Leukocyte adhesion: **High-speed cells with ABS**

P. Anton van der Merwe

In order to decide where to exit blood vessels and enter tissues, leukocytes roll along endothelial surfaces. Recent studies suggest that an 'automatic braking system' (ABS), involving selectin cell-adhesion molecules, enables leukocytes to roll at a fairly constant velocity despite large variations in blood flow rate.

Address: Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK.
E-mail: anton.vandermerwe@path.ox.ac.uk

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Leukocytes circulating in the blood need to exit the bloodstream to enter specific tissues or areas of inflammation. This highly regulated process is initiated when the leukocytes tether to, and subsequently roll along, the surface of the endothelium. Rolling enables leukocytes to survey the endothelial surface for chemotactic signals, which stimulate the leukocyte to stop rolling (arrest) and migrate through the endothelium and its supporting basement membrane. Recent work by Chen and Springer [1] has provided new insights into leukocyte rolling, revealing the existence of a mechanism that allows leukocytes to maintain a constant rolling velocity in the face of wide variations in blood flow rates.

The tethering and rolling steps are mediated by a highly specialized group of cell-adhesion molecules called selectins [2]. L-selectin is expressed on leukocytes, whereas E- and P-selectin are expressed on activated endothelial cells. These molecules bind to carbohydrate ligands presented by cell-surface glycoproteins. Compared with other molecular interactions, selectin–ligand interactions are particularly effective at mediating tethering and rolling [3], raising the question of what special features equip them for this highly dynamic form of adhesion. The features that need to be considered include various mechanical properties of molecules and their interactions (see Box 1).

Properties of selectin–ligand interactions

Rolling is a state of dynamic equilibrium in which there is rapid breaking of bonds at the trailing edge of the leukocyte–endothelium contact zone, matched by rapid formation of new bonds at the leading edge. It was therefore suggested that selectin–ligand interactions might have very fast association (k_{on}) and dissociation (k_{off}) rate constants [4]. Recently, it has been confirmed that the interactions of soluble forms of P-selectin and L-selectin with their natural glycoprotein ligands — P-selectin glycoprotein ligand-1 (PSGL-1) and glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1), respectively — are characterized by very fast k_{off} values [5,6]. These values agree remarkably well with the k_{off} of unstressed cell–substrate tethers mediated by P- and L-selectin (Figure 1). The ~10-fold difference in these k_{off} values also agrees well with the ~10-fold difference in the velocity of leukocyte rolling mediated by P- and L-selectin (Figure 1), strongly suggesting that the k_{off} is an important determinant of rolling velocity.

Although a fast k_{off} may be required for rolling, it is clearly not the only requirement, because most adhesion interactions have a fast k_{off} and yet are unable to mediate rolling [4,7]. This raises the question of whether selectin–ligand bonds form unusually quickly. Although the P-selectin–PSGL-1 interaction does indeed have a very fast k_{on} [5], the k_{on} of the L-selectin–GlyCAM-1 interaction is unremarkable [6]. The rate at which new bonds are formed, however, is dependent on the density and accessibility of ligands, in addition to the k_{on} value. It may therefore be significant that all L-selectin glycoprotein ligands are mucins, which can present many copies of a particular carbohydrate structure, thereby increasing the ligand density [2]. Indeed, GlyCAM-1 has been shown to carry more than one L-selectin-binding site [6]. E- and P-selectin do not appear to have particularly high ligand densities; the major E-selectin ligand is not a mucin and, although PSGL-1 is a mucin, it has only one P-selectin-binding site [2]. In this context, it is noteworthy that rolling mediated by L-selectin will require a higher rate of bond formation because it is approximately 10-fold faster than rolling mediated by P- and E-selectin.

Selectins and their ligands are concentrated on the tips of leukocyte microvilli [2]. This exposed positioning is likely to facilitate the formation of bonds. Indeed, targeting of L-selectin away from microvilli decreases the tethering efficiency *in vitro* and *in vivo* [8]. Localization to microvilli is less important for tethering in small vessels, possibly because the leukocyte is forced into close proximity with the endothelium in these vessels [8]. Curiously, however, unlike tethering, rolling does not appear to be dependent on either the presence of microvilli or the localization of selectins or selectin ligands to microvillus tips [8].

