ABO and Rh blood group systems

The molecular structure, physiological and clinical role of red cell antigens

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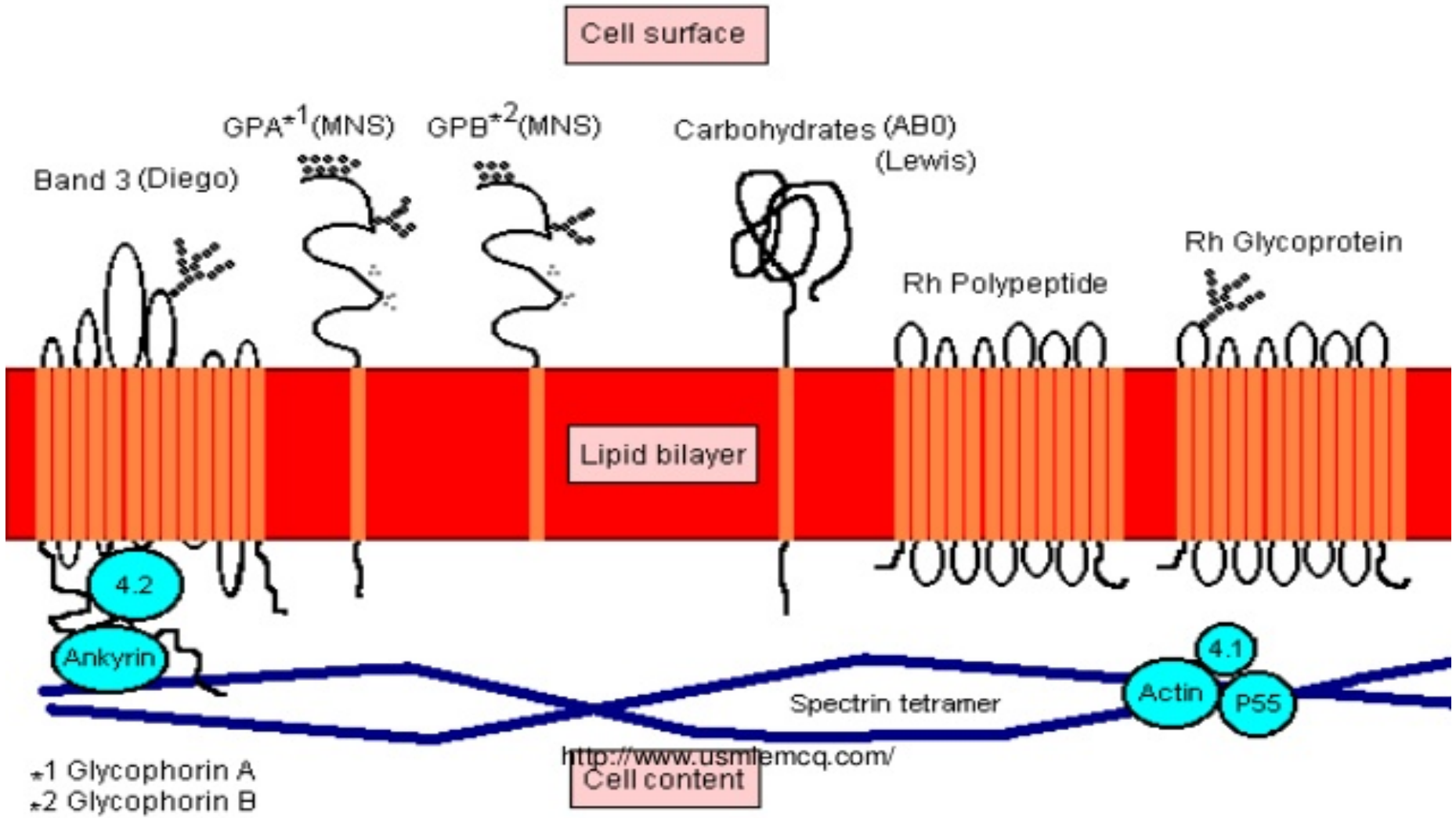
Structure of the Red Cell Membrane



Blood group system

- International Society of Blood Transfusion (ISBT)
  - http://www.isbtweb.org
  - Genetic classification
  - 43 formally registered blood group systems

No.	System name	System symbol	Gene name(s)*	Number of antigens
001	ABO	ABO	*ABO*	4
002	MNS	MNS	*GYPA, GYPB, (GYPE)*	50
003	P1PK	P1PK	*A4GALT*	3
004	Rh	RH	*RHD, RHCE*	55
005	Lutheran	LU	*BCAM*	27
006	Kell	KEL	*KEL*	36
007	Lewis	LE	*FUT3*	6
008	Duffy	FY	*ACKR1*	5
009	Kidd	JK	*SLC14A1*	3

ABO blood group system

- Discovered by Karl Landsteiner in 1900, 1901
  - Three groups: A, B and O (he called C)
  - The fourth group, AB was discovered by Decastello and Sturli in 1902

