In silico imaging of the dynamics of antigen presentation and cellular activation in a lymph node

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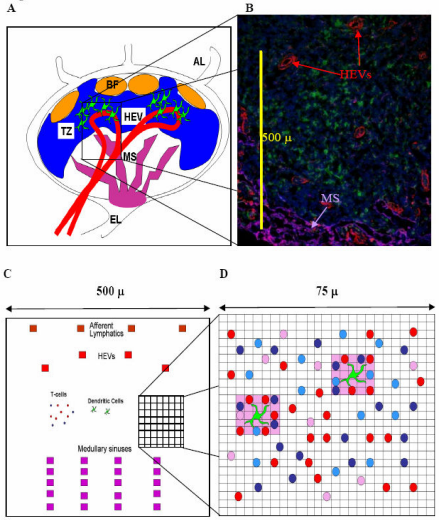
Abstract

The immune system and its process of antigen presentation in particular encompass events that occur at multiple length and time scales. Despite a wealth of information in the biological literature regarding each of these scales, no single representation synthesizes this information into a model that incorporates antigen presentation into the overall adaptive immune response. We have begun an approach for integrating information over relevant biological and temporal scales to generate such a representation for MHC class II-mediated antigen presentation. Here we focus on one of the single-scale models to give sufficient details of our approach. In particular, antigen-presenting cells, or dendritic cells (DCs), present antigen to matching cognate T cells, which is a critical step in the generation of adaptive immunity. These dynamics occur within lymph nodes (LN), where many thousands of T cells briefly interact with antigen bearing DCs, looking for that rare peptide-MHC match. The resulting activation and proliferation of a specific T cell line generates an appropriate immune response. To study these interactions, we developed an agent-based computational model that describes the dynamics of antigen presentation within a LN by individual cellular interactions, governed by known rules of behavior. We used this model to investigate how the physical structure of the LN enhances the relevant cell-cell contacts and the extent to which random motion vs. chemoattraction might enhance stochastic scanning by DCs of many T cells to find a rare cognate match.

Introduction

An appropriate immune response requires a complex interaction of antigens, antigen-presenting cells (APCs) and T-cell populations that modulate, regulate and effectuate an adaptive immune response. Most antigen presentation events occur in secondary lymphoid tissues (e.g., lymph nodes (LNs)) whose anatomy intersects the circulatory and lymphatic systems enhancing the likelihood of interaction between antigens, APCs and T-cells specific for the antigen being presented. Our goals are to create a model system of a LN that will: recapitulate antigen presentation dynamics observed through current imaging techniques and enable understanding of the anatomic and cellular motion features which contribute to efficient contact between T-cells and DCs and optimal production of non-naïve CD4+ and CD8+ T cells.

Figure 1



Anatomic basis for our model of the T zone within a lymph node (LN). (A) Schematic of the LN, focusing on the T-zone, where dendritic cells entering from the afferent lymphatics (AL) encounter T cells that are entering via the high endothelial venules (HEV); the latter exit via medullary sinuses to the efferent lymphatics. (B) Corresponding photomicrograph of the T zone showing the distance from HEV to MS. (C) Our model lattice representation of the T-zone, represented by a 100x100 lattice of 5 micron squares, representing 1/400th of the LN volume. (D) Smaller section is shown of two DCs with their surrounding "sweep area" around each DC. T4 = CD4+ T cells are shown in shades of red, CD8+ are shades of blue, DCs are green.

Methods

Agent-based Model

- ***Agents*** consist of discrete T cells and DCs, which move around the environment according to known rules
- ***Environment*** where agents reside, representing a 2-D cross-section of a single murine LN (see Figure 1)
- ***Rules*** that govern the interaction of the agents within the LN. i.e., how they change state
- ***Time step*** that updates cell position, entry into and exit from the LN, completion of lifespan of a cell, change of its state etc.

Rules of T-cell motion

- T-cells enter via HEV and leave via EL; MDCs enter via AL and almost always would complete their lifespan on LN
- T-cell position is updated every 30 seconds; potentially can move to next grid position (speed = 10 µm/min)
- Does not make 180 degree turn
- Once in motion, tends to continue in that direction (persistence), modeled as "probability of straight path"
- Constrained to not leave North-South boundaries (East-West is toroidal) or enter occupied position on the grid
- DC motion is updated every 2 minutes: potentially can move to next grid position (2.5 µ/min)
- DC can bind maximum of 16 adjacent T-cells; once bound, all move at DC speed

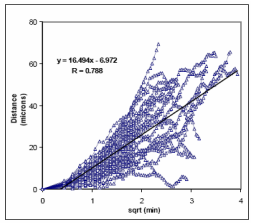
Uncertainty and Sensitivity Analysis

Statistical techniques of Latin hypercube sampling (LHS) and partial rank correlation (PRC) guided our understanding as to how and what extent variability in parameter values affects infection outcome. The t-test and its p-value (based on multiple hypothesis testing) were used to infer statistical significance.

