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Neutrophil rolling at high shear: flattening, catch bond behavior, tethers and slings

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Abstract

Neutrophil recruitment to sites of inflammation involves neutrophil rolling along the inflamed endothelium in the presence of shear stress imposed by blood flow. Neutrophil rolling in post-capillary venules *in vivo* is primarily mediated by P-selectin on the endothelium binding to P-selectin glycoprotein ligand-1 (PSGL-1) constitutively expressed on neutrophils. Blood flow exerts a hydrodynamic drag on the rolling neutrophil which is partially or fully balanced by the adhesive forces generated in the P-selectin-PSGL-1 bonds. Rolling is the result of rapid formation and dissociation of P-selectin-PSGL-1 bonds at the center and rear of the rolling cell, respectively. Neutrophils roll stably on P-selectin in post-capillary venules *in vivo* and flow chambers *in vitro* at wall shear stresses greater than 6 dyn cm⁻². However, the mechanisms that enable neutrophils to roll at such high shear stress are not completely understood. *In vitro* and *in vivo* studies have led to the discovery of four potential mechanisms, *viz.* cell flattening, catch bond behavior, membrane tethers, and slings. Rolling neutrophils undergo flattening at high shear stress, which not only increases the size of the cell footprint but also reduces the hydrodynamic drag experienced by the rolling cell. P-selectin-PSGL-1 bonds behave as catch bonds at small detachment forces and thus become stronger with increasing force. Neutrophils rolling at high shear stress form membrane tethers which can be longer than the cell diameter and promote the survival of P-selectin-PSGL-1 bonds. Finally, neutrophils rolling at high shear stress form slings, which act as cell autonomous adhesive substrates and support step-wise peeling. Tethers and slings act together and contribute to the forces balancing the hydrodynamic drag. How the synergy between the four mechanisms leads to stable rolling at high shear stress is an area that needs further investigation.

Keywords

Slings; PSGL-1; tethers; qDF; step-wise peeling

1. Introduction

Neutrophil recruitment to sites of inflammation is a hallmark of the inflammatory response (Ley et al., 2007; Phillipson and Kubes, 2011). Collisions with red blood cells (RBCs) cause neutrophils to marginate towards the venular wall (Goldsmith and Spain, 1984.; Nobis et al., 1985; Schmid-Schonbein et al., 1980), where they adhere to the vascular endothelium *via* an

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adhesion cascade which consists of sequential but overlapping steps of capture and rolling, followed by slow rolling, activation, and firm arrest (Ley et al., 2007; Springer, 1994). The primary steps of capture and rolling are mediated by rapid but reversible bonds between the selectin family of adhesion molecules (P- and E-selectin) expressed on endothelial cells (ECs) or neutrophils (L-selectin) and their glycoprotein ligands expressed on leukocytes and ECs (McEver and Zhu, 2010; Sundd et al., 2011). Selectins are type I transmembrane glycoproteins with an N-terminal Ca^{2+} -dependent-carbohydrate-recognizing lectin domain, followed by an epidermal growth factor (EGF) like domain, a series of short consensus repeats, a transmembrane domain, and a short cytoplasmic domain (Kansas, 1996; McEver, 2002; McEver et al., 1995; Springer, 1990; Tedder et al., 1995; Vestweber and Blanks, 1999). Neutrophil rolling in post-capillary venules *in vivo* is primarily mediated by P-selectin on the ECs binding to P-selectin glycoprotein ligand-1 (PSGL-1) (Norman et al., 2000; Norman et al., 1995; Sperandio et al., 2001) constitutively localized around the tips of neutrophil microvilli (Li et al., 1996; Moore et al., 1995; Sundd et al., 2012; Yago et al., 2002). PSGL-1 is a transmembrane disulfide-linked homodimeric sialo-mucin which is a ligand for all three selectins (P-, E- and L-selectin) (Carlow et al., 2009; Martinez et al., 2005; McEver, 2002; McEver and Zhu, 2010; Miner et al., 2011; Moore et al., 1995; Norman et al., 2000; Sperandio et al., 2003; Sperandio et al., 2001; Springer, 1990; Sundd et al., 2011; Vestweber and Blanks, 1999; Walcheck et al., 1996; Xia et al., 2002; Yago et al., 2010; Zarbock et al., 2011; Zarbock et al., 2009). The extracellular domain of PSGL-1 is rich in threonine and serine residues which are glycosylated to bear sialylated, fucosylated O-glycans presenting the sialyl Lewis-x (sLe^x) tetrasaccharide determinant (McEver, 2002; McEver et al., 1995; McEver and Zhu, 2010; Moore et al., 1994; Vestweber and Blanks, 1999). The P-selectin-PSGL-1 bond is the result of lectin domain residues and the Ca^{2+} ion in the lectin domain interacting with the sLe^x determinant on a short core 2 O-glycan expressed on the N-terminal threonine adjacent to sulfated tyrosine residues (Carlow et al., 2009; Lepänen et al., 1999; Leppänen et al., 2000; Liu et al., 1998; Pouyani and Seed, 1995; Somers et al., 2000; Xia et al., 2003). The structure, expression, and ligands of different selectins (L-, P- and E-selectin) have been described in detail elsewhere (Carlow et al., 2009; Hidalgo et al., 2007; McEver, 2002; McEver and Zhu, 2010; Sundd et al., 2011; Zarbock et al., 2011).

Blood vessels are distensible tubes and the velocity of blood changes in the radial direction with maximum velocity at the center and zero at the vessel wall (Long et al., 2004; Pries and Secomb, 2008). The ratio of the change in velocity (meters per second; m s^{-1}) to the radial distance (meters; m) is the shear rate (per second; s^{-1}). Due to the shear rate and fluid viscosity (defined later), adjacent layers of fluid flow at different velocities giving rise to a drag force (Newtons; N) between them. The ratio of the drag force to the surface area of contact between adjacent layers is defined as shear stress (Newtons per meter square; N m^{-2} or Pascals; Pa) between the two layers. In the field of biorheology, shear stress is often expressed in the cgs unit of dynes per centimeter square (dyn cm^{-2} ; $1 \text{ Pa} = 10 \text{ dyn cm}^{-2}$). The ratio of shear stress to the shear rate represents the viscosity of the fluid (Pascal seconds; Pa s) which is a measure of the resistance or unwillingness to flow offered by the fluid. Viscosity is also expressed in the cgs unit of centipoises (cP; $1 \text{ cP} = 10^{-3} \text{ Pa s}$). Blood is predominantly a suspension of RBC (45% v/v) in plasma (54% v/v) and undergoes phase separation along the radius of the blood vessel with RBC concentrated towards the center and depleted close to the vessel wall (Alberts et al., 2008; Pries and Secomb, 2008). As a result, the viscosity of blood is not constant but changes along the radius of the blood vessel with maximum viscosity towards the center and minimum (same as viscosity of plasma) close to the vessel wall (Long et al., 2004). This means that neutrophils rolling along the vessel wall are largely surrounded by plasma with few blood cells. As described elsewhere (Sundd et al., 2011), blood flowing through a venule affects the motion of a rolling neutrophil by two physical phenomena. The blood not only exerts a forward force which

pushes the cell forward but also a torque (about the cell center) which tends to rotate the cell like a wheel along the vessel wall. This is because the fluid flow velocity at the “top” of the cell is higher than near the vessel wall. For the convenience of the readers, the forward force and torque are collectively referred throughout the remaining text as hydrodynamic drag acting on the rolling cell. Shear stress acting near the blood vessel wall is referred as wall shear stress, which is the maximum shear stress in any given vessel. Shear stress reaches zero in the center of each vessel. The hydrodynamic drag acting on a rolling neutrophil increases with increasing wall shear stress. The hydrodynamic drag is partially or fully balanced by the adhesive forces generated in the P-selectin-PSGL-1 non-covalent bonds at the rear of the rolling cell (Dembo et al., 1988; Hammer and Apte, 1992; Pospieszalska et al., 2009; Tözeren and Ley, 1992). The characteristic stop and go jerky motion referred as rolling is the result of rapid formation and dissociation of P-selectin-PSGL-1 bonds at the center and rear of the rolling cell, respectively, which either fully (stop) or partially (go) balances the hydrodynamic drag (Caputo and Hammer, 2005; Pospieszalska and Ley, 2009a; Pospieszalska and Ley, 2009b).

Neutrophil rolling has been studied in post-capillary venules *in vivo* (Atherton and Born, 1972; Atherton and Born, 1973; Chiang et al., 2007; Damiano et al., 1996; Firrell and Lipowsky, 1989; Jung and Ley, 1997; Jung and Ley, 1999; Jung et al., 1998; Kunkel et al., 1996; Ley et al., 1995; Phillipson et al., 2006; Sperandio et al., 2003) and in flow chambers *in vitro* (Alon et al., 1995; Chesnutt et al., 2006; Goetz et al., 1994; Lawrence and Springer, 1991; Park et al., 2002; Ramachandran et al., 2004; Schaff et al., 2007; Schmidtke and Diamond, 2000; Smith et al., 2004; Sundd et al., 2010). *In vivo*, neutrophils roll stably in venules with wall shear stress ranging from 1.2 to 35 dyn/cm² (Damiano et al., 1996; Smith et al., 2002). Similarly, neutrophils rolling on P-selectin in an *in vitro* flow chamber continue to roll stably at relatively constant velocity when the wall shear stress is gradually increased from 2–30 dyn cm⁻² (Yago et al., 2002). Based on the experimentally measured molecular and cellular parameters, *in silico* models have been developed (Caputo and Hammer, 2005; Pawar et al., 2008; Pospieszalska and Ley, 2009a; Tözeren and Ley, 1992). However, these models fail to predict stable rolling at shear stresses in excess of about 3 dyn cm⁻². A plausible explanation for this discrepancy is that these studies consider rolling to be the result of a balance between the hydrodynamic drag acting on the cell (nondeformable (Caputo and Hammer, 2005; Pospieszalska and Ley, 2009a; Tözeren and Ley, 1992) or deformable (Khismatullin and Truskey, 2005; Pappu and Bagchi, 2008; Pawar et al., 2008)) and the adhesive forces acting on the P-selectin-PSGL-1 bonds clustered on the tips of few deformable and extendable microvilli in the cell footprint (contact zone with the P-selectin substrate). Although this mechanism is predominant at small shear stresses of 0–3 dyn cm⁻², it is not the only mechanism that enables neutrophil rolling at shear stresses in excess of 6 dyn cm⁻². *In vitro* and *in vivo* studies over the last decade have led to the discovery of four potential mechanisms that could possibly explain how neutrophils can roll at high shear stress (Fig. 1). 1.)

