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Inoculation Dose of *Mycobacterium tuberculosis* Does Not Influence Priming of T Cell Responses in Lymph Nodes

Amy J. Myers,* Simeone Marino, ‡ Denise E. Kirschner, ‡‡ and JoAnne L. Flynn*

The effect of *Mycobacterium tuberculosis* inocula size on T cell priming in the lymph node and effector T cells in the lung remains controversial. In this study, we used a naive mouse model, without the transfer of transgenic T cells, in conjunction with mathematical model to test whether infection with higher aerosolized inocula would lead to increased priming of *M. tuberculosis*-specific T cells in the lung-draining lymph node. Our data do not support that inoculum size has a measurable influence on T cell priming in the lymph nodes but is associated with more cells overall in the lung, including T cells. To account for increased T cells in the lungs, we tested several possible mechanisms, and recruitment of T cells to the lungs was most influenced by inoculum dose. We also identified IL-10 as a possible mechanism to explain the lack of influence of inoculum dose on priming of T cells in the lymph node. *The Journal of Immunology*, 2013, 190: 000–000.

*Mycobacterium tuberculosis* is a leading global health concern causing 1.4 million deaths in 2011 (1). A small percentage of infected individuals do not generate an initial protective response and progress to primary disease within 2 y, whereas 90% of immunocompetent people are able to control infection, often for life. Although a vaccine (bacillus Calmette-Guérin) is widely administered, it does not provide adequate immunity against infection or disease. Various animal models and human studies have demonstrated that certain cytokines, including IFN-γ, TNF, and IL-12, as well as CD4 and CD8 T cells and activated macrophages are essential for protection against tuberculosis (2).

Initiation of the immune response against *M. tuberculosis* infection is a slow process. In humans, very little is known about the events that occur during transmission and initial stages of infection, because these events are “silent.” Exposure is likely to be to a very small numbers of organisms, and in some settings, it is probable that it is repeated exposure that results in successful transmission events.

Animal studies demonstrated that the microbe is inhaled into the airways where it encounters alveolar macrophages and dendritic cells (DC), which transport bacteria to draining lymph nodes for the purpose of priming T cells (3, 4). These primed T cells migrate back to the infected lung to participate in granuloma formation, but the lymph nodes also remain infected. Studies have demonstrated that after low-dose aerosol infection of mice, *M. tuberculosis* bacilli appear in the lymph node between days 9 and 11, with variation among even inbred mice; bacteria in lymph nodes are necessary to initiate a priming response (4). A recent study (5) using mice without appreciable lymph nodes suggested that priming of T cells can also occur in the lung. In normal mice, bacteria arrive in the spleen 2–3 wk postinfection, and this is also a potential site for priming T cells. Using adoptive transfer systems with large numbers of *M. tuberculosis* Ag–specific transgenic T cells, priming of T cells in lymph nodes (as determined by CD69 expression) occurred between days 11 and 12, but significant T cell proliferation in the lymph nodes began only at day 14 (6). T cell responses can be detected in the lungs by ∼2 wk postinfection (p.i.), and by 4 wk p.i., bacterial growth in lungs is stabilized (7); the level of bacilli in lungs remains at high levels for months as the mouse experiences progressive chronic tuberculosis. This relatively long period of time between infection and induction of T cell responses may allow *M. tuberculosis* to gain a foothold in the lungs without facing an adaptive immune response (4). This was also observed in a computational model of the immune response in lungs to *M. tuberculosis* (8). Understanding factors involved in priming of T cells in response to *M. tuberculosis* infection may improve our ability to design vaccines that enhance rapid recall responses in the lungs and lymph node to improve protection against disease.

Our previous studies in CD40<sup>−/−</sup> mice indicated that a 2- to 3-fold higher aerosol inoculum resulted in an increase of IFN-γ–producing cells in the lymph node by 3 wk and in the lungs at 4 and 5 wk, thus improving survival of these mice (9). This suggested that Ag or bacterial load could influence priming of T cells in the lymph nodes. Two other studies, using adoptive transfer of transgenic T cells, demonstrated that the numbers of bacilli within the draining lymph nodes were positively correlated with robustness of priming (as defined by activation and proliferation of the transgenic T cells) (6, 10). The studies were conflicting in their findings of effects of inoculum size on timing of priming: one study supported that a higher inoculum could cause earlier priming of T cells (6); however, effects were minimal even though large inocula (1200 CFU via aerosol) were used. The other study showed an effect of dose on magnitude of responses but not on timing of induction (10).

In the current study, we addressed the influence of inoculum size on timing and magnitude of T cell priming in lymph nodes in a naive...
mouse model without transfer of transgenic T cells to determine how normal naive frequencies of *M. tuberculosis* Ag–specific T cells respond to different doses of infection. We integrated mathematical modeling of the priming response in lymph nodes with our experimental data and determined that, in an intact mouse, there was minimal effect of inoculum size on priming in the lymph node and only a modest effect on the *M. tuberculosis*–specific number of T cells in the lung. There were, however, higher bacterial numbers and total cell numbers (including T cells) in mice with higher inocula compared with those inoculated with fewer bacteria. We addressed three hypotheses for these observed increases using wet-laboratory and modeling approaches. Our mathematical modeling approach predicts additional factors, such as a key role for IL-10 and DCs in regulating T cell priming in the lymph node and in driving effector T cell numbers in the lung in a high-dose scenario.

