How many TCR clones does the body maintain?

Competition model of T-cell repertoire

Grant Lythe and Carmen Molina-París (Leeds)
Robin Callard and Rollo Hoare (UCL)

Applied Mathematics, University of Leeds
http://maths.leeds.ac.uk/~grant

Ko te Moana-nui-a-Kiwa te moana

How many TCRβ chains are there in a body?

Early estimates Casrouge et al (2000), Arstila et al (1999)

10^6 distinct β chains in the human blood

extract and reverse transcribe mRNA from a pool of 10^8 cells, amplify VB18 from a sample of cDNA, analyse the subfraction that has VJ1.4 and 12-aa-long CDR3. 17 different β chains found.

$17/(0.093 \times 0.03 \times 0.008) \simeq 10^6$. 25 α chains per β chain?

More recent estimates Robins et al (2009), Warren et al (2011)

4×10^6 distinct β chains in the human blood

Direct counting of more than 10^6 distinct β chains

single-molecule DNA sequencing, millions of TCRβ determined, "unseen species" analysis. relative abundance, naive and memory repertoires compared

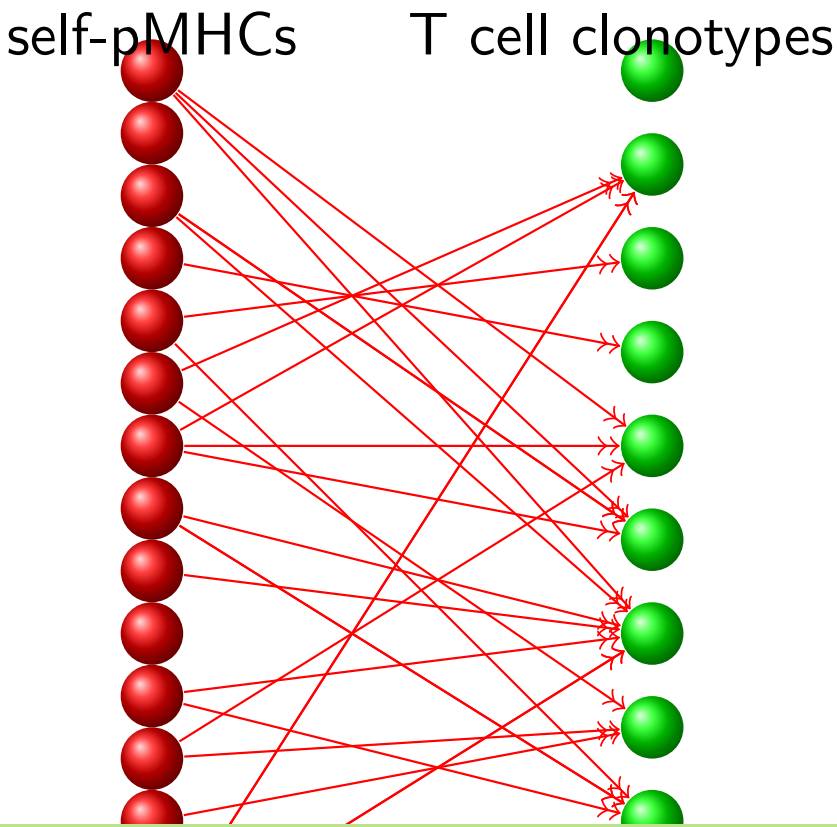
Single-cell analysis?

