Dynamics of CD8⁺ T Cell Responses during Acute and Chronic Lymphocytic Choriomeningitis Virus Infection

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Dynamics of CD8+ T Cell Responses during Acute and Chronic Lymphocytic Choriomeningitis Virus Infection

Christian L. Althaus, Vitaly V. Ganusov, and Rob J. De Boer

Infection of mice with lymphocytic choriomeningitis virus (LCMV) is frequently used to study the underlying principles of viral infections and immune responses. We fit a mathematical model to recently published data characterizing Ag-specific CD8+ T cell responses during acute (Armstrong) and chronic (clone 13) LCMV infection. This allows us to analyze the differences in the dynamics of CD8+ T cell responses against different types of LCMV infections. For the four CD8+ T cell responses studied, we find that, compared with the responses against acute infection, responses against chronic infection are generally characterized by an earlier peak and a faster contraction phase thereafter. Furthermore, the model allows us to give a new interpretation of the effect of thymectomy on the dynamics of CD8+ T cell responses during chronic LCMV infection: a smaller number of naive precursor cells is sufficient to account for the observed differences in the responses in thymectomized mice. Finally, we compare data characterizing LCMV-specific CD8+ T cell responses from different laboratories. Although the data were derived from the same experimental model, we find quantitative differences that can be solved by introducing a scaling factor. Also, we find kinetic differences that are at least partly due to the infrequent measurements of CD8+ T cells in the different laboratories. The Journal of Immunology, 2007, 179: 2944–2951.

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2 Address correspondence and reprint requests to Christian L. Althaus, Theoretical Biology, Utrecht University, 3584 CH Utrecht, The Netherlands. E-mail address: c.l.althaus@uu.nl
3 Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; p.i., postinfection; SThx, sham-thymectomized; Thx, thymectomized; CI, confidence interval.

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Materials and Methods

The data used in our analysis have been published recently (4, 14). In the study by Wherry et al. (4), 4- to 6-wk-old female C57BL/6 mice were infected with LCMV Armstrong (2 × 10^6 PFU, i.p.) or LCMV clone 13 (2 × 10^6 PFU, i.v.) causing acute and chronic infection, respectively. The numbers of Ag-specific CD8^+ T cells/spleen were determined by MHC tetramer staining at several time points after infection against the following epitopes: NP396, GP33, GP34, and GP276. Mice infected with LCMV Armstrong were able to control the virus effectively in serum and tissues, whereas in mice infected with LCMV clone 13, virus persisted at high levels in the brain and kidneys (4). The study by Miller et al. (14) used Thx and SThx C57BL/6 mice. Surgeries were performed when the mice were ~6 wk old. Two to 3 wk after surgery, mice were infected with 2 × 10^6 PFU of LCMV clone 13 i.v. Specific CD8^+ T cells against the epitopes NP396, GP33, and GP276 were measured as cells per spleen by MHC tetramer staining. At day 80 p.i., virus was cleared in the liver but persisted in the lung. For a more detailed description of how data were obtained, we refer to the original studies (4, 14). The data were provided to us by the authors of these papers.

Different models have been proposed to describe the dynamics of CD8^+ T cells after viral infections (8, 9, 12, 15). De Boer et al. (12) published a mathematical model that was fitted to CD8^+ T cell responses following LCMV Armstrong infection. In this study, we used the same model to estimate the dynamics of CD8^+ T cell responses during acute and chronic LCMV infection. The model considers clones of activated (A) and effector/memory (M) cells. Because the proliferation of T cells after antigen stimulation seems to be programmed (16–19), the dynamics are split into two distinct phases. Before the peak of the response at time T_{off}, there is rapid proliferation. After the peak, activated cells die or turn into effector/memory cells.

