Original Article

Identifying Key Pathways and Components in Chemokine-Triggered T Lymphocyte Arrest Dynamics Using a Multi-Parametric Global Sensitivity Analysis

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Abstract

Introduction—The arrest of rolling T lymphocytes at specific locations is crucial to proper immune response function. We previously developed a model of chemokine-driven integrin activation, termed integrative signaling adhesive dynamics (ISAD). In addition, we have shown that loss of diacylglycerol kinase (DGK) leads to a gain of function regarding adhesion under shear flow. We undertook this study to understand the sensitivity of adhesion to perturbations in other signaling molecules.

Methods—We adapted multi-parametric sensitivity analysis (MPSA) for use in our ISAD model to identify important parameters, including initial protein concentrations and kinetic rate constants, for T lymphocyte arrest. We also compared MPSA results to those obtained from a single parametric sensitivity analysis.

Results—In addition to the previously shown importance of DGK in lymphocyte arrest, PIP₂ cleavage and Rap1 activation are crucial in determining T cell arrest dynamics, which agree with previous experimental findings. The L-selectin density on the T lymphocyte surface also plays a large role in determining the distance rolled before arrest. Both the MPSA and single-parametric method returned similar results regarding the most sensitive kinetic rate constants.

Conclusion—We show here that the regulation of the amount of second messengers are, in general, more critical for determining T lymphocyte arrest over the initial signaling proteins, highlighting the importance of amplification of signaling in cell adhesion responses. Overall, this work provides a mechanistic insight of the contribution of key

pathways and components, thus may help to identify potential therapeutic targets for drug development against immune disorders.

Keywords—Sensitivity analysis, Chemokine, T lymphocyte, Firm arrest, LFA-1.

ABBREVIATIONS

DAG	Diacylglycerol
DGK	Diacylglycerol kinase
ICAM-1	Intracellular adhesion molecule-1
ISAD	Integrative-signaling adhesive dynamics
LFA-1	Lymphocyte function-associated protein-1
MPSA	Multi-parametric sensitivity analysis
GPCR	G protein coupled receptor
IP3	Inositol triphosphate
PIP ₂	Phosphatidylinositol 4,5-bisphosphate
PLC	Phospholipase C
GEF	Guanine nucleotide exchange factor

INTRODUCTION

Rapid and precise stopping of T lymphocytes at specific vascular sites is crucial to directed transvascular migration to tissues to achieve a successful immune response.^{7,25} T lymphocytes circulating in the blood search for activated endothelium by tethering

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and rolling along the surface. These early interactions are mediated by the relatively weak bonds between selectins and their ligands on the opposing surface.^{10,17} However, stronger linkages to the surface, in the form of integrin-ligand bonds, are needed to arrest on the surface and fully resist the shear forces applied to the cell.^{5,18} Lymphocyte function-associated antigen-1 (also known as $\alpha_L\beta_2$; LFA-1) is an integrin receptor on T lymphocytes which is able to modulate its affinity in response to activation signals sent through complex intracellular networks.¹² It is known that chemokine receptor signaling following chemokine binding is one of the activation pathways able to enhance the affinity of LFA-1 on the T lymphocyte surface.¹³ Because of the highly nonlinear nature of the signaling cascades, it is difficult to investigate the importance of individual signaling steps in influencing the phenotypic adhesion of T lymphocytes solely through experimental methods. Therefore, it is useful to employ mathematical models in order to facilitate our understanding of factors controlling cell adhesion decisions.

The basic structure of the intracellular signaling cascade governing LFA-1 activation is roughly conserved across all leukocytes.²¹ The cascade is initiated by the binding of chemokines on the endothelial surface to chemokine receptors on the leukocyte. These chemokine receptors are typically G protein coupled receptors (GPCRs), and the binding of receptor and ligand releases the G_α subunit and causes the activation of the β and γ subunits. The activation of the G proteins leads to the creation of second messengers, including (but not limited to) inositol triphosphate (IP₃) and diacylglycerol (DAG) from phosphatidylinositol 4,5-bisphosphate (PIP₂), through the activity of members of the phospholipase C (PLC) family. DAG then triggers the GTPase cycle of downstream signaling molecules, including Rap1, by activating their associated guanine nucleotide exchange factors (GEFs). Rap1 interaction with molecules such as talin then leads to activation of LFA-1.^{3,19}

