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Sickle cell vasoocclusion: many issues and some answers

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Abstract. The pathophysiology of sickle (SS) cell vasoocclusion is derived from the presence of hemoglobin S (HbS) which forms polymeric fibers in the deoxygenated state. Nevertheless, phenotypic expression of sickle cell disease (i.e., clinical severity) shows marked individual variations and is influenced by genetic modifiers such as epistatic effects of linked and unlinked genes. Furthermore, the polymerization of HbS is central but not the only event, and is more likely a consequence of disruptions of the steady state of flow. The available evidence indicates that the vasoocclusive crisis is a microcirculatory event in which multiple factors could be involved. We present a model of vasoocclusion as a two step process in which adhesion of deformable cells occurs first, followed by obstruction induced by less deformable SS cells. This review discusses, in addition, rheologic and microcirculatory behavior of SS erythrocytes and the interacting role of vascular factors, red cell heterogeneity, deoxygenation rates, and red cell–endothelial interactions in the pathophysiology of SS cell vasoocclusion.
Key words. Sickle erythrocyte; vasoocclusion; adhesion; von Willebrand factor; epistatic effects; abnormal rheology; vascular factors; deoxygenation rates; red cell heterogeneity.

General background

Hemoglobin S (HbS) forms polymeric fibers in red cells, which are helical arrangements of the Wishner-Love double-strand[79,106]. This double strand of HbS tetramers is also the basic unit of HbS crystals[106], but in the case of the HbS polymer it arranges itself in a different, quintic structure. Contacts *within* the tetramers forming the double strand, both up and down as well as lateral, are well defined[9,79]. In contrast, contacts *between* the double strands are not yet fully understood.

The pioneering work of Hofrichter and Eaton[30] has elucidated the formation of the HbS polymer. The polymer is generated by a nucleation-dependent reaction, which involves first the formation of reversible and unstable aggregates of isolated hemoglobin molecules, until a critical nucleus is achieved. At that point the polymerization reaction becomes irreversible, and it takes off as a rapid exponential event. The polymerization reaction occurs both by homogenous (no preexisting polymer to serve as anchor for nucleation) and heterogenous mechanisms (in which the new polymer is formed 'piggy-back' on another fiber). These two types of reaction have been dramatically shown by DIC-image enhanced microscopy by Samuel et al.[93] confirming Hofrichter and Eaton's model.

Polymerization of Hb in the red cell is influenced by a variety of factors: red cell heterogeneity, changes in cell volume, pH, cycles of oxygenation/deoxygenation, and osmolarity [as reviewed by Eaton and Hofrichter[30]]. The presence of inhibitors such as fetal hemoglobin (HbF) can alter contact sites in such a way that polymer formation is not possible. Shear stress applied to the red cell[16] can also have an effect on sickling. Red cell volume changes (secondary to K:Cl cotransport, Ca^{++} dependent K^+ efflux and Na^+/H^+ exchange) can facilitate sickling (when they shrink the cell), or inhibit it (when they swell the cell), as can membrane changes, for example changes in cell transport systems, recently reviewed by Canessa[19].

A sickle cell endowed with the capacity for rapid deoxy HbS polymerization is indispensable to, but not sufficient for vasoocclusion. Sickle cell adhesion to venular endothelium is another important factor[51], and there are several other modulating effects. However, the irreversibly sickled cells (ISCs) can, by themselves, obstruct a precapillary sphincter. Lighter red cells tend to adhere, and when this is followed by trapping of less deformable cells, obstruction occurs. Thus the two mechanisms may work together in succession, first

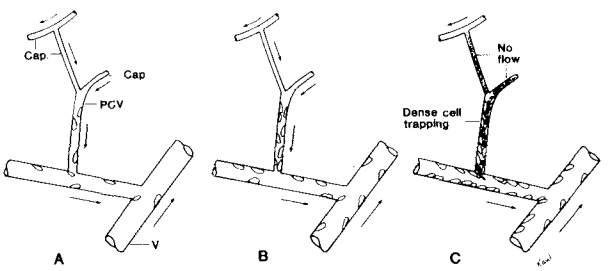

Figure 1. The sequential model for sickle vasoocclusion: *A* initial adhesion of young deformable SS red cells to the venular endothelium, flow remains patent; *B* increase in the number of adhered cells, until a critical level is reached in which *C* dense SS cells (including ISCs) become the trapped in low diameter venules bedecked with adhered SS cells. Only then is obstruction to the flow present. Cap: capillaries; PCV: postcapillary venule; V: vein. Arrows: direction of the flow. (From Kaul, Fabry and Nagel[57].)

adherence and then obstruction, as proposed by Kaul, Fabry and Nagel[56] (fig. 1). We will return to this point. A key protein in adhesion is, in all likelihood, the von Willebrand factor (vWF)[56,60,103,105]. Preparation of anti-vWF can almost completely abolish adherence in microcirculatory preparations[60]. Nevertheless, other adherent proteins may be found to be participants in this phenomenon when it has been more completely studied in different organs.

