Strong associations between RFLP and protein polymorphisms for CD46


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Abstract. Human CD46 (membrane cofactor protein) is a cell surface glycoprotein with cofactor activity for the factor I mediated cleavage of components C3b and C4b. Using a CD46 cDNA clone, three restriction enzymes give simple two allele restriction fragment length polymorphisms (RFLPs) in samples of over 300 Caucasians. For PvuII, P1 with a 16.5 kilobase (kb) fragment and P2 with 14.8 kb + 1.9 kb fragments have frequencies of .40 and .60. For HincII, H1 with a 4.3 kb fragment and H2 with a 2.3 kb fragment have similar frequencies. For BglII, B1 with a 10 kb fragment and B2 with 8.3 kb + 1.8 kb fragments have frequencies of 0.08 and 0.92. There is strong linkage disequilibrium between these polymorphic sites. Designating haplotypes by HincII, PvuII, BglII alleles, there are two common haplotypes P2, H2, B2 and P1, H1, B2, expected at frequencies of .62 and .32, one less common haplotype P1, H1, B1 expected at a frequency .08. The two major protein isoforms of CD46, as detected on peripheral blood lymphocytes by western blot, of Mr 66 000 (α) and 56 000 (β) are determined by differential splicing in production of the mRNA.

Introduction

Human CD46 [membrane cofactor protein (MCP)] is a cell surface glycoprotein and is a regulator of complement activity (RCA; Seya et al. 1986; Lublin et al. 1988; Purcell et al. 1989). The gene coding for CD46 has been mapped to chromosome 1 at a site which contains several closely linked genes for other proteins involved in complement binding: complement component receptor 1, 1-like, and 2 (CR1, CR1L, CR2), decay accelerating factor (DAF), C4 binding protein α and β (C4BPA, C4BPB) and complement component H (HF; Carroll et al. 1988; Lublin et al. 1988; McKusick 1991). CD46 assists in the regulation of the complement cascade by binding C3b and C4b, and acting as a cofactor for their cleavage by factor I (Holers et al. 1985; Ross and Medof 1985).

CD46 is expressed in all cells except erythrocytes (Seya et al. 1988; 1990; McNearley et al. 1989; Purcell et al. 1989) but variations in M, have been observed in various cell types (Sparrow et al. 1985; Ballard et al. 1987; Purcell et al. 1990) resulting from the differential splicing of the 14 exons producing many different mRNAs (Russell et al. 1991). Variation is found between subjects in the predominance of the CD46 protein isoforms on peripheral blood lymphocytes (PBLs) by using western blots of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) which show three protein patterns (Sparrow et al. 1985; Ballard et al. 1987; Russell et al. 1991). Some subjects have predominantly the M, 66 000 isoform, α, some predominantly the M, 56 000 isoform, β, and others have approximately equal amounts of each. This could result from a simple polymorphism within CD46 with two alleles, each determining a different predominant form of its splicing product as discussed elsewhere (Bora et al. 1991).

CD46 is strongly expressed by the syncytiotrophoblast layer of the placenta where it was originally characterized as the TLX molecule (McIntyre et al. 1983). Polymorphism of TLX has been postulated to be involved in the protection from immune-rejection of the developing fetus during pregnancy (Johnson and Stern 1986; McIntyre 1988). Several studies have sought associations between CD46 variation and pregnancy: in pre-eclampsia (A. N. Wilton, D. W. Cooper, S. P. Brennecke, unpublished...
data) where there was none, and recurrent spontaneous abortion (Risk et al. 1991) in which a significant association occurred.

Using the PBLs from 495 subjects, we have examined the CD46 gene for restriction fragment length polymorphisms (RFLPs) and further examined 30 of these subjects for protein polymorphism. The genetic variation detectable by RFLPs closely correlates with the protein phenotype of CD46 and may have a major influence in determining the mRNA splicing preference of the CD46 gene in a cis fashion.

**Materials and methods**

Using the Triton X-100 method (Kunkel et al. 1977), DNA was isolated from PBLs from 495 individuals, mostly of Caucasian ancestry, including 139 subjects with hemophilia, 97 with human immunodeficiency virus (HIV) infection (Donald et al. 1991), 131 members of ten families in a study on pre-eclampsia (Wilton et al. 1990), 111 subjects used elsewhere as a control group for the pre-eclampsia study (Wilton et al. 1991), and a three generation family of 17. Southern blots were made of 10 μg DNA samples after digestion with specific restriction endonucleases and after electrophoresis on 0.8-1% agarose gels. Blots were hybridized with the cDNA clone of CD46, pm5.1, which is a full length clone of 1.66 kilobases (kb) with exon 7 deleted (Purcell et al. 1991). [32P] was incorporated into the probe using random priming. Filters were hybridized either in plastic boxes with 10 ml of prehybridization solution per filter (Maniatis et al. 1982) with 10% dextran sulphate and 1 x 10^6 cpm/ml, in lots of up to 40 filters, or in rotating glass bottles for 1-5 filters with 10^7 cpm/ml in 10-15 ml. Washes were to a stringency of 2 x standard sodium citrate (SSC) at 65 °C. Filters were exposed to X-ray film at ~70 °C with intensifying screens for at least one week for low activity hybridizations and overnight for high activity hybridizations. For immunoblotting the PBLs from 10 ml blood samples were separated using Lympho-paque (Nygaard, Oslo, Norway) or Ficoll-Paque (Pharmacia, Uppsala, Sweden) and membrane lysates prepared in 200 μl of lysis buffer [5% Nonidet P-40 in 1 mM Tris pH7.4, 0.15 M NaCl, 1 mM ethylenediaminetetraacetate (EDTA), 1 mM phenylmethylsulphonyl fluoride (PMSF)]. Lysates were acid/base dissociated and 30 μg aliquots were separated by SDS-PAGE under nonreducing conditions, blotted onto Immobilon-P membranes (Millipore, Bedford, MA) and remaining protein binding sites were blocked with 2.5% casein. The CD46 isoforms were detected with the anti-CD46 monoclonal antibody (mAb) E4.3, followed with alkaline-phosphatase-conjugated anti-mouse IgG and visualized with 5-bromo-4-chloro-3-indolyl-phosphate and nitro blue tetrazolium chloride. Three different assessors independently determined the protein phenotype, predominance of Mr 66000, predominant of Mr 56000 isoform (>α), predominance of Mr 56000 isoform (>β), and equal quantities of the Mr 66000 and Mr 56000 isoforms (αβ). No discrepancies between these typings occurred. This technique has proved to produce equivalent results to using densitometric measurements and determining the ratio of α and β bands with cut offs at 0.5 and 2.0 separating >β, αβ, and >α.