Previously, we have built an advanced Adhesive Dynamics model, Integrative-Signaling Adhesive Dynamics (ISAD), which allows us to estimate integrin affinity modulation due to stochastic chemokine signal transduction.² We previously found that total chemokine receptor occupancy is important to induce increased adhesion. Based on these results, we predicted that the activity of diacylglycerol kinase (DGK), which converts the second messenger DAG to inactive phosphatidic acid (PA), is important to the T cell arrest under chemokine stimulation. This prediction was experimentally confirmed through a flow-driven cell adhesion assay with DGK ζ -deficient mouse T lymphocytes.¹⁶ Despite this success, it is still necessary to systematically identify key signaling pathways and

components in T lymphocytes to perturb functional behaviors because T cell integrin activation signaling consists of tightly connected networks.

Sensitivity analysis in biological modeling is an *in silico* study to determine how changing inputs (e.g., kinetic parameters; initial molecule concentration) in mathematical models correlate to model outputs (e.g., signal propagation; cell phenotype).²⁸ Therefore, sensitivity analysis can provide valuable information not only on key parameters governing the model system but also for model development for designing further mathematical models and experiments. Typical sensitivity analysis consists of altering the concentration or activity of a single molecule at a time. However, this approach of analysis may not be appropriate in a highly interconnected network, such as the signaling cascade governing integrin activation. Therefore, instead of applying a local sensitivity analysis, we employed a Multi-Parametric Sensitivity Analysis (MPSA) in which all input parameters are systematically perturbed to investigate how cell arrest dynamics in the ISAD model are regulated by systemic change in kinetic parameters and components.^{27,29} The ability to change multiple parameters at the same time can find parameters critical to the model that are not found in a local sensitivity analysis.²⁴

In this study, we explore which signaling pathways and components play a role in CXCR4-mediated T cell adhesion under shear flow. We show that DGK-catalyzed degradation of DAG, Rap1 activation, and PIP₂ cleavage to IP₃ and DAG are the most sensitive signaling steps to determine when a rolling T lymphocyte stop under shear flow. The L-selectin density on the T cell surface is also an important factor to affect the T cell arrest dynamics, indicating the importance of cell rolling on the arrest of lymphocytes under flow. Overall, we show regulation of second messengers appears to be one of the most important factors controlling leukocyte adhesion.

METHODS

Integrative Signaling Adhesive Dynamics Model

Here, we employ an ISAD model implemented by Beste *et al.* for simulating LFA-1-mediated naïve T cell homing in high endothelial venules triggered by chemokine engagement.² ISAD is a multiscale stochastic mathematical model which simulates how chemokine signaling during integrin activation determines T cell stopping dynamics by integrating a spatio-temporal kinetic model of inside-out integrin activation processes with a stochastic simulator of leukocyte mechanical adhesion. We have also advanced the model to simulate an adverse role of DGK in effector

T cell stopping in response to CXCL12 stimulation.¹⁶ As shown in the schematic of Fig. 1a, the recognition of chemokines presented on a planar surface through G-protein-coupled receptors expressed on the lymphocyte membrane initiates inside-out activation of the lymphocyte integrins while the cell rolls via the binding of L-selectin with sLe^x under a venular shear rate. To simulate the intracellular integrin activation signaling cascade, a spherical model cell was divided into subvolumes that are so small that the concentrations of signaling molecules in a subvolume are near-homogeneous in space (Fig. 1b).⁸ The pathway of chemokine-triggered inside-out activation signaling of LFA-1 and all the biochemical reactions included in the model were illustrated in Fig. 1c. The signaling model is constructed by ordinary differential equations composed of 18 major kinetic and 4 initial values that are greater than zero. Reaction and diffusion of intracellular signaling and adhesion molecules were calculated

every time step (10^{-7} s) and corresponding molecular adhesive binding and cell location were tracked during calculation. The time (Δt) and distance to stop (Δx) (i.e., time and distance prior to stopping) were eventually determined as model outputs, as those values are simple but important metrics to represent how sensitive the cell responds dynamically to the chemokine signaling and an applied shear force (Figs. 1d and 1f).

