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Simulating Leukocyte-Venule Interactions – A Novel Agent-Oriented Approach

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Abstract—**Leukocyte recruitment into sites of inflammation involves a complex cascade of molecular interactions between the leukocyte and the endothelial cells of the inflamed venule. This report proposes a novel agent-oriented approach for simulating leukocyte-venule interactions during inflammation. We focus on modeling and simulating the initial steps of rolling, activation, and firm adhesion of neutrophils on TNF-α-treated mouse cremaster muscle venules.**

Keywords—**Adhesion, Agent-Based Model, Endothelium, Inflammation, Leukocyte, Rolling, Transmigration**

I. Introduction

Leukocyte recruitment into sites of inflammation involves a complex cascade of molecular interactions between the leukocyte and endothelial cells of the inflamed venule. Initial interactions are primarily mediated by the selectin family of receptors and their respective carbohydrate ligands found on the membranes of both leukocytes and endothelial cells. Selectin-ligand interactions are transient due to the fluid force the leukocyte experiences within the venule. As selectin-ligand bonds are constantly formed at the leading edge of the leukocyte, bonds at the trailing edge are broken, which causes the leukocyte to undergo rolling [1]. Each selectin molecule is believed to have a characteristic rate of bond association and dissociation and thus rolling velocities are different when mediated by different selectin molecules.

As leukocytes roll along the venular surface, they can detect chemokines presented on the membrane of activated endothelial cells via chemokine receptors. Upon chemokine detection, intracellular signaling events are triggered that activate the leukocyte. The result is an increased affinity and avidity (clustering) of the integrins, another class of receptors found on leukocytes. When in a high affinity state, these integrins are capable of forming strong interactions with endothelial cell adhesion molecules that are believed to enable leukocytes to decrease their rolling velocity and eventually firmly adhere to the vessel wall [2]. In neutrophils, it has also been observed that engagement of the selectins and integrins with their ligands can lead to intracellular signaling events and activate the leukocyte [3]. Once firmly adhered to an endothelial cell, a leukocyte undergoes diapedesis: they apparently crawl between adjacent endothelial cells and into the target tissue.

Here, we propose a novel computational approach for simulating the events and interactions described here. We use an agent-oriented approach and represent this system as a collection of autonomous (software) entities, or agents, making decisions on how to interact with their local environment based on a set of encoded rules. Our initial simulation experiments are designed to represent neutrophil rolling, activation, and firm adhesion on TNF-α-treated mouse cremaster muscle venules.

II. Model Structure

The proposed agent-based model contains *Ligand*, *Cell, Membrane,* and *Space* interfaces[1]. Classes that implement the *Ligand* interface represent biological molecules that can form bonds with other macromolecules. Classes that implement the *Membrane* interface represent cellular membranes. Cells within the biological model will correspond to classes that implement the *Cell* interface. Lastly, classes that implement the *Space* interface represent environments within which the objects can interact.

A. Ligand

The *Ligand* interface is implemented by *AdhesMolecule*, *Chemok, and ChemokReceptor* classes. *AdhesMolecule* objects represent the behaviors of adhesion molecules such as the selectins, integrins, and cellular adhesion molecules. Each *AdhesMolecule* object has parameters that are used to calculate a probability that it will form an interaction with its *AdhesMolecule* LIGAND[2]. Such parameters include the total number of molecules that the *AdhesMolecule* object represents, as well as an affinity and avidity value. *AdhesMolecules* also contain parameters that are used to calculate probabilities that they will break their interaction with their LIGAND. The values for these parameters are dynamic and may change during the course of a simulation to reflect the events that occur in the biological model during cell activation by chemokine detection or by engagement of the selectins and integrins with their ligands. Rules that determine how these parameters will be updated are encoded within the *AdhesMolecule* objects.

Chemok objects represent chemokine signaling molecules, and *ChemokReceptor*s correspond to the chemokine receptors that bind them. Knowledge of which *AdhesMolecules* are affected upon formation of

[1] We use Sun Microsystem's definition of an interface used in the Java programming language, which is a named collection of method definitions. An interface defines a set of methods but does not implement them. A class that implements an interface agrees to implement all the methods in that interface, thereby agreeing to certain behavior [4].
[2] The same word may be used to refer to a biological molecule, component, or event and the in silico object or event that represents them. In the latter case the word is written using small caps.

