Blood Group Antigens:
principles and practice

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3-95

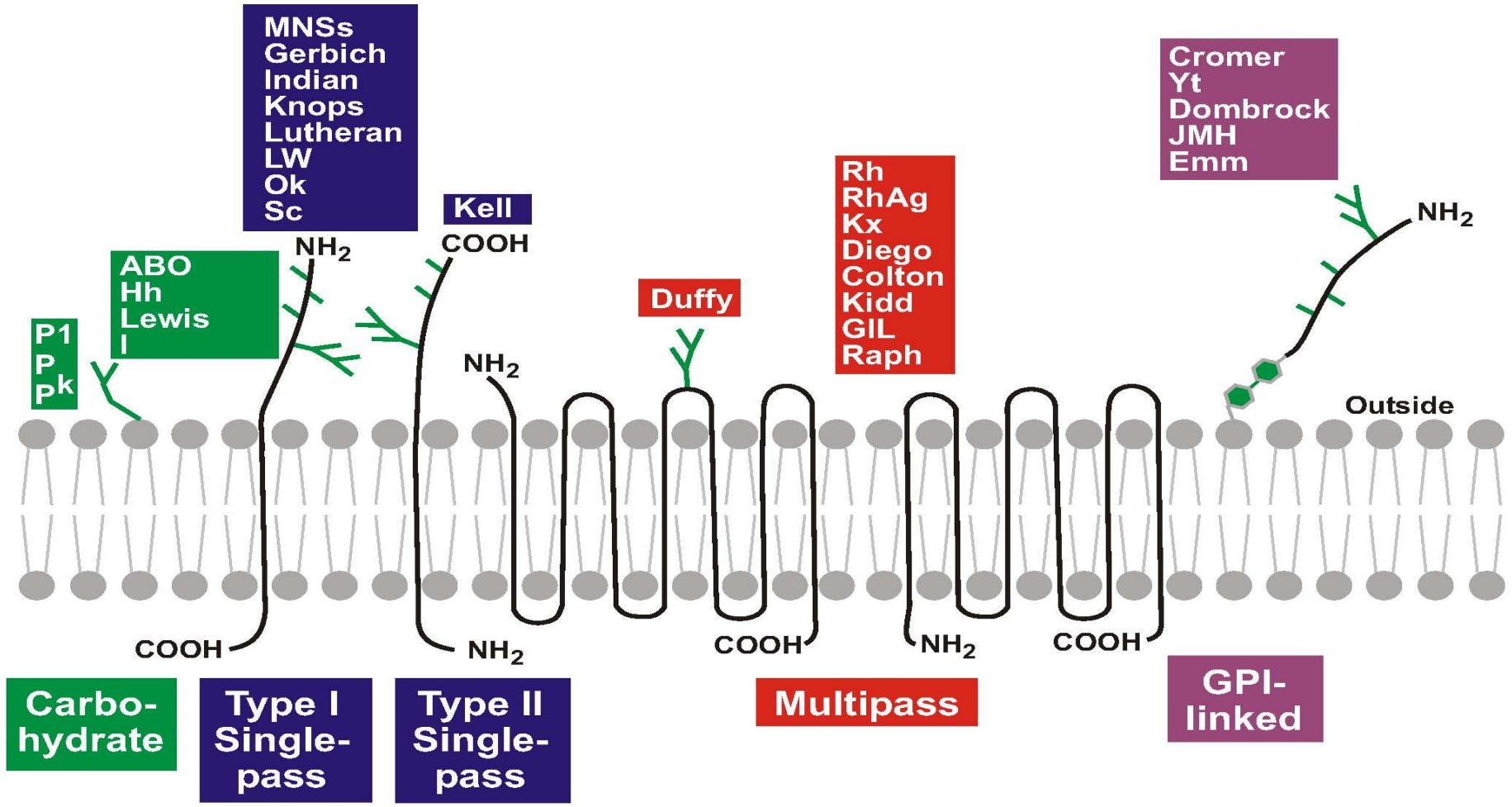
References

1. ***NATHAN AND OSKI'S HEMATOLOGY AND ONCOLOGY OF INFANCY AND CHILDHOOD, 8 th ed.,2015***
2. ***Christopher D. Hillyer,et al.,Handbook of PEDIATRIC TRANSFUSION MEDICINE,2004***
3. ***Rossi'sPrinciplesofTransfusionMedicine,5th ed.,2015***

- More than 250 Ags
- Erythrocyte antigens are polymorphic inherited structural characteristics located on proteins, glycoproteins, or glycolipids on the **outside surface of the RBC membrane.**
- Erythrocyte antigens are clinically important in the **immune destruction of RBCs** in allogeneic blood transfusions, maternal-fetal blood group incompatibility, autoimmune hemolytic anemia, and organ transplantation

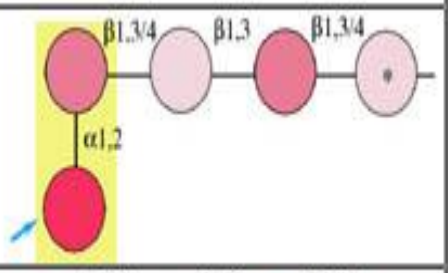
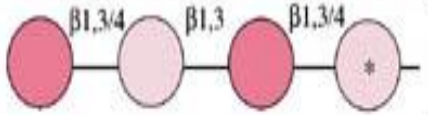
TABLE 36-1 Blood Group Systems with Associated Gene Product