**Materials and Methods**

**Aerosol infection of mice**

C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) were aerosol infected with *M. tuberculosis* (H37Rv or Erdman strain) using a flat chamber system (Infors, Mortsel, NY). A low dose of *8 × 10^6–3 × 10^7/ml* or a high dose of *8 × 10^6–3 × 10^7/ml* in the nebulizer was used to deliver 10–40 or 130–550 CFU, respectively, to the lung as determined by plating whole-lung homogenates from four to six mice per group on 7H10 agar (Difco, Sparks, MD) 24 h after aerosolization. Colonies were counted after incubation of plates for 21 d at 37°C and 5% CO2. The experiments were performed six times, using different doses and timing of necropsies. Representative data are provided in this study. Mice were kept under specific pathogen-free conditions in a biosafety level 3 facility, and all studies were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

**Single-cell suspensions and flow cytometry**

Whole lungs and lymph nodes were crushed, and single-cell suspensions were obtained as described previously (11). Live cells were counted by trypan blue exclusion and × 5 × 10^6 cells were washed in FACS buffer (1× PBS and 0.01% BSA; SerCare Life Sciences, Milford, MA). The following cell surface Abs were added in staining buffer (FACS buffer, 20% mouse serum [Gemini Bio-Products, West Sacramento, CA], anti-CD69 (clone H1.2F3), anti-CD11b (clone M1/70), anti–GR-1 (clone RB6-8CS), anti-CD69 (clone H1.2F3), anti-CD11b (clone M1/70), anti–GR-1 (clone RB6-8CS), anti-CD3 (clone RM4-5), and anti-CD8 (clone 53-6.7) from BD Biosciences (San Diego, CA) and anti-CD11c (clone N418) from eBioscience (San Diego, CA), and anti-CD11c (clone N418) from eBioscience (San Diego, CA), and anti-CD11c (clone N418) from eBioscience (San Diego, CA). MHC class I GAP tetramer (recognizing the GAPN1SAL6 amino acids of Rv0125) (Beckman Coulter, Fullerton, CA) and NIH Tetramer Facility, Atlanta, GA and MHC class II multimer ESAT-6 (recognizing aa 1–20 (MTEQQWNFAGIEAAASAIQG) (M. Jenkins, University of Minnesota, Minneapolis, MN, and NIH Tetramer Facility) were also added at 1.67 or 1.100 (respectively) final concentration. Cells were incubated with Abs for 30 min at room temperature then washed with FACS buffer and fixed in 2% paraformaldehyde (Sigma-Aldrich, St. Louis, MO). Samples were collected using an LSRII flow cytometer (BD Biosciences, San Jose, CA) and analyzed with FlowJo (Tree Star, Ashland, OR).

**Statistical analysis**

Data were compared among high- and low-dose mice at each time point. Mann–Whitney analysis was used for flow cytometry data, and Student t test was used for log-transformed CFU data and ELISPOT data. Analyses were performed in Prism (GraphPad) or Excel (Microsoft), including the linear regression analyses. Mean and SD is shown in each figure.

**Mathematical model**

We developed a series of mathematical models that qualitatively and quantitatively characterize the cellular and cytokine network during infection in lung and lymph node (12, 13). In this study, we used our most recent generation, two-compartment mathematical model of lung and lymph node dynamics (13) to capture the behavior of T cell priming in lymph nodes and T cell migration to lungs during *M. tuberculosis* infection. The model was built on our experimental data generated in normal mice. A mathematical modeling approach allows us to address a number of questions that are challenging to address in an in vivo system, and because this model was built on data from immune competent C57BL/6 mice infected with different inocula, it is directly relevant to our studies here. The model captures the dynamics of many cell types, such as macrophage (resting; infected; classical; and alternatively activated [AAM]), DC (immature [IDC], Ag-bearing, or mature [MDC]) and lymphocyte populations in both lung and lymph node (naive, precursor, and effector CD4 and CD8 T cells). Table I lists the T cell phenotypes used in the model (macrophages not shown, for a full explanation of model, see Ref. 13). We also model four cytokine concentrations (TNF, IFN-γ, IL-10, and IL-12) in both lymph node and lung compartments. Details of the nonlinear ordinary differential equation system are shown in Ref. 13. Fig. 1 shows a diagram of the main immune mechanisms captured in the two-compartment mathematical model.

The model describes macrophage recruitment, infection, activation and death (by age, apoptosis, bursting, and cytotoxic T cell killing). DC mechanisms captured in the model are recruitment, maturation, trafficking to the lymph node, and death (by age). DCs become mature after bacterial uptake and they are the only cell types that migrate to the lymph node upon maturation. We also describe mechanisms of T cell recruitment, differentiation, proliferation, migration, and death (by apoptosis and age), both in the lung and lymph node. To exactly replicate experimental conditions, we use data on CFU dynamics as input functions for the model in the lung and lymph node. Details on how the input functions are calculated and implemented are shown in Ref. 13.

