Supplement for:  
Characterizing the dynamics of CD4+ T cell priming within a lymph node

The rules included in our model capture more than that we report on. The focus of this current paper is on the dynamics of priming of CD4+ T cells. However, we include priming and memory events in the model, even though it is not reported for completeness.

Supplement 1: Agent Based Model Rules

S1.1 Details of Agent Based Model

S1.1.1 Environment

Environment of our model is a 200 X 25, two dimensional grid that represents a 1/20 section of mouse lymph node (LN); each micro compartment of the grid (grid unit) is a 20 µm X 20 µm square. 40 high endothelial venules (HEV) are located in row 7, 100 afferent lymphatics (AL) are located in rows 4 and 5, and 160 medullary sinuses (MS) are located in rows 21, 22, 24 and 25 (Figure 2). Each HEV, AL and MS occupies one micro compartment.

S1.1.2 Agents

Agents in the model are CD4+ (helper) T cells, CD8+ (killer) T cells and dendritic cells (DCs). CD4+ T cells can become primed and then activated. CD8+ T cells can become primed, activated and also memory cells. We only focus on the priming events in this paper.

All agents have following attributes:

i. Id: Unique identifier used to track each cell
ii. Birth time: This attribute is used to keep track a cell’s age.
iii. State: Represents cell type; CD4+ T cells states are resting, activated or effector. CD8+ T cells are resting (naïve),
activated, effector, or memory. Naïve T cells are either free or bound to antigen bearing (Ab-DC). DC states are immature (IDC), mature (AB-DC), licensed (LDC).

iv. Time in: Time when the cell first appears on the grid.

DCs have following additional attributes:

i. pMHC level: Amount of peptide-major histocompatibility complex (pMHC) on the surface of DC is used to represent amount of antigen that the DC carries.

T cells have following additional attributes:

i. Cognate: a naïve T cell is either antigen recognizing (cognate) or non cognate.
ii. Birth row: row where the T cell first appears on the grid
iii. Birth column: column where the T cell first appears on the grid
iv. Division counter: Number of divisions T cell has undergone
v. Bound to DC: Id of DC to which T cell is bound
vi. Direction: Direction taken by the T cell at the last update

S1.1.3 Rules

a. Each micro compartment can hold up to four T cells or one DC. The grid is a cylindrical torus; cells that leave from one edge enter the grid at the other edge.

b. Cell Density: Ratio of CD4+ : CD8+ T cells is 6:5; ratio of DC:T cells is 1:160 (T. Riggs et al., 2008).

c. T cell recruitment: Naïve T cells are recruited (enter the grid via HEV) during simulation. Every 2 minutes, with 99% probability of recruitment. one naïve CD4+ T cell can enter the grid per available HEV location Following CD4+ T cell recruitment and their subsequent movement, CD8+ T cells are similarly recruited, one per available HEV location with 95% probability of recruitment. Recruitment probabilities for naïve CD4+ T cells and naïve CD8+ T cells are determined such that these cells maintain a steady state population over the duration of simulation. Among these recruited T cells,
some are randomly chosen to be cognate (antigen recognizing); total number of cognate CD4+ T cells and cognate CD8+ T cells is determined by specified cognate frequency for CD4+ T cells and CD8+ T cells respectively.

d. DC recruitment: Antigen is introduced via DCs that enter the LN via AL.

i. DC recruitment is designed to achieve a certain distribution of DC population on the grid during the simulation. Eight DCs enter the grid at time = 0. Subsequently, DCs are recruited every hour such that the number of DCs on the grid reaches the peak level (~100) at 33 hours. After reaching peak, DC recruitment pattern differs in acute infection scenario and in chronic infection scenario. In acute infection scenario, hourly DC recruitment is continued until 96 hours such that the number of DCs on the grid taper off after 33 hours and number of antigen bearing DCs (AB-DC+LDC) reaches zero by day 7. In chronic infection scenario, hourly DC recruitment is continued in order to maintain a steady state level population of DCs (at the peak level) throughout the rest of the simulation duration.

ii. pMHC level distribution: pMHC level on the surface of a DC is chosen from a normal distribution. pMHC level on 60% (randomly selected) of the total DCs recruited is chosen from $N(250, 25)$, pMHC level on the remaining 40% DCs is chosen from $N(50, 20)$. A DC with pMHC level below a defined threshold (50) is considered an immature DC (IDC). DCs with pMHC level above the threshold are considered antigen bearing DCs (Ab-DC) or mature DCs (Ab-DC). pMHC level on the surface of DCs undergoes decay; this value is updated at each 2 minute interval for every DC based on chosen half-life value for pMHC level. Base line value for pMHC half life is 60 hours.

