Supplementary Material for "On the role of CD8 T cells in the control of persistent infections," by S.P. Stromberg and R. Antia

The modeling of complex systems typically involves reducing the number of free parameters in a model of the system, to a tractable quantity, while keeping the essential features of the phenomena under study (1). Ecological models frequently start with an assumption of functional forms such as the logistic term in Eq. 4 or the saturating function in Eq. 1 as these functional forms can arise in many ways. Here we present our derivation of these functional forms using more detailed models of the T cell, pathogen dynamics, and innate immunity, and derive the equations presented in the main text from them. The T cell subsection considers the integration of a within cell model for PD-1 expression in T cells, and the population dynamics of T cells, dendritic cells, and pathogen. The pathogen subsection considers a common model of free pathogen, uninfected target cell, and infected target cell and reduces it to the logistic equation used in the main text. The innate immunity model considers an alternate derivation of the logistic equation starting from an explicit innate immunity model, underscoring the ability of the logistic term to describe either resource limitations or innate immunity. These reductions are performed using quasi-static approximations. The quasi-static approximations become exact in the behavior of the steady states of the model, which much of the main text is focused on. The quasi-static approximations also yield a model in qualitative agreement with experimental results (2–4).

T cell Dynamics

Here we derive Eq. 1 from a mechanistic, population expression model, which combines within cell dynamics of chemical expression with population dynamics. In the full model, T cells are described by two variables, a binary variable denoting stimulated or unstimulated, and a continuous variable denoting PD-1 expression. We do not consider differentiation of T cells to memory cells as this plays an insignificant role in the dynamics of chronic infections.

In the unstimulated state, the within cell dynamics of PD-1 expression (A) are given by production rate α_1 and decay rate δ giving:

$$\frac{dA}{dt} = \alpha_1 - \delta A. \tag{1}$$

Once stimulated the production rate of PD-1 increases to $\alpha_2 > \alpha_1$, giving the within cell model:

$$\frac{dA}{dt} = \alpha_2 - \delta A. \tag{2}$$

Here production has increased instantaneously, but δ is slow, and reaching the equilibrium PD-1 expression level is gradual.

We model unstimulated T cells as not dividing. Stimulated T cells divide with rate $G(A)$, which is dependent on PD-1 expression, into two cells that are also in the stimulated state and the daughter cells inherit the PD-1 expression level of the parent. T cells in the full model transition from unstimulated to stimulated upon encounter with activated dendritic cells D^*. Dendritic cells transition from inactivated D, to activated D^*, by encounter with pathogen.

Thymic influx generates diversity. Unstimulated naive cells with low PD-1 expression enter the system while other cells may already be expressing high levels of PD-1. This diversity requires partial differential equation to model the continuum of PD-1 expression levels. This can be performed using standard techniques to integrate the within cell model equations above, with the population dynamics. This extension to a partial differential equation is typically performed using tools from structured population dynamics (5) which consist of constructing a non-conservative advection equation. This generates the following set of equations:

$$\frac{\partial \rho_1(A,t)}{\partial t} = -\frac{\partial}{\partial A}\left[(\alpha_1 - \delta A)\rho_1(A,t)\right] - d\rho_1(A,t)$$
$$- k_1 D^* \rho_1(A,t) + k_2 \rho_2(A,t) \tag{3}$$

$$\frac{\partial \rho_2(A,t)}{\partial t} = -\frac{\partial}{\partial A}\left[(\alpha_2 - \delta A)\rho_2(A,t)\right]$$
$$+ (G(A) - d)\,\rho_2(A,t)$$
$$+ k_1 D^* \rho_1(A,t) - k_2 \rho_2(A,t) \tag{4}$$

$$\frac{dD}{dt} = -k_3 PD + k_4 D^* \tag{5}$$

$$\frac{dD^*}{dt} = +k_3 PD - k_4 D^*, \tag{6}$$

where there is additionally an influx of cells at $\rho_2(0,t)$ (not shown), and the dynamics of the pathogen are presented separately below. This advection equation is analogous to the density of cells acting like a fluid, with flow rates given by Eq. 1 and 2. When pathogen P is introduced to the system, dendritic cells become activated and present antigen. T cells that are presented with antigen become stimulated, increasing production of PD-1, and

begin to divide. When pathogen is cleared from the system, T cells transition back to the unstimulated state and PD-1 expression decays back to the initial level. This relaxation of PD-1 expression is based on data showing that after an acute LCMV infection is cleared, PD-1 expression returns to the naive expression level (3).