Blood Group System	Gene Product
Carbohydrate Antigens	
ABO	Glycosyltransferase
P	Glycosyltransferase
Lewis	Glycosyltransferase
Hh	Glycosyltransferase
Protein Antigens	
MNS	Glycophorin A, glycophorin B
Rh	D polypeptide *RH*CE polypeptide CcEe polypeptide
Lutheran	Lutheran glycoprotein
Kell	Kell glycoprotein
Kx	Xk glycoprotein
Duffy	Fy glycoprotein
Kidd	Jk glycoprotein
Diego	Band 3 (AE1)
Yt	Acetylcholinesterase
Xg	Xg^a glycoprotein
Scianna	Sc glycoprotein
Dombrock	Glycoprotein (possibly adenosine 5′-diphosphate[ADP]-ribosyltransferase)
Colton	Channel-forming integral protein
LW	Glycoprotein
Chido/Rodgers	C′ component 4 (C4)
Gerbich	Glycophorin C, glycophorin D
Cromer	CD55 (DAF)
Knops	CD35 (CR1)
Indian	CD44



Carbohydrate blood groups

- ABO
- LEWIS
- P
- Hh

Phenotype	Structure	Minimal determinant structure
H		Fuc–α1→2–Gal–β1–R
h		Gal–β1–R

Gal GalNAc Fuc GlcNAc *: residue could be glucose in case of glycolipids; yellow shade: minimal determinant or core structure; blue arrow: residue added by blood group gene product;examples of type 1 and 2 core structures are illustrated above but they can vary widely, as they can be assembled on at least six possible types of carbohydrate chains; also they can reside on a variety of protein or lipid glycan structures containing branches, repeats, etc.

ABO Antigens & Enzymes Table

Antigen	Structure	Enzyme
"O" - H	Galactose — G1cNAC — Galactose ..... Fructose	alpha - 2 - L fucosyltransferase
A	N-acetyl-galactosamine — Galactose — G1cNAC — Galactose ..... Fructose	1, 3 acetylgalactosaminyl-transferase
B	Galactose — Galactose — G1cNAC — Galactose ..... Fructose	glycosyltransferase

(A)