**Uncertainty and sensitivity analysis**

To further investigate the impact of inocula dose on priming dynamics, we performed uncertainty and sensitivity analyses (US/A) on simulations generated after varying the dose of infection. This method allows us to determine the amount of variability in the system and also which mechanisms predominately induce it. More generally, we seek to identify immune mechanisms that affect a change in priming and/or effector cell numbers in lung and lymph node during infection with *M. tuberculosis*. Uncertainty analysis is performed by an extensive and efficient statistical approach. The number of parameters is 30, and the model is solved for each. For the purposes of the analysis, we label all the effector T cells in the lung as primed T cells and all the effector T cells in the lung as effector T cells. The results are grouped by T cell phenotype and physiological compartment (lung or lymph node) and are all shown in
Tables II and III. We also looked at what mechanisms can impact total cell numbers in the lung.

Results

Higher inocula results in higher bacterial numbers in lymph node and lung but higher cell numbers only in the lungs

C57BL/6 mice were infected via aerosol with M. tuberculosis (Erdman strain) (Fig. 1). Inoculum dose was modulated by the concentration of bacteria placed in the nebulizer; exposure time in the aerosolizer was kept constant. In this experiment, one group was exposed to a low dose of \(2.7 \times 10^6\) CFU and the other group a high dose of \(2.4 \times 10^7\) CFU/ml. At day 1, four mice per group were harvested to determine actual dose of bacteria delivered to the lung. The low-dose mice received an average of 20 ± 5 CFU, and the high-dose mice received an average of 555 ± 150 CFU in the lungs. Mice were harvested at early time points (four mice per group at days 9, 14, 18, 22, 25, and 28 p.i.). Mice infected with the higher inoculum had significantly higher bacterial burdens at 14 and 28 d in the lymph nodes (Fig. 2A). In the lungs, bacterial numbers were 10-fold higher in the group with the higher inoculum at every time point sampled through 28 d (Fig. 2A). In a separate experiment, mice were followed to 14 wk and those infected with a higher inoculum maintained significantly higher bacterial burdens in the lungs (data not shown).

Total cell numbers upon homogenization were counted in the lung and lymph node using trypan blue exclusion; most of the living cells were lymphocytes or of monocytic lineage. No difference in total cell numbers was seen in the lymph nodes between the mice inoculated with low- or high-dose M. tuberculosis, but after 3 wk, there were significantly more cells in lungs of mice inoculated with the higher dose. (Fig. 2B) These findings were consistent across six separate experiments with different inocula, with low dose defined as <130 CFU and high dose as ≥130 CFU; matched low- and high-dose groups were inoculated with ~10-fold differences in CFU.

IFN-γ production in lungs, but not lymph node, is associated with inoculation dose

M. tuberculosis infection results in the presence of IFN-γ–producing T cells in the lung by 3–4 wk p.i. in mice (7). If an inoculation dose was a factor in priming immune responses to M. tuberculosis, we would expect an increase in IFN-γ–producing T cells in lymph nodes in mice that received a higher inoculum, because more bacteria (and therefore Ag) should increase priming and cytokine-producing T cells. This should then cause a subsequent increase in IFN-γ–producing cells in the lungs. By IFN-γ ELISPOT, lymph node cells from mice infected with a low or high dose had similar frequencies of M. tuberculosis–specific IFN-γ responses from T cells (Fig. 3). In contrast, in high-dose infected mice, there was a higher frequency of IFN-γ–producing T cells in lungs between 3 and 14 wk p.i. (Fig. 3), compared with low-dose infected mice. For this assay, we stimulated lung and lymph node cells with either M. tuberculosis–infected DCs (Fig. 3A) or DCs pulsed with ESAT-6 peptides (Fig. 3B). Although there were higher responses to the M. tuberculosis–infected DCs, the pattern was similar when ESAT-6 peptides were used.

ELISPOT relies on stimulation of T cells for 2 d and may provide additional signals for T cell differentiation and cytokine production ex vivo; thus, one may be measuring the potential for cells to produce cytokines, rather than the status of cytokine production in the tissue. To assess the T cell populations more directly, we used flow cytometry and fluorescent-tagged M. tuberculosis Ag–specific tetramers for T cells recognizing ESAT-6 and Rv0125 (i.e., GAP) (11) to characterize M. tuberculosis–specific CD4 and CD8 T cells (Fig. 4). Ki67 and CD69 were markers used to assess proliferation and activation, respectively (Figs. 5, 6). Intracellular cytokine staining to assess TNF, IFN-γ, and IL-2 production by specific T cells in response to ESAT-6 and Rv0125 was also performed (data not shown). Unexpectedly, no differences in the frequency of ESAT6+CD4 cells and GAP+CD8 cells in the lung or lymph node were observed between mice inoculated with high or low dose (Fig. 4A). In terms of actual numbers of ESAT-6+CD4 and GAP+CD8 T cells, there were no differences in the lymph node; in lungs, there were more of these specific T cells in the high-dose animals at days 22 and 25 (Fig. 4B), reflecting increased numbers of overall cells. No dose-dependent differences were observed in the functions (cytokines) of ESAT-6– or GAP–specific T cells in the lungs or lymph nodes by intracellular cytokine staining (data not shown). We also stimulated with anti-CD3/anti-CD28 Abs to assess total cells capable of producing cytokines and again found no differences between mice infected with high or low dose (data not shown) in terms of function of responding cells.

