The molecular basis of the Rhesus antigen E\textsuperscript{w}

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BACKGROUND: The Rhesus antigen E\textsuperscript{w} (ISBT designation 004 011) was first described in 1955. It is defined by a specific antibody, but its molecular genetic basis has not yet been resolved.

STUDY DESIGN AND METHODS: Two individuals serologically characterized to express the rare Rhesus antigen E\textsuperscript{w} were analyzed by sequencing of all 10 exons of the RHCE gene.

RESULTS: A nucleotide exchange at position 500 (T500A) resulting in a Met167Lys amino acid substitution was found in both individuals. Moreover, we show that an individual carrying the E\textsuperscript{w} antigen is capable to produce an alloantibody against the wild-type E antigen.

CONCLUSION: The single-point mutation T500A in exon 4 of the RHCE gene is a molecular basis of the rare Rhesus antigen E\textsuperscript{w}.

The Rhesus antigens represent the most complex blood group system on human RBCs, with at least 48 serologic entities described so far.\textsuperscript{1} It is encoded by two highly homologous genes (RHD and RHCE) located on chromosome 1p34 through p36 that are inherited together.\textsuperscript{2,3} The RHCE gene product carries the C or c antigen together with either the E or e antigen.\textsuperscript{4} In addition, several closely related variant antigens, recognized serologically by their corresponding antibodies, are known. One of them, the Rhesus antigen E\textsuperscript{w} (ISBT designation 004 011) was first described by Greenwalt and Sanger in 1955.\textsuperscript{5} E\textsuperscript{w} seems to be a rare antigen (<0.1% in Caucasians) and only few reports were published so far.\textsuperscript{5-9} It is defined by a specific antibody, which in some cases is able to cause HDN.\textsuperscript{5,8} Furthermore, it has been reported, that some, though not all polyclonal anti-E sera and some anti-E MoAbs can recognize RBCs carrying the E\textsuperscript{w} antigen.\textsuperscript{5,7,9,10} In this study, we investigated the molecular genetic basis of the rare E\textsuperscript{w} antigen in two individuals.

MATERIALS AND METHODS

Patients

Two unrelated adult male individuals showing the E\textsuperscript{w} antigen were examined on the molecular level. Individual 1 had been recognized some years ago in a family study, when his newborn daughter was found to be E\textsuperscript{w} + 1.\textsuperscript{11} She had shown discrepant results upon testing the Rhesus status using an incomplete polyclonal anti-E reagent and an anti-E MoAb reagent of the IgM-class. The presence of the E\textsuperscript{w} antigen in both, father and daughter, was confirmed using a specific anti-E\textsuperscript{w} serum (serum no. 861, Bavarian Red Cross Blood Donation Service, Munich, Germany, containing anti-E\textsuperscript{w} and weak anti-E, the latter absorbed by E + RBCs).\textsuperscript{11} The blood group of Individual 1 was established to be O and D+, C–, E+, c+, e–, C\textsuperscript{w}–, E\textsuperscript{w}+.

The DAT of his RBCs and the antibody screening test of his serum were negative. Individual 2 was hospitalized due to a gastrointestinal bleeding. In his serum we found a weak irregular antibody of anti-E specificity, although in routine typing he seemed to be E+. Using the gel-
Molecular analyses

Total DNA was isolated from peripheral EDTA blood using a standard extraction method (Qiagen, Hilden, Germany). Genomic fragments encompassing the entire coding region of the RHCE gene were PCR-amplified using specific intron primers as shown in Table 1. Two different primer pairs were designed for specific amplification of exon 2 of either the C or c allele. Accordingly, exon 2 of Individual 1 was investigated only with c-specific primers, whereas for Individual 2 the primer pair specific for the C region of the gene was additionally used.

PCR reactions were performed by 1 cycle (2 min, 94°C, 30 cycles (30 sec, 94°C; 30 sec, 55°C for Ex1-Ex6, 50°C for Ex7, 60°C for Ex8, 50°C for Ex9, 56°C for Ex10; 45 sec, 72°C) and 1 cycle (10 min, 72°C). After electrophoresis on a 2-percent agarose gel and purification using a purification kit (Amersham Biosciences Corp, Piscataway, NJ), the products of all PCR reactions were sequenced on both strands with the above-mentioned primers using a commercially available kit (GFX PCR DNA and Gel Band Purification Kit, Amersham Biosciences Corp, Piscataway, NJ), the products of all PCR reactions were sequenced on both strands with the above-mentioned primers using a sequencing kit (ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit, PE Biosystems, Weiterstadt, Germany) on an analyzer (ABI PRISM™ 310 Genetic Analyzer).

RESULTS

Molecular analysis of the RHCE gene of Individuals 1 and 2 revealed a single-point mutation in exon 4 (T500A) (Fig. 1). No other mutations except the specific polymorphisms encoding the C/c and E/e alleles could be detected. Thus, the T500A nucleotide substitution leading to a methionine-to-lysine exchange at amino acid position 167 (Met167Lys) is a molecular basis of the rare Rhesus antigen E*. 

DISCUSSION

The E and e antigens differ by a single nucleotide substitution in the RHCE gene at position C676G, leading to an amino acid exchange Pro226Ala.12 Several E variants have been identified by serologic means in the past decades,13-16 one them, E1 (RH24), now being obsolete because the specific antibody is no longer available.17 Recently, five E variants have been resolved on the molecular level.18-20 One of these, previously named E variant I (E var I), is

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<th>Specificity</th>
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* The positions of the primers are indicated relative to their distance from the first nucleotide or the last nucleotide of each exon, respectively.
† In the list of the primers, the nucleotides specific for RHCE exons are underlined.
‡ Temperature indicates annealing temperature.
characterized by a single-point mutation at nucleotide position 500 (T500A) of the RHCE gene. This mutation was also detected in our Ew individuals. Thus, we conclude that the T500A exchange is a molecular basis for the Rhesus antigen Ew, which might be identical with E var I published in 1998. Because no c-DNA analysis could be performed, the presence of an additional gene conversion could not be excluded.

Moreover, Individual 2 is the first Ew patient reported to have produced an anti-E. This may be of practical importance for transfusion medicine because not only an alloantibody can be made by E– individuals against the rare variant Ew, but that also individuals with the rare variant Ew can produce an alloantibody against the wild-type E. This situation resembles that found in rare individuals carrying distinct partial D antigens presenting with low-incidence marker antigens and sometimes making an allo-anti-D.

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REFERENCES