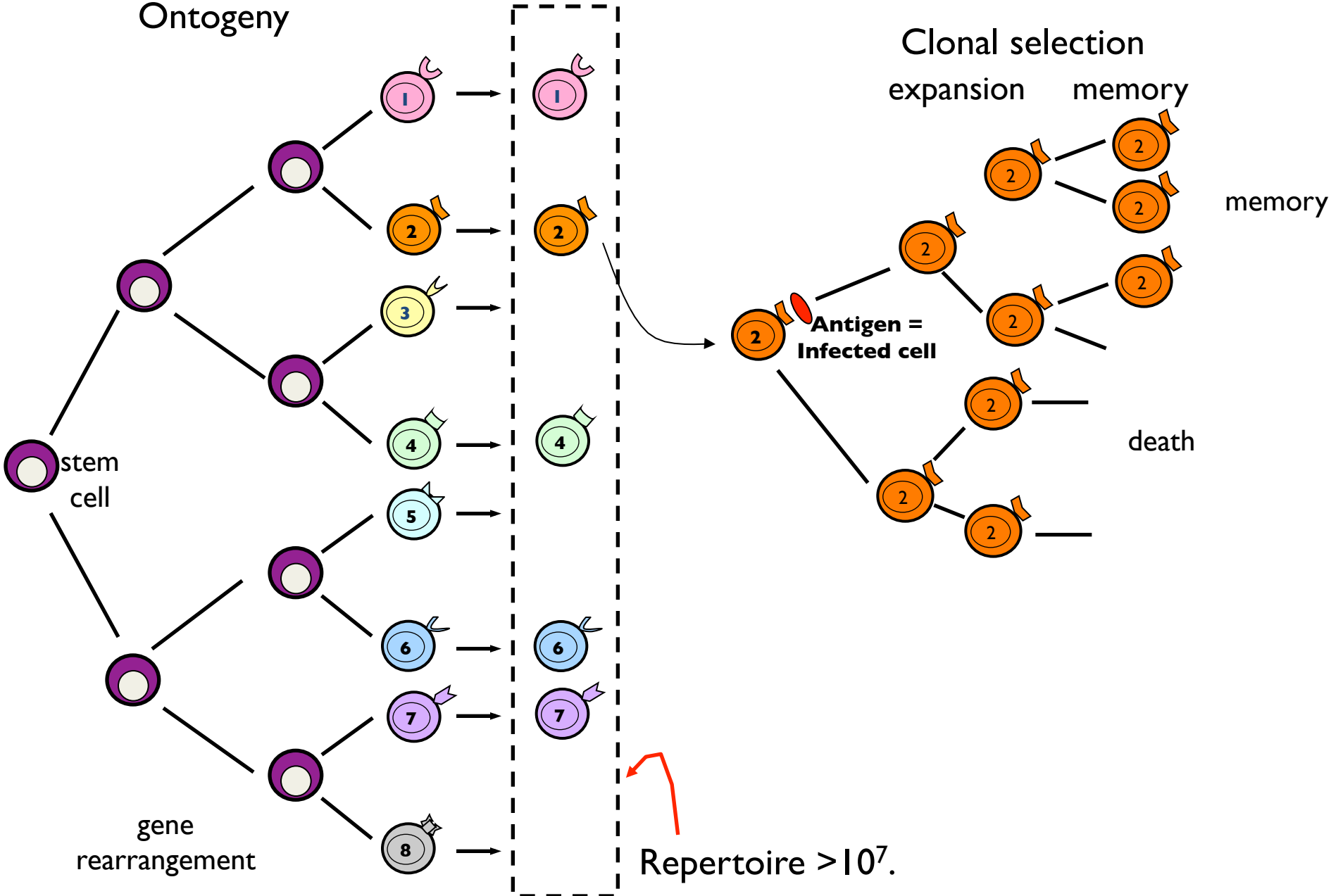
Maintenance of memory and a diverse repertoire.

Rafi Ahmed
Joseph Blattman
Jorg Goronzy
Philip Johnson
Andrew Yates

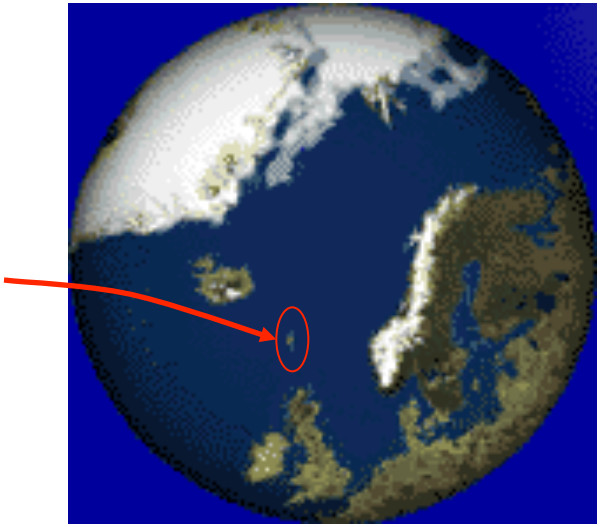
Clonal selection theory for adaptive responses



A historical introduction

- Thucididies (430 BC)
  Those who had successfully recovered from disease were able to take care of the ill during a plague in Athens.

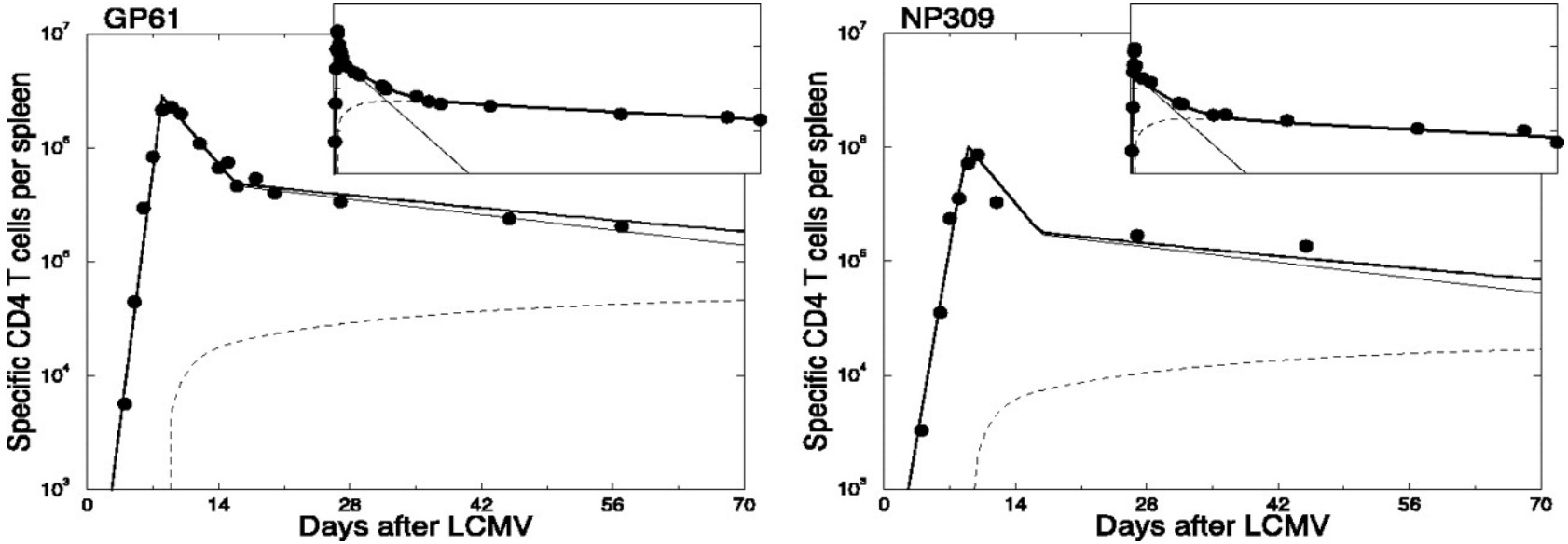
- Panum (1847)
  Memory lasted between 1781 & 1846 measles epidemics in the Faroe islands.



- Yellow fever (1931)
  Antibody titers persisted for decades following a 1855 epidemic in Norfolk, VA. Measured by protection of monkeys conferred by transferred immune sera.

Memory in mice

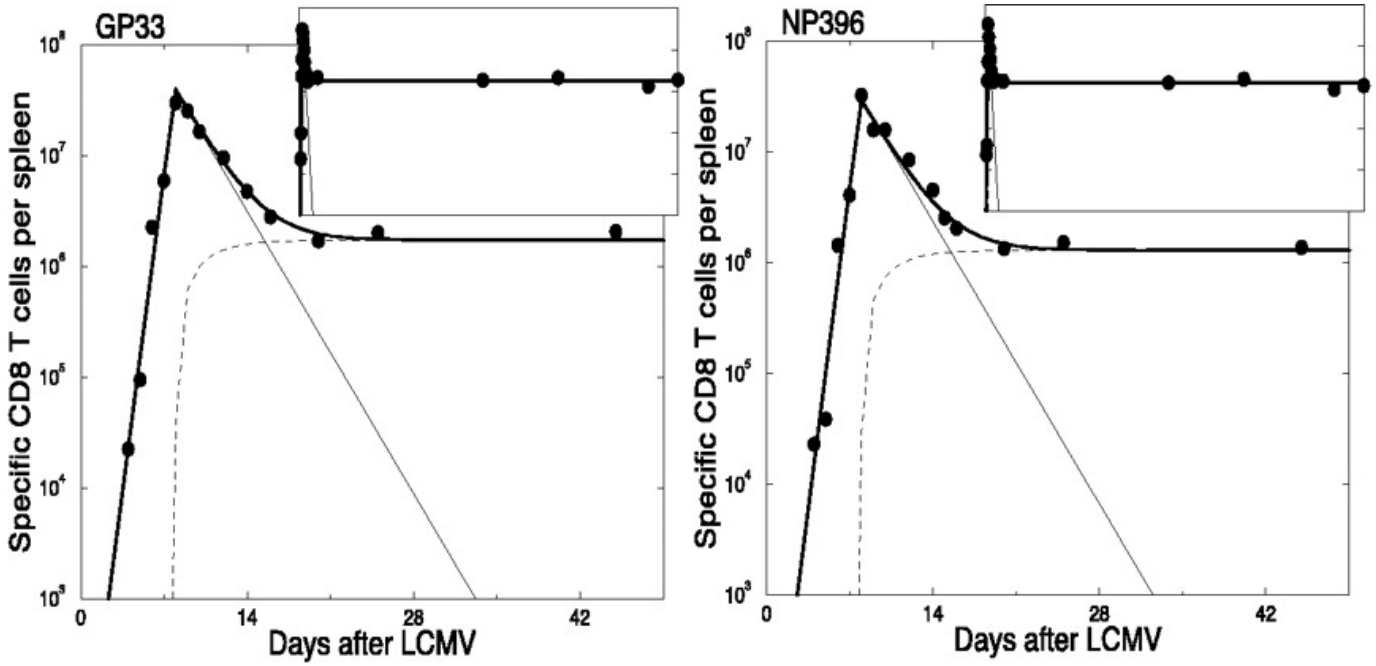
Mice: CD4 T cell responses following LCMV infection ……. De Boer et al JI (2003)



CD4 T cells exhibit biphasic contraction

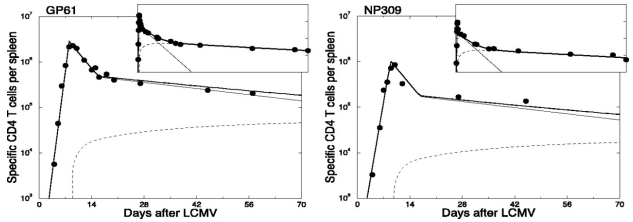
Memory in mice

Mice: CD8 T cell responses following LCMV infection ……. De Boer et al JI (2003)