Perfusion pressure, which may be especially important in the lung, is another critical factor in occlusion[59]. In the presence of low perfusion pressure occlusion is much more likely. Regulatory vasomotor substances are also involved, but little is known about their role in vascular occlusion. Endothelin, a vasoconstrictor, and nitric oxide, a vasodilator, are currently under study.

The above description demonstrates that while the β^S mutation is the product of a single abnormal gene, sickle cell anemia is a multigene disease. The genes other than the β^S gene may be polymorphic (that is, they are not identical in all individuals) and contribute greatly to the variability in phenotypic expression of the disease. This is not a unique situation: all genetic diseases of man have variable phenotypes which are the consequence of the effect of other genes, which are mostly normal but polymorphic. The 'other' genes that participate in the phenotypic definition of genetic disease are called epistatic genes and the phenomenon is referred to as 'epistasis'. Of course, epistasis is the main basis for the extreme variability in the phenotype of sickle cell anemia, in which some patients can have a relatively benign course, others a particularly malignant course, and most patients fall between these two extremes.

An integrated model of sickle cell anemia must start with the notion that in the pathogenesis of sickle cell anemia, the primary effect is the presence of the β_6 Glu → Val mutation. The immediate consequence of this mutation is that HbS polymerizes in the deoxygenated state[30]. As stated above, the primary effect is modified by epistatic effects which can come either from genes linked to the abnormal gene, or unlinked genes far away in the same chromosome, or in other chromosomes. These effects do not complete the picture since there are also secondary effects, which are the result of the abnormal gene and the polymerization of deoxy HbS, and give rise to important pathophysiological consequences of their own (preponderance of young red cells, oxidative stress, etc). Finally, there are also environmental effects. These are likely to be partially genetic, since there is very probably an innate variable susceptibility to these exogenous influences.

Among unlinked epistatic effects we find the modulating effect of co-existing α-thalassemia in sickle cell anemia[33,34,38,101]. Evidence is also mounting for the presence of an X-linked factor affecting the expression of HbF[1,28,72,80]. Other potential candidates (not yet proven) for unlinked epistatic effects include the volume regulatory membrane transport systems expressed in SS red cells (as mentioned before)[17,20,22]. These have been shown to have inter-individual variability in expression in SS patients (although there is no formal evidence of hereditability). The genes involved in marrow proliferative regulation, yet to be identified, could have an impact on the level of anemia, as well as the genes defining enzymes and proteins which are involved in the 'mopping-up' of free radicals in red cells[50].

Epistatic effects linked to the β^S gene involved genetic determinants found within the β-like gene cluster. Recent analysis of a large multicenter sample of adult SS patients definitely established that β-gene cluster haplotypes do indeed serve as markers or stand-ins for genetic factor/s (to which they are linked) that contribute to the determination of the level of HbF expression[16]. The Los Angeles group of Schroeder and Powars[86,96] has, for some time, claimed the same for a sample largely composed of pediatric patients. In effect, gender and haplotype interact so that patients bearing the homozygous Senegal haplotype and the compound heterozygote Senegal/Benin have more HbF and less anemia than other haplotypes, and this is particularly true among females. In addition, the same haplotype group has the least osteonecrosis, while those with at least one chromosome with the Bantu haplotype exhibit doubling of the incidence of this complication[16].

The possibility of down-regulation of the β^S gene in the Arab-Indian haplotype has been proposed[10,32], but it has not been definitively proven. Nevertheless, further suggestive evidence has been presented[89]. Finally, very recent data from Oner et al.[84] suggests that sequences in the hypersensitivity site 2 of the locus control region (LCR)[103] may be involved in defining HbF expression.

What are the secondary effects? These do not directly involve sickling, but are derived from the presence of the β^S gene. Because of their importance, these secondary effects take on a life of their own. They fall into two groups. Firstly, owing to chronic hemolysis most of the SS red cells are younger than normal. Three immediate repercussions: 1) increased expression of K:Cl cotransport[21,46], 2) increased adhesion of SS cells to venular endothelium[40,55], and 3) increased red cell turnover and hemopoietic stress creates changes in the properties of circulating BFU-E. These changes include an increase in the number of these cells, a modification of the type of cytokine/s produced by accessory mononuclear adherent cells, an alteration in their responsiveness to cytokine, and an increase in their capacity to proliferate[25–27]. This is particularly true in SS patients with lower HbF and consequently higher hemolysis.

Another class of secondary effects, not related to the presence of young cells, is the increased oxidative stress

found in SS red cells, most likely the consequence of increased cycles of methemoglobin formation and reduction[52]. The consequences are oxidative damage to cytoskeletal proteins[48,49,97], a phenomenon that has the potential to induce cell rigidity, as well as alteration of transport proteins.

Finally, environmental effects play a clear role in the phenotypic expression of sickle cell anemia. For example, there is direct evidence that low temperatures induce dactylitis[70], and cold has been proposed as a major mechanism in the induction of painful crises[99]. Parvovirus B19 infections produce aplastic crises in sickle cell anemia[92], but secondary effects (increases in the compartment of BFU-E in the marrow) are a necessary condition for infection and significant aplasia. Finally, there is general accord, but not too much data, that dehydration has a negative effect on these patients.

