Influence of human leukocyte antigen and tumour necrosis factor genes on the development of pre-eclampsia

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Pre-eclampsia is a syndrome with a strong familial component. Autosomal recessive inheritance acting only in the mother is not consistent with the epidemiological data, and a more complex genetic susceptibility, involving interactions between maternal and fetal genomes, seems likely. The human leukocyte antigen (HLA) system has been implicated, but many of the findings reported have been inconsistent or contradictory. Pre-eclampsia is unlikely to be the simple result of excessive HLA-class II antigen sharing between mother and fetus, as was first thought, but a more complex mechanism involving feto-maternal compatibility cannot be excluded. The reported increase in HLA-DR4 in mothers and babies from pre-eclamptic pregnancies has not been independently confirmed for mothers, and no further studies have been conducted with babies. Consequently, the allegedly stronger relationship with HLA-DR4 sharing between mother and fetus has neither been confirmed nor refuted. Certain (B44-DR7)-containing haplotypes appear to confer increased risk for pre-eclampsia on the basis of independent analyses of American and Scottish populations. HLA-DR53 may be associated with the antiphospholipid antibody syndrome, which is itself a strong risk factor for pre-eclampsia. The tumour necrosis factor (TNF) α allele, TNF1, may be associated with pre-eclampsia and certainly elevated concentrations of the cytokine appear to be a feature of the disease. The inducibility of TNF-α is HLA-class II-dependent, and the relevance of HLA-class II genes might be entirely in relation to TNF-α synthesis and secretion.

Key words: HLA-DR/pre-eclampsia/pregnancy hypertension/tumour necrosis factor

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Introduction

Pre-eclampsia is a mysterious yet relatively common disease of pregnancy for which the only cure is delivery of the placenta. Reduced trophoblast invasion of decidual arteries during the first half of pregnancy is typical of pre-eclampsia and is associated with reduced placental perfusion (Meekins et al., 1994a). Circumstantial evidence points to an excessive release of placental products contributing to maternal organ non-specific endothelial cell dysfunction, but it is well to remember that ideas about causation that were popular in the past have subsequently lost favour. As the name suggests, the condition often precedes eclamptic seizures, but most cases of pre-eclampsia do not progress to eclampsia, and a substantial proportion of eclampsia cases are not preceded by pre-eclampsia (Douglas and Redman, 1994). No definite diagnostic test is available. It is believed that the histopathological demonstration of glomerular endotheliosis is the most specific sign of the disease, but renal biopsy would not usually be justified in suspected cases and could never be used as a routine antenatal screening test. Instead, the variable diagnostic criteria applied usually involve some degree of hypertension combined with some significant degree of proteinuria. It is likely that such definitions exclude some genuine patients with the disease and include others inappropriately. This diagnostic imprecision complicates genetic studies of the disorder. Nevertheless, there is compelling evidence that susceptibility to pre-eclampsia is, at least in part, inherited. Family studies conducted by Chesley and co-workers with American eclamptic index cases demonstrated an increased incidence of pre-eclampsia in sisters and daughters (Chesley et al., 1961, 1968; Chesley and Cooper, 1986). With pre-eclamptic pro-
bands, a significantly increased prevalence in sisters has received convincing confirmation from several Scottish (Adams and Findlayson, 1961; Cooper and Liston, 1979; Kilpatrick et al., 1989) and an Icelandic (Arngrimsson et al., 1990) population study. That pre-eclampsia 'runs in families' is now firmly established.

