Paroxysmal Nocturnal Hemoglobinuria (PNH)

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Introduction

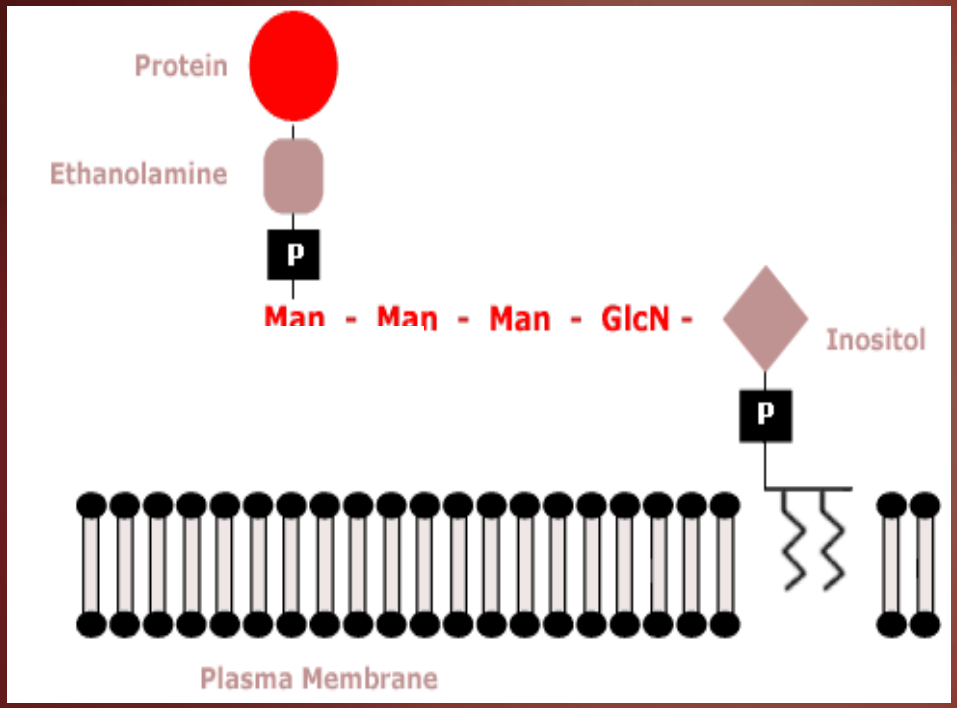
- Recurrent, episodic
  - Intravascular hemolysis
  - Hemoglobinuria
  - Venous thrombosis
- May not be
  - Paroxysmal
  - Nocturnal
  - hemoglobinuric

Introduction

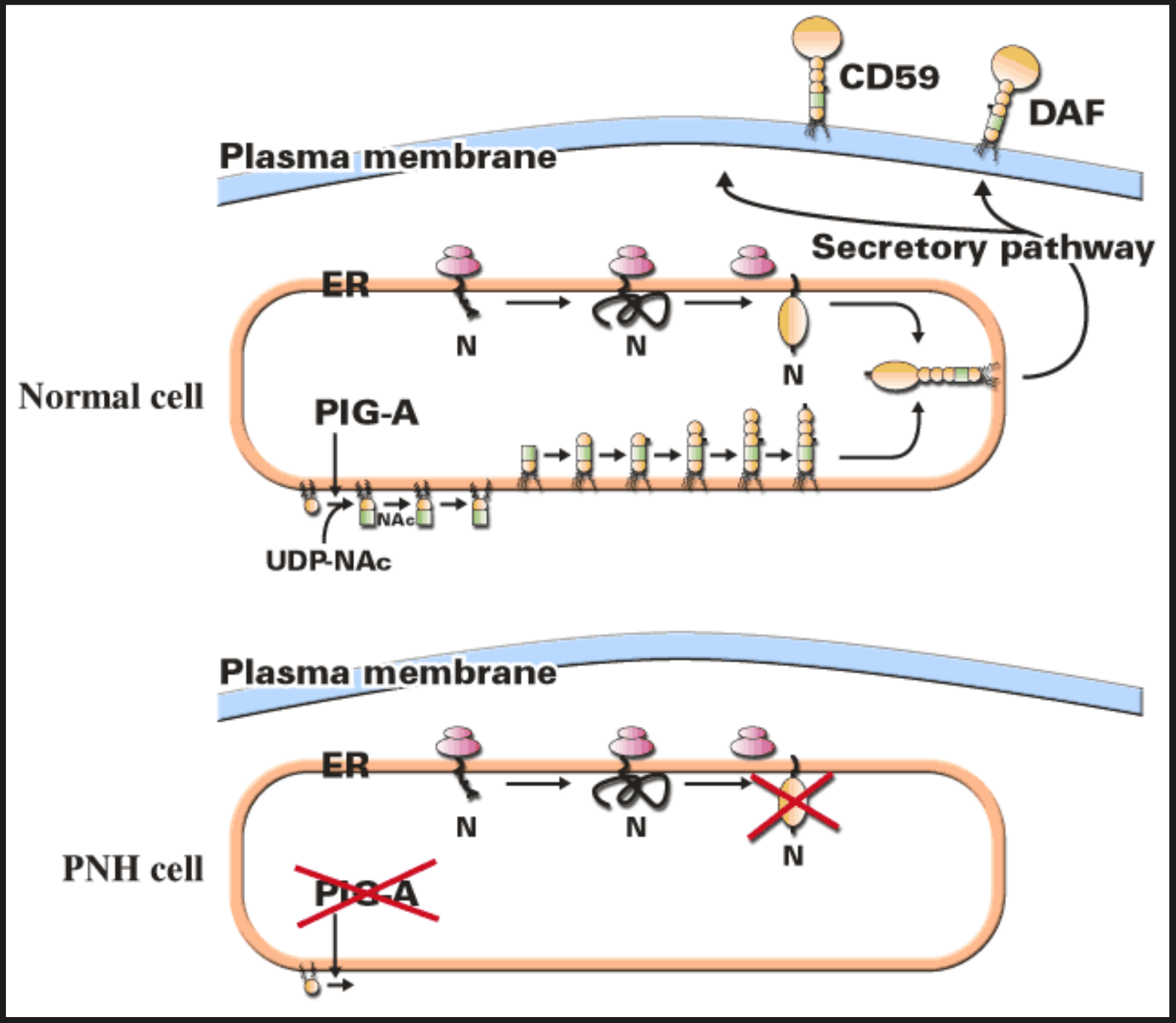
- Rare acquired stem cell disorder
  - Spontaneous somatic mutation in the hematopoietic stem cell
- PIG-A gene (phosphatidylinositol glycan complementation class A)
  - X chromosome encodes GPI (glycophosphatidylinositol) anchor protein
  - Partial or complete loss of linkage of cell surface proteins to the membrane by (GPI) anchor proteins
- Defect seen in all blood cells
- Clinically evident due to complement pathway

PIG-A Function

- Encodes for GPI protein
- Inability to synthesize GPI anchor protein
  - –Deficiency of cell surface proteins
  - –Serves as attachment for approx. 20 cell surface proteins



GPI, glycosyl-phosphatidylinositol


CD59
DAF
Plasma membrane
Secretory pathway
ER
N
N
N
Normal cell
PIG-A
NAc
UDP-NAc
Plasma membrane
ER
N
N
N
PNH cell
PIG-A

t11.1 Hematopoietic Cell Surface Proteins Decreased or Absent in PNH Patients

Complement regulatory proteins
- Decay accelerating factor (CD55)
- Homologous restriction factor
- Membrane inhibitor of reactive lysis (CD59)

Proteins associated with immune function
- Lymphocyte function antigen-3 (LFA-3, CD58)
- Fc receptor gamma III (CD16)
- Endotoxin-binding protein receptor (CD14)