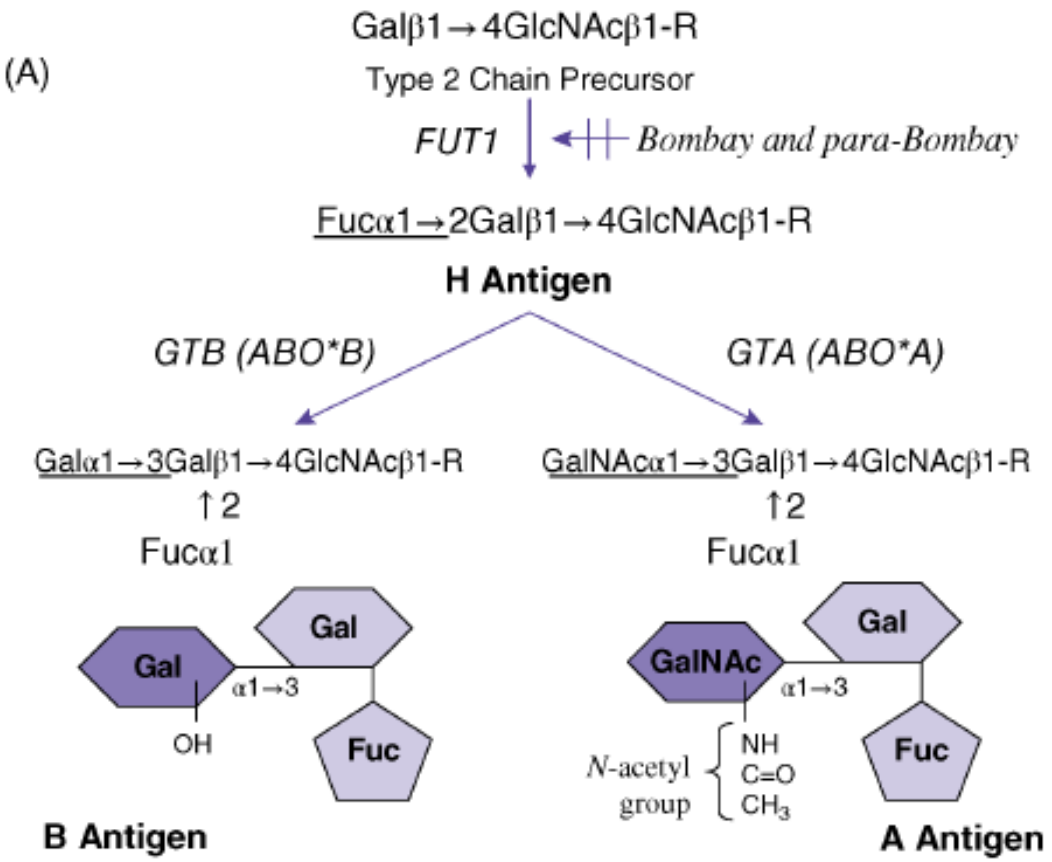


Table 13.1 Group A structures in humans

Name	Structure
Type 1 A (A-1)	GalNAcα1 → 3Galβ1 → 3GlcNAc → R^b ↑2 Fucα1
Type 1, ALe^b	GalNAcα1 → 3Galβ1 → 3GlcNAc → R ↑2 ↑4 Fucα1 Fucα1
Type 2 A (A-2)	GalNAcα1 → 3Galβ1 → 4GlcNAc → R ↑2 Fucα1
Type 3 A	(mucinous A) GalNAcα1 → 3Galβ1 → 3GalNAcβ1 → 3Galβ1 → R ↑2 ↑2 Fucα 1Fucα1
Type 4 A (globo-A, A_1)	GalNAcα1 → 3Galβ1 → 3GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glc → Cer ↑2 Fucα1

Cer, ceramide; Fuc, fucose; Gal, galactose; GalNAc, n-acetylgalactosamine; Glc, glucose; GlcNAc, n-acetylglucosamine.

Table 13.2 ABO serology

	GT Genes*		RBC Grouping (Forward or Antigen Type)			Serum Grouping (Back Type)		
ABO Type	***FUT1***	***ABO***	**Anti-A**	**Anti-B**	**UEA-1†**	**A_1 RBC**	**B RBC**	**O RBC**
A_1	+	+	++	0	0	0	+	0
A_2	+	+	+	0	+	+/0	+	0
B	+	+	0	++	0	+	0	0
O	+	0	0	0	++	+	+	0
O_h (Bombay)	0 (*hh*)	+	0	0	0	+	+	+

*Inheritance of at least one functional *FUT1* or *ABO* gene.

†Testing for H-antigen with lectin *Ulex europeaus* (UEA-1). Not routinely performed except to resolve ABO typing discrepancies.

- The fucosyltransferase (and thus the H antigen) is present in all persons except those with the rare Bombay (Oh) phenotype
- The genes for the A and B blood group antigens are codominant
- Antigens A&B are not fully developed until 2 to 4 years of age:ABO hemolytic disease of the newborn (HDN) is usually a mild disease
- Isohemagglutinins from group A and B individuals are predominantly immunoglobulin M (IgM) that do not usually cross the placenta and cause HDN.
- However, as group O serum contains IgG isohemagglutins, **ABO HDN is most frequently seen in non–group O infants of group O mothers**.

Molecular basis of ABH

- Three genes control the expression of the ABO antigens:
  - *ABO*, *Hh*, and *Se*.
- The *H* gene attaches L-fucose to the RBC membrane-anchored polypeptide
  - On red cells, platelets ,and endothelium ,ABH is primarily expressed on **type 2 chain or lactosamine** based structures.
- The secretor gene (*Se*) controls the individual's ability to secrete soluble
  - Genitourinary and gastrointestinal tissues, are rich in **type1 chain ABH antigens** : depends on secretor gen FUT2
- **The classic Bombay phenotype**: is an H-deficient nonsecretor(*hh,se/se*), with an absence of both type1 and type2 chain ABH antigens. As nonsecretors , **will also type as Le(b**)
- ***para*-Bombay** : H-deficient secretors(*hh*,*Se/Se*,or*Se/se*) and retain synthesis of type1 H antigen on mucosa and in secretions
  - Unlike Bombay cells, *para*-Bombay **red cells may have trace amounts of ABH antigen on red cells due to adsorption of soluble type1 ABH from plasma**
- **Acquired B phenotype** : group A patients transiently type as group B due to infection by deacetylase producing bacteria