We reduce this model, removing the binary variable, by considering the fractions of cells in the stimulated and unstimulated states. We further reduce it by a substitution of variables, considering PD-1 expression (A) as a measure of "exhaustion" (a) ranging from $a = 0$ for non-exhausted (low PD-1 expression) to $a = 1$ for completely exhausted (high PD-1 expression). The reduction from PD-1 expression to exhaustion removes two model parameters.

Dendritic cell activation is assumed rapid compared to the other processes in the system (T cell exhaustion evolves over 1-2 weeks for example). A quasi-static approximation for the dendritic cell dynamics, with total dendritic cells $D_0 = D + D^*$, yields:

$$D^* = \frac{k_3 D_0 P}{k_4 + k_3 P}.$$ (7)

We make a second quasi-static approximation noting that stimulation of T cells is rapid compared to the chemical expression dynamics of PD-1. This is inferred from the fact that the PD-1 expression profile in a persistent LCMV infection evolves slowly as a unimodal distribution (3). The opposite case, gradual activation with rapid PD-1 shifts, would give a shifting bimodal distribution. This quasi-static approximation coupled with the dendritic cell approximation gives us:

$$\rho_2(A, t) \approx \frac{k_1}{k_2} \frac{k_3 D_0 P}{k_4 + k_3 P} \rho_1(A, t)$$ (8)

$$= \frac{\mu P}{\phi + P} \rho(A, t)$$ (9)

$$\rho_1(A, t) \approx \left(1 - \frac{\mu P}{\phi + P}\right) \rho(A, t)$$ (10)

where we have introduced the total density of T cells $\rho(A, t) = \rho_1(A, t) + \rho_2(A, t)$ and defined the parameters:

$$\mu = \frac{k_1 D_0}{k_2 + k_1 D_0}$$ (11)

$$\phi = \frac{k_2 k_4}{k_3(k_2 + k_1 D_0)}.$$ (12)

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Eq. 9 and 10 describe the fraction of time T cells spend stimulated or unstimulated. In the model, the PD-1 timescale δ is much slower than the rates of T cells binding and unbinding antigen presenting cells. PD-1 expression therefore reflects the average amount of time spent in the stimulated state.

We now obtain a partial differential equation for the total T cell density $\rho(A, t)$ by summing Eq. 3 and 4 and using the approximations in Eq. 9 and 10:

$$
\begin{aligned}
\frac{\partial \rho(A, t)}{\partial t} &= -\frac{\partial}{\partial A}\left[\alpha_1 \rho_1(A, t) + \alpha_2 \rho_2(A, t) - \delta A \rho(A, t)\right] \\
&\quad - d\rho(A, t) + G(A)\rho_2(A, t) \qquad (13) \\
&= -\frac{\partial}{\partial A}\left[\left\{\alpha_1 - \delta A + (\alpha_2 - \alpha_1)\frac{\mu P}{\phi + P}\right\}\rho(A, t)\right] \\
&\quad + \left[G(A)\frac{\mu P}{\phi + P} - d\right]\rho(A, t). \qquad (14)
\end{aligned}
$$

This relationship is further simplified by the substitution of variables:

$$
a = \frac{\alpha_1 - \delta A}{\mu(\alpha_1 - \alpha_2)}. \qquad (15)
$$

and the replacement of the density $\rho(A, t)$ a function of PD-1 expression, with the density $U(a, t)$ a function of exhaustion level. These replacements yield the equation:

$$
\begin{aligned}
\frac{\partial U(a, t)}{\partial t} &= -\frac{\partial}{\partial a}\left[\delta\left(\frac{P}{\phi + P} - a\right)U(a, t)\right] \\
&\quad + \left[g(a)\frac{P}{\phi + P} - d\right]U(a, t), \qquad (16)
\end{aligned}
$$

where we have also defined $g(a) = \mu G(A)$. This equation implicitly contains the dynamics of pathogen uptake, activation of dendritic cells, and PD-1 expression. It illustrates why the proliferation term and the exhaustion term are expected to have similar functional dependence, both arising from stimulation of T cells by activated dendritic cells. While we have derived the saturating function for proliferation rate from a simple mechanistic model, the functional form has been empirically observed (4). The exhaustion dynamics of this model, i.e.:

$$
\frac{da}{dt} = \delta\left(\frac{P}{\phi + P} - a\right), \qquad (17)
$$

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are mathematically equivalent to the dynamics of exhaustion in previous models (6).