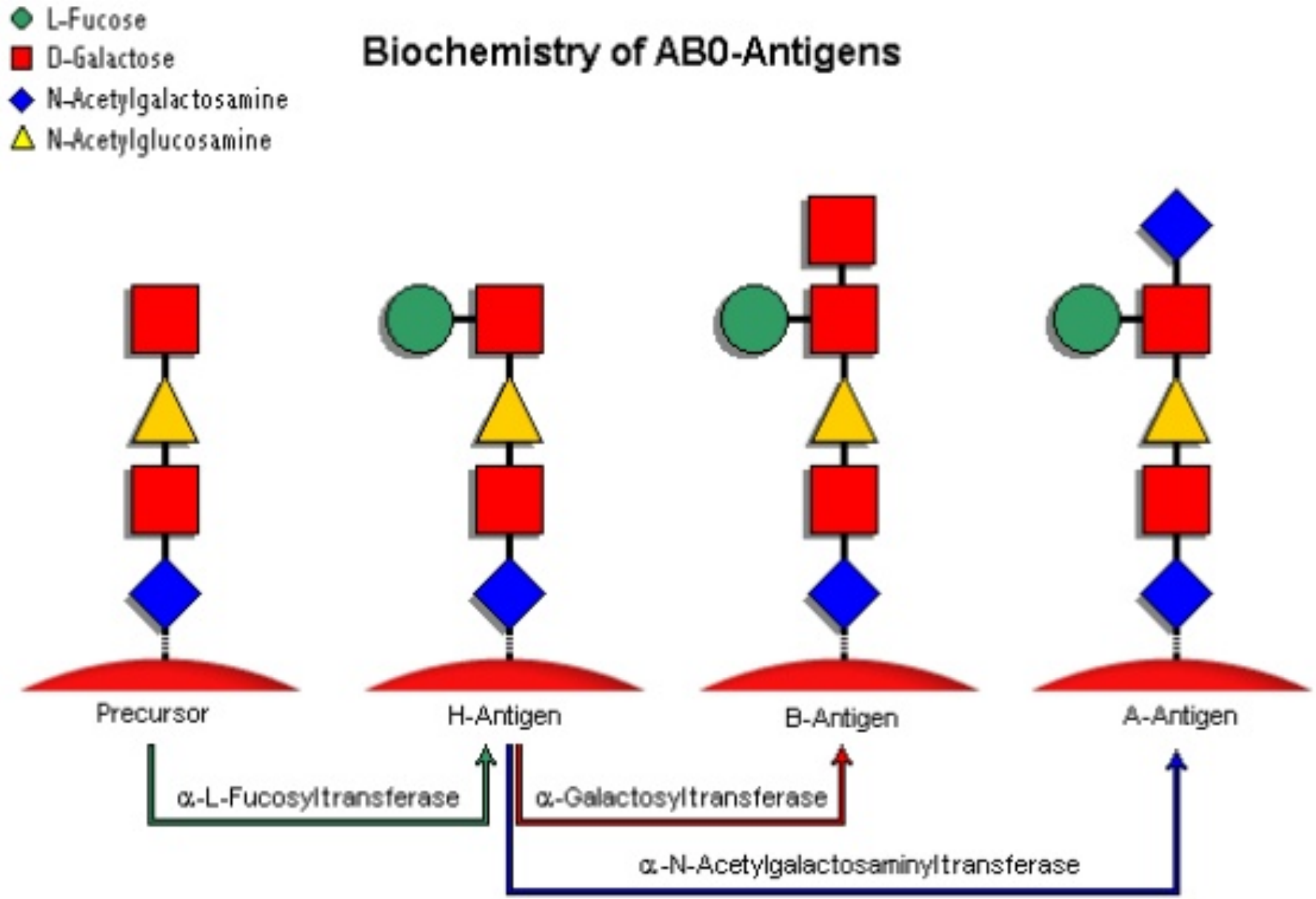
- Most important system in transfusion practice
  - natural occurrence of anti-A and/or anti-B antibodies that react at 37 °C
  - ABO incompatible transfusion causes an acute hemolytic transfusion reaction (AHTR)
    - Intravascular hemolysis of red blood cells

Genetics of ABO blood groups

- Mendelian inheritance
- The direct products of genes are glycosyl-transferases
  - these enzymes transport and add the appropriate sugars to the cell membrane precursor glycoproteins and determine antigen specificity
- *H/h* gene:
  - *H* gene plays a major role in ABO phenotypes
  - *H* allele (dominant) codes indirectly the H antigen, the precursor of A and B antigen
  - *h* allele (recessive)
    - hh genotype - Bombay phenotype (Oh)
    - no H antigen on red cells

Genetics of ABO blood group system

- *ABO* gene:
- located on chromosome number 9
  - *A* allele codes the A-transferase:
    - H antigen ⟶ A antigen
  - *B* allele codes the B-transferase:
    - H antigen ⟶ B antigen
  - Alleles *A* and *B* are codominant
  - *O* allele is recessive


L-Fucose
D-Galactose
N-Acetylgalactosamine
N-Acetylglucosamine
Biochemistry of ABO-Antigens
Precursor
H-Antigen
B-Antigen
A-Antigen
α-L-Fucosyltransferase
α-Galactosyltransferase
α-N-Acetylgalactosaminyltransferase

ABO phenotype	ABO genotype	Surface antigens on cells
A	*A/A* or *A/O*	A antigen
B	*B/B* or *B/O*	B antigen
AB	*A/B*	A and B antigens
O	*O/O*	H antigen

Antigens of the ABO system

- **A**, **B** and **H** antigen
- Carbohydrate structures (glycoproteins)
- Antigens can be detected on the red cells of 5-6-week-old embryos
  - at birth the number of antigen sites is less , maximum level are reached by the age of 2-4 years
- Present on most tissues: RBCs, lymphocytes, platelets, endothelial and epithelial cells (histocompatibility system)
- Soluble form in secretions: saliva, tears, urine, milk...
  - Only in secretors (controlled by Se gene)

Table 4.4 Various estimates of the number of A, B and H sites on red cells of different phenotypes from adults and newborn infants.

	Sites × 10^6 per red cell	Reference*
A sites†		
A_1 adults	0.83	(1)
A_1 adults	0.81–1.17	(2)
A_1 adults	0.85	(3)
Newborn	0.25–0.37	(2)
A_2 adults	0.24–0.29	(2)
A_2 adults	0.24	(3)
Newborn	0.14	(2)
A_1B adults	0.46–0.85	(2)
Newborn	0.22	(2)
A_2B adults	0.14	(2)
B sites†		
B adults	0.75	(2)
Newborn	0.2–0.32	(4)
A_1B adults	0.43	(2)
H sites		
O adults	1.7	(5)
Newborn	0.325	(5)
A, B, AB newborn	0.07	(5)

Number of ABO antigens:

A antigens:

- A1 adults:
  800.000 – 1.000.000/RBC
- A1 newborns:
  200.000 – 300.000/RBC
- A2 adults:
  200.000 – 300.000/RBC

B antigens:

- B adults:
  700.000 – 800.000/RBC

H antigens:

- 0 adults:
  1.500.000– 2.000.000/RBC

Frequencies of ABO groups

Table 4.2 Frequencies of ABO groups in a few selected populations.

Population* (no. tested)	Percentage of various phenotypes						Special characteristics
	O	A_1	A_2	B	A_1B	A_2B	
South American Indians (539)	100	0	0	0	0	0	All O
Vietnamese (220)	45	21.4	0	29.1	4.5	0	No A_2; B commoner than A
Australian aborigines (126)	44.4	55.6	0	0	0	0	No A_2 or B
Germans (100 000)	42.8	32.5	9.4	11.0	3.1	1.1	
Bengalese (241)	22	22.2	1.8	38.2	14.8	0.9	B commonest
Lapps (324)	18.2	36.1	18.5	4.8	6.2	6.2	Very high A_2

*The figures are for selected populations and do not necessarily apply to the racial group as a whole.
Source: Data from Mourant *et al.* 1976.