Multi-Parametric Sensitivity Analysis

MPSA is a global sensitivity approach based on Monte-Carlo filtering. The general methodology of MPSA begins with a set of randomly generated parameter values. These values are put into the model and the output determined. By using a large number of parameter sets, the sensitivity of the system to a single parameter can be determined by calculating the Kolmogorov–Smirnov (K–S) statistic. The K–S statistic

FIGURE 1. Simulation of chemokine-triggered T lymphocyte arrest dynamics *in silico*. (a) Schematic of T lymphocyte arrest dynamics cascade under shear flow. Chemokine recognition through chemokine receptors expressed on the lymphocyte surface initiates integrin activation signaling, which results in the cell stopping against flow. (b) A model lymphocyte was discretized into small subvolumes and contact area is highlighted with red color. (c) Entire signaling pathway of chemokine-triggered LFA-1 activation in the model. 18 kinetic parameters and 6 components were considered in MPSA. (d) Simulated cell trajectories in which cells undergo in a sequence of tethering, rolling and arrest. Time (e) and distance to stop (f) were obtained as model outputs.

quantifies, in our case, the distance between two empirical distribution functions, one as the reference case and the other as the perturbed case. Larger values of the K-S statistic indicate increased sensitivity compared to smaller values.²⁸ The MPSA method has been used to analyze the key pathway elements in several biological models including NF- κ B, JAK-STAT, and MAPK signaling pathways.^{6,27,29} The detailed procedure of MPSA in this study is described as follows:

- Step 1: Select the parameters to be tested.
- Step 2: Set the range of each selected parameter large enough to cover all feasible variations. Kinetic parameters were varied 2 orders of magnitude above and below the initial value. Protein concentrations were varied over a single order of magnitude. These changes allow us to determine the sensitivity of the parameters over biologically relevant parameter spaces.¹
- Step 3: For each parameter, generate a series of independent random numbers from a uniform linear distribution within the defined range from Step 2 and obtain parameter combinations using the Latin hypercube sampling method. Latin hypercube sampling is an efficient parameter sampling method while ensuring maximal sampling through each parameter dimension.²⁰
- Step 4: Simulate the model for each set of parameter values and calculate the corresponding objective function. The objective function is defined as the sum of squared errors of the time (Δt) and distance to stop (Δx) between the observed and perturbed system as follows:

$$f_{\text{obj}}(k) = \sum_i^n (x_{\text{obs}}(i) - x_{\text{per}}(i, k))^2$$

where f_{obj} is the objective function that describes how much the system output deviates from the observed data by varying the parameters, $x_{\text{obs}}(i)$ denotes an observed system output at the i th sampling time, $x_{\text{per}}(i, k)$ denotes that perturbed system output value at the i th sampling time for the parameter variation set k , and n is the number of sample points. We set a single sampling time of 30 s in our analysis.

- Step 5: Determine whether the chosen set of parameter values is acceptable or unacceptable by comparing the objective function value to a given threshold. If the objective function for a specific set of parameters is less than the threshold, the set of parameters is classified as

acceptable; otherwise it is classified as unacceptable. Here, the threshold is defined as the average of the objective functions for entire range of parameters.

- Step 6: Statistically evaluate the parameter sensitivity by comparing two distributions of the parameter values associated with the acceptable and the unacceptable results. For each parameter, the cumulative frequency is calculated for both acceptable and unacceptable cases. We evaluate the sensitivity by a direct measure of the separation of the two cumulative frequency distributions. We use the following K-S statistic:

$$\text{K-S} = \sup_x |S_a(x) - S_u(x)|$$

where S_a and S_u are the cumulative frequency functions corresponding to acceptable and unacceptable cases, respectively, and x is the given parameter. The statistic K-S is determined by the maximum vertical distance between the cumulative frequency distribution curves for n acceptable and m unacceptable cases. A large value of K-S value indicates that the system is sensitive to variation in the given parameter.

