Sickle-cell disease

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Lancet 2004; 364: 1343–60

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Summary

With the global scope of sickle-cell disease, knowledge of the countless clinical presentations and treatment of this disorder need to be familiar to generalists, haematologists, internists, and paediatricians alike. Additionally, an underlying grasp of sickle-cell pathophysiology, which has rapidly accrued new knowledge in areas related to erythrocyte and extra-erythrocyte events, is crucial to an understanding of the complexity of this molecular disease with protean manifestations. We highlight studies from past decades related to such translational research as the use of hydroxyurea in treatment, as well as the therapeutic promise of red-cell ion-channel blockers, and antiadhesion and anti-inflammatory therapy. The novel role of nitric oxide in sickle-cell pathophysiology and the range of its potential use in treatment are also reviewed. Understanding of disease as the result of a continuing interaction between basic scientists and clinical researchers is best exemplified by this entity.

Introduction

Knowledge of a disease heralded by painful episodes and leading to early death has existed in Africa for over a century.[1] James Herrick,[2] a physician and Chaucer scholar, first identified sickle cells in a medical student from Grenada. Several seminal observations related to sickle-cell disease dot the landscape of the first half of the 20th century: Linus Pauling[3] showed the abnormal electrophoretic mobility of haemoglobin in an affected individual; Vernon Ingram[4] discovered that the defect of the disease was a single aminoacid substitution in the haemoglobin molecule of sickle cells (HbS); Max Perutz,[5] who deciphered the structure of haemoglobin, elucidated the molecular basis of its function; and Janet Watson,[6] who noted that symptoms appeared in infants only after concentrations of fetal haemoglobin (HbF) had fallen, established the notion of the beneficial effect of HbF on disease manifestations.

Genetic epidemiology of the sickle gene

The sickle gene has a genetic advantage: it protects heterozygous carriers from succumbing to endemic *Plasmodium falciparum* malaria infection.[7] However, with the increased premature death rate of homozygous individuals, the sickle gene is an example of balanced polymorphism.[7] Globin haplotypes of the gene are based on a series of restriction-endonuclease-defined polymorphisms in the globin-gene cluster on chromosome 11.[8,9] The β^S-globin gene is present on three major distinct African haplotypes, all localised exclusively to one of three separate geographical areas (figure 1). The vagaries of war and Atlantic and Arab slave trades have been responsible for gene dissemination in the diaspora. A fourth major Indo-European sickle mutation (Arab-India) probably originated in the Indus Valley Harappa culture, and by gene flow it was distributed to Saudi Arabia, Bahrain, Kuwait, and Oman. This haplotype is also linked to the sickle gene in populations from the eastern oasis of Saudi Arabia and the Adivasis tribe of India. Whether gene conversion explains haplotype diversity because of their strict geographic segregation is unlikely.[9]

Sickle-cell disease denotes all genotypes containing at least one sickle gene, in which HbS makes up at least half the haemoglobin present. In addition to the homozygotic HbSS disease (sickle-cell anaemia), five other major sickle genotypes are linked to the disease (panel 1). Generation of HbS is a monogenic event, determining the polymerisation of the deoxygenated conformer of sickle haemoglobin. The process is an indispensable but insufficient determinant of phenotype. By contrast, the phenotype of sickle-cell anaemia is multigenic.[10] Other genes, unlinked to the β-globin locus, participate in relevant pathological events (eg, rapid destruction of sickle cells, dense cell formation, adhesion to endothelium) that are controlled by many genes, known as pleiotropic or secondary effector genes. Severity of sickle-cell anaemia varies greatly between individuals, since not all patients have identical pleiotropic genes. Some carriers have mutated genes that can either ameliorate or exacerbate the phenotype. Expression microarrays are being used to identify upregulated or downregulated genes in several organs affected by the disease in man and in sickle transgenic mice. After pleiotropic genes are located, polymorphisms can be searched for to identify epistatic or modifier genes that will help to define individual risk, allowing for rationale-based interventions before the onset of organ damage.

The presence of epistatic effects is not theoretical, although our knowledge of these factors is far from complete. Known epistatic or modifier genes include: copresence of α-thalassaemia that ameliorates the

Search strategy and selection criteria

The Medline database was searched from 1966 to June, 2004 for specific topics in relation to sickle-cell disease pathophysiology, complications, and treatment. We prioritised articles published in high-quality journals, natural history studies, and randomised controlled trials. Personal knowledge and clinical experience was finally used to complete the picture, where gaps in knowledge still remain.

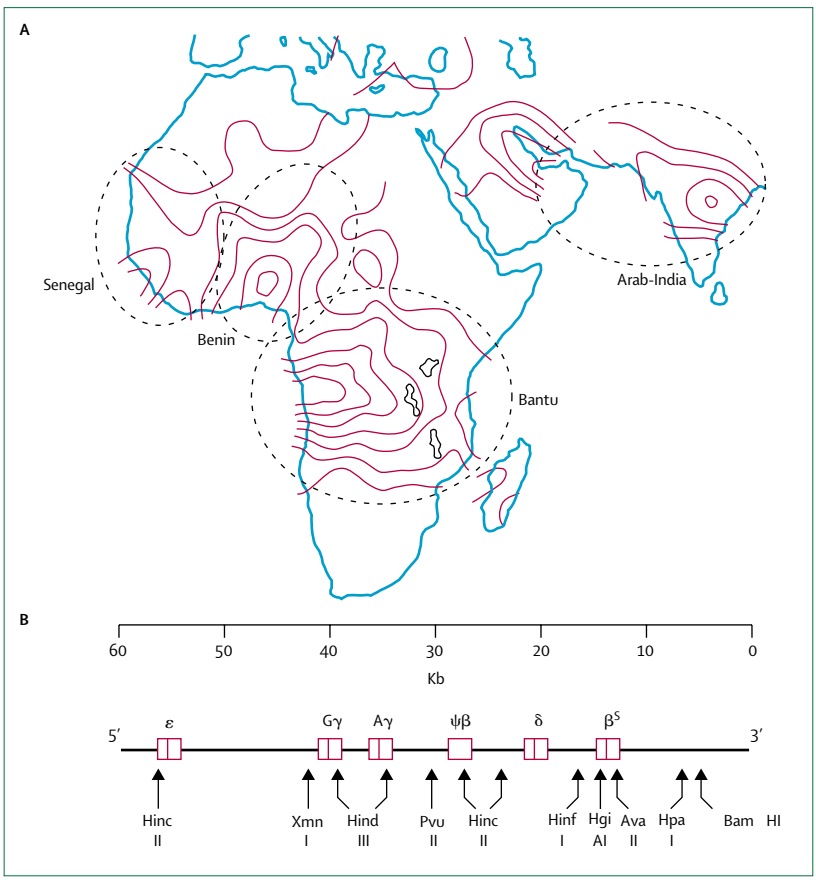

Figure 1: **Geographical distribution and schematic representation of the sickle gene**
(A) Map identifies the three distinct areas in Africa and one in the Arab-India region where the sickle gene is present (dotted lines). Numbers of individuals with sickle-cell disease (red lines) in Senegal, Benin, and Bantu are higher near the coast, and falls concentrically inland. (B) The β-globin gene cluster haplotype is determined by DNA polymorphic sites (boxes) that are identified by endonuclease enzymes. With this information, haplotypes are constructed as shown.

***Panel 1:* Sickle-cell disease genotypes**

- HbSS disease or sickle-cell anaemia: homozygote for the β^s globin with usually a severe or moderately severe phenotype.
- HbS/β° thalassaemia: severe double heterozygote for HbS and β° thalassaemia, and almost indistinguishable from sickle-cell anaemia phenotypically.
- HbSC disease: double heterozygote for HbS and HbC with intermediate clinical severity.
- HbS/β^+ thalassaemia: mild to moderate severity, but variable in different ethnic groups.
- HbS/hereditary persistence of fetal Hb (S/HPHP): very mild phenotype or symptom-free.
- HbS/HbE syndrome: very rare and generally very mild clinical course.
- Rare combinations of HbS with HbD Los Angeles, HbO Arab, G-Philadelphia, among others.

phenotype (by reduction of mean corpuscular haemoglobin concentration, dense cell numbers, and haemolytic rate); the .158C→T mutation upstream of the $^G\gamma$ gene (that enhances HbF expression, especially in the Senegal and Arab-Indian globin-cluster haplotypes); and the female population, in whom as yet unidentified epistatic genes ameliorate phenotype. DNA-based diagnosis has become the standard for prenatal diagnosis. Fetal tissue is obtained by chorionic villus sampling in the first trimester, or amniocentesis in the second. Isolation of fetal cells from the maternal circulation and preimplantation diagnosis have also been successfully used.[11,12]

Erythrocyte-related pathophysiological considerations

HbS polymerisation

A single nucleotide substitution (GTG for GAG) in the sixth codon of the β-globin gene results in the substitution of valine for glutamic acid on the surface of the variant β-globin (β^s globin) chain (figure 2A).[13] This change allows HbS to polymerise when deoxygenated, since valine can dock with complementary sites on adjacent globin chains. The polymerisation of deoxygenated HbS is the primary indispensable event in the molecular pathogenesis of sickle-cell disease. It is dependent on intraerythrocytic HbS concentration, degree of cell deoxygenation, pH, and the intracellular concentration of HbF. Inhibition of HbS polymerisation by HbF requires the formation of asymmetrical HbS/HbF hybrid forms ($\alpha_2\delta\beta^s$).[14] Polymerisation tendencies of mixtures of HbS and several Hb variants show that residues 22, 80, and especially 87 of the γ chain are implicated in intermolecular contact sites that stabilise the deoxygenated-HbS polymers (figure 2B).[15]

The polymer is a rope-like fibre that aligns with others to form a bundle, distorting the red cell into classic crescent or sickled forms. These shapes interfere with a critical erythrocyte feature; its deformability. The polymerisation of HbS is a nucleation-initiated reaction with a delay time, during which no polymer is detectable. At the end of this period, the critical nucleus is formed, and exponential polymer formation follows.[16] The kinetics of this reaction have a critical role in the rheology and morphology of circulating red cells.[16,17] Because the range of transit times in the microcirculation is short relative to the range of delay times of HbSS red cells, most cells fail to undergo HbS polymerisation. If, however, these red cells are subjected to a prolongation of their transit times, due to local microcirculatory conditions, then almost all the HbSS red cells, because of equilibration at the lower oxygen tension, would contain HbS polymer and become less deformable (figure 2C).

