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Characterizing the Dynamics of CD4+ T Cell Priming within a Lymph Node

Jennifer J. Linderman,*,†‡ Thomas Riggs,§ Manjusha Pande,§ Mark Miller,¶ Simeone Marino,§ and Denise E. Kirschner*†§

Generating adaptive immunity postinfection or immunization requires physical interaction within a lymph node T zone between Ag-bearing dendritic cells (DCs) and rare cognate T cells. Many fundamental questions remain regarding the dynamics of DC–CD4+ T cell interactions leading to priming. For example, it is not known how the production of primed CD4+ T cells relates to the numbers of cognate T cells, Ag-bearing DCs, or peptide-MHCII level on the DC. To address these questions, we developed an agent-based model of a lymph node to examine the relationships among cognate T cell frequency, DC density, parameters characterizing DC–T cell interactions, and the output of primed T cells. We found that the output of primed CD4+ T cells is linearly related to cognate frequency, but nonlinearly related to the number of Ag-bearing DCs present during infection. This addresses the applicability of two photon microscopy studies to understanding actual infection dynamics, because these types of experiments increase the cognate frequency by orders of magnitude compared with physiologic levels. We found a trade-off between the quantity of peptide-major histocompatibility class II on the surface of individual DCs and number of Ag-bearing DCs present in the lymph node in contributing to the production of primed CD4+ T cells. Interestingly, peptide-major histocompatibility class II $t_{1/2}$ plays a minor, although still significant, role in determining CD4+ T cell priming, unlike the primary role that has been suggested for CD8+ T cell priming. Finally, we identify several pathogen-targeted mechanisms that, if altered in their efficiency, can significantly affect the generation of primed CD4+ T cells. The Journal of Immunology, 2010, 184: 2873–2885.

The adaptive immune response to pathogen infection is dependent on generation of primed CD4+ T cells in the lymph node (LN). Dendritic cells (DCs) in tissues take up pathogens and present antigenic peptides on their surface in the context of major histocompatibility class II molecules (peptide-MHCII complexes [pMHCII]) (1–3). These Ag-bearing DCs migrate via afferent lymphatics to the closest tissue-draining LN. T cells constantly transit between the circulatory and lymphatic systems, entering individual LNs via high endothelial venules and re-entering the circulatory system via the thoracic duct from different lymphatics (4). Generation of primed CD4+ T cells requires contact between these Ag-bearing DCs and specific (cognate) CD4+ T cells; their successful binding leads to CD4+ T cell priming (5). Whereas DCs rarely leave the LN, primed CD4+ T cells exit the LN and return to the site of infection to participate in the immune response.

Despite a wealth of information on the immune response, including studies on the dynamics of DC–T cell interactions in a LN via two-photon microscopy (2PM) (3, 6, 7), many fundamental questions remain regarding the functioning of the LN. For example, it is not known how the production of primed CD4+ T cells is related to the numbers of cognate CD4+ T cells, Ag-bearing DCs, or pMHCII level on the DC surface. Will doubling the number of cognate CD4+ T cells or Ag-bearing DCs double the primed CD4+ T cell output? Experimental preparations such as 2PM often increase the density of cognate CD4+ T cells or DCs beyond the physiologic range to facilitate observation (8, 9), so answering this question will indicate how information from these studies can be interpreted. Do high numbers of DCs with fewer pMHCIIIs give equivalent CD4+ T cell priming as low numbers of DCs with more pMHCIIIs? Answering this question would give insight into the relationship between Ag dose and T cell priming. A few recent studies have explored these questions for CD8+ T cells (10, 11). However, CD8+ T cells recognize Ag presented on DCs in the context of peptide-MHC class I (pMHCII) molecules. The $t_{1/2}$ of pMHCI is on the order of a few hours; it is not surprising then that pMHCI $t_{1/2}$ profoundly affects the extent and dynamics of CD8+ T cell priming (10, 11). Interestingly, the $t_{1/2}$ of pMHCI is on the order of days (12–14)—that is, it is extended beyond the lifespan of the DC in most cases. Therefore, additional processes and parameters might play important and undiscovered roles in driving CD4+ T cell priming dynamics. Several recent computational studies have focused on the details of cell movement within a lymphoid organ (10, 15–17). In contrast, our study focuses on functionality of the LN during exposure to Ag.

Pathogenic microbes can interfere with the immune response via several mechanisms related to DC–T cell interactions (e.g., reduction of pMHCI on the DC surface during infection with Mycobacterium tuberculosis or interference with trafficking of DCs to the LN) (18, 19). Therefore, understanding the relationships between the quantities outlined above—numbers of CD4+ T cell and...
DC cell levels entering the LN, pMHCII levels on DCs, and numbers of primed CD4+ T cell levels exiting the LN—can give us insight into the mechanisms that pathogens have evolved to evade the immune response at the level of CD4+ T cell priming.

A systems biology approach that incorporates in silico modeling to generate and test hypotheses, run virtual experiments, and make experimentally testable predictions is uniquely suited to address these questions. For example, modeling can be used to overcome the significant time scale (minutes to a few hours) and length scale (a few micrometers) limitations of 2PM experiments, allowing us to predict how the observed local cell behavior will translate into the behavior of an entire LN. Modeling can also enable prediction of the outcome of numerous and simultaneous processes in cases where it is too difficult to intuit the results. Specifically, agent-based models (ABMs) have been used in immunology and other types of models as applied to biological systems, see two recent reviews (26, 31). ABMs can be used to simultaneously explore low frequencies of cognate T cells (∼10−4 in this study), to track the history of each individual cell, to incorporate probabilistic events such as cell motion, and to follow the evolution of T cell priming in both space and time (15, 20). Furthermore, we have developed analytical tools both for uncertainty and sensitivity analysis and for assessing compensatory relationships (i.e., tradeoffs) between parameters (32). We have previously used an ABM to explore the representation of the detailed movement of T cells within the T zone of an LN via comparison of simulated motion and motion captured in 2PM studies (15). We now extend that framework to allow us to simulate physiologic or near-physiologic LN cell numbers and cognate frequencies over much longer time periods than 2PM studies capture, enabling us to both compare model results with infection scenarios and address some open questions regarding the dynamics of CD4+ T cell priming.