The nature of this genetic susceptibility, however, is far from clear. Chesley and Cooper came out strongly in favour of a single recessive gene present in the mother (Chesley and Cooper, 1986), but in a later study (Cooper et al., 1988) the same authors conceded some evidence for a fetal genetic contribution. Cooper and Liston argued that pre-eclampsia could be a simple recessive trait, but could not distinguish whether the mother or the fetus had the crucial genotype (Cooper and Liston, 1979). The latter seemed to be excluded by the finding of a >3-fold higher incidence of the disease in mothers of pre- eclamptics compared to mothers-in-law (Sutherland et al., 1981), a finding later confirmed in relation to daughters versus daughters-in-law (Arngrimsson et al., 1990). The latter authors favoured simple maternal inheritance via a single recessive gene or a dominant gene with incomplete penetrance. Liston and Kilpatrick considered six models of inheritance and compared the theoretical expectations to previously published data (Liston and Kilpatrick, 1991). On this basis, all dominant gene models and all models of simple recessive inheritance acting either in the mother alone or acting in the fetus alone were excluded. The only model to survive this analysis was one in which both mother and fetus expressed the same recessive gene. Arngrimsson undertook a similar theoretical approach, and concluded that the best fit was with a single dominant gene with incomplete penetrance (Arngrimsson, 1990), with the penetrance depending partly or wholly on the genotype of the fetus. A subsequent analysis was also most consistent with a materno-fetal genetic interaction or multi-factorial inheritance (Arngrimsson et al., 1995). Finally, the finding of discordance for pre-eclampsia amongst identical twins (Thornton and Onwude, 1991) is simply incompatible with the originally favoured concept of a single recessive gene acting in the mother. Moreover, a population-based study from Norway has since provided additional evidence that both maternal and fetal genes contribute to the risk of pre-eclampsia (Lie et al., 1998).

Indeed, a variety of possible genetic influences on pre-eclampsia have appeared (Table I), all outside the major histocompatibility complex (MHC). Moreover, pre-eclampsia, however defined, is likely to be a heterogeneous syndrome, not a single disease. Ness and Roberts have helpfully tried to classify pre-eclampsia into two main aetiological groups: one has its origin in reduced placental perfusion (placental pre-eclampsia), while the other results from a miscellany of pre-existing maternal disorders (maternal pre-eclampsia; due to diabetes, renal diseases etc.) (Ness and Roberts, 1996). If many primary causes lead to a similar syndrome, many genetic influences may act independently or in combinations to confer susceptibility. It is against this background that the possible influence of the MHC and human leukocyte antigen (HLA)-related genes has to be considered. It is no longer plausible to take the view that polymorphisms on the short arm of chromosome 6 alone can explain the genetics of pre-eclampsia.

### HLA sharing and compatibility

The early literature on HLA in pre-eclampsia was confusing and inconsistent, a description which characterizes the literature up to the present time. Perhaps the most influential of those early studies was described in a paper by Redman et al. (1978). Those workers investigated HLA class I antigens in 80 pre-eclamptic couples and 83 control couples, and their major finding was a significant increase in presumed homozygosity at the HLA-B locus in the pre-eclampsia patients and their male partners. A similar, but weaker, relationship with the HLA-A locus was noted and, moreover, A-locus blanks were combined with B-locus blanks more often than expected. The study was conducted in two stages, the first involving 24 cases and controls, and the observed difference persisted when the next 56 cases were prospectively investigated. There was no greater antigen sharing observed between the pre-eclampsia couples, but the increased homozygosity had the effect of decreasing the number of antigenic disparities between pre-eclamptic spouses compared to control spouses. Although class II specificities were not investigated in this study, the authors conjectured that their results might reflect greater homozygosity for immune response (D-region) genes and suggested that increased HLA compatibility between couples might at least contribute to the development of pre-eclampsia, perhaps as a result of an impaired ability to generate a protective immune response. These concepts,
based on a methodologically and statistically sound study, were persuasive and attracted much support.

Moreover, the above findings were supported by two complementary studies from another part of England (Leeds). The first (Jenkins et al., 1977) reported an absence of lymphocytotoxins in pre-eclamptic women, which could be interpreted as an indication of decreased feto-maternal histocompatibility in pre-eclamptic pregnancies. A later paper (Jenkins et al., 1978) provided more direct evidence. Comparing 38 pre-eclamptic women and their husbands with 39 normal couples, an increase in class I HLA matching between spouses was observed in pre-eclampsia, and, more importantly, one-way mixed lymphocyte cultures revealed a diminished reactivity on the part of maternal responder cells to paternal or fetal (cord) stimulator lymphocytes in pre-eclampsia compared to control families. The patients chosen for both those studies were women with very high blood pressure and heavy proteinuria, a severe subgroup constituting perhaps <5% of the pre-eclamptic population.

