*RHCE*ceAR* encodes a partial c (RH4) antigen

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The Rh blood group system is highly complex both in the number of discrete antigens and in the existence of partial antigens, especially D and e. Recently, several partial c antigens have been reported. Here we report findings on an African American man with sickle cell disease whose RBCs typed C+c+ and whose plasma contained anti-c. Hemagglutination tests, DNA extraction, PCR-RFLP, reticulocyte RNA isolation, RT-PCR cDNA analyses, cloning, and sequencing were performed by standard procedures. RBCs from the patient typed C+c+ but his plasma contained alloanti-c. DNA analyses showed the presence of *RHCE*Ce* in *trans* to *RHCE*ceAR* with *RHD*D* and *RHD*Weak D Type 4.2.2*. The amino acid changes on RhceAR are such that a C+c+ patient made alloanti-c. This case shows that RhceAR carries a partial c antigen and illustrates the value of DNA testing as an adjunct to hemagglutination to aid in antibody identification in unusual cases. ***Immunohematology* 2010;26:57–59.**

Key Words: blood groups, Rh blood group system, blood transfusion, partial antigen

The Rh blood group system is the most complex of the 30 human blood group systems.[1,2] This is attributable not only to the 50 discrete antigens[3] but also to the fact that some of the antigens, notably D, C, and e, have numerous altered forms, the so-called partial antigens.[4] Partial c antigens have also been described. The first example of alloanti-c in a c+ (presumed phenotype R_1r) person was reported in 1982.[5] Anti-Rh26, which can appear as anti-c, has been made by an Rh26–, c– person[6] and also by an RH26–, c+ person.[7] Molecular studies revealed that Rh26 is antithetical to the low-prevalence antigen LOCR, and serologic studies have shown that the LOCR+ phenotype encodes altered (weakened) expression of c.[8] Recently, it has been shown that *RHCE*ce^S(340)* and *RHCE*(C)ce^S* each encode a partial c antigen.[9,10] Each of these alleles encodes a different haplotype, and the alloanti-c may not be of identical specificity.

One of a growing number of *RHCE*ce* alleles that encode an Rhce protein with an altered c antigen is *RHCE*ceAR*. *RHCE*ceAR* has six nucleotide changes (48G>C, 712A>G, 733C>G, 787A>G, 800T>A, and 916A>G), which predict the amino acid changes of Trp16Cys, Met238Val, Leu245Val, Arg263Gly, Met267Lys, and Ile306Val, respectively.[11] The *RHCE*ceAR* allele encodes an altered e, a weak V, but no Rh18 or hr^S.[12] In this article, we describe serologic and DNA testing on blood from a C+c+ African American patient whose plasma contains alloanti-c, thereby revealing that *RHCE*ceAR* encodes a partial c antigen. We published this finding in an abstract,[13] and while this manuscript was in preparation, a report by Peyrard and coworkers[14] appeared and thus there are two such cases.

Materials and Methods

Blood samples from a 17-year-old multi-transfused African American man with sickle cell disease were analyzed.

Hemagglutination

Reagents were from our libraries and obtained from numerous colleagues and commercial sources. Hemagglutination was performed in test tubes using the method best suited to the antibody being tested. Eluates were prepared using Gamma Elu-Kit II (Gamma/Immucor, Norcross, GA).