Defining the epistatic, secondary and environmental factors for phenotypic expression of sickle cell anemia is not an academic exercise. It has implications for diagnosis and management.

In other words, in each complication of sickle cell anemia, a particular set of epistatic genes and a particular mechanism of pathogenesis are active. The increase in the incidence of osteonecrosis and retinopathies in otherwise mild sickle diseases (SC and S/β thalassemia) speak of the same phenomena.

Conceptually, this model allows for the different complications of sickle cell anemia *not* to be identical in their pathophysiological mechanisms. For example, hemolysis is determined by how 'bad' the SS red cells are in terms of tendency to polymerize, but epistatic effects that include marrow response (including erythropoietin response), and HbF expression are modulators. In painful crises, the incidence does not have a positive correlation with dense cells/ISC count[12], but it correlates better with the percentage of deformable SS cells[6]. Hence, in this case the properties of the SS red cells (as to polymerization) do not play a central role. Of course, SS red cells are indispensable, but they are clearly far from sufficient. The tendency of deformable cells to adhere may be more important and it would be a logical necessity in a sequence in which SS cell adhesion precedes vasoocclusion by dense cells as described above[56,37]. The data of Fabry et al.[36], recently confirmed and expanded by Ballas et al.[5], are consistent with this sequence of events.

Diagnostically, it is no longer sufficient to ascertain only the genotype, but it is also necessary to define the future course of the disease in terms of severity. As is apparent from the previous analysis, this is not yet possible. Although progress has been made in defining severity factors (α-thalassemia, gender, β-gene haplotypes), more progress is needed before patients can be confidently classified in severity categories.

The ability to ascertain future severity would be useful in deciding on proper treatment when risk/benefit ratio is an important consideration (i.e. bone marrow transplantation), and in providing proper information for mothers requesting prenatal counseling. To attain this goal, we need to know more about the genetic polymorphisms involving SS red cell volume regulation, those involving SS red cell adhesion (including vWF polymorphism), and those controlling microcirculatory tone regulation.

There are also therapeutic implications of this analysis. It is clear that any treatment, short of the magic bullet, should be aimed at attacking all components of the disease. Hence, we need to develop not only anti-polymerizing drugs, but also anti-volume reduction drugs (which are also anti-polymerizing) and anti-adhesion drugs.

The analysis is probably applicable to most human genetic diseases, and we suspect that sickle cell anemia will again serve as a ground-breaking leader in the understanding of molecular diseases. It is noteworthy that significant advances in our understanding of the disease have occurred since this subject was last reviewed[78]. In conclusion, the central point to be emphasized when considering the pathophysiology of sickle cell anemia is that while β^S is the product of a single gene, sickle cell anemia is the product of many genes. This is a concept with diagnostic and therapeutic implications. In addition, it explains why all sickle cell anemia patients are not 'equal', in spite of the fact that they share the same defect.

Rheologic and microcirculatory abnormalities

Although multiple factors that may trigger a painful vasoocclusive crisis remain to be fully elucidated, it is clear from the foregoing account that the polymerization of HbS is the central but not the only event. Furthermore, the available evidence indicates that the vasoocclusive crisis is a microcirculatory event precipitated by the impedance of capillary blood flow. Disruption of the steady state at the microvascular level could involve vascular, red cell and hemostatic factors, and result in an increased transit time of sickling of HbS-containing red cells, thereby initiating a vasoocclusive event. It was recognized long ago that HbS polymerization results in morphological deformation or sickling of SS red cells, which in turn causes an increase in cellular rigidity, as well as in the viscosity of sickle cell suspensions. Thus, the loss of red cell deformability (abnormal rheology) under deoxy conditions is the net result of HbS polymerization. The initial blockage may drastically alter the local hemodynamics (pressure gradients and wall shear rates), oxygen tension and pH, and intravascular sickling may progress to involve a large area of tissue, causing a painful vasoocclusive crisis.

Vasoocclusive potential of sickle cells

In vitro studies have shown that the rheological behavior of whole SS blood is sensitive to oxygen tension (pO_2). Even in the oxygenated state, SS blood is more viscous than normal AA blood due to the presence of ISCs. Deoxygenation results in a reduced red cell deformability and in a dramatic rise in the bulk viscosity of whole SS blood[23,98]. Abnormal rheologic behavior indicates the vasoocclusive potential of sickle cells. The reduced deformability of individual sickle cells has been elucidated by micropipette studies. The studies of Evans et al.[35] have demonstrated that in the oxygenated condition, individual sickle cells show an increase in both static and dynamic rigidity. The rigidity of sickle cells is significantly influenced by the state of dehydration or mean corpuscular hemoglobin concentration (MCHC). In the deoxygenated condition, abnormalities in the deformation response of sickle cells have been associated with morphologic sickling at low pO_2 (below 35 mm Hg)[81]. The static rigidity or membrane extensional rigidity increases with decreasing pO_2 and is 5 to 50 times higher than that of oxygenated sickle cells. These results indicate a profound influence of HbS polymerization on the cytoplasmic viscoelasticity. Direct visualization of deformation and alignment characteristics of sickle cells under defined shear stresses has been carried out in vitro using a rheoscope[94,95] or by interpretation of laser-diffraction images from an ekacytometer[11,73]. These studies show that oxygenated discoid sickle cells deform into an ellipsoidal shape and align in the direction of flow similarly to AA red cells. However, the subpopulation of ISCs shows a tendency to rotate in the flow and their orientation is variable.

