Alteration of RH Gene Structure and Expression in Human dCCee and DC\textsuperscript{w}— Red Blood Cells: Phenotypic Homozygosity Versus Genotypic Heterozygosity

By Cheng-Han Huang

This report describes a comparative study on the dCCee and DC\textsuperscript{w}— red blood cells devoid of RhD and CeE antigens, respectively. Southern blots showed that the two variants carried opposite deletions in the D and non-D (CeEe) genes. Rh haplotyping and exon polymerase chain reaction (PCR) assay indicated that the deletions did not extend beyond the 5' region upstream from exon 1 or the 3' region downstream from exon 10 of the respective genes. This was confirmed by fixing intact promoters and 3' untranslated regions in both D and non-D genes in each variant. Reverse transcriptase-PCR and cDNA sequencing showed the expression of two transcripts in each cell type. In dCCee cells, one transcript was the regular Ce form and the other occurred as a D-Ce-D hybrid whose Ce sequence spanned exons 2 through 9. In DC\textsuperscript{w}— cells, the two transcripts were derived from reversely arranged hybrid genes, i.e., the Ce\textsuperscript{wD} gene was formed by fusion of Ce\textsuperscript{w} exon 1 with D exons 2 through 10, whereas the reverse product was formed by fusion of D exons 1 through 9 with non-D exon 10. These results indicated that DNA deletion and recombination had occurred in either cis or trans configuration and involved both RH loci in the dCCee or DC\textsuperscript{w}— genome. Identification of such compound alterations correlates the genotypes with phenotypes and explains the lost Rh antigenic expression. A reinvestigation of gene organization also led to the reassignment of several 5' and 3' splice sites. Together, this study not only shows the complexity of Rh phenotypic diversity, but also points to the importance of concurrent analysis of genomic structure and transcript expression in deciphering the underlying genetic mechanisms.

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The Red Blood Cell (RBC) Rh antigens constitute a clinically important blood group system mainly because of their involvement in hemolytic reactions as extremely potent immunogens.\textsuperscript{1} Besides the major D, C/c, and E/e antigens, a large array of qualitative and/or quantitative Rh polymorphisms are encountered in the human population.\textsuperscript{2} These surface-active markers are carried on Rh polypeptides, a family of transmembrane proteins that possess two unusual defining features, nonglycosylation and palmitylation.\textsuperscript{3,4} The Rh polypeptides appear to play important roles in RBC membrane structure and physiologic processes, as highlighted by the occurrence of chronic but moderate hemolytic anemia and stomatocytosis in patients with Rh deficiency syndrome.\textsuperscript{5}

Since the cloning of two Rh cDNAs, much information has been obtained about their primary structure, erythroid expression, and complex interaction with other membrane components.\textsuperscript{6,10} Whereas biochemical analyses showed that the D, C/c, and E/e antigens reside in at least three distinct polypeptides, molecular studies indicated that only two structural genes, D and non-D (including ce, Ce, cE, and CE alleles), occur in the human genome. Nevertheless, it has remained unclear whether C/c is coexpressed with or derived from the E/e allelic series by differential RNA splicing.\textsuperscript{11} It is also yet to be established whether the Rh protein(s) alone would be sufficient or whether additional factor(s) would be required for the antigenic presentation on the RBC membrane.

Despite these uncertainties, studies on a number of Rh phenotypes at the molecular level have provided insight into the underlying genetic mechanisms. Analysis of the D category variants indicated that they have taken place via homologous recombinations between the D and non-D genes.\textsuperscript{12,13} However, the molecular basis for those variants lacking some of the major Rh antigens appears to be more heterogeneous. For instance, the absence of D antigen from RBCs, a status referred to as Rh-negative, may or may not be associated with D locus deletion.\textsuperscript{14,15} In addition, whereas the D— variants occurred on the background of partial deletion or nondeletion of the non-D locus,\textsuperscript{16,21} the Dc-and DC\textsuperscript{w}— phenotypes were proposed to result from segmental gene conversion events.\textsuperscript{21} I describe here the structure and expression of Rh polypeptide genes in two unrelated individuals exhibiting the dCCee and DC\textsuperscript{w}— phenotypes. The results show that the dCCee and DC\textsuperscript{w}— variants each carry two differently altered Rh haplotypes in their genome. These detailed studies have also led to the reassignment of two exon-exon junctions misidentified in previous studies.\textsuperscript{22}