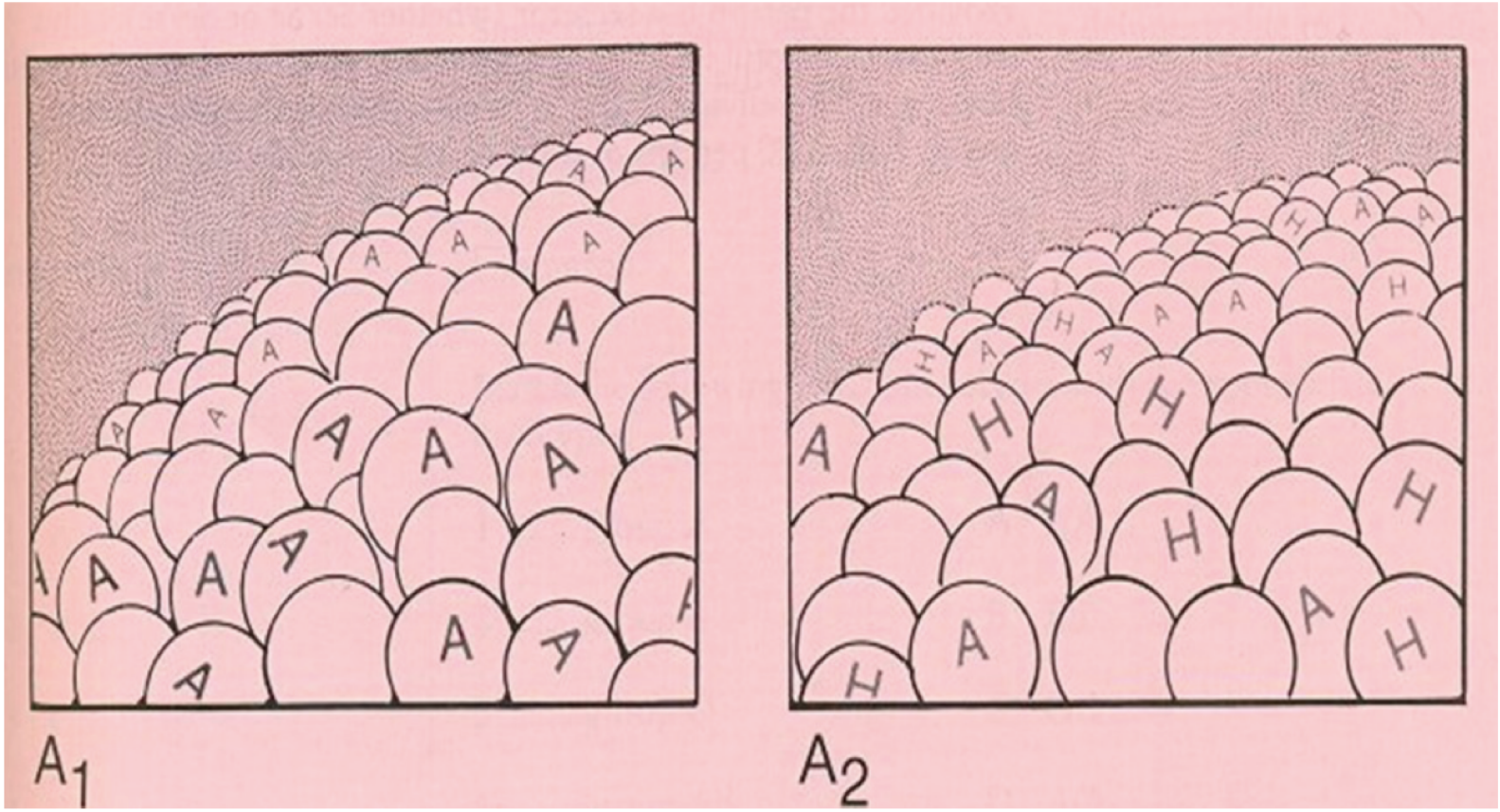
Hungarians (1004916)**	31,05	42,38	17,9	8,67