In Step 3 for the 18 rate constants, we sampled 1000 random parameter vectors while evenly covering individual parameter ranges using Latin hypercube sampling as a previous work indicated. For initial protein concentrations, 100 random parameter vectors were sampled. This allows us to fully explore the range for each parameter while remaining computationally feasible to simulate and analyze.

Local Sensitivity Analysis

To confirm our MPSA findings regarding key control points within the inside-out signaling pathway governing chemokine-induced LFA-1 integrin activation, we evaluated the parametric sensitivities of the ODE model using calibrated kinetic parameters. Sensitivity coefficients S_i for each of the rate parameters were calculated by evaluating the change in the time-integrated induction of high-affinity LFA-1 by 1 nM soluble chemokine when each calibrated parameter was varied logarithmically tenfold around its calibrated value, p_i . Specifically, the normalized sensitivity coefficients were numerically computed as:

$S_i = \frac{d}{d \ln p_i} \ln_0^{t_{ss}} \text{LFA} - 1_{\text{HIGH}}(t) dt$ where the integrand is evaluated over the time-scale t_{ss} required for the system to reach steady-state.

RESULTS

The nominal kinetic parameter sets, initial conditions, and their perturbation test ranges are described in Tables 1 and 2. Note the nominal parameter values and initial conditions were used to generate T cell arrest dynamics similar to *in vitro* results as depicted in our previous work.²

Critical Signaling Pathways to Determine T Lymphocyte Arrest Dynamics

We investigated the kinetic rate constants and protein concentrations controlling T lymphocyte arrest dynamics following chemokine stimulation using MPSA and ISAD. A K-S statistic was calculated for each kinetic parameter to evaluate the sensitivity of the

system to perturbation in the given parameter. Because the significant parameters were not known *a priori*, we varied all 18 kinetic constants and ran 1000 simulations randomly as described in Methods. As shown in Figs. 2 and 4a, reactions involved in the DGK-catalyzed conversion of DAG to phosphatidic acid (PA), the conversion of Rap1^{GDP} to Rap1^{GTP}, the association of Rap1^{GTP} with LFA-1, and the cleavage of PIP₂ to IP₃ and DAG had significant effects on the arrest dynamics of T lymphocytes under chemokine CXCL12 stimulation. Specifically, these parameters controlled the time to stop of the cells. Qualitatively similar results were obtained when using distance to stop as the metric of interest (Supplemental Fig. 1). However, variations of the parameters concerning the formation of G_{αβγ}, G_α^{GDP}, and CC:CCRp had minor effects on the outputs of the system.

TABLE 1. Ranges for kinetic parameter values.

Parameter	Reaction	Reference value ²	Range	Unit
k_1	CC + CCR → CC:CCR	1×10^{-6}	1×10^{-8} – 1×10^{-4}	$\mu\text{M}^{-1}\text{s}^{-1}$
k_2	CC:CCR → CC + CCR	5×10^{-1}	5×10^{-3} – 5×10^1	s^{-1}
k_3	CC:CCR → CC:CCRp	1	1×10^{-2} – 1×10^2	$\mu\text{M}^{-1}\text{s}^{-1}$
k_4	G _{αβγ} → G _α ^{GTP} + G _{βγ}	8	8×10^{-2} – 8×10^2	$\mu\text{M}^{-1}\text{s}^{-1}$
k_5	G _α GDP + G _{βγ} → G _{αβγ}	1×10^2	1×10^0 – 1×10^4	$\mu\text{M}^{-1}\text{s}^{-1}$
k_6	G _α ^{GTP} → G _α ^{GDP}	1	1×10^{-2} – 1×10^2	s^{-1}
k_7	G _{βγ} + GRK → GRK:G _{βγ}	10	1×10^{-1} – 1×10^3	$\mu\text{M}^{-1}\text{s}^{-1}$
k_8	GRK:G _{βγ} → G _{βγ} + GRK	1	1×10^{-2} – 1×10^2	s^{-1}
k_9	PIP ₂ → IP ₃	3.25×10^1	3.25×10^{-1} – 3.25×10^3	$\mu\text{M}^{-1}\text{s}^{-1}$
k_{10}	DAG → PA	5	5×10^{-2} – 5×10^2	s^{-1}
k_{11}	Rap1 ^{GDP} → Rap1 ^{GTP}	5×10^{-1}	5×10^{-3} – 5×10^1	s^{-1}
k_{12}	Rap1 ^{GTP} → Rap1 ^{GDP}	1×10^{-1}	1×10^{-3} – 1×10^1	s^{-1}
k_{13}	LFA-1 _{LOW} → LFA-1 _{HIGH}	1×10^{-1}	1×10^{-3} – 1×10^1	$\mu\text{M}^{-1}\text{s}^{-1}$
k_{14}	LFA-1 _{HIGH} → LFA-1 _{LOW}	5×10^{-3}	5×10^{-5} – 5×10^{-1}	s^{-1}
Km ₁	PIP ₂ → IP ₃	1	1×10^{-2} – 1×10^2	μM
Km ₂	DAG + GEF → DAG:GEF	1	1×10^{-2} – 1×10^2	μM
Km ₃	Rap1 ^{GDP} → Rap1 ^{GTP}	1×10^{-2}	1×10^{-4} – 1×10^0	μM
Km ₄	Rap1 ^{GTP} → Rap1 ^{GDP}	1×10^{-2}	1×10^{-4} – 1×10^0	μM