Materials and Methods

Agent-based model

ABMs are computational tools used to model behavior of a system resulting from interactions between individual components. ABMs consist of an environment, autonomous objects (agents), time steps, and rules governing the behavior of individual agents and interactions between agents. We implemented an ABM to simulate the immune response arising from cellular interactions within an LN (Fig. 1). This two-dimensional (2D) ABM accurately captures random encounters between T cells and DCs as observed via 2PM, with which it is possible to track individual cells as they interact with other cells and change their location and state (Fig. 1B). Previously, we developed an ABM that represented a small portion of an LN T zone and had sufficient spatial and temporal resolution to make observations at the level of individual cells (15). We found that chemotaxis-directed motion of T cells toward DCs increased the total number of contacts, but decreased unique contacts and thus inhibited efficient repertoire scanning of T cells, thereby decreasing production of primed T cells. That is, our model supported the principle that a persistent random walk strategy of T cells to DCs was more effective than random motion and motion captured in 2PM studies (15). We now extend this framework to allow us to simulate physiologic or near-physiologic events such as cell motion, and to follow the evolution of T cell priming in both space and time (15, 20). Furthermore, we have developed analytical tools both for uncertainty and sensitivity analysis and for assessing compensatory relationships (i.e., tradeoffs) between parameters (32). We have previously used an ABM to explore the representation of the detailed movement of T cells within the T zone of an LN via comparison of simulated motion and motion captured in 2PM studies (15). We now extend that framework to allow us to simulate physiologic or near-physiologic LN cell numbers and cognate frequencies over much longer time periods than 2PM studies capture, enabling us to both compare model results with infection scenarios and address some open questions regarding the dynamics of CD4+ T cell priming.

CD4+ T cells. CD4+ T cells are naive or primed. T cells are either cognate (able to recognize Ag presented on MHC class II molecules on Ag-bearing DCs) or non-cognate. Only cognate cells can be primed.

DCs. DCs sample their environment and take up Ag to present on their surface (35). We assigned a pMHC level to each DC as it enters the grid. We represented a continuum of Ag-bearing DCs by tracking the quantity of peptide-MHC class complexes (pMHC) on the surface of DCs with time. DCs whose pMHCII value is below a threshold are incapable of priming naive, cognate CD4+ T cells and are designated as immature DCs (IDCs), and those capable of priming are designated as antigen-bearing DCs (Ab-DCs). The fraction of the DC population entering the LN that is Ag-bearing is varied in some of the simulations, and is referred to as percent Ab-DC’s. Here we assume that ∼60% of all DCs are Ag-bearing as inferred from data (36). We also assume that the levels of pMHC on the surface and the numbers of Ab-DCs reflect the levels of Ag in a system, as previously described (10, 11). A third class of DCs (licensed DCs (LDCs)) have interacted with a primed CD4+ T cell and have increased numbers of pMHC (see below) (37, 38).

Cognate frequency. The frequency of cognate T cells is estimated to be on the order of 1010 to 1016 in actual infection scenarios (39, 40). In 2PM experiments in which is introduced by Ab-DCs that have been exposed to antigen (OVA), to increase the number of observable events during the time frame of an experiment, the frequency of cognate cells is enhanced to ~1:300 by using transgenic T cells and recipient mice (7). In our simulations, we vary the cognate frequency from 1:300 to 1:10,000. We are limited to a minimum frequency of 1:10,000 to ensure that enough events occur during a simulation to allow statistical significance. This method allows us to predict how well the 1:300 ratio represents other more biologically relevant scenarios.

T cell recruitment. Cognate and noncognate T cells enter the LN via HEVs. Rates of entry of T cells, their levels, and the ratio of CD4+ to CD8+ T cells were inferred based on the number of T cells present in a mouse LN (15) and the rate of their exit (<24 h for noncognate T cells) (4). See http://malthus.micro.med.umich.edu/lab/movies/FullLN/ for more details. Recruitment rates via HEV allowed us to maintain the number of T cells in the LN at a steady state for the negative control simulations—that is, with no Ab-DC present or entering the LN (data not shown) and is referred to as percent Ab-DC’s. Here we assume that ∼60% of all DCs are Ag-bearing as inferred from data (36). We also assume that the levels of pMHC on the surface and the numbers of Ab-DCs reflect the levels of Ag in a system, as previously described (10, 11). A third class of DCs (licensed DCs (LDCs)) have interacted with a primed CD4+ T cell and have increased numbers of pMHC (see below) (37, 38).

DC recruitment. DCs enter the LN via afferent lymphatics; estimates of the number of DCs are based on murine data (16). DC entry into the LN is...
described via two distinct infection scenarios (see below). DCs are not allowed to leave the LN in our simulations, as this rarely occurs in the natural system. CD8+ T cells. Although not the focus of this paper, the model also included CD8+ T cells (naive and primed). Naive CD8+ T cells can be activated by contact with an LDC only. Including these cells, which like CD4+ T cells can bind to a DC surface, ensures that we do not overestimate the available DC area available to CD4+ T cells. More detail on CD8+ T cells and the rules relevant to them can be found at http://malthus.micro.med. umich.edu/lab/movies/FullLNI. Below we briefly describe key rules of our model that represent biologic mechanisms of key interest in this study.

