

# **DYNAMICS OF CD8 IMMUNE RESPONSES**

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*Outline:*

Recent experimental results show that even brief stimulation with antigen can cause antigen-specific CD8 T-cells to undergo sustained proliferation followed by differentiation into memory cells. These results show that the dynamics of these immune responses are not governed by constant monitoring of antigen levels, but rather that following stimulation immune cells commit to a “program”.

In this talk, I will

- (i) develop the mathematical framework for modeling immune responses with antigen-independent proliferation phase;
- (ii) use this framework together with experimental data to describe basic principles of the immune program.

*Dynamics of the CD8 response:*

*Traditional modeling approach:*

Traditional models of CD8 and other immune responses are formally similar to predator-prey models in ecology. In these models, the proliferation of immune cells (predators) is continuously updated according to the abundance of the pathogen (prey) present in the body (ecosystem).

These models include subpopulations of naive, activated/effector and memory cells and successfully reproduce various dynamic features of the immune response

- (i) basic expansion-contraction dynamics;
- (ii) generation of immunodominance;
- (iii) synchrony in the contraction phase;
- (iv) generation of memory.

*However...*

*Predator-prey models fail to explain:*

*Antigen-independent expansion*

Full scale immune responses can be induced even by brief antigenic stimulation (Murali-Krishna *et. al.*, 1998; Mercado *et. al.*, 2000; Wong and Pamer, 2001; vanStipdonk *et. al.* 2001; Kaech and Ahmed, 2001; Bevan and Fink, 2001). These studies suggest the immune response is "programmed" early during the infection.

*Lack of compensation by subdominant response*

The immunodominant and subdominant responses appear to behave nearly independently of each other and the removal of the dominant epitope does not result in a compensatory increase in the subdominant response (Vijh *et al.*, 1999; van der Most *et al.*, 1996).

*The simplest possible program: formulation*

Following initial stimulation, the CD8 cells progress through a fixed program of expansion, contraction, and differentiation to memory. In the simplest case, all cells undergo a fixed number of divisions and then a fixed fraction of cells is converted to memory.

*Nomenclature:*  $P$  - pathogen,  $N$ - naive CD8,  $E$  - proliferating / effector CD8,  $M$  - memory CD8,  $\tau$  -time since recruitment.

*Strict program:*

$$\frac{dN_i}{dt} = -b_i N_i(t) P(t), \quad N_i(0) > 0,$$

$$y_i(t, 0) = b_i N_i(t) P(t),$$

$$\left( \frac{\partial}{\partial t} + \frac{\partial}{\partial \tau} \right) y_i(t, \tau) = F(\tau) y_i(t, \tau),$$

$$\frac{dP(t)}{dt} = rP(t) \left( 1 - \frac{P(t)}{c} \right) - P(t) \sum_i h_i E_i(t),$$

where

$$E_i(t) = \int_0^\infty (1 - \mu(\tau)) y_i(t, \tau) d\tau,$$

$$M_i(t) = \int_0^\infty \mu(\tau) y_i(t, \tau) d\tau.$$

*The simplest possible program: results*

1. For severe infections (such as LCMV), the process of recruitment is relatively short. The magnitude of the response to each epitope will be proportional to the frequency of precursor cells.
2. Differences in timing of recruitment for different epitopes will result in *different timing* of the peak of the response.
3. Responses to different epitopes are nearly independent. Thus removal (or change) of the response to one epitope will not affect the responses to other epitopes.

*The strict program fails on three main counts:*

1. Kaech and Ahmed (2001) showed that infections with higher doses result in both greater fraction of cells recruited and a larger per capita expansion of recruited cells.
2. Precursor frequency is not the only factor contributing to immunodominance. The time of recruitment also contributes to immunodominance (Yewdell and Bennik, 1999; DeBoer *et. al.*, 2001).
3. The data suggests that peaks of responses to different epitopes are synchronous (Murali-Krishna *et. al.*, 1998; DeBoer *et. al.*, 2001).