The use of artificially perfused ex vivo microvascular beds has allowed insight into the more complex interplay between sickle red cells and vascular factors. In the oxygenated state, hemodynamic abnormalities of SS blood can likewise be attributed to a higher-than-normal MCHC and the presence of ISCs[54,104]. This results in an increased vascular resistance to sickle cells. Furthermore, vascular topographical features such as the angle of branching may play an important role in ISC-induced obstruction at the junction of the precapillary arteriole and a capillary (or a precapillary sphincter)[3,63]. This is a potentially vasoocclusive event. However, in the microcirculatory flow ISCs usually align parallel to the direction of flow[64]; this behavior is different from the rotating motion and erratic orientation reported for rheoscopic flow. Both pO_2 and perfusion pressure have a significant effect on the microvascular flow of sickle cells[15]. A decline in microvascular red cell velocities (Vrbc) and wall shear rates occurs at a relatively higher pO_2 of 60 mm Hg. Deoxygenation may induce arteriolar dilation which is not sufficient to overcome the increased capillary resistance to deoxygenated SS red cells[66]. In addition, microvascular obstruction by SS blood is accompanied by selective trapping of less deformable red cells with high MCHC[55].

As can be seen in a wet preparation of sickle blood, there is a marked morphological heterogeneity among sickle red cells. In addition to ISCs, one also encounters multilobulated stress reticulocytes, discocytic cells with an irregular contour, and smooth discocytes; the latter form the bulk of sickle blood[39,55]. Density gradient separation of sickle blood shows that this morphological variability reflects a marked heterogeneity of MCHC (density) (31–46 g/dl) among sickle cells, the reticulocytes being the lightest while dense discocytes and ISC are the most dense[24,39,58]. The proportion of these cells varies among SS patients[58]. Gradual deoxygenation of light density SS red cells (reticulocytes and discocytes) with low MCHC results in the formation of typical sickle cells and holly leaf forms, while under the same deoxy conditions, dense cells (dense discocytes and ISC) with high MCHC polymerize more rapidly and undergo minimal shape changes[58]. Since the delay time of the polymerization is extremely sensitive to MCHC, the presence of dense cells (dense discocytes and ISC) with the shortest delay times could mean a greater obstructive potential for these cells under hypoxic conditions, as these cells will be the first to undergo polymerization. The question arises: what are the hemodynamic characteristics and vasoocclusive potential of these subpopulations of red cells in the microcirculation?

Studies by Kaul et al.[58] have revealed distinct rheologic and hemodynamic characteristics of density classes of sickle cells in oxy and deoxy states. When deoxygenated, all density cell classes nearly doubled their viscosities and the oxy-deoxy differences remained constant. In contrast, in the isolated vasculature, infusion of different classes of deoxy cells resulted in a dramatically increased peripheral resistance (PRU), and the oxy-deoxy differences were directly proportional to cell density or MCHC. Thus the infusion of dense cell classes (dense discocytes and ISC) results in a pronounced increase in PRU, which is indicative of the widespread capillary blockage. The differences between the viscosity and hemodynamic measurements are most probably due to the different sensitivity of the two methods not only to the extent of intracellular polymerization of HbS but also to a shape factor (polymer characteristics) of deoxy density cell classes. The extent of abnormal viscoelastic behavior of individual deoxy red cells has also been shown to correlate with the amount of intracellular polymer, which is determined by MCHC[59].

The studies of Fabry et al.[37,40], in a perfused rat leg model, have also provided evidence of the different roles of the distinct classes of SS red cells. In this preparation, neural and humoral phenomena are present, and the results appropriately complement the findings in isolated mesocecum.

Rate of deoxygenation

Another issue of physiological significance concerns the rate of deoxygenation and how it affects the polymer characteristics and rheology of sickle cells. In other words, what are the rheologic differences between rapidly deoxygenated cells and gradually deoxygenated cells? Recent studies of Kaul and Xue[61] have shown that gradual deoxygenation of sickle blood results in the formation of morphologic cell types based on MCHC differences, as noted above. This is accompanied by gradual increase in the viscosity of SS whole blood over a given period of deoxygenation. In contrast, rapid deoxygenation of SS whole blood results in the transformation of most cells into granular forms, *irrespective* of MCHC differences among the cell classes[61]. The rapidly deoxygenated SS suspensions show two time-dependent distinct phases in the viscosity. First, there is an initial rapid rise in viscosity to a peak value, which is characterized by the presence of mostly granular forms. In the next phase, there is a time-dependent significant decrease in the whole blood viscosity. This phase is characterized by the appearance of a large percentage of elongated cells. Transmission electron microscopy shows that cells that develop long processes after prolonged deoxygenation contain regions of aligned polymers in addition to regions of very small polymer domains or hemoglobin aggregates. This is in accordance with earlier kinetic, morphologic and ultrastructural analyses of rapidly deoxygenated sickle cells[2,29,45]. Prolonged deoxygenation would then result in an elongated shape caused by the growth of the aligned domains. The viscosity decrease is probably due to the alignment of elongated cells along the direction of flow. This is reminiscent of the viscosity decrease observed in deoxy HbS solutions following the polymer alignment[16].

