IL-2 Regulates Expansion of CD4$^+$ T Cell Populations by Affecting Cell Death: Insights from Modeling CFSE Data[1]

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It is generally accepted that IL-2 influences the dynamics of populations of T cells in vitro and in vivo. However, which parameters for cell division and/or death are affected by IL-2 is not well understood. To get better insights into the potential ways of how IL-2 may influence the population dynamics of T cells, we analyze data on the dynamics of CFSE-labeled polyclonal CD4$^+$ T lymphocytes in vitro after anti-CD3 stimulation at different concentrations of exogenous IL-2. Inferring cell division and death rates from CFSE-delabeling experiments is not straightforward and requires the use of mathematical models. We find that to adequately describe the dynamics of T cells at low concentrations of exogenous IL-2, the death rate of divided cells has to increase with the number of divisions cells have undergone. IL-2 hardly affects the average interdivision time. At low IL-2 concentrations 1) fewer cells are recruited into the response and successfully complete their first division; 2) the stochasticity of cell division is increased; and 3) the rate, at which the death rate increases with the division number, increases. Summarizing, our mathematical reinterpretation suggests that the main effect of IL-2 on the in vitro dynamics of naive CD4$^+$ T cells occurs by affecting the rate of cell death and not by changing the rate of cell division. *The Journal of Immunology,* **2007, 179: 950–957.**

Generally T cells require several stimuli for rapid expansion, among which triggering the specific TCR, costimulation, and signal 3 cytokine (IL-12 or type I IFNs for CD8$^+$ T cells) are the most important (1, 2). Several additional factors may also influence the magnitude and duration of T cell responses; it has been known for long time that IL-2 is generally required for expansion of populations of T lymphocytes in vitro (3). In many cases IL-2 appears to be dispensable for proliferation of Ag-specific CD8$^+$ T cells in vivo (4–7). How IL-2 regulates expansion and magnitude of the CD8$^+$ and CD4$^+$ T responses is not well understood, however. For example, administration of IL-2 in mice infected with lymphocytic choriomeningitis virus may enhance survival, or increase apoptosis, of both CD8$^+$ and CD4$^+$ T cells depending on the time of IL-2 administration (8). Whether such changes occur via affecting the rate of cell proliferation or cell death is generally unknown. In most cases, discriminating between the effects of IL-2 on cell division and cell death is difficult because changes in the total population size only delivers an estimate of the net difference between the rates of proliferation and death and not their individual values.

CFSE labeling (or delabeling) is one of the most informative tools used in immunology to detect cell division in various settings in vitro and in vivo (9). Generally, CFSE allows one to determine the number of divisions a cell has undergone, and up to 6 to 10 divisions can be monitored with CFSE with good accuracy (9, 10). By multiplying the fraction of cells that have undergone different numbers of division by the total number of cells, one finds the absolute number of cells that have undergone a given number of divisions at different time instances. This we call CFSE data. In principle, CFSE data allow one to separate the contribution of proliferation and death processes to the net change of the population size (10–21).

A previous study addressed the role of IL-2 in proliferation of polyclonal CD4$^+$ T lymphocytes in vitro (22). In this study, CFSE-labeled naive CD4$^+$ T cells were stimulated in vitro with anti-CD3 Abs at different concentrations of exogenous IL-2. A simple mathematical model was developed to fit these CFSE data. The model assumed a lognormal distribution of division times for undivided cells and a deterministic division with a fixed interdivision time for divided cells (20, 22). The authors found that IL-2 mainly affects the fraction of cells recruited into the response, the interdivision time, and the probability of cell death during the division for divided cells. Here we extend this previous study and reanalyze the dynamics of CFSE-labeled CD4$^+$ T cells in vitro using several mathematical models fitted to the same data (22). The reanalysis was prompted by two important observations we have made in the data.

First, there is change in the symmetry of the time course of cell cohorts having completed the same number of divisions. If one looks at the time course of the number of cells in a cohort of undivided cells (Fig. 1), or cells having undergone one division (e.g., in Fig. 3*A*), this time course can be described by an asymmetric, lognormal distribution. This time course becomes more symmetric (i.e., can be described by a normal distribution) for cohorts of cells having undergone more divisions (e.g., 3 divisions, see Fig. 3*A*). This observation indicates that division of cells cannot be strictly deterministic, because in a deterministically dividing cell population, the time course of cell cohorts is simply shifted in time and scaled in amplitude relative to each other (20).

Second, the dynamics of cell cohorts at low IL-2 concentrations (e.g., at IL-2 = 2.5 U/ml in Fig. 3*C*) suggest that parameters for cell division and/or death should change with time, or with the number of divisions cells have undergone. This stems from the

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[3] Abbreviations used in this paper: SM, Smith-Martin; TLE, thymidine-labeling experiment.

observation that the maximum density is reached by the cell cohort having undergone 2 divisions. This cannot be achieved with the current homogeneous models (i.e., models with division- and time-independent parameters (12–14). Because the previous interpretation of these data was performed with a model with deterministic division and constant parameters (22), here we study how these two added features, stochasticity of cell division and time or division dependence of parameters, influence interpretation of the data.

In our study, we improve the analysis of these CFSE data in several directions. First, we explicitly account for the data on the recruitment on undivided $CD4^+$ T cells into the S phase of the cell cycle after initial stimulation (see *Materials and Methods*). We show that neglecting these data can lead to a possibly incorrect interpretation of the data (20). Second, we use a more general model for cell division, the Smith-Martin $(SM)^3$ model, that includes both a deterministic and a stochastic component, because the latter is required for the changes in symmetry of the time course of cell cohorts in the CFSE data (see above). Finally, we investigate several alternative models explaining these data (division-dependent death rate model and IL-2 consumption model, see *Materials and Methods*) and suggest experimental tests allowing discrimination between different models. Our study demonstrates 1) the wealth of information that can be obtained from CFSE-delabeling experiments, and 2) potential challenges one faces with the analysis of these data. The main goal of this study is to investigate which parameters for cell division and death are affected by IL-2 during expansion of populations of naive $CD4^+$ T cells in vitro.