*Incorporating antigen-dependent expansion:*

We modify the strict program by introducing the window of antigen dependent expansion prior to the onset of antigen independent program. During this window, expansion of immune cells may be enhanced if antigen is present.

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial \tau}\right) y_i(t, \tau) = F(P(t), t, \tau) y_i(t, \tau).$$

The window of antigen dependent proliferation must be relatively short and it must end prior to the clearance of the pathogen because

- (i) duration and/or magnitude of the exposure to pathogen affects the magnitude of the expansion;
- (ii) there must be a lack of compensation by subdominant epitope(s) when the dominant epitope is removed.

We assume that this window starts immediately after recruitment. Then we consider two alternative models in which the duration of this window is determined by an *internal signal* or an *external signal*.



*Internal vs. external signal:*

*Internal signal:*

$$F(P(t), t, \tau) = \frac{sP(t)}{k + P(t)} + F(\tau), \quad \tau \in [\tau_{\text{on}}, \tau_{\text{off}}],$$

and

$$F(P(t), t, \tau) = F(\tau), \quad \tau \notin [\tau_{\text{on}}, \tau_{\text{off}}].$$

The window of antigen dependent expansion is determined by the time since *recruitment*, it turns on at  $\tau = \tau_{\text{on}}$  and turns off at  $\tau = \tau_{\text{off}}$ . (In our simulations, we used  $\tau_{\text{on}} = 0$  and  $\tau_{\text{off}} = 1$  day.)

*External signal:*

$$F(P(t), t, \tau) = \frac{sP(t)}{k + P(t)} + F(\tau), \quad t \in [t_{\text{on}}, t_{\text{off}}],$$

and

$$F(P(t), t, \tau) = F(\tau), \quad t \notin [t_{\text{on}}, t_{\text{off}}].$$

The window of antigen dependent expansion is determined by the time since *infection*, it turns on at  $t = t_{\text{on}}$  and turns off at  $t = t_{\text{off}}$ . (In our simulations, we used  $t_{\text{on}} = 0$  and  $t_{\text{off}} = 2$  days.)

*Summary of different models:*

<b>Model</b>	<b>Predator</b>	<b>Strict</b>	<b>Internal</b>	<b>External</b>
<b>General features</b>	<b>Prey</b>	<b>Program</b>	<b>Signal</b>	<b>Signal</b>
1. Expansion, contraction, memory phases	yes	yes	yes	yes
2. Antigen independent expansion phase	no	yes	yes	yes
3. Magnitude of expansion regulated by antigen	yes	no	yes	yes
<b>Epitope-specific features</b>				
4. Responses to different epitopes are independent	no	yes	yes	yes
5. Synchrony at peak	yes	no	no	yes
6. Immunodominance				
(a) precursor frequency	yes	yes	yes	yes
(b) timing of recruitment	yes	no	no	yes

*Discussion: why have a program?*

At first glance, continual updating might seem to be a more efficient way to deal with pathogen challenge, in that the immune response can be more finely tuned and optimized to the current infection. However, viruses and bacteria employ an extensive array of mechanisms that serve to subvert immune responses, and potentially this sensing apparatus (Gooding, 1992; Evans and Desrosiers, 2001). This sort of subversion could be avoided if an antigen-independent program is set before the pathogen has the opportunity to alter it, i.e., early in infection while pathogen density is low. Program responses, while less efficient, are likely to be more robust in that they will be less prone to interference from the pathogen.

Given a programmed response, what would we expect its features to be? Since the pathogen environment is highly variable (unpredictable) and there is no fine-tuning possible, the expansion program must err on the side of caution, typically overshooting the necessary number of CD8 cells required to clear the pathogen. This is indeed what is observed. CD8 proliferation continues well beyond the point of pathogen clearance in many infections. Immunodominance experiments illustrate just how markedly the immune system overshoots most infections: following removal of the immunodominant response to LCMV or *Listeria*, the subdominant responses do not increase substantially and despite their far lower peak densities, suffice to control these pathogens.

