Immunohematology

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Outline

- Blood groups
- Introduction immune mechanisms
- Hemolytic disease of newborns
- Pathphysiology of transfusion reactions
- Hemolytic anemias caused by immune mechanisms
- Summary

Blood groups

Blood groups

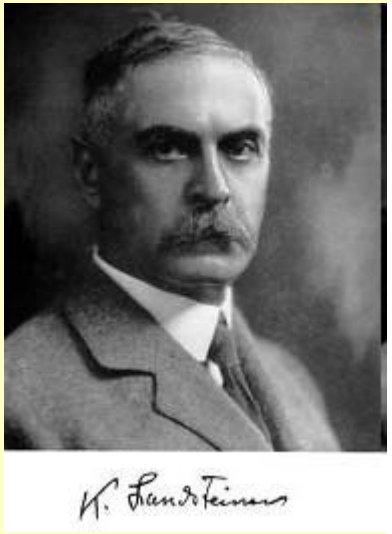
- AB0 system (A,B,0, Bombay)
- Rh system
- >30 other blood group systems (~500 antigens)
  - Lewis
  - I system
  - P system
  - MNSsU
  - Kell
  - Duffy
  - Kidd

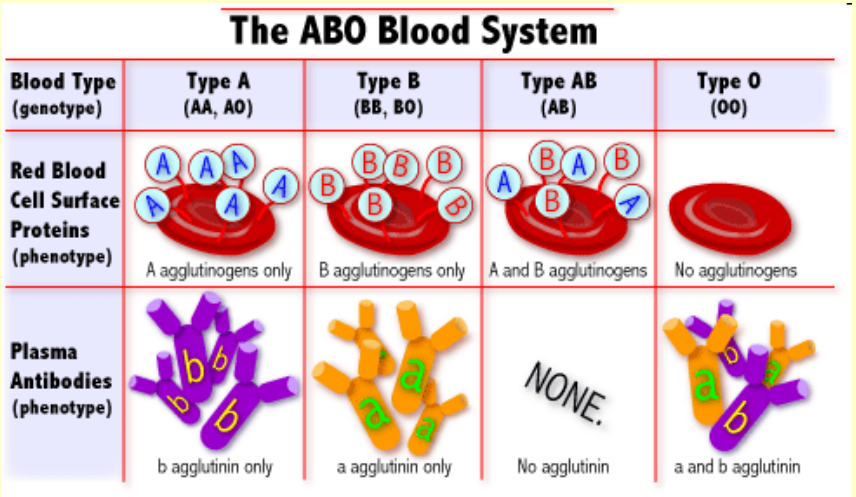
Antigenic determinats of blood groups

- Glycans (poly- or oligo-sacharides)
  - e.g. AB0 blood groups, Lewis,
  - bound on proteins (glyco-peptides/proteins)
  - bound on lipids (glycolipids)
  - T cell independent immune response
- Peptides
  - e.g. Rh blood group
  - T cell dependent immune response

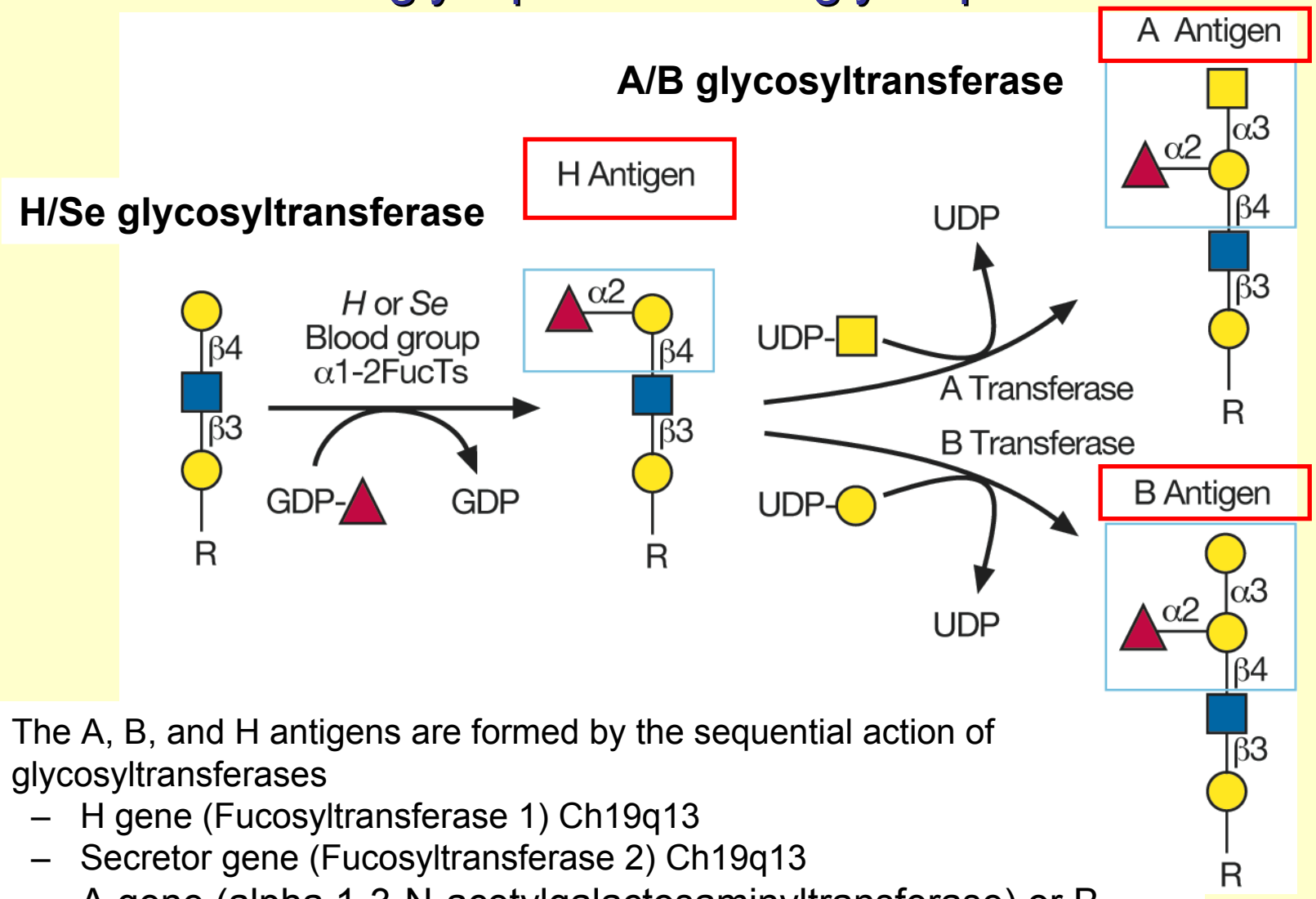
The ABO blood group system

- 1901 by Karl Landsteiner
  - humans could be divided into classes according to the presence or absence of serum factors (antibodies) that would agglutinate red cells from other humans by recognition of the corresponding antigens (glycan epitopes)
- 1907 Jan Janský
  - confirmed the presence of fourth blood group, currently known as type AB (classification groups I-IV)



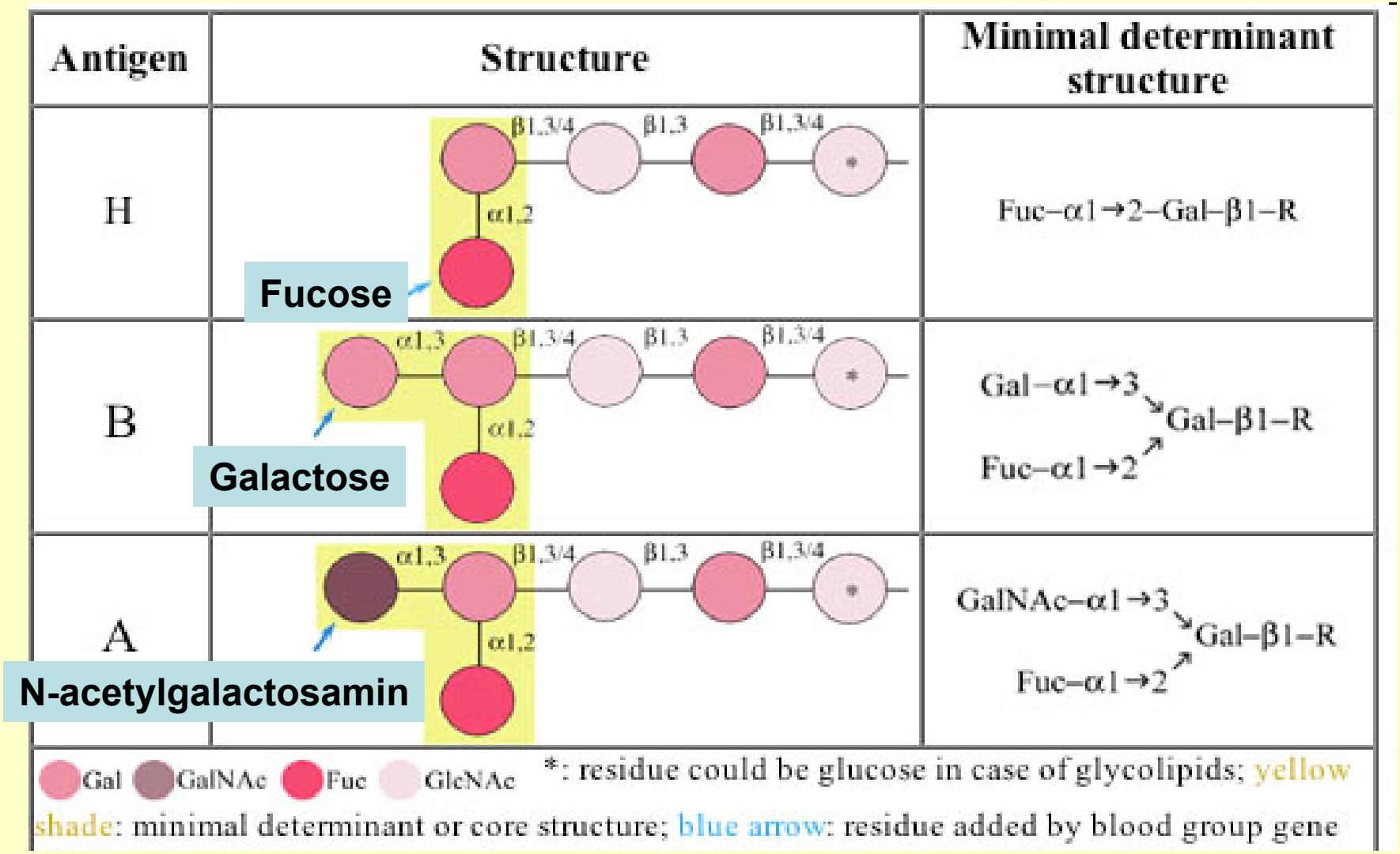


The ABO antigens are expressed on membrane glycoproteins and glycolipids



- The A, B, and H antigens are formed by the sequential action of glycosyltransferases
  - H gene (Fucosyltransferase 1) Ch19q13
  - Secretor gene (Fucosyltransferase 2) Ch19q13
  - A gene (alpha 1-3-N-acetylgalactosaminyltransferase) or B gene (alpha 1-3-galactosyltransferase)