Proliferation of T cells is similar between high and low dose infection mice in lungs and lymph nodes

Although there appeared to be no dose-related response in terms of numbers of cells or priming in the lymph nodes, there were more cells, including T cells, in the lungs by 3 wk p.i. in the high-dose group compared with the low-dose group. There are at least three possibilities that could account for this increase: increased migration from lymph node to lung, increased proliferation of T cells in the lungs, or decreased cell death, all possibly related to Ag load.
We addressed these possibilities by integrating our math modeling and mouse experimental approaches (Table I). We tested whether increased proliferation could account for the increases using the mouse model, whereas we tested increased recruitment and decreased cell death with the mathematical model.

To assess proliferation in the lymph nodes and lungs, Ki67, a nuclear Ag expressed in actively cycling cells, but not in resting G0 cells (15), was measured on T cells by flow cytometry. ESAT6+ CD4 T cells expressing Ki67 in the lymph node peaked in the high-dose group at day 14 but not until day 18 in the low-dose group. (B) Total cell number in the lymph node and lung with of four mice per group at time point. This experiment was independently performed six times, and a representative experiment is shown here. Statistical significance was determined by the unpaired Student t test; *p < 0.05, **p < 0.01, ***p < 0.001. SD reported.

Activation of T cells is increased by inoculum dose in lungs but not lymph node

CD69 expression has been used as a marker for early activation. Previously published data using mice where large numbers of transgenic T cells were adoptively transferred suggested that a higher dose of infection caused a faster and more robust priming of T cells in the lymph nodes, based primarily on CD69 expression (6). However, in our unmanipulated mouse model, there was no dependence on inoculum dose in frequency of CD69+ CD4 T cells (Fig. 6A) or CD8 T cells (Fig. 6B), either in the total population or in M. tuberculosis–specific T cells (Fig. 6C, 6D) in the lymph node, except at day 25. However, the frequency of activated M. tuberculosis–specific T cells was higher than the activation of the total T cell population (Fig. 6C, 6D). In the lung, high-dose mice had significantly higher percentages of CD69+ CD4 T cells at day 25 (Fig. 6A) and of CD69+ CD8 T cells at days 14 and 25 (Fig. 6B) compared with low-dose infection mice. In the lung, the total number of activated CD4 T cells (ESAT6-specific and nonspecific) from the high-dose mice increased over time and are significantly higher than in low-dose mice at days 21 and 25 p.i. (Supplemental Fig. 1). The total number of activated CD8 T cells (GAP-specific and nonspecific) from the high-dose group also increases over time and is significantly higher at every time point than the low-dose group. There was no difference in the lymph node total number of lymph node CD69+ CD4 or CD8 T cells (both specific and nonspecific) at any time point examined (Supplemental Fig. 1). In our unmanipulated mouse model, we do not see a dose-dependent difference in priming of T cells based on CD69 expression.
Increased migration could explain higher cell numbers in lungs in the high-dose infection group

In an effort to explain the increased number of total cells measured experimentally in the lung during a high-dose scenario, we used our mathematical model to test whether increased inocula have a significant impact on cell death, T cell proliferation, or cell recruitment. We used a baseline trajectory that fits our experimental data and varied only the dose (as a forcing function). We grouped together all the mechanisms captured in the mathematical model that affect cell death and cell killing in the lung, namely TNF-induced apoptosis of macrophages and T cells, Fas/Fas ligand–induced apoptosis of macrophages, cytotoxic killing, and bursting of macrophages. Our analysis indicates that inoculum dose does have a marginal effect on cell death/killing in the lung. We predict that increased inocula result in increased, and not decreased, level of cell death/killing in the first 10 d. Our US/A shows a significant positive correlation between Ag dose and cell death/killing within the first 7–10 d.p.i. (PRCC ∼ 0.8; p < 0.001). The correlation becomes negative from day 10 until day 30 (PRCC ∼ −0.8; p < 0.001). The mathematical model also confirms our experimental finding that dose does not affect T cell proliferation in lung. In contrast, our US/A results support a dose-dependent recruitment driving higher cell numbers in the lung during infection. Both recruitment terms (i.e., TNF-dependent and TNF-independent recruitment) show a positive correlation to dose (PRCC ∼ 0.5; p < 0.001 in the first 10 d). Recruitment to the lung increases levels of macrophages and precursor and effector T cells (both CD4 and CD8) at higher bacterial doses. DC numbers are negatively affected by higher doses (PRCC ∼ −0.75; p < 0.001). Fig. 7A shows model simulations of total cell numbers in the lung during increased dose challenges (in line with the data of Fig. 2B). Fig. 7B apportions the
increased cell numbers to three different mechanisms, namely cell death/killing, cell recruitment and cell proliferation. Recruitment is by far the most important mechanism in driving cell numbers in the lung (as determined by comparing the total fluxes of cells to the lung because of either recruitment, killing/death, and proliferation; Fig. 7B), where cell death/killing and T cell proliferation are influencing total cell numbers in week 1 (death/killing ∼ 13%, mainly for macrophages) and weeks 2 and 3 p.i. (T cell proliferation ∼ 10%), respectively. Higher doses result in higher cell recruitment and death/killing in the first 10 d p.i., likely driving the higher total cell numbers in the lung later during infection.