Clinical Significance

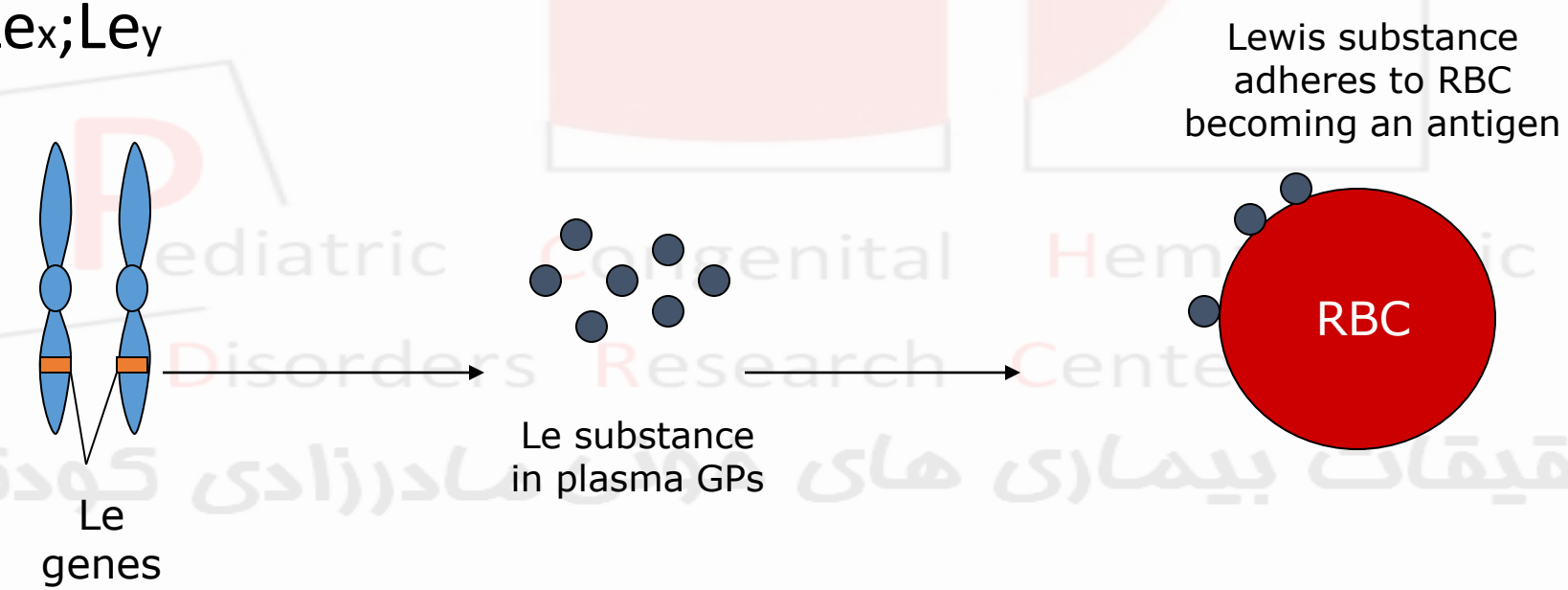
- The antibodies of the ABO system are “naturally occurring” in that they are formed as a result of exposure to ABH-like substances from the gastrointestinal tract,
- Intravascular hemolysis due to incorrect blood transfusion
- Mild HDN ; most often found in nongroup O infants of group O mothers because anti-A and anti-B from group O individuals often have a significant IgG component.

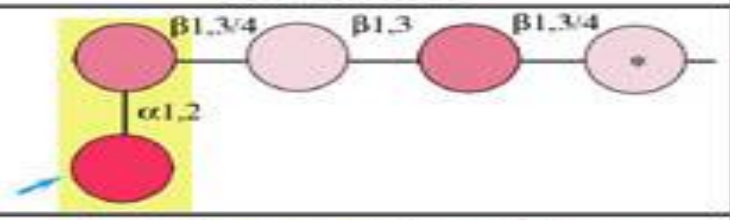
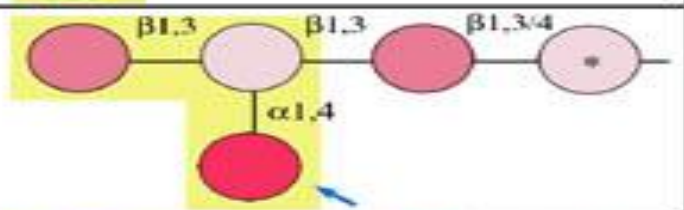
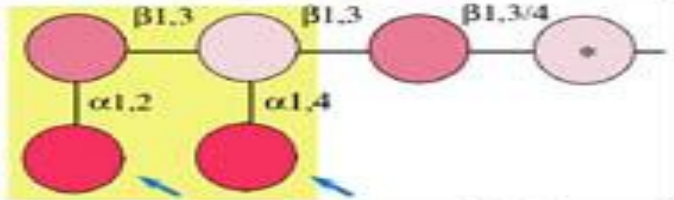
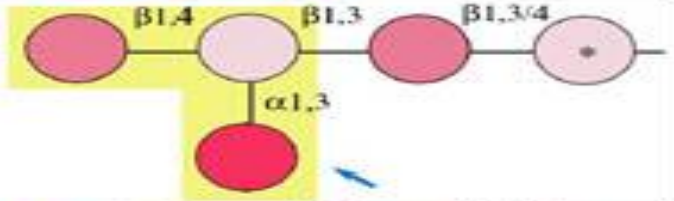
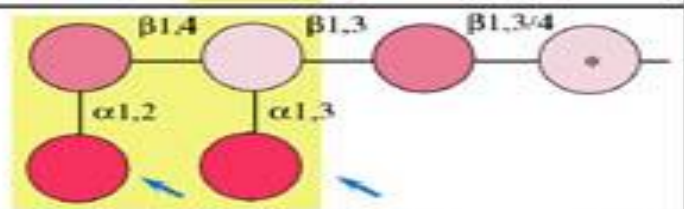
TABLE 4.3 ABO Blood Group Phenotypes and Prevalence

Phenotypes	Prevalence	
	Caucasians	African-Americans
A	40%	27%
B	11%	20%
AB	4%	4%
O	45%	49%

Lewis Antigens

- *Soluble* antigens produced by tissues and found in body fluids (plasma)
- Adsorbed on the RBC
- Le^a;Le^b; Le^x;Le^y