Neutrophils are deformable cells and thus, rolling neutrophils undergo flattening at high shear stress (Damiano et al., 1996; Firrell and Lipowsky, 1989; Sundd et al., 2010). Cell flattening not only increases the size of the cell footprint (Sundd et al., 2010) but also reduces the hydrodynamic drag experienced by the rolling cell (House and Lipowsky, 1988; Jadhav et al., 2005; Khismatullin, 2009; Sundd et al., 2011). 2.) Selectin-ligand bonds behave as catch bonds at small detachment forces and thus tend to become stronger with increasing force (Marshall et al., 2003). Modeling shows that the presence of such bonds can enhance the ability of neutrophils to roll at small shear stresses of 0–3 dyn cm⁻² (Caputo et al., 2007; Pawar et al., 2008). 3.) Differential interference contrast (DIC) microscopy (Ramachandran et al., 2004) and quantitative dynamic footprinting (qDF) (Sundd et al., 2010) have revealed that neutrophils rolling at a shear stress of 6 dyn cm⁻² or higher form

3–4 membrane tethers which can extend up to 16 μm behind the cell footprint. 4.) A recently discovered cell autonomous adhesive structure called sling is formed by neutrophils rolling at high shear stress (Sundd et al., 2012). Slings enable rolling by contributing to the forces balancing the hydrodynamic drag acting on the rolling cell. Slings and tethers together improve that balance by at least 50% (Sundd et al., 2012).

This review provides an overview of the current understanding of the mechanisms that enable neutrophils to roll at high shear stress ($> 6 \text{ dyn cm}^{-2}$) both *in vivo* and *in vitro*.

2. Role of cell flattening

Neutrophils can roll on P-selectin over a wide range of shear stress ($0.5\text{--}30 \text{ dyn cm}^{-2}$) both *in vitro* (Ramachandran et al., 2004; Sundd et al., 2010; Yago et al., 2002) and *in vivo* (Damiano et al., 1996; Dong et al., 1999; Firrell and Lipowsky, 1989; House and Lipowsky, 1988; Smith et al., 2002). Neutrophils rolling in the post-capillary venules *in vivo* at a shear stress of 6 dyn cm^{-2} or higher are known to deform into a tear drop shape (Damiano et al., 1996; Dong et al., 1999; Firrell and Lipowsky, 1989; Smith et al., 2002) which is the result of elongation in the direction of flow imposed by the hydrodynamic drag acting on the rolling cell (Firrell and Lipowsky, 1989). Neutrophils have a constant volume and thus, elongation of the cell also causes the cell to flatten (Firrell and Lipowsky, 1989; House and Lipowsky, 1988; Lipowsky et al., 1991). The flattening of the cell facilitates rolling at high shear *via* two biophysical mechanisms. First, the hydrodynamic drag acting on a rolling neutrophil positively correlates with the ratio (h/D) of the height of the cell, h , to vessel diameter, D (Fig. 1), i.e. the fraction of the vessel lumen obstructed by the rolling neutrophil (Dong et al., 1999; House and Lipowsky, 1988; Khismatullin and Truskey, 2004). As the cell flattens, the height decreases and thus reduces the hydrodynamic drag acting on the rolling cell. Second, a flattened and elongated cell has a much larger contact area or footprint which increases the probability of P-selectin-PSGL-1 bond formation. The size of the neutrophil footprint touching a flat P-selectin substrate was recently measured in an *in vitro* microfluidic flow chamber (Sundd et al., 2010). qDF revealed that neutrophils rolling at a shear stress of 6 dyn cm^{-2} have a four-fold larger footprint than if the cell remained spherical (Sundd et al., 2010). Since the load-bearing PSGL-1-P-selectin bonds reside in a half-moon-shaped area at the rear of the cell (Pospieszalska et al., 2009), we can estimate that the increased footprint doubles the number of load-bearing bonds. Thus, the four fold increase in contact area and reduced hydrodynamic drag following flattening enable neutrophils to roll on P-selectin both *in vivo* and *in vitro* at high shear stress.

In silico models are great tools that provide profound understanding of biological phenomena at a time resolution which cannot be achieved experimentally. However, *in silico* modeling of neutrophil rolling which can predict cell deformation in response to hydrodynamic drag is not trivial. A cell has a complex structural organization consisting of cell membrane, cytoplasm, nucleus and different organelles in the cytoplasm and the biophysical properties of a cell are influenced by the properties of these individual compartments (Alberts et al., 2008). Recently, the effect of cell flattening on rolling was explored using *in silico* studies which made use of a simplified approximation of the complex cellular structure (Jadhav et al., 2005; Khismatullin, 2009; Khismatullin and Truskey, 2005; Pappu and Bagchi, 2008; Pawar et al., 2008). In one approach (Jadhav et al., 2005; Pawar et al., 2008), a rolling neutrophil was approximated as a spherical capsule of liquid enclosed by an elastic membrane. The deformability of the cell was varied by changing the deformability of the elastic membrane. In a different approach (Khismatullin, 2009; Khismatullin and Truskey, 2005), the leukocyte was modeled as a sphere made of a viscoelastic nucleus surrounded by a slightly less viscoelastic cytoplasm which is enclosed by a thin plasma membrane with a constant surface tension. A liquid which shows resistance

to flow is known as viscous and a solid which shows resistance to pulling or stretching is known as elastic, however, several biological fluids including cytoplasm, show resistance to both flow and to stretch or compression and are known as viscoelastic (Fung, 2004). The deformability of the viscoelastic cell was varied by varying the viscosity of the cytoplasm (Khismatullin, 2009). Simulations from their *in silico* studies revealed that at an elevated shear stress a more deformable leukocyte has a more flattened profile, rolls more stably, has a larger contact area with greater number of P-selectin-PSGL-1 bonds and experiences smaller hydrodynamic drag compared to a less deformable leukocyte. In addition to *in silico* studies, several *in vitro* (Finger et al., 1996; Ramachandran et al., 2004; Sheikh and Nash, 1998; Yago et al., 2002) and *in vivo* (Lipowsky et al., 1991; Lipowsky et al., 1996) studies have experimentally confirmed that cell deformability is critical for the ability of neutrophils to roll at elevated shear stresses. The neutrophils were rendered less deformable by either fixing the cells with paraformaldehyde (Park et al., 2002; Ramachandran et al., 2004; Yago et al., 2002) or depleting membrane cholesterol using methyl- β -cyclodextrin (Yago et al., 2002). In the same studies, neutrophils were also rendered more deformable by treating the cells with cytochalasins (Finger et al., 1996; Lipowsky et al., 1991; Lipowsky et al., 1996; Yago et al., 2002) which depolymerize the actin cytoskeleton (Sheikh and Nash, 1998). Comparison of the rolling behavior of control, less deformable and more deformable neutrophils at high shear stress revealed that more deformable cells were more flattened, had larger contact area, smaller mean rolling velocities and demonstrated more stable rolling compared to less deformable or control cells. In these studies, potential contribution of altered tether and sling behavior (see below) were not considered.

3. Role of catch bond behavior

Neutrophil rolling on P-selectin along the vascular endothelium *in vivo* or in flow chambers *in vitro* is a result of rapid formation and dissociation of P-selectin-PSGL-1 bonds which balance the hydrodynamic drag (Sundd et al., 2011). Receptor-ligand bonds were traditionally thought to dissociate faster under a pulling force and such bonds are called slip bonds (Alon et al., 1995; Dembo et al., 1988). The rate of dissociation of slip bonds increases in response to a pulling force (Alon et al., 1995; Dembo et al., 1988). However, Dembo et al. (1988) theoretically proposed a counterintuitive phenomenon known as catch bonds which become stronger with increasing force. Marshall et al. (2003), showed, using atomic force microscopy (AFM) and flow chamber rolling assays, that P-selectin-PSGL-1 bonds have biphasic responses to applied force. The bonds behaved like catch bonds in the force range of 0–30 pN and slip bonds at forces greater than 30 pN (Marshall et al., 2003). Thus, the mean lifetime of P-selectin-PSGL-1 bonds increased with increasing force reaching a maximum around 20–30 pN and then decreasing with further increase in pulling force (Marshall et al., 2003). This behavior change at ~30 pN was referred to as catch-slip transition (Marshall et al., 2003). In a later study (Sarangapani et al., 2004), L-selectin-PSGL-1 bonds were also shown to undergo catch-slip transition, however, at ~50 pN. Based on the molecular structures and the non-covalent inter- and intra-molecular atomic interactions (hydrogen bonds, van der Waals interactions, electrostatic interactions and dipole-dipole interactions) between P-selectin and PSGL-1 or L-selectin and PSGL-1, two models for catch bond behavior have been proposed, the allosteric model (Springer, 2009; Waldron and Springer, 2009) and the sliding-rebinding model (Lou et al., 2006; Lou and Zhu, 2007; McEver and Zhu, 2010). Both models are inspired by the x-ray crystal structure of P-selectin either unliganded or bound to the sLe^x carbohydrate determinant or the PSGL-1 N-terminal glycopeptide (Somers et al., 2000). Based on crystal structures, molecular dynamic simulations and point mutations, both models propose that P-selectin has two conformations, bent and extended, represented by the closed and open state of lectin-EGF domain interface hinge, respectively. The bent and extended conformations of P-selectin have low and high affinity for PSGL-1, respectively. Both models propose the

