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Predicting lymph node output efficiency using systems biology

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Abstract

Dendritic cells (DCs) capture pathogens and foreign antigen (Ag) in peripheral tissues and migrate to secondary lymphoid tissues, such as lymph nodes (LNs), where they present processed Ag as MHC-bound peptide (pMHC) to naïve T cells. Interactions between DCs and T cells result, over periods of hours, in activation, clonal expansion and differentiation of antigen-specific T cells, leading to primed cells that can now participate in immune responses. Two-photon microscopy (2PM) has been widely adopted to analyze lymphocyte dynamics and can serve as a powerful in vivo assay for cell trafficking and activation over short length and time scales. Linking biological phenomena between vastly different spatiotemporal scales can be achieved using a systems biology approach. We developed a 3D agent-based cellular model of a LN that allows for the simultaneous in silico simulation of T cell trafficking, activation and production of effector cells under different antigen (Ag) conditions. The model anatomy is based on in situ analysis of LN sections (from primates and mice) and cell dynamics based on quantitative measurements from 2PM imaging of mice. Our simulations make three important predictions. First, T cell encounters by DCs and T cell receptor (TCR) repertoire scanning are more efficient in a 3D model compared with 2D, suggesting that a 3D model is needed to analyze LN function. Second, LNs are able to produce primed CD4⁺T cells at the same efficiency over broad ranges of cognate frequencies (from 10⁻⁵ to 10⁻²). Third, reducing the time that naïve T cells are required to bind DCs before becoming activated will increase the rate at which effector cells are produced. This 3D model provides a robust platform to study how T cell trafficking and activation dynamics relate to the efficiency of T cell priming and clonal expansion. We envision that this systems biology approach will provide novel insights for guiding vaccine development and understanding immune responses to infection.

Keywords

Agent based model; 3D; Priming; Effector; T cells

1. Introduction

Adaptive immunity develops in response to pathogens that cannot be cleared by first line defenses. It relies on the selective recognition of foreign antigens (Ags) to direct the immune response and can lead to long-lived immunological memory. Secondary lymphoid tissues such as the Peyer's patches, lymph nodes (LNs) and spleen are the canonical sites where adaptive immune responses are initiated. Here, naïve lymphocytes (both CD4 and CD8 T cells) encounter antigen-presenting cells (APCs), such as dendritic cells (DCs), bearing foreign Ag. Each naïve T cell clone expresses a T cell receptor (TCR) with unique specificity for antigenic peptides bound to major histocompatibility (MHC) molecules on the surface of an APC. The clonal frequency of T cells for any given Ag varies, but is typically low, with about one in a million T cells specific for each unique Ag epitope (referred to as *cognate frequency*).

Two-photon microscopy (2PM) allows single-cell lymphocyte dynamics to be analyzed deep within intact lymphoid tissues and in living mice (Miller et al., 2002, 2004; Germain and Jenkins, 2004). However, a comprehensive understanding of immune cell interactions during infection is still lacking, and, in particular, the fate of individual cells over longer timeframes is difficult to track in vivo. Systems biology approaches can be used to integrate knowledge into a discrete and stochastic representation of LNs and simulate cellular dynamics occurring within a LN (Mirsky et al., 2011). Agent-based models (ABM) are widely used to study emergent properties of complex systems with interacting heterogeneous components, making them ideal for studying immune cell dynamics and lymphoid tissue function.

ABMs have been used to examine important aspects of LN dynamics, including naïve T cell motility and behavior in the LN (Bogle and Dunbar, 2008; Riggs et al., 2008), the physical interaction between DCs and T cells (Beltman et al., 2007; Zheng et al., 2008) and the role of chemotaxis facilitating cognate T cell–DC interactions (Riggs et al., 2008; Vroomans et al., 2012). In particular, we (Linderman et al., 2010) used an ABM to study factors influencing CD4 T cell priming and proliferation; our two dimensional (2D) model predicted a *LN efficiency*, the ratio of the number of effector T cells leaving the LN to the number of naïve cognate T cells entering the LN. Our model indicated that the primed CD4 T cell output is linearly correlated with cognate frequency, but not with the number of Ag-bearing DCs. Bogle and Dunbar (2010) developed a 3D ABM to simulate T cell activation in the LN during an immune response, and a recent extension of this model (Bogle and Dunbar, 2012) incorporated detailed mechanisms regarding T trafficking. However, infection dynamics and the capacity of LN to produce effector T cells has yet to be analyzed in the context of realistic LN structure and T cell density.

New data presented here regarding LN anatomy allow us to update our previous model framework (Riggs et al., 2008; Linderman et al., 2010). As new experimental data increase our understanding of lymphoid tissue structure and function, this information provides an opportunity to refine, expand and validate our models and simulations. In addition, we are interested in using a 3D ABM model to test how LN output (defined by the generation of primed T cells) responds to changes in numbers of APCs and cognate T cells during different infection scenarios. Furthermore, we address a debate within the modeling community: do 2D computational models of LN have predictive value for understanding 3D cell dynamics and LN function in vivo or are 3D models required?

In this study, we present a novel 3D ABM that captures T cell and DC dynamics, can accommodate low (physiological) cognate T cell frequencies, and includes T cell activation and expansion kinetics. We built and validated this model by comparing our model

simulations to data generated by in situ imaging approaches. We use this model to address questions that are currently intractable with in vivo imaging, due to the limits of tracking individual cells over long time scales. Our model allows us to test quantitative predictions for how perturbations to LNs affect not only single-cell dynamics, but more importantly T cell activation and expansion kinetics, which has direct relevance for vaccine design and guiding therapeutic interventions for infection.

2. Methods

2.1. Fluorescent immunohistochemistry of LN sections

All animal procedures and husbandry practices were included in protocols approved by the University of Pittsburgh's Institutional Animal Use and Care Committee (IACUC). Axillary or inguinal lymph nodes from Mtb-negative cynomolgus macaques were biopsied and fixed in 10% neutral buffered formalin using previously reported procedures (Capuano et al., 2003; Lin et al., 2009) before being embedded in paraffin and cut into 5 μ m sections by the University of Pittsburgh Medical Center's in situ laboratory. Multicolor fluorescent immunohistochemistry was performed on serial sections. Briefly, Ag retrieval was performed in a pressure cooker (Mantra, Piscataway, NJ) containing boiling Ag retrieval buffer (20 mM Tris/820 mM EDTA/0.00005% Tween 20 [pH 9.0]) followed by blocking with 2% FBS-PBS.

