

HEMATOLOGY# 15 -- Hemolytic Anemias II

HEMOGLOBIN DEFECTS --SICKLE CELL ANEMIA/OTHER HEMAGLOBINOPATHIES

- I. Introduction
 - A. Genetic control of hemoglobin synthesis
 1. synthesis of α -like, β -like globin chains controlled by separate structural genes
 - a. α and ζ genes located on chromosome 16
 - b. β, γ, δ , others located on chromosome 11
 2. normal adult blood contains
 - a. 90-98% Hb A1 ($\alpha_2\beta_2$)
 - b. 3.5% HbA2- ($\alpha_2\delta_2$)
 - c. 1% HbF - ($\alpha_2\gamma_2$)
 3. proportion of Hb formed related to rate of synthesis of different chains
 - B. Results of mutations at the genetic level (3 types)
 1. mutations in structure (a.a. substitution or deletion)
 - a. unstable hemoglobin disease
 - b. decrease molecular stability of molecule
 - c. >90% of Hb variants are single a.a. substitutions
 - i. result from single base substitution in triplet code
 - ii. Hb inherited as autosomal codominant trait
 2. Mutations altering O₂ binding (interface) sites.
 - a. decrease cooperativity (esp if alter α 1\$2 interface)
 - b. result in Hb with abnormal O₂ affinity
 - c. interfere with allosteric properties
 3. Abnormal chain synthesis (thalassemias)
 - C. Hemaglobinopathies (diseases of Hb)
 1. >500 variants of Hb --- (not all result in disease)
 2. many result from single a.a. substitution on one chain
 3. diagnoses
 - a. mild to severe anemia which is refractory to treatment
 - b. blood smear
 - i. microcytosis, hypochromia, target cells
 - ii. normoblastosis, poikilocytosis, [anisocytosis](#)
 - iii. Hb crystals in RBC, reticulocytosis
 - c. erythrocytosis d/t Hb with >O₂ affinity or cyanosis with decreased O₂ affinity or methemoglobinemia
 4. inheritance of hemoglobinopathies
 - a. autosomal dominant (heterozygote)
 - i. symptoms (disease)
 - ii. no symptoms (trait)
 - b. autosomal recessive
 - c. homozygosity may be lethal

II. Sickle Cell Anemia

- A. Introduction
 1. hemoglobinopathy found in 8% American Blacks (heterozygote)
 2. 2-4/1000 homozygous (death by age 30) (0.2-0.4%)
- B. Inheritance of Sickle cell syndromes (and other hemoglobinopathies)

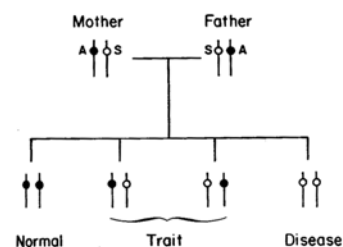


Fig. 11-8. Parents with sickle cell trait can have children with sickle cell anemia.

HEMATOLOGY# 15 -- Hemolytic Anemias II

C. Nomenclature

1. sickle cell disease: HbS only ($\alpha_2 \beta^S_2$ -- no HbA1)
2. sickle cell trait: HbS and HbA1 ($\alpha_2 \beta^S_2, \alpha_2 \beta^A_2$, no $\alpha_2 \beta^S \beta^A$)
(β^A, β^S : refers to WT and Sickle globin genes or chains respectively)
3. HbS = tetrameric Hb molecule containing β^S chains
4. HbA = tetrameric Hb molecule containing β^A chains (HbA or HbS refers to tetrameric structure)

D. Basis of RBC sickling

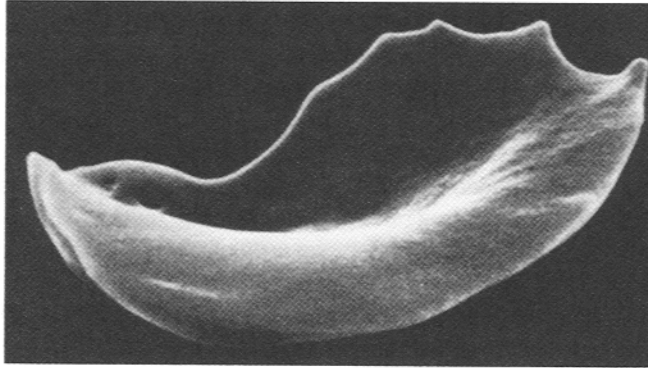


Fig. 11-3. A sickle cell. (From Bessis M [1973] Living Blood Cells and Their Ultrastructure. Springer-Verlag, New York, with permission.)

