Blood Reviews (2006) **20**, 93—110

ELSEVIER

www.elsevierhealth.com/journals/blre

REVIEW

Rh proteins: Key structural and functional components of the red cell membrane

Caroline Le Van Kim *, Yves Colin [1], Jean-Pierre Cartron [2]

Inserm U76; Institut National de la Transfusion Sanguine, 6 Rue Alexandre Cabanel, 75015 Paris, France

KEYWORDS
Rh;
Blood group;
HDN;
Red cell membrane;
Spectrin-based skeleton;
Ammonium channel

Summary Rh (Rhesus) proteins (D, CcEe) are expressed in red cells (RBC) in association with other membrane proteins (RhAG, LW, CD47 and GPB). By interacting with the spectrin-based skeleton through protein 4.2 and ankyrin, the Rh complex contributes to the maintenance of the mechanical properties of the erythrocyte membrane. The RH system is one of the most immunogenic and polymorphic human blood group system. Molecular basis of most Rh phenotypes, including the Rh_{null} phenotype associated with hemolytic anemia, have been determined. The demonstration that the *RHD*-positive locus is composed of the *RHD* and *RHCE* genes, whereas the *RHD* gene is deleted in most RhD-negative individuals, allowed fetal RhD genotyping by non-invasive PCR assays for antenatal diagnosis of pregnancy at risk for Rh hemolytic disease of the newborn. In mammals, the Rh protein family includes two non-erythroid members, RhBG and RhCG, mainly expressed in liver and kidney, two organs specialized in ammonia genesis and excretion. Functional analyses in heterologous systems revealed that RhAG, RhBG and RhCG can mediate ammonium (NH_3 and/or NH_4^+) transport across the cell membrane and might represent mammalian specific ammonium transporters. Furthermore, recent studies performed in human and murine red blood cells (RBC) indicate that RhAG facilitates CH_3NH_2/NH_3 movement across the membrane and represents a potential example of gas channel. The crystallographic structure of the bacterial ammonia channel AmtB and functional studies showing that AmtB conducts NH_3 into reconstituted vesicles is fully consistent with these latter studies. In RBCs, RhAG may transport NH_3 to detoxifying organs like kidney and liver and with non-erythroid tissues orthologs may contribute to regulation of the acid-base balance.

* Corresponding author. Tel.: +33 1 44 49 30 46; fax: +00 33 1 43 06 50 19.
E-mail addresses: levankim@idf.inserm.fr (C.L. Van Kim), colin@idf.inserm.fr (Y. Colin), cartron@idf.inserm.fr (J.-P. Cartron).
[1] Tel.: +33 1 44 49 30 93.
[2] Tel.: +33 1 44 49 30 44.

RH (Rhesus) is a highly complex blood group system in man deeply rooted in transfusion medicine, through implications in alloimmune transfusion reactions, hemolytic disease of the newborn, auto-immune hemolytic anemia and through the

0268-960X/$ - see front matter
doi:10.1016/j.blre.2005.04.002

non-immune hemolytic condition associated with Rh-deficiency syndrome.[1] The basic serology of the RH system has been largely deciphered following biochemical and molecular analysis which revealed the primary structure of the genes and proteins and of the major polymorphisms.[2–5]. Since these initial studies, extensive analysis have contributed to clarify the molecular basis of most common and rare Rh polymorphisms occuring in man[6–11] including the molecular background of the Rh-deficiency syndrome.[12,13] The purpose of this review is to summarize current knowledge on the genetical and biochemical basis of this highly polymorphic system and to focus onto most recent findings regarding clinical application of *RHD* genotyping, the structural organization of the Rh protein complex within the cell membrane and the transport function of Rh proteins in red cells and in tissues where homologues have been recently identified.

