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Perspective

Perspectives Series: Cell Adhesion in Vascular Biology

Adhesive Interactions of Sickle Erythrocytes with Endothelium

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The pathophysiologic hallmark of sickle cell disease is episodic occurrence of vasoocclusive events that precipitate acute painful episodes and lead, ultimately, to organ failure and death. Based upon studies from multiple laboratories over the last two decades, it is widely believed now that a key participant in this process may be the sickle erythrocyte's predilection for adhesiveness to endothelium. Initial studies of this abnormal cell–cell interaction were stimulated by skepticism regarding the extant dogma that ascribed sickle vasoocclusion solely to deoxygenation-induced polymerization of the mutant hemoglobin and resultant cell sickling. In fact, emerging data were revealing that the time required for the development of cell sickling is, for most red cells, actually longer than the microvascular transit time. Thus, anything serving to delay microvascular passage of red cells might allow sickling to occur, and thereby would be a critical participant in evolution of occlusive manifestations. Hence, consideration was given to the possibility that abnormal adhesion of sickle red cells to endothelium might be such a factor (1, 2). Ensuing studies have provided convincing support for this notion (3), with the caveat that definitive proof of the pathophysiologic importance of this cell–cell interaction in the human model, per se, has not yet been obtained (or sought).

The interaction of sickle red cells with endothelial cells has been studied in a great variety of experimental systems using various suspending media (serum, plasma, culture media, buffers) and a variety of adhesion assays (static, vessel perfusion, flow cell, in vivo). These studies, most using human umbilical vein endothelial cells as the target cell type, clearly establish that oxygenated sickle red cells are abnormally adherent to endothelium. Development of effective adhesive interaction requires some degree of intimate contact, so red cells that are stiffened or actually sickled before contact with endothelium are less able to become attached. Perplexingly, the magnitude of the observed difference in adhesivity between normal and sickle red cells has varied markedly from study to study, reflecting, in part, interpatient differences; in part, the specific mechanism of adhesion being studied; and in large part, the particular nuances of the experimental systems used. Among the numerous studies of sickle red cell interaction with endothelium that followed the seminal observations of this phenomenon, a few milestones clearly stand out.

Milestones in the study of red cell adhesion to endothelial cells

Significantly, a strong correlation between red cell adhesiveness to endothelial cells and clinical vasoocclusive severity was identified (4), although it was not definitively established whether this adhesiveness means tenacity of adhesion or size of an adhesive subpopulation of red cells, or both. Nevertheless, measurements using elegant micropipette techniques indicated that, compared to normal cells, sickle red cells establish more endothelial contacts, each of which has a greater adhesive strength (5). Although this difference, as initially reported, between normal and sickle red cells was small, subsequent refinement of the original calculations (necessitated by discovery that the sickle cell membrane is abnormally stiff) suggested that sickle cells are perhaps an order of magnitude more adhesive than normal cells (3). It is not known, however, which specific mechanism of adhesion (see below) was operative in these tenacity measurements.

Studies conducted using endothelialized flow chambers documented the occurrence of sickle red cell adhesion under flowing conditions (6), although this was observed at relatively low shear rates (< 1 dyn/cm^2), predicting only microvascular relevance of this interaction. In fact, when adhesion of sickle red cells was later observed under flow in vivo, it was seen only in the postcapillary venules, corroborating its relevance to vascular areas characterized by a low shear regime. This has been found to be true when human sickle red cells are infused into the rat mesocecum (7), as well as within the cremaster muscle of sickle transgenic mice (8) where there need be no concern about cross-species compatibilities of ligands and receptors.

Complex studies comparing the behavior of different human sickle red cell subpopulations when infused into rats revealed that initiation of vasoocclusion is a two step process, with the abnormally adhesive red cells providing a triggering function, and the poorly deformable cells subsequently participating in a propagation phase via a log jamming mechanism (9). Extrapolation of this to the human situation, in principle, seems reasonable, and this concept certainly fits the stochastic occurrence of painful crises, so that variations in red cell adhesivity (or likelihood for adhesion) could explain episodic occurrence of the triggering mechanism.

