SICKLE CELL DISEASE;
BASIC PATHOPHYSIOLOGY and
NEW THERAPEUTIC OPTIONS

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HbS
$\alpha_2\beta^s_2$

dehoxyHbS polymer
Normal red blood cells  Sickled Red Blood Cells
The SICKLE CELL CRISIS

- Bone pain of varying severity; mild to unbearable; usually low back; also in the tibias and arms
- Abdominal pain, often intractable; must be differentiated from pain due to surgical causes (cholecystitis, appendicitis etc.)
- Left upper abdominal quadrant pain resulting from splenic infarcts and, the most dreadful, splenic red cell sequestration
- Lumbar pain often accompanied by hematuria resulting from necrosis of the papilla
- Acute Chest Syndrome; often leading to death (thoracic Pain, dyspnea, ARDS)
- Neurologic Complications (focal signs, blindness etc)
β-globin chain

Chromosome 11

β^s gene

GAG codon 6

GTG

β^s globin chain

β^6 Glu → Val

Red blood cell

Soluble oxy HbS

polymerized deoxy HbS

Blood vessel

Sickle cell
deformed rigid fragile

Haemolytic anaemia

Vaso-occlusion
A key observation is that the kinetics of the intracellular polymerisation of hemoglobin S and the resulting deformation of red cells cannot satisfactorily explain the mechanical obstruction of the microvessels (mainly at the post-capillary venule level) because the time required for the development of cell sickling is actually longer than the time required for the transit of the red cells (including those carrying HbS only) cells through the micro-vasculature.

Therefore, the passage must be delayed by other causes which contribute to the development of sickling within the microvessels. The identification of these causes has now become a challenging step.
MECHANISMS PARTICIPATING IN THE VASO-OCCLUSIVE EVENT

- Intracellular polymerisation of HbS and mechanical obstruction; mechanical vaso-occlusion
- Retardation of the blood flow through the microcirculation
  - Adhesion of young red cells on the endothelial wall
  - Activation of the passing-by leucocytes and platelets and adhesion on the endothelial wall
  - Erythrocytic membrane damage; Changes in antigen density
  - Activation of the endothelial cells
- Vasoconstriction
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INTRACELLULAR POLYMERISATION OF HbS

Depends on:

- The amount of oxygen carried by each red cell
  - Decreased at high altitude
  - Decreased in bad condition of the pulmonary vascular bed (a common finding in SCD because of frequent infarcts and lung infections)
  - Decreased when excessively delivered (high temperature, fever or intensive exercise, acidosis)

- The intracellular hemoglobin S concentration
INTRACELLULAR HbS CONCENTRATION

Determined by:

- Cellular hydration, which depends on the rate of potassium and water flux across the red cell membrane. The latter is mediated by the function of the
  - Potassium/chloride co-transport, Mg\(^{++}\) activated pump, and the
  - Ca\(^{++}\) dependent potassium pump (Gardos channel)

- Intracellular HbS quantity and concentration
  - iron deficiency
  - simultaneous presence of \(a\)-thalassemia (although the available clinical evidence does not confirm it, probably because of other additional factors)
  - presence of other hemoglobin types which “dilute” HbS
POTENTIAL AND CLINICALLY EFFECTIVE THERAPIES

Decreasing the intracellular HbS concentration
  Repeated blood lettings aiming to create an iron deficiency hypochromic anemia; controversial results.

Prevention of the cellular dehydration
  DDAVP. To decrease plasma osmolality; not used anymore.
  Magnesium pidolate (or aspartate). To improve the function of the Mg$^{++}$ activated K$^{+}$ pump, thereby preventing potassium and water loss.
  Clotrimazole, Miconazole, charybdotoxine. To improve the function of the Ca$^{++}$ activated K$^{+}$-pump.
INTRACELLULAR CONCENTRATION OF HBS.
May decrease in the presence of other types of hemoglobin molecules which spread among those of HbS and diminish their chances of contact.

HbF (and the hybrid α2βSγ) produced by simultaneously present genes for the
  o hereditary persistence of HbF,
  o various δβ-thalassemia genes or
  o various β-thalassemia genes which are associated with a varying degree of HbF synthesis
  o other modifying genes (-157 XmnI site, the x-linked HPFH etc)
Adding intracellular HbF to reduce the chances of contact and polymerisation by dispersing HbF ($\alpha_2\gamma_2$ molecules and $\alpha_2\beta^S\gamma$ hybrids among the molecules of HbS.

