

**BLOOD GROUP SYSTEMS**  
**SHORT OUTLINES OF ESSENTIAL FACTS**

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## Rh BLOOD GROUP SYSTEM

- I. **ANTIGENS:** There are 49 antigens in the Rh system recognized by the ISBT.
- A. "Positivity" indicates presence of "D" Ag or "Rho"
1. 85% of the European-origin population is positive.
  2. Some Rh-positive individuals make a weak form of D termed "weak D" (formerly "D<sup>u</sup>").
    - a. Weak D is operationally defined; if an IAT is required to agglutinate the RBCs, they are weak D. The weak D test (formerly "D<sup>u</sup> test") is an IAT using IgG anti-D.
    - b. Weak D RBCs may stimulate anti-D formation in D-neg recipients, so weak D testing is required when testing blood donors.
    - c. Errors in typing an Rh-pos RBC recipient as Rh-neg are of no consequence except unnecessary use of Rh-neg RBCs, so weak D tests do not have to be done on recipients.
  3. The D antigen is a "mosaic" of multiple epitopes.
    - a. Occasional individuals lack one or more D epitopes and are termed "partial D".
    - b. Some, but not all, partial D's have a weak D phenotype.
    - c. Partial D individuals can make antibodies against the epitopes they lack. Such antibodies react with most other D positive individuals (i.e. they appear as a normal anti-D).
- B. Two other pairs of antithetical antigens, products of the *RHCE* gene, in close genetic linkage exist:

Antigen	Frequency	
	Caucasian	Black
C	67	33
c	80	97
E	30	21
e	98	99

- C. Rh antigens are carried on 2 multi-pass (12 membrane-spanning regions) membrane proteins, one of which carries the D antigen, and the other the C/c and E/e polymorphisms. The proteins are fatty acylated, but not glycosylated. They exist in the membrane associated with multiple other proteins including the band 3, LW, Duffy, and glycophorin proteins.
- D. Rh antigens are well developed in early fetal life.
- E. Rh associated glycoprotein (RhAG) must be present for RhD and RhCcEe antigens to be expressed. RhAG does not carry Rh antigens but associates with the Rh proteins forming part of the Rh complex.
- F. A rare "null" phenotype, lacking expression all Rh antigens, exists.
1. Rh-null cells have reduced survival and a "stomatocyte" morphology.
  2. 2 genetic bases
    - a. Regulator type: mutation in the *RHAG* gene which causes abnormal RhAG protein.
    - b. Amorphic type: most often found in individuals lacking the *RHD* gene and all or part of the *RHCE* gene.
- G. Other Rh or related antigens:
1. C<sup>w</sup>, a low prevalence antigen present in 2% of individuals.
  2. Compound antigens, made whenever a C/c and E/e antigen is inherited on the same protein, i.e. ce ("f") made by *r* allele (see below), cE made by *R*<sup>2</sup> allele, Ce made by *R*<sup>1</sup>, and CE made by *R*<sup>2</sup>.
  1. G, an antigen on both the D and CE proteins, on the latter of which it is usually present with C-determining alleles. Only rare C-negative individuals make G, so anti-G appears as an inseparable form of anti-D plus anti-C.

4. LW; a very high prevalence antigen, phenotypically related to the Rh system, but controlled by genes which segregate independently from those controlling the production of the DCE antigens.
  - a. Rh-positive RBCs typically express more LW than Rh-negative RBCs.
  - b. Rh<sub>null</sub> RBCs lack LW antigens.

II. **GENETICS:** phenotype is determined by two closely linked genes on the 1<sup>st</sup> chromosome, *RHD* and *RHCE*.

- A. The Rh negative phenotype is due to a deletion of the *RHD* gene in most Caucasians, but in other populations may be due to an inactive or partial *RHD* gene.
- B. The Rh system alleles exhibit "linkage disequilibrium", in that not all combinations are as likely as would be predicted were they to segregate independently.