The dynamics can be described by a set of ordinary differential equations. During the expansion phase, when t ≤ T_{off}, we assume that there are no effector/memory cells and activated T cells A proliferate according to the following:

\[ \frac{dA}{dt} = \rho A, \]  

(1)

where \( \rho \) is the net expansion rate, and \( A_0 \) is the initial number of cells at \( t = 0 \). More realistically, naive precursor cells should start proliferating after they have been recruited through antigen stimulation. For simplicity, we lump these precursor numbers and the recruitment time together, and consider a proliferation that starts at \( t = 0 \). Therefore, our estimates of \( A_0 \) are lower than the precursor frequency of naive cells that has been estimated by Blattman et al. (20). After the peak at time \( T_{off} \), activated cells A die with a net death rate of \( \delta_A \) (representing activation-induced cell death and normal death) and turn into effector/memory cells M at a rate \( r \). Therefore, the dynamics of activated and effector/memory cells for \( t > T_{off} \) can be described as follows:

\[ \frac{dA}{dt} = - (r + \delta_A)A, \]  

(2)

\[ \frac{dM}{dt} = rA - \delta_AM, \]  

(3)

where \( \delta_A \) represents the net death rate of effector/memory cells, consisting of a balance between cell renewal and death. This balance is maintained through ongoing cell division, which has been shown with BrdU labeling for cells that persist after acute and chronic LCMV infection (5, 14).

Using the number of activated cells at the peak of the response, \( A(T_{off}) \), as the initial condition for Equation 2, the solution of Equations 1–3 gives a continuous function for the total cell number \( N = A + M \). Parameter estimates of the differential equations were obtained by fitting the prediction of the total cell number, \( N \), to the data (taking the natural logarithm) based on the Levenberg-Marquardt algorithm (21) for solving nonlinear least-squares problems. The 95% confidence intervals (CIs) for the inferred parameters were determined using a bootstrap method (22), where the residuals to the optimal fit were resampled 500 times. When we compare a smaller model with restricted parameters to a bigger model with a higher number of free parameters, we perform the F test to compare the two nested models by the difference between their residual sum of squares per additional parameter, divided by the residual mean square of the larger of the two models (23). Given this value, the F distribution contains two parameters. The one in the numerator is the difference in the number of parameters between the two models. The one in the denominator is the number of degrees of freedom of the larger model (i.e., the number of data points minus the number of parameters). Throughout the paper, we accepted the hypothesis that the model with the larger number of parameters provides the best fit with a significance level of \( p < 0.05 \).

To compare data sets of CD8^+ T cell responses from different laboratories, we introduced a scaling factor, \( s \). First, the mathematical model was fitted to a specific CD8^+ T cell response from laboratory 1. Then, the estimated total cell numbers, \( N_i \), were scaled and used as a prediction for the specific CD8^+ T cell response from laboratory 2 (\( N_2 = sN_1 \)). Regression analysis and Student’s t test were performed using Gnumeric (version 1.0.12) and the R statistical software package (24). Significance was defined as \( p < 0.05 \).

Results

CD8^+ T cell responses during acute and chronic LCMV infection

We fit the data of Wherry et al. (4) with a simple mathematical model described in Materials and Methods to detect differences between the dynamics of CD8^+ T cell responses during acute and chronic LCMV infection (Fig. 1, Tables I and II). Due to the limited number of data points before the peak of the response, one cannot estimate the expansion rate, the time of the peak, and the initial cell number from these data. We therefore used previous estimates of the net expansion rate that were obtained by fitting the same mathematical model to CD8^+ T cell responses against LCMV Armstrong in the same mouse strain (13). The data used in this study had several measurements before the peak of the response and therefore allowed to estimate the expansion rates (25). Because clone 13 differs from LCMV Armstrong by 2 aa not affecting the relevant CD8^+ T cell epitopes (26, 27), we consider the initial expansion rate of the corresponding CD8^+ T cell responses to be similar for both acute and chronic LCMV infection (see also Discussion). We also force the number of naive precursor cells for each epitope to be the same for acute and chronic responses. Because there are no data points during the expansion phase for the GP34-specific CD8^+ T cell response during acute infection, we only fit the contraction phase of this response.

Fitting the model to all data sets of CD8^+ T cell responses during acute and chronic infection gave reasonable fits (Fig. 1). As observed in De Boer et al. (13), allowing the net death rate of effector/memory cells, \( \delta_{M} \), to be a free parameter failed to improve the quality of the fits and converged to a value close to zero for both acute and chronic infection. Therefore, we set \( \delta_{M} \) to be zero. The time of the peak, \( T_{off} \), appeared to be different between responses during acute and chronic infection but similar for the epitope-specific responses for one type of infection. Based on an F test, we could indeed restrict the model to a single \( T_{off} \) for the responses during acute infection, and another single \( T_{off} \) for the responses during chronic infection. Furthermore, the responses during acute infection could be fitted with the same effector/memory formation rate, \( r \). In combination, this means that we restricted the model having eight \( T_{off} \) parameters and eight \( r \) parameters for the four responses against both types of infection to a smaller model having five \( r \) parameters and two \( T_{off} \) parameters (i.e., reducing the number of parameters by nine, which gives \( F(9,225) = 1.6 \) and \( p = 0.13 \)).