Main mechanisms:

- Promoting the promoters by improved binding of various agents on specific sequences 5' to the $^\alpha\gamma$ gene (BRE)
  - Butyrates and derivatives; all short chain fatty acids;
  - Valproic acid; other compounds.

- Preventing histone de-acetylation, thus keeping the $\gamma$ genes in active state. Butyrates and derivatives; all short chain fatty acids; Valproic acid; Trichostatin; other compounds

- Putting back in cycle (proliferation and differentiation) the population of early erythroid precursors which maintain the capacity for $\gamma$-chain synthesis but remain "dormant" in the marrow, unless "recruited" to this effect in conditions of acute erythropoietic stress.
  - 5'-Azacytidine; Aracytine; HYDROXYUREA
A : baseline values
B : values after 6 months of treatment with HU
C : maximal values throughout therapy with HU
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- Vasoconstriction
Abnormal adhesion of SS-RBC to the endothelium

Adhesion of normal and sickle erythrocytes to endothelial monolayer cultures. 
*Blood* 1979, 54:872-876
Hebbel RP, Yamada O, Moldow CF, Jacob HS, White JG, Eaton JW

Reticulocyte adhering on endothelial cell
Activated endothelial cells are major contributors to the pathophysiology of SCD.

Conran & Costa, Clin Biochem, 2009, 1824-38
Activated endothelial cells are major contributors to the pathophysiology of SCD

Conran & Costa, Clin Biochem, 2009, 1824-38
RBC are activable cells and activation participates to RBC adhesion to the endothelium

El Nemer, Le Van Kim, Colin
Blood. 2007
Transfus Clin Biol. 2008
Transfus Clin Biol. 2010
Blood Flow

Increased Erythropoiesis

Oxidation and Dehydration

Aged RBC

Endothelial Cells

Stress Reticulocyte

GP1b/2b3a-like
HMW vWF

CD36

Thrombospondin

GP1b-like

FCR

Platelets

VLA4

VCAM1

Blood Flow

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VLA4

VCAM1

Reversal of membrane phospholipids;
Appearance of phosphatidyl-serine
promoting coagulation

Activation of Endothelial Cells
Uncreased expression of the $\alpha 4\beta 1$ integrin (and CD36) on reticulocytes in SCD (VOC/non-VOC Controls (n=20) HU (n=20) VOC (n=18) Non VOC (n=23)
ADHESION MOLECULES

INTEGRINS
A superfamily of α and β heterodimeric molecules which associate in various combinations to give rise to large integrin families. Of these, most common are the VLA (very late antigen) or β₁-integrins. The β₁-family includes integrins α₄β₁ (VLA 4) and α₅β₁, expressed on the surface of immature erythroid cells, and integrins αᵥβ₁ and αᵥβ₃, expressed on the erythroid but also on endothelial cellular surface.

ANTIGEN CD 36
Expressed on the surface of immature erythroid cells, and less on the surface of normal reticulocytes. Expressed abnormally high on “stress” reticulocytes, those of SCD and splenectomized β-thalassemia patients.
THE VASO-OCCLUSIVE EVENT IS EXTREMELY COMPLEX. CONTRIBUTING MECHANISMS INCLUDE:

- Mechanical trapping of the Irreversibly Sickled Cells
- The sickle cell membranes display abnormal charge topography, most probably caused by increased lipid peroxidation
- Sickle Reticulocytes show abnormally high expression of antigen CD36 and integrin α₄β₁, which cause high adhesiveness in flow; in contrast, Dense Sickle Cells show high static adhesiveness.
- Sickle cells are coated with an excess of immunoglobulins, the Fc segment of which is promptly taken up by the endothelial cells and monocytes.
- Several plasmatic proteins mediate adhesion between sickle cells and endothelial cells.
- Thrombospondin released from activated platelets, elevated fibrinogen, and exposure to subendothelial tissues cause perturbation of the hemostatic mechanism and bring it into play.
THE VASO-OCCLUSIVE EVENT IS EXTREMELY COMPLEX. CONTRIBUTING MECHANISMS INCLUDE:

- **The role of Granulocytes**: Substantially larger and less deformable, they adhere to the activated endothelium (inflammation) and cause flow retardation, which favors low affinity adhesion.