On the other hand, an Israeli study reported no increased HLA sharing (including HLA-DR sharing) in pre-eclampsia, nor any difference in the pattern of one-way mixed lymphocyte reactions in either direction when pre-eclamptic couples were compared to control couples (Persitz et al., 1983). African women, surprisingly, were found to have significantly decreased homozygosity at the B locus compared with control women (Johnson et al., 1988), while HLA-B35 was almost four times more common than expected in their babies (Johnson and Moodley, 1989). In both these populations, relatively demanding diagnostic criteria (>110 mm Hg diastolic blood pressure; moderate to heavy proteinuria) were applied, which presumably excluded many typical pre-eclamptic patients. However, a much larger and diagnostically unbiased series found no increase in spousal HLA sharing, no increase in maternal homozygosity at HLA-A or B loci, and only a modest, statistically non-significant increase in feto-maternal HLA-DR compatibility (Kilpatrick et al., 1990). This takes us back full circle to one of the earliest reports which found no increase in HLA-homozygosity or antigen sharing in pre-eclampsia (Scott et al., 1976).

There are a number of factors which could have contributed to these inconsistencies, including the study of geographically (and therefore ethnically) different populations and the limitations of serological tissue-typing. I suspect the main factors were the small numbers of patients in many of the studies, and the exclusive nature of diagnostic criteria chosen in some cases, both of which may have contributed to statistically freak results. It seems fairly likely now that there is no increased HLA-B locus homozygosity in pre-eclamptic patients, and the hypothesis that couples associated with pre-eclamptic pregnancies show increased compatibility for HLA in general, especially class II antigens, must be considered very doubtful. Having stated that, a more recent study of 48 Swedish pre-eclamptic patients and their male partners provides evidence for increased feto-maternal HLA sharing. An increased homozygosity at the HLA-B locus was found in the babies born to these pre-eclamptic mothers (Schneider et al., 1994).

**Putative association with HLA-DR4**

None of the early (pre-1980) investigations found any association between pre-eclampsia and specific HLA, but the patient numbers were small while the number of specificities tested for was large. Only a very strong relationship would achieve statistical significance under these circumstances. The most practical way to investigate this topic is to conduct a small preliminary study, from which a provisional hypothesis may be formed which can then be examined prospectively in a subsequent larger study. This approach was conducted independently in France and Scotland, with to some extent similar results. Simon et al. were the first to report an increase in HLA-DR4 in 26 pre-eclamptic mothers compared to 16 control women (Simon et al., 1980), while Kilpatrick et al. found a similar association and were the first to report an equally strong association with HLA-DR4 in the cord blood of babies born to 20 pre-eclamptic mothers (Kilpatrick et al., 1987a).

The French group expanded their study (Simon et al., 1988) to cover 67 patient couples, 31 classified as having proteinuric pre-eclampsia and 36 couples with latent hypertension (although in the latter group, one patient developed eclampsia and the perinatal mortality rate was remarkably high at 11%). The patient data were compared to those obtained from 40 fertile, normotensive control couples and 200 blood donors. Although the authors stressed an association of HLA-DR4 with latent pre-eclampsia, particularly recurrent pregnancy-induced hypertension, there was also a significant ($P < 0.05$) association between HLA-DR4 and proteinuric pre-eclampsia mothers.

The number of patients in each category was small, however, and several aspects of Simon et al.’s data give grounds for unease: (i) For proteinuric pre-eclampsia, HLA-DR4 was three times more common in the patients than in their husbands, and the reverse was found for the control group. (ii) For latent hypertension, HLA-DR5 in the female patients was significantly protective. (iii) For proteinuric pre-eclampsia, but not latent hypertension, HLA-DR2 was positively associated with the disease in both female patients and their husbands. None of these findings has been confirmed elsewhere, and therefore it is a reasonable suspicion that they represent statistical flukes. If so, obviously less reliance can be placed on the HLA-DR4 findings.