A watershed experiment revealed consequential endothelial cell heterogeneity by demonstrating differences in the relative robustness of different adhesion mechanisms, depending upon whether red cell adhesion was being measured to large vessel or microvascular endothelial cells (10). Likewise, the

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ability of the endothelium to modulate its display of adhesion molecules in response to biologic modifiers adds another layer of complexity, in that it predicts interindividual, temporal, and perhaps even regional variability in endothelial receptiveness to different mechanisms of red cell adhesion. For example, expression of vascular cell adhesion molecule on endothelial cells stimulated with tissue necrosis factor creates a red cell adhesion mechanism not relevant to quiescent endothelium (11).

Mechanisms of red cell adhesion to endothelial cells

Most investigative attention has been devoted to identification of specific mechanisms underlying the adhesion of sickle red cells to endothelium (3). Initial studies supported the hypothesis that abnormal surface charge topography on the sickle cell membrane could underlie enhanced adhesiveness. Yet, related studies also suggested that something in patient plasma could augment adhesiveness in conjunction with acute painful crisis. Thus, these early observations established the principle, supported by subsequent observations (5), that abnormal adhesiveness of sickle cells reflects not only inherent features of the red cell membrane but also factors in the red cell's environment.

Analysis of mechanisms has been complicated greatly by the heterogeneity of red cells themselves, as subpopulations of different character coexist in sickle blood. Most studies support the notion that all sickle cells are abnormally adherent, although the youngest and least dense cells (i.e., reticulocytes) clearly are more so in some studies, while the most dense cell population (but not the dense irreversibly sickled cells) are most adherent in others. These findings seem to be model dependent, with flow studies generally recognizing adhesion of reticulocytes, and static adhesion assays revealing adhesion of the more dense cells. This disparity has led to the reasonable assumption that the former type of adhesive interactions reflect high affinity adhesion mechanisms, while the latter type reflect low affinity mechanisms. Although reasonable, this assumption has not been verified experimentally by direct measurement of affinity.

Notwithstanding these uncertainties, in vitro studies now have identified multiple mechanisms of red cell–endothelial interaction (3). The specific mechanisms that have been convincingly identified to date are shown schematically in Fig. 1. The best defined mechanisms are those involving unique endothelial expression of viral receptors for the F_c end of immunoglobulin coating sickle red cells, and those used by reticulocytes to adhere under flowing conditions. For most of these latter mechanisms, which generally involve adhesogenic proteins and/or known cell surface adhesion molecules, the participating structures on both red cells and endothelial cells have been identified. Because these mechanisms involve receptor molecules, which have not yet been completely lost from reticulocytes, they apparently are relevant only to this youngest subpopulation of red cells. Whether sickle reticulocytes are materially different from normal reticulocytes in these characteristics has been explored only in two cases, with the finding that sickle reticulocytes do have abnormally increased expression of $\alpha_4\beta_1$ and CD36 (12, 13).

Less well-defined mechanisms mediated by fibrinogen or dehydration or seen in the absence of adhesogenic proteins seem to be real but are presumed to be of lower affinity. Utilization of these latter mechanisms apparently is not restricted to the reticulocyte subpopulation. In flow systems, both in vitro and in vivo, rolling of red cells on endothelium has been recorded, but the mechanism has not been identified.