TABLE 2. Ranges for initial conditions.

Initial condition	Reference value ²	Test range	Unit
CCR	4.8×10^4	4.8×10^3 – 4.8×10^5	Molecule cell ⁻¹
$G_{\alpha\beta\gamma}$	4.8×10^4	4.8×10^3 – 4.8×10^5	Molecule cell ⁻¹
Rap1 ^{GDP}	1.44×10^5	1.44×10^4 – 1.44×10^6	Molecule cell ⁻¹
LFA-1	4.8×10^4	4.8×10^3 – 4.8×10^5	Molecule cell ⁻¹
L-selectin	1.25×10^4	1.25×10^3 – 1.25×10^5	Molecule cell ⁻¹
GRK	4.8×10^3	4.8×10^2 – 4.8×10^4	Molecule cell ⁻¹

FIGURE 2. Cumulative frequency of MPSA with respect to kinetic parameters in chemokine-triggered integrin LFA-1 activation signaling during T lymphocyte trafficking under flow using time to stop as metric of interest. Red curves represent unacceptable cases and blue curves denote acceptable cases. The K-S statistic is calculated with the maximal vertical difference between the two curves.

Critical Components to Determine T Lymphocyte Arrest Dynamics

In addition to kinetic rate parameters, we also used MPSA and ISAD to determine whether the system is sensitive to the initial concentration of adhesion receptors or signaling molecules. As shown in Figs. 3

and 4b, the results of MPSA indicated that L-selectin, a surface adhesion receptor for mediating rolling adhesion and $G_{\alpha\beta\gamma}$, a G-protein subtype, are relatively important components in T lymphocyte arrest dynamics. On the other hand, LFA-1, an integrin receptor for mediating cell arrest, and GRK, G-protein

FIGURE 3. Cumulative frequency of MPSA with respect to initial concentrations of components in chemokine-triggered integrin LFA-1 activation signaling during T lymphocyte trafficking under flow. Red curves represent unacceptable cases and blue curves denote acceptable cases.

FIGURE 4. K-S statistic in MPSA. Heat bar of K-S statistic for each kinetic parameters (a) and initial concentrations of components (b).

coupled receptor kinase, are less important for the output of the system when using time to stop as the metric of interest. Using distance to stop gives qualitatively similar results (Supplemental Fig. 1).

Comparison to Local Sensitivity Analysis

We also used a traditional single parameter local sensitivity analysis to compare results to the MPSA. We varied a single kinetic parameter at a time while

keeping the others fixed at reference values. To identify controlling pathways that contribute to increase in LFA-1 affinity, we calculated the change in the time-integrated induction of high-affinity LFA-1 upon 1 nM chemokine stimulation. As shown in Fig. 5, the increase in LFA-1 affinity is sensitive to reactions involved in the cleavage of PIP_2 to IP_3 and DAG, the activation and inactivation of Rap1^{GTP} , and deactivation of the GEF, suggesting that LFA-1 affinity is strongly related to T lymphocyte arrest dynamics. However, the pathway of DGK-catalyzed conversion of DAG to PA that is most sensitive for triggering T lymphocyte arrest dynamics in the MPSA is less sensitive in the single parameter analysis.