The study of a Scottish population based in the environs of Edinburgh was the largest of its kind at the time. The 22 patients from the preliminary study were excluded, and 92 new patients were investigated (Kilpatrick et al., 1990). A total of 56 of these patients who had parous sisters were the subjects of a separate family sub-study (Kilpatrick et al., 1989). Normal control data were taken from a previous study of HLA types from the same geographical area (Jazwinska and Kilpatrick, 1987; Jazwinska et al., 1987). The major findings were as follows: (i) HLA-DR4
was approximately twice as frequent in pre-eclamptic mothers and their babies than in women and babies from normotensive first pregnancies. (ii) The strongest association, though not the most significant statistically, was with sharing of HLA-DR4 between mother and fetus. (iii) The frequency of HLA-DR4 was high in sisters of probands with proteinuric pre-eclampsia who had some kind of hypertensive pregnancies compared to sisters with normotensive pregnancies, and more of the former group shared HLA-DR4 with their spouses. Therefore, both within families and throughout the general population, HLA-DR4 conferred a risk of pre-eclampsia whether present in the mother or her fetus.

The only obvious potential weakness in the Scottish study was the use of control data obtained at an earlier time, and defined with different anti-HLA sera. This should not have mattered, but tissue typing laboratories in other parts of Britain detected HLA-DR4 in typically 30–40% of their normal groups, often healthy close relatives of patients with renal failure. Over the same period, similar antisera defined several non-pre-eclampsia Edinburgh populations: HLA-DR was found in 19% of 31 endometriosis patients (Maxwell et al., 1989), 28% of 47 babies born to HIV-infected mothers (Kilpatrick et al., 1991) and 32% of 108 female patients with recurrent spontaneous abortion (unpublished data from Kilpatrick and Liston, 1993). These figures, while variable, are all lower than the 36% reported for pre-eclamptic women, but such populations can hardly be considered normal controls.

Two reports published immediately afterwards cast much doubt on the putative HLA-DR4 association. Hoff et al. (1990) found HLA-DR4 to be significantly under-represented in a large group (n = 715) of American (50% black) antenatal subjects. Groups of patients with various types and severity of hypertension in pregnancy all had HLA-DR4 frequencies of 10–11%, while the corresponding figure for normotensive women was 25%. Hayward et al. compared 50 Scottish pre-eclamptic patients (some of whom were included in the earlier Edinburgh study) and 100 normotensive controls by a polymerase chain reaction-oligotyping method (Hayward et al., 1990). These workers found little difference between patients and controls (14 versus 13% respectively); however, the remarkably low value for controls must provoke some suspicion about accuracy, and if the ‘fault’ of the original work was a spuriously low control HLA-DR4 frequency, such data cannot be regarded as a refutation. Therefore, although both of these independent investigations are inconsistent with the putative DR4 association, neither can be regarded as constituting completely damning evidence.

However, two papers from Australia seemed to exclude any major contribution from maternal HLA genes. The first (Wilton et al., 1990) was a linkage study of 10 pedigrees which were investigated using restriction fragment length polymorphisms (RFLP), a typing method generally considered to correlate well with, and be more accurate than, serological typing. Various analyses of the data failed to support the concept of a maternal susceptibility gene close to the HLA-DRB region. A complimentary study (Wilton et al., 1991) of unrelated pre-eclamptic and eclampsia patients (50 of each) by RFLP revealed no difference in HLA-DR4 frequency between patients and controls, although a bizarre feature of this study was the control group: only 14% of the 210 ‘controls’ were truly appropriate (normotensive pregnant women), while 65% were haemophiliacs! The latter were presumably male and unable to manifest the disease phenotype if carrying any disease susceptibility genes. Nevertheless, these papers taken together more or less exclude the possibility that the major maternal susceptibility gene is identical or closely linked to HLA-DR4. Finally, a recent small-scale study (35 patients) reported a negative association with HLA-DR4 (Takakuwa et al., 1997).

It is ironic that the best evidence for an HLA association, and the only relationship which has been found independently in two centres, is with HLA-DR4, but also that the best evidence against a specific HLA is the group of negative reports concerning HLA-DR4. Can any conclusions or even insights be drawn from these conflicting reports?

I think we may now conclude that the HLA region is not the only genetic region involved in pre-eclampsia, and is most unlikely to be the most important susceptibility region in the mother. A minor role for maternal HLA-DR4 has not been rigorously ruled out, especially in relation to maternal–fetal interaction. The association with HLA-DR4 in the fetus and the possible importance of maternal–fetal HLA-DR4 sharing has not been confirmed or refuted by independent studies. The suggestion that pre-eclampsia is a syndrome of heterogeneous origins, falling into two main categories of maternally derived and fetally derived diseases, is consistent with the concept of fetally expressed HLA-associated genes acting alone or by interacting with non-HLA-associated susceptibility genes in the mother.

