Discriminating between Different Pathways of Memory CD8$^+$ T Cell Differentiation[1]

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Despite the rapid accumulation of quantitative data on the dynamics of CD8$^+$ T cell responses following acute viral or bacterial infections of mice, the pathways of differentiation of naive CD8$^+$ T cells into memory during an immune response remain controversial. Currently, three models have been proposed. In the "stem cell-associated differentiation" model, following activation, naive T cells differentiate into stem cell-like memory cells, which then convert into terminally differentiated short-lived effector cells. In the "linear differentiation" model, following activation, naive T cells first differentiate into effectors, and after Ag clearance, effectors convert into memory cells. Finally, in the "progressive differentiation" model, naive T cells differentiate into memory or effector cells depending on the amount of specific stimulation received, with weaker stimulation resulting in formation of memory cells. This study investigates whether the mathematical models formulated from these hypotheses are consistent with the data on the dynamics of the CD8$^+$ T cell response to lymphocytic choriomeningitis virus during acute infection of mice. Findings indicate that two models, the stem cell-associated differentiation model and the progressive differentiation model, in which differentiation of cells is strongly linked to the number of cell divisions, fail to describe the data at biologically reasonable parameter values. This work suggests additional experimental tests that may allow for further discrimination between different models of CD8$^+$ T cell differentiation in acute infections. *The Journal of Immunology,* **2007, 179: 5006–5013.**

Great advances in techniques allowing ex vivo enumeration of Ag-specific T cells (using tetramers, ELISPOT, or intracellular cytokine straining) have led to quantification of CD8$^+$ T cell responses to several viral pathogens including lymphocytic choriomeningitis virus (LCMV)[3] and influenza virus (1–5). From these and other studies, it has become clear that a CD8$^+$ T cell response during acute infections is comprised of three main phases (6, 7). First, there is an initial expansion phase during which a few Ag-specific naive T cells proliferate and reach maximum density of up to 10^6–10^7 cells per spleen of a mouse within 1 wk after the infection (6–10). The expansion is followed by the contraction phase where 90–95% of activated T cells undergo apoptosis leaving a small population of memory cells. This phase is followed by the maintenance of memory cells at which the epitope-specific CD8$^+$ T cells remain approximately at a constant level essentially for the life of a mouse (1, 4).

Although the basic quantitative features of the CD8$^+$ T cell responses during acute viral infections have been elucidated, the pathways of T cell differentiation during acute infections remain controversial. In particular, how memory CD8$^+$ T cells are produced during an infection, namely from the effector cells or independently of them, is still debated (11). The question remains largely unresolved partly due to difficulty of discriminating between effector and memory CD8$^+$ T lymphocytes during an immune response. Currently, three models on the differentiation of memory CD8$^+$ T cells exist in the literature (12–14).

The first model, "stem cell-associated differentiation" (SCAD), assumes that memory cells have properties of stem cells. In this model, during the expansion phase of an immune response, terminally differentiated effector cells are produced by continuously turning over memory lymphocytes (Refs. 12, 15, 16; see Fig. 1*A*). Following the contraction phase, effectors die due to apoptosis, leaving a pool of long-lived memory cells with an increased frequency. Indirect experimental evidence for this model has come from 1) the analogy with the humoral immune response in which proliferating plasmablasts differentiate into effectors (plasma cells) and memory B cells (precursors); and 2) the observation that in vitro, several days of culture are required to generate effector CTL from the CTL precursors (16, 17). This model was intensively used in theoretical research by several authors studying the dynamics of CTL responses to viral infections (15, 18–21).

In the "linear differentiation" (LD) model, the expansion phase is driven by T cells with an activated/effector phenotype, and these cells either become memory cells or die during the contraction phase (Refs. 6, 7, 10, 14; see Fig. 1*B*). Experimental support for this model came from observations including the following: 1) effectors "marked" using the CRE/LOX system at the peak of the response are also found in the memory population (22); 2) memory CD8$^+$ T cells are generated only after extensive proliferation of CTLs (23); 3) there are gradual changes in the expression of genes of cells recovered at different times after the infection with LCMV (24); and 4) cells with a high expression level of the IL-7Rα chain survive from the peak of the response into the memory population (25).

Finally, in the "progressive differentiation" (PD) model (also called the "decreasing potential" model; Refs. 6 and 13), proliferating CD8$^+$ T cells progress through several stages of differentiation during the expansion phase (Refs. 12, 14, 26; see Fig. 1*C*).

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[3] Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; SCAD, stem cell-associated differentiation; LD, linear differentiation; PD, progressive differentiation; AIC, Akaike's An Information Criterion; CI, confidence interval; T_{CM}, central memory CD8$^+$ T cell; T_{EM}, effector memory CD8$^+$ T cell.

