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*RHCE*ceCF* encodes partial c and partial e but not CELO an antigen antithetical to Crawford

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Abstract

Background—RH43 (Crawford) is encoded by *RHCE*ce* with nucleotide changes 48G>C, 697C>G, and 733C>G (*RHCE*ceCF*). We investigated the Rh antigen expression and antibody specificities in four patients with this allele.

Methods—Hemagglutination tests, DNA extraction, PCR-RFLP, AS-PCR, reticulocyte RNA isolation, RT-PCR cDNA analyses, cloning, and sequencing were performed by standard procedures.

Results—RBCs from two patients typed D+C−E−c+e+/−, $hr^{S}-/+^{W}$, $hr^{B}-$ and their serum was reactive (3+) with all RBC samples of common Rh phenotype tested, but non reactive with Rh_{null} or D- - RBCs (apparent alloanti-Rh17). At the RHCE locus, Patient 1 was homozygous for *RHCE*ceCF*, and Patient 2 inherited *RHCE*ceCF in trans* to a silenced *RHCE*cE*. Cross testing of serum and RBCs from these two samples showed mutual compatibility, indicating that both antibodies define the same novel high prevalence antigen on Rhce. Two additional patients, one whose serum contained alloanti-c but the RBCs typed C+c+, and one whose serum contained anti-e but the RBCs typed E+e+, also had *RHCE*ceCF*. *RHCE*Ce* was present *in trans* in the former and *RHCE*cE* in the latter patient.

Conclusion—We report that amino acid changes on RhceCF (Trp16Cys, Gln233Glu, and Leu245Val) alter the protein to the extent that c and e antigens are partial, and a high prevalence antigen, we have named CELO (provisional ISBT number 004058; RH58) is not expressed. CELO is antithetical to RH43 (Crawford).

Keywords

Blood groups; Rh blood group system; blood transfusion; partial antigen

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Introduction

The Rh blood group system is the most complex of the 30 human blood group systems.1 This is due not only to the 50 discrete antigens2 but also to the fact that some of the antigens, notably D, C, c, and e, are carried on proteins that have numerous changes resulting in qualitatively altered forms of the antigens; the so-called partial antigens.

A low prevalence antigen, Crawford (RH43), was first reported in 1980 in an abstract.3 The antigen was detected by a separable antibody in a polyclonal anti-D reagent. No sera containing a monospecific anti-Crawford have been described; RBCs with the Crawford phenotype ,if from a perrson with a silenced *RHD* (D-negative phenotype) are not agglutinated by most anti-D but are strongly reactive with monoclonal anti-D (GAMA401).4 In addition to GAMA401, two IgM monoclonal, anti-D RUM-1, D175-2, and three IgG monoclonal anti-D, F5S, H2D5D2F5, MCAD-6, also react with D− Crawford+ RBCs.5 The molecular basis associated with expression of Crawford was elucidated in 2006. The Crawford antigen is encoded by *RHCE*ce* with the nucleotide changes 48G>C, 697C>G, and 733C>G and the allele is referred to as *RHCE*ceCF*. *RHD* has nucleotide G at position 697 and the *RHCE* change of 697C>G is thought to be responsible for the D epitope detected by certain anti-D.5 As Crawford cannot serologically be readily distinguished on D + cells, knowledge of the molecular background allows for detection of Crawford when the RBCs are D+.

The RhD protein is well known to have numerous epitopes that were first recognized by testing anti-D made by D+ people against RBCs from each other.6 These altered RhD were classified into six distinct categories, but introduction of monoclonal anti-D expanded the number of epitopes to 16,7 and finally, as many as 30 different epitopes appear to be recognized by monoclonal anti-D.8 Alteration of D epitopes results from single or multiple amino acid changes in the RhD protein. When a patient makes alloanti-D but has RBCs that type as D+, the RBCs are considered to express a partial D antigen. Partial D antigens were given names to designate different reactivity patterns, (e.g., DIIIa, DIV, DVI, DBT) but the antibody that people with partial D make is simply called anti-D. Locating “antigen-negative blood”, i.e., D− blood, is not difficult and serves all the many different partial D individuals equally well for transfusion purposes. If more precise specificity is required, statements such as “anti-D made by a DVI person” are used.

