Immunological memory and protection

Theory

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Experiments

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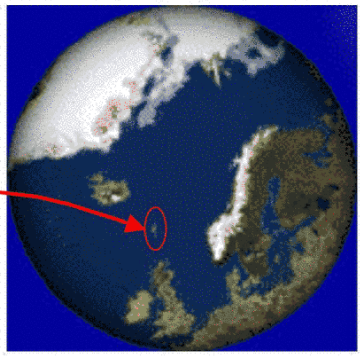
Outline

- Modeling the longevity of CD8 memory
  - A basic model.
  - Application to vaccines.
  - How can the model be tested?
- Testing the model: are all memory cells equal?
  - Are memory cells of different specificities equal?
  - CFSE turnover experiment
- Memory and protection from pathogenesis
  - A preliminary framework

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.

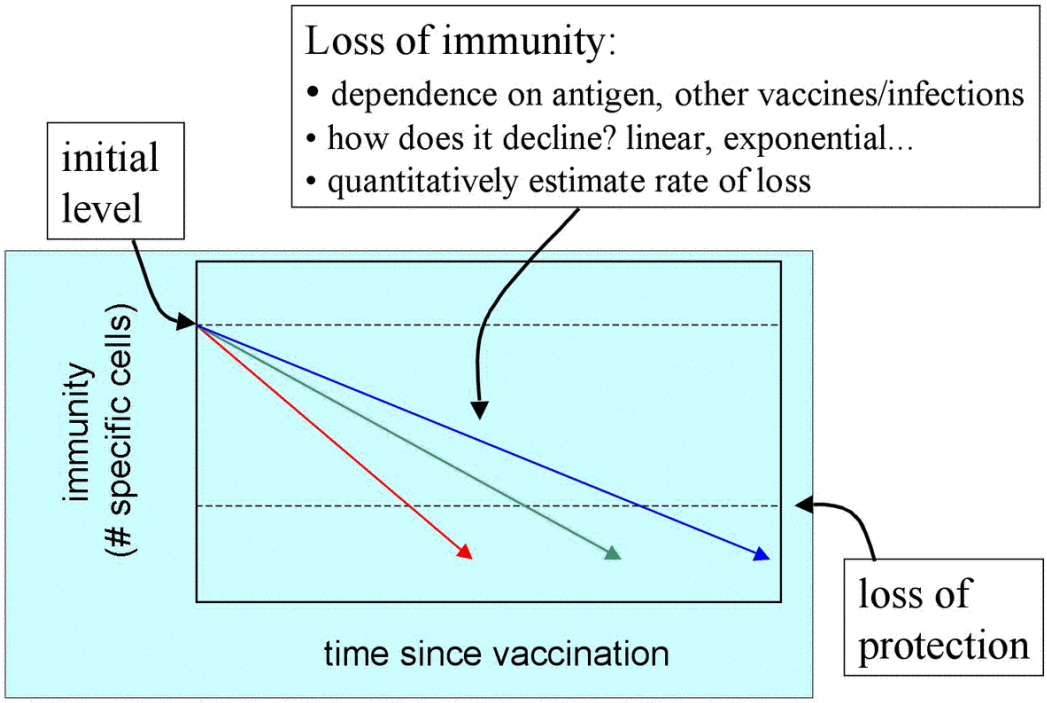
- Panum (1847)
  Memory lasted between 1781 & 1846 measles epidemics in the Faroe islands.



- 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.

What do we want to know?

The dynamics of the CD8 population after immunization

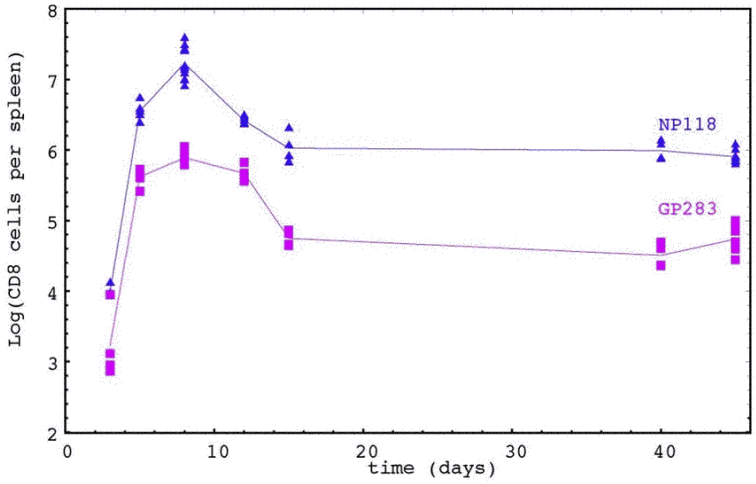


Hypothesis for immune memory

- Immortal memory cells
- 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
  - cross-reactive or bystander stimulation
  - homeostasis

CD8 memory is long-lived

Dynamics of CD8 response during acute infections:



Basic features:
- expansion (>10^4x)
- contraction (10-100x)
- memory phases

Virus is cleared by 1 week following infection

Adoptive transfer studies suggest CD8 memory does not require antigen.

