Leukocyte adhesion to the vascular endothelium involves a disruptive force exerted on the leukocyte by the flow of the blood and an adhesive force that forms at the leukocyte-endothelial interface. The relative strengths of these two competing forces govern leukocyte adhesion.

Leukocyte adhesion to the vascular endothelium (the layer of cells that lines the blood vessel walls) plays a central role in normal and pathological inflammation (e.g., host response to infection, wound healing, atherosclerosis, inflammatory bowel disease, and arthritis). This process involves a cascade of adhesive events commonly referred to as initial tethering, rolling, firm adhesion, and extravasation (16). Initially, circulating leukocytes attach or tether to the endothelium. The initial contact between leukocytes and the endothelium is due, in part, to the interaction of the leukocytes with red blood cells, leading to margination of the leukocytes toward the vessel wall (7). Although the initial contact may be strictly physical in nature, the initial tethering requires a molecular interaction that occurs between the leukocyte and the endothelium. After initial tethering, the leukocyte may detach back into the free stream or begin to roll in the direction of the blood flow. This rolling behavior is characterized by a velocity that is typically 10- to 100-fold lower than a nonadherent leukocyte moving next to the vessel wall (5). There is also considerable fluctuation in the rolling velocity (i.e., leukocytes tend to speed up and slow down as they roll on the endothelium) (5). After rolling begins, the leukocyte may become “activated” and proceed to adhere firmly to the endothelium and migrate across the endothelium into the extravascular space (i.e., extravasate). As with initial tethering, the other steps of the adhesion cascade involve molecular interactions between the leukocyte and the endothelium.

It is easiest to gain an appreciation for the biophysics of this process by considering in greater detail the tethering, rolling, and firm adhesion steps of the adhesion cascade. Figure 1 shows that the flow of the blood exerts a force and torque on a leukocyte that is adherent to the endothelium. The force and torque combine to displace the leukocyte in the direction of flow. These clearly constitute a disruptive (counteradhesive) force. Basic physics dictates that for the leukocyte to be adherent (i.e., not moving), this disruptive force and torque must be balanced by an opposing force (i.e., the sum of the forces and torques must be zero.) The counterforce is an adhesive force that forms at the leukocyte-endothelial interface.

Cells carry a significant negative charge on their surfaces. This leads to a variety of weak, nonspecific colloidal forces (electrostatic and whole cell van der Waals) between cells. These forces, however, tend to be small, and an additional “specific” force, due to bonds that form between surface moieties present on the leukocyte (ligands) and complementary moieties on the endothelium (receptors), is necessary for robust adhesion. Ligand-receptor bonds are noncovalent, yet they are much stronger than the nonspecific whole cell colloidal forces. An aggregate of ligand-receptor bonds at the interface between the leukocyte and the endothelium applies an adhesive force to the leukocyte that counters the disruptive force exerted on the leukocyte by the flow of the blood. The relative strength of these two competing forces (the disruptive hydrodynamic force and the adhesive force) dictates the nature of the adhesion of the leukocyte to the endothelium (i.e., whether the leukocyte will tether, roll, or firmly adhere).

Over the past 20 years, many of the ligand-receptor pairs that form the adhesive bonds have been identified. These molecules are typically proteins or carbohydrates present on membrane proteins and perhaps lipids. For the purposes of this article it is sufficient to know that, for the most part, selectins (L-, E-, and P-selectin) bind to their cognate molecules (e.g., PSGL-1 and sialyl Lewis^a^like carbohydrates) to mediate the initial tethering and rolling, whereas the later steps of the adhesion cascade involve integrins (e.g., CD11a/CD18) binding to immunoglobulin superfamily adhesion molecules (e.g., ICAM-1). For further details regarding the biochemistry of leukocyte adhesion, the reader is referred to several excellent reviews (10, 11, 16).

Equally important to identifying the ligand-receptor pairs is elucidating the details of the underlying biophysics of this dynamic adhesion process. Although there are many aspects to this issue, we focus our attention on the role of the cell size and ligand-receptor bond properties in adhesion because these two areas have recently received significant attention and are ideal vehicles for conveying the biophysical principles involved in leukocyte adhesion to the endothelium.

The size of an adherent cell influences both the disruptive and the adhesive force

In addition to leukocytes, other formed elements within the bloodstream can also adhere to the endothelium through mechanisms similar to that described for leukocytes. Examples include platelet adhesion during the latter stages of atherosclerosis and cancer cell adhesion during metastasis. The diameter of adhering cells can thus span two orders of magnitude, ranging from ~2 μm (a platelet) to 20 μm (a cancer cell),...
with leukocytes (7–10 μm) falling in the middle of this range. We have recently demonstrated (15) that the size of the cell will influence adhesion under flow.

