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Computer Lab Exercise

Quantifying immune responses to LCMV

Handout for the *Immunobiology* lecture, at Utrecht University.

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Objectives of this exercise:

1. Learn to think in a quantitative manner about immune reactions.
2. Learn to fit mathematical models to experimental data to obtain quantitative parameter estimates.
3. Identify the mechanisms underlying the immunodominance ranking of primary immune reactions.

This handout and all files can be downloaded from the LCMV directory.

Programmed immune responses

Vigorous infections with rapidly replicating bacteria or viruses trigger strong adaptive immune responses. A few days after the initial exposure, antigen specific naive T cells will become activated to undergo rapid clonal expansion, until the peak of the response, after which most of the activated cells die by apoptosis. The clonal expansion in mice infected with bacteria typically continues if the antigenic stimulus is removed by treating the mice with antibiotics, but the peak response is somewhat lower (Badovinac *et al.*, 2002). Similarly, the contraction after the peak will take place even if the antigen persists (Badovinac *et al.*, 2002). It has therefore been suggested that after proper antigenic stimulation the acute immune response of T cells is "programmed", and only partly regulated by the current concentration of antigen. Such a program can conveniently be modeled with piece-wise models (De Boer *et al.*, 2001, 2003; Kohler, 2007; De Boer and Perelson, 2013), that can be fitted to the experimental data from acute immune responses to viruses and bacteria. These models differ in the timing when memory T cells are formed. Classically it was assumed that memory T cells are formed during the contraction phase (De Boer and Perelson, 2013), but recent data suggest that they are also formed during the expansion phase (Kohler, 2007; Kretschmer and Buchholz, 2022; Abadie *et al.*, 2024).

To model this most recent view, consider antigen specific activated T cells, A, and memory cells, M,

$$\begin{cases} \mathrm{d}A/\mathrm{d}t = 0 \quad \text{and} \quad \mathrm{d}M/\mathrm{d}t = 0\,, & \text{if } t \le T_{\text{on}}\,, \\ \mathrm{d}A/\mathrm{d}t = (p-q)A \quad \text{and} \quad \mathrm{d}M/\mathrm{d}t = qA\,, & \text{if } T_{\text{on}} < t \le T_{\text{off}}\,, \\ \mathrm{d}A/\mathrm{d}t = -d_A A \quad \text{and} \quad \mathrm{d}M/\mathrm{d}t = -d_M M, & \text{otherwise}\,, \end{cases} \tag{1}$$

where p is the division rate (per day), d_A and d_M are death rates (per day), and q is the rate at which activated cells stop dividing and revert to quiescent memory T cells (also per day). The parameter T_{on} and T_{off} define the time points at which clonal expansion starts and ends, respectively. $A(0)$ is the initial number of cells specific for the epitope of interest (and $M(0) = 0$). This initial number of cells reflects the number of naive T cells specific for the epitope of interest. These cells are also called the 'precursor cells' of the cognate immune response, and their frequency in the naive T cell pool is called the precursor frequency. Kotturi *et al.* (2008) determined the precursor frequencies of several $CD8^+$ T cell responses to LCMV and report that naive B6 mice contain on average 445 precursors for the GP33 epitope, and 118 precursors for NP396, 42 precursors for the GP118, and 57 precursors for the NP205 epitopes, respectively.

We will use an R-script called Grind to fit this model to the data. For this practical you will need just two Grind

functions:

- `run()` integrates a model numerically and provides a time plot,
- `fit()` fits a model to data by estimating its parameters, and also depicts the results in a time plot.

The R-code to call these functions is provided and explained on the lcmv webpage. We have made things convenient for you by providing the functions `myrun()` and `myfit()` that call Grind's `run()` and `fit()` with parameters that are most appropriate for this exercise. We provide a zip-file containing four text files, each describing a $CD8^+$ T cell response to LCMV, and the R-scripts `grind.R` and `lcmv.R`. Use the link to the `lcmv.zip` file on the lcmv webpage to download the file, and unzip that file in a local folder on your device. The four text files, `gp33.txt`, `np396.txt`, `gp118.txt`, and `np205.txt`, contain data of the $CD8^+$ T cell responses to these four LCMV epitopes (Homann *et al.* (2001) and Kotturi *et al.* (2008)). If this fails copy these files one-by-one from the LCMV directory.

`grind.R` is a wrapper around R-libraries developed by Karline Soetaert and colleagues (Soetaert and Herman, 2009; Soetaert and Petzoldt, 2010; Soetaert *et al.*, 2010; Soetaert, 2009). If you have never used Grind before, you need to install these libraries. This is explained in the section **Installation** on this webpage. Open RStudio, install the "deSolve", "rootSolve", "coda" and "FME" libraries, and set the working directory to the folder containing the text files and the `grind.R` script (by Set working directory in the Session menu of RStudio). If you need help on this please watch the clip installGrind.mp4. We also provide a short clip on using Grind, grind.mp4.

Check how the model of Eq. (1) is defined in the function `model()` on the lcmv webpage. The parameters are defined in the vector `p` and the initial state is defined in the vector `s`. Note the model defines dA/dt, dM/dt, and dT/dt as the sum of the two. Only this total, T, is be fitted to the data. Once you understand the model function, open a new R-script in RStudio, and start copying gray R-chunks from the webpage into RStudio's main window. Place you cursor at the start of the function, and execute it by clicking the `Run` button (or by typing `Control Enter`). Be sure you understand every individual statement in each chunk and make notes!

