Blood Groups – Rh Systems

Qun Lu, MD
Assistant Medical Director
Division of Transfusion Medicine
Department of Pathology and Lab Medicine
UCLA Medical Center
Los Angeles, CA

07/20/2009
History of Rh Blood Group

- 1900: Blood type A, B, O, AB discovered by Karl Landsteiner and others

- 1939 (reported by Levin 1941):
  - A woman suffered a severe hemorrhage after delivery of a stillborn child with hydrops.
  - Her husband’s blood transfused to the wife. They had same blood type
  - Patient suffered a severe acute hemolytic transfusion reaction.
  - Wife’s serum agglutinated her husband’s RBCs and 85% of Caucasian ABO-compatible donors RBCs.
  - Mother lacked antigens shared by the fetus and father. Mother produced Ab against the fetus and father’s RBCs
History of Rh Blood Group

- **1940s:**
  - Karl Landsteiner and Wiener transfused rhesus monkey RBCs to guinea pigs and rabbits. The pigs and rabbits made an antibody.
  - This antibody agglutinated 85% of human RBCs, named it “Rh” antibody, thought was the cause of AHTR.
  - Later understood “Rh” antibody is not human antibody, but still use the name Rh for the blood group.
  - Original rabbit/pig anti-rhesus antibody renamed as anti-LW

- **1945:** D, E, e, C, c —— 5 major antigens defined

- **Today:** 56 antigens in the system
Nomenclatures of the Rh Antigens

- **Wiener vs Fisher-Race**

  - $R^1 = DCe, \quad r' = dCe$
  - $R^2 = DcE, \quad r'' = dcE$
  - $R^0 = Dce, \quad r = dce$
  - $R^z = DCE, \quad r^y = dCE$
  - $R = D, r = d, 1' = Ce, 2'' = cE$

- **White:** $R^1 > r > R^2 > R^0$
- **Black:** $R^0 > r > R^1 > R^2$
- $R^1$ is most common in whites
- $R^0$ is most common in blacks, least common in whites
- $r$ is always the second most common
- $R^1$ is always comes before $R^2$.
- In Whites: most common antigen is $e$ (98%), least common antigen is $E$ (30%)
ISBT Numeric Terminology

- 1990/International Society of Blood Transfusion
- D antigen is 004001
- Six digit number for each authenticated blood group specificity
  - First 3 digits – blood system. Remaining 3 digits - antigenic specificity
- Rhnull – no Rh antigens

Review article for Rh blood group system:
  - http://bloodjournal.hematologylibrary.org/cgi/content/full/95/2/375#F3
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Biochemistry – Rh Complex

- Rh Complex: a tetramer with 2 RhAG molecules and 2 RhCcEe or RhD protein stabilized by accessory proteins

- Rh protein family:
  - Rh proteins: RhD, RhCcEe proteins, gene RH located on chromosome 1p34-p36
  - Rh associated glycoprotein (RhAG): 40% of amino acid sequence in RhAG is the same as in Rh proteins, gene RHAG located on chromosome 6p11-p21.1

- Rh accessory proteins: other glycoproteins that are associated with the Rh protein family
Biochemistry – Rh Complex

- Expression of Rh proteins and RhAG during erythropoiesis: expressed on RBCs from the 6-week conceptus – HDFN tends to be severe and early, but anti-K is associated with the most severe and early HDF (anti-D causes the most severe HDN).

- Possible function of Rh protein family: remains unclear, but possibly involved in ammonium transmembrane transportation.
Rh Proteins

- Rh proteins (RhD, RhCcEe): 417 amino acids traverse the cell membrane 12 times (6 extracellular loops), form an integral part of the RBC membranes.
- The first 41 N-terminal amino acids are identical.
- Rh-null phenotype
  - Total lack of all Rh antigens, make anti-Rh29, react with any Rh antigen – transfuse only with Rh null blood
  - Associated with a hemolytic anemia with stomatocytes on smear
Model of RhAG, RhCE, RhD.

The N-glycan on the first loop of RhAG is indicated by the branched structure of red circles.

**RhCE:** Red ovals represent amino acids that are critical for C/c (Ser103Pro) and E/e (Pro226Ala) antigens.

**RhD:** The domain of the RhD protein encoded by each exon is depicted by numbered boxes, which represent the start and finish of each exon. Of the D-specific amino acids, 8 are on the exofacial surface (yellow ovals), and 24 are predicted to reside in the transmembrane and cytoplasmic domains (black ovals). Purple ovals represent Ser103 and Ala226. The zigzag lines represent the Cys-Leu-Pro motifs that are probably involved in the palmitoylation sites.
Genotype

- Gene coding for Rh antigens is \textit{RH (RH30)}
- \textit{RH (RH30)} is located on chromosome 1p34-p36,
  - \textit{RHD}- gene coding for D antigen
  - \textit{RHCE}- gene coding for C,c, E,e antigens
  - They are closely linked
- \textit{RHD, and RHCE}, \textit{RHcE, RHce, RHCE} genes are very similar, only differ by 44 base pair
- Co-dominant alleles
Chromosomal organization of the RHCE and RHD genes

Genomic DNA: __________

Exons:  50 aa  Translated  Untranslated

Introns:  5 kb

Chromosome 1, short arm
**Structure of RHCE and RHD Genes**

Located on chromosome 1p34-p36, 10 exons on each gene.