existence of an equilibrium between the two states which shifts towards the high affinity extended state in response to pulling forces, resulting in increased lifetime. Although both models predict that application of pulling force results in opening of the lectin-EGF domain hinge which increases the overall affinity of the binding pocket for PSGL-1, they have different but not mutually exclusive explanations for the underlying mechanism (Lou and Zhu, 2007; McEver and Zhu, 2010; Springer, 2009). The allosteric model proposes that in response to a pulling force, the opening of the lectin-EGF domain hinge causes changes in the atomic interactions at the lectin-EGF interface which are allosterically transmitted *via* the lectin domain to the binding pocket which undergoes a conformational shift to recruit additional binding surfaces for ligand binding (Waldron and Springer, 2009). It was shown that insertion of a glycan wedge between the lectin-EGF hinge by introducing an *N*-glycosylation site by point mutations (Q30N and K32T) stabilized the extended conformation which increased the adhesiveness of P-selectin for PSGL-1 both in the absence and presence of pulling force (Phan et al., 2006). The concept of conformational change in the lectin domain being transmitted allosterically to the binding pocket was confirmed by testing the effect of point mutations in the lectin domain on the conformation of the PSGL-1 binding site (Waldron and Springer, 2009). Substitution of alanine at residue 28 in the lectin domain with a much larger histidine (A28H) forced P-selectin to acquire an extended conformation with high affinity for PSGL-1 both in absence and presence of pulling force (Waldron and Springer, 2009). In contrast, the sliding-rebinding model proposes that the opening of the lectin-EGF hinge in response to increasing pulling force tends to align the binding site in the direction of pulling force allowing the ligand to slide along the binding site (Lou et al., 2006; Lou and Zhu, 2007). As the ligand slides, old interactions break, however, new interactions form that prevent dissociation leading to increased life time of the bond (McEver and Zhu, 2010). As the force increases beyond the catch-slip transition boundary, the lectin-EGF hinge cannot open any further and the lifetime of the bond starts to decrease, resulting in the bond entering the slip regime. According to the sliding-rebinding model, the flexibility of the lectin-EGF domain should dictate the catch bond behavior (Lou et al., 2006; Lou and Zhu, 2007). A more flexible lectin-EGF hinge should enable catch-slip transition at much lower pulling forces than a less flexible hinge. The L-selectin-PSGL-1 bond exhibits catch bond behavior at much larger forces than the P-selectin-PSGL-1 bond, which implies that the lectin-EGF domain of L-selectin is less flexible than P-selectin. Indeed, the residue 138 in the EGF domain proximal to lectin-EGF hinge is Asn (N) in L-selectin and Gly (G) in P-selectin. Lou et al. (2006) used molecular dynamic simulations to show that the reduced flexibility of the lectin-EGF hinge of L-selectin is due to the presence of a hydrogen bond between N138 in the L-selectin EGF domain and Y37 in the L-selectin lectin domain. This hydrogen bond was absent in P-selectin which has a G instead of N as residue 138 in the EGF domain. Substitution of N138G in L-selectin resulted in increased flexibility of lectin-EGF hinge and elicited catch-behaving bonds with PSGL-1 at much lower forces showing that opening of inter-domain hinge dictates the high affinity conformation of selectins (Lou et al., 2006; McEver and Zhu, 2010).

Recently, the sliding-rebinding model was extended to include the effect of history of force application on catch bond behavior by L-selectin-PSGL-1 bonds (Sarangapani et al., 2011). In these experiments, the force (N) on a bond was gradually increased to a certain value and then maintained constant until the bond failed. The rate of increase of force over the application process was called ramp rate or loading rate (N s^{-1}) and the constant force was called hold force (Sarangapani et al., 2011). In the study by Sarangapani et al. (2011), it was shown that the hold force required to elicit the catch bond behavior by L-selectin-PSGL-1 bond correlates negatively with the ramp rate. In the same study, a point mutation at residue 108 in the lectin domain of P-selectin from His to Ala (H108A) abolished an interaction of the lectin domain with the peptide chain of PSGL-1 which elicited catch-slip transition by P-

selectin-PSGL-1 bond at much larger force than wild type P-selectin and also increased the sensitivity of P-selectin-PSGL-1 bond to ramp rate.

In vitro rolling (Lou et al., 2006; Yago et al., 2004; Zhu et al., 2008) as well as *in silico* studies (Beste and Hammer, 2008; Caputo et al., 2007) have shown that catch-behaving bonds stabilize neutrophil rolling at small shear stresses of less than 1 dyn cm^{-2} . Such bonds can also explain the shear-threshold phenomenon: rolling requires a (small) shear stress below which the cells detach. This is described in detail elsewhere (Lawrence et al., 1997; McEver and Zhu, 2010; Yago et al., 2004; Zhu et al., 2008). However, there is no experimental or modeling study showing a role of catch-behaving bonds in facilitating neutrophil rolling at shear stresses greater than 6 dyn cm^{-2} . Recent studies (Sundd et al., 2012; Sundd et al., 2010) have shown that neutrophil rolling on P-selectin at high shear stress ($> 6 \text{ dyn cm}^{-2}$) is facilitated by long membrane tethers and slings which are attached to the substrate *via* highly strained P-selectin-PSGL-1 bonds. We have shown that slings and tethers behind a neutrophil rolling at $6\text{--}8 \text{ dyn cm}^{-2}$ bear an average force of 60 pN (Sundd et al., 2012) which when distributed over 3–4 bonds attaching the tether or sling to the substrate (15–20 pN per bond) can effectively contribute to catch bond behavior of the system. Such bonds will tend to increase their lifetime in response to pulling force, thus allowing the tethers and slings to stay attached to the P-selectin substrate for longer times and grow longer in length. Whether catch-behaving bonds are responsible for formation of long tethers (up to 16 μm long) and slings needs to be investigated both by experimental and *in silico* studies.

4. Role of membrane tethers

Membrane tethers are nano-tubes extruded from the lipid bilayer membranes of blood cells under the application of a pulling force (Waugh, 2009; Waugh and Hochmuth, 1987). Excess membrane on the surface of neutrophils and other leukocytes is folded into cytoskeleton-linked projections known as microvilli (Finger et al., 1996; Shao, 2009) which are about 270 nm in diameter and up to 700 nm long (Sundd et al., 2012). The mechanism of tether formation and the response of tether to pulling forces have been described in detail elsewhere (Borghi and Brochard-Wyart, 2007; Brochard-Wyart et al., 2006; Pospieszalska et al., 2011; Sundd et al., 2011). A tether is formed when a microvillus on the surface of a neutrophil is pulled with a force (pico-Newton; pN) which is increasing over time. Once the pulling force exceeds a threshold force of about 31 pN (Waugh, 2009), the lipid membrane of the microvillus eventually dissociates from the underlying cytoskeleton and the tether is referred to have reached a crossover (Pospieszalska et al., 2011). In some studies the growing structure is not referred as a tether until it reaches the crossover (Evans et al., 2005; Heinrich et al., 2005; Shao et al., 1998; Xu and Shao, 2008). Following the crossover, the lipid membrane on the cell surface starts to flow around the membrane integral proteins linked to the cytoskeleton and into the tether causing it to elongate more easily over time. Based on the current understanding of the theory of tether formation (Pospieszalska et al., 2011; Sundd et al., 2011), the biorheological properties of membrane tethers undergo transition during the phase of crossover. Before the occurrence of crossover, a membrane tether is viscoelastic in nature which enables the lipid to flow (viscous) into the tether like a liquid and at the same time allows the tether to stretch or retract (elastic) like a spring. Once the crossover is reached, the elastic stretch/retract ability of the tether starts to diminish over time as the pulling force increases. Eventually (below or at 92 pN for neutrophils), the tether becomes viscous (Pospieszalska et al., 2011), almost like a tube of liquid which can easily elongate but has no or minimal ability to retract. This diminished ability of tethers to retract at higher pulling forces is thought to contribute to the formation of slings (Sundd et al., 2012) which are discussed in the next section. The gradual loss of elasticity (loss of spring) after crossover is most likely due to the dissociation of the microvillus membrane from the

cytoskeleton at crossover. Using a micropipette aspiration set-up, Shao et al. (Shao and Hochmuth, 1996; Shao et al., 1998) showed that a neutrophil adhered to an antibody-coated bead formed membrane tethers when moved away from the bead under a known pulling force. It was shown that under the application of the pulling force neutrophil microvilli were extended to form membrane tethers. The first evidence of tether formation by rolling neutrophils was provided by the *in vitro* flow chamber studies by Park et al. (2002) and Schmidtke and Diamond (2000). Although done at smaller shear stresses of less than 3 dyn cm⁻², these studies showed that neutrophil rolling on P-selectin is associated with tether formation at the rear of the rolling cell. Tethers were also observed to be formed by neutrophils rolling in the postcapillary venules of mice *in vivo* (Sperandio et al., 2003). Ramachandran et al. (2004) addressed the issue of neutrophils rolling at high shear stress and showed that neutrophils rolling on P-selectin at a shear stress of 8 dyn cm⁻² formed long membrane tethers. This study also showed that the tethers become longer and more complex (branched) as the rolling progresses, resulting in more stable rolling over time. Recently, qDF coupled to *in vitro* microfluidic flow chamber assay provided a quantitative analysis of the role of tethers in facilitating neutrophil rolling at high shear stress (Sundd et al., 2010). qDF is an adaptation of total internal reflection fluorescence (TIRF) microscopy and allows estimation of *z*-distances from the cover slip in the footprints of rolling neutrophils (Sundd et al., 2010). We showed that a neutrophil rolling on P-selectin under high shear stress (6–8 dyn/cm²) forms 3–4 long tethers which can extend up to 16 μm behind the rolling cell (Sundd et al., 2010). The anchorage points of long tethers were visible in the qDF micrographs of rolling neutrophils and the 3D reconstruction of the qDF footprints revealed that the tether anchorage points were held to the substrate through highly stressed P-selectin-PSGL-1 bonds (Sundd et al., 2010). 3D-reconstruction was based on the known drop in the intensity of the evanescent wave, which decreases exponentially with *z* distance from the cover slip and becomes effectively undetectable within 200 nm from the cover slip (Sundd et al., 2010). Figure 2A demonstrates the stretching of P-selectin-PSGL-1 bonds in a tether anchorage point at the rear of a rolling neutrophil. PSGL-1 stained with AlexaFluor568-conjugated-nonblocking-anti-mouse-PSGL-1 mAb (red) is clustered in the anchorage point of a membrane tether (membrane labeled with DiO; green). As the cell rolls forward (*t* = 0.6 s), the PSGL-1 staining in the anchorage point decreases because the anti-PSGL-1 mAb bound to the membrane proximal region of PSGL-1 is pulled out of the evanescent wave as the P-selectin-PSGL-1 bonds are stretched.