The ABO blood group system



- Group O is formed in idividuals with a functionally inactive A/B glycosyltransferase alleles

A/B glycosyltransferases

- 7 exons (spans over 18kb of genomic DNA)
  - The single nucleotide deletion, found in a large number (but not all) of O alleles and responsible for the loss of the activity of the enzyme, is located in exon 6.
  - Seven nucleotide substitutions distinguish the A and B glycosyltransferases
    - The first resides in coding exon 6
    - other six result in four amino acid substitutions reside in coding exon 7
  - Substitutions at two sites (Leu266Met and Gly268Ala) determine the A or B specificity of the enzyme
    - results in an alteration of the shape of the active site pocket
      - smaller size UDP-Gal (B) rather than UDP-GalNAc (A) becomes preferentially accommodated as a substrate
- The function of ABH antigens remains unknown.

Tissue distribution of ABH antigens

- The ABH antigens are expressed on RBC and other cells in many tissues as:
  - membrane glycoproteins
  - membrane glycolipids
- ABH determinants on each human RBC
  - ~ 1–2 mil. (~80%) attached to the protein, band 3
  - ~ 0.5 mil. attached to glucose transport protein, band 4.5
  - ~ 0.5 mil. attached to glycolipids
- Secreted by some people in their body fluids, including saliva, tears, and urine

The ABO blood group system

- Early in the postnatal period, the immune system generates IgM antibodies (isoagglutinins) against ABO antigen(s) when they are absent from an individual's red cells
  - Group O individuals exhibit circulating IgM antibodies against A or B blood group antigens
  - Group B individuals exhibit circulating IgM anti-A antibodies
  - Group A individuals exhibit circulating IgM anti-B
  - Group AB individuals do not make either anti-A or anti-B IgM antibodies

Antibodies to alien antigens in the ABO group are usually present in our plasma **prior to the first contact with blood of a different ABO type**

The ABO blood group system

- Bombay phenotype: rare ABO blood group phenotype
  - Bombay people have inactive H and Se genes
    - Red cells deficient in H, A, and B antigens
    - Their sera contain IgM antibodies that react with red cells from virtually all donors (H antigen-positive) except those of the same Bombay (H-deficient) blood type

Population distribution of ABO antigens

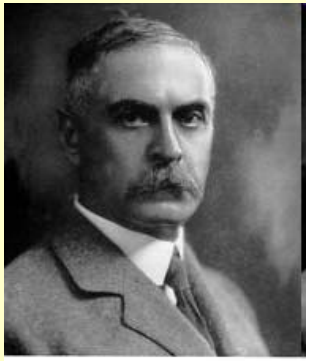
Allele	Protein	%cauc|black|orient
• A1	A1 transferase	22 | 12 | 18
• A2	A2 transferase	7 | 6 | rare
• B	B transferase	6 | 12 | 17
• O	non-functional	65 | 70 | 65

anti-A / -B IgM antibodies

- Trigger the complement cascade
- Circulate in human plasma at titers sufficient to cause complement-dependent lysis of transfused erythrocytes that display the corresponding blood group antigens
- Red cell lysis is associated with an acute transfusion reaction:
  - hypotension, shock
  - acute renal failure
  - death from circulatory collapse

Rh system

- Protein antigen
- Landsteiner and Wiener in 1940
- Consists of 50 defined blood-group antigens
- 5 antigens D, C, c, E, e are the most important ones
- Rh factor
  - Rh positive and Rh negative refer to the D antigen only
  - Cause of the hemolytic disease of the newborn (erythroblastosis fetalis)



Immune mechanisms

The immune system is a complex cellular and humoral system able to:

- recognize foreign antigens (a specific **sensory function**)
- organize defense (**self-controling, regulatory, mechanisms**)
- effectuate suppression or elimination of „the invader“ (**effector functions**)

- Innate immunity subsystem
- Adaptive immunity subsystem

Innate immunity

- Pattern recognition receptors (PRR)
  - type C lectins, Integrins, Lipid transferase, Leucine-rich proteins (TLR, CD14)
- Antimicrobial peptides
  - defensins, cathelin, histatin
- Cells
  - phagocytes (neutrofils, monocytes, makrophages),
  - cells producing proinflammatory mediators (bazophils, mastocytes, eozinophils), NK cells, DC
- Complement components
- Cytokines

Adaptiv immune response

- Cellular
  - T lymphocytes, B lymphocytes
- Humoral
  - Antibodies
- Cytokines
  - specific regulation of T and B cells function

Circulating antibodies

- Activation of complement when bound on cell surface
- Antibody dependent cell cytotoxic reaction

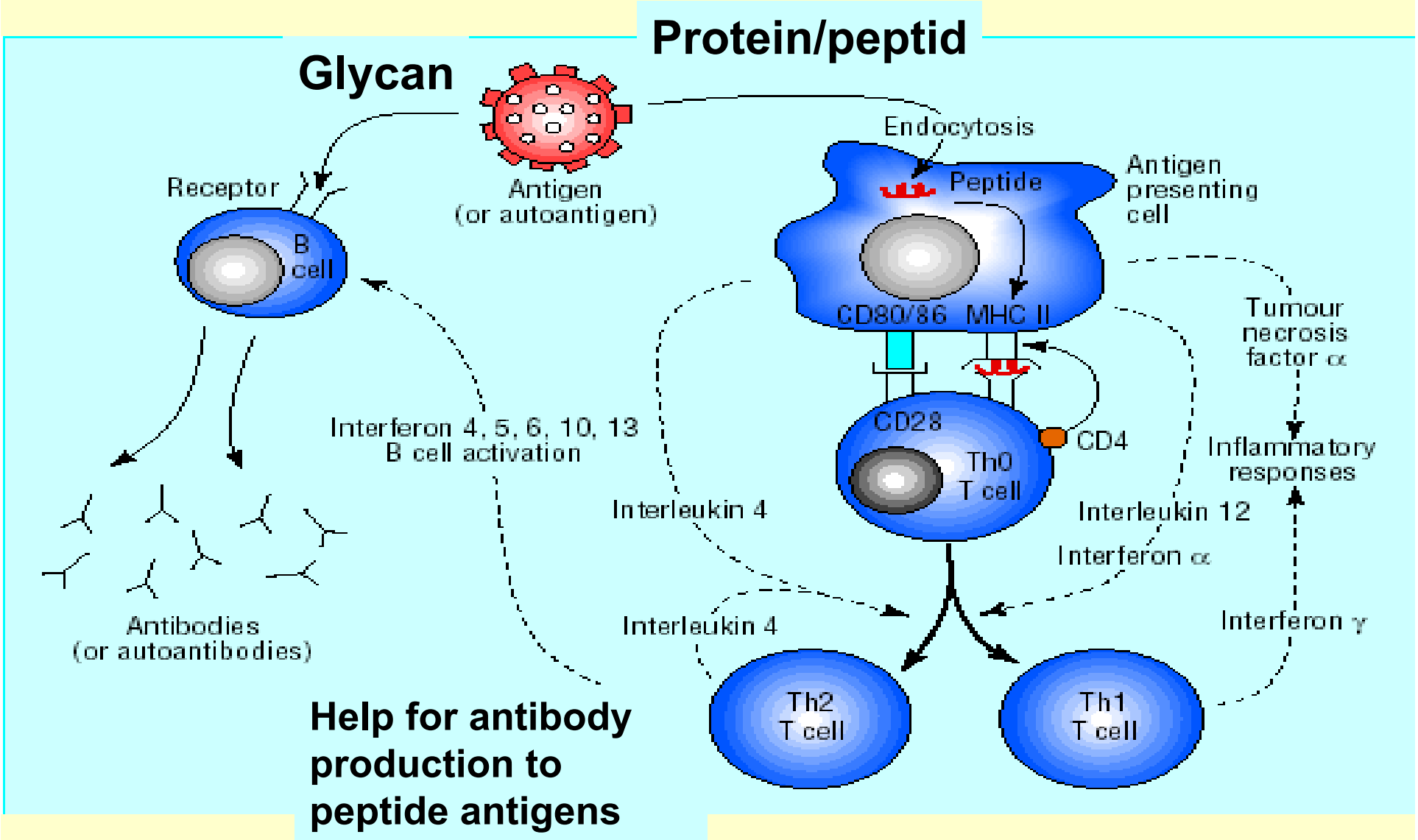
Complement

- Plasma proteins
- Cascade activation
- Individual activated complement components have various functions