Although mechanical properties, such as the ability to withstand force, are an important property of cell-adhesion interactions, they have only been studied very recently [9–11]. In general, when two interacting molecules are

Box 1. Mechanical properties of molecular interactions.

When leukocytes attach to the endothelium, they are subjected to mechanical forces that originate from the hydrodynamic force of flowing blood. The familiar properties of affinity and kinetics are not adequate for describing the effects of mechanical force on molecular interactions. Some of the relevant properties are introduced here.

1. The unbinding force required to break an intermolecular interaction – that is, its **tensile strength** – is only indirectly related to the affinity constant. Adhesion strength has been shown to be proportional to the binding free energy, which is proportional to the logarithm of the affinity constant. The binding free energy, however, includes contributions from enthalpic and entropic changes, and there is evidence that tensile strength correlates better with the enthalpic component [13]. This seems plausible because the enthalpic component arises from the formation of atomic bonds, such as hydrogen bonds and van der Waals contacts.

2. The force needed to break an intermolecular bond is also dependent on an intrinsic property termed the **reactive compliance**, which is unrelated to equilibrium properties such as binding energy or enthalpy. This may be understood intuitively by considering the binding energy as being equivalent to a potential energy well. The work required to break the bond, equivalent to the bond energy, is represented by the depth of the well. In contrast, the force required to break the bond is represented by the slope or gradient of the well, being greater if the gradient is steep. The gradient depends on the distance over which the work is done, a property referred to as the **mechanical bond length**, which is inversely proportional to reactive compliance [12]. In reality, the unbinding force is determined by the steepest gradient on the path of least resistance through a 'mountainous' energy landscape from a low energy state (bound) to a high energy state (unbound) [14].

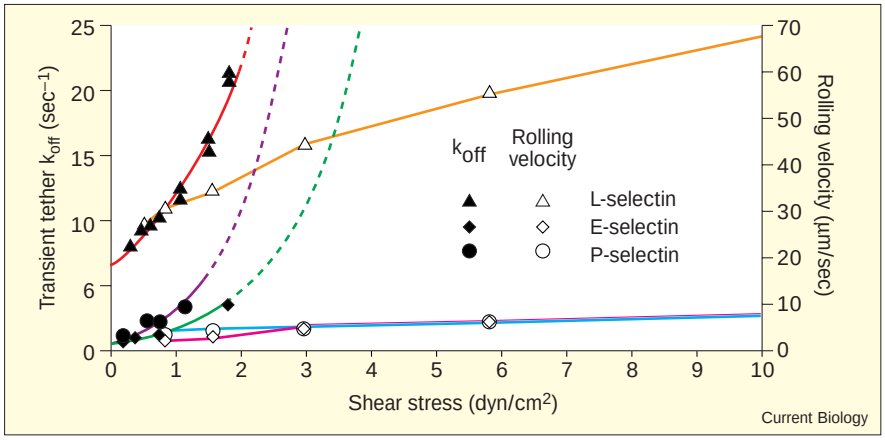
3. For weak non-covalent bonds the tensile strength of the bond depends on the rate at which the force is increased, the **loading rate** [11,14]. This is a consequence of the fact that all interactions are subject to thermal instability, which drives spontaneous fluctuations in the depths of the energy well. Large fluctuations will be much rarer than small ones. Consequently, slowly ramping up the unbinding force provides time for the rare large fluctuations in bond energy to reach levels that match the unbinding force. The relationship between tensile strength and loading rate is also an intrinsic property of the bond, intuitively equivalent to the mechanical bond length [14].

