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Abstract
White blood cell recruitment from the bloodstream to surrounding tissues is an essential component of the immune response. Capture Of hlood-borne Ieuk'Wks onto vascular endothelium proceeds via a two-step mechanism, with each step mediated by a distinct receptor-ligand pair. Cells first transiently adhere, or "roll" (via interactions between selectins and sialyl-Lewis-x), and then firmly adhere to the vascular wall (via interactions between integrins and ICAM-1). We have reported that a eomputatiokl method called Adhesive Dynamics (AD) accurately reproduces the fine scale dynamics of selectin-mediated rolling [1]. This paper extends the use of AD simulations to model the dynamics of cell adhesion when two classes of receptors are simultaneously active: one class (selectins) with weakly adhesive properties, and the other (integrins) with strongly adhesive properties. AD simulations predict synergistic functions of the two receptors in mediating adhesion. We present this relationship in a two-receptor state diagram, a map that relates the densities and properties of adhesion molecules to various adhesive behaviors that they code, such as rolling or firm adhesion. The predictions of two-receptor adhesive dynamics are validated by the ability of the model to reproduce experimental neutrophil rolling velocities.

Comments
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Dynamic Simulations of Inflammatory Cell Recruitment: The State Diagram for Cell Adhesion Mediated by Two Receptors

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White blood cell recruitment from the bloodstream to surrounding tissues is an essential component of the immune response. Capture of blood-borne leukocytes onto vascular endothelium proceeds via a two-step mechanism, with each step mediated by a distinct receptor-ligand pair. Cells first transiently adhere, or "roll" (via interactions between selectins and sialyl-Lewis-x), and then firmly adhere to the vascular wall (via interactions between integrins and ICAM-1). We have reported that a computational method called Adhesive Dynamics (AD) accurately reproduces the fine scale dynamics of selectin-mediated rolling [1]. This paper extends the use of AD simulations to model the dynamics of cell adhesion when two classes of receptors are simultaneously active: one class (selectins) with weakly adhesive properties, and the other (integrins) with strongly adhesive properties. AD simulations predict synergistic functions of the two receptors in mediating adhesion. We present this relationship in a two-receptor state diagram, a map that relates the densities and properties of adhesion molecules to various adhesive behaviors that they code, such as rolling or firm adhesion. The predictions of two-receptor adhesive dynamics are validated by the ability of the model to reproduce experimental neutrophil rolling velocities.

I. Introduction

Trafficking of blood-borne cells to tissues and organs is necessary for numerous immune functions such as inflammation [2]. Leukocyte recruitment to the vessel wall proceeds via a multi-step process, with each step mediated by distinct cell adhesion molecules. Initially, E- and P-selectin expressed on vascular endothelium interact with cell surface molecules bearing the carbohydrate sialyl-Lewis-x (sLe^x). Selectin-sLe^x binding and force-driven unbinding generates transient adhesion, or "rolling," of leukocytes along the vessel wall. Once leukocytes have been slowed by selectin-mediated rolling, they become activated to firmly adhere to the vessel wall [3]. Firm adhesion is mediated by interactions between cell surface β_2 integrins and endothelial intercellular adhesion molecule-1 (ICAM-1). Different dynamic states of adhesion are thus mediated by different classes of molecules.

While the adhesive states that selectins and integrins support are known, the mechanism by which leukocytes go from rolling to firm adhesion, as well as the precise molecular requirements for leukocyte arrest, remain unclear. Studies in double knockout mice show that deficiency in E- and P-selectin can eliminate the granulocyte-mediated inflammatory response, even when integrins and ICAM-1 are available for firm adhesion [4]. Selectin-mediated rolling is thus necessary for integrin-mediated firm adhesion. However, granulocyte rolling velocities during inflammation are significantly increased in ICAM-1-deficient mice [5], suggesting that ICAM-1 is also required for optimal selectin-mediated rolling.

To clarify the relationship between the molecular properties of adhesive molecules and the macroscopic behavior such as rolling or firm adhesion that they mediate, our laboratory has devised a direct numerical simulation of a spherical cell interacting with a reactive surface under flow [6]. Our model incorporates the hydrodynamics of a sphere rotating and translating near a plane under shear flow [7,8], and force-dependent binding kinetics [9]. The resulting Adhesive Dynamics (AD) simulation accurately reproduces the dynamics of selectin-mediated rolling [10].

This paper extends AD simulations to determine the dynamics of adhesion when two receptor-ligand systems are simultaneously active. One system is characterized by relatively weak adhesive properties, such as a low association rate and high unstressed dissociation rate which suggest a propensity for rolling interactions, as are experimentally observed with the selectin-sLe^x system. The second system is characterized by relatively strong adhesive properties, such as a high association rate and low unstressed dissociation rate, as are experimentally observed with the activated integrin-ICAM-1 system. AD simulations predict that the two receptor-ligand systems have synergistic roles in promoting cell adhesion.

II. Methods

The AD method has been extensively described [1]. The simulation begins with a freely moving "cell" or receptor-coated particle, modeled as a sphere with receptors distributed over its surface. A large number of adhesion molecules are randomly placed on the surface of the sphere and a bounding wall. The tip of each free adhesion molecule reacts with its counter-receptor with association rate k_f and dissociation rate k_r. We use the Bell model [9]:

$$k_r = k_r^0 \exp\left[\frac{\gamma_0 F}{k_b T}\right] \qquad (1)$$

which relates the rate of dissociation k_r to the magnitude of the force on the bond F.