Sections imaged for T cells, B cells and dendritic cells were stained first with antibodies against CD3 (rabbit polyclonal; Dako, Carpinteria, CA) and CD11c (mouse monoclonal, clone 5D11; Leica Microsystems, Buffalo Grove, IL) followed by fluoroconjugated donkey anti-rabbit (Jackson ImmunoResearch, West Grove, PA) and donkey anti-mouse (Life Technologies, Grand Island, NY) secondary antibodies. After incubation with secondary antibodies, B cells were stained with fluoroconjugated anti-CD20 antibodies (rabbit polyclonal; Thermo Fisher Scientific, Kalamazoo, MI) that were labeled with Zenon antibody labeling reagents (Life Technologies). A separate section was stained for high endothelial venules (HEV) with antibodies against peripheral lymph node addressin (PNAd; clone MECA-79; BioLegend, San Diego, CA) and lymphatic vessels stained with antibodies against LYVE-1 (goat polyclonal; R&D Systems, Minneapolis, MN) followed by fluoroconjugated donkey anti-rat, donkey anti-rabbit and donkey anti-goat secondary antibodies (Jackson ImmunoResearch). Between incubation all steps, and before mounting coverslips, the sections were washed 3–5 times with PBS containing 0.2% Tween-20. Coverslips were mounted with Prolong Gold Antifade mounting medium containing DAPI (Life Technologies) and the sections were imaged with either an Olympus Fluoview 500 or Fluoview 1000 laser scanning confocal microscope (Olympus, Center Valley, PA) maintained by the University of Pittsburgh's Center for Biologic Imaging. Three-color images (red, green, far red [pseudocolored as blue]) were acquired sequentially at 200–400 $\times$ magnification, followed by a DAPI image (gray) showing nuclei. Because the lymph nodes were too large to image in a single field, multiple overlapping fields were acquired and assembled into a single composite image with Photoshop (Adobe Systems Incorporated, San Jose, CA).

2.2. Two-photon microscopy to track T cell movement

CD4⁺ T cells were isolated from spleens and LNs of C57BL/6 mice (The Jackson Laboratories) by magnetic negative selection (Stem-Sep). T cell purity was >95% CD4⁺, CD3⁺ as assessed by flow cytometry. T cells were labeled with 5 μ M CFSE (Molecular Probes) for 30 min at 37 °C, washed once, and 5–10 million cells were transferred into B6 mice by tail vein injection. Mice were euthanized by CO₂ asphyxiation 18 h after T cell transfer and cervical LNs removed for 2PM as previously described (Miller et al., 2002,

2004). Briefly, LNs were secured in the flow chamber with a thin film of VetBond (3M) and maintained at 37 °C by superfusing the chamber with warm RPMI bubbled with a mixture of 95% O₂/5% CO₂ (Carbogen gas). Time-lapse imaging was performed using a custom built two-photon microscope at the Washington University School of Medicine. CFSE labeled T cells were excited by a Chameleon Ti:sapphire laser (Coherent) tuned to 780 nm and fluorescence at 490–550 nm was collected. ImageWarp (A&B software) was used to control the various hardware devices during real-time acquisition and to process and archive the image data. Images were acquired every 27 s, with each time point consisting of a z-stack of 21 sequential optical sections 2.5 μm apart to yield a 200 μm by 250 μm by 50 μm volume (x and $y=2$ pix/μm). In some experiments we increased time resolution to 11 s by acquiring 15 z-steps, yielding an imaging volume of 200 μm by 250 μm by 35 μm volume (x and $y=2$ pix/μm). To increase signal contrast, we averaged 10 video frames for each z-slice. Multi-dimensional rendering and cell tracking were performed with Imaris (Bitplane).

2.3. Model geometry and cell density

Our 3D model represents a trabecular region of a generalized LN. Fig. 1 shows an idealized LN structure. Afferent lymphatic vessels deliver lymph fluid and DCs into the subcapsular sinus, where they enter into LN parenchyma. LN parenchyma is organized into B cell follicles and T cell zones. Trabeculae further divide the parenchyma into incompletely separate compartments. T cells are recruited from high endothelial venules (HEV). Within the T cell zone, our region of focus, DCs and T cells interact. T cells drain into the medullary sinuses and exit the LN via efferent lymphatic vessels.

In order to determine the size and structure of a LN (and the T cell zone in particular), we used immunofluorescence images of LN sections from non-human primates (NHPs). As shown in Fig. 2, the deeper cortex of an LN mainly consists of T cells (blue). In this LN section, the area is bean-shaped with a diameter of 2.6 mm. The T cell zone is a shell about 800 μm thick surrounding the medulla. PNAd is a marker for High Endothelial Venules (HEVs), the sites where CD4 and CD8 T cells enter the LN. From the image we can see that HEVs are distributed throughout the T cell zone. LYVE-1 is a marker of medullary and cortical sinuses, from which T cells are collected into efferent lymphatics and exit a LN. Medullary sinuses are distributed at the bottom of T cell zones. Cortical sinuses can be found near the structures that divide T cell zones laterally, and are typically in vicinity of HEV. This distribution pattern of cortical sinuses is also observed in mouse studies (Grigorova et al., 2010).

We also analyzed the T cell zones of these images and calculated the T cell density. We measured the cell density from the NHP LN images using the software CellProfiler (Carpenter et al., 2006). We sampled five 200 × 200 μm² regions of the T cell zone. The depth of the sections is 5 μm, which is approximately the diameter of a T cell. We calculated the density of CD3+ cells (both CD4+ and CD8+ T cells) to be $4.0 \pm 0.4 \times 10^6 \text{ mm}^{-3}$.

Based on these first time data from primate LNs (Fig. 2) and other studies (Grigorova et al., 2010), we designed the grid structure of our 3D LN ABM (Fig. 3). We abstracted the shape of the T zone in the LN to be part of a spherical shell, with thickness of 800 μm. Accounting for symmetry within T zones (Figs. 1 and 2), we chose a section of the LN to model by defining a computational grid in the shape of a cone-plug similar to a trabecular section (Fig. 3). This volume represents roughly 0.5% of a LN and balances biological relevance and computational performance. Volumes up to 2% of a LN were tested in simulations to confirm that model behavior is preserved. We define our grid compartments as cubes of edge length 5 μm. The entire LN grid has 344148 grid compartments, and the total volume is 0.043 μL. The number of T cells on the grid in steady state is about 1.7×10^5 .

We also generated a fibroblastic reticular cell (FRC) network, which occupies 17% of the entire LN grid (Beltman et al., 2007). This network has a wide range of functions in LNs, but in our current model it serves to block movement of cells. We explore the role of the FRC further in other work.

2.4. Model agents and interactions

Within the T cell zone, DCs display peptide-MHC (pMHC) on their surfaces and move randomly. T cells migrate through the T cell zone until they encounter a DC bearing cognate Ag. T cells recognizing pMHC are activated over several hours while bound to DCs (Celli et al., 2007). After detaching from the DC, T cells are primed and undergo several rounds of division before exiting the LN.

Our model is based on a stochastic cellular automaton, in which different types of cells (T cells and DCs) are represented as agents on a 3D grid. In discrete time steps, the agents move on the grid. Agents interact with their environment as well as other agents following a defined set of rules with stochastic components. These rules are based on current understanding of immunology, or in the case where experimental evidence is insufficient, assumptions were made. For a complete list of agent rules see the on-line Supplement. Fig. 4 depicts the model and below we give more details regarding how data were used to build and validate the model, with data from both mice and NHP studies (Fig. 2). The emergent behavior of our virtual LN is quantitatively analyzed in real-time simulations.

The LN ABM includes three sets of cells: CD4+T cells, CD8+T cells and DCs, each with different activation states: CD4+ and CD8+T cells can be resting, bound or proliferating. When cells have undergone a sufficient number of divisions, they can exit the LN and we refer to them as effector T cells, even though they need continual cytokine or Ag stimulation to remain fully activated. DCs can be immature, Ag-bearing (Ag-DC), or licensed (LDC). We briefly summarize the rules for the cell agent here, but a full list of rules is given in the on-line supplement.