e. Cell movement: T cell movement is modeled as random motion (T. Riggs et al., 2008). Unbound naïve CD4+ T cells and naïve CD8+ T cells attempt to move every two minutes; primed T cells (activated or effector CD4+ T cells, activated, effector or memory CD8+ T cells) attempt to move every 4 minutes. A T cell moves to a chosen adjacent micro compartment to the east, west,
north or south. Direction of movement is modeled as short-term persistence i.e. direction of movement is decided by direction taken at the previous step. T cells do not make 180° turns, and have equal probability of moving in one of the remaining three directions. T cell’s initial direction is chosen randomly and when a T cell pauses because the chosen location is occupied, its direction at the new update time is likewise chosen randomly. A cognate T cell bound to a DC moves with the DC at its slower speed. After being released from DC, T cell resumes its usual speed depending upon its state. T cells move sequentially to avoid collision; the order is randomized at each update step. Since the grid is a cylindrical torus, T cell moving off one edge of the grid appears on the other side. A DC attempts to move along with it’s the bound T cells every 8 minutes to an adjacent available micro compartment. DCs are moved sequentially; the order is randomized at each update step. DCs do not move off the edge of the grid.

f. Cell interactions: T cell - Ab-DC interactions are checked at every 2 minute time step.

i. Contact: A free T cell (i.e. T cell not bound to a DC) contacts a DC if it is in any of the DC’s 8 adjacent micro compartments. Up to 32 T cells can contact a DC at a time.

ii. Binding: Contact between a naïve cognate is updated every two minutes, binding probability is calculated for each naïve cognate CD4+ T cell that contacts an Ab-DC or LDC or naïve cognate CD8+ T cell that contacts an LDC. Binding probability is calculated as a logistic function (Figure 1b).

\[
\text{Binding probability} = \frac{1}{1 + e^{\left(\frac{x - a}{b}\right)}}
\]

- \(a\) = pMHC level for median binding probability \((binding\ threshold)\)
- \(b\) = shape of binding curve \((binding\ shape)\)
- \(x\) = pMHC level on DC

A maximum of 32 T cells can bind to a DC at a time.
Release: After a T cell binds to Ab-DC, T cell’s binding status is checked every hour. At the end of each hour, the average pMHC value on the surface of the DC over that hour is added to the priming sensitivity history of the T cell. Thus the T cell accumulates “priming signal” as a product of average pMHC concentration and binding duration. Bound T cell is released if one of the following conditions is true:

1. Priming sensitivity history of the T cell exceeds the priming threshold value (200).
2. pMHC level on the DC to which the T cell is bound, falls below unbinding threshold value (100).
3. The T cell has been bound to the DC for the maximum binding period (7 hours).
4. Ab-DC dies because its lifespan is over.

g. Cell Priming: priming probability is calculated for a T cell released after binding with an Ab-DC, as a logistic function (Figure 1c).

\[
\text{Priming probability} = \frac{1}{1 + e^{-\frac{(x - c)}{d}}}
\]

- \(c\) = time for median priming probability (priming threshold)
- \(d\) = shape of priming curve (priming shape)
- \(x\) = product of pMHC level and duration of binding

To investigate if repeated binding events between a cognate T cell and Ab-DCs increased priming sensitivity of the T cell, two versions were implemented; in base line version or no history version, priming probability of a T cell is independent of previous unsuccessful binding events (binding events which did not result in priming) and priming
probability of a T cell released from Ab-DC depends only on the priming signal accumulated for the current binding event. In the second version *priming sensitivity history version*, T cell’s priming sensitivity history from previous unsuccessful binding events is accumulated and is added to priming sensitivity history which is used to calculate the priming probability; thus incrementing the priming probability of each subsequent binding event.

h. Cell Proliferation: Activated T cells divide every 8 hours (T cell doubling time). Activated CD4+ T cells undergo up to four divisions; activated CD8+ T cells undergo up to 8 divisions. An Activated CD4+ T cell that has undergone four divisions becomes an effector CD4+ T cell; an activated CD8+ T cell that has undergone 8 divisions has 50% probability of becoming an effector CD8+ T cell and 50% probability of becoming a memory CD8+ T cell.

