In Silico White Blood Cell: Mechanisms Underlying Leukocyte Rolling and Adhesion During Inflammation

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Abstract

We have used the synthetic modeling method to construct a multilevel, agent oriented, in silico analogue of an in vitro experimental system for studying leukocyte rolling, activation, and adhesion during inflammatory conditions. We specify capabilities that the envisioned analogues must have to achieve long-term goals. Here, we report progress towards our goal of using variants of this model as experimental systems for exploring the potential role of hypothesized mechanisms that are thought to mediate leukocyte rolling, activation, and adhesion. We focus initially on diffusion and clustering events of the LFA-1 integrin receptor on the leukocyte membrane during rolling and adhesion.

1. INTRODUCTION

Rolling, activation, and adhesion on endothelial cell surfaces are necessary steps for the proper recruitment of leukocytes from blood to sites of inflammation [1]. Leukocyte adhesion has been modeled using inductive, equation-based methods. These models have brought insight into how putative biophysical properties of leukocyte receptors can affect cell rolling and adhesion [2-3]. However, new models and methods are needed to better understand the complex set of internal interactions between leukocyte components and endothelial cells and the resulting leukocyte behaviors.

A goal in systems biology research is to understand how molecular level interactions give rise to system level behaviors and properties. That task requires having plausible, adequately detailed design plans for how components at various system levels are thought to fit and function together. Advanced modeling and simulation methods are needed that can be used to test the plausibility of candidate ideas and design plans. Demonstrating that a design plan is functionally plausible requires assembling individual components according to that design plan, and then showing that the constructed device, an analogue – on its own – exhibits behaviors that match those observed in the original biological system. This can be done in silico using the synthetic modeling method in which object-oriented software components are designed, verified, plugged together, and operated in ways that represent the mechanisms and processes according to the design plan. We show that some components will need to function autonomously. The greater the similarity between the measured behaviors of our in silico devices and the observed behaviors in the original biological system, the more useful that in silico system will become as a scientific research tool and as an expression of the coalesced, relevant knowledge of the system. The expectation is that increasing behavioral similarity between actual leukocytes in context and or analogue systems will require, and can be achieved in part through, similarities in design plan and in generative mechanisms.

We previously constructed, verified, and validated an in silico synthetic model of leukocyte rolling, activation, and adhesion [4]. Here, we report progress towards our goal of using the model as an experimental system for exploring the potential role of hypothesized mechanisms that are thought to mediate leukocyte rolling, activation, and adhesion. In this report we have focused initially on the potential role of LFA-1 diffusion and clustering events on the leukocyte membrane during rolling and adhesion. How important a role could each of these hypothesized mechanisms play in determining leukocyte adhesion? Answers to such questions will help us to gain deeper insight into the key events that may determine when and how leukocytes are able to adhere to endothelial cell surfaces during inflammatory conditions.

2. BIOLOGICAL BACKGROUND

Rolling, activation, and adhesion are a complex set of processes. A key feature involves concurrent ligand binding events between leukocyte and the endothelial cells (Fig 1) that involve several different receptor-ligand pairs along with local activation signals between receptors found on the leukocyte membrane.

