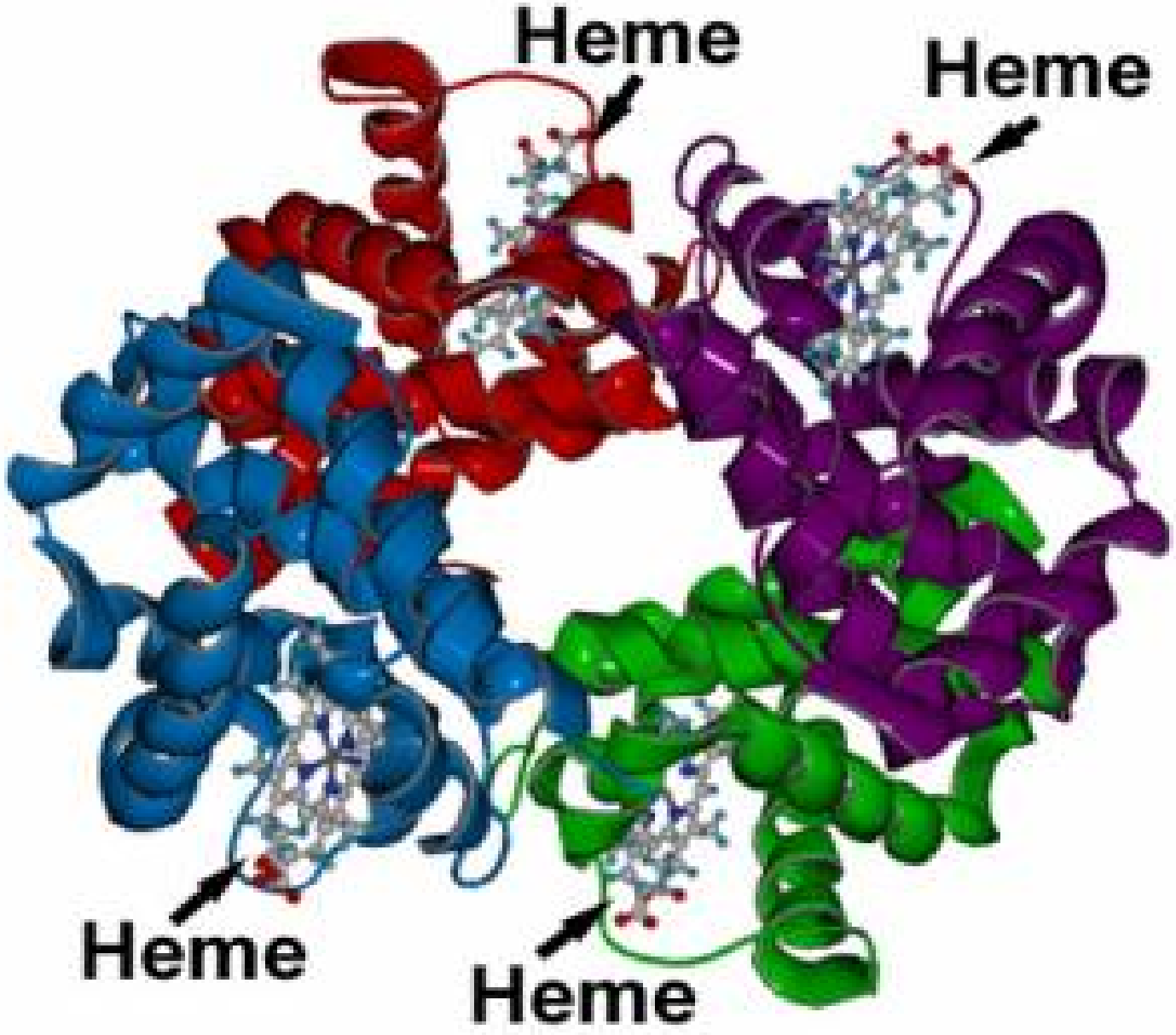
PATHOPHYSIOLOGY OF SICKLE CELL DISEASE

Definition

- Sickle cell anaemia is a haemoglobinopathy that results from a point mutation of the beta polypeptide chain of haemoglobin(Hb).
- Haemoglobinopathy: disorders affecting the structure, fxn and prdn. of Hb. They are usually inherited.