2.4.1. CD4+T cells—CD4+T cells are $5 \times 5 \times 5 \mu\text{m}^3$ in size, which is equal to the size of one grid compartment. These cells are recruited via HEVs with probability specified in the parameter file (see Appendix). At each time step, free CD4+T cells move one grid compartment in a randomly selected direction. We define preferred and non-preferred movement directions relative to the previous movement direction (based on the idea of random walk with persistence (Riggs et al., 2008)), and assign probabilities to the possible 26 different direction options in 3D. Movement of CD4+T cells are periodic or toroidal at boundaries, so if a cell steps off the grid, the same type of cell emerges on the opposite side of the grid.

Cognate CD4+T cells can be primed by Ag-DCs and LDCs. When a T cell enters the LN grid, it will be either a cognate or non-cognate cell based on the cognate frequency specified (ranging from 1.6×10^{-5} to 1×10^{-2} in our simulations). At each time step, we check whether there are Ag-DCs or LDCs in the binding radius of a cognate CD4+ T cell. If there are DCs available for binding, one is chosen randomly and attempts binding with a probability based on a logistic function that accounts for pMHC-II levels (see Supplementary). Before unbinding from the DC (Miller et al., 2004), the amount of accumulated stimulation is calculated, and a T cell will either be primed and become activated, or remain in the resting naïve state. Activated T cells proceed through a series of cell divisions. Daughter cells are identical to mother cells in most attributes. After 4 divisions (as estimated in a previous study (Linderman et al., 2010)), the CD4+T cells are classified as effector cells and can leave the LN.

CD4+T cells can die anytime during these dynamics when they exhaust their life span. They can also leave the grid by draining through medullary sinuses and exiting via efferent lymphatics (ELs) in both resting and effector states. The rate of T cells exiting the grid is controlled by the parameter specifying the number of the EL elements generated the grid (See Appendix). Actively dividing cells are excluded from exiting because of down regulated S1P1 on the surface of T cells needed for their egress (Shiow et al., 2006).

2.4.2. CD8+T cells—The rules and dynamics of CD8+T cells are similar to those outlined above for CD4+T cells. However, only LDCs can prime CD8+T cells. This rule represents a requirement for CD4+T cell help for CD8+T cell expansion (Smith et al., 2004), and the binding and priming are based on pMHC-I molecule level on the surfaces of these LDCs. The model is flexible to account for testing other CD8/CD4 dependencies for activation.

2.4.3. Dendritic cells—DCs are larger in size than T cells; they occupy 8 compartments ($2 \times 2 \times 2$) on our grid (see Fig. 4). DCs are recruited from the top of the grid (representing afferent lymphatics, Fig. 1). The region of entry is controlled by two parameters, specifying top and bottom grid slices (See Appendix). Unlike T cells, DCs are not allowed to leave the LN. This assumption is based on experimental data showing that DCs are not typically found in the medullary sinuses. When recruited, DCs can be immature (IDC) or Ag-DCs. At each time step, one DC is recruited with a given probability. DCs can move in any of 26 directions (defined as the Moore neighborhood) with the same probability. To capture DC movement around other cells, we assume that if there are T cells in the destination grid space that a DC is moving into, the T cells will be relocated to neighboring compartments. We also assume that DCs exhibit some plasticity, and can bend around the FRC structure. We assume reflecting boundary conditions for DCs (i.e. DCs cannot cross the LN boundary). This assumption is appropriate because DCs rarely reach the boundaries as their movement is slow compared to T cells and then accumulate around HEVs (Miller et al., 2004, 2004; Girard et al., 2012). It also simplifies computations, as DCs simultaneously occupy 8 model compartments.

At the time of entry into the LN, each DC is assigned a number of both surface pMHC-I and pMHC-II molecules from a given range of values. The number of pMHCs decrease over time according to a value for the pMHC-I and pMHC-II half-life decay values (Zehn et al., 2004). IDCs can be activated if they come into contact with an Ag-DC or LDC. Primed CD4+T cells in contact with an Ag-DC can license that Ag-DC to become an LDC with a given probability. The numbers of pMHC I and II on the surface of a DC increase after activation or licensing (Brophy et al., 2007). DCs die when their ages exceed the limit drawn from a distribution.

2.5. Model parameter estimation

There are a total of 74 parameters in our 3D LN model, governing the behavior of CD4+T cells, CD8+T cells, DCs and the environment of the LN. We grouped parameters into different functional sets such as anatomical structure or based on mechanisms they are involved in, for example: motility, population size, priming related, etc. Values of parameters were taken from experimental studies when available, or adopted from previous models. For those parameters for which we are unable to find an experimental value, we estimate a range based on biological knowledge. These values and ranges are shown in Appendix. All parameters were studied using uncertainty analysis to assess how changes in their values affect simulation outputs and to help fine tune their values based on validation with data. Values of parameters related with physiological structure and cell movement are estimated with an uncertainty analysis. A baseline situation (“no infection/no antigen”) in which no Ag-DCs are recruited to the LN is established with these parameter values.

2.6. Definition of model outputs

We define several outputs that we use to explore model behavior in detail:

Transit time: average time for a T cell to travel through a LN (Linderman et al., 2010). A non-cognate T cell requires ~24 h to travel through the entire lymph system and exit into the blood, based on data from cannulated thoracic ducts (Sprent and Tough, 2001). Since there are 700 LNs distributed throughout the body, we estimate that it should take on average less than 24 h but more than 1 h for a T cell to traverse a LN. For each T cell, we record when it enters the grid from HEV and when it exits through an EL.

Search time: average time for a T cell to find the first matching Ag-DC or LDC.

Match percentage: percentage of cognate T cells that detected Ag-DC or LDC before exiting the LN.

Scanning rate: average number of T cells contacted by a DC per unit time. Mature or licensed DCs constantly probe their surroundings for cognate T cells. A scanning rate is calculated by dividing the increment of a scanning event during unit time by the average Ag-DC/LDC ratio. In mice the T cell scanning rate has been reported to be between 500 and 4000, depending on the experimental system (Miller et al., 2004; Bousso and Robey, 2003). We keep a record of both the total number of scanning (*apparent scanning rate*), as well as the number of T cells that have not been previously scanned by the same DC (*unique scanning rate*).

Displacement: for both T cells and DCs, we record the number of steps taken by cells (maintained within vectors). By vector addition, we get the displacement of the cell over any given time interval; and by summing the length of these vectors we calculate the distance that the cell travels in a time interval.

Motility coefficient: assuming a random walk, the motility coefficient M (Miller et al., 2004) is calculated from $M = x^2/6t$ where x is the displacement of a T cell and t is time. We also calculated the **velocity** of these cells by obtaining the regression coefficient of distance versus time. We used the same algorithm to calculate motility coefficient and velocity for both 2PM data from mouse experiments and model simulations.

Free path: the average length of a cell traveling without changing its direction is a description of T cell movement in a LN (Miller et al., 2003). In our model, we implemented explicit criteria for a free path called **current trajectory free path**, which compares the direction of each single step in a current free path to the direction of the vector from the initial point to current location of cell. If a step is longer than 5 μm and the angle is larger than 45°, we assume the current free path ends and a new free path starts. This algorithm was developed to enable the comparison between simulated results and 2PM data (see Supplementary rules for more information).

LN priming efficiency: the ratio of the number of effector T cells leaving the LN to the number of naïve cognate T cells entering the LN (Linderman et al., 2010). We use this measurement as an indicator of the capacity of LN to expand the cognate T cell population during an immune response. As a primary model output, we record the number of effector T cells exiting the LN and the numbers of incoming naïve cognate T cells during a period of time and calculate their ratio. We apply this method to chronic infection scenarios where different cell populations are in steady-state, so that the start/end time points of the simulation will not introduce bias.