Exercise 1 The LCMV primary immune response

a. Source the `grind.R` script, and copy-paste the chunks containing the model and the definition of `myrun()` and `myfit()` into RStudio, The chunk starting with `kotturi <- c()` defines the initial state, $A(0)$, and the parameters. Check in the model how these parameters define the kinetics of the expansion and the contraction phase. Next, run the model for the initial parameter values by calling `myrun()`. Explain the time plot on your screen in your own words (this plot should be identical to the first time plot on the lcmv webpage).

b. The next chunk let's you read the gp33 data (`read.table()`) and plot them. (If the reading of the data fails, you may have to set the working directory to the folder containing the text files and the `grind.R` script, by `Set working directory` in the `Session` menu of RStudio).

c. Next define which parameters of the model should be estimated during the fitting procedure, i.e., define which parameters are `free` to be fitted. Subsequently call the `myfit()` function to fit (the log of) the data. Interpret the parameter values: how fast do cells divide, when do they start to divide, etcetera. How good do you find this fit?

d. To check whether or not all parameters can be identified, ask for a summary of the fit by calling `summary(fit33)`.

e. Why is it better to fit the log of the data?

f. Next fit the np396 data set. Which parameters differ most between the gp33 and np396 responses? Which of the two responses is the largest? How can that be?

g. Next fit the gp118 data set (uncomment the lines in the R-chunk before executing them). Which parameters differ most between the three responses?

h. Finally fit the np205 response and interpret your results.

i. How would you explain the immunodominance ranking of the gp33, np396, gp118 and np205 responses?

Exercise 2 Formation of quiescent cells

In Eq. (1) memory T cells are formed during the expansion phase at a rate q. The value of q therefore seems to determine how many memory cells are formed. Study this with a simplified model where we say that a

fraction, f, of the divisions leads to a memory T cell during the expansion phase,

$$\frac{\mathrm{d}A}{\mathrm{d}t} = p(1-f)A \quad \text{and} \quad \frac{\mathrm{d}M}{\mathrm{d}t} = pfA\,, \tag{2}$$

and consider a typical expansion phase of about five days. This fraction, f, can also be interpreted as the probability that a dividing cell gives rise to a quiescent daughter cell. Note that during the expansion phase this model is identical to that of Eq. (1) because one can define $q = fp$. The model is provided on the quiescence webpage, with R-chunks that can be copy-pasted into RStudio (The R-script `quiescence.R` is also provided in the zip-file). Use the R-chunks to answer the following questions:

a. We estimated above that cells divide about two times per day, $p \simeq 2\ \mathrm{d}^{-1}$. What do you think is the optimal value of f that maximizes the number of memory cells that are formed?
b. Check your answer by running the model of Eq. (2) using the R-chunks on the quiescence webpage.
c. Does this optimum depend on the length of the expansion phase?
d. Does this optimum depend on the proliferation rate p?
e. Would evolution be able to select for an optimal value of the rate, q, at which memory T cells are formed?

Project

If you choose to do a project on the primary LCMV response you could start with studying the alternative model of Eq. (3); see the Question below. Afterwards it would be good to study the question which parameter(s) should truly be different between the four immune response. This can be done by improving the way the data are fitted. Fitting the four LCMV immune responses simultaneously their data, rather than one by one, it is easier to study which parameters best define the immuno-dominance ranking. At the bottom of the lcmv webpage you will find a a few lines showing how one can fit all four data sets together, allowing a subset of parameters to be shared, and others to be different, among the four responses. Because we have direct estimates of the initial number of cells, one can fix the $A(0) = T(0)$ values to those observed in the Kotturi data. This is achieved via the list `fixed`. The data can be fitted simultaneously to the model of Eq. (1), and/or to Eq. (3), and/or to novel variants you develop yourselves. When you fit Eq. (3) to the data, do compare your estimates of those in Fig. 2 of De Boer and Perelson (2013). Actually, the recent paper by Abadie *et al.* (2024) demonstrates that memory T cells are formed during both the expansion and contraction phase.

Exercise 3 Memory cells formed during the contraction phase

Previously memory T cells were assumed to be formed to be formed during the contraction phase (De Boer and Perelson, 2013), and the same data were fitted with the following model

$$\begin{cases} \mathrm{d}A/\mathrm{d}t = 0 \quad \text{and} \quad \mathrm{d}M/\mathrm{d}t = 0\,, & \text{if } t \le T_{\mathrm{on}}\,, \\ \mathrm{d}A/\mathrm{d}t = pA \quad \text{and} \quad \mathrm{d}M/\mathrm{d}t = 0\,, & \text{if } T_{\mathrm{on}} < t \le T_{\mathrm{off}}\,, \\ \mathrm{d}A/\mathrm{d}t = -(d_A + r)A \quad \text{and} \quad \mathrm{d}M/\mathrm{d}t = rA - d_M M, & \text{otherwise}\,, \end{cases} \tag{3}$$

where r is the rate at which activated cells revert to quiescent memory T cells.

a. Change the model to allow memory cells to be formed from activated cells during the contraction phase.
b. Fit the new model to the same data, and compare how the parameter estimates change.
c. Does your conclusion on the mechanisms explaining the immunodominance-ranking of these four responses depend on the timing when memory cells are formed?
d. Can this data be used to discriminate between these two mechanisms of forming memory cells? If not, what kind of data would be required?

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