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Vox Sang. Author manuscript; available in PMC 2012 July 1.

Published in final edited form as:

Vox Sang. 2011 July ; 101(1): 61–64. doi:10.1111/j.1423-0410.2010.01465.x.

Red cells from the original JAL+ proband are also DAK+ and STEM+

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Abstract

Background—The low prevalence Rh antigen, JAL, was named after the index case, Mr. J. Allen. Based on reactivity of seven multi-specific sera with his RBCs, it was apparent that they express at least one additional low prevalence antigen. The purpose of this study was to investigate the other low prevalence antigen(s) on J. Allen's RBCs.

Methods—Blood samples and reagents were from our collections. Hemagglutination and DNA analyses were performed by standard methods.

Results—Our DNA analyses confirmed the presence of *RHCE*ceS(340T)* in J. Allen and revealed the presence of *RHCE*ceBI* (ce 48C, 712G, 818T, 1132G) and *RHD*DOL* (509T, 667T). RBCs from J. Allen were agglutinated by anti-JAL, anti-STEM, and anti-DAK. Two of the reactive multi-specific sera tested in the original paper reacted with RBCs from J. Allen, and with RBCs from four other people with *RHCE*ceBI*, including the original STEM+ index case (P. Stemper) but not with RBCs with the DIIIa, DAK+ phenotype. We conclude that they contain anti-STEM.

Conclusion—J. Allen's RBCs express the low prevalence Rh antigens, JAL, V/VS (extremely weakly), STEM, and DAK. The presence of JAL on the variant Rhce, RhceJAL (16Cys, 114Trp, 245Val), STEM on the variant Rhce, RhceBI (16Cys, 238Val, 273Val, 378Val), and DAK on the variant RhD (170Thr, 223Val), encoded by *RHD*DOL in trans* to *RHCE*ceBI* is consistent with expression of these antigens. When J. Allen RBCs are used to detect and identify an anti-JAL, it is important to remember that they also express STEM and DAK.

Keywords

Blood groups; low prevalence antigens; Rh blood groups

Introduction

The low prevalence Rh antigen, JAL (RH48), was named after the index case, Mr. J. Allen, who was on African American. Prior to naming this antigen, Lomas et al., noted that the antigen had been reported as 'S. Allen', 'J. Allen', and 'Allen', even though that the majority of 'Allen+' RBCs carried a different antigen [1]. Based on reactivity with his red blood cells (RBCs) and seven so-called multi-specific sera, it was apparent that they express at least one additional low prevalence antigen. Three sera (S. Allen, J. McD., and J. Pas)

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agglutinated RBCs from J. Allen and from six other JAL+ probands (SA2, E.H., de S., de L., Sa6, and D. Pas); these sera contained a single specificity, anti-JAL. Four sera (McN., Pear., Wr., and Wad.) agglutinated RBCs from J. Allen but not from the six other JAL+ probands. The reactions with these sera could not be attributed to any blood group specificity known in 1990 [1].

Anti-JAL is clinically significant and has caused severe hemolytic disease of the fetus and newborn [1,2]. The presence of the unidentified antigen(s) on RBCs from J. Allen, precluded Lomas et al., from concluding that anti-JAL in S. Allen's (wife of J. Allen) plasma was the cause of a strongly positive direct antiglobulin test in their child. Using two anti-JAL (S. Allen and J. Pas.), recent reports have correlated the molecular basis of the JAL antigen [3–5]. Hustinx and colleagues [3] tested three Rhce JAL+ samples without an *in trans* RHCE*ce^{S} allele with anti-V/VS; two were non-reactive and one was weakly reactive. Lomas-Francis and colleagues [4] tested nine such samples; three [including J. Allen and a homozygous *RHCE*ceJAL* (proband)] were non-reactive and six (including the homozygous *RHCE*ceJAL* sister of the homozygous *RHCE*ceJAL* proband) were weakly reactive with some anti-V/VS, and non-reactive with others. Clearly, Rhce JAL+ RBCs express the V/VS antigen but only extremely weakly. A paucity of anti-JAL and JAL+ RBCs has prevented extensive serological studies.