Similar to RhD described above, numerous epitopes of RhCE exist. The most familiar are C/c and E/e which represent distinct antigenic epitopes on Rhce, RhCe, RhcE, and rare RhCE proteins. In addition to these well-defined antigens a parallel scenario to partial D with anti-D exists for partial Cc and Ee antigens and antibodies to the RhCE protein, i.e., anti-Rh17. Just as different anti-D are directed at numerous epitopes on the RhD protein, anti-Rh17 made by people with altered or partial C/c and E/e phenotypes are directed at different epitopes on the RhCE protein that result from single or multiple amino acid changes in the proteins. The RBCs type C or c and E or e positive, but the antigens are revealed to be partial when the patient makes alloanti-C, -c, -E, -e and/or anti-ce (f), -Ce (rh_i), or −cE (Rh27). Antibodies made by people with partial “RhCE” could be simply called anti-Rh17, but locating “antigen-negative blood”, i.e., D- -, which lacks expression of C/c and E/e is difficult or impossible. Therefore, in contrast to calling the alloantibody made by D+ people simply anti-D, giving specific names to high prevalence antigens expressed on RhCE proteins, recognized by anti-Rh17, would enhance communication of the specific epitopes altered, and aid location of compatible blood.

In this article, we describe serological and DNA testing on blood from four patients with the Crawford allele, *RHCE*ceCF*. Two Rh17+ patients, one African American and one

Hispanic, whose serum reacted with all RBCs of common Rh phenotype tested, but was non-reactive with Rh_{null} or D- - RBCs, had apparent alloanti-Rh17. Two other African American patients were also studied; one c+ with alloanti-c, and the other e+ with alloanti-e. Our study reveals the amino acid changes on RhceCF are such that the associated c and e are partial antigens, and that the protein does not express a high prevalence antigen, which we have named CELO (provisional ISBT number 004058; RH58). CELO is antithetical to RH43 (Crawford) and is also absent from RBCs with the Rh_{null}, D- -, or $DC^{W}-$ phenotype, and is markedly depressed on RBCs with Dc(e) phenotype encoded by *RHCE*ceBP*.9

Case Reports

Patient #1 was a 27 year-old African American female, pregnant with her second child. Her RBCs typed A, D+C−E−c+e+/−, G+, $hr^{S+W}/-$, $hr^{B}-$, V/VS+, Rh17+, Rh29+, where +/− means positive or negative result according to the reagent used. Her serum contained an apparent anti-Rh17 that reacted strongly (3+) by the indirect antiglobulin test (IAT) with all RBC samples tested, including those with $hr^{S}-$ and/or $hr^{B}-$ phenotypes, but was non reactive with her own RBCs and those with the Rh_{null}, D− -, or $DC^{W}-$ phenotype. No family members were available for testing.

Patient #2 was a non-transfused 23 year-old Hispanic female, gravida 2, para 0 whose RBCs typed O, D+C−E−c+e+/−, $hr^{S}-$ (with one source), $hr^{B}-$, Rh17+. Her serum contained anti-Rh17 (IAT 3+) and alloadsorption studies suggested the possibility of an underlying anti-E. RBCs from the patient's ABO-compatible husband and two brothers were agglutinated by her serum. Her baby, whose group O, E− RBCs were positive (2+) in the direct antiglobulin test, was born with a hematocrit of 56%, reticulocyte count of 3.5%, bilirubin 9.4 μmol/L. Phototherapy was effective and the baby did not require transfusion.

Patient #3 was a 53 year-old African American female with sickle cell disease (SCD) whose RBCs typed B, D+C+ E−c+e+ and whose serum contained antibodies to c, E, M, S, Le^{a}, Le^{b}, Fy^{a}, and Jk^{b} antigens. She had no history of pregnancies but had received numerous transfusions. When the anti-c was first identified in 2004, the patient had been transfused with c+ RBCs and the anti-c was present in her plasma as well as in an eluate. A month later, when the transfused RBCs were no longer present, the autologous control was non-reactive. The patient has been transfused with c-negative RBC components and the anti-c is now below detectable levels.

Patient #4 was a 66 year-old African American female with a history of four pregnancies who was admitted with gastrointestinal bleeding and received multiple transfusions. Her RBCs typed O, D+ and reticulocytes10 typed C−E+c+ and e+ with one reagent but e− with another. Her serum contained anti-Jk^{a} and anti-e.