ChemokReceptor-Chemok LIGAND interactions are encoded within the *ChemokReceptors*.

A precondition for any *Ligand-Ligand* interaction to occur is that the two *Ligands* in question map to an observed biological receptor-ligand pair. A complete set of such receptor-ligand pair rules will be specified during implementation.

B. *Cell and Membrane*

The two classes that implement the *Cell* interface within our model are the *ECell* and the *Leuk*. They represent endothelial cells and leukocytes, respectively.

The *Membrane* interface is implemented by two classes, *ECellMem* and *LeukMem*. The *LeukMem*, which represents the leukocyte membrane, is a 2-D array data structure. As shown in Fig. 1-A, each unit in the array corresponds to a section of the leukocyte membrane. When a *Leuk* object is created, a unique, corresponding *LeukMem* object will also be created, which involves randomly choosing items from this array. These chosen items represent locations on the membrane where the *Ligands* on the *Leuk* object are exposed to the environment. At each chosen section in the *LeukMem*, there will be *AdhesMolecule* and *ChemokReceptor* objects.

Each *ECell* object has an *ECellMem* to represent the entire luminal surface of the referent membrane. We simplify our model by assuming that this section is rectangular in shape. As shown in Fig 1-B, it is implemented as a 2-D array. Similar to *LeukMem* objects, unique *ECellMem* objects are created for every *ECell*. Units within the array will be uniquely chosen to indicate where the *AdhesMolecule* objects are exposed to the environment. At each such location there will be an *AdhesMolecule* object for each adhesion molecule found on endothelial cells in the biological model. In addition, if the *ECell* has a *Chemok* object to present to *Leuk* objects, it will be positioned at one of these specified locations.

D. *Space*

The first class to implement the Space *interface* is the *FreeStreamSpace*, which represents the central region of a venule where the free blood stream is located. As shown in Fig. 2, it is a 1-D array, where each unit corresponds to a particular cross-section of the venule. Each unit will contain information about that particular cross-section, such as pointers to *Leuk* objects within that region, shear rate, and cross-sectional diameter. In addition, each unit will have a parameter that determines the probability that a *Leuk* object within that unit will move out of the *FreeStreamSpace*.

The *MembraneSpace* is the last space to implement the *Space* interface. It represents the endothelial cell membrane. As shown in Fig. 2, it is a 2-D grid that is circular in one dimension, and contains *ECell* objects.

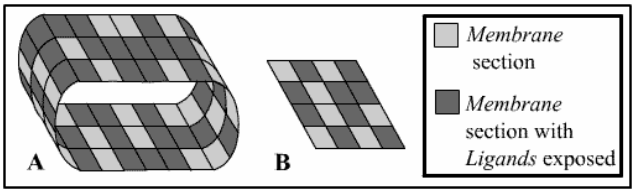

Fig. 1. Illustration our representation of the leukocyte membrane (A) and the luminal endothelial cell membrane (B) in our model.

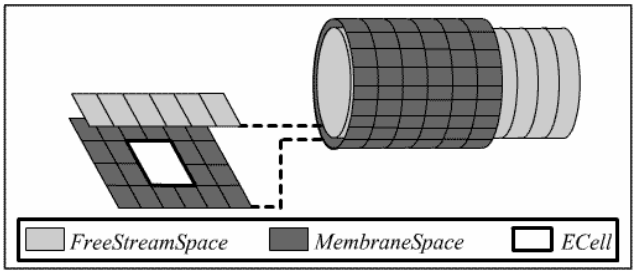

Fig 2. Illustration of the *FreeStreamSpace* and the *MembraneSpace* classes in our model.

III. MODELING BEHAVIORS

A. *AdhesMolecule-AdhesMolecule* Interactions

From the total number, affinity, and avidity parameters of both the *Leuk AdhesMolecule* object and *ECell AdhesMolecule* object, a probability that an interaction will form can be calculated. Interaction formation will be Monte Carlo determined. The disengagement of interactions between *AdhesMolecules* will also be determined in a similar manner.