BACKGROUND

- Hb found in RBCs
- A complex protein consisting of an Fe. containing heme group and protein chains
- It’s a tetramer made of two pairs of PP. chains each having a heme group attached
- Heme :protopophyrin 1X and Ferrous iron


Heme
Heme
Heme
Heme

Development/types of Hb

- Embryonic Hb:predominates wks.4-8, disappear by the 3rd month
- Gower 1: ζ_2 ε_2
- Gower 2: α_2 ε_2
- Portland: ζ_2 γ_2
- The zeta(ζ) chains are structurally similar to alpha chains

Fetal Hb

- Contains α_2 γ_2
- Predominates by the 10-11th wk, by 24th wk it constitutes 90%, declines in 3rd trimester :makes 70% of Hb at birth.Synthesis declines rapidly postnatally only traces found by 6-12 mo.
- Adults have less than 2% fetal Hb
- Its heterogeneous coz the γchain synthesis is directed by 2 sets of genes.Chains differ at position 136 in presence of either glycine or alanine.ratio at birth 3:1

Adult Hb

- Hb A_1: α_2 β_2
- Hb A_2: α_2 δ_2
- Hb A_1 makes 5-10% of the total by wk 24. At term 30%, by 6-12 mo. The adult pattern appears.
- Hb A_2 makes 2-3%
- Ratio of A_1:A_2=30:1
- Alpa chains 141 AA, beta 146AA

Alterations of Hb by disease

- Gower found in trisomy 13/15
- Portland found in stillborn infants with homozygous α thalassemia
- Hb F elevation occurs with haematologic stress
- HbA_2 elevated in beta thal., vit.B_{12} def. and folate def. Decreased levels in Fe. Def. and alpha thal.

Genetics

- Alpha like chains encoded by two sets of genes on Ch.16
- Beta,gamma and delta chains encoded by genes on Ch. 11

Classification of hemoglobinopathies

- Structural ;mutation of amino acids of globin chain leading to abn.polymerization,altered solubility and O2 binding
- Thalassemia syndromes:impaired prdn. of globin chains
- Thalassemia hemoglobin variants: combine features of thal. and structural Hbpathies

Cotd

- Hereditary persistence of fetal Hb(HPFH)
- Acquired Hbpathies: modification of Hb molecule by toxins eg.methemoglobinaemia

Epidemiology

The disease is common in Africa, Middle east, Mediterranean countries and in India.

- In the US the disease is common among the blacks with 8% of them being carriers and 0.8% being homozygous
- In Kenya SCD is common among the Luo(53.4%) and Luhyia(24.2%) tribes in western Kenya and among the Miji Kenda(18.7%) at the coast.

Cotd

- Other tribes including the Kisii's and the Kamba's have been found to have the disease
- The sickle cell trait however has been found in nearly all the tribes of Kenya with the Miji Kenda leading at 35%
- There are no significant differences between the males and females
- Majority of the cases are between 1-20 years of age
- 2003:26% of Kenyans in malaria holoendemic regions had the sickle cell genotype opposed to only 3% in the highland regions
-

Haplotypes

- Senegal –found commonly along the Atlantic coast of W. Africa
- Benin-Found in Central, West Africa, Angola and Kenya
- Bantu/Central Africa Republic-common in Zaire,CAR,Kenya
- Asia-Common in Saudi Arabia and India

Sickle cell Disease

- Major forms:
- Homozygous SS-most common, worse
- clinical presentation
- Sickle-beta thalassemia-β^0 and β^+
- Hb SC
- Rare forms
- Sickle cell Hb D $_{punjab}$ dse
- Sickle cell Hb O_{Arab}dse

History

- 1st described by Herrick in 1910
- Syndenstericker described the first paediatrics case.He recognized that it's a hemolytic dse.
- 1949-Pauling identified it as an autosomal disorder
- 1959-Ingram described the substitution of valine for glutamate
- Was the 1st dse to be described to be due to molecular mutation