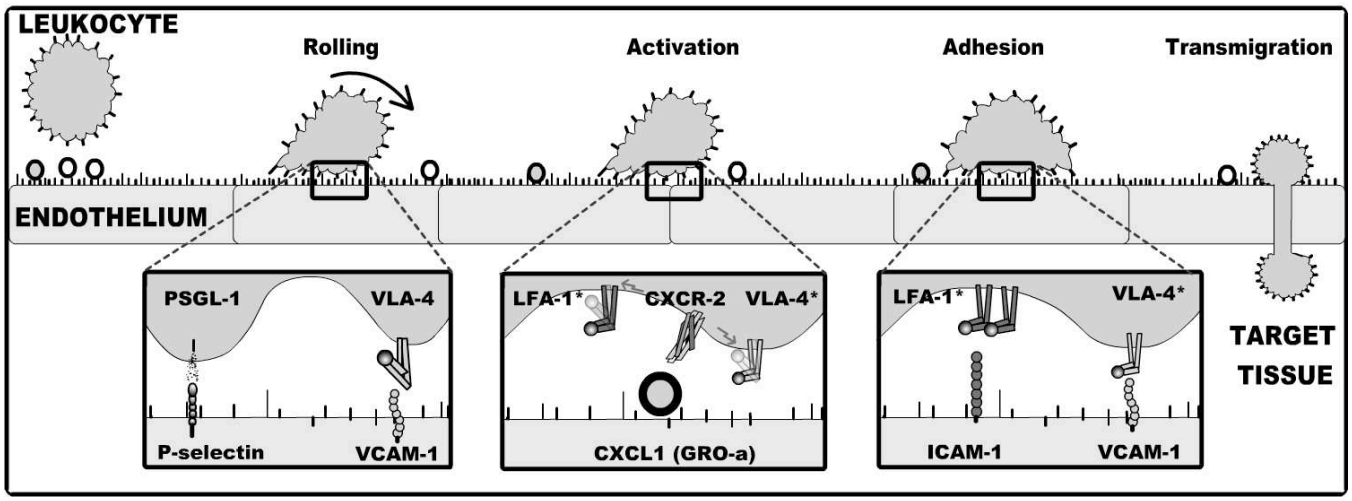

Figure 1. Cartoon Depicting the Roles of the Relevant Receptor-Ligand Pairs During Rolling, Activation, and Adhesion. Integrins labeled with asterisks, as in LFA-1* and VLA-4*, indicate integrins in a high-affinity conformational state.

The selectin family of receptors is primarily responsible for leukocyte rolling, while integrin receptors exclusively mediate transitions from rolling to adhesion [5]. The integrins exist natively in low avidity[1] states to prevent leukocytes from sticking non-specifically to blood vessel walls. Under inflammatory conditions, endothelial cells present immobilized chemokine molecules on their apical surface. As leukocytes roll along the activated endothelial cells, they detect these chemokines via chemokine receptors. Chemokine receptors initiate spatially restricted intracellular signaling events that trigger local integrins into high avidity states that allow the leukocyte to firmly adhere to the vessel wall.

Two non-mutually exclusive mechanisms have been proposed to explain how integrins transition into high avidity states. The first mechanism involves a change in the affinity of the integrin for its ligand. An integrin molecule can exist in one of multiple discrete conformational states, each with distinct ligand-binding properties. Chemokine receptor signaling can initiate the switching of nearby integrins into higher affinity states enabling them to form longer lasting bonds. The second mechanism is through a dynamic reorganization of the integrins into multi-molecular assemblies (called nanoclusters and polar patches) that are thought to increase the chance of forming bonds. This second mechanism is still conceptual because the effects of different integrin spatial distributions on bond formation rates at the interface cannot be visualized and quantified. Using the modeling and simulation approach described below, our goal has been to separately instantiate each mechanism in silico and then ascertain which one provides the best representation of the available observations.

[1] Avidity is a term that describes the combined strength of multiple bond interactions.

3. METHODS

We have used RePast as our modeling and simulation framework. It is a java-based software toolkit developed at the University of Chicago for creating and exercising agent-based models (http://repast.sourceforge.net/). The libraries provided are used to create, run, display, and collect data.

3.1 Argument for an Agent-Oriented Approach

Informative, mechanistically realistic, physiologically based, synthetic models like the ISWBC will need to exhibit several capabilities. The following nine have been adapted from [4].

1. *Turing test*: ISWBC data are, to a domain expert (in a type of Turing test), experimentally indistinguishable from the referent wet-lab leukocyte rolling and adhesion data; achieving this capability provides validation evidence, and requires the in silico device to be suitable for experimentation.
2. *Phenotype overlap*: Attributes of an abstract, but mechanistically realistic analogue should overlap attributes of the referent wet-lab system. Measures can be used to document the attributes exhibited by the analogue and the referent system. As the analogue evolves, measurements of it are expected to increasingly overlap corresponding measures of the referent wet-lab system's attributes.
3. *Mappings*: Observables at any in silico level, whether measured or not, are designed to be consistent with those of the referent system. Doing so enables iteratively concretizable mappings between referent and in silico components and mechanisms.