Cell motion. Cells in our model follow a persistent random walk (Fig. 1B). Detailed data from 2PM tracking of individual T cell motion within an LN are available (7, 8, 41). Our model matches these data on cell speed, mean free path before changing direction, and cell motility (relationship between displacement and time from original tracking point; see http://malthus.micro.med.umich.edu/lab/movies/FullLNI/ for details on model validation).

Similarly, data are available for validation of our model regarding DCs, which move more slowly, as do T cells that have become bound to a DC (7, 8). Our model also matches these data (15).

pMHC display. The $t_{1/2}$ of pMHCII complexes on IDCs is typically on the order of 24 h; however, Ag loaded onto MHC II molecules and presented on the surface of Ab-DCs (or LDCs) extends that $t_{1/2}$ to the lifespan of the cell itself (days) (12–14). In our simulations, pMHCII level is represented on the surface of Ab-DCs (or LDCs) extends that $t_{1/2}$ to the lifespan of the cell itself (days) (12–14). In our simulations, pMHCII level is allowed to decrease according to a given $t_{1/2}$; we used a range of reported $t_{1/2}$ values for our analysis to determine the effects this parameter has on the system (34–41). As mentioned above, the $t_{1/2}$ of pMHCII is much shorter than that of pMHCII, although it may also be extended (12–14), and this affects the generation of primed CD8+ T cells. Because LDCs are efficient Ag presentation, we assume all the costimulatory molecules and pMHCII are necessary for presenting CD8+ T cells.

Contact. Contact between cells occurs when immune cells find themselves within the same or adjacent microcompartments (Fig. 1B). DCs use a strategy to allow rapid and efficient scanning of many T cells to locate the rare cognate T cell whose TCR has sufficient affinity with the pMHC on a DC to result in binding, priming, and proliferation of that T cell lineage. DCs may contact up to 5000 T cells per hour by moving their dendrites within a local “sweep area” (44, 45). We implemented this local sweep area by defining DC-T cell contact as a T cell occupying any of eight compartments adjacent to a DC, with a maximum of 32 simultaneous T-cell contacts per DC (because up to 4 T cells can fit into a microcompartment). A rather than 75–250 has been suggested in the literature (7, 44); thus, in 2D the value of 32 is reasonable.

Binding and priming of CD4+ T cells. Following physical contact between a CD4+ T cell and an Ab-DC or LDC, binding occurs only if both the quantity of pMHCI on the DC surface and the TCR affinity are sufficient (43), whereas priming requires prolonged binding duration (average of 6 h) and the minimum duration has been reported to vary inversely with pMHC level (43). Although the $t_{1/2}$ is much shorter than that of pMHCII, it may also be extended (12–14), and this affects the generation of primed CD8+ T cells. Because LDCs are efficient Ag presenters, we assume all the costimulatory molecules and pMHCII are necessary for presenting CD8+ T cells.

Metrics used to analyze simulations. For each simulation, we track all properties for each cell (agent), including location (within the LN or exiting the LN). We use these data to compute the cumulative output of primed CD4+ T cells; cumulative refers to the summing of exiting cell numbers up to the time point plotted. In addition, we also compute three metrics to help us understand T cell–DC contact dynamics within the LN. Search time is defined as the time elapsed from when a cognate CD4+ T cell enters the LN until it first contacts an Ab-DC. Transit time is defined as the time required for a cognate T cell to migrate through the LN T zone, including any priming and division steps. Match percentage is defined as the percentage of entering cognate CD4+ T cells that contact an Ab-DC during their time in the LN.

Uncertainty and sensitivity analysis. Uncertainty analysis measures the variation in model output based on the variation of inputs (i.e., parameter values). Sensitivity analysis involves the correlation of these variations in parameter values to variances in model output and is particularly useful when parameter values are not known with certainty. We use Latin hypercube sampling (LHS) to sample from a large parameter space and partial rank correlation coefficients to determine sensitivity, as we previously described for application to ABMs (32). Parameters varied during sensitivity and uncertainty analysis are indicated in Table I along with their default values, ranges tested, and references based on experimental data; this was done for acute infection scenario. In the LHS, the initial number of pMHCI on the surface was not varied, because the parameters that describe all the binding and priming curves in Fig. 6 are sufficient to capture the effect without redundancy. If initial numbers of pMHCI were included in the LHS, parameter values are expected to vary slightly from one cell to another; this is captured in the ABM by choosing parameters for each cell from a biologically reasonable defined range that is normally distributed. In addition, because there is uncertainty in the values of many of the parameters, it is important to examine closely the influence of parameter variation on the model outputs of interest, as described in the sensitivity analysis.

Virtual infection simulation. Both Ab-DCs and IDCs enter the LN; the entry of Ab-DCs simulates an infection. We considered two infection scenarios. The first, which we term the acute infection scenario, captures a multiday pulse of Ab-DCs entering the LN and the production of primed T cells that is based on existing data (62) regarding Ag transport of (FTTC) from the airways to thoracic LNs via airway-derived DC1c1-MHC-II DCs (Fig. 2B). Transport of Ag by DCs begins within 6 h after introduction of FTTC Ag into the airways, peaks after 24 h, and declines to zero by day 7. We used these data to infer the rate of entry of DCs (Fig. 2A; note that the cumulative number of DCs entering is shown).

We also considered a chronic infection scenario, an artificial construct to allow us to do a steady state analysis, thus avoiding confounding effects of changing numbers of Ab-DCs. In these simulations, we maintain a steady-state level of Ab-DCs post peak influx (~36 h) through day 14 of the simulation (Fig. 2E, 2F). This scenario was used for investigating the effect of varying cognate frequency and Ag-DC frequency. For both infection scenarios, simulations are first run for 15 h to achieve a steady state of ~9000 naïve CD4+ T cells via transmigration of naïve CD8+ T cells before infection. Infection is then introduced by adding DCs in a given ratio (termed % Ab-DCs) of IDCs and Ab-DCs (Fig. 2A).
number of primed CD4+ T cells during a chronic infection scenario. We used the chronic scenario so that varying levels of DCs would not effect the results. The distribution for initial levels of pMHC on the surface of the DCs was chosen from a much smaller range than the rest of the simulations, with a small SD of 5. An average of 10 runs were performed, and a target value of cumulative CD4+ T cells was achieved (100 for cognate frequency 1/3000 and 500 for cognate frequency 1/300) and the parameters yielding those values (within 10% of the target values) were obtained. These pairs of parameter values that yielded the same number of cumulative (14 d) primed CD4+ T cells leaving the LN were compiled and plotted. Because such plots yield information on how a change in one parameter can compensate for a change in the other parameter, we term these plots trade-off plots (48, 63).