Sickle forms were originally thought to cause microcirculatory obstruction because of impaired erythrocyte deformability during capillary transit, resulting in the vaso-occlusive crisis. The actual mechanism is much more complicated than expected (panel 2, figure 2D),

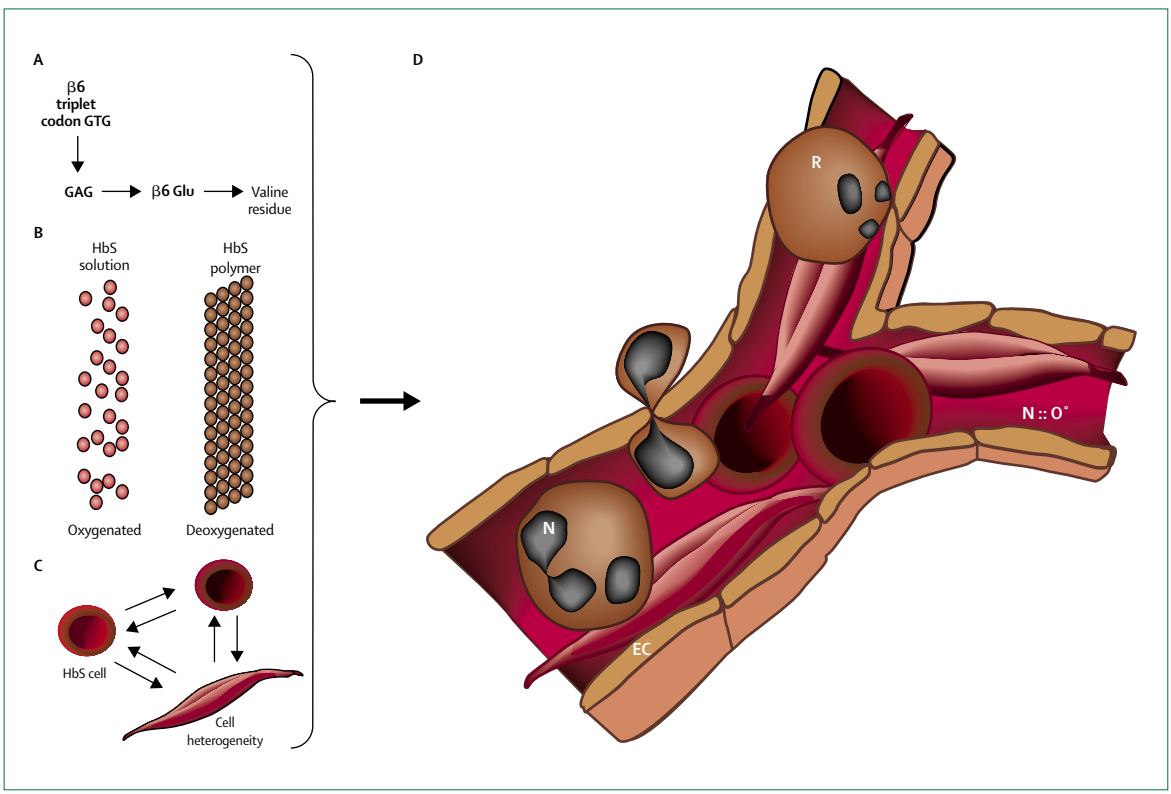

Figure 2: **Pathophysiology of vaso-occlusion**
(A) Single nucleotide substitution (GTG for GAG). (B) HbS polymerisation. (C) Cell shape changes of HbS-polymer-containing erythrocyte. (D) Cross-section of microvascular bifurcation. EC=endothelium. R=reticulocyte. ISC=irreversibly sickled cell. N=leucocyte. N::O·=NO bioavailability. RBC=red blood cell. Luminal obstruction has been initiated by attachment of proadhesive reticulocyte to endothelium with secondary trapping of irreversible sickled cells. Leucocytes participate in formation of heterocellular aggregates, and NO bioavailability crucial to vasodilation is impaired. Figure adapted from reference 13, by permission of M H Steinberg.

with polymer-containing sickled cells being trapped predominantly in the slow flowing venular side of the microcirculation. Primary events crucial to vaso-occlusion include adhesion of erythrocytes (reticulocytes and poorly deformable dense cells) to the endothelium of the postcapillary venule.[18,19] Leucocyte-endothelial adhesion with formation of heterocellular aggregates (leucocytes and irreversibly sickled cells) also contribute to obstruction,[20] resulting in local hypoxia, increased HbS polymer formation, and propagation of the occlusion to the adjacent vasculature. Neutrophil transmigration (figure 2D) through endothelial gap junctions also adds to increased inflammation in the microvasculature. Recent attention has also focused on dysregulation of vasomotor tone by perturbation in vasodilator mediators such as nitric oxide (NO).

Cation homeostasis

Abnormal cation homoeostasis is implicated in a pathogenic pleiotropic event: it leads to formation of dehydrated dense sickle cells (in general), and short-lived, irreversibly sickle cells (in particular). Irreversibly sickled cells are the most dense, are fixed in their deformed shape, and do not return to normal contours even when oxygenated because of irreversible membrane damage. In addition to their role in the initiation of vaso-occlusion, these cells are a crucial causative factor for anaemia and a raised haemolytic rate. In-vivo dehydration of sickle erythrocytes takes

Panel 2: **Basic pathophysiology of vaso-occlusion**

1 Prolongation of the erythrocyte microvascular transit time caused by:
- Enhanced red cell adhesion to endothelium and heterocellular aggregate formation
- Abnormal cation homeostasis with cell dehydration, dense-cell formation, and irreversibly sickled cell formation
- Abnormal vasomotor tone favouring vasoconstriction (via NO, endothelin-1, and eicosanoid dysregulation)

2 Reduction in delay time to HbS polymer formation caused by:
- Red-cell deoxygenation
- Increase in intracellular HbS concentration
- Low concentrations of protective Hb types (eg, HbF, HbA_2)
- Fall in pH

3 Miscellaneous potential modulators
- Free-radical release and reperfusion injury
- Coagulation activation with proadhesive thrombin formation

***Panel 3:* Cation-transport mechanisms that induce sickle erythrocyte dehydration**

- Increased erythrocyte membrane permeability: general abnormality in sickle cells induced by deoxygenated HbS polymers that affects several cations (sodium, potassium, calcium, magnesium); referred to as P^{sickle}. It allows calcium-ion entry, which activates the Gardos channel.
- Calcium-ion-sensitive potassium-ion-efflux channel (Gardos): activation triggers the loss of potassium ions and water, leading to cell dehydration and dense-cell formation.
- Potassium-chloride cotransporter: activated by low pH and cell swelling. In normal erythrocytes, this transporter is only active in reticulocytes; HbSS, HbSC (heterozygous with HbS and HbC), and HbCC (homozygous with HbC) cells manifest substantially high levels of expression. The combined activation of this transporter and the Gardos channel leads to rapid dehydration of a young subpopulation of sickle cells, forming irreversibly sickled cells.

place mainly by activation of one or more of the cation-transport pathways described in panel 3.[21,22]

Proinflammatory molecules induce activation of the Gardos channel, which might explain the association between inflammation, vaso-occlusion, and increased haemolysis sometimes seen during infection. Cation homoeostasis is relevant to therapy, since inhibition of these transporter channels prevents sickle erythrocyte dehydration, formation of dense and irreversibly sickled cells, with ameliorative effects on both haemolysis and adhesion. Table 1 shows a selection of ion-channel blockers being investigated in sickle-cell treatment.[23]

Adhesion reactions

The seminal observation that sickle erythrocytes adhere to endothelium in vitro,[24,25] and that this adherence was thought to correlate with disease severity,[25] made necessary the delineation of the mechanisms involved. These adhesion reactions are mainly mediated by interaction between receptors on erythrocytes and endothelial cells. Interactions have also been shown between sickle cells and immobilised extracellular matrix components (exposed after vascular injury, or thrombin-induced endothelial retraction; figure 3).