4. The **elasticity** of the adhesion molecules and their supporting structures (such as microvilli) moderates the force that is transduced to the bond. This force decreases with increased elasticity because some of the unbinding energy is used for stretching.

drawn apart by a force (referred to as the unbinding force), there is an exponential increase in the k_{off}, as predicted by Bell two decades ago [12]. This is illustrated by the effect of the force exerted by flowing blood (known as the shear stress) on the k_{off} of transient leukocyte tethers (Figure 1). The ability of a bond to resist an unbinding force has been termed its 'reactive compliance'. Interestingly, selectin–ligand interactions appear to be better at resisting unbinding forces than several interactions (such as avidin–biotin [13,14] or antibody–antigen [3]) that have much higher affinities. This higher reactive compliance cannot explain the observed slow increase in rolling velocity with shear stress, however, because increasing the shear stress should still lead to an exponential increase in the rolling velocity, and this is clearly not the case (Figure 1).

Recently, it has been demonstrated that the amount of force required to break a bond is dependent on the rate at which the force is increased (the loading rate) [11,14]. At very high loading rates the P-selectin–PSGL-1 interaction can tolerate forces that are similar to the force required to break the very high affinity avidin–biotin interaction [14], and only about an order of magnitude lower than the

Figure 1



The contrasting effects of shear stress on the k_{off} of transient tethers and the rolling velocity. Leukocytes adhere transiently to surfaces coated with very low selectin (or selectin ligand) densities. These transient tethers may be mediated by single selectin–ligand bonds [9,10]. The k_{off} of transient tethers increases exponentially with shear stress, as predicted by Bell [12]. In contrast, when rolling (at higher ligand densities) the rolling velocity increases very little with shear stress. The transient tether k_{off} in the absence of shear stress (intrinsic or unstressed k_{off}) is obtained by extrapolation to zero shear stress. Both the tether k_{off} and the rolling velocities are approximately 10-fold faster when mediated by L-selectin compared with P- and E-selectin. Adapted from [1], with permission.

strength of covalent bonds [15]. These forces are greater than those required to extract membrane-anchored molecules from the membrane [12,16]. This implies that selectins or their ligands must be anchored to the cytoskeleton to be able to resist extraction from the membrane. In support of this, L-selectin-mediated tethering is abrogated by disruption of the actin cytoskeleton (using cytochalasins) or by truncation of the L-selectin cytoplasmic domain [8,17].

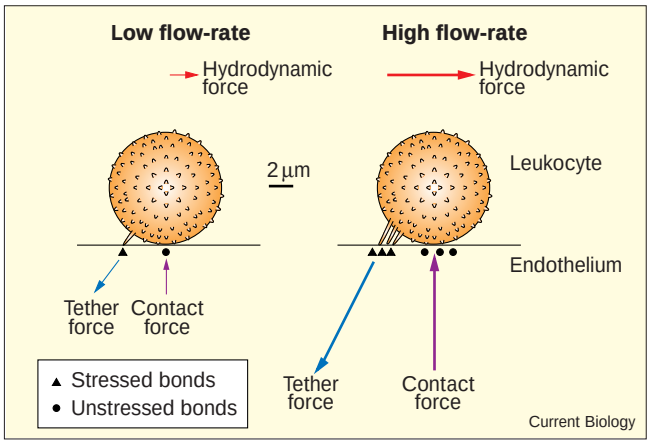
The elasticity of adhesion molecules and their supporting cellular structures — such as microvilli — should significantly influence tethering and rolling. Recent studies have confirmed that P-selectin and/or its ligand PSGL-1 do stretch when loaded [11], as do microvilli [3,10]. The effects of stretching on tethering and rolling are likely to be complex. By diverting energy into the elongated molecule or microvillus, stretching reduces the force on the selectin–ligand bond. The potential for stretching provides an additional mechanism by which presentation of selectins or selectin ligands on microvilli enhances tethering [8].