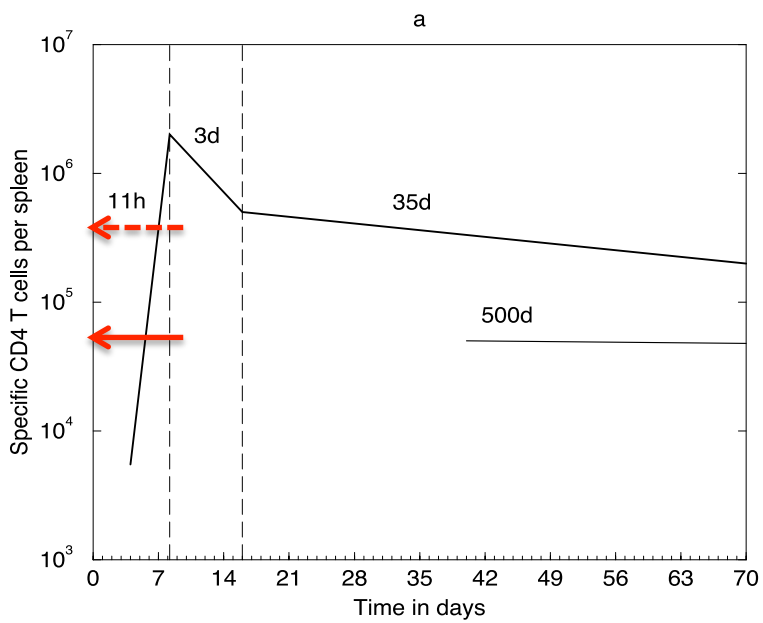
CD8 T cells exhibit no discernable loss of memory

Biphasic decay of CD4 memory

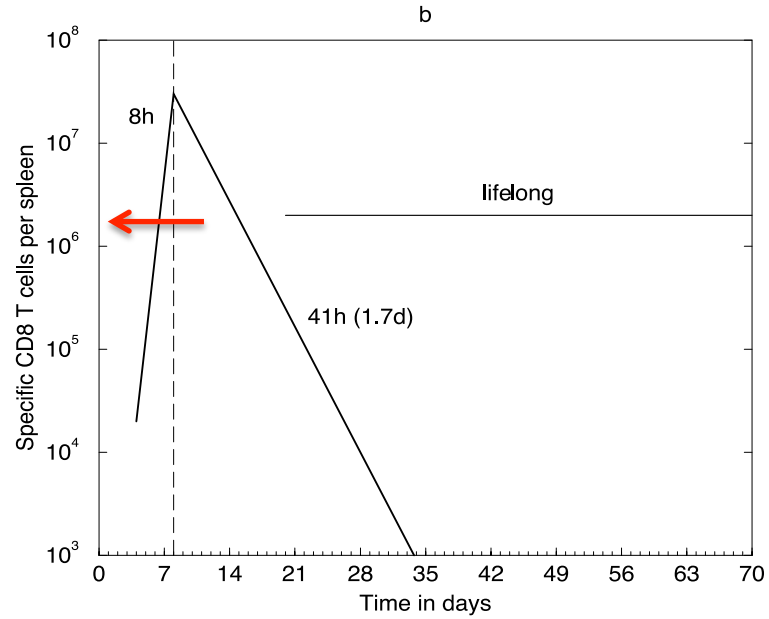


Memory in mice

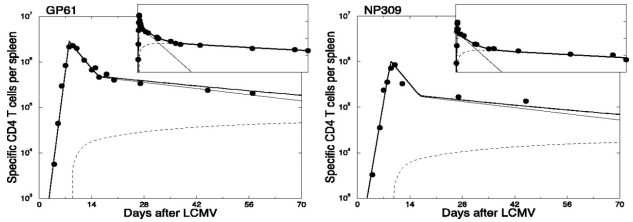
CD4 T-cells



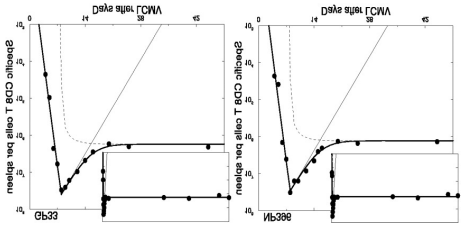
CD8 T-cells



Biphasic decay of CD4 memory



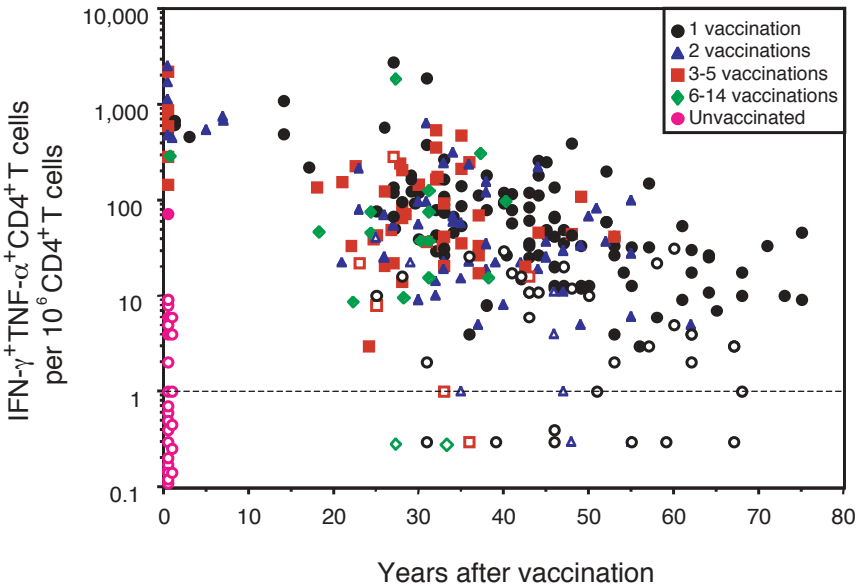
No decay of CD8 memory



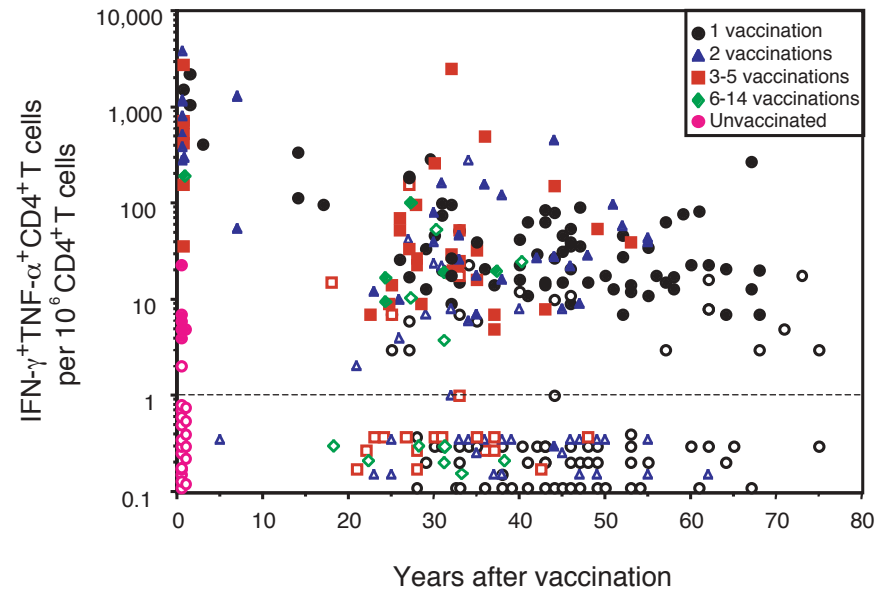
Memory in mice and men

Hammarlund … and Slifka (2003) Nat. Med

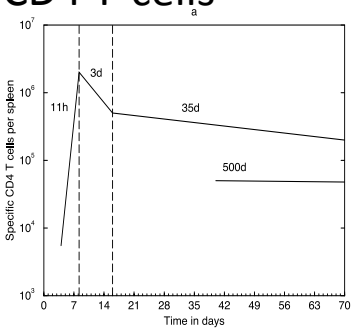
CD4+ T cells ($t_{1/2}$≈ 10 years)



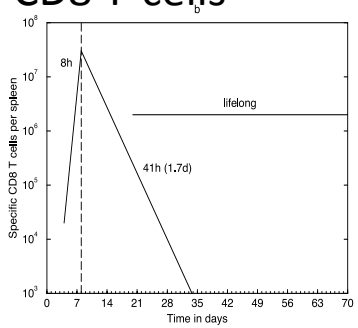
CD8+ T cells ($t_{1/2}$≈ 15years)



CD4 T-cells



CD8 T-cells

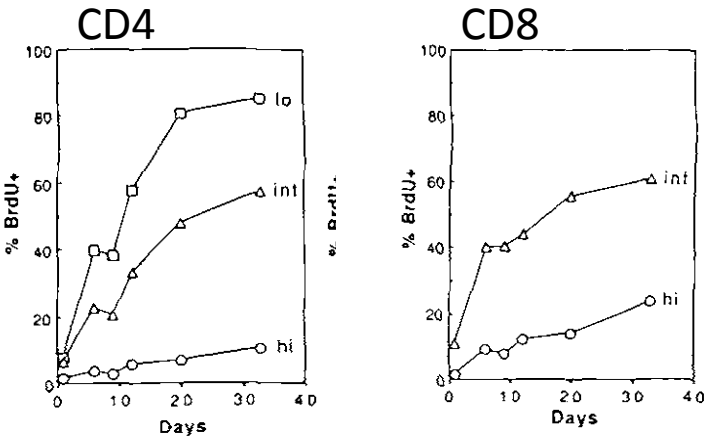


1. Magnitude of response
2. Rate of decay

Hypothesis for immune memory

- Immortal memory cells

  Tough and Sprent
  rejected this 20 years ago
  JEM 1994



"long-lived memory from short lived cells"

1. How?
2. Rules for the longevity of memory?

- Maintenance of memory requires antigen:
  association of antigen with memory
  - persistent antigen (as antigen or live pathogen)
  - reexposure to antigen (infection)
  - anti-idiotypic networks

- Memory does not require antigen:
  adoptive transfer experiments
  - bystander stimulation
  - homeostasis: (active and passive attrition models)

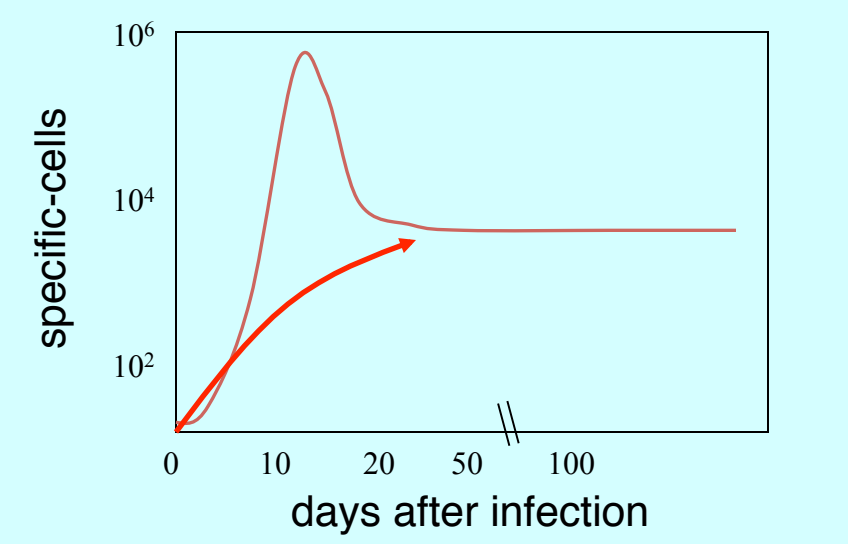
Constructing a simple model

- Define memory
  as the number of antigen-specific cells following stimulation

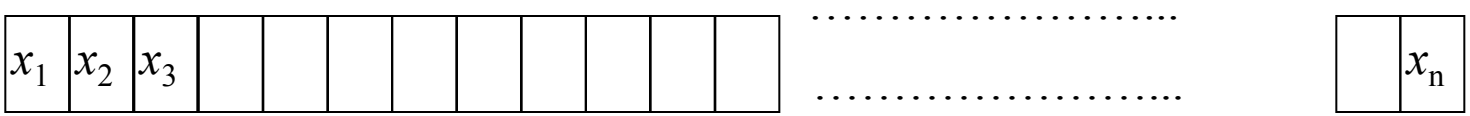
- Memory is a general phenomenon
  (i.e. it is possible to make a general model for memory)

- Include the relevant biology
  Repertoire of lineages with
  (i) input (from thymus),
  (ii) specific-stimulation,
  (iii) cross-reactivity,
  (iv)homeostasis (total population)
  (v) turnover/death,

- Memory >> acute infection.
  On the timescale of memory, an acute infection is approximated by a jump in the # of pathogen-specific immune cells.