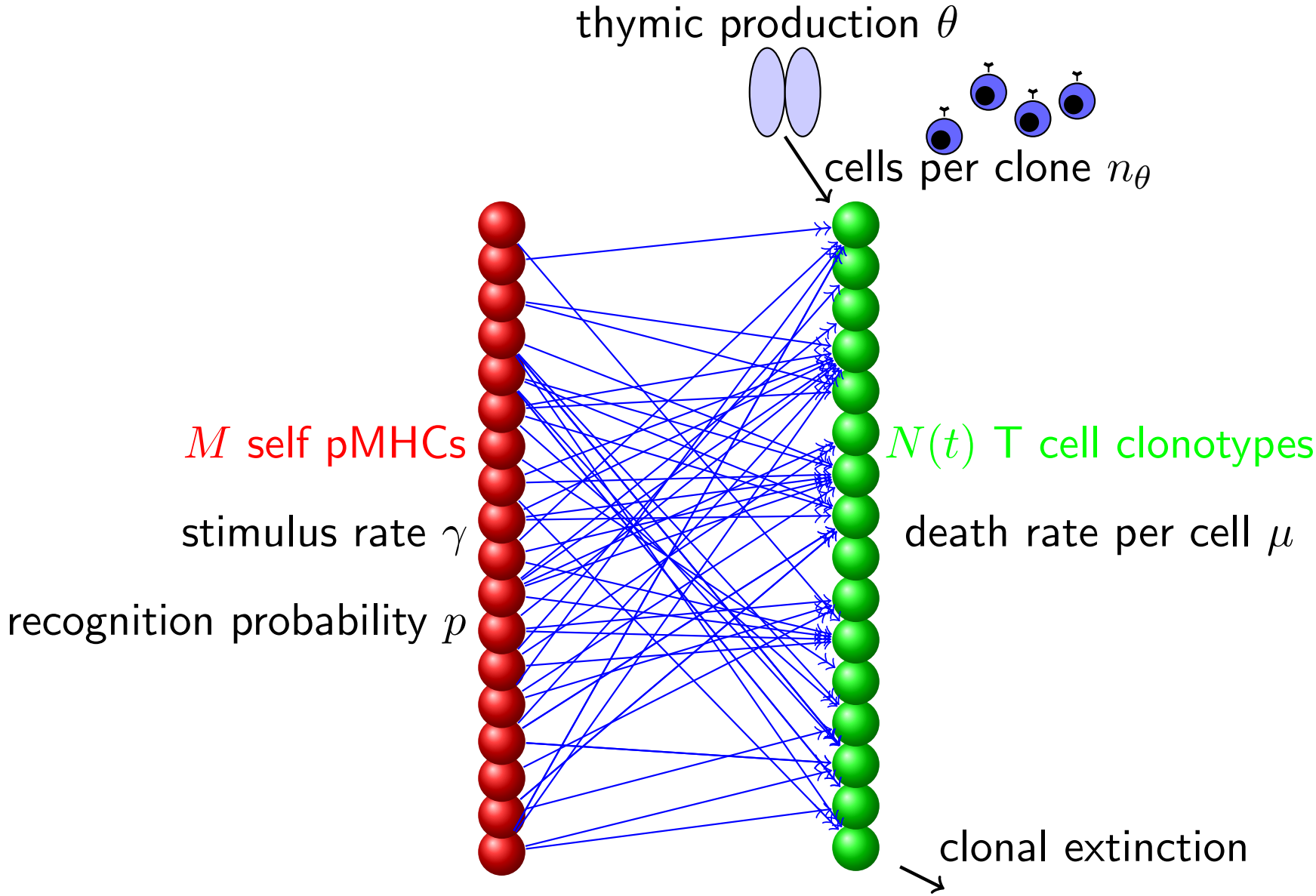
Homeostasis of naive T cells: size and TCR diversity

"The peripheral T-cell compartment is kept at a constant size as a consequence of homeostatic regulation, which requires the activity of cytokine and TCR signals." (Seddon and Zamoyska, 2003).

Simple idea:

- IL-7, produced by stromal cells in lymph nodes, determines the overall size of the (naive) T-cell population. (Link et al 2007)
- TCR diversity of the T-cell population is determined by competition for (many different) self-pMHC ligands. (Mason 1998)





Singh, Bando and Schwartz, Immunity **37** (2012)
Sewell, Nature Reviews Immunology **12** (2012)
Nikolich-Žugich et al Nature Reviews Immunology **4** (2004)

Stochastic system dynamics

Death

Every T cell has a constant probability per unit time μ of dying, independent of all others.