	Modes of action	Results of in-vivo studies
Magnesium pidolate	Originally proposed as potassium-chloride cotransport inhibitor. However, effects of magnesium ions are more complex and remain indeterminate.	In transgenic mice: improved red-cell hydration and haemolytic rates. In humans: more variable effects on dense cells and reticulocyte counts noted, although pain days reduced in pilot studies.
Clotrimazole	Gardos-channel inhibitor. Also inhibits cytochrome P450 activity because of imidazole moiety.	In mouse and man: increased potassium ion content in red cells and decreased dense cells, irreversibly sickled cells, and haemolytic rate. Side-effects present because of P450 inhibition.
ICA-17043	Potent and selective Gardos-channel inhibitor without P450 inhibition.	In transgenic models: reduced red-cell dehydration by increase of potassium-ion content. Raised Hb concentrations due to a fall in haemolytic rate. Trials in progress.
L-arginine	Lowers Gardos-channel activity.	In transgenic mice: reduced red-cell density and dense-cell formation by increase of potassium-ion content. In thalassaemia mouse: no effect. Trials in progress.
Dipyridamole	Inhibition of P^{sickle} and Gardos channels.	In patients taking dipyridamole for its antithrombogenic effects membrane levels of drug were sufficient for ion-flux inhibition. Small pilot study of dipyridamole and aspirin in sickle-cell disease might have shown a beneficial effect on pain. Trials in progress.

Table 1: **Ion-channel blockers for modulation of sickle-cell disease phenotype**

Two of the earliest red-cell adhesion molecules to be identified were very-late-activation-antigen-4 (VLA-4/$\alpha_4\beta_1$) and CD36.[26–28] The integrin $\alpha_4\beta_1$ binds to its endothelial ligand vascular cell adhesion molecule-1 (VCAM-1). Agonist-induced alterations in $\alpha_4\beta_1$ conformation also allows for additional binding to fibronectin (figure 3). Although VCAM-1 is not constitutively expressed on the endothelial surface, expression takes place after exposure to several agonists, including cytokines and hypoxia. Hypoxia also increases VCAM-1 adhesion to the endothelium via $\alpha_4\beta_1$.[29] Another well characterised mechanism is the bridging role of the soluble ligand thrombospondin between erythrocyte CD36 and several constitutively expressed endothelial receptors, including $\alpha_v\beta_3$ (vitronectin receptor), CD36, and heparan sulphate proteoglycans.[30] Sickle red cells also bind to immobilised thrombospondin via the integrin-associated protein CD47,[31] a molecule that is associated with the rhesus complex. High-molecular-weight multimers of von Willebrand factor promote red-cell adhesion to endothelial $\alpha_v\beta_3$, and the glycoprotein Ib (GPIb)-IX-V complex, although the interactive site on the erythrocyte is unknown.[32] Non-receptor mechanisms include proadhesive roles for both red-cell sulphated glycolipids and phosphatidylserine.[33–35]

Of particular interest, laminin binds strongly to sickle erythrocytes via B-CAM/Lu,[36] the protein that carries Lutheran blood-group antigens. Epinephrine increases this adhesion, concomitant with rises in intraerythrocyte cAMP concentrations and with BCAM/Lu as the target receptor for cAMP signalling.[37] Since stress is a potential initiation factor for vaso-occlusion, epinephrine modulation of adhesion provides a powerful biological link between intraerythrocytic signalling pathways and the external milieu. Another important interaction is mediated via thrombin, which causes endothelial retraction[38] with exposure of proadhesive extracellular matrix components and the endothelial expression of P-selectin, involved in erythrocyte, white-cell, and platelet-endothelial interactions.[39] The present availability of transgenic and knockout animal models have modernised sickle-cell treatment, since individual adhesive interactions can be better delineated in vivo, thus forming the foundation for future antiadhesion therapy.

Lipid bilayer dysfunction

In steady state, the choline-containing phospholipids, sphingomyelin and phosphatidylcholine, are mostly located on the outside of the membrane bilayer whereas the aminophospholipids are either mainly (phosphatidyl-ethanolamine) or exclusively (phosphatidylserine), located in the inner monolayer. This membrane structure is maintained by an active set of transport systems that

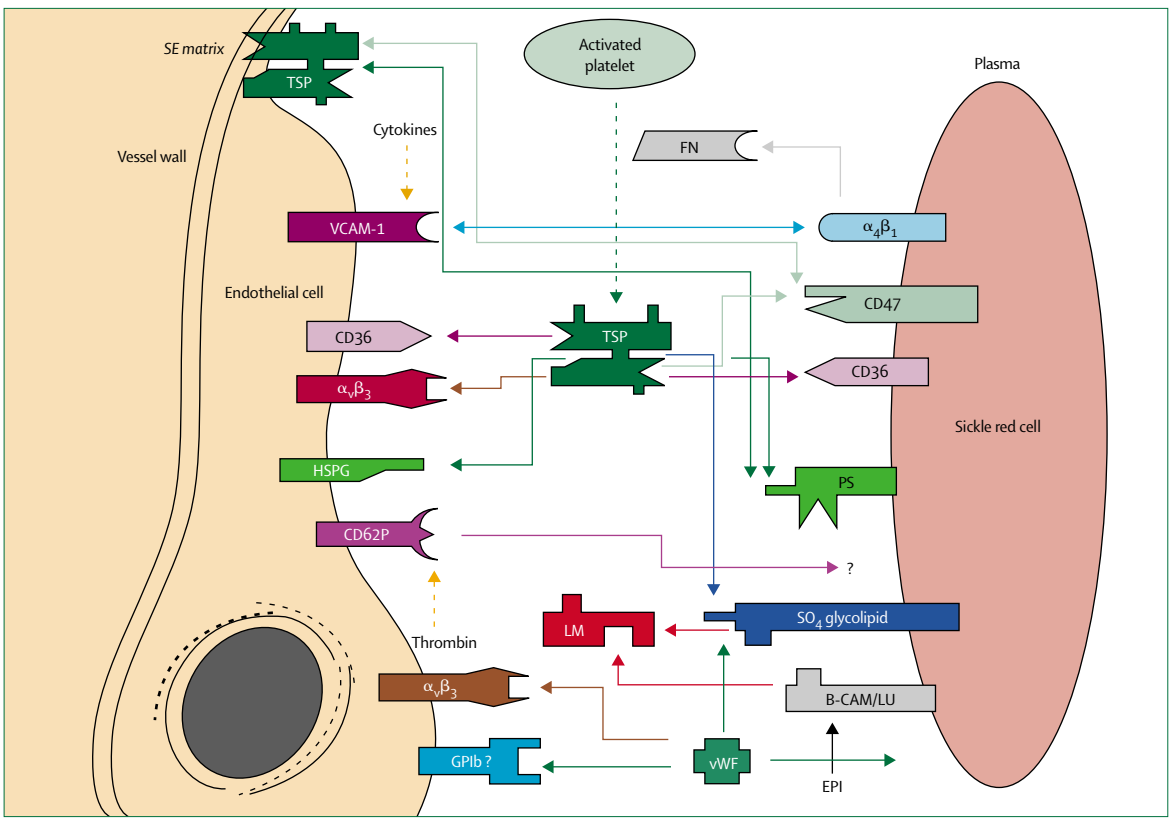

Figure 3: **Adhesive interactions between sickle erythrocytes and the endothelium, subendothelial matrix, and plasma ligands**
SO_4 glycolipid=sulphated glycolipids. PS=phosphatidylserine. TSP=thrombospondin. FN=fibronectin. LM=laminin. VWF=von Willebrand factor. SE matrix=subendothelial matrix. HSPG=heparan sulphate proteoglycans. EPI=epinephrine. CD62P=P-selectin. The red cell receptors associated with adhesion are either present in increased numbers or sickle reticulocytes ($\alpha_4\beta_1$ and CD36), on mature sickle cells, when compared with normal erythrocyte (B-CAM/LU) or exhibit abnormal structure-function activity in sickle red cells (B-CAM/LU and integrin-associated protein). Phosphatidylserine levels are also raised on sickle when compared with control HbAA red cells. Figure adapted from reference 38, by permission of B N Y Setty.

pass phospholipids across the membrane. The flipase actively transports aminophospholipids from the outer to the inner monolayer, whereas the scramblase, when activated, moves all phospholipids in both directions, thereby scrambling phospholipid distribution.

Loss of normal phospholipid asymmetry with the appearance of anionic phosphatidylserine on the erythrocyte surface happens in various haemolytic anaemias. Phosphatidylserine exposure is heterogeneous, occurring on mature erythrocytes and transferrin-positive stress reticulocytes.[40,41] Causes for this exposure are inactivation of ATP-dependent aminophospholipid translocase (which transports phosphatidylserine from the outer to inner membrane surface), premature activation of apoptosis in marrow cells, and sickling-induced membrane damage.[42] High HbF concentrations protect sickle erythrocytes from phosphatidylserine externalisation.[43,44] Consequences of such exposure are: exacerbation of anaemia because of enhanced phagocytic recognition and removal; increased adhesion to the endothelium and extracellular matrix components; and development of a pro-coagulant erythrocyte phenotype, since phosphatidylserine promotes assembly of clotting factors on cell surfaces.[45,46] The dysfunctional lipid bilayer thus contributes greatly to sickle-cell pathophysiology (figure 4).