LCMV (Arm) infections of Balb/c mice (Murali-Krishna 1999)

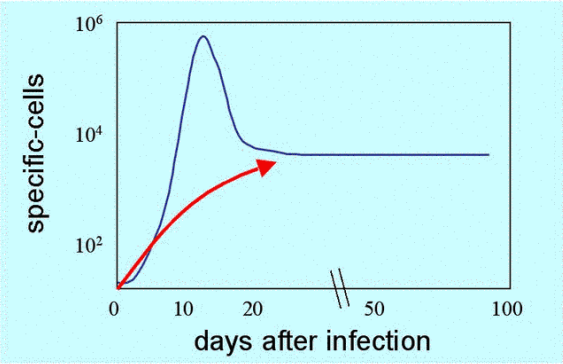
Questions we would like to answer

- The requirement of antigen for memory is a quantitative question. We will estimate the duration of memory in the absence of antigen.
- Use models to examine the relative contributions of cross-reactive/bystander stimulation and homeostasis for the maintenance of memory.
- Generate experimentally testable predictions.

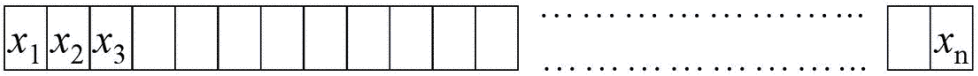
We will focus on CD8+ T-cell memory

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 i^{th} lineage
$X=\Sigma x_i$ = *total* number of cells

deterministic terms — cross-rx, homeostasis, death

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

stochastic terms — input — stimulation

Model 1

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

Sum all lineages x_i: replace stochastic terms for a^* and q^* by averages na and nmq

$$\frac{dX}{dt} = na + nmq + X(cq + S(X) - d)$$

Homeostasis for total population $\tilde{X}$: set total population to steady state.

$$cq + S(\tilde{X}) - d = \frac{n(a + mq)}{\tilde{X}}$$

Rate of loss of memory: Consider a long timescale (hold X at $\tilde{X}$). Evaluate x_i after a single specific stimulation and in absence of further specific stimulation ($q_i^*=0$)

$$\frac{dx_i}{dt} = a_i^* + \left(cq + S(\tilde{X}) - d\right)x_i = a_i^* - \frac{n(a + mq)}{\tilde{X}}x_i$$

To study the long-term average behavior: x_i exponentially approaches value $\tilde{x}$ at rate R

$$\tilde{x} = \tilde{X}\frac{a}{n(a + mq)} \qquad R = -\frac{n(a + mq)}{\tilde{X}}$$

Results - either memory or a diverse repertoire

The decline of memory is exponential at rate

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

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

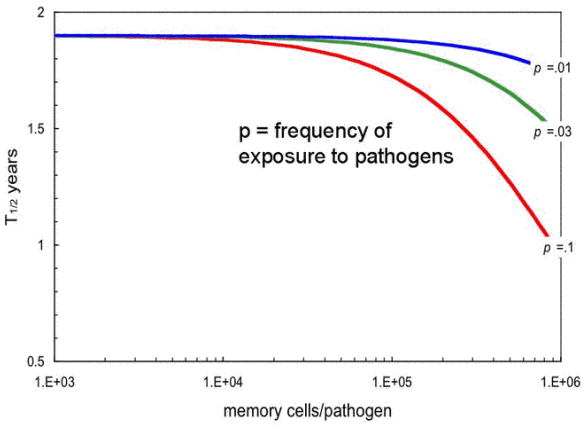
The repertoire is maintained only if the input from the thymus is sufficiently large that