*Further studies:*

1. Generate a cell-cycle based model for immune program;
2. Incorporate a lag of about one day (vanStipdonk *et. al.*,2001) following recruitment of naive cells during which the cells do not proliferate.
3. Study the effect of gradual change from effector to memory function  $\mu(t)$ .
4. Explicitly consider the program for memory cells.
5. Consider how the presence of antigen during the contraction phase may result in the generation of anergy rather than memory cells, and apply this to consider persistent infections in more detail.
6. Consider the how antigen independent proliferation may alter the dynamics of CD4 T cells and B cells.

**THE METHOD OF RESCALING FOR  
AGE STRUCTURED MODELS**

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*Problem:*

We would like to estimate various kinetic parameters of the cell cycle from experimental data. Specifically, we need a reliable analytical tool for estimating the rates of cell division and cell death that govern the rate of change in the total cell population.

*Example:*

We would like to understand the mechanism of homeostatic regulation for immune memory which results in a nearly constant cell population. Does such population consist of quiescent cells or there is a balanced turnover of cells? If turnover occurs, how do cells progress through the cell cycle so that division and death processes balance each other?

*Available data:*

Development of CFSE dye dilution experiments allows for accurate tracking of the number of divisions that a given cell has undergone following transfer *in vivo*.

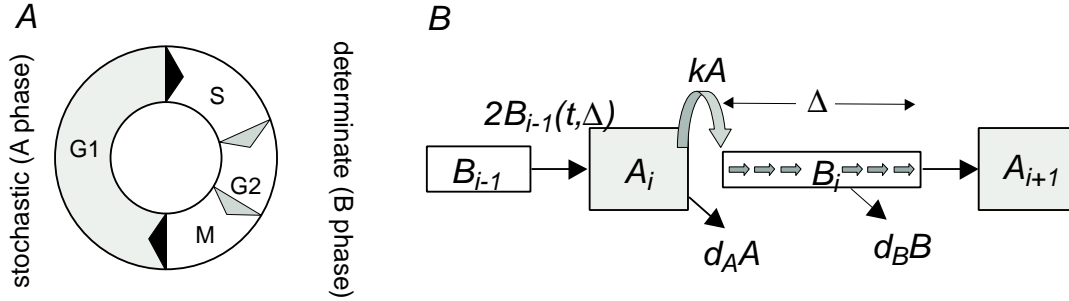
*Traditional method:*

Formulate a specific model for cell division and death and fit this model to the data.

*Problems with traditional method:*

1. We cannot be sure of the specific model. Two different models may produce equally good fits to the data.
2. The data may be insufficient to unambiguously determine parameters of the model. Several parameter combinations may fit the data equally well.
3. If the specific model involves distributed variables, then we have to make additional assumptions on the form of initial conditions.

*Illustration: Smith-Martin model*



*Model 1:*  $\Delta = 0$ ,  $d_B = 0$ ,  $d = d_A$ ;

$$\frac{dx_n(t)}{dt} = 2\lambda x_{n-1}(t) - (\lambda + d)x_n(t),$$

$$x_n(t) = \underbrace{\frac{(2\lambda t)^n}{n!}}_{\text{distribution}} \underbrace{e^{-2\lambda t} [x_0 e^{(\lambda-d)t}]}_{\text{total \#}}.$$

Predicted age distribution: Poisson with  $2\lambda t$ .

*Model 2:*  $\Delta \rightarrow 0$ ,  $\exp(-d_B \Delta) = 0.5$ ,  $d_A = 0$ ;

$$\frac{dx_n}{dt} = 2\lambda(1-f)x_{n-1} - \lambda x_n, \quad f = 0.5,$$

$$x_n(t) = \underbrace{\frac{(2\lambda(1-f)t)^n}{n!}}_{\text{distribution}} \underbrace{e^{-2\lambda(1-f)t} [x_0 e^{\lambda(1-2f)t}]}_{\text{total \#}}.$$

Predicted age distribution: Poisson with  $\lambda t$ .