History
We considered the scenario in which T cells retain memory of previous interactions with DCs, and this memory accumulates to assist in priming. We term this memory history. A control baseline condition had no history—that is, each T cell binding event is independent of prior contacts of that individual T cell, and T cell priming depends solely on its binding with a particular DC (all results shown in this article were done for this case). Second, we considered a scenario of binding sensitivity history for T cells in which prior binding events that did not lead to priming can increase the probability that a DC–T cell contact leads to successful binding. In this case, the pMHC value used to compute the binding probability (Fig. 6) is calculated by summing pMHC values from prior contacts (still decreasing with a given $t_{1/2}$). Finally, we considered the case of priming sensitivity history, in which prior T cell binding events (that failed to prime) increase the probability that subsequent binding events result in priming. In this case, the pMHC duration values used to compute the priming probability (Fig. 6) are calculated by summing the pMHC duration values from prior contacts (still decreasing with a given $t_{1/2}$). Implementations of history were evaluated independently as well as a scenario in which priming and binding sensitivity occurred simultaneously.

Numerical methods and visualization
The ABM was implemented in C++. Code was developed on a Linux Mandriva 2007 operating system and compiled using a GCC compiler. The simulation time step was 6 s. To visualize results, we generated text files that record the state and location of every cell on the grid at 2 min intervals during the simulation. These text files were then converted to .png image files using Java 3D module (Sun Microsystems, Palo Alto, CA). Image frames were converted into movies using QuickTime Pro player (Apple Computer, Cupertino, CA). Movies of our ABM simulations are available at http://malthus.micro.med.umich.edu/lab/movies/FullLN.

Results
Virtual infections track cell behavior and LN output of primed cells
To understand the dynamics of immune cells in the LN during both acute and chronic infections, we developed a computer model known as an ABM. DCs and naive T cells migrate to a single LN, interact according to a set of rules determined from known biological data, and T cells then leave the LN primed and ready to combat infection (Fig. 1). The cumulative number of primed CD4+ T cells exiting the LN via the MSs serves as a measure of the strength of the immune response generated for particular infection scenarios.

We first describe the dynamics of the acute infection scenario (Fig. 2A–D). Fig. 2A shows the cumulative number of DCs (60% decreasing with a given $t_{1/2}$). Implementations of history were evaluated independently as well as a scenario in which priming and binding sensitivity occurred simultaneously.
Ab-DCs and 40% IDCs) that have entered the LN. The dynamics of all the DC populations in the LN are shown in Fig. 2B; the total DC population increases to a maximum of 100 by 36 h and then declines. Initially, the population of total DCs in the LN is composed of IDCs and Ab-DCs; as these DCs become licensed, the number of Ab-DCs declines and the number of LDCs increases. Although data are not knowledge available on the rate of entry of DCs into a draining LN, we can assume that for different infections, the dynamics of both timing and magnitude will be different. In this study, we chose the influx of DC dynamics to represent total numbers of DCs in the LN (the sum of Ab-DCs, LDCs, and IDCs) that represent experimentally determined values from one human study (Fig. 2B) (62). The cumulative predicted numbers of primed CD4+ and CD8+ T cells leaving the LN are shown in Fig. 2C and 2D. There are no data available on the number of CD4+ T cells leaving a single LN. However, a spleen is a secondary lymph organ, and there are data from one group looking at the expansion of cells during LCMV infection (64). The data show a 3 order-of-magnitude expansion in CD4+ T cell numbers and a 4 order-of-magnitude expansion in CD8+ T cell numbers over a 2 wk period. Our lymph node outputs (from just a portion of the LN) are similar. The magnitude and timing of the simulated expansion phase are similar to the data; the output from our simulated LN precedes that of the spleen data by a few days, as expected. Thus, our simulation captures essential features of an acute infection scenario in an LN. Time-lapse movies of the acute infection scenario for two different cognate frequencies are available at http://malthus.micro.med.edu/lab/movies/FullLN.

For comparison with acute infection, we also simulated a chronic infection scenario (Fig. 2E–H). The entry of IDCs and Ab-DCs into the LN is set to reach a steady rate by ~1 d, as shown by a nearly constant rate of increase in the number of DCs that have entered the LN (Fig. 2E). In a manner similar to that seen for the acute case, the number of total DCs in the LN is initially composed of IDCs and Ab-DCs, but later switches to predominantly LDCs (Fig. 2F). The corresponding (cumulative) output of primed CD4+ and CD8+ T cells is shown in Fig. 2G and 2H. The cumulative cell numbers are initially similar to the acute case, but continue to increase slowly with time. Although this scenario is an artificial construct and not meant to be compared with experiment, chronic infection simulations will serve as a tool to observe the effects of varying the number of Ab-DC or cognate frequency without the confounding effect of varying levels of entering DCs.

