Competition for antigen between Th1 and Th2 responses determines the timing of the immune response switch during *Mycobacterium avium* subspecies *paratuberculosis* infection in ruminants

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**Supplemental Information**

**Model analysis**

Starting with non-negative initial conditions the structure of the model gives non-negative solutions. The model disease free state (DFE), \( E_0 \) is given by

\[
E_0 = (\hat{M}_\phi, \hat{I}_m, \hat{B}, \hat{T}_{h_0}, \hat{T}_{h_1}, \hat{T}_{h_2}) = \left( \frac{\sigma_m}{\mu_m}, 0, 0, \frac{\sigma_0}{\mu_0}, 0, 0 \right). 
\]

The model disease equilibrium state (DEE), \( E_1 \) (we could not express the DEE in a closed form) is given by

\[
E_1 = (\bar{M}_\phi, \bar{I}_m, \bar{B}, \bar{T}_{h_0}, \bar{T}_{h_1}, \bar{T}_{h_2}). 
\]

**Computation of the basic model disease reproduction number \((R_0)\)**

The basic model disease reproduction number was obtained using the next generation method by Castillo-Chavez \textit{et al} \cite{1}. The basic model can be written in the form:

\[
\begin{align*}
\frac{dX}{dt} &= f(X,Y,Z), \\
\frac{dY}{dt} &= g(X,Y,Z), \\
\frac{dZ}{dt} &= h(X,Y,Z),
\end{align*}
\]

where \( X = (M_\phi,T_{h_0},T_{h_1},T_{h_2}) \in \mathbb{R}^4 \), \( Y = I_m \in \mathbb{R} \) and \( Z = B \in \mathbb{R} \) and \( h(X,0,0) = 0 \). \( X \) denote uninfected macrophages and T cell sub-types, \( Y \) denotes infected macrophages and \( Z \) denotes free
bacteria (the pathogen causing infection), therefore \( E_0 = (X^*, 0, 0) \). Assuming that \( \tilde{g}(X^*, Y, Z) = 0 \) implicitly determines a function \( Y = \tilde{g}(X^*, Y) \). Let \( A = D_Z h(X^*, \tilde{g}(X^*, 0), 0) \) and further assume that \( A \) can be written in the form \( A = M - D \), with \( M \geq 0 \) (that is \( m_{ij} \geq 0 \)) and \( D > 0 \), a diagonal matrix. Then, the basic model disease reproduction number is defined as the spectral radius (dominant eigenvalue) of the matrix \( MD^{-1} \), that is \( R_0 = \rho(MD^{-1}) \).

Now, with \( X = (M\phi, T_{h_1}, T_{h_2}) \), \( Y = I_m \), and \( Z = B \), we evaluate that
\[
g(X^*, Y) = \frac{k_i M\phi B}{(k_b + k_i T_{h_1} + \mu_I)} \quad \text{and} \quad h(X^*, \tilde{g}(X^*, Y), Z) = \frac{N_o k_b k_i M\phi B}{(k_b + k_i T_{h_1} + \mu_I)} B(k_i M\phi + k_m M\phi + \mu_B).
\]

Therefore, \( A = D_Z h(X^*, \tilde{g}(X^*, 0), 0) = \frac{N_o k_b k_i M\phi}{(k_b + \mu_I)} - (k_i M\phi + k_m M\phi + \mu_B) \). Hence \( M = \frac{N_o k_b k_i M\phi}{(k_b + \mu_I)} . \)

\[
D = (k_i M\phi + k_m M\phi + \mu_B) \quad \text{and} \quad R_0 = \frac{k_b k_i M\phi N_o}{(k_i M\phi + k_m M\phi + \mu_B)(k_b + \mu_I)} \quad \text{or} \quad R_0 = \frac{k_b k_i M\phi N_o}{(k_i M\phi + k_m M\phi + \mu_B)(k_b + \mu_I)} = \frac{(k_i M\phi + k_m M\phi + \mu_B)(k_b + \mu_I)}{k_b k_i M\phi N_o} . \]

(S.1)