Antigens defined	Allele Designation	Frequency	
		Caucasian	Black
Dce	<i>R<sup>o</sup></i>	.04	.44
DCe	<i>R<sup>1</sup></i>	.42	.17
DcE	<i>R<sup>2</sup></i>	.14	.11
DCE	<i>R<sup>c</sup></i>	rare	rare
-ce	<i>r</i>	.37	.26
-Ce	<i>r<sup>'</sup></i>	.02	.02
-cE	<i>r<sup>''</sup></i>	rare	rare
-CE	<i>r<sup>y</sup></i>	rare	rare

III. **ANTIBODIES**

- A. D is highly immunogenic; > 50% of Rh-neg recipients of 1 Unit Rh-pos RBCs make anti-D.
- B. Red cell immune: virtually all are formed after transfusion or pregnancy.
- C. IgG antibodies which do not fix C'
- D. Reactions are generally enhanced by: enzyme treatment of RBCs, albumin, LISS, PEG.
- E. Dosage can sometimes be seen with anti-E, -c, and -e.
- F. Anti-E and anti-C<sup>w</sup> can be non-red cell stimulated, in which case they are usually IgM.

IV. **CLINICAL SIGNIFICANCE**

- A. Recipient antibody predictably destroys incompatible donor cells in vivo but clinical consequences are milder than those of ABO incompatibility.
- B. Rh system antibodies are relatively common due to the antigens' high immunogenicity.
- C. Rh system antibodies cross the placenta and can cause severe fetal hemolysis.

## KELL BLOOD GROUP SYSTEM

- I. **ANTIGENS:** There are 28 antigens in the Kell system.
- A. Most important antigen is K (KEL1; often referred to as "Kell")
    1. Relatively low prevalence (see table below)
    2. Antithetical high prevalence antigen is "k" (KEL2; sometimes referred to as "Cellano")
  - B. Two additional pairs of high and low prevalence antigens defined by antithetical alleles in close genetic linkage to  $K/k$ :  $Kp^a/Kp^b$  and  $Js^a/Js^b$
  - C. A rare "null" phenotype,  $K_o$ , lacking all Kell system antigens exists.
    1. Inherited as an autosomal recessive
    2. No RBC abnormalities
    3. Enhanced expression of antigen "Kx"

Phenotype	Frequency	
	Caucasians	Blacks
K+k-	0.2	Rare
K+k+	8.8	2
K-k+	91	98
$Kp(a+b-)$	rare	0
$Kp(a+b+)$	2.3	Rare
$Kp(a-b+)$	97.7	100
$Js(a+b-)$	0	1
$Js(a+b+)$	rare	19
$Js(a-b+)$	100	80
$K_o$	very rare	

- D. Kell antigens are well developed in fetal life.
  - E. Kell antigens are destroyed by sulfhydryl reducing agents such as AET (2-aminoethylisothiuroniumbromide), 2-ME (2-mercaptoethanol) and DTT (dithiothreitol).
  - F. Antigenic activity is carried by a single-pass transmembrane protein with multiple intra-chain sulfhydryl bonds: structurally similar to zinc-binding neutral endopeptidases such as common acute lymphoblastic leukemia antigen (CALLA, CD10).
  - G. McLeod phenotype
    1. Decreased expression of all Kell-system antigens
    2. Acanthocytic RBCs and mild hemolytic anemia
    3. Kx antigen negative due to a variety of inactivating mutations or deletions
    4. Kx antigen is carried by Xk, a multi-pass membrane protein (10 membrane spanning regions) linked to the Kell protein by a disulfide bond and coded by the XK gene
    5. Some X chromosome deletions that include XK may also cause chronic granulomatous disease.
- II. **GENETICS:** alleles  $K$ ,  $k$ ,  $Kp^a$ ,  $Kp^b$ ,  $Js^a$ , and  $Js^b$  are in "linkage disequilibrium"; no gene exists which carries both  $K$  and  $Kp^a$  or  $Js^a$ , or both  $Kp^a$  and  $Js^a$ .
- A.  $K/k$ ,  $Kp^a/Kp^b$  and  $Js^a/Js^b$  inherited in autosomal, codominant fashion.
  - B. McLeod syndrome is inherited in X-linked fashion.