Model-1



"effective repertoire" n very large ~ 10^7

x_i = number of cells in the ith lineage
$X = \Sigma x_i$ = *total* number of cells

deterministic terms bystander homeostasis death

$$\frac{dx_i}{dt} = a_i^* + mq_i^* + cqx_i + S(X)x_i - dx_i$$

stochastic terms input stimulation

Results - either memory or a diverse repertoire

The decline of memory is exponential at rate

$$R = -\frac{na + nmq}{\tilde{X}}$$

$$= \frac{\text{input} + \text{expansion due to other pathogens}}{\text{total population size}}$$

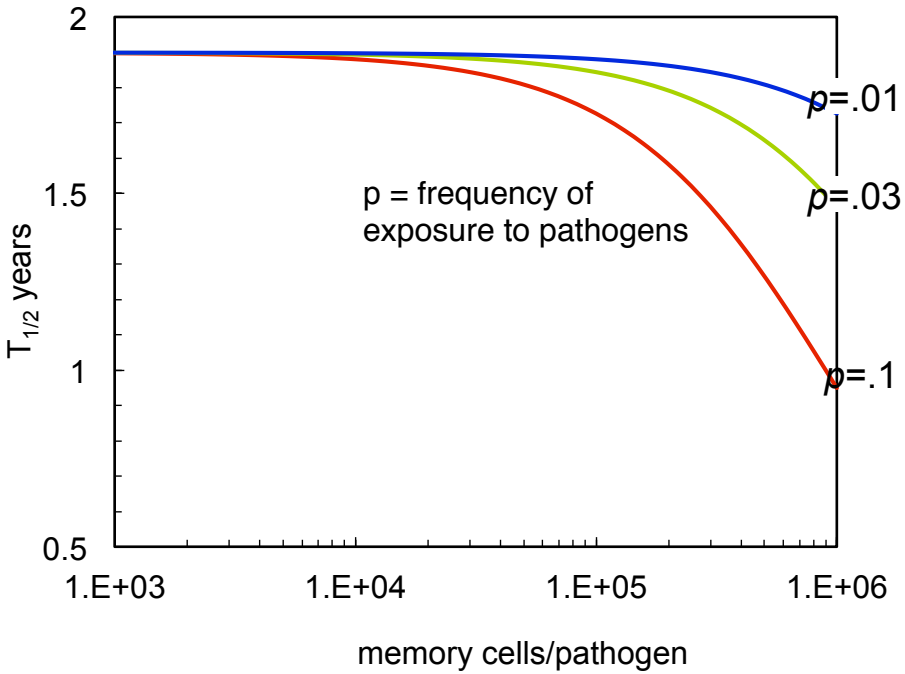
The repertoire is maintained only if the input from the thymus is sufficiently large that

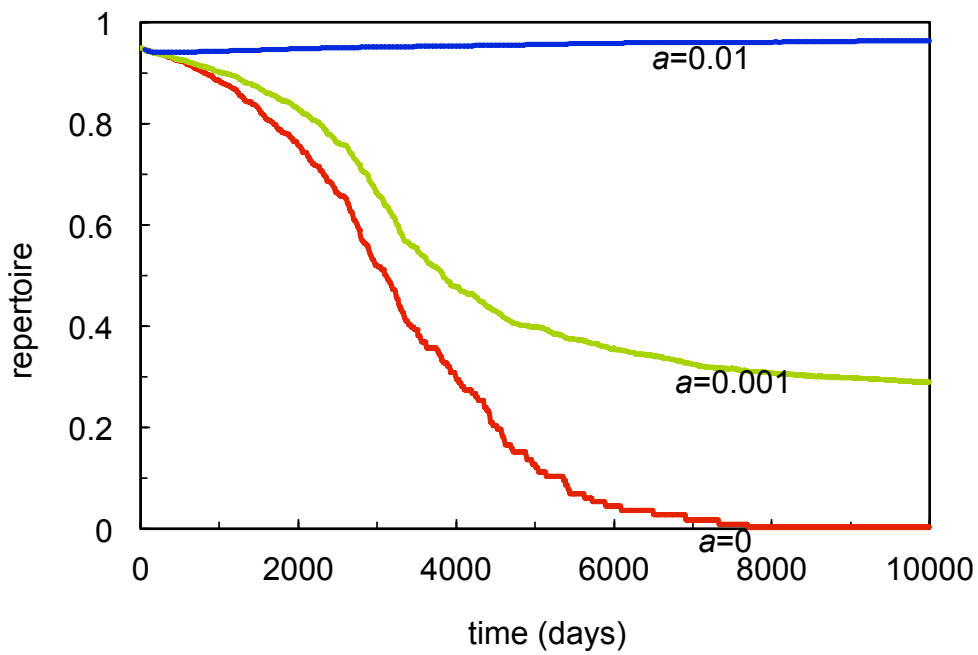
$$\tilde{x} = \tilde{X}\frac{a}{n(a+mq)} > 1$$

The longevity of memory is

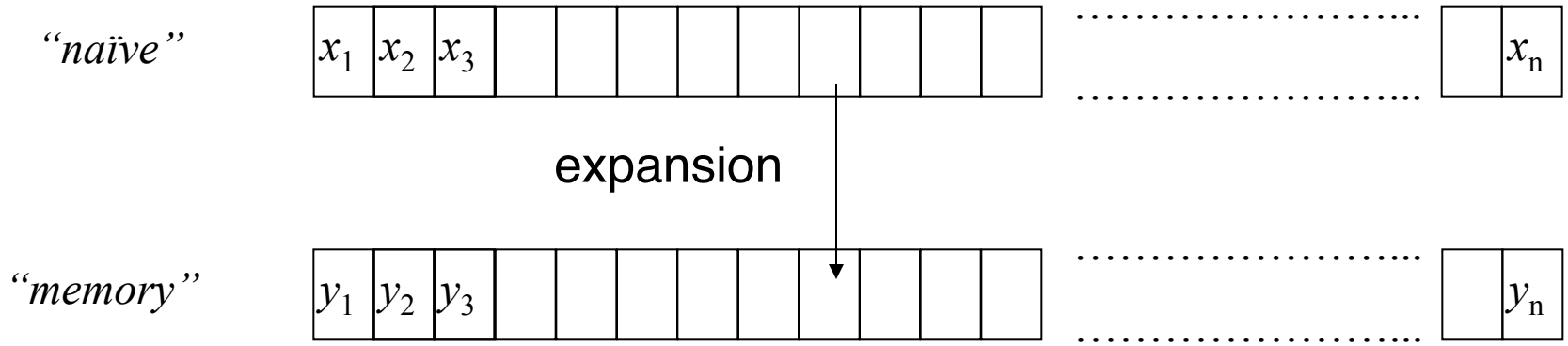
(i) independent of cross-reactivity

(ii) relatively long if thymic input small





Model 2



input

"naïve"

$$\frac{dx_i}{dt} = a_i^* - q_i^* x_i + S_X(X,Y)x_i - dx_i$$

expansion homeostasis death

bystander

"memory"

$$\frac{dy_i}{dt} = mq_i^* + S_Y(X,Y)y_i + cqy_i - dy_i$$

Results - memory

Independent homeostasis of naïve and memory populations results in the maintenance of memory and the naïve repertoire

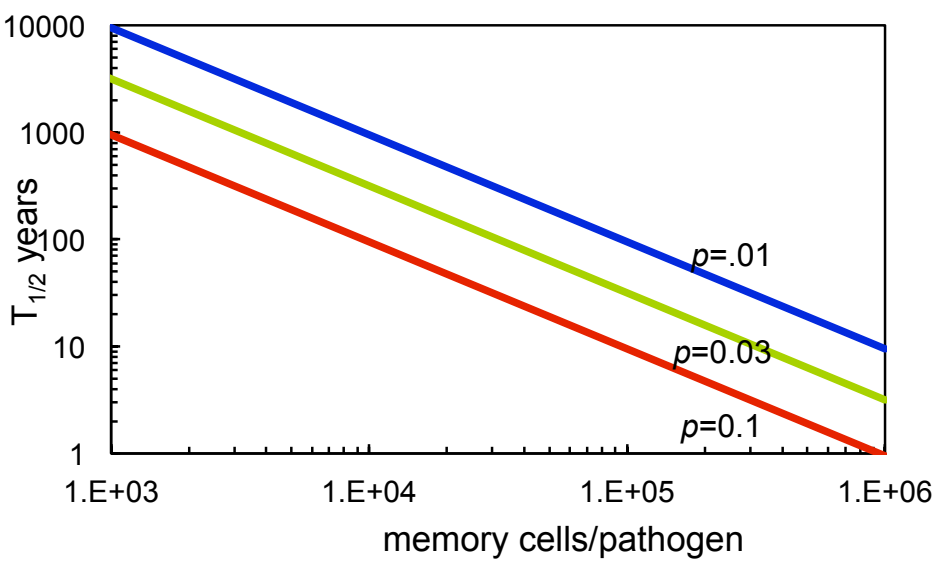
Loss of memory at rate

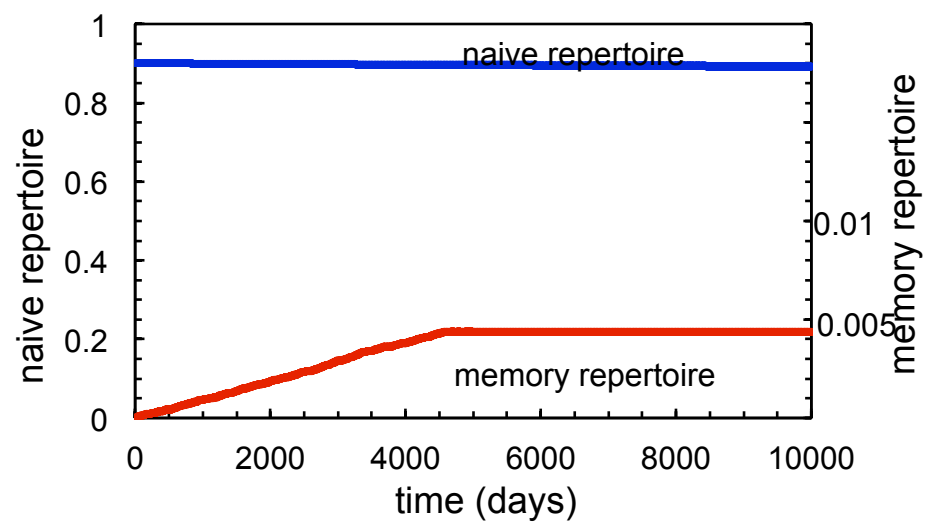
$$R = -\frac{nmq}{Y} = \frac{\text{expansion to other pathogens}}{\text{size of memory compartment}}$$