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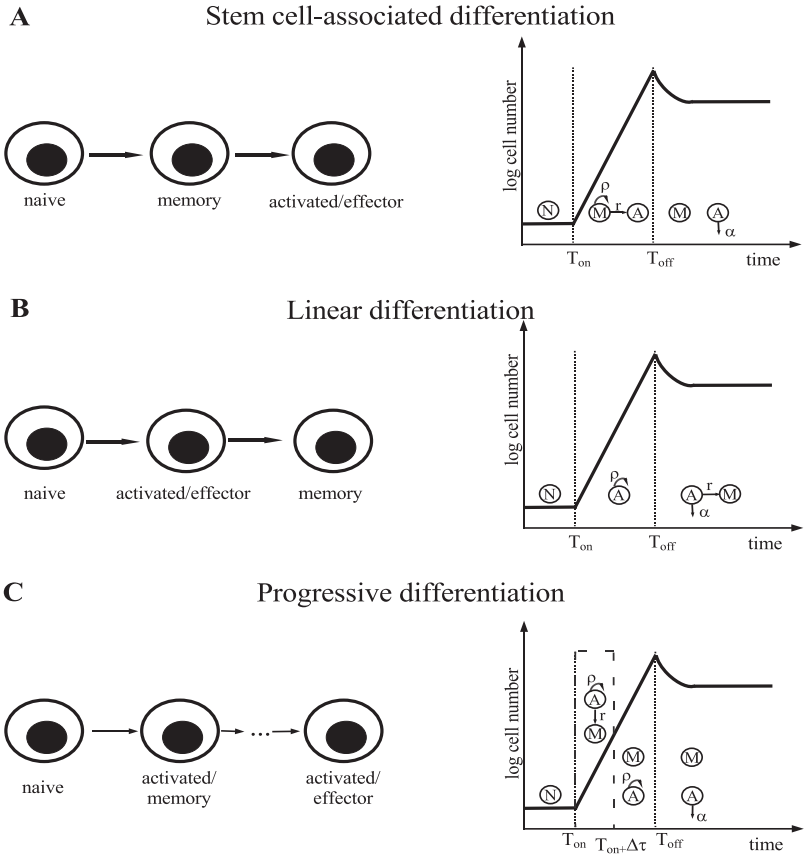

FIGURE 1. Three main models for differentiation of $CD8^+$ T cells during acute infections. In all models, the expansion phase starts at time T_{on}, and the peak of the response occurs at time T_{off} (see *Materials and Methods* for more detail). At $t = T_{on}$, naive cells N become activated and start proliferating. In the SCAD model (A), during the expansion phase, memory "stem" cells M divide at the rate ρ and differentiate into nondividing activated effectors A at the rate r. During the contraction phase, effectors die at the rate α. In the LD model (B), during the expansion phase, activated cells A proliferate at the rate ρ. During the contraction phase, the activated A (having phenotype of effector cells) die at the per capita rate α and differentiate into memory cells M at the rate r. In the PD model (C), activated cells A proliferate at the rate ρ. Within a time window $\Delta\tau$ after initial activation, activated cells differentiate into memory cells M at the rate r. During the contraction phase, activated effectors A die at the per capita rate α. In all models, memory cells are assumed to have zero death rate.

Cells receiving a weak signal will differentiate into memory cells, while cells receiving a strong signal will differentiate into effector cells. It is believed that differentiation of lymphocytes is linked to the number of divisions cells have undergone (14, 23, 27). This link between phenotype and the number of cell divisions so far has been best demonstrated for B and $CD4^+$ T cells (28, 29). Therefore, according to this model, it is assumed that activated, proliferating cells differentiate into memory cells early during the response, when activated cells have undergone only a few divisions. Following the contraction phase, activated/effector cells are prone to die while memory cells survive (Fig. 1*C*). Although many studies are cited for support of this model (reviewed in Refs. 14, 26, 30), evidence for progressive generation of memory and effector $CD8^+$ T lymphocytes in vivo is relatively limited. For example, several studies have reported that memory $CD8^+$ T cells in some situations can be already formed a few days after initial stimulation (31–33).

This report reformulates these proposed hypotheses for T cell differentiation as mathematical models, and investigates whether all models can adequately describe data on the dynamics of a $CD8^+$ T response to a viral infection in vivo.

Materials and Methods

The used modeling approach is an extension of the approach suggested in De Boer et al. (34). It is assumed that before infection, only a few naive cells, specific to a particular epitope of the virus, are present. Following infection, cells remain naive for times $t < T_{on}$. At the time $t = T_{on}$, the expansion phase begins: naive cells become activated and start proliferating. The peak of the response occurs at the time $t = T_{off}$, and all cells stop proliferating. After the peak of the response, the contraction phase starts and apoptosis of activated cells takes place. To describe the dynamics of the $CD8^+$ T cell response, the following "step" function was used

$$f(t) = \begin{cases} 1, & \text{if } T_{on} < t < T_{off}, \\ 0, & \text{otherwise.} \end{cases} \quad (1)$$

For the analysis, a previously published data set of Homann et al. (4) on the dynamics of the $CD8^+$ T cell response to LCMV measured by intracellular cytokine staining (IFN-γ) in spleens of B6 mice was used. Data for the first 45 days were taken for the analysis, and in the main text, the analysis is restricted to the gp33-specific $CD8^+$ T cells. Analysis of the responses to other LCMV epitopes in B6 or BALB/c mice (1) gave similar results (Ref. 7 and results not shown). The initial number of Ag-specific $CD8^+$ T cells present in a spleen of a mouse has been estimated previously and is in the range 60–200 gp33-specific $CD8^+$ T cells (9). For these fits, the initial cell number was chosen to be 100. Changing the precursor number to other realistic values (50 and 200) did not affect the main conclusions of this article (results not shown). The population of memory cells is assumed to have a zero rate of loss (1, 4, 35).