DISCUSSION

Identifying key regulatory factors in controlling immune cell function is essential to the development of therapeutic biomarkers against immune disorders and inflammatory diseases. However, integrin activation in

the immune system is a highly complicated and interconnected network, making it difficult to discern the perturbation effects of an individual parameter on cell phenotypic responses. As a step to improve our understanding of how an immune cell interprets a chemotactic stimulus transmitted through complex signaling networks, we assessed which kinetic parameters and protein concentrations in chemokine-triggered integrin activation signaling are crucial in controlling cellular adhesion outputs under shear flow. Using MPSA, all of the signaling kinetic rate constants were systematically perturbed and cellular adhesion outputs investigated through ISAD simulations. To our knowledge, this work is the first study to integrate molecular-level pathway analysis into spatio-temporal single cell *in silico* modeling.

Through our MPSA, we discovered several key pathways regulating T cell arrest under flow. The crucial steps were: (i) conversion of DAG to PA by DGK catalysis; (ii) Rap1^{GTP} activation from Rap1^{GDP} by DAG:GEF complexes; (iii) IP_3 and DAG production through PIP_2 hydrolysis; and iv) LFA-1 activation by its association with Rap1^{GTP} . Interestingly, we have previously identified that T lymphocyte arrest is controlled by the DGK concentration through an integrative approach of computational modeling with experimental assays.¹⁶ Other identified critical pathways in our sensitivity analysis have already been experimentally validated.^{11,14,23,26} However, we found that upstream kinetic reactions of chemokine signaling such as the formation of $\text{G}_{\alpha\beta\gamma}$, $\text{G}_{\alpha}^{\text{GDP}}$, and CC:CCRp had minor effects on the outputs of the system, suggesting that the amplification of the initial signal is more important than the signal itself.

We also systematically varied initial protein concentrations among model inputs to identify the proteins which are most critical for ensuring cell adhesion under shear flow. Interestingly, the density of L-selectin is the most critical component governing how long a T cell rolls before stopping under chemokine stimulation, even though L-selectin is not typically considered to be involved in chemokine-triggered integrin activation. This result implies that rolling of T lymphocytes on vascular endothelium is essential in determining the dynamics of T cell arrest by providing the opportunities for chemokine receptors to recognize surface-bound chemokines and for LFA-1 to bind to ICAM-1 under flow. An experimental study by Galkina *et al.*, based on *in vivo* tracking of transgenic mouse T lymphocytes expressing varying levels of L-selectin, supported a significant role of the L-selectin density in regulating T cell homing to lymph nodes.⁹ The concentrations of CCR and $\text{G}_{\alpha\beta\gamma}$ are also important components in controlling T cell arrest. However, T cell arrest is less sensitive to the LFA-1 level on the

FIGURE 5. Sensitivity coefficient in local sensitivity analysis. Heat bar of sensitivity coefficient for each kinetic parameters obtained from local sensitivity analysis.

lymphocyte surface, which agrees with previous findings that a small number of activated LFA-1 bonds are required to make T cells arrest under flow.^{2,4,22}

A single-parametric sensitivity analysis was also performed to explore the effect of a single variation of kinetic parameters on the perturbation of LFA-1 affinity. Since LFA-1 affinity increase is closely related to rapid T cell arrest under flow,¹⁵ it is worthwhile to investigate which signaling pathways are the dominant reactions in the integrin activation. Since the local sensitivity analysis deals with only small perturbations of the reference model with varying a single parameter for each simulation, it may not detect the full range of system behaviors that are possible by simultaneous perturbations of multiple parameters of arbitrary magnitudes, like in MPSA. In fact, we confirmed this limitation of the local sensitivity analysis in the DGK-catalyzed degradation of DAG, as the local sensitivity analysis did not consider this to be a critical step, while the MPSA and experimental results suggest a large role in the regulation of LFA-1 affinity.

