Maternal CD46H*2 and IL1B-511*1 homozygosity in T helper 1-type immunity to trophoblast antigens in recurrent pregnancy loss

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BACKGROUND: Women with recurrent pregnancy loss (RPL) and T-helper (Th)1-type immunity to trophoblast antigens have an increased frequency of the IL1B-511*1 promoter variant. Since CD46 gene products also regulate maternal immune responses including Th1 immunity, we investigated whether CD46 gene polymorphisms are also associated with RPL in women with and without Th1 immunity to trophoblast, and the possibility of a synergistic effect with the IL1B-511*1 promoter variant. METHODS: A case-controlled study was performed to document HindIII site polymorphism in intron 1 of the CD46 gene in 131 women with RPL and 72 fertile controls. Clinical information, Th1-type immune responsiveness to trophoblast in women with RPL history, and IL1B promoter allele types for this cohort were documented in a previous study. RESULTS: The frequency of the CD46H*2 allele and CD46H*2 homozygosity were significantly increased in women with RPL compared with fertile controls (P < 0.028 and P < 0.011). CD46H*2 homozygosity was highly associated with RPL-Th1(+) (32.4 versus 9.7% in fertile controls, P < 0.0045). Logistic regression analysis revealed that women homozygous for both the IL1B-511*1 and CD46H*2 alleles had an extremely high risk of RPL-Th1(+) [exponential coefficients (EC) = 24]. Among women with RPL, homozygosity at both alleles, but not each alone, significantly increased the risk of Th1 immunity to trophoblast antigens (EC = 16), suggesting a possible genetic interaction between these two alleles in the development of Th1 immunity. CONCLUSIONS: The combination of homozygosity for both IL1B-511*1 and CD46H*2 alleles is a high risk factor for RPL-Th1(+).

Key words: CD46/IL1B/Th1 immunity/recurrent pregnancy loss

Introduction

Recurrent pregnancy loss (RPL) is a common complication of early pregnancy affecting ~1 in 300 pregnancies. Its aetiology is known in ~50% of cases (Regan, 1991; Hill, 1998). T helper (Th)1-type immunity to trophoblast has been implicated in this disorder since Th1-type cytokines such as interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) may disrupt a number of normal reproductive processes (Hill et al., 1992; Yui et al., 1994; Raghupathy, 1997). Previous studies have revealed that Th1-type immunity to trophoblast antigens and defective Th2-type cytokine production by decidual T cells are associated with unexplained RPL (Hill et al., 1995; Piccinni et al., 1998; Raghupathy et al., 1999). Decreased IL-1β and increased IL-12 mRNA levels in the decidua were found to be correlated with a bias towards Th1-type immunity in the decidua of women with unexplained RPL (Wang et al., 2001). Increased serum IL-12 levels have also been reported in women experiencing RPL (Wilson et al., 1997). We recently reported an association between polymorphism in the promoter region of the IL-1β gene (IL1B) and RPL with evidence of Th1 immunity to trophoblast (Wang et al., 2002). Homozygosity for the variants IL1B-511*1 and IL1B-31*2 in the IL1B promoter region, which are in almost complete linkage disequilibrium, may confer susceptibility to this disorder. However, it remains unknown whether there are other genetic factor(s) potentially involved in regulation of Th1 immunity in reproduction.

Human CD46, a membrane cofactor protein of the complement system, regulates interleukin-12 (IL-12) production by monocytes and thus influences Th1 immune responsiveness (Karp et al., 1996). CD46 is also involved in the protection of cells from complement-mediated lysis (Liszewski et al., 1991), and in sperm–oocyte interactions (Anderson et al., 1993). The gene for CD46 is polymorphic with a biallelic polymorphism at the restriction enzyme HindIII site in its first intron (Bora
Genetic polymorphism and Th1 immunity in recurrent pregnancy loss

Polymorphisms at the isofoms of CD46 and HindIII restriction fragment length polymorphism in the intron 1 of the CD46 gene (Bora et al., 1991). Genomic and protein analysis has established a correlation between the isoforms of CD46 and HindIII restriction fragment length polymorphism in the intron 1 of the CD46 gene (Bora et al., 1991). A previous study has attempted to link polymorphism of CD46 gene to RPL, but the sample size in that study was too small to define an association between RPL and the allele at the HindIII restriction site in intron 1 of the CD46 gene (Risk et al., 1991). In the present study we used a large cohort of cases with RPL to investigate the potential association between CD46 gene polymorphism and susceptibility to RPL with or without Th1 immunity to trophoblast antigens. We also examined the possibility of combinatorial effects of alleles of the CD46 gene and the IL1B-511 locus of the IL1B promoter region on these parameters.