T cells can potentially contact multiple DCs during their transit through an LN. The extent to which separate contacts might lead to subsequent priming and proliferation has been previously studied in models of CD8+ T cell priming (11, 46, 65). To explore whether previous contacts between naive CD4+ T cells and DCs influence CD4+ T cell binding and/or priming, we implemented various history scenarios (see Materials and Methods; binding sensitivity history, priming sensitivity history, and the sum of the

![FIGURE 2. Acute and chronic infection scenarios. The cartoons along the top indicate the flow of cells into, on, and out of the LN and correspond to the plots below. A and E, Cumulative number of DCs that have entered the LN. B and F, Numbers of each population of DCs in the LN during the infection. In B, experimental data on the total number of DCs in a human LN at particular times (66) is shown for comparison. C and G, Cumulative number of primed CD4+ T cells exiting the LN. D and H, Cumulative number of primed CD8+ T cells exiting the LN. In C and D, experimental data from a mouse spleen are shown for comparison (64). No measure of variability (e.g., SD) is available for these data. Model parameter values used are from Table 1 with 60% Ab-DCs, cognate frequency 1:300, pMHC t1/2 of 60 h.](http://malthus.micro.med.edu/lab/movies/FullLN)
We set the “memory” of these contacts to decay with a similar time-frame as pMHC themselves are lost (60 h $t_{1/2}$). We found no significant differences in generation of primed CD4+ T cells when we performed the experiments in any of the history cases versus no history control (http://malthus.micro.med.umich.edu/lab/movies/FullLN/). This finding provides a strong argument that, at least for priming of CD4+ T cells, this concept of history may not be playing a significant role in the generation of primed CD4+ T cells that leave the LN. We note that if the pMHCII $t_{1/2}$ is reduced by an order of magnitude to that of pMHCI, we do find an effect of history, in agreement with previous work by Zheng et al. (10) on the priming of CD8+ T cells.

**LN efficiency in producing primed CD4+ T cells is primarily a function of the number of Ab-DCs**

The influx of Ab-DCs and cognate CD4+ T cells to an LN is central to the generation of primed T cells; however, the relationship between the numbers of Ab-DCs and cognate T cells entering an LN and primed CD4+ T cells generated and exiting an LN is not known. We investigated a range of cognate frequencies, from the elevated frequency (1:300) typical of 2PM experiments to a frequency approaching physiological (1:10,000). Similarly, we varied the fraction of entering DCs carrying sufficient levels of Ag to prime a T cell (percent Ab-DCs). Under basal conditions, DCs represent <1% of cells within LNs (15, 66); infections likely generate increased and varying levels of Ab-DCs. To study the influence of these inputs on primed CD4+ T cell production, we used the chronic infection scenario (Fig. 2).

Fig. 3A shows the simulation of cumulative output (through day 14) of primed CD4+ T cells from an LN for five different cognate frequencies and a range of percent Ab-DCs. For a given cognate frequency, increasing the percent Ab-DC increases the cumulative output of primed CD4+ T cell, as expected. Similarly, for a given percent Ab-DC, increasing the cognate frequency increases the output of cumulative primed CD4+ T cell.

To determine whether the LN efficiency (i.e., the generation of primed T cells leaving the LN) varies with cognate frequency or percent Ab-DC, we plotted the ratio of the cumulative primed CD4+ T cell output to the number of naive cognate CD4+ T cells entering the LN (primed CD4/cognate CD4 T cell; Fig. 3B). As the percent Ab-DCs is increased, a saturation effect is observed; this is anticipated because DCs can act catalytically—that is, one DC can activate more than one T cell. The curves for each cognate frequency fall almost on top of each other, indicating that LN efficiency is nearly independent of cognate frequency. The separation between the curves in Fig. 3B at low values of percent Ab-DC suggests that

![Cumulative output](image1.png)  
**A** Cumulative output of primed CD4+ T cells from the LN as a function of percent Ab-DC by differing cognate frequencies (Cog).

![Primed CD4/cognate CD4 Tcell](image2.png)  
**B** Data in A replotted as the ratio of the cumulative primed CD4+ T cell output to the number of naive cognate CD4+ T cells entering the LN for different cognate frequencies. (The numbers are nearly identical if you use data from the simulations used to construct C.)

![Primed CD4/cognate CD4, no LDC](image3.png)  
**C** Simulations results as in B, but for no licensing of DCs.

![Match Percentage](image4.png)  
**D** Average match percentage, search time and transit time for cognate CD4+ T cells entering the LN for the simulations shown in A and B. Standard deviations are also given. Data in D are averaged across all cognate frequencies, because differences between cognate frequency were not statistically significant for these outputs. Simulation parameter values are found in Table I.
there is a slight effect of cognate frequency on the output of primed CD4+ T cells per cognate CD4+ T cell entering the LN. This effect is due to the positive feedback provided by LDCs: primed CD4+ T cells interact with Ab-DCs, leading to production of LDCs which, by virtue of their higher pMHC levels, are efficient activators of CD4+ T cells. This is apparent only at low values of percent Ab-DCs, where contact between T cells and DCs is less frequent. When we remove the pathway leading to LDC production, the data show no significant effect of cognate frequency on the ratio plot (Fig. 3C).

Our finding that LN efficiency is nearly independent of cognate frequency is consistent with the data (67) showing that CD4+ T cell populations expanded in proportion to their naive progenitors. Thus, increasing the percent Ab-DC increases the likelihood that a naive T cell will meet its DC match, but changing the cognate frequency has no effect on LN efficiency. Although there is certainly some crowding on the grid (Fig. 4), we do not find that Ab-DCs are always completely surrounded by cognate T cells and thus unable to activate additional T cells. The evidence for this finding is that as the cognate CD4+ T cell frequency is increased, approximately the same percentage of T cells become activated (Fig. 3B). Fig. 3B also provides a quick assessment of the function of the LN in priming CD4+ T cells, in that for physiologically realistic values of the numbers of entering Ab-DCs during an infection, on the order of 1–4 primed CD4+ T cells will be produced for every cognate CD4+ T cell entering the LN.