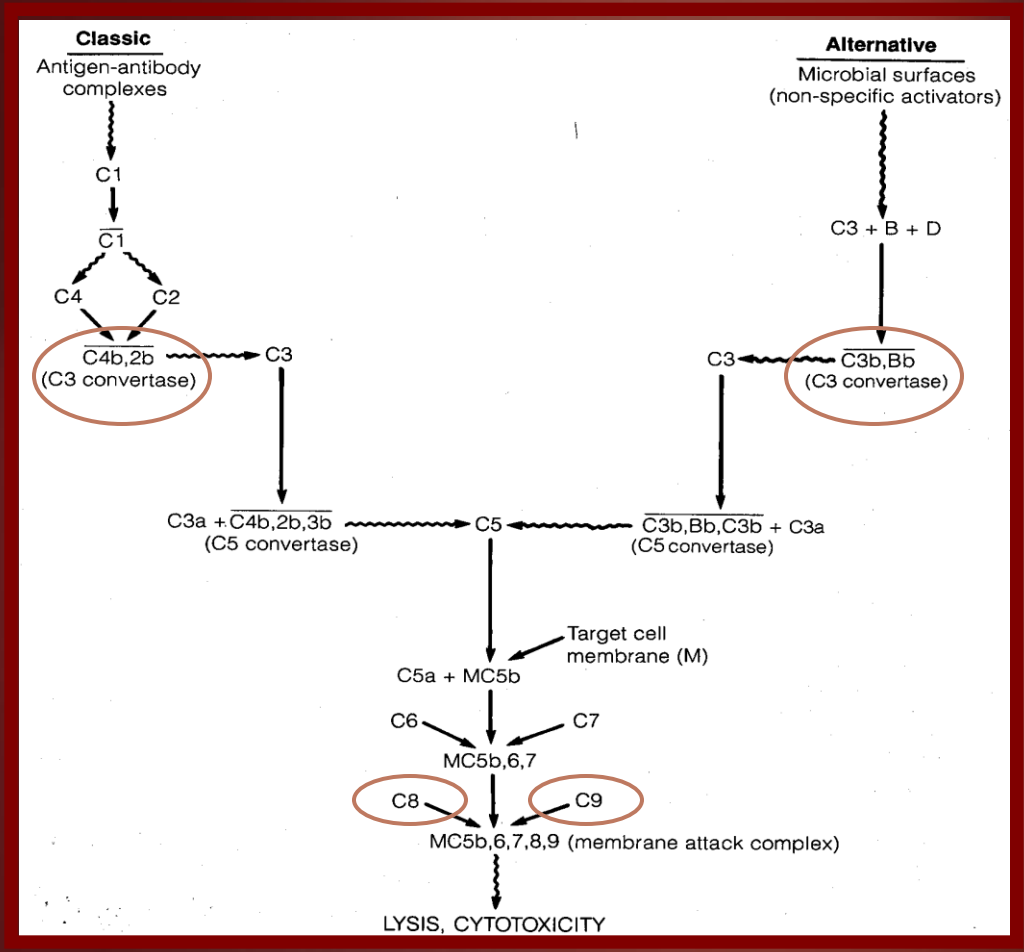
Other receptors
- Urokinase receptor
- Folate receptor

Enzymes
- Alkaline phosphatase
- Acetylcholinesterase
- 5'-ectonucleotidase

Other proteins
- CD24
- CD48
- CD52 (campath-1)
- CD66c
- CD67
- JMH-bearing protein

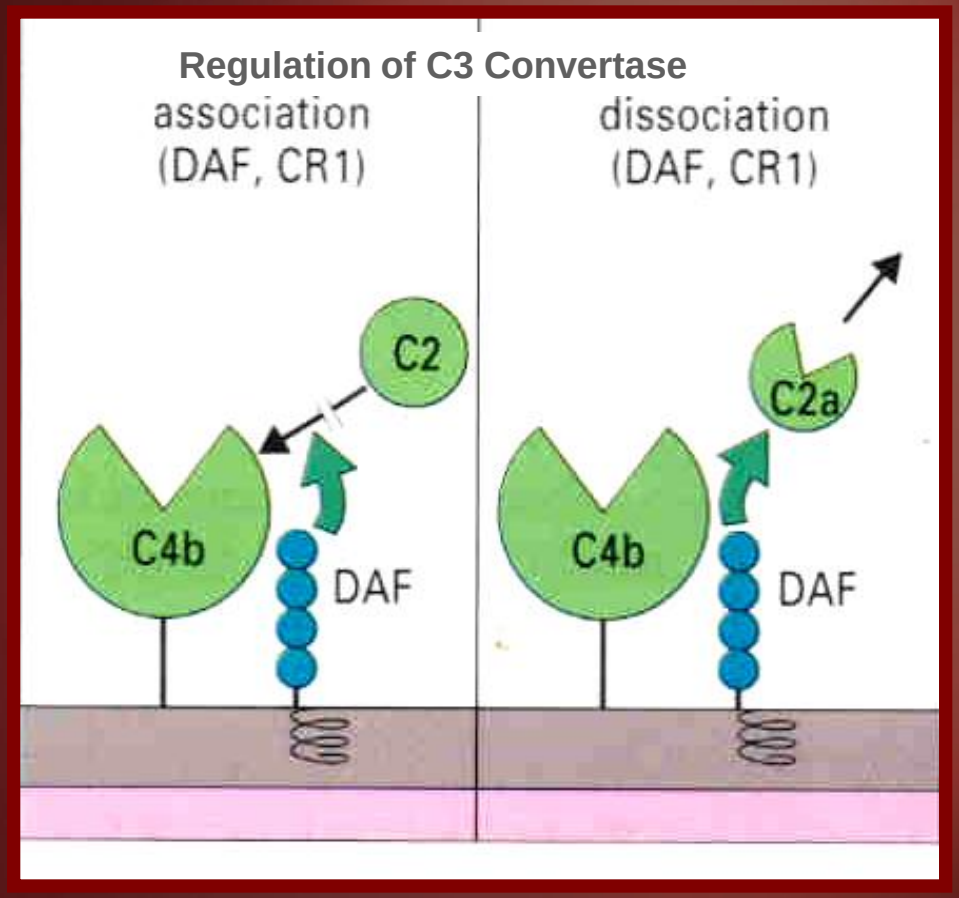
Complement Control Proteins

- CD55 (DAF)
  - –Regulates C3 convertase
- HRF
  - –Regulates C8 binding
- CD59 (MIRL)
  - –Modulates complement-mediated lysis