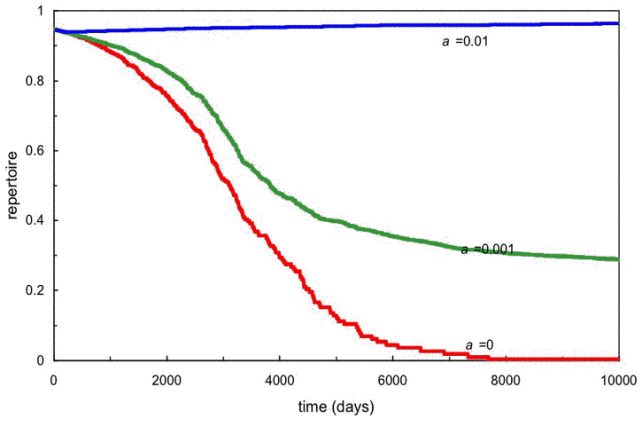
$$\tilde{X} = X\frac{a}{n(a+mq)} > 1$$

The longevity of memory is

(i) independent of cross-reactivity

(ii) relatively long if thymic input small

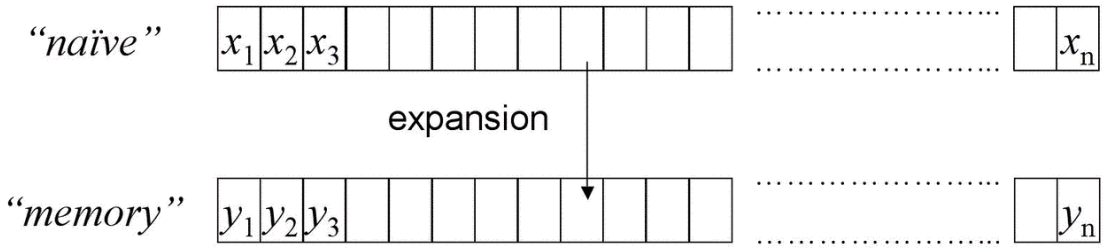




Incorporate naïve and memory populations

- CD8 cell lineages with a given antigen-specificity can be of "naïve" or "memory" phenotypes with different properties.
- On specific stimulation cells move from the naïve to memory phenotype and remain in the memory phenotype (e.g. CD8+ cells go from $CD44^{lo}$ to $CD44^{hi}$)
- Homeostasis operates independently in the "naïve" and "memory" pool.

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

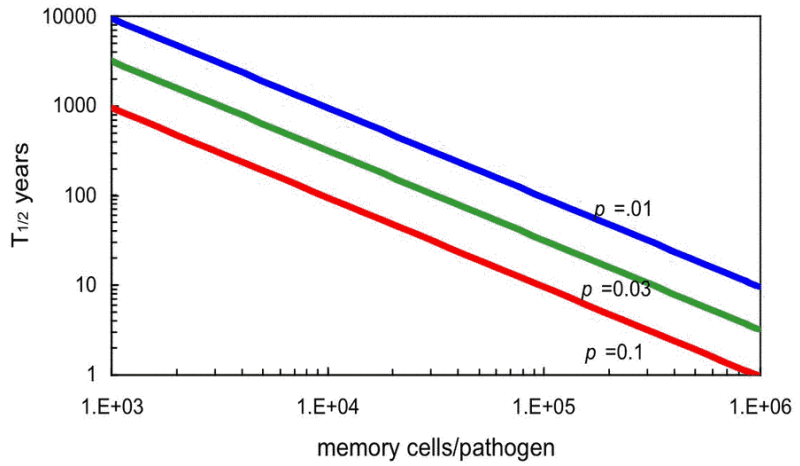
cross rx

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

Results - memory

The decline of memory is exponential at rate R equal to

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

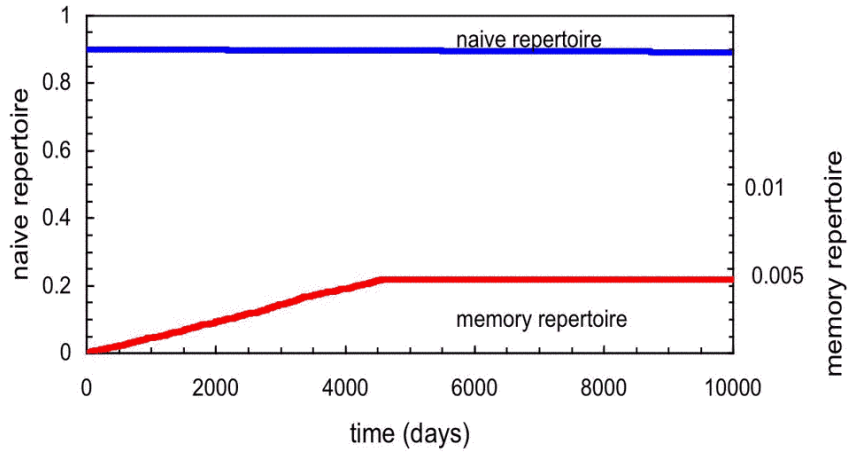


total expansion
||
average frequency of exposure to pathogens (p)
x
average # of memory cells per pathogen (m)