*General model: assumptions*

- (i) Cells proliferate by binary fission, which is viewed as an event when one mother cell leaves its generation and at the same time two identical daughter cells enter the next generation.
- (ii) The cell population is homogeneous, that is, cells in different age classes (generations) exhibit identical behavior which is independent of the behavior of other cells or a given cell's genealogy.
- (iii) The generation time (defined as the time required for a cell to complete the cell cycle) is a random variable that depends only on the time since the cell entered the generation as a newborn daughter.
- (iv) Cell death (the removal of cells from the population) is a random event whose probability of occurrence depends only on the time since the birth of a given cell.
- (v) The probability that division and death events occur simultaneously is negligibly small.
- (vi) The system is closed, so that new cells enter the population only through division.

*General model: equations*

We let  $x_n(t, s)$  denote the density of cells in the  $n$ -th generation at time  $t$  which have already spent  $s$  time units in this age class. We call  $s$  the age of cells inside the generation. We let  $\lambda(s)$  denote the probability rate of cell division at age  $s$  and  $d(s)$  denote the probability rate of cell death at age  $s$  inside the generation.

$$\frac{\partial x_n(t, s)}{\partial t} + \frac{\partial x_n(t, s)}{\partial s} = -(\lambda(s) + d(s))x_n(t, s), \quad n \geq 0. \quad (1)$$

The total number of cells that divide anywhere between the times  $t$  and  $t + dt$  is given by

$$\left( \int_0^\infty \lambda(s)x_n(t, s) ds \right) dt,$$

and therefore twice the number of cells enter the next generation between  $t$  and  $t + dt$ . The dynamics of consecutive generations are coupled through the boundary condition

$$x_n(t, 0) = 2 \int_0^\infty \lambda(s)x_{n-1}(t, s) ds, \quad n \geq 1. \quad (2)$$

We let  $X_n(t) = \int_0^\infty x_n(t, s)ds$  denote the total number of cells in  $n$ -th generation at time  $t$ . A typical data set is a table of values

$$X_n(t_m), \quad n = 0, 1, \dots, N_{\max}, \quad t_m \in \{t_1, t_2, \dots, t_k\}.$$

*Rescaled model: equations*

The boundary condition given by equation (2) can be considered as the rate of external input of cells into the  $n$ -th generation. Equation (1) is linear, therefore rescaling the external input by a factor of  $a \geq 0$  will result in the identical rescaling of the cell density  $x_n(t, s)$ . The dynamics of the rescaled cell densities  $x_n(t, s, a) = a^n x_n(t, s)$  satisfy the equations

$$\frac{\partial x_n(t, s, a)}{\partial t} + \frac{\partial x_n(t, s, a)}{\partial s} = -(\lambda(s) + d(s))x_n(t, s, a), \quad (3)$$

$$x_n(t, 0, a) = 2a \int_0^\infty \lambda(s)x_{n-1}(t, s, a) ds. \quad (4)$$

We let  $X_n(t, a) = a^n X_n(t) = \int_0^\infty x_n(t, s, a) ds$ . The total number of cells in the rescaled population is

$$X(t, a) = \sum_{n=0}^{\infty} X_n(t, a).$$

*Rescaled model: characteristic equation*

Adding up equations in (3) and (4), we obtain

$$\frac{\partial x(t, s, a)}{\partial t} + \frac{\partial x(t, s, a)}{\partial s} = -(\lambda(s) + d(s))x(t, s, a),$$

$$x(t, 0, a) = 2a \int_0^\infty \lambda(s)x(t, s, a) ds.$$

Substitution  $x = e^{r(a)t}U(s)$  yields the eigenfunctions

$$U(s) = \exp\left(-\int_0^s (\lambda(z) + d(z)) dz\right) = \exp(-\Lambda(s) - D(s)),$$

and the characteristic equation

$$1 = 2a \int_0^\infty \lambda(s)e^{-\Lambda(s)-D(s)}e^{-r(a)s}ds. \quad (5)$$

*Method of rescaling:*

For a given experimental time series  $X_n(t)$ , we generate a family of rescaled time series  $X_n(t, a)$ , calculate the change in the total population size  $X(t, a)$  with time, and evaluate the exponential proliferation rate  $r(a)$  for each value of  $a$ . Theoretically, we can obtain the function  $r(a)$  by manipulating a single time series.