### III. ANTIBODIES

- A. K is next most immunogenic after D; 5-10% of K-neg recipients of 1 Unit K-pos RBCs make anti-K.
- B. Anti-Kp<sup>a</sup> and anti-Js<sup>a</sup> are uncommon due to the low prevalence of the inciting antigen.
- C. Anti-k, anti-Kp<sup>b</sup> and anti-Js<sup>b</sup> are rare because of the rarity of potential antibody formers (individuals lacking the antigen).
- D. Anti-Js<sup>b</sup> is found in individuals of African descent (e.g. patients with sickle cell disease).
- E. Most are red cell stimulated (formed after transfusion or pregnancy); rare examples of anti-K are formed transiently during bacterial infection (E. coli, TB).
- F. Occasionally occur as autoantibodies in K-pos OR K-neg individuals.
- G. IgG antibodies, esp. IgG1, many of which fix C'
- H. Reactions are not enhanced by enzyme treatment of RBCs.
- G. Fetuses of mothers with relatively low antibody titers may have severe anemia, possibly as a result of suppression of erythropoiesis.

### IV. CLINICAL SIGNIFICANCE

- A. Recipient antibody predictably destroys incompatible donor cells in vivo, sometimes with severe clinical consequences including renal failure.
- B. Anti-K is relatively common due to its high immunogenicity (of 100 K-neg recipients receiving K-pos RBCs, 10 will make anti-K) in Caucasian populations where the K antigen is prevalent.
- C. Kell system antibodies cross the placenta and can cause severe fetal anemia.

## KIDD BLOOD GROUP SYSTEM

### I. ANTIGENS/GENETICS: 2 important antigens Jk<sup>a</sup> and Jk<sup>b</sup> defined by 2 common alleles

Phenotype	Frequency*		
	Caucasian	Black	Asian
Jk(a+b-)	26	52	23
Jk(a+b+)	50	40	50
Jk(a-b+)	24	8	27
Jk(a-b-)	very rare		0.9 (Polynesians)

\*AABB Technical Manual, 16<sup>th</sup> ed.

- A. Kidd antigens are well developed at birth.
- B. Antigens are carried by a multi-pass membrane glycoprotein (10 membrane-spanning regions) that transports urea preventing RBCs from shrinking in the high urea concentration region of the renal medulla,
- C. NOT destroyed by ficin and papain.
- D. 2 mechanisms for Jk(a-b-) phenotype
  - 1. "Silent allele" *JK*, found in Pacific islanders (e.g. Polynesians, Japanese).
    - a. Make anti-Jk3, reactive with Jk<sup>a</sup> & Jk<sup>b</sup>
    - b. Associated with resistance to RBC lysis by 2M Urea.
  - 2. Dominant "suppressor" gene (Japanese)

### II. ANTIBODIES

- A. IgG antibodies, frequently IgG3,
- B. 50% fix C' (weak anti-Jk antisera may require anti-C' in AHG for detection).
- C. Often show dosage.
- D. Red cell immune: virtually all are formed after transfusion or pregnancy.
- E. Antibody expression is often transient, but returns on re-exposure to antigen.

### III. CLINICAL SIGNIFICANCE

- A. Expected to cause immediate hemolytic transfusion reactions; common cause of delayed hemolytic transfusion reactions.
- B. Antibodies uncommon but not rare.
- C. Anti-Jk antibodies can cause HDFN, but usually it is mild.

## DUFFY BLOOD GROUP SYSTEM

- I. ANTIGENS/GENETICS: 2 important antigens, Fy<sup>a</sup> and Fy<sup>b</sup>, determined by 2 codominant alleles (*FYA* and *FYB*) and one recessive allele (*FY*).

Frequency	Frequency*		
	Whites	Blacks	Japanese
Fy(a+b-)	20	10	81
Fy(a+b+)	48	3	15
Fy(a-b+)	32	20	4
Fy(a-b-)	0	67	0

\*AABB Technical Manual, 16<sup>th</sup> ed.