Materials and Methods

Hemagglutination

Reagents were from our libraries and 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 (Immucor-Gamma, Norcross, GA).

DNA and RNA isolation, reverse transcription-polymerase chain reaction, cloning and sequencing

Genomic DNA was prepared from 200μL of the buffy coat layer of peripheral blood using a DNA extraction kit (QIAamp DNA Blood Mini Kit, Qiagen, Valenica, CA, USA). RNA

was isolated from the reticulocytes (TRIzol and PureLink Micro-to-Midi Total RNA Purfication System, Invitrogen, Carlsbad, CA). For Patients #1 and #3, reverse transcription was carried out with the 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, Carlsbad, CA). 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* and *RHCE* cDNA using HotStarTaq Master Mix Kit, according to manufacturer's instructions (Qiagen, Valencia, CA). PCR products were checked for purity on agarose gels, purified using ExoSAP-IT according to manufacturer's instructions (USB Corporation, Cleveland, Ohio) and directly sequenced by GeneWiz Inc. (South Plainfield, NJ). Sequences were aligned, and protein sequence comparisons were performed using Sequencher 4.8 (GeneCodes, Ann Arbor, MI). In Patient #1, and Patient #3, RT-PCR products of *RHCE* were also cloned by Genewiz Inc. (South Plainfield, NJ) and sequenced to verify direct cDNA sequencing results. For Patients #2 and #4, RNA isolation and reverse transcription was performed as above, but poly A and random hexamers were used to generate cDNA. Rh transcripts were amplified, purified, cloned by TA cloning (Invitrogen) and sequenced. Sequences were aligned and comparisons performed with CLUSTALX. Exon-specific *RHD* and *RHCE* amplification and sequencing was performed to confirm cDNA results.

Results

RHCE*ceCF: altered e and the absence of an antigen of high prevalence

RBCs from Patients #1 and #2 typed D+C−E−c+e+/−, $hr^{S+W}/hr^{S}-$, $hr^{B}-$, Rh17+. RBCs from both patients were non-reactive with monoclonal anti-e MS16 and MS69 and reactive with other reagent anti-e. Polyclonal anti-e reacted weaker with the RBCs than with r"r control RBCs (Table 2).

Serum from both patients contained a strongly reactive (3+) alloantibody to a high prevalence antigen in the Rh system (apparent alloanti-Rh17) in as much as the antibody reacted with RBCs of common Rh phenotype but did not react with RBCs with the Rh_{null} or D- - phenotype. Due to ABO incompatibility, cross-testing using serum could not be done so eluates (prepared following incubation of serum with antigen-positive (rr) RBCs) were prepared. Cross-testing with eluates and RBCs from these two cases showed mutual compatibility. (Table 3) Molecular testing showed that Patient #1 was *RHCE*ceCF/RHCE*ceCF* and Patient #2 had *RHCE*ceCF/RHCE*cE*^{silenced}. (Table 4) The eluates from Patient #1 and Patient #2 were tested against other Crawford+ RBCs with Ce/ceCF or ce/ceCF backgrounds. Weaker reactivity was obtained with the eluate from Patient #2 with the Ce RBCs (1+) than with the ce RBCs (2+) when compared to ce/ce control RBCs (3+). The weakening was not so obvious with the eluate from Patient #1. (Table 3)

Anti-CELO was tested against RBCs of various unusual phenotypes defined by molecular analysis, including ceAR/ceAR, ceEK/ceEK, ceMO/ceMO, ceTI/ceTI, $ce^{S}(340)/ce^{S}(340)$, $(C)ce^{S}/(C)ce^{S}$, and $R^{=N}/R^{=N}$; all were strongly agglutinated. (Table 3) The antibody from both patients did not agglutinate, by the IAT, RBCs with the Dc(e) phenotype encoded by *RHCE*ceBP*, which express Rhce that lacks Arg229.9 Adsorption of serum from Patient #1 onto these Dc(e) RBCs and elution of antibody demonstrated that they express the CELO antigen extremely weakly.

RHCE*ceCF encodes partial c and partial e

Serum from Patient #3 contained alloanti-c but her RBCs typed C+c+. RH genotyping revealed she was *RHCE*ceCF/RHCE*Ce* (Table 4).