*Which parameters can we estimate?*

(1) In theory, we can find the generating kernel  $\lambda(s)e^{-\Lambda(s)-D(s)}$  by inverting the Laplace transform given by (5). Nevertheless, we *cannot* infer  $\Lambda(s)$  or  $D(s)$  without some additional assumptions. Roughly speaking, we cannot estimate birth or death rate *within* the cell cycle.

(2) The parameters that we can estimate must therefore describe the kinetics of a complete cell cycle. For example, we can estimate the probability that a cell dies before dividing ( $\mathcal{D}$ ), or the mean generation time for surviving cells ( $\mathcal{T}$ ) and its higher order moments.

(3) The probability that a cell divides before dying is

$$1 - \mathcal{D} = \int_0^{\infty} \lambda(s)e^{-\Lambda(s)-D(s)} ds. \quad (6)$$

Here we assume that all cells eventually divide, i.e.  $\Lambda(s) \rightarrow \infty$ .

(4) The mean generation time for surviving cells is

$$\mathcal{T} = \frac{1}{1 - \mathcal{D}} \int_0^{\infty} s\lambda(s)e^{-\Lambda(s)-D(s)} ds. \quad (7)$$

(5) The variance of generation times for surviving cells is

$$\sigma_{\mathcal{T}}^2 = \frac{1}{1 - \mathcal{D}} \int_0^{\infty} s^2\lambda(s)e^{-\Lambda(s)-D(s)} ds - \mathcal{T}^2. \quad (8)$$

*Evaluating  $\mathcal{D}$  and  $1 - \mathcal{D}$ :*

Compare equations (5) and (6):

$$\frac{1}{2a} = \int_0^{\infty} \lambda(s) e^{-\Lambda(s)-D(s)} e^{-r(a)s} ds. \quad (5)$$

$$1 - \mathcal{D} = \int_0^{\infty} \lambda(s) e^{-\Lambda(s)-D(s)} ds. \quad (6)$$

Let  $a^*$  be such that  $r(a^*) = 0$ . Equations (5) and (6) imply that

$$1 - \mathcal{D} = (2a^*)^{-1}, \quad \mathcal{D} = 1 - (2a^*)^{-1}.$$

*Evaluating  $\mathcal{T}$ :*

We implicitly differentiate (5)

$$1 = 2a \int_0^\infty \lambda(s) e^{-\Lambda(s)-D(s)} e^{-r(a)s} ds. \quad (5)$$

with respect to  $a$  at the point  $a = a^*$  and substitute  $r(a^*) = 0$  to obtain

$$0 = 2 \int_0^\infty \lambda(s) e^{-\Lambda(s)-D(s)} ds - 2a^* r'(a^*) \int_0^\infty s \lambda(s) e^{-\Lambda(s)-D(s)} ds.$$