The molecular basis associated with expression of JAL in Blacks is a nucleotide (nt) change 340C>T in an RHCE*ce^{S} allele [*RHCE*ceS(340T)*, *RHCE*ceJAL*, or *RHCE*ceVS.07*, i.e., nt 340C>T (Arg114Trp) and nt 733C>G (Leu245Val)].[3,5] The study by Westhoff, et al. [5], included analysis of blood from J. Allen and revealed that a variant *RHCE*ce* was present *in trans* to the *RHCE*ceJAL*. The purpose of the study presented here was to analyze the RHCE alleles in J. Allen and to test for expression of low prevalence antigens predicted by DNA analyses to be present on J. Allen's RBCs.

One challenge of working with low prevalence antigens in the Rh blood group system is that they may be expressed on more than one phenotype (e.g., the low prevalence Rh antigen, DAK, is expressed on RBCs with either DOL, DIIIa, or R^{N} phenotype [6]) and that more than one antigen may be expressed by the same phenotype (e.g., Rh33 and FPTT are both expressed on RBCs with the D^{Har} phenotype) [7]. Furthermore, antibodies to more than one low prevalence antigen may occur in the same plasma (the so-called multi-specific lows). Studies are further complicated by the inheritance of variant alleles *in cis* (e.g., *RHD*DOL* is often *in cis* to *RHCE*ceBI* or *RHCE*ceSM* [8], and *RHD*DIIIa* is often *in cis* to RHCE*ce alleles encoding V/VS [9].

Materials and methods

Standard hemagglutination tests were used throughout. Testing was performed in tubes by the method of optimal reactivity for the antibody being used and appropriately controlled. Blood from J. Allen was recovered from storage in liquid nitrogen. RBC samples and antisera were from our collection and had been identified by us or obtained from numerous sources. None of the reagents were obtained specifically for this study. Hemagglutination and DNA extraction were performed by standard methods. Genomic DNA was isolated from the stored blood sample using the QIAamp DNA Blood Mini Kit (QIAGEN, Inc. Valenica, CA). Primers, annealing temperatures, amplicon sizes (in bp), and restriction enzymes are listed in Table 1. PCR amplicons for *RHD* exons 4 and 8 were analyzed by direct sequencing to infer the presence of *RHD*DOL*.

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Results

DNA analysis

PCR-RFLP analyses of DNA from J. Allen showed *RHCE*48G/*C (exon 1), *RHCE*340C/T* (exon 3), *RHCE*712A/G, 733C/G* (exon 5), *RHCE*818C/T* (exon 6), and *RHCE*1132G/G* (exon 8). The nucleotide changes 340T, and 733G are associated with *RHCE*ceJAL*, whereas 712G, 818T, and 1132G are associated with *RHCE*ceBI* [10]. Direct sequencing of exon 8 confirmed the nt 1132G/G result, which is unexpected, and is likely due to allele drop-out [11–13] rather than to the presence of a RHCE*ceJAL allele with a nt 1132C>G change. Allele dropout is the failure to PCR amplify one allele, and can result from low quantity or poor quality DNA associated with the age of the sample. Alternatively, failure to amplify could be the result of nucleotide change(s) in the intron region complementary to the location of the primers for one of the alleles. We conclude that the second RHCE*ce allele in J. Allen is *RHce*ceBI* (*RHCE*01.08 or RHCE*ce.08*), which is reported to encode STEM.[14] *RHCE*ceBI* is often *in cis* to *RHD*DOL,* and direct sequencing of *RHD* exons 4, and 5, revealed, respectively, *RHD*509T/C* (Met170Thr), and 667T/G (Phe223Val). This is consistent with the presence of an RHD*DOL allele [15].

Hemagglutination

Historically, RBCs from J. Allen were agglutinated by anti-JAL (S. Allen and J. Pas) and their reactivity with these sera was confirmed prior to our study. They were also agglutinated (this study) by anti-STEM (TS95), anti-DAK (AK, Riz), but not by anti-V and anti-VS. This V/VS typing is consistent with results previously reported [1,4]. Two of the reactive multi-specific sera tested by Lomas, et al. [1], McN. and Pear., reacted with J. Allen's RBCs, and with RBCs from four other people with a RHCE*ceBI allele, including the original STEM+ index case (P. Stemper). As these two sera did not react with an RBC sample with the DIIIa, DAK+ phenotype, we conclude that they contain anti-STEM. Consistent with the original findings [1], and those of Hustinx, et al. [3], we found one batch of the multi-specific serum, Hor., agglutinated RBCs from J. Allen; however, a second batch did not agglutinate his RBCs. As has been described for sera containing multiple antibodies to low-prevalence antigens (@@Daniels GL; Human Blood Groups (2002) page 501)[16] the at serum collected from Hor. on different occasions contains different specificities. (Personal observations) By testing STEM+ and DAK+ RBCs, we demonstrated that serum from Hor. (current batch) and from S. Allen do not contain anti-STEM or anti-DAK.