i. DC licensing: When an effector CD4+ T cell contacts an AB-DC (i.e. an effector CD4 T cell is in one of the eight neighboring compartments of the Ab-DC), the effector CD4+ T cell licenses the AB-DC with probability of 50%. pMHC level on the surface of licensed DC is set to be 150 above the unbinding threshold (250).

j. IDC maturation: When an IDC contacts an LDC (i.e. IDC is in one of the eight neighboring compartments of an LDC), the LDC converts the IDC into AB-DC. pMHC level on the of the newly matured DC is set to an average of binding threshold (200) and pMHC level on the surface of the LDC.

k. Cell Exit: T cells that land on MS location exit the LN. DCs do not exit the LN.

l. Cell Lifespan: Cells of different types have different lifespan. A cell dies after its lifespan is over and is removed from the grid. Age of a naïve T cell is chosen randomly (when the cell is placed on the grid) between minimum and maximum age specified below. Once a naïve T cell becomes primed i.e. becomes activated, effector or memory T cell, it lives for the lifespan specified for that particular cell type. When an Ab-DC dies, all T cells bound to it are released.
i. Naïve CD4+ T cell age: 165-365 days (McCune et al., 2000)
ii. Activated CD4+ T cell maximum lifespan: 48 hours
iii. Effector CD4+ T cell maximum lifespan: 60 hours (Sprent and Tough, 2001).
iv. Naïve CD8+ T cell age: 165-365 days (McCune et al., 2000)
v. Activated CD8+ T cell maximum lifespan: 48 h (Sprent and Tough, 2001).
vi. Effector CD8+ T cell maximum lifespan: 60 h (Sprent and Tough, 2001)
vii. IDC: maximum life span is 11 days.
viii. Ab-DC maximum lifespan: 60 h (Kamath et al., 2002)
ix. LDC maximum lifespan is chosen from normal distribution: \(N(36, 4)\) hours (Lanzavecchia and Sallusto, 2004, Lindquist et al., 2004).

**S1.2 Simulation**

A simulation is run either with acute infection scenario or chronic infection scenario (see rules for details). Simulation sets are run with either base line parameter values where fixed parameter values are used for all simulations in a set or with Latin Hypercube Sampling (LHS) method where parameter values are sampled over a range and each simulation is run with a different set of parameters. Range for each parameter is centered at the base line value for that parameter (see Table 1 for base line parameter values and ranges). The basic time step of the simulation is 6 seconds.

**S1.2.1 Parameters**

a. Values of following parameters are set before starting the simulation:
   i. Number of days: Duration of simulation.
   ii. Cognate Frequency: Proportion of cognate naïve CD4+ and CD8+ T cells is chosen from 1:100 -1:10000.

b. Values of following parameters are varied over a range during LHS simulations.
   i. pMHC halflife: Half life of pMHC level on DC surface.
ii. Unbinding threshold: Minimum level of pMHC on Ab-DC surface at which a T cell can bind to DC. If the pMHC level falls below the unbinding threshold, the T cell is released.

iii. pMHC for median binding probability for CD4+ T cell: pMHC level on Ab-DC surface that corresponds to 50% binding probability for CD4+ T cells bound to that Ab-DC.

iv. Shape of binding probability curve for CD4+ T cell: Slope of binding probability curve for CD4+ T cells.

v. Time for median priming probability (priming threshold) for CD4+ T cell: Duration of binding of CD4+ T cell to Ab-DC that corresponds to 50% priming probability for the bound CD4+ T cell.

vi. Shape of priming probability curve (priming shape) for CD4+ T cell: slope of priming probability curve for CD4+ T cells.

vii. pMHC for median binding probability for CD8+ T cell: pMHC level on LDC surface that corresponds to 50% binding probability for CD8+ T cells bound to that LDC.

viii. Shape of binding probability curve for CD8+ T cell: Slope of binding probability curve for CD8+ T cells.

ix. Time for median priming probability (priming threshold (k cell)) for CD8+ T cell: Duration of binding of CD8+ T cell to LDC that corresponds to 50% priming probability for the bound CD8+ T cell.

x. Shape of priming probability curve (priming shape (k cell)) for CD8+ T cell: slope of priming probability curve for CD8+ T cells.