2.7. Simulated acute and chronic infection scenarios

We introduce Ag-DCs to the system in two ways to simulate 2 different types of generalized infection scenarios: acute infection (brief Ag input and then waning after a specified number of days) and chronic infection (constant Ag input). We first simulate cellular dynamics within a LN in the absence of Ag-DC until a steady state is reached. Then, Ag-DCs are recruited to the system. To mimic an acute infection dynamic, we chose a DC recruitment period of 5 days to model a generalized host response. To mimic chronic infection, Ag-DCs are introduced into the system at a constant rate until the end of the simulation.

2.8. Uncertainty and sensitivity analysis

A complete list of all model parameters and their values are listed in the Appendix. We use uncertainty analysis to study how parameter variability induces variability of model outputs. Latin Hypercube Sampling (LHS) is employed in order to sample the high dimensional parameter space efficiently (Marino et al., 2008). In cases where a distribution for a parameter is unknown, we assume a uniform distribution within a physiologically plausible, but large, range. For each parameter value combination, simulations are performed with multiple replications (to account for stochastic uncertainty, i.e. aleatory uncertainty) and statistics are recorded. Partial Rank Correlation Coefficients (PRCCs) are calculated to evaluate global parameter sensitivity (Marino et al., 2008). The variability of each recorded output statistic is apportioned to the variability of input parameter values, and non-linear monotonic relationships are detected. We use this correlation coefficient as an indicator of sensitivity of a specific model output to different model mechanisms and inputs. This allows us to predict factors in the model that are key to driving system dynamics.

After defining a baseline simulation, we use sensitivity analysis to focus on a specific set of parameters involved in T cell priming events to study how they affect model output. This allows us to make predictions for how LN output will change if we modify corresponding mechanisms in vivo. 500 experiments are performed, and each experiment is simulated with 5 replications. After the simulation, the model outputs of each experiment were averaged, and the PRCC of average model output of interest (e.g. scanning rate, match percentage and output of effector cells) was calculated against the parameters that were varied. Then, PRCCs were tested for significance with significance level of $\alpha=0.001$.

To complement the uncertainty and sensitivity performed above, we also apply, for the first time in the setting of an ABM, an additional tool known as the extended Fourier amplitude sensitivity test (eFAST) (Marino et al., 2008; Saltelli and Bolado, 1998; Saltelli et al., 1999; Saltelli, 2004; Cukier et al., 1973; Collins and Aivissar, 1994). This method is more general than PRCC (i.e. it applies to non-linear non-monotonic relationship between input and output variability) and will help confirm the sensitivity of parameters as determined using the LHS/PRCC method. eFAST is a variance decomposition method: parameter values are varied at different frequencies, and variability of output is quantified and partitioned by Fourier analysis. Therefore, the fraction of the variance explained by each input parameter is an indication of the model's sensitivity to this parameter. We picked parameters related to priming and simulated the chronic infection with 65 sampling, 2 re-sampling and 4 replications focusing on each of these parameters. We use the first order effect (S_j) and total effect (S_{Ti} , sum of first order and higher order effects) of eFAST protocol to evaluate sensitivity. Details of the implementation can be found in an earlier publication (Marino et al., 2008).

To understand the robustness of the model, we examined the source of uncertainty by comparing results from replications and results from different parameter sets. As discussed in previous studies (Marino et al., 2008), in ABMs, it is not possible to entirely separate the

aleatory uncertainty that is generated from stochastic components of simulations from epistemic uncertainty that is generated from variations in parameter values. Although for some of the outcomes the two effects can be distinguished (Fig. 5A), for others, aleatory uncertainty could partially mask epistemic uncertainty (Fig. 5B). We address this by using the mean of multiple simulations as the input into the PRCC program (Marino et al., 2008).

2.9. Computational simulations and implementation

Our ABM is written in C++ and runs on Linux/Mac/Windows. Pseudo code for the agent rules is available in the online Supplement (<http://malthus.micro.med.umich.edu/lab/movies/3dLN/>). Simulations require ~6–10 h depending on the total number of time steps and the size of LN grid. Each simulation is repeated 5 to 10 times in the sensitivity analysis.

3. Results

The key goals of this work are predictions about the efficiency of LNs in priming T cells and determination of how the model simulation environment dimension (i.e., 2D vs 3D) affects those predictions. We built, calibrated and validated an ABM model based on available data and data generated herein as described in Section 2. A sample snap shot simulation is shown in Fig. 3C.

3.1. T cell motility validation

Fig. 6A and B shows T cell track data measured by 2PM. We generated 2 sets of data describing T cell tracks from 3D live tissue, with imaging intervals of 11 and 27 s respectively, and calculated the mean free path of the recorded T cell tracks (Fig. 5A and B). We then calculated the motility coefficient and velocity of these data (Table 1). Any discrepancy between velocities calculated from these two datasets arises as a consequence of a more detailed track from a finer imaging interval in the 2PM.

We used these data to calibrate our model before introduction of Ag-DCs. Our simulations agree with these data as well as with data from other modeling studies regarding duration of T cells traveling through a LN (Bogle and Dunbar, 2010) and the rate of T cells exiting the LN (~700–1400 per minute per 10^6 cells) (Bogle and Dunbar, 2010) (Table 1).

We also create time-lapse simulations of cell movement in our 3D LN model. Simulations in the absence of Ag-DCs are available at <http://malthus.micro.med.umich.edu/lab/movies/3dLN/> (On-line Supplement Movie 1).

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.jtbi.2013.06.016>.

3.2. Simulating acute infection dynamics

The temporal dynamics of an acute infection scenario are shown in Fig. 7 and spatiotemporal dynamics are shown in a time-lapse simulation (Supplement Movie 3, <http://malthus.micro.med.umich.edu/lab/movies/3dLN/>). The number of Ag-DCs begins to increase from day 0. CD4+T cells are activated after binding with Ag-DCs, and then further differentiate into fully primed T cells. Ag-DCs are gradually activated to LDC phenotypes after about 2 days, in the presence of these newly primed CD4+T cells. Licensed DCs are able to prime CD8+T cells. The priming of CD8+T cells and the initiation of proliferation and generation of activated cells exhibit a delay of about 2.5 days compared to CD4+T cells (Fig. 7B and C.) After DC recruitment ceases, primed T cell counts drop while Ag-DCs and LDCs die out (they have a very short half-life), and the system gradually returns to a baseline uninfected scenario. When Ag-DCs and LDCs are present, we observed almost a

constant rate of CD4+ and CD8+ primed T cell output from the LN, where primed CD8+T cells appear a few days later, and more of them are produced during the process (Fig. 7D). The dynamics generated in this case is representative of what happens in an acute infection where an adaptive immune response to a generalized pathogen clears infection (reduces Ag to zero) within a couple of weeks.

3.3. Simulating chronic infection dynamics

In a chronic scenario, the initial pattern of cellular dynamics follows closely what is observed during our acute infection simulations (compare Fig. 7 with Fig. 8 for early points by day 8). However, after about day 8, numbers of different subpopulations of DCs (Fig. 8A) and T cells (Fig. 8B and C) reach a new steady state, resulting in a constant rate of output of fully primed (effector-like) CD4+ and CD8+T cells (Fig. 8D). The dynamics generated above reflect the response of an immune system when faced with a chronic infection, constantly challenging host defense mechanisms.