For division rate we choose a functional form which is simple yet captures observed T cell behavior for total numbers (2). The functional form $g(a) = s(1-a)$ satisfies this requirement yielding Eq. 1:

$$\frac{\partial U(a,t)}{\partial t} = -\frac{\partial}{\partial a}\left[\delta\left(\frac{P}{\phi+P}-a\right)U(a,t)\right]$$
$$+\left[s(1-a)\frac{P}{\phi+P}-d\right]U(a,t). \tag{18}$$

Below we discuss the implications of this choice of $g(a)$ for T cells under a high pathogen load.

No Influx and Constant Pathogen

To explore the functional form $g(a) = s(1-a)$, we study the behavior of the model for T cells under high pathogen load (i.e. $P \gg \phi$,) and no thymic influx. The condition that $P \gg \phi$ is satisfied when T cells have a constant proliferation rate while pathogen density changes over many orders of magnitude. This is observed for example in LCMV infection where peak viral density is two orders of magnitude larger than when T cells reach maximal growth rate (4). The lack of thymic influx may represent a thymectomized animal or distinct transplanted cells.

Without thymic influx there is no diversity. In this case the initial population, tightly peaked around $a = 0$, remains tightly peaked while changing in a. In this case we can rewrite 18 with an ODE for cells having internal variable $a(t)$, and a separate ODE for da/dt. In Eq. 18 exhaustion a was conceptually similar to a spatial variable where cells flowed toward higher values of a as they became exhausted. We now have a as a function of time $a(t)$. This approach was used in modeling persistent infections without thymic influx (6). In the absence of thymic influx and with high pathogen density, our T cell model is reduced to:

$$\frac{dX}{dt} = s(1-a)X - dX \tag{19}$$

$$\frac{da}{dt} = \delta - \delta a. \tag{20}$$

The solution to the a equation with initial condition $a(0) = 0$ is:

$$a(t) = 1 - e^{-\delta t}. \tag{21}$$

Which, when substituted into the equation for total T cell number, yields:

$$\frac{dX}{dt} = se^{-\delta t}X - dX. \tag{22}$$

This illustrates that high pathogen load yields an exponentially declining T cell proliferation rate in our model.

Pathogen Dynamics

The use of a logistic growth term is common in ecological models with resource limitations and models are often presented with these terms without derivation. Here we show how a common, more detailed model of viral dynamics, can be reduced to the equation used in the text.

We take as a starting point the most common form for an explicit target cell model (7–11):

$$\frac{dT}{dt} = \lambda - d_V T - k_V PT \tag{23}$$

$$\frac{dI}{dt} = k_V PT - \delta_V I \tag{24}$$

$$\frac{dP}{dt} = p_V I - c_V P \tag{25}$$

Where T is uninfected target cells, I is infected target cells, and P is free virus. This model does not include adaptive or innate immunity explicitly. This model assumes that innate immunity sets these values rapidly; decreasing k_V, increasing δ_V, reducing p_V, and/or increasing c_V. We incorporate adaptive immunity with an additional term introduced below.

To reduce the model from three equations to one equation we need to identify the relative rates of all the terms in these equations. It has been found that the $k_V PT$ terms are orders of magnitude slower than the other terms in these equations (7). While this term is present in two of the equations, taking linear combinations of the equations allows us to obtain one slow equation and one fast equation. We introduce $M = I - T$ and $N = I + T$, giving us the new system:

$$\frac{dM}{dt} = k_V P(N - M) - \frac{\delta_V}{2}(N + M) - \lambda$$
$$- \frac{d_V}{2}(N - M) \tag{26}$$

$$\frac{dN}{dt} = \lambda - \frac{d_V}{2}(N - M) - \frac{\delta_V}{2}(N + M) \tag{27}$$

$$\frac{dP}{dt} = \frac{p_V}{2}(N + M) - c_V P. \tag{28}$$

Now we can use a quasi-static approximation, as Eq. 26 is the only equation with the slow term $k_V PT = k_V P(N + M)$. Setting Eq. 27 and 28 equal to zero and solving for N and M in terms of V we obtain:

$$N = \frac{d_V - \delta_V}{d_V p_V} c_V P + \frac{\lambda}{d_V} \tag{29}$$

$$M = \frac{d_V + \delta_V}{d_V p_V} c_V P - \frac{\lambda}{d_V}. \tag{30}$$

Substituting these two expressions for N and M, and the derivative of the M term:

$$\frac{dM}{dt} = \frac{d_V + \delta_V}{d_V p_V} c_V \frac{dP}{dt}, \tag{31}$$

into Eq. 26 we obtain:

$$\frac{dP}{dt} = \frac{2(k_V p_V \lambda - d_V \delta_V c_V)}{(d_V + \delta_V) c_V} P \left(1 - \frac{k_V \delta_V c_V P}{k_V p_V \lambda - d_V \delta_V c_V} \right), \tag{32}$$

or:

$$\frac{dP}{dt} = rP \left(1 - \frac{P}{C} \right), \tag{33}$$

where we have set:

$$r = \frac{2(k_V p_V \lambda - d_V \delta_V c_V)}{(d_V + \delta_V) c_V} \tag{34}$$

$$C = \frac{k_V p_V \lambda - d_V \delta_V c_V}{k_V \delta_V c_V}. \tag{35}$$

We see that C corresponds to the equilibrium value of P in the full system of equations. These values can also be written in terms of R_0, the approximate number of cells a single infected cell will infect in an otherwise uninfected host. We find from the full system that R_0 is well approximated by:

$$R_0 = \frac{\lambda k_V p_V}{c_V d_V \delta_V}, \tag{36}$$

yielding:

$$r = \frac{2 d_V \delta_V}{d_V + \delta_V} (R_0 - 1) \tag{37}$$

$$C = \frac{d_V}{k_V} (R_0 - 1). \tag{38}$$

T cell killing is modeled with a mass action term for T cells interacting with infected target cells:

$$k'IX = k'\frac{p_V}{c_V}PX = kPX, \tag{39}$$

where we have used the relationship $I = (c_V/p_V)P$ from the quasi-static approximation and rescaled the killing rate to relate killing to free pathogen concentration, rather than infected cell concentration. Incorporating this killing term we obtain the functional form found in Eq. 4 of the main text:

$$\frac{dP}{dt} = rP\left(1 - \frac{P}{C}\right) - kPX. \tag{40}$$

We have reduced the three equations for uninfected target cell, infected target cell, and free virus to the single equation presented in the main body of the paper. Though we started with an explicit equation for pathogen density (Eq. 25), because the cell infection rate is by far the slowest rate in the system, the pathogen dynamics are best described by the difference of the infected and target cell rate equations.

Alternate Innate Immunity Model

Above, we have considered that innate immunity rapidly sets the values of the parameters for virus infecting cells, viral proliferation rates, and free virus survival rates. There are many ways of modeling innate immunity which could include activated cells, cytokines and other factors. Here we consider a previously used alternate model of innate immunity (12) and show that while the functional form differs, the qualitative behavior and the conclusions of this paper are preserved.

The model considers innate immunity described by a single variable Y with a negative feedback regulating the total magnitude of the innate response (12). The equations are given by:

$$\frac{dY}{dt} = \sigma_Y P(j - Y) - d_Y Y, \tag{41}$$

$$\frac{dP}{dt} = rP - hPY - kPX, \tag{42}$$

where X is the antigen specific response having the same dynamics as is described in the main text, and j represents complete activation of the

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innate immune system. As the innate response is rapid we have a quasi-static equilibrium (i.e. $dY/dt \approx 0$) for the pathogen dynamics giving:

$$\frac{dP}{dt} = rP\left(1 - \frac{P}{\frac{r}{hj}(\frac{d_Y}{\sigma_Y} + P)}\right) - kPX. \tag{43}$$

Here we see instead of a logistic equation, we have a first degree hill function. For comparing with other models we define:

$$C = \frac{d_Y}{\sigma_Y}\frac{r}{hj - r}, \tag{44}$$

which is the pathogen density expected in the absence of an antigen specific immune response. The product hj gives the maximum clearance (not the peak clearance of an acute infection, but a theoretical maximum if the totality of the innate immune system is activated) and we see that if innate immunity is to control the infection that $hj > r$. We can rewrite Eq. 43 using C obtaining:

$$\frac{dP}{dt} = rP\left(1 - \frac{P}{C - \frac{r}{hj}(C - P)}\right) - kPX. \tag{45}$$

Since we know that for innate immunity to control the pathogen that $hj > r$, and that $C > P$, we can accurately approximate this equation with the logistic function used above:

$$\frac{dP}{dt} = rP\left(1 - \frac{P}{C}\right) - kPX. \tag{46}$$

The fact that the two very different models from resource limitation and innate immunity yield the same approximate functional form underscores the difficulty of discriminating between them with experimental data (12). In general the carrying capacity C can be assumed to be a combination of innate immunity (Eq. 43) and resource limitation (Eq. 35).

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