As noted in the introduction, a cell that is adherent to the endothelium in the circulation will experience a force and a torque exerted by the flow of the blood (Fig. 1). As might be expected, the magnitude of this force and torque is a function of the local fluid-dynamic environment, in particular the shear stress at the vessel wall. To gain an intuitive feel for the concept of shear stress, it is instructive to imagine standing on the banks of a rapidly flowing river. At the bank of the river, one might see silt being “sheared off” the bank and entering the river. Thus it appears that the flow of the river is exerting a stress (a “shear stress”) on the bank of the river. The force on the bank of the river is directly related to the shear stress; the greater the shear stress at the bank of the river, the greater the tendency for silt to be sheared off of the bank and enter the river. Similarly, a mathematical model of a cell adherent to the endothelium under flow conditions reveals that the force ($F$) and torque ($T$) exerted on the adherent cell is related to the shear stress ($S$) at the vessel wall. Specifically

$$F = 1.7(6\pi rhS)$$  \hspace{1cm} (1)$$

and

$$T = 0.94(4\pi r^3S)$$  \hspace{1cm} (2)$$

where $r$ is the radius of the cell and $h$ is the distance from the cell center to the vessel wall ($h$ is almost equal to $r$ for cells) (4, 6). As the shear stress increases, the force and torque increase as well. Importantly, note that the force and torque also increase with increasing cell size. A larger cell will experience a greater force and torque relative to a smaller cell. The disruptive force thus increases with increasing cell size.

The size of the cell will also influence the contact area between the adhesive cell and the endothelium. If it is assumed that a given ligand-receptor bond can form over a certain distance (typically ~50 nm), one can estimate the adhesive contact area from geometric considerations [the derivation can be found in Cozens-Roberts et al. (4)]. The final result is that as the cell size increases, the contact area increases. A larger cell will be in contact with a greater area of the endothelium compared with a smaller cell, and thus, all else being equal, the larger cell will presumably have a greater adhesive force.

The discussion in the previous two paragraphs reveals that increasing the size of the cell increases the disruptive hydrodynamic force as well as the adhesive force. Cozens-Roberts et al. (4) introduced a parameter termed the critical shear stress ($S_c$), which is defined as the shear stress required to remove adherent cells from an adhesive surface (e.g., the endothelium). By incorporating the ideas of the above two paragraphs and the model of Hammer and Lauffenburger (9), they derived an expression that relates $S_c$ to the size of the adherent cell. Specifically, they found that

$$S_c = Kg$$  \hspace{1cm} (3)$$

where $K$ incorporates the thermodynamic properties of the ligand-receptor pair, the temperature, and the surface densities of the ligand and receptor and $g$ is a function of the radius of the cell. Although $g$ is a rather complex function of $r$, it is sufficient to know that $g$ decreases with increasing particle size, causing $S_c$ to decrease with increasing particle size. Hence, the analysis of Cozens-Roberts et al. (4) suggests that the larger the cell, the lower the shear stress required to remove the adherent cell from the endothelium. Recall that this analysis took into account the competing effects of increased fluid force (an antiadhesive effect) and increased contact area (a proadhesive effect) for the larger cell relative to the smaller cell. Thus the analysis predicts that the effect of the increased fluid force wins out; although a larger-sized cell may be in contact with more of the endothelium, it will experience a greater disruptive force, which will overwhelm the proadhesive effect of increased contact area, leading to a less adhesive situation relative to a smaller cell.

This analysis was recently verified experimentally (15). Polystyrene particles ranging in diameter from 5 to 20 μm were coated with a recombinant leukocyte ligand. The particles were allowed to adhere to a surface coated with a cognate endothelial receptor for a set amount of time. Subsequently, the adherent particles were subjected to increasing levels of fluid shear. At each level of shear, the number of particles remaining firmly adherent to the receptor-coated surface was determined. The shear stress at which 50% of the adherent particles were no longer firmly adherent was determined to be $S_c$. As predicted by the model of Cozens-Roberts et al. (4), $S_c$ (the shear stress required to remove the particles) decreases with increasing particle diameter (Fig. 2). Thus changing the size of an adherent particle alters the balance of the disruptive force and the adhesive force, clearly illustrating that tipping the balance of forces can dramatically affect adhesion.