Identification of important intracellular signaling reactions can help to discover new drug targets to help control T cell recruitment in abnormal immune states. Here, we identified four key reactions governing T cell arrest dynamics: (i) conversion of DAG to PA by DGK catalysis; (ii) Rap1^{GTP} activation from Rap1^{GDP} by DAG:GEF complexes; (iii) IP₃ and DAG production through PIP₂ hydrolysis; and (iv) LFA-1 activation by its association with Rap1^{GTP}. Understanding how these reactions work together and through the rest of the system will allow for improved hypothesis generation and possibly improved therapies in the future.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (<https://doi.org/10.1007/s12195-019-00575-2>) contains supplementary material, which is available to authorized users.

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AUTHOR CONTRIBUTIONS

DL designed the study, performed simulations, analyzed the data and wrote the paper. MTB designed the study, performed simulations and analyzed the data. NRA wrote the paper. GAK designed the study. DAH designed the study and wrote the paper.

CONFLICT OF INTEREST

Dooyoung Lee, Michael T. Beste, Nicholas R. Anderson, Gary A. Koretzky and Daniel A. Hammer declare that they have no conflicts of interest.

DATA AVAILABILITY

The codes used to perform the calculations in this paper are available upon request to the corresponding author, Daniel A. Hammer, hammer@seas.upenn.edu, in accordance with the data sharing policy of the National Institutes of Health which funded this work.

ETHICAL STANDARDS

No human studies were carried out by the authors for this article. No animal studies were carried out by the authors for this article.

REFERENCES

- ¹Aldridge, B. B., J. M. Burke, D. A. Lauffenburger, and P. K. Sorger. Physicochemical modelling of cell signalling pathways. *Nat. Cell Biol.* 8:1195–1203, 2006.
- ²Beste, M. T., D. Lee, M. R. King, G. A. Koretzky, and D. A. Hammer. An integrated stochastic model of “inside-out” integrin activation and selective T-lymphocyte recruitment. *Langmuir* 28:2225–2237, 2012.
- ³Bialkowska, K., *et al.* The integrin co-activator kindlin-3 is expressed and functional in a non-hematopoietic cell, the endothelial cell. *J. Biol. Chem.* 285:18640–18649, 2010.
- ⁴Caputo, K. E., and D. A. Hammer. Adhesive dynamics simulation of G-protein-mediated chemokine-activated neutrophil adhesion. *Biophys. J. Biophys. Soc.* 96:2989–3004, 2009.
- ⁵Chen, W., and C. Zhu. Mechanical regulation of T-cell functions. *Immunol. Rev.* 256:160–176, 2013.
- ⁶Cho, K.-H., S.-Y. Shin, W. Kolch, and O. Wolkenhauer. Experimental design in systems biology, based on parameter sensitivity analysis using a monte carlo method: a case study for the TNF α -mediated NF- κ B signal transduction pathway. *Simulation* 79:726–739, 2003.
- ⁷DeNucci, C. C., J. S. Mitchell, and Y. Shimizu. Integrin function in T-cell homing to lymphoid and nonlymphoid sites: getting there and staying there. *Crit. Rev. Immunol.* 29:87–109, 2009.
- ⁸Elf, J., A. Donicic, and M. Ehrenberg. Mesoscopic Reaction-Diffusion in Intracellular Signaling. Bellingham: International Society for Optics and Photonics, p. 114, 2003.
- ⁹Galkina, E., *et al.* T lymphocyte rolling and recruitment into peripheral lymph nodes is regulated by a saturable density of L-selectin (CD62L). *Eur. J. Immunol.* 37:1243–1253, 2007.
- ¹⁰Gallatin, W. M., I. L. Weissman, and E. C. Butcher. A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature* 304:30–34, 1983.