SCAD model

This model assumes that during the expansion phase, activated effectors A are being produced from proliferating memory "stem" cells M. Therefore, initial conditions used in this model are $M(0) = 100$ and $A(0) = 0$. During the expansion phase, memory $CD8^+$ T cells proliferate at a rate ρ and differentiate at a rate r into nondividing activated effectors. During the contraction phase (i.e., at $t > T_{off}$), activated cells die at the constant rate α (15). The dynamics of both populations at $t > T_{on}$ are given by equations

$$\begin{aligned} \frac{dA(t)}{dt} &= f(t)rM(t) - [1 - f(t)]\alpha A(t), \\ \frac{dM(t)}{dt} &= f(t)(\rho - r)M(t). \end{aligned} \quad (2)$$

In "*Alternative models*", the SCAD model was extended to allow for 1) conversion of terminally differentiated effectors into memory cells during the contraction phase, and 2) proliferation of effectors in the expansion phase of the immune response.

LD model

In this model, memory cells are only formed from activated/effector cells A after the peak of the immune response. The initial conditions are then $A(0) = 100$ and $M(0) = 0$. During the expansion phase, activated cells proliferate at the rate ρ. After the peak of the immune response, activated cells die at the rate α due to apoptosis and differentiate into memory cells at the rate r. For $t > T_{on}$, the dynamics of the model are given by

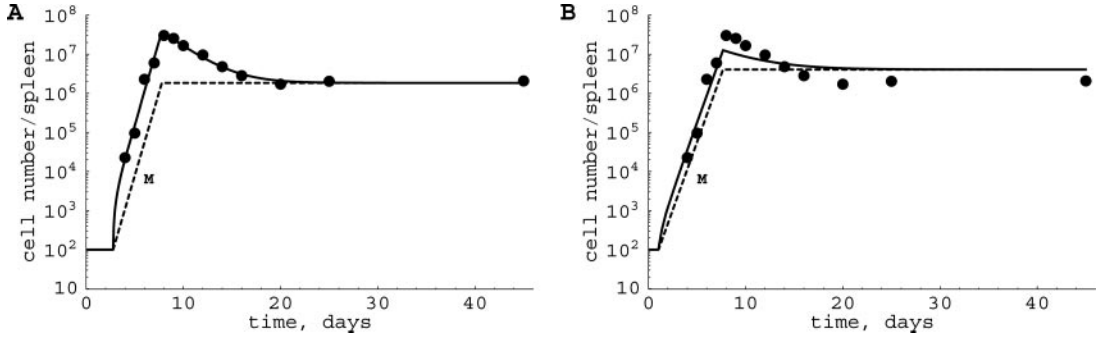

FIGURE 2. Fitting the SCAD model to the data. In the model, proliferating activated cells convert into terminally differentiated, nondividing effectors. *A*, The fit when all parameters of the model are varied freely. *B*, The fit when the division rate for activated (memory stem) cells is fixed at $\rho = 5\ \text{day}^{-1}$. The quality of the fit of the constrained model is significantly worse than that of the unconstrained model ($F_{1,8} = 42.7$, $p < 0.001$, F test for the nested models). In this and in other figures, dots represent the data, and solid lines represent the prediction of the model (i.e., the total number of epitope-specific CD8$^+$ T cells). Dashed lines show the dynamics of memory lymphocytes. The parameters providing the best fits are shown in Table I.

$$\begin{aligned}\frac{dA(t)}{dt} &= f(t)\rho A(t) - [1-f(t)](\alpha + r)A(t),\\ \frac{dM(t)}{dt} &= [1-f(t)]rA(t).\end{aligned} \tag{3}$$

In "*Alternative models*", the LD model was also extended to allow memory cells to be generated within a short time window before the peak of the response.

PD model

In this model, proliferating activated cells A differentiate into memory cells M starting at time $t = T_{on}$ for $\Delta\tau$ days at the rate r. The time window for differentiation of activated cells into memory $g(t)$ is given by

$$g(t) = \begin{cases} 1, & \text{if } T_{on} < t < T_{on} + \Delta\tau,\\ 0, & \text{otherwise.}\end{cases} \tag{4}$$

The initial conditions for the PD model are $A(0) = 100$ and $M(0) = 0$. For times $t > T_{on}$, the model equations are given by

$$\begin{aligned}\frac{dA(t)}{dt} &= f(t)\rho A(t) - g(t)rA(t) - [1-f(t)]\alpha A(t),\\ \frac{dM(t)}{dt} &= g(t)rA(t).\end{aligned} \tag{5}$$

In "*Alternative models*", the results of the analysis of another variant of the PD model in which differentiation of activated cells into effectors explicitly depends on the number of divisions that cells have undergone during an immune response were also presented. In the model, the time taken by cells to complete their first division is distributed lognormally, and the number of cells completing their first division per unit of time is given by the recruitment function $R(t)$ (36, 37):

$$R(t) = \begin{cases} \dfrac{C}{\sqrt{2\pi}\sigma(t-\Delta_0)} \exp\left(-\dfrac{(\log(t-\Delta_0) - \log(\mu))^2}{2\sigma^2}\right), & \text{if } t > \Delta_0,\\ 0, & \text{otherwise,}\end{cases} \tag{6}$$

where C is the total number of cells entered their second division ($C = 200$), Δ_0 is the minimal time of the first division, μ is mean of the distribution, and σ is the shape parameter (36, 37). In vivo, it takes 24 to 48 h for naive T cells to complete their first division (38, 39), and therefore the first cell division was allowed to be at least 1 day (i.e., $\Delta_0 = 1$ day). Variance of the recruitment function $R(t)$ was set to $\sigma = 1.4$ to ensure recruitment of most epitope-specific CD8$^+$ T cells within 4 days after the infection. At these parameter values, over 50% of naive T cells undergo their first division in 2 days. The second and following cell divisions occur at fixed periods of time given by Δ (i.e., occur deterministically). In vitro, CD4$^+$ and CD8$^+$ T cells stimulated with anti-CD3 Abs in appropriate conditions appear to divide in accord with these assumptions (36, 37, and data not shown). For $t > T_{on}$, the model is formulated as the system of differential equations (36):