So how does a membrane tether of the order of 10–16 μm long facilitate neutrophil rolling at a shear stress of greater than 6 dyn cm⁻²? As mentioned in the Introduction, the blood flow imposes a hydrodynamic drag on the rolling cell which enables the cell to move forward and also rotate like a wheel along the vessel wall. In order to continue rolling, the cell needs to at least partially balance both the forward and rotating components of the hydrodynamic drag. Membrane tethers in cooperation with the catch phenomenon (described in previous section) are crucial in this process. Membrane tethers extend under pulling force and contribute to balancing of rotating component of the hydrodynamic drag. Catch bond behavior allows bonds to live longer under pulling force. When a neutrophil rolls along the vessel wall or P-selectin substrate, the hydrodynamic drag imposed on the cell causes P-selectin-PSGL-1 bonds clustered on the tips of microvilli in the cell footprint to stretch like a spring. As the cell rolls forward, the pulling force acting on few bonds on the tip of any individual microvillus increases, enabling the spring-like bonds to enter a critical period of increasing stress during which they can break. Some of the bonds indeed break, however, the majority survive the initial pulling forces because they act as catch bonds and become stronger with increasing pulling force. As described in the previous section, when the pulling force reaches ~30 pN, the P-selectin-PSGL-1 bonds undergo the catch-slip transition. Also, 31 pN is the lowest pulling force at which the involved tether can undergo a crossover (Waugh, 2009). As each tether elongates after its crossover, it becomes

more viscous (like a tube of liquid) and visible at the rear of the cell (tether anchor point visible in Fig. 2B and Supplementary movie 1). As new bonds under the cell footprint become load-bearing, the cell slows down, which reduces the rate of elongation of the tether. According to the theory of tether formation (Pospieszalska et al., 2011), the rate of tether elongation correlates positively with pulling force. Thus, reduction in the rate of tether elongation translates into reduced pulling force acting on the bonds attaching the tether to the vessel wall or P-selectin substrate (refer force trace in Fig. 2C and Supplementary movie 1). The reduction in pulling force prolongs the lifetime of these bonds. Thus, long tethers enable neutrophil rolling through four mechanisms. First, the long tethers preserve load-bearing bonds, which stabilizes rolling. Second, longer living bonds allow the tether to stay attached to the P-selectin substrate, and continue to extend and participate in balancing the hydrodynamic drag. As the cell rolls forward causing the tether arm (Fig. 1) to elongate, the point of origin of the tether on the cell surface moves up in the *z*-direction generating bigger counter torque which acts against the torque imposed by the flowing blood or buffer (Pospieszalska and Ley, 2009a). Third, according to the theory of tether formation (Pospieszalska et al., 2011) a dynamic equilibrium of the tether is a post-crossover state in which the tether force remains constant. In a dynamic equilibrium, the tether keeps on elongating at a constant extension rate under a constant pulling force. Thus, the force on individual bonds attaching the tether to the P-selectin substrate changes very little because the total pulling force on a tether in dynamic equilibrium is constant. The theory predicts that the likelihood for a tether to enter a state of dynamic equilibrium is very high provided the rolling is stable. Whether this proposed mechanism leads to stable rolling at high shear stresses needs to be verified using more detailed *in silico* computational models. Fourth, after their detachment from the substrate, the long tethers do not retract but rather rotate with the rotating cell giving rise to slings. The role of slings in cell rolling is discussed in the next section.

Although current models (Caputo and Hammer, 2005; King et al., 2005; Pawar et al., 2008; Pospieszalska and Ley, 2009a) can predict formation of tethers less than 1 μm long by neutrophils rolling at shear stresses of less than 6 dyn cm^{-2} , a model which can predict stable rolling at high shear stress and formation of tethers greater than the diameter of the cell does not exist. A computational approach using experimental qDF data (Sundd et al., 2012) has shown that four 10 μm long tethers can balance up to 100% of the torque acting on a neutrophil rolling at 6–8 dyn cm^{-2} . A study using micropipette aspiration has shown that tethers can also be pulled from ECs when a neutrophil and an EC adhered to each other are moved apart under known force (Girdhar and Shao, 2007). Thus, a tether formed at the rear of a neutrophil rolling in a post-capillary venule *in vivo* may be a composite tether with contributions from both ECs and neutrophils. However, experimental evidence either *in vitro* or *in vivo* showing tether formation by ECs at the rear of rolling neutrophils has not been presented. Membrane tethers which facilitate neutrophils to roll at high shear stresses may also have a role in stabilizing integrin mediated neutrophil arrest under high shear stresses. In a recent study (Rullo et al., 2012), monocyte arrest on VCAM-1 under shear flow was shown to be stabilized by the formation of F-actin-rich anchoring tethers at the rear of the cell. These anchors, which were attached to the VCAM-1 substrate by VLA-4-VCAM-1 bonds allowed monocytes to resist detachment in presence of shear flow (Rullo et al., 2012). Whether neutrophils use similar anchoring tethers to arrest on ICAM-1 at high shear stress is not known.

5. Role of slings

Recently, we have shown that neutrophil rolling at shear stresses of 6–10 dyn cm^{-2} is facilitated by slings which are cell-autonomous adhesive structures extended at the front of rolling neutrophils (Sundd et al., 2012). As described in the previous section, neutrophils

rolling on P-selectin at a shear stress of 6 dyn cm^{-2} or higher form long membrane tethers at the rear. qDF revealed that following detachment these long tethers do not retract as previously postulated (Schmidtke and Diamond, 2000; Shao, 2009) but instead, persist and land in the front of rolling neutrophils as slings (Fig. 3A and Supplementary movie 2). Figure 3A shows a tether to sling transformation in a DiI-stained mouse bone marrow neutrophil rolling on P-selectin coated on the glass cover slip that forms the floor of a microfluidic device. In the same study (Sundd et al., 2012), epifluorescence intravital microscopy revealed that neutrophils rolling in the cremaster venules of mice also form slings in the front (Fig. 3B). qDF revealed that as the cell rolls over the sling laid in front of it, the sling wraps around the rolling neutrophil which was also confirmed by scanning electron micrographs (SEMs) of neutrophils fixed while rolling on P-selectin in a microfluidic device (Fig. 3C). SEMs revealed (Sundd et al., 2012) that slings are membrane nano-tubes 200 nm in diameter and their mean length increases with increasing pulling forces generated by wall shear stresses in the range of $6\text{--}10 \text{ dyn cm}^{-2}$. Tracking of individual rolling neutrophils over time using qDF revealed that both the length of slings and frequency of sling formation increases as the cell rolls for a longer time and distance (Sundd et al., 2012). When a rolling neutrophil was tracked over time starting from the instant when it made first contact with the P-selectin substrate, both the length and frequency of visible slings were small for the first 25–50 s of rolling and then progressively increased over time (Sundd et al., 2012).

Dual-color qDF (DqDF) is an improvement of qDF and allows simultaneous visualization of two fluorochromes in the footprints of rolling neutrophils (Sundd et al., 2012). In the same study (Sundd et al., 2012), DqDF of rolling neutrophils stained with membrane dye DiO (green) and AlexaFluor568-conjugated-nonblocking anti-PSGL-1 mAb (red) revealed that PSGL-1 was presented as clusters on the tips of microvilli and as discrete sticky patches (about $1.6 \text{ }\mu\text{m}$ apart) on the slings (Fig. 3D). In addition, SEMs of neutrophils fixed while rolling on P-selectin revealed that microvilli tips were preserved as lobes (estimated to be $1.3 \text{ }\mu\text{m}$ apart) on the slings showing that membrane from several microvilli is used to form a tether which following detachment becomes a sling and the tips of microvilli appear as PSGL-1 rich patches or lobes on the sling (Fig. 3E) (Sundd et al., 2012).

We have shown in the previous section how tethers facilitate neutrophil rolling at high shear stress. Now the question is how slings facilitate neutrophil rolling at high shear stress. Rigorous analysis of DqDF images of rolling neutrophils revealed that as the cell rolls over the sling laid in front of it, the sling starts to wrap around the rolling cell (Supplementary movie 2) undergoing a step-wise peeling at the rear of the cell enabled by the tandem failure of PSGL-1 patches under hydrodynamic drag (Sundd et al., 2012) (Fig. 3F). When a PSGL-1 patch on a peeling sling fails, the cell tries to jump forward, but only for a short distance until the next patch downstream of the first patch on the same sling becomes load-bearing (Fig. 3F) (Sundd et al., 2012). This step-wise peeling distinguishes a tether from a sling because unlike a sling, failure of a tether is catastrophic as there are no other bonds available which can keep the tether attached to the substrate and thus the cell accelerates forward. However, failure of sling patches allows the cell to roll forward in only small increments, thus stabilizing rolling at high shear stress (Fig. 3F). Failure of the last patch on a sling is similar to failure of a tether because it results in complete detachment of the sling from the P-selectin substrate (step 6 in Fig. 3F). Estimation of the vertical z distance of the membrane in the PSGL-1 patches from the cover slip revealed that the loading of a PSGL-1 patch results in significant increase in the z -distance of the membrane from the cover slip (Sundd et al., 2012). This is accompanied by a decrease in intensity of PSGL-1 staining (red) in the patch because pulling of the membrane causes the anti-PSGL-1 mAb (red stain), which binds to the membrane proximal region of PSGL-1, to be pulled out of the evanescent wave (Sundd et al., 2012). Thus, similar to tether anchor points, the load-bearing patches of

slings are also attached to the P-selectin substrate by highly strained P-selectin-PSGL-1 bonds.