CD8^+ T cell numbers specific for GP33 measured by intracellular cytokine staining for IFN-γ roughly correspond to the sum of GP33- and GP34-specific CD8^+ T cells obtained by MHC tetramer staining (25). To compare the dominance ranking for the four responses to the results in De Boer et al. (13), which were obtained by fitting the model to CD8^+ T cell numbers measured by intracellular cytokine staining, we use the sum of GP33- and GP34-specific CD8^+ T cells. The results are in agreement with that study. For responses during acute LCMV infection, the data shows the same dominance ranking for the epitopes at the peak and in the memory phase, given as GP33 + GP34 > NP396 > GP276.
We find the same hierarchy at the peak of the response during chronic LCMV infection, but the hierarchy is changed after the peak because the GP276 response becomes the largest, i.e., GP276 > GP33 + GP34 > NP396. The change in immunodominance can be explained by different apoptosis rates and different effector/memory formation rates between the responses during chronic infection (Fig. 2 and Table II). Apoptosis rates also differ between responses during acute infection, which is in contrast to the study by De Boer et al. (13); however, the differences are minor (Fig. 2A and Table I). Comparing the obtained parameters between acute and chronic responses shows two major differences. The studied responses during chronic infection generally show an earlier peak (6.5 days for chronic and 7.3 days for acute infection) and a faster contraction phase thereafter. For parameters, see Tables I and II.

Table I. Parameter estimates obtained by fitting the model to the data on CD8<sup>+</sup> T cell responses to four different epitopes during acute LCMV infection (Armstrong)

<table>
<thead>
<tr>
<th></th>
<th>NP396</th>
<th>GP33</th>
<th>GP34</th>
<th>GP276</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Value</td>
<td>95% CI</td>
<td>Value</td>
<td>95% CI</td>
</tr>
<tr>
<td>$\delta_t$</td>
<td>0.13</td>
<td>0.10–0.17</td>
<td>0.23</td>
<td>0.18–0.31</td>
</tr>
<tr>
<td>$r$</td>
<td>0.004</td>
<td>0.003–0.006</td>
<td>0.004</td>
<td>0.003–0.006</td>
</tr>
<tr>
<td>$T_{off}$</td>
<td>7.3</td>
<td>7.2–7.5</td>
<td>7.3</td>
<td>7.2–7.5</td>
</tr>
<tr>
<td>$A_0$</td>
<td>4.9</td>
<td>3.8–6.2</td>
<td>8.6</td>
<td>6.9–11.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Previous estimates of proliferation rates were used: 1.92 day<sup>-1</sup> for NP396, 1.89 day<sup>-1</sup> for GP33 and GP34, and 1.87 day<sup>-1</sup> for GP276 (13). The death rate of effector/memory cells is $\delta_t = 0$. 

FIGURE 1. A–H. The epitope-specific CD8<sup>+</sup> T cell responses during acute (A–D) and chronic (E–H) LCMV infection against the following epitopes: NP396, GP33, GP34, and GP276. Each data point represents one mouse as measured by Wherry et al. (4). The solid lines represent the best fit of the model to the data. Compared with the CD8<sup>+</sup> T cell responses against acute infection, responses against chronic infection are generally characterized by an earlier peak (6.5 days for chronic and 7.3 days for acute infection) and a faster contraction phase thereafter.
after the peak. During the contraction phase of responses during chronic LCMV infection, both the death rate of activated cells, and the effector/memory formation rate are faster (Fig. 2, A and B).