Materials and Methods

Blood samples. Peripheral whole blood samples were obtained from normal donors or variant individuals and their Rh blood group phenotypes were determined by standard serologic methods.\textsuperscript{18} The DCCee blood was drawn from a known Dc/DcE homozygote. The Rh-negative blood samples were from a white patient (genotype, dcE/dcE) and an Asian patient (genotype, deE/dcE). The dCCee blood was from a black patient (presumed genotype, De/cDeCe). The DC\textsuperscript{w}— sample was from a white patient (presumed genotype, DC\textsuperscript{w}—/DC\textsuperscript{w}—) whose RBCs carried D and C\textsuperscript{w}— antigens, but not the C/c and E/e antigens. Phenotypically, the expression of C\textsuperscript{w}— antigen, which is considered an allelic variant of C,\textsuperscript{7} was slightly depressed, whereas that of the D antigen was elevated.

Preparation of genomic DNA and total RNA. High molecular weight chromosomal DNA was isolated from leukocytes pelleted inuffy coat, as previously described.\textsuperscript{23} Total RNA was isolated from hemolysates by preferential lysis of erythroid cells followed by acetic acid titration.\textsuperscript{24} The RNA precipitate was then solubilized and ex-
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Table 1. RH Gene Synthetic Primers

<table>
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<tr>
<th>Category</th>
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<th>X-mer</th>
<th>Location</th>
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<tr>
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<td>r</td>
<td>CTGCTCTGATCCGTGTGTGATTAC</td>
<td>24</td>
<td>Non-D 3'-UT</td>
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</table>

* The adenyl nucleotide of initiation codon ATG is numbered position 1.

Fig. 1. Analysis of dCcee and dCW- genomic DNAs by Southern blot hybridization. Genomic DNAs from dCcee (Rh-positive), dCcee (Rh-negative), and dCW- individuals (lanes 1 through 3) were digested with restriction endonucleases BamHI, HindIII, and PstI. (A) Southern blots hybridized with the Rh cDNA (5') probe encompassing exons 1 through 4. Note that the exon 4 portion of this probe contains only 52 bp. The size markers of HindIII-cleaved λ DNA are shown at the left margin. (B) Southern blots hybridized with the Rh cDNA (3') probe encompassing exons 4 through 9. The RH bands that display an opposite pattern of deletion between dCcee and dCW- individuals are indicated by arrows at the right margin.

The nucleic acid probes used were described previously. The Rh cDNA probes included the 5' and 3' coding sequences that encompass exons 1 through 4 (539 bp) and exons 4 through 9 (710 bp), respectively. The regional probes specific for the individual exons were selectively amplified from Rh cDNAs and then purified by native PAGE. Parameters for the exonic probes, including their size, nucleotide (nt) position, and primer location, were as detailed in our previous studies.

Synthetic oligonucleotide primers used in genomic amplification and cDNA synthesis are listed in Table 1. They were synthesized on a 380A automated DNA synthesizer (Applied Biosystems, Foster City, CA) and purified by denaturing 15% PAGE.

Amplification and restriction analyses of genomic sequences. Rh genomic sequences were amplified by the polymerase chain reaction (PCR) using as templates total genomic DNA or electroeluted restriction fragments, as previously described. Amplification was run for 30 cycles and at a volume of 50 µL, using AmpliTaq polymerase (Perkin Elmer, Norwalk, CT) and gene-specific primers (Table 1). Cycling on a Power Block System (Erieomp, San Diego, CA) was programmed as follows: denaturation at 94°C for 60 seconds, primer annealing at 55°C for 45 seconds, and chain extension at 72°C for 30 to 60 seconds, depending on the length of fragments to be amplified. The products were digested with restriction enzymes and analyzed by agarose gel electrophoresis.