**Association with HLA-DR7-containing haplotypes**

In the course of a prospective study of HLA and pregnancy outcome, Peterson et al. (1994) reported an increased prevalence of pre-eclampsia and/or intrauterine growth retardation (40 versus 17%) in 20 primigravid women possessing the haplotypes A23/A29-B44-DR7 compared to 692 primigravid women without these haplotypes. For pre-eclampsia alone the relative risk was 3.3 (P < 0.02).

Peterson et al. (1994) compared A23/A29-B44-DR7-positive and -negative individuals for the occurrence of pre-eclampsia, and, to the author’s knowledge, no other group has confirmed or disputed this association by a similar analysis. However, the converse aspect of this relationship has been investigated in the 92 Edinburgh patients previously referred to (unpublished data). The haplotypes A23-B44-DR7 (3.3 versus 2.3%), A24-B44-DR7 (2.1 versus 0.4%) and A29-B44-DR7 (6.5 versus...
1.5%) were all commoner in the 92 pre-eclampsia patients than in the control group of 132 normotensive mothers ($\chi^2 = 4.2$; $P < 0.05$).

It is therefore a real possibility that certain extended haplotypes bearing B44 and DR7 predispose to pre-eclampsia. Nevertheless, both in Alabama and in Scotland these haplotypes were found in <5% of the white population (and a considerably lower proportion of the African-Americans who made up half the group studied by Peterson and co-workers). Therefore, this association cannot be a major contributor to maternal genetic susceptibility. It is possible that the genetic susceptibility is heterogeneous and this is one kind giving rise to a small minority of pre-eclampsia cases. It is also possible that it is an indication of a disease-susceptibility gene linked to alleles at the HLA class II loci which have a minor influence on the development of symptomatic disease. It is noteworthy that HLA-DR4 and HLA-DR7 are included in the supertypic specificity HLA-DR53, which can be indirectly implicated in some cases of pre-eclampsia, as discussed in the next section.

**Indirect associations of HLA class II antigens and pre-eclampsia**

The antiphospholipid antibody syndrome (APAS; Shapiro, 1996) is strongly associated with recurrent miscarriage but also with intrauterine growth retardation (IUGR), which is a common problem in pre-eclampsia. Although a minority of pre-eclampsia patients have antiphospholipid antibodies (Kilpatrick, 1991), women possessing such antibodies are at very high risk of developing pre-eclampsia. Although many of pre-eclampsia patients have antiphospholipid antibodies (Kilpatrick and Liston, 1992; Pattison et al., 1993; Yasuda et al., 1995; Katano et al., 1996). Could there be some genetic factor in common between pre-eclampsia, IUGR, spontaneous abortion and autoimmunity? Although not all investigations have found HLA associations with APAS, a reasonable conclusion to be derived from several independent studies is that there is a weak association with HLA-DR4 and more definitely with HLA-DR53 (Camps et al., 1995).

A rather different HLA class II association has been postulated for pre-eclampsia and IUGR (Hoff et al., 1992, 1993). In a cohort of 659 primigravidas, both pre-eclamptic mothers and lower birthweight babies were associated with the combination of a single HLA-DR antigen in the fetus with the maternal expression of a second HLA-DR antigen incompatible with the fetal HLA-DR type (‘maternal allogenicity’). In an interesting variation of the old lack-of-maternal-exposure-to-fetal-antigen hypothesis, the authors suggest the opposite sequence of events initiates the process. They speculate that the fetus mounts an antibody response to its mother, and when such antibodies, bearing an incompatible allelotype, enter the maternal circulation, the mother in turn mounts an anti-allelotype antibody response, leading to deleterious immune complex deposition in uteroplacental blood vessels. On this occasion, our data (unpublished) do not support the hypothesis. Examination of the birthweights of the 51 infants born to primigravidas in the normal Edinburgh cohort (Jazwinska et al., 1987) revealed only five pregnancies exhibiting HLA-DR ‘maternal allogenicity’. The birthweights in this group were actually slightly higher on average than for the other types of maternal–fetal relationships combined (mean + SD: 3.42 + 0.22 versus 3.33 + 0.42 kg). This interesting hypothesis therefore requires further independent investigation.