Pathophysiology

- Basic defect:Replacement of glutamate by valine at position 6 of the beta globin chain
- The clinical presentation is due to either of these main pathophysiologic processes
  - vaso-occlusion
  - hemolysis
  - Infections

Molecular pathogenesis

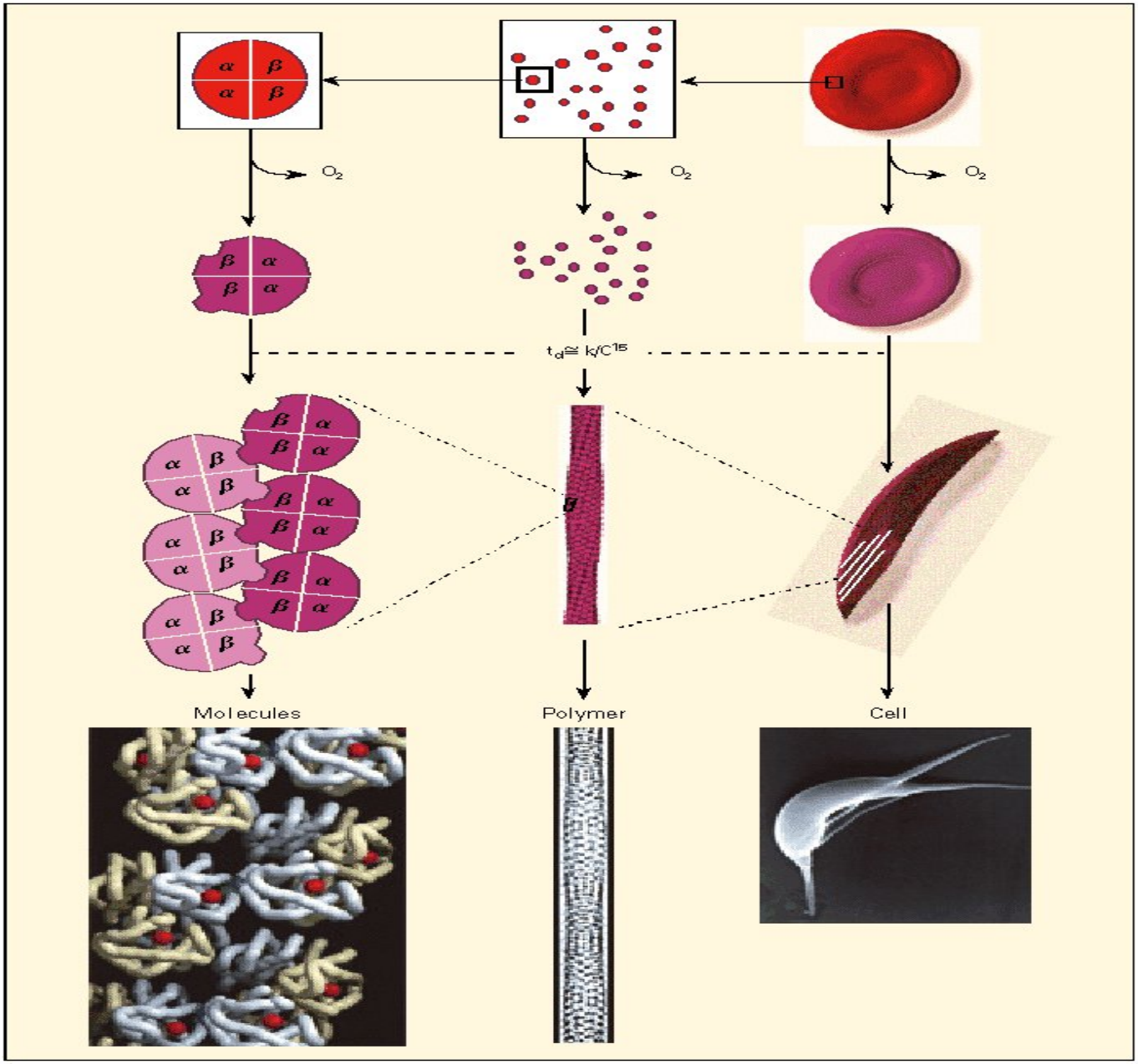
- The presence of valine at position 6 creates a hydrophobic chain that also has a high propensity of binding with other globin chains
- This leads to interaction btwn. different Hb molecules leading to aggregation into large polymers.
- The polymerization is the 1° event that leads to distortion of shape and decreased deformability of the cells