- A. Carried by multipass membrane glycoprotein (7 membrane-spanning regions); receptor for pro-inflammatory chemokines
- B. Fy<sup>a</sup> and Fy<sup>b</sup> antigens are destroyed by proteolytic enzymes (ficin, papain, and bromelin, but not trypsin).
- C. Fy<sup>3</sup>, made by all Fy<sup>a</sup> or Fy<sup>b</sup> positive individuals, is NOT protease sensitive.
- D. Duffy antigens are well developed at birth.
- E. Allele *FY*, common in individuals of West African descent is a variant of *FYB*.
  - 1. Directs synthesis of Fy<sup>b</sup> antigen on other cells but not RBCs.
  - 2. Fy(a-b-) RBC phenotype is associated with resistance to RBC invasion by *Plasmodium vivax*.
  - 3. Homozygotes (Fy{a-b-}) do not make anti-Fy<sup>b</sup>, but occasionally make anti-Fy<sup>3</sup>.

### II. ANTIBODIES

- A. Fy<sup>a</sup> is much more immunogenic than Fy<sup>b</sup>
- B. Red cell immune: virtually all are formed after transfusion or pregnancy.
- C. IgG antibodies, many of which fix C'
- D. Weak examples show dosage.
- E. Anti-Fy<sup>3</sup> acts as an inseparable form of anti-Fy<sup>a</sup> + anti-Fy<sup>b</sup>, formed by Fy(a-b-) individuals.

### III. CLINICAL SIGNIFICANCE

- A. Antibodies cause immediate and delayed hemolytic transfusion reactions.
- B. Antibodies uncommon but not rare.
- C. Anti-Fy<sup>a</sup> can cause HDFN.

## MNSs BLOOD GROUP SYSTEM

### I. History

Landsteiner and Levine described heterologous (rabbits immunized with human RBCs) anti-M and -N in 1928.

### III. Antigens

Phenotypes and Frequencies in the MNSs System							
Reactions with Anti-					Phenotype	Phenotype Frequency %	
M	N	S	s	U		Black	Caucasian
+	0				M	26	28
+	+				MN	44	50
0	+				N	30	22
		+	0	+	S	3	11
		+	+	+	Ss	28	44
		0	+	+	s	69	45
		0	0	0	S-s-U-	<2	0
		0	0	+	S-s-U+	rare	0

- A. M/N and S/s sites closely linked
  1. "Blood group system"
  2. Linkage disequilibrium exists;  $Ns$  5x more likely than  $NS$  than predicted by individual frequencies.
- B. Approximately 40 other linked antigens described; most are low prevalence.
  1. Mur – product of a gene that is a hybrid between the  $M/N$  and  $S/s$  genes ( $GYP A$  and  $GYP B$  resp.) that is rare in Europeans and Africans but common in East Asia (e.g. 7% of Chinese, 10% of Thais). Anti-Mur is common in some locales and can cause HTRs and HDFN.
  2.  $M^g$  - low prevalence allele, non-reactive with anti-M and -N (i.e. alternate allele to M and N)
  3.  $M^c$  - low frequency allele crossreacting with most anti-M, some anti-N
- C. Multiple deleted phenotypes exist.
  1. U neg individuals lack S and s
  2.  $En^a$  neg individuals lack M and N
  3.  $M^k M^k$  individuals lack M, N,  $En^a$ , S, s, and U
  4. "U-negative" individuals are heterogeneous (i.e. some make an antibody that reacts with other "U-negative" RBCs)
- D. Antigens are protease sensitive.
  1. All M and N are destroyed by ficin, papain, bromelin and pronase; trypsin sensitive, chymotrypsin partially sensitive.
  2. S and s sensitivity to papain, ficin, bromelin, and pronase is variable; trypsin resistant, chymotrypsin sensitive,
  3. U is protease resistant
- E. 'N' reflects weak agglutination of M pos, N neg RBCs with anti-N (see biochemistry)