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**RHCE**

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**RHD**

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Exon sizes are indicated above the line as number of nucleotides, and intron sizes are indicated below the line. A c-specific short tandem repeat (STR) is located in intron 2 of RHCE and another in intron 6 of RHD. The information used to compile this figure came from the database accession numbers given in the figure.

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**GenBank accession numbers:**

*RH:* U66340; U66341; X54534; Z97364; L08429; M34015; X63094; X63097

*RHD:* Intron 1 (part) Z97363; Intron 3 (part) Z97031; intron 4 Y10605; introns 5 & 6 Z97334; U77078; U83206.

*RHCE:* Intron 1 (part) Z97362; Intron 3 (part) Z97030; Intron 4 Y10604; Introns 5 & 6 Z97333; U77078; U83205.

3'-UT: see Huang C-H. *Blood* 1996;88:2326-2333.

Genotyping

- Not available at UCLA Blood Bank
- Our reference lab is American Red Cross
- Genotyping is generally not needed for routine patient care.
- Indication:
  - Unusual phenotype cases such as D+ patients with anti-D or e+ patients with anti-e
  - Donor genotyping to have a donor database
  - Fetal genotyping from amniotic fluid or fetal blood or other fetal tissue when the mother is positive for RBC alloantibody
  - RhD zygosity for husband of D= antiD+ women – to predict future baby’s phenotype.
- Specimen will be sent to ARC Pomona. They will ship specimen to their Philadelphia Lab. Takes 2 weeks to get results
D Antigen

- Rh positive generally means D positive.
- C decreases the expression of D antigen. So R2R2 (DcE/DcE) cells have more D than R1R1 (DCe/DCe).
- Elevated D expression in D--, Dc-, Dcw- (the dashes represent missing antigens).
- \(D_{el}\) phenotype: extremely low level of D antigen expression, usually will be typed as D neg, but these RBCs can adsorb and elute anti-D, common among Asian D neg donors.
- D and D-like epitopes on RhCE: react with monoclonal anti-D, but no reaction with polyclonal anti-D, such as \(D^{har}\) in German, Crawford (ceCF) in African.
- Weak D and partial D→
Weak D phenotype

- **Causes:**
  - Partial D: altered D antigen, will make anti-D, caused by missense mutation of *RHD* gene (hybrid gene of *RHD* and *RHCE*).
  - Weak D: lower # of D antigen on RBCs, will not make anti-D. Severely reduced expression of D, but normal *RHD* gene.

- **Who should get weak D test?**
  - All D-donors, weak D donors should be labeled as D positive donor.
  - Newborn from Rh neg mother, if baby is weak D +, mom should receive RhIG prophylaxis.
  - Patient with first time anti-D identified.

- **Weak D testing:** Patient’s RBCs incubated with anti-D at 37°C for 15 min, wash w NS 3 times, add anti-IgG, mix, centrifuge, re-suspend, exam for agglutination.

- **D- patients do not need weak D test.**
- **Weak D pregnant women also need RhIG (some can make anti-D).**
Molecular basis of weak D phenotypes

- Checkered ovals - locations of mutations on the RhD protein
- Open ovals - the D-specific amino acids
- Gray zones - missense mutations are located within nonconserved membrane spans and cytoplasmic regions.
- Black rectangles - Regions of conserved Rh protein family sequence
D Negative

- Rh negative means no D antigen (use d, but d antigen does not exist)
  - In 15% of Whites, 3-5% of Blacks, <0.1% of Asians
  - Caucasians: deletion of \textit{RHD} \textit{gene}

- D\textsubscript{el} phenotype: extremely low level of D antigen expression, usually will be typed as D neg, but these RBCs can adsorb and elute anti-D, common among Asian D neg donors
## Incidence of the D- Blood Group

