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HLA-DEPENDENT TNF SECRETORY RESPONSE MAY PROVIDE AN IMMUNOGENETIC LINK BETWEEN PRE-ECLAMPSIA AND TYPE 1 DIABETES MELLITUS

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SUMMARY

Tumour necrosis factor (TNF) may be relevant to the pathogenesis of both pre-eclampsia and type 1 diabetes, and there is evidence than human TNFα responses to stimuli are HLA-DR dependent. To test the hypothesis that pre-eclampsia and diabetes may share a common immunogenetic susceptibility, 92 pre-eclampsia patients were compared with 264 general population controls. The relative frequencies of individual HLA-DR antigens in pre-eclamptics were found to correlate with reported relative TNFα responses for those antigens. Moreover, putative high responder HLA-DR1, DR3 and DR4 alleles were significantly ($p<0.001$) more frequent in pre-eclampsia patients (79%) than in controls (59%). This hypothesis could explain the weak association between pre-eclampsia and diabetes and may help resolve the apparently conflicting literature on HLA in pre-eclampsia.

KEY WORDS Tumour necrosis factor HLA-DR Diabetes Pre-eclampsia

INTRODUCTION

Studies on pre-eclampsia have thrown up many conflicting ideas. There is undoubtedly a genetic component, but its nature us unclear (Cooper *et al.*, 1993). Data on HLA relationships have been particularly inconsistent (Cooper *et al.*, 1993). Our own finding of an association between pre-eclampsia and sharing of HLA-DR4 between fetus and mother (Kilpatrick *et al.*, 1990) has never been confirmed or refuted, but others have failed to confirm an association with maternal HLA-DR4 or to implicate linkage disequilibrium between maternal HLA-DR4 and the putative pre-eclampsia susceptibility gene (Wilton *et al.*, 1991; Hayward *et al.*, 1990). Nevertheless, the association with maternal HLA-DR4 is the only HLA association that has been reported from two independent laboratories (Cooper *et al.*, 1993; Simon *et al.*, 1988).

Diabetics are at increased risk of pre-eclampsia (Garner *et al.*, 1990), and conversely, women with pre-eclampsia are more likely than normotensive women to experience type 1 diabetes in later life (Dahlquist and Kallen, 1992). It is well established that HLA-DR4 is positively associated with insulin dependent diabetes mellitus, and it is possible these clinically unrelated diseases share a common immunogenetic susceptibility marker.

A totally separate line of investigation could also link pre-eclampsia with diabetes. The oxidative stress hypothesis attributes a central role to tumour necrosis factor (TNF) in the pathogenesis of pre-eclampsia (Stark, 1993). Human TNF secretion in response to lipopolysaccharide is HLA-Class II dependent (Santamaria *et al.*, 1989). Pociot *et al.* (1993) have investigated the relationship between TNF release and individual HLA-DR

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alleles. HLA-DR1, 3 and 4 individuals were found to be high responders, while HLA-DR2 and 5 individuals were low responders. Pociot *et al.* suggested that TNF plays a role in the pathogenesis of type 1 diabetes and is linked to the HLA associations of that disease. I have therefore re-analysed HLA-DR data for pre-eclampsia patients and controls to test the hypothesis that pre-eclampsia may also be associated with the DR alleles linked to a high TNF response.

METHODS

HLA-DR typing was previously carried out on 92 unrelated women whose pregnancies were complicated by proteinuric pre-eclampsia meeting the criteria for gestational proteinuric hypertension (Kilpatrick *et al.,* 1990). Control data representing the general population were obtained from 132 women and their husbands after normal pregnancies (Jazwinska *et al.,* 1987). Data on TNFα secretion as a function of HLA-DR type was taken from Pociot *et al.* (1993).

Regression analysis was performed by Student's t-test. 2 x 2 analyses were conducted by the x^2 test and Fisher's exact test.

RESULTS

The relative TNFα secretory capacity calculated from Pociot *et al.* (1993) closely resembles the relative frequency of HLA-DR alleles in pre-eclamptic patients (Table 1). The DR 5,6 and 8 antigens have been grouped together because serological typing with the antisera available at the time did not distinguish accurately between those specificities, and all three were associated with a below-average TNFα response. Thus analysed, there was a striking correlation ($r=0.78$) which approached statistical significance ($p<0.07$).

When the HLA data were analysed to compare the proportion of women with pre-eclampsia ($n=92$) with HLA DR1 or 3 or 4 (79%) to the corresponding proportion in the general population (59% of 264 controls), the difference was highly significant ($x^2 = 12.6$; $p<0.001$). Even when all patients and controls possessing HLA-DR4 were excluded from the analysis, the putative high TNFα responders were still significantly over-represented (Table 2).

DISCUSSION

These results clearly confirm an association between pre-eclampsia and the group of HLA-DR specificities found in association with high TNFα responders. This analysis and interpretation relies on the data of Pociot and coworkers, but other groups have also found DR3 to be associated with high, and DR2 to be associated with low, TNFα responders (Bendtzen *et al.,* 1988; Jacob *et al.,* 1990; Peces *et al.,* 1995).

This relationship not only supports a role for TNF in the pathogenesis of pre-eclampsia, but may help explain some of the confusion in the literature of HLA and pre-eclampsia. It is not a single HLA-DR specificity that would be over-represented in the disease, but a group of antigens. which might occur in varying proportions in different series of patients. The putative HLA-DR4 association in particular has been called into question, and therefore the data were analysed separately after the withdrawal of all DR-

Table 1. Pre-eclampsia and TNF response related to HLA-DR.

HLA-DR specificity	Relative frequency in pre-eclampsia*	Relative TNFα response#
1	1.81	1.34
2	0.86	0.86
3	1.21	1.52
4	1.43	1.28
7	0.93	0.94
5/6/8	0.68	0.80

*Calculated as the ratio of the antigen frequency in pre-eclampsia patients to controls.
#Ratio of TNFα secretory capacity to the average value, calculated from data provided by Pociot *et al.* (1993).

Table 2. Putative high TNF responders in pre-eclampsia patients and controls after exclusion of all subjects with HLA-DR4.

	DR1 or DR3 positive (%)	Significance
Pre-eclampsia patients (n=58)	69	–
Female controls (n=51)	48	$p<0.01$
Total (male+female) controls (n=91)	46	$p<0.002$

4 possessing subjects. It is noteworthy that the positive association was still evident after this amendment.

Although there is an unexplained association between pre-eclampsia and type 1 diabetes, most diabetics do not experience pre-eclampsia during pregnancy and only a small proportion of pre-eclampsia patients subsequently develop diabetes. These circumstances suggest that pre-eclampsia and diabetes are distinct diseases with separate causes, yet with some relevant common factor linking the two. A tendency to produce a high level of TNF in response to (presumably different) stimuli could be the common factor.

We found no single DR4-bearing haplotype to be over-represented in pre-eclampsia (Liston and Kilpatrick, 1991) and others have failed to find any linkage between pre-eclampsia and the maternal HLA-DR region (Wilton *et al.*, 1991; Hayworth *et al.*, 1992). If the suggestion made here be true, it is not necessary to invoke HLA-DR as being linked to a disease susceptibility gene. Instead, HLA-DR4 (and other DR_β alleles) would function as immune response genes, modulating the TNFα response to (unspecified) stimulation. Such HLA associations would presumably be in addition to, and independ-

ent of, other disease susceptibility genes. HLA would be part of a more complex genetic susceptibility, which would also include a fetal contribution (Liston and Kilpatrick, 1991). It might be worthwhile for other workers with HLA data in pre-eclampsia to re-examine their HLA-DR data in the light of this analysis.

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