We also investigated the nature of LFA-1 expression on slings (Sundd et al., 2012). Mouse LFA-1 ($\alpha_L\beta_2$) is the main β_2 -integrin responsible for arrest of rolling neutrophils during inflammation and binds to inter-cellular adhesion-molecule-1 and 2 (ICAM-1 and ICAM-2) expressed on ECs (Lawrence and Springer, 1991; Ley et al., 2007; Springer, 1994; Xu et al., 1996). It has been shown that PSGL-1-dependent rolling on P-selectin or E-selectin allows LFA-1 to assume an extended conformation with intermediate affinity which can support rolling type interactions with ICAM-1 (Kuwano et al., 2010; Yago et al., 2010; Zarbock et al., 2007). DqDF experiments revealed that unlike PSGL-1, LFA-1 is expressed all over the neutrophil surface and the entire length of sling (Sundd et al., 2012) (Fig. 3B and 4A). Flow cytometry revealed that ICAM-1 was absent, however, ICAM-2 was expressed on the surface of mouse bone marrow neutrophils (Sundd et al., 2012). To test whether LFA-1 binding to ICAM-2 influences wrapping of slings around the rolling cell, rolling experiments were conducted using bone marrow neutrophils from WT mice or LFA-1 deficient (*Itgal*^{-/-}) mice or neutrophils from WT mice blocked with function blocking antibodies against LFA-1 or ICAM-2. Surprisingly, absence of LFA-1 or blocking LFA-1 or ICAM-2 resulted in a small but reproducible decrease in rolling velocity (Sundd et al., 2012). Using DqDF (Sundd et al., 2012), it was shown that unlike LFA-1, which was present both on cell surface and sling, ICAM-2 was primarily present on the cell surface of neutrophils rolling on P-selectin, suggesting that LFA-1 on the sling binds to ICAM-2 on the cell surface in *trans*. Measurements of the sling arm which is the distance '*d*' (Fig. 4B) of the inflection point of the sling from the center of the cell when the rearmost patch on the sling gets loaded or pulled revealed that the distance *d* was larger for LFA-1 deficient (*Itgal*^{-/-}) than WT mouse bone marrow neutrophils rolling on P-selectin at 10 dyn cm⁻² (Sundd et al., 2012). As explained in Section 4, the longer the tether arm, the larger is the counter torque which acts against the torque imposed by the flowing blood resulting in longer lifetime of bonds and slower rolling. Thus, LFA-1-ICAM-2 interaction in *trans* enables tighter wrapping of each sling, which results in a smaller sling arm (the loaded part of the sling) (WT in Fig. 4B) and faster rolling. In the absence of LFA-1-ICAM-2 interaction (*Itgal*^{-/-} in Fig. 4B), the wrapping of each sling is less tight, resulting in a longer sling arm and slower rolling. Although, LFA-1-ICAM-1 bond has been shown to behave as a catch-like bond at small bond forces (Chen et al., 2010), there is no report of such behavior by LFA-1-ICAM-2 bond. However, a catch-like LFA-1-ICAM-2 interaction will probably result in even more tight wrapping of sling at smaller bond forces compared to a slip bond.

In the same study (Sundd et al., 2012), we used a computational model (Pospieszalska et al., 2011) coupled with qDF measurements and found that the force on a tether or a sling varies over time and has a mean value of 60 pN at a wall shear stress of 6 dyn cm⁻². It was also shown that if a neutrophil rolling on P-selectin at 6 dyn cm⁻² has two slings and two tethers with inflection points and anchor points, respectively at 10 μ m from the cell center then the two tethers and two slings together can balance 64–88% of forward pushing force and up to 100% of the torque imposed on the rolling cell by the flowing blood or buffer (Sundd et al., 2012). Although stepwise peeling of slings is distinct from the pulling of tethers (discussed in section 4), slings and tethers work synergistically to facilitate neutrophil rolling on P-selectin at shear stresses greater than 6 dyn cm⁻². Microvilli are pulled to form tethers under pulling forces greater than the threshold force of 31 pN (Pospieszalska et al., 2011; Waugh, 2009) and elongate over time to lengths greater than one cell diameter (Ramachandran et al., 2004; Sundd et al., 2010). Eventually, the long tethers detach from the substrate and transform into slings, which stabilize rolling by undergoing step-wise peeling (Sundd et al., 2012). As rolling progresses, the number of slings increases, which may explain the well-

known phenomenon that rolling becomes more stable over time (Ramachandran et al., 2004; Sundd et al., 2012).

6. Conclusion and future directions

Here, we describe four mechanisms (cell flattening, catch bond behavior, tethers and slings) that enable neutrophils to roll on P-selectin at high wall shear stress (6 dyn cm^{-2} or higher) during inflammation. The synergy between these mechanisms is an area that needs further investigation and requires computer models that can integrate all four mechanisms to test their relative role in stabilizing neutrophil rolling at high shear stress. Tethers are believed to be devoid of cytoskeleton, based on studies of tethers pulled from RBCs under known forces (Waugh, 2009; Waugh and Bauserman, 1995). However, there is no experimental evidence confirming that tethers pulled from neutrophils are indeed devoid of cytoskeleton. The finding that microvilli are preserved as lobes on slings and that PSGL-1 is clustered on these lobes (Sundd et al., 2012) suggests that some sort of cytoskeletal structure may exist in the slings to restrict PSGL-1 in clusters and maintain the shape of lobes. The PSGL-1 cytoplasmic tail is known to bind ezrin/radixin/moesin (ERM) proteins, which are responsible for linking PSGL-1 to the actin cytoskeleton (Serrador et al., 2002; Shao et al., 2012; Snapp et al., 2002; Spertini et al., 2012). Also, a recent study has shown that tethers pulled from fibroblasts contain F-actin (Pontes et al., 2011). Thus, experimental studies are needed to elucidate the composition and nature of cytoskeletal organization of neutrophil slings and to address the role of the sling cytoskeleton, if it exists, in maintaining sling integrity at high shear stress. We have observed slings in neutrophils and T-helper1 lymphocytes (Sundd et al., 2012). Future investigations may find that other leukocytes may also be able to produce slings.

Monocytes have been shown to arrest on VCAM-1 and resist detachment to hydrodynamic drag using tether-like anchors, which are rich in F-actin (Rullo et al., 2012). What happens to membrane tethers and slings when a rolling neutrophil arrests on ICAM-1? Will tethers become anchors? How anchors form and how they stabilize adhesion at high shear stress are questions which deserve experimental investigation. Also, we have shown that LFA-1 is expressed over the entire surface of neutrophils including slings. Previous work has shown that LFA-1 assumes an extended conformation (Kuwano et al., 2010; Zarbock et al., 2007), which enables it to bind ICAM-2 on cell surface in *trans* (Sundd et al., 2012), presumably with intermediate affinity. DqDF experiments using fluorochrome-conjugated conformation reporter Abs (Kuwano et al., 2010) may elucidate the dynamics of LFA-1 activation in the footprint of arresting neutrophils, including the spatio-temporal distribution of different conformations (bent-low affinity, extended-intermediate affinity, and extended-high affinity (Springer and Dustin, 2012)) of LFA-1 in the footprint of arresting neutrophils. qDF is also suitable to study how the dynamics of LFA-1 activation and post-arrest clustering cooperate to prevent detachment at high shear stress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Bibliography & References Cited

- Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular biology of the cell*. 5. Garland Publishing, Inc; New York: 2008.
- Alon R, Hammer DA, Springer TA. Lifetime of the P-selectin-carbohydrate bond and its response to tensile force in hydrodynamic flow. *Nature*. 1995; 374:539–542. [PubMed: 7535385]
- Atherton A, Born GVR. Quantitative investigation of the adhesiveness of circulating polymorphonuclear leucocytes to blood vessel walls. *Journal of Physiology*. 1972; 222:447–474. [PubMed: 4624453]
- Atherton A, Born GVR. Relationship between the velocity of rolling granulocytes and that of the blood flow in venules. *Journal of Physiology*. 1973; 233:157–165. [PubMed: 4759098]
- Beste MT, Hammer DA. Selectin catch-slip kinetics encode shear threshold adhesive behavior of rolling leukocytes. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:20716–20721. [PubMed: 19095798]
- Borghi N, Brochard-Wyart F. Tether extrusion from red blood cells: integral proteins unbinding from cytoskeleton. *Biophys J*. 2007; 93:1369–1379. [PubMed: 17526591]
- Brochard-Wyart F, Borghi N, Cuvelier D, Nassoy P. Hydrodynamic narrowing of tubes extruded from cells. *Proc Natl Acad Sci U S A*. 2006; 103:7660–7663. \. [PubMed: 16679410]
- Caputo KE, Hammer DA. Effect of microvillus deformability on leukocyte adhesion explored using adhesive dynamics simulations. *Biophys J*. 2005; 89:187–200. [PubMed: 15879471]
- Caputo KE, Lee D, King MR, Hammer DA. Adhesive Dynamics Simulations of the Shear Threshold Effect for Leukocytes. *Biophys J*. 2007; 92:787–797. [PubMed: 17085490]
- Carlow DA, Gossens K, Naus S, Veerman KM, Seo W, Ziltener HJ. PSGL-1 function in immunity and steady state homeostasis. *Immunol Rev*. 2009; 230:75–96. [PubMed: 19594630]
- Chen W, Lou J, Zhu C. Forcing switch from short- to intermediate- and long-lived states of the alphaA domain generates LFA-1/ICAM-1 catch bonds. *The Journal of biological chemistry*. 2010; 285:35967–35978. [PubMed: 20819952]
- Chesnutt BC, Smith DF, Raffler NA, Smith ML, White EJ, Ley K. Induction of LFA-1-dependent neutrophil rolling on ICAM-1 by engagement of E-selectin. *Microcirculation*. 2006; 13:99–109. [PubMed: 16459323]
- Chiang EY, Hidalgo A, Chang J, Frenette PS. Imaging receptor microdomains on leukocyte subsets in live mice. *Nat Methods*. 2007; 4:219–222. [PubMed: 17322889]
- Damiano ER, Westheider J, Tozeren A, Ley K. Variation in the velocity, deformation, and adhesion energy density of leukocytes rolling within venules. *Circ Res*. 1996; 79:1122–1130. [PubMed: 8943950]
- Dembo M, Torney DC, Saxman K, Hammer D. The reaction limited kinetics of membrane-to-surface adhesion and detachment. *Proceedings of the Royal Society of London B Biological Sciences*. 1988; 234:55–83.
- Dong C, Cao J, Struble EJ, Lipowsky HH. Mechanics of leukocyte deformation and adhesion to endothelium in shear flow. *Ann Biomed Eng*. 1999; 27:298–312. [PubMed: 10374723]
- Evans E, Heinrich V, Leung A, Kinoshita K. Nano- to microscale dynamics of P-selectin detachment from leukocyte interfaces. I. Membrane separation from the cytoskeleton. *Biophysical Journal*. 2005; 88:2288–2298. [PubMed: 15653718]
- Finger E, Bruehl R, Bainton D, Springer T. A differential role for cell shape in neutrophil tethering and rolling on endothelial selectins under flow. *J Immunol*. 1996; 157:5085–5096. [PubMed: 8943418]
- Firrell JC, Lipowsky HH. Leukocyte margination and deformation in mesenteric venules of rat. *Am J Physiol Heart Circ Physiol*. 1989; 256:H1667–1674.
- Fung, YC. *Biomechanics: Mechanical properties of living tissues*. Springer; New York: 2004.
- Girdhar G, Shao JY. Simultaneous tether extraction from endothelial cells and leukocytes: observation, mechanics, and significance. *Biophys J*. 2007; 93:4041–4052. [PubMed: 17704170]
- Goetz DJ, El-Sabban ME, Pauli BU, Hammer DA. Dynamics of neutrophil rolling over stimulated endothelium in vitro. *Biophysical Journal*. 1994; 66:2202–2209. [PubMed: 7521229]