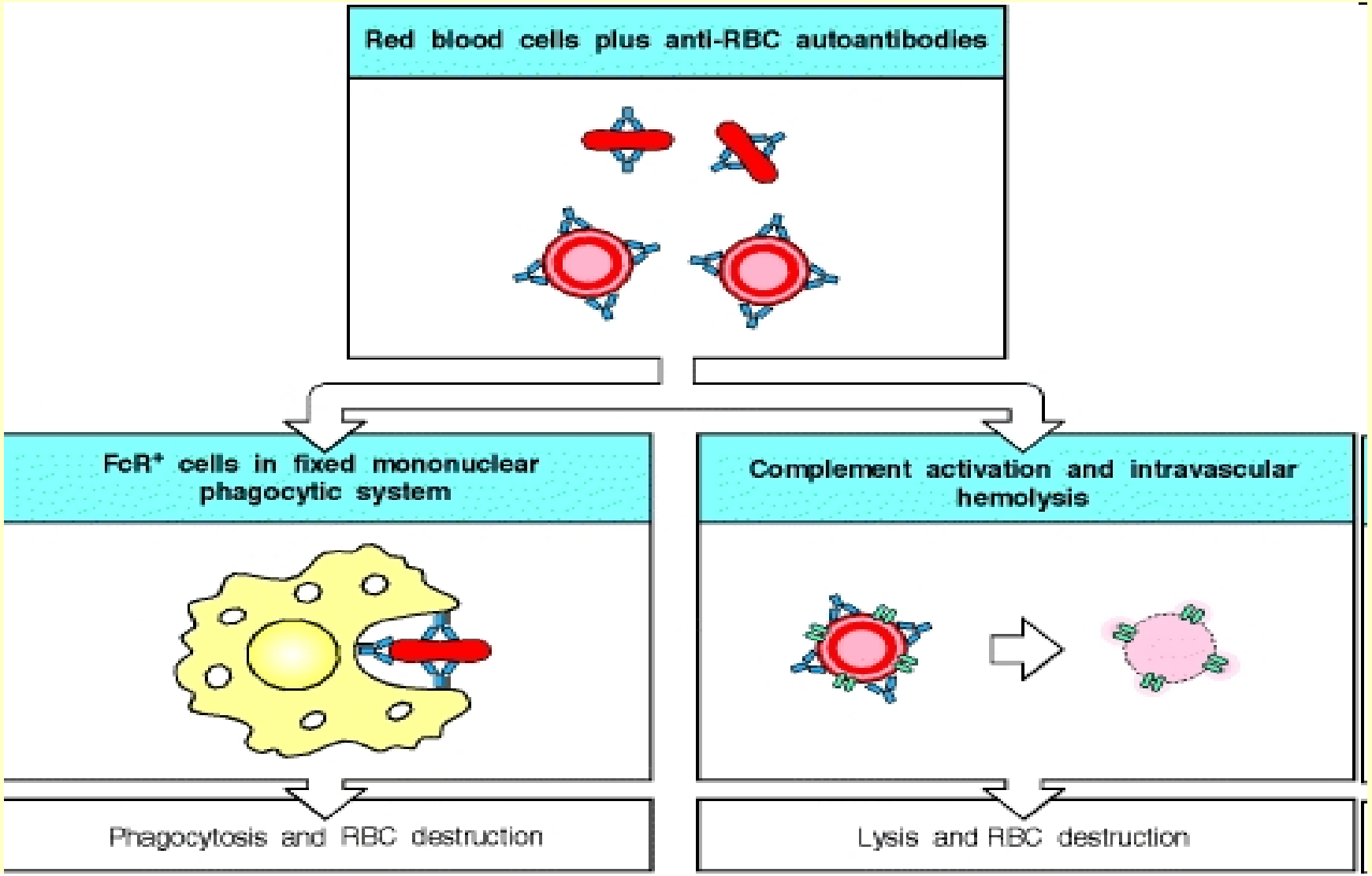
Function:

- Cell lysis
- Amplification of phagocytosis (opsonisation)
- Leukocyte chemotaxis (migration)

key componets of immune response



Antibodies specific for cell-surface antigens can destroy cells



Hemolytic disease of the fetus/newborn

HDN

HDN

- ~ 50 different red cell surface antigens have been found to be responsible for hemolytic disease of fetus and newborn
- Clinically important antibodies
  - anti-A, -B (most often, generally mild)
  - anti-D (1. may cause severe HDN)
  - anti-K (Kell) (2. may cause severe HDN)
  - anti-c (3. may cause severe HDN)
  - anti-other (rare: Kidd, Duffy, MNSs)

Antibodies directed against the P and Lewis blood groups have not been associated with HDN

Etiology of HDN

- Protein RBC antigens
  - Fetal RBCs possess an antigen that the mother lacks cross the placenta into the maternal circulation, where they stimulate antibody production (e.g. Rh D, Rhe, or Kell-K1)
- Glycan RBC antigens
  - Blood group type “O” mother has naturally occurring titers of IgM anti-A and B antigens but they don’t cross placental barrier. In some cases IgG anti-A and B antigens develop.

HDN: History

- 1609
  - French midwife was the first to report hemolytic disease of the newborn (HDN) in a set of twins
- 1953
  - Chown confirmed the pathogenesis of Rh alloimmunization to be the result of passage of Rh-positive fetal RBCs after transplacental hemorrhage into maternal circulation that lacked this antigen
- 1966
  - 2 groups from UK and USA demonstrated that anti-D IgG prophylaxis during the pregnancy prevented sensitization in Rh-negative women
- 1971
  - The World Health Organization (WHO) recommended anti-D immunoglobulin G (IgG) should be given as prophylaxis to Rh negative mother of Rh-positive child
- Now:
  - Routine use of Rh IgG prophylaxis
  - Significant decline in the incidence of RhD alloimmunization

BUT also other causes of HDN

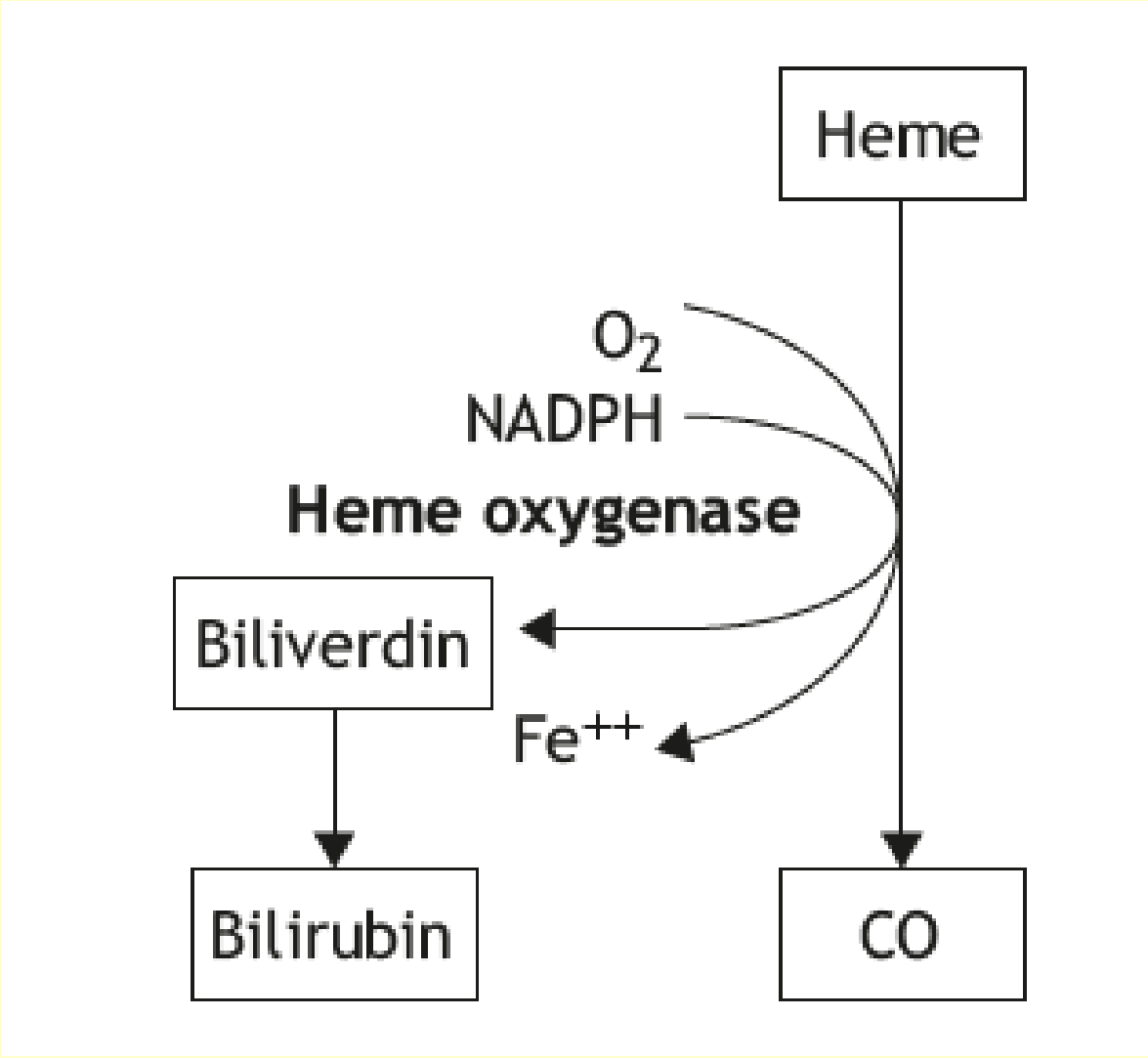
General pathogenesis of HDN

- Mothers antibodies (IgG) to fetal blood group antigens cross to the fetal circulation and interact with fetal RBC antigens and cause RBC destruction

Manifestation of HDN

- Elevated (unconjugated) bilirubin levels
  - neonatal jaundice
    - only in the delivered newborn because the placenta effectively metabolizes bilirubin

Heme degradation pathway



Manifestation of HDN

- Elevated (unconjugated) bilirubin levels
  - neonatal jaundice
  - kernikterus (brain damage)

Mechanism of bilirubin induced neurotoxicity ??