We find that IL-2 has the most profound effect on the death rate of dividing $CD4^+$ T cells, and specifically, on the rate of increase of the death rate with the division number. IL-2 also affects the fraction of cells recruited into the response and surviving their first division. In contrast, IL-2 does not affect the average interdivision time of dividing cells. Summarizing, our results suggest that magnitude of expansion of populations of naive $CD4^+$ T cells in vitro is largely controlled by regulating the rates of cell death, and hardly the rates of cell division.

Materials and Methods

Data

The data used in this study have been provided by Dr. P. Hodgkin and have been published previously (22); the data are available at http://theory.bio.uu.nl/vitaly/data/Ganusov07_ji_il2_data.zip. In brief, mouse lymph nodes naive $CD4^+$ T lymphocytes have been labeled with CFSE and then stimulated in vitro with anti-CD3 Abs in the presence of different concentrations of exogenous human (h)IL-2 (IL-2 = 1.25, 2.5, 5, 50 U/ml). Endogenously produced mouse (m)IL-2 was effectively blocked using anti-mIL-2 Abs because in cultures where no hIL-2 was added, no cell proliferation was observed (E. Deenick, unpublished observations). The initial number of cells in cultures at all IL-2 concentrations was 3×10^4. In a separate experiment, cell cultures were pulsed at day 4 with 100 μg/ml BrdU for 4 h before harvesting. BrdU incorporation was detected using an anti-BrdU Ab. In the thymidine-labeling experiments (TLE), cells were stimulated in the presence of 5 ng/ml demecolcine. After various times, cultures were pulsed with ^{3}H[TdR] for 2 h before harvesting.

Dynamics of undivided cells

Previous studies have suggested that in some situations division and death of undivided and divided cells needs to be described separately (15, 22). In particular, it may take 24–48 h for a cell to divide for the first time whereas subsequent divisions typically occur much faster (15, 23–25). Some cells may die in vitro soon after their recovery from hosts due to different reasons including cytokine deprivation (15), and not all cells are stimulated to divide. To overcome these problems with describing the dynamics of undivided cells, we focus on the dynamics of *divided* cells only and introduce a recruitment function $R(t)$ which describes the number of cells successfully completing their first division (and entering their second division) per unit of time (Fig. 2). In independent experiments, recruitment of cells into the first S phase has been estimated by pulsing cell cultures with radioactive thymidine at various times and preventing mitosis with demecolcine (22). We refitted these data using a log normal distribution with a delay and estimated the mean of the distribution μ', shape parameter σ', and the delay Δ_0' (Fig. 1 and Table I). The form of the recruitment function used then in the fits of the CFSE data was simply a scaled and shifted log normal distribution with estimated parameters μ' and σ':

$$R(t) = \begin{cases} \dfrac{C}{\sqrt{2\pi}\sigma'(t-\Delta_0)} \times \\ \qquad \exp\left(-\dfrac{(\log(t-\Delta_0)-\log(\mu'))^2}{2\sigma'^2}\right), & \text{if } t < \Delta_0 \\ 0, & \text{otherwise,} \end{cases} \quad (1)$$

where C is the total number of cells entered their second division, and Δ_0 is the total delay in the recruitment function resulted after adding an extra delay to the estimated parameter Δ_0' ($\Delta_0 \geq \Delta_0'$). Because demecolcine may induce apoptosis in cells (26, 27), only the mean μ' and shape parameter σ' of the log normal distribution in the recruitment function $R(t)$ were used in fitting CFSE data and not the total number of cells entering their first S phase after stimulation (proportional to the area under the curve in Fig. 1).

Models for cell division and death of divided cells

We have extended the SM model for cell division for the analysis of CFSE data (14, 28). In the SM model, the cell cycle of proliferating cells can be divided in two main parts: the variable A-state and the deterministic B phase. The waiting time in the A-state is exponentially distributed (with parameter λ), and different cells will take different times to leave the A-state. In contrast, the B phase has a fixed duration Δ, and cells completing the B phase, divide and deliver two daughter cells into the A-state. In this model, the average interdivision time is $T = \Delta + 1/\lambda$ (Fig. 2). Biologically, the A-state and the B phase correspond approximately to the G_1 phase and $S + G_2 + M$ phases of the cell cycle, respectively. In our model thus the progression through the cell cycle is determined by two parameters, λ and Δ. In more complex models, however, this progression can be also affected by other factors, for example, by cell size (29–31). Cells in the A-state and the B phase die at a constant rate d. The model can be formulated as a system of ordinary and partial differential equations (14, 18).

As discussed in the Introduction, there are quantitative changes in the dynamics of cell cohorts that imply changes in the parameters for cell division/death with the division number and/or time. Changes in the parameters with the division number are intuitive and come from the general observation that changes in cell phenotype are often (but not always) associated with cell division (32–36). The SM model with division-dependent parameters can be reformulated as a set of delayed differential

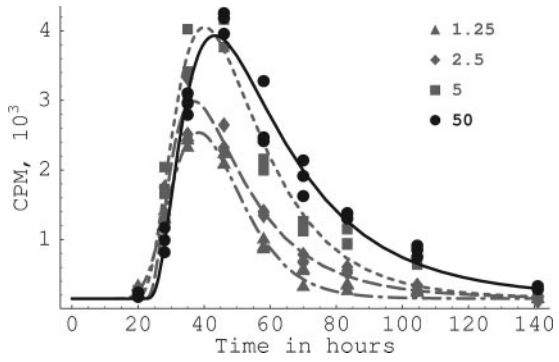

FIGURE 1. Recruitment of naive $CD4^+$ T cells into the S phase of the cell cycle following anti-CD3 stimulation in the presence of demecolcine. At various times after stimulation, cell cultures were pulsed with [^{3}H]TdR (thymidine) for 2 h before harvesting. The resulting radioactivity is plotted as the function of time of harvesting. Experiments were done with four different IL-2 concentrations (concentrations of IL-2 in U/ml are marked). A log normal distribution with a delay was fitted to these data. The data are presented by the symbols and the fits of the distribution as lines. Estimates of the parameters for the log normal distribution are given in Table I.