**Data from Rex-Kiss, Szabó 1978.

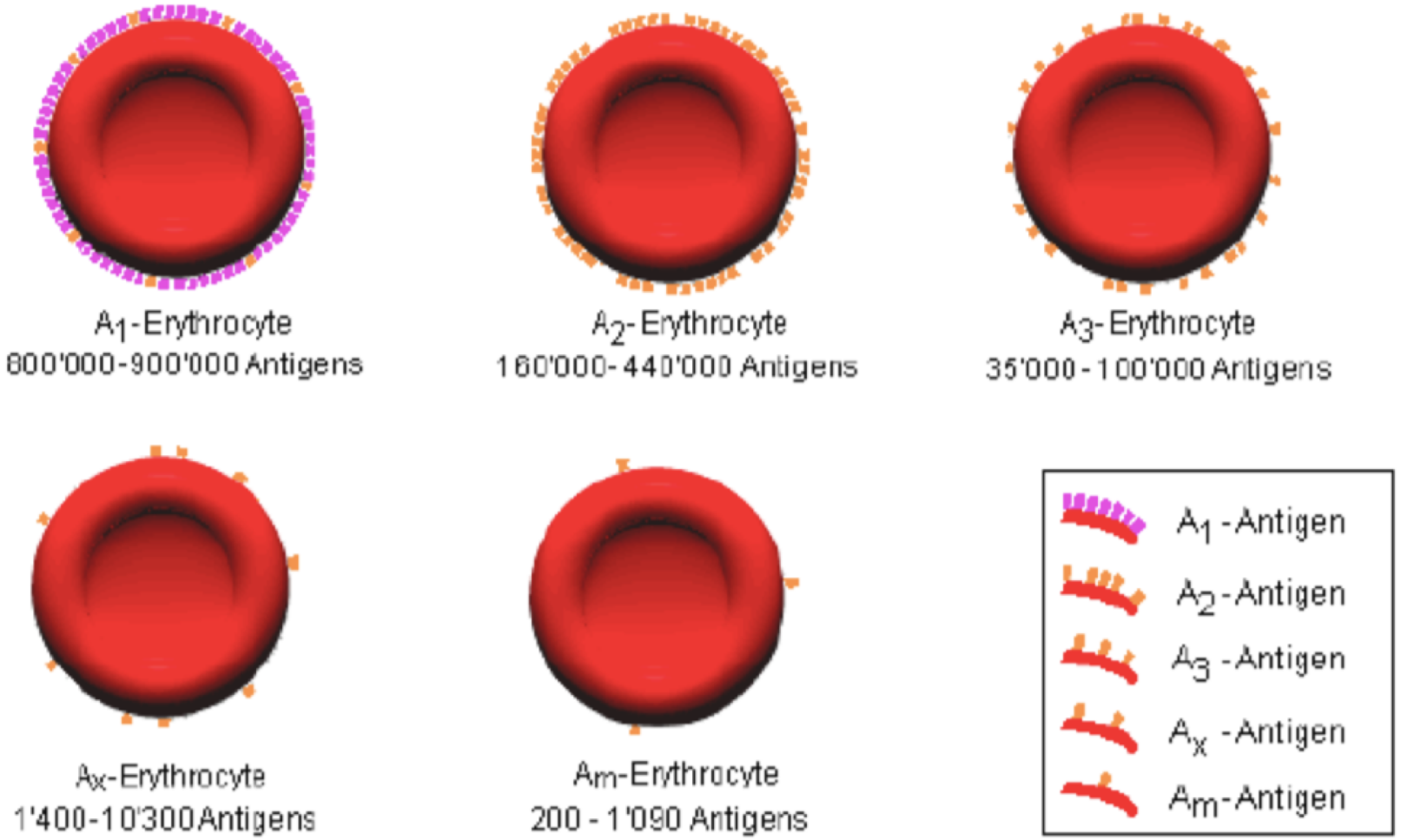
Subgroups of A

- Most common:
  - A1:
    - Frequency: 80 %
    - Nearly all H antigens are transformed into A antigens by A1-transferase
    - Strong agglutination with anti-A reagent
  - A2:
    - Frequency: 19 % (in Europeans)
    - Single nucleotide deletion in A allele
    - A2-transferase is less effective than A1-transferase
    - the number of A sites is substantially lower on A2 than A1 red cells
    - Less A antigens mean more H antigen sites, so agglutination is weak with anti-A reagent

Subgroups of A



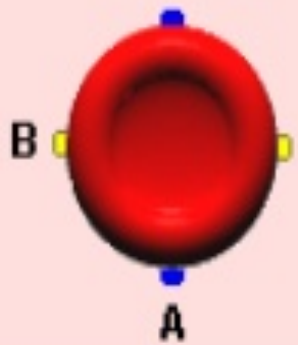
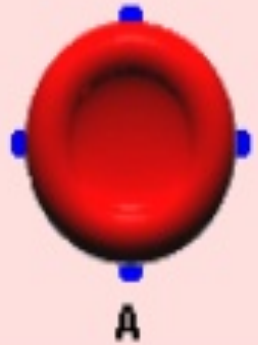
Antigen Frequency in Blood Group A Subgroups



Rare subgroups are e.g.: A3, Ax, Am, Ael and B3, Bx, Bm
Minimum of 250.000– 400.000 A sites/red cell are needed for agglutination by anti-A reagent.

Antibodies of the ABO system

- **Anti-A** and **anti-B** (anti-A,B in group O)
  - Regular antibodies= natural occurence in plasma
    - only at ABO system, thus ABO is the most important blood group system
    - they are non-red cell stimulated
  - Mainly IgM (small amount of IgG, IgA)
  - develop between 3-6 months of age due to unknown antigenic stimuli, presumably bacteria and foodstuffs

Isoagglutinins		Anti-B	Anti- A	Anti-B Anti- A
Erythrocytes with surface antigens				
Blood groups	AB	A	B	0

Landsteiner's law

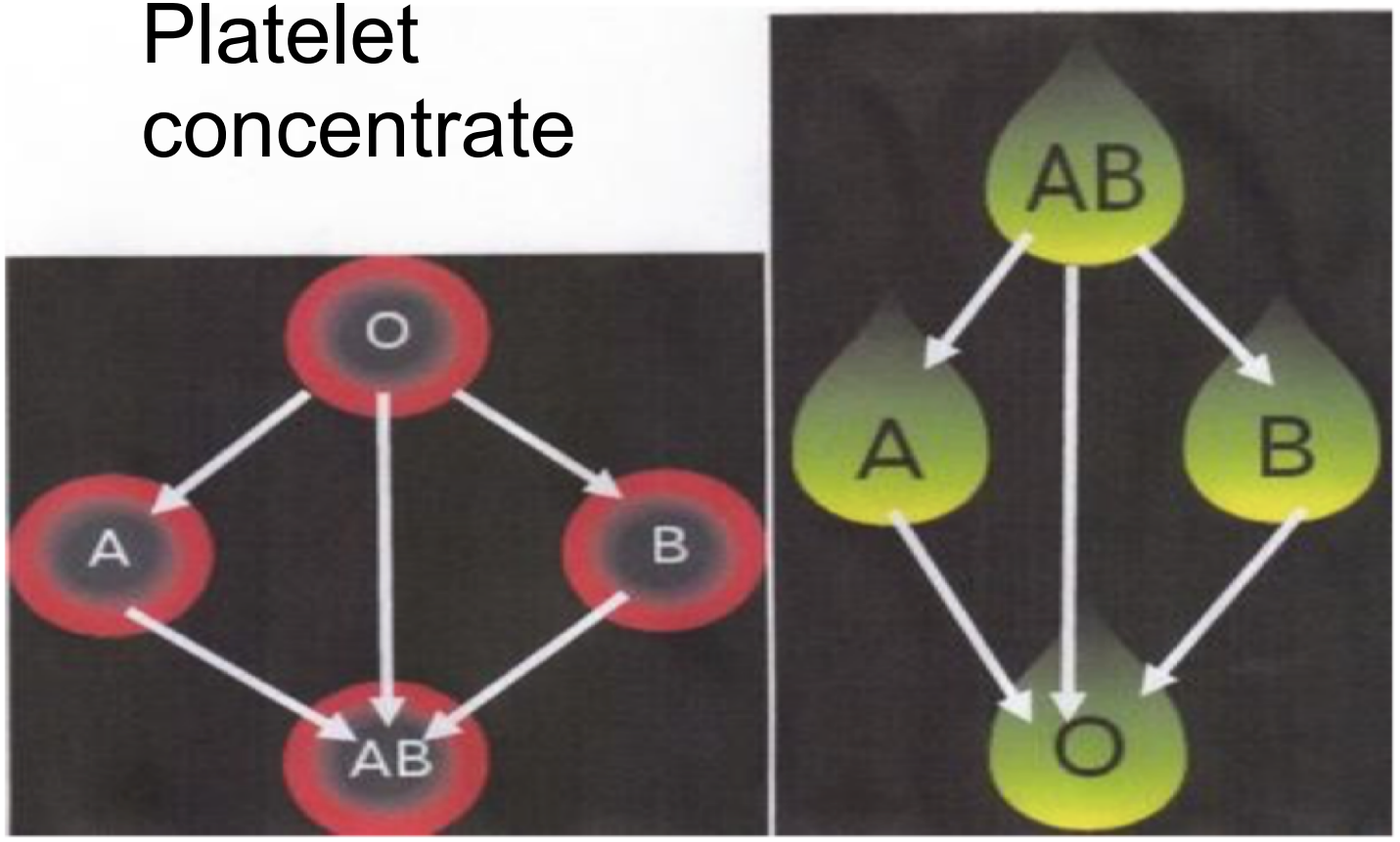
- If an agglutinogen (A or B antigen) is present on the RBCs, then the corresponding agglutinin (anti-A or anti-B) must be absent in the plasma