4. *Local mechanisms*: The behaviors that emerge during a simulation are the consequences of local mechanisms—local component interactions.
5. *Transparency*: Simulation details, as they unfold, need to be visualizable, measurable, and comparable where feasible to those of the wet-lab system.
6. *Adaptability*: In addition to flexibility and reusability, an analogue's components must be constructed so that they can be adapted to function as components in other physiologically based models, such as organ and whole organism models.
7. *Reusability*: Analogue components can be designed to be autonomous and thus can be easily reconfigured to represent different cell types, experimental conditions, and compounds, *alone or in combination*. It must be easy to simulate and analyze outcomes of several different in silico experiments in a fraction of the time (and at a fraction of the cost) required to complete the wet-lab experiments.
8. *Flexibility*: It must be relatively simple to increase or decrease detail in order to simulate an additional phenotypic attribute or change usage and assumptions, without requiring significant reengineering. Achieving this capability will enable cycles of scientific testing and analogue refinement, as new wet-lab data becomes available. Having flexible methods will enable improved, more realistic and heuristic analogues.
9. *Components articulate*: Because system attributes of interest are expected to result from interactions of autonomous components, to study alternative mechanisms it must be easy to join, disconnect, and replace them without having to reengineer the in silico analogue or its components.

Everything except *Local mechanisms* can be achieved without agents. *Local mechanisms* and *Adaptability* both strongly suggest using an agent-oriented approach. *Phenotype overlap*, *Mappings*, *Transparency*, *Reusability*, *Flexibility*, and *Articulation* only weakly suggest agency but strongly suggest that the modeling approach be object-oriented.

3.1 The In Silico Analogue

The in silico system we have constructed is designed to represent and be an experimentally useful analogue of the parallel plate flow chamber system that is used to study leukocyte rolling and adhesion *in vitro* (Fig. 2). The surface of the bottom plate of the flow chamber is coated with uniform densities of various ligands of interest. Leukocytes in solution are perfused through the chamber and their interactions with the substrate-coated surface are recorded using videomicroscopy. Our in silico system is a discrete event, discrete space, discrete time analogue of the entire *in vitro* experimental system (Fig. 2). We use the synthetic modeling method. Object-oriented software components were designed, verified, plugged together, and then operated in ways