Table I. *Parameter estimates with 95% confidence intervals (CI) obtained by fitting the SM model with division-dependent death rate to CFSE data*[a]

		IL-2 = 1.25 U/ml		IL-2 = 2.5 U/ml		IL-2 = 5 U/ml		IL-2 = 50 U/ml	
		Mean	95% CIs	Mean	95% CIs	Mean	95% CIs	Mean	95% CIs
μ'	h	36.76	31.49–41.40	24.42	22.28–28.13	31.68	27.31–40.20	34.11	31.77–37.36
σ'	–	0.32	0.28–0.39	0.65	0.51–0.80	0.53	0.38–0.71	0.66	0.56–0.76
Δ_0'	h	5.30	0.46–11.05	21.03	16.46–23.79	16.12	6.53–21.57	21.25	17.39–23.64
$C \times 10^{-4}$	Cells	0.4	0.32–0.51	0.83	0.67–1.04	0.87	0.83–0.98	0.99	0.76–1.29
Δ_0	h	20.65	17.71–23.1	29.47	26.21–32.14	25.57	23.9–27.06	21.25	21.25–24.94
λ	1/h	0.088	0.065–0.123	0.115	0.084–0.166	0.191	0.163–0.229	0.23	0.159–0.412
Δ	h	4.25	2.43–6.05	6.24	4.47–7.76	6.78	6.3–7.28	9.2	7.9–10.35
d_1	1/h	0.016	0.0–0.035	0.016	0.003–0.028	0.0	0.–0.007	0.023	0.012–0.034
α	1/h	0.039	0.025–0.054	0.023	0.015–0.031	0.021	0.017–0.023	0.003	-0.001–0.008
T	h	15.66	12.82–19.12	14.91	12.82–17.55	12.01	11.26–12.86	13.55	12.23–14.37

[a] Parameters estimated by fitting the TLE data (Fig. 1) are the mean μ', shape parameter σ', and delay Δ_0'. Parameters estimated by fitting the CFSE data are the total number of cells completing their first division C, the delay in the recruitment function Δ_0 the rate of recruitment into the B-phase of the cell cycleλ , the duration of the B-phaseΔ , the death rate d_1 of cells having undergone one division, the slope of the change of the death rate with the division number α, and the average interdivision time $T = \Delta + 1/\lambda$.

equations (for derivation, see Supplementary Material available online at http://theory.bio.uu.nl/vitaly/publ/Ganusov07_ji_appendix.pdf):

$$\frac{dA_1(t)}{dt} = R(t) - (\lambda_1 + d_1)A_1(t), \tag{2}$$

$$\frac{dA_n(t)}{dt} = 2\lambda_{n-1}e^{-d_{n-1}\Delta_{n-1}}A_{n-1}(t-\Delta_{n-1}) - (\lambda_n + d_n)A_n(t), \quad n > 1, \tag{3}$$

$$\frac{dB_n(t)}{dt} = \lambda_n A_n(t) - \lambda_n e^{-d_n\Delta_n}A_n(t-\Delta_n) - d_n B_n(t), \quad n \geq 1 \tag{4}$$

where $A_n(t)$ and $B_n(t)$ are the numbers of cells in the A-state and the B phase, respectively, having undergone n divisions by time t; $R(t)$ is the recruitment function denoting the number of cells that successfully complete their first division per unit of time, λ_n, Δ_n, and d_n are the rate of recruitment into the B phase, the duration of the deterministic B phase, and the death rate, respectively, dependent on the division number n. In the SM model, we calculate the probability of cell death per division as $\delta_n = 1 - \lambda_n/(\lambda_n + d_n) \times e^{-d_n\Delta_n}$, where $\lambda_n/\lambda_n + d_n)$ is the probability that a cell survives during the A-state, and $e^{-d_n\Delta_n}$ is the probability that a cell survives during the B phase.

Additional experiments undertaken by Deenick et al. (22) involving short-pulse BrdU labeling of cultures of CD4$^+$ T cells stimulated in vitro at different concentrations of IL-2 suggested that parameters for cell division are unlikely to change with the division number. This comes from the observation that there is only a minimal change in the fraction of BrdU$^+$ cells with the cell division number (for $n > 2$) across all IL-2 concentrations (22). Therefore, in our "best guess" model we assumed constant parameters for cell proliferation (λ and Δ), and let only the death rate change linearly with the division number n, $d_n = d_1 + a(n-1)$. The model was fitted to each individual CFSE dataset for a given IL-2 concentration.

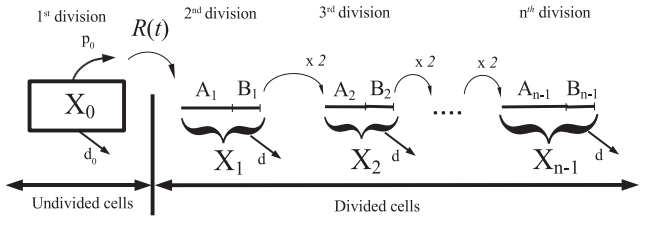

FIGURE 2. A cartoon of the modified SM model for cell dynamics. We focus on the dynamics of divided cells (i.e., cells having undergone at least one division). We define the recruitment function $R(t)$ which is equal to the number of cells entering their second division per unit of time. Cell division is described using the SM model (28). In this model, the cell cycle of cells consists of two parts: a stochastic A-state in which the waiting time is exponentially distributed (with parameter λ), and a deterministic B phase of fixed duration (equal to Δ). The total number of cells in the nth division is $X_n = A_n + B_n$. The rate of cell death during the A-state and the B phase is d.