**Dynamics of CD8\(^+\) T cell responses in Thx mice**

Miller et al. (14) investigated the role of thymic output in the dynamics of Ag-specific CD8\(^+\) T cell responses during chronic LCMV infection (Fig. 3). The authors argue that there is a transient CD8\(^+\) T cell defect in Thx mice compared with SThx mice. The argument is based on the observation that there is a significantly lower CD8\(^+\) T cell response in Thx mice at day 30 p.i. compared with SThx mice for all measured epitopes. Because there is no significant difference in the responses at later time points after day 30 p.i., Miller et al. (14) conclude that cells in Thx mice later rebound to levels similar to those in SThx mice, which suggests that there is an increase of the responses in Thx mice between days 30 and 80 p.i. BrdU labeling between days 15 and 25 p.i., and between days 30 and 40 p.i. with subsequent flow cytometry at the end of each pulse showed no difference in the proliferation rates between Thx and SThx mice, however. Another explanation for the reduced number of T cells in day 30 p.i. for Thx mice is that T cells are more sequestered to peripheral tissues because of higher viral load at these sites, compared with SThx mice (14).

To test the hypothesis of a transient CD8\(^+\) T cell deficit in Thx mice, we performed a Student \( t \) test to check whether the CD8\(^+\) T cell numbers at days 30, 45, and 80 p.i. are significantly different from each other but found no evidence \((p > 0.05)\). Furthermore, we performed a linear regression between all data points at days 30, 45, and 80 p.i., and found no evidence for a slope significantly different from zero \((p > 0.05)\). Both these analyses argue against the increase in T cell responses in Thx mice from day 30 p.i.

This statistical analysis is in agreement with the results of our dynamical model: the stable phase of effector/memory cells given \(\delta_{eff} = 0\) indicates that there is no CD8\(^+\) T cell rebound after day 30 p.i. Therefore, we fit our model to the data describing CD8\(^+\) T cell responses against three epitopes for SThx and Thx mice concurrently. Again, there is only one time point of measurements before the expected peak of the response (at day 5 p.i.). Hence, we set the proliferation rates to previously estimated parameters (13), and assume the time of the peak, \(T_{off}\), to be equal for Thx and SThx mice because both of them are infected with the same type of virus. The only parameters that can vary between Thx and SThx mice are the initial number of naive cells that become activated, \(A_0\), the rate of effector/memory cell formation, \(r\), and the net death rate of activated cells, \(\delta_{A}\). Because young Thx mice are expected to have markedly lower naive T cell numbers \((\sim 50\%\) compared with control mice\) (I. den Barber, manuscript in preparation), a basic hypothesis to test is whether we can account for the difference between the responses by assuming a difference in the initial number of T cells \(A_0\) only. Indeed, the quality of the fit is not reduced when we restrict \(r\) and \(\delta_{A}\) to be the same in Thx and SThx mice \((F_{2,185} = 0.75; p = 0.48)\), which is not the case for restricting identical \(A_0\) with a free \(r\). Furthermore, we are able to estimate a constant fraction of 62\% for the initial number of specific T cells in Thx mice compared with SThx mice (Table III). Given this result, we are able to explain the differences in T cell responses for SThx only by a lower precursor frequency of naive T cells.

**Comparing data of CD8\(^+\) T cell responses between laboratories**

The availability of data characterizing CD8\(^+\) T cell responses against LCMV infections from different laboratories allows us to examine whether the published data are consistent with each other. We can compare the dynamics of T cell responses during acute LCMV infection between the study of Homann et al. (25) and Wherry et al. (4). Additionally, we have two studies that quantify T cell responses against chronic LCMV infection (4, 14). It is important to note that these studies measure CD8\(^+\) T cell responses after the same challenge with virus in the same mouse strain (see Materials and Methods). The only difference is that, compared with normal C57BL/6 mice in Wherry et al. (4), Miller et al. (14) use SThx mice. However, it is typically assumed that CD8\(^+\) T cell dynamics do not show a different behavior due to surgery without thymus removal.

First, we compared the data on CD8\(^+\) T cell responses against NP396 and GP276 during acute LCMV infection from Wherry et al. (4) to previously estimated parameters by De Boer et al. (13). Those parameters were obtained by fitting the model to

![Figure 2](http://www.jimmunol.org/DownloadedFrom/fig2.jpg)

