451c Shear-Induced Mechanical Shedding of L-Selectin on Neutrophils Can Explain the Shear Threshold Effect at Higher Shear

Dooyoung Lee and Michael R. King

L-selectin is constitutively present on the surface of all varieties of leukocytes and plays an important role in tethering and subsequent rolling of leukocytes on the endothelial wall, facilitating their migration into sites of inflammation or secondary lymphoid tissues. It has been reported that the initial inflammation steps via surface L-selectin show a shear threshold effect in which leukocytes are unable to bind to a ligand-presenting surface below a critical level of shear; as the shear increases through a threshold, adhesion of cells increases to a maximum, beyond which it decreases sharply. Other studies have shown that leukocyte activation by chemotactic factors, cross-linking of L-selectin, or hypotonicity results in rapid shedding of L-selectin from the cell surface. We have studied the shear threshold effect in L-selectin-mediated neutrophil rolling on sLe^x-coated surfaces in a parallel plate flow chamber. It was verified using ELISA that higher shear induces neutrophil activation-independent mechanical shedding of L-selectin. In order to investigate the effect of L-selectin shedding on neutrophil rolling, we have tracked individual rolling cells for several millimeters within flow chambers. At 4.0 dyn/cm^2 of wall shear stress, the average rolling velocity of neutrophils increases with time, but not at 1.5 dyn/cm^2. Inhibition of L-selectin shedding using a hydroxamic acid-based protease inhibitor significantly increased the number of rolling cells and the length of time that each neutrophil rolled, while decreasing the average rolling velocity and the variability in cell rolling velocity. We have also performed a computational simulation in which the adhesive dynamics algorithm was modified to include catch-slip bond kinetics, which was able to accurately reproduce the experimentally observed behavior over the entire range of physiologically relevant wall shear stresses. We conclude that the higher shear force applied to neutrophils induces L-selectin shedding mechanically and therefore decreases the adhesion of neutrophils to a ligand-presenting surface in flow. These observations help to resolve the reason why the rolling flux decreases sharply after a maximal level of adhesion as wall shear stress increases in *in vitro* flow chamber experiments.