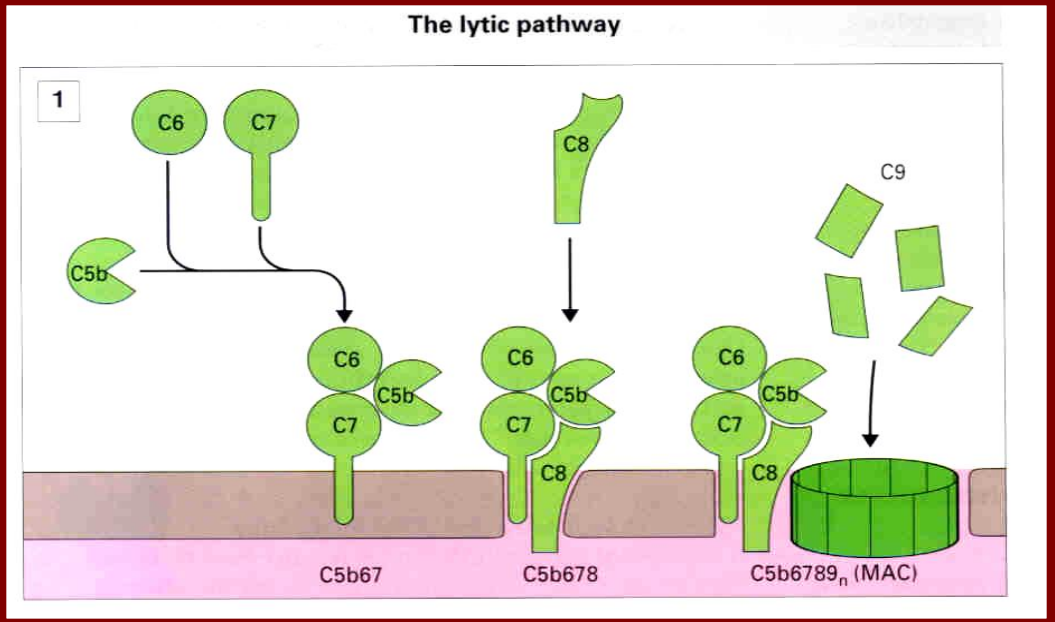


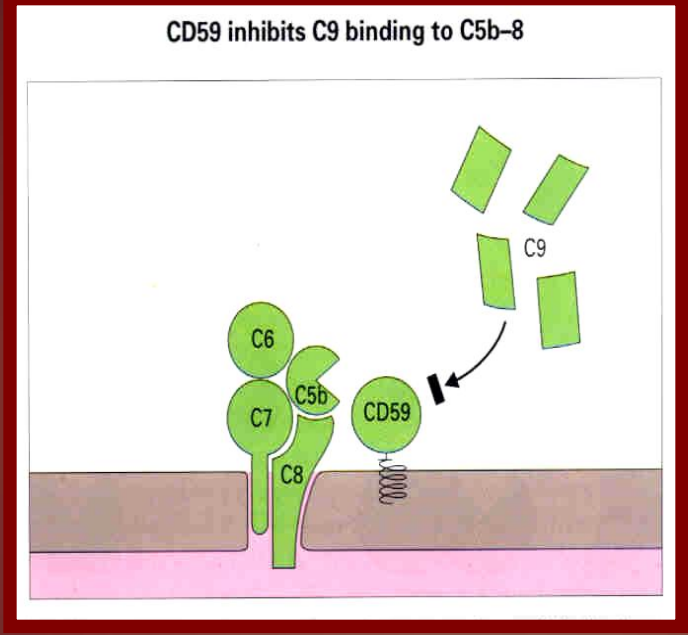
Complement Control Proteins

- CD55 (DAF)
  - –Inhibits association of C4b and C2
  - –Promotes dissociation of C4bC2a complex (C3 Convertase)

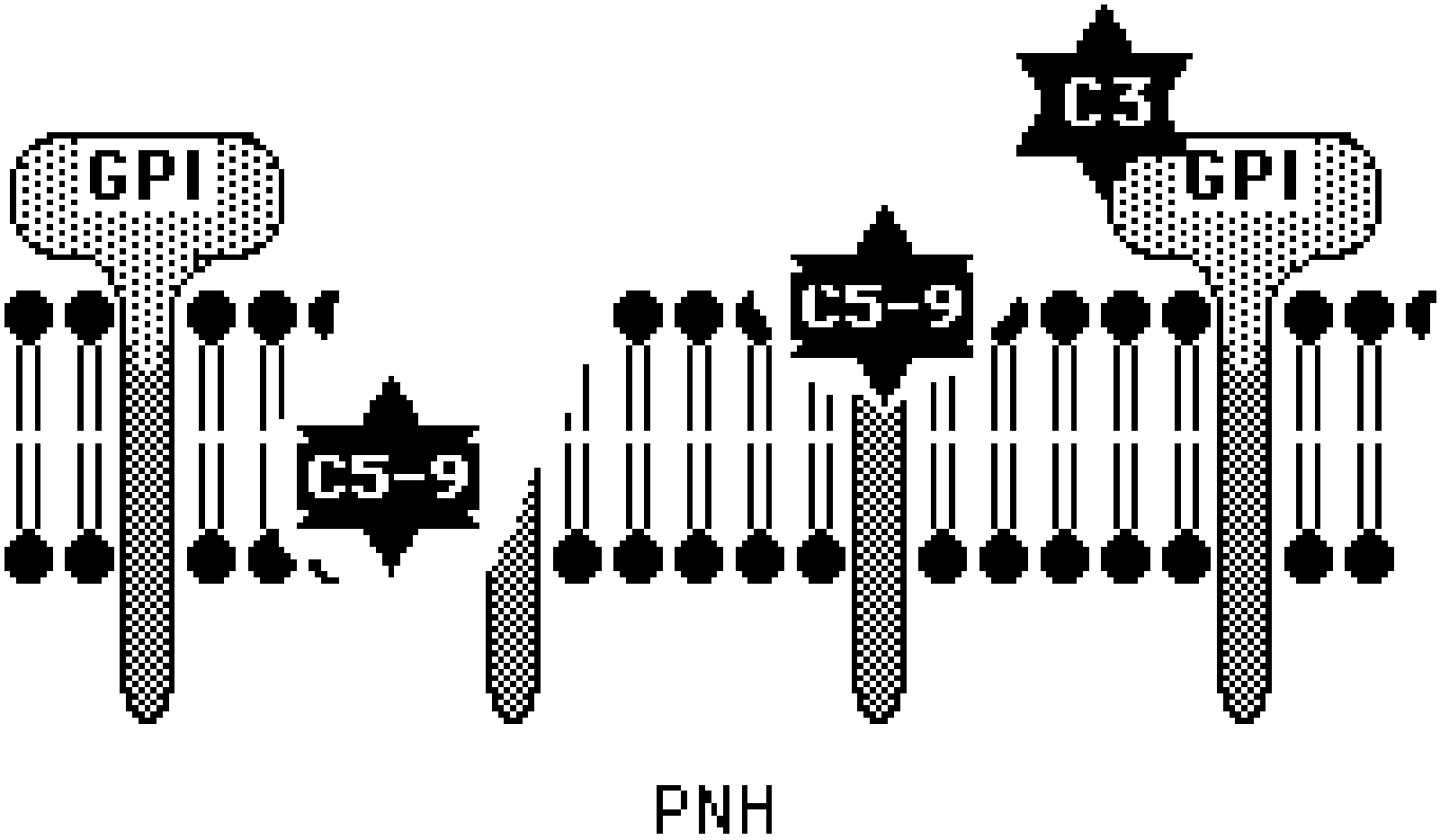


Complement Control Proteins





- CD59 (MIRL) and HRF prevent formation of Membrane Attack Complex and lytic action