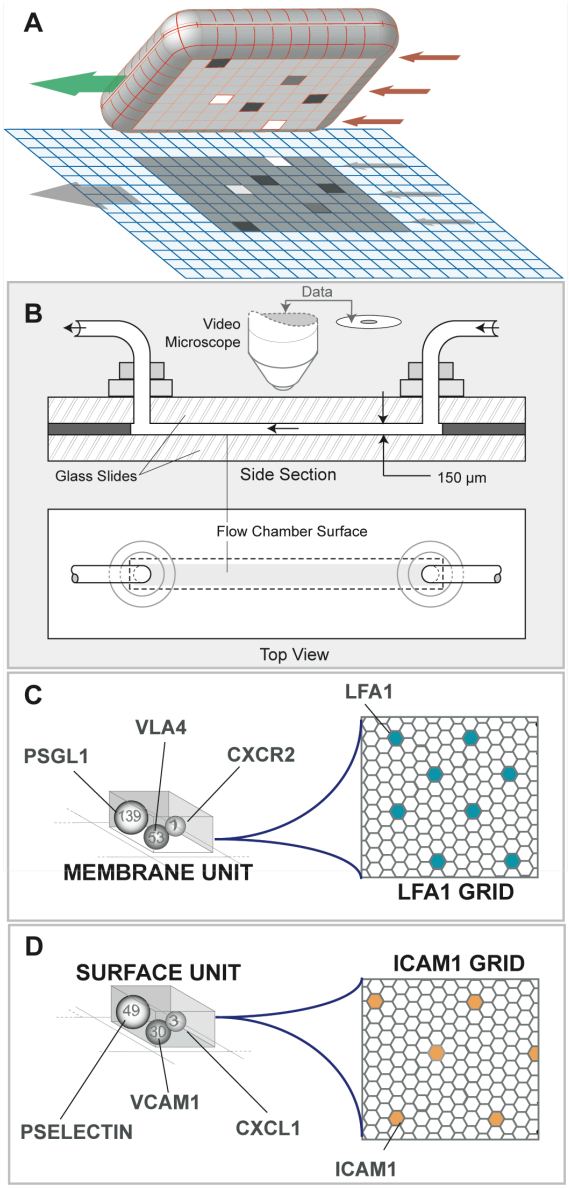

Figure 2. Sketches of in vitro and in silico experimental system components. (A) A LEUKOCYTE object is shown pulled away from the simulated flow chamber surface to which it was attached. The left arrow indicates ROLL direction; the three right arrows indicate SHEAR resulting from the simulated flow. The simulated flow chamber surface is discretized into independent units of function called SURFACE UNITS. The LEUKOCYTE'S MEMBRANE is similarly discretized into matching units of function called MEMBRANE UNITS: 600 total (20 x 30). The 8 x10-shaded region on the SURFACE and on the underside of the LEUKOCYTE identifies the CONTACT ZONE. The UNITS within the CONTACT ZONES that are shaded differently indicate different numbers of BONDS had formed between LIGAND–LIGAND pairs in overlapping UNITS; otherwise, no BONDS formed. ROLLING is the result of a sequence of forward ratchet events. One ratchet event is the result of one row of MEMBRANE UNITS being released at the rear of the CONTACT ZONE along with engagement of a new row of at

the front of the CONTACT ZONE. One ratchet event maps to a leukocyte rolling 1 μm (relative to the flow chamber surface). (B) A shop drawing of a typical parallel plate flow chamber used for in vitro studies of leukocyte rolling and adhesion. A video microscope is used to record leukocyte behaviors. (C) A MEMBRANE UNIT is illustrated. Each MEMBRANE UNIT is simulated using a software object functioning as a container. All leukocyte membrane functionality (relevant to these studies) within each UNIT is represented by four objects functioning as agents: PSGL1, VLA4, CXCR2, and LFA1. The microvillar receptor agents PSGL1, VLA4, CXCR2 are shown as spheres, where each number on the sphere indicates the number of receptors each agent represents. (D) A SURFACE UNIT is illustrated. Similar to MEMBRANE UNITS, each SURFACE UNIT is simulated using a software object functioning as a container. All flow chamber surface functionality (relevant to these studies) within each UNIT is represented by four objects functioning as agents: PSELECTIN, VCAM1, CXCL1, and ICAM1.

that represent the mechanisms and processes believed responsible for leukocyte rolling and adhesion. To avoid confusion and clearly distinguish in silico components, features, measurements, and events from their *in vitro* counterparts, such as a leukocyte and bonds, we use SMALL CAPS when referring to the in silico analogues (Table 1).

3.2 Computational Spaces

Details of the model have been reported [4]. MEMBRANE and SURFACE objects represent the leukocyte membrane and the surface of the chamber, respectively. For simplicity, each is implemented as a 2D toroidal lattice. With our current parameter values, one SURFACE grid space maps to 1 μm^2 of parallel plate surface area and when rolling or adhered, one MEMBRANE grid space maps to the same amount of surface area on the cell membrane.

For exploration of the role of LFA-1 integrin lateral mobility and clustering, we have increased the spatial resolution of the model by placing a hexagonal grid within each MEMBRANE and SURFACE UNIT. So doing represents the cell body surface where LFA-1 and ICAM-1 are located in vitro (Fig. 2C-D). Each hexagonal grid space maps to approximately 100 nm^2 of leukocyte membrane or flow chamber surface space.

3.3 RECEPTOR Objects

In previous versions [4], the receptors found at the tips of microvilli (PSGL-1, VLA-4, and CXCR-2) were represented by the RECEPTOR objects PSGL1, VLA4, AND CXCR2. Each one represented several binding molecules of the same type that may be found within a discrete area within the referent system. For example, a PSGL1 RECEPTOR represented several PSGL-1 adhesion molecules found within a specified area. The number represented by that object is determined by its parameter, *TotalNumber*.