- Goldsmith HL, Spain S. Margination of leukocytes in blood flow through small tubes. *Microvascular Research*. 1984; 27:204–222. [PubMed: 6708830]
- Hammer DA, Apte SM. Simulation of cell rolling and adhesion on surfaces in shear flow: general results and analysis of selectin-mediated neutrophil adhesion. *Biophysical Journal*. 1992; 63:35–57. [PubMed: 1384734]
- Heinrich V, Leung A, Evans E. Nano- to microscale dynamics of P-selectin detachment from leukocyte interfaces. II. Tether flow terminated by P-selectin dissociation from PSGL-1. *Biophysical Journal*. 2005; 88:2299–2308. [PubMed: 15653735]
- Hidalgo A, Peired AJ, Wild MK, Vestweber D, Frenette PS. Complete identification of E-selectin ligands on neutrophils reveals distinct functions of PSGL-1, ESL-1, and CD44. *Immunity*. 2007; 26:477–489. [PubMed: 17442598]
- House S, Lipowsky H. In vivo determination of the force of leukocyte-endothelium adhesion in the mesenteric microvasculature of the cat. *Circ Res*. 1988; 63:658–668. [PubMed: 3409492]
- Jadhav S, Eggleton CD, Konstantopoulos K. A 3-D computational model predicts that cell deformation affects selectin-mediated leukocyte rolling. *Biophysical Journal*. 2005; 88:96–104. [PubMed: 15489302]
- Jung U, Ley K. Regulation of E-selectin, P-selectin, and intercellular adhesion molecule 1 expression in mouse cremaster muscle vasculature. *Microcirculation*. 1997; 4:311–319. [PubMed: 9219223]
- Jung U, Ley K. Mice lacking two or all three selectins demonstrate overlapping and distinct functions for each selectin. *Journal of Immunology*. 1999; 162:6755–6762.
- Jung U, Norman KE, Scharffetter-Kochanek K, Beaudet AL, Ley K. Transit time of leukocytes rolling through venules controls cytokine-induced inflammatory cell recruitment in vivo. *Journal of Clinical Investigation*. 1998; 102:1526–1533. [PubMed: 9788965]
- Kansas GS. Selectins and their ligands: current concepts and controversies. *Blood*. 1996; 88:3259–3287. [PubMed: 8896391]
- Khismatullin, DB. The cytoskeleton and deformability of white blood cells. In: Ley, K., editor. *Leukocyte adhesion*. Academic press; San Diego: 2009. p. 47-111.
- Khismatullin DB, Truskey GA. A 3D numerical study of the effect of channel height on leukocyte deformation and adhesion in parallel-plate flow chambers. *Microvasc Res*. 2004; 68:188–202. [PubMed: 15501238]
- Khismatullin DB, Truskey GA. Three-dimensional numerical simulation of receptor-mediated leukocyte adhesion to surfaces: Effects of cell deformability and viscoelasticity. *Physics of Fluids*. 2005:17.
- King MR, Heinrich V, Evans E, Hammer DA. Nano-to-micro scale dynamics of P-selectin detachment from leukocyte interfaces. III. Numerical simulation of tethering under flow. *Biophys J*. 2005; 88:1676–1683. [PubMed: 15574709]
- Kunkel EJ, Jung U, Bullard DC, Norman KE, Wolitzky BA, Vestweber D, Beaudet AL, Ley K. Absence of trauma-induced leukocyte rolling in mice deficient in both P-selectin and intercellular adhesion molecule 1. *Journal of Experimental Medicine*. 1996; 183:57–65. [PubMed: 8551244]
- Kuwano Y, Spelten O, Zhang H, Ley K, Zarbock A. Rolling on E- or P-selectin induces the extended but not high-affinity conformation of LFA-1 in neutrophils. *Blood*. 2010; 116:617–624. [PubMed: 20445017]
- Lawrence MB, Kansas GS, Kunkel EJ, Ley K. Threshold levels of fluid shear promote leukocyte adhesion through selectins (CD62L,P,E). *J Cell Biol*. 1997; 136:717–727. [PubMed: 9024700]
- Lawrence MB, Springer TA. Leukocytes roll on a selectin at physiological flow rates: distinction from and prerequisite for adhesion through integrins. *Cell*. 1991; 65:859–874. [PubMed: 1710173]
- Lepänen A, Mehta P, Ouyang Y-B, Ju T, Helin J, Moore KL, Die Iv, Canfield WM, McEver RP, Cummings RD. A novel glycosulfopeptide binds to P-selectin and inhibits leukocyte adhesion to P-selectin. *Journal of Biological Chemistry*. 1999; 274:24838–24848. [PubMed: 10455156]
- Leppänen A, White SP, Helin J, McEver RP, Cummings RD. Binding of glycosulfopeptides to P-selectin requires stereospecific contributions of individual tyrosine sulfate and sugar residues. *Journal of Biological Chemistry*. 2000; 275:39569–39578. [PubMed: 10978329]

- Ley K, Bullard DC, Arbones ML, Bosse R, Vestweber D, Tedder TF, Beaudet AL. Sequential contribution of L- and P-selectin to leukocyte rolling in vivo. *J Exp Med*. 1995; 181:669–675. [PubMed: 7530761]
- Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol*. 2007; 7:678–689. [PubMed: 17717539]
- Li F, Erickson HP, James JA, Moore KL, Cummings RD, McEver RP. Visualization of P-selectin glycoprotein ligand-1 as a highly extended molecule and mapping of protein epitopes for monoclonal antibodies. *Journal of Biological Chemistry*. 1996; 271:6342–6348. [PubMed: 8626430]
- Lipowsky HH, Riedel D, Shi GS. In vivo mechanical properties of leukocytes during adhesion to venular endothelium. *Biorheology*. 1991; 28:53–64. [PubMed: 2049532]
- Lipowsky HH, Scott DA, Cartmell JS. Leukocyte rolling velocity and its relation to leukocyte-endothelium adhesion and cell deformability. *American Journal of Physiology-Heart and Circulatory Physiology*. 1996; 270 (39):H1371–H1380.
- Liu W-J, Ramachandran V, Kang J, Kishimoto TK, Cummings RD, McEver RP. Identification of N-terminal residues on P-selectin glycoprotein ligand-1 required for binding to P-selectin. *Journal of Biological Chemistry*. 1998; 273:7078–7087. [PubMed: 9507018]
- Long DS, Smith ML, Pries AR, Ley K, Damiano ER. Microviscometry reveals reduced blood viscosity and altered shear rate and shear stress profiles in microvessels after hemodilution. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:10060–10065. [PubMed: 15220478]
- Lou J, Yago T, Klopocki AG, Mehta P, Chen W, Zarnitsyna VI, Bovin NV, Zhu C, McEver RP. Flow-enhanced adhesion regulated by a selectin interdomain hinge. *J Cell Biol*. 2006; 174:1107–1117. [PubMed: 17000883]
- Lou J, Zhu C. A structure-based sliding-rebinding mechanism for catch bonds. *Biophys J*. 2007; 92:1471–1485. [PubMed: 17142266]
- Marshall BT, Long M, Piper JW, Yago T, McEver RP, Zhu C. Direct observation of catch bonds involving cell-adhesion molecules. *Nature*. 2003; 423:190–193. [PubMed: 12736689]
- Martinez M, Joffraud M, Giraud S, Baisse B, Bernimoulin MP, Schapira M, Spertini O. Regulation of PSGL-1 interactions with L-selectin, P-selectin, and E-selectin: role of human fucosyltransferase-IV and -VII. *J Biol Chem*. 2005; 280:5378–5390. [PubMed: 15579466]
- McEver RP. Selectins: lectins that initiate cell adhesion under flow. *Curr Opin Cell Biol*. 2002; 14:581–586. [PubMed: 12231353]
- McEver RP, Moore KL, Cummings RD. Leukocyte trafficking mediated by selectin-carbohydrate interactions. *Journal of Biological Chemistry*. 1995; 270:11025–11028. [PubMed: 7538108]
- McEver RP, Zhu C. Rolling cell adhesion. *Annu Rev Cell Dev Biol*. 2010; 26:363–396. [PubMed: 19575676]
- Miner JJ, Shao B, Wang Y, Chichili GR, Liu Z, Klopocki AG, Yago T, McDaniel JM, Rodgers W, Xia L, McEver RP. Cytoplasmic domain of P-selectin glycoprotein ligand-1 facilitates dimerization and export from the endoplasmic reticulum. *The Journal of biological chemistry*. 2011; 286:9577–9586. [PubMed: 21220419]
- Moore KL, Eaton SF, Lyons DE, Lichenstein HS, Cummings RD, McEver RP. The P-selectin glycoprotein ligand from human neutrophils displays sialylated, fucosylated, O-linked poly-N-acetyllactosamine. *Journal of Biological Chemistry*. 1994; 269:23318–23327. [PubMed: 7521878]
- Moore KL, Patel KD, Bruehl RE, Li F, Johnson DA, Lichenstein HS, Cummings RD, Bainton DF, McEver RP. P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. *Journal of Cell Biology*. 1995; 128:661–671. [PubMed: 7532174]
- Nobis U, Pries AR, Cokelet GR, Gaehtgens P. Radial distribution of white cells during blood flow in small tubes. *Microvasc Res*. 1985; 29:295–304. [PubMed: 3999988]
- Norman KE, Katopodis AG, Thoma G, Kolbinger F, Hicks AE, Cotter MJ, Pockley AG, Hellewell PG. P-selectin glycoprotein ligand-1 supports rolling on E- and P-selectin in vivo. *Blood*. 2000; 96:3585–3591. [PubMed: 11071658]
- Norman KE, Moore KL, McEver RP, Ley K. Leukocyte rolling in vivo is mediated by P-selectin glycoprotein ligand-1. *Blood*. 1995; 86:4417–4421. [PubMed: 8541529]