**Theorem 1.** \( E_0 \) is locally asymptotically stable if \( R_0 < 1 \) and unstable if \( R_0 > 1 \).

**Proof of local stability of \( E_0 \):**

The Jacobian matrix of the system of equations (1)-(6) (which represent the basic model) evaluated at the DFE is
\[
J = \begin{bmatrix}
-\mu_m & 0 & -k_i M\phi & 0 & 0 & 0 \\
0 & -(k_b + \mu_I) & 0 & 0 & 0 & 0 \\
0 & 0 & -(k_i M\phi + k_m M\phi + \mu_B) & 0 & 0 & 0 \\
0 & -\delta_m T_{h_0} & -\delta_B T_{h_0} & -\mu_0 & 0 & 0 \\
0 & \theta_1 \delta_m T_{h_0} & 0 & 0 & -\mu_1 & 0 \\
0 & 0 & \theta_2 \delta_B T_{h_0} & 0 & 0 & -\mu_2
\end{bmatrix}.
\]

The eigenvalues of the Jacobian matrix, \( J \), can be determined by solving the characteristic equation \( |J - \lambda I| = 0 \), which yeilds the following eigenvalues \( \lambda_1 = -\mu_m, \lambda_2 = -\mu_0, \lambda_3 = -\mu_1, \lambda_4 = -\mu_2 \), and the polynomial
\[
\lambda^2 + \lambda \left((k_b + \mu_I) + (k_i M\phi + k_m M\phi + \mu_B)\right) + (k_b + \mu_I)(k_i M\phi + k_m M\phi + \mu_B) - k_i M\phi k_b N_o = 0 \quad (S.2)
\]

With \( \lambda_{1,2,3,4} < 0 \), to complete the proof for local stability we apply the Routh Hurwitz criterion, which requires that, in polynomial equation (S.2)

(i) \((k_b + \mu_I) + (k_i M\phi + k_m M\phi + \mu_B) > 0, \)

(ii) \((k_b + \mu_I)(k_i M\phi + k_m M\phi + \mu_B) - k_i M\phi k_b N_o > 0. \)

These conditions are satisfied if \((k_b + \mu_I)(k_i M\phi + k_m M\phi + \mu_B) > k_i M\phi k_b N_o \), that is if
\[
1 > \frac{k_b k_i M\phi N_o}{(k_i M\phi + k_m M\phi + \mu_B)(k_b + \mu_I)},
\]

hence \( 1 > R_0, \) therefore \( R_0 < 1 \). Thus, the DFE is locally asymptotically stable \( \square \)
Global stability of $E_0$

Using the method by Castillo-Chavez et al [1] to establish the global stability of the DFE.

Set $X = (M_\phi, T_{h0}, T_{h1}, T_{h2})$ and $Z = (I_m, B)$. The model equations are rewritten as follows:

$$
\dot{X} = F(X, 0),
\dot{Z} = G(X, Z).
$$

**Theorem 2.** The DFE is globally asymptotically stable when $R_0 < 1$, if (i) $E_0 = (X^*, 0)$ is locally asymptotically stable and (ii) $\dot{G}(X, Z) = AZ - G(X, Z) \geq 0$ in the biological feasible region, where $A = D_Z(X^*, 0)$ is the Jacobian of $G(X, Z)$ evaluated at $(X^*, 0)$.

**Proof of global stability of $E_0$:**

Therefore, Condition (ii)

$$
F(X, 0) = \begin{bmatrix}
\sigma_m - \mu_m M_\phi \\
\sigma_0 - \mu_m T_{h0} \\
0 \\
0
\end{bmatrix}, \quad \text{and} \quad \dot{G}(X, Z) = \begin{bmatrix}
k_0 T_{h1} \\
0
\end{bmatrix} \geq 0.
$$

Therefore, $\dot{G}(X, Z) \geq 0$. Condition (i) follows from Theorem 1. Thus, the DFE is globally asymptotically stable $\blacksquare$