Synthesis and amplification of Rh cDNAs. Rh polypeptide mRNAs were converted to single-stranded cDNAs by the avian myeloblastosis virus (AMV) reverse transcriptase (RT; Promega, Madison, WI) and then amplified by PCR, as previously described. Total RNA (2 to 3 µg), together with 25 to 50 ng of 3'-UT primer (Table 1) specific to the D or non-D gene, was incubated at 65°C for 5 minutes and on ice for 5 minutes. The total volume was brought to 20 µL with 15 µL of RT, 1× cDNA synthesis buffer, 20 U of RNase.

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Fig 3. Amplification and sequence analysis of the 5' promoter regions of RH genes. (A) Specific amplification of D and non-D (ie, C, c, and C') 5' sequences with primers p and b (Table 1). The large and small HindIII fragments were identified by Southern blots, eluted, and used as PCR templates. Note that, although DCw showed reduced intensity in both bands, dCw displayed reduced intensity in the large one only. (B) Comparison of the promoter and exon 1 sequences amplified from the D and Ce genes, except the comigrating bands containing exon 3 or exon 8 of both genes, is indicated at the right margin. Note that, in comparison with DCw, only one set of SpH bands containing exons 2, 4, 5, 6, 7, and 9 is seen on either dCw or DCw' bands. Also of note, the exon-1- and exon-10-containing fragments of both D and Ce are present in the two variants, despite an apparent reduction in band intensity (see also Fig 3A).
inhibitor, and water. The solution was incubated at 42°C for 75 minutes to allow cDNA synthesis and then at 72°C for 10 minutes to inactivate the enzyme. From this cDNA pool, 5 µL was taken and added to 45 µL of premade PCR mixture containing 1× reaction buffer, 2.5 U of AmpliTaq, and 250 ng each of the forward and reverse primers that were internal to the respective 3'UT primers (Table 1). The cDNA product was amplified for 35 cycles using similar conditions mentioned above and analyzed by electrophoresis and restriction digestion.

Direct nucleotide sequence determination. Amplified Rh cDNA and genomic DNA products were directly sequenced on both strands by automated procedure. Before sequence determination, the DNA templates were purified by native PAGE and eluted in 1× TE buffer. PCR cycle sequencing with fluorescent tags as chain terminators was performed on an automated 373A DNA sequencer (Applied Biosystems).

RESULTS

Southern blot analysis of dCCee and DCw- genomic DNAs. To determine whether the RH loci specifying the dCCee and DCw- phenotypes were associated with structural alteration, genomic DNAs were digested with restriction enzymes and analyzed by Southern blotting (Fig 1). Compared with DCCee control, the dCCee and DCw- variants both displayed reduced band intensity and gross DNA deletion. The dosage reduction was apparently involved in S' exons (exons 1 through 3, Fig 1A), whereas the deletion had enclosed a relatively large stretch of genomic sequence encompassing exons 4 through 9 (Fig 1B). Moreover, the latter alteration appeared to be opposite between the dCCee and DCw- variants with respect to the distribution of various RH gene fragments. This mutually exclusive pattern suggested that the RH locus of the dCCee variant contained a D gene deletion, whereas that of DCw- harbored a Ce or CE gene deletion on both homologous chromosomes.

Rh haplotyping by Sph I digestion and exon mapping. To further delineate the deletion, Rh haplotype analysis with the Sph I enzyme was performed. This procedure can discriminate the origin and haploid combination of D and non-D genes because Sph I is diagnostic of all fragments except those containing exons 3 and 8,19 When probed with Ex1, Ex3, and Ex10, no change in the banding pattern, except reduction of band intensity, was noted in the two variants (Fig 2, panels I, III, and VIII). However, the pattern of other blots differed, namely, those bands that were seen in dCCee were missing in DCw- and vice versa (Fig 2, panels II and IV-VII). Accordingly, the D exons 2, 4, 5, 6, 7, and 9 were absent in dCCee, whereas the Ce exons 2, 4, 5, 6, 7, and 9 were absent in DCw- DNA. Taken together, these results indicated that the deletion of the respective RH gene was either partial or internal, not extending beyond the S' region upstream of exon 1 or the 3' region downstream of exon 10.

