesis is provided by reports indicating that increased levels of apoptosis, both in placental bed cytotrophoblasts and in the syncytiotrophoblast layer, occur in pre-eclampsia (Di Federico *et al*., 1999; Leung *et al*., 2001b). It is, therefore, possible, that such increased levels of cell death may lead to corresponding increased liberation of cell-free fetal DNA.

Since it has been shown that the interaction of placental microparticles with the endothelium leads to the destruction of endothelial cells (Knight *et al*., 1998), it is feasible that this step would responsible for the elevated release of free maternal DNA (Figure 1B). In this manner, the measurement of cell-free fetal DNA would be indicative of the amount of inflammatory subcellular debris released by the placenta, whereas the measurement of maternal cell-free DNA would be indicative of the extent of maternal endothelial cell damage (Figure 1A and B). Of interest is the fact that our data predict two distinct stages in this cascade: (i) an initiating asymptomatic phase involving a placental lesion responsible for the release of placental microparticles; and (ii) a secondary symptomatic phase involving the destruction of the maternal endothelium. Therefore, our model supports proposals made previously regarding the aetiology and initiation of pre-eclampsia (Redman and Sargent, 2001).

Further experiments will be necessary to test this new hypothesis, and should include in-vitro systems to test the release of the microparticles and cell-free DNA by placental explants under different conditions, as well as an examination of these two parameters in clinical samples.

Could enhanced fetal cell traffic aggravate the 'immune maladaptation' of pre-eclampsia?

A recent reinterpretation of epidemiological data has indicated that pre-eclampsia might be associated with primipaternity rather than primiparity, in that a change of partner leads to a similar increased risk of pre-eclampsia as for firstborn (Robillard *et al*., 1999). In pursuing these observations, a hypothesis was developed that pre-eclampsia might result from an incorrect immune response by the mother to paternal antigens (Dekker *et al*., 1998). Support for this hypothesis of 'immune maladaptation' was obtained from several epidemiological studies. The data indicated, among other things, that prolonged exposure to the partner's semen can have a protective effect and reduce the risk of pre-eclampsia, whereas the