Conclusions

J. Allen's RBCs express five low prevalence Rh antigens: the previously reported JAL and extremely weak V and VS antigens, and the two low prevalance antigens reported here, STEM, and DAK. In the absence of long-distance PCR, it is not possible to be certain of the nucleotide alignments; thus, we presume JAL, V, and VS are carried on Rhce^{S}(340T) (114Trp, 245Val), STEM on RhceBI (16Cys, 238Val, 273Val, 378Val), and DAK on the variant RhD (170Thr, 223Val), encoded by *RHD*DOL.* When J. Allen RBCs are used to detect and identify an anti-JAL, it is important to remember that they also express V, VS (albeit extremely weakly), STEM and DAK. Furthermore, during antibody identification, when a serum reacts with DAK+ RBCs and the molecular basis of that sample is unknown, the presence of anti-STEM should also be considered because RBCs with the DOL phenotype (DAK+) are usually STEM+ (*RHD*DOL* and *RHCE*cBI* or *RHCE*ceSM* often travel together) [8]. The dearth of antibodies to certain low prevalence antigens (e.g., anti-JAL, anti-DAK, and anti-STEM) make DNA analysis a desirable approach to more fully characterize donors especially those used in an antibody identification panel.

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The possibility of allele dropout must be considered whenever using DNA testing for clinical purposes. Allele dropout, an artefact that occurs during PCR amplification, can lead to genotyping errors [11–13]. The problem can occur as a result of inherited nucleotide changes close to the 3′ end of the amplimer (i.e., primers fail to anneal correctly), or can be due to poor quality DNA where, due to DNA fragmentation, there are significantly more contiguous small targets than larger ones (i.e., PCR cannot amplify larger fragments as efficiently as smaller fragments). Thus, in allele-specific PCR assays, the presence of control band(s) and the absence of an allele-specific band does not universally mean that the allele is absent.

Acknowledgments

This study was supported in part by grant NIH HL091030 (MER). The authors thank the following colleagues for generously sharing the following samples over the years: (the late) Sally Frank and Karen Kirkley, Charity Hospital, New Orleans for samples from J. Allen and S. Allen; Beat Fry (address/affiliation) for the J. Pas serum; Elizabeth Smart, Durban, South Africa for anti-STEM (TS95) and RBCs from the original STEM+ proband. We thank Robert Ratner for help in preparing the manuscript.

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Table 1

Oligonucleotide primers used in the analysis of selected exons

Name	Primer sequence (5′ to 3′)	Annealing temperature	Exon (amplicon size in bp) RFLP if stated
RHDI3S	ggcttgccccgggcagagg	64°C	*RHD* Exon 4 (321) Sequenced for nt 509
RHDI4AS	gcttcagacacccaggggaac		
RHDI7F	ctggaggctctgagaggttgag	62°C	*RHD* Exon 8 (516) Sequenced for nt 1132
RHDI8R	catagacatccagccacacggca		
CEex1S	gcacacaggatgagctctaa	62°C	*RHCE* Exon 1 (283) *Hha*I for nt 48
CEint1R	agatgggggaatcttttcctc		
CEI4	ggcaacagagcaagagtcca	60°C	*RHCE* Exon 5 (521) *Nla*III for nt 712
RHDCEIn5R	gtgtgctagtcctgttagacc		
RH-I5-F	gagtgtgatgggtgcctaggatgctgagcacct	58°C	*RHCE* Exon 6 (478) *Mwo*I for nt 818
RHCE-I6-R	cctgctggccttcagccaaagcagag<u>agca</u>		
RhCE-I7-F	ctggaggctctgagaggttaaagg	60°C	*RHCE* Exon 8 (516) *Tsp*45I for nt 1132
Rh-I8-R	catagacatccagccacacggca		