The above rheologic studies[61] were the first to demonstrate that HbS polymer characteristics could significantly affect the bulk flow behavior of SS red cells. These observations have a potential physiological significance, since disruption of the steady state could lead to variable hypoxia, red cell residence times and rates of HbS polymerization. Acute hypoxic conditions in microcirculation could result from vasoconstriction, or from a narrowing of the vessel lumen by adhesion of RBCs. Both of these could affect the transit time and deoxygenation rate of sickle cells within the capillaries. Although the polymerization of HbS and sickling could occur under hypoxic conditions, the kinetics of polymerization suggest that in the steady state, most cells do not sickle in vivo, because of the capillary transit times involved[76]. This is because the delay time of polymerization is generally longer than the capillary transit time (< 1 s). Also, some intravascular sickling ('microcrises') could be tolerated without ill effects[41]. The question arises: what are the potential vascular and cellular factors that could trigger a major episode of vasoocclusion leading to a painful crisis?

Sickle cell-endothelial interactions

As mentioned earlier, one potential factor is increased adhesion of sickle cells to the vascular endothelium. This phenomenon was first demonstrated by Hebbel and co-workers[51] and later confirmed by others, using cultured endothelial monolayers[7,18,42,53,74,100]. Microvascular characteristics and sites of sickle cell adhesion were first elucidated in the ex vivo mesocecum microvasculature by Kaul et al.[56]. Three novel findings emerged from these studies. First, sickle cells adhered exclusively to the venular endothelium. Second, adhesion was inversely correlated with the venular diameter, and maximal adhesion (per 100 μm^2) was recorded in the immediate postcapillary venules, the sites of lowest wall shear rates in the microcirculation. Also, there was frequent blockage of postcapillary venules. Third, light density deformable sickle cells (reticulocytes and discocytes) were the most adhesive while dense cells (dense discocytes and ISCs) were the least adhesive but contributed maximally to microvascular obstruction (fig. 2). Direct intravital observations by Kaul et al.[56], involving selective fluorescent labeling of density cell classes, showed a high concentration of dense cells trapped in the area of total obstruction, and their absence from areas with adhesion but without lumen obstruction.

Kaul et al.[57] proposed that selective trapping of dense cells in the areas of adhesion may play an important role in the initiation of a vasoocclusive episode as shown in figure 1. In this scenario, preferential adherence of deformable sickle cells in postcapillary venules would cause a narrowing of the vessel and a further decrease in the local velocities and wall shear rates. Next, selective trapping of rigid dense cells would occur in postcapillary venules where deformable sickle cells have preferentially adhered. Since the immediate postcapillary venules are a part of the exchange compartment, any such adhesion and trapping would also promote a hypoxic environment, favoring polymerization in both adhered and trapped cells. This initial obstruction could then extend to involve the whole capillary network, and other vessels.

Important support for this hypothesis has been provided by the perfused rat leg model of Fabry et al.[40]. Using density-separated SS red cells it was demonstrated that dense cells are capable of causing obstruction by themselves, as measured by technetium (^{99m}Tc) labeled cells as well as by NMR metabolic studies[37,40]. More importantly, low-density SS red cells were seen adhering to the endothelium of the microcirculatory bed downstream from the site of the injection. Nevertheless, no obstruction was observed by metabolic criteria when the only cells injected were SS discocytes with normal