This equation can be simply written as

$$\frac{1}{a^*} - 2a^* r'(a^*) \mathcal{T} (1 - \mathcal{D}) = 0,$$

and since  $2a^*(1 - \mathcal{D}) = 1$ , we derive that

$$\mathcal{T} = \frac{1}{a^* r'(a^*)}.$$

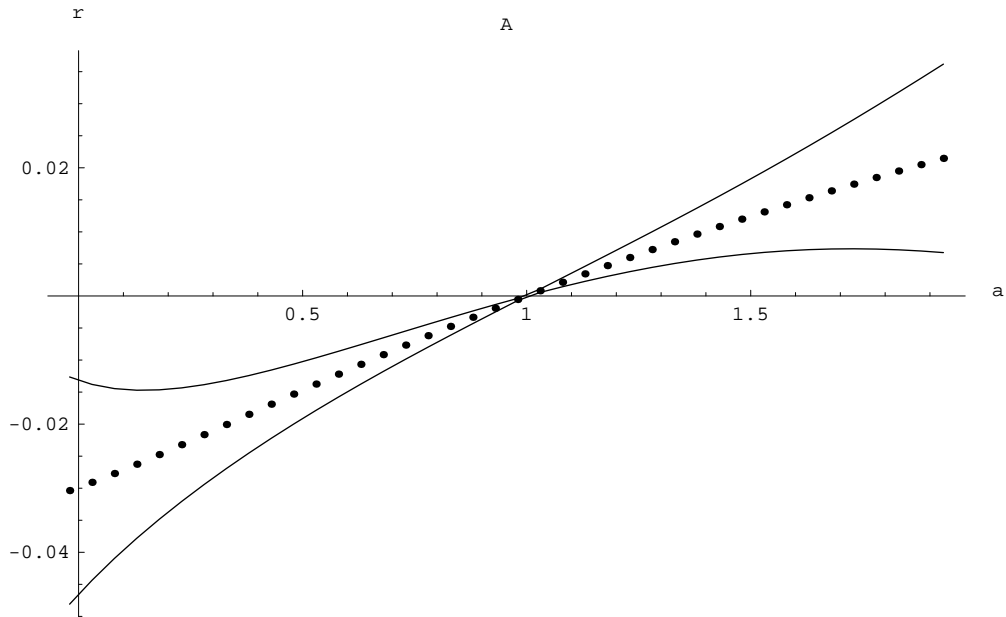
*Evaluating  $\sigma_{\mathcal{T}}^2$ :*

Repeated implicit differentiation of (5) yields

$$\sigma_{\mathcal{T}}^2 = \mathcal{T}^2 \left( 1 + (a^*)^2 r''(a^*) \mathcal{T} \right).$$

*Analysis of memory cell data:*

	$X_n(t)$	$t$ (days)						
		2	4	7	29	60	105	
$n$	0	99.6	99.0	93.0	22.4	14.8	4.9	
	1	0.3	1.0	4.0	62.5	33.7	12.0	
	2	0.0	0.0	1.0	8.9	29.7	27.0	(T)
	3	0.0	0.0	0.8	1.8	14.1	26.3	
	4	0.0	0.0	0.0	0.6	4.2	19.1	
	5	0.0	0.0	0.0	0.0	2.4	10.1	
	6+	0.0	0.0	0.0	0.0	1.1	0.6	



The dotted line is the graph  $r = r(a)$  obtained from the data. The 99% confidence intervals for  $r(a)$  are represented by the thin solid lines. From this graph, we estimated the average survival as  $1 - \mathcal{D} = 0.5$  and the mean generation time as  $\mathcal{T} = 36.9$  days.



*Limitations of the rescaling technique:*

(1) Truncation errors: we can only detect a maximum of between 5-10 divisions. Therefore, the data excludes the cells with higher generation numbers and the truncation errors occur. If we use the explicit solutions for a specific underlying model to fit the truncated data, then we are likely to obtain a better fit to the data; unfortunately, such estimates would depend on the underlying model.

(2) The rescaling method evaluates the function  $r(a)$  assuming that the population size changes exponentially. To increase the accuracy of the method, the population must be allowed to acquire the phase of exponential growth while having undergone a limited number of divisions (when the truncation errors are minimal, see above).

(3) The key assumption of our model (which may be invalid in some biological cases) is that the cell turnover is independent of both time and number of divisions.

*Advantages of the rescaling technique:*

(1) No dependence on specific model, no extra assumptions on the cell cycle, no ambiguity in parameter estimation.

(2) In theory, we can obtain the characteristic equation (5) of the model from a single time series.