Extra-erythrocyte-related pathophysiological changes

Natural history studies and animal data implicate the leucocyte as of major importance in the pathophysiology of sickle-cell disease. Raised white cell counts predict disease severity[47] and mortality,[48] whereas an increased baseline white cell count is an independent risk factor for acute chest syndrome[49] and cerebral infarction.[50] The syndromes of acute chest and multiple organ failure have also occurred after administration of myeloid colony stimulating factors.[51] Qualitative abnormalities in circulating leucocytes indicative of an activated phenotype include evidence for degranulation, down-regulation of L-selectin (a surface membrane glycoprotein that initiates leucocyte-endothelial attachment), activation of the respiratory burst, and raised leukotriene B4 concentrations.[52–55] Leucocyte size, rigidity, and adhesive characteristics are relevant to microvascular blood flow, with transgenic mouse models providing evidence of vascular inflammation, and leucocyte involvement in the

vaso-occlusive event.[20,56] Models of hypoxia-reoxygenation also lend support to the hypothesis that microvessel occlusion is a form of reperfusion injury, in which oxidant stress and inflammation lead to chronic end-organ damage.[57] Quantitative and qualitative reductions in leucocytes during hydroxyurea treatment correlate with amelioration of disease severity.[58,59]

The platelet does not seem to be critical to the pathophysiology of acute microvascular occlusion, although its role in large-vessel disease (eg, cerebral vasculopathy) has yet to be determined. Qualitative platelet abnormalities are secondary to activation (presumably, because of in-vivo thrombin generation). Quantitative changes are a result of functional asplenia. Activation-related changes occur during steady state, with further modulations during vaso-occlusive crises.[60,61] However, available data do not forge a primary link between platelets and either haemostatic perturbations related to sickle-cell disease,[62] or vaso-occlusive changes.[20,47] These studies corroborate trials of antiplatelet drugs, in which there was no beneficial effect.[63,64] A recently postulated correlation between platelet activation and pain episode frequency is difficult to interpret since platelet and pain assessments were not concurrent.[65]

Evidence for perturbation of the endothelium include histological studies of vascular changes,[66,67] circulating endothelial cells during painful crises,[68] and increased levels of circulating adhesion molecules, such as soluble VCAM-1.[69,70] Solovey and colleagues[71,72] have conclusively proven that the endothelium in sickle-cell disease is activated by showing increased numbers of microvascular circulating endothelial cells that express tissue factor, VCAM-1, intercellular adhesion molecule-1 (ICAM-1), E-selectin, and P-selectin (signifying their procoagulant, proadhesive, and proinflammatory phenotype). Data from animal studies also support the notion that the phenotype of circulating endothelial cells reflects in-situ microvasculature.[73] Panel 4 shows the main agonists in sickle-cell disease that result in an activated endothelial phenotype. Activation frequently takes place via nuclear factor (NF)κB, a transcription factor that upregulates many proinflammatory, proadhesive, and procoagulant endothelial molecules in response to inflammatory stimuli and cytokines. Other transcription factors might include early growth response (EGR)-1 and activator protein (AP)-1. Thus, the endothelium is under a constant barrage of stimuli, resulting in a state of chronic activation and providing a dysfunctional template on which microvessel occlusion and large-vessel vasculopathy occurs.

Fluid-phase coagulation in sickle-cell disease is a process of perturbed and activated haemostasis with in-vivo thrombin generation and thromboses.[60,65,74] In general, patients with the HbSC disease have milder abnormalities than their homozygous HbSS counterparts.[62,74] Key and colleagues[72,75] recorded both a rise in whole-blood tissue factor in sickle-cell disease and the presence of circulating endothelial cells expressing a tissue factor phenotype.[72] These findings suggest that the activated endothelium is one pathophysiological source for coagulation activation. An additional trigger for the thrombophilic state is the phosphatidylserine-positive sickle erythrocyte,[62] an idea first postulated by Lubin and Zwaal.[45,46]

Thrombin mimics many cytokine-associated vascular effects, and could provide a crucial link between coagulation activation and adhesion. Thrombin mediates endothelial cell retraction with exposure of proadhesive matrix elements,[26] and causes endothelial expression of P-selectin (that modulates leucocyte rolling,[76] red-cell-endothelial interactions,[35] and platelet-endothelial interactions[77]). Although initial trials of anticoagulation treatment were not beneficial in vaso-occlusive crises, they were not well controlled.[60] Notably, in-vitro studies have shown that heparin inhibits erythrocyte-endothelial adhesion via inhibition of P-selectin,[78] and that n-3 fatty-acid dietary supplementation reduces pain episode rates, with concomitant evidence for a reduction in thrombin activity.[79]

Haemostatic activation could be implicated in the genesis of macrovasculopathy. A preliminary study[80] reported increases in prothrombin fragment F1.2 and erythrocyte phosphatidylserine in a paediatric population—these biomarkers correlated with increased transcranial doppler-flow velocities. The time is ripe for studies investigating the prevalence of thrombosis and

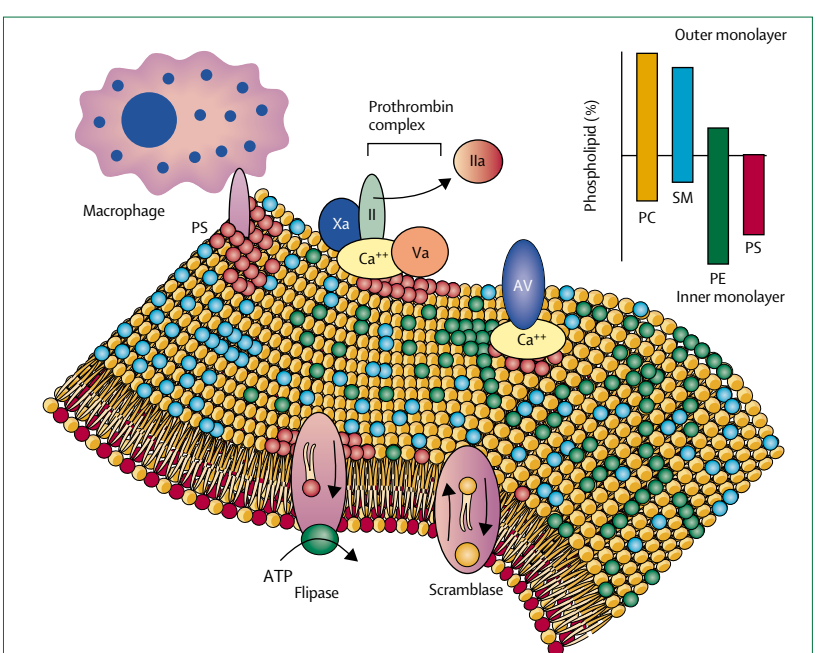

Figure 4: **Perturbations in erythrocyte membrane lipids in sickle-cell disease**
SM=sphingomyelin. PC=phosphatidylcholine. PE=phosphatidylethanolamine. PS=phosphatidylserine. AV=annexin V. Ca++=calcium ions. Phosphatidylserine exposed on the cell surface forms a docking site for haemostatic factors, such as the prothrombinase complex (factor Xa, Va, and II). Additionally, phosphatidylserine is recognised by macrophages and interacts with proteins such as annexin V, allowing its measurement by flow cytometry. Cell-surface phosphatidylserine also aids erythrocyte adhesion to the vascular endothelium and thrombospondin, an extracellular matrix component. Figure provided by F A Kuypers.

Panel 4: **Causes of endothelial activation in sickle-cell disease**

Cell–related causes
- Sickle erythrocyte: membrane oxidation products, surface phosphatidylserine, cell-free haemoglobin
- White cell: Mainly cytokine and free-radical-mediated
- Platelet microparticles

Fluid-phase–associated causes
- Thrombin
- Cytokines: TNFα, interleukin1β
- Reactive oxygen species from reperfusion injury
- Other biological modifiers: vascular endothelial cell growth factor (VEGF), platelet-activating factor, and erythropoietin
- Deficiency of cytoprotective mediators including NO, and antioxidants (such as vitamin E)
- Eicosanoids
- Raised homocysteine concentrations
- Shear stress

Miscellaneous causes
- Hypoxia
- Iron overload
- Bacterial infection via leucocytosis, endotoxin, and humoral mechanisms
- Viruses

for clinical trials highlighting the antiadhesive properties of heparin or other antithrombins in the modulation of vaso-occlusion.

Transgenic mouse models

Transgenic mouse models aid our understanding of sickle-cell pathophysiology, development of diagnostic imaging techniques and new treatments, and provide data on potential pleiotropic and epistatic genes affecting phenotype. Previous models expressed a combination of murine and human globin chains, whereas second-generation knockout mice exclusively express human globin chains. Advantages and disadvantages for use of individual models are discussed in a recent review.[81]

Selected acute clinical syndromes

The Cooperative Study of Sickle Cell Disease, formed in 1979 among US institutions, started a new era in clinical sickle-cell research. Another cohort that has provided much clinical information is the Jamaican cohort.

The vaso-occlusive crisis

The protean manifestation is the vaso-occlusive or painful crisis, in which episodic microvessel occlusion at one or many sites induces pain and disability, accompanied by local inflammation. Burgeoning evidence has shown the multifactorial and heterocellular nature of this event. The multifactorial cause is dependent mainly on whether the delay time—ie, rate of HbS polymer formation[16]—is within the range of microcirculatory transit time. As previously discussed (panel 2, figures 2 and 3), events that slow the transit of sickle red cells through the microcirculation (including factors that enhance cell-endothelial adhesion, erythrocyte dehydration, and vasomotor dysregulation), have a critical role in the genesis of vaso-occlusion.[18–20,82]

Microvascular occlusion arises predominantly in localised areas of marrow, leading to necrosis. Inflammatory mediators activate nociceptive afferent nerve fibres, evoking the pain response.[83] Affected areas are long bones, ribs, sternum, spine, and pelvis, often with multiple-site involvement. Microvascular occlusion of the cortex and periosteum could occasionally mimic osteomyelitis. Hand-foot syndrome is a painful swelling of the hands, feet (or both) due to dactylitis that affects children younger than 3 years old. Microvascular occlusion in mesenteric vessels with impaired intestinal mobility is characteristic of the abdominal crisis that can mimic acute abdomen. Heightened pain rates are associated with increased haematocrits and reduced HbF.[84] In general, individuals with sickle-cell anaemia manifest the highest rate of vaso-occlusive events (panel 1). However, some with HbSC or sickle-β^+ thalassaemia might have higher pain rates than those with more severe syndromes of HbSS and sickle-β^0 thalassaemia, suggesting that epistatic genetic factors have a role in clinical severity. A third of patients are asymptomatic, with a small cohort (about 5%) accounting for a third of all admissions. Platt and colleagues[83] calculated a vaso-occlusion rate of 0·8 episodes per patient per year (data derived from hospital visits). Adults with high pain rates tend to die earlier than those with low pain rates, suggesting that reperfusion-injury-induced oxidant stress and inflammation accelerate end-organ dysfunction.