1. 50% cells sickled in homozygous state (sickle cell disease)
2. 1% of cells sickled in heterozygous state
 - a. sickling occurs during decreased pO₂
 - b. exertion or exposure to increased altitude
 - c.
3. molecular basis
 - a. $\alpha_2 \beta^S_2$ 6 val: substitution at position 6 of β chain
 - i. HbA: Glu6; HbS: - Val6
 - ii. HbA2 ($\alpha_2 \beta_2$) and HbF ($\alpha_2 \beta_2$) normal
 - b. deoxyHbS less soluble than oxyHbS and Hb nucleates → polymerizes
 - c. 70-98% of Hb is polymerized HbS (in sickles cell crisis)
 - d. sickling occurs when hydrophobic bonds form between HbS (and other Hb molecules) in low pO₂
 - e. HbS molecules in RBC form crystals which lie parallel and elongate the cell

HEMATOLOGY# 15 -- Hemolytic Anemias II

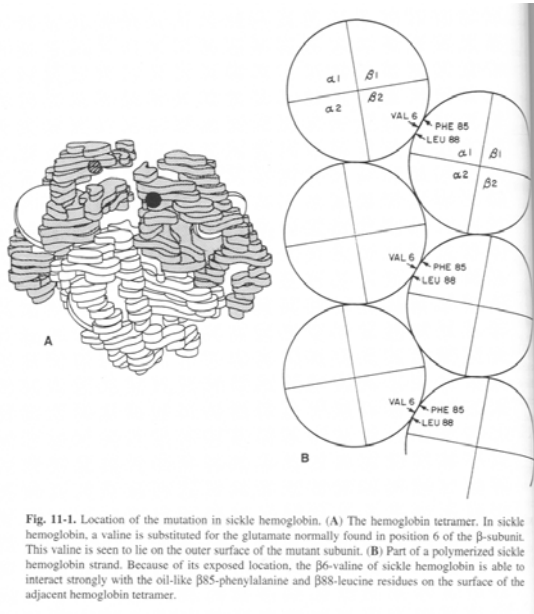


Fig. 11-1. Location of the mutation in sickle hemoglobin. (A) The hemoglobin tetramer. In sickle hemoglobin, a valine is substituted for the glutamate normally found in position 6 of the β -subunit. This valine is seen to lie on the outer surface of the mutant subunit. (B) Part of a polymerized sickle hemoglobin strand. Because of its exposed location, the β -valine of sickle hemoglobin is able to interact strongly with the oil-like β 85-phenylalanine and β 88-leucine residues on the surface of the adjacent hemoglobin tetramer.

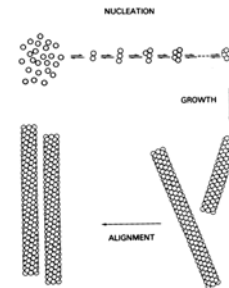


Fig. 11-4. The three stages in the polymerization of deoxyhemoglobin S. (From Dean J. Schechter AN (1978) Sickle-cell anemia: molecular and cellular bases of therapeutic approaches. *N Engl J Med* 299:752, with permission.)

4. HbS co-polymerizes better with some types of Hb molecules than with others
 - a. severity of disease dependent on persons genetic makeup (\$^S\$^X)
 - increasing severity of disease in association with Hb
 - HbS > HbD > HbC > HbE > HbA > HbF
 - [S,C,D,E are abnormal Hb variants]
 - [A and F are normal but in certain cases F can be very high
 - hereditary persistence of fetal hemoglobin
 - e.g. patients doubly heterozygous for S-C have serious disease almost identical to sickle cell disease
 - HbS + HbF (newborn or HPFH) rarely have disease.
 - A-S usually have no disease

5. Cellular events
 - a. cells can sickle. unsickle, resickle
 - b. 1/3 of sickled cells hemolyze intravascularly due to fragmentation in microcirculation and membrane damage

166 • Hematology: A Pathophysiological Approach

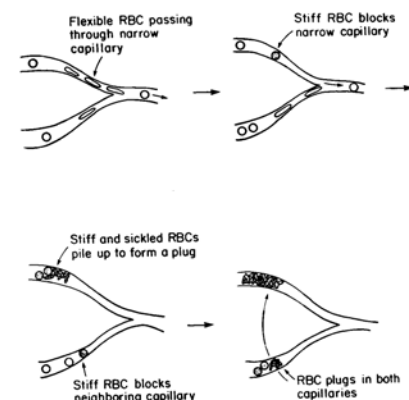


Fig. 11-5. How sickle cells block the microcirculation.