**FIGURE 2.** A and B. Estimated death rates of activated cells, \(\delta_{A}\) (A), and estimates of the rate, \(r\), at which effector/memory cells are formed for CD8\(^+\) T cell responses during acute and chronic LCMV infection (B). Error bars represent 95\% CI of parameter estimates. The rates are largely increased for responses during chronic infection except for the specific response against GP33.
data from Homann et al. (25), which resulted in very accurate fits. In Fig. 4, A and B, the data are shown together with the previously estimated dynamics depicted by the solid line. For both responses, the data are described well around the peak, whereas it drops below the previous estimate after 30 days p.i. We also compared responses during chronic infection. NP396- and GP276-specific CD8\(^+\) T cell responses as measured in Miller et al. (14) are shown together with the prediction of the model fitted to data by Wherry et al. (4) (Fig. 4, C and D). In this study, the estimated dynamics are far below the data of CD8\(^+\) T cell responses from the other laboratory. This comparison raises the question why T cell measurements using the same experimental protocol lead to different results. On the one hand, it is important to note that the T cell measurements in Homann et al. (25) were done using intracellular cytokine staining, whereas the other studies used MHC tetramer technology. However, a previous study using both techniques to count Ag-specific CD8\(^+\) T cells shows more consistent measurements (3). In contrast, it is likely that the total numbers of T cells that are measured depend on certain experimental procedures. For example, the preparation of a single-cell suspension of splenocytes is done by squeezing the spleen through a mesh. In this study, some laboratories use stainless steel, whereas others use meshes made of plastic. To account for problems with eliminating total cell numbers, we introduce a scaling factor where the prediction of cell numbers from one laboratory are given relative to the estimated cell numbers from another laboratory.

Fig. 4, E and F, again shows data from Wherry et al. (4) together with the CD8\(^+\) T cell dynamics as estimated in De Boer et al. (13). However, by fitting a scaling factor to account for differences in total numbers of T cells, the given lines describe the data much better (\(s_{H11005} = 0.41\)). Also in Fig. 4, G and H, the estimated parameters from one study describe the data from another study much better, given the application of a scaling factor (\(s_{H11005} = 6.4\)). Nevertheless,

**FIGURE 3.** A–F, Comparison of CD8\(^+\) T cell responses during chronic LCMV infection (clone 13) in SThx (A–C) and Thx (D–F) mice. Each data point represents one mouse as measured by Miller et al. (14). Statistical analysis using Student’s t test and linear regression (dashed line) showed no significant change in T cell numbers after day 30 p.i. for Thx mice and therefore no evidence for a rebound. The solid lines represent the best fit of the model to the data for each epitope. \(r\) and \(\delta_0\) are restricted to be the same in SThx and Thx mice, \(\delta_0 = 0\) and \(T_{off} = 5.6\) days. The difference in Thx mice can be explained by a reduced number of naive precursor cells (62%) compared with SThx mice. Other parameters are given in Table III.

**Table III.** Parameter estimates obtained by fitting the model to the data on CD8\(^+\) T cell responses against three epitopes in SThx mice and Thx mice during chronic LCMV infection (clone 13)\(^a\)

<table>
<thead>
<tr>
<th>Name</th>
<th>Units</th>
<th>NP396 Value 95% CI</th>
<th>Value 95% CI</th>
<th>GP33 Value 95% CI</th>
<th>Value 95% CI</th>
<th>GP276 Value 95% CI</th>
<th>Value 95% CI</th>
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<tbody>
<tr>
<td>(\delta_0)</td>
<td>day(^{-1})</td>
<td>0.23 0.15–0.35</td>
<td>0.19 0.11–0.31</td>
<td>0.072 0.026–0.215</td>
<td>0.035 0.010–0.080</td>
<td>0.62 0.54–0.72</td>
<td>0.60 0.54–0.72</td>
</tr>
<tr>
<td>(r)</td>
<td>day(^{-1})</td>
<td>0.017 0.012–0.026</td>
<td>0.024 0.015–0.035</td>
<td>0.010–0.100</td>
<td>0.010–0.080</td>
<td>5.6 5.4–5.8</td>
<td>5.6 5.4–5.8</td>
</tr>
<tr>
<td>(T_{off})</td>
<td>days</td>
<td>5.6 5.4–5.8</td>
<td>5.6 5.4–5.8</td>
<td>5.6 5.4–5.8</td>
<td>5.4–5.8</td>
<td>5.6 5.4–5.8</td>
<td>5.4–5.8</td>
</tr>
<tr>
<td>(A_0) in SThx</td>
<td>Cells</td>
<td>42.8 28.4–58.9</td>
<td>95.5 62.3–136.9</td>
<td>46.0 30.0–65.0</td>
<td>30.0–65.0</td>
<td>46.0 30.0–65.0</td>
<td>30.0–65.0</td>
</tr>
<tr>
<td>(\alpha)</td>
<td></td>
<td>0.62 0.54–0.72</td>
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<td>0.62 0.54–0.72</td>
<td>0.62 0.54–0.72</td>
</tr>
</tbody>
</table>