Once k_r is set by Eq. 1, the association rate k_f can be calculated from the Boltzmann distribution for affinity:

$$\frac{k_f}{k_r} = \frac{k_f^0}{k_r^0} \exp\left[\frac{-\sigma|\mathbf{x}_b - \boldsymbol{\lambda}|^2}{2k_b T}\right] \qquad (2)$$

where σ is the Hookean spring constant and $|\mathbf{x}_b - \boldsymbol{\lambda}|$ is the deviation bond length.

During each time step of the simulation, bond formation and breakage are simulated by a Monte Carlo lottery, in which random numbers are compared with the probabilities for binding and unbinding to determine whether a bond will form or break in the time interval. The bond stresses, f, are

calculated from the distance between the end points of the attachment, L, using Hooke's law, $f = \sigma(L - \lambda)$. The stress contributed by each bond is summed to determine the total force and torque exerted by the bonds on the cell. In addition to the bonding forces, we include colloidal forces, and the external force and torque imparted to the cell by fluid shear to compute the net force and torque acting on the cell. The motion of the particle is obtained from the mobility matrix for a sphere near a plane wall in a viscous fluid [7,8]. The new positions of bond tethers at $t + dt$ are updated from their positions at t, using the cell velocity.

III. Results

To determine the adhesive behavior expected for a given combination of receptor densities, we calculated a state diagram for two-receptor adhesion (Fig. 1), in which observed adhesive behaviors are plotted for a range of selectin and ICAM-1 surface densities. The boundary separating the states of rolling adhesion and firm adhesion is parametrized by a mean velocity of $0.02V_R$. In this state diagram, the rate constants and mechano-chemical properties are fixed; the shear rate is 100 s^{-1}. For integrin-ICAM-1 interactions, rate constants reflecting activated β_2 integrin are used. The boundary separating rolling and firm adhesion is calculated for three different integrin-ICAM-1 association rates, $k_{f,integrin}^0$ = 1000, 100, and 10 s^{-1}.

The state diagram demonstrates synergistic functions of the two receptor-ligand systems in promoting cell adhesion and arrest. For example, at a selectin site density of 30 molecules/μm^2, the state diagram predicts rolling adhesive behavior if no ICAM-1 is present on the surface. At an ICAM-1 site density of 3 molecules/μm^2, the state diagram predicts rolling adhesion if no selectin is present on the surface. However, the combination of 30 molecules/μm^2 selectin and 3 molecules/μm^2 ICAM-1 results in firm adhesion, when $k_{f,integrin}^0$ is greater than 100 s^{-1}. As $k_{f,integrin}^0$ decreases, the location of the firm adhesion envelope shifts to slightly higher ICAM-1 densities, and the synergy between the two receptors is somewhat less pronounced though still present. Instead, at low $k_{f,integrin}^0$, there are critical values of both selectin and ICAM-1 density necessary for the transition to firm adhesion.

Therefore, addition of ICAM-1 on the surface for a cell rolling on a fixed level of selectin facilitates firm binding, and the presence of selectin facilitates firm binding at fixed levels of integrin and ICAM-1. Clearly, the two molecular pairs work synergistically to support firm binding.

We were interested in examining the validity of our model, by comparing simulation predictions to experimental results from the literature. Kunkel and coworkers [11] used intravital microscopy to track individual leukocytes in the microcirculation of wild-type mice, CD18 (β_2 integrin) -/- knockout mice, and E-selectin -/- knockout mice following TNF-α induced inflammation. These investigators found that neutrophil rolling velocities in wild-type mice were significantly lower than rolling velocities in CD18-/- or E-/- mice. AD simulations reproduce this result (Fig. 2).

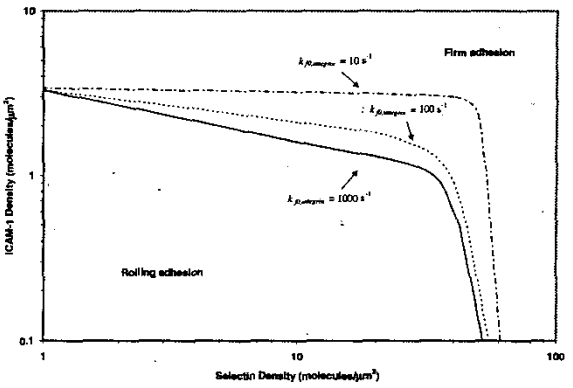

Fig. 1. The state diagram for adhesion mediated by two receptors. For each rolling state, the boundary represents a mean velocity of $0.02V_R$.

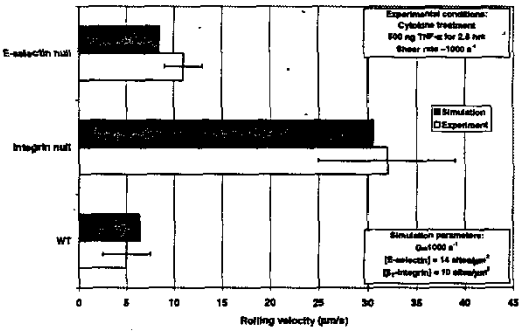

Fig. 2. Comparison of two-receptor adhesive dynamic simulations to experimental data from Kunkel and coworkers [11]. This calculation is performed at $k_{f0,integrin}$ = 1000 s^{-1}.

IV. Discussion

Using two-receptor adhesive dynamics, we have constructed a state diagram for two-receptor adhesion. The state diagram illustrates that selectin-sLe^x interactions and integrin-ICAM-1 interactions have synergistic functions in promoting cell adhesion and arrest. The predictions of two-receptor adhesive dynamics are validated by the ability of the model to reproduce, both qualitatively and quantitatively, in vivo neutrophil rolling velocities. Simulation parameters that independently reproduce neutrophil rolling velocities from E-/- and CD18-/- knockout mice can be combined to reproduce the two-receptor-mediated rolling of neutrophils in wild-type mice.

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