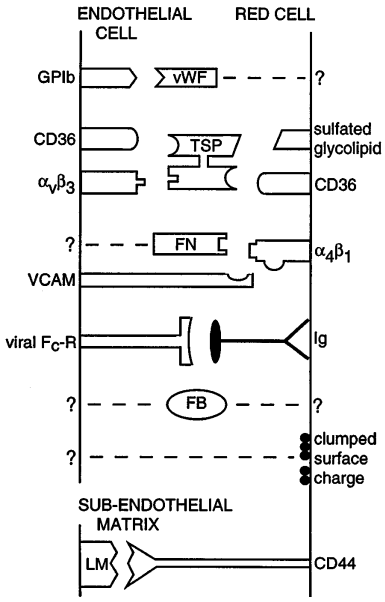

Figure 1. Identified mechanisms by which sickle red cells adhere to endothelial cells or subendothelial matrix. *FB*, fibrinogen; *F_c-R*, F_c receptor; *FN*, fibronectin; *LM*, laminin; *Ig*, immunoglobulin; *TSP*, thrombospondin; *VCAM*, vascular cell adhesion molecule.

Of particular interest, some adhesive interactions establish a means by which concurrent illness can impact upon sickle vascular pathobiology. For example, thrombospondin is derived from the platelet release reaction, thus potentially linking red cell adhesion and hemostatic perturbation. It is likely that a number of the described adhesive mechanisms would also serve to mediate adhesion of sickle red cells to subendothelial matrix should it become exposed, but this possibility is just starting to be evaluated.

How important is ligand/receptor affinity in pathophysiologic red cell adhesion?

It often is assumed that only the red cell adhesion mechanisms identified under flowing conditions, those believed to be higher affinity mechanisms, are of pathophysiologic relevance. However, this view does not take into account the fact that microvascular blood flow can be intermittent. In addition, red cell passage may be delayed by slowly moving granulocytes, which are substantially larger and much less deformable than red cells and which might pause upon the inflamed endothelium of the sickle patient. Under such circumstances, it is conceivable that lower affinity mechanisms might become important. Indeed, it may be a critical clue that the described correlation between sickle cell adhesiveness and clinical vasoocclusive severity (4) was identified using an experimental model that probably was measuring one of the low affinity adhesion mechanisms.

Perhaps it is useful to recall that a stably attached red cell must be in mechanical equilibrium, so that the forces promoting detachment (fluid shear force and a peeling torque) must be overcome by the bonding force (the product of bond number and strength per bond). By analogy to white cell–endothelial interactions, red cell adhesion observable under flowing conditions probably comprises a regime in which adhesion requires only a small number of higher affinity receptors and is rate controlled, mostly determined by the on-rate for the ligand–receptor interaction. Conversely, mechanisms only ob-

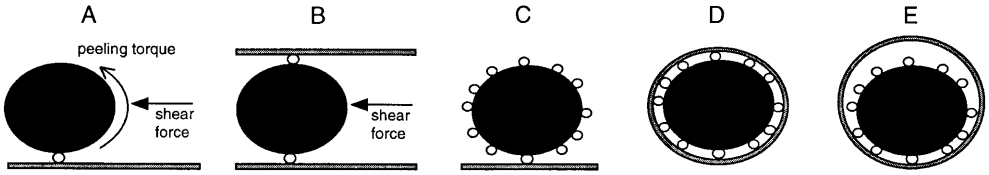

Figure 2. Conceptual models illustrating the significance of circumferential contact between a red cell and vascular wall. Here, the red cell is conceived as being sufficiently rounded (rendered as a black oval) so that adhesion can represent a single point-of-contact phenomenon. Blood flow is from right to left in *A* and *B*, and it is perpendicular to the plane of the page in *C–E.* In large vessels precluding circumferential contact (*A*), detachment forces countering the adhesive bond are derived from shear flow and a peeling torque. If the vessel is small enough to allow adhesive contacts on opposite sides of the red cell (*B*), the effect of the peeling torque is lost. Also, since twice as many attachments to endothelium exist in *B*, the avidity of each can be lower than in *A* to still allow the red cell to maintain endothelial adhesion. The potential influence of multiple adhesion molecules is not realized as long as the red cell makes contact in a large vessel (*C*), so adhesion there is most likely to develop via high affinity mechanisms. However, in a microcirculatory vessel that allows complete (*D*) or partial (*E*) circumferential contact, the forces promoting detachment can be countered by development of multiple contacts, even if they individually are of much lower affinity than that required to allow attachment via a single point. In the case of complete circumferential contact (*D*), the effect of peeling torque is lost as well. This highly simplified model can be greatly complicated by inclusion of a wide variety of parameters relevant to real physiology, but this does not materially change the basic conclusions illustrated here.