In the current version of the ISWBC, we introduce an additional RECEPTOR object, LFA1. It represents the integrin adhesion molecule LFA-1 that is found on cell membranes between microvilli. Distinct from the other RECEPTOR objects, each LFA1object will represent single LFA-1 molecule.

Table 1. Table of biological aspects from the in vitro flow chamber system and their in silico analogue counterparts.

Biological Aspects	Model Components
Substrate-Coated Surface	Lattice Grid: SURFACE
Functional Unit of the Substrate-Coated Surface	Lattice Grid Unit: SURFACE UNIT
Leukocyte	Object: LEUKOCYTE
Leukocyte Membrane	Lattice Grid: MEMBRANE
Functional Unit of Leukocyte Membrane	Lattice Grid Unit: MEMBRANE UNIT
Chemokine	Object: CXCL1
Chemokine Receptor	Object: CXCR2
Adhesion Molecule Receptors	Object: ADHESION MOLECULE
Tensile Force on Rear Bonds Due to Shear	Parameter: *RearForce*
Hypothesized Biological Mechanisms	Algorithm: Operating Principles

3.4 The Behaviors and Interactions

The ISWBC experiments are analogous to those performed using an in vitro flow chamber system. While in the FLOW CHAMBER, LEUKOCYTES use their RECEPTORS to interact and form BONDS with the SUBSTRATE-coated surface. Those interactions are recorded and measured.

The ISWBC consists of three levels of spatial resolution: LEUKOCYTE-level, MEMBRANE/SURFACE UNIT-level, and LFA1 grid/ICAM1 grid-level. High-level behaviors are dependent upon the collective operation of agents at each of the lower levels contained within. For example, the behavior of MEMBRANE and SURFACE UNITS are emergent properties of the agents contained within each. Similarly, the behavior at the LEUKOCYTE-level is dependent upon the collective events that occur within the underlying MEMBRANE/SURFACE UNITS (Fig. 3A-C). Conversely, events at the highest level impose constraints upon lower levels. For example, the positioning and movement of the LEUKOCYTE on the SURFACE dictate which MEMBRANE and SURFACE UNITS are overlapping and can interact.

3.5 MEMBRANE/SURFACE UNIT-level (BOND Formation and Dissociation)

BOND formation events occur when a RECEPTOR in a MEMBRANE UNIT encounters its partner in an overlapping SURFACE grid subsection in the CONTACT ZONE. For each potential BOND, the value of parameter Pon for that RECEPTOR-LIGAND pair is compared to a randomly generated number between 0 and 1 to determine if that potential