E. Pathophysiology

1. asymptomatic if not in crisis (more infection prone)
2. complications of sickling
3. hemolytic anemia (minor)
 - a. due to intra and extravascular hemolysis of irreversibly sickled cells causes anemia, jaundice
 - b. hyperplasia of b.m. with enlarged, marrow spaces, thinning of cortices, demineralization, osteoporosis
 - c. vascular occlusion (major)
 - d. sickled RBCs (rigid) cause sluggish flow in microcirculation

HEMATOLOGY# 15 -- Hemolytic Anemias II

- e. sluggish flow leads to hypoxia and acidosis
 - f. hypox & acid increases sickling
 - g. local hypoxia (O₂ starvation) → ischemic necrosis, thrombosis (platelets adhere), infarction
 - h. affect spleen, marrow, liver, kidney, lung, neurons
 - i. associated with "crises" painful episodes precipitated by acute infections, fever, dehydration, acidosis
4. Course of HbS disease
 - a. death by age 30
 - b. adults have periodic vaso-occlusive crises, but hemolytic crises rare (common in childhood)
 - c. chronic hemolytic anemia
 - d. cardiomegaly due to compensation for anemia
 - e. hepatic enlargement, renal dysfunction, bone lesions ischemic necrosis)
 5. Diagnosis
 - a. clinical appearance of patient
 - b. Blood smear
 - i. sickled and holly-leaf cells, Howell-Jolly bodies
 - ii. nucleated RBC, basophilic stippling
 - iii. hyperplastic bone marrow
 6. Lab tests of HbS
 - a. Sickling test: add O₂ consumer (metabisulfite)
 - b. Solubility test: HbS relatively insoluble in >1.0 M phosphate buffers
 - c. Hb electrophoresis (70-98% HbS + 0-20% HbF)

F. Sickle Trait

1. prevalence: 8% of American Blacks
2. heterozygous for HbS and HbA1
3. asymptomatic with no anemia
4. balanced polymorphism
 - SS = sickle cell disease
 - AS = slight resistance to falciparum malaria
 - a. Why? perhaps parasite can't metabolize HbS; sickling interrupts life cycle or passage to tissue phase
 - b. also protection in Hbt trait (HbA1 + HbC)
AA = susceptible to malaria
5. pathophysiology
 - sickling can occur during severe hypoxia
 - also during air flights even in pressurized cabins
 - although combination of HbS with HbA1 is benign, with double heterozygosity with second hemoglobinopathy → severe disease
5. Diagnosis
- D. sickling test (requires much lower pO₂ than HbS)
- E. Hb electrophoresis (35% HbS + 60% HbA)

III. Other Hb variants

A. HbC

1. mostly in blacks,
2. rare: 22/100,000 in U.S.
3. CC-homozygote has mild disease
 - a. episodic abdominal pain, slight jaundice, splenomegaly, mild hemolytic anemia
 - b. lysine substituted for glu at position 6 of β chain

HEMATOLOGY# 15 -- Hemolytic Anemias II

- c. HbC/HbS heterozygote
 - i. 2 of most common hemoglobinopathies may combine
 - ii. (prevalence = 80/100,000 in U.S.)
 - mild - severe symptoms, but less- severe than sickle disease
- B. HbD (no sickling: hematuria, mild hemolytic anemia, target cells)
 - 1. both the disease and trait are very rare and asymptomatic
 - 2. HbD + HbS (or thalassemia) heterozygote may have severe disease
- C. HbE occurs largely in SE Asia
 - 1. Heterozygote asymptomatic
 - 2. Homozygote has mild hemolytic anemia too
- F. Unstable hemoglobins
 - 1. due to aa substitutions in globin; some asymptomatic
 - 2. in others, heme may detach and cause Heinz bodies ppt formation and severe hemolytic anemia
 - 3. most are autosomal dominants which are lethal in homozygous state
 - 4. symptom if anemia is present
 - a. jaundice induced by acute infection
 - b. dark urine d/t heme breakdown products
 - c. splenomegaly
 - d. reticulocytosis, anisocytosis, poikilocytosis
 - e. Heinz bodies numerous in acute attacks, otherwise they are normally removed by spleen and only occasional instability is caused by heme dissociation
 - f. precipitation of heme caused by mild chemical or thermal trauma (fever)
- G. Hemoglobinopathies associated with abnormal O₂ transport
 - 1. Increased O₂ affinity with erythrocytosis
 - a. e.g. Hb Bethesda
 - b. increased O₂ affinity sufficient to inhibit 'optimum release of O₂ in tissue
 - c. symptoms
 - i. hypoxia
 - ii. erythrocytosis d/t stimulation of erythropoietin
 - d. pathophysiology
 - i. location of a.a. substitution impairs 'heme-heme interaction resulting
 - ii. in shift- to the left
 - iii. some substitutions prevent binding of 2,3DPG in deoxy Hb
 - iv. inherited as autosomal dominant
 - 2. Decreased O₂ affinity
 - a. less common than increased affinity e.g. HbKansas
 - b. symptoms of cyanosis
- H. Hb M
 - 1. most common cause of cyanosis (familial)
 - 2. a.a. substitutions in alpha or beta chains at the site of heme attachment (Tyr → His residue replacement)
 - a. stabilizes heme of abnormal chains in Fe⁺⁺⁺
 - b. alters oxygenation of normal chain
 - 3. heterozygotes asymptomatic except for cyanosis
 - 4. probably lethal in homozygote