**Derivation of the Th1/Th2 ratio equation**

We define the ratio, $R$, to be given by $\frac{T_{h1}}{T_{h2}} (R = \frac{T_{h1}}{T_{h2}})$. Differentiating $R$ with respect to time gives

$$
\frac{dR}{dt} = \left( \frac{1}{T_{h2}} \right) \dot{T}_{h1} - \left( \frac{T_{h1}}{T_{h2}^2} \right) \dot{T}_{h2}.
$$

Substituting $\dot{T}_{h1}$ and $\dot{T}_{h2}$ with the differential equation representing the time kinetics of Th1 cells and Th2 cells, respectively, gives

$$
\frac{dR}{dt} = \left( \frac{1}{T_{h2}} \right) \left( \dot{T}_{h1} - \frac{T_{h1}}{T_{h2}} \dot{T}_{h2} \right),
$$

$$
= \frac{1}{T_{h2}} \left( \theta_1 \delta_m I_m T_{h0} - \mu_1 T_{h1} \right) - \frac{T_{h1}}{T_{h2}^2} \left( \theta_2 \delta_B T_{h0} - \mu_2 T_{h2} \right),
$$

$$
= \theta_1 \delta_m I_m \left( \frac{T_{h0}}{T_{h2}} \right) - \mu_1 R - R \theta_2 \delta_B B \left( \frac{T_{h0}}{T_{h2}} \right) + R \mu_2,
$$

$$
= (\theta_1 \delta_m I_m - \theta_2 \delta_B B R) \left( \frac{T_{h0}}{T_{h2}} \right) - R(\mu_1 - \mu_2). \quad (S.3)
$$

**Sensitivity analysis of the immune response parameters**

Additional insights into the dynamics of the basic mathematical model can be obtained from the basic model disease reproduction number, $R_0$ (expression S.1), and carrying out sensitivity analysis. The value of $R_0$ determines whether infection will persist or is cleared. We find that the rate of infection of macrophages by bacteria ($k_1$), bursting rate of infected macrophages ($k_0$) and the
amount of bacteria released in the extracellular environment \((N_o)\) are the main factors that define kinetics of disease progression during MAP infection. The parameter \(k_m\) which models the killing of bacteria by macrophages prevent infection progression. This result can easily be derived through sensitivity analysis of \(R_0\) with respect to these parameters, \(\frac{X_i \partial R_0}{R_0 \partial X_i}\), where \(X_i\) are the parameters in the \(R_0\) expression [2]. A positive normalised derivative indicates that increasing the value of the corresponding parameters will increase disease progression, while a negative value implies suppression of infection progression.

Sensitivity analysis for infection \((k_i, k_b, \mu_B)\) and immune \((k_m, k_i, \theta_1, \theta_2, \delta_m, \delta_B, \mu_1, \mu_2)\) parameters was carried out using the LHS method [3] at the time when the ratio of Th1/Th2 response reaches 1 as the output variable (Figure S1). Sensitivity analysis identified parameters that contribute the most to the timing of the Th1/Th2 switch including the decay rates of Th1 and Th2 cells \((\mu_1\) and \(\mu_2)\), rates at which Th0 cells differentiate into either Th1 or Th2 cells \((\delta_m\) and \(\delta_B)\), clonal expansion factors \((\theta_1\) and \(\theta_2)\), and rate of bursting of infected macrophages, \(k_b\).

Using the equation for \(R\) (S.3), sensitivity analysis was carried out to determine parameters that have significant influence to the Th1 to Th2 switch (Figure S1).

**Sensitivity Analysis of \(R_0\)**

Sensitivity indices for the basic model disease reproduction number, \(R_0\), were evaluated. Sensitivity indices allows us to measure the relative change in \(R_0\) with respect to its parameters. The normalised forward sensitivity of a variable to a parameter is the ratio of the relative change in the variable to the relative change in the parameter [2]. The normalised forward sensitivity index of a variable, \(u\), that depends differentiably on a parameter, \(p\), is defined as

\[
T_u^p := \frac{\partial u}{\partial p} \times \frac{p}{u}.
\]