p = frequency of exposure to pathogens

Results - repertoire

- If homeostasis acts independently on the naïve and memory compartments the repertoire is maintained in the naïve compartment even in the absence of input from the thymus.
- If turnover is stochastic then drift will be proportional to the rate of turnover and a low rate of turnover will minimize drift.
- The memory repertoire has a finite capacity given by $r_m = \frac{\rho' \ln(m)}{m}$



Some problems

- The stimulation of new lineages (exposure to new pathogens) may be dependent on the age of the individual.
- The size of the total population of memory cells changes over time.
- What happens during very early life (i.e. following exposure to the first few antigens? The assumption that the timescale for the generation of memory cells following infection is fast compared with the timescale for the change in the total population of immune cells is not met. What rules apply here?

Some refinements

What is the longevity of memory for the general case when

- the input of new memory cells (specific for other antigens) occurs at rate *a(t)*.
- the size *Y(t)* of the memory compartment changes over time.

$$\frac{dy(t)}{dt} = -\frac{a(t)}{Y(t)}y(t)$$

$$\frac{dy(t)}{y(t)} = -\frac{a(t)}{Y(t)}dt$$

integrating we get

$$\ln y(t)\Big]_{t=0}^{t} = -\int_{t=0}^{t}\frac{a(t)}{Y(t)}dt$$

$$\frac{y(t)}{y(0)} = \frac{Y(t)}{Y(0)}\exp\left(\int_{0}^{t}\frac{a(s)}{Y(s)}ds\right)$$

Conclusions

- The requirement of antigen for memory is a quantitative question: memory is likely to be relatively long-lived with $T_{1/2}$ of several years to decades in the absence of specific antigen.
- The duration of memory is independent of the extent of cross-reactive stimulation. (Cross reactive may only help in the maintenance of homeostasis).
- Homeostasis is the most important factor in the longevity of memory. (Freitas/Rocha hypothesis correct).
  - Rate of loss of memory independent of the mechanism or rate of maintenance of homeostasis.
  - Independent homeostasis in the naïve and memory compartments gives a reasonable tradeoff between memory and the repertoire
  - Memory is lost exponentially at rate

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

Testing the model

- Assumptions:
  - All "memory cells" equal
  - Turnover/homeostasis is independent of
    (i) the number of divisions a cell has undergone
    (ii) its antigenic specificity
- Predictions:
  - Memory independent of extent of cross-reactivity
  - Loss of memory is exponential at rate R given by

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

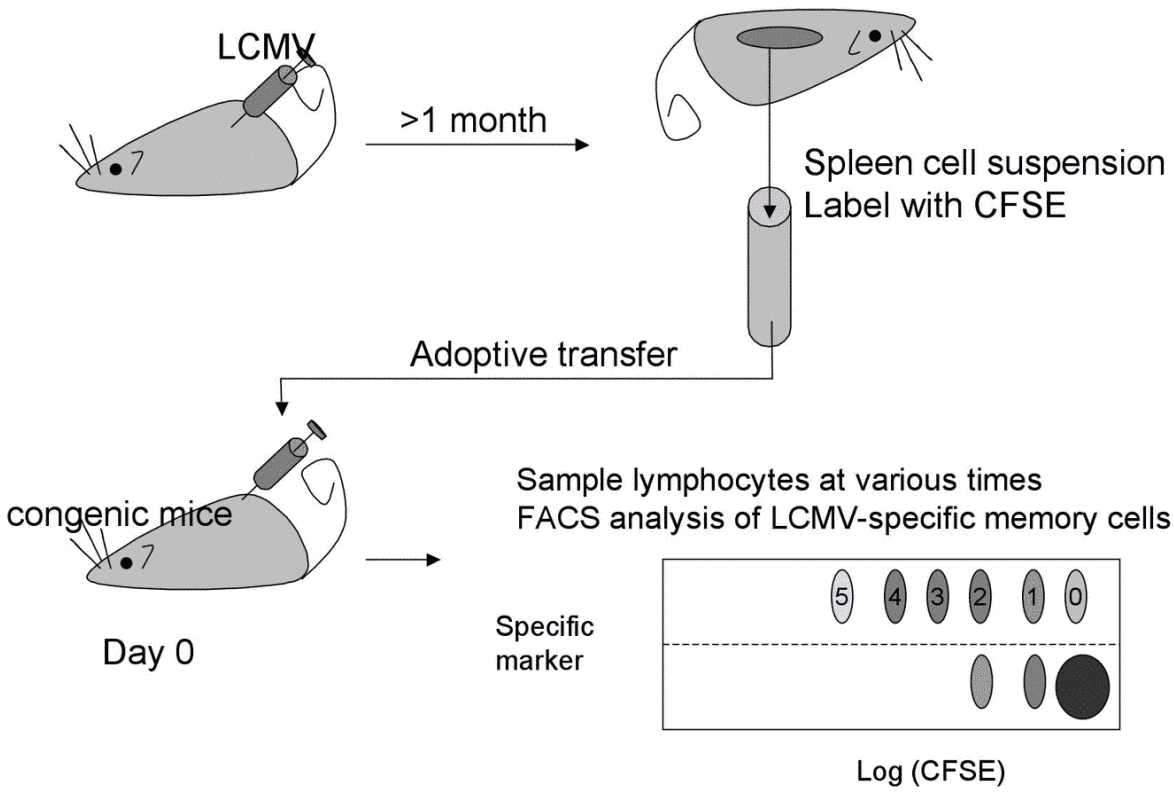
Are all memory cells "equal"

