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The Shear Threshold Effect for Particle Adhesion to Bioreactive Surfaces: Influence of Receptor and Ligand Site Density

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Abstract
Selectins are cell adhesion molecules that mediate capture and rolling adhesion of white blood cells to vascular walls, an essential component of the inflammatory response. Adhesion through L-selectin requires a hydrodynamic shear stress above a threshold level, a phenomenon known as the shear threshold effect. We have reported that the shear threshold effect can he re-created in cell-free systems, in which microspheres coated with the carbohydrate ligand sialyl Lewis x (sLe^x) are perfused over L-selectin-coated surfaces. This paper extends the use of the cell-free system to determine the concurrent influence of receptor and ligand site density on the shear threshold effect. We find that the shear threshold effect diminishes with increasing levels of either L-selectin or sLe^x. At reduced site densities of either L-selectin or sLe^x, the shear threshold effect is present, with maximal rolling observed at a shear stress of 1.2 dynes/cm^2. At higher site densities of L-selectin and sLe^x, the shear threshold effect disappears. These results suggest that the shear threshold relies on the formation of low numbers of receptor-ligand bonds.

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Selectins are cell adhesion molecules that mediate capture and rolling adhesion of white blood cells to vascular walls, an essential component of the inflammatory response. Adhesion through L-selectin requires a hydrodynamic shear stress above a threshold level, a phenomenon known as the shear threshold effect. We have reported that the shear threshold effect can be re-created in cell-free systems, in which microspheres coated with the carbohydrate ligand sialyl Lewis x (sLe^x) are perfused over L-selectin-coated surfaces. This paper extends the use of the cell-free system to determine the concurrent influence of receptor and ligand site density on the shear threshold effect. We find that the shear threshold effect diminishes with increasing levels of either L-selectin or sLe^x. At reduced site densities of either L-selectin or sLe^x, the shear threshold effect is present, with maximal rolling observed at a shear stress of 1.2 dynes/cm^2. At higher site densities of L-selectin and sLe^x, the shear threshold effect disappears. These results suggest that the shear threshold relies on the formation of low numbers of receptor-ligand bonds.

I. Introduction

Selectins are cell adhesion receptors that initiate cell recruitment to a site of inflammation, an essential component of the inflammatory response. The selectin family of cell adhesion molecules, consisting of E-, P-, and L-selectin, mediates capture and dynamic adhesion (referred to as "rolling") of blood borne cells onto activated vascular walls [1]. E- and P-selectin expressed on the vascular wall interact with carbohydrate-presenting ligands on white blood cells; L-selectin expressed on white blood cells binds carbohydrate-presenting ligands on the vessel wall [2,3]. The carbohydrate sialyl-Lewis^x (sLe^x) has been shown to bind all selectins in static and dynamic studies [4-7]. Selectin-sLe^x binding and force-driven unbinding generates transient adhesion and rolling of cells on the vascular wall.

Adhesion through L-selectin has been studied in rolling experiments using neutrophils and the L-selectin ligand CD34. L-selectin-mediated rolling is unique in that a threshold level of hydrodynamic shear is required for initiation of rolling interactions [8]. At wall shear stresses below 0.6 dynes/cm^2, rolling flux is decreased; rolling is maximal at wall shear stresses of ~1.0 dynes/cm^2. A shear stress of 1.0 dynes/cm^2 is physiologically relevant, as this is within the range of measured shear stresses *in vivo* of the venular microvasculature. It has been speculated that the shear threshold effect provides a hydrodynamic switch for regulating initial attachment or accumulation of cells at a site of inflammation. The shear requirement may also help prevent inappropriate leukocyte aggregation in vessels with inherently low wall shear stresses or in hypoperfusion [9].

Cell-free systems are an ideal method for studying receptor-ligand binding under flow, without confounding variables such as cell morphology or rheology. In cell-free experiments, microspheres coated with carbohydrate ligands, such as sLe^x, are perfused over selectin-coated surfaces. We have shown that microspheres coated with sLe^x interact specifically and roll over E-, P-, and L-selectin under hydrodynamic flow [5-7]. The dynamics of rolling in cell-free systems is similar to that observed with cells. Cell-free rolling mediated by L-selectin recreates the shear threshold effect [7], with maximal rolling observed at shear stresses of 0.7 to 1.0 dynes/cm^2. The ability of cell-free systems to capture the shear threshold effect suggests that the origin of the effect is in the interaction between the hydrodynamic forces acting on the particle and the physical chemistry of selectin-ligand interactions, rather than cellular features such as deformability or topography.

In this paper, we extend the use of the cell-free system to determine whether site densities of L-selectin and sLe^x may modulate the shear threshold effect. We find that the appearance of the shear threshold effect may be controlled via either receptor or ligand density.

II. Methods

To prepare surfaces, L-selectin (5.0-15.0 μg/ml) in PBS was incubated on silanated glass slides. L-selectin site densities were estimated by immunolabeling with anti-human L-selectin. To prepare microspheres, a 100 μl solution of 10.0 μg/ml biotinylated carbohydrate in PBS+ was added to 10^6 Superavidin beads and incubated for 1 h at room temp. To vary the site densities of sLe^x on the particle surface, microspheres were incubated with 70% sLe^x/30% Le^x, 90% sLe^x/10% Le^x, or 100% sLe^x. The site density of sLe^x on microspheres was measured by flow cytometry.

Experiments were conducted in a parallel plate flow chamber. Microspheres at a concentration of 5 x 10^5/ml in PBS+ were drawn through the flow chamber by a withdrawal syringe pump. Microsphere interactions with the surface were recorded at 300X magnification.