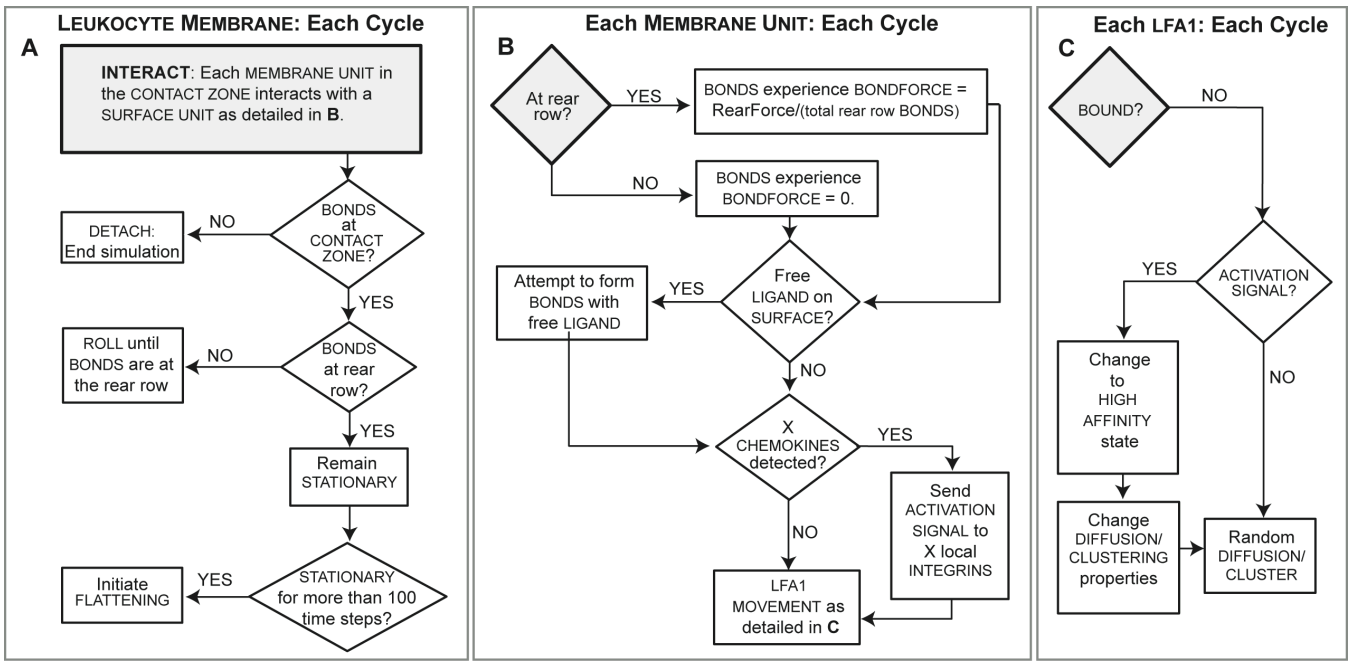

Figure 3. The decisional process for the LEUKOCYTE MEMBRANE, each MEMBRANE UNIT, and each LFA1 agent during a simulation cycle. (A) The LEUKOCYTE steps through its decisional process only once during a simulation cycle. At the start of the cycle, the MEMBRANE instructs all MEMBRANE UNITS within the CONTACT ZONE to follow the decisional process in B. Once that process is complete, the MEMBRANE completes its process by selecting and following the one applicable action option. (B) The state of each MEMBRANE UNIT depends on the properties of the RECEPTOR objects contained within. During each simulation cycle, each MEMBRANE UNIT, selected at random, uses this decisional process to update its status relative to the SURFACE UNIT over which it is positioned. (C) The LIGAND-BINDING properties and spatial movement of an LFA1 agent are dependent on its state, which may change upon local ACTIVATION events.

BOND becomes an actual BOND. The effect of shear on the rear of a leukocyte is represented by the variable *RearForce*. BONDS at the rear experience a *bondforce* that is calculated each simulation cycle by dividing the *RearForce* value by the total number of BONDS in the rear row of the CONTACT ZONE. BONDS within the rest of the CONTACT ZONE experience no *bondforce*. Drawing from in vitro data, we have assumed a simple linear relationship between *bondforce* and the probability of BOND dissociation. It is calculated as (probability of dissociation) = b_0 + (*bondforce*) x b_1, where b_1 and b_0 are the slope and intercept, respectively, of the line segment associated with a specific *bondforce*. Each type of simulated adhesion molecule pair uses a unique set of b_0 and b_1 values.

Local ACTIVATION of INTEGRIN agents occurs when a CHEMOKINE RECEPTOR in a MEMBRANE UNIT detects a CHEMOKINE in an overlapping SURFACE UNIT. An ACTIVATION SIGNAL is sent to local INTEGRIN agents.

3.6 LFA1 Grid/ICAM1 Grid-level (LFA1 Diffusion)

If an ACTIVATION SIGNAL is detected, the LIGAND-BINDING and DISSOCIATION properties of a local LFA1 agent changes from a state with low affinity properties to one of higher affinity through a change in the parameter values of *Pon*, b_0, and b_1.

The DIFFUSIVE/CLUSTERING properties of LFA1 are also dependent on its state. The parameter, *Pmove*, determines the probability that an LFA1 object will move into one a neighboring space within the LFA1 grid. The value of *Pmove* is dependent on its state. At each simulation cycle, *Pmove* is compared to a randomly generated number between 0 and 1 to determine if the move event occurs. LFA1 has an equal probability of moving into any of its six neighboring spaces, but cannot move into an already occupied grid space.