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

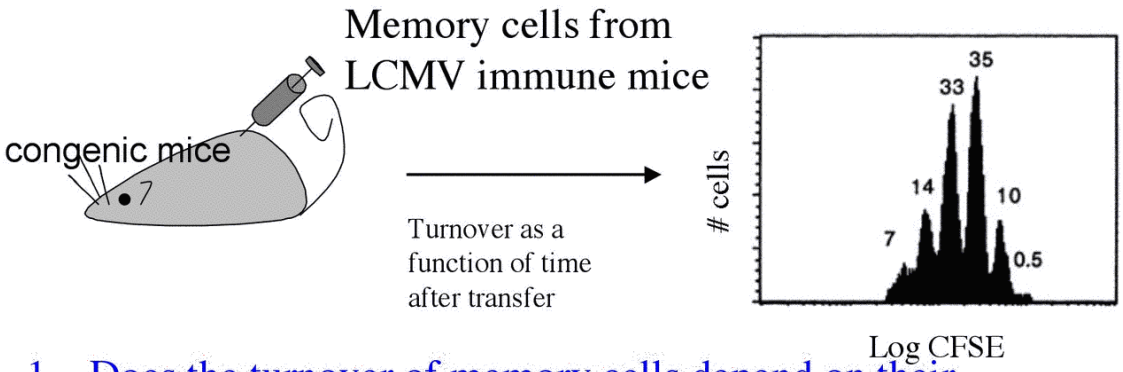
Using this assay we would like to test the assumptions of the model, namely

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?

CFSE experiments



CFSE experiments

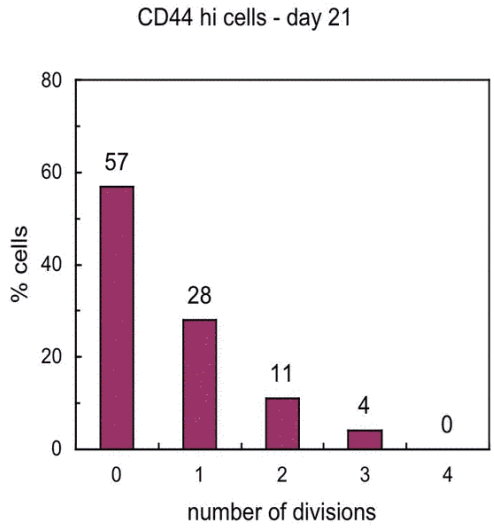


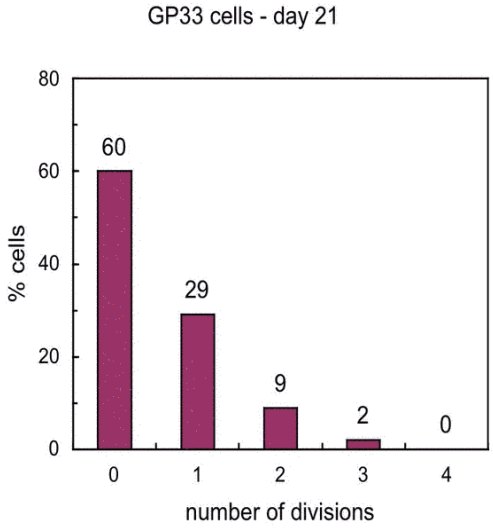
1. Does the turnover of memory cells depend on their antigenic specificity?
   Compare turnover of cells specific to a given LCMV epitope with that of all memory cells taken together.