We pooled the data from the simulations shown in Fig. 3B to calculate the average match percentage, search time, and transit time for cognate CD4+ T cells entering the LN (Fig. 3D). As the percent Ab-DCs increases, more CD4+ T cells are able to find their DC match, and more quickly, by random walk. Transit times increase with percent Ab-DC because many T cells are now binding to DCs for several hours on their path through the LN. These transit times are consistent with data from whole animal experiments (68).

The calculated quantities from Fig. 3D can help us to understand the values of the ratio-primed CD4/cognate CD4 T cell in Fig. 3B. For example, note that at 50% Ab-DCs, approximately half of the naive cognate CD4+ T cells find their DC match. Those T cells take an average of 3 h to find their DC match and after binding have time for two to three divisions. The other half of the T cells do not find a match, and thus the primed CD4+/cognate CD4+ T cell ratio of ~3 reflects that a significant part of the population of entering T cells do not contribute to generation of primed CD4+ T cells leaving the LN.

Peptide-MHC levels and numbers of Ag-bearing dendritic cells trade off to give similar levels of T cell output

There are two key parameters that characterize the role of the Ab-DC in CD4+ T cell priming: the number of Ab-DCs that enter and the ability of each to successfully prime CD4+ T cells as quantified in this study by pMHCII levels. Together, these parameters serve as a measure of the Ag in the system. To determine whether these parameters play equivalent roles (i.e., whether a deficiency in the number of Ab-DCs can be overcome by introducing only Ab-DCs with high levels of pMHCII) we performed simulation experiments with our ABM. Pairs of values of pMHCII and percent Ab-DC that gave similar cumulative (14 d) numbers of primed CD4+ T cells exiting the LN were plotted (Fig. 5). These plots demonstrate that efficiency in one process (e.g., the display of many pMHCII by
each Ab-DC can compensate for a deficiency in the other process (e.g., entry of Ab-DCs into the LN). Interestingly, the slopes of the trade-off curves for two different cognate frequencies (1:300 versus 1:3000) are different. At high cognate frequency (1:300), many cognate CD4+ T cells are present. Opportunities for multiple T-DC contacts mean that, once a minimum number of pMHC (∼110 pMHC) are present on the Ab-DC, there are sufficient opportunities for T cell activation, even given the low probabilities of binding and priming implied (Fig. 6). However, at low cognate frequency (1:3000), there are fewer opportunities for DC-T cell contacting, allowing the DC parameters (percent DCs, pMHC levels) to play equivalent roles (indicated by the less steep slope of the trade-off curve). This trade-off between percent Ab-DCs and levels of pMHC has also been observed for CD8+ T cells as well, although they showed that pMHC concentration is more important, as expected because the $t_{1/2}$ of pMHCI is much less than for the pMHCII in this study (10, 11).

Identification of key mechanisms that control LN output of primed CD4+ T cells

Our analysis up to this point has focused on the effect of varying the numbers of entering cells (cognate frequency, Ab-DC frequency) and the quality of those cells (pMHC number on an Ab-DC) on the LN output of primed CD4+ T cells. However, parameters that describe the efficiency and kinetics of various processes that occur within the LN also play an important role in generating primed CD4+ T cells. To identify key parameters and processes, we performed a model sensitivity analysis using a technique that allows multiple parameters to be simultaneously varied over physiologically reasonable ranges (see Materials and Methods and http://malthus.micro.med.umich.edu/lab/movies/FullLN/). When model parameters were simultaneously varied (parameters 1–16 in Table I), sensitivity analysis identified four parameters that have the strongest influence on the generation of primed CD4+ T cells (Table II, Tier 1 Parameters). As expected from earlier results (Fig. 3), these parameters include both cognate frequency and Ab-DC frequency. The maximum number of divisions a primed cell can undergo is also significantly and positively correlated with LN output. The time it takes for a T cell to divide (division time) is significantly but negatively correlated with LN output. Note that these tier 1 parameters identified by sensitivity analysis can be classified as either inputs into the LN or cellular properties that at first glance appear unrelated to DC–T cell interactions and more related to cell physiology.

To determine whether parameters describing DC–T cell interactions also significantly influence the LN output, but perhaps at a more subtle level than the tier 1 parameters, we performed a second sensitivity analysis fixing the tier 1 parameters at biologically relevant values, but allowing all other model parameters to vary over physiologically reasonable ranges (Table I). In this way, we identified four additional parameters that have a significant effect on the generation of primed CD4+ T cells exiting the LN (Table II, Tier 2 Parameters). The tier 2 parameters identified are all related directly to the interactions of the DCs and T cells in the LN. Recall from Fig. 6 that the binding and priming thresholds determine the probability that a particular Ab-DC (or LDC) is able to bind and ultimately prime a cognate T cell. Both the binding and priming thresholds are negatively correlated with primed CD4+ T cell output, because increasing either threshold lowers the probability...
that a T cell will be primed. Similarly, the unbinding threshold is negatively correlated with primed T cell output. At low cognate frequencies (1:3000; Table II), these three tier 2 parameters lose significance, indicating that there are differences between dynamic mechanisms identified in the sensitivity analysis (see previous tables). Finally, of the tier 2 parameters, pMHC \( t_{1/2} \) is strongly correlated with primed CD4+ T cell generation and exits from the LN at both cognate frequencies. This means that, if the pMHCII complexes are short-lived (the lower end of the range we tested was 30 h), there is a significant reduction in the generation of primed CD4+ T cells. However, it is well-documented that Ab-DCs (and similarly LDCs) can extend the lifespan of the DC (12–14, 69). This mechanism is evolutionarily beneficial, because extending the \( t_{1/2} \) of pMHC allows for continual priming of cognate T cells by DCs. Kinetic stability of pMHCII complexes has also been found to be a key parameter of immunodominance, as complexes with a \( t_{1/2} < 10 \) h were usually cryptic, whereas dominant immune peptides had a long \( t_{1/2} (> 150 \) h) (69, 70).