Most vaso-occlusive episodes are managed at home, with a combination of anti-inflammatory agents, and opioid or non-opioid analgesics.[85] Published work from the USA shows a well recognised bias against opioid use in pain management in sickle-cell disease, leading to undertreatment. This non-use of opioid is accentuated in patients with chronic or recurrent pain, or in specific racial groups, including African-Americans and Hispanic-Americans.[86,87] Aggressive regimens are recommended for the management of pain severe enough to require hospital care,[13,83,87] and include intravenous use of opioids at full therapeutic dose, with morphine as the drug of choice. Treatment is initially continuous, with increased doses in patients on chronic opioids; frequent pain checks by use of assessment instruments; additional opioids for breakthrough pain; and hydration. Well established protocols and guidelines are essential for safe and successful continuous opioid use. Such aggressive therapy in a day hospital or day-care facility results in a striking reduction in emergency room visits and

admission rates, at reduced cost.[87,88] Day hospital management can be followed by home administration of controlled-release opioids for limited periods.[89] A painful vaso-occlusive crisis might be associated with fever. Since increased susceptibility to gram-positive and gram-negative organisms characterises this patient cohort, infection and vaso-occlusion might coexist, such that rigorous assessment and initiation of empirical antibiotic treatment—until culture results are available—should be routine. Recent standards of care guidelines for management of acute vaso-occlusive crises have been widely disseminated in the UK.[90]

For the long-term, oral hydroxyurea is presently the accepted treatment for decreasing the pain rate in HbSS patients who have frequent and severe vaso-occlusive crises.[58] Ion-channel blockers (table 1) and specific endothelial-targeted antiadhesive and anti-inflammatory approaches (to be discussed subsequently) are also potential treatments. Less desirable therapeutic alternatives include chronic transfusions for limited periods in hydroxyurea non-responders.[13]

Acute chest syndrome

Acute chest syndrome is a frequent cause of admission, and the leading cause of mortality in young adults.[48] Repeated episodes predispose to chronic pulmonary disease, including pulmonary hypertension.[91–93] The syndrome is the radiological appearance of a new pulmonary infiltrate of at least one complete lung segment, accompanied by fever and a combination of respiratory symptoms. Hypoxia is often present. Risk factors include HbSS genotype, low HbF concentrations, and high steady-state leucocyte and Hb concentrations.[49] Nearly half the patients are admitted for a diagnosis other than acute chest syndrome, with superimposition of the disorder during hospital care.[92] A decrease in steady-state haemoglobin values and a platelet count of less than 200 000 cells per μL (especially when associated with neurological symptoms and confusion, sternal pain, or rib infarct), suggests the most severe form of the syndrome—ie, marrow infarction with concomitant fat embolism.

In a landmark study of 30 participating centres,[92] specific causes were identified in 38% of patients, which were infections (29%) and fat embolisms (9%). Infections were equally distributed between bacterial, viral, mycoplasma, and chlamydial infections, suggesting potential treatment with a macrolide antibiotic. Parvovirus B19 infection causes marrow necrosis and a severe form of acute chest syndrome.[94] Since hypoventilation from pain or narcotic analgesics (or both) leads to the syndrome, judicious use of analgesics without respiratory compromise—as well as prophylactic incentive spirometry during vaso-occlusive crises or surgery-associated admissions—should be standard.[95] Rates of complications and mortality figures are age-dependent, with individuals aged 20 years or older faring worse than their younger counterparts.[92]

The syndrome is a hypoxia-driven event in the lung, a uniquely vulnerable target organ; the vasculature constricts with hypoxia, by contrast with other vascular beds. In sickle-cell disease, the combination of regional hypoxia and vasoconstriction will not only increase HbS polymerisation and sickling, but slow capillary transit time

Panel 5: Overall strategies for management of acute chest syndrome

Prophylaxis

- Judicious use of opioid analgesics
- Incentive spirometry and periodic ambulation in patients admitted for vaso-occlusive crises, surgery, or febrile episodes
- Hydroxyurea, (especially in patients with previous history of acute chest syndrome), or pronounced baseline pulmonary disease)
- Watchful expectancy in any hospitalised child or adult with sickle-cell disease (pulse oximetry monitoring and frequent respiratory assessments)
- Avoidance of overhydration
- Intense education and optimum care of patients with sickle-cell disease with reactive airway disease or sleep apnoea
- Pneumococcal, influenza, and parvovirus B19 immunisations

Diagnostic testing and laboratory monitoring

- Blood cultures and serology (chlamydia, mycoplasma, Epstein-Barr virus, parvovirus B19)
- Nasopharyngeal samples for viral culture (respiratory syncytial virus, legionella)
- Deep sputum for bacterial and viral culture
- Blood counts every day and appropriate chemistries
- Continuous pulse oximetry
- Chest radiographs (probably at least every day during initial period)
- Blood gases and pulmonary function testing

Treatment

- Life-saving blood transfusion (simple or exchange with Multicenter Acute Chest Syndrome Study guidelines for phenotypical matching of units)
- Supplemental O_2 for drop in pulse oximetry by 4% over baseline, or values <92%
- Empirical antibiotics (cephalosporin and macrolide)
- Continued respiratory therapy (incentive spirometry and chest physiotherapy as necessary)
- Bronchodilators for patients with reactive airway disease
- Optimum pain control and fluid management
- Miscellaneous: NO inhalation, systemic steroids, mechanical ventilation, and extracorporeal membrane oxygenation
- Follow-up assessments: pulmonary function tests and brain MRIs

with exacerbation of agonist-induced endothelial activation via mediators such as hypoxia, cytokines, and free-radical species (generated by sickle erythrocytes and the increased leucocyte numbers that accompany acute chest syndrome). Infectious agents and fat embolism might further enhance upregulation of proinflammatory and proadhesive molecules.[70,93] Further microcirculatory slowing and polymerisation takes place, resulting in sequestration or adhesion (or both) of circulating cellular elements.[93,96] Intrapulmonary shunting exacerbates desaturation. The substantially raised concentrations of secretory phospholipase A_2[97] (which could predict the syndrome),[98] soluble VCAM-1,[70] and F2 isoprostanes[99] might be evidence for perturbations induced by multiple secondary effectors leading to heterocellular activation, and oxidative stress in a vascular bed in which hypoxia induces a vasoconstrictor response of particular detriment.

Clinical studies suggest that concentrations of the cytoprotective mediator NO are reduced substantially during acute chest syndrome.[70,100] Since NO can downregulate agonist-induced endothelial activation, and inhibit the enhanced red-cell endothelial adhesion induced by hypoxia,[70] it could have a critical role in pathogenesis.[82,93] In acute chest syndrome complicated by respiratory failure, inhaled NO improved alveolar-arterial oxygen gradients, reduced pulmonary arterial pressure, and reversed a downhill course. Beneficial effects were also seen in a transgenic mouse model in which lung injury induced by hypoxia/reoxygenation was reduced by NO inhalation.[101] Clinical trials would be the next logical step to test NO efficacy in acute chest syndrome. A report showing that exhaled NO levels are diminished in children who previously had the syndrome and correlate with a polymorphism in the NO synthase I gene, could provide a marker for the prediction of susceptibility to the disorder.[102]

Panel 5 shows management strategies for acute chest syndrome. Exchange transfusion in severe cases and simple transfusion in moderate cases can be life saving. Treatment with a macrolide antibiotic is an important adjunct. Rapid increase in desaturation is an indication for aggressive intervention. In instances of substantial marrow necrosis (besides pulmonary fat embolisation), fat can traverse the pulmonary microvasculature, gaining access to the systemic circulation with dissemination of embolisms to multiple sites, including the CNS. This multiorgan failure syndrome is often lethal, and warrants immediate exchange transfusion.

Splenic sequestration

Hyposplenism and autoinfarction in HbSS disease arises during the first 2 years of life, with slowed onset in HbSC disease.[103] Splenic sequestration thus occurs in HbSS disease, usually within 5 years, with episodes in the other sickling syndromes continuing into adulthood. The spectrum of severity is wide, with rare instances of acute splenic enlargement accompanied by circulatory collapse and death from anaemia and hypovolaemic shock. This constellation of features must be kept in mind, both for children and for adults in emergency room settings, in which knowledge of the haemoglobinopathy might not be part of the initial presentation. Present definition of a sequestration event includes an enlarged organ concomitant with a decrease in haemoglobin concentration ($\geqslant$20 g/L) with substantial reticulocytosis. Thrombocytopenia might also be present. In cohort studies, sequestration has been reported at rates varying from 7 to 30%. Protection is provided by high concentrations of HbF.[104]

Immediate treatment includes correction of hypovolaemia with transfusion. Since the rate of recurrence is high (near to 50%), follow-up management is crucial. For children older than 2–3 years, splenectomy is recommended shortly after the acute episode. Results of chronic transfusion regimens for younger children have only been partly successful in the prevention of recurrence.[104,105] Older patients with chronic hypersplenism need to be considered for elective splenectomy. All new mothers should be educated to do splenic palpation on their infants, as well as to recognise symptoms of this potentially life-threatening event, since such measures have reduced the number of deaths.