No correlation between CFU and M. tuberculosis–specific T cells

We studied whether a correlation exists between bacterial numbers and numbers of M. tuberculosis–specific CD4 and CD8 T cells in the lymph nodes and lungs from individual mice, regardless of dose of infection or time point, to determine whether bacterial numbers were affecting T cell priming. There was no correlation with bacterial numbers in the lymph nodes of individual mice with either ESAT-6+ CD4 T cells (Fig. 8A) or GAP+ CD8 T cells (Fig. 8B) in those same mice, which does not support an influence of inoculum on priming of specific T cells. There was a very modest correlation in the lungs because of either recruitment, killing/death, and proliferation; cell recruitment and cell proliferation. Recruitment is by far the most important mechanism in driving cell numbers in the lung (as determined by comparing the total fluxes of cells to the lung because of either recruitment, killing/death, and proliferation; Fig. 7B), where cell death/killing and T cell proliferation are influencing total cell numbers in week 1 (death/killing ∼ 13%, mainly for macrophages) and weeks 2 and 3 p.i. (T cell proliferation ∼ 10%), respectively. Higher doses result in higher cell recruitment and death/killing in the first 10 d p.i., likely driving the higher total cell numbers in the lung later during infection.

Mathematical modeling can replicate adoptively transferred transgenic T cell experiments

We used our mathematical model (13) to address the differences in our experimental findings compared with published data on adoptively transferred transgenic T cells that show an effect of inoculum size on priming of T cells in the lymph node, either in terms of timing or magnitude. The goal was to recapitulate in silico those experiments where adoptively transferred transgenic T cells were injected into mice prior to challenge (with pools of up to 5 × 10^6 transgenic T cells). In those mouse experiments, inoculum dose did increase priming in lymph nodes (6, 10). To observe an effect of inoculation dose on priming (Fig. 7C, 7D), we had to increase in the model the rates at which CD4 and CD8 T cells were primed (a.k.a. the priming probabilities of both naive CD4 Ts [k_{14}; Fig. 7C] and naive CD8 Ts [k_{17}; Fig. 8D] in the model) by ∼10-fold from model baseline values (estimated in Ref. 13 to match experimental data from our unmanipulated mice). Thus, the mathematical model is able to recapitulate the adoptively transferred transgenic T cells experiments (6, 10), accommodating the effect of higher cognate frequencies of naive cells (e.g., ESAT-6+ CD4 T cells or GAP+ CD8 T cells) by increasing priming probabilities that eventually influence T cell priming in lymph nodes in a dose-dependent manner.

Modeling predicts a key role for IL-10 and DCs in T cell priming and in driving effector T cell numbers

We then used the tested mathematical model to explore additional factors that could affect T cell priming in the lymph node and effector T cell numbers in the lungs to help explain the increase of cells in the lungs by 3 wk p.i. in the high-dose infected mice compared with the low-dose infected mice, seen experimentally. Our uncertainty and sensitivity results are shown in detail in Tables II and III.

![Figure 6](http://jimmunol.org/Downloadedfrom/)

**FIGURE 6.** CD69 expression on T cells is not dependent on inoculum dose in lymph nodes. Lymph node or lung cells were gated on CD4 (A) or ESAT-6+ CD4 (C) T cells and CD8 (B) or GAP+ CD8 (D) T cells and assessed for CD69 expression. ●, low dose; ■, high dose. Graphs summarize the data of four mice per group where low dose (n = 20) received a nebulizer concentration of 3.3 × 10^7/ml and the high group (n = 20) 2.7 × 10^7/ml. The average day 1 CFU was 40 in the low-dose group and 550 in the high-dose group. Experiment was independently performed four times. Representative data are shown here. Statistics used, Mann–Whitney t test; *p < 0.05.