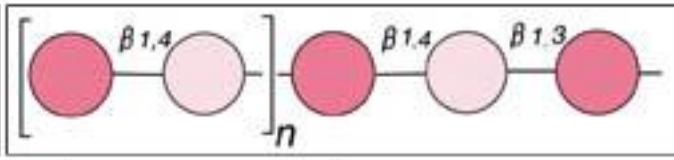
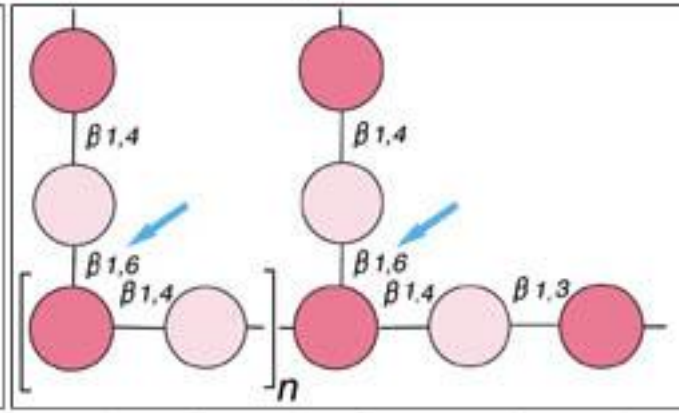
Phenotype	Structure	Minimal determinant structure
H		Fuc–α1→2–Gal–β1–R
Le^a		Gal–β1→3 GlcNAc–β1–R Fuc–α1→4
Le^b		Fuc–α1→2–Gal–β1→3 GlcNAc–β1–R Fuc–α1→4
Le^x		Gal–β1→4 GlcNAc–β1–R Fuc–α1→3
Le^y		Fuc–α1→2–Gal–β1→4 GlcNAc–β1–R Fuc–α1→3

Gal GalNAc Fuc GlcNAc *: residue could be glucose in case of glycolipids; yellow shade: minimal determinant or core structure; blue arrow: residue added by blood group gene product;examples of type 1 and 2 core structures are illustrated above but they can vary widely, as they can be assembled on at least six possible types of carbohydrate chains; also they can reside on a variety of protein or lipid glycan structures containing branches, repeats, etc.

The Lewis System

- Le^a individuals rarely make anti-Le^b. Hence in most cases Lewis antibodies are made only in individuals who are Le(a−b−)
- May be detected soon after pregnancy because **pregnant women may temporarily become Le(a-b-)**
- Le antibodies in a patient can be neutralized by the Lewis antigens in the donor's plasma (cancel each other out):
  - do not cause hemolysis except rarely when the antibody reacts at 37° C.
  - No HDN (usualy IgM)
- Expressed on H.Pylori

The Ii Collection

i epitope	
I epitope	

Gal GlcNAc ; *n* is the number of repeating lactosamine units - average of 6 for i antigen, and from 8-25 for I antigen. The structure is attached to glucose-ceramide in case of glycolipids or to N-linked sugars of the band 3 glycoprotein on red blood cells. The blue arrow designates the linkage added by the product of *IGnT*.

I antigens

- These antigens may be I or i
- They form on the precursor chain of RBC
- Newborns have i antigen
- Adults have I antigen
- i antigen (linear) converts to I (branched) as the child matures (precursor chain is more linear at birth) at about 18 months

Ii antibodies

- Auto Ab:
  - Most people have Autoanti-I (RT or 4°C)
  - Pathologic autoantibodies often react at 30° C in albumin.
  - The titer and thermal range of anti-I is often increased after infection with ***Mycoplasma pneumonia***
  - anti-i antibodies are sometimes present ***in Epstein-Barr virus infection*** (neither a sensitive nor a specific)
- Alloanti-I:
  - **Very rare**
  - Cold-reacting (RT or below) IgM antibody
  - Clinically insignificant
  - Can attach complement (no hemolysis unless it reacts at 37°)
- **Anti-I often occurs as anti-IH :**This means it will react at different strengths with reagent cells (depending on the amount of H antigen on the RBC)
  - O cells would have a strong reaction
  - A cells would have a weaker reaction

Clinical Significance

- Levels of the i antigen can aid in differentiating Diamond-Blackfan anemia from transient erythroblastopenia of childhood:
  - Is enhanced on RBCs from patients with Diamond-Blackfan anemia (reflects stress hematopoiesis)
  - Is absent or of reduced strength on RBCs from children with transient erythroblastopenia of childhood(selective suppression of erythropoiesis) /enhanced during recovery

P Antigen

- Similar to the ABO system
- The most common phenotypes are P_1 and P_2
  - P_1 – consists of P_1 and P antigens
  - P_2 – consists of only P antigens
- Like the A_2 subgroup, P_2 groups can produce anti-P_1
- The P1 antigen expression:
  - is more strongly expressed on fetal cells than on neonatal cells
  - Adult levels are not reached until 7 years of age.
  - 75% of adults have P_1
  - Strength of the antigen **decreases** upon storage
- Found in secretions like plasma and **hydatid cyst fluid**

P antibodies

- Anti-P_1
  - Naturally occurring IgM
  - Not clinically significant: (cold reacting Ab) no hemolysis; no HDN
  - Can be neutralized by hydatid cyst fluid to reveal more clinically significant antibodies
- Anti-P
  - Produced in individuals with **paroxysmal cold hemoglobinuria (PCH)**
  - This PCH antibody is also called the **Donath-Landsteiner antibody**
  - most frequently seen in children
  - This IgG **autoantibody** is a **biphasic hemolysin** that binds to RBCs in the cold and then hemolyzes them when warmed:IgG auto-anti-P attaches complement when cold (fingers, toes). As the red cells circulate, they begin to lyse (releasing Hgb)
  - This antibody should be considered when the patient has hemoglobinuria or anemia (or both) and C3 alone is present on the RBCs.