- Immature blood–brain barrier result in the penetration of unconjugated bilirubin to the brain cells (e.g. basal ganglia, hippocampus).
- Irreversible damage is thought to be caused by elevated intracelular levels of bilirubin by unknown mechanism
- Hypothesis:
  - Induction of apoptosis
    - interference with mitochondrial membrane functions
    - increase intracellular Ca2+
  - Interference withthe function of enzymes
  - Aberant activation of MAP kinase pathway

Manifestation of HDN

- Elevated (unconjugated) bilirubin levels
  - neonatal jaundice
  - kernikterus (brain damage)
- Anemia

Severe anemia

- Tissue hypoxia
  - Rise in umbilical arterial lactate occurs when the hemoglobin (Hb) level drops below 80 g/L
  - Stimulation of fetal erythropoiesis
    - in the liver, spleen, bone marrow
      - possible destruction of hepatic parenchyma
    - other extramedullary sites (skin, placenta)
  - Increase of cardiac output
    - heart failure
  - Respiratory distress

Manifestation of HDN

- Elevated (unconjugated) bilirubin levels
  - neonatal jaundice
  - kernikterus (brain damage)
- Anemia
  - reticulocytosis - erythroblastosis
  - high-output heart failure
  - respiratory distress
- Generalized swelling – hydrops fetalis

Mechanisms responsible for swelling and hydrops

- Hypoalbuminemia secondary to depressed liver function
- Increased venous pressures due to poor cardiac function
- Increased capillary permeability (hypoxia)

Pathophysiology of Rh immunization (and other protein blood group antigens)

- Primary response (sensitisation)
  - Fetomaternal hemorrhage during pregnancy
    - usually less than 0.1 mL (symptomatic)
    - ~75% of all pregnancies
    - Can occur at any stage of pregnancy (documented in 7% during the first trimester)
  - Establishment of B-lymphocyte anti-D clones

- Secondary immune response (repeat exposure) to the same antigen
  - Rapidly induces the production of **anti-D IgG**
  - 0.03 mL of Rh-positive RBCs is sufficient

Pathophysiology of Rh immunization (and other protein blood group antigens)

- **Maternal anti-D** IgG cross the placenta into fetal circulation and attach to Rh antigen on fetal RBCs
  - Antibody antibody-coated RBCs form rosettes on macrophages in the reticuloendothelial system (spleen)
  - RBCs are lysed by lysosomal enzymes released by macrophages and natural killer lymphocytes
- Independent of the activation of the complement system

Pathophysiology of neonatal hemolysis associated with ABO incompatibility

- In type-O mothers with type A or type B fetuses (rarely in type-A mothers with type-B infants)
  - IgM anti-A or -B antigens do not cross the placenta
  - 1% of type-O mothers **IgG anti-A and –B**
    - cross the placenta and cause hemolysis in fetus
- Massive hemolysis is rare
  - A and B antigens are expressed in various tissues (consumption)
  - Fetal RBCs have less surface expression of A or B antigen

HDN due to Kell sensitization

- Hemolysis
- Suppression of erythropoiesis
  - Kell antigen is on the surface of erythroid progenitors (BM inhibition)
    - Low reticulocytes and normoblasts
- Low bilirubin compared with HDN due to anti-D

Prognosis

- 50% of the affected newborns do not require treatment, (mild anemia and hyperbilirubinemia)
- 25% are born near term but become extremely jaundiced
  - without treatment and either die (90%) or become severely affected by kernicterus (10%)
- 25% of affected newborns are severely affected in utero and become hydropic

Before any interventions were available

- the perinatal mortality rate was 50%
- Introduction of exchange transfusion in 1945 reduced the perinatal mortality rate

Therapy

- Fototherapy
- Blood exchange transfusion

Pathophysiological aspects of blood trasfusion

History of blood transfusion

- Blood transfused in humans since mid-1600's
- 1828 – First successful transfusion
- 1900 – Landsteiner described ABO groups
- 1916 – First transfusion of stored blood
- 1939 – Rh factor discovered

Blood products to transfuse

- Whole blood
- Packed RBC
- Platelets
- Fresh-frozen plasma
- Cryoprecipitate
- Plasma derivatives

Adverse reactions to blood transfusion

- Immune mediated reactions
- Non-immune mediated reactions

- Acute reactions (within 24 hours)
- Delayed reactions
- Chronic reactions

Usually present in complex clinical situations

Immune mediated reactions

- Acute hemolytic transfusion reaction
- Delayed hemolytic and serologic reaction
- Febrile nonhemolytic transfusion reaction
- Allergic reaction
- Anaphylactic reaction
- GvHD
- TRALI
- Postransfusion purpura
- Alloimunization
- Immunomodulation

Non-immune mediated reactions

- Hypothermia
- Electrolyte toxicity
- Iron overload
- Volume overload
- Hypotensive reaction
- Infections

Hemolytic transfusion reaction

- The result of antibodies in the recipient's plasma directed against antigens on the donor's erythrocytes
- Intravascular hemolysis of the donor red blood cells
  - activation of complement
  - e.g. ABO incompatibility
- Extravascular hemolysis of the donor red blood cells
  - The RBCs are tagged for removal by splenic macrophages
  - e.g. Rh or some non-ABO antigens

Manifestation of intravascular hemolysis

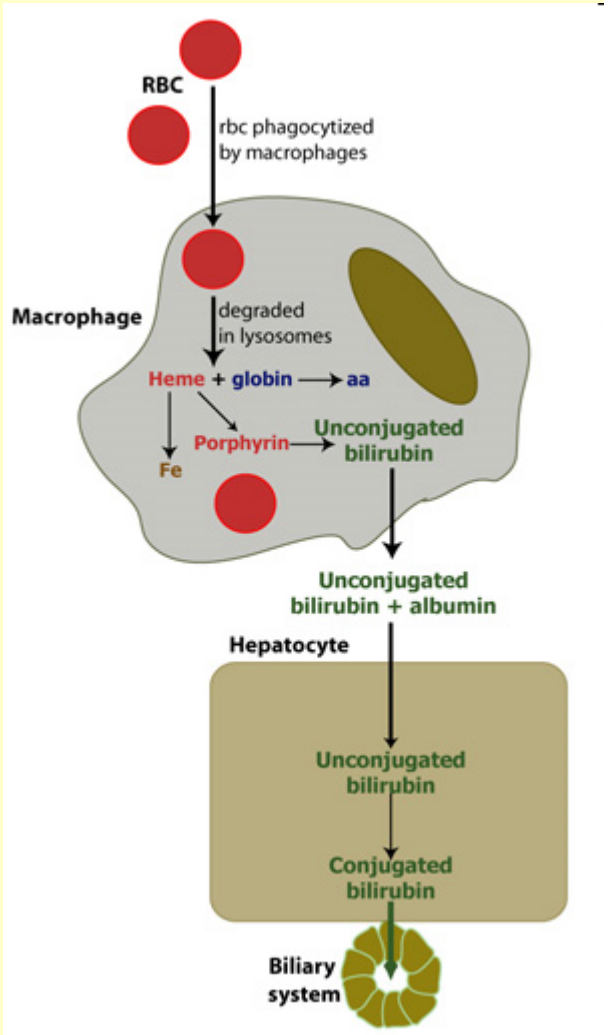
- Hemoglobinemia
- Hemoglobinuria
- Disseminated intravascular coagulation (DIC)
- Renal failure
- Complement-mediated cardiovascular collapse

Extravascular hemolysis

- Antibody titers often are too low to be detected through routine antibody screening
- History of previous antigen exposure – priming (pregnancies, transplantation, transfusions)
- Reexposure
  - Amplification of antibody production

Extravascular hemolysis

- Antibodies do not activate complement
- Hemoglobinemia is absent

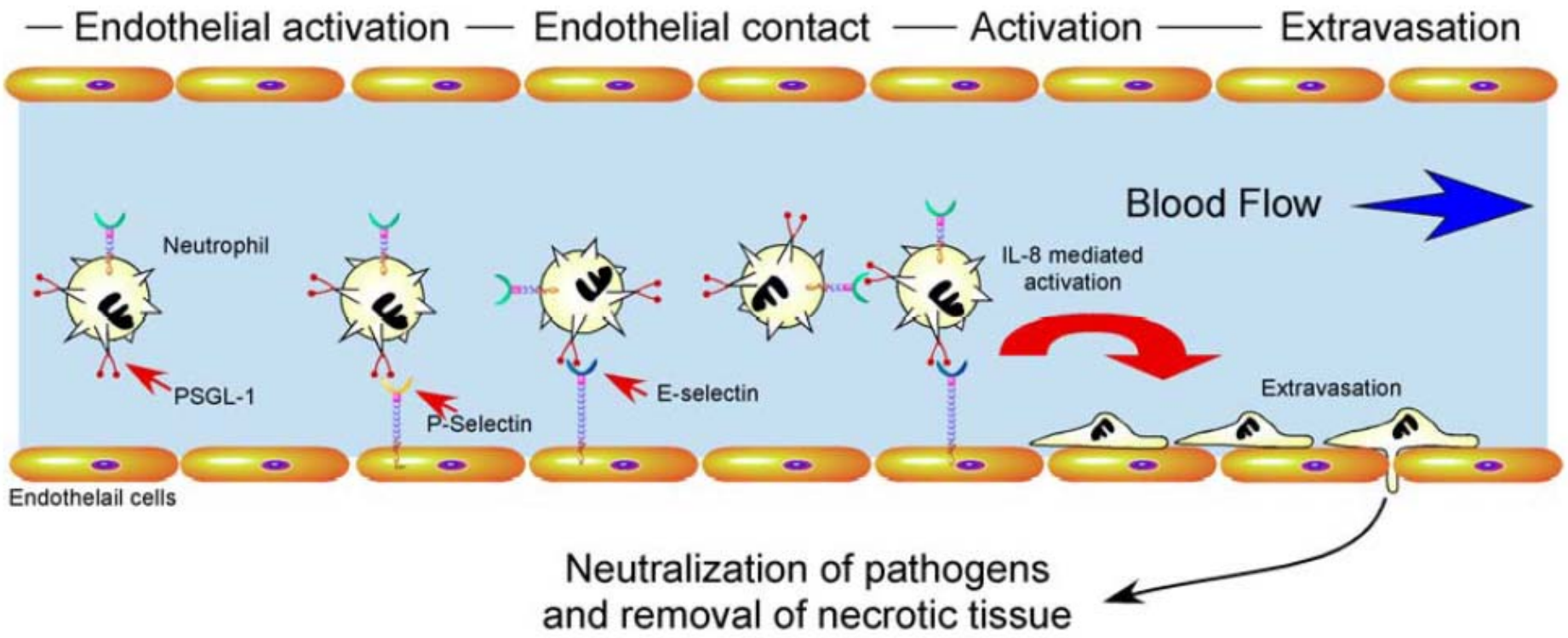


Transfusion-related acute lung injury (TRALI)