- **The role of monocytes**: Activated monocytes cause further activation of the endothelial cells by releasing TNF

- **The role of the endothelial cells**
  - Quiscent
  - Activated
  - NF-κB
  - Expression of pro-adhesion molecules such as: VCAM, ICAM, E-selectin, Tissue Factor (TF) and pro-coagulant molecules
  - Hypoxia
  - Cytokines
  - Reperfusion injury; free radicals
ANTI-ADHESION THERAPY

- Hydroxyurea: decreases number of granulocytes; decreases number of (ineffective) reticulocytes; decreases expression of CD36 and α₄β₁
  Styles et al, Blood 89:2554, 1997

- Monoclonal Antibodies blocking integrin αᵥβ₃ and glycoprotein Iβ/III.
  Kaul et al, NEJM 342:1910, 2000

- Sulfasalazine: inhibiting expression of Nuclear Factor κB (NF-κB)
  Solovey et al, Blood 97:1937, 2001

- Pluronium F-68: a vascular “lubricant” now entering clinical trials
  Smith et al, Blood 69:1631, 1987
in vivo, HC reduces the expression of the α4β1 integrin (and CD36) on reticulocytes
In vivo results are mimicked in vitro on erythroid progenitors in culture.

α4β1 integrin (VLA-4)

Odièvre et al, Haematologica, 2008
<table>
<thead>
<tr>
<th>Quantitative expression of clinical severity (Arbitrary Scale)</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-3 d</td>
</tr>
<tr>
<td>Mild bone and joint aches with no restriction of physical activity</td>
<td>0.2</td>
</tr>
<tr>
<td>Pain necessitating conventional analgesics; limitation of physical activity</td>
<td>0.4</td>
</tr>
<tr>
<td>Severe pain necessitating major analgesics and/or admission to hospital</td>
<td>0.8</td>
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### Quantitative Expression of Clinical Severity in 59 SCD patients during the 52 weeks prior to starting HU (black letters) compared to the clinical condition throughout the study (12,018 weeks)(dark red letters)

<table>
<thead>
<tr>
<th>Duration of event</th>
<th>1-3 days</th>
<th>4-7 days</th>
<th>&gt; 7 days</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild bone and joint pain with no restriction of physical activity</td>
<td>63.6</td>
<td>56.1</td>
<td>6.0</td>
<td>125.7</td>
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<tr>
<td>Pain necessitating conventional analgesics; limitation of physical activity</td>
<td>1.4</td>
<td>2.7</td>
<td>0</td>
<td>4.1</td>
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<tr>
<td>Severe pain necessitating major analgesics and/or admission to Hospital</td>
<td>71.6</td>
<td>255.0</td>
<td>286.0</td>
<td>612.6</td>
</tr>
<tr>
<td></td>
<td>10.4</td>
<td>5.2</td>
<td>2.0</td>
<td>17.4</td>
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<tr>
<td></td>
<td>45.0</td>
<td>142.0</td>
<td>256.0</td>
<td>1181.6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>10.0</td>
<td>50.2*</td>
<td>60.2</td>
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<tr>
<td>Total</td>
<td>63.4</td>
<td>432.3</td>
<td>502.0</td>
<td>1181.6</td>
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<tr>
<td></td>
<td>11.8</td>
<td>17.7</td>
<td>52.2</td>
<td>81.7</td>
</tr>
</tbody>
</table>

* mostly infections
EFFECTS OF MoAbs

Rat mesoecum venules pretreated (activated) with PAF

A: Venule perfused with Ringer’s solution. Clear

B: Venules perfused with SS blood; red cells adhere to endothelial wall

C: Perfusion with Ringer’s solution; several cells remain stuck on the endothelial wall

Rat mesoecum venules pretreated with PAF + MoAb7E3

D: as in A.