### Table I. T cell phenotypes described in the mathematical model

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Lung</th>
<th>Lymph Node</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>Precursor Th1 [T^L_1], Th1 [T^L_1]</td>
<td>Precursor Th1 [T^LN_1], Th1 [T^LN_2]</td>
</tr>
<tr>
<td></td>
<td>Precursor effector CD8 [T^L_8]</td>
<td>Precursor effector CD8 [T^LN_8]</td>
</tr>
<tr>
<td></td>
<td>IFN-γ-producing CD8 [T^L_8γ], CTL [T^L_C]</td>
<td>IFN-γ-producing CD8 [T^LN_8γ], CTL [T^LN_C]</td>
</tr>
</tbody>
</table>

List of all the lymphocyte phenotypes represented in the ordinary differential equations (ODEs) system capturing lung–lymph node dynamics as described in Ref. 13. Measure units are number of cells in the whole organ (lung or lymph node). The variable labels are shown in square brackets to help the reader in cross-referencing all the model details illustrated in Ref. 13.
Modeling analyses of our two-compartment model predict two mechanisms that have a strong impact on T cell priming (in lymph node) and on effector T cell numbers (in lung), regardless of Ag dose and T cell type (CD4 or CD8). The first mechanism predicted by the model is the rate of bacterial uptake by, and maturation of, IDCs in the lung (k12; Table II, positive correlation). The second mechanism predicted is the rate at which resident/resting macrophages differentiate into alternatively activated macrophages in the lymph node (k7a; Table II, negative correlation), although the latter affects primarily an increase in CD8 T cells. Both mecha-

![FIGURE 7](image)

Mathematical model simulations of the effect of dose on total number of cells in the lung (A and B) and on T cell priming in the lymph node (C and D). The x-axes represent days postinfection and the y-axes cell counts. (A) Total cell numbers in the lung resulting from a range of CFU forcing functions, spanning from the low- to the high-dose time courses of Fig. 2A. (B) Mechanisms contributing to total cell numbers in the lung. Each curve represents the percentage of a single mechanism over the sum of all three. (C) CD4 effector T cell dynamics, after increasing CD4 T cell priming probability (k14) from the baseline value 1e−4 up to 1e−3. (D) CD8 effector T cell dynamics, after increasing CD8 T cell priming probability (k17) from the baseline value 1e−3 up to 1e−2 (see Ref. 18 for details on model equations and parameter values).

![FIGURE 8](image)

There is no correlation between CFU and *M. tuberculosis*-specific T cells in the lymph nodes or lungs. Correlation was measured by plotting the linear regression of CFU versus number of ESAT-6+ CD4 (A) T cells or GAP+ CD8 (B) T cells in the LN and lung. $R^2$ values are lower in the lymph node than the lung. There does not seem to be a difference in priming in the lymph node as seen by *M. tuberculosis*-specific CD4 and CD8 T cells; this does not support the hypothesis that priming in the lymph node is dose dependent. Lines on the graphs are fitted by linear regression. $R^2$ represents the goodness of fit, and $p$ value represents the significance of slope from non-zero.
Higher rates of IDC uptake/maturation increase Ag-bearing DC (MDC) numbers in the lung and induce higher rates of migration of MDCs to the lymph node. In contrast, a higher rate of AAM activation in the lymph node (k9) likely increases IL-10 concentrations (AAMs are a major producer of IL-10). More IL-10 in the lymph node has a dual negative impact on priming: the first effect is direct by reducing the proliferation of CD4 and CD8 precursor effector T cells (namely, precursor Th1 and precursor CTLs) (17), whereas the second one is indirect by decreasing/delaying DC maturation (as shown in Refs. 16 and 17).

Overall, the model predicts that IL-10 plays a key regulatory role in priming during a high Ag dose scenario. This is consistently observed for both CD4 and CD8 T cell priming in the lymph node, as well as for effector CD4 and CD8 T cell numbers in the lung. Two IL-10–related mechanisms are significantly affecting priming and effector T cell numbers (Table III): 1) regulation of DC maturation rates in the lung (i.e., half-saturation of IL-10 concentration on DC maturation in the lung, hsI10-DC) and 2) regulation of precursor Th1 proliferation rates in the lymph node (i.e., half-saturation of IL-10 concentration on precursor Th1 proliferation in the lymph node, hsI10-T0LN). By decreasing regulation rates of DC maturation or precursor Th1 proliferation, a negative impact on priming occurs for both CD4 and CD8 T cells in lymph nodes, as well as on effector T cell numbers in lung (Fig. 7C, Table III). We postulate that this occurs primarily in a high-dose inoculation scenario, because more initial uptake by and subsequent infection of AAMs occurs. Increased AAM infection results in a further boost toward an IL-10–dominated environment, which slows down DC maturation as well as T cell proliferation. Alternatively, IL-10 related mechanisms do not affect priming and effector T cell numbers in a low-dose scenario. At low Ag doses (Table III), only CD8+ T cells are affected, and the mechanisms predicted as significant are in the lymph node and pertain to the efficiency of CD4 T cell priming by an MDC (k14) and the rate of