### III. Antibodies

#### A. Anti-M and -N

1. Naturally occurring, cold-reactive agglutinins
2. 50 - 80% of antisera are partially or solely IgG
3. Essentially all antibodies show dosage
4. Anti-M reactions are enhanced by low pH
5. Little C' fixation
6. No IHTRs if not reactive at 37°C, so only "respect" such warm reactive examples (Respect = transfuse RBCs negative for antigen using reagent antisera).
7. Rare DHTRs (little increase in thermal amplitude with transfusion of incompatible RBCs)
8. HDFN can occur if reactive at 37°
9. May occur as autoantibodies
10. Anti-N may react weakly with *MM* individuals (see IVB4. below).
11. Anti-N common in dialysis-patients using formaldehyde sterilized dialyzers
  - a. Equally common in N-pos and N-neg individuals
  - b. Anti-N<sub>form</sub> reacts more strongly with formaldehyde treated, N-pos RBCs
  - c. Anti-N<sub>form</sub> reacts with formaldehyde treated, N-neg RBCs

#### B. Anti-S, -s, -U

1. Less common than anti-M and -N
2. Many anti-S and most anti-s react at 37°, so "respect" all with regard to RBC selection
3. HDFN more common and more severe than with anti-M and -N

### IV. Biochemistry

#### A. M, N, and En<sup>a</sup> carried on glycophorin A (GPA)

1. 31,000 D (131 amino acid{aa}) single-pass, membrane glycoprotein
2. 10<sup>6</sup> copies per cell
3. Major sialoglycoprotein carrying 60% of RBC negative charge on 15 tetrasaccharides
  - a. Removing sialic acid x 2 yields T antigen
  - b. Removing sialic acid x 2 and gal yields Tn antigen
4. Polymorphism is based on aa differences at positions 1 and 5 of the N-terminus
  - a. M<sup>c</sup> has an intermediate aa structure
  - b. M<sup>g</sup> varies from N at position 4

#### B. S, s, and U carried on glycophorin B (GPB)

1. 100 aa single-pass, membrane glycoprotein
2. 170 - 250,000 copies per cell
3. Minor sialoglycoprotein carrying 15% of RBC negative charge
4. S and s differ by one aa at position 29
5. First 26 N-terminal aa sequence is identical to 26 N-terminal aa of GPB (N' antigen), but most anti-N do not agglutinate N-negative RBCs, presumably because of the low number of GPB copies

## LEWIS BLOOD GROUP SYSTEM

I. ANTIGENS: 2 antigens,  $Le^a$  and  $Le^b$ , define 3 phenotypes.

Phenotype	Genotype	Frequency	
		Caucasian	Black
$Le(a+b-)$	<i>Lele</i> or <i>LeLe</i> , <i>sese</i>	22	23
$Le(a-b+)$	<i>Lele</i> or <i>LeLe</i> , <i>Sese</i> or <i>Sese</i>	72	55
$Le(a-b-)$	<i>lele</i> , <i>sese</i> , <i>Sese</i> , or <i>SeSe</i>	6	22

- A. Plasma antigens which adsorb to the RBC membrane; transfused RBCs adopt the Lewis phenotype of the recipient within days.
- B. Lewis antigens are NOT developed in fetal life.
- C. Assembled in plasma on type 1 polysaccharide chains.
- D. "Lewis substance" present in secretions neutralizes antibodies (as does plasma antigen).

### II. ANTIBODIES

- A. IgM or IgM + IgG antibodies, which usually fix C'
- B. Naturally occurring antibodies with little increase in titer after transfusion.
- C. Anti- $Le^a$  may be hemolytic *in vitro*.
- D. Reactions enhanced by proteolytic enzymes
- E. Specificity demonstrated by neutralization with Lewis substance
- F. Anti- $Le^a$ 
  - 1. More common than anti- $Le^b$ , particularly in pregnant, African-American woman
  - 2. Only found in individuals who are  $Le(a-b-)$
- G. Anti- $Le^b$ 
  - 1. Fairly common weak antibody, often combined with anti- $Le^a$
  - 2. Made by  $Le(a-b-)$ , and rarely  $Le(a+b-)$ , individuals
  - 3. Anti- $Le^{bH}$  - made by A1 individual and reacts with group O and A<sub>2</sub>  $Le(b+)$  RBCs
  - 4. Anti- $Le^{bL}$  - reacts with all  $Le(b+)$  red cells