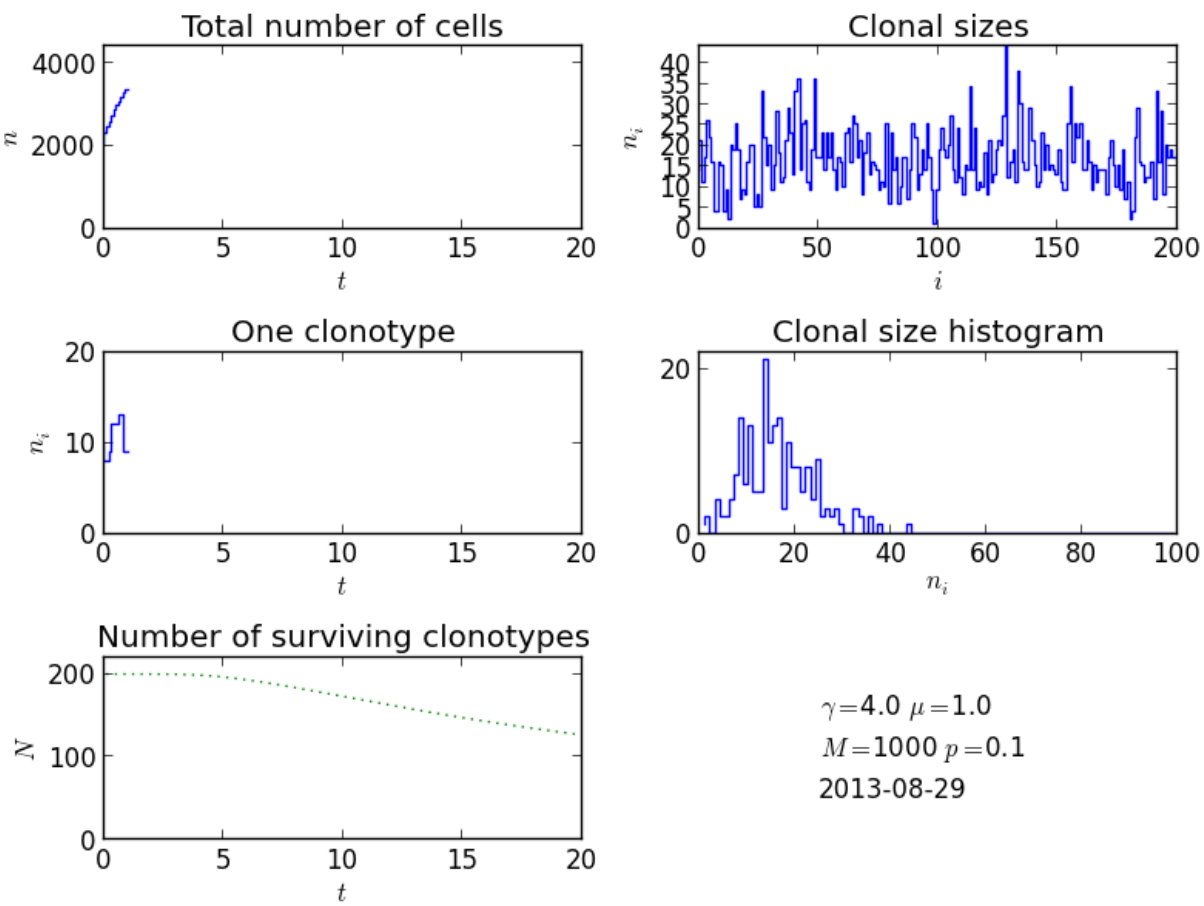
Division

Each pMHC set stimulates at rate γ. The stimulus is equally likely to cause one round of cell division in any of the T cells capable of recognising it.

The stimulus is divided into M subsets.
The number of T cells of type i at time t is $n_i(t) \geq 0$.
A clonotype has survived to time t if $n_i(t) > 0$.
The number of surviving clonotypes at time t is $N(t)$.