3.7 LEUKOCYTE-level (LEUKOCYTE ROLLING)

LEUKOCYTE behavior is determined by the number and location of BONDS at the MEMBRANE UNIT-level. If there are BONDS within the rear column of the CONTACT ZONE, the LEUKOCYTE PAUSES, or remains STATIONARY, until the next simulation cycle. If there are no BONDS within the rear column of the CONTACT ZONE, the LEUKOCYTE, influenced by the simulated shear force, performs a forward ROLLING movement. ROLLING is the result of a sequence of forward ratchet events. The process involves removing a column from the rear of the rectangular interaction zone of the MEMBRANE while a new one is placed at the front of the zone above the SURFACE.

3.8 The In Silico Experimental Method

An in silico experiment consists of building the software into an executable, creating and editing the parameter vector, and beginning the simulation. An experiment on an individual leukocyte consists of a single run with the output representing the results of the experiment. An experiment on a population of leukocytes can also be executed by performing a batch run [4]. Depending on the experiment type, simulation output can consist of LEUKOCYTE positions, BONDS and RECEPTOR information at each time step. At the end of the experiment, one of several data reduction scripts may be run to process and analyze the simulation results. Examination and processing of data from simulations used a combination of Matlab (7.0.0) and Microsoft Excel.

3.9 ISWBC Development

Code is managed using CVS with a single HEAD branch and ChangeLogs for each commit. Experiments are conducted using the last stable version of the HEAD branch. As an experiment runs, simulation data is kept in memory until the end of the experiment, at which time it is written to comma separated files indexed by filename.

Development follows a loose and iterative specification, implementation, test, and commit process. Each specific change is documented in the ChangeLog. The changes are then committed to CVS; there is one CVS module for the entire framework.

4. RESULTS

An initial objective in determining the role of LFA1 lateral diffusion and clustering on leukocyte rolling and adhesion is to first determine lateral mobility parameters of our LFA1 objects such that they have similar diffusive properties as observed in vitro. We explored several parameter values for *Pmove* and calculated the diffusion coefficients using Eq 1. $<r^2>$ is the MSD, t is the time interval, D is the time-dependent diffusion coefficient, and the α coefficient classifies the mode of anomalous diffusion.

$$<r^2> = 4Dt^{\alpha} \qquad \text{[Eq. 1]}$$

Cairo et al. used single-particle tracking to determine the diffusion coefficients of LFA-1 on peripheral blood lymphocytes prior to and after activation with phorbol-12-myristate-13-acetate [6]. They observed in all cells before and after activation the existence of two subpopulations of LFA-1, an immobile and a mobile subpopulation, distinguished by their diffusive properties.

We first sought *Pmove* parameter values that would allow our LFA1 objects to exhibit similar diffusive properties as those in the mobile and immobile subpopulations. In their calculations of the diffusion coefficients, Cairo et al. observed values of α that were consistent with Brownian diffusion ($0.7 < \alpha < 1.2$), and therefore we calculated D values using an α value of 1. We determined that a *Pmove* of 0.1 gave similar diffusive properties to those of the immobile subpopulation, while a *Pmove* of 0.5 yielded similar diffusive properties to the mobile subpopulation (Fig. 4).

5. DISCUSSION

The primary objective of this project has been to develop novel in silico models that can be used for experimentation and for studying and exploring how components at the molecular level may interact to yield white blood cell-level and population-level behaviors during inflammation. Leukocyte rolling and adhesion has been modeled using inductive, equation-based methods. The discrete-time models by Hammer and co-workers are the most developed to date [2,3]. In their models, leukocytes are idealized as solid spheres decorated with rod-like microvilli containing receptors at their tips. With their simulations they have