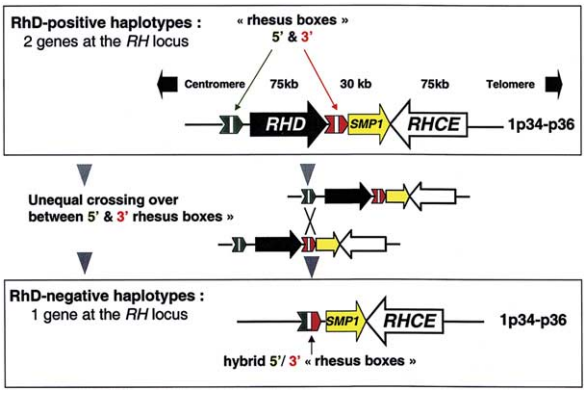

Figure 1 ***RhD-positive/RhD-negative polymorphism in whites.*** In RhD-positive haplotypes, the RH locus is composed of the *RHD* and *RHCE* genes in opposite orientation, whereas the *RHD* gene is absent from RhD-negative haplotypes. The presence of an hybrid 5′/3′ rhesus box in *RhD*-negative locus strongly suggests that *RHD* gene deletion occurred by unequal crossing-over between 5′ and 3′ rhesus box of two *RhD*-positive chromosomes (adapted from[16,18]).

Rh system and molecular basis of the polymorphisms

Rh blood group system was discovered 65 years ago by its role in feto-maternal incompatibilities responsible for the hemolytic disease of the newborn (HDN).[14] It is currently the most immunogenic and the most polymorphic blood group system in man with more than 45 serologically-defined antigens.[15]

RhD polymorphism. The *RH* locus on chromosome 1p34-p36 is composed of the homologous *RHD* and *RHCE* genes (96% identity), which are tandemly organized in opposite orientation {RHCE (5′->3′)-(3′ < -5′) *RHD*} and interspersed by a third gene, *SMP1*, whose function is presently unknown. Furthermore, the *RHD* gene is flanked by two repeated sequences (9,000 bp) exhibiting 98.6% homology called ''rhesus boxes''[16] (Fig. 1). In the caucasian population, the major antigenic polymorphism of the Rh system known as RhD-positive/RhD-negative is associated in most cases with the presence or the absence of the *RHD* gene.[17] The deletion of the *RHD* gene in the RhD-negative haplotypes is most probably the result of an unequal crossing over in the rhesus boxes, as deduced from the presence of a hybrid rhesus box.[16] In few cases, the RhD-negative phenotype in Caucasians can result from gene rearrangements or point mutations leading to stop codons.[18] The RhD negative phenotypes in Africans is in most cases due to the presence of a pseudogene, *RHDψ*, exhibiting different mutations, including a 37 bp duplication in intron 3 and exon 4 which disrupt the open reading frame.[19]

RhD variants. RhD and RhCE proteins differ by 34 to 37 amino acid substitutions,[20] depending on which *RHCE* allele is considered (*RHce, cE, Ce, CE*), but their respective role in the D specificities remain unknown (Fig. 2). This was explored through the studies of partial D phenotypes[21,22] and by site-directed mutagenesis analysis in recombinant K562 cells.[23,24] Partial D individuals can produce alloanti-D antibodies and carry a normal *RHCE* gene and a modified *RHD*. This phenotype may result from either a gene conversion or double crossing-over between the two *RHD* and *RHCE* genes[25,26] or a single point mutation in *RHD* gene causing amino acid change with subsequent loss of some D epitopes and/or expression of a low-incidence antigen.[8,11,27] Weak D variants (quantitative D variants) exhibit a weak level of D antigens but do not generally make anti-D,[28] although in rare cases, anti-D immunization was observed in variants with very low expression of RhD.[29] These phenotypes are due to point mutations leading to amino acid changes in predicted intramembraneous or intracytoplasmic domains of the RhD polypeptide,[30–32] potentially altering protein transport to the cell surface. To date, 31 weak D have been characterized at the molecular level. A compilation of the weak D variants as well as that of all other *RHD* variants can be found on the mutation database at the web sites http://www.uni-ulm.de/~wflegel/RH/ and http://www.bioc.aecom.yu.edu/bgmut/rh.htm.