3.4. Baseline LN statistics and LN priming efficiency scales with grid size

We compare simulations using a LN model of original size (Fig. 3) to those run on a larger grid to test whether cell dynamics are independent of grid size. The larger grid has the same height, but twice the length and width to yield a grid representing four times the volume of our standard simulated LN region. The number of HEVs, ELs and the DC recruitment rate are increased proportionally on the larger grid. For each grid, 10 simulations were performed and the results are shown in Table 2. T cell density, motility and transit time are preserved over the two LN grid sizes. Furthermore, the priming process and related results were not sensitive to LN grid size, as scanning rate, LN priming efficiency and search time all remain unchanged. Thus, the section of the LN T zone we model (Fig. 3) is sufficient to capture larger scale LN dynamics. These results also suggest that using reflecting boundary conditions (as opposed to toroidal boundary conditions) for DCs does not affect model output.

3.5. LNs maintain a high priming efficiency when cognate frequency increases

In our previous studies, we found that LN efficiency related to priming of CD4+ T cells is unaffected by cognate frequency in a 2D ABM model of a LN but increased and then plateaued with the number of Ag-DCs (Riggs et al., 2008; Linderman et al., 2010). To test whether this also holds true in our 3D model, we uncoupled cognate frequency of CD4+ and CD8+T cells to study how priming of each cell type is affected. For each set of simulations, we varied the maximum number of Ag-DCs in the simulation from 50 to 550 (total or per time point), together with the DC recruitment rate (Fig. 9A and B). This allows us to test how varying levels of APC affects priming. In addition, we simultaneously varied cognate frequency of CD8+T cells from 1.6×10^{-5} (in the range of true physiological condition) to 1.0×10^{-2} (a boosted high frequency, similar to that used in 2PM studies). Simulations are carried out with low and high CD4+ cognate frequencies. Under both conditions, CD8+T cell priming efficiency increases as DC recruitment is increased, until LN efficiency plateaus near 350 Ag-DCs. Additional Ag-DCs do not lead to additional T cell priming, indicating that saturating the LN with Ag-DCs would not be effective. For each given Ag-DC level, the increase of cognate frequency only resulted in slight reduction of LN efficiency, indicating the effector output is almost linear to the precursor cognate frequencies. We also tested how CD4+T cell cognate frequency affects LN efficiency for priming of CD4+T cells. Our results show that across different DC numbers, LN efficiency of CD4+T cells is also only altered in a relatively small range when CD4+ cognate frequency increases in orders of magnitudes, from 1.6×10^{-5} to 1.0×10^{-2} (Fig. 9C). These results agree with our previous study (Linderman et al., 2010); LNs have a high theoretical T cell priming capacity over a wide range of cognate frequencies. In other words, LNs

potentially maintain T cell priming efficiency for cognate frequencies that range from high physiological values to over 3 orders of magnitude larger.

We also calculated the CD8+T cell priming efficiency when CD4+T cell cognate frequencies vary between low and high ranges, and observed that CD8+T cell LN efficiency is higher when more cognate CD4+T cells are present, as expected. In the case of high CD4+ cognate frequency, the differences in efficiency across various CD8+ cognate inputs are even smaller (Fig. 9A vs. B), and the plateau is reached earlier. These simulations suggest that if the LN system is augmented by introducing more cognate CD8+T cells to the system (e.g. by vaccination), LN efficiency will remain almost unaffected. In addition, LN efficiency can be enhanced by adding exogenous Ag-DCs and/or cognate CD4+T cells.

3.6. LN scanning is more efficient in 3D than 2D

In our previous 2D study, we calculated a LN efficiency for CD4+T cells of ~4–5 (Linderman et al., 2010). Using our 3D ABM of a LN T zone, we now find LN efficiencies for CD4+T cells of up to 15. We also assessed the match percentage and search time, and results are shown in Table 3. The match percentage in our 3D model is ~90%, higher than the 40–60% measured in our 2D model (Linderman et al., 2010). The average 3D search time is only 10 min, less than the 2–5 h measured in our 2D model.

We next “flattened” our 3D LN grid to mimic a 2D model (which preserves dimensions in the previous 2D simulations as well). Using the same set of parameters, simulations yield a match percentage of 50% and a search time of 150 min (Table 3), similar to those obtained in our previous 2D model (Linderman et al., 2010). Thus, match percentage and search time are highly dependent on model dimensionality.

Why is it easier for cognate T cells to find their matched Ag-DC in 3D compared to 2D? To understand this, we calculated the average distance to the nearest neighbor among DCs in 2D and 3D (Clark and Evans, 1954, 1979) using

$$\mu(r) = \frac{[\Gamma(k/2+1)]^{1/k} \Gamma(1/k+1)}{\rho^{1/k} \pi^{1/2}} \quad (1)$$

$\mu(r)$ is the expected mean distance between individuals when a population is randomly distributed; k is the dimension and ρ is the population mean density. For the DC density used in our model, the average nearest neighbor distance between DCs calculated by Eq. (1) is 29.5 μm in 3D, and 82.5 μm in 2D. Thus a large part of the increase in the T cell search efficiency in 3D is due to shorter neighbor distances between DCs.

The frequency of T cells visiting the same DC was less in 3D than 2D. We tested this by tracking how many T cells a DC can scan on average per hour. As shown in Table 3, the apparent scanning rate is about 2.5 times the unique scanning rate for both CD4 and CD8 T cells in 3D, but about 4.5 in 2D. Compared to 2D, DCs in 3D are more likely to encounter T cells that they have not previously contacted. This also contributes to the observed differences between 2D and 3D models.

3.7. Sensitivity analysis detects mechanisms that correlate with higher effector T cell output

Cognate frequency and Ag-DC recruitment correlate with the production of primed T cells leaving a LN (Fig. 9). However, other parameters may significantly influence LN output as well. In order to identify such correlations, we used sensitivity analyses.

We examine 16 parameters that are likely to either directly or indirectly influence T cell priming (See Appendix). The parameters that show significant correlation are shown in Tables 4 and 5, for acute and chronic scenarios, respectively.

For the acute scenario, we chose day 4 (when the total DC count is maximum, and priming is ongoing) to examine the influence of various mechanisms on outcomes such as scanning rate and match percentage, as well as effector T cell output on day 14 (after the immune response is dampened). The result shows that in an acute scenario, both CD4 and CD8 T cells scanning rates were correlated positively with Ag-DC licensing probability, and correlated negatively with CD4 T cell binding time and the pMHC level needed for 50% priming probability (Table 4). The match percentage is correlated negatively with Ag-DC licensing probability, and correlated positively with T cell binding time and pMHC stimulation requirements (Table 4). These results indicate that faster DC licensing and a more sensitive priming system induces high levels of scanning, but that they negatively affect the percentage of T cells that have been scanned by DCs before they exit. This effect is likely due to a reduced average lifespan of the DCs, since both Ag-DC (with an average lifespan of 60 h) and LDC (with an average lifespan of 36 h) can prime CD4+T cells. The production of primed cells also has a significant negative correlation with the Ag-DC licensing probability, as well as the average binding of T cells to DCs. Because a higher Ag-DC licensing probability leads to reduced DC lifespan, it also reduces effector T cell output. However, a shorter CD4+T cell binding time, as long as it is productive, permits more T cell binding opportunities and therefore results in more primed T cells.