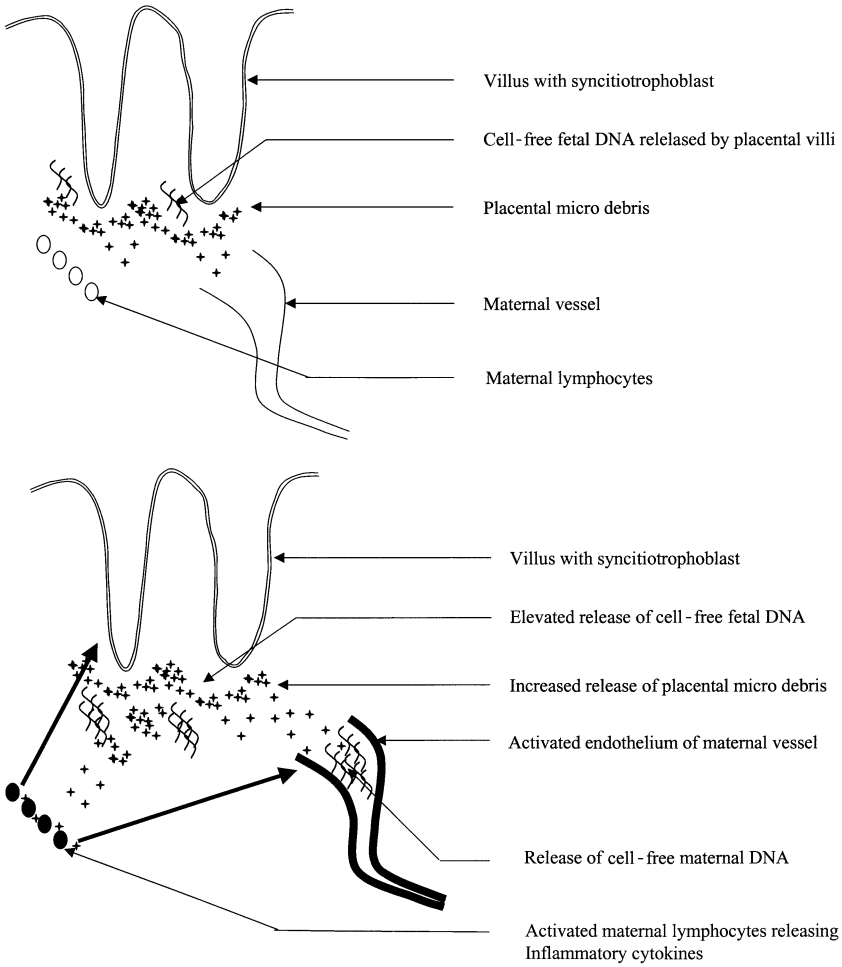

Figure 1. Model reconciling the release of placental microdebris and endothelial disruption with the elevated release of cell-free fetal and maternal DNA in pre-eclampsia. (**A**) The release of placental microdebris by the placental villi would be accompanied by the concomitant release of cell-free fetal DNA. (**B**) In pre-eclampsia, the increased release of these placental subcellular particles, accompanied by an increased release of cell-free fetal DNA, would lead to dysfunction of the maternal endothelium, leading to the release of cell-free maternal DNA. This system would be exasperated by the parallel overt activation of the maternal immune system, which would have negative effects both on the placental trophoblast and on the maternal endothelium (B).

use of condoms, by which the mother is shielded from exposure to paternal antigens, can increase the risk (Dekker, 1999; Tubbergen *et al*., 1999; Koelman *et al*., 2000). Elegant proof for this concept was obtained recently when pregnancies resulting from IVF involving ICSI were examined (Wang *et al*., 2002). Here, the rate of pre-eclampsia was found to be significantly higher in those pregnancies where the partner was aspermic (and where as a consequence the mother was never exposed to the partner's semen) than in other comparable IVF pregnancies where the mother was exposed to paternal semen. Additional pertinent evidence indicating that pre-eclampsia might result from an incorrect immune response to the semi-allogeneic fetus (foreign paternal antigens) is provided by data obtained from pregnancies resulting from oocyte donation, where the entire fetus is foreign (both the male and female complements). In these instances almost 50% of these pregnancies developed pre-eclampsia (Salha *et al*., 1999). It appears therefore that pre-eclampsia is associated with a breakdown in the exquisitely controlled tolerance that exists between mother and fetus during pregnancy.

The question arises, therefore, as to what might be the effect on a potentially imbalanced maternal immune response to foreign fetal antigens, under conditions which lead to a significantly higher influx of semi-allogeneic fetal cells into the maternal circulation. It is to be expected that the presence of such foreign cells will be deleterious. It has been shown that the transplacental trafficking of fetal cells is elevated early during gestation, before the onset of pre-eclamptic symptoms; consequently, it is possible that this influx of foreign cells might provide a vital trigger in the development of the disorder. In this manner, the increase in foreign fetal material might contribute to the overt inflammatory response of the maternal immune system, which has been postulated to occur in pre-eclampsia (Redman *et al*., 1999).

It is unclear whether the presence of increased amounts of fetal DNA could also contribute to the development of this pathology. Although foreign DNA has been shown to be capable of integrating into host cells and thereby permitting the expression of foreign genes (Garcia-Olmo *et al*., 1999, 2000), it should be borne in mind that the quantity of fetal DNA present in the

maternal circulation represents only a very small fraction (1–5%) of the total cell-free DNA (Lo *et al*., 1998). It is therefore possible that this large reservoir of cell-free maternal DNA acts as a sink, effectively diluting out the potential deleterious effects of foreign fetal DNA. It is clear that further studies will be necessary to clarify these pertinent issues.

Is enhanced fetal cell traffic associated with disorders during pregnancy or post partum?