Memory repertoire

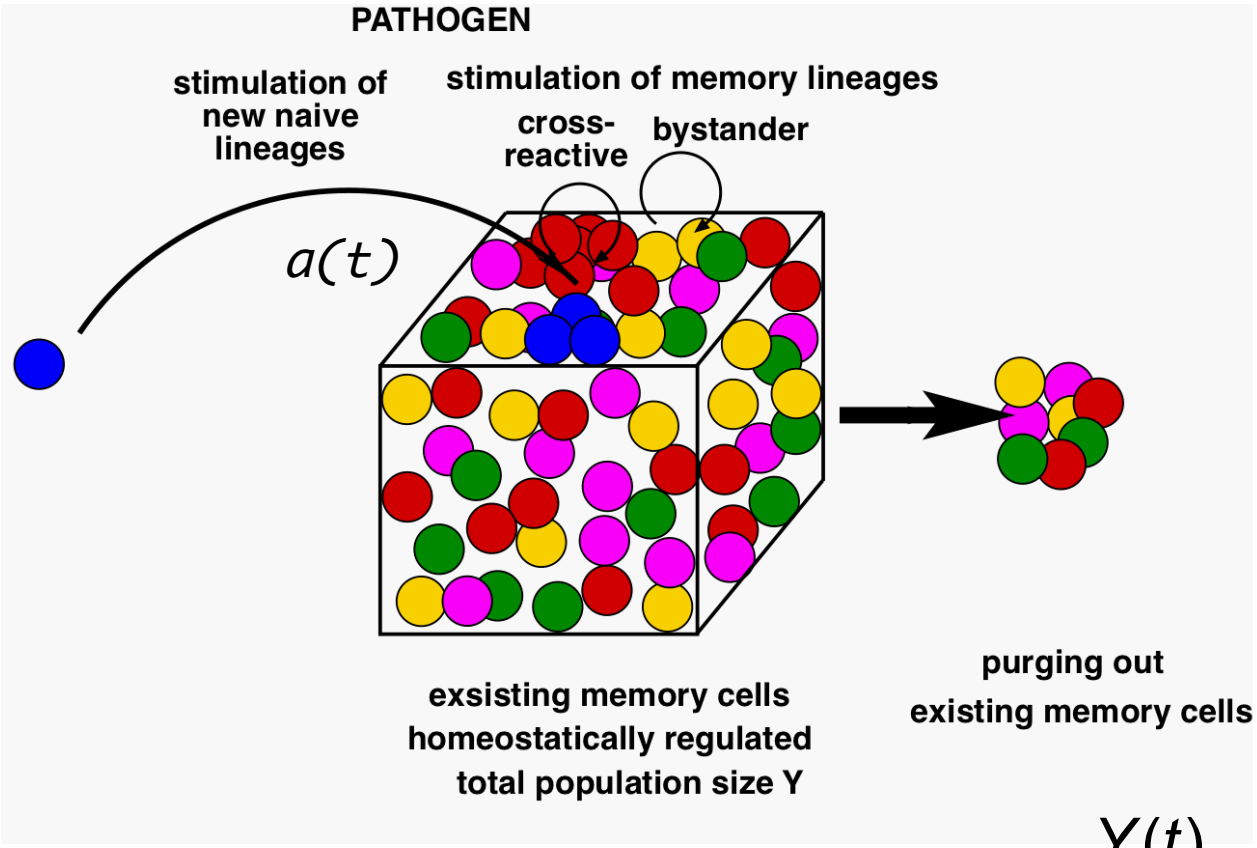
$$r_m = \frac{Y \ln(m)}{m}$$

m = expansion due to new pathogens; $p = nq$ = frequency of exposure to new pathogens





A simple model for immune memory



1. Incorporation of "new" memory cells
2. Change in size of memory compartment

$$y(t) = y(0)\underbrace{\frac{Y(t)}{Y(0)}}_{\text{Change in size of memory compartment}}\underbrace{\exp\left(-\int_0^t \frac{a(s)}{Y(s)}ds\right)}_{\text{Purging due to addition of memory cells to new pathogens}}$$

$y(t)$ = number of memory cells of a given specificity

$a(t)$ = influx of new memory cells

$Y(t)$ = total population size of all memory cells

Testing the model

- Assumptions:
  - All “memory cells” equal
  - Turnover/homeostasis is independent of:
    - antigenic specificity
    - previous division history
- Predictions:
  - Loss of preexisting memory on exposure to novel pathogens

Are all memory cells “equal”

The CFSE dye dilution assay allows us to look at the turnover of memory cells specific for different lineages with unprecedented accuracy.

Using this assay we would like to test the assumptions of the model, namely

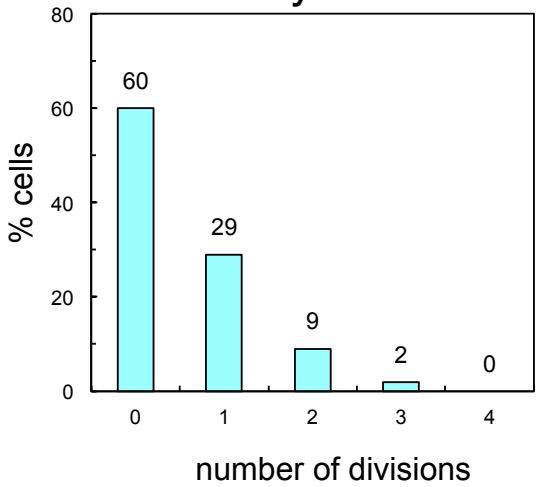
1. Does the turnover of memory cells depend on their antigenic specificity?

2. Does it depend on time since the primary response or the number of divisions a cell has undergone?

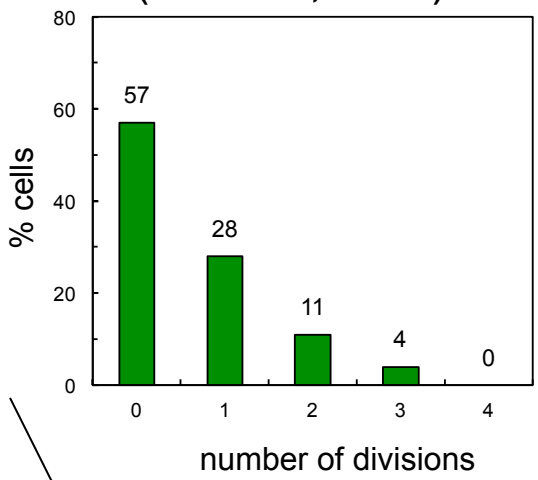
Turnover of memory cells of different specificities

turnover @ 21 days after transfer

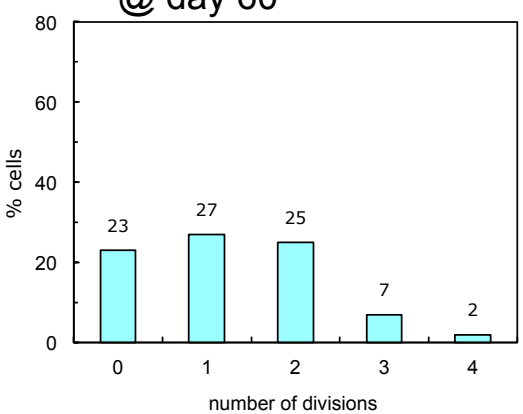
NP396-specific memory cells



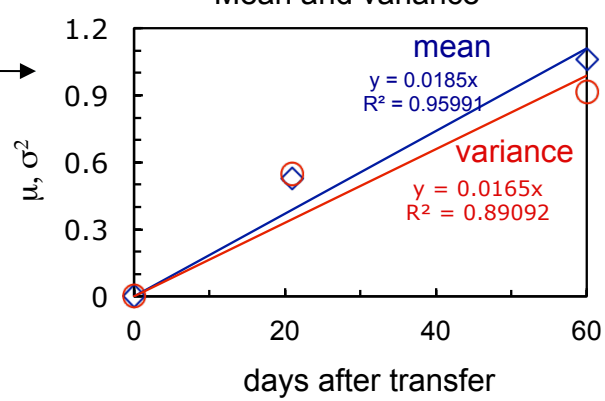
all memory cells (CD44hi, CD8)



NP396 cells @ day 60



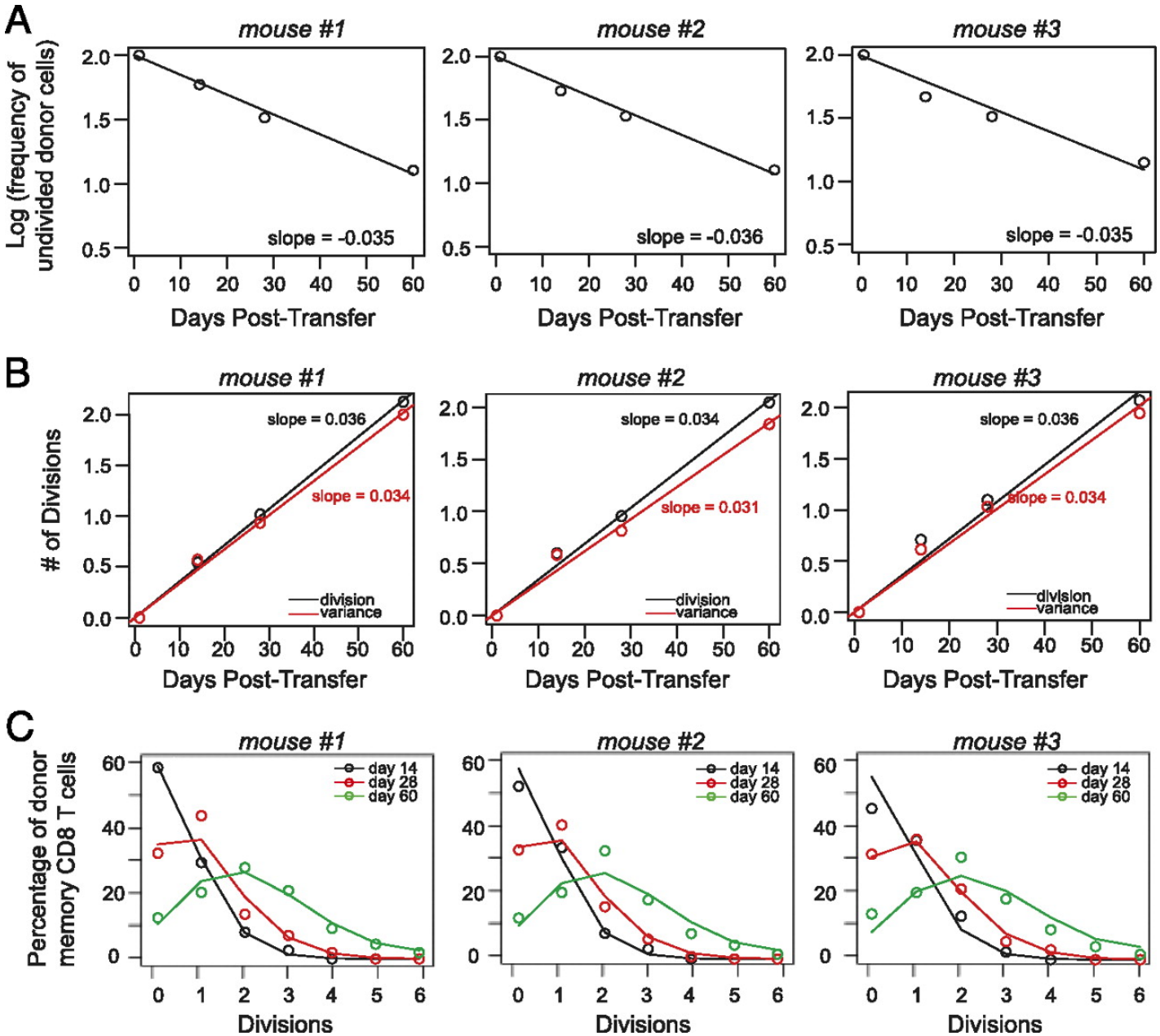
Mean and variance



Memory cell turnover is independent of specificity

Poisson distribution?