Serum from Patient #4 contained alloanti-e but her E+ RBCs typed e+ with Gamma-Clone monoclonal anti-e but they were non-reactive with the Ortho Bioclone monoclonal reagent (Table 2). DNA testing indicated she was *RHCE*ceCF/RHCE*cE* (Table 4).

RBCs from all four cases were agglutinated by 12 anti-c reagents (polyclonal and monoclonal, all commercial with expiration dates ranging from 2002 to 2010) to the same strength as control RBCs expressing a single dose of c antigen (data not shown). This result was surprising given that Patient #3 had a single dose of c antigen and made anti-c. However, in titration studies, RBCs from Patient #3 showed a slightly weakened expression of c.

Discussion

We report four patients with the *RHCE*ceCF* allele, who collectively reveal that the encoded c and e are partial antigens and that Crawford (RH43) is antithetical to a high prevalence antigen, which we have named, CELO ('CE' of Rhce and 'LO' from the names of the two probands). CELO has been assigned the ISBT provisional antigen number 004058 (RH58). CELO is absent from RBCs with the Rh_{null}, D- -, or $DC^{W}-$ phenotype and is markedly depressed on RBCs with the Dc(e) phenotype encoded by *RHCE*ceBP*. Using the antibody (in an eluate) from Patient #2, CELO clearly has a weaker expression on Ce/ceCF RBCs (1+) than on ce/ceCF RBCs (2+) or ce/ce RBCs (3+). RBCs from Patient #1 (*RHCE*ceCF/RHCE*ceCF*) and Patient #2 (*RHCE*ceCF/RHCE*cE*^{silent}) were strongly agglutinated by some anti-e but not by other anti-e consistent with partial e antigen expression. In contrast, they were strongly agglutinated by all the anti-c reagents tested. This is consistent with the prediction that the two proline residues that comprise the c epitope may be more resistant to downstream amino acid changes in Rhce protein.11

We also report findings on two African Americans with sickle cell disease who had been transfused on numerous occasions. RBCs from Patient #3 typed C+c+ and her serum contained anti-c reactive by the indirect antiglobulin test. Thus, the amino acid changes on the RhceCF protein (16Trp to Cys, 233Gln to Glu, and 245Leu to Val) are such that the C+c+ patient made alloanti-c, and thus the c antigen is a partial antigen. However, the strong reactivity of anti-c reagents with RBCs expressing the Ce/ceCF phenotype prevents recognition of the partial nature of this c antigen by hemagglutination. In addition, the paucity of specific antibody limits the ability to detect Crawford so that the presence of Crawford+ is only readily recognized by hemagglutination in D-negative people using selective anti-D reagents. RBCs from Patient #4 were E+e+ and her serum contained anti-e, showing that the e antigen on the RhceCF is also a partial antigen.

Many specific antigens are carried on altered forms of the RhCE protein.1 Partial e phenotypes initially were thought to lack the high prevalence antigens hr^{S} and/or hr^{B}. DNA-based analyses have confirmed the previously suspected heterogeneity among phenotypes considered to be $hr^{S}-$ or $hr^{B}-$. RBCs with altered Rhce and considered to be $hr^{S}-$ included ceAR, ceBI, ceEK, and ceMO, and those considered to be $hr^{B}-$ included ce^{S} (encoded by *RHCE*ce 48C, 733G, 1006T*), ceMO,1›12 and (C)ce^{S} (r'^{S}).1 Partial c antigens have been reported to be associated with, RH:−26, (C)ce^{S}, ce^{S}(340), and ceAR.13-20 We have shown here that RhceCF expresses partial c and e antigens. Each of the previously mentioned alleles encodes a different phenotype and the alloanti-c and/or anti-e are not expected to be mutually compatible.

Although RBCs and immune sera from people with partially deleted phenotypes (D- -, Dc-, DC^{W}-) are mutually compatible, apparent anti-Rh17 made by people with other rare phenotypes are not necessarily so. Heterogeneity of anti-Rh17 was originally noted by

variable reactions with RBCs expressing the following rare phenotypes: DIVa(C)-, D··, r^G, R_0^{Har}, and Rh_{mod}.21,22 Only RBCs with the Rh_{null} D- -, or DC^W- phenotype lack the Rh17 mosaic. The fact that anti-Rh17 made by D- - people agglutinated RBCs from CELO-negative individuals further confirms heterogeneity among so-called apparent anti-Rh17. Giving specific names to high prevalence antigens expressed on RhCE proteins could enhance communication of the specific epitopes altered, and thus aid location of compatible blood.