We tested whether these same mechanisms were at work during a simulated chronic infection. We chose day 14 for our analysis since cell dynamics approach a steady-state. In these simulations, we found that higher licensing probability by Ag-DCs still increases scanning rates and reduces match percentages of T cells, but it has a less significant role on the production of primed CD4+ and CD8+T cells (Table 5). This could be explained by the continuous influx of Ag-DCs in the chronic infection scenario. Even though the average number of Ag-DCs and LDCs will be lower when licensing probabilities are high, the impact is still much smaller considering the high LN efficiency achieved when DC density is low, as discussed in previous sections.

As shown by the PRCC results, the parameters that most strongly affect our 3D model outputs are different from those in the 2D model (Linderman et al., 2010). To test whether this is due to dimensionality, we performed the same PRCC analysis using the 2D version of the 3D model. The results for the chronic scenario (Table 6) are consistent with the previous 2D results. CD4 T cell output is sensitive to the priming threshold and binding threshold. This discrepancy might be explained by the more efficient searching in 3D. Because Ag-DCs are comparatively easier for cognate T cells to find, T cells have a higher chance of being primed, so the threshold for binding or priming is no longer a bottleneck for the production of effector cells.

We used eFAST to confirm our sensitivity analysis results. We varied the values for a focused list of parameters, including binding threshold and maximum binding time for CD4+T cells, maximum binding time for CD8+T cells, and Ag-DC licensing probability. Results are shown in Table 7. These results are consistent with the PRCC analysis performed earlier (see Table 5). Using this method, CD4+ and CD8 T cell outputs are most sensitive to binding time, and sensitivity is induced mainly by first order effects (i.e. explained by this parameter alone and in a linear manner). In addition, scanning rates of both CD4 and CD8 T cells are sensitive to binding time of CD4 T cells, while match percentage of CD4 T cell is sensitive to the licensing probability of Ag-DCs. In summary, using two

distinct analysis tools (PRCC and eFAST), we confirmed that the mechanisms stated above strongly influence the LN functionality.

4. Discussion

In this work, we created a 3D ABM model of a LN T zone that embodies the physiological structure of non-human primate LNs and is calibrated with cell motility data obtained with 2PM. We used the model to learn how LN output efficiency is affected by the number of entering cognate T cells and DCs and by model dimensionality (2D vs. 3D). We performed computational simulations of T cell repertoire scanning followed by clonal expansion in the LN under various conditions. We found that a high LN efficiency can be induced by small numbers of DCs and is almost unaffected by increased cognate frequency, as was found in an earlier 2D model (Linderman et al., 2010). However, the efficiency of cognate T cell searching is highly dependent on model dimensionality, with the 3D model being more efficient.

We varied the cognate T cell frequency over three orders of magnitude in our simulations and found that LN efficiency (effector T cell output/naïve cognate T cell input) remains fairly constant at any given DC number. A proportional relationship between endogenous naïve cognate frequency and expanded primed T cell populations has been shown in recent studies (Moon et al., 2007; Obar et al., 2008). Our model results not only agree with this, but also predict that this correlation is maintained when the initial cognate frequency is further increased. This theoretical excess capacity in the system has important implications for vaccine design: large increases in cognate T cell numbers in the LN are still expected to yield an almost linear increase in effector T cell output. This result evinces both promise and caution. In many infectious disease studies, extremely high levels of transgenic T cells (up to 10^7), all of which are naïve but specific for a single epitope, are transferred to animals, and the priming and effector functions of these T cells are analyzed. Our results suggest that increasing the number of cognate T cells in a system may lead to an increase in priming compared to an un-manipulated system, and therefore could be misleading when comparing to non-manipulated scenarios. Compared with CD4 T cells, LN efficiency of CD8 T cells is less straightforward. LN efficiency of CD8 T cell priming drops when CD4 cognate frequency or DC recruitment is low, since in our model LDCs are necessary for CD8 T cell priming and effector cell survival as observed in vivo (Smith et al., 2004). This result provides another explanation regarding the disconnect between naïve and expanded T primed T cell population as observed in some studies examining CD8 T cell expansion (La Gruta et al., 2010; Ruckwardt et al., 2011). This is in addition to a threshold effect and differentially sustained proliferation as suggested by Jenkins and Moon (2012). Therefore, when cognate CD8 T cells are abundant, such as after a viral infection, immunotherapy that increases an influx of antigen presenting DCs and CD4 T cells into LNs may be helpful for taking full advantage of the excess priming capacity and increasing CD8 T cell output.

By comparing results from our 3D model to the 2D model by Linderman et al. (2010) and also a 2D version of our 3D model performed herein, we found that a T cell search for Ag-DCs is more efficient in 3D. We tested several possible explanations for this, and showed that the average minimum distance between DCs is shorter in 3D, and also that T cells are less likely to revisit the same DCs and therefore the proportion of unique T cell DC interactions increases. Both of these factors are likely to contribute to a more efficient repertoire scanning in an actual 3D LN. This result suggests that 2D LN models underestimate LN efficiency and may not properly reflect the nature of LN function in vivo. T cell search times in 3D are significantly shorter, but this assumes random T cell migration. In ongoing work, we are investigating the effects of chemotaxis on T cell repertoire scanning. Since steric inhibition of T cell–DCs contact is less of a barrier in 3D than on 2D

grids, it will be interesting to test whether chemotaxis can enhance T cell search times and unique DC contacts in our 3D model.

The intention of this model is to build a generic NHP LN that allows computational simulation and analysis of the core functions in T cell priming. For this reason, we did not fit the model to infection specific data, but instead we assumed that all the dynamics are based on a generalized antigen, like ovalbumin, as is used in the 2PM studies; all calibrations we made to our model based on cell movement used data from 2PM studies (Miller et al., 2004). We used our LN ABM to produce dynamics that qualitatively match immune responses, and to study how dynamics are affected when the dimensionality of the environment changes. We are now ready to use the model to test specific infection scenarios that will require the LN be connected with compartments such as blood, lymph and peripheral tissues. Furthermore, LNs can be seeded with pathogens and become a site of infection (Golden and Vikram, 2005).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix: Parameter values or ranges

Appendix

Parameter values or ranges

Parameter	Baseline LHS	Priming LHS	Description	
<i>General</i>				
$\$$	probMovePreferredDir	0.6-0.9	0.8	Probability of moving into preferred directions
$\$$	probMoveNonOppositeDir	0.95-0.99	0.95	Probability not moving into opposite directions
$\$$	probMovePreferredDirBlock	0.5-0.8	0.6	Probability of moving into preferred directions after being blocked
$\$$	probMoveNonOppositeDirBlock	0.9-0.95	0.92	Probability not moving into opposite directions after being blocked
	ratio CD8 to CD4	NA	0.5 (Latif et al., 2001)	Ratio of the number of CD4 T cells to that of CD8 T cells
	binding radius	2 (Miller et al., 2004)	2	T cells within this range can bind to DCs
<i>CD4 T cell</i>				

