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CORRESPONDENCE

Refining the DNA Polymorphisms That Associate With the Rhesus c Phenotype

To the Editor:

We have recently reported restriction fragment length polymorphism (RFLP) studies showing a number of DNA polymorphisms that have close, though not perfect, associations with the Rhesus (Rh) C and Rhc antigens.[1] Mouro et al[2] have also reported DNA polymorphisms that associate with the RhC/c antigens. Comparing these results and our RFLP studies with additional sequence data we wish to further refine the Rhc DNA polymorphisms that have complete antigen associations, and therefore, are critical to Rhc antigen expression.

Mouro et al reported six Rh C/c-associated nucleotide substitutions, four of which encode amino acid changes that occur within the first and second exons of the Rh C/c gene locus, as shown in Fig 1A. Particularly important to this study is the C to G substitution at position 48 in exon 1 that encodes a C or W amino acid associated with the Rh C and Rh c phenotypes, respectively. Hydropathy analysis of Rh cDNA sequences predicts this amino acid will lie intracellularly. It has been postulated that this substitution contributes to the Rh C/c antigen specificity by introducing a conformation change rather than by direct involvement.[3,4]

We have recently described an RFLP analysis in which DNA from 102 randomly selected donors were examined using the *Hae*III restriction enzyme and an exon 1 DNA probe.[1] The *Hae*III enzyme cuts the Rh c gene, but not the Rh C gene at position 48 because of the G to C base substitution. Among the donors with Rh c phenotypes, all but six Rh c genes were cut as expected with *Hae*III at the exon 1 DNA polymorphism. Sequence data obtained from one of these six discrepant DNA (ccee) confirmed that the exon 1 sequence at position 48 was a cytosine residue, previously associated with the Rh C rather than the observed Rh c phenotype (Fig 1B). Further sequencing data of exon 2 from five of the six aberrant DNA types showed the expected Rh polymorphisms associated with the Rh c phenotype.

We have also examined DNA from these 102 random donors using the restriction enzyme *Msp* I, which results in a highly associated Rh C/c polymorphism located within the intron following exon 1.[1] This *Msp* I analysis correctly predicted all Rh C and Rh c phenotypes except for the same six discrepant DNA types that were again incorrectly predicted to have an Rh C phenotype where an Rh c phenotype was present (Fig 1B).

Therefore, analysis of these individuals has shown six Rh c phenotypes whose genomic DNA sequence within both exon 1 and intron 1 is identical to the Rh C phenotype, although exon 2 sequence data and serologic testing indicates an Rh c phenotype to be present. Because we have observed these six individuals who do not have the previously described exon 1 polymorphism associated with Rh c, we conclude this G nucleotide at position 48 is not exclusively indicative of Rh c phenotypes. Therefore, because the W amino acid at position 16 is not always present for Rh c phenotypes, we can also conclude it is not critical for expression of the Rh c antigen.

Therefore, these data indicate that the C to W amino acid substitution does not contribute either directly or indirectly through conformational effects to the Rh c antigen. Apparently, the Rh c specificity is entirely coded by polymorphisms contained within exon 2. Further sequence data from larger numbers of samples or development of a Rh cDNA expression/transfection system are required before the importance of each of the exon 2 amino acid substitutions can be determined.

The mechanisms responsible for these six aberrant DNA types are unknown, although interallelic recombination between the Rh C and Rh c genes would account for both the polymorphisms in exon 1 and intron 1 corresponding to the Rh C gene, whereas the remaining polymorphisms correspond to the Rh c gene. Sequence analysis of the intron following exon 1 may show these processes thereby providing more details of the events that have made Rhesus one of the most polymorphic blood group systems known.

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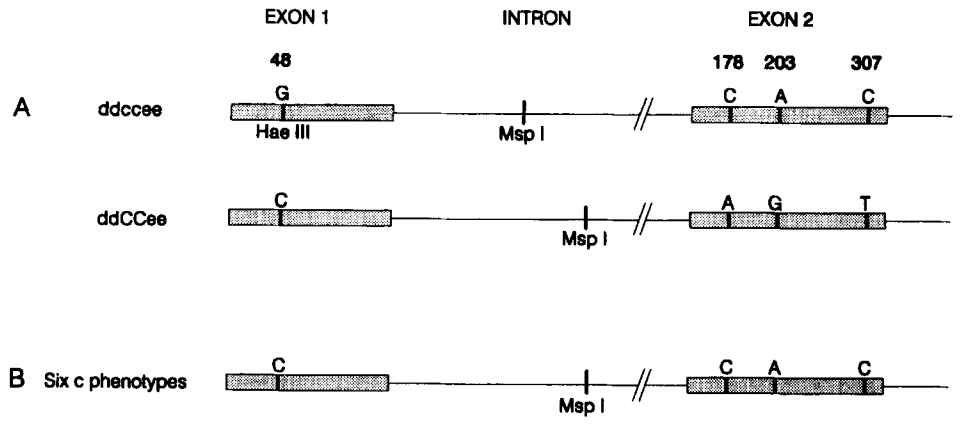

Fig 1. Exon 1 to exon 2 schematic of the Rh C/c genomic DNA sequence showing, in A, the polymorphisms previously associated with the Rhesus c and C phenotypes, and B, the observed polymorphisms within six random blood donors with Rh c phenotypes.

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