2. Does it depend on time since the primary response or the number of divisions a cell has undergone?
   Analysis of the turnover of memory cells as a function of time.

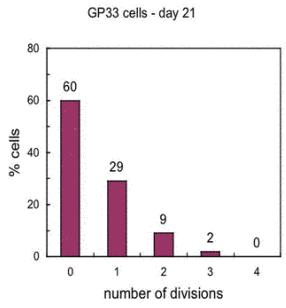
Turnover of cells with different specificities

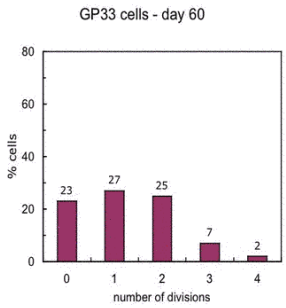
Memory cells specific to the GP33 exhibit similar turnover at 21 days after transfer as the total population of CD44hi memory cells.

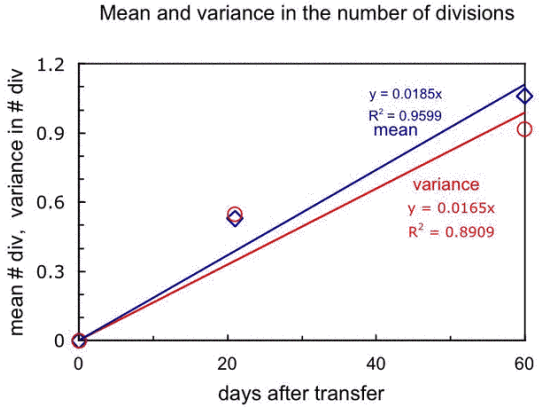




Turnover of cells as a function of time

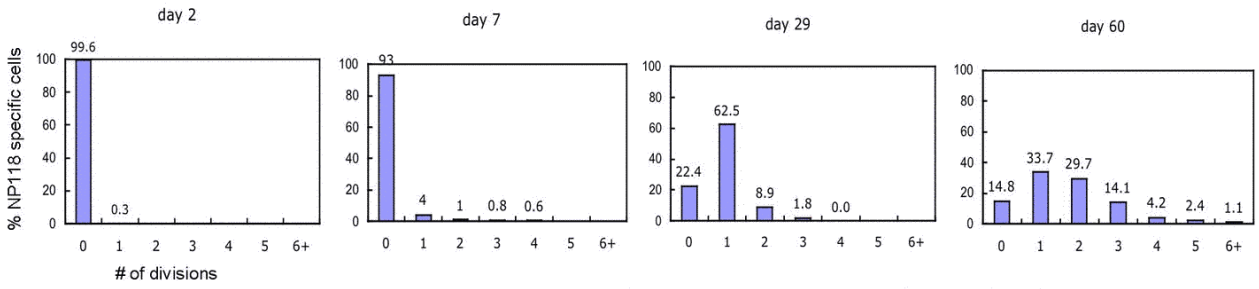


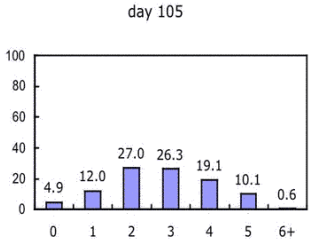




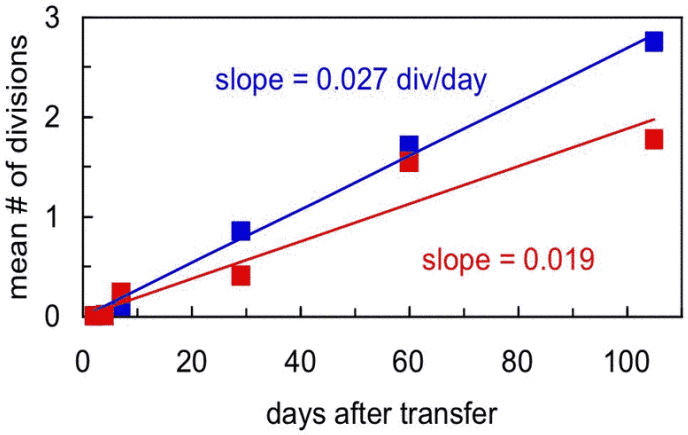
The mean number of divisions and the variance in the number of divisions increase linearly with time at approximately the same rate.