- Typically within 6 hours of transfusion

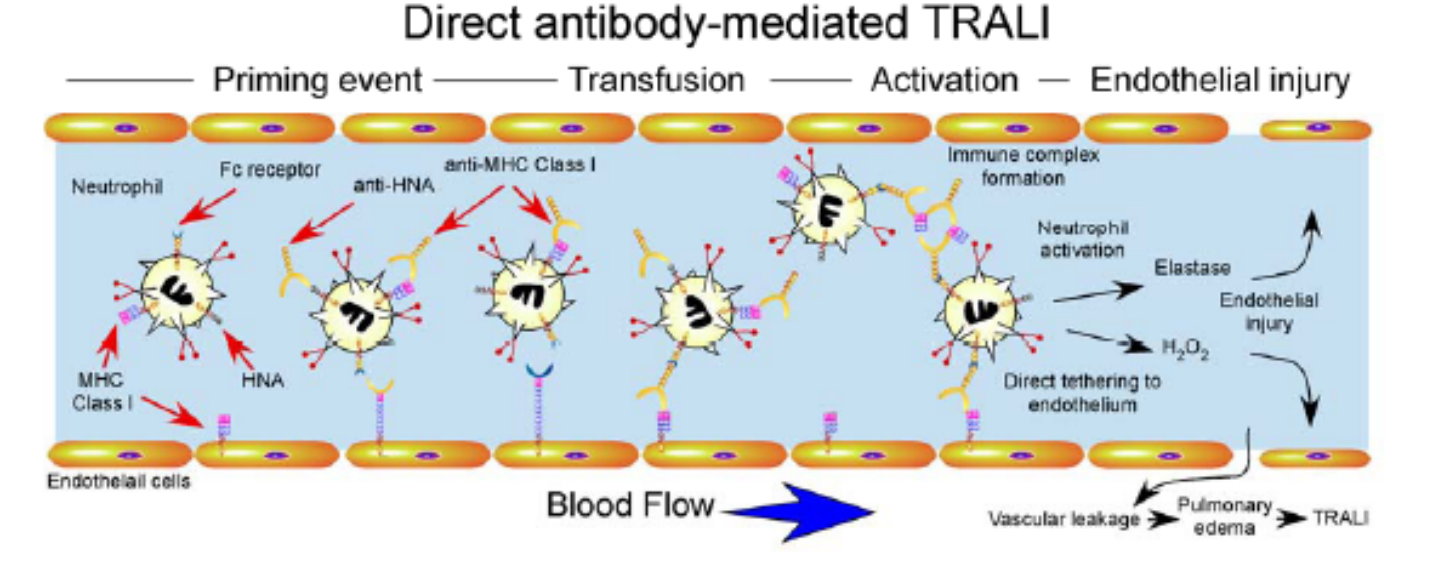
- Pathophysiologic mechanisms:
  - the antibody hypothesis
  - the neutrophil priming hypothesis

Regulation of leukocyte activation and extravasation



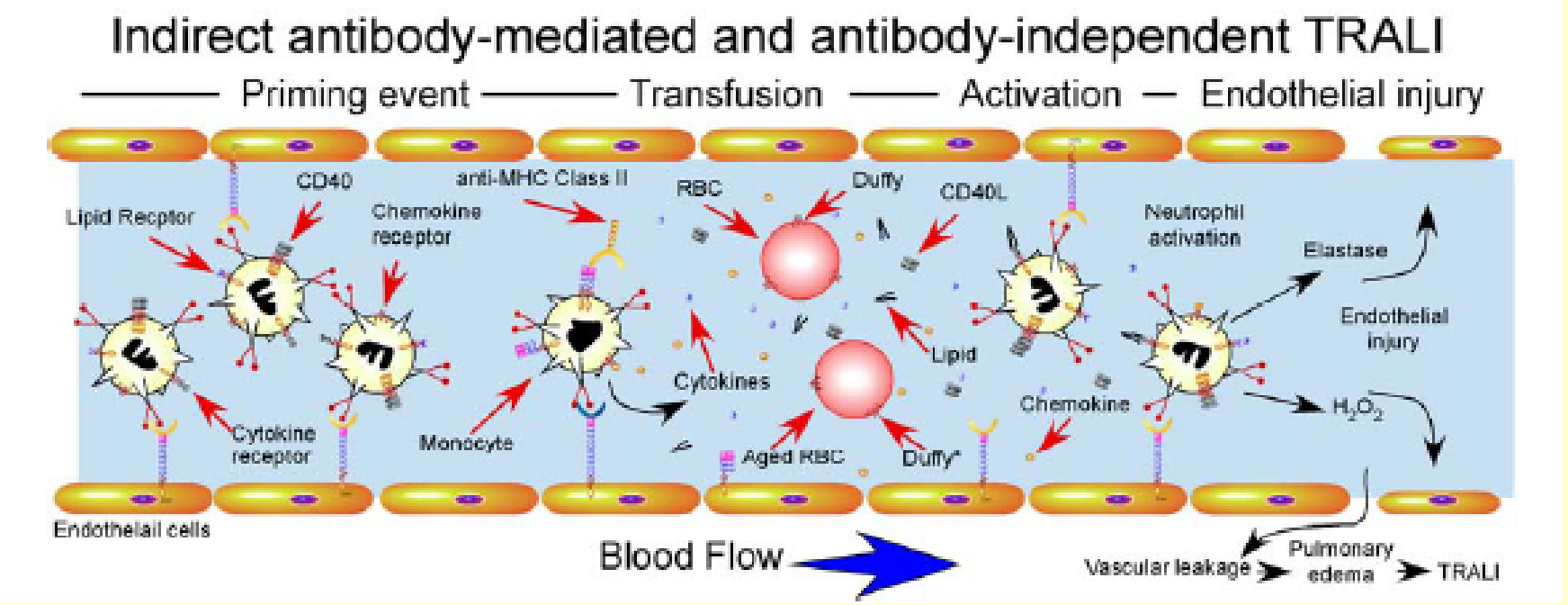
The antibody hypothesis

- Antibodies to HLA class I, HLA class II or human neutrophil antigen (HNA) in the transfused component reacts with neutrophil antigens in the recipient
- The recipient's neutrophils lodge in the pulmonary capillaries (transient leukopenia) and release mediators that cause pulmonary endothelia damage and capillary leakage



The neutrophil priming hypothesis

- Occurs in patients with primed neutrophils and endothelial activation (e.g. infection, surgery, inflammation)
- Bioactive substances in the transfused component further activate the primed neutropils on pulmonary endothelial cells to relese mediators and cause pulmonary endothelial damage



Pulmonary capilary endothelial dammage

- Lead to pulmonary edema in the absence of circulatory overload
- Alveolar damage

- Pulmonary hypertension
- Hypoxia

Nonhemolytic febrile transfusion reactions

- 3-4% of all transfusions
- usually caused by cytokines from leukocytes in transfused red cell or platelet components
- causing fever, chills, or rigors
- a fever is defined as a temperature elevation of 1º Celsius (Centigrade) or 2º Fahrenheit

Allergic reactions

- Manifested as rash, urticaria, or pruritus
- Mediated by alergen specific IgE
- Usually attributed to hypersensitivity to soluble allergens found in the transfused blood component
- Anaphylactic reactions
  - associated with anti-IgA in recipients who are IgA deficient

Graft-versus-host disease

- 80-90% mortality rate
- When donor lymphocytes mount an immune response against the recipient's HLA antigens
- Normally
  - the donor lymphocytes are recognized as being foreign and are destroyed.
- immunocompromised recipient
- donor is homozygous and the recipient is heterozygous for an HLA haplotype

Autoimmune hemolytic anemia (AIHA)

Incidence ~ 0.8/100 000/year

Prevalence 17/100 000

Etiology of (AIHA)

Consequence of immune tolerance failure

- Primary (idiopathic) AIHA (less frequent)
  - cause unknown
- Secondary AIHA
  - systemic lupus erythematosus
  - CLL-associated
  - non-Hodgkin lymphomas
  - Drug-related (fludarabine)
  - Infection-related
    - e.g. hepatitis C, A, and E and cytomegalovirus

AIHA blood tests finding

- Normocytic or macrocytic anemia
- Reticulocytosis
- Low serum haptoglobin levels
- Elevated lactate dehydrogenase (LDH) level
- Increased unconjugated (indirect) bilirubin level
- Positive direct Coombs test (direct antiglobulin test = DAT)

AIHA pathogenesis

- Antibodies and associated complement system components become fixed onto the RBC surface

What type of the antibody is involved?