Because in experiments of Deenick et al. (22), cell cultures were started with different initial concentrations of IL-2, it seems natural to consider models where the IL-2 concentration changes over time and that affects the rates of proliferation and/or death of T cells. Indeed, it has been shown that proliferating T lymphocytes reduce the amount of IL-2 present in the culture medium (3, 37, 38), and it seems likely that changes we observe in the dynamics of CD4$^+$ T cells at low IL-2 concentrations are due to "consumption" of IL-2 by T cells. Therefore, in an alternative, IL-2 "consumption" model, we let CD4$^+$ T cells reduce the medium concentration of IL-2. Changes in the IL-2 concentration affect parameters for cell division and death (see Supplementary Material for more details). Shortly, the dynamics of the IL-2 concentration in the medium, $I(t)$, are described by

$$\frac{dI(t)}{dt} = -[c_1X(t) + c_2R(t)]\frac{I(t)}{h_I + I(t)}, \tag{5}$$

where c_1 and c_2 are parameters describing consumption of IL-2 by divided cells $X(t) = \Sigma_{n=1}^{\infty} X_n(t)$, and by undivided cells, respectively; h_I is a half-saturation constant defining that at high IL-2 concentrations, cells have a maximum rate of IL-2 consumption. Dependencies of the parameters for divided cells on the medium concentration of IL-2 were chosen to be simple saturating functions

$$\lambda(I) = \lambda_1 + \frac{(\lambda_2 - \lambda_1)I}{h_\lambda + I}, \quad \Delta(I) = \Delta_1 + \frac{(\Delta_2 - \Delta_1)I}{h_\Delta + I}, \quad d(I) = d_1 + \frac{(d_2 - d_1)I}{h_d + I}, \tag{6}$$

with different half-saturation constants h_λ, h_Δ, and h_d (see Fig. 7). The dynamics of T cells having undergone different numbers of divisions is given by the modified SM model reformulated as a system of delayed differential equations (shown in Supplementary Material). The model was fitted to the CFSE data at all IL-2 concentrations simultaneously, with different initial IL-2 concentrations for different datasets.

Finally, there could be changes in the parameters for cell division and/or death with time since stimulation, for example, due to worsening of medium conditions. This seems more unlikely than our IL-2 consumption model because one expects medium conditions to worsen fastest when cell numbers are high. Cell numbers are highest at IL-2 = 50 U/ml, and cells continue proliferating over the entire time period observed. At the lowest IL-2 concentration, there are fewer cells and yet, the total cell number declines with time (20). Therefore, in this work we restrict our main analysis to the model with division-dependent parameters and a more mechanistic IL-2 consumption model (see *Results*).

Numerical procedures

We use numerical solutions of the mathematical models for fitting the CFSE data. The fitting is done by minimizing the difference between the model predictions on the total cell number $X_n(t) = A_n(t) + B_n(t)$ and the data using least squares nonlinear regression (assuming normally distributed errors with zero mean and constant variance). Because the CFSE concentration is diluted 2-fold following cell division, it is often difficult to estimate the number of cells having undergone many divisions due to autofluorescence of CFSE-unlabeled cells. To reduce errors associated with the estimation of the number of cells having undergone >6 divisions, we have created a new division class of cells, called "6+", by

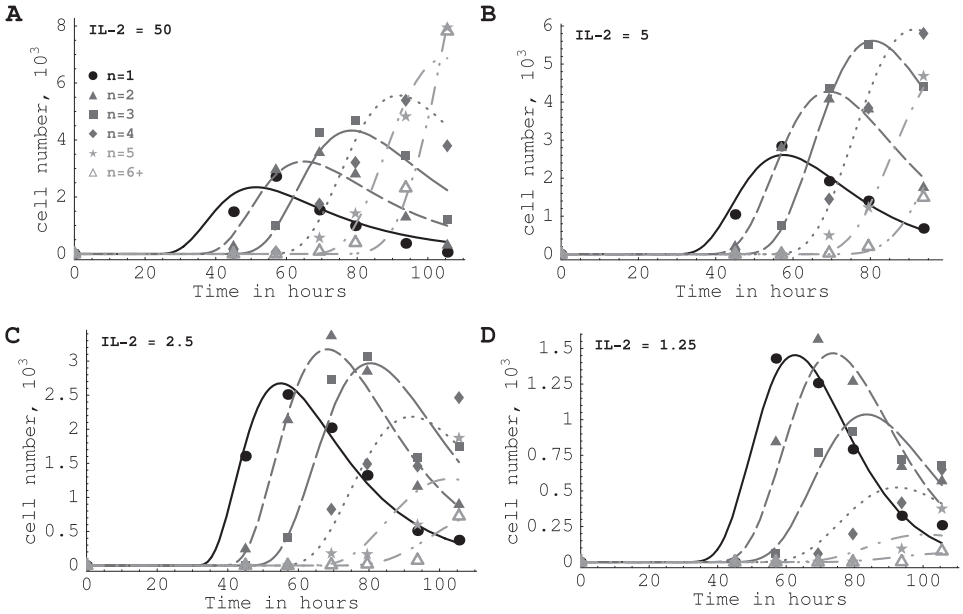

FIGURE 3. Best fit of the SM model with division-dependent death rate to the CFSE data of $CD4^+$ T cells stimulated in vitro with anti-CD3 Abs at four concentrations of exogenous IL-2: IL-2 = 50 (*A*), 5 (*B*), 2.5 (*C*), and 1.25 (*D*) U/ml. We plot the number of cells having undergone the same number of divisions as a function of time: cohort having completed one division (solid line with circles), two divisions (short-dashed line with triangles), three (long-dashed line with squares), four (dotted line with diamonds), five (dash-dotted line with stars), to six or more (double dash-dotted line with open triangles). A lognormal distribution with the mean and shape parameters estimated from the TLE data, were used to describe the recruitment function $R(t)$. Note that the graphs are plotted on different scales on the y-axes for different IL-2 concentrations; this is done to demonstrate the quality of the mode fits to data. The sums of squared residuals for the fits are: 7.28×10^6 (IL-2 = 50 U/ml), 1.03×10^6 (IL-2 = 5 U/ml), 3.06×10^6 (IL-2 = 2.5 U/ml), 5.23×10^5 (IL-2 = 1.25 U/ml). Parameter values providing the best fits are given in Table I.

which we denote the number of cells undergone 6 or more divisions. Confidence intervals have been calculated by bootstrapping the residuals with 500 simulations (39). Statistical comparison of nested models was done using the F-test (40, 41). Simulations were done in *Mathematica* 4.0 using FindMinimum routine and external functions coded in C. Solutions of delay differential equations have been obtained using routine retard.f described by Hairer et al. (42) and ported to C. *Mathematica* notebooks and relevant C codes are available from the authors upon request. Additional results and mathematical derivations are given as Supplementary Material (available online at http://theory.bio.uu.nl/vitaly/publ/Ganusov07_ji_appendix.pdf).