### S1.2.2 Initialization

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HEVs, ALs and MS are placed on a 200 X 25 grid. ~6000 naïve CD4+ T cells and ~5000 naïve CD8+ T cells are placed on the grid. The simulation is run for 15 hours to reach steady state levels which is 5000 CD4T Cells and 4000 CD8 T cells. The level of DCs is chosen to be 1% of Total T cells (as identified in our previous work from mouse data [Riggs et al]). For placing the T cells, for each cell a location (micro compartment) is chosen randomly and the T cell is placed there.
if the chosen location is available and is not an AL. Among these initial T cells, some are randomly chosen to be cognate (antigen recognizing); number of cognate CD4+ T cells and cognate CD8+ T cells is determined by specified cognate frequency for CD4+ T cells and CD8+ T cells respectively.

**S1.2.3 Order of Events**

Simulation is run for 15 hours before introducing DCs. This step is done to achieve a steady state number of T cells (~9600) on the grid before antigen is introduced. After running for 15 hours to reach the T cell steady state (this is considered time = 0), the simulation is run for 14 days.

Following events occur during simulation (See rules for details):

a. Events occurring every 2 minutes:
   i. naïve CD4+ T cells are recruited
   ii. CD4+ T cells are moved
   iii. naïve CD8+ T cells are recruited
   iv. CD8+ T cells are moved
   v. State of T cells on the grid is checked and updated if changed due to binding to DC, priming, differentiation or death. This check and update of state is done sequentially, first for CD4+ T cells and then for CD8+ T cells.
   vi. State of each of DCs on the grid is checked and updated if changed due to maturation, licensing or death. This check and update is done sequentially.
   vii. pMHC level on each DC is updated based on specified half life.
   viii. Dead cells are removed

b. Event occurring every 8 minutes:
   i. Dendritic cells are moved

At time = 0, 8 DCs enter the grid via randomly chosen AL locations. DCs are recruited hourly from first hour onwards as per the DC recruitment rule for acute and chronic infection scenarios.

**S1.2.4 Metrics**

a. Time series: Number of cells of different types; resting CD4+ T cells, activated CD4+ T cells, effector CD4+ T cells, resting CD8+ T cells,
activated CD8+ T cells, memory CD8+ T cell, immature DCs, mature DCs and licensed DCs is recorded every 2 minutes. Populations of different cell types over time are displayed using graphs and grid snapshots.

b. T cell motility: Movement of naïve T cells from point of entry (HEV) over time. Positions of 50 naïve T cells are tracked for 25 minutes after their entry into the LN.

c. Life time displacement: Total distance traveled by first 1000 recruited T cells from entry until they exit or die or until the end of simulation.

d. Mean free path: The weighted average of straight path lengths (in µm) for naïve T cells is determined by frequency count of ~10^5 path lengths between turns from 1000 CD4+ T cells per simulation.

e. Search time: Mean time that a naïve cognate T cell takes from entry via the HEV to make first contact with an Ab-DC.

f. T cell – DC contacts: Contacts between Ab-DCs and T cell (counted separately for CD4+ T cells and CD8+ T cells for each AB-DC and LDC) as total number of contacts with all T cells and total contacts with different types of T cells over the entire lifespan of each antigen bearing DC.

   xi. Total CD4+ (/CD8+) T cell contacts
   xii. Naïve CD4+ (/CD8+) T cell contacts
   xiii. Cognate CD4+ (/CD8+) T cell contacts
   xiv. Non cognate CD4+ (/CD8+) T cell contacts
   xv. Unique naïve CD4+ (/CD8+) T cell contacts i.e. how many different naïve CD4+ (/CD8+) T cells contacted the Ab-DC.
   xvi. Activated CD4+ (/CD8+) T cell contacts
   xvii. Unique activated CD4+ (/CD8+) T cell contacts

g. Match percentage: Fraction of total cognate T cells that contact Ab-DC.

h. Proportion of binding and priming: For each simulation, proportion of cognate CD4+ T cells that bind to Ab-DC and proportion that get activated is recorded.
i. Transit time: Mean time taken by a naïve cognate T cell to traverse the entire LN (from entry via HEV to exit via MS) is recorded.

j. Cumulative output of primed CD4+ and CD8+ T cells: All non-naïve T cells are considered primed T cells. Output of the system is measured as cumulative number of primed CD4+ T cells (activated CD4+ T cells and effector CD4+ T cells) and primed CD8+ T cells (activated CD8+ T cells, effector CD8+ T cells and memory CD8+ T cells) that leave the LN via MS is recorded every 2 minutes.

S1 References