$$\begin{aligned}\frac{dA_0(t)}{dt} &= -R(t)/2,\\ \frac{dA_n(t)}{dt} &= 2^{n-1}R(t-(n-1)\Delta) - 2^{n-1}R(t-n\Delta), \quad n > 0,\end{aligned} \tag{7}$$

where $A_n(t)$ is the number of activated cells having undergone n divisions by time t, Δ is the duration of the cell cycle for divided cells, and $A_0(0) = 100$ and $A_n(0) = 0$ for $n > 0$. In this formulation of the model, it was assumed that activated cells do not die during the expansion phase. During the contraction phase (for $t > T_{off}$), activated cells that have divided more than n_{cr} times die, and other cells instantaneously become memory cells. The dynamics of activated cells are given by equations

$$\frac{dA_n(t)}{dt} = -\alpha A_n(t) H(n - n_{cr}), \quad n \geq 0, \tag{8}$$

where $H(x)$ is the Heaviside step function, and

$$M(t) = \sum_{n=0}^{n_{cr}} A_n(t) \tag{9}$$

Importantly, extending the model for cell division to a more complex Smith-Martin model (37, 40, 41) led to qualitatively similar results (data not shown).

Numerical procedures

The models were fitted to the data by applying the $\log_{10}$ transformation to the measured cell number and the model prediction for the total cell number ($A+M$), and by minimizing the residual sum of squares. Each model in the text is formulated as a system of ordinary differential equations, and due to their simplicity, the model solutions can be found in an analytical form (results not shown). For models that describe data with reasonable quality, the 95% confidence intervals (CIs) were calculated. CIs for parameters were determined by bootstrapping the residuals with 500 simulations (42). Statistical comparison was done using the F test for nested models (43, 44) or using Akaike's An Information Criterion (AIC) for non-nested models (45). Shortly, AIC for a given model is calculated as

$$\text{AIC} = N \log\left(\sum_{i=1}^{N} \varepsilon_i^2/N\right) + 2(p + 1 + (p+1)(p+2)/(N-p-2)) \tag{10}$$

where N is the number of data points, ε_i is the ith residual of the best fit of the model to data, and p is the number of model parameters. Both the F test and AIC take into account the quality of the model fits to data and the number of parameters of the model (i.e., the model complexity). Note that for model selection, not the absolute value of AIC, but the difference between AIC for different models, is important. An AIC difference that is <3 is considered to be insignificant, while a difference of 10 is considered to be large, making the model with a larger AIC a very poor descriptor of the data (as compared with the model with a lower AIC). Fittings were done in Mathematica 5.2 using the FindMinimum routine.

Table I. *Parameter estimates obtained by fitting the SCAD, LD, and PD models to data*[a]

Parameters	SCAD Model		LD Model	PD Model	
95% CIs	Uncon	Constr	Uncon	Uncon	Constr
ρ, day^{-1}	39.98	**5**	1.99	2.56	2.37
	24.85–64.21		1.77–2.22	2.28–5.60	
T_{on}, day	2.77	1.04	1.32	1.44	1.3
	2.37–3.18		0.83–1.82	0.98–1.88	
T_{off}, day	7.79	7.67	7.77	7.69	6.79
	7.49–8.15		7.51–8.11	7.26–8.0	
$\Delta\tau$, day	—	—	—	5.56	**2**
				4.87–6.18	
r, day^{-1}	38.02	3.40	0.02	0.57	0.68
	22.99–62.26		0.01–0.03	0.24–3.69	
α, day^{-1}	0.41	0.27	0.39	0.41	0.06
	0.28–0.59		0.28–0.58	0.28–0.60	
AIC	−29.66	−13.08	−29.74	−19.35	−5.74

[a] Estimates are obtained by fitting the model predictions to the data on the CD8$^+$ T cell response to the gp33 epitope of LCMV. For unconstrained fits (column "uncon"), all parameters are allowed to be any positive number. For the constrained fit (column "constr"), in the SCAD the rate of division of memory cells was set to the maximum $r = 5$ day^{-1}, and in the PD model, the window for differentiation was set to $\Delta\tau = 2$ days (shown in bold). AIC is calculated as described in *Materials and Methods.* Model fits and the data are shown in Figs. 2–4. Dash "—" stands for nonapplicable.

Results

SCAD model

The formulated mathematical model describes the data on the dynamics of the CD8$^+$ T cell response to the gp33 epitope of LCMV with excellent quality (Fig. 2*A*). However, such a good correspondence between model predictions and the data was achieved only at extremely fast rates of cell proliferation and differentiation (Table I), with the doubling time of the population of proliferating lymphocytes $T_{1/2} \approx \ln(2)/40 \approx 0.017$ days or 25 min. Indeed, in this model, to produce large numbers of nonproliferating effector cells, which are doomed to die after the peak of the response, the rate of cell differentiation r from the memory precursors into effectors must be high. Furthermore, to maintain the observed rate of increase for the total population size, which is given by the difference $\rho - r$, the rate of cell division ρ must be also unrealistically high. Constraining the rate of T cell replication to $\rho = 5$ per day (i.e., a doubling time of ~3 h, which is already likely to be too fast), leads to a significantly worse fit of the model to the data ($p < 0.001$, F test). The latter fit cannot adequately describe the contraction phase of the immune response during which 90–95% of cells present at the peak of the response die. Reducing the rate of cell proliferation during the expansion phase to lower, more realistic values, leads to fits of even lower quality (results not shown). Due to these two reasons (nonbiologically fast proliferation and a poor quality of the constrained fit), this form of the SCAD model can be rejected.