- If an agglutinogen (A or B antigen) is absent on the RBCs, then the corresponding agglutinin (anti-A or anti-B) must be present in the plasma

ABO compatibility

Red cell or Platelet concentrate

FFP



Donor → Recipient

BLOOD TYPES

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TYPE	YOU CAN GIVE BLOOD TO	YOU CAN RECEIVE BLOOD FROM
A+	A+ , AB+	A+ , A- , O+ , O-
O+	O+ , A+ , B+ , AB+	O+ , O-
B+	B+ , AB+	B+ , B- , O+ , O-
AB+	AB+	Everyone
A-	A+ , A- , AB+ , AB-	A- , O-
O-	Everyone	O-
B-	B+ , B- , AB+ , AB-	B- , O-
AB-	AB+ , AB-	AB- , A- , B- , O-

Bombay phenotype

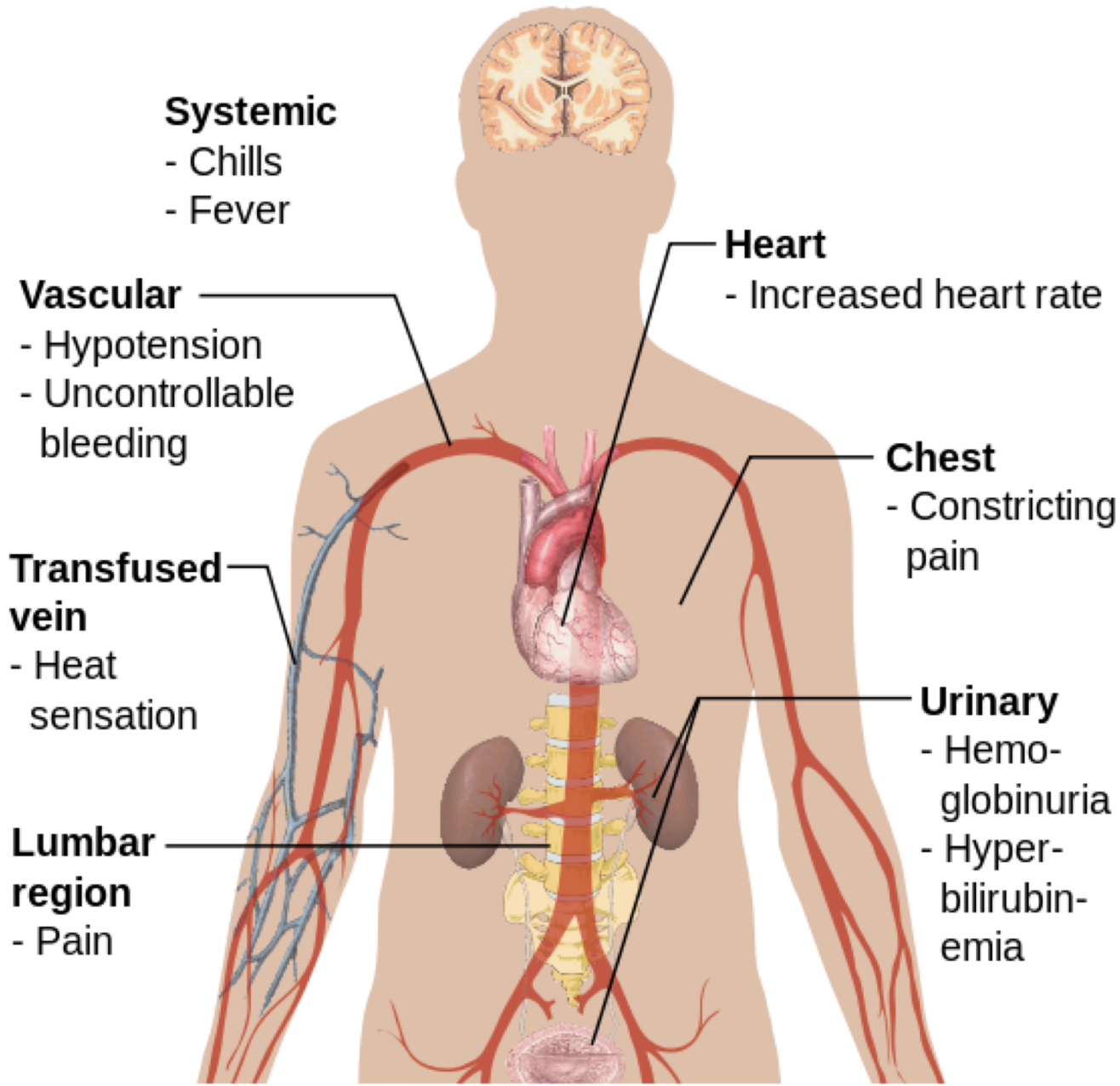
- 1952 Bombay – first case
- *hh* genotype, Oh phenotype:
  - Very rare (1:250000)
  - No H-transferase
  - No H antigen is made on RBCs
  - Therefore A and B antigen are also lacking
  - Not only anti-A and anti-B antibodies are present in the serum, but also anti-H as regular antibody
  - Because of anti-H, O red cells are not compatible, only Oh donor or autotransfusion