Table II. Sensitivity analysis results varying Ag dose

<table>
<thead>
<tr>
<th>Output</th>
<th>Positive Correlation</th>
<th>Negative Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 in the lymph node</td>
<td>1) Max infection/uptake rate by IDCs in the lung [k12]</td>
<td>1) Resting M0alternative activation rate (LN) [k2a]</td>
</tr>
<tr>
<td></td>
<td>2) Max TNF-dependent IDC recruitment rate [rc4]</td>
<td>2) Precursor Th1 cells migration rate out of the LN into the blood [ε1]</td>
</tr>
<tr>
<td></td>
<td>3) Rate (likelihood) of naive CD4+ T cell priming when encountering a MDC (LN) [k1a]</td>
<td>3) Max rate of TNF-dependent apoptosis of Th1 cells (LN) [k22a]</td>
</tr>
<tr>
<td></td>
<td>4) Precursor Th1 proliferation rate (LN) [k13]</td>
<td>4) Max Th1 differentiation rate, IL-12 and MΦ dependent (LN) [k26a]</td>
</tr>
<tr>
<td>CD8 in the lymph node</td>
<td>1) Max IDC infection/uptake rate in the lung [k12]</td>
<td>1) MΦ alternative activation rate (LN) [k2a]</td>
</tr>
<tr>
<td></td>
<td>2) Max TNF-dependent IDC recruitment rate [rc3]</td>
<td>2) Max T8/CTL differentiation rate, IL-12/MDC dependent (LN) [k3a]</td>
</tr>
<tr>
<td></td>
<td>3) Max rate (likelihood) of naive CD8+ T cell priming when encountering a MDC (LN) [k1a]</td>
<td>3) Max rate of TNF-dependent apoptosis of CTL cells (LN) [k22a]</td>
</tr>
<tr>
<td></td>
<td>4) Precursor T8/CTL proliferation rate (LN) [k18]</td>
<td>4) Max rate of TNF-dependent apoptosis of T8 cells (LN) [k26a]</td>
</tr>
<tr>
<td>CD4 in the lung</td>
<td>1) Max IDC infection/uptake rate in the lung [k12]</td>
<td>1) Maximum Th1 differentiation rate, IL-12/MΦs dependent (LN) [k23a]</td>
</tr>
<tr>
<td></td>
<td>2) Precursor Th1 proliferation rate (LN) [k15]</td>
<td>2) Max Th1 differentiation rate, IL-12/MDC dependent (LN) [k23a]</td>
</tr>
<tr>
<td></td>
<td>3) Rate (likelihood) of naive CD4+ T cell priming when encountering a MDC (LN) [k1a]</td>
<td>3) Max rate of TNF-dependent apoptosis of Th1 cells (LN) [k22a]</td>
</tr>
<tr>
<td></td>
<td>4) Scaling factor between lung and LN compartments [Υ]</td>
<td>4) Max CD8+ T cells max MDC-dependent recruitment rate (LN) [k26a]</td>
</tr>
<tr>
<td></td>
<td>5) Max TNF-independent precursor Th1 recruitment rate (Lung) [rc5]</td>
<td>5) Naive CD8+ T cells max MDC-dependent recruitment rate (LN) [k26a]</td>
</tr>
<tr>
<td></td>
<td>6) Precursor Th1 cells migration rate out of the LN into the blood [ε1]</td>
<td>6) Max TNF-independent Th1 recruitment rate (Lung) [rc6a]</td>
</tr>
<tr>
<td></td>
<td>7) Max TNF-independent Th1 recruitment rate (lung) [rc8a]</td>
<td>7) Max TNF-dependent Th1 recruitment rate (lung) [rc6a]</td>
</tr>
<tr>
<td></td>
<td>8) Max TNF-dependent Th1 recruitment rate (lung) [rc6a]</td>
<td></td>
</tr>
<tr>
<td>CD8 in the lung</td>
<td>1) Max IDC infection/uptake rate in the lung [k12]</td>
<td>1) MΦ alternative activation rate (LN) [k2a]</td>
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<td></td>
<td>2) Max TNF-independent IDC recruitment rate [rc3]</td>
<td>2) Max T8/CTL differentiation rate, IL-12/MDC dependent (LN) [k3a]</td>
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<td></td>
<td>3) Max rate (likelihood) of naive CD8+ T cell priming when encountering a MDC (LN) [k1a]</td>
<td>3) Max rate of TNF-dependent apoptosis of T8 cells (LN) [k26a]</td>
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<tr>
<td></td>
<td>4) Naive CD8+ T cells max MDC-dependent recruitment rate (LN) [k26a]</td>
<td>4) Max TNF-dependent apoptosis of T8 cells (LN) [k26a]</td>
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<td></td>
<td>5) Precursor T8/CTL proliferation rate (LN) [k18]</td>
<td>5) Max TNF-dependent apoptosis of T8 cells (LN) [k26a]</td>
</tr>
<tr>
<td></td>
<td>6) Max TNF-independent precursor T8/CTL recruitment rate (lung) [rc7]</td>
<td>6) Resting MΦ alternative activation rate (LN) [k2a]</td>
</tr>
<tr>
<td></td>
<td>7) Scaling factor between lung and LN compartments [Υ]</td>
<td>7) Max T8/CTL differentiation rate, IL-12/MΦs dependent (LN) [k3a]</td>
</tr>
<tr>
<td></td>
<td>8) Precursor T8/CTL cells migration rate out of the LN into the blood [ε1]</td>
<td>8) Maximum Th1 differentiation rate, IL-12/MΦs dependent (LN) [k23a]</td>
</tr>
</tbody>
</table>

Most important mechanisms affecting priming (CD4s and CD8+ Ts) that resulted consistently significant (p < 0.001) between high- and low-dose LHS experiments. Only either PRCCs > 0.15 or PRCCs < −0.15 are shown. The parameter labels in Tables II and III are shown in square brackets to help the reader in cross-referencing all the model details illustrated in Ref. 13.