RBCs of people who inherit two RHCE*ce^S(340) alleles lack a high prevalence antigen (CEST; RH57) that is antithetical to JAL and, if immunized, their plasma contains what appears to be anti-Rh17. However, on more comprehensive testing this can be shown to actually contain anti-CEST.12,23 The Rh17-like antibodies, anti-CEST and anti-CELO, are not mutually compatible (Table 3).

We have shown that CELO is expressed on RBCs with other hr^S-negative and hr^B-negative phenotypes. (Table 3) The collective terms anti-Rh17, hr^S-negative/anti-hr^S, and hr^B-negative/anti-hr^B have limited value in clinical practice. To make communication easier and help locate compatible blood it is important to know the specific altered Rhce protein involved, as defined by DNA analysis. As suitable reagents for hemagglutination tests are neither robust nor readily available, matching of sensitized patients with RBCs lacking high prevalence RhCE antigens at the DNA level is becoming a feasible and desirable option.

Importantly, the clinical relevance of these antibodies has not been clearly established. Depending on the allele *in trans*, people with *RHCE*ceCF* can make alloanti-c (when *in trans* to *RHCE*Ce*) as well as anti-E, or alloanti-e (when *in trans* to *RHCE*cE*) as well as anti-C. Table 5 summarizes some examples of RH alleles if found together with *RHCE*ceCF*, the predicted RBC phenotype and possible alloantibodies that could be present. Pham and colleagues reported recently that RBCs from people homozygous for (C)ceS (r'^S) or DIIIa-ce^S are hr^B-, Hr^B- and RBCs from (C)ce^S-DcE people are hr^B-, Hr^B+.24 Whether or not RhceCF expresses Hr^B is not known.

In conclusion, RhceCF carries altered c, e, and hr^S and does not express CELO or hr^B. The cases reported here show the value of DNA testing as an adjunct to hemagglutination to aid in antibody identification in unusual cases. As shown in Table 4, in these four patients, four of the five RHCE*ceCF alleles were assumed to be *in trans* to a RHD*weak D Type 4.0 allele that encodes a partial D antigen. Thus, *RHCE*ceCF* is associated with partial *RHD*weak D Type 4.0*, *RHD*DIIIa-CE-D*, *RHD*pseudo D*, *RHD* deletion, and *RHD*.5 (personal observations). The existence of partial antigens causes a problem in regard to issuing suitable blood products to a certain patient. A component prepared from a blood donor with a partial antigen will be labeled as antigen-positive. For example, a DIIIa-ce^S/(C)ce^S patient with anti-D, anti-E, and anti-hr^B (if clinically significant) could be transfused with D−, E−, hr^B-. However, such blood is extremely rare and usually not available; donor blood of the same phenotype (DIIIa-ce^S/(C)ce^S) would be desirable. It will be labeled as D+, e+, so thought needs to be given to future terminology and labeling as the field moves toward combining genetic matching with serology for finding compatible blood for transfusion.

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Table 1

Sequence, location, and use of primers

Primer Name	Primer Sequence (5′ to 3′)	Location	Use
cDx10R	gtattctacagtgcataataaatggtg	Exon 10 3′ UTR	RHD gene-specific RT reaction (create RHD-specific cDNA)
cCEx10R	ctgtctctgaccttgtttcattatac	Exon 10 3′ UTR	RHCE gene-specific RT reaction (create RHCE-specific cDNA)
cRHx1F	agctctaagtacccgcggtctgtcc	Exon 1	Amplify Exons 1-4 in gene-specific cDNA
cRHx5R	tggccagaacatccacaagaagag	Exon 5	
cRHx4F	acgatacccagtttgtctgccatg	Exon 4	Amplify Exons 5-10 in gene-specific cDNA
cRHx10R	tgaacaggccttgtttttcttggatgc	Exon 10 3′ UTR	