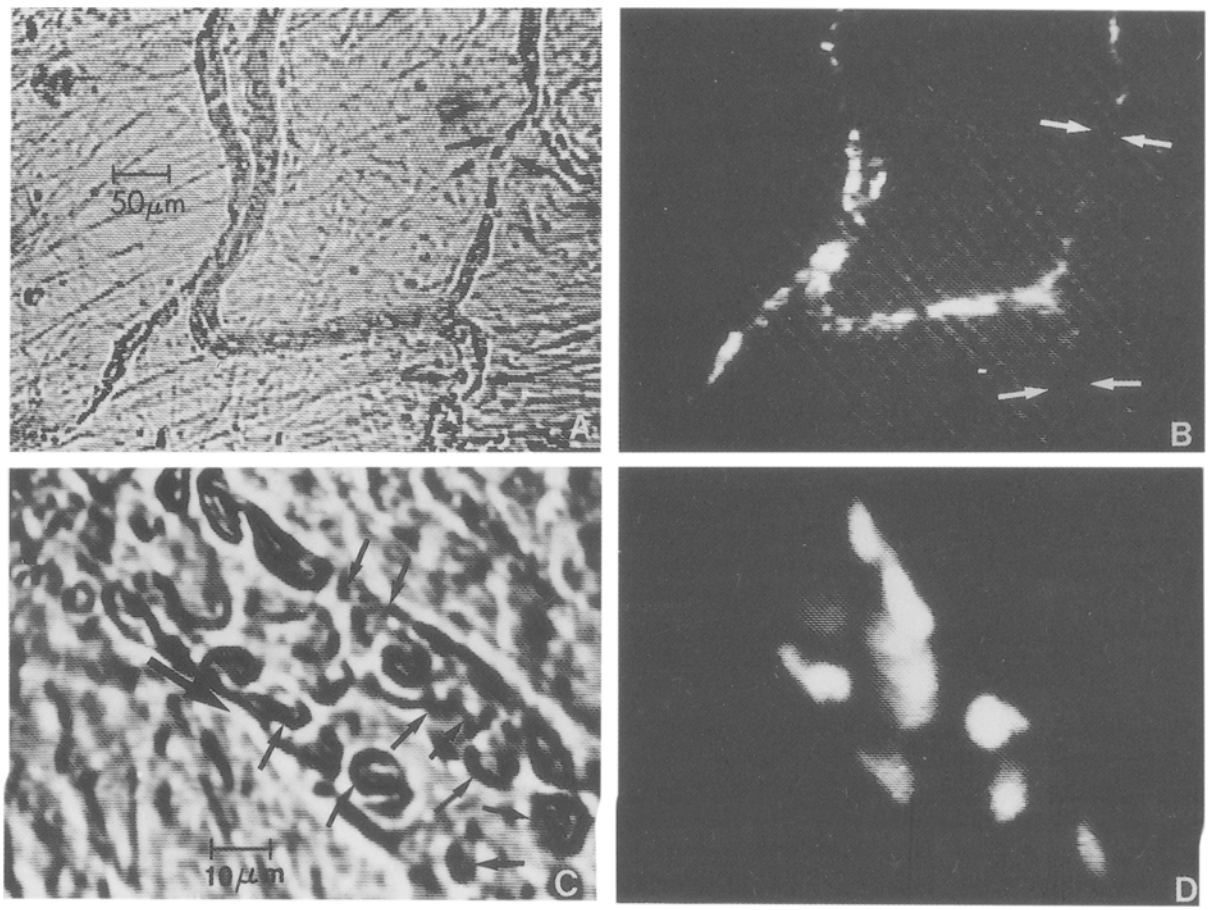

Figure 2. Relative adherence and obstructive behavior of density-defined red cell classes of sickle blood. *A* and *B* Selective trapping of dense SS cels in postcapillary venules of isolated mesocecum vasculature of the rat following infusion of a mixture of discocyte fraction (SS2) and FITC-labelled dense cells (dense discocytes and ISCs, SS4) mixed in a ratio of 3:1. *A* Areas of venular obstruction. Arrows indicate unobstructed areas with adherent cells. *B* The same area under epifluorescence illumination showing localization of FITC-labelled dense SS cells trapped in the obstructed venules and their absence in areas with adhesion (arrows). *C* and *D* Relative adherence of the least dense SS cells (reticulocytes and young discocytes, SS1) and discocytes (the predominant fraction or SS2). SS1 (FITC-labelled) and SS2 (unlabelled) were mixed in 1:1 ratio and infused into the isolated vasculature. *C* High magnification videomicrograph showing individually adhered cells following the passage of bolus. The cells are aligned in the direction of the flow (large arrow). *D* The same area viewed under epifluorescence illumination illustrates that majority of adhered cells are the fluorescent SS1 reticulocytes and young discocytes. The unlabelled cells (small arrows) in frame *C* are from SS2 discocytic fraction.

MCHC. These results are entirely consistent with the model described in figure 1. Another interesting observation of Fabry et al.[37] is that adhesion of deformable SS cells is a dynamic event: cells first adhere and are then released gradually. In the presence of desmopressin, cells have much stronger adhesive properties so the release is slowed considerably. In addition, even when there is obstruction, flow might continue in a partial fashion, a fact that might suggest that pharmacological intervention has a chance.

The selective dense cell trapping as observed in studies based on the mesocecum as well as the rat leg preparation, explains the disappearance of the densest cells from the peripheral circulation during painful crisis[36]. This observation has been confirmed by Ballas et al.[6], who have added the observation of an apparent increase in dense cells preceding or in coincidence with the initiation of crises. This last event might represent the disappearance from the circulation of low density SS cells (due to adhesion preceding obstruction) with a concomitant apparent increase of dense cells.