Results

Fitting CFSE data

We fitted the CFSE data with the modified version of the SM model (see *Materials and Methods*, Equations 2–4) with division-dependent death rate (Fig. 3). These data have been fitted previously by several models (20, 22), but the extended SM model for the first time accurately describes the dynamics of cells having undergone a few divisions at low IL-2 concentrations. In particular, at IL-2 = 2.5 U/ml, the maximum density of cells in each cohort is correctly predicted to be reached by the cohort of cells having completed two divisions (Fig. 3*C*).

The total number of cells successfully completing their first division, which is the total area under the recruitment function $R(t)$, is similar for the three highest IL-2 concentrations ($C \approx 9 \times 10^3$, see Figs. 4*A* and 5*A* and Table I). At these conditions, ~15% of the initial cell number ($9 \times 10^3/(2 \times 3 \times 10^4) \approx 15\%$) is completing their first division. At the lowest IL-2 concentration (IL-2 = 1.25 U/ml), only half of that number is completing the first division ($C \approx 4.0 \times 10^3$). The mean time cells require to successfully complete their first division, $T_1 = \mu' + \Delta_0$, is hardly affected by the IL-2 concentration (Fig. 5*B*).

IL-2 only moderately affects the average interdivision time of dividing cells. At IL-2 concentrations above 2.5 U/ml, it is roughly constant ($T \approx 13.6$ h), and increases by 15% at the lowest IL-2 concentration ($T \approx 15.7$ h at IL-2 = 1.25 U/ml, Fig. 5*C* and Table I). This change is not statistically significant ($p > 0.05$, calculated from the bootstrap estimates). The minor role that IL-2 plays in setting the interdivision time was further supported by fitting the model to the CFSE data for all IL-2 concentrations simultaneously; fixing the interdivision time T for all IL-2 concentrations hardly affected the quality of the fits ($p = 0.19$, F-test). Our analysis also

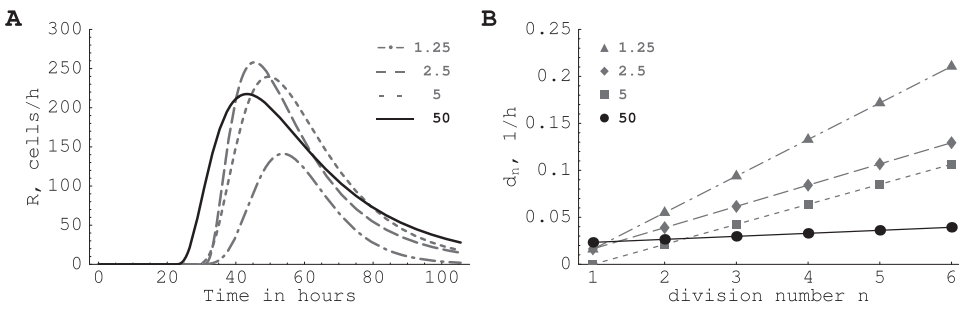

FIGURE 4. Estimated recruitment function $R(t)$ (*A*) and the death rate of divided cells (*B*) for the four IL-2 concentrations (IL-2 = 50 U/ml: continuous lines, 5 U/ml: small dashing lines, 2.5 U/ml: large dashing lines, and 1.25 U/ml: dot-dashed lines). The death rate of dividing cells is linearly changing with the division number n, $d_n = d_1 + a(n - 1)$.

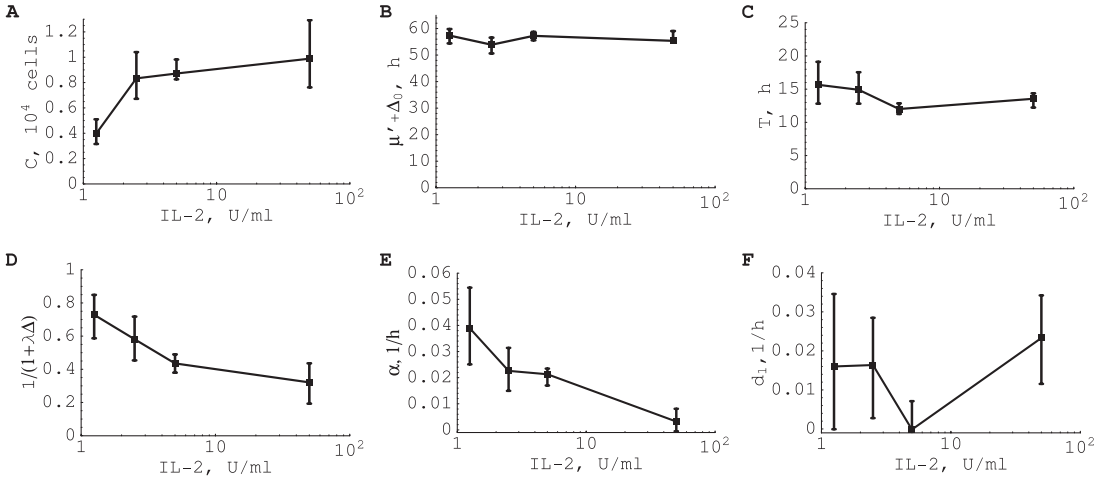

FIGURE 5. Parameters estimated by fitting the SM model with division-dependent death rate d_n plotted as a function of the IL-2 concentration. The total number of cells successfully completed first division C (*A*), the average time required to complete first division by surviving cells $\mu' + \Delta_0$ (*B*), the average cell cycle time of dividing cells $T = \Delta + 1/\lambda$ (*C*), the degree of stochasticity of the cell cycle (*D*), the slope of the death rate α (*E*), and death rate d_1 of cells having completed one division (*F*). Error bars, 95% confidence limits as obtained by bootstrapping the residuals with 500 simulations.

allows us to address the question of whether cell division becomes more or less stochastic at increasing IL-2 concentrations. As it was pointed out by Smith and Martin (28), stochasticity in the interdivision time of cells may arise due to variation in the length of the G_1 phase of the cell cycle. In the SM model the stochasticity of the division, time is calculated as the ratio of the average duration of the stochastic A-state λ^{-1} (which is approximately the duration of the G_1 phase of the cell cycle) to the average interdivision time $T = \Delta + \lambda^{-1}$. This ratio, $1/(1 + \Delta\lambda)$, is depicted as a function of the IL-2 concentration in Fig. 5*D*. Cell division becomes significantly more deterministic at higher IL-2 concentrations, i.e., at high IL-2 concentrations, cells have a shorter G_1 phase, and given a constant interdivision time, a larger S + G_2 + M phase.