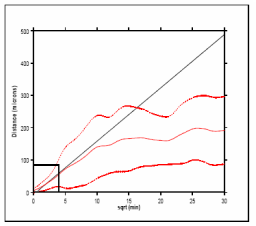
Parameter Range for Uncertainty/Sensitivity analyses

Range	Min	Max	Usual
Probability DC binds cognate CD4 (%)	50	100	75
Probability CD4 activated by MDC (%)	90	100	95
Prob. recruitment of naïve CD4 (%)	5	11	8
Number divisions for activated CD4	2	6	4
Doubling period CD4 (hrs)	7	9	8
Effector CD4 lifespan (hrs)	36	60	48
Prob MDC licensed by effector CD4 (%)	90	100	95
Prob LDC matures an IDC (%)	50	90	70
Prob non-naïve T cell matures IDC (%)	90	100	95
MDC lifespan (hrs)	36	60	48
LDC lifespan (hrs) (uniform dist)	0	72	36
Probability LDC binds cognate CD8 (%)	50	100	75
Probability CD8 activated by LDC (%)	90	100	95
Prob. recruitment of naïve CD8 (%)	4	10	7
No. divisions for activated CD8	2	6	4
CD8 doubling period (hrs)	7	9	8
Effector CD8 lifespan (hrs)	36	60	48
Probability of straight path (%)	50	95	90

Matching in vivo cell data to Simulations

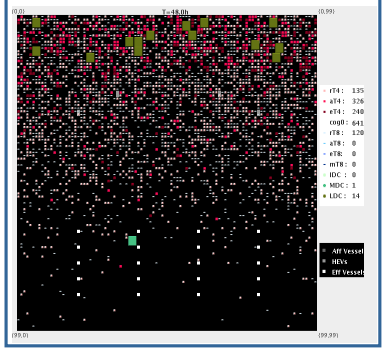


In vivo tracking of 46 CD4+ T cells, with a superimposed regression line, showing the relationship between displacements (in microns) vs. min$^{-1/2}$. Under random motion, this relationship is linear.

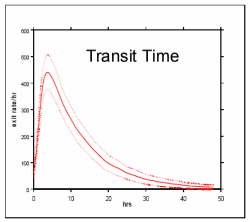


Mean displacement (solid line is the mean ± 1 S.D. shown as dashed lines) vs. min$^{-1/2}$ is plotted from 25 independent simulations using a total of 1250 CD4+ T cell trajectories, superimposed on the regression line from the left panel. Means from simulations and in vivo data match for ~ 1 hour, after which T cell motility decreases as some become bound to DCs. Insert box marks the range for distance and time shown in the left panel.

This "snapshot" of the entire 500x500 micron grid, taken at 48 hours, shows the population of CD4 (shown in shades of red), CD8 (shades of blue) and larger dendritic cells (in shades of green). Efferent vessels are arranged along the bottom of the grid, T cells and dendritic cells enter from the top of the grid



Results

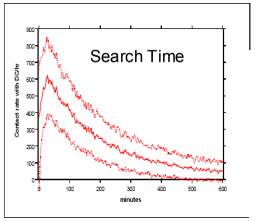


Distribution of mean exit rate (mean ± 1 S.D.) from T zone vs. time, based on 25 independent simulations involving > 250,000 T cells. The mean "transit time" from HEV to MS is a weighted average. In this example, the mean is ~ 13 hrs, but with rightward skewing.