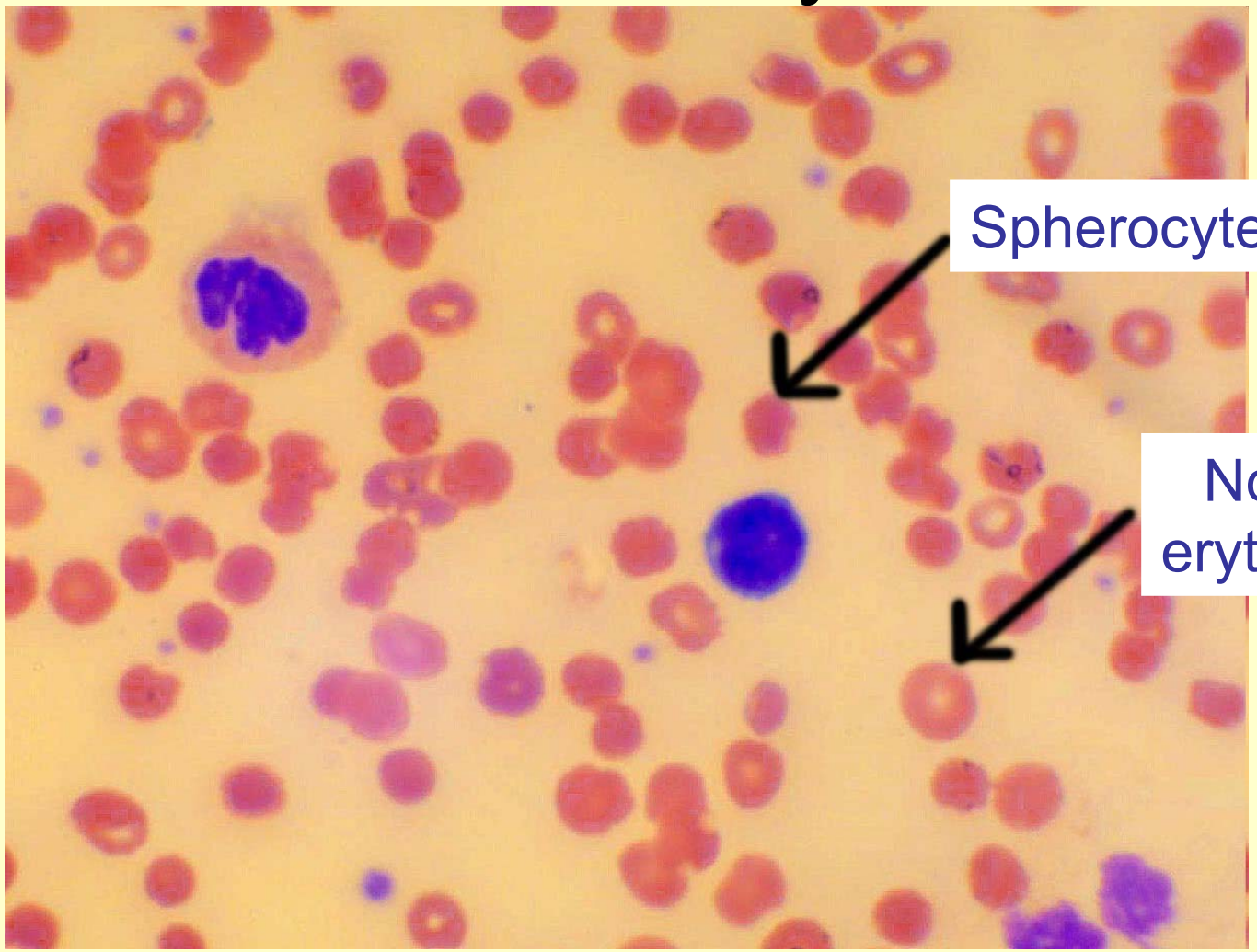
- Warm antibody AIHA (WAIHA)
  - Erythrocytes are coated with IgG or IgG plus C3d
  - usually a warm antibody
- Cold antibody (CAIHA)
  - Coated with C3d only the IgM are dissociated
  - Coombs test negative
- Paroxysmal „cold“ hemoglobinuria
  - Donath-Landsteiner antibodies IgG (biphasic)
  - P blood group

Mechanisms of AIHA

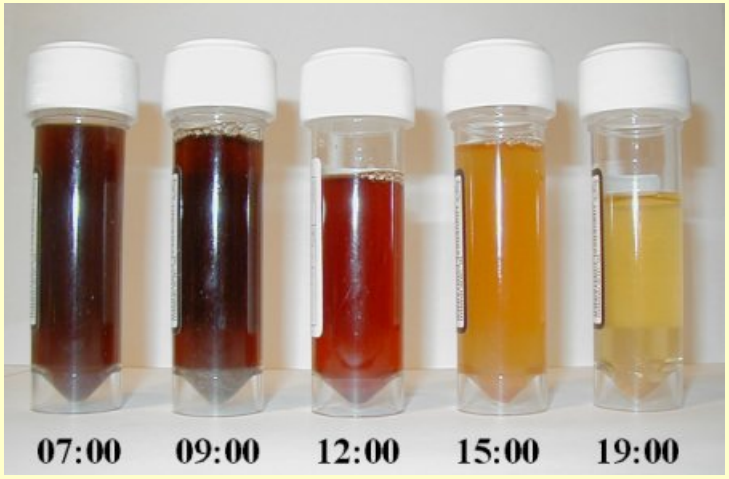
- Intravascular haemolysis:
  - Red blood cell lysis occurs in the circulation as a result of activation of the complement system cascade.

- Extravascular haemolysis:
  - Red blood cells that are coated with antibodies are specifically recognized in the reticuloendothelial system and destroyed by macrophages

Spherocytes: congenital spherocytosis or autoimmune hemolytic anemia



Paroxysmální noční hemoglobinurie (PNH)



Primary Clinical Manifestation

- Hemolytic anemia
- Bone marrow failure
- Thrombophilia

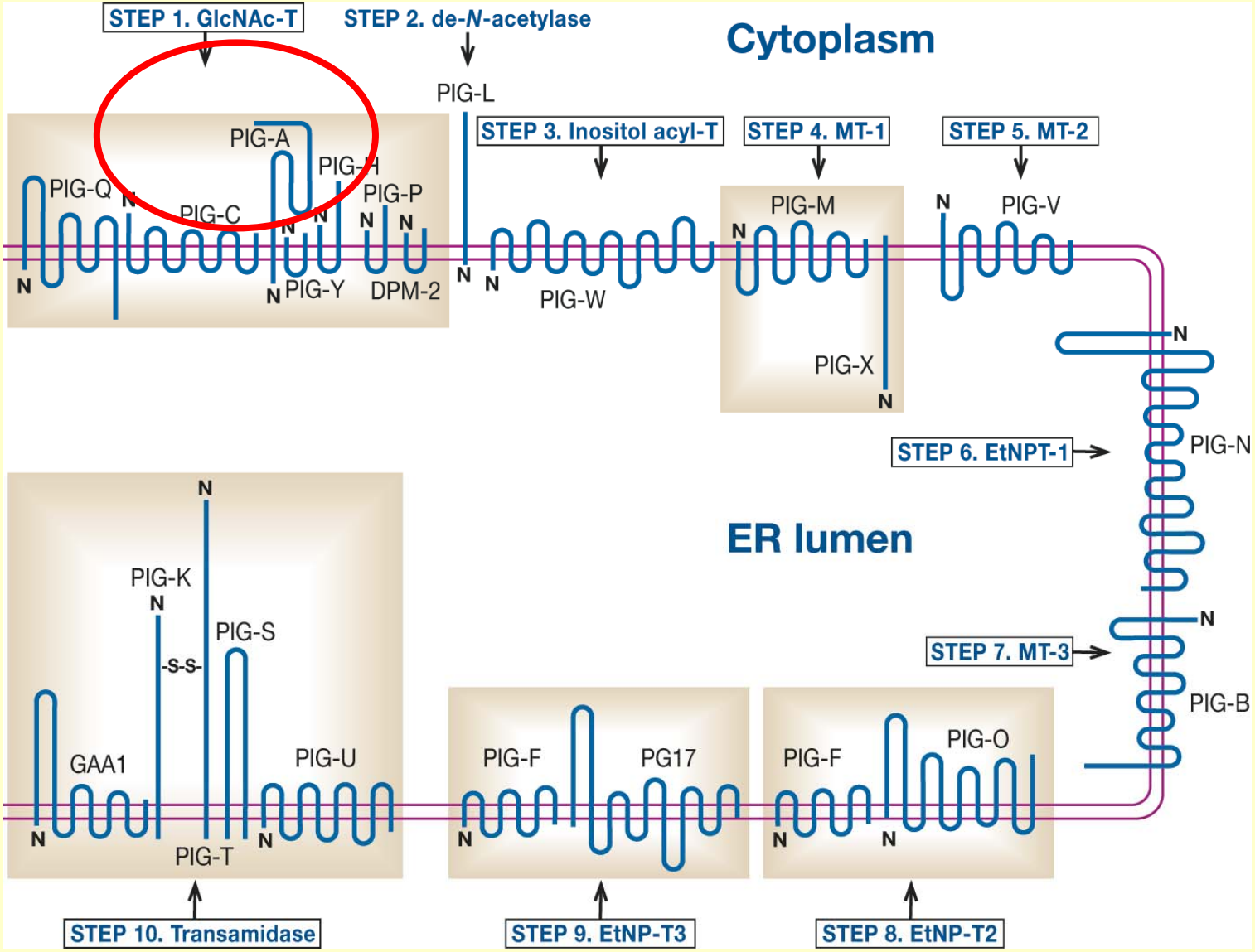
Classification of PNH

- Classic PNH
- PNH in the setting of another bone marrow disorder
  - e.g. PNH/aplastic anemia or PNH/refractory anemia-MDS
- PNH-subclinical (PNH-sc)
  - small PNH clone
  - no significant hemolysis

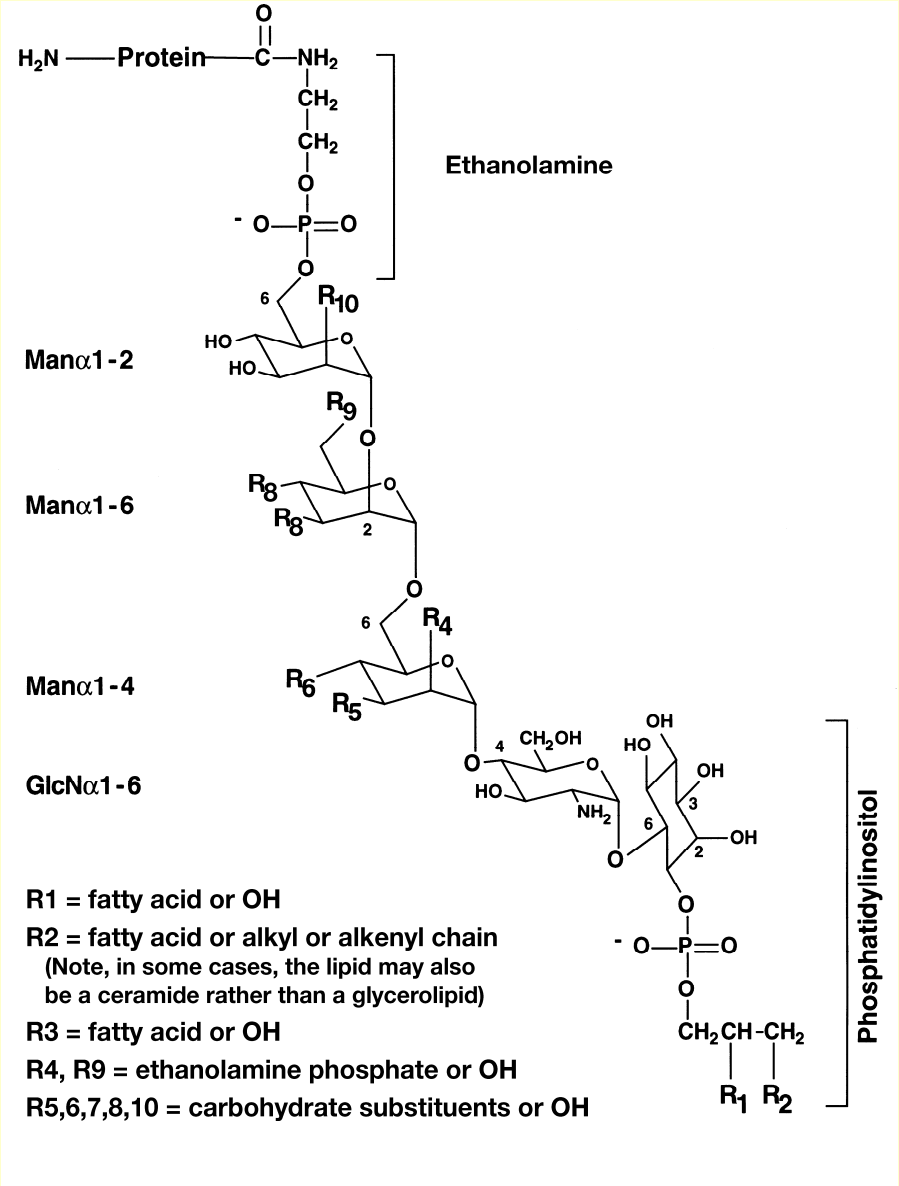
Etiology of PNH

- Acquired somatic mutation of the PIG-A gene in bone marrow stem cells
- GPI is involved in the first stage of biosynthesis of glycosylphosphatidylinositol (GPI) anchors