The latter result is surprising because intuitively one expects a longer B phase when IL-2 concentration is low. Because T cells at low IL-2 concentration have a longer A-state (see Table I), such cells could be better "prepared" for division and could traverse through the B phase faster. For example, such cells could have a higher RNA content at the end of the G_1 phase (i.e., A-state), which is known to affect the duration of the S phase of the cell cycle (43, 44). In contrast, this result may be an artifact of the SM model because an increase in stochasticity of cell division at the fixed average interdivision time can only be accounted for with a shorter B phase. Indeed, our estimate for the length of the B phase at IL-2 = 1.25 U/ml appears to be somewhat shorter than previous estimates of S + G_2 + M phases for lymphocytes (43). More complex models for cell division incorporating extra parameters for stochasticity of cell division will have to be used to investigate this result further. Several variants of such models are currently being developed (20, 45). Whatever the precise mechanism, our results do suggest that at high concentrations of IL-2, CD4$^+$ T cell division becomes fairly deterministic, as was proposed earlier (12).

In BrdU-labeling experiments, Deenick et al. (22) have observed that IL-2 has strong influence on the fraction of BrdU$^+$ cells in the culture. At the lowest IL-2 concentration, 40% of cells were BrdU$^+$ in each division while at IL-2 = 50 U/ml, around 80% of cells were BrdU$^+$. This observation is consistent with the parameter estimates of our model. In the SM model, recruitment of cells into the S phase of the cell cycle is determined by the rate parameter λ. This parameter is strongly affected by the IL-2 concentration such that at a low IL-2 concentration a longer time is required for a cell to be recruited into the S phase (Table I).

Confirming our previous results (20), we find that IL-2 has the most profound effect on the death rate of dividing CD4$^+$ T cells and, specifically, on the increase of the death rate with the division number. The rate α, at which the death rate increases with the division number, increases with the decreasing IL-2 concentration (Figs. 4*B* and 5*E* and Table I). At IL-2 = 50 U/ml, the death rate hardly changes with the division number, and 20–40% of cells die per cell cycle. At the lowest IL-2 concentration the change in the death rate with the division number is significantly different from zero resulting in 20% and 90% of cells dying in the first and the sixth divisions, respectively. Note that decreasing the IL-2 concentration from IL-2 = 50 U/ml to 1.25 U/ml increases the slope α >10-fold, whereas the change in the relative number of cells recruited into the response given by the parameter C over the same range of IL-2 concentrations is more modest (little over 2-fold). Although the number of cells recruited into the response does depend on the IL-2 concentration (Ref. 22; see Fig. 1), the magnitude of the CD4$^+$ T cell response is mainly determined by the changes in the death rate with the division number.

The death rate of cells that have divided once, d_1, is approximately independent of IL-2 concentration (Fig. 5*F* and Table I); the low estimate for d_1 at IL-2 = 5 U/ml is probably due to an experimental error in estimation of total cell numbers as discussed previously (20). Given that there are only small changes in the average interdivision time with the IL-2 concentration, these results suggest that magnitude of expansion of populations of naive CD4$^+$ T cells in vitro is largely controlled by regulating the rates of cell death, and hardly by the rates of cell division.

Comparison to other models

The SM model with a death rate linearly changing with the division number gives an excellent description of the dynamics of the CFSE-labeled CD4$^+$ T lymphocytes. We have also tested whether simpler models with a smaller number of parameters can describe these CFSE data. Two simpler models have been tested: a model with random division in which interdivision times are exponentially distributed (obtained by letting $\Delta \to 0$), and a model with deterministic division in which division takes a fixed time period (by letting $\lambda \to \infty$). Both models gave rather poor fits at all IL-2 concentrations ($p < 0.001$ for all models, F-test). The poor fits of the random division model are due to a very rapid cell division of some cells since the model lacks a minimum interdivision time (19). Poor

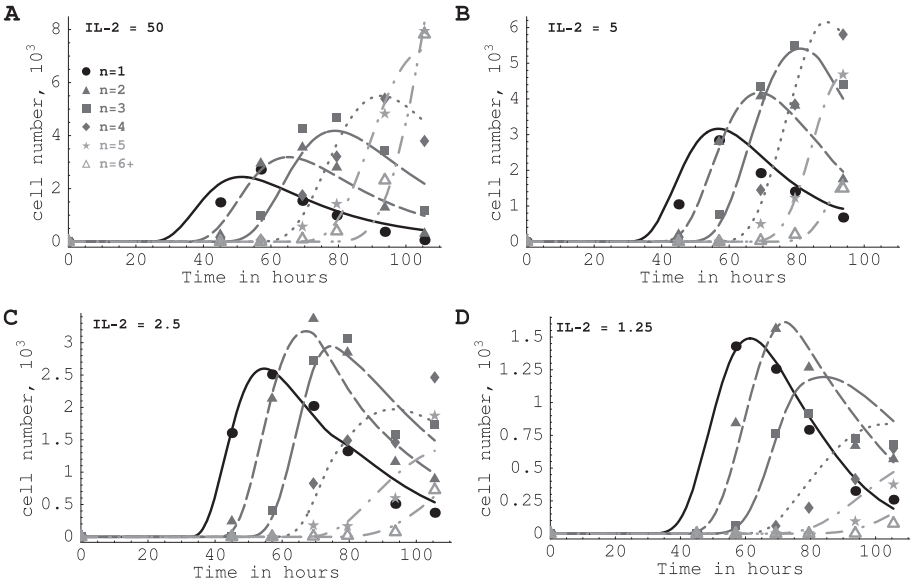

FIGURE 6. Best fit of the IL-2 consumption model to the CFSE data of CD4$^+$ T cells stimulated in vitro with anti-CD3 Abs at four initial concentrations of exogenous IL-2: IL-2 = 50 (*A*), 5 (*B*), 2.5 (*C*), and 1.25 (*D*) U/ml. Notations for symbols and lines are the same as in Fig. 3. Parameters providing the best fit are: C = (4.2, 9.0, 12.1, 10.5) $\times$ 10^3 cells and Δ_0 = (20.13, 30.53, 53.25.49, 21.25) hours for IL-2 = (1.25, 2.5, 5, 50) U/ml, respectively; λ_1 = 0.046 1/h, λ_2 = 0.24 1/h, Δ_1 = 7.24 h, Δ_2 = 12.71 h, d_1 = 0.04 1/h, d_2 = 0.029 1/h, h_λ = 0.97 U/ml, h_Δ = 66.1 U/ml, $h_d = 4.5 \times 10^{-3}$ U/ml, $h_I = 1.6 \times 10^{-2}$ U/ml, $c_1 = 2.8 \times 10^{-4}$ U/(ml $\cdot$ cell $\cdot$ h), and $c_2 = 1.8 \times 10^{-2}$ U/(ml $\cdot$ cell). The sum of squared residuals is 1.36×10^7.