Parameter	Baseline LHS	Priming LHS	Description
	min age	165 (McCune et al., 2000)	165 Minimum age of naïve CD4 T cell when recruited (days)
	max age	365 (McCune et al., 2000)	365 Maximum age of naïve CD4 T cell (days)
	max age stdev	15	15 Standard deviation of max age (days)
+	binding threshold	150 (Demotz et al., 1990)	100-300 pMHC-II level corresponding to 50% CD4 T cell binding probability
+	binding shape	15	10-50 Shape parameter for CD4 T cell binding probability curve
+	max bind time max bind time stdev	16 4	5-20 4 Max time CD4 T cell bind to DCs (h) Standard deviation of max bind time (h)
+	priming threshold	2000	1000-3000 pMHC-II level corresponding to 50% CD4 T cell priming probability (h)
+	priming shape	200	100-300 Shape parameter for CD4 T cell priming probability curve (h)
	max time active	96	96 Maximum lifetime of active CD4 T cells (h)
	max time active stdev	4	4 Standard deviation of max time active (h)
	Number of division	4 (Miller et al., 2004; Foulds et al., 2002)	4 Number divisions CD4 T cells are allowed before exiting the LN
	double time	8 (Linderman et al., 2010)	8 Time it takes to divide (h)
	max time effector	60 (Sprent and Tough, 2001)	60 Maximum lifetime of effector CD4 T cells (h)
	max time effector stdev	1	1 Standard deviation of max time effector (h)
§	recruitment prob.	0.15-0.4	0.26 Probability of naïve CD4 T cell recruitment each time step
	initializing prob.	0.422 *	0.422 Probability that an empty grid compartment is initialized as naïve CD4 T cell
	cognate frequency	1/300	1/10,000 (Casrouge et al., 2000; Blattman et al., 2002) Frequency of cognate CD4 T cells

Parameter	Baseline LHS	Priming LHS	Description	
CD8 T cell				
	min age	165 (McCune et al., 2000)	165	Minimum age of naïve CD8 T cell when recruited (days)
	max age	365 (McCune et al., 2000)	365	Maximum age of naïve CD8 T cell (days)
	max age stdev	15	15	Standard deviation of max age (days)
+	binding threshold	150 (Henrickson et al., 2008)	100-300	pMHC-I level corresponding to 50% CD8 T cell binding probability
+	binding shape	15	10-50	Shape parameter for CD8 T cell binding probability curve
+	max bind time	16	5-20	Max time CD8 T cell bind to DCs (h)
	max bind time stdev	4	4	Standard deviation of max bind time (hrs)
+	priming threshold	2000	1000-3000	pMHC-I level corresponding to 50% CD8 T cell priming probability (h)
+	priming shape	200	100-300	Shape parameter for CD8 T cell priming probability curve (h)
	max time active	96	96	Maximum lifetime of active CD8 T cells (h)
	max time active stdev	4	4	Standard deviation of max time active (h)
	max division	8 (Miller et al., 2004)	8	Number divisions CD8 T cells are allowed before exiting the LN
	double time	8 (Linderman et al., 2010)	8	Time it takes to divide (h)
	max time effector	60 (Sprent and Tough, 2001)	60	Maximum lifetime of effector CD8 T cells (h)
	max time effector stdev	1	1	Standard deviation of max time effector (h)
§	recruitment prob.	0.075-0.2	0.13	Probability of naïve CD8 T cell recruitment each time step
	initializing prob.	0.211 *	0.211	Probability that an empty grid compartment is initialized as naïve CD8 T cell

Parameter	Baseline LHS	Priming LHS	Description
<i>DC</i>	cognate frequency	1/300	1/10,000 (Casrouge et al., 2000; Blattman et al., 2002)
	min age	1 (Kamath et al., 2002)	1
	max age	11 (Kamath et al., 2002)	11
	max age stdev	2	2
<i>+</i>	IDC mature pMHC increment	50	50-200
	IDC MDC threshold	50 (Linderman et al., 2010)	50
<i>+</i>	pMHC-I half life	25 (Cella et al., 1997)	10-100
<i>+</i>	pMHC-II half life	100 (Cella et al., 1997)	20-200
<i>+</i>	unbinding threshold	100	75-150
	mature ave age entry	20	20
	max time mature	60 (Kamath et al., 2002)	60
	max time mature stdev	5	5
<i>+</i>	licensing prob.	0.005	0.001-0.1
	max time licensed	36 (Lanzavecchia and Sallusto, 2004; Lindquist et al., 2004)	36
	max time licensed stdev	4	4
	recruitment prob.	1	0.06
	initialization prob.	0.00115	0.00115
<i>+</i>	IDC activation prob.	0.01	0.001-0.1
	movement interval	4 (Miller et al., 2004)	4
	Ag-DC recruit start	NA	5

Parameter	Baseline LHS	Priming LHS	Description
	Ag-DC recruit end	NA	10 (acute)/0 (chronic) recruited The day when Ag-DC stops being recruited
	recruit interval	35	1 Time step interval to recruit DCs
	recruit top slice	159	159 Top grid slice that DC is recruited
	recruit bottom slice	85	85 Bottom grid slice that DC is recruited
	recruit count	2	1 Number of DCs recruited each time
	size	2	2 Size of DC in each dimension
	max number DC	400	400 DC recruitment pauses when DC number exceeds this point
	number DC resting	40	40 Expected number of IDC at resting stage
<i>Ports</i>			
$\S$	HEV count	100-300	180 Number of HEV port
	HEV top	157 ⁺	157 Top slice allowed to generate HEV ports
	HEV bottom	50 [*]	50 Bottom slice allowed to generate HEV ports
$\S$	EL count	100-300	300 Number of exit ports
$\S$	EL top	50-100	77 Top slice allowed to generate exit ports

$\S$ Varied in baseline LHS
⁺ Varied in priming focused LHS
^{*} Estimated from data.

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HIGHLIGHTS

- First 3D ABM model predicting LN output during a fully simulated infection response in the LN.
- 3D ABMs, and not 2D ABMs accurately predict LN cellular dynamics.
- LN efficiency is not dependent on cognate frequency.
- Generation of effector cells leaving LNs depends on cognate frequency.

Fig. 1. Structure of a human lymph node. Afferent lymphatics (AL) bring lymph to the lymph node. Parenchyma is organized into B cell follicles (BCF) and T cell zone (TCZ). Trabeculae further divide the parenchyma into incompletely separated compartments. Lymphocytes drain through cortical sinuses (CS) to medullary sinuses (MS), and eventually exit the lymph node via efferent lymphatics (EL).

Fig. 2. LN images. (A and B) Cross-sectional images of LN from *Cynomolgus macaques*. (A) Stained for CD3 (blue; T cells), CD11c (green; DCs) and CD20 (red; B cells). White bar in lower left corner indicates the length 200 μm . (B) Stained for PNAd (green; HEV) and LYVE1 (red; medullary and cortical sinuses). White box indicates size of the modeled region.

Fig. 3.

Structure of computational model. (A) Abstracted shape of the T cell zone (blue) and modeled portion (white outline). The T cell zone is a spherical shell with thickness 800 μm. (B) Approximately 1/200 of the T cell zone volume is modeled, shaped as a cone plug. Dimensions as indicated. The modeled section of a LN has 344148 compartments, each a cube of edge length 5 μm (roughly the size of a T cell), and total volume of 0.043 μL. (C) Simulation image showing LN populated with cells. As in 2PM studies, for clarity, not all cells are displayed.

Fig. 4.