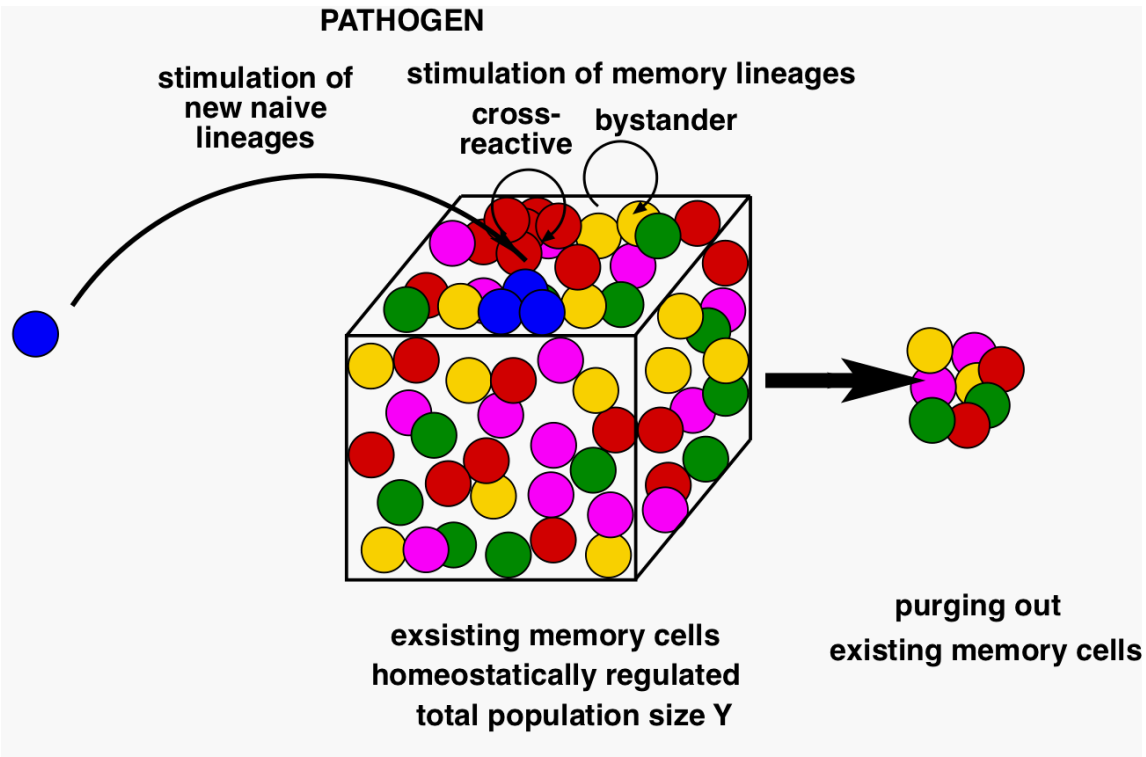
Turnover of antigen-specific memory cells

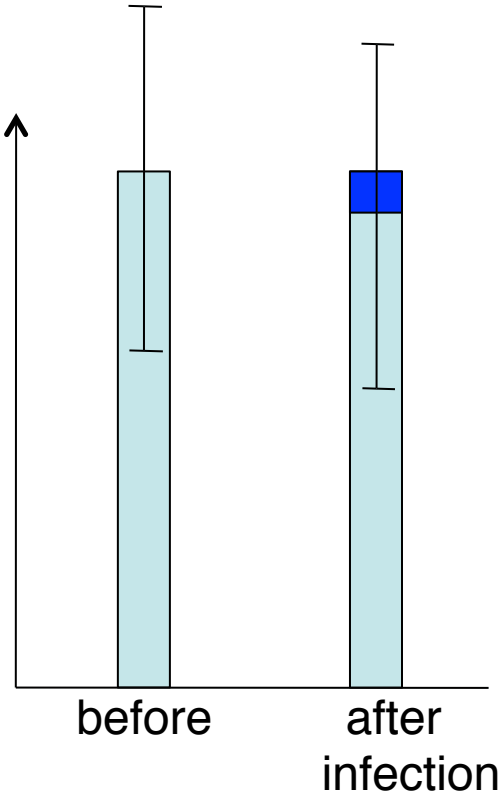


Prediction – loss of memory following exp. to new pathogens

First suggested by Selin and Welsh. Some potential problems

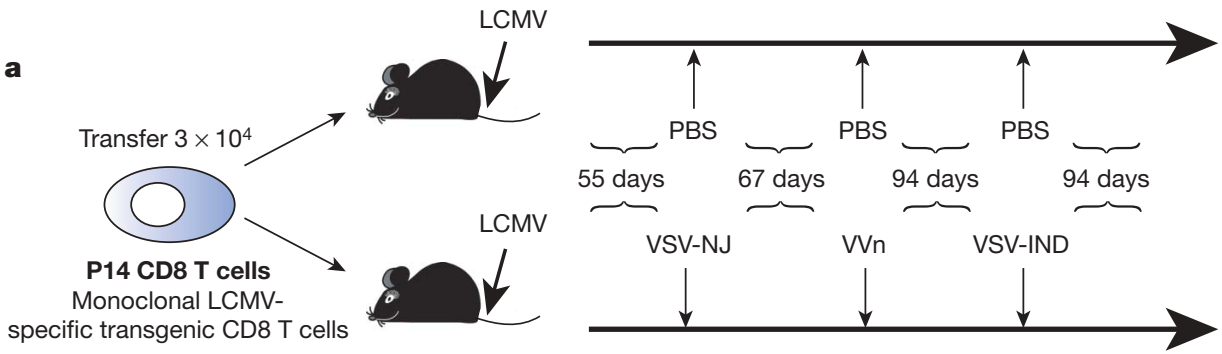
1. Need to measure total cell numbers (not percentages).
2. Inter mouse variation in numbers of cells about 50%.

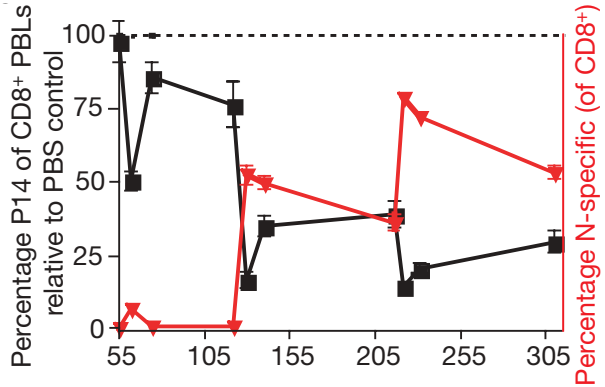


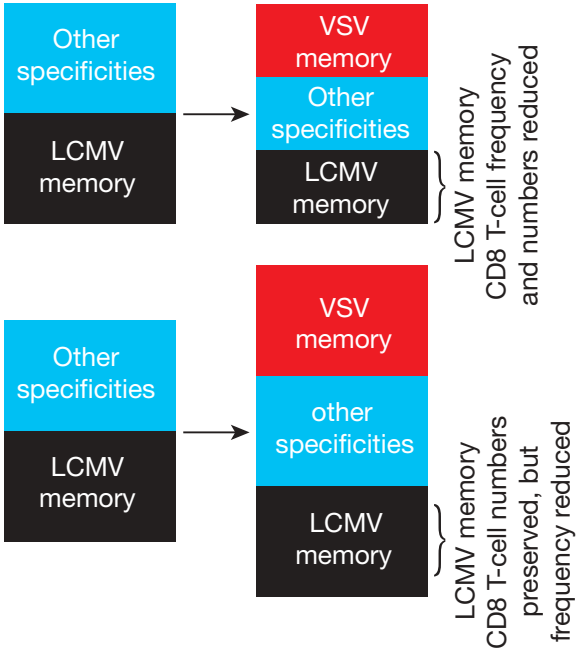


Experimental design

Immunization regime results in over half the CD8 memory population being specific for new pathogens.