Aplastic crises

In chronic haemolytic anaemias, temporary cessation of erythropoiesis leads to severe anaemia (known as aplastic crises), in which sickle-cell disease is no exception. Although most individuals spontaneously recover in a few days, the anaemia can be so severe that it causes cardiac decompensation, with rare deaths (if anaemia and reticulocytopenia are unrecognised and untreated). Parvovirus B19 infection is responsible for most cases.[106] Cytotoxicity of erythroid precursors by the virus accounts for aplasia, with reticulocytopenia lasting for 7–10 days.

Cerebrovascular accidents

Stroke is usually infarctive in children. In a large natural history study,[107] first-time stroke was highest in early childhood (1·02 per 100 patient years in 2–5-year-olds), with a decrease in incidence in 10–19-year-olds (0·41 per 100 patient-years). Arterial disease commonly involves the internal carotids, anterior and middle cerebral arteries, and the circle of Willis, with histological tests showing intimal hyperplasia, fibroblast and smooth muscle proliferation, and thrombus formation.[66,67,108] Risk factors include the HbSS phenotype, previous transient ischaemic attacks, low steady-state Hb concentrations, high leucocyte counts, raised systolic blood pressure, and previous acute chest syndrome.[107] An increased risk in siblings suggests that genetic factors might be involved.[109] Distinct HLA associations with small-vessel compared with large-vessel CNS abnormalities have been recorded,[110] as well as correlations with VCAM-1

	Major clinical manifestations	Underpinning mechanisms	Management issues
Eye	Retinitis proliferans occurs with greatest frequency in HbSC disease and can lead to visual impairment.	Peripheral retinal vascular occlusion due to red-cell and leucocyte-endothelial adhesion (via surface adhesion molecules). Angiogenic factors seem crucial to seafan formation.	Periodical ophthalmic assessment with early identification of peripheral retinal disease.
Kidney	Hypertrophy: occurs in all sickle genotypes, especially HbSS. Also age-dependent.	Renal enlargement is due to glomerular hypertrophy and increased renal blood volume.	..
	Alterations in distal nephron function, resulting in hyposthenuria and nocturia. Tubular acidosis (type IV or incomplete distal acidosis) can also arise.	Hyposthenuria mainly due to hyperosmolar-induced loss of deep juxtamedullary nephrons, which also causes acidosis due to perturbation in bicarbonate reabsorption.	Hyposthenuria can lead to childhood enuresis. Urine-specific gravity is a poor index of hydration status.
	Tubular deficiencies in adults include increased secretion of creatinine and uric acid. Heightened reabsorption of phosphates (aldosterone-independent) leading to hyperphosphataemia can also take place.	Proximal tubular dysfunction.	Creatinine clearance might overestimate GFR.
	Glomerular abnormalities: increases in GFR and ERPF occur in children, preceded by microalbuminuria. GFR and ERPF decline towards normal rates in adolescence, and fall to subnormal rate levels in older individuals. Proteinuria could progress to nephrotic syndrome, and end-stage renal disease. HbSS patients develop renal failure earlier than those with HbSC disease (median age of onset 23 *vs* 50 years).	Mesangial phagocytosis of sickle-cells, glomerular hypertrophy, immune-complex glomerulonephritis, and hyperfiltration-induced glomerular injury have all been implicated. NSAID used for pain control further impairs renal function.	ACE inhibitors reduce microalbuminuria and reduce glomerular damage. NSAID to be used with caution in patients with sickle nephropathy. Transplantation is a recourse in some patients with end-stage renal insufficiency.
Lung	Most serious complication is PH (mean pulmonary artery pressure of >25 mm Hg, and/or a tricuspid jet velocity on echocardiogram >2·5 m/s). PH occurs in 5–30% of patients, with a median survival time of 2 years.	Recurrent ACS is a predisposing factor for SCD-related PH. Chronic anaemia with hypoxia, pulmonary release of inflammatory cytokines, reduction in NO synthase in small pulmonary arterioles with increase in endothelin-1, and platelet-derived factors have been implicated in pathogenesis.	Often asymptomatic in early stages. Should be suspected in those with unexplained 0_2 desaturation, syncope, or fixed dyspnoea. Ominous prognosis justifies experimental treatments including epoprostenol infusions, NO inhalation, oral L-arginine, or use of the endothelin antagonist bosentan.
Chronic leg ulcers	Usually occur over medial malleoli in chronic haemolytic anaemias, including HbSS, thalassaemia, and spherocytosis.	Possible incompetence of venous valves draining ankle region and a reduction in venous refilling time. Hydroxyurea treatment in SCD can cause leg ulcers.	Unna boot (gauze impregnated with zinc oxide) is effective. IV arginine butyrate also reported to cause rapid healing. Hydroxyurea to be used with caution in SCD individuals with previous history of leg ulcers.
Osteonecrosis	Osteonecrosis of the femoral and humeral heads occur in all sickle genotypes, most commonly in SS α-thalassaemia and in HbSS individuals with a high haemoglobin concentration.	Expansion of red-cell marrow with increased pressure or end-arterial vascular occlusion of the femoral and humeral heads have been postulated.	Disease frequently asymptomatic; T1 and T2-weighted images on MRI detect early lesions not seen on radiographs. Hip coring used for early disease; surgical hip replacement indicated for more advanced lesions.
Spleen	Autoinfarction in patients with HbSS before age 2 years. Hyposplenism has slower onset in those with HbSC disease.	Distinct sinusoidal blood flow, high rates of oxygen extraction, and acidosis provide ideal conditions for HbS polymerisation, leading to autoinfarction.	Elevated susceptibility to infection. Use of prophylactic penicillin and pneumococcal vaccine standard.

GFR=glomerular filtration rate. ERPF=effective renal plasma flow. NSAID=non-steroidal anti-inflammatory drugs. ACE=angiotensin-converting enzyme. PH=pulmonary hypertension. ACS=acute chest syndrome. SCD=sickle-cell disease.

Table 2: **Select chronic organ dysfunctions leading to pronounced morbidity and mortality**

variants.[111] Hypoxaemia could also be an additional factor contributing to stroke.[112,113]

In the acute stage of ischaemic stroke, immediate transfusion is required to reduce HbS to less than 30%, with a follow-up transfusion regimen to maintain the HbS concentration at less than 30%. Such therapy has reduced the rate of recurrence from 50% in the 3-year follow-up to about 10%.[114] Standard treatment includes the continuation of blood transfusions (with chelation therapy) for at least 5 years. Whether a longer period of transfusion therapy is necessary remains unclear. Transfused blood should be leucocyte-depleted and sickle-negative. Extended red-blood-cell phenotype matching (for antigens E, C, and Kell) is associated with a decrease in transfusion reactions, and allo-immunisation.[115,116] Maintenance of HbS proportions at 50%, after an initial period of rigorous HbS reduction, has proven as successful in prevention of stroke recurrence as conservative regimens.[117] Since withdrawal of transfusion treatment might be followed by recurrent stroke, therapy with hydroxyurea has been suggested to be used before transfusions are stopped.[118] Some cases of stroke intractable to other treatments have received marrow transplants with stabilisation of vasculopathy.[119] In adults with previous stroke, although the risk of recurrent stroke is also increased,[107] its haemorrhagical nature, unlike the infarctive stroke seen in young people, rules out the types of prophylaxis discussed for children. Treatment for adults is similar to that available in a non-sickle stroke population.

Stroke prevention has benefited from non-invasive testing to assess cerebral blood flow by transcranial doppler velocity measurements that detect areas of vascular narrowing. An increased rate of stroke (10–15% per year) was seen in children with HbSS disease and abnormal velocity measurements, compared with a risk of about 0·5–1% per year in an age-matched cohort with HbSS disease and normal velocity measurements.[120] On the basis of this finding, the Stroke Prevention in Sickle Cell Anemia (STOP) study[121] established that prophylactic transfusions aimed to reduce HbS to less than 30% in children with HbSS and persistently raised

velocity measurements prevented initial stroke (risk reduction to <1% per year). Transcranial doppler screening every year is recommended for children aged 2–16 years with HbSS disease. An editorial[122] accompanying the results of STOP[121] interpreted the findings from a different perspective; the probability of remaining stroke-free 40 months after one abnormal velocity measurement was roughly 60%, with substantial numbers of patients stroke-free, without intervention, after 10 years. Thus, some children who would not show CNS disease progression would be exposed to the risks of chronic transfusion. Alternative treatment to prophylactic transfusions include hydroxyurea.[123]

Sophisticated imaging techniques have uncovered silent brain lesions on MRI in the microcirculation of the grey matter in 10–20% of children with sickle-cell disease.[120] Since these lesions might be accompanied by neuropsychometric deficits, MRI abnormalities should alert caregivers that learning and cognitive problems could arise in this vulnerable group. Although evidence[124] suggests that silent infarcts in children might predict propensity for stroke, no prophylactic intervention is presently recommended; a multicentre trial[120] is in progress.

Priapism

Priapism is a painful failure of detumescence which could be due to excess release of contractile neurotransmitters, obstruction of draining venules, malfunction of the intrinsic detumescence mechanism, or longlasting relaxation of intracavernosal smooth muscle.[125] Two main types of priapism exist: high flow (non-ischaemic) and low flow (ischaemic). Low-flow priapism is more common, and is associated with a reduction in venous outflow, hypoxia, acidosis, stasis, and tissue ischaemia. Clinical presentation of priapism involves either scattered episodes, or a stuttering pattern, usually nocturnal, in which progressively more intense episodes cluster over a short time. Both presentations can lead to impotence.