The ABS phenomenon

Although perhaps unusual, the properties of selectin–ligand interactions described above — fast kinetics, high tensile strength, high reactive compliance, and elasticity — fail to explain two characteristic features of selectin-mediated rolling. Firstly, rolling requires a minimum level of shear stress, termed the shear threshold. This was first noted for L-selectin [18], but has recently been demonstrated for E- and P-selectin as well [19]. Secondly, as the shear stress is increased above this threshold, the velocity of rolling cells reaches a plateau and is then remarkably resistant to further increases in shear force (Figure 1). A recent study by Chen and Springer [1] has provided a plausible explanation for these puzzling features. Using automated image analysis, together with some elegant experimental manoeuvres, Chen and Springer [1] were able to estimate the number of bonds (and microvilli tethers) between rolling cells and substrate over a range of shear stresses. The key finding was that, as shear stress increased, the number of bonds (and the number of microvillus tethers) increased. Because the rate of bond breakage increases with shear stress, the unavoidable conclusion is that the rate of bond formation must also increase as the shear stress increases.

This finding provides a simple explanation for both the shear threshold and the resistance of rolling to increases in shear stress. When the shear stress is below the shear threshold, bonds cannot form as quickly as they break, and so rolling is not possible. As the shear stress increases, more bonds form and become available to tether the cell. Because the additional bonds share the increased load, the force exerted through each bond will not increase in proportion to shear stress. Consequently, the dissociation rate and hence the rolling velocity will not increase exponentially and may remain constant. Chen and Springer [1] term this an 'automatic braking system' (ABS), analogous to the ABS deployed in modern automobiles. Interestingly, these authors also demonstrate that, under certain conditions, selectin-mediated leukocyte rolling exhibits oscillatory behaviour, slowing down and speeding up at regular intervals, with the velocity remaining within certain limits [1]. Such behaviour is typical of an ABS, experienced as a low-frequency vibration or 'juddering' by drivers when applying ABS-controlled brakes on slippery road surfaces.

Figure 2



Automatic braking system. During rolling there is a hydrodynamic force in the direction of blood flow and a tethering force at the rear of the cell. The tether 'swings' the cell against the endothelium, which consequently exerts a contact force. As the flow rate – and therefore the hydrodynamic force – increases, there is a proportional increase in both the tethering force and the contact force. Chen and Springer propose that the rate of new bond formation increases as the contact force increases. Because more bonds become available for tethering, the cell is able to resist higher shear stresses. Adapted from [1], with permission.

A major challenge is to determine what mechanism(s) accounts for the increase in the rate of bond formation as hydrodynamic shear is increased. A notable effect of any increase in shear stress is an increase in the contact force between the leukocyte and the endothelium (Figure 2). This force could enhance bond formation by cellular mechanisms, by molecular mechanisms, or by a combination of the two. A possible cellular mechanism is that leukocytes and/or microvilli flatten as the contact force increases, thereby increasing the contact area and therefore the number of bonds. Chen and Springer [1] argue that such deformations of the leukocytes themselves would be too slow, although they do show that microvilli can deform very rapidly. Also, if the mechanism were cellular, it should be possible to reproduce the ABS phenomenon with a different molecular interaction, such as an antibody that binds antigen on leukocyte microvilli. Instead, Chen and Springer favour a molecular mechanism, postulating that selectin–ligand interactions are

inhibited by steric and/or electrostatic barriers arising from the mucin-like portions of the glycoprotein ligands and that these barriers are more easily overcome if the molecules are driven together by an increased contact force.

A dependence of the rate of bond formation on shear stress has significant biological advantages. Firstly, leukocytes will not adhere spontaneously to each other or to endothelial surfaces under conditions of low shear. This avoids inappropriate adhesion in normal and pathological states where blood flow is slow or absent. Secondly, an ABS mechanism ensures that the rolling velocity of leukocytes falls within a narrow range. This is likely to be important because the rolling leukocyte scans endothelial surfaces for chemotactic signals, which stimulate integrin-mediated arrest and firm adhesion of the leukocyte to the endothelium. This process is likely to depend on the duration of contact and therefore on the rolling velocity. The ABS mechanism thus ensures that the duration of contact remains fairly constant in the face of wide variations in shear rate.

One of the most important and challenging areas of research in the post-genome era of molecular biology will be working out exactly how molecules and multi-molecular 'machines' function. It has been pointed out that this line of research will require greater familiarity with the mathematical and physical sciences [20]. The work reported here regarding selectin–ligand interactions provides a clear illustration of why this is so.

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