**Tumour necrosis factor and pre-eclampsia**

The so-called class III HLA genes lie between the HLA-B and DR loci and include those for certain complement components and for tumour necrosis factor (TNF). Both TNF-α and TNF-β (lymphocytotoxin) genes exhibit polymorphisms associated with differential production of those cytokines. The secretion of TNF-α is regulated both at the transcriptional level and at the level of translation. Dimorphism at position 308 in the promoter region of the TNF-α gene results in two alleles, TNF1 and TNF2; linkage disequilibrium with certain HLA-DR specificities has been observed, although the strongest association reported was with an extended haplotype (Wilson et al., 1993; Bouma et al., 1996). Inducibility of TNF seems to be strongly influenced by HLA-DR type. Although the literature is not entirely consistent, it would certainly seem that high levels of TNF secretion are associated with HLA-DR3 (Jacob et al., 1990; Pociot et al., 1993; Peces et al., 1995) and low levels with HLA-DR2 (Bendtzen et al., 1988; Jacob et al., 1990; Pociot et al., 1993; Peces et al., 1995).

It has been suggested that normal pregnancy is a T helper 2 (Th-2) phenomenon (Wegmann et al., 1993; Raghupathy, 1997; Fried et al., 1998). That is to say, it is characterized by a bias towards humoral relative to cellular immunity and a greater tendency to secrete cytokines such as interleukins 4, 5 and 10. The cytokines associated with Th-1 responses, of which TNF is highly typical, may be harmful to pregnancy. Several groups have investigated TNF in relation to pre-eclampsia. Although some inconsistent data have emerged, the majority finding has been a significant increase in TNF-α production in pre-eclamptic compared to control pregnancies (Table II). There is conflicting evidence concerning whether TNF-α could initiate the disease process or is simply a result of the disease. A role for TNF-α in the pathogenesis of the disease was predicted by the oxidative stress hypothesis (Stark, 1993) and is consistent with the possibility that excessive TNF release from the placenta could contribute to systemic maternal endothelial cell dysfunction. Soluble TNF receptors were also found to be elevated in the sera of pre-eclamptic women (Opsjon et al., 1995a; Vince et al., 1995). Two studies have examined TNF in amniotic fluid, but unfortunately with inconsistent results: Opsjon et al. (1995b) found no difference between pre-eclamptic and normal pregnancies at delivery, whereas Stallmack et al. found high levels in association with IUGR (Stallmack et al., 1995).
**Main findings**

No association with pre-eclampsia; decreased TNF-α concentration associated with idiopathic small-for-age neonates

TNF detected in 36% of 19 pre-eclampsia patients after the syndrome was clinically apparent, but only 1 of 22 normal pregnancies

TNF detected in 80% of 65 pre-eclampsia patients, but only 44% of 25 controls. Values were significantly higher in 18 patients with HELLP syndrome

TNF detected in 39% of 19 pre-eclampsia patients, but only 19% of controls, with significantly higher levels in the pre-eclampsia group

TNF levels elevated in first-trimester pre-eclamptics (and therefore before the onset of clinical manifestations).

**References**

Schiff et al. (1994)

Meekins et al. (1994b)

Visser et al. (1994)

Vince et al. (1995)

Hamai et al. (1997)

HELLP = Haemolysis, elevated liver enzymes and low platelets.

Whatever the precise role of TNF in pre-eclampsia, there is a sound experimental basis for suspecting some involvement of TNF in the pathogenesis of the disease. In addition to the various reports of plasma/serum concentrations of TNF-α in pre-eclampsia, TNF-α mRNA expression was detected in pre-eclamptic but not in normal placentas (Wang and Walsh, 1996) and was found to be significantly increased in the leukocytes from pre-eclamptic patients relative to those of normotensive pregnant women (Chen et al., 1996).