Although many treatments have been attempted, none have had controlled assessment. In one report, aspirations and irrigation of the corpora cavernosa with dilute epinephrine within the first 24 h caused rapid involution of tumefaction,[126] whereas another suggested similar success with the α-adrenergic agonist etilefrine.[127] No basis for the use of exchange transfusion for acute episodes exists. In intractable cases, surgery (Winter shunt) has been undertaken with success. No evidence-based prophylaxis has been proved for this complication. Oral pseudoephedrine, α-adrenergic and β-adrenergic agonists (etilefrine and terbutaline), and diethylstilboestrol, have been used as preventive treatment. Restriction of fluids at bedtime to avoid bladder distention is advisable. Sildenafil has been associated with the development of priapism in sickle-cell trait,[128] although another report suggests resolution of priapism with this drug.[129]

Chronic organ dysfunctions

Neonatal screening and the introduction of prophylactic penicillin in early childhood has reduced mortality to less than 2% by 10 years of age.[130] The average lifespan in the USA for men and women with HbSS has increased to 42 and 48 years, compared with ages of 60 and 68 years in those with HbSC disease.[48] These changes in life expectancy have shifted the spectrum of clinical problems to an increased focus on chronic organ dysfunction in developed countries. Table 2 describes the salient features of organ dysfunction that contribute to disease-associated complications. However, a disparity exists in mortality and morbidity related to sickle-cell disease in developing countries with large affected populations, where the economic environment is not conducive to optimum medical care. For instance, in most parts of Africa, life expectancy for affected individuals is less than 30 years, with sickle-cell disease being the third leading cause of mortality (after malaria and diarrhoea) in children receiving hospital care.[131]

Select management issues

Management of sickle-cell disease needs a concerted team effort (panel 6). Fragmented care can be disastrous, since life-threatening complications might not be recognised in time by practitioners unfamiliar with the nuances of acute sickle presentations. Chronic problems could also be left unidentified. To complement another review,[132] we will discuss specific management issues, and summarise standard and promising new treatments.

Panel 6: Comprehensive care profile for sickle-cell disease

Medical care coordinated by haematology team

- General paediatric or adult medical care
- Specialist care with experience with sickle-cell disease: pulmonology, neurology, infectious disease, renal care, orthopedics, ophthalmology, surgery, anaesthesia, prenatal care
- Pain management
- Preventive care: immunisations, prophylactic penicillin, prospective transcranial doppler velocity measurements, assessments and screening for pulmonary hypertension
- Prenatal and newborn screening, and genetic counselling
- Blood-bank support
- Nutritional services
- Transition from paediatric to adult care

Other medical and non-medical services

- Social services
- Psychotherapy
- Drug dependency
- Physiotherapy
- Patient-parent information and peer-parent support groups
- Job training and vocational services
- Supportive community agencies

Perioperative care

Frequency of both perioperative and postoperative complications is greatly increased in sickle-cell disease. Factors precipitating vaso-occlusion include intraoperative hypoxaemia and hypoperfusion, with postoperative-pain-induced immobility leading to hypoventilation and acute chest syndrome. A multiinstitutional trial[133] that randomly assigned patients to an aggressive regimen (part exchange transfusions to reduce HbS concentration to about 30%), did not show any advantage over conservative transfusion (preoperative Hb of about 100 g/L). Frequency of serious complications (non-transfusion-related) was similar, with acute chest syndrome developing in 10% of both groups. History of pulmonary disease and high-risk surgical procedures were predictors of this syndrome. Other reports have supported the preoperative use of less intensive transfusion regimens.[134] A top-up transfusion might not be indicated because of the absence of a non-transfused control group in studies advocating transfusion.[135] Further, support for this stance is the low perioperative rate of serious complications reported during elective surgery in children without preoperative transfusion.[136] Simple transfusion therapy, if used, should not increase the Hb value by greater than 20 g/L over steady-state concentrations in HbSS, since blood viscosity will be increased with potentially undesirable consequences. Decisions on transfusion therapy should be made on an individual basis. Watchful expectancy, and use of such prophylactic measures as prevention of hypoxia and acidosis, appropriate hydration, incentive spirometry, early ambulation, and optimum pain control without narcotic sedation should be standard.

Panel 7: Indications for transfusion therapy in sickle-cell disease

Acute

- Acute on chronic anaemias: splenic sequestration and severe or longlasting aplastic crises
- CNS: acute stroke
- Pulmonary: acute chest syndrome (with hypoxia or chest radiography with multisegment involvement)
- Acute multiple-organ-failure syndrome
- Preoperative (in select cases)
- Malaria-associated severe haemolytic anaemia with impending cardiac decompensation

Chronic

- CNS
  - -Prophylaxis against recurrent stroke
  - -For stroke prevention when transcranial doppler velocities are abnormal
- Cardiopulmonary
  - - Chronic pulmonary hypertension (unresponsive to other modalities)
  - - Refractory congestive heart failure
- Hydroxyurea non-responders to tide over an interim period of severe recurrent vaso-occlusive crises or acute chest syndrome
- Previous splenic sequestration in a child aged ≤2–3 years (in anticipation of later splenectomy)

Pregnancy-related issues

In both the USA and Jamaica, pregnancy-associated complications have fallen over the decades, although substantial maternal mortality (1·7 and 2·1%, respectively), spontaneous abortions, and perinatal deaths still occur.[137,138] An increased rate of urinary tract infections has been seen, with major complications such as septicaemia, toxaemia, and thrombophlebitis arising close to the time of expected delivery. Fetal death was three times higher during the first trimester, than late in gestation, with substantial increases in prematurity and intrauterine growth retardation.[137,138] Previous studies reported maternal mortality rates as high as 11·5% in west Africa, with evidence of the positive effect of prenatal programmes.[139] Prophylactic intrapartum transfusions have not proven uniformly beneficial.[140] In women with previous fetal loss, or in pregnancy complicated by multiple gestations, the early use of a transfusion regimen to maintain haemoglobin concentrations at about 100 g/L has been suggested. However, the data supporting this recommendation are tenuous.

Transfusion therapy

An aggressive approach to transfusion therapy in the USA with blood-bank and chelation support has been met with caution.[135] Panel 7 depicts a conservative approach to transfusion therapy in sickle-cell disease; when a simple transfusion is chosen, overtransfusion (ie, haemoglobin concentrations of more than 110 g/L) should be avoided. Recommendations are based on a few evidence-based clinical trials (for CNS), with other indications identified by consensus among haematologists caring for patients with the disease.[115,141]

Hydroxyurea in sickle-cell anaemia

In-vivo evidence of mild disease in individuals with sickle-cell disease with high concentrations of HbF, paved the way for a search for compounds that would increase HbF in vivo.[84] Hydroxyurea, an S-phase-cytotoxic drug, is the only one that has been widely used to increase HbF concentrations in sickle-cell disease. Its effect on HbF synthesis is ascribed to the premature commitment of erythroid precursors during the marrow regeneration that follows drug-related cytoreduction.[142] Thus, effectiveness of increasing HbF concentrations is tied to the cytoreductive property of hydroxyurea, and is response dependent on the capacity of the marrow to withstand moderate drug dosages. In responders, the number of F cells (red cells containing HbF) and the amount of HbF

per F cell increase, and the number of dense cells and reticulocytes decrease, with improved erythrocyte survival. Other beneficial effects of hydroxyurea include modulation of sickle erythrocyte adhesive properties and enhanced NO production (panel 8). In a landmark study[58] in adults with homozygous HbSS disease, hydroxyurea therapy reduced the frequency of painful crises, acute chest syndrome, admission rates, and the need for blood transfusions. A 9-year follow-up of the study has shown reduced mortality in the hydroxyurea-treated cohort.[143]

In children with HbSS disease, data are similar to those for adults.[144,145] Hydroxyurea-related toxic effects are mild and reversible with no evidence of growth failure.[144] Notably, a beneficial effect on preservation of splenic function was seen in very young children given hydroxyurea.[146] A placebo-controlled pilot trial of hydroxyurea treatment in young children (aged 12–18 months at enrolment) has begun in the USA, in an attempt to prevent chronic organ dysfunction. Until results are available, the drug is not recommended for this age group. However, hydroxyurea can be cautiously considered in infants at high risk, as identified by Miller and co-workers.[47] Three early life predictors of adverse outcomes included hand-foot syndrome, severe anaemia (Hb concentration <70 g/L), and leucocytosis. An additional indication is the child with stroke, who for various reasons might need to discontinue prophylactic transfusion therapy. In this situation hydroxyurea could decrease the rate of stroke recurrence.[118] The drug also seemed to relieve pain in children with HbSC disease, although its use in children and adults with HbSC disease is fairly limited. Hydroxyurea should be given in a structured environment of medical care, attention to compliance, and initial escalating therapy with maximum tolerated doses between 20–30 mg/kg.[13] Optimum doses provide a balance between haematological toxic effects and increased concentrations of HbF, with the highest tolerated dose yielding the greatest response.[149]

Disadvantages include a large number of non-responders, and potential long-term carcinogenic or leukaemogenic effects.[58] Additionally, women and men on hydroxyurea should use contraception, discontinuing use of the drug before conception. The effects of the drug in human fetuses are unknown, although teratogenic effects are seen in animals. As well as hydroxyurea, other inducers of HbF synthesis include butyric acid (a short-chain fatty acid given either alone or in combination with hydroxyurea).[150] Decitabine, a DNA hypomethylating drug that greatly increases HbF concentrations at non-cytotoxic doses, also is worthy of further investigation.[151,152]

Other promising treatments

Ion-channel blockers

As discussed earlier, cation homoeostasis is important in sickle pathogenesis (table 1).[23] Combination therapy with additional agents (acting synergistically) merits further consideration.