Detailed results of our sensitivity analyses are given at http://malthus.micro.med.umich.edu/lab/movies/FullLN/. We also note that model parameters not identified as tier 1 or tier 2 parameters can still affect LN outcome, but not as significantly, at least within the ranges tested.

Identification of pathogen-affected processes that may influence generation of primed CD4+ T cells

Pathogens are known to interfere with the immune system via several mechanisms that affect DC–T cell interactions in the LN. If the biologic mechanisms identified in the sensitivity analysis (see previous tables) can still affect LN outcome, but not as significantly, at least within the ranges tested.

Identification of pathogen-affected processes that may influence generation of primed CD4+ T cells

Pathogens are known to interfere with the immune system via several mechanisms that affect DC–T cell interactions in the LN. If the biologic mechanisms identified in the sensitivity analysis (see previous tables)
section) were targets with which pathogens evolved to interfere during infection, would the generation of primed CD4+ T cells be affected? To explore this further, we simulated these “pathogen affects” on DC–T cell interactions by choosing four key mechanisms known to have an effect on primed CD4+ T cell generation, as identified in the sensitivity analysis (Table II): pMHCII t1/2, unbinding threshold, binding threshold, and priming threshold. Although it is known that pathogens can affect aspects of immune system function, the extent to which individual mechanisms influence LN output of primed CD4+ T cells is unknown and not possible to quantify experimentally. To simulate these pathogen-related factors, we reduced (or increased) the mechanism under study by a standardized amount to observe the effects these changes have on the output of primed CD4+ T cells. We did this not only for each parameter independently, but also for the four chosen mechanisms (parameters) in different combinations.

Fig. 7 shows the cumulative (14 d) totals for primed CD4+ T cells leaving the LN. Our results show that changes in the values for each of the four mechanisms (parameters) individually reduce the output of both primed CD4+ T cells. It is interesting to consider whether pathogens would adopt a strategy that affects only a single process in the DC–T cell interaction, but what if pathogens evolved to affect multiple processes simultaneously? We explored this possibility by first combining effects to the four processes in groups of two. The overall effect on primed CD4+ T cell output can be both greater than or less than the sum of the two processes acting individually. For example, when we affect both pMHC t1/2 and the unbinding threshold (processes 1 and 2 in Fig. 7), we get a greater than additive affect. However, when both pMHC t1/2 and the binding threshold (processes 1 and 3 in Fig. 7) are affected, we get a less than additive effect, on average. This finding follows from the nonlinear relationship between binding and priming parameters (Fig. 6). We can also consider a pathogen affecting three or four processes simultaneously. When mechanisms 1, 2, and 4 are simultaneously pathogen-affected, the generation of primed T cells is completely abrogated. This suggests that a synergy exists between the mechanisms operating during DC–T cell dynamics that, if disrupted, leads to a dramatic effect. Our result provides a strong argument for selection of pathogens that can interfere with multiple elements of DC–T cell interaction dynamics. In other words, a pathogen that can reduce the numbers of pMHCII (perhaps by reducing pMHC t1/2) and in addition can shift the binding threshold, priming threshold, or unbinding threshold (Fig. 6; perhaps by affecting costimulatory molecules) should be more successful than a pathogen that affects only one of these mechanisms.

### Table II. Results of model sensitivity analysis

| Parameter                      | +/− Correlation |
|-------------------------------|-----------------|
| Tier 1                        |                 |
| Cognate frequency             | −               |
| Number of divisions           | +               |
| Division time                 | −               |
| Percent Ab-DC                 | +               |
| Tier 2                        |                 |
| pMHC t1/2                     | +               |
| Unbinding threshold           | −               |
| Binding threshold             | −               |
| Priming threshold             | −               |

Tier 1 parameters are those identified as the most significant factors affecting the cumulative (14 d) output of primed CD4+ T cells when 16 of the model parameters (from Table I) are varied (http://malthus.micro.med.umich.edu/lab/movies/FullLN/ and data not shown). Tier 2 parameters are identified as significant when 12 parameters from Table I are varied. Results are listed for two sample cognate frequencies, 1:300 and 1:3000. See http://malthus.micro.med.umich.edu/lab/movies/FullLN/ for details. All values shown are p < 0.01.

### Discussion

Generation of an adaptive immune response begins with physical encounters between Ab-DCs and T cells whose receptors have high affinity for a specific peptide-MHC complex. Trafficking of T cells and DCs into the LN leads to multiple opportunities for contact between Ab-DCs and cognate T cells. Unfortunately, many of the fundamental processes and parameters that describe the functioning of an LN are difficult to measure experimentally. For example, 2PM studies are by necessity limited to small areas of observation (micrometers) and short times frames (minutes to only a few hours). Most significantly, the relationship between Ab dose (in terms of density of Ab-DCs or how laden individual DCs are with Ag), frequency of matching cognate T cells, and generation of primed CD4+ T cells are not known. Many of these questions have been recently explored for CD8+ T cells (10, 11), but they remain open for CD4+ T cells. In addition, experimental protocols to investigate such phenomena often involve infusion of cognate T cell clones or introduction of large numbers of DCs, both necessary for measurable results, but often outside the usual biological range of cell frequency or density (9). There is often an underlying assumption of proportionality, either explicitly stated in models or implicitly understood while interpreting results. For example, one might assume that if the density of Ab-DCs were reduced to 10 or 1% of the original amount, then the output would be proportionately reduced, but this is currently an open question. The implications of these relationships are important for understanding the roles that pathogens, pharmaceutical interventions, and vaccines can have on the immune response.
In this study, we develop a 2D computational model of a complete T cell zone of an LN and its ability to generate primed CD4+ T cells, and we use it to simulate the dynamics of immune cell interactions in an LN during a virtual infection (Fig. 2) over long time frames (days). The timing and the magnitude of the generation of primed CD4+ and CD8+ T cells are consistent with the scant data available, with more primed CD8+ than CD4+ T cells generated (64). Thus, these simulations provide a first look at how infection (characterized by the introduction of Ab-DCs into the LN) translates into generation of primed CD4+ T cells exiting from a single LN.