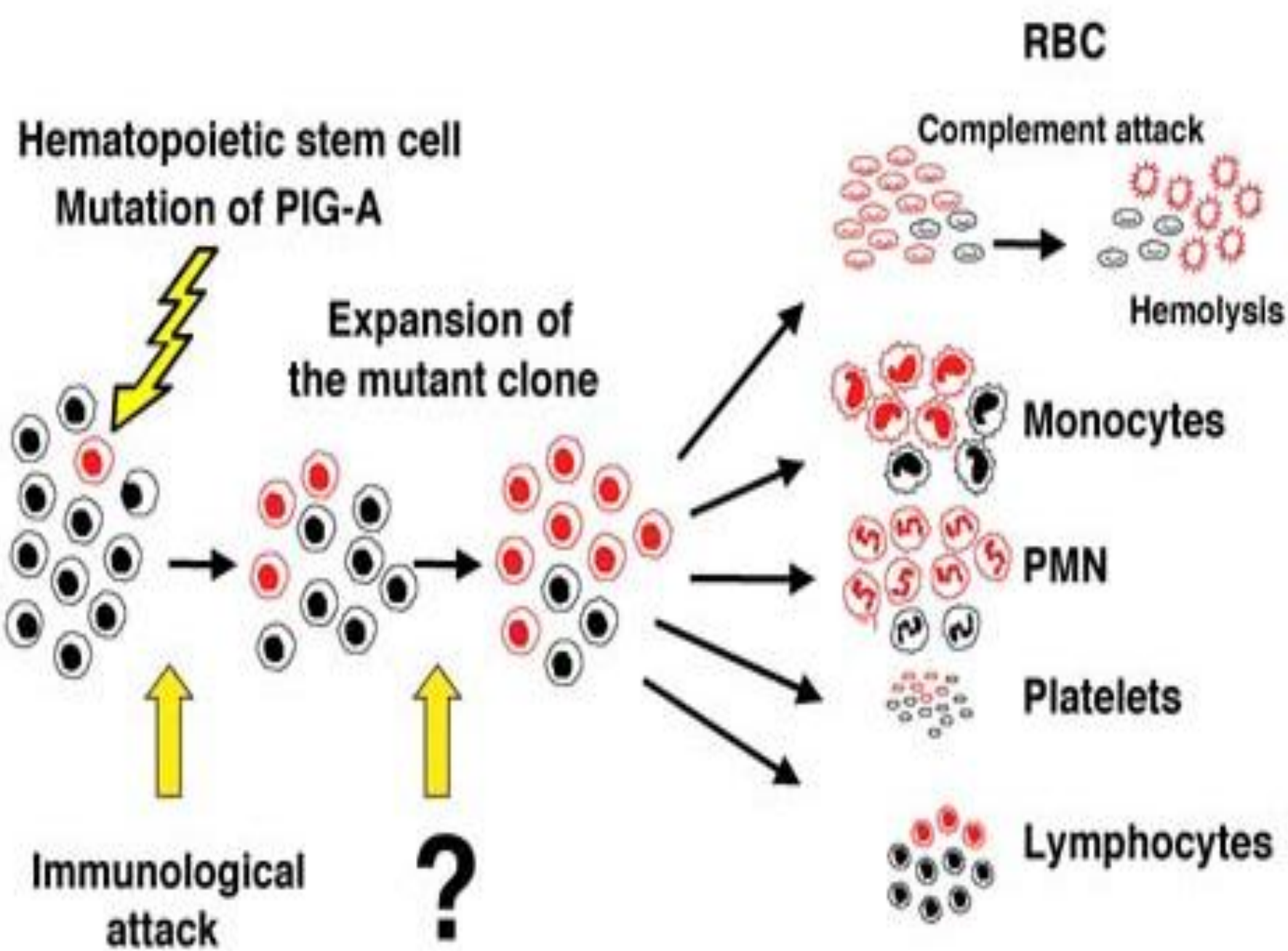


- Without important GPI anchored membrane-bound regulatory proteins such as DAF and CD59, complement molecules can bind to the target cell membrane and lyse the cell.

Mutations

- Hemolytic PNH
  - De novo
  - Hemolytic PNH evolves into aplasia
- Aplastic anemia/PNH

  Abnormal RBC clones develop during aplastic anemia


RBC
Hematopoietic stem cell
Mutation of PIG-A
Complement attack
Hemolysis
Expansion of
the mutant clone
Monocytes
PMN
Platelets
Lymphocytes
Immunological
attack
?

t11.2 Types of Cells Observed in PNH

PNH Cell Type	Sensitivity to Complement	Observed Complement Pathway Defects	GPI Protein Expression	Associated PIG-A Mutations
I	Normal to near normal	Near normal lytic behavior	Near normal to mild deficiency; partial lack of DAF (CD55) and/or MIRL (CD59)	None
II	Intermediate (10-15 times more sensitive)	Increased C3 binding to cell; increased C3/C5 convertase activity	Partial lack of DAF (CD55) and/or MIRL (CD59) (DAF deficiency appears most significant)	Missense (partial)
III	Highly sensitive (25 times more sensitive)	Increased binding of C3 to cell; increased C3/C5 convertase activity; increased binding of C5b67 complexes; increased C9 binding	Near total lack of DAF (CD55), MIRL (CD59), HRF	Nonsense, frameshift, deletion or insertion causing gene inactivation

PNH = paroxysmal nocturnal hemoglobinuria; GPI = glycophasphotidylinositol; PIG-A = phosphatidylinositolgycan A; DAF= decay accelerating factor; MIRL= membrane inhibitor of reactive lysis; HRF= homologous restriction factor

- Variable expression – genetic mosaicism

Additional Points

- Clonal disorder
  - Not a malignant clone
- Deficiency of complement-control proteins
  - On all affected hematopoietic cells
- Increased susceptibility to complement lysis
  - Erythrocytes not able to endocytose MAC as opposed to nucleated cells

Clinical Findings

- Uncommon, 1 to 10 cases per million, M=F
- Adults, insidious onset of anemia
  - often severe
- Episodic hemolysis
  - Classic: increased at night – drop in pH, dark urine on wakening
  - May occur chronically throughout the day
  - Precipitated by events – infection, surgery and transfusions
  - Not frequently seen, hemoglobinuria may be absent
  - Hemosiderinuria – chronic urinary iron loss
    - Constant feature
    - Iron deficiency anemia
  - Chronic renal failure due to renal tubular damage

Clinical Findings

- Thrombotic events
  - Complement activity creates a pro-thrombotic state
    - Damaged, dysfunctional platelets
  - Venous thrombosis in 33%, arterial rare
  - Major cause of death
- Abnormal platelet function
  - Often refractory to thrombolytic therapies
  - Severe episodes of abdominal or back pain
  - Most common
    - Budd-Chiari syndrome (hepatic vein thrombosis)
    - mesenteric vein
    - cerebral vein
- Thrombophlebitis in arms and legs → PE

Clinical Findings

- Leukopenia
  - Increase susceptibility for infection
    - Sinopulmonary
    - Blood borne infections
- Frequently progress to severe cytopenia
  - Transfusions other other therapeutic interventions

Clinical

t11.3 Clinical Manifestations of PNH

Intravascular hemolysis–Anemia associated with dark urine, hemoglobinuria, hemosiderinuria and possible iron deficiency. May develop chronic renal failure.

Increased thrombosis (1/3 of patients)