Nucleotide sequence analysis of the S' regions of RH genes. By taking advantage of the presence of S' regions in separate HindIII fragments, the promoter and exon 1 of both D and non-D genes were selectively amplified and directly sequenced (Fig 3). Concerning the intensity, dCCee showed a decrease in the D gene band only, whereas DCw- displayed an apparent reduction in both D and non-D fragments (Fig 3A). With regard to the S' regions pertaining to DCw-, only six nucleotide differences were noted between the two sequences shown (Fig 3B), and none of them was involved in the cis-regulatory elements described for the non-D gene.22 Of the exonic changes (Fig 3B), the G-to-C transition (nt48, Tyr16Cys) distinguished the status of D from C,13 whereas the A-to-G transition (nt122, Gln40Arg) was associated with C and was identical to the point change shown to correlate the Cw specificity.21 The D and Ce 5' sequences of dCCee contained, respectively, G and C at nt48, but lacked the nt122G substitution (data not shown). These results showed that the structure of both D and C or Cw promoters remains intact, thus confirming the nature of partial deletion in the two variants.

Amplification and analyses of exonic and intronic sequences. To locate the deletion endpoints and exclude possible exonic rearrangements, exons 1, 2, 4, 5, and 9 were elected for restriction analysis (Fig 4) and exon 3 for nucleotide sequencing (gels not shown). As shown in Fig 4, two types of exon 1 (Apa I panel) were present in both variants, but only DCw- contained the Taq I site in one of its RH genes (Taq I panel), thus confirming the occurrence of the
The cleavage pattern of exon 4 and 5 (Fig 4) indicated that dCCee and DCW− should differ in the size of their intron 4 sequences. Amplification indeed produced two bands in DCCee, but only one in the dCCee or DCW− variant (Fig 5A, left). Hybridization with Alu probe showed that the Ce intron 4 harbored, whereas the D intron 4 lacked, the Alu repeat sequence (Fig 5A, right). By nucleotide sequencing, the exon 4/5 boundary of Ce gene was shown to be determined by nt634/635 (Fig 5B and C) rather than by nt635/636. Sequence analyses of D, ce, cE, Ce, and CE genes from 15 unrelated individuals showed the same nt 634/635 assignment and showed further that the exon 6/7 boundary is defined by nt9401941 (gels not shown) but not by nt9391940. Such differences in exon/intron assignment were most likely due to G, C band compressions, because the relevant regions are GC-rich (Fig 5B). Complete analysis of the two intron 4 sequences also led to the localization of breakpoints for the insertion event. As shown in Fig 5C, the D intron 4 is 426 bp in size, whereas the Ce intron 4 contains an extra sequence (including the Alu repeat) that was inserted between nt179 and 180 downstream of the donor splice site. Analyses of Rh transcripts expressed in dCCee and DCW− erythroid cells. To delineate the pattern of gene expression, the composition and structure of Rh transcripts were characterized. The cDNAs were synthesized separately with D and non-D specific primers and then amplified with two pairs of internal primers (Fig 6A). Both D and non-D full-length cDNAs were present and were of expected size (gels not shown), thus showing the expression of both D and Ce exon 10s in the dCCee and DCW− variants. To determine whether the two types of exon 1 were also expressed, segments a through n, the cDNA products encompassing exons 1 through 5, were digested with Apa I and Taq I. Notably, the restriction cleavage of dCCee cDNAs was identical with that of DCCee, whereas the pattern of DCW− cDNAs was reversed with respect to Apa I digestion and differed in the distribution of Taq I sites (Fig 6B). These results indicated that the dCCee and DCW− variants each expressed two distinct Rh transcripts in their erythroid cells.
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A

DCCee

dCCee

dCW-

B

DCCee
dCCee
dCW-

C

1

2

Fig 6. RT-PCR and sequence analysis of Rh transcripts expressed in dCCee and DCW- erythroid cells. (A) The strategy for synthesis and amplification of Rh cDNAs by RT-PCR. The Rh cDNA was synthesized separately with D and non-D gene-specific primers and then amplified with two pairs of internal primers (see Table 1 for sequences). The cDNA synthesis primers q and r are located downstream of the homology breakpoint (nt1358) in the 3' untranslated region. (B) Restriction analysis of cDNA segments a through n encompassing exons 1 through 5. U, undigested; A, Apa I; T, Taq I. The expected size of the various fragments is indicated. Note the difference between DCW- and other lanes in Taq I digestion. (C) Diagram of the Rh transcript structures as determined by cDNA sequencing. In DCCee, dCCee, and DCW-, each cell type expressed two different transcripts that encode distinct Rh polypeptides. The D and non-D identities of exons are illustrated by solid and open bars, respectively. The hybrid structures are denoted by exon numbering.