Mathematical Models and Immune Cell Biology, Springer (2011)

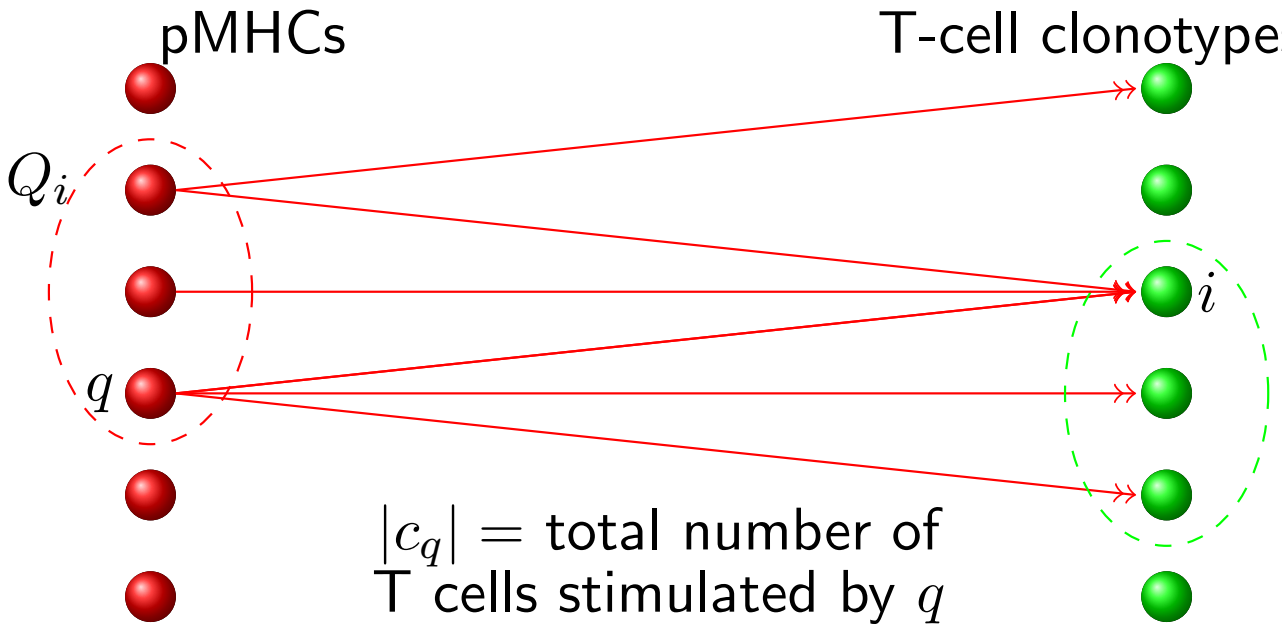
Model of large-scale clonal competition



- Transient timescale: the mean total number of T cells finds the level $\frac{M\gamma}{\mu}$.
- Extinction timescale: the probability that a clone, initially with n_0 cells, survives up to time t is

$$\Pr(\text{survival}) = 1 - \exp(-\frac{n_0}{\mu t})$$

Stimuli and cell division



Birth rate for T cells of type i

$$\Lambda_i = \gamma \sum_{q \in Q_i} \frac{n_i}{|c_q|} \quad \leq \gamma \phi_i \text{ where } \phi_i = \text{number of pMHCs in } Q_i.$$

Stirk et al, Mathematical Biosciences **224** (2010)

Distribution of clonal extinction times

Now, if we assume $n(t) \simeq \frac{\gamma}{\mu}$, then $\lambda_i(t) =\simeq \mu n_i$, so that birth rates and death rates are in balance.
Let us, further, approximate $n_i(t)$ by a diffusion process, $\mathbf{X}_t$.

$$\mathrm{d}\mathbf{X}_t = \sqrt{2\mu\mathbf{X}_t}\mathrm{d}\mathbf{W}_t.$$

If $F(t,b)$ is the probability of hitting 0 before time t, starting with $\mathbf{X}_0 = b$, then