Hepatic vein, mesenteric vein or cerebral vein thrombosis

Thrombophlebitis and pulmonary embolism

Thrombocytopenia

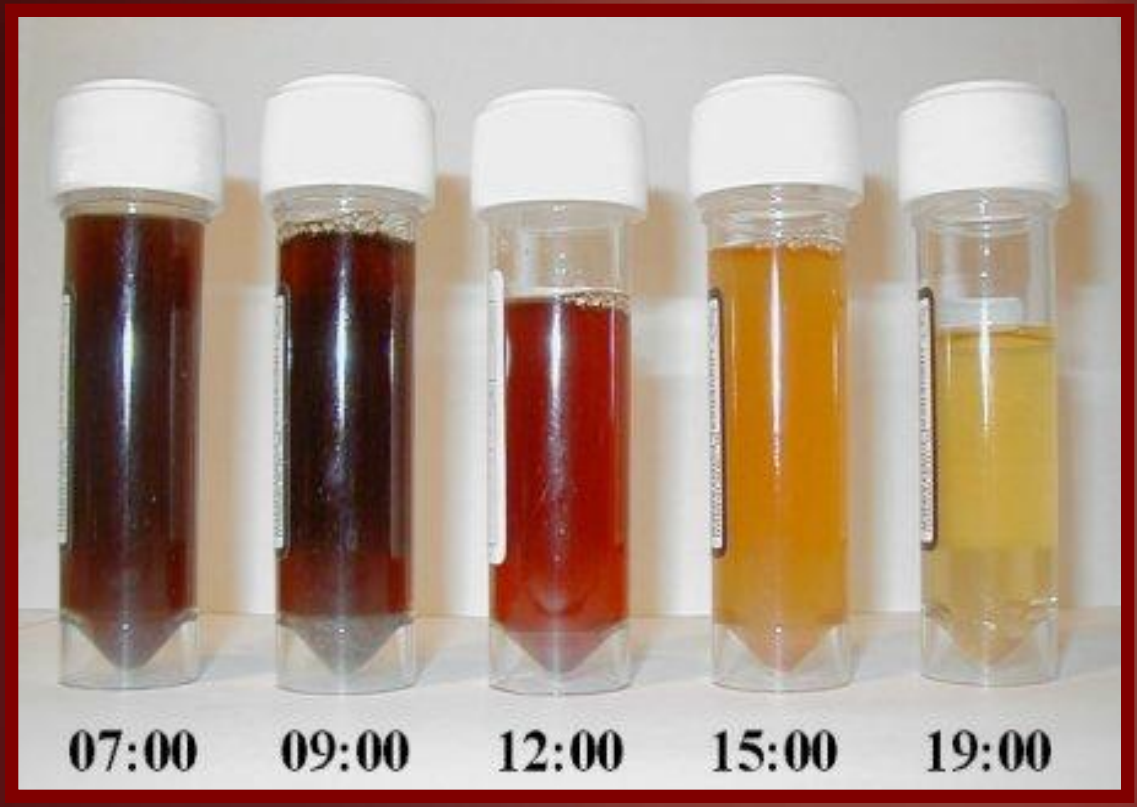
Leukopenia–sinopulmonary and blood infections

Bone marrow failure or aplastic anemia

Transformation to acute myelogenous leukemia or myelodysplastic syndrome

Nocturnal Hemoglobinuria

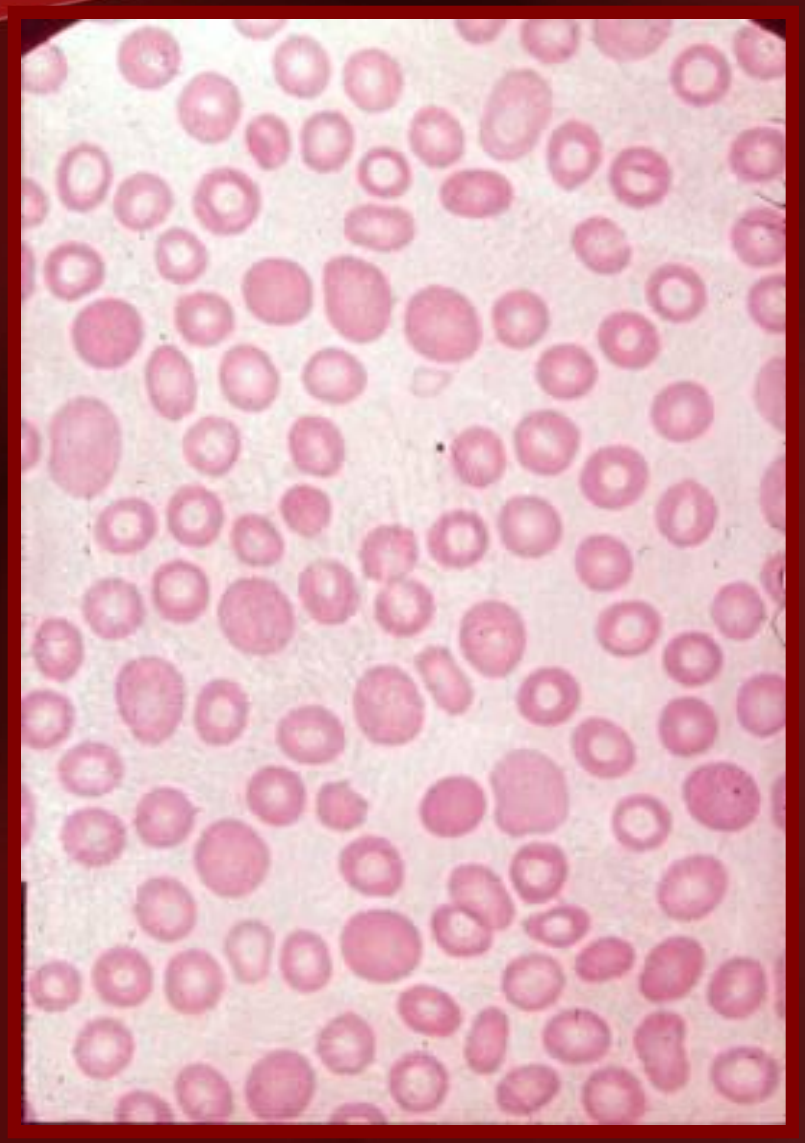
- Classical description
  - –Dark urine in morning that clears through the day
  - –Sleep decreases pH, increases complement activity
  - -Accumulation of urine



PNH Testing

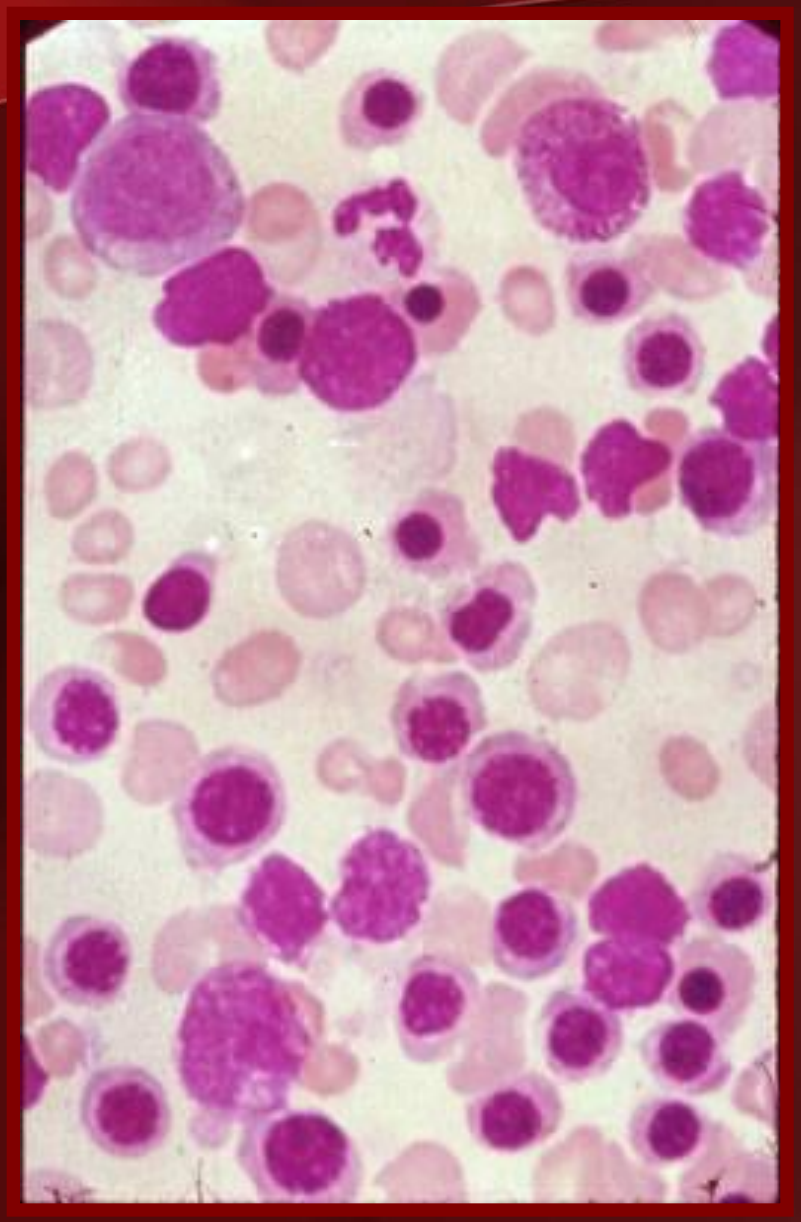
- Hematologic evaluation
  - CBC
  - Peripheral smear
  - Bone marrow examination
- Screening
  - Sucrose lysis test
  - Urine hemosiderin (Rous) test
  - LAP
- Confirmatory
  - Acidified serum (Ham's) test
  - Flow cytometry (most common now)

