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Predicting lymph node output efficiency using systems biology

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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.

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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.

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, naive 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

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(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 μm 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 (Manttra, 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 flurochrome-conjugated 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 fluorochrome-labeled 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 fluorochrome-conjugated 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 × 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 C57BL6 mice (The Jackson Laboratories) by magnetic negative selection (StemSep). T cell purity was $>95\%$ CD4+, CD3+ as assessed by flow cytometry. T cells were labeled with 5 μM 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 CO_2 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