<table>
<thead>
<tr>
<th>Population</th>
<th>Incidence</th>
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<tr>
<td>Chinese and Japanese</td>
<td>0.4%</td>
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<tr>
<td>North American Indian and Inuit</td>
<td>1 - 2%</td>
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<tr>
<td>Indo-Eurasian</td>
<td>2%</td>
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<tr>
<td>African American</td>
<td>4 - 8%</td>
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<tr>
<td>Caucasian</td>
<td>15%</td>
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<tr>
<td>Basque North-Central Spain and the adjoining region of South-Western France</td>
<td>30 - 35%</td>
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Fig 3. Rearrangements at the Rh locus giving rise to D-negative and Rh deletion haplotypes. The structures of the RH locus (located at 1p34-36) that has been defined in various D-negative phenotypes and rare Rh antigen deletion phenotypes are depicted. Each RH gene is represented as 10 boxes, each box representing an exon, where RHCE is shown as gray, RHD as black. Crosshatched boxes depict silent RHD alleles (eg, RHD Q41 X). The positions of microinsertions or deletions of DNA that cause or are indicative of D-negative phenotypes are shown as triangles. Because exon 8 of RHCE and RHD are of identical sequence and their origins are not possible to define, they are shaded according to the gene loci position. The significance of these rearrangements, and their impact in particular on molecular genotyping, is discussed within the text. Sources for the information in this figure: DCW (AM)184; DCW (Glo)185; D (LM)186; D (Gou)186; D (SH)72; D and Evans+ D (JD)187; Evans+ D (AT)69; Evans+ D (Dav)186; Dc (Bol)186; Dc (LZ)188; Ce70,74,149; r"G (SF)105,140; (Ce)Ce184; (C)ceS VS+ (Donor 1077) V r'S 29;98;104; Amorph Rhnull (BK/DR)176,189; Amorph Rhnull (DAA)177; CML.179
Other Rh Antigens

- **CcEc Antigens: CE, Ce, cE, ce antigens**
  - Product of \textit{RHCE} gene
  - C, c differ only by 4 amino acids
  - E, e differ only by 1 amino acid
  - C found in 70\% of the population and c found in 80\% of the population
  - E found in 30\% of the population and e found in 98\% of the population

- **G antigen:**
  - D and C antigens carry the G antigen which is in the second extracellular loop encoded by exon 2.
  - Anti-G react with D or C antigen, have to give D and C neg blood if patient has anti-G
  - Pregnant woman with anti-G, but not anti-D should receive RhIG prophylaxis
  - Women <50 y o should have work up to differentiate anti-G from anti-D or anti-C by adsorption and elution test
  - Anti-G will be adsorbed by D-C+ cells or D+C- cells, anti-D only will be adsorbed by D+ cells.
Other Rh Antigens

- **f antigen:**
  - a compound antigen when both c and e genes are next to each other on the same chromosome (R^0 or r haplotype)
  - Patient with anti-f can receive blood with e or c, but not both, usually choose e+c- blood, because e – blood is only 2% of inventory

- **Other compound antigens:** Ce(rh_i), cE (Rh27), CE (Rh22) antigens

- **C^w antigen:**
  - low frequency antigen, in 2% of Whites and 1% of Blacks.
  - Anti- C^w maybe naturally occurring and may cause HDN and HTR.
  - No need to search for antigen negative units since most of the units are negative. Crossmatch will pick up antigen+ units

- **VS, V antigens:**
  - low frequency alleles found in 1% or less of the Whites, but are more common in Blacks. V is found in 30% of the Blacks and VS in 32%.
  - No need to search for antigen negative units since most of the units are negative. Crossmatch will pick up antigen+ units
Rh Antibodies

- Warm-reacting, exposure-requiring IgG antibodies that are clinically significant
- Cause HTR, delayed HTR
- IgG antibody can cross placenta and cause hemolytic disease of fetus/newborn
- Potent immunogens: 80% of Rh- patients will develop anti-D after 1 unit of transfusion
- Anti-E, 70% of population (Caucasian) E-
- Anti-e, 2% of population e-
- Anti-C, 30% of population C-
- Anti-c, 20% of population c-
RhIG prophylaxis

- Ig G antibody – with 21 days half life, from anti-D + donor plasma
- Australia: transfuse D+ blood to D – volunteer donors in order to provide enough RhIG for pregnant women

Dosage:
- 1 vial (300 mcg), around the 28th week of pregnancy
- at time when vaginal bleeding occurs (calculate)
- at the time of delivery within 72 hours - calculate

At time of delivery,
- type baby umbilical cord blood, if D -, do weak D test, if D truly negative
  - no RhIG is required
- If cord blood D +, do maternal-fetal bleeding screen test (rosette test),
  - If -, give 1 vial
  - if +, give 2 vials immediately, then order Kleihauer-Betke test
- Based on the results of the test, calculate dosage, if more than 2 vials are needed, call OB to order more
Calculating Dosage of RhoGam

First perform the Kleihauer-Betke test to get the percentage of fetal red cells in the mother

- **Mechanism**
  - Blood smear stained with acid elution
  - Hb F is more acid resistant
  - Fetal RBC darkly stained, Maternal RBC "ghosts"
Calculating Dosage of RhoGam

Hemoglobin electrophoresis on cellulose acetate

Hemoglobin HPLC
Calculating Dosage of RhoGam

Method 1:
% of fetal RBC x 50 / 30

Method 2:

\[
\text{Vials of RhIG} = \frac{\text{Wt (kg)} \times 70 \text{ ml/kg} \times \% \text{ of fetal RBC}}{30}
\]

If the decimal < 0.5, round up; if the decimal is > 0.5, round up and then round up again
References