Our model allows us to quantify the relationship between cognate frequency and total number of primed T cells leaving the LN over a 14 d period. Interestingly, generation and output of primed CD4+ T cells from an LN scales with naive cognate CD4+ T cell input for a fixed Ab-DC level, with a small, positive feedback effect from the LDC pathway (Fig. 3) and one to four primed CD4+ T cells produced per entering naive cognate CD4+ T cell.

We can also quantify the relationship between Ag dose and generation of primed CD4+ T cells by the LN. Ag dose is composed of two different factors: how many Ab-DCs enter the LN and how many pMHCs are present on the surface of these Ab-DCs (11). Increasing either the number of Ab-DC or the number of pMHC on those Ab-DCs increases the output of primed CD4+ T cells, as expected (Figs. 3, 5). This can be visualized as a trade-off (Fig. S5) between the number of Ab-DC and their ability to present Ag. High numbers of Ab-DCs can compensate for low values of pMHC, and vice versa.

One wonders whether the 2PM experiments with their necessarily high cognate frequency and high numbers of Ab-DCs can give insight into a more physiologic lower frequency situation. Because extrapolation of results to situations outside the range of an experiment is fraught with error, supplementation of experimental results with the in silico simulations is useful. Thus, our results suggest how to extrapolate results obtained using 2PM to understand actual infection dynamics: the output of primed CD4+ T cells scales linearly with cognate frequency, but nonlinearly with the number of Ab-DCs present (Fig. 3).

Physiologic cognate frequency is not known definitively, but recent estimates in mouse (67), suggest ~20–200 cognate naive CD4+ T cells out of a total population of 10^7, thus giving an estimate of cognate of 1:10^5; if one considers multiple epitopes from a single Ag being presented by a single DC, one can imagine that the effective cognate frequency is increased by an order of magnitude (i.e., 1:10^6). We are able to consider in our model cognate frequencies as low as 1:10^4. Unfortunately, estimates of the number of Ab-DCs present in an actual infection are scarce. The number of Ab-DCs tested in this study is consistent with the scant data available, but more data are needed on this point.

Our simulations offer interesting and novel statistics on the passage of a cognate T cell through an LN (Fig. 3). The number of CD4+ T cells that find their DC match is significantly less than 100%, even at the numbers of Ab-DCs tested in this study, which are likely at the high end of the true physiologic range. CD4+ T cells require ~2–5 h to find their DC match and 16–23 h to transit through the LN, consistent with an earlier calculation for a smaller section of an LN (15) and experimental (i.e., 2PM) data (6–8).

Our analysis allows us to quantify the degree to which mechanisms that are part of the DC–T cell interactions contribute to the generation of primed CD4+ T cells (Fig. 7; Table II; http://malthus.micro.med.umich.edu/lab/movies/FullLN/). Interestingly, these factors are precisely those that pathogens have been identified to inhibit. Microbes are known to alter DC–T cell interactions in multiple and even overlapping ways. Data support the effects of both bacteria and viruses on DC recruitment, maturation, and survival. Herpes virus and poxvirus can encode homologs of chemokine receptors that function as chemokine antagonists and prevent recruitment of additional DCs to the site of an infection (18, 19). Shigella and Salmonella sp. can activate DC apoptosis via caspase 1 (71, 72). Vaccinia can inhibit DC maturation and induce DC death by apoptosis (73). Inhibition of DC maturation has been shown to occur via 1) secretion of altered receptors that block interferon, TNF-α, or IL-1; 2) secretion of regulatory cytokines such as viral IL-10s from EBV; 3) inactivation of intracellular pathways such as HCMV to prevent surface expression of MHC-peptide complexes; or 4) blocking chemokine receptors (e.g., CCR7), resulting in impairment of DC migration to the draining LN (18). These effects on recruitment, maturation, and survival map to our model parameters of Ab-DC frequency, licensing of DCs, and DC life span.

Similarly, data suggest the role of pathogens on inhibition of T cell dynamics. Bordetella pertussis generates T regulatory cells that secrete IL-10 and inhibit DC secretion of IL-12, which helps to evade an immune response for whooping cough and suppresses the immune response toward unrelated pathogens (74); this maps in our model to parameters that lead to priming of T cells. Viruses, such as measles or CMV (18, 75, 76), can also interfere with T cell priming, proliferation, and differentiation, probably via blocking or altering cytokine secretion. Each of these types of mechanism is represented in some aspect of our model, and our model suggests that the acquisition of these inhibitory mechanisms by pathogens evolved in the most optimal of ways, because all the tier 1 and tier 2 parameters are used by pathogens to suppress immunity. Although the details of all these mechanisms are not specifically represented in our model structure, they would act to shift the priming and binding curves in Fig. 6 right and/or down. In contrast, if a vaccine or drug were developed to enhance aspects of the DC–T cell interaction, then the curves would move up and/or to the right.

We show here that an in silico model can extend knowledge to spatial and temporal scales that fall well outside the range of what is feasible in actual 2PM studies. Specifically, we describe the efficiency of an LN in producing primed CD4+ T cells, demonstrate the influence of particular pathogen-influenced processes on that priming, and we offer insight into the extent to which 2PM studies reflect in vivo infections. Our work compliments other computational studies on understanding priming and dynamics of CD8+ T cells (12–14) and the motion of lymphocytes on the fibroblastic reticular cell network within the LN (16, 33). We have now reached the point at which systems biology approaches can simulate the functioning of an entire LN during infection.

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Disclosures

The authors have no financial conflicts of interest.

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