A role for collagen-binding proteins in sickle cell adhesion was first suggested by Mohandas and Evans[74]. Evidence for the involvement of von Willebrand factor (vWF), a collagen binding protein, in this interaction came recently from the work of Wick and co-workers[105]. Using endothelial monolayer cultures in a flow chamber, Wick et al.[105] demonstrated that endothelial-conditioned media greatly enhanced (2 to 27-fold) sickle cell adhesion to the endothelium. Later, Tsai et al.[102] showed that the desmopressin (DDAVP) treatment (which causes release of extra large vWF) of the ex vivo mesocecum vasculature induced adhesion of normal (AA) cells but no obstruction. On the other hand,

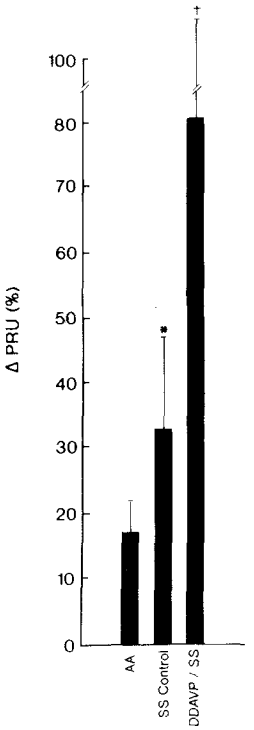

Figure 3. The effect of desmopressin (DDAVP) on the hemodynamic behavior of oxy SS red cells in the isolated mesocecum microvasculature of the rat. In the desmopressin-treated vasculature, the infusion of SS cells (DDAVP/SS) caused widespread microvascular blockage, and about 2.4-fold increase in the peripheral resistance (ΔPRU) over that for the control SS cells in the untreated vasculature ($^{+}p < 0.01$). Infusion of SS cells in the untreated vasculature also resulted in a significantly higher PRU compared with normal (AA) red cells ($^{*}p < 0.01$).

desmopressin treatment caused a much more extensive increase in adhesion and microvascular blockage of sickle cells[60]. The microvascular blockage in the presence of desmopressin was accompanied by a more than two-fold increase in the peripheral resistance as compared with the control (untreated) group (fig. 3). This interaction was significantly abolished by anti-vWF antibodies, suggesting involvement of endothelial vWF[60]. However, microvascular obstruction required the presence of dense cells, as sickle discocytes alone did not result in postcapillary blockage. The exact mechanism of action of the receptors involved in vWF-mediated adhesion are yet to be investigated. Several physiological relevant substances and their analogues can cause almost immediate release of stored endothelial vWF[44]. Among such substances are vasopressin (and its analogue desmopressin)[69], histamine[47], thrombin[68], epinephrine[88], and fibrin[90]. At sites of vascular injury, thrombin and fibrin are formed, and at sites of inflammation histamine may be generated. These agents may cause an increase in the cytosolic Ca^{2+} and almost simultaneous vWF release from the endothelial cells[47], and could have an important role in adhesion-initiated vasoocclusion.

Vascular tone and vasomotion

Another critical factor in the initiation of vasoocclusion could be a deterioration of the local vascular tone. The phenomenon of vasomotion consists of rhythmic contraction and dilation of precapillary arterioles, which facilitates the flow of blood cells through capillaries[82,107]. In normal conditions, tissue metabolic activity and oxygen tension significantly affect vasomotion. The vascular tone is controlled by neural, humoral and vascular (smooth muscle cells and endothelium) factors. In sickle cell patients, as noted by the use of noninvasive laser-Doppler velocimetry, the increased intravascular pressure caused by capillary passage of less deformable sickle cells appears to trigger an oscillatory vasomotion pattern[91]. In the steady state, these oscillations may help to maintain the microvascular flow of rheologically abnormal SS blood. However, a loss of local vascular tone due to endothelial injury or some other factor would abolish this advantage. In fact, Lipowsky et al.[65] have demonstrated a diminished vasodilatory reserve in sickle cell patients in crisis, on the basis of a delayed onset of post-occlusive reactive hyperemia. In addition, the role of potential factors such as the endothelial-derived vasoactive factors (NO, endothelin) in microvascular flow of SS red cells is yet to be examined.

Vasoocclusive painful crises

No case for multigene participation is stronger than that of painful crises. These are recurrent episodes of pain that involve the vasoocclusion of marrow and/or bone microcirculation, which are characteristic of this disease, and that greatly influence the perception of severity and the quality of life.

From the purely clinical realm, the following issues pertaining to painful crises have to be clarified: 1) what precipitates a painful crisis? 2) why is the pain commonly symmetrical? That is, it involves both knees, both elbows, both thighs, both shoulders, etc.; 3) why do the crises begin in one place, and over hours or days, extend contiguously and/or distally? In the latter case, the new site of pain may be remote from the initial site; and 4) why are painful crises stereotypical? (pain recurs very frequently in the same site/s, and many times, with the same sequence). It is not hard to postulate that vascular factors play a major role. We will return to this point later.

It is clear from this simple analysis that the properties of SS red cells alone do not explain this constellation of events, in spite of proposals to the contrary[31,88]. The pathophysiology of painful crises must involve mechanisms that trigger the event, that make it symmetrical, that will explain the distal extension of the sites involved in vasoocclusion, and finally, factors that make it more likely for the event to repeat itself in the same site. Most of these are likely not to be properties of the

red cells and are likely to include the anatomical, physiological, humoral and neural factors involved in vascular regulation. In addition, most of these factors must be polymorphic (that is, exhibit inter-individual differences) to explain the enormous variability of the incidence of painful crises within genotypes of sickle diseases.