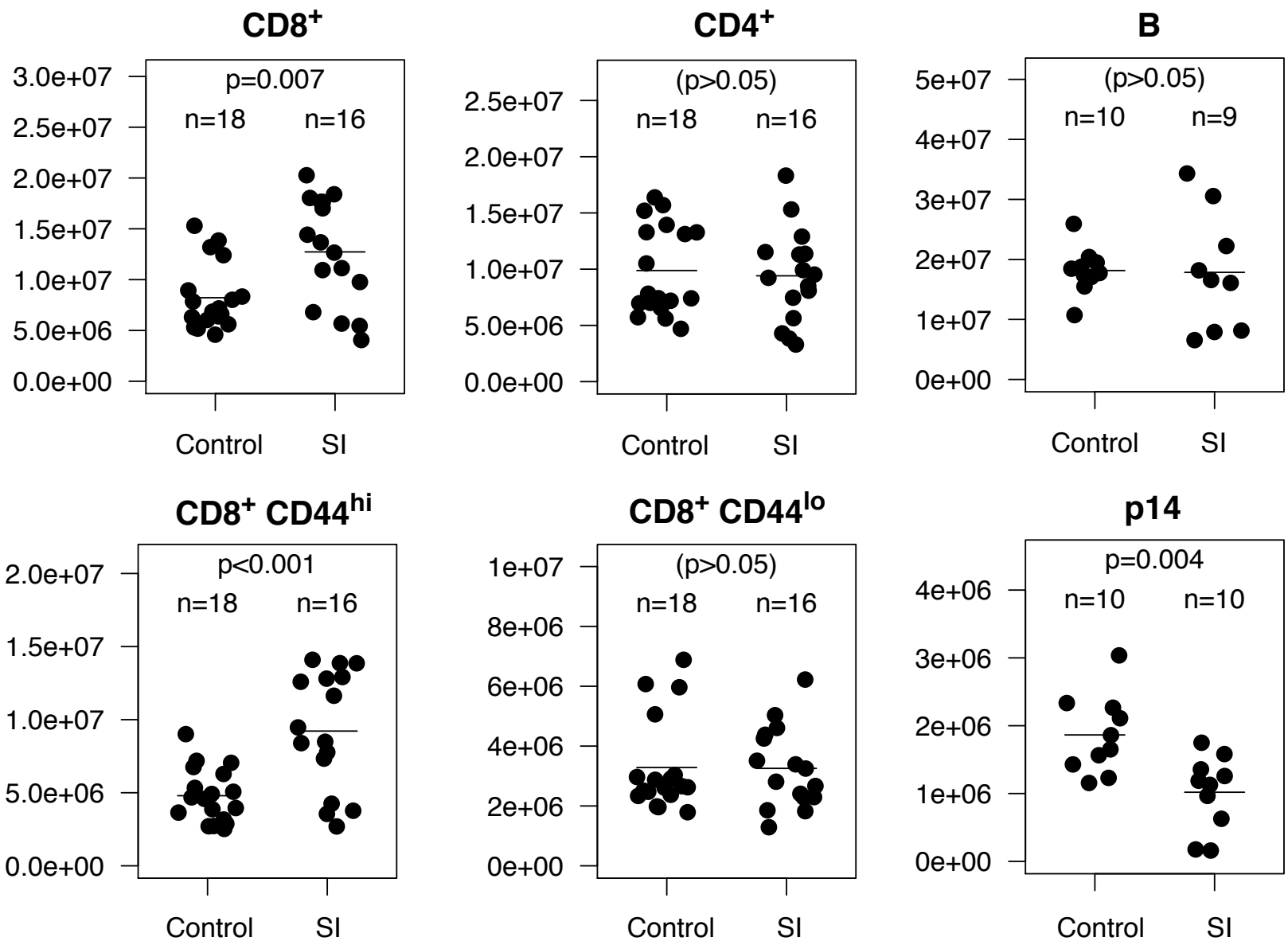




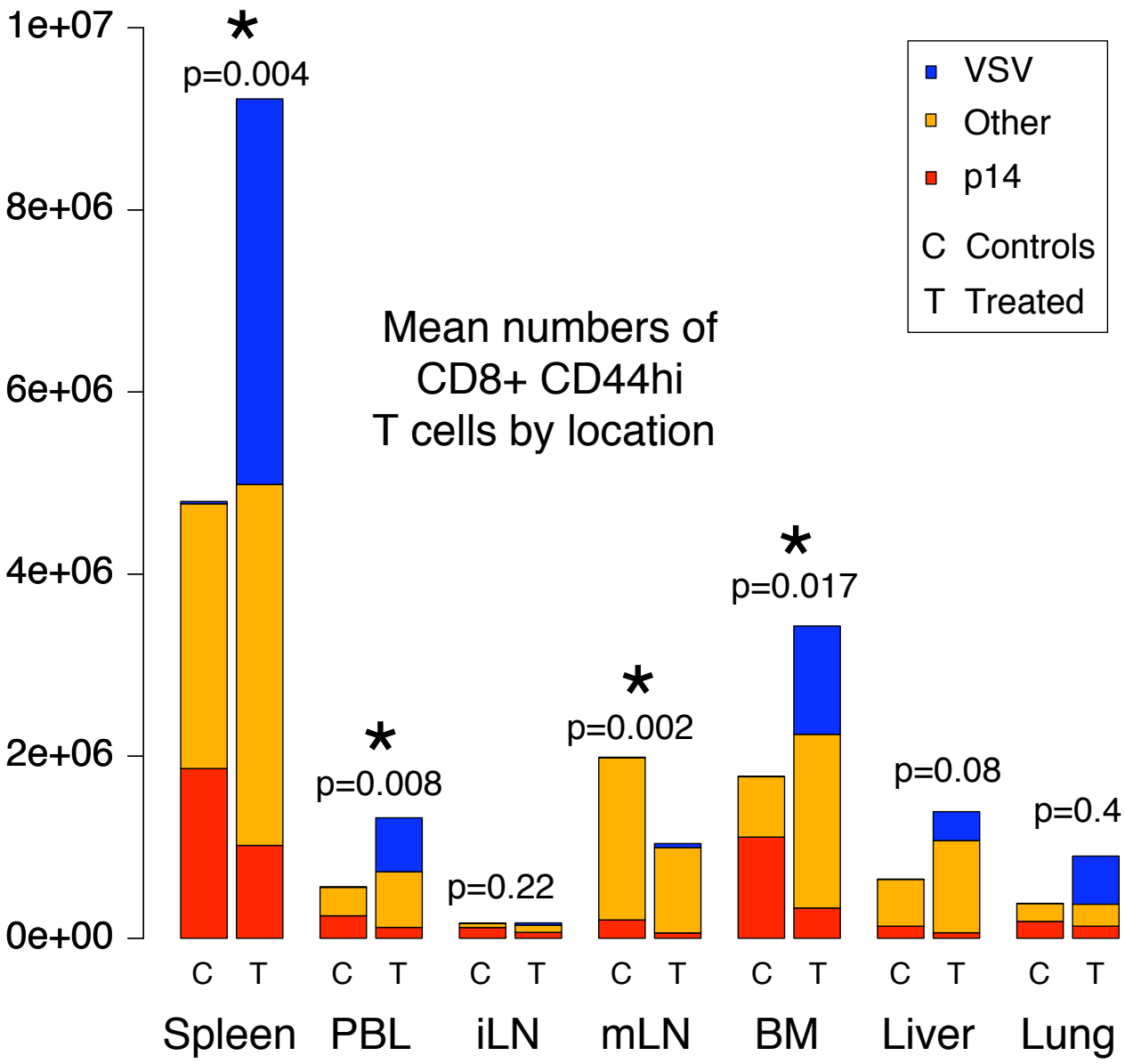
What is the relative contribution of
– attrition in existing memory
– increase in size of memory pool

Vezyz et al Nature 2009

Change in the numbers of cells in the spleen



Specificity of memory CD8 cells



Open questions

Discrepancy between memory in mice and men
(numbers of CD4 and CD8, and rates of decay)

Flexibility in size of the memory compartment
(causes, limits and consequences …)

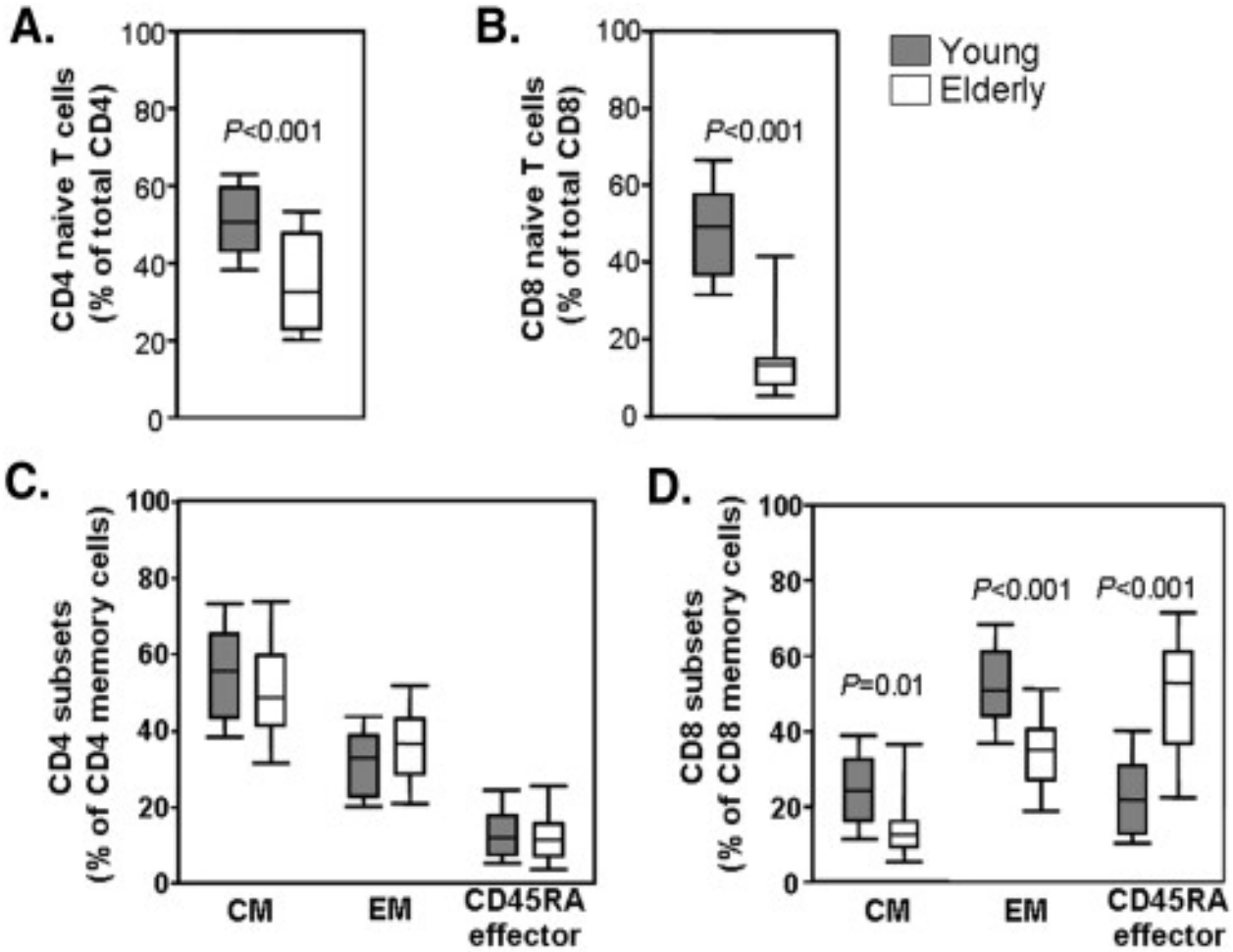
Potential heterogeneity in memory
(Rob's talk)

The role of cross-reactivity in the maintenance of memory.
(Matzinger, Selin and Welsh, Ganusov …)

Heterogeneity in protection by vaccination.

Repertoire and aging

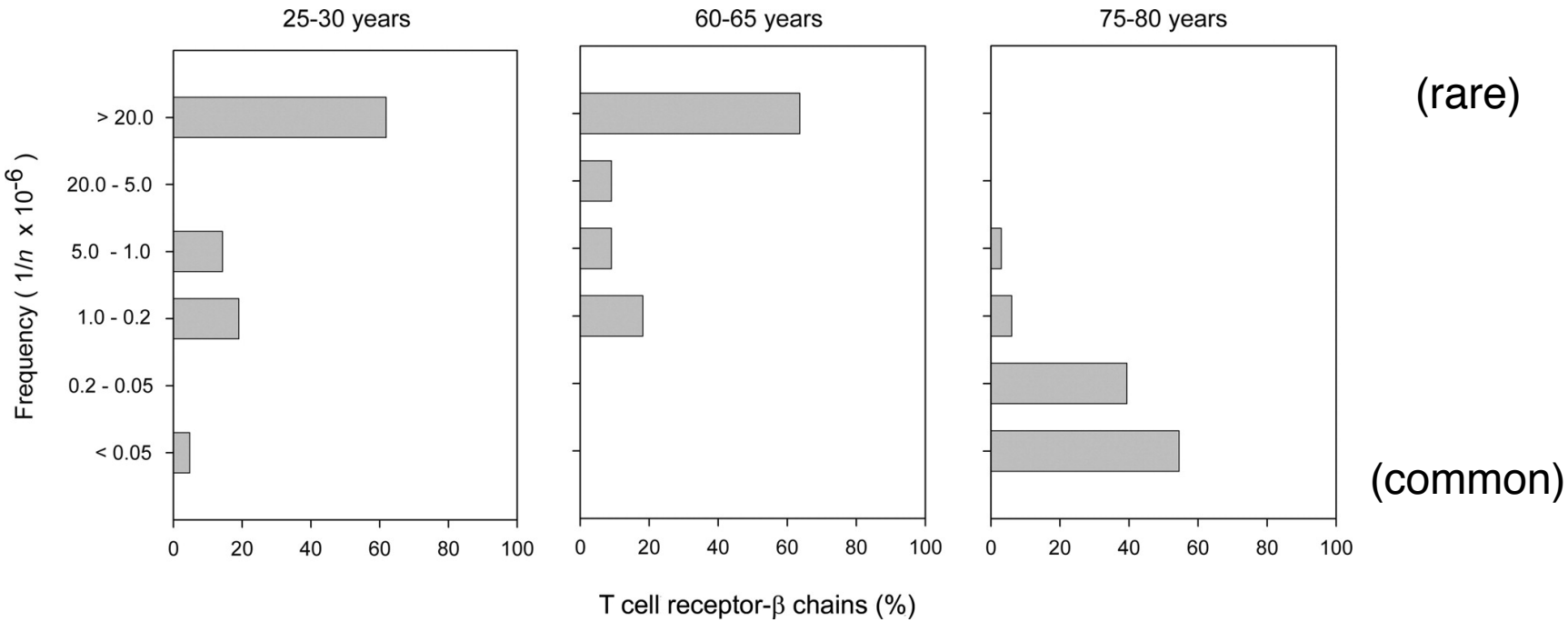
What happens during aging?



Sampling restricted to blood

Czesnikiewicz-Guzik et al
(2008) Clinical Immunology

Naïve CD4 T cell repertoire



Relatively severe loss of the naïve CD4 T cell repertoire @ around 70 yrs

(results qualitative … pairing problem etc.)

Naylor et al (2005) J. Immunology

Thymic output declines much earlier

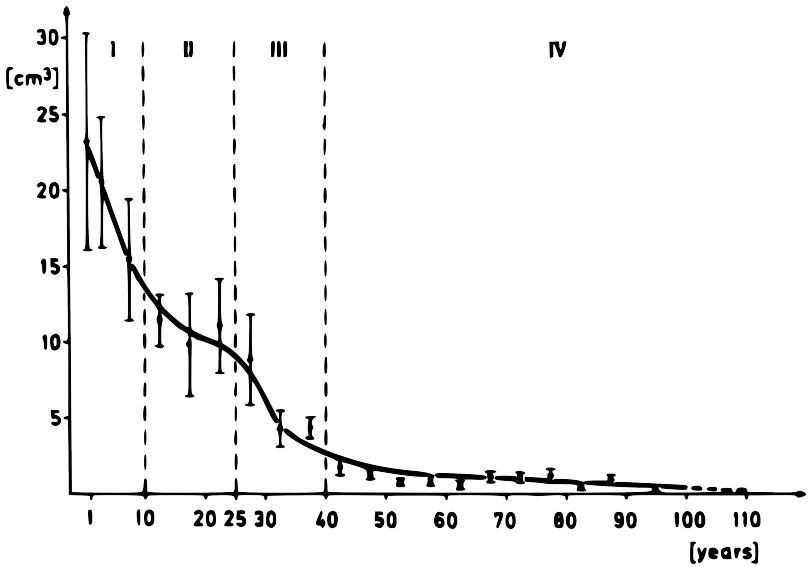
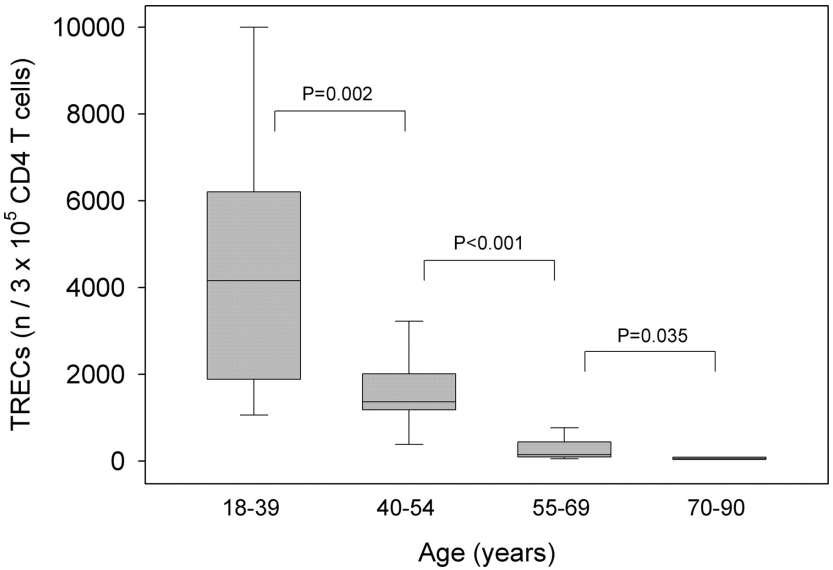


Fig 7 from Steinmann et al. (1985)



Naylor et al (2005) J. Immunology

What causes the crash in the repertoire?