LD model

In contrast with the SCAD model, the LD model describes the data well at biologically reasonable parameter values (Fig. 3 and Table I). This is expected because the expansion phase is driven by activated cells, and from the large population of activated cells at the peak of the response, a small subpopulation of memory cells can easily be generated. Thus, when comparing the SCAD and LD models, it was concluded that the LD model describes these data more adequately.

PD model

The PD model also describes the data with excellent quality (Fig. 4*A*). This, however, requires a relatively wide time window for memory T cell differentiation after initial activation (in Table I, the estimated differentiation window $\Delta\tau \approx 5.6$ days after initial activation occurring at $T_{on} = 1.4$ days). Because during the expansion phase, the cell population doubles at least every ln (2)/2 ≈ 0.35 days = 8 h, activated cells will undergo at least 15 divisions in 5 days. This requirement stems from the constraint put by the data in which a few naive Ag-specific T cells have to lead to generation of $>10^6$ memory cells. The model prediction for the differentiation window, therefore, is in contrast with the assumptions of the PD model proposing that extensive cell division is likely to result in terminally differentiated and prone to apoptosis effectors (see also "*Alternative models*").

Constraining the differentiation window to shorter periods, for example, to 2 days, corresponding to approximately six cell divisions, leads to a much poorer fit of the model to the data (Fig. 4*B*, $p < 0.001$, F test). The poor quality of the model fit is due to the very few memory cells generated during the response, which in turn arises due to a short window for differentiation of activated cells into memory. A constant decline in cell numbers after the peak occurs due to apoptosis of activated effectors, and does not show the constant level of memory observed in data (Fig. 4). These results suggest that if there is early differentiation of activated cells into memory, it cannot occur only in a short time window after cell activation.

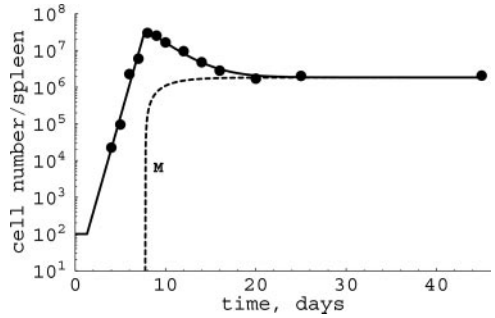

FIGURE 3. Fitting the LD model to the data. In this model, differentiation of activated cells into memory occurs after the peak of the immune response. Note that in this formulation of the LD model, memory cells appear only during the contraction phase (and thus were absent before the peak of the immune response). The parameters providing the best fits are shown in Table I.

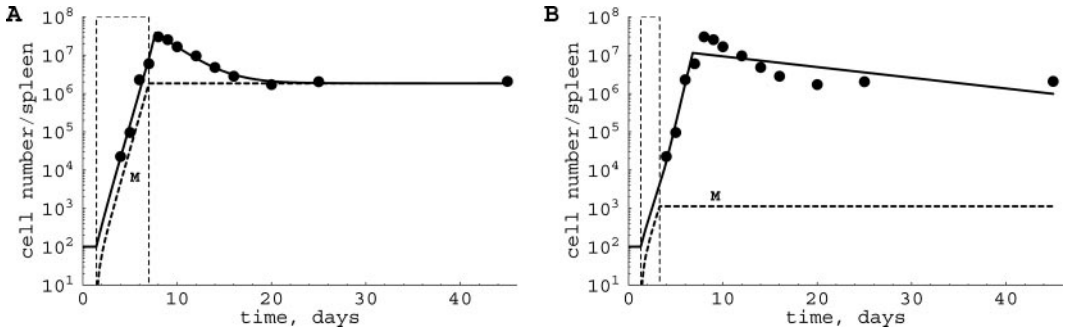

FIGURE 4. Fitting the PD model to the data. *A*, The fit when the window for differentiation $\Delta\tau$ is fitted. *B*, The fit with the window for differentiation constrained to $\Delta\tau = 2$ days (corresponding to approximately six divisions). Dashed rectangulars show the window for differentiation of activated cells into memory. The quality of the model fit in *B* is significantly lower than that in *A* ($F_{1,7} = 37.36$, $p < 0.001$, *F* test for the nested models). The parameters providing the best fits are shown in Table I.