\(^a\) The death rate of effector/memory cells, \(\delta_0\), is zero. The number of naive precursor cells in Thx mice are 62% of that in SThx mice. The fraction is given as \(\alpha\).
there are still kinetic differences in the data between laboratories as can be seen at 15 days p.i. in Fig. 4, E and F, and at days 15 and 80 p.i. in Fig. 4H. Together, this comparison shows distinct differences between measurements of CD8<sup>+</sup> T cell responses from different laboratories.

**Discussion**

Our analysis of CD8<sup>+</sup> T cell responses allowed us to get new insights into their dynamics. The parameter that differs between Ag-specific responses is the apoptosis rate $\delta_A$. For responses during chronic infection, also the effector/memory formation rate, $r$, varies. Comparing acute to chronic infection, we observed that the time of the peak of the response, $T_{off}$, is different. All other parameters are the same. Zajac et al. (5) already observed that immunodominance changes during chronic LCMV infection. We can now further discriminate the CD8<sup>+</sup> T cell responses between acute and chronic LCMV infection. The four studied responses during chronic infection generally show an earlier peak and a faster contraction of the response. In our model, at the time of the peak of the response, cells stop to proliferate. Virus replication and dynamics could affect this process. Although LCMV Armstrong and clone 13 differ by only 2 aa (26, 27), virus replication differs dramatically for chronic infection compared with acute infection. Between days 5 and 8 p.i., acute LCMV infection shows a decrease in serum viral load compared with chronic LCMV infection where virus load is increasing (Fig. 5) (data from Wherry et al. (4)). Ongoing expansion of CD8<sup>+</sup> T cells may cause immunopathology when the virus is not cleared. Therefore, the CD8<sup>+</sup> T cell proliferation might be stopped to reduce acute symptoms (28).

In Results, we assumed the expansion rates to be the same for an epitope-specific CD8<sup>+</sup> T cell response during acute and chronic LCMV infection. We also wanted to test two alternative hypotheses for the different dynamics during proliferation. First, we fitted the model to the data with a free expansion rate for CD8<sup>+</sup> T cell responses during chronic infection but a restricted $T_{off}$ and $A_0$ for responses during acute and chronic infection. Second, CD8<sup>+</sup> T cells might start to proliferate earlier in one type of infection, i.e., the recruitment time could be different. As mentioned in Materials and Methods, in our model, the recruitment time is at $t = 0$ and lumped together with the precursor frequency $A_0$. We can also
introduce a $T_{\text{on}} \neq 0$ for later recruitment and a constant $\Delta T = T_{\text{off}} - T_{\text{on}}$ for the time window of proliferation. Therefore, we fitted the model to the data with the same expansion rates and a single $\Delta T$ but a different $T_{\text{on}}$ for the responses during acute and chronic infection. However, the quality of both fits proved to be worse than the one described in Results (based on mean squares; results not shown), and therefore we do not favor these alternative explanations. In Fig. 5, it can be seen that the serum viral load during the first 5 days is higher for chronic than acute infection. This is likely to be due to the higher infectious dose of clone 13. Indeed, there appears to be a constant log difference in serum viral load during the first 5 days, which suggests a similar replication rate of both virus strains during that time. The higher serum viral load for chronic infection could result in an earlier recruitment and/or a faster proliferation of the CD8$^+$ T cell responses. However, the fitting of the two alternative models described the chronic responses during proliferation with a later recruitment or a slower proliferation, which would disagree with the higher viral load. In combination with the lower quality of the fits of the alternative models, this analysis suggests that the earlier time of the peak during chronic infection is the most likely explanation. More measurements of CD8$^+$ T cell responses during the expansion phase (in between days 3 and 6 p.i.) would be helpful to check whether the proliferation rates for responses during acute and chronic infection are the same, and whether our hypothesis of an earlier peak is valid. The faster contraction rate after the peak derives from differences in the net death rate of activated cells, $\delta_2$. It was recently shown that the cytokine IL-10 is involved in the immune suppression induced by LCMV clone 13 infection (29, 30). Both the net death rate of activated cells and the time of the peak might be influenced by the levels of secreted IL-10. Another recent study by Grayson et al. (31) supports our estimates of the apoptosis rates, $\delta_3$. Their observation that Bim mutant (apoptosis gene knockout) mice exhibit a similar delay in the contraction of CD8$^+$ T cell responses to all epitopes during acute infection is in line with our finding that $\delta_3$ does not differ markedly between the different epitopes. Furthermore, the immunodominance is altered during chronic viral infection in Bim mutant mice, with the loss of Bim preferentially affecting the deletion of CD8$^+$ T cells to epitope NP396. Our estimates for chronic infection show clearly that differences in $\delta_3$ between epitopes vary largely, and that the apoptosis rate is largest for NP396.