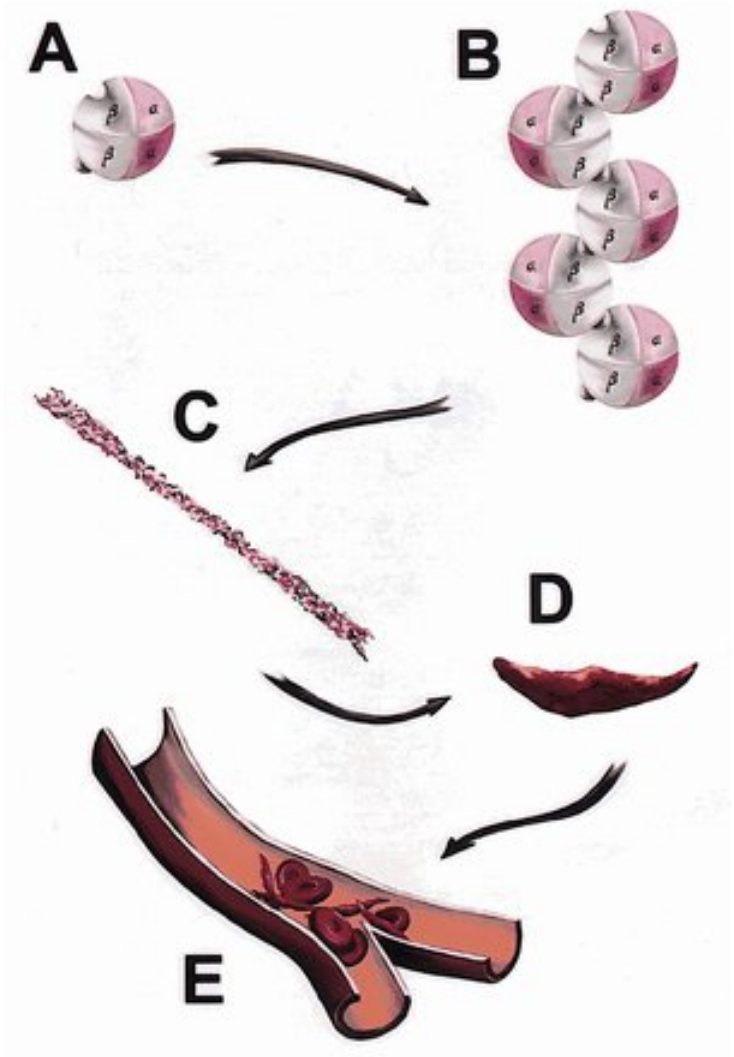
Cotd.

- The formation of a single nucleus of aggregated molecules is followed by growth and alignment of fibers
- Distortion of shape follows leading to disturbance of structure and function of the membranes

Rate /Extent of polymer formation depends on:

- Degree and rate of deoxygenation
- Hb conc.
- Intracellular Hb type.
- pH
- Temperature
- Extacellular osmolarity


α β
α β
O2
β α
β α
td≅ k/C15
Molecules
Polymer
Cell


A
B
C
D
E

Kinetics of HbS polymerization

- Rapid deoxygenation leads to formation of multiple independent polymers.This leads to granular or cobblestone texture that does not alter the cell shape
- Partial or slow deoxygenation leads to formation of a single nucleus.This is followed by growth and alignment of more fibers.

Dysregulation of red cell volume

- Mean intracellular Hb.conc.and mean density of SS RBCs is close to that of normal RBC.However the density distribution is broad.Less dense cells are due to reticulocytosis with low Hb conc. Very dense cells are due to polymerization induced membrane damage leading to enhanced dehydration.This usually leads to irreversibly sickled cells.