Peripheral Smear



- Macrocytic cells and polychromasia with
  - Reticulocytosis
  - B12/folate deficiency
- Microcytic, hypochromic cells with
  - Iron deficiency anemia
- Variable leukopenia & thrombocytopenia

Bone Marrow



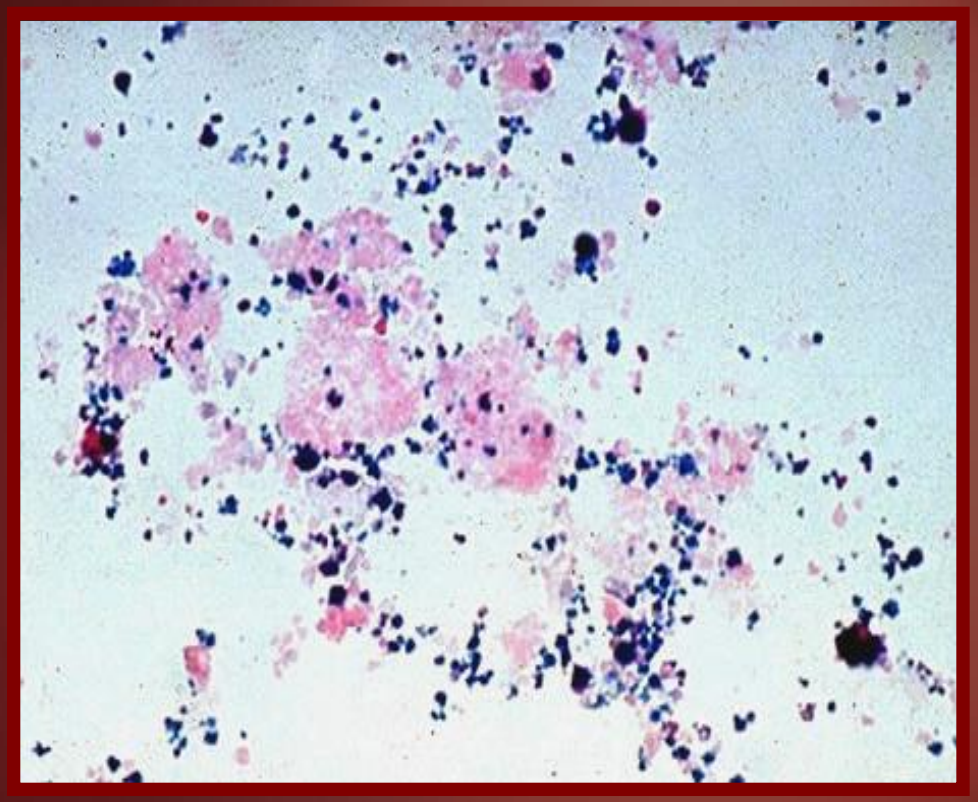
- Bone Marrow
  - Most often hypercellular
  - Normoblastic erythroid hyperplasia, consistent with intravascular hemolysis
  - Or, aplastic anemia

Sucrose Lysis Test

- Screening (outdated test)
- Pts. RBCs added to solution of isotonic sucrose and serum
  - Serum - complement protein source
  - Sucrose - aggregates globins onto RBC
  - Promotes binding and activation of complement on RBC surface
- Positive result if >5% RBC are lysed
  - Detected by the eye, visible red color to supernatant

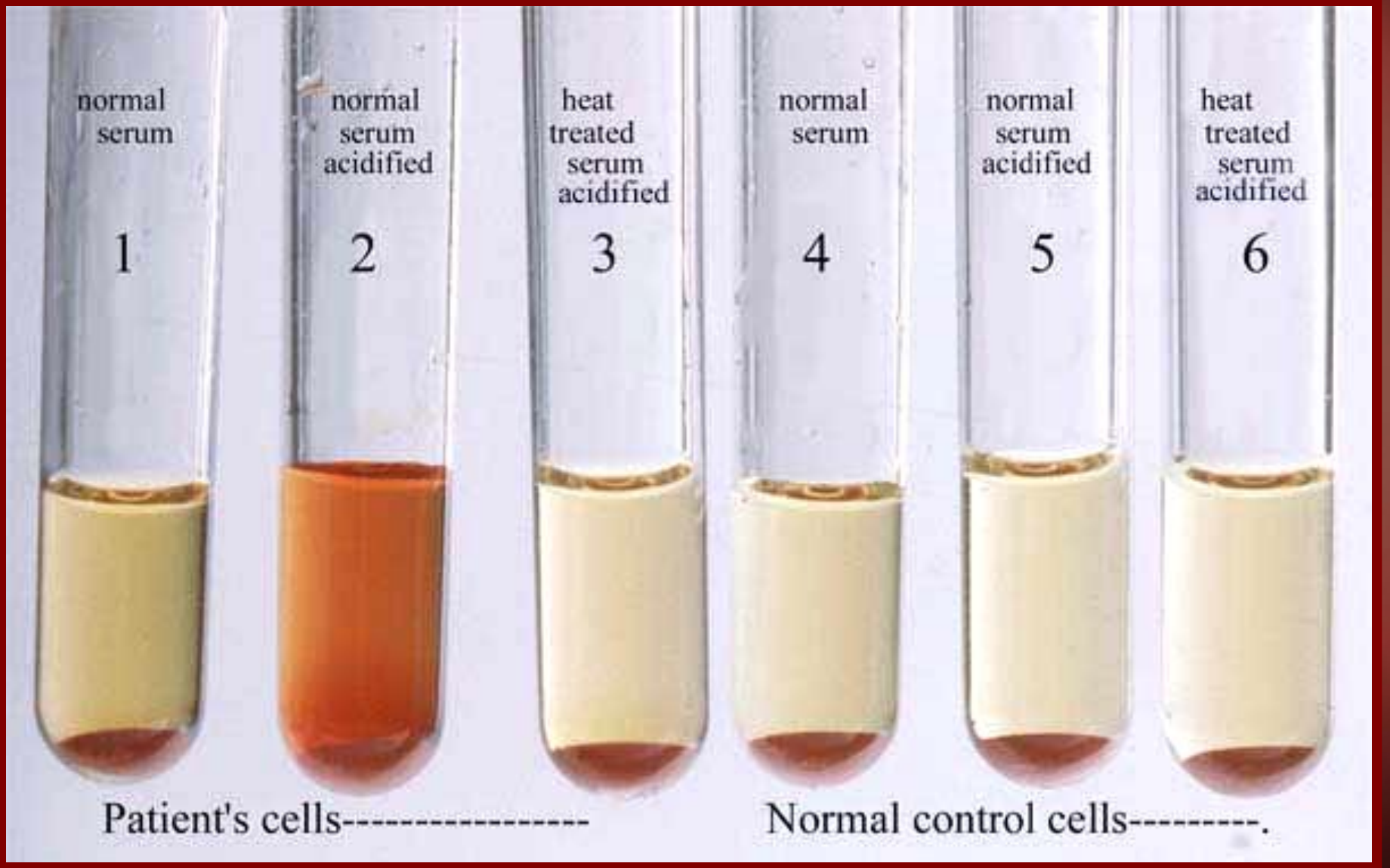
Additional Testing

- Urine hemosiderin
  - Chronic intravascular hemolysis
- Low LAP
  - Similar to CML (not specific)