servable in static adhesion assay systems, which do not have the time constraint imposed by flow, would most likely be relevant to a low affinity regime where adhesion is equilibrium controlled. Then, adhesion is time dependent, and the number of receptors required is inversely related to affinity. During transiently interrupted microcirculatory flow, low affinity mechanisms certainly could participate, particularly in the smallest vessels of critical diameter where a red cell might establish some degree of circumferential contact with endothelium. In that case, a greater number of adhesive contacts is allowed, and the removal tendency conferred by peeling torque could be lost (Fig. 2).

Antiadhesive therapy for sickle disease

Insofar as red cell adhesion to endothelium provides a triggering mechanism for occlusion, therapeusis directed at this interaction should be beneficial. Thus, investigators have exhibited interest in identification of "the" mechanism of red cell adhesion in vivo. While this is an understandable question, is it relevant? Pathophysiology of this disorder is exceedingly complex. It seems entirely possible that adhesion-mediated triggering of occlusion could involve different processes from time to time, or from patient to patient, or perhaps even from organ to organ. Considering all the adhesogens potentially operative in the physiologic plasma environment, even a single adhesive event may be complex, very possibly involving multiple mechanisms at once. This perhaps justifies some pessimism whether therapy delicately targeted to a specific adhesive interaction would ever be sufficiently effective. On the other hand, it may be worth emphasizing nonspecific, global antiadhesive approaches. One that has been identified in vitro and has now reached clinical trials uses a "vascular lubricant" (14), and others may be forthcoming. Certainly there will be relevant advances in unexpected areas. For example, clinical trials of hydroxyurea, given in an attempt to pharmacologically influence hemoglobin type, have revealed an unexpected amelioration of red cell adhesiveness and decreased expression of CD36 and $\alpha_4\beta_1$ to endothelium in response to therapy (15, 16). Whether the observed clinical benefits of hydroxyurea therapy derive, at least in part, from this effect remains to be proven.

Needed studies

A limitation of flow chamber studies is that, as they are conducted currently, mechanisms of lower affinity adhesion (including those allowing red cell rolling) are not identified. Moreover, in vitro studies have thus far examined adhesion only on flat surfaces, corresponding best to a vessel having an infinite radius of curvature. Yet pathophysiologic red cell interaction with endothelium probably occurs in very small vessels that would allow some degree of circumferential contact to develop. Beyond the importance of this to adhesion alone (Fig. 2), the potential interplay between cell deformability and adhesivity in the context of constraining vascular diameter is particularly interesting to contemplate. Clearly, studies examining these nuances are necessary to bring adhesion science closer to microcirculatory reality.

We need to know where occlusion actually is initiated in humans. Clearly, adhesion investigations will be most relevant if the endothelial cells used experimentally correspond in character to those that participate in vascular pathobiology. It would be helpful to know what the activation status of endothelium is in situ, in general and in reference to the adhesion molecules specifically relevant to red cell adhesion in vivo. A first step in this direction has just been taken, with observation of an activated endothelial phenotype in humans with sickle disease (17). A more complete understanding of the role of biologic modifiers of adhesion receptor expression and/or affinity is necessary.

Additionally, the pathophysiologic consequence of endothelial contact by sickle red cells needs further definition. Already described are an inhibition of endothelial DNA synthesis, stimulation of prostacyclin release, and upregulation of endothelin-1 gene expression (3). Yet, significant clinical questions remain. For example, does modulation of NO production by red cell adhesion play a role in vascular pathobiology? Does repeated endothelial molestation by adherent cells lead to manifest endothelial dysfunction in these patients? Clearly, there is great potential for abnormal adhesivity of sickle red cells to contribute to many facets of sickle disease pathobiology, a concept that requires exploration.

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