### Results

**RFLPs.** Eighteen different restriction enzymes, Bam HI, Bgl I, Bgl II, Bst EII, Cfo I, Dra I, Eco RI, Hin dIII, Kpn I, Msp I, Nci I, Nsi I, Pst I, Pvu II, Sac I, Sca I, Taq I, and Xba I, were tested on 15 control subjects and fragment sizes calculated from known standards. Allelic polymorphic fragments were found with three enzymes, HindIII, Pvu II, and Bgl II. There are no recognition sites for these enzymes in the sequences cDNA so the restriction site are probably within the introns or regions flanking the gene. HindIII gives a simple two allele polymorphism in the 5' flanking region with either a 4.3 kb fragment (H1) or a 2.3 kb fragment (H2) among many other constant fragments (Fig. 1a) as previously reported (Bora et al. 1990, 1991). Pvu II gives two alleles, one with a 16.5 kb fragment (P1) and the other with a 14.8 kb fragment and a 1.9 kb fragment (P2; Fig. 1b). Bgl II gives two alleles, one with a 10 kb fragment (B1) and the other with an 8.3 kb fragment and a 1.8 kb fragment (B2; Fig. 1c). No homozygotes for B1 have been observed but the 8.3 kb and 1.8 kb fragments appear less dense in heterozygotes, B1/B2, than in homozygotes, B2/B2. B1 segregated as codominant allele to B2 in all four informative pre-eclampsia families. Contrary to one published report (Risk et al. 1991), we find that there are no polymorphic Eco RI sites (Eco RI gives 9.5, 8, 6.6, 4.5, 4.0, and 2.2 kb fragments) and that there are only two allelic HindIII variants as described previously (Bora et al. 1990, 1991). Extra fragments found by Risk and coworkers (1991) with Eco RI and HindIII could be result of plasmid contamination of DNA samples and probing with insert plus plasmid vector.

For each enzyme the frequencies of the polymorphic fragments were analyzed in members of the hemophilic cohort, HIV infected the acquired immunodeficiency syndrome (AIDS) cohort, and others, and all gave similar results. Combined results are shown in Table 1. There is no departure from expectations for Hardy-Weinberg equilibrium. Pvu II and HindIII are highly polymorphic with gene frequencies of approximately .60 and Bgl II digests are less polymorphic. Note that the Hin dIII gives two alleles, one with a 10 kb fragment (B1) and the other with an 8.3 kb fragment and a 1.8 kb fragment (B2; Fig. 1c). No homozygotes for B1 have been observed but the 8.3 kb and 1.8 kb fragments appear less dense in heterozygotes, B1/B2, than in homozygotes, B2/B2. B1 segregated as codominant allele to B2 in all four informative pre-eclampsia families. Contrary to one published report (Risk et al. 1991), we find that there are no polymorphic Eco RI sites (Eco RI gives 9.5, 8, 6.6, 4.5, 4.0, and 2.2 kb fragments) and that there are only two allelic HindIII variants as described previously (Bora et al. 1990, 1991). Extra fragments found by Risk and coworkers (1991) with Eco RI and HindIII could be result of plasmid contamination of DNA samples and probing with insert plus plasmid vector.

### Table 1. Frequency of RFLPs detected with CD46 probe, pm5.1, in Caucasians and chi square testing goodness of fit to Hardy-Weinberg equilibrium.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Numbers per Genotype*</th>
<th>Total</th>
<th>(p2)</th>
<th>x²</th>
<th>Prob</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
<td>12</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pvu II</td>
<td>52</td>
<td>137</td>
<td>111</td>
<td>300</td>
<td>0.598</td>
</tr>
<tr>
<td>HindIII</td>
<td>36</td>
<td>74</td>
<td>43</td>
<td>153</td>
<td>0.523</td>
</tr>
<tr>
<td>Bgl II</td>
<td>0</td>
<td>33</td>
<td>187</td>
<td>220</td>
<td>0.075</td>
</tr>
</tbody>
</table>

* 11 and 22 are homozygotes for the largest fragment and smallest fragment respectively while 12 is the heterozygotes.

| (p2) is the frequency of the smallest restriction fragment. | Prob, probability |