Recent studies have indicated that fetal cells which have entered the maternal circulation could potentially be involved in a variety of maternal disorders (Bianchi, 2000). These include the pregnancy-associated disorders, polymorphic eruptions of pregnancy (PEP) (Aractingi *et al*., 1998) or thyroiditis (Srivatsa *et al*., 2001). It has also been suggested that persisting fetal lymphoid progenitor cells could play a role in the development of certain autoimmune disorders such as systemic sclerosis (Nelson *et al*., 1998) or systemic lupus erythematosus (SLE) (Johnson *et al*., 2001). It is also possible that possible that maternal cells which have entered the fetal circulation may play a role in certain juvenile or adult disorders (Artlett *et al*., 2000, 2001).

As several studies have shown that elevated numbers of fetal cells enter the maternal circulation in pre-eclampsia, it is enticing to speculate that this may increase the predisposition of such women or their children to these disorders with autoimmune character. In this regard, it is worth noting that the incidence of both pre-eclampsia (Knuist *et al*., 1998) and scleroderma (Burns *et al*., 1996; Laing *et al*., 1997) is increased in certain ethnic population groups, such as Afro-Americans when compared with Caucasians. In addition, the onset of scleroderma occurs earlier and in a more severe form among the Afro-American population. Thus, might a link exist between these two disorders?

By contrast, the finding that enhanced trafficking of maternal cells into the fetal periphery may occur in pre-eclampsia, and that this may have deleterious effects on the child, has been indirectly suggested by two high-profile case reports. In the first, an instance of early-onset sarcoidosis (an autoimmune disorder) was reported in a 4-year-old child (Falcini *et al*., 1999), whilst in the second case the transplacental transmission from mother to child of a natural killer lymphoma was reported (Catlin *et al*., 1999). Of interest was that both pregnancies were affected by pre-eclampsia, and it is therefore open to speculation whether an increased release of maternal cells occurred into the fetal circulation in these situations.

These questions are very difficult to address due to the relatively low incidence of pre-eclampsia, and especially of autoimmune disorders such as scleroderma, and will require the analysis of large data sets, perhaps of specific ethnic groups. Nevertheless, it is felt that such studies may yield an unexpected insight into the aetiology of certain autoimmune disorders, in the same manner that complications of the fetus during pregnancy or childbirth have been suggested to be involved in the development of numerous disorders in adult life, for example hypertension (Barker, 2001).

Conclusions

The association of fetal cell traffic and pre-eclampsia has been both long and tenuous, and only recent developments in technology that permit the reliable isolation and characterization of rare fetal cells from the maternal periphery have allowed the substantiation and extension of descriptive reports and speculations first made during the 19th century. In this regard, much of the data which have recently been obtained are an expected consequence of research performed on the possible use of fetal cells and cell-free fetal DNA for non-invasive prenatal diagnosis.

During the time since our original chance finding, several independent studies have shown that pre-eclampsia is associated with an underlying placental lesion which facilitates the increased trafficking of fetal cells, and the release of cell-free fetal DNA. These studies have also shown that this placental defect occurs early in pregnancy, long before the onset of any clinical symptoms. Interestingly, this preclinical phase is only associated with an increased release of cell-free fetal DNA, whereas the later clinically manifest stage is also associated with an increased release of cell-free maternal DNA. It is proposed that these data could be used to support a model whereby pre-eclampsia results from the release of placental microparticles which cause widespread disruption of the maternal endothelium. In this regard, the present data indicate that this process involves two distinct stages: an initiating placental lesion, associated with the release of cell-free fetal DNA and toxic debris; this subsequently leads to the clinical maternal syndrome in a second stage involving the maternal endothelium, which is associated with the increased release of free maternal DNA.

One feature which will need to be addressed more carefully in future is the impact of increased numbers of fetal cells expressing foreign (paternal) antigens on a maladapted maternal immune system, and whether this leads to the development of an overt inflammatory response. Indeed, such an imbalance of the maternal immune response has been postulated to be a key event in the development of pre-eclampsia. It will also be necessary to determine the role that cell-free fetal DNA plays in the manifestation of this disorder. It is therefore, essential to appreciate how rapidly these new insights into this enigmatic disorder have been obtained from apparently unrelated events, namely the trafficking of fetal cells and the release of cell-free DNA.

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