To establish the transcript structure, the full-length cDNA products from each cell type were completely sequenced on both strands and their amino acid sequences were deduced. The results showed the presence in each variant of two, and only two, types of Rh transcripts. Regarding the transcripts detected in dCCee cells, one corresponded to the regular Ce and the other occurred as the altered form of Ce, with its exons 1 and 10 being derived from D and exons 2 through 9 from Ce, respectively (Fig 6C). Thus, the latter transcript retained a chimeric structure of D-Ce-D with two novel exon-exon junctions. The 5' D-Ce junction resulted in the fusion of D and non-D sequences, whereas the 3' Ce-D junction did not affect the coding sequence, because codons 411 through 417 in exon 10 are identical between the D and non-D genes. To determine whether this D-Ce-D gene occurred in unrelated individuals, six more samples were studied. The hybrid gene and its transcript were detected in three individuals of whom two were heterozygotes and one was a homozygote, based on the restriction analysis of cDNAs and genomic mapping with exon 1 and 10 probes (gels not shown). Thus, 5 of the 14 chromosomes analyzed (7 subjects altogether) were the irregular dCe haplotype (an incidence of 0.36).

The cDNA sequencing results also confirmed the expression of two different Rh transcripts in the DCCee cells, but both of them were present as chimeric forms of RH genes, with each retaining a single hybrid junction (Fig 6C). In one form, exons 1 through 9 of D were fused with exon 10 of Ce, thus generating a hybrid junction that was the same as the 3' D-Ce junction in the D-Ce-D transcript without altering the D coding sequence. In the other form, exon 1 was derived from the Ce gene containing the n122G mutation and was fused with exons 2 through 10 of the D transcript. This alteration brought the Ce and D coding sequences into a cis configuration, producing a hybrid junction that was reverse to the 5' D-Ce arrangement of the D-C-D transcript.

DISCUSSION

In this report, multiple structural changes involving the Rh polypeptide genes have been identified and their effect on transcript expression in the dCCee and DCW- RBCs have been defined. Although Rh phenotyping suggested that the two propositi are homozygotes, molecular studies showed that they are heterozygous for two differently altered haplotypes. The alterations include both DNA deletion and gene recombination that have occurred in either cis or trans configuration. Elucidation of the molecular basis for these two variants correlates the genotypes with phenotypes and provides an explanation for the lost expression of some common Rh antigens (Fig 7). A reinvestigation of gene organization has led to the reassignment of several splicing sites and the localization of the insertion point of intron 4 for the non-D genes. These results establish that the D and non-D genes bear identical exon-intron junction structures.

The dCe haplotype occurs with an incidence of less than 1% in American whites and blacks. Some of its related DNA polymorphisms were recently described, but their relationship with RH locus structure and expression has remained unclear. For the dCCee variant studied here, one change was shown to be a deletion that removed the entire D locus, giving rise to a regular dCe haplotype on the altered chromosome (Fig 7b, H2). In contrast, the other change appeared not to vary the gene number, but had taken place as a recombination event. By replacing D exons 2 through 9 with the Ce homologous counterpart, that event transforms the D locus into a D-Ce-D fusion locus, resulting in an irregular dCe haplotype that harbors both a hybrid and a normal Ce gene (Fig 7b, H1). This variant haplotype occurred with an incidence of 0.36 among seven unrelated dCCee individuals. Although the estimation may not reflect its true population frequency due to a small number of cases studied, the finding of a D-Ce-D fusion gene is significant with regard to the evolution of Rh-negative haplotypes and
prenatal assessment of Rh alloimmunizations. As shown by serologic typing, this fusion gene does not produce the D antigen; yet, its D-Ce junction formed by D exon 1 and Ce exon 2 has created a new positional link that differs from either ce or Ce association. Whether this difference would cause the disposition of a variant antigen on the surface of the dCccee cells requires further investigation.