This increase in TNF-α gene expression may be associated with TNF gene polymorphism which in turn could exist in linkage disequilibrium with HLA specificities. The TNF1 allele was markedly over-represented (64 versus 17% for homozygotes) in 14 pre-eclampsia patients, and the TNF1 homozygotes produced significantly more TNF mRNA than TNF2 homozygotes, with heterozygotes occupying an intermediate position (Chen et al., 1996). This TNF1 allele is associated with HLA-DR4 (Wilson et al., 1993).

This connection between TNF-α inducibility and production, TNF-α gene polymorphism and HLA polymorphism prompted a reconsideration of the nature of the possible HLA-class II association with pre-eclampsia. Kilpatrick (1996), noting the quantitative relationships between LPS-induced TNF-α release and individual HLA-DR specificities (Pociot et al., 1993), used two independent sets of previously obtained data to test the hypothesis that pre-eclampsia and diabetes mellitus have a common immunogenetic link which could explain the weak association between the two diseases. A striking correlation was obtained when the relative TNF-α secretory capacity calculated from the data of Pociot et al. (1993) was related to the relative frequency of individual HLA-DR specificities in the Edinburgh pre-eclampsia population. Moreover, when the HLA data were reanalysed to compare the 92 pre-eclampsia patients with 264 normal controls with regard to the high responder HLA-DR specificities (DR 1, 3 or 4), the difference was statistically significant (79 versus 59%; P < 0.001). Indeed, even when all subjects possessing HLA-DR4 were excluded from the analysis, the putative high TNF responders were still over-represented amongst the pre-eclamptics (69 versus 46%; P < 0.002). It was suggested that HLA-DR4 (and other DRB alleles) may function in pre-eclampsia as immune response genes, influencing the TNF-α response to an unknown stimulus (Kilpatrick, 1996).

It would obviously be helpful if other workers would re-analyse their HLA-DR data in a comparable way to confirm or otherwise this relationship. Some limited support is provided by the table of gene frequencies published in Wilton et al. (1991). The combined gene frequencies of high-responding DR1 and DR3 was higher in pre-eclampsia patients (0.267 versus 0.217), while the combined low-responding DR2 and DR5 frequencies were lower (0.233 versus 0.286).

**Concluding comments**

The pioneering work of Chesley and coworkers (Chesley and Cooper, 1986; Cooper et al., 1988) was undoubtedly a distinguished and important contribution to the genetics of pre-eclampsia, but it may also have hindered further research. The concept of a single recessive gene expressed in the mother conferring susceptibility was so simple and attractive that other possibilities were largely ignored, even in the face of evidence to the contrary. Numerous strands of circumstantial evidence indicated a fetal contribution; the epidemiological data did not really fit the model of maternal recessive inheritance very well; and the best fit between a theoretical model and reported data was with a model in which both mother and fetus shared a recessive gene. Yet, for example, we have Arnagrimsson et al. (1990) interpreting their findings in a manner consistent with the established dogma, and Hayward et al. (1992) performing their exclusion map analyses only on the assumption of maternal autosomal recessive inheritance. Even before twin studies provided conclusive refutation of this genetic hypothesis, there were good grounds for believing some kind of fetal–maternal interaction leads to the development of at least some cases of pre-eclampsia. Although conclusive proof still eludes us, at present it seems reasonable to...
conclude that pre-eclampsia is a syndrome of heterogeneous origin, possibly sharing a common pathway(s) of pathogenesis, on which a variety of genetic influences might be brought to bear.

Does the HLA system contribute at all to that variety? Perhaps not, for it is possible that each of the reported associations could have arisen purely by chance. Moreover, the concept of excessive HLA (especially HLA class II) compatibility between mother and fetus is another popular and seductive idea which many workers are reluctant to discard. Certainly, the balance of evidence accumulated does not provide much support for the hypothesis, at least in its original form.