- Pappu V, Bagchi P. 3D computational modeling and simulation of leukocyte rolling adhesion and deformation. *Computers in Biology and Medicine*. 2008; 38:738–753. [PubMed: 18499093]
- Park EYH, Smith MJ, Stropp ES, Snapp KR, DiVietro JA, Walker WF, Schmidtke DW, Diamond SL, Lawrence MB. Comparison of PSGL-1 microbead and neutrophil rolling: microvillus elongation stabilizes P-selectin bond clusters. *Biophysical Journal*. 2002; 82:1835–1847. [PubMed: 11916843]
- Pawar P, Jadhav S, Eggleton CD, Konstantopoulos K. Roles of cell and microvillus deformation and receptor-ligand binding kinetics in cell rolling. *American Journal of Physiology-Heart and Circulatory Physiology*. 2008; 295:H1439–H1450. [PubMed: 18660437]
- Phan UT, Waldron TT, Springer TA. Remodeling of the lectin-EGF-like domain interface in P- and L-selectin increases adhesiveness and shear resistance under hydrodynamic force. *Nat Immunol*. 2006; 7:883–889. [PubMed: 16845394]
- Phillipson M, Heit B, Colarusso P, Liu L, Ballantyne CM, Kubes P. Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. *J Exp Med*. 2006; 203:2569–2575. [PubMed: 17116736]
- Phillipson M, Kubes P. The neutrophil in vascular inflammation. *Nature medicine*. 2011; 17:1381–1390.
- Pontes B, Viana NB, Salgado LT, Farina M, Moura Neto V, Nussenzveig HM. Cell cytoskeleton and tether extraction. *Biophysical Journal*. 2011; 101:43–52. [PubMed: 21723813]
- Pospieszalska MK, Lasiecka I, Ley K. Cell protrusions and tethers: a unified approach. *Biophys J*. 2011; 100:1697–1707. [PubMed: 21463583]
- Pospieszalska MK, Ley K. Dynamics of microvillus extension and tether formation in rolling leukocytes. *Cellular and Molecular Bioengineering*. 2009a; 2:207–217. [PubMed: 20046963]
- Pospieszalska, MK.; Ley, K. Modeling leukocyte rolling. In: Ley, K., editor. *Leukocyte adhesion*. Elsevier; San Diego: 2009b. p. 221-296.
- Pospieszalska MK, Zarbock A, Pickard JE, Ley K. Event-tracking model of adhesion identifies load-bearing bonds in rolling leukocytes. *Microcirculation*. 2009; 16:115–130. [PubMed: 19023690]
- Pouyani T, Seed B. PSGL-1 recognition of P-selectin is controlled by a tyrosine sulfation consensus at the PSGL-1 amino terminus. *Cell*. 1995; 83:333–343. [PubMed: 7585950]
- Pries, AR.; Secomb, TW. Blood flow in microvascular networks. In: Tuma, RF.; Duran, WN.; Ley, K., editors. *Handbook of Physiology: Microcirculation*. Academic Press; San Diego: 2008. p. 3-36.
- Ramachandran V, Williams M, Yago T, Schmidtke DW, McEver RP. Dynamic alterations of membrane tethers stabilize leukocyte rolling on P-selectin. *Proceedings of the National Academy of Sciences USA*. 2004; 101:13519–13524.
- Rullo J, Becker H, Hyduk SJ, Wong JC, Digby G, Arora PD, Cano AP, Hartwig J, McCulloch CA, Cybulsky MI. Actin polymerization stabilizes $\alpha 4 \beta 1$ integrin anchors that mediate monocyte adhesion. *The Journal of cell biology*. 2012; 197:115–129. [PubMed: 22472442]
- Sarangapani KK, Qian J, Chen W, Zarnitsyna VI, Mehta P, Yago T, McEver RP, Zhu C. Regulation of catch bonds by rate of force application. *The Journal of biological chemistry*. 2011; 286:32749–32761. [PubMed: 21775439]
- Sarangapani KK, Yago T, Klopocki AG, Lawrence MB, Fieger CB, Rosen SD, McEver RP, Zhu C. Low force decelerates L-selectin dissociation from P-selectin glycoprotein ligand-1 and endoglycan. *Journal of Biological Chemistry*. 2004; 279:2291–2298. [PubMed: 14573602]
- Schaff UY, Xing MM, Lin KK, Pan N, Jeon NL, Simon SI. Vascular mimetics based on microfluidics for imaging the leukocyte--endothelial inflammatory response. *Lab Chip*. 2007; 7:448–456. [PubMed: 17389960]
- Schmid-Schonbein GW, Usami S, Skalak R, Chien S. The interaction of leukocytes and erythrocytes in capillary and postcapillary vessels. *Microvasc Res*. 1980; 19:45–70. [PubMed: 7360047]
- Schmidtke DW, Diamond SL. Direct observation of membrane tethers formed during neutrophil attachment to platelets or P-selectin under physiological flow. *Journal of Cell Biology*. 2000; 149:719–729. [PubMed: 10791984]
- Serrador JM, Urzainqui A, Alonso-Lebrero JL, Cabrero JR, Montoya MC, Vicente-Manzanares M, Yanez-Mo M, Sanchez-Madrid F. A juxta-membrane amino acid sequence of P-selectin glycoprotein ligand-1 is involved in moesin binding and ezrin/radixin/moesin-directed targeting at

- the trailing edge of migrating lymphocytes. *European Journal of Immunology*. 2002; 32:1560–1566. [PubMed: 12115638]
- Shao B, Yago T, Coghil PA, Klopocki AG, Mehta-D'souza P, Schmidtke DW, Rodgers W, McEver RP. Signal-dependent Slow Leukocyte Rolling Does Not Require Cytoskeletal Anchorage of P-selectin Glycoprotein Ligand-1 (PSGL-1) or Integrin α L β 2. *The Journal of biological chemistry*. 2012; 287:19585–19598. [PubMed: 22511754]
- Shao, J-Y. Biomechanics of leukocyte and endothelial cell surface. In: Ley, K., editor. *Leukocyte adhesion*. Academic press; San Diego: 2009. p. 25-45.
- Shao J-Y, Hochmuth RM. Micropipette suction for measuring piconewton forces of adhesion and tether formation from neutrophil membranes. *Biophysical Journal*. 1996; 71:2892–2901. [PubMed: 8913626]
- Shao J-Y, Ting-Beall HP, Hochmuth RM. Static and dynamic lengths of neutrophil microvilli. *Proceedings of the National Academy of Sciences USA*. 1998; 95:6797–6802.
- Sheikh S, Nash GB. Treatment of neutrophils with cytochalasins converts rolling to stationary adhesion on P-selectin. *Journal of Cellular Physiology*. 1998; 174:206–216. [PubMed: 9428807]
- Smith ML, Smith MJ, Lawrence MB, Ley K. Viscosity-independent velocity of neutrophils rolling on P-selectin *in vitro* or *in vivo*. *Microcirculation*. 2002; 9:523–536. [PubMed: 12483549]
- Smith ML, Sperandio M, Galkina EV, Ley K. Autoperfused mouse flow chamber reveals synergistic neutrophil accumulation through P-selectin and E-selectin. *J Leukoc Biol*. 2004; 76:985–993. [PubMed: 15075351]
- Snapp KR, Heitzig CE, Kansas GS. Attachment of the PSGL-1 cytoplasmic domain to the actin cytoskeleton is essential for leukocyte rolling on P-selectin. *Blood*. 2002; 99:4494–4502. [PubMed: 12036880]
- Somers WS, Tang J, Shaw GD, Camphausen RT. Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to sLe^x and PSGL-1. *Cell*. 2000; 103:467–479. [PubMed: 11081633]
- Sperandio M, Smith ML, Forlow SB, Olson TS, Xia L, McEver RP, Ley K. P-selectin glycoprotein ligand-1 mediates L-selectin-dependent leukocyte rolling in venules. *Journal of Experimental Medicine*. 2003; 197:1355–1363. [PubMed: 12756271]
- Sperandio M, Thatte A, Foy D, Ellies LG, Marth JD, Ley K. Severe impairment of leukocyte rolling in venules of core 2 glucosaminyltransferase-deficient mice. *Blood*. 2001; 97:3812–3819. [PubMed: 11389021]
- Spertini C, Baisse B, Spertini O. Ezrin-radixin-moesin-binding sequence of PSGL-1 glycoprotein regulates leukocyte rolling on selectins and activation of extracellular signal-regulated kinases. *The Journal of biological chemistry*. 2012; 287:10693–10702. [PubMed: 22311979]
- Springer TA. Adhesion receptors of the immune system. *Nature*. 1990; 346:425–434. [PubMed: 1974032]
- Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell*. 1994; 76:301–314. [PubMed: 7507411]
- Springer TA. Structural basis for selectin mechanochemistry. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:91–96. [PubMed: 19118197]
- Springer TA, Dustin ML. Integrin inside-out signaling and the immunological synapse. *Current Opinion in Cell Biology*. 2012; 24:107–115. [PubMed: 22129583]
- Sundd P, Gutierrez E, Koltsova EK, Kuwano Y, Fukuda S, Pospieszalska MK, Groisman A, Ley K. 'Slings' enable neutrophil rolling at high shear. *Nature*. 2012; 488:399–403. [PubMed: 22763437]
- Sundd P, Gutierrez E, Pospieszalska MK, Zhang H, Groisman A, Ley K. Quantitative dynamic footprinting microscopy reveals mechanisms of neutrophil rolling. *Nature Methods*. 2010; 7:821–824. [PubMed: 20871617]
- Sundd P, Pospieszalska MK, Cheung LS, Konstantopoulos K, Ley K. Biomechanics of leukocyte rolling. *Biorheology*. 2011; 48:1–35. [PubMed: 21515934]
- Tedder TF, Steeber DA, Chen A, Engel P. The selectins: vascular adhesion molecules. *Faseb J*. 1995; 9:866–873. [PubMed: 7542213]