Alternative models

One of the uses of mathematical modeling is to discriminate between plausible biological hypotheses (7), and one of the main goals of this work is to investigate which hypotheses for memory CD8$^+$ T cell differentiation are least consistent with the analyzed data. However, there is always a chance that the current mathematical formulation of a hypothesis is incomplete or biased in some way. Whether the models that poorly describe the data can be modified to improve the quality of model fits to the data was therefore tested. Finding such modifications may indicate potential areas for future experiments. In some cases, such model modifications were indeed possible. The SCAD model has two main assumptions that lead to poor quality fits of the data: lack of reversion of effectors to memory cells during the contraction phase and inability of terminally differentiated effectors to proliferate during the expansion phase. If either of these assumptions is relaxed, then the model can describe the data with excellent quality (results not shown; see also Ref. 46). However, either of the

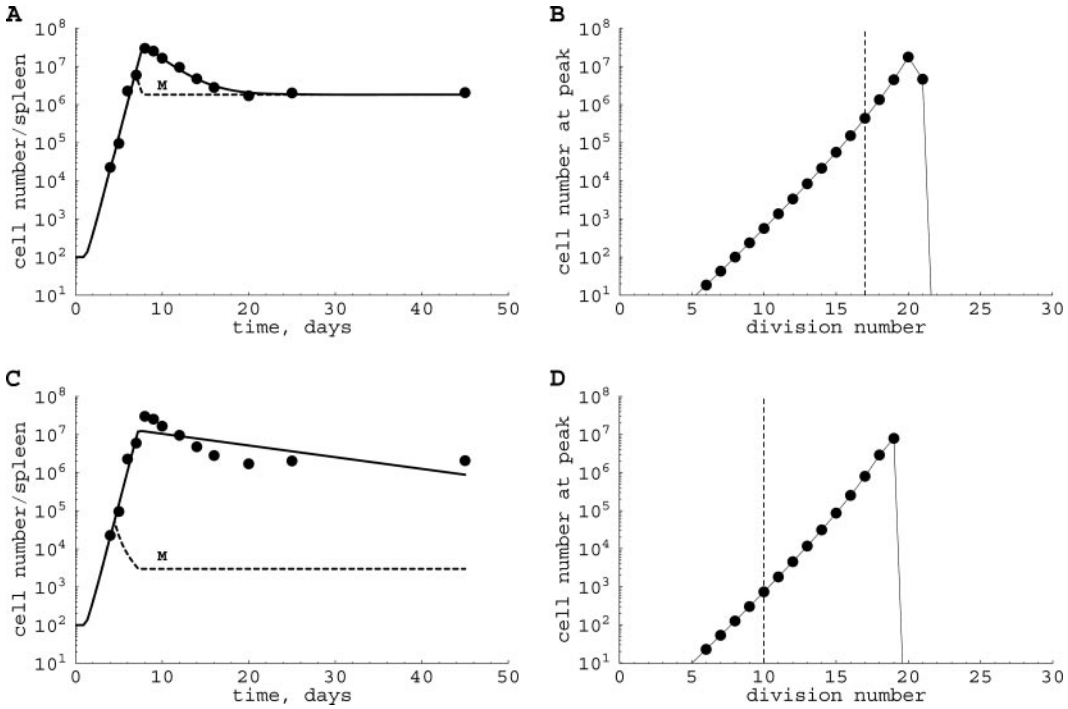

FIGURE 5. Fitting the PD model in which likelihood of differentiation of activated cells into effectors depends on the number of cell divisions. In the model, activated cells that have undergone more than n_{cr} divisions become terminally differentiated effectors and die during the contraction phase. Activated cells that have divided no more than n_{cr} times retain properties of memory cells and survive during the contraction phase. *Left panels*, The fits of the model to data; *right panels*, the predicted number of gp33-specific CD8$^+$ T cells at the peak of the response as the function of number of divisions that cells have undergone. The vertical dashed lines in *B* and *D* show the critical number of cell divisions n_{cr} either predicted by the model fit to the data (*B*) or fixed in fitting (*D*). *A*, The model fit to the data when all parameters of the model are varied freely. *C*, The model fit to the data when the critical division number is set to $n_{cr} = 10$. The quality of the fit of the constrained model is significantly worse than that of the unconstrained model ($F_{1,8} = 42.0$, $p < 0.001$, *F* test for the nested models). Dashed lines in *A* and *C* show the dynamics of activated lymphocytes that have undergone n_{cr} or less divisions (and which have properties of memory cells). The loss of these memory cells before the peak of the response seen in *A* and *C* is due to further division of activated cells. In this model, cells that have divided n_{cr} times after one more division are lost from the memory population. The time of the first cell division is lognormally distributed with mean μ, the shape parameter $\sigma = 1.4$, and the initial delay (see *Materials and Methods*). Other parameters providing the best fits are shown in Table II.

extensions blend the main difference between stem-like memory cells and terminally differentiated effectors, namely that these different cell types do not interconvert and have different proliferative properties. A possibility of interconversion of terminally differentiated effectors into effector memory $CD8^+$ T cells has indeed been proposed (12), and further studies are needed to investigate whether this extension allows for a better agreement with the data on the dynamics of central and effector $CD8^+$ memory T cells during an acute viral infection (see *Discussion*). The extension of the SCAD model to allow effectors to proliferate similarly to the memory cells, however, is hard to justify biologically because it is unclear why memory and effector cell populations undergoing a similar expansion would have dramatically different properties in the contraction phase (i.e., effectors die and memory cells survive). Therefore, it may be concluded that although extending the SCAD model to let it be more consistent with the analyzed data is possible, these extensions require additional experimental and theoretical validations.