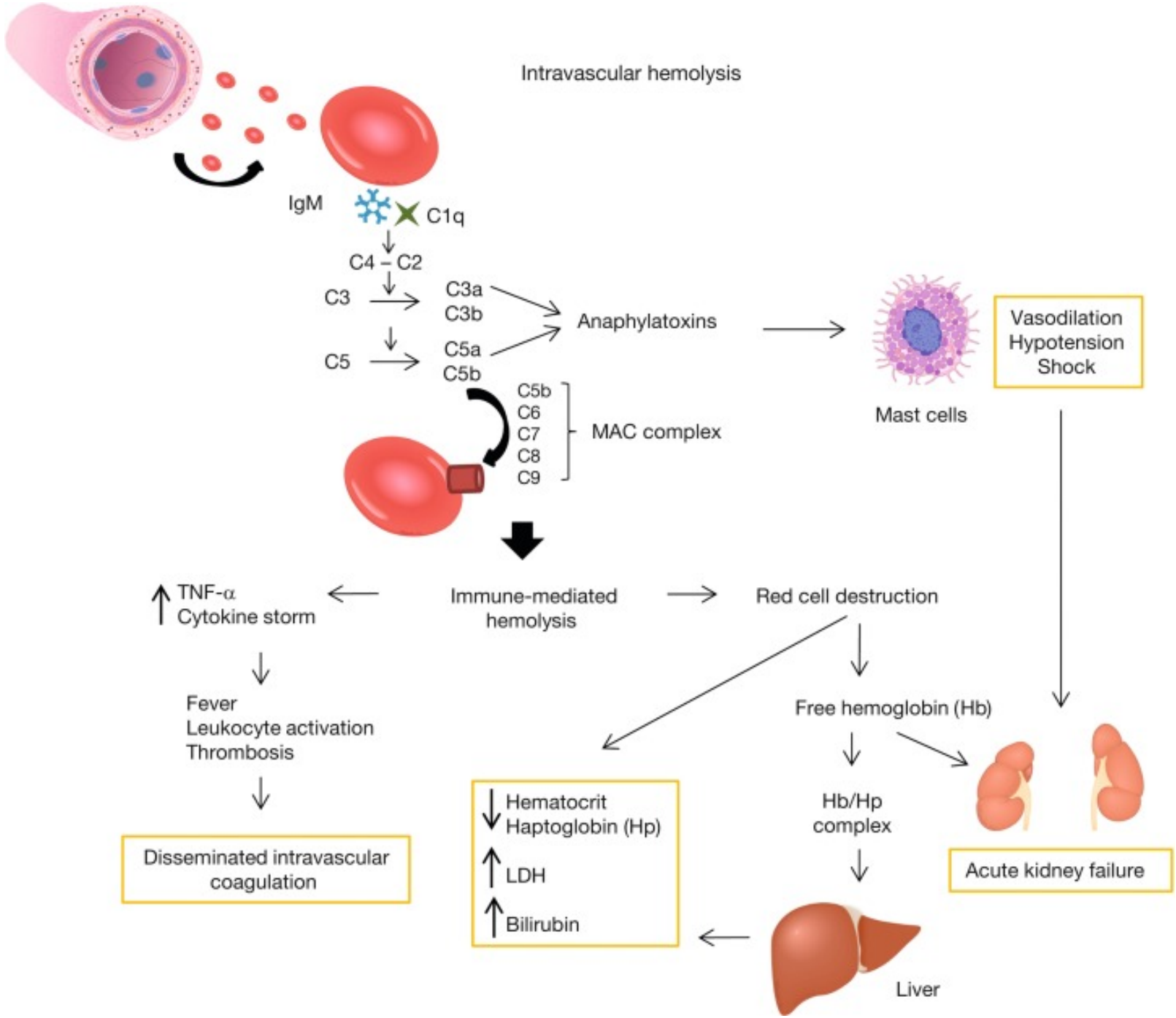
Clinical relevance of ABO system

- ABO incompatible transfusion causes acute immune-mediated hemolytic transfusion reaction
  - Transfusion of incompatible red cells:
    - Recipient has ABO antibodies to donor’s ABO antigens (major incompatibility)
    - intravascular hemolysis of donor's RBCs
    - e.g.: A ⟶ O
  - Transfusion of incompatible FFP:
    - Donor has ABO antibodies to recipient’s ABO antigens (minor incompatibility)
    - intravascular hemolysis of recipient's red cells
    - e.g.: O ⟶ A

Main symptoms of

Acute hemolytic reaction




Intravascular hemolysis
IgM
C1q
C4 – C2
C3
C3a
C3b
C5
C5a
C5b
Anaphylatoxins
C5b
C6
C7
C8
C9
MAC complex
Mast cells
Vasodilation
Hypotension
Shock
TNF-α
Cytokine storm
Immune-mediated hemolysis
Red cell destruction
Fever
Leukocyte activation
Thrombosis
Free hemoglobin (Hb)
Hb/Hp complex
Hematocrit
Haptoglobin (Hp)
LDH
Bilirubin
Disseminated intravascular coagulation
Acute kidney failure
Liver

Clinical relevance of ABO system

- Transplant aspects:
  - In solid organ transplantations:
    - ABO compatibility plays the major role in donor selection as ABO is also a histocompatibility system
    - HLA matching is not required
  - In allogenic HSCTs:
    - HLA matching is the most important
    - Pure red cell aplasia in major ABO-incompatible HSCT is a common complication

The RhD blood group system

- The second clinically significant blood group after the ABO system
- 55 antigens
- Antigens can be detected in the red cell membrane of 5-6-week-old embryos