B. Rolling

Leuk-ECell interactions can only occur when the *Leuk* object is on the *MembraneSpace* near *ECell* objects. *Leuk* objects are placed on the *MembraneSpace* in an orientation that allows the *LeukMem* to rotate around its axis of rotation and ROLL on the *MembraneSpace* in the direction of flow. Only a small rectangular region of the *LeukMem* will be placed on the *MembraneSpace*. *Leuk AdhesMolecule* and *ECell AdhesMolecule* interactions occur only at overlapping regions of the *LeukMem* and *ECellMem* where both have been labeled as locations where *AdhesMolecules* are exposed to the environment.

A *Leuk* ROLLING event consists of a partial rotation of *LeukMem* around its axis of rotation and in the direction of flow such that a new column of the 2-D array is placed on top of the *MembraneSpace*, while an old one is removed. If there is an overlapping region of *LeukMem* and *ECellMem* where both regions have been labeled as locations where *AdhesMolecules* are exposed to the environment, then all *Leuk AdhesMolecules* will attempt to form interactions with their respective *AdhesMolecule* LIGANDS on the *ECell*. A prerequisite for a ROLLING event to occur is that all *AdhesMolecule-AdhesMolecule* interactions dissociate at the rear column of the 2-D array that is removed from the *MembraneSpace*.

If at any time, a *Leuk* object is on the *MembraneSpace*, but a sufficient number of *AdhesMolecule-AdhesMolecule* interactions are not present, then the *Leuk* will disengage and move back into the *FreeStreamSpace.*

B. Activation and Firm Adhesion

A *Leuk* object can encounter a *Chemok* object with its *ChemokReceptors* when ROLLING on the *MembraneSpace*. The *Chemok* object will be removed and SIGNALS (messages) will be sent to all relevant *AdhesMolecule* objects at nearby regions on the *LeukMem* to modify their parameters appropriately. When ROLLING, engagement of *AdhesMolecule* objects with their LIGANDS can also result in SIGNALS being sent to relevant and nearby *AdhesMolecule* objects to modify their parameters in an appropriate manner.

FIRM ADHESION will only occur when *AdhesMolecule-AdhesMolecule* interactions have been formed and will not disengage when they are within the very last column of the *LeukMem* that is on the *MembraneSpace*.

IV. MODEL SIMULATION

A. Rolling

Our initial simulation experiments explore the parameter space for the ADHESION MOLECULES that participate in the process of NEUTROPHIL ROLLING. These will include the parameters representing the selectin, α4 integrin, and β2 (CD18) integrin adhesion molecules. We use data from intravital microscopy experiments [5-6], which observed neutrophil rolling on cremaster muscle venules in mice that lacked different combinations of the selectin and integrin adhesion molecules after treatment of TNF-α.

The first simulated experimental condition is based on short term TNF-α treatment [5]: TNF-α is injected intrascrotally 2 h before the beginning of the intravital microscopic experiment. In the in vivo model, the treatment has been shown to induce E-selectin expression and increase the expression of P-selectin [5]. Mice lacking E-selectin ($E^{-/-}$), P-selectin ($P^{-/-}$), L-selectin ($L^{-/-}$), L- and E- selectin ($L/E^{-/-}$), E- and P- selectin ($E/P^{-/-}$), and L- and P-selectin ($L/P^{-/-}$) were generated by bone marrow transplantation. Experimental observations for these mice are summarized in Table I. E, L, and P-selectin deficient mice were generated, but no rolling was observed for these mice.

Under short term TNF-α treatment conditions, it has been shown that the β2 integrins play a cooperative role with E-selectin in mediating slow rolling of neutrophils at velocities below 5 μm/s. To refine the parameter space for these adhesion molecules, we use experimental data from [6]. Neutrophil rolling was observed under the same short term TNF-α treatment conditions, but in wild-type mice (WT) and in mutant mice lacking E-selectin ($CD62E^{-/-}$), β2 integrins ($CD18^{-/-}$), or β2 integrins and E-selectin ($CD18^{-/-}$ $CD62E^{-/-}$). Observations are summarized in Table II.