DNA and RNA Isolation, RT-PCR, Sequencing, and Cloning

Genomic DNA was prepared from 200 µL of the buffy coat layer of peripheral blood using a DNA extraction kit (QIAamp DNA Blood Mini Kit, Qiagen, Valencia, CA). RNA was isolated from the reticulocytes (TriZol and PureLink Micro-to-Midi Total RNA Purification System, Invitrogen, Carlsbad, CA). Reverse transcription was carried out with gene-specific *RHD* and *RHCE* primers listed in Table 1 and Superscript III, according to manufacturer's instructions (Supercript III First Strand Synthesis SuperMix, Invitrogen). PCR amplification was carried out with primers cRHx1F and cRHx5R to amplify exons 1–4; and cRHx4F and cRHx10R to amplify exons 5–10 on *RHD* (RefSeq accession NM_016124) and *RHCE* (RefSeq accession NM_020485) cDNA using HotStarTaq Master Mix Kit (Qiagen). PCR amplicons were checked for purity on agarose gels, cleaned using ExoSAP-IT (USB Corporation, Cleveland, OH) according to manufacturer's instructions and directly sequenced by GeneWiz Inc. (South Plainfield, NJ). Cloning reactions were carried out and sequenced by GeneWiz Inc. Sequences were aligned, and protein sequence comparisons were performed using Sequencher v4.8 (GeneCodes, Ann Arbor, MI). The complete sequences of *RHCE* and *RHD* were analyzed using gene-specific cDNA direct sequencing, and to verify the results and determine which alleles carry which alterations, all RT-PCR products of *RHCE* and *RHD* were also cloned and sequenced. The sequence of *RHD* exon 7 was determined by amplifying and sequencing exon 7 individually using genomic DNA as described previously. To verify the *RHCE*ceAR* nucleotide change 48G>C that does not always sequence well owing to its early position in exon 1, PCR-RFLP was conducted on genomic DNA.

Table 1. Sequence and location of primers

Primer name	Primer sequence (5′–3′)	Location
cDx10R	gtattctacagtgcataataaatggtg	Exon 10
cCEx10R	ctgtctctgaccttgtttcattatac	Exon 10
cRHx1F	agctctaagtacccgcggtctgtcc	Exon 1
cRHx5R	tggccagaacatccacaagaagag	Exon 5
cRHx4F	acgatacccagtttgtctgccatg	Exon 4
cRHx10R	tgaacaggccttgtttttcttggatgc	Exon 10

Results

Hemagglutination

The patient's RBCs typed as group B, D+C+E–c+e+, and his serum contained anti-c. His RBCs were agglutinated by 12 commercial anti-c reagents (reagents included monoclonal and polyclonal antibodies) to the same strength as control RBCs expressing a single dose of c antigen. As this was a surprising result given that he had made anti-c, we performed DNA analyses.

RH cDNA Sequence Analysis

Collectively, the results of DNA analyses showed the presence of the following genes: *RHD*D, RHD*Weak D Type 4.2.2, RHCE*Ce*, and *RHCE*ceAR*. The weak D Type 4.2.2 allele is the same as the *RHD*DAR* allele, except it harbors a 744C>T and a 957G>A nucleotide change.[15] It is likely that the two haplotypes in this patient are *RHD*D/RHCE*Ce* and *RHD*Weak D Type 4.2.2/RHCE*ceAR*.

Discussion

We report findings on an African American with sickle cell disease who had been transfused on numerous occasions. His RBCs typed C+c+, and his serum contained anti-c reactive by the IAT. This study reveals that the amino acid changes on RhceAR (Trp16Cys, Met238Val, Leu245Val, Arg263Gly, Met267Lys, and Ile306Val) are such that a C+c+ patient can make alloanti-c. Thus, ceAR carries a partial c antigen. The strong reactivity of anti-c reagents with the patient's RBCs that express Ce/ceAR and the absence of a known low-prevalence antigen on RBCs expressing ceAR preclude detection of the altered c antigen associated with ceAR.

This case shows the value of DNA testing as an adjunct to hemagglutination to aid in antibody identification in unusual cases. After we published our findings in a preliminary form[13] and while this manuscript was in preparation, Peyrard and coworkers reported a case of anti-c in a person with the C+/ceAR phenotype.[14] Thus, there are two such published cases. The clinical relevance of the alloanti-c in this latter case is unknown, because after it was identified, the patient received c– RBC components. Interestingly, to date, the majority of partial c antigens have been in persons of African or Hispanic ancestry.

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