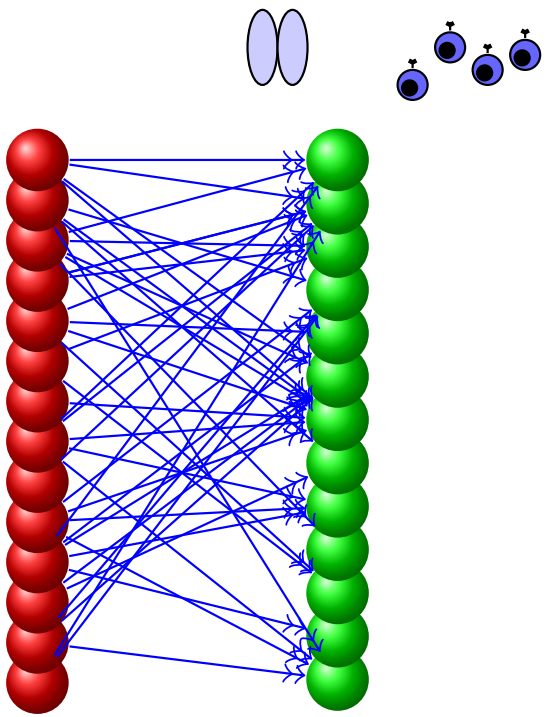
$$\frac{\partial}{\partial t}F(t,b) = \frac{1}{2}\mu b\frac{\partial^2}{\partial b^2}F(t,b),$$

with $F(t,0) = 1$. Thus $F(t,b) = 1 - \exp(-\frac{b}{\mu t})$ and

$$\Pr[\mathbf{X}_t = 0|\mathbf{X}_0 = b] = \exp(-\frac{b}{\mu t}).$$

Thymic production

At rate θ, new clonotypes are created with n_θ cells. The steady-state value of N is the product of the rate of production of new clonotypes and the mean lifetime of a clonotype.



The steady-state fraction of T cells that are thymic emigrants is

$$\frac{n_\theta \theta}{\gamma M + n_\theta \theta}.$$

The parameter $\alpha = \frac{n_\theta \theta}{\gamma M}$ measures the strength of thymic production relative to peripheral division.

Berzins et al, Trends in Molecular Medicine **10** (2002). den Braber et al, Immunity **36** 288–297 (2012)

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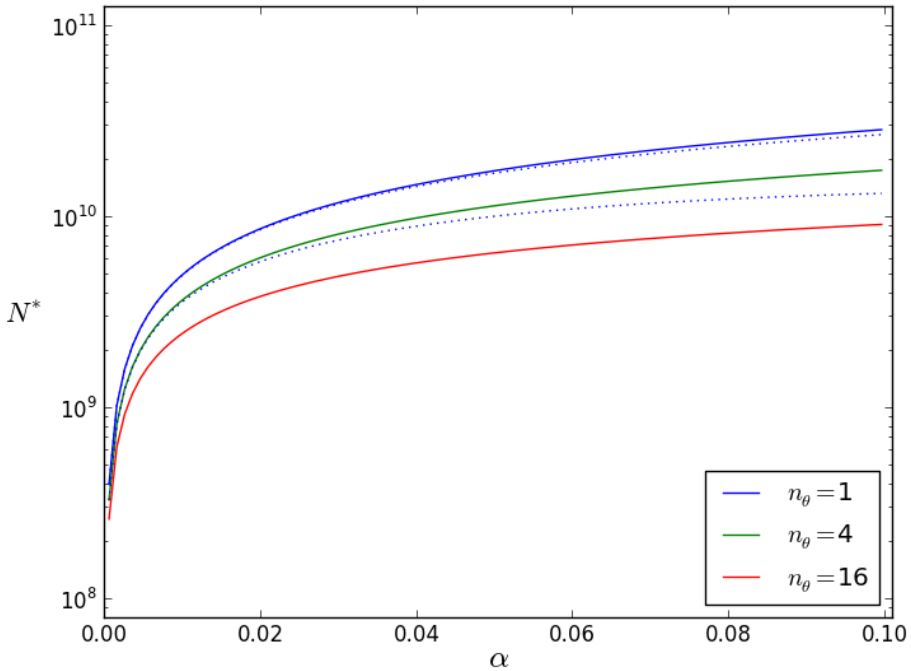


Figure: Predicted steady-state number of distinct clonotypes. The dotted lines are valid in the weak-thymus limit. Three values of n_θ are shown. We use $\gamma M/\mu = 10^{11}$ cells, approximately equal to the number of naive CD4$^+$ T cells in a human.