fits of the deterministic division model are due to the inability of the model to "convert" an asymmetric recruitment function $R(t)$ into the later symmetric time course for cohorts of cells having undergone three and more divisions (see Introduction and Ref. 20).

Allowing the parameters for cell division such as λ and Δ to change linearly with the division number in the SM model with a linearly changing death rate d_n did not lead to an improvement of the quality of the fits ($p > 0.05$, F-test). Surprisingly, allowing only the commitment rate λ or the duration of the B phase Δ, to change linearly with the division number n led to fits of a similar quality as those with division-dependent death rate (results not shown). These fits predicted a relatively fast increase in the average interdivision time $T_n = \Delta_n + 1/\lambda_n$ with the division number n (either by increasing Δ_n or decreasing λ_n with n, see Supplementary Material). This is not in agreement with the BrdU-labeling experiments suggesting that there are limited, if any, changes in the cell division parameters with the IL-2 concentration (22).

Changes in the dynamics of lymphocytes may have also occurred due to reduction in the amount of IL-2 in the medium due to "consumption" of IL-2 by dividing T cells (37, 38). How IL-2 concentration would affect the rates of cell division and death, and how cells "consume" IL-2 is not well understood. A simple SM model in which lymphocytes could reduce the medium concentration of IL-2 was fitted to data (see *Materials and Methods* for model description). This model describes the dynamics of CFSE-labeled CD4$^+$ T cells very well (Fig. 6), although with somewhat lower quality than the SM model with division-dependent death rate (based on sum of squared residuals). The fits predict that the rate of recruitment into the B phase λ is affected the most by changes in the IL-2 concentration (Fig. 7). This is in disagreement with the prediction of the SM model with division-dependent death rate suggesting that the main effects of IL-2 is on the rate of cell death.

Importantly, the fits also predict that when the initial IL-2 concentration is low (IL-2 $\leq$ 2.5 U/ml), IL-2 should be depleted in the medium by 70 h after initial stimulation (Fig. 8). This is well supported by the observation in the CFSE data that at 70 h after stimulation the maximum density reached by cell cohorts is decreasing with the division number (Fig. 6, *C* and *D* and Introduction). The latter in turn suggests that at 96 h, recruitment of cells into the S phase of the cell cycle, which is determined by the parameter λ, should be slow (see Fig. 7*A*). By this lower λ, the prediction of the model is that 5 times fewer BrdU$^+$ cells should be found in BrdU-labeling experiments at IL-2 = 1.25 U/ml than at IL-2 = 50 U/ml (Fig. 7*A*). This prediction is not in agreement with the data of Deenick et al. (22) demonstrating that the difference in the fraction of BrdU$^+$ cells at IL-2 = 1.25 U/ml and IL-2 = 50 U/ml was only 2-fold at 96 h (22). We cannot rule out the possibility that changing the structure of the IL-2 consumption model may lead to a slower rate of IL-2 depletion, but it seems that the data argue against it. Summarizing, the SM model with division-dependent death rate does the best job at describing both CFSE data and explaining BrdU-labeling experiments.

In addition, the parameters governing cell division and death could be affected by the time since stimulation, for example, due

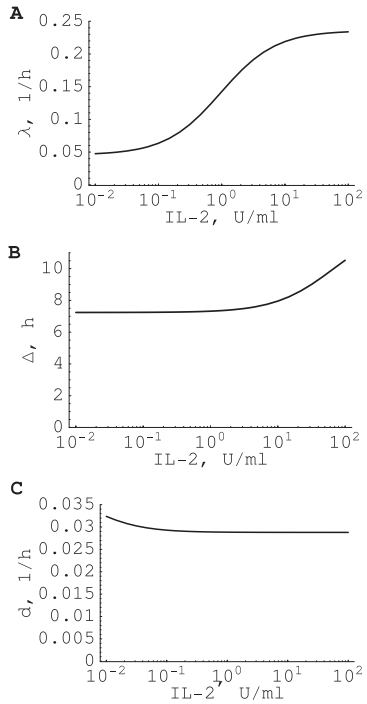

FIGURE 7. Estimated parameters for cell division and death in the IL-2 "consumption" model as the function of the IL-2 concentration. Changes in the rate of commitment to division λ (*A*), the duration of the B phase Δ (*B*), and the death rate d (*C*) with the IL-2 concentration given in Equation 6 are plotted. Estimates of coefficients determining changes in these parameters with the IL-2 concentration are listed in the legend of Fig. 6.

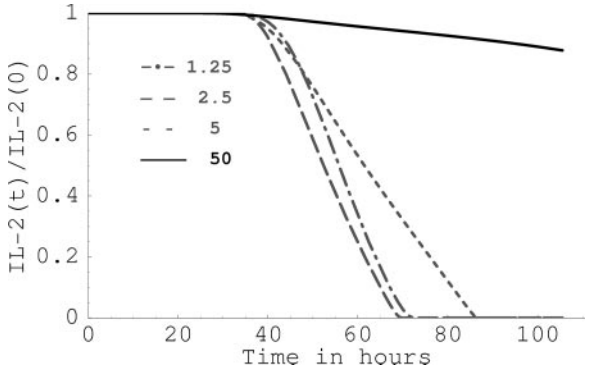

FIGURE 8. Predicted medium IL-2 concentration as the function of time since stimulation in the IL-2 "consumption" model. The plotted IL-2 concentration is normalized to its initial value. Predictions are for the initial concentration of IL-2 = 50 U/ml (continuous lines), 5 U/ml (small dashing lines), 2.5 U/ml (large dashing lines), and 1.25 U/ml (dot-dashed lines). Estimates of the model parameters are listed in the legend of Fig. 6.

to worsening of medium conditions. Although such changes are not very likely to occur (see *Materials and Methods*), we have tested whether the SM model with parameters changing with time since stimulation, can also describe the CFSE data. Allowing for a linear change in parameters with time, or a linear change starting at some critical time, did not lead to high quality fits of the model to the data (results not shown). Obviously, we cannot rule out that parameters are changing over time since more complex functions could have provided better fits. However, in the absence of experimental data we prefer the IL-2 consumption model over general time-dependent models because it is more mechanistic and easily testable.