Model schematics and rules. (A) Cells undergo a biased random walk; probabilities of movement in different directions are unequal and depend on previous movement. (B) T cells (orange) occupy one grid compartment ($5 \times 5 \times 5 \mu\text{m}^3$). DCs (green) occupy 8 grid compartments (i.e., a cube of edge length 2). A DC can push a T cell away to make room for its movement. (C) When encountering the FRC network (grey), DCs are allowed to overlap, but T cells move around the FRC. (D) Cell rules are labeled with numbers on the diagram. (1) DCs are recruited from top of grid (via ALs). (2) Naive CD4 and CD8 T cells are recruited from HEVs. (3) Ag-DCs and LDCs can induce IDC maturation. (4) Ag-DCs prime CD4 T cells. (5) Primed CD4/CD8 T cells proliferate. (6) After a given number of divisions, both kinds of T cell become effector cells. (7) Effector CD4 T cells license Ag-DCs, turning them into LDCs. (8) LDCs prime CD8 and CD4 T cells. (9) Effector CD4/CD8 T cells exit LNs from ELs. (10) Cells may die. The distribution of life spans is cell-type specific. See text and online supplement for more detail.

Fig. 5.

Aleatory and epistemic uncertainties of the model. Two parameter sets from one LHS analysis are selected, with low or high maximum binding times for CD4 T cells. The number of Ag-DCs(A) and effector CD4 T cells output(B) are chosen as model readouts. (A) Aleatory uncertainty is small compared to epistemic uncertainty. (B) The two sources of uncertainty are partially convoluted.

Fig. 6.

Cell tracks for comparison of experiments and simulations. (A) Cell tracks from 2PM experiment with an imaging interval of 11 s. 15 tracks were obtained over a period of 6.7 min and are plotted. (B) Cell tracks from 2PM experiment with an imaging interval of 27 s. 15 tracks were obtained over a period of 10.8 min and are plotted. (C) Simulated cell tracks with 3D LN ABM. 15 tracks were obtained over a simulated period of 12.5 min and are plotted.

Fig. 7.

Simulation of an acute infection scenario. The introduction of Ag-bearing DCs stops on day 5. (A) Simulated numbers of DCs on the LN grid. (B) Simulated numbers of CD4 T cells (naïve, primed, effector) on the LN grid. (C) Simulated numbers of CD8 T cells in different states on the LN grid. (D) Simulated output of effector CD4 and CD8 T cells exiting from the LN.

Fig. 8.

Simulation of a chronic infection scenario. The introduction of Ag-bearing DCs does not stop after day 5. (A) Simulated numbers of DCs on the LN grid. (B) Simulated numbers of CD4 T cells in different states on the LN grid. (C) Simulated numbers of CD8 T cells in different state on the LN grid. (D) Simulated output of effector CD4 and CD8 T cells exiting from the LN.

Fig. 9.

Simulations of lymph node efficiency in generating primed T cells by Ag-DCs in a LN. Different colors represent different cognate frequencies (CF). (A) CD8 T cell LN efficiency in the context of high CD4 T cell cognate frequency. (B) CD8 T cell LN efficiency in the context of low CD4 T cell cognate frequency. (C) CD4 T cell LN efficiency for different cognate frequencies and different levels of Ag-bearing DCs on the grid.

Table 1

Model calibration

Source	Mean free path μm	Motility coefficient $\mu^2 \cdot \text{min}^{-1}$	Velocity $\mu\text{m} \cdot \text{min}^{-1}$	Transit time h	Exit rate $\text{min}^{-1} \cdot (10^6 \text{cells})^{-1}$
Simulation	15.96	45.87	13.0	18	915
2PM 11 s	17.48	47.18	14.6	-	-
data 27 s	15.6	42.86	9.8	-	-
Literature	20	-	12	12-24	700-1400

Table 2

Simulation outputs when model size is scaled from ~0.5% to ~2% of an entire LN

	1 × Grid	4 × Grid
Size (μL)	0.043	0.172
Density (10 ⁶ cell/μL)	4.20±0.08	4.16±0.05
Mean free path (μm)	15.88±0.07	15.93±0.05
Transit time (h)	18.0±0.6	17.7±0.3
CD4 scan rate (h ⁻¹)	2.93 ±0.03 × 10 ³	2.89 ±0.02 × 10 ³
CD8 scan rate (h ⁻¹)	1.46±0.01 × 10 ³	1.43±0.01 × 10 ³
CD4 priming efficiency	13.5±0.1	13.±±0.1
CD8 priming efficiency	2.10 ±0.02 × 10 ²	2.14±0.01 × 10 ²
Search time (h)	0.316±0.006	0.311 ±0.003

Table 3

Comparing search times, match percentages and scanning rates from 2-D and 3-D simulations

		2D		3D	
		CD4	CD8	CD4	CD8
Match %		55.77±0.73	49.75±1.34	92.64±0.87	86.41±1.19
Search time (min)		140.2±4.2		10.64±0.26	
Scan rate (cell/h)	Apparent	562±6	277±3	2977±26	1479±13
	Unique	125±2	64±1	1189±9	592±5
	Ratio	4.488±0.018	4.335±0.018	2.504±0.003	2.498±0.003

Table 4

Sensitivity analysis for parameters involved in priming performed during an acute infection scenario

	CD4		CD8	
	Mechanism	PRCC	Mechanism	PRCC
Scanning rate	CD4 T cell binding time	−0.57	CD4 T cell binding time	−0.61
	Priming threshold for pMHC-II	−0.49	Priming threshold for pMHC-II	−0.48
	Ag-DC licensing probability	0.73	Ag-DC licensing probability	0.8
Match %	Ag-DC licensing probability	−0.76	Ag-DC licensing probability	−0.62
	CD4 T cell binding time	0.4	CD4 T cell binding time	0.38
	Priming threshold for pMHC-II	0.42	Priming threshold for pMHC-II	0.39
Effector output	CD4 T cell binding time	−0.71	CD8 T cell binding time	−0.66
	Ag-DC licensing probability	−0.44	CD4 T cell binding time	−0.22
			Priming threshold for pMHC-II	−0.17

Table 5

Sensitivity analysis for parameters involved in priming performed during a chronic infection scenario

	CD4		CD8	
	Mechanism	PRCC	Mechanism	PRCC
Scanning rate	Ag-DC licensing probability	0.66	Ag-DC licensing probability	0.69
Match %	Ag-DC licensing probability	−0.48	-	-
Effector output	CD4 T cell binding time	−0.68	CD8 T cell binding time	−0.64
	Ag-DC licensing probability	−0.23	CD4 T cell binding time	−0.21

Table 6

Sensitivity analysis of parameters involved in priming during a chronic infection scenario. 2-D case

	CD4		CD8	
	Mechanism	PRCC	Mechanism	PRCC
Scanning rate	Ag-DC licensing probability	0.2	Ag-DC licensing probability	0.34
Match %	Ag-DC licensing probability	−0.19	Ag-DC licensing probability	0.61
	IDC activation probability	0.36	IDC activation probability	0.28
Effector output	CD4 T cell binding time	−0.44	CD8 T cell binding time	−0.52
	Priming threshold for pMHC-II	−0.31	Ag-DC licensing probability	0.49
	Binding threshold for pMHC-II	−0.15	IDC activation probability	0.19
	IDC activation probability	0.28		

Table 7Significant (p -value < 0.05) eFAST results for parameters during a chronic infection scenario

	CD4			CD8		
	Mechanism	S_i	S_{ii}	Mechanism	S_i	S_{ii}
Scanning rate	CD4 T cell binding time	0.44	0.89	CD4 T cell binding time	0.44	0.86
Match %	Ag-DC licensing probability	0.19	0.88			
Effector output	CD4 T cell binding time	0.76	0.91	CD8 T cell binding time	0.61	0.89