The DCw− phenotype is extremely rare and has been only found in Caucasians. Albeit being phenotypically homozygous, the propositus of this study appeared to be genotypically heterozygous, inheriting from each parent a differently altered RH haplotype. On one chromosome, the RH locus exists as a deleted haplotype and is occupied by a D-CE hybrid whose fusion does not change the coding sequence of the D gene (Fig 7Ca, H1). On the other chromosome, the RH locus also exists as a deleted haplotype but is structurally reversed with respect to hybrid arrangement (Fig 7Ca, H2). Overall, these two RH loci are essentially D-like, because all of their coding sequences were derived from the D gene except exon 1 or 10 in each complement. A second possibility would be the heterozygosity for a variant haplotype bearing both hybrid genes and a null haplotype lacking all RH genes (Fig 7Cb). Nevertheless, this is less likely, because no true deletion as such has been found in humans. Accordingly, although the resulting D-CE and Cw-D fusion genes contain no Eε specific sequence, they both are expressed, with the former encoding D and the latter cotransmitting the Cw and D antigens, respectively. Furthermore, the expression of D antigen on the DCw− RBCs is elevated when compared with Dce/Dce homozygotes. Because the non-D gene is largely missing from the DCw− diploid genome, the display of this quantitative polymorphism relates, at least in part, to the loss of the known competitive effect of C on D that may be exerted in either cis or trans configuration.

Based on the results of genomic and transcript analyses, mechanisms may be proposed to account for the origin of the dCccee and DCw− phenotypes. In the dCccee cells, a novel cDNA form with two hybrid exon-exon junctions was identified in addition to the regular Ce transcript. This form to the formation of a cognate D-Ce-D hybrid gene whose 5' and 3' breakpoints were localized to intron 1 and 9, respectively. Although behaving as an internal deletion, such a chimeric structure was not accompanied by decrease of gene number on its resident chromosome. This finding suggests the occurrence of a segmental gene conversion event in which Ce acted as a donor while D served as the recipient. This type of mechanism has been implicated to play a major role in generating the antigenic polymorphisms of human MNSs and RH blood group systems. Because the D exons 2 through 9 were ultimately replaced and lost, the directional transfer from Ce to D along with strand synapsis and heteroduplex repair must have involved large stretches of sequences of the two genes. Nevertheless, whether the donor Ce gene involved was derived from a DCe or a dCe haplotype cannot be unequivocally determined. In addition, the entire deletion of the D gene on the opposite chromosome appears to be independent of this recombination event, because unrelated individuals homozygous for the regular or irregular dCe haplotype were found.

It is noteworthy that the present DCw− variant differs from that previously reported, suggesting that the molecular basis for the DCw− phenotype may be heterogeneous. As inferred from their inheritance and structural differences, the two hybrid genes expressed in the present DCw− variant are likely to have originated from independent recombination events. With regard to the formation of Cw-D hybrid, the recombinational break and reunion apparently proceeded at a single site of intron 1 and resulted in partial loss of both original RH genes. The mode and consequence of such a DNA rearrangement event would be compatible with the occurrence of a single crossing-over by the mechanism of unequal homologous recombination. Nevertheless, the other deleted haplotype was configuratively reversed as a D-CE fusion hybrid and therefore would be expected to regain copies of the participating genes. The observation that this hybrid locus was also accompanied by the absence of intact D and non-D genes raises the possible involvement of an alternative event or mechanism in addition to crossover, be
it a gene conversion, an intragenic looping, or an illegitimate recombination. More studies will be required to decipher the mechanism underlying this paradox, because a range of issues relevant to the RH locus as a whole remains to be resolved, such as the physical order of gene members, their transcriptional orientation, and the spatial distribution of multiple exon-10-containing modules.

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REFERENCES
31. Mouro I, Colin Y, Sistonen P, Le Pennec PY, Cartron JP, Le Van Kim C: Molecular basis of the RhCc (Rh8) and RhCc (Rh9) blood group specificities. Blood 86:1196, 1995
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