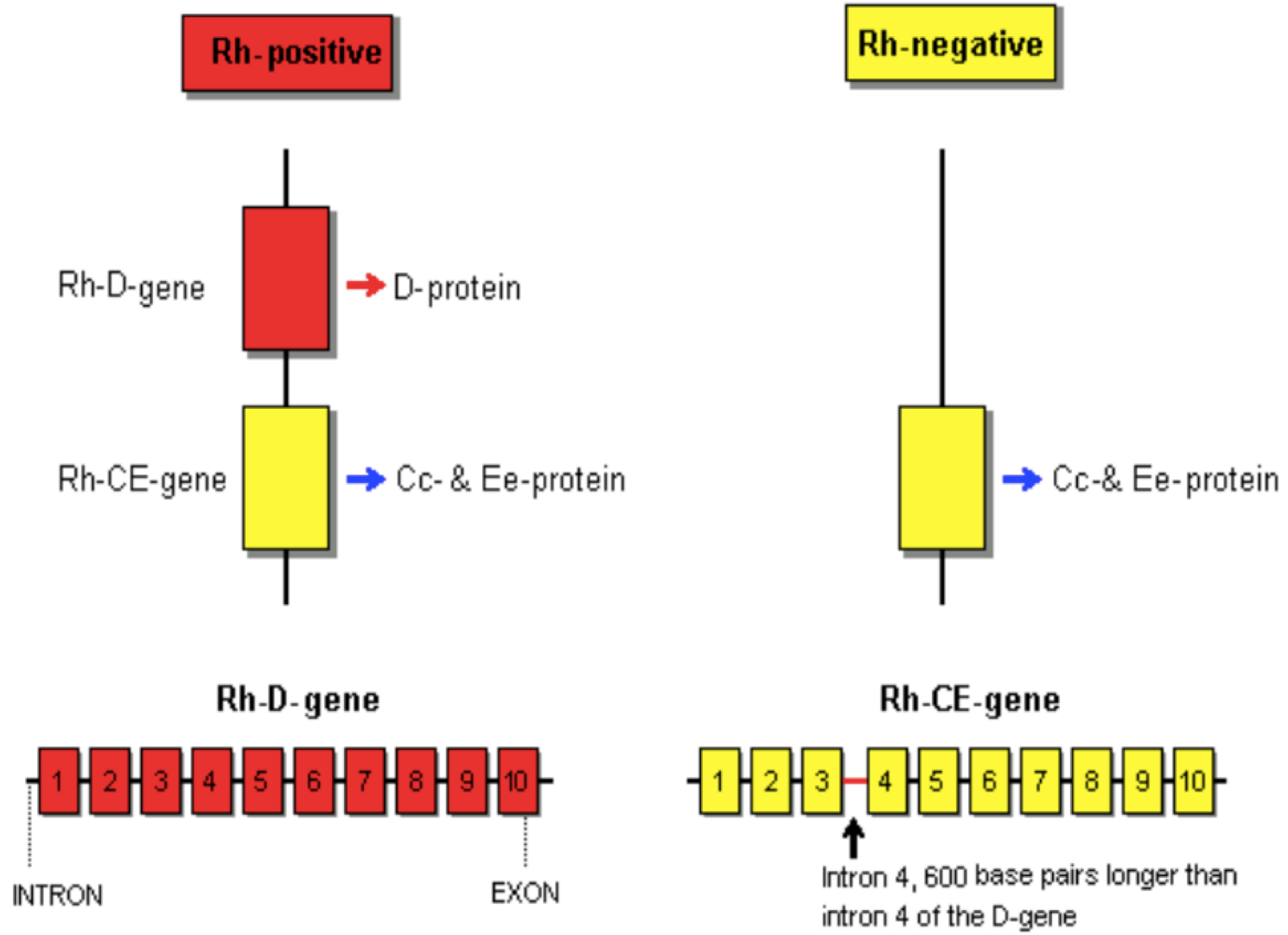
Rh blood group system

- Clinical importance:
  - The D antigen is highly immunogenic
    - If D-positive red cells are transfused to D-negative recipient, anti-D alloantibodies are formed in some 70-90% of cases
  - Rh antibodies cause
    - hemolytic transfusion reaction (HTR)
    - hemolytic disease of the fetus and newborn (HDFN)

Genetics of the Rh blood group system

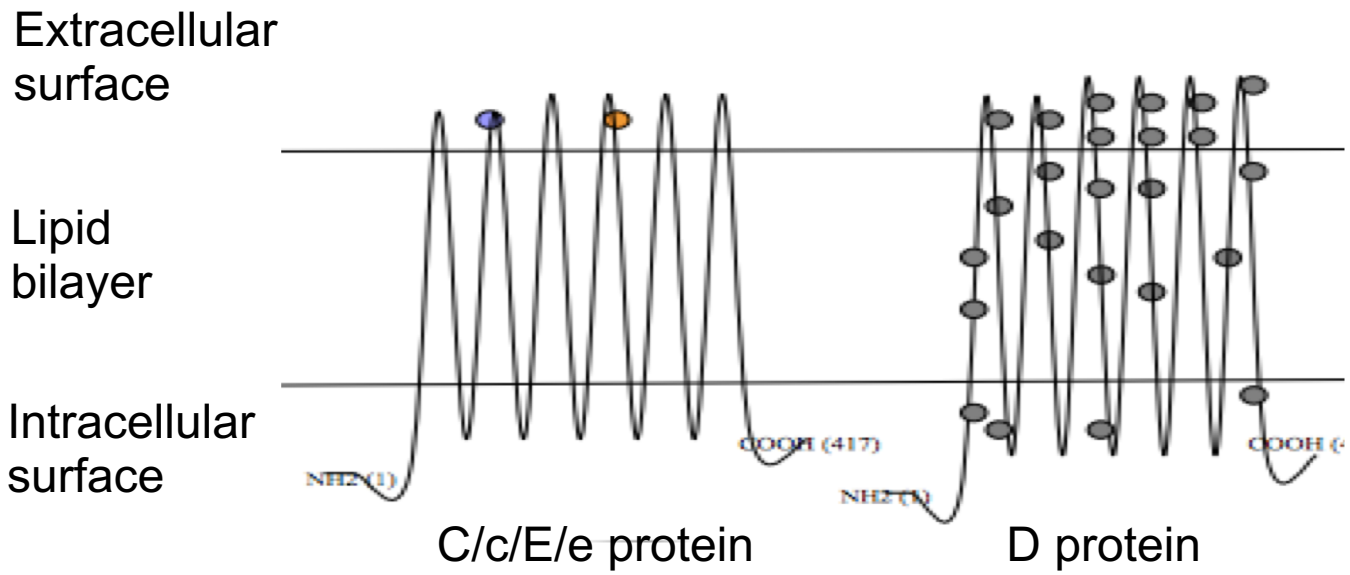
- 2 closely linked genes:
  - *RHD* gene has no allele
    - the RhD-positive or -negative phenotype is associated with the presence or the absence of the D gene
  - *RHCE* gene has many alleles
    - CE, Ce, cE, ce
    - Intermediate inheritance:
      - Rh antigens are expressed in both homozygous and heterozygous forms

In Caucasians D negative phenotype is caused by the deletion of D gene, in black Africans it commonly results from RHD pseudogene. In Asians the frequency of this phenotype is less than 1 %.