Table 2

Tests with anti-e against RBCs from Patients #1, 2, and 4

Anti-e reagent (clone/s)	**Patient #1**	**Patient #2**	**Patient #4**	**Positive control E+e+(r”r)**	**Negative contol E+e−(R_2R_2)**
Gamma-Clone (MS16, MS21, MS63)	4+	4+	3+	4+	0
Immucor polyclonal	2+	3+	NT	4+	0
Immucor Series 1 (MS16)	0	0	NT	3+	0
Ortho Bioclone (MS16)	0	0	0	4+	0
Polyclonal single source (70820)	$1+^{S}$	NT	NT	4+	0
MS62, supernatant fluid	3+	3+	NT	3+	0
MS69,supernatant fluid)	0	0	NT	4+	0

Table 3

Testing ABO-compatible RBCs ,with rare Rh phenotypes, with anti-CELO from Patient #1 and Patient #2

RBCs defined serologically and type confirmed by DNA analysis	Patient #1		Patient #2	
	Serum	Eluate	Serum	Eluate
Patient #1 ceCF/ceCF	0	0	NT	0
Patient #2 ceCF/cE^{silenced}	0	0	0	0
ce/ceCF	3+	3+	3+	2+
Ce/ceCF	NT	3+^{W}	NT	1+
ce/ce(control)	3+	3+	3+	3+
Rh$_{null}$	0		0	
D- -	0		0	
DC^{W}–	0		NT	
ceBP/ceBP	0$^{\wedge}$		NT	
ceAR/ceAR	3+		NT	
ceEK/ceEK	NT		3+	
ceMO/ceMO	NT		3+	
ceTI/ceTI^{25}	NT		3+	
ceS(340)/ceS(340) (CEST−)	3+		NT	
(C)ce^{S}/(C)ce^{S}	3+		NT	
R$^{=N}$/R$^{=N}$ (Rh46−)	3+		NT	

$^{\wedge}$= ceBP /ceBP RBCs absorbed and eluted anti-CELO; the eluate reacted 2+ with antigen-positive RBCs

Table 4

Summary of alleles and antibody specificity

Case	RHD alleles	RHCE alleles	Antibody
Patient #1 African American	*RHD* weak D type 4.0*[§] *RHD*	*RHCE*ceCF*[^] *RHCE*ceCF*	Anti-CELO
Patient #2 Hispanic	*RHD* weak D type 4.0* *RHD*	*RHCE*ceCF* *RHCE*cE*^{Silenced}	Anti-CELO
Patient #3 African American	*RHD* weak D type 4.0* *RHD*	*RHCE*ceCF* *RHCE*Ce*	Anti-c
Patient #4 African American	*RHD* weak D type 4.0* *RHD*	*RHCE*ceCF* *RHCE*cE*	Anti-e

[^] = RHCE*ceCF: *48C, 697G, 733G*

[§] *RHD*weak D type 4.0* encodes a partial D antigen

Table 5

RBC phenotypes and possible antibodies that people with a RHCE*ceCF allele, if immunized, can make

***RHCE*ceCF* together with**	**RBC typing^ of phenotype**	**Possible RhCE alloantibody in any combination**
*RHCE*ceCF*	C−, E−, c+#, e+/−, hr^{S+W}/−, hr^{B}−, Rh43+, CELO−	Anti-CELO (+ anti-C, -E, -c, -e, -hr^{B})
*RHCE*cE^{Silenced}*		
*RHCE*Ce*	C+, E−, c+#, e+ hr^{S}+, hr^{B}+, Rh43+	Anti-E, -c
*RHCE*cE*	C−, E+, c+, e+/−, hr^{S+W}/−, hr^{B}−, Rh43+	Anti-C, -e, -hr^{B}
(C)ce^{S}†(r'^{S})	C+/−@, E−, c+#, e+/−, hr^{S}+, hr^{B}− Rh43+,	Anti-C, -E, -c, -e, -hr^{B}
*DIIIa-RHCE*ce* ^{S} †	C−, E−, c+, e+/−, hr^{S}+, hr^{B}−, Rh43+	Anti-C, -E, -e, -hr^{B}

^ = Hr^{S} and Hr^{B} status of ceCF are not known

= partial c but all currently available anti-c agglutinate these RBCs strongly

@ = C+/−due to the RhD-CE-D hybrid protein associated with the (C)ce^{S} haplotype

† = *RHCE*ce 48C, 733G, 1006T*