The second experimental condition that will be simulated uses longer-term treatment with TNF-α: it is injected intrascrotally 6 h before the beginning of the intravital microscopic experiment. Evidence has shown that the roles of L-selectin and α4 integrin in rolling are different in long term TNF-α treatment than in short term treatment [5]. In E- and P-selectin deficient mice, L-selectin and α4 integrin-dependent rolling is induced after long term TNF-α treatment. $L^{-/-}$, $L/E^{-/-}$, $L/P^{-/-}$, E- and P-selectin deficient ($E/P^{-/-}$), and E-, L-, and P-selectin deficient ($E/L/P^{-/-}$) mice were generated by bone marrow transplantation. Table III summarizes observations for this experiment.

Parameter vectors for the *AdhesMolecules* will be identified such that ROLLING behaviors in our model are calibrated to the rolling behaviors observed from these in vivo experiments.

B. Activation And Firm Adhesion

The third experimental condition being simulated is aimed at refining parameters so that the model adequately represents the steps of activation and firm adhesion. For this

TABLE I *
HEMODYNAMICS AND MICROVASCULATURE PARAMETERS FOR BONE MARROW-TRANSPLANTED MICE AFTER SHORT TERM TREATMENT WITH TNF-α (2 h)

Mouse	Avg. Vessel Diameter (μm)	Wall Shear Rate (s^{-1})	Rolling Flux Fraction (%)	Avg. Rolling Velocity (μm/s)
L	37.3 ± 1.4	680 ± 30	10.1 ± 1.7	5.0 ± 0.3
$E^{-/-}$	40.3 ± 1.4	710 ± 30	48.1 ± 5.5	31.1 ± 0.9
$P^{-/-}$	40.5 ± 1.7	610 ± 30	11.5 ± 2.2	5.9 ± 0.3
$L/E^{-/-}$	41.6 ± 1.6	720 ± 20	11.3 ± 1.3	14.8 ± 0.4
$L/P^{-/-}$	49.1 ± 2.1	730 ± 30	1.3 ± 0.2	3.5 ± 0.2

* Reproduced from [5].

TABLE II **
HEMODYNAMICS AND MICROVASCULATURE PARAMETERS FOR MUTANT MICE AFTER SHORT TERM TREATMENT WITH TNF-α (2 h)

Mouse	Avg. Vessel Diameter (μm)	Wall Shear Rate (s^{-1})	Avg. Rolling Velocity (μm/s)
WT	47 ± 2	470 ± 30	6.9 ± 0.2
$CD62E^{-/-}$	37 ± 1	600 ± 40	21.1 ± 0.5
$CD18^{-/-}$	43 ± 2	460 ± 30	22.7 ± 0.8
$CD18^{-/-}$, $CD62E^{-/-}$	51 ± 2	580 ± 50	50.1 ± 1.4

** Reproduced from [6].

TABLE III *
HEMODYNAMICS AND MICROVASCULATURE PARAMETERS FOR BONE MARROW-TRANSPLANTED MICE AFTER LONG TERM TREATMENT WITH TNF-α (6 h)

Mouse	Avg. Vessel Diameter (μm)	Wall Shear Rate (s^{-1})	Rolling Flux Fraction (%)	Avg. Rolling Velocity (μm/s)
$L^{-/-}$	36.8 ± 1.1	560 ± 20	1.2 ± 0.3	10.7 ± 1.2
$L/E^{-/-}$	39.5 ± 1.7	660 ± 30	5.0 ± 1.0	15.2 ± 0.9
$L/P^{-/-}$	42.6 ± 2.2	590 ± 30	0.9 ± 0.3	4.8 ± 0.4
$E/P^{-/-}$	43.4 ± 2.0	480 ± 40	0.9 ± 0.2	15.7 ± 1.2
$E/L/P^{-/-}$	45.1 ± 2.1	540 ± 30	0.4 ± 0.06	13.6 ± 1.2

* Reproduced from [5].

we use experimental data from [7], which tracked individual neutrophils rolling along a TNF-α-treated mouse cremaster muscle venular tree. Reported cumulative distance versus time profiles are shown in Fig. 3. Values for average velocity and average distance rolled before firm adhesion were calculated for $CD18^{-/-}$, $E^{-/-}$, and WT mice. Reported values are shown in Table IV.