Dehydration

- Main factors that lead to dehydration: K^{+},Cl^{-} co transport and Ca^{++} activated K efflux (Gardos channels)
- In normal RBCs the K^{+},Cl^{-} co transporter is found only in reticulocytes
- Rates of co transport in SS and CC RBCs high

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- The co transport is induced by cell swelling and low pH (may occur with stagnant circulation)
- This co transport system is inhibited by magnesium
- SS RBCs have increased Ca^{++} in intracellular vesicles but its normal in cytosol.

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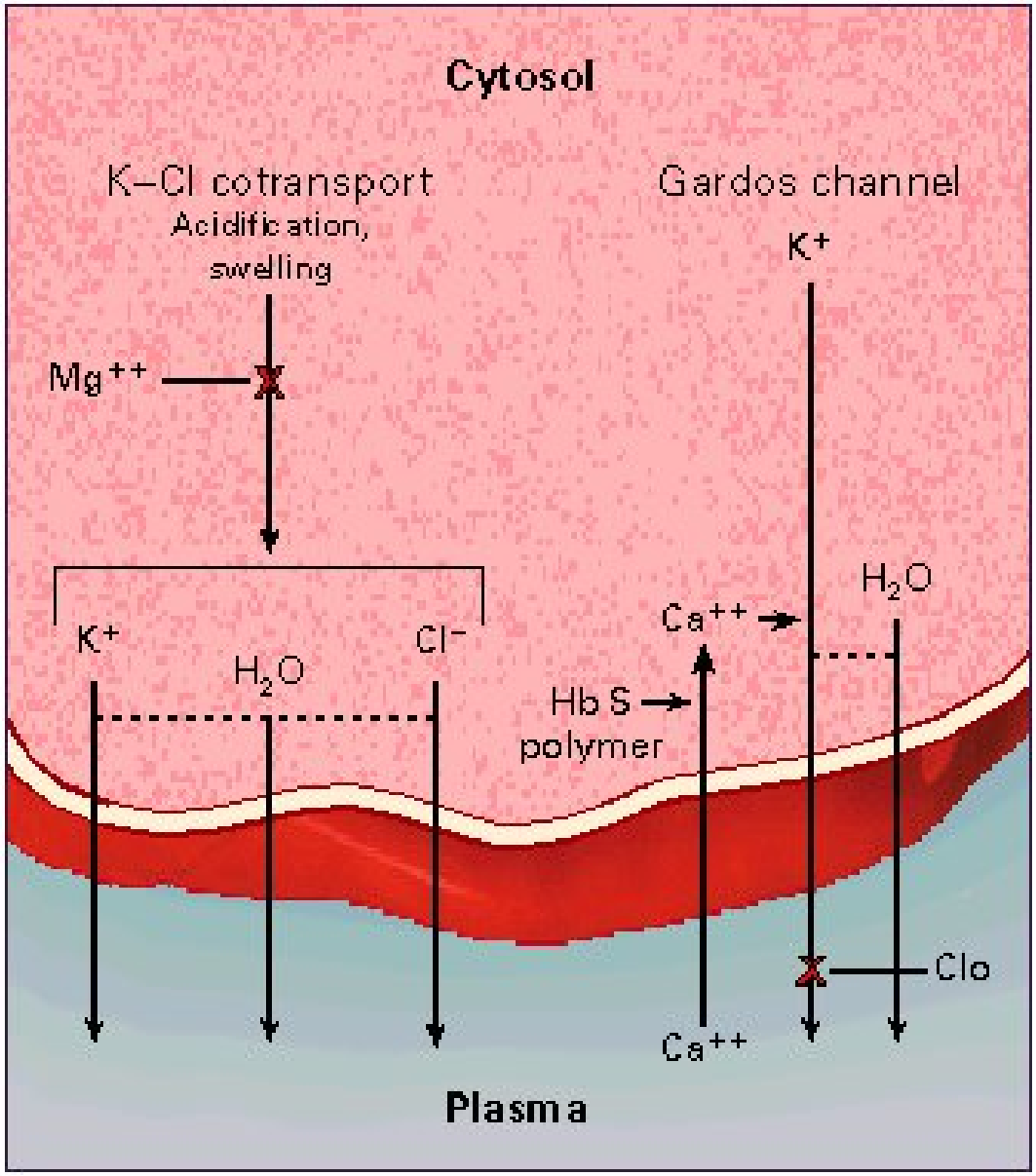
- Membrane destruction leads to transient increase in cytosolic calcium .This opens the Gardos channels leading to loss of water and K^+
- The Gardos channels are inhibited by clotrimazole