- ¹¹Ghandour, H., X. Cullere, A. Alvarez, F. W. Luscinskas, and T. N. Mayadas. Essential role for Rap1 GTPase and its guanine exchange factor CalDAG-GEFI in LFA-1 but not VLA-4 integrin mediated human T-cell adhesion essential role for Rap1 GTPase and its guanine exchange factor CalDAG-GEFI in LFA-1 but not VLA-4 integrin—mediated. *Regulation* 110:3682–3690, 2009.
- ¹²Herter, J., and A. Zarbock. Integrin regulation during leukocyte recruitment. *J. Immunol.* 190:4451–4457, 2013.
- ¹³Hogg, N., I. Patzak, and F. Willenbrock. The insider's guide to leukocyte integrin signalling and function. *Nat. Rev. Immunol.* 11:416–426, 2011.
- ¹⁴Katagiri, K., A. Maeda, M. Shimonaka, and T. Kinashi. RAPL, a Rap1-binding molecule that mediates Rap1-induced adhesion through spatial regulation of LFA-1. *Nat. Immunol.* 4:741–748, 2003.
- ¹⁵Kim, M., C. V. Carman, W. Yang, A. Salas, and T. A. Springer. The primacy of affinity over clustering in regulation of adhesiveness of the integrin α L β 2. *J. Cell Biol.* 167:1241–1253, 2004.
- ¹⁶Lee, D., J. Kim, M. T. Beste, G. A. Koretzky, and D. A. Hammer. Diacylglycerol kinase zeta negatively regulates CXCR16-stimulated T lymphocyte firm arrest to ICAM-1 under shear flow. *Integr. Biol.* 4:606, 2012.
- ¹⁷Ley, K., and G. S. Kansas. Selectins in T-cell recruitment to non-lymphoid tissues and sites of inflammation. *Nat. Rev. Immunol.* 4:325–336, 2004.
- ¹⁸Ley, K., C. Laudanna, M. I. Cybulsky, and S. Nourshargh. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat. Rev. Immunol.* 7:678–689, 2007.
- ¹⁹Luo, B.-H., C. V. Carman, and T. A. Springer. Structural basis of integrin regulation and signaling. *Annu. Rev. Immunol.* 25:619–647, 2007.
- ²⁰McKay, M. D., R. J. Beckman, and W. J. Canover. A comparison of three methods for selecting values of input variables in the analysis of output from a computer code. *Technometrics* 21:239–245, 1979.
- ²¹Montresor, A., L. Toffali, G. Constantin, and C. Laudanna. Chemokines and the signaling modules regulating integrin affinity. *Front. Immunol.* 3:1–10, 2012.
- ²²Shamri, R., *et al.* Lymphocyte arrest requires instantaneous induction of an extended LFA-1 conformation mediated by endothelium-bound chemokines. *Nat. Immunol.* 6:497–506, 2005.
- ²³Shimonaka, M., *et al.* Rap1 translates chemokine signals to integrin activation, cell polarization, and motility across vascular endothelium under flow. *J. Cell Biol.* 161:417–427, 2003.
- ²⁴Tiemann, C. A., J. Vanlier, M. H. Oosterveer, A. K. Groen, P. A. J. Hilbers, and N. A. W. van Riel. Parameter trajectory analysis to identify treatment effects of pharmacological interventions. *PLoS Comput. Biol.* 9(8):e1003166, 2013.
- ²⁵von Andrian, U. H., and C. R. Mackay. T-cell function and migration. Two sides of the same coin. *N. Engl. J. Med.* 343:1020–1034, 2000.
- ²⁶Weiss, A., and J. B. Imboden. Cell surface molecules and early events involved in human T lymphocyte activation. Cambridge: Academic Press, pp. 1–38, 1987.
- ²⁷Yoon, J., and T. S. Deisboeck. Investigating differential dynamics of the MAPK signaling cascade using a multi-parametric global sensitivity analysis. *PLoS ONE* 4(2):e4560, 2009.
- ²⁸Zi, Z. Sensitivity analysis approaches applied to systems biology models. *IET Syst. Biol.* 5:336–346, 2011.
- ²⁹Zi, Z., K. H. Cho, M. H. Sung, X. Xia, J. Zheng, and Z. Sun. In silico identification of the key components and steps in IFN- γ induced JAK-STAT signaling pathway. *FEBS Lett.* 579:1101–1108, 2005.

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