*Significantly only in the first 10 d p.i.  
LN, Lymph node; MΦ, macrophage; Max, maximum; MΦ, resting macrophage.
bacterial uptake by immature DCs and consequent DCs maturation in the lymph node (k_{12a}).

Overall, IL-10 concentration is positively correlated with dose. AAM development is initially positively correlated with dose, then at approximately day 35–40, the correlation becomes increasingly negative on a very fast time scale (data not shown). This suggests that day 35 could be the switching time for an AMM–dominated to a classically activated macrophage–dominated lung environment (18).

**Discussion**

Priming of T cells and migration of those cells to the site of *M. tuberculosis* infection is crucial for preventing primary tuberculosis. Previous studies in mice indicated that the numbers of bacilli infecting a host could influence the strength or timing of initiation of the T cell response against this pathogen (4, 6, 9, 10). In this study, we sought to address this issue in an unmanipulated mouse model, to determine whether inoculum dose could affect priming of T cell responses in lymph nodes in the presence of the normal but low frequency of naive precursor T cells. Our data support that day 35 could be the switching time for an AMM–dominated to a classically activated macrophage–dominated lung environment (18).

We also used the mathematical model to address why our results differ from previously published studies (6, 10). It was reported that increased inocula led to increased T cell priming in lymph nodes. However, these studies were performed by adoptively transferring large numbers of naive *M. tuberculosis*–specific transgenic CD4 T cells prior to infection, and analysis of transferred T cells. In contrast, our studies were performed by examining native populations of T cells that were induced upon infection. Transfer of transgenic T cells greatly increases (10,000–1,000,000-fold) the precursor frequency of CD4 T cells. Although this certainly makes it easier to detect specific responses using flow cytometry, it may also skew the responses seen. To address whether increasing the precursor frequency could lead to an effect of inoculum dose on priming, we used our mathematical model. This model validation step was designed to show the power of our in silico representation recapitulating the transgenic T cells transfer experiment described in (6, 10). We showed how the model can capture a dose-dependent impact on T cell priming by increasing the priming probabilities of both naive CD4 T cells (k_{14}) and naive CD8 (k_{17}) Ts: these probabilities are proxies for many mechanisms related to Ag presentation and priming, one of which is cognate frequency. Thus, higher priming probabilities captures a scenario where transgenic (“cognate”) T cells are present in the system and the mathematical model successfully reproduced the dose-dependent transgenic T cells transfer experiment results, where priming becomes sensitive to Ag dose.

In summary, our data support that although inoculum dose of *M. tuberculosis* does not substantially influence T cell priming in the

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**Table III. Sensitivity analysis results fixing Ag dose**

<table>
<thead>
<tr>
<th>Name</th>
<th>Low Dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 in the lymph node</td>
<td>1) Max IDC infection/uptake rate in the LN (+) [k_{12a}]</td>
<td>1) IL-10 half saturation on delaying IDC maturation (lung) [hsI10-DC] (+)</td>
</tr>
<tr>
<td>CD8 in the lymph node</td>
<td>2) Rate (likelihood) of Naive CD4+ T cell priming when encountering a MDC (LN) [k_{14}] (+)</td>
<td>2) IL-10 half saturation on delaying precursor Th1 proliferation (LN) [hsI10-T0LN] (+)</td>
</tr>
<tr>
<td>CD4 in the lung</td>
<td>3) Scaling factor between lung and LN compartments [Y] (+)</td>
<td>3) MDC migration rate from the lung to the LN [p] (+)</td>
</tr>
<tr>
<td>CD8 in the lung</td>
<td>1) Max IDC infection/uptake rate in the LN (+) [k_{12a}]</td>
<td>1) IL-10 half saturation on delaying IDC maturation (lung) [hsI10-DC] (+)</td>
</tr>
</tbody>
</table>

Important mechanisms affecting priming (CD4s and CD8’ Ts) that resulted uniquely significant (p < 0.001) for either high- or low-dose LHS experiments. Only either PRCCs > 0.15 or PRCCs < −0.15 are shown. The sign of the correlation is in parentheses.

*Significant only in the first 10 d p.i.*

LN, Lymph node; Max, maximum.
lymph nodes, it does affect cell numbers and bacterial burden in the lungs. It is possible that in a vaccinated host, a higher dose challenge may more quickly recall T cell responses to the lungs, because in that setting priming of new T cells is less important. Thus, in vaccine challenge models, it is important to replicate a low-dose challenge, since humans are likely exposed to low levels of *M. tuberculosis* (often repeatedly), rather than large inocula.

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**Disclosures**

The authors have no financial conflicts of interest.

**References**