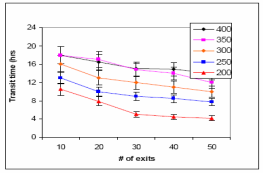
Table I: Variation in HEV vs. AL Placement Affects T cell search time, transit time, number of contacts per DC and output of non-naïve T cells

Placement of HEV with AL	Original	HEV same level with AL	AL below HEV
Transit time (hrs)	13.2 ± 0.14	13.4 ± 0.15 NS	13.3 ± 0.17 NS
Search time (hrs)	2.80 ± 0.23	2.47 ± 0.17 *	3.89 ± 0.16 ***
Average # T cell contacts per DC	18,500 ± 2700	11,800 ± 1270 ***	6730 ± 1030 ***
Cumulative output of T cells			
naïve CD4+ T cells	15,300 ± 3300	15,100 ± 3100 NS	15,500 ± 3200 NS
CD8+ T cells	13,500 ± 2600	13,400 ± 2600 NS	13,700 ± 2800 NS
non-naïve CD4+ T cells	2130 ± 310	2240 ± 300 NS	1560 ± 390 ***
CD8+ T cells	660 ± 150	540 ± 130 **	325 ± 130 ***

All comparisons are vs. original placement (1st column) as the control, using Student's t-test to compute significance levels: NS = p > 0.05, * = p < 0.05; ** = p < 0.01, *** = p < 0.001. Distance from HEV to MS was constant. 25 independent simulations for each scenario resulted in column means that were each based on 500 DCs, > 250,000 individual transit times and > 50,000 search times.

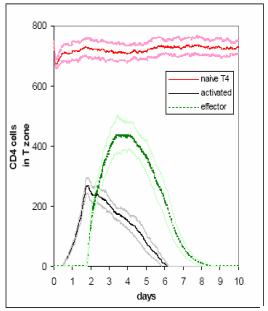


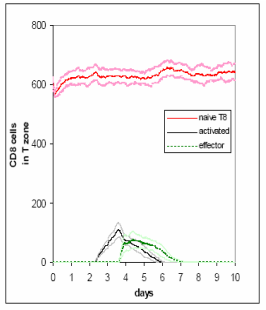
Distribution of mean contact rate with DC (mean ± 1 S.D.) for T cells entering the T zone vs. elapsed time to first encounter a DC, based on 25 independent simulations involving > 50,000 T cells. The mean "search time" from HEV to DC is a weighted average. In this example, the mean is ~ 2 hrs, but again with positive skewness.



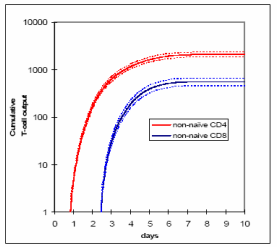
Transit time was affected by both mean distance from HEV to MS and the number of MS exits. The mean ± 1 S.D. for transit time is shown as mean distance varied from 200 to 400 microns and the number of exits increased from 10 to 50. Our simulations had 30 exits and a mean HEV-MS distance of 325 µm, based on measurements from 84 sections as shown in figure 1 b.

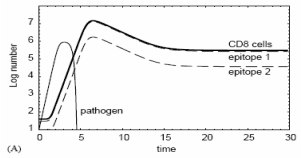
Magnitude and time course of CD4+ and CD8+ T cell production





Results from 25 simulations showing mean ± 1 SD of the CD4+ and CD8+ T cell classes, after introduction of a single pulse of antigen into the LN T zone. The CD4+ response begins with activated CD4+ T cells appearing by 24 hours and peaking by about 48 hours, followed by effector CD4+ T cells reaching a peak around 3-4 days. The CD8+ response, dependent on CD4+ helper cells, is delayed about 1 day. Cumulative output of all non-naïve T cells is shown in panel C, plotted on a log scale. Maximum outputs are achieved by about 4 and 6 days, respectively. These outputs are from cross section of a murine LN that represents about 1/400 of its volume.





Time course of immune response to LCMV infection, as measured by CD8+ counts in mouse spleen. *Antia et al, J Theoret Biol 2003: 221(4):585-598.*

Conclusions

- An agent-based model of LN dynamics can simulate the known patterns of cellular motility, velocity and LN transit times and represent the structural anatomy of a LN
- The magnitude and temporal sequence of the cellular immune response can be recapitulated
- Anatomic placement of HEVs, afferent and efferent lymphatics can be varied in simulations
- Agent-based models can capture the stochasticity of encounters between rare cognate T cells and dendritic cells in the LN.
- Varying degrees of complexity of interaction can be implemented in a rules-based model; hypotheses can be tested in silico to help in design of experiments.
- Multiple parameters can be varied to assess their association with the immune response
- This modeling approach is adaptable to implement new rules of interaction of dendritic cells with T-cells as they become elucidated by experimental data.

Future directions

- Adapt model to specific infectious agents
- Adapt model to immune response to vaccination
- Contrast chemotaxis with random T cell motion
- Assess effect of drugs that change recruitment to LN or exit from LN