The above analysis was based on a mathematical model that described firm adhesion. More sophisticated models have been developed that describe a range of adhesive behaviors, includ-

![FIGURE 1. Leukocyte adhesion to the endothelium involves a competition between an adhesive and a disruptive force. The flow of the blood exerts a disruptive force on the leukocyte in the direction of flow as well as a torque (which is also disruptive). An adhesive force at the interface (i.e., the contact area) between the leukocyte and the endothelium counters the disruptive force. The source of the adhesive force is noncovalent bonds that form between complementary moieties on the surface of the leukocyte (ligands) and the surface of the endothelium (receptors). These ligand-receptor bonds are typically protein-protein or carbohydrate-protein interactions. It is thought that ~10–20 such bonds are enough to counter the disruptive force exerted by the flow of the blood. To illustrate the principles of the biophysics of adhesion, a review of recent studies regarding the role cell size plays in adhesion and the nature of the adhesive bonds is given.](http://physiologyonline.physiology.org/)
The shear stress required to set in motion 50% of a population of ligand-coated microspheres initially firmly adherent to a cognate receptor-coated surface was measured experimentally (15). This shear stress, termed the critical shear \( (S_c) \), was determined for 5-, 10-, 15-, and 20-\( \mu \)m-diameter microspheres. \( S_c \) is plotted as a function of the radius of the microsphere. Cozens-Roberts et al. (4) derived a relationship between \( S_c \) and the radius of the adherent particle by using a force balance on a theoretical particle adherent to a surface under shear flow. The theoretical \( S_c \) is plotted as a function of the microsphere radius. Clearly the size of the microsphere influences the adhesion, and the model based on the force balance gives a reasonable prediction of the experimental results.

The first important observation about bond strength is that, given enough time, any individual noncovalent ligand-receptor bond will dissociate on its own. Bonds are known to form and dissociate with reaction rates that can be measured by using techniques like Scatchard analysis and surface plasmon resonance (12). If all possible adhesion molecules (including antibody-antigen binding and nonphysiological binding pairs like avidin-biotin) are considered, dissociation rates can range from milliseconds to months. Typical dissociation rate values measured for molecules involved in leukocyte adhesion correspond to unstressed bond lifetimes of seconds to tens of seconds.

If it is true that bonds will dissociate in the absence of external forces, what then is the role of applied force in adhesion? The full answer to this question is complex, but applied force on a bond will affect dissociation rates, presumably by changing the conformation of the nanometer-scale binding interface between ligand and receptor molecules and reducing (or, conceivably, augmenting) the energy barrier for dissociation. The details of how an applied force should change dissociation rate or dissociation rate of the bond \( (k_o) \) as a function of applied force \( (f) \) is given by

\[
k_o = k_o^{\exp}f(k_BT)
\]

where \( k_o^{\exp} \) is the dissociation rate constant in the absence of any applied force [i.e., with \( f = 0 \), as might be measured with surface plasmon resonance (12) or by using radioactive tracers]. \( k_BT \) is Boltzmann’s constant multiplied by absolute temperature (e.g., body temperature) and is easily determined. The Bell model given in Eq. 4 predicts that the product of \( r_o f \) dictates the response of the bond to force. Clearly, for positive values of \( r_o f \) applying a force to a bond increases the rate of dissociation of the bond. Exactly how the bond responds to force is captured in the parameter \( r_o \) (also known as \( \gamma, \sigma, x_p \), or \( x_0 \) in different references). This parameter has units of length and relates the reactivity of the bond to the details of the ligand-receptor binding potential. A small value for \( r_o \) means that reaction rates depend weakly on force, whereas a large value means that reaction rate depends strongly on force. Thus characterizing the relationship between a given ligand-receptor bond and applied force reduces to the problem of determining \( r_o \) and \( k_o^{\exp} \) for that given ligand-receptor bond.

The details of where the Bell equation comes from and how it might be improved have been well described elsewhere (13). Although the Bell model makes a number of assumptions about the behavior of the binding potential, it has become the de facto standard for analyzing the force dependence of reaction rates in leukocyte adhesion. This standardization thus allows for intercomparison of measurements from different methods. Techniques for measuring \( k_o^{\exp} \) have existed for many years. It is only in the past 10 years, however, that a number of methods have been developed to measure the \( r_o \) parameter in the Bell model.