Parameter value guesses for mice and humans

The steady mean total number of cells is $\mu^{-1}(\gamma M + n_\theta \theta)$.
Let $\alpha = \frac{n_\theta \theta}{\gamma M}$.
As $\alpha \to 0$, $N^* \to \frac{\gamma M}{\mu}\alpha(1 - 0.577 - \log(n_\theta \alpha))$.

Mice

$\mu = 1\text{month}^{-1}$

Total (naive CD4$^+$) T cells: 4×10^7

Thymic production:

$n_\theta \theta = 4 \times 10^7 \text{month}^{-1}$

$p = 10^{-6}$, $M = 10^9$

$\gamma = 10^{-3}\text{month}^{-1}$

$N \simeq 2 \times 10^7 / n_\theta$

Humans

$\mu = 1\text{year}^{-1}$.

Total (naive CD4$^+$) T cells: 4×10^{11}.

Thymic production:

$n_\theta \theta = 10^{10}\text{year}^{-1}$

$p = 10^{-6}$, $M = 10^{10}$

$\gamma = 10\text{year}^{-1}$

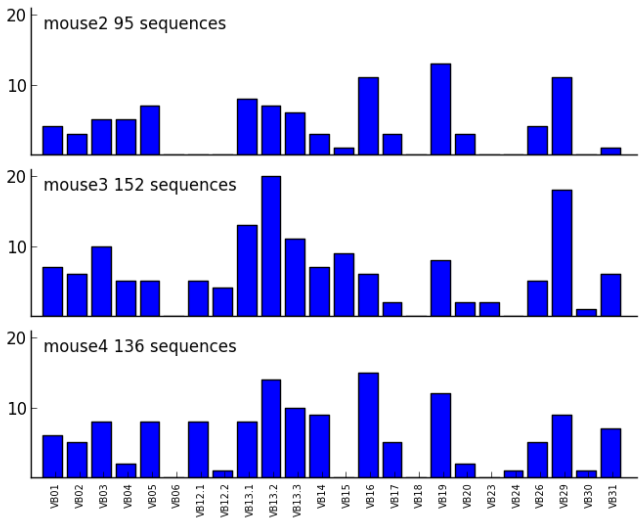
$N \simeq 10^{10}$.

Bains, Antia, Callard and Yates, Blood **113** (2009)
Westera et al Blood (2013)
Vrisekoop et al PNAS **105** (2008)
Murray et al Immunology and Cell Biology (2003)
de Boer and Perelson, J Theoretical Biology (2013)

Cell by cell TCR analysis (Gonçalves and Rocha)

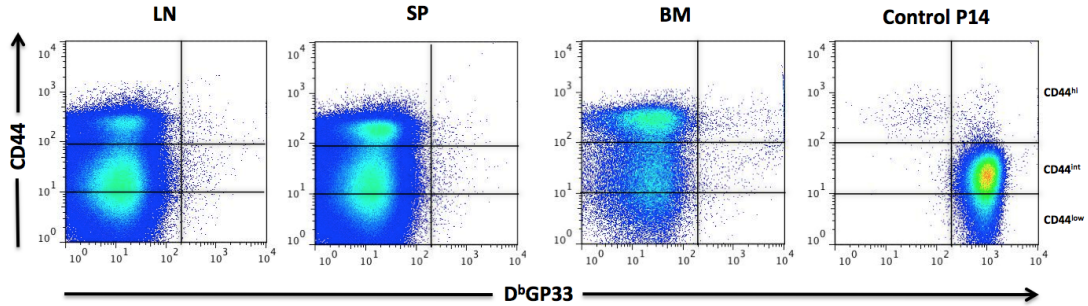
Using material obtained from T cell pools makes direct evaluation of clone sizes difficult, because identical Tcrs may correspond to different cells expressing the same receptor, or to several amplicons of the same T cell.
Gonçalves and Rocha (INSERM and Pasteur): TCRB expression in individual CD8 naive T cells. from specific-pathogen-free adult mice.
In each individual cell, a single primer pair is used for the PCR amplification of the Tcrb.
187 of the 188 single cells from mouse 1 expressed unique Tcrb chains, even though this cohort included $CD44^{+}$ cells.
All sequences had nibbling at the V-D-J junctions and 90% also had N additions
2.9% of individual cells expressed two in-frame Tcrb chains,
No sequences were shared between different mice.