The fitting of the model to data from Miller et al. (14) allowed us to give a reinterpretation of the effect of thymectomy on CD8$^+$ T cell responses during chronic LCMV infection. The only parameter that varied between SThx and Thx mice was the initial number of cells, $A_0$. Hence, we can account for the differences in the responses between SThx and Thx mice simply by differences in the number of naive T cells. This hypothesis is supported by findings that naive T cell numbers in Thx young mice are reduced by $\sim50\%$ (I. den Barber, manuscript in preparation). To test our prediction, one could also perform experiments infecting Thx mice with LCMV Armstrong to see whether cell numbers around the peak of the CD8$^+$ T cell response are lower compared with SThx mice. An alternative explanation for the difference in the CD8$^+$ T cell responses is the lack of continued thymic output of naive T cells in Thx mice. It has indeed been shown that there is continuous recruitment of naive T cells during persistent polyoma virus infection (32). Our model can also allow for the lower cell numbers in Thx mice by a difference in the net death rate of activated cells, $\delta_2$ (results not shown). A decrease in a net death rate can be interpreted as missing recruitment of naive cells into the activated T cell pool of Thx mice.

Because the analyzed data are derived by infecting mice of the same strain with LCMV, it is interesting to compare the different studies. We show that the data sets from different laboratories are kinetically and quantitatively different. Comparing the T cell measurements of a recent study by Grayson et al. (31) by digitizing their published data also shows large differences to the data sets we used for our analysis (results not shown). Upon introducing a scaling factor between laboratories to account for differences in the total number of cells, the data sets become more consistent. However, there are still kinetic differences. Indeed, several factors could influence the CD8$^+$ T cell responses in these experiments. For example, the environment where the mice are kept in laboratories might be different, and cause a distinct state of the mice immune systems. Especially SThx mice could have a more inflammatory environment due to surgery that might change the dynamics of CD8$^+$ T cell responses. Furthermore, the mouse age at the time of infection differs between the study by Wherry et al. (4) and that by Miller et al. (14), which might affect the number of CD8$^+$ T cells in the spleen. Even within a laboratory, data fluctuations are expected due to the intrinsic stochasticity of the immune response, the so-called process noise (33). Alternatively, the estimated kinetic differences could derive from the number of measurements over time. Again, several measurements around the peak of the response (i.e., between days 5 and 15 p.i.) appear to be important to precisely model the contraction phase of the response. However, some kinetic differences remain that seem to suggest different biological rates in different laboratories, e.g., different apoptosis rates (Tables I and II). Nevertheless, we would like to highlight the importance for multiple measurements around the peak to provide a better understanding of CD8$^+$ T cell dynamics.

Our analysis proved to be useful in discriminating the dynamics between CD8$^+$ T cell responses during acute and chronic LCMV infection. However, it remains to be established whether our results can be generalized for other LCMV-specific CD8$^+$ T cell responses not studied here. Although the analysis was done for responses that are dominant during acute or chronic infection, several other epitope-specific responses are likely to be important in clearance or control of viral replication. Indeed, a recent study by Kotturi et al. (34) identified 19 novel epitopes in LCMV that have not been investigated so far. Additional insight into the dynamics of CD8$^+$ T cell responses could be provided by knockout experiments of mice. The recent study by Grayson et al. (31) looks promising to get a more detailed knowledge on the influence of apoptosis during the contraction phase of the response. Further analysis in this direction can shed more light on the nature of acute and chronic infections and their accompanying cellular immune responses.
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Disclosures
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References