- Tözeren A, Ley K. How do selectins mediate leukocyte rolling in venules? *Biophysical Journal*. 1992; 63:700–709. [PubMed: 1420908]
- Vestweber D, Blanks JE. Mechanisms that regulate the function of the selectins and their ligands. *Physiol Rev*. 1999; 79:181–213. [PubMed: 9922371]
- Walcheck B, Moore KL, McEver RP, Kishimoto TK. Neutrophil-neutrophil interactions under hydrodynamic shear stress involve L-selectin and PSGL-1. *Journal of Clinical Investigation*. 1996; 98:1081–1087. [PubMed: 8787668]
- Waldron TT, Springer TA. Transmission of allostery through the lectin domain in selectin-mediated cell adhesion. *Proc Natl Acad Sci U S A*. 2009; 106:85–90. [PubMed: 19118202]
- Waugh, RE. Membrane tethers. In: Ley, K., editor. *Leukocyte adhesion*. Academic press; San Diego: 2009. p. 3-24.
- Waugh RE, Bauserman RG. Physical measurement of bilayer-skeletal separation forces. *Annals of Biomedical Engineering*. 1995; 23:308–321. [PubMed: 7631984]
- Waugh RE, Hochmuth RM. Mechanical equilibrium of thick, hollow, liquid membrane cylinders. *Biophys J*. 1987; 52:391–400. [PubMed: 3651558]
- Xia L, Ramachandran V, McDaniel JM, Nguyen KN, Cummings RD, McEver RP. N-terminal residues in murine P-selectin glycoprotein ligand-1 required for binding to murine P-selectin. *Blood*. 2003; 101:552–559. [PubMed: 12393631]
- Xia L, Sperandio M, Yago T, McDaniel JM, Cummings RD, Pearson-White S, Ley K, McEver RP. P-selectin glycoprotein ligand-1-deficient mice have impaired leukocyte tethering to E-selectin under flow. *Journal of Clinical Investigation*. 2002; 109:939–950. [PubMed: 11927621]
- Xu G, Shao JY. Human neutrophil surface protrusion under a point load: location independence and viscoelasticity. *Am J Physiol Cell Physiol*. 2008; 295:C1434–1444. [PubMed: 18815230]
- Xu H, Bickford JK, Luther E, Carpenito C, Takei F, Springer TA. Characterization of murine intercellular adhesion molecule-2. *Journal of Immunology*. 1996; 156:4909–4914.
- Yago T, Leppanen A, Qiu H, Marcus WD, Nollert MU, Zhu C, Cummings RD, McEver RP. Distinct molecular and cellular contributions to stabilizing selectin-mediated rolling under flow. *J Cell Biol*. 2002; 158:787–799. [PubMed: 12177042]
- Yago T, Shao B, Miner JJ, Yao L, Klopocki AG, Maeda K, Coggeshall KM, McEver RP. E-selectin engages PSGL-1 and CD44 through a common signaling pathway to induce integrin α L β 2-mediated slow leukocyte rolling. *Blood*. 2010; 116:485–494. [PubMed: 20299514]
- Yago T, Wu J, Wey CD, Klopocki AG, Zhu C, McEver RP. Catch bonds govern adhesion through L-selectin at threshold shear. *Journal of Cell Biology*. 2004; 166:913–923. [PubMed: 15364963]
- Zarbock A, Ley K, McEver RP, Hidalgo A. Leukocyte ligands for endothelial selectins: specialized glycoconjugates that mediate rolling and signaling under flow. *Blood*. 2011; 118:6743–6751. [PubMed: 22021370]
- Zarbock A, Lowell CA, Ley K. Spleen tyrosine kinase Syk is necessary for E-selectin-induced α L β 2 integrin-mediated rolling on intercellular adhesion molecule-1. *Immunity*. 2007; 26:773–783. [PubMed: 17543554]
- Zarbock A, Muller H, Kuwano Y, Ley K. PSGL-1-dependent myeloid leukocyte activation. *J Leukoc Biol*. 2009; 86:1119–1124. [PubMed: 19703898]
- Zhu C, Yago T, Lou J, Zarnitsyna VI, McEver RP. Mechanisms for flow-enhanced cell adhesion. *Ann Biomed Eng*. 2008; 36:604–621. [PubMed: 18299992]

Highlights

- Neutrophils flatten against the substrate, thus increasing adhesion
- While rolling, neutrophils form long tethers that favorably influence bond lifetimes
- Selectin bonds with their glycoprotein ligands are catch bonds
- Rolling neutrophils form slings, self-adhesive structures that stabilize rolling

Figure 1.

Schematic of a neutrophil rolling along a blood vessel at a wall shear stress of 6 dyn cm^{-2} or higher. Cell is flattened and has a tether and a peeling sling. Cell membrane, green; P-selectin, blue; PSGL-1, red; D , diameter of vessel; h , height of cell; tether arm, distance of the tether anchor point from cell center; sling arm, distance of the sling inflection point from cell center. To avoid clutter, only one P-selectin-PSGL-1 complex is shown under the tether anchor point and sling inflection point.

Figure 2.

Pulling force on a tether. (A) DqDF footprints of an isolated mouse bone marrow neutrophil rolling on P-selectin in a microfluidic device. Neutrophil stained with DiO (green) and AlexaFluor568-nonblocking-anti-mouse-PSGL-1 mAb (red). As the cell rolls forward between $t = 0$ s and 0.6 s, the P-selectin-PSGL-1 bonds attaching tether anchor point (marked with white vertical arrow) to P-selectin substrate are stretched and the PSGL-1 signal (red) decreases in the tether anchor point at $t = 0.6$ s. Scale bars 5 μm . (B) qDF image of a DiI-stained mouse bone marrow neutrophil rolling on P-selectin in a microfluidic device. Wall shear stress 10 dyn cm^{-2} (A and B). P-selectin 20 molecules μm^{-2} (A and B). Thick horizontal arrows in A and B denote direction of rolling. Images in A and B were processed (saturated) to reveal tether anchor points. TIRF excitation 488 and 561 nm lasers and incidence angle, $\theta = 70^\circ$. (C) Pulling force F acting on a tether (anchor point marked with a circle in B) during 1.5 s before tether detachment. The tether detaches at $t = 0$ s. Black diamonds represent qDF data. Dotted curve is based on theory of tether formation (Pospieszalska et al., 2011). The complete force trace is shown in Supplementary movie 1.

Figure 3.

Step-wise peeling of slings (A–D, F adapted from Sundt et al. (2012)). (A) Processed qDF images of a DiI-stained-isolated mouse bone marrow neutrophil rolling on P-selectin in a microfluidic device. Following detachment at the rear, the membrane tether (anchor point marked with white arrowhead at $t = 0$ s) swings around to the front of the cell as a sling (marked with white arrow at $t = 3$ s). (B) Epifluorescence-intravital-microscopic images demonstrating sling formation by two different leukocytes rolling in venules of the cremaster muscle of WT (C57BL/6J) mice. Leukocytes were stained by intravenous injection of DyLight-488-conjugated-anti-LFA-1 mAb (green). The slings in front of the leukocytes are marked with white arrows. (C) Scanning electron micrograph showing a sling (red arrow) wrapped around a rolling neutrophil. A tether at the rear is marked with a white arrowhead. View from top of the cell. (D) Isolated mouse bone marrow neutrophils stained with membrane dye DiO (green) and Alexa-Fluor-568-conjugated non-blocking mAb (red) to PSGL-1 were allowed to roll on P-selectin in a microfluidic device and footprints visualized using DqDF. PSGL-1 is expressed in patches (red spots) on the sling (green; marked with white arrow). TIRF excitation 488 and 561 nm lasers and incidence angle, $\theta = 70^\circ$. DqDF images were processed (saturated). Scale bars 5 μm . Wall shear stress 10 dyn cm^{-2} (A, C–D); P-selectin 20 molecules μm^{-2} (A, C–D). Thick horizontal arrows denote rolling direction. (E) Schematic showing how membrane from several microvilli is pulled to form a tether (step I–III). Three representative microvilli are marked as 1, 2 and 3. Tether following detachment becomes a sling (step IV). Microvilli tips are preserved as lobes on the tether or sling. Cell membrane, green. PSGL-1 on microvilli tips and lobes is shown as red circles. (F) Schematic of step-wise peeling mechanism that enables slings to stabilize neutrophil rolling at high shear stress. Cell (green), PSGL-1 (red) and P-selectin on the substrate (blue). PSGL-1 rich patches on the sling shown as clusters of PSGL-1.

Figure 4.

Slings enable LFA-1-ICAM-2 interactions in *trans* (adapted from Sundd et al. (2012)). **(A)** Isolated mouse bone marrow neutrophils stained with membrane dye DiI and DyLight-488-conjugated-anti-mouse-LFA-1 mAb were allowed to roll on P-selectin in a microfluidic device and footprints visualized using DqDF. The DiI footprint was analyzed to generate a 3D-reconstruction of the footprint and the LFA-1 image overlaid to reveal distribution of LFA-1 (green) on sling, tether anchorage points, and microvilli (grey hills) vs. cell surface (valleys). Wall shear stress 10 dyn cm^{-2} . P-selectin $20 \text{ molecules } \mu\text{m}^{-2}$. Thick horizontal arrow denotes the rolling direction. **(B)** Schematics showing the effect of LFA-1-ICAM-2 interactions in *trans* between rolling neutrophils and their slings. Double sided arrows denote the distance, d , of the rearmost load-bearing patch at the inflection point of the sling from the cell center. The distance is smaller in WT than LFA-1 deficient (*Itgal*^{-/-}) neutrophils. LFA-1 on sling (red); ICAM-2 on cell (green); PSGL-1 on sling (violet); P-selectin on substrate (blue).