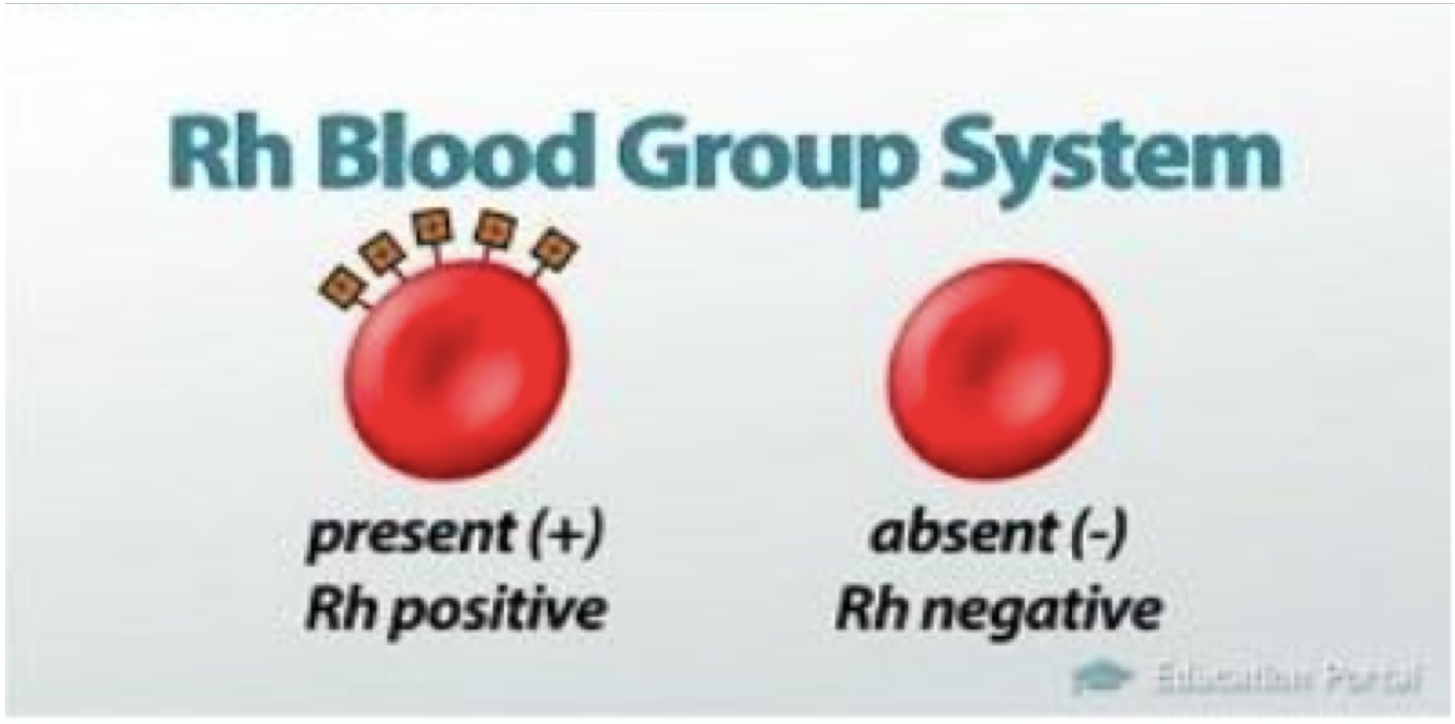
Structure of Rh antigens

- Genes encode two integral membrane proteins
  - one carries the D antigen, and the other carries CE antigens in various combinations (ce, Ce, cE, or CE)
  - 12 transmembrane domains (6 extra- and 5 intracellular loops)
  - 9 RhD epitopes on extracellular surface



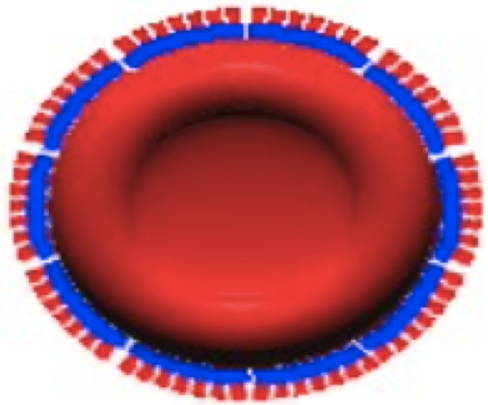
D antigen

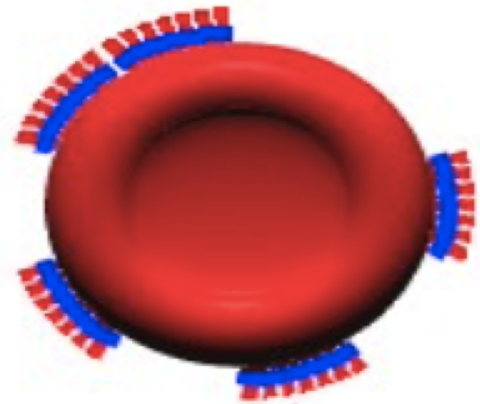
- The number of D-sites is 10,000-30,000/RBC.
- Presence only in red cells membrane
- 3 phenotypes:
  - RhD positive
  - RhD negative
  - RhD variant
    - Weak D
    - Partial D

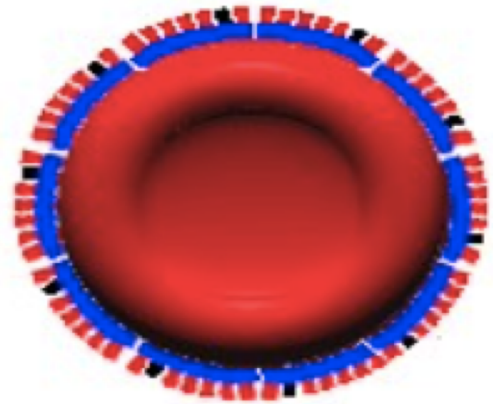


D variants > 1%

- Weak D
  - primarily results from single point mutations in *RHD*
  - the expression of D antigen is quantitatively weaker than normal
  - D antigen is normal
    - A weak D patient does not form anti-D antibody after being transfused with D-positive blood
- Partial D
  - Is qualitatively different from normal D
  - lack one or more of the D epitopes
  - a partial D patient has the potential to produce anti-D to normal D antigen and should therefore be given D-negative blood







	Normal D-Antigen	D-weak	D -Partial
Epitope:	Normal	Normal	Mutated
Antigen frequency:	Normal	Reduced	Normal

Legend:

Epitope 

Antigen 

D variants in transfusion practice

- If the donor or the newborn has D variant phenotype, they are managed like RhD positive individuals
  - Prevent the alloimmunization:
    - RhD negative patient does not receive D variant red cells
    - RhD-negative gravida is given anti-D prophylaxis if the newborn is a D variant

D variants in transfusion practice

- If the patient or the gravida has D variant phenotype, they are managed like RhD negative individuals
  - Prevent the alloimmunization:
    - D variant patient won't get RhD positive blood
    - D variant gravida will recieve anti-D prophylaxis, if her newborn is RhD positive

Other Rh antigens

- C, c, E and e antigens
  - Homozygotes have greater number of antigenic sites on red cells than heterozygotes
- Rh phenotype: D, C, c, E, e antigens are „together”
- The commonest three geno- and phenotypes:
  - DCe/dce 32% → D+ C+ c+ E- e+
  - DCe/DCe 18% → D+ C+ c- E- e+
  - dce/dce 15% → D- C- c+ E- e+

There is no d gene, d is use to indicate the absence of D.

Antibodies of Rh system

- Anti-D, anti-C, anti-c, anti-E and anti-e are significant (there is no anti-d)
- Irregular antibodies – alloimmunization due to the exposure of non-self Rh antigen
- The most frequently detected antibodies at antibody screening
- Type **IgG**
  - transferred across the placenta – severe HDFN
- HTR is also severe, but in delayed form

Biological function of Rh antigens

- Assist in the transport of carbon dioxide across the cell membrane
- Rh proteins are expressed on red cells in association with other membrane proteins (RhAG, LW, CD47 and GPB) = Rh complex
  - Maintaining the mechanical properties of the membrane
  - Rhnull is a rare phenotype characterized by the lack of expression of all Rh antigens
    - Osmotically fragile red cells (stomatocytes)
    - Chronic haemolytic anaemia
- RhAG can mediate ammonium transport

Thank you for your attention!