On the other hand, the number of reported HLA associations, however inconsistent, may be due to some weak, indirect but nonetheless genuine influence from the MHC region. Also the weak, obscure but apparently real connection between pre-eclampsia, recurrent miscarriage and the antiphospholipid antibody syndrome may have its basis in an HLA class II immunogenetic link. Then there is the undoubted exchange of nucleated cells between mother and fetus during pregnancy: CD34+ leukocytes of fetal origin can be detected in parous women up to 27 years after their last pregnancies (Bianchi et al., 1996), while nucleated maternal cells have been found in the fetal circulation as early as the 13th week of gestation (Lo, 1998). It is possible this exchange has important immunological consequences during pregnancy, and, if so, some malfunction of this physiological process could contribute to disorders such as pre-eclampsia. [After all, these phenomena have been implicated in rheumatoid arthritis (Wolde et al., 1993), scleroderma (Arlett et al., 1998; Nelson et al., 1998) and transplantation tolerance (Claas et al., 1988) long after birth.] A role for some kind of unknown compatibility/incompatibility associated with HLA could explain some of the apparent inconsistencies in the literature.

Both HLA-C (King et al., 1996) and the non-classical HLA-G (Carosella et al., 1996; Loke et al., 1997) are expressed on extravillous trophoblast and may have important functions in pregnancy, possibly by interacting with decidual NK cells. The highly restricted tissue distribution of the latter, its polymorphism (at least at the nucleic acid level), and the linkage disequilibria of HLA-G alleles with HLA-A (Morales et al., 1993) raise the possibility that HLA-G might be involved in pre-eclampsia and that classical HLA associations could be derived from linkage disequilibrium with HLA-G. However, the only study in this area found no relationship between susceptibility to pre-eclampsia and HLA-G genotypes ( Humphrey et al., 1995).

We can probably now conclude that any association with HLA-DR4 in the mother is at most very weak, and not a major contributor to genetic susceptibility. Moreover, the possible significance of HLA-DR4 in the fetus, and indeed fetal antigens generally, requires further investigation. Moreover, HLA-DR4 is not monomorphic: there are at least 20 HLA-DR4 subtypes known (Cassidy et al., 1995), of which two, 0401 and 0404, are relatively common in European populations. It is worth noting that HLA-B27, the serological specificity with one of the strongest disease associations, consists of at least 11 subtypes: most are associated with ankylosing spondylitis, but one (B*2706) is not, while the status of another (B*2703) is uncertain in this regard (Nuki, 1998). Therefore, it would be helpful, if not essential, to perform HLA-DR subtyping studies on mothers, fathers and babies before reaching firm conclusions about any association or its absence.

Some of the confusion in the literature could be dispersed if TNF-α were proved to play a significant role in the pathogenesis of the disease. An unknown primary cause (fetal–maternal incompatibility? infection?) could lead to increased placental production of TNF; its secretion into the maternal circulation could explain the perception of illness experienced by some pre-eclampsia patients and is consistent with disease-related pathological changes in the liver, kidneys and blood as well as in the placenta itself. The control of TNF-α production is complex and depends on TNF allele polymorphism (existing in linkage disequilibrium with HLA) and on HLA-DR polymorphism (where HLA-DR acts as an immune response modifier). It is conceivable that the same HLA-DR specificity could even be associated with opposite tendencies, promoting TNF production as an immune response to a stimulus, while depressing TNF production as part of a low responder extended haplotype. If indeed the same HLA-DR specificity in different individuals, depending on the rest of the haplotype, were associated with a different TNF response to the same stimulus, this would certainly confound the usual kind of HLA association study. For example, HLA-DR7 in general is not associated with pre-eclampsia and may tend to confer a below average TNF-α response to lipopolysaccharide, but, as discussed earlier, the minority of DR7-positive individuals with certain (B44-DR7)-containing haplotypes may be at increased risk of the disease.

At present it seems that TNF production is increased in at least some cases of pre-eclampsia, and it is a plausible suggestion that circulating TNF contributes to the maternal features in conjunction with other placental products. The influence of HLA, presumably only a secondary component of genetic susceptibility, would be related to TNF production. Further investigations on the role of TNF and its possible inducers, and further studies on fetal HLA and non-HLA are needed. This author would like to think the infectious agent conjectured to cause pre-eclampsia on the basis of circumstantial and anecdotal evidence (Kilpatrick et al., 1987b) induces TNF-α production in an endotoxin-like manner, which, with enhanced trophoblast deportation, leads to endothelial cell dysfunction in maternal tissues. Such, however, must remain for now as just another speculation about the ‘disease of theories’ (Zweifel, 1916; MacGillivray, 1983).

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