Clinical Significance

- The P antigen serves as a receptor for parvovirus B19 and pyelonephritic *Escherichia coli:*
- People with the rare p phenotype:
  - may produce anti-P1+P+Pk, a potent hemolytic antibody that can cause immediate hemolytic transfusion reactions; HDN; fetal death; and miscarriages during early pregnancy in women whose RBCs have the p phenotype
  - p phenotype lack the antigens P1, P, and Pk and are not susceptible to infection by parvovirus B19

Protein blood groups

- Rh system
- MNS system
- Kell sustem
- Duffy system
- Kidd system
- Lutheran system

Rh system

- Includes D, C, c, E, e, and 40 other antigens
- Rh Associated Glycoprotein (RHAG) is required for cell surface expression of the Rh antigens. D, C, c, E, and e antigens , encoded by at least two genes on chromosome 1
- Only approximately 22% of D-negative patients transfused with D-positive RBCs make an anti-D antibody
- Weak D:
  - 0.2% to 1% of white individuals
  - Fewer D Ag on RBCs
  - Safely recieve D+
  - Weak D donor RBCs are labeled as D+
  - All D-negative donor blood is tested for weak D by the antiglobulin test
- Partial D:
  - Truncated D
  - Can make Anti-D ,despite of their phenotype which may be interpreted as weak D or D+
- Rh deficient syndrom(Rh Null):
  - Lack of RHAG
  - Mild spherocytic hemolytic anemia

Rh Antibodies

- Rh(D) antigen has greater immunogenicity than virtually any other RBC antigen, followed by Rh(c) and Rh(E).
- Most Rh antibodies result from exposure to human RBCs through pregnancy or transfusion.
- Are almost always IgG and do not bind complement:
  - Extravascular RBC destruction ; HDN; mild to severe delayed transfusion reactions

TABLE 4.5 Prevalence of Rh Antigen-Negative Phenotypes

Phenotypes	Prevalence		
	Caucasians	African-Americans	Asians
D-negative	15%	8%	1%
C-negative	32%	73%	7%
E-negative	71%	78%	61%
c-negative	20%	4%	53%
e-negative	2%	2%	4%

R1=CDe
R2=cDE
R0=cDe
Rz=CDE
r=ce
r`=Ce
r``=cE

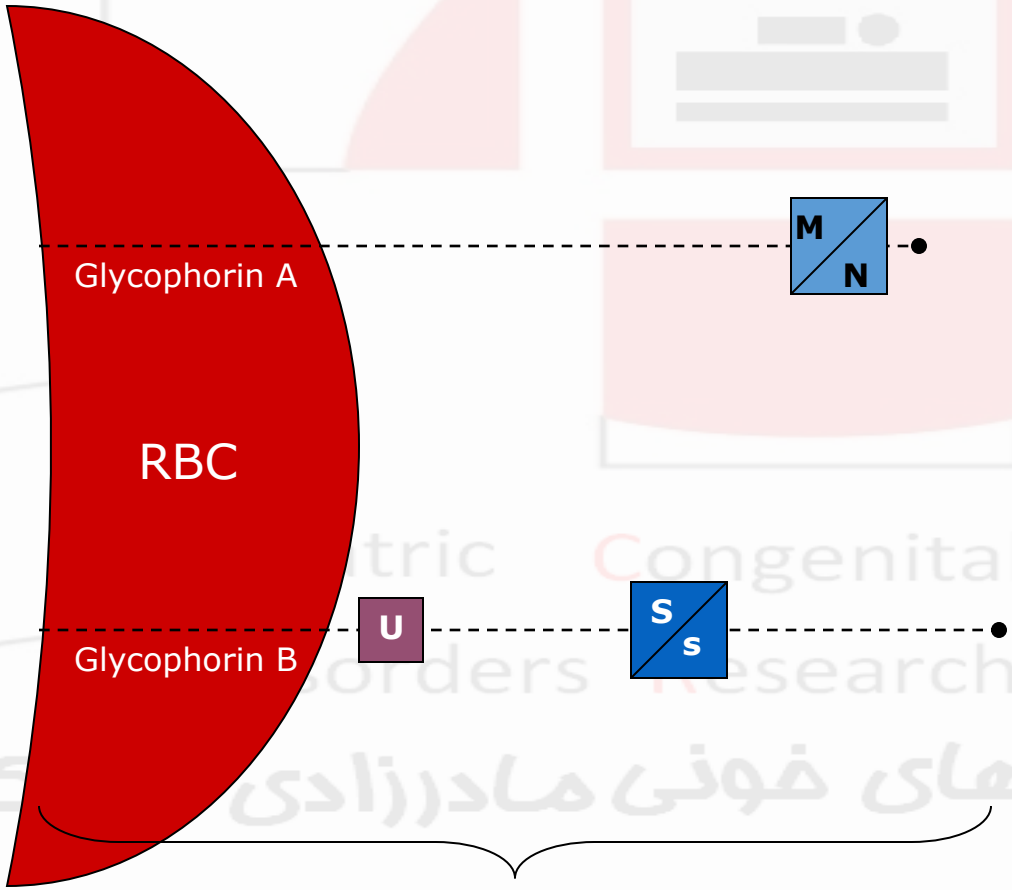
TABLE 4.4 Rh Blood Group Phenotypes and Prevalence