Repertoire subsets: TCRBV and epitope-specific



CD44^{-} T cells expressing only TRBV13 or only TRBV19.

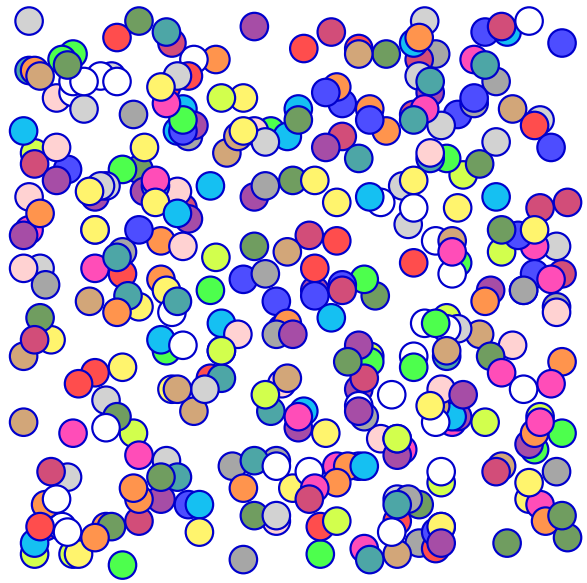
CD44- CD8 T cells recognising the GP33 peptide from the Lymphocytic Choriomeningitis Virus (LCMV) (CD44-GP33+ CD8+ T cells), separated using GP33 dextramers



Sampling from repertoires (in silico)

Three types of hypotheses are:

- that each clone has the same number of cells
- that the clonal sizes follow a simple geometric distribution, where the probability of finding clones with small size is higher than that of finding large clones
- that there are two types of clones in the repertoire, the majority of clones made up of only a few cells, and a small minority of clones that contain many cells



The observed distribution of clonal sizes

Our goal is to find the probability distribution of the number of instances of k copies of a TCR in a random sample of m cells. Firstly, consider the point of view of one cell in the total of S cells in the repertoire. The probability, which we denote q, that this cell is one of the m cells in the sample is equal to m/S. Next, let us define the Bernoulli random variable B:

$$\Pr[B = 0] = 1 - q \quad \text{and} \quad \Pr[B = 1] = q, \qquad \text{where} \qquad q = \frac{m}{S}.$$

The probability generating function (pgf) of B is $\phi_B(z) = 1 - q + qz$. If n_i is the number of cells of a clonotype labelled i, then the number of cells of type i in the sample is the random variable Y_i, which can be written $Y_i = B_1 + \cdots + B_{n_i}$, With the approximation that the B_j are **independent** random variables, the pgf of Y_i is

$$\phi_{Y_i}(z) = \phi_B(z)^{n_i} = (1 - q + qz)^{n_i}.$$

a word from . . .

- Quantitative T cell Immunology (ITN)
- Mathematics for Health and Disease (FP7 IRSES)

`http://www1.maths.leeds.ac.uk/Applied/QUANTI`
`http://www1.maths.leeds.ac.uk/Applied/INDOMATH`

See you at . . .

- BSI mathematical modelling. Cambridge, june 2016.
  `http://www1.maths.leeds.ac.uk/applied/BSI/`
- ICI. Melbourne, august 2016

- Joint BSI and NVVI Congress. Liverpool, december 2016

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