To measure the \( r_o \) parameter, a method of applying force to a single bond is needed. Most methods today use some form of highly flexible spring (with spring constant \( k_{spring} \) ) coupled with nanometer-precision position sensing \( (x) \) to obtain forces on bonds from Hooke’s Law \( (f = k_{spring} \times) \). The best-known
example of such a technique is probably atomic force microscopy (AFM). In AFM, a flexible cantilever beam (the spring) is brought into contact with a surface. If both cantilever and surface are coated with adhesion molecules (i.e., ligand-receptor pairs), bonds between beam and surface form, leading to an adhesive event or deflection of the beam as one tries to move the beam away from the surface. When the bond breaks, the force at the moment of dissociation is observed. Other similar techniques include the biomembrane force probe (which uses a red blood cell as the elastic sensing element), the microcantilever technique (in which a thin glass cantilever is the elastic sensing element), and optical tweezers (in which radiation pressure from a laser beam is the force sensing element). Alternatively, hydrodynamic methods (flow chambers) can be used to apply force to adherent leukocytes on surfaces coated with low densities of endothelial receptors (1). Although the exact analysis depends on the technique used, for all of the techniques a curve is ultimately generated that relates the measured rate of bond disruption as a function of the applied force (see, for example, Fig. 3). The slope and intercepts of the line segments that make up this curve (Fig. 3) are directly related to $r_o$ and $k_r^o$.

The two categories of molecules involved in leukocyte adhesion, selectins and integrins, have attracted considerable experimental interest from the forced unbinding biophysics community. Most of the work so far has focused on the selectins (13). Values for $k_r^o$ measured for selectins fall in the range of 0.5–2 s, consistent with the range of results determined from surface plasmon resonance. The measured $r_o$ values range from 0.2 to 0.8 Å. Some experiments have shown larger values for $r_o$ (2–3 Å) over at least some range of force application rate. It is possible to reconcile these results if one posits that some measurements (particularly in the flow chambers) can be used to apply force to adherent leukocytes on surfaces coated with low densities of endothelial receptors (1). Although the exact analysis depends on the technique used, for all of the techniques a curve is ultimately generated that relates the measured rate of bond disruption as a function of the applied force (see, for example, Fig. 3). The slope and intercepts of the line segments that make up this curve (Fig. 3) are directly related to $r_o$ and $k_r^o$.

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cyte rolling.

It is important to note that for clarity we have simplified the relationship between ligand-receptor bonds and force in the above discussion. In particular, most forced unbinding studies show that several sets of Bell model parameters are needed to fit the data over the whole range of applied force. To understand this observation, one must consider the nature of the nanometer-scale ligand-receptor binding interface. Structural studies from X-ray crystallography, nuclear magnetic resonance, and mutations of binding interfaces show that specific amino acid residues are often critical for the binding. This means that for a ligand-receptor bond to break, a number of subbonds in the binding interface must be broken. Each of these subbonds is an activation energy barrier. The different sets of Bell model parameters represent these different barriers in the ligand-receptor interaction (14). Since the $r_o$ parameter in the Bell model is a distance, one can think of each of the barriers as being at ligand-receptor separation distances ($r = r_o$). Not all of these barriers have the same height (that is related to the $k_o$ parameter). Applied force can change the heights of these energy barriers differentially. Outer activation barriers (i.e., those with relatively large $r_o$) at low forces and thus control the process in the absence of applied force. Outer barriers are suppressed more strongly by applied forces than inner barriers, and thus inner barriers control dissociation above a certain critical force. The multiple barriers to dissociation lead to multiple curves of dissociation rate versus applied force. Figure 3 shows such a curve from a recent forced unbinding experiment on E-selectin and its carbohydrate ligand sialyl Lewis$^\alpha$ (17). Note that there is a kink in the curve that indicates the presence of more than one energy barrier, and hence the unbinding needs to be modeled by more than one set of Bell parameters. It is an eventual goal of forced unbinding research to relate Bell model parameters to specific structures in the ligand-receptor binding interface. An example of such an analysis (for integrin-ICAM-1 binding) was recently performed by Zhang et al. (18).

**Conclusion**

At the most fundamental level, a force balance dictates the adhesive interactions between leukocytes and the endothelium. Over the past 10 years, the balance between hydrodynamic and adhesive forces at work during leukocyte adhesion to the endothelium has been intensely investigated. These studies have revealed and continue to reveal the intricate relationship between the disruptive and adhesive forces as well as the ligand-receptor bond properties that govern leukocyte adhesion.

**References**