Classic population genetics problem?

1. **Involution of the thymus** and decline in production of new naïve cells (loss of immigration)

2. **Stochastic extinction** during homeostatic replication or conversion into memory T cells (genetic drift or emigration)

3. **Decline in total number** of naïve T cells with age (population shrinkage)

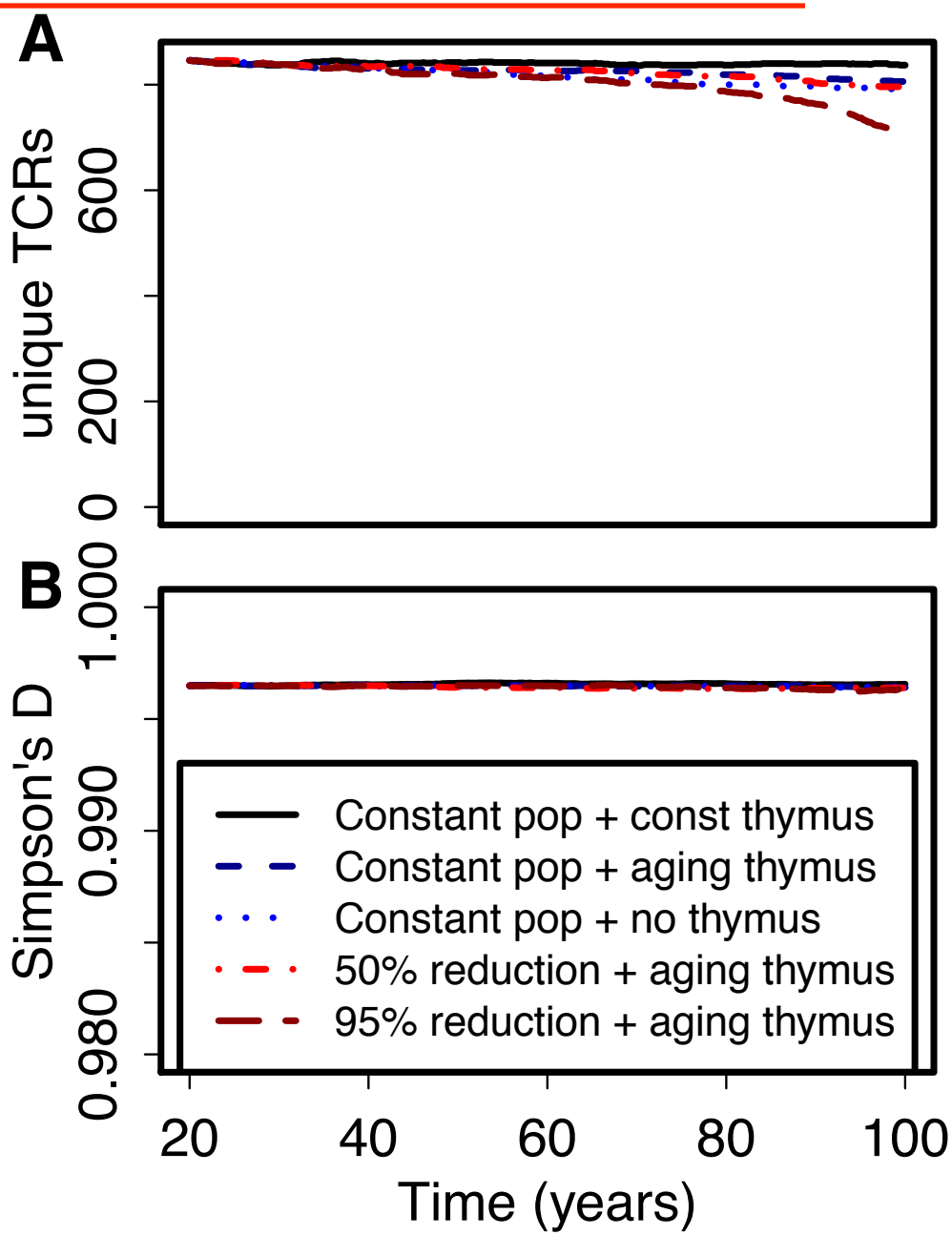
Conventional “neutral” model

Testing the conventional "neutral" model

We perform a forward simulation of the naïve T cell population and track the following transitions:

- Emigration from the thymus
  with a TCR chosen at random from the potential repertoire. New lineages emigrate at time-dependent rate $\nu[t]$ and start with clone size C=500.
- Homeostatic division
  at rate $\lambda(1\{N[t]/K[t])$, where $\lambda=1$, N[t] is the total population size and K[t] is the age-dependent carrying capacity. Due to computational constraints, $K[0]=5\times10^5$.
- Cell death / conversion to memory at rate $\delta=0.001$/day

For parameters in a biologically reasonable regime or how the total naïve population size changes, this neutral model cannot reproduce the abrupt decline of the repertoire

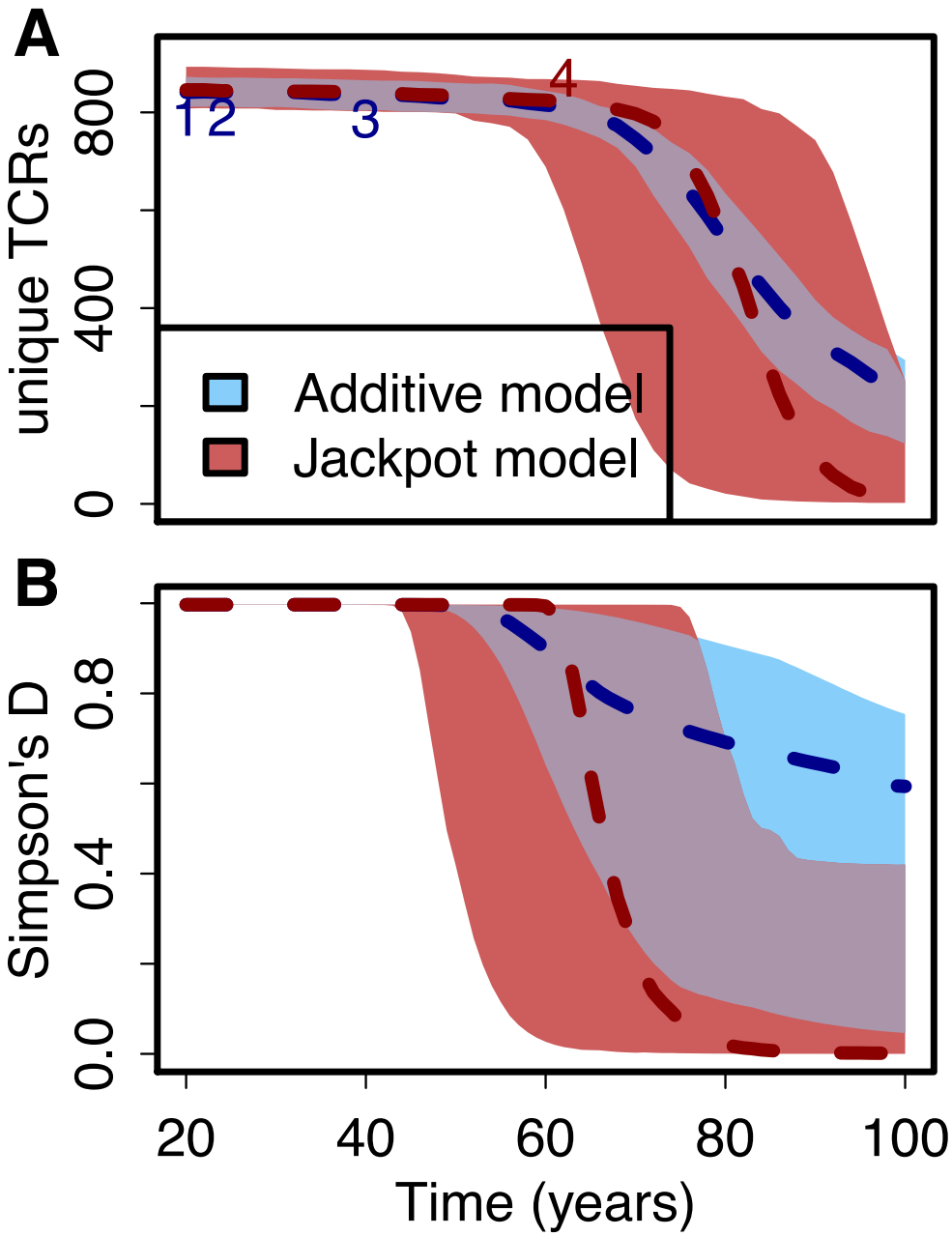


Alternative “selection” models

We now modify our neutral model to consider the effect of `mutations` (either genetic or epigenetic) that might lead to a heritable change in a lineage's homeostatic division rate. We implement this change using two different models:

- “additive” in which each additional mutation, *m*, increases the division rate by $(1+C_1 \min[m,3])$
- “jackpot” in which multiple mutations must accrue before any benefit arises and increases the division rate by $(1+C_2)$

Since mutations arise stochastically, we shade regions where 90% of simulated trajectories fall.