Materials and methods

We performed a case-controlled study in 131 Caucasian women previously evaluated in the Recurrent Pregnancy Loss Clinic within the Center for Reproductive Medicine at Brigham and Women’s Hospital. All women had a history of three or more first trimester spontaneous abortions without defined aetiology following a thorough clinical evaluation and laboratory testing, including normal parental chromosomes, a normal uterine structural study and endometrial biopsy, and mean 3.95, range 3–7 for Th1(–) groups. Controls included 72 Caucasian women who had a history of at least two prior successful pregnancies with no history of a prior pregnancy loss. Research described in this study was conducted under a human subjects Institutional Review Board.

Polymorphisms of the CD46 and IL1B genes were typed using a PCR–restriction fragment length polymorphism method (Bora et al., 1991; di Giovine et al., 1993). A region (243 bp) containing the HindIII site in intron 1 of the CD46 gene was amplified by PCR for 35 cycles with 5′ primer AGAGACCCCTGTCTCATAAACAAAACAC and 3′ primer CACCTTCTCTAGGTTTCATACCTG. The PCR products were subsequently subjected to digestion with restriction enzyme HindIII and analysed by 3% agarose gel electrophoresis. The sequences at the HindIII locus are AAGCTT for allele 1, and AAGGTG for allele 2. Digestion of PCR products with HindIII yielded two segments (188 bp and 55 bp) for CD46H*1 and a single segment (243 bp) for CD46H*2. The IL1B locus located in the promoter region at the position –511 bp from transcription start site of IL1B gene had been allelotyped for this cohort in a previous study (Wang et al., 2002). The primers for PCR and digestion of PCR products with restriction enzyme AvaI have been described in detail (di Giovine et al., 1993).

Data were analysed statistically by Fisher’s exact test (two tailed) with INStat software (Graphpad, San Diego, CA, USA) and by logistic regression analysis with Statview statistic software (SAS Institute Inc. Cary, NC, USA). In logistic regression analysis, the odds ratio is represented as exponential coefficients. Levels of significance were reported as P value. Alleles at the HindIII site in intron 1 of the CD46 gene and at the IL1B-511 locus in our fertile women group were in Hardy–Weinberg equilibrium.

Results

As shown in Table I, the frequency of allele 2 at the HindIII site in intron 1 of the CD46 gene (CD46H*2) was increased in the women with a history of RPL, compared with fertile controls. Analysis of multiple genotypes together in a 2×3 table showed a difference between the women with RPL and fertile controls (P < 0.02). To further analyse individual genotypes, we used CD46H*1 homozygosity as a reference genotype for comparison since the allele frequency of CD46H*1 was decreased in women with RPL and did not appear to be a predisposing allele. The relative frequency of CD46H*2 homozygotes was also elevated in these women with RPL (Table I).

We asked whether these changes reflected an association between CD46H*2 and Th1 immunity to trophoblast antigens. To answer this, we separated RPL cases into RPL-Th1(+) and RPL-Th1(–) groups and compared these groups with fertile controls for polymorphisms in the CD46H locus. For the analysis of multiple groups and genotypes, we first performed

| Table I. Polymorphism at the HindIII site in intron 1 of the CD46 gene in women with a history of recurrent pregnancy loss (RPL) and fertile controls |
|---------------------------------|-----------------|-----------------|-----------------|
|                              | Fertile          | RPL              | P<sup>b</sup> | OR<sup>c</sup>, 95% CI<sup>d</sup> |
| Allele frequency             | (n = 72)         | (n = 131)        |               |                                      |
| CD46H*1                       | 92 (63.9)        | 137 (52.3)       | 0.028          | 1.6, 1.04–2.50                    |
|                              | 52 (36.1)        | 125 (47.7)       |               |                                      |
| Genotype                      |                  |                  |               |                                      |
| CD46H*1/*1                   | 27 (37.5)        | 42 (32.1)        | 1             |                                      |
|                              | 27 (9.7)         | 36 (27.5)        | 0.011          | 3.2, 1.2–9.99                     |
| CD46H*1/*2                   | 38 (52.7)        | 53 (40.4)        | 0.74           | 0.9, 0.44–1.77                    |

<sup>a</sup>Number of cases.<br><sup>b</sup>P-value from Fisher’s exact test.<br><sup>c</sup>Odds ratio; for genotype, CD46H*1/*1 was chosen as the reference genotype for comparison.<br><sup>d</sup>CI = confidence interval.
Polymorphism at the HindIII sites of the CD46 gene in RPL-Th1(+) and RPL-Th1(−) women