Similar data from different mice (NP118 Balb/c)





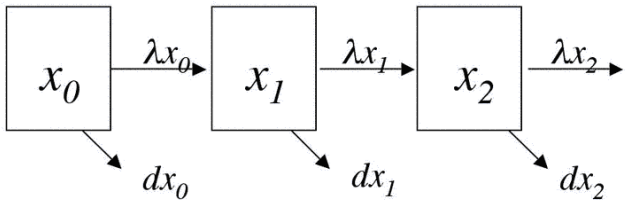
Mean and variance in the # of divisions



A simple model of cell turnover that fits the data

- Stochastic model
- x_i equals the number of cells having undergone i divisions
- The probability of division λ and death d are independent of the number of divisions the cells have undergone.

model



$$\frac{dx_0}{dt} = -\lambda x_0 - dx_0$$

$$\frac{dx_i}{dt} = 2\lambda x_{i-1} - \lambda x_i - dx_i$$

solution

$$X = \sum x_i = X(0)e^{(\lambda - d)t}$$

$$x_n(t) = x_0(0)\left\{\frac{(2\lambda t)^n}{n!}e^{-2\lambda t}\right\}e^{(\lambda - d)t}$$

Poisson distribution
mean=$2\lambda t$
variance=$2\lambda t$

exponential growth
(λ-d)

Accurate estimation of turnover rates is harder

Model A: death (d) occurs stochastically any time during the cell cycle

equations

$$\frac{dx_i}{dt} = 2\lambda x_{i-1} - \lambda x_i - dx_i$$

solution

$$x_n(t) = x_0(0)\left\{\frac{(2\lambda t)^n}{n!}e^{-2\lambda t}\right\}e^{(\lambda-d)t}$$

Poisson distribution mean=variance=$2\lambda t$

exponential growth (λ-d)

Model B: death kills a fraction f of cells at the time of division.

$$\frac{dx_i}{dt} = 2\lambda(1-f)x_{i-1} - \lambda x_i \qquad x_n(t) = x_0(0)\left\{\frac{(2\lambda(1-f)t)^n}{n!}e^{-2\lambda(1-f)t}\right\}e^{\lambda(1-2f)t}$$

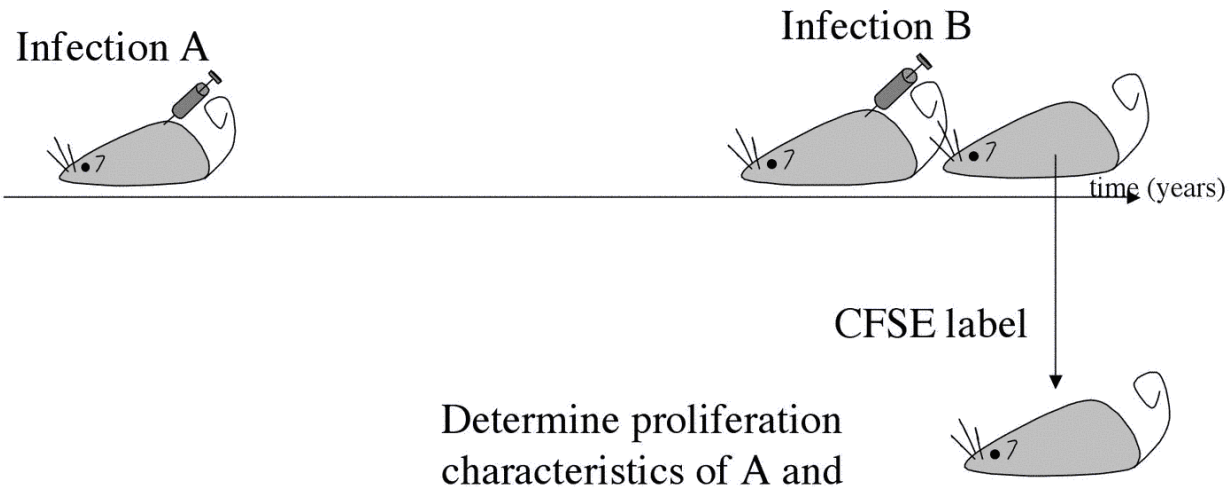
Poisson distribution mean=variance=$2\lambda(1-f)t$

exponential growth (λ-d)

Homeostatic population: λ=d, f=0.5
The estimate of λ from model A will be half that from model B

Designing a better experimental tests -1

Is the turnover of immune cells of different specificities independent of time since they were generated?