Similarities with cancer models

Armitage-Doll model

$$E_0 \xrightarrow{\lambda_0} E_1 \xrightarrow{\lambda_1} \dots \xrightarrow{\lambda_{n-1}} E_n$$

$$h(t) \approx \frac{N \lambda_0 \lambda_1 \dots \lambda_{n-1} t^{n-1}}{(n-1)!}$$

Lubek-Moolgavkar model

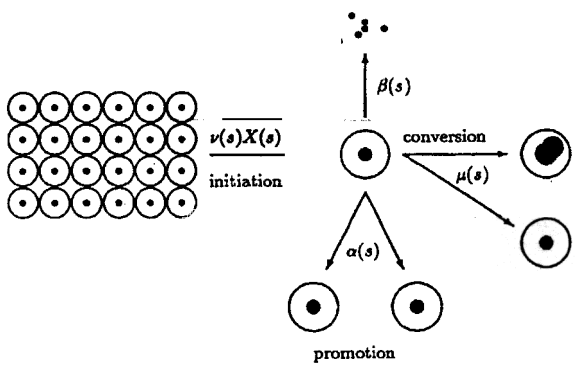
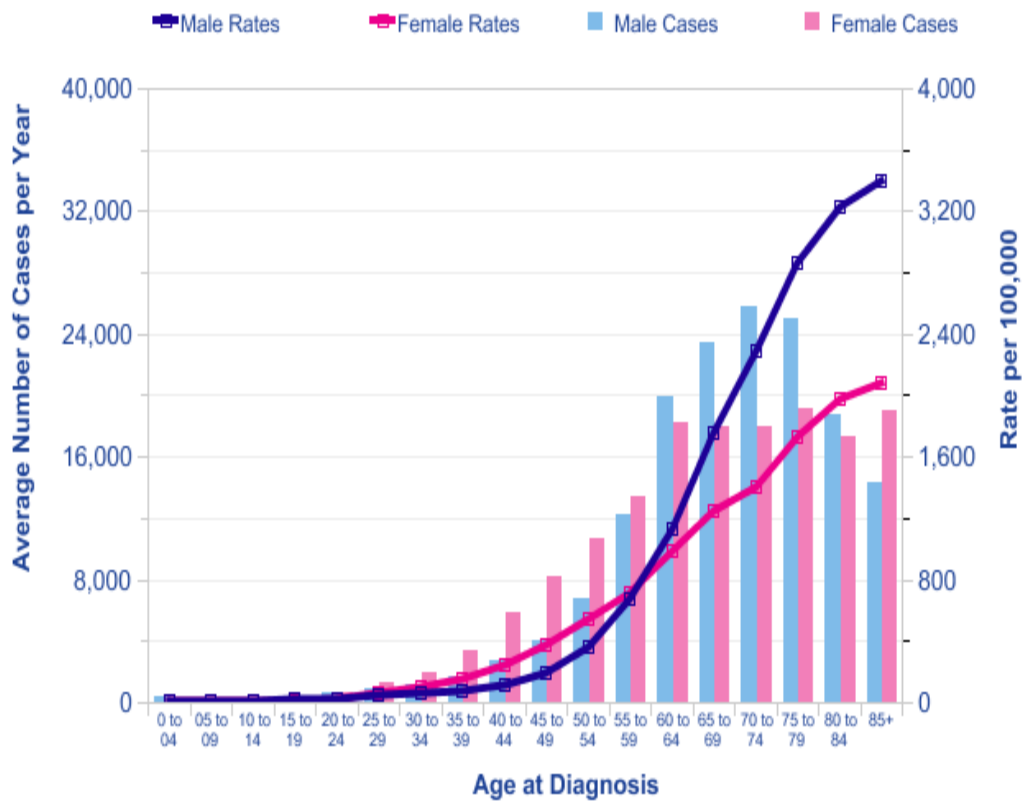


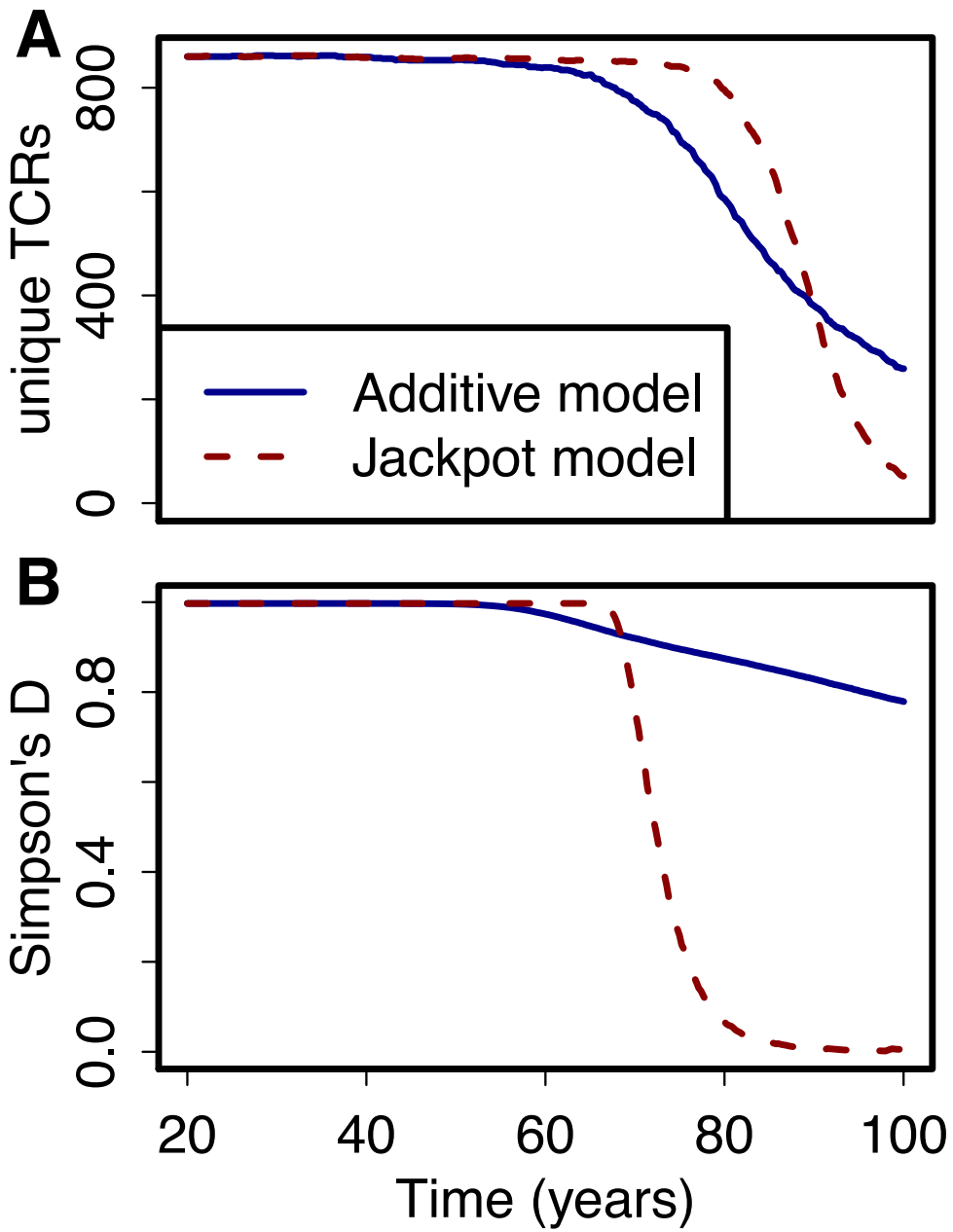
Figure 1. Two-mutation model for carcinogenesis.



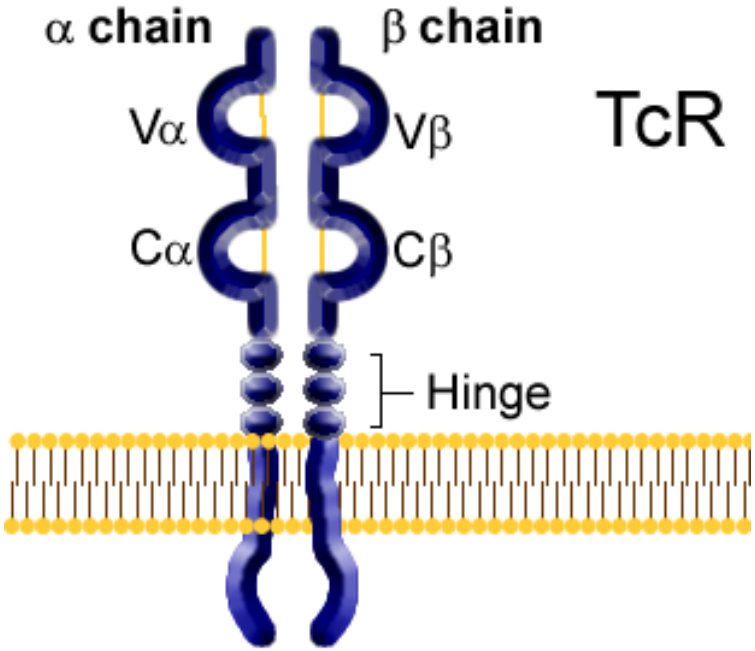
Implications and conclusions

While the form of selection has yet to be determined, these alternative “selection” models fit the observed data much better than the conventional model.

Our finding has implications for immunosenescence therapy: **thymic rejuvenation will have little effect under a selection model,** since new TCR lineages from the thymus will be less fit than the mutated lineage(s) in the population.



An open problem : measuring the immunological repertoire



How diverse is the immune system?

We can count the number of distinct α and β chains

The problem is determining the association between TCR α and β chains

Limitations of simple diversity measures

Exhaustive T-cell repertoire sequencing of human peripheral blood samples reveals signatures of antigen selection and a directly measured repertoire size of at least 1 million clonotypes

René L. Warren, J. Douglas Freeman, Thomas Zeng, et al.

Genome Res. published online February 24, 2011

repertoire between 10^6 - 10^{12}

29 OCTOBER 1999 VOL 286 SCIENCE

A Direct Estimate of the Human αβ T Cell Receptor Diversity

T. Petteri Arstila,*† Armanda Casrouge, Véronique Baron, Jos Even, Jean Kanellopoulos, Philippe Kourilsky

# of α chains 106

αβ diversity > 2.5 10^7 ?

comment by Kesmir, Borgans &DeBoer

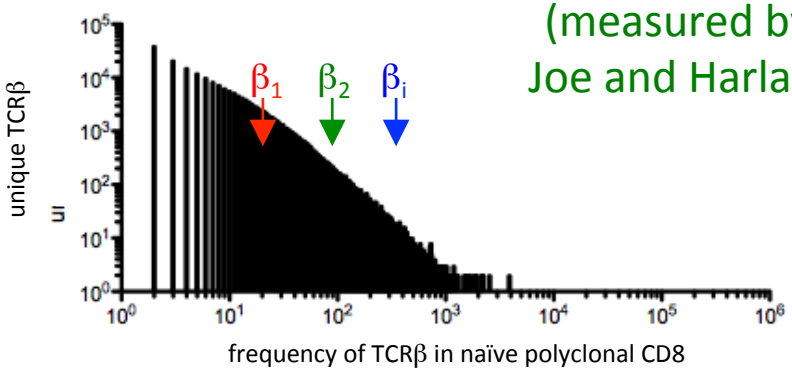
A potential solution

wild-type (H-2^b)

sequence TCRβ

marginal frequency distribution of TCRβ in naïve H-2^b polyclonal

(measured by Joe and Harlan)



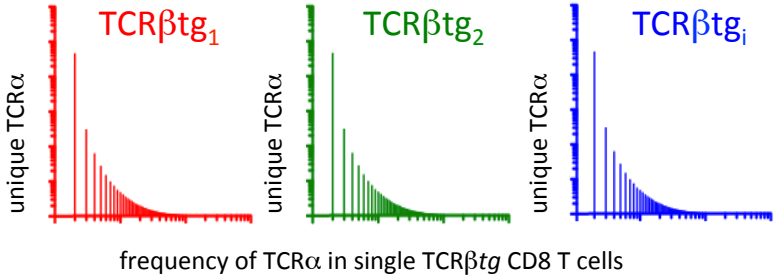
TCRβ*tg* (H-2^b)

($TCR\beta tg_1$, $TCR\beta tg_2$…$TCR\beta tg_i$)

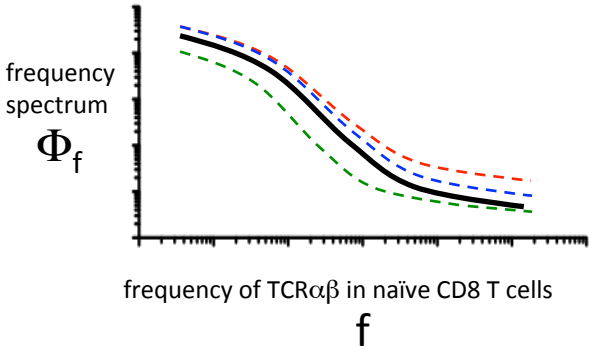
sequence TCRα

conditional frequency distribution of TCRα in each TCRβtg

(proposed)



joint frequency distribution of TCRαβ



also compute:

species richness

Simpson's diversity ($D = 1 - \Sigma\Phi_f^2$)

& other composite measures

Colleagues and collaborators

Theory

Carl Bergstrom
Vitaly Ganusov
Andreas Handel
Philip Johnson
Beth Kochin
Sergei Pilyugin
Roland Regoes
Sean Stromberg
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Epidemiology

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Experiments

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Viva Vezys

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Ageing

Jorg Goronzy

Transplantation

Chris Larsen

Malaria

Jaap de Roode
Mary Stevenson

SIV/HIV

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