V. Discussion

We have made many assumptions about the biological system that will need to be iteratively revisited during the course of these studies. For example, our initial in silico experiments use one *ECell* object that spans the entire *MembraneSpace* and we assume that the distribution of *AdhesMolecules* is uniform on the *ECellMem*. The distribution of adhesion molecules on the surface of the endothelial cells has not been fully characterized, but it is known that it is not uniform [8].

In addition, it has been shown that LFA-1 and Mac-1, the two major β2 integrins involved in neutrophil rolling and firm adhesion, have distinct and cooperative roles [9]. Our initial experiments use a simplified model that represents all β2 integrins as a single agent. Our future models will contain separate agents for each of the β2 integrins and their ligands.

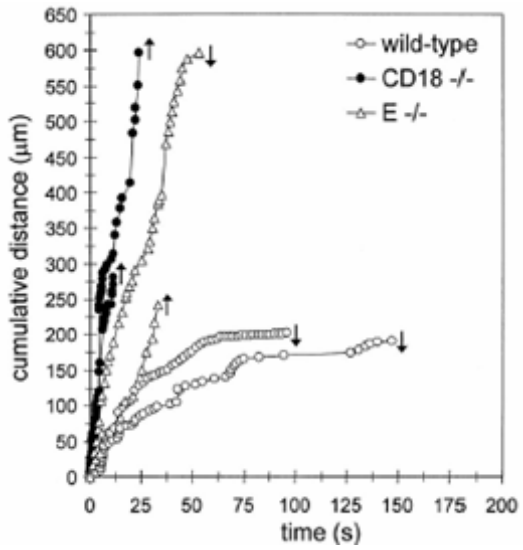

Fig 3. Distance-time curves for typical rolling leukocytes. Typical distance-time tracings for individual leukocytes from WT (open circles), $CD18^{-/-}$ (dark circles), and $E^{-/-}$ (triangles) mice. Leukocyte outcome is indicated by arrows: up (detach), down (attach). Reproduced from [7].

TABLE IV *
AVERAGE TIME ROLLED, DISTANCE ROLLED, AND AVERAGE ROLLING VELOCITY

Mouse Genotype	Outcome	Time Rolled (s)	Distance Rolled (μm)	Avg. Rolling Velocity (μm/s)
WT	Adhere	86 ± 18	270 ± 58	3.8 ± 0.4
WT	Detach	140 ± 29	610 ± 200	5.0 ± 2.5
CD18-/-	Adhere	---	---	---
CD18-/-	Detach	12 ± 2	270 ± 26	32 ± 7
E-/-	Adhere	40 ± 14	280 ± 49	14 ± 2
E-/-	Detach	41 ± 13	350 ± 110	11 ± 2

* Reproduced from [7].

Finally, the dynamics of leukocyte activation by chemokine detection and by engagement of selectins and integrins with their ligands is not understood. An issue of debate is whether each chemokine detection event or selectin and integrin-ligand engagement event sends global or local activation signals to the integrins [10]. We are testing the latter hypothesis.

VI. Conclusion

We present a novel, agent-oriented approach to modeling and simulating leukocyte-venule interactions during inflammation. The leukocyte adhesion cascade involves overlapping and intertwined processes [3]. An advantage of this new approach to modeling and simulation is that it is straightforward to systematically represent non-linear systems, which makes it well suited for studying leukocyte-venule interactions.

We anticipate that our models will be able to progressively integrate and organize the knowledge that has amassed on this system, thereby helping us to understand how such a system functions under normal, stressed, and diseased conditions. Furthermore, we anticipate that these new models can provide an in silico environment to help inspire and test novel cellular engineering approaches for creating therapeutics.

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