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>Fertile (n = 72)*</th>
<th>RPL-Th1(+) (n = 71)</th>
<th>RPL-Th1(−) (n = 60)</th>
<th>OR, 95% CI</th>
<th>OR, 95% CI</th>
<th>OR, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD46H*1</td>
<td>92 (63.9)</td>
<td>68 (47.9)</td>
<td>69 (57.5)</td>
<td>1.9, 1.1–3.1</td>
<td>1.3, 0.7–2.2</td>
<td>1.4, 0.8–2.4</td>
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<tr>
<td>CD46H*2</td>
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<td>74 (52.1)</td>
<td>51 (42.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fertile (n = 72)*</th>
<th>RPL-Th1(+) (n = 71)</th>
<th>RPL-Th1(−) (n = 60)</th>
<th>OR, 95% CI</th>
<th>OR, 95% CI</th>
<th>OR, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD46H*1/*2</td>
<td>27 (37.5)</td>
<td>22 (36.7)</td>
<td>13 (21.6)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CD46H*2/*2</td>
<td>7 (9.7)</td>
<td>23 (32.4)</td>
<td>25 (41.7)</td>
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<td>0.8, 0.3–1.8</td>
<td>0.8, 0.3–1.9</td>
</tr>
<tr>
<td>CD46H*1/*1</td>
<td>38 (52.7)</td>
<td>28 (39.4)</td>
<td>12 (20)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Case number.

a significant association was only found with CD46H*2 homozygosity (CD46H*2/CD46H*2) were found only in the RPL-Th1(+) group when compared with fertile controls (Table II). The significant association was only found with CD46H*2 homozygosity, not with its heterozygosity (i.e. CD46H*1/CD46H*2). In contrast, the frequency of the CD46H*2 allele and its homozygosity was not significantly different between the RPL-Th1(−) group and the fertile control or between RPL-Th1(+) versus (−) groups. These results suggest an association between maternal CD46H*2 homozygosity and susceptibility to RPL-Th1(+).

As an association between IL1B-511*1 and RPL-Th1(+) was found in this cohort in a previous study (Wang et al., 2002), we investigated the possibility of an interaction between CD46H*2 and IL1B-511*1 alleles in the susceptibility to RPL and Th1 immunity to trophoblast antigens. To test this, we stratified the data into nine variables with different genotype combinations of the two loci, and first performed a multiple comparison for all the genotypic combination variables of the two loci between groups in a 2×9 table to test whether there was any difference. We found significant differences between RPL-Th1(+) and fertile controls (P = 0.0198) and RPL-Th1(−) (P = 0.028), but no difference between RPL-Th1(−) and fertile controls (P = 0.12). We then performed a logistic regression analysis to determine which genotypic combination of the two loci is most significantly associated with the disorder. Assuming that alleles IL1B-511*1 and CD46H*2 were risk factors for RPL associated with Th1 immunity to trophoblast, we used the genotype without IL1B-511*1 and CD46H*2 alleles as reference for a baseline. As shown in Table III, women homozygous for both predisposing alleles IL1B-511*1 and CD46H*2 had the highest risk for RPL-Th1(+) when comparing the RPL-Th1(+) group with fertile controls (EC = 24; Table III). This effect was only observed in the RPL-Th1(+) group; no difference was found between the RPL-Th1(−) group and fertile controls.

Although the risk of Th1(+) immunity in RPL patients was significantly increased in women with either IL1B-511*1 or CD46H*2 homozygosity (P = 0.023 and P = 0.037 respectively), there was a much stronger association between the presence of homozygosity for both of these alleles and the susceptibility to RPL-Th1(+) (P = 0.0015). Furthermore, among women with RPL, a significant increase in the risk of development of Th1 immunity to trophoblast was found only in those women homozygous for both IL1B-511*1 and CD46H*2 alleles (EC = 16, P = 0.015; Table III). These results suggest that homozygosity for both CD46H*2 and IL1B-511*1 alleles produces conditions highly favourable for the induction of Th1 immunity to trophoblast in women with RPL.

We next examined whether homozygosity for both IL1B-511*1 and CD46H*2 could be used to predict Th1 immunity to trophoblast in women with RPL. In our RPL cohort, 16 out of 18 women with this genotype were Th1(+) (i.e. true positive,
recent study demonstrated that the interleukin-1 receptor levels in RPL women (Wang et al., 1999) were low \([TP/(TP + FN) \times 100 = 22.5\%]\) for Th1 immunity to trophoblast antigens in women with RPL. But its sensitivity and negative predictive value were low \([TP/(TP + FN) \times 100 = 22.5\%\) and \(TN/(TN + FN) \times 100 = 51.3\%\) respectively]. The combination of homozygosity at the \(IL1B-511^*1\) and \(CD46H^*2\) alleles was highly predictive and specific for RPL-Th1(+) but was only found in 22.5% of the cases with RPL-Th1(+)..