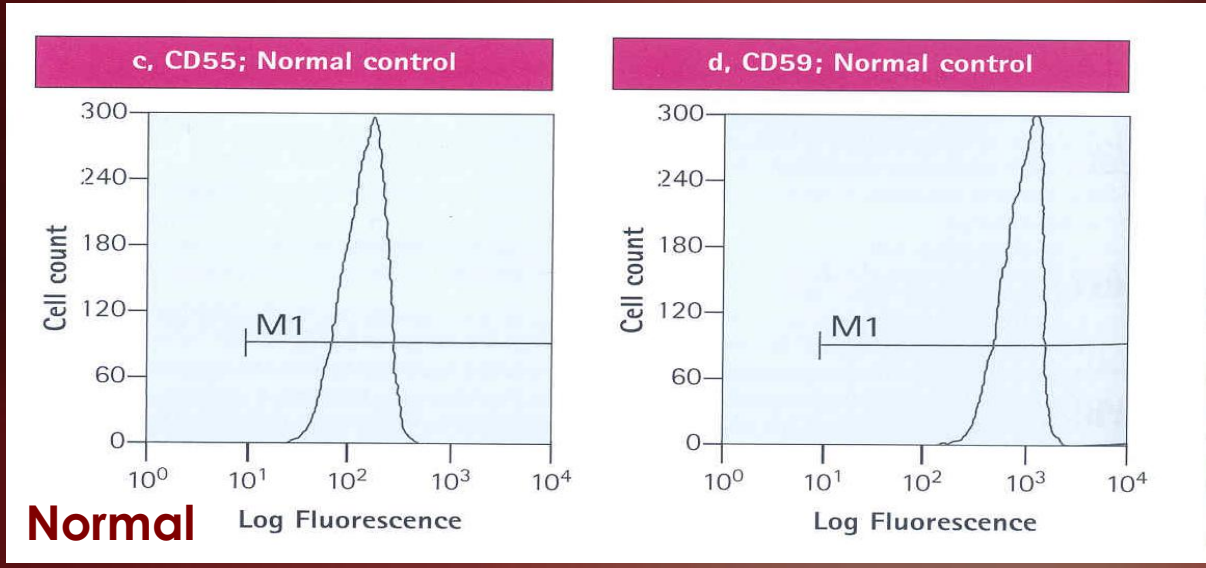
Acidified Serum (Ham's) Test

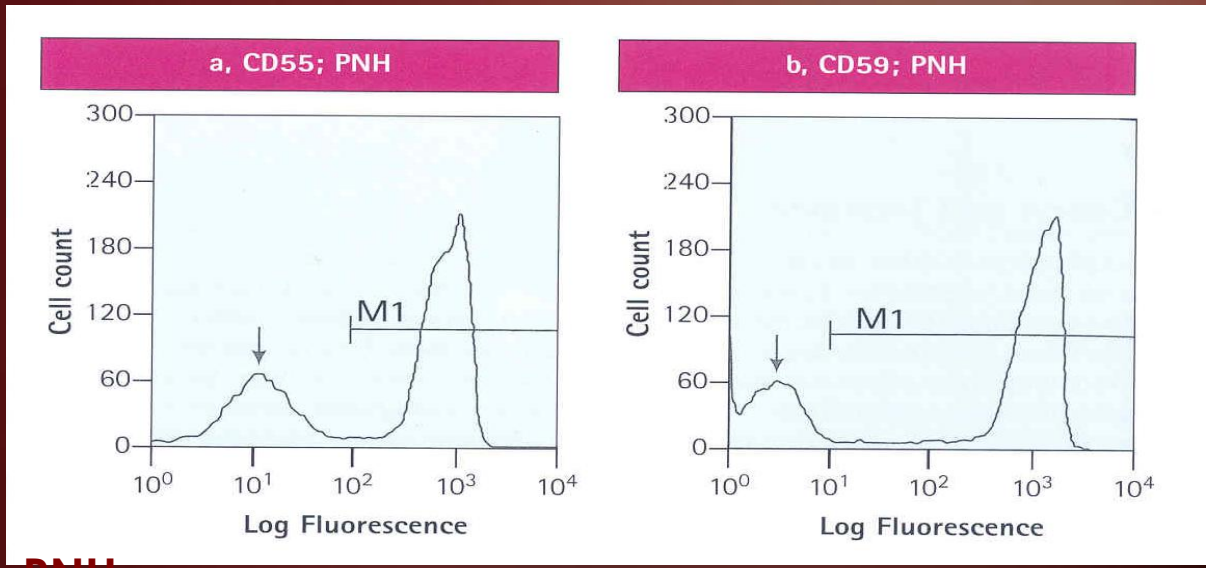
outdated test

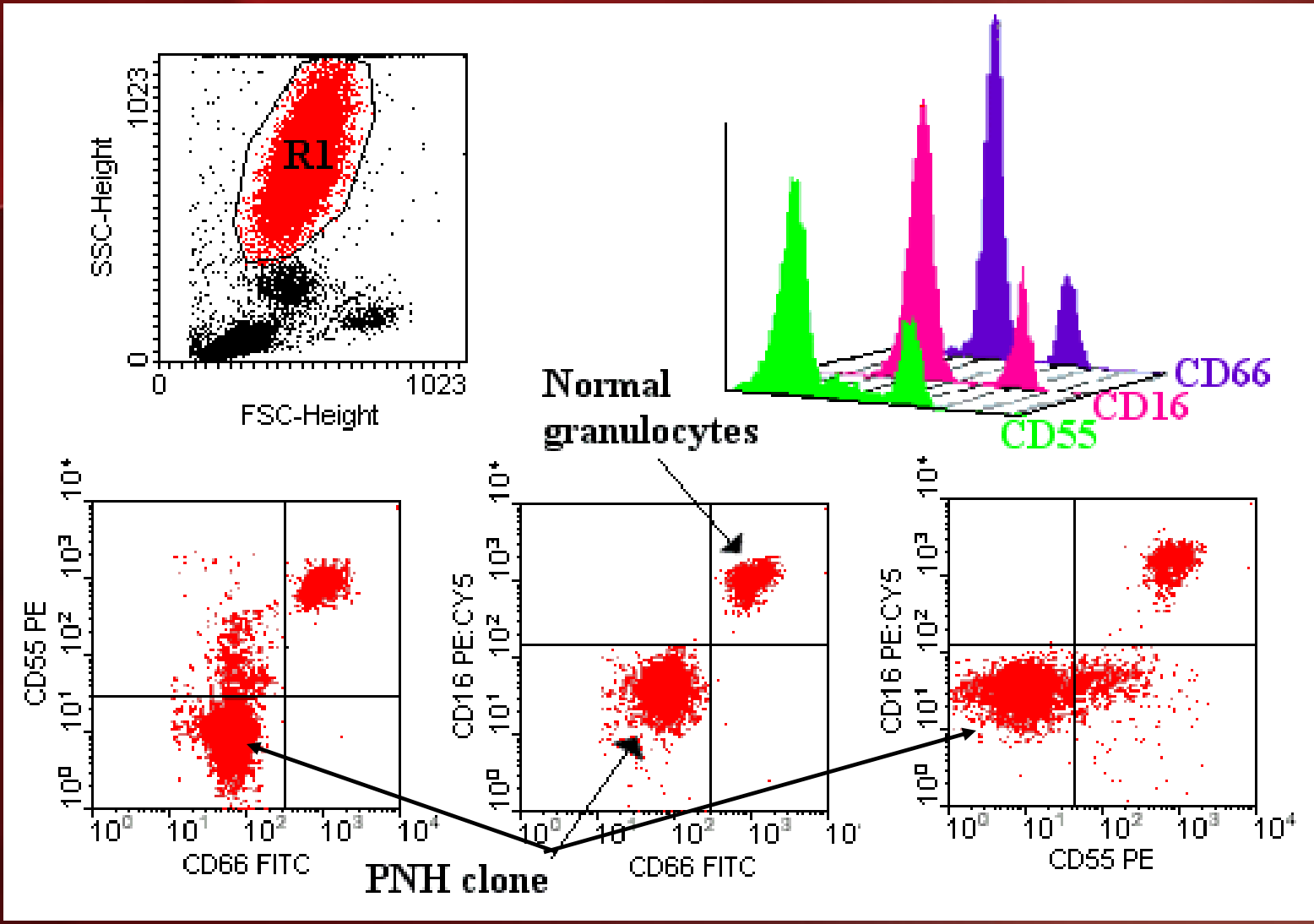


Flow Cytometry

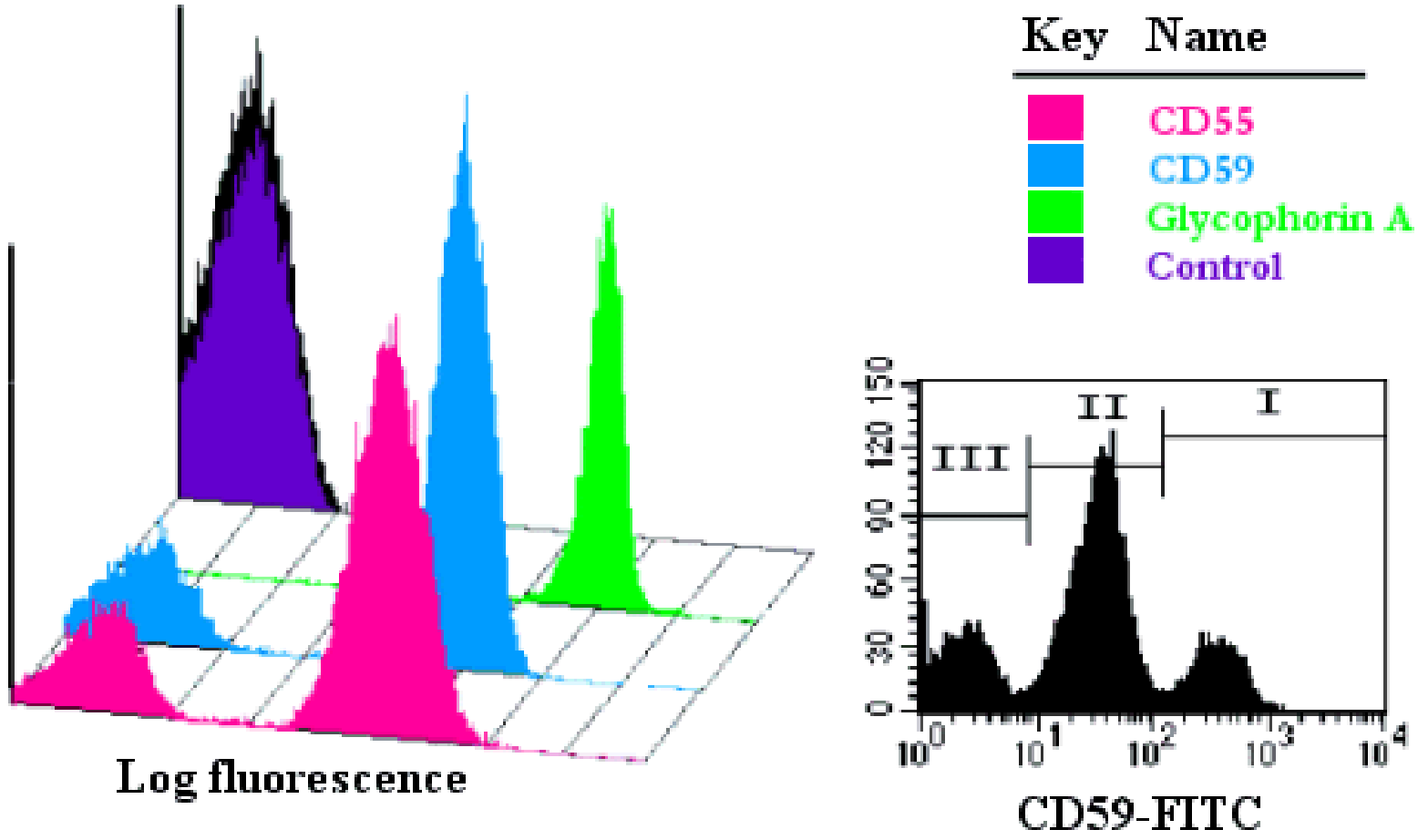
- Preferred confirmatory test
- Decreased expression of CD55 and/or CD59







Granulocyte flow cytometry in PNH. Granulocytes are electronically selected (upper left plot: red R1 region), and analyzed for expression of CD16, CD55 and CD66 cell-membrane proteins (lower dot-plots). Two cell populations are visible, a residual normal and the GPI-deficient PNH clone.



Red-cell flow cytometry in PNH. Red-cells are analysed for expression of CD55, CD59 and Glycophorin-A (CD235a; red-cell marker). The normal and GPI-deficient PNH red-cell populations (defined by CD55 and CD59) are visible in the histogram overlay plot. The lower right histogram shows three CD59-defined red-cell populations, Types I (normal), II (partial deficiency) and III (complete deficiency).

Clinical Course

- Fulminating or chronic
- Median survival 10 -15 years
- 25% surviving >25 years
- 33% spontaneous remission
- 33% Thrombotic complications
  - may be rapidly fatal
- MDS in 5%
- AML in 1-5%

Treatment

- Symptomatic blood transfusions
- Androgenic steroids → hematopoiesis
- Corticosteroids
  - complement modulation during episodes of increased hemolysis
- Thrombolytic or anticoagulant agents
- Bone marrow transplant (curative)

Table 4-1 Types of Constitutional and Acquired Aplastic Anemia and Red Cell Aplasia