Therefore, we can derive analytical expressions of the sensitivity indices of \(R_0\) to be given by

\[
T_{R_0}^{X_i} = \frac{\partial R_0}{\partial X_i} \times \frac{X_i}{R_0},
\]

where \(X_i\) are the eight parameters in the expression of \(R_0\) and are evaluated to be

\[
\begin{align*}
T_{N_o}^{R_0} &= 1, & T_{k_b}^{R_0} &= \frac{\mu_i}{k_b + \mu_i}, \\
T_{k_m}^{R_0} &= -\frac{\mu_i}{k_b + \mu_i}, & T_{k_m}^{R_0} &= \frac{\sigma_m k_m + \mu_m \mu_B}{\sigma_m (k_i + k_m) + \mu_m \mu_B}, \\
T_{k_B}^{R_0} &= -\frac{\mu_B \mu_m}{\sigma_m (k_i + k_m) + \mu_m \mu_B}, & T_{k_m}^{R_0} &= \frac{-\sigma_m k_m}{\sigma_m (k_i + k_m) + \mu_m \mu_B}, \\
T_{\theta_1}^{R_0} &= \frac{-\mu_B \mu_m}{\sigma_m (k_i + k_m) + \mu_m \mu_B}, & T_{\theta_2}^{R_0} &= \frac{\mu_B \mu_m}{\sigma_m (k_i + k_m) + \mu_m \mu_B}, \\
T_{\delta_m}^{R_0} &= \frac{-\mu_B \mu_m}{\sigma_m (k_i + k_m) + \mu_m \mu_B}, & T_{\delta_B}^{R_0} &= \frac{\mu_B \mu_m}{\sigma_m (k_i + k_m) + \mu_m \mu_B}.
\end{align*}
\]

(S.4)
Table S1: Sensitivity indices for $R_0$. The parameters are ranked from the most sensitivity to the least. Increasing the value of a parameter with a positive index results in the increase of the $R_0$ value, hence infection progression, while increasing the numeric value of a parameter with a negative index will reduce infection progression. Parameters values used to calculate the sensitivity indices are given in Table 1.

$$N_0, k_b, k_i$$ are the most sensitivity parameters that favour infection progression. Since $\frac{I_{N_0}}{R_0} = 1$, therefore increasing (or decreasing) $N_0$ by 10% will increase (or decrease) $R_0$ by 10%, while increasing $k_b$ by 10% will increase $R_0$ by 9.6%.

Alternative models

The basic mathematical model of the MAP infection predicts that the classical Th1 to Th2 switch occurs late in infection when the rate of removal of extracellular bacteria is relatively slow, the burst size $N_0$ is small ($N_0 \approx 100$), and the decay rates of the Th1/Th2 responses are similar ($\mu_1 \approx \mu_2$). Under these circumstances there is a slow build-up of bacteria in the host. Here we investigate how additional immunological mechanisms may influence the timing of the Th1/Th2 switch if extracellular bacteria are short-lived. These mechanisms include inhibition of the Th1 cell differentiation by Th2 effectors, proliferation of effector T cells at the site of infection, and functional exhaustion of MAP-specific Th1 responses (Figure S2).

Differentiation cross inhibition and Th1/Th2 switch

There is strong evidence that cytokines produced by Th2 effectors skew differentiation of Th0 cells towards Th2 phenotype and suppress differentiation of cells into Th1 effectors (and vice versa) [4–7]. It is therefore possible that the switch from Th1 to Th2 response during MAP infection is due to suppression of the initially dominant Th1 response by Th2 effectors. To investigate this hypothesis we modified the terms for the generation of Th1 and Th2 subsets in Eqns. (4)-6 to 

$$\frac{\delta_m I_m T_{h_0}}{1 + h_2 T_{h_2}} \text{ and } \frac{\delta_B B T_{h_0}}{1 + h_1 T_{h_1}}$$,