Antigens	Phenotypes	Prevalence		
		Caucasians	African-Americans	Asians
CcDe	R_1r	34.9%	21%	8.5%
CDe	R_1R_1	18.5%	2%	51.8%
CcDEe	R_1R_2	13.3%	4%	30%
cDe	R_or	2.1%	45.8%	0.3%
cDEe	R_2r	11.8%	18.6%	2.5%
cDE	R_2R_2	2.3%	0.2%	4.4%
CDEe	R_1R_z	0.2%	Rare	1.4%
CcDe	R_2R_z	0.1%	Rare	0.4%
CDE	R_zR_z	0.01%	Rare	Rare
cde	rr	15.1%	6.8%	0.1%
Cce	r′r	0.8%	Rare	0.1%
cEe	r″r	0.9%	Rare	Rare
CcEe	r′r″	0.05%	Rare	Rare

MNSs Blood System

- 4 important antigens (more exist):
  - M
  - N
  - S
  - s
  - U :
    - ALWAYS present when S & s are inherited
    - U-negative cells are only found in the Black population
- M & N located on Glycophorin A
- S & s and U located on Glycophorin B
  - Remember: Glycophorin is a protein that carries many RBC antigens
- RBCs lacking GPA, GPB, or GPC are resistant to invasion by *Plasmodium falciparum* to varying degrees

MNSs Antigens



M & N only differ in their amino acid sequence at positions 1 and 5

S & s only differ in their amino acid sequence at position 29

Frequency of MNSs antigens

Phenotypes	Blacks (%)	Whites (%)
M+	74	78
N+	75	72
S+	30.5	55
s+	94	89
U+	**99**	**99.9**

High-incidence antigen

Antibodies

- Anti-M and anti-N
  - IgM (rarely IgG): often naturally occurred
  - Clinically insignificant
  - If IgG, that react at 37° C may cause hemolysis of transfused cells and could be implicated in HDN (RARE)
- Anti-S, Anti-s, and Anti-U:
  - **Clinically significant**
  - **IgG ; occur after stimulation**
  - Can cause RBC destruction and HDN
  - Anti-U
    - will react with S+ or s+ red cells
    - Usually occurs in S-s- cells
    - Can only give U-negative blood units found in <1% of Black population
    - Contact rare donor registry

MNSs Antibody Characteristics

Antibody	Ig Class	Clinically significant
Anti-M	IgM (rare IgG)	No
Anti-N	IgM	No
Anti-S	IgG	Yes
Anti-s	IgG	Yes
Anti-U	IgG	Yes

Kell System

- Similar to the Rh system
- over 20 Ags exist
- 2 major antigens
  - K (Kell), <9% of population (K-Neg donors are easy to find)
  - k (cellano), >90% of population (k-Neg donors are not easy to find)
- The *K* and *k* genes are codominant alleles on chromosome 7 that code for the antigens
- Well developed at birth
- The K antigen is *very* immunogenic (2nd to the D antigen) in stimulating antibody production: acute and delayed transfusion reactions ; hemolysis; HDN

Other Kell antigens

- Other sets of alleles also exist in the Kell system: Analogous to the Rh system: *C/c* and *E/e*
- **Kp antigens**
  - Kp^a is a low frequency antigen (only 2%)
  - Kp^b is a high frequency antigen (99.9%)
- **Js antigens**
  - Js^a (20% in Blacks, 0.1% in Whites)
  - Js^b is high frequency (80-100%)
- Antibodies to other antigens on the Kell protein, such as Kp^a, Kp^b, Js^a, and Js^b, are less common but are also clinically significant.

Kx antigen

- Not a part of the Kell system, but is related
  - The XK protein is encoded by a gene on the X chromosome
  - Kx antigens are present in small amounts in individuals with normal Kell antigens
  - Kx antigens are increased in those who have no expression of Kell antigens($K_{0)}$

$Kell_{null}$ or K_0

- No expression of Kell antigens except a related antigen called **Kx**
- As a result of transfusion, K_0 individuals can develop anti-Ku (Ku is on RBCs that have Kell antigens)
- Rare Kell negative units should be given

Kell antibodies

- IgG (react well at AHG)
- Produced as a result of immune stimulation (transfusion, pregnancy)
- Clinically significant
- **Anti-K is most common** because the K antigen is extremely immunogenic
- k, Kp^b, and Js^b antibodies are rare (many individuals have these antigens and won't develop an antibody) since few donors have the antigen **and accordingly are not easy to find compatible blood for them.**