Constitutional aplastic anemia	Constitutional red cell aplasia
Fanconi's anemia	Diamond-Blackfan anemia
Dyskeratosis congenita	
Shwachman-Diamond syndrome	
Acquired aplastic anemia	Acquired red cell aplasia
Idiopathic	Transient erythroblastopenia of childhood
Secondary to drugs, toxins, infections, and miscellaneous disorders/conditions	Parvovirus infection* (usually transient)
Paroxysmal nocturnal hemoglobinuria (clonal)	Idiopathic pure red cell aplasia
	Sustained pure red cell aplasia secondary to neoplasms, immune disorders, infections, and drug treatment

*Parvovirus infection may be sustained in an immunocompromised host.

Aplastic Anemia

- Reduction of
  - Erythroid
  - Granulocytic/monocytic
  - Megakaryocytic cell lines in the bone marrow and their progeny in the peripheral blood

Blood Findings

- Pancytopenia
  - Normocytic/normochromic anemia
  - RBC, platelets, granulocytes have normal morphology
- Elevated erythropoietin
- Decreased reticulocytes

Clinical Findings

- Anemia- weakness, fatigue, pallor
- Granulocytopenia- fever, infection
- Thrombocytopenia - petechiae, ecchymosis, mucosal bleeding
- No hepatosplenomegaly or LAD

- Phenotypic abnormalities- bony defects, mental retardation, skin/nail abnormalities

Bone Marrow Cellularity

- Hypocellular bone marrow
  - Rare residual hematopoietic elements
  - Replaced by fat

- Normal cellularity=100 - age

- Newborn 75-100%
- Adolescent 50-90%
- Adult 30-80%
- >65 years old 20-50%

- Hypocellular: <20%