respectively, where $h_1$ and $h_2$ are inhibition constants. Interestingly, under conditions of a rapid removal of bacteria from extracellular environment when the classical switch is not observed (Figure 3B), inhibition of Th0 cell differentiation into Th1 subset by Th2 effectors allow for the loss of the protective Th1 response (Figure 3A). Increasing differentiation inhibition of Th1 response by Th2 effectors results in reduced Th1 cell population and increased growth of the Th2 subset of immune response. Reduced production of Th1 cells from differentiation will gradually weaken the protective immunity, which allows bacteria accumulation and disease progression. It should be noted, however, that if efficiency of suppression of Th2 cell differentiation by Th1 cells is high ($h_1$ is large) then the switch will not be observed even under the conditions of slow removal of extracellular.
bacteria (results not shown). Thus, influence of Th1 and Th2 responses on differentiation of naïve CD4 T cells into effectors has a large impact on the kinetics of the Th1/Th2 switch.

**Maintenance of committed effectors at the site of infection by proliferation**

In our basic mathematical model we assumed that MAP-specific effector T cell responses are maintained at the site of infection by continuous recruitment of differentiated cells to the site of infection. However, it is possible that on site proliferation of effector T cells may contribute to the size of the Th1 and Th2 responses [4,7-9]. To investigate whether effector T cell proliferation at the site of infection may influence the kinetics of the Th1/Th2 switch we extended the mathematical model. As in the case with differentiation of Th0 cells into Th1/Th2 effectors, we assume that proliferation of MAP-specific Th1 cells is mainly driven by the density of infected macrophages and that of Th2 cells by the density of extracellular bacteria. We thus add the following proliferation terms \( \frac{\alpha_1 I_m T_h_1}{I_m + T_1} \) and \( \frac{\alpha_2 B T_h_2}{B + T_2} \) to Eqns. (5) and (6) for Th1 and Th2 responses, respectively. Here \( \alpha_1 \) and \( \alpha_2 \) are the maximal rates of proliferation of Th1 and Th2 cells and \( T_1 \) and \( T_2 \) are half-saturation constants.

Proliferation of effector Th cells at the site of infection has a large impact on the kinetics of the switch, and sensitivity of the Th1 and Th2 subsets to the local antigen concentrations determined by the parameters \( T_1 \) and \( T_2 \), plays the major role. In the case of short-lived extracellular bacteria, if only minute amounts of free bacteria are sufficient to drive Th2 cell proliferation, the Th1/Th2 switch will occur (Figure S3B). On the other hand, if Th1 cell sensitivity for the antigen is low (low value of \( T_1 \)), the Th1/Th2 switch may not occur even if extracellular bacteria are long-lived (results not shown). Thus, the specific details of how antigen availability influence the rate of proliferation of MAP-specific CD4 T cells are important in determining the likelihood and kinetics of the Th1/Th2 switch.

**Exhaustion of the Th1 response**

In chronic infections, T cells may become dysfunctional or exhausted [10-12]. Exhaustion has been mainly documented for virus-specific CD8 T cell responses and has been thought to arise when immune cells receive persistent stimulation. Exhausted T cells often fail to produce cytokines upon recognition of pathogen-infected cells. While the mechanistic details of how exhaustion develops especially for antigen-specific CD4 T cells are not fully understood, this mechanism may explain why Th1 responses are lost over the course of MAP infection. We investigated if exhaustion of the MAP-specific Th1 response may be responsible for the Th1/Th2 switch and disease progression. To model cell exhaustion, we assume an additional death term in Eqn. (5) for Th1 cells, \( \nu T_h_1 F(I_m(t)) \), where \( F(I_m(t)) = \int_0^t I_m(\tau)d\tau \) [12,13]. The parameter, \( \nu \), is the rate of Th1 immune cells exhaustion and \( F(I_m) \) is the memory that is associated with accumulation of the number of times that a Th1 cell encounters an infected macrophage [13].

As expected, the possibility of exhaustion of MAP-specific Th1 response naturally leads to the loss of Th1 cells and, as the consequence, accumulation of ineffective Th2 response (Figure S3C). This occurs irrespectively of the death rate of extracellular bacteria suggesting that in this model the loss of protective Th1 response is the consequence of the disease progression in MAP-infected animals.
References