Cytosol
K–Cl cotransport
Acidification,
swelling
Mg++
Gardos channel
K+
H2O
K+
Cl−
H2O
Ca++
Hb S
polymer
Clo
Ca++
Plasma

Interaction of SS cells and vascular endothelium

- Most challenging aspect in SCD is the unpredictable and episodic nature of vasoocclusion
- Potential for a sickled cell to initiate a vaso-occlusive event depends primarily on whether the rate of polymer formation is within the range of capillary transit time

Cotd

- Its been shown that SS red cells have a sticky surface and attach more readily than normal cells to endothelium
- Degree of adherence strongly correlated to severity of disease

Molecular interaction for adhesion

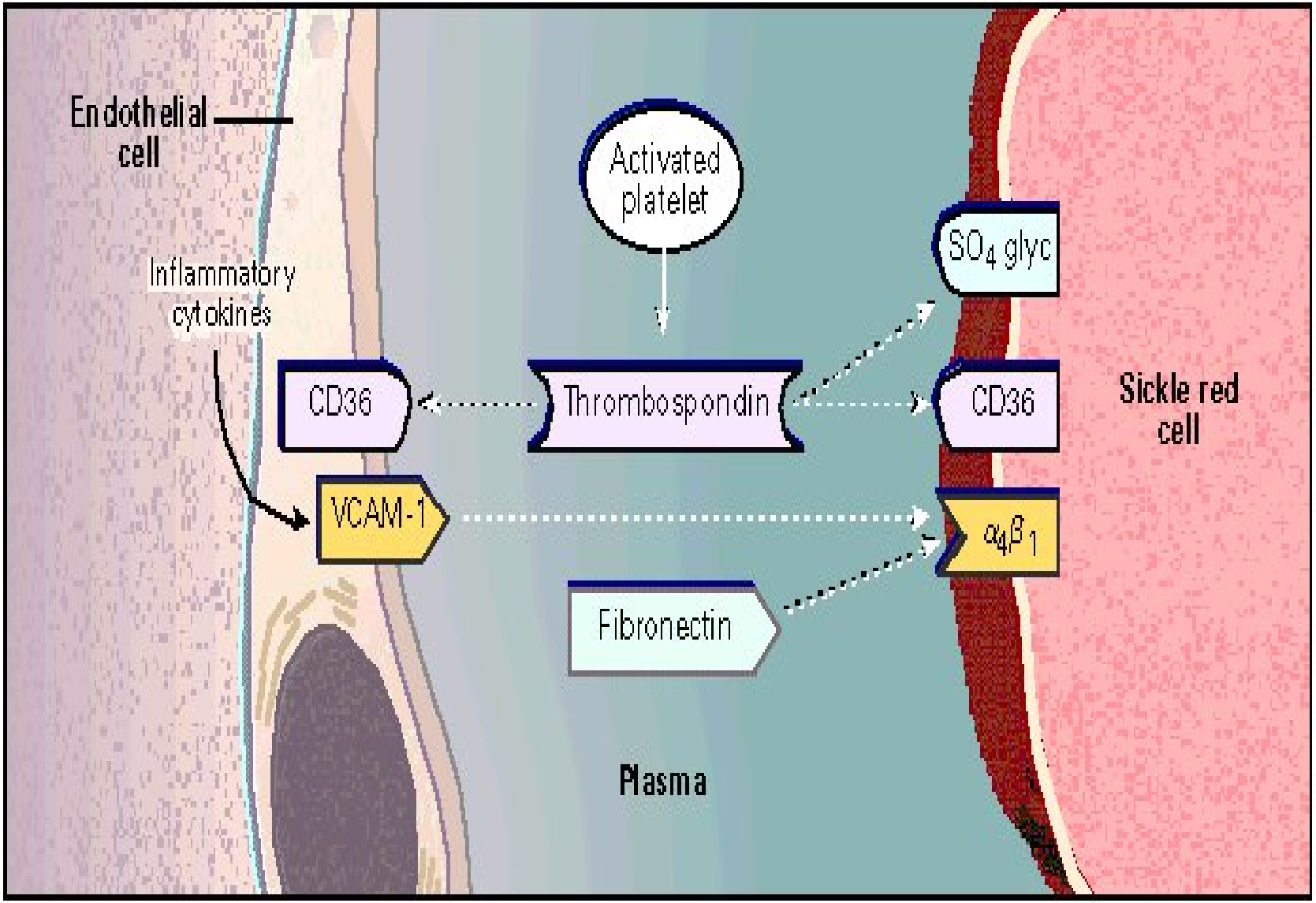
- Reticulocytes esp. those of pts. With SCD have on their surface the integrin complex α4β1 which binds to fibronectin and VCAM-1
- VCAM-1 usually expressed by endothelial cells after activation by inflammatory cytokines eg TNFα.