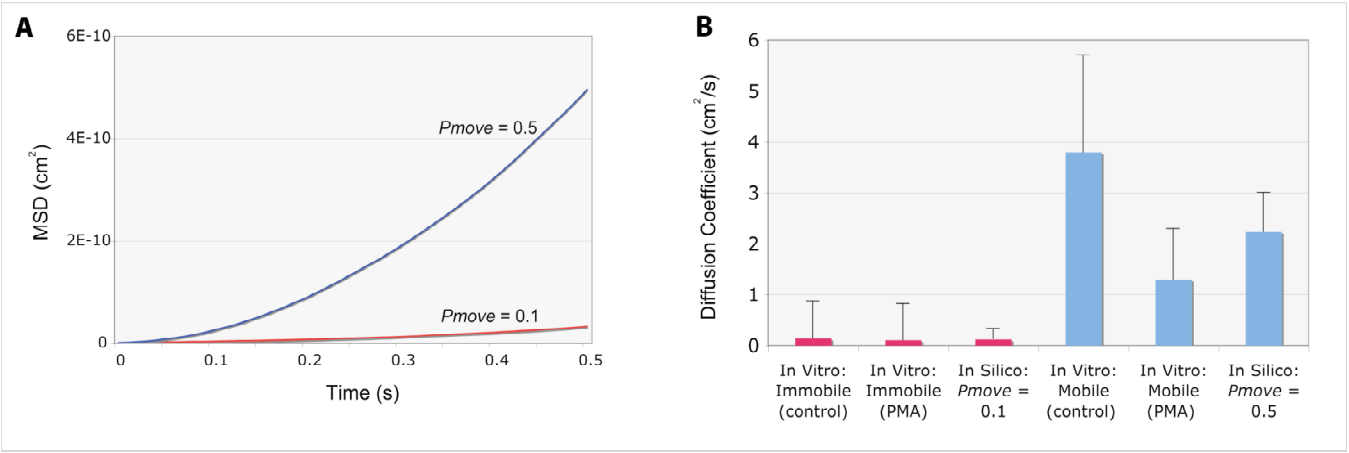

Figure 4. Simulation results. A) Shown is a plot of Mean Square Displacement with time interval for two example-simulation-runs using *Pmove* values of 0.1 and 0.5. B) Comparison of in vitro LFA-1 diffusion coefficients for the immobile and mobile subpopulations (with and without PMA stimulation) with calculated diffusion coefficients for in silico LFA1.

explored how putative molecular properties of adhesion molecules, such as reaction rates and bond elasticity, may relate to macroscopic behavior such as rolling and adhesion [2,3].

Such inductive models are often fragile to context. When they target specific phenomena and constrain their use to account for specific sets of data, it becomes difficult to extend the model to different experimental circumstances or to help explain different phenomena or particular examples of individual behavior. It becomes difficult to explore potential mechanistic differences between individual cell behaviors, for example. Our ISWBC was designed in part to help circumvent those problems.

We constructed and refined our ISWBC using an iterative method, within which we target key phenotypic attributes from a list of desired leukocyte behaviors and properties. The ISWBC is modified, refined, and revalidated such that it can reproduce these targeted attributes. This process is repeated so that each iteration results in increased overlap between ISWBC behaviors, properties, and characteristics with leukocyte behaviors observed from in vitro and in vivo experiments. The current ISWBC is capable of mimicking only a few of a long list of desired phenotypic attributes of leukocytes observed in vivo and in vitro.

We have previously shown that the ISWBC can successfully mimic in vitro data of rolling, activation, and adhesion on P-selectin, VCAM-1, and CXCL1 substrate at the cell-level and population-level [4]. Here, we report progress towards refining and extending the ISWBCs so that they can be used as experimental systems for exploring the potential role of hypothesized mechanisms that are thought to mediate leukocyte rolling, activation, and adhesion. Our initial focus has been on the potential role of LFA-1 diffusion and clustering events on the leukocyte membrane during rolling and adhesion. Does the rate of diffusion affect bond formation or bond reformation rates? What is the relative time scale for LFA-1 clustering such that these events would be relevant to leukocyte rolling and adhesion? Answers to these questions will help us to gain a better understanding of the spatiotemporal events and key rules of engagement that may determine when and how leukocytes are able to adhere to endothelial cell surfaces during inflammatory conditions.

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