In the PD model, it was assumed that activated cells differentiate into memory cells at a constant rate without explicit dependence on the number of divisions that activated cells have undergone. An alternative PD model was formulated in which the number of cell divisions was tracked (see *Materials and Methods*). The model assumes that cells are recruited into the response within 1–4 days after the infection and divide deterministically thereafter (Refs. 36 and 37; see Fig. 5). Importantly, the best fit of the model to data also predicts that activated cells must have undergone up to 17 divisions to generate enough memory cells (Fig. 5*A* and Table II). In comparison, the majority of effectors that die during the contraction phase have undergone 20 divisions which is only 3 divisions more (Fig. 5*B*). Distribution of T cells at the peak of the response as the function of the division number (shown in Fig. 5*B*) is in part determined by the recruitment function $R(t)$ describing the distribution of times of the first cell division. At current parameter values, over 50% of Ag-specific naive $CD8^+$ T cells undergo their first division within 48 h after infection. Changing parameters of the recruitment function to other realistic values affected very little estimates for the critical division number n_{cr} and the number of cell divisions reached by most cells in the population shown in Fig. 5 and Table II (results not shown). Constraining the critical number of divisions n_{cr}, at which activated cells terminally differentiate into effectors, to a lower value, $n_{cr} = 10$, leads to fits of a significantly lower quality ($p < 0.001$, F test, Fig. 5*B*) supporting conclusions reached with a simpler model analyzed above.

In the PD model, it was also assumed that differentiation of activated cells into memory starts immediately after cells became activated (i.e., at $t = T_{on}$). It has been suggested, however, that activation of a fraction of Ag-specific naive T cells may occur later in the response, and these cells due to weak or limited stimulation will preferentially develop into memory cells (14). An alternative model was formulated in which a fraction q of naive cells becomes activated at time T_{on} after the infection and a fraction $1 - q$ is activated at a later time $T_{off} - \Delta\tau > T_{on}$. The former cells are assumed to have undergone extensive division and differentiate into effectors prone to death in the contraction phase. The latter cells, however, are assumed to undergo fewer divisions and differentiate into memory cells. Fitting this model to the data (with several fixed values of q) suggests that for the best description of the data, activation of cells differentiating into memory cells still has to start very early to generate a large population of memory cells observed in the data (results not shown). During this period, activated cells are expected to undergo many (~15) divisions. This, again, contradicts the main assumption of the PD model, proposing that only cells receiving weak stimulation and having undergone few divisions will preferentially differentiate into memory cells. The PD model, however, can be made consistent with the data if it is assumed that the likelihood of differentiation of dividing cells into effectors does not strongly depend on the number of divisions that cells have undergone. If activated cells that have divided even 15 times could still remain undifferentiated and become memory cells by surviving during the contraction phase, the model can perfectly well describe the data (Figs. 4*A* and 5*A*). Indeed, in a recent study, it was found that a few HY- or OVA-specific $CD8^+$ T cells present at the peak of the immune response in vivo, while having undergone >8 divisions, can still remain undifferentiated (47).

Table II. *Parameter estimates obtained by fitting an alternative PD model, in which differentiation of activated cells into effectors depends on the number of divisions that cells have undergone (fits are shown in Fig. 5)*[a]

Parameters (95%CIs)	Uncon	Constr
Δ, day	0.35 (0.33–0.36)	0.35
μ, day	0.57 (0.27–1.19)	0.58
T_{off}, day	7.76 (7.57–7.92)	7.25
n_{cr}	17.0 (17–18)	**10**
α, day^{-1}	0.40 (0.32–0.51)	0.07
AIC	−29.87	−13.48

[a] Parameters for two fits are shown: unconstrained fit (when all parameters are allowed to be fitted), and constrained fit (when the critical division number n_{cr} is set to 10) (shown in bold). Parameters are: Δ, interdivision time; μ, mean of the recruitment function $R(t)$ (and $\mu + \Delta_0$ is approximately the average time of the first division, $\Delta_0 = 1$ day); n_{cr}, the critical number of division after which cells become terminally differentiated and prone to apoptosis effectors; and α, the rate of apoptosis of effectors during the contraction phase.

In the LD model, it is assumed that memory cells are formed from activated cells after the peak of the immune response. An alternative model was formulated and analyzed in which activated cells start differentiating into memory a few (1–2) days before the peak of the response. This model also gave excellent fits to the data (results not shown). Therefore, this extended LD model can also predict the presence of memory cells at the peak of the response if differentiation of activated cells into memory occurs a few days before the peak (see *Discussion*). Moreover, the LD model can be extended with generation of memory cells from the beginning of the immune response. However, such an extension will make the LD and PD models essentially identical precluding a possibility to discriminate between these alternative hypotheses.

Discussion

Mathematical models have been used to estimate the rates of expansion and contraction of T cell populations during an immune response (34, 35), as well as to get better insights into mechanisms of the loss of $CD8^+$ T cell memory (7, 15, 48–50). These previous studies were extended by investigating the likelihood of different pathways of differentiation of $CD8^+$ T cells following an acute viral infection using mathematical modeling.

All analyzed models were found to describe the data well, but some fits would lead to biologically unreasonable parameters values. To compare the quality of fits of all models together, AIC (Ref. 45 and see *Materials and Methods*) was used. Such comparison suggests (see Table I) that of the tested models, the LD model in which differentiation occurs after the peak of the immune response can best describe the data with a minimal number of parameters (and as the result, this model has the lowest AIC). The fit also provides biologically reasonable estimates for parameters of the model. The next "best" model is a variant of the LD model in which activated cells differentiate into memory just before the peak of the immune response (results not shown). This analysis, therefore, suggests that of the three models tested, the data are most consistent with

a model in which differentiation of activated cells into memory following acute LCMV infection is likely to occur just before or after the peak of the T cell response. Interestingly, a qualitatively similar conclusion for the differentiation of CD4$^+$ T cells has been recently obtained also using mathematical modeling (51).