Cotd.

- Microvascular endothelial cells and sickle erythrocytes have CD36which binds to thrombospondin secreted by activated platelets
- Thrombospondin also binds to sulfated glycans on SS cells
- Other molecules that contribute to the adhesion include the very high molecular weight form of von willebrand factor
- Increased binding of SS reticulocytes to fibronectin also contributes

Cotd.

- VCAM is upregulated by hypoxia and inhibited by nitrous oxide
- Low nitrous oxide levels also promote adhesiveness


Endothelial cell
Activated platelet
Inflammatory cytokines
SO_4 glyc
CD36
Thrombospondin
CD36
Sickle red cell
VCAM-1
$\alpha_4\beta_1$
Fibronectin
Plasma

Other factors

- Adherent SS RBCs are known to inhibit vasorelaxation
- Macrocirculation of pts. With SCA dvp. Intimal hyperplasia
- With repeated episodes of polymerization heme is released from Hb .The Fe. In heme promotes formation of reactive oxygen species which damage the lipids and proteins of the RBCs membrane.
- The damaged proteins form abn.clusters which lead to dvp of Ab.leading to hemolysis.

Cotd.

- The membrane outer leaflet has increased amounts of phosphatidylethanolamine and phosphatidylserine which promote thrombosis

Haemolysis

- One third of the haemolysis occurs intravascularly
- This occurs due to membrane damage and fragmentation in the microvasculature
- The SS RBCs also adhere to macrophages and undergo phagocytosis
- Rest of haemolysis occurs in the spleen and in the liver

Acute chest syndrome

- Infections
- Fat embolism
- Pulmonary thromboembolism
- Vaso occlusion

Reduced immunity

- Asplenia
- Reduced IgM
- Impaired opsonization
- Sluggish alternate complement pathway

Sickle-cell trait confers protection against mortality between 2-16 months of life in western Kenya

CDC

Aplastic crises

- Infections
- Folate deficiency
- Toxins

Sickle cell nephropathy

- Renal haemodynamics usually normal upto thirty years of age
- Inability to conc. Urine the commonest disorder
- In sicklers usually there’s increased renal plasma flow .This is accompanied by increased GFR.

Cotd. Nephropathy

- The increased GFR leads to glomeruloscrerosis and you may get proteinuria
- The vasa recta pass thro. A hypertonic interstitium which promotes sickling and vascular obstruction.
- The obstruction interferes with the countercurrent mechanism leading to decreased ability to conc. urine

Pulmonary hypertension

- One out of every three adults with SCD develop pulmonary hypertension
- This is related to low levels of NO
- Low NO has been attributed to decreased levels of AA arginine
- During haemolysis the enzyme arginase which is normally in high amounts in the RBCs is released destroying the arginine
- Free Hb is also a scavenger of NO