E: as in B

F: as in C; only rare SS cells remain on the endothelial wall

Kaul et al, Blood 95:368, 2000
Kaul et al,
Proc Natl Acad Sci USA 1989

Adherent red blood cells/100μm²

Vessel diameter (μm)
VASOCONTRICTION/VASODILATATION

Two main factors

- **Endothelin 1** Vasoconstriction
- **Nitrogen Oxide** Vasodilatation

Synthesized within the endothelial cells from arginine; it then diffuses rapidly into the smooth muscle cells and through an enzymatic cascade reduces the intracellular calcium thus resulting in vasodilatation. It is also readily bound to hemoglobin (in the red cells or in the plasma) forming various NO-compounds.
L-Arginine → cNO-Synthase → NO → Nitrogen Oxide

ENDOTHELIAL CELL:
- L-Arginine
- Citrulline
- cNO-Synthase
- NO

PLASMA:
- Nitrogen Oxide
- Guanyl-cyclase
- Guanosine Triphosphate (GTP)
- Cyclic GMP; Cyclic guanosyl-monophosphate (cGMP)
- cGMP depending protein kinases
- Decrease of intracellular Ca^{++}
- DILATATION OF SMOOTH MUSCLE; VASODILATATION

RED CELL:
- Free hemoglobin binds to NO
- The latter forms nitrosyl-Hb
- It also forms nitro-thiols
- The NO increases the affinity of the SS cells towards the O_2, thus decreasing the intracellular HbS polymerization

SMOOTH MUSCULAR CELL OF THE VASCULAR WALL
L-Arginine is converted to Citrullin by cNO-Synthase.

Nitrogen oxide (NO) interacts with plasma:
- Free hemoglobin binds NO.
- NO increases the affinity of the SS cells to Oxygen and decreases intracellular polymerization.

In the endothelial cell:
- cNO-Synthase converts NO to Citrullin.
- NO binds to the SS cells, increasing their affinity to Oxygen and decreasing intracellular polymerization.

In the red cell:
- Hb binds NO.
- NO forms nitrosyl-Hb.
- NO forms nitro-thiols.

In the smooth muscle cell:
- NO binds to guanylyl cyclase, forming cyclic GMP (cGMP).
- cGMP dependent protein kinase is activated.
- Intracellular Ca remains unchanged.
- MINIMAL OR NO DILATATION OF THE SMOOTH MUSCLE; NO VASODILATION.

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ACTIONS OF NITROGEN MONOXIDE

Vessels
- Smooth muscular cell dilatation
- Decreased expression of both the endothelin-1 gene and the respective receptor

Adhesion Molecules
- Decreased synthesis (VEGF and others)

Thrombosis
- Decreased platelet activation
- Decreased activity of the tissue factor
- Decreased thrombin synthesis

Oxidative phenomena
- Neutralization of free oxidative radicals
EVIDENCE THAT THE DECREASED PLASMA NO THROUGH ITS BINDING TO FREE HEMOGLOBIN PREVENTS VASODILATATION

- Under normal conditions, administration of the NO-synthase inhibitor L-NMMA is followed by vasoconstriction. This is not observed when the plasma contains free hemoglobin as a result of significant hemolysis.

- Under normal conditions, administration of sodium nitroprusside (NO donor) is followed by vasodilatation. However, this change does not occur in presence of free hemoglobin in the plasma.

- On measuring the blood flow in the microcirculation, the administration of nitroglycerin is followed by vasodilatation. However, this does not occur when the plasma contains free hemoglobin.

- Normally, administration of exogenous NO (by inhalation) results in vasodilatation. In contrast, this effect is not reproduced when the plasma contains free hemoglobin.
These indications leave no doubt that the presence of free hemoglobin in the plasma diminishes the available NO, prevents vasodilatation and gradually leads to development of angiopathy characterized by:

- vasoconstriction
- hyperplasia of the muscular vascular sheath, and
- local thrombosis
THERAPEUTIC TRIALS

- NO Inhalations
- Administration of arginine (NO donor)
- Administration of sildenafil (inhibition of the GMP phosphodiesterase mediated dephosphorylation of GMP)
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- Vasoconstriction
CONCLUSIONS

Proper management of the vaso-occlusive crisis in sickle cell disease requires our complete understanding of the underlying pathophysiology, and indeed, this is a most complicated but also challenging field, which still hides many questions to be answered.