III. Results

Both receptor site density on surfaces and ligand site density on microspheres were systematically varied to determine the influence of receptor and ligand site density on the shear threshold effect. Overall rolling flux decreases with decreasing L-selectin site density ; overall rolling flux also decreases with decreasing sLe^x site density (Fig. 1). The appearance of the shear threshold effect is dependent on both receptor and ligand site density. At reduced L-selectin site density (3300 molecules/μm^2), the shear threshold effect is present at all sLe^x site densities tested (Fig. 1A). At an L-selectin site density of 4700 molecules/μm^2, the shear threshold effect is not present when sLe^x site density is 95

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molecules/μm^2. When the sLe^x site density is lowered to 70 molecules/μm^2 under these conditions, the shear threshold effect appears (Fig. 1B). At an L-selectin site density of 5200 molecules/μm^2, the shear threshold effect is not evident at sLe^x densities of 95 or 70 molecules/μm^2. When the sLe^x density is lowered to 30 molecules/μm^2 under these conditions, the shear threshold effect appears (Fig. 1C). Taken together, these results suggest that the magnitude of the shear threshold effect diminishes with increasing receptor site density and increasing ligand site density.

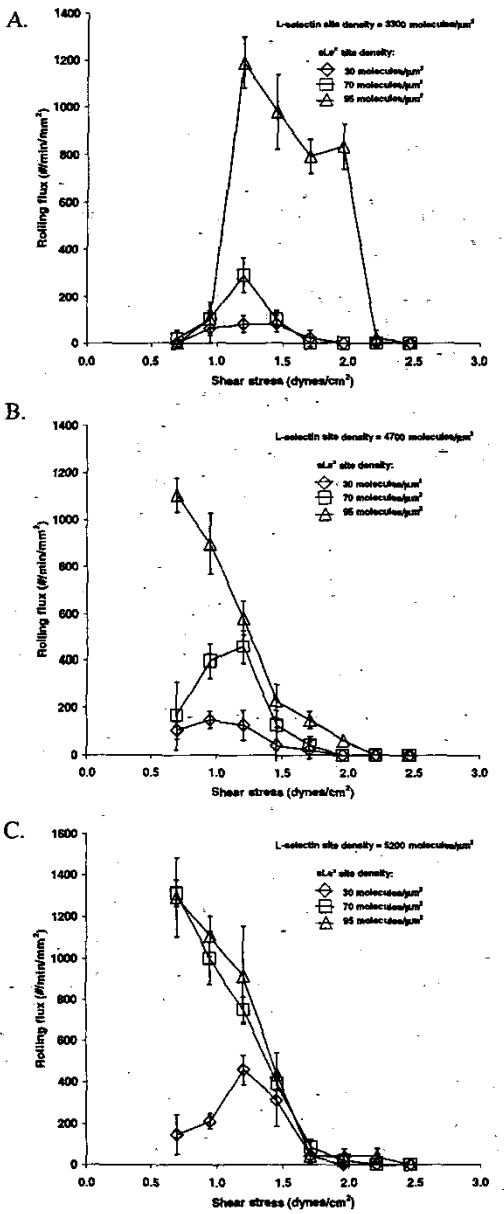

Fig. 1. Rolling flux of sLe^x-coated microspheres over human L-selectin as a function of wall shear stress, receptor site density, and ligand site density. The L-selectin site density is (A) 3300, (B) 4700, (C) 5200 molecules/μm^2.

IV. Discussion

Using the cell-free system, we have shown that the rolling behavior of sLe^x-coated microspheres over L-selectin surfaces may be tuned via receptor and ligand site densities. Rolling exhibits a shear threshold effect, with maximum rolling interactions at a wall shear stress of 1.2 dynes/cm^2. When the shear threshold is observed, the location of the threshold does not change with receptor or ligand site density; maximal rolling is always observed at a shear stress of 1.2 dynes/cm^2, despite changes in molecular site densities. The shear threshold effect is only evident when either receptor or ligand site density is low. At a reduced L-selectin site density of 3300 molecules/μm^2, the shear threshold effect is present regardless of sLe^x site density. At a reduced sLe^x site density of 30 molecules/μm^2, the shear threshold effect is present regardless of L-selectin site density. However, when both receptor and ligand site densities are increased, the shear threshold effect disappears. The ability to reproduce the shear threshold effect in a cell-free system proves that the effect is due to the physical chemistry of receptor-ligand binding.

We propose a mechanism to explain the shear threshold effect and its dependence on molecular binding; the mechanism originates in a recent study from our lab [10], which models the overall reaction rate of species bound to surfaces under relative motion. The study shows that the rate of collision between receptor and ligand increases with shear rate, and the encounter duration decreases. Depending on the rate of bimolecular reaction, increases in shear rate can increase adhesion (by increasing the encounter frequency) or decrease adhesion (by decreasing the encounter time). The shear threshold may occur at shear rates at which these effects are counterbalanced. This suggested mechanism also explains why the shear threshold effect disappears when both L-selectin and sLe^x densities are high. At very high site densities of receptor and ligand, the collision rate is very high, even at low shear stresses, resulting in an increased probability of bond formation.

The shear threshold effect is a potential physiologic mechanism for the regulation of leukocyte attachment and the prevention of inappropriate leukocyte accumulation. The dependence of the shear threshold effect on receptor and ligand density may be of physiologic significance, as it suggests that the effect can be modulated *in vivo* to regulate cell attachment and accumulation at a site of inflammation.

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