Predicted topologies of the components of GPI biosynthesis in mammalian cells

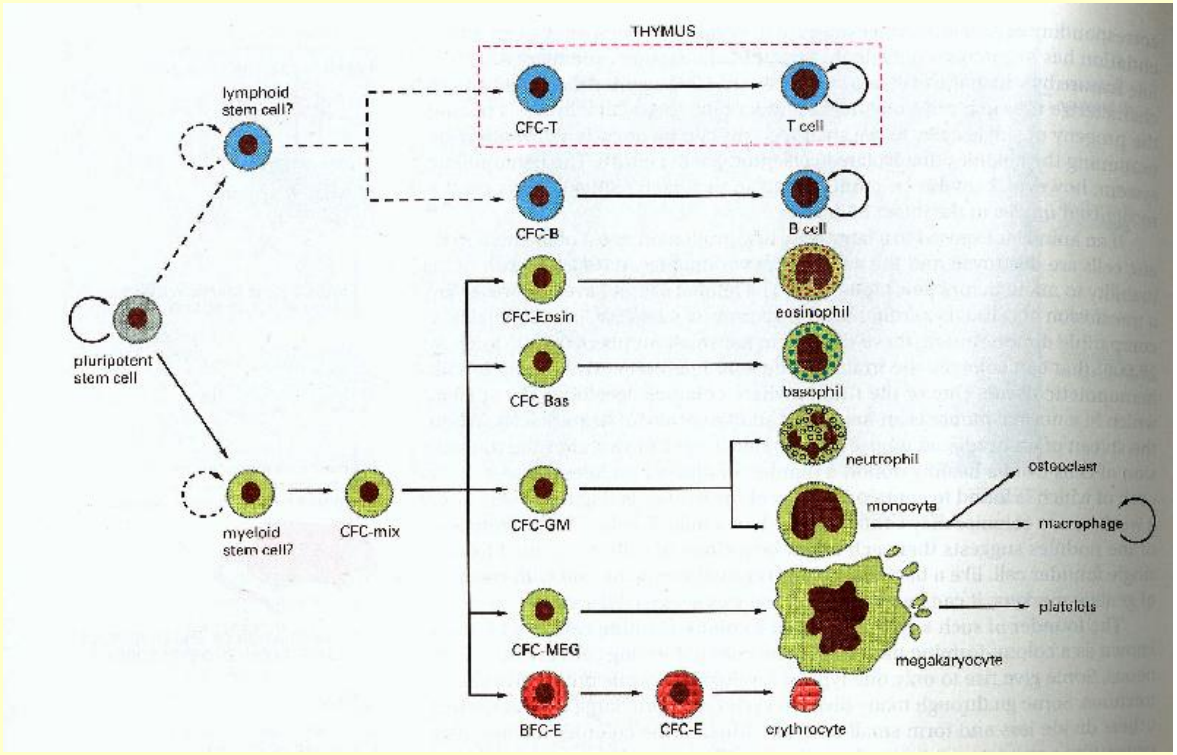


General structure of GPI anchors

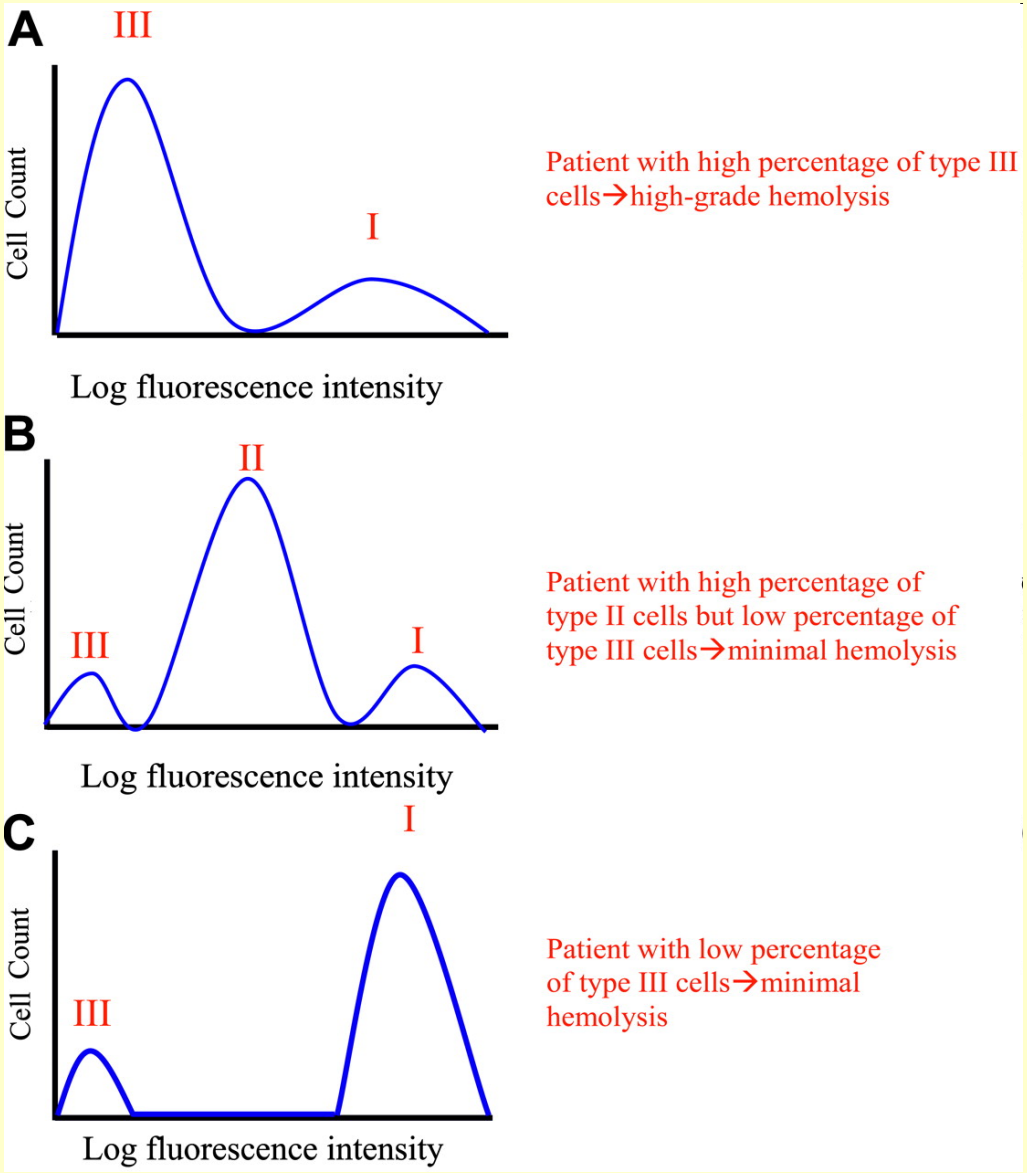


Patogenesis of PNH

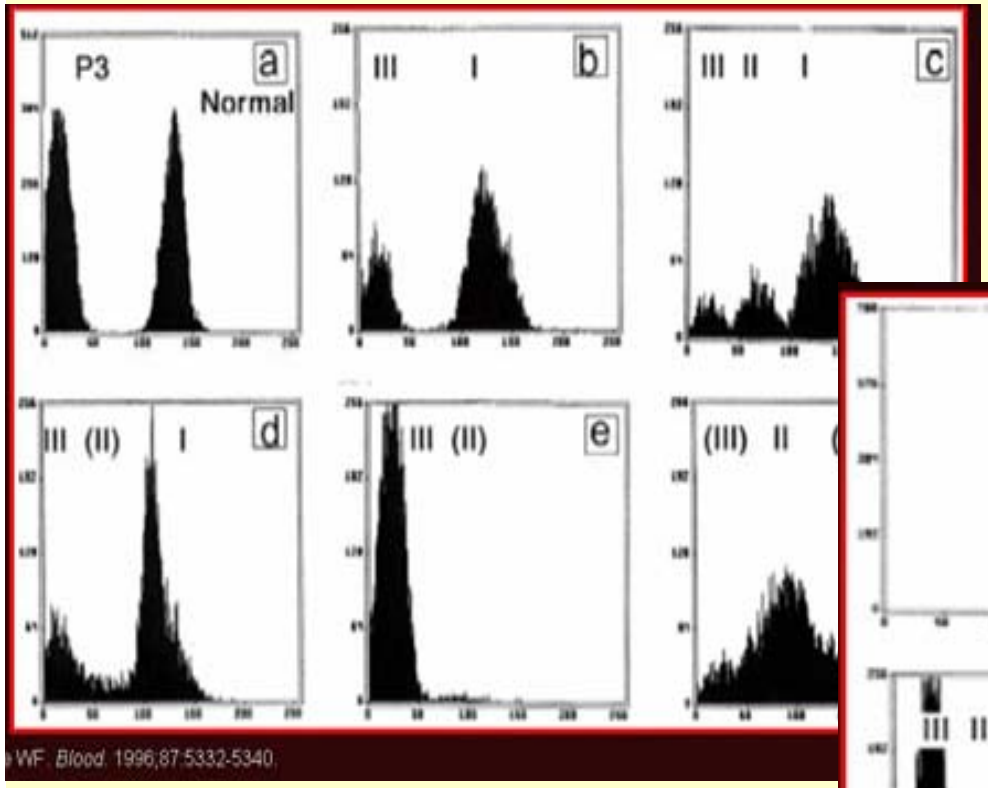
- GPI-dependent proteins are not exposed on the membrane of myeloid cells
  - erythrocytes
  - granulocytes
  - monocytes