### III. CLINICAL SIGNIFICANCE

- A. 37°C reactive anti- $Le^a$  may hemolyze  $Le^a$  positive RBCs *in vivo* (immediate hemolytic reaction).
  - 1. NO delayed hemolytic transfusion reactions
  - 2. NO hemolysis by anti- $Le^b$
  - 3. Transfuse RBCs compatible at 37°C and AHG
- B. Antibodies common (nuisance)
- C. Do NOT cause HDFN

## P BLOOD GROUP SYSTEM

- I. **ANTIGENS:** Three antigens P, P<sub>1</sub>, P<sup>k</sup> on two membrane glycolipid structures (officially 2 blood group systems) define five phenotypes.

Phenotype designation	Blood group antigens expressed		Antibody(ies) formed	Frequency	
	P system	Globoside system		Caucasian	Black
P <sub>1</sub>	P <sub>1</sub>	P <sup>k</sup> , P	none	79	94
P <sub>2</sub>		P <sup>k</sup> , P	anti-P <sub>1</sub>	21	6
P <sub>1</sub> <sup>k</sup>	P <sub>1</sub>	P <sup>k</sup>	anti-P	rare	
P <sub>2</sub> <sup>k</sup>		P <sup>k</sup>	anti-P <sub>1</sub> + -P	rare	
p			anti-P <sub>1</sub> , -P <sub>1</sub> , -P <sup>k</sup> ("anti-Tj <sup>a</sup> ")	rare	

- A. P<sub>1</sub> antigen is only expressed on RBCs.
- B. P<sub>1</sub> antigen strength varies from individual to individual and deteriorates on storage.
- C. P<sub>1</sub> [neutralizing] substance is present in hydatid cyst fluid.
- D. P and P<sup>k</sup> are high frequency antigens expressed on non-erythroid cells.
- E. P constitutes 6% of RBC membrane lipid.
- F. P<sub>1</sub> antigen is NOT fully developed at birth, P antigen is.

## II. ANTIBODIES

- A. Anti-P<sub>1</sub>
  1. Common antibody formed by P<sub>2</sub> individuals
  2. Most antisera are cold-reactive, IgM, naturally occurring, clinically insignificant antibodies.
  3. Occasionally IgG, warm reactive, or complement fixing
  4. High titers occur in individuals with hydatid disease and Fasciola hepatica (liver fluke), and in pigeon breeders.
    1. Neutralizable with P<sub>1</sub> substance
- B. Anti-P
  1. Rare alloantibody made by P<sup>k</sup> individuals (P<sub>1</sub><sup>k</sup> & P<sub>2</sub><sup>k</sup>)
    - a. Reacts with P<sub>1</sub> and P<sub>2</sub> individuals
    - b. Naturally occurring, IgM or IgM + IgG
    - c. Potent hemolysins causing HTR's and HDFN
  2. Autoantibody causing Paroxysmal Cold Hemoglobinuria (PCH); "Donath-Landsteiner antibody"
    - a. "Biphasic hemolysin": binds and initiates C' cascade in vitro at <32°C, and C'-mediated hemolysis is completed on rewarming
    - b. Stimulated by prior infection: syphilis, mumps, measles (incl. vaccination), non-specific
      - c. 5% of AIHA in children
- C. Anti-P, P<sub>1</sub>, P<sup>k</sup> ("anti-Tj<sup>a</sup>")
  1. Made by p phenotype individuals (rare)
  2. Most are IgM and fix C'
  3. Cause severe transfusion reactions
  4. IgG anti-P, P<sub>1</sub>, P<sup>k</sup> causes spontaneous abortion.

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- III. **CLINICAL SIGNIFICANCE [OF ANTI-P1]:** see above for significance of the remaining, rare antibodies
  - A. 37°C reactive anti-P1 can cause decreased RBC survival and HTRs
  - B. Antibodies common (nuisance)
  - C. Do NOT cause HDFN