This analysis provides little support for the SCAD model—the model widely used in mathematical modeling for understanding T cell dynamics during acute and chronic viral infections (reviewed in Ref. 21). Furthermore, it was shown that the PD model, in which only cells that have divided 10 times or less are capable of surviving during the contraction phase and can become memory cells, cannot describe the data well. It is important to stress that finding consistency of a model, describing a particular biological process, with data does not prove that the underlying biological hypothesis is the correct one. However, showing that a model is not consistent with data allows one to reject the underlying biological hypothesis.

These results should be applicable to other viral and bacterial acute infections with two main properties: 1) expansion of Ag-specific CD8$^+$ T cell populations must be rapid and large in magnitude (4–5 orders of magnitude), and 2) a moderate contraction phase with 90–95% of activated cells dying. On the one hand, a rapid expansion of Ag-specific CD8$^+$ T cell populations and a relatively large contraction phase restricts the SCAD model which is unable to generate many effectors at the peak of the response at biologically reasonable parameters. On the other hand, a large expansion of Ag-specific CD8$^+$ T cell populations from few naive precursors to thousands of memory cells requires extensive cell division that constrains the PD model.

It is important to realize, however, that infection of mice with LCMV, as far as is known, leads to a generation of the largest murine CD8$^+$ T cell response. Many other infections of mice invoke CD8$^+$ T cell responses of lower magnitude, and therefore, the proposed methodology may not be able to reject some of the models, for example, the PD model, for such infections. Therefore, although this analysis clearly demonstrates that of the models tested, the LD model is best consistent with the data on the dynamics of the LCMV-specific CD8$^+$ T cell response, other data may not be sufficient to reject alternative models.

There is at least one important observation that seemingly contradicts the LD model of CD8$^+$ T cell differentiation. Several authors found that memory CD8$^+$ T cells under some circumstances can be already present at the peak of the immune response (32, 33). It has been shown that these data are consistent with a variant of the LD model in which differentiation of activated effectors into memory starts already before the peak of the response, thus resolving this potential discrepancy between these and other data.

Changing the duration of the infection, for example, by antibiotics, may affect the generation of memory CD8$^+$ T cells, and therefore, may provide additional means for testing different models of cell differentiation. However, it was found that treatment of *Listeria monocytogenes*-infected mice with antibiotics 24 h after the infection yielded conflicting results. In several studies from two groups, treated BALB/c mice had a lower peak listeriolysin O-specific CD8$^+$ T cell response, and a proportionally lower number of memory CD8$^+$ T cells as measured 3–4 wk postinfection such that the ratio of the number of effectors to memory cells was unaffected by the treatment (52–54). Similarly, the ratio of the peak to memory number of listeriolysin O- and OVA-specific CD4$^+$ T cells was largely unaffected by the treatment in B6 mice (55, 56). In contrast, in another study, the authors found a similar number of OVA-specific CD8$^+$ effectors at the peak in control and treated B6 mice, and disproportionately fewer memory CD8$^+$ T cells in treated than in control mice (56). Therefore, from these data, it is not yet clear whether a shorter duration of infection affects the ratio of the number of effectors present at the peak to the number of memory cells generated. The result seems to be dependent on the mouse strain used and the T cell specificity analyzed. Furthermore, the current mathematical models will also have to be extended to incorporate the potential dependence of the model parameters on the duration of Ag display. For example, longer exposure to an Ag may reduce the rate of differentiation of effectors into memory cells and the rate of apoptosis of effectors during the contraction phase in the LD model.

It is important to note that in all models analyzed in this article, cells differentiate, in some sense, in accord with a "linear" pathway. That is, cells in these models "convert" from one type to another type in one direction. For example, in the LD model, cells differentiate from effectors to memory, and in the SCAD model, cells differentiate from memory to effectors. An alternative "branching" model for cell differentiation assumes that during encounter with an Ag, a naive CD8$^+$ T cell is instructed in some way to become either a memory or an effector cell depending on circumstances (13). For example, several observations are consistent with the "instructive" (branching) model for cell differentiation. These include differentiation of naive CD8$^+$T cells directly to the memory phenotype bypassing the effector stage in vitro (31, 57), or after homeostatic proliferation in lymphopenic hosts in vivo (58). A recent study suggests that unequal partition of key cell proteins at first cell division can imprint the future faith of T lymphocytes to become memory or effector cells (59). Construction and analysis of branching models of cell differentiation is a topic for future research.

One of the best ways of testing the predictions of different models for cell differentiation is to look for markers that are restricted only to memory or effector CD8$^+$ T cells. Recent studies suggest that during viral infections of mice, high levels of expression of the killer cell lectin-like receptor G1 on Ag-specific CD8$^+$ T cells, marks effector cells during the expansion phase (60–62). Future studies therefore will need to include this information for finer discrimination between the discussed and additional models for cell differentiation.

One important limitation of this study needs to be mentioned. Previous studies have suggested that the memory CD8$^+$ T cell population consists of two different subsets: T_{CM} and T_{EM} cells (26). In this analysis, these two subsets were not explicitly modeled, partly because currently no data have been published on the dynamics of these subsets during a CD8$^+$ T cell response to LCMV in mice. Investigating whether the data on the dynamics of CD8$^+$ T cell responses can be used to understand differentiation of CD8$^+$ T cells into T_{CM} and T_{EM} cells is a topic for future research. Nevertheless, this approach demonstrates potential strength and limitations of mathematical modeling for discrimination between different hypotheses on T cell differentiation and identifies the potential ways this analysis can guide future experiments.

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