Phenotypic mosaicism in PNH

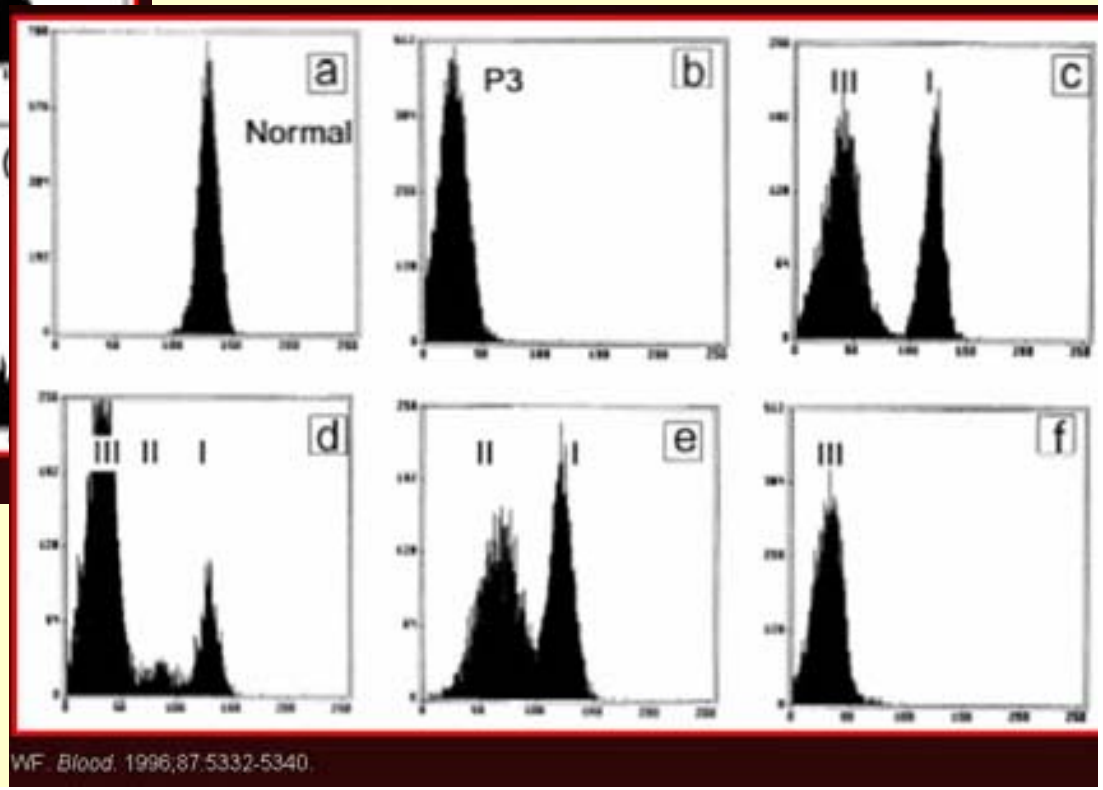


Expression of CD59 in patients with PNH (flow cytometry)



Erythrocytes

Granulocytes



Example of GPI anchored protein

• Erythrocyte CD59 and decay acceleration factor (DAF)	complement regulation
• Alkaline phosphatase	cell-surface hydrolase
• 5′-Nucleotidase	cell-surface hydrolase
• Renal dipeptidase	cell-surface hydrolase
• Trehalase	cell-surface hydrolase
• Neural cell adhesion molecule 120 (NCAM-120)	adhesion molecule
• Neural cell adhesion molecule TAG-1	adhesion molecule
• CD58	adhesion molecule
• FcγIII receptor	Fc receptor
• Ciliary neurotrophic factor receptor (CNTFR)	
• α subunit	neural receptor
• Glial-cell-derived neurotrophic factor receptor	
• (GDNFR) α subunit	neural receptor
• CD14	LPS receptor
• Prion protein (PrP)	unknown
• Glypican family of GPI-anchored proteoglycans component	extracellular matrix

Patogenesis of PNH

- GPI-dependent proteins
  - inhibitors of complement cascade
    - CD55/DAF= decay accelerating factor
    - CD59/MIRL= membrane inhibitor of reactive lysis

- As a result of complement inhibition defect erythrocytes are susceptible to intravascular hemolysis by activated complement mechanisms

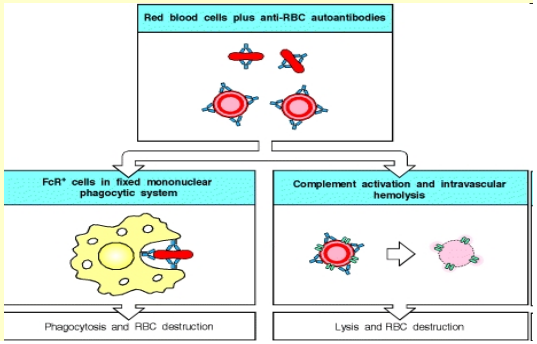
Prognosis

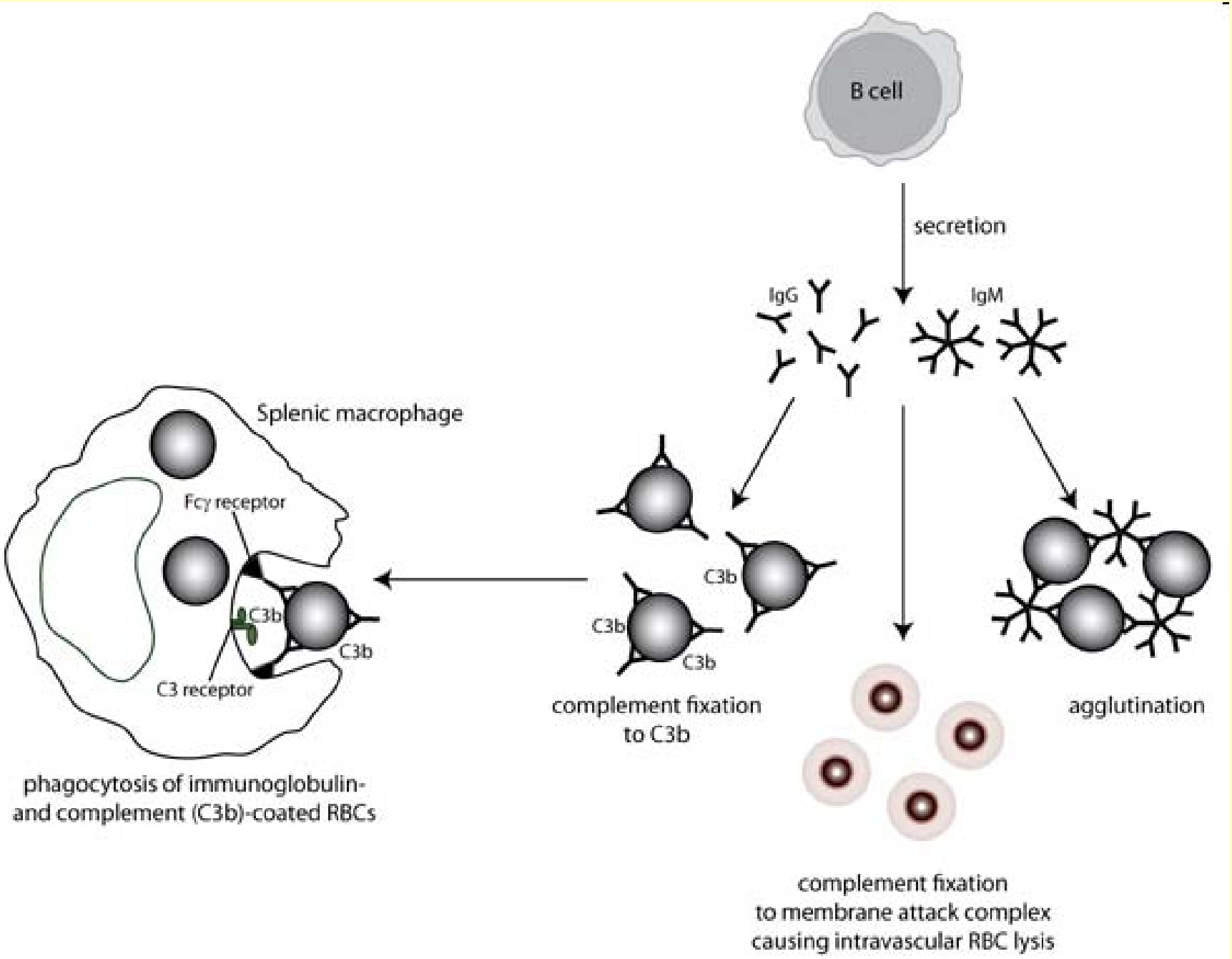
- Survival varies among patients varies (~10 – 15 years fron diagnosis)

- Thromboembolic complications
- Renal failure
- Aplastic anemia (imune reaction to PNH clone?)
- MDS/Leukemie

Summary

- IgM antibodies to ABO system antigens (glycans) are generated shortly after birth (3-5 month)
- IgG anti-D antibodies (Rh system protein antigen) are generated after the exposition of Rh negative individual
- Hemolysis/clearence of antibody taged or complement susceptible erythrocytes is executed through activation of complement (intravascular hemolysis) or macrophage lysis (extravascular hemolysis)




B cell
secretion
IgG
IgM
Splenic macrophage
Fcγ receptor
C3b
C3b
C3 receptor
C3b
C3b
C3b
phagocytosis of immunoglobulin-
and complement (C3b)-coated RBCs
complement fixation
to C3b
agglutination
complement fixation
to membrane attack complex
causing intravascular RBC lysis

Thank you!