Panel 8: Beneficial erythrocyte and extra-erythrocyte effects of hydroxyurea treatment in homozygous HbSS disease

Erythrocyte effects

- Increase in F-cell numbers and HbF concentration per F cell
- Inhibition of cation depletion and dense-cell formation
- Reduction in stress reticulocytes and haemolytic rate
- Increased deformability with improved rheology
- Inhibition of sickle red cell-endothelium adhesion
- Inhibition of sickle erythrocyte adhesion to extracellular matrix components, including fibronectin, thrombospondin, and laminin

Extra-erythrocyte effects

- Quantitative reduction in leucocyte count
- Qualitative changes in leucocytes, including reduction in leucocyte-free-radical production and activation marker L-selectin
- Reduction in soluble VCAM-1 concentrations (indicative of decreased endothelial activation)
- In-vivo NO release

Antiadhesion and anti-inflammatory treatment: future prospects

Antiadhesion and anti-inflammatory treatments also have potential prospects to ameliorate sickle-cell disease. Since the endothelium is the template on which adhesion occurs, inhibition strategies providing maximum benefit might include the targeting of single molecules (such as P-selectin), that mediate heterotypic cell-endothelial interactions.[35,76,77] Anti-P-selectin antibodies and heparin have shown promising results that lend support to this theory.[57,78] Transcription factor activation can lead to inflammatory, proadhesive, and procoagulant gene expression. Since these effectors, such as NFκB, are increased in sickle transgenic mice models, another targeted approach is the inhibition of transcription.[153] A pilot investigation of the NFκB inhibitor sulfasalazine in human beings showed downregulation of endothelial expression of proinflammatory and proadhesive molecules.[154] Glucocorticoids inhibit NFκB and reduce cytokine production.[155] Although such anti-inflammatory effects have proven beneficial in the acute management of vaso-occlusion[156] and acute chest syndrome,[157] the rebound of symptoms after discontinuation of steroid use, and the potentially harmful effect of a further increase of leucocyte counts must be considered. A vascular lubricant (poloxamer 188; a non-ionic surfactant copolymer) has been assessed, with a small reduction in the duration of acute vaso-occlusion.[158]

Antiadhesive treatment might be targeted at specific erythrocyte or leucocyte-endothelial interactions. High fetal Hb levels that occur in infancy, or can be induced by drugs such as hydroxyurea, decrease sickle red cell adhesion molecule expression,[159,160] protect

against the adhesion process, and are associated with a decrease in vaso-occlusive crises and chronic organ damage.[6,143] Monoclonal antibodies against endothelial integrin $\alpha_v\beta_3$ prevented vascular obstruction in an animal model of vaso-occlusion.[19] Abciximab (antibody against platelet integrin $\alpha_{IIb}\beta_3$) used in the treatment of acute coronary syndromes, cross-reacts with $\alpha_v\beta_3$ integrin. Thus, future studies on transgenic animal models assessing its effectiveness (or other specific $\alpha_v\beta_3$ inhibitors) in the modulation of vaso-occlusion might be desirable. Antagonists to leucocyte integrins of the β_2 or CD18 subfamily, and antioxidants such as the xanthine oxidase inhibitor allopurinol, deserve further investigation as modulators of both reperfusion injury and chronic inflammation.[161,162] Thus while preliminary studies on animals show that pharmacological inhibition of endothelial cell activation or heterocellular adhesion (or both) is feasible, the route of administration, and haemostatic-related effects of some compounds (monoclonal antibodies against $\alpha_v\beta_3$ and heparin), might make oral antioxidants, and NFκB antagonists better suited for initial clinical trials.

Nitric oxide

Reference has been made to a role for NO in the acute and chronic complications of sickle-cell disease. This signalling molecule, whose precursor is L-arginine, is continuously produced in the endothelium by a constitutive NO synthase, which is functionally calcium-dependent. NO increases cyclic guanosine monophosphate production, leading to dephosphorylation of myosin light chains. Thus, in the vessel wall, NO induces relaxation of smooth muscle and vasodilation. Other mechanisms related to sickle-cell disease include the role of NO as a cytoprotective mediator, inhibiting gene transcription of proadhesive and proinflammatory molecules such as endothelial VCAM-1 and P-selectin. Effects on circulating cellular elements include inhibition of platelet aggregation, leucocyte adhesion and migration, quenching of superoxide, and inhibition of erythrocyte-endothelial adhesion.[82]

NO bioavailability is maintained by a balance between endothelial production and consumption; this balance is disrupted in sickle-cell disease.[70,82,100] Although NO synthase is upregulated by anaemia, shear stress, and tissue hypoxia, NO bioavailability is impaired, especially in male individuals with the disease.[163] This unavailability is due to rapid scavenging of NO by cell-free haemoglobin[164] and free-oxygen radicals,[165] together with low concentrations of substrate L-arginine.[100] Additionally, cell-free haemoglobin amounts are also higher in men with sickle-cell disease than in women, which partly explains the recorded differences in NO bioavailability between the sexes. The lung is most affected by perturbations in NO, in which a reduction in NO seems to be the mechanism underlying hypoxic pulmonary vasoconstriction, and a predisposition to acute chest syndrome.[82,93,102] Reduced NO with raised concentrations of endothelin-1 and chronic haemolysis have been implicated in pulmonary hypertension associated with sickle-cell disease.[82,166,167] Furthermore, dysregulation of microcirculatory vascular tone, partly due to reduced NO bioavailability, is thought to have a role in the pathophysiology of vaso-occlusion.[82]

Transgenic models and case reports suggest a beneficial effect of NO inhalation in acute chest syndrome.[93,101] A pilot study of children with vaso-occlusion also showed a trend towards lower opioid use and pain scores than controls.[168] A larger study is needed for definitive results. Clinical trials of NO in pulmonary hypertension are also in progress. Although NO inhalation is cumbersome, use of oral L-arginine improves endothelial function,[169] and seems to modify pulmonary hypertension related to sickle-cell disease.[170] Concentrations of this aminoacid are reduced during vaso-occlusive crises, with increases in NO after L-arginine is given.[100,171] Gardos-channel inhibition has been shown in transgenic mice fed with arginine with concomitant beneficial effects on haematological indices.[172]

Haemopoietic cell transplantation

Haemopoietic cell transplantation is the only available potentially curative therapy for sickle-cell disease. Estimated risk of death from HLA-identical-stem-cell transplantation in the disease is 5%. Rapid development of procedures such as non-myeloablative conditioning regimens that lead to stable mixed chimerism, cord-blood transplantation, and transplantation from unrelated stem-cell donors holds promise.[132,173,174] Although initial transplantations were restricted by eligibility criteria that included evidence for substantial single-organ dysfunction, less restrictive criteria will apply as mortality rates improve. The goal is to successfully replace the host's marrow with normal genotype cells before development of organ dysfunction. Choice of appropriate candidates will be helped by identification of epistatic genes that improve the definition of risk.

Gene-therapy advances

Gene therapy has been successful in the sickle transgenic mouse.[175] A lentivirus construct containing a β^{A-T87Q} globin gene variant to resemble HbF was generated. This vector was made optimum for transfer to haemopoietic stem cells and gene expression in the erythroid lineage. Transduced haemopoietic stem cells were transplanted into mice in two mouse models with sickle-cell disease by marrow ablation. Long-term expression was achieved, without preselection, in all transplanted mice. Erythroid-specific accumulation of the anti-sickling protein was noted for up to 52% of total haemoglobin and in virtually all circulating erythrocytes. The mouse models showed inhibition of red-cell dehydration and sickling, in addition to correction of

haematological indices, splenomegaly, and hyposthenuria. Clinical trials are being considered, although unexpected difficulties could arise before gene therapy becomes a reality. Nevertheless, there are good reasons to be optimistic.

Future areas of research

With respect to sickle-cell research, natural history studies and clinical trials have provided incremental increases in our knowledge about this monogenic event that results in a systemic disorder of monumental complexity. Although some issues have been successfully tackled, and gene therapy holds promise, many questions remain. What are the genetic and environmental modifiers of clinical phenotype? Will genetic determinants of the inflammatory response, adhesion, haemostatic activation or related surrogate biomarker values be able to determine future treatment, or identify fetuses at risk for severe disease? Can we unravel CNS vasculopathy; or improve pregnancy-related complication rates? Can we do anything about acute chest syndrome and chronic pulmonary disease? Is the fully functional spleen or kidney a realistic therapeutic goal? In countries where stem-cell transplantation or gene therapy will be initially impractical, can we develop safe, easily administered, and effective treatments that will revolutionise care without straining resources? The challenges remain for this archetype of the single-gene disease in the genomic era of medical research.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

M J Stuart wishes to acknowledge her deceased mentor, Frank A Oski, who fostered her spirit of scientific inquiry. R L Nagel acknowledges his debt to Helen M Ranney and Quentin Gibson, who introduced him to haemoglobin and haemoglobinopathies. We thank Lorenzo Thomas for manuscript preparation, and M H Steinberg, B N Y Setty, and F A Kuypers, who kindly gave consent to our use of figures 2–4. This manuscript was funded by grants HL62148, HL70585, HL73944 (M J Stuart); HL709994, HL55435, HL68962, RR12248 (R L Nagel) from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA. The funding source had no role in preparing this Seminar.

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