In effect, there are also data that demonstrate that the tendency of SS cells to polymerize is not the main determinant of painful crises: sickling is indispensable, but far from sufficient. For example, the incidence of painful crises is not related to the level of SS dense cells[12], and is more frequent in SS with concomitant α-thalassemia (a phenotype which is in many other aspects more benign than SS with a full complement of α-genes)[4]. The relation of the incidence of painful crises to the level of HbF (which affects the tendency of red cells to polymerize) is also unclear. The studies find no relationship[8,87]. Two other studies claim a correlation between HbF and the incidence of painful crises. Nevertheless, in one study[67] the correlation coefficient is 0.31, r^2 is 0.10, which means that only 10% of the variance in the incidence of painful crises can be attributed to HbF. In other words, 90% of the variance has an origin in another parameter or parameters. The second study, by Platt et al.[85] is more substantial since it covers a large sample and a wide span of ages, and finds a correlation of HbF with incidence of painful episodes, but only if the effect of total hemoglobin one controls for. Nevertheless, these two factors co-vary with a direct relationship, making the pathophysiological consequences difficult to interpret. Most interestingly, the authors also find a significant inverse effect of the total hemoglobin level on incidence of painful episodes. This is another instance of a non-sickling factor, probably operating through its effect on bulk viscosity.

The past analysis notwithstanding, if sickling is dramatically increased, the consequence is painful crises. Evidence of its effect comes from an unethical experiment of Greenberg and Kass[43], in which low pH solutions were given i.v. to steady-state sickle cell anemia patients, and the effect was the generation of painful crises. Also, low environmental O_2 can induce painful crises in sickle trait individuals. A balanced view would conclude that a situation favoring sickling alone, per se, is not the only, or even not the common mechanism of the initiation of painful crises.

Our knowledge of sickle-cell vasoocclusion in animal models suggests that adherence of SS cells to venular endothelium could precede the trapping of dense cells in these areas, an interaction which would generate obstruction[55,56,60]. Figure 1 shows the sequence of events proposed for the first time in 1988 by Kaul et al.[56]. Hence, the phenomena of adhesion could be critical in the induction of painful crises. In support of this point of view is the correlation between painful crises and deformable SS cells (not dense cells), and the apparent increase of dense cells observed by Ballas et al.[6] in the early periods of the crises, which is followed by the decrease in dense cells observed first by Fabry et al.[36] and confirmed now by Ballas et al.[5,6] in the middle of the vasoocclusive episode. At first, during the early periods of the crises, adhesion of deformable cells would make the dense cells appear to increase in number, followed by their decrease when sequestration in the site of adherence follows.

Serjeant and Chalmers[99] have proposed that painful crises could be a 'steal syndrome' induced by cold on the skin, which then suddenly directs the circulation to already heavily expanded marrow tissue and nearby bone. While this mechanism might be operative in some instances, it is likely to be limited to regions of the world in which there is little protection from climatic hardships. After all, seasonal variations of the incidence of crises is not observed in the United States. The Serjeant and Chalmers proposal explains the symmetry of painful sites, but not distal expansion, nor stereotypicality.

To explain the several clinical features of painful crises we need to postulate vascular factors that include anatomical/physiological variation in the microcirculatory beds of marrow/bone in an individual (to explain stereotypical recurrence), humoral factors released from the first vasoocclusion site that favors further vasoocclusion in a distal site, and finally vascular factors which are responsive to fever, infection, emotions, etc. (which might favor adhesion?) to explain the sudden triggering of crises.

It is tempting to speculate that, for example, DNA sequences involved in the expression of von Willebrand factor on the surface of endothelium cells (the gene products involved in the adhesion of deformable SS cells), and those involved in vascular tone control (regulation of endothelin and NO synthesis or release), could be the polymorphic mechanisms that determine to what extent each SS patient is at risk of painful crises.

Neutrophils, other leukocytes, and platelets may play an important role in the microvascular pathophysiology of ACS and chronic sickle cell lung disease. The number of circulating white cells is above normal in SS patients, yet investigators tend to ignore them. In addition, sickle cell patients are more susceptible to infections[13]. Most people carrying out research on sickle cell disease focus on red cells, despite evidence that interactions with endothelium and white cells may play a role. Increased activation of leukocytes during a delayed hypersensitivity response following infection could increase the leukocyte-endothelial interaction in postcapillary venules, which are also the site of increased SS red cell adhesion in ex vivo preparations. In sickle cell patients, there is also evidence of endothelial damage[62], probably inflicted by circulating rigid dense sickle erythrocytes. Furthermore endothelial injury itself could release sub-

stances such as P-selectin (GMP-140) in addition to vWF[71]. Also, both vWF and P-selectin are localized in Weible-Palade bodies and are released upon stimulation with histamine[107]. While vWF has been shown to be active in sickle red cell adhesion, P-selectin is implicated as one of the adhesion molecules in neutrophil adhesion[75]. Thus under inflammatory conditions, an increased interaction of neutrophils with the endothelium may also play a significant role in vasoocclusion.

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