Discussion

There is a rapidly growing body of literature quantifying the dynamics of T cell responses in mice (46); nevertheless, in most cases we do not have clear understanding of relative contribution of proliferation and death processes during this dynamic (47). In this work we have demonstrated that one can retrieve both division and death rate from experimental data obtained using CFSE. This was done by building a set of mathematical models with different levels of complexity and fitting these models to the data. Statistical comparison of the fits produced by simple and more complex models is important for selection of a simplest model that can explain the data while making a minimal number of assumptions (48).

By using a rigorous theoretical framework, we have obtained a number of new results that complement and reevaluate the previous analysis of Deenick et al. (22), and emphasize the importance of testing several alternative models in how well they describe the data. Our main new result is that expansion of the population of CD4$^+$ T cells can be regulated by IL-2 largely by changing the rate of cell death. IL-2 has the most dramatic influence on the rate at which the death rate increases with the division number. IL-2 only moderately affects the cell interdivision time, but reducing the IL-2 concentration increases the degree of stochasticity of the cell division. Interestingly, a recent study has also found dependence of the rate of cell death and proliferation on the division number for lymphocytes stimulated with PMA in vitro (21).

IL-2 also has an influence on the dynamics of undivided cells by reducing the fraction of cells recruited into the response and successfully completing their first division. Furthermore, using statistical methods, we also have shown that the SM model is a minimal model required for the description of the CFSE data. Simpler models such as widely used random division model (20) or deterministic division model (12, 22), fail to adequately describe these data. In contrast, to discriminate between alternative SM models, additional experimental data from BrdU-labeling experiments, were required. This argues in favor of using the SM model and not more complex models (29–31) for the analysis of these CFSE data.

Our results suggest that IL-2 affects the magnitude of CD4$^+$ T cell expansion by affecting the rate of cell death. This does not contradict the notion that IL-2 is also necessary for cell division since we observed a tendency for longer cell interdivision times, and fewer cells were recruited into the response at low IL-2 concentrations.

In our analysis we have assumed that the rates of cell proliferation and death are identical for all CD4$^+$ T cells. Alternatively, there might be inherent heterogeneity in propensity to proliferate and/or die for some particular T cells. For example, distribution times of first division of T cells stimulated in vitro may dependent on the distribution of IL-2 receptors (49). More work is clearly required to investigate whether such inherent heterogeneity extends beyond the first cell division. We have assumed that the death rate of divided cells is exponentially distributed. Restricting death events to occur only in the A-state, or only in the B phase generally led to fits of similar quality as presented above (results not shown). Previous work suggested that changing the distribution of cell death over the cell cycle may affect the estimates for the rates of cell division and death, especially for declining cell populations (14, 18). CFSE data typically is not sufficient for estimating the distribution of death rates over the cell cycle (18). Future work on additional measurements (such as the fraction of cells in the S/G_2/M phases of the cell cycle), and combining CFSE and BrdU-labeling experiments may allow for a more precise estimation of the proliferation and death rates of proliferating T cells.

Our preference of the SM model with division-dependent death rate as the best description of the CFSE data was in part due to using additional, independent experimental data on labeling T lymphocytes with BrdU (see above). This clearly demonstrates that even for such large CFSE datasets (there are 6 time points in the CFSE data, much more than the majority of CFSE data in the field), several alternative models can describe the data relatively well, and additional information is required to reduce the number of likely models. However, it is not convincing that only one experimental observation is used to discriminate between distinct models. Indeed, once we have a set of candidate models that reasonably describe the data, additional measurements/experiments (in addition to BrdU labeling) may help to support/reject alternative models. Discriminating between the model with division-dependent death rate and the model with IL-2 consumption is crucial since they predict an opposite effect of IL-2 on parameters for cell division and death. The SM model with division-dependent death predicts that IL-2 affects mainly the cell death rate, while the IL-2 consumption model predicts that IL-2 largely affects the rate of cell proliferation (or the average interdivision time).

There are several ways in which predictions of different models analyzed in this paper can be tested. First, we have tried to test the prediction of our model that the death rate of cells changes with the division number, but were plagued by difficulties in measuring rates of cell death. Measuring the fraction of cells in early or late stages of apoptosis (by caspase-3, Annexin V, or PI staining) at one time instance does not deliver an estimate of the rate at cell death (50, 51). We have extended the current SM model to include the dynamics of cells in an proapoptotic state, for example, caspase-3+ cells, making it possible to test the prediction of the model if the relevant data become available. However, this requires additional kinetic assumptions on how cells become proapoptotic and how proapoptotic cells actually die (results not shown).

Alternatively, one could try to directly measure the dynamics of dead cells having undergone different numbers of divisions. A simple extension of the SM model to include the dynamics of dead cells suggests that having such data allows one to discriminate between the SM model with division-dependent death rate d_n and division-dependent commitment rate λ_n (see Supplementary Material). At the present, however, it is too ambiguous to determine the number of dead cells per division class because 1) CFSE peaks cannot be clearly identified; 2) the variance in the CFSE distribution of dead cells is quite different from that of divided cells; and 3) it is not known how much CFSE cells lose when they die. Measuring the medium IL-2 concentration, or adding IL-2 at late time points to see whether such interventions affect cell dynamics is a direct test of the IL-2 "consumption" model. Addressing these experimental issues, as well as measuring the rate of accumulation of dying cells by markers of apoptosis, will allow for a major advance in understanding kinetics of cell proliferation, and could be used to examine the predictions of our and other mathematical models.

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Disclosures

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