Discussion

Our study demonstrates an association between the \(CD46H^*2\) allele and recurrent pregnancy loss. This observation is consistent with data in a previous report showing a slightly increased frequency of a \(CD46\) allele, now referred to \(CD46H^*2\), identified by genomic Southern hybridization in RPL women (Risk et al., 1991). In that study the difference did not reach statistical significance due to the small number of cases. Our study, which included 131 women with RPL, has confirmed and expanded upon this early observation. We found that this allele is associated only with RPL-Th1(+), not RPL-Th1(-). Furthermore, we also found that homozygosity, but not heterozygosity, of \(CD46H^*2\) was associated with RPL-Th1(+). This type of association indicates that a recessive trait affects this disorder.

In a previous report (Wang et al., 2002) we described a similar association between the \(IL1B\) promoter variant \(IL1B-511^*1\) and RPL-Th1(+). Another study of \(IL1B\) promoter variants in RPL did not find a relationship between \(IL1B\) polymorphisms and RPL (Reid et al., 2001), but the effect may have been masked because the patients were not separated into Th1(+) and Th1(-) groups. Since both \(CD46H^*2\) and \(IL1B-511^*1\) alleles were detected at an increased frequency in the RPL-Th1(+) group, we next investigated the potential of a synergistic effect of these two genotypes. When we analysed the genotypes of \(CD46H^*2\) and \(ILI-511\) loci together, the strongest association with RPL-Th1(+) was found when there was homozygosity for both \(CD46H^*2\) and \(IL1B-511^*1\) alleles. These results indicate a potential genetic interaction between these two alleles in the induction of Th1 immtnity to trophoblast antigens in women with RPL. This finding warrants further clinical studies in large RPL cohorts to confirm this association, and studies to define the biological effects of these genotypes in RPL.

Because homozygosity of the \(CD46H^*2\) and \(IL1B-511^*1\) alleles is associated with RPL-Th1(+), it is possible that recessive traits associated with low function (either lower expression or activity) underlies their effect. Prior research on these two alleles provides information on immunological processes that could predispose women to Th1 immunity to trophoblast antigens. The \(IL1B-511^*1\) allele has been associated with lower production of IL-1β (Santtila et al., 1998; Wilkinson et al., 1999), and there are decreased decidual IL-1β mRNA levels in RPL women (Wang et al., 2001). Furthermore, a recent study demonstrated that the interleukin-1 receptor antagonist allele 1 was associated with lower concentrations of IL-1β in mid-trimester fetal amniotic fluid from women with a history of RPL (Perni et al., 2004). This allele is in linkage disequilibrium with the \(IL1B31*1\) and \(IL1B-511^*1\) alleles (El-Omar et al., 2000). IL-1 is a co-stimulator for Th2 cell generation (Manetti et al., 1994; Huber et al., 1996; Oriss et al., 1997), suggesting that low levels of IL-1 at the fetal–maternal interface could predispose women to a Th1 immune response to trophoblast antigens. \(CD46\) gene products down-regulate Th1 immune responses via inhibition of IL-12 production, a key inducer of Th1 immune responses (Karp et al., 1996). Whereas it is not yet known whether the \(CD46H^*2\) allele has a lower expression rate or whether its associated isoform has lower biological activity, it can be predicted that decreased CD46 production or function would favour a Th1 immune response. If the \(CD46H^*2\) allele is associated with lack of suppression of Th1 immunity, and the \(IL1B-511^*1\) allele is associated with decreased Th2 activity, these two effects could very well synergize to produce Th1 immunity to trophoblast in women with RPL.

The combination of homozygosity of both \(IL1B-511^*1\) and \(CD46H^*2\) alleles has high specificity and a relatively high predictive value for Th1 immunity women with RPL. However, this combination has low sensitivity and negative predictive value because only a proportion of RPL-Th1(+) women carry this risk factor. This suggests that RPL-Th1(+) has a heterogeneous aetiology. Homozygosity at both the \(CD46H^*2\) and \(IL1B511^*1\) alleles was associated with Th1 immunity to trophoblast in only 22.5% of RPL-Th1(+) cases, and therefore does not provide a good candidate marker for a screening test for Th1 immunity in RPL women. However, these markers may prove useful for the clinical management and use of immunomodulation therapy for a subset of RPL women with defined predisposing factors underlying Th1 immunity to trophoblast.

Other cytokine gene variants, such as those of the IL1RN, IL-10 and TGFβ genes, have been associated with susceptibility to RPL (Karhukorpi et al., 2003; Costea et al., 2004). In addition to a potential role in immunoregulation during pregnancy, these cytokines may also have effects on the early placenta and fetus development; for example, IL-1β affects trophoblast proliferation and invasion (Librach et al., 1994; Nikaeen et al., 2000), and IL-10 has effects on fetal development (Rivera et al., 1998; Hanna et al., 2000). Local angiogenesis and tissue remodelling are also regulated by cytokines (for review see Chaouat et al., 2004). Therefore, the Th1/Th2 paradigm may be only part of the role of cytokines in early pregnancy, and cytokine genetic factor(s) could influence the fate of early pregnancy through both immune and non-immune mechanisms.

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References


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