McLeod Syndrome

- No XK Ag and diminished other K antigens:
  - **do not make anti-Kx and** can be transfused with McLeod (KX neg) or Ko type blood
- Almost exclusive in White males
- Causes abnormal red cell morphologies and decreased red cell survival:
  - Acanthocytes – spur cells (defected cell membrane)
  - teardrop erythrocytes, and bizarre poikilocytes
  - Reticulocytes – immature red cells
- Other systemic problems:
  - subclinical myopathy
  - Progressive neuropathy
  - Psychiatric symptoms, and cognitive changes.
  - Cardiac symptoms, such as dilated cardiomyopathy and arrhythmias.
- Associated McLeod Syndrome with **chronic granulomatous disease (CGD):**
  - usually make antibodies to the XK and Kell protein in case of stimulation: **should receive McLeod RBC(K0 & KX neg)**

Kidd Blood Group

- 2 antigens :Jk^a and Jk^b (codominant alleles)
- Well developed at birth

Genotype	Phenotype	Whites (%)	Blacks (%)
Jk^aJk^a	Jk(a+b-)	26.3	51.1
Jk^aJk^b	Jk(a+b+	50.3	40.8
Jk^bJk^b	Jk(a-b+)	23.4	8.1
JkJk	Jk(a-b-)	rare	rare

Kidd antibodies

- Anti-Jk^{a} and Anti-Jk^{b}
  - usually IgG, but may be a mixture of IgG and IgM. *They often bind complement lead to sever Intravascular* HTR
  - **Common cause of delayed HTR**
  - HDN
  - **Anamnestic or "rebound" phenomenon** :
    - **Are often weak** and may become undetectable over time,
    - they may **escape detection**
    - **increase in the titer of the antibody and hemolysis of the transfused antigen-positive RBCs**.
  - Usually appears with other antibodies when detected
- Anti-Jk3
  - Found in some individuals who are Jk(a-b-)
  - Far East and Pacific Islanders (RARE)

Duffy Blood Group

- Predominant genes (codominant alleles):
  - *Fy*^{a} and *Fy*^{b} code for antigens that are well developed at birth
  - Antigens are destroyed by enzymes
- **Most African-Americans are Fy(a-b-)**
  - Interestingly, certain malarial parasites (*Plasmodium knowlesi* and *P. vivax*) will not invade Fy^{a} and Fy^{b} negative cells

Duffy antibodies

- IgG
- Do not bind complement
- Clinically significant :HTR
- Stimulated by transfusion or pregnancy
- Anti-Fya has caused mild HDN, but anti-Fyb has not been implicated
- Do not react with enzyme treated RBCs

Lutheran Blood Group System

- 2 codominant alleles: Lu^a and Lu^b
- Weakly expressed on cord blood cells
- Most individuals (92%) have the Lu^b antigen, Lu(a-b+)
- The Lu(a-b-) phenotype is RARE ; associated with acanthocytosis but no hemolysis
- Lu glycoproteins may be involved in the pathogenesis of sickle cell vaso-occlusive crises and may also be involved in the metastasis of certain types of malignancy.

Lutheran antibodies

- Anti-Lu^{a}
  - IgM and IgG
  - Not clinically significant
  - Reacts at room temperature
  - Mild HDN
  - Naturally occurring or immune stimulated
- Anti-Lu^{b}
  - Rare because Lu^{b} is high incidence antigen
  - IgG
  - Associated with transfusion reactions (rare HDN)

Maturation of Blood Group Antigens

- Several blood group antigens are not expressed or are only weakly expressed on cord RBCs and usually reach adult levels by 2 years of age
  - Antibodies to these antigens are unlikely to cause HDN.
- **No cord blood express:Le$_a$**, Sd$_a$, Ch, Rg, or AnWj antigens.
- **Weak cord blood express:** A, B, H, I, Le$_b$, P1, Lu$_a$ (but not Lu$_b$), Yt$_a$, Vel, Do$_a$, Do$_b$, Gy$_a$, Hy, Jo$_a$, Xg$_a$, and Bg

TABLE 4.2 Clinical Significance of Antibodies to the Major Blood Group Antigens

Usually Clinically Significant	Sometimes Clinically Significant	Clinically Insignificant If Not Reactive at 37°C	Generally Clinically Insignificant
A and B	At[a]	A_1	Bg
Diego	Colton	H	Chido/Rogers
Duffy	Cromer	Le[a]	Cost
H in O_h	Dombrock	Lutheran	JMH
Kell	Gerbich	M and N	Knops
Kidd	Indian	P1	Le[b]
P, PP1P[k]	Jr[a]	Sd[a]	Xg[a]
Rh	Lan		
S, s, and U	LW		
Vel	Scianna		
	Yt		

Importance of the Abs

Clinically significant antibodies occur in the following order, **from most commonly to least commonly** encountered in transfusion practice:

1. anti-D,
2. anti-K,
3. anti-E,
4. anti-c,
5. anti-Fya,
6. anti-C,
7. anti-Jka,
8. anti-S,
9. anti-Jkb

clinically insignificant unless the alloantibodies are reactive in tests performed at 37 0 :

- Anti-P1,
- anti-M,
- anti-N,
- anti-Lua (Lutheran),
- anti-Lea,
- anti-Leb,
- anti-Sda