Determine proliferation characteristics of A and B sepecific cells

Designing a better experimental tests -2

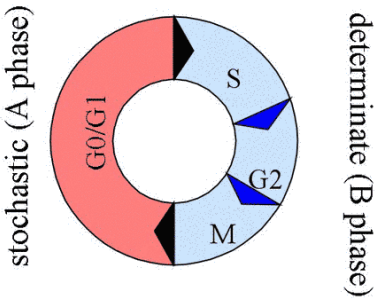
Determine the rate of loss of memory cells to a given antigen after exposure to other antigens (basic idea of Ray Welsh and colleagues).

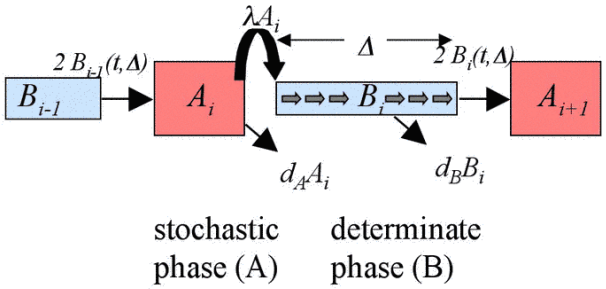
Problem with measuring small changes in numbers when different mice have different numbers of cells.

Immunize first with the smallest infection, subsequently immunize with the largest possible infection and use multiple infections.

Two approaches to measuring cell turnover

1. Smith-Martin based cell-cycle model





2. Estimate parameters independent of the specific model for cell division and death.
   NP118 (BALB/c) memory cells
   mean time for division of surviving cells T= 37 days (31-45: 95%CI)
   fraction of cells which die per cell cycle 0.5

From memory to protection

We have defined memory as the maintenance of increased numbers of functional antigen-specific cells.

Under what circumstances does memory lead to protection from disease?

Immunopathology

Pathology can occur if (i) the virus kills infected cells or (ii) if the immune system kills infected cells, and is defined by the reduction in host target cells.

Simple models have been used to investigate immunopathology..

$$\text{uninfected cells} \quad \frac{dX}{dt} = d(X_0 - X) - \beta XV$$

$$\text{infected cells} \quad \frac{dY}{dt} = \beta XV - (d + \alpha)Y - hYZ$$

$$\text{free virus} \quad \frac{dV}{dt} = pY - cV$$

$$\text{CD8 response} \quad \frac{dZ}{dt} = sY - \delta Z$$

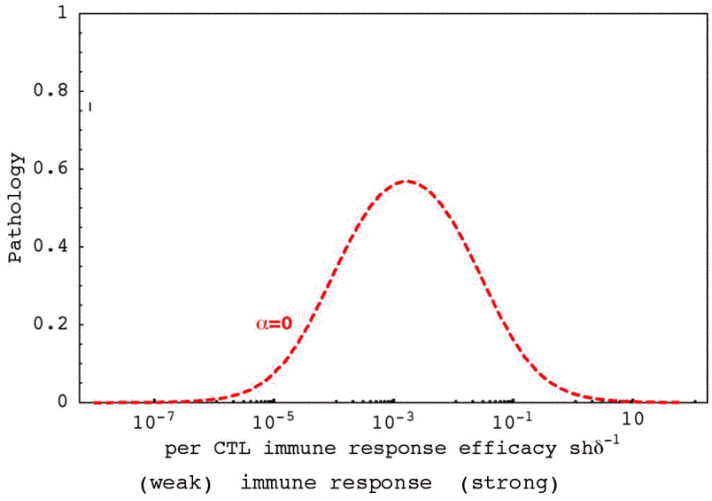
Pathology is proportional to the drop in total target cell numbers (X+Y)

* See Krakauer, Wodarz and Nowak

Immunopathology

Krakauer and colleagues have considered immunopathology during persistent infections by determining the drop in the number of target cells.

They found that pathology to a non-cytopathic virus (and to a lesser extent to a cytopathic viruses) is most severe for intermediate efficacies of the immune responses



Immunopathology during acute infections

To consider immunopathology during acute infections we need to consider the pathology during the course of infection and define immunopathology in terms of the reductions in target cells during this time.

Some possible ways are

1. Pathology equals the maximum reduction in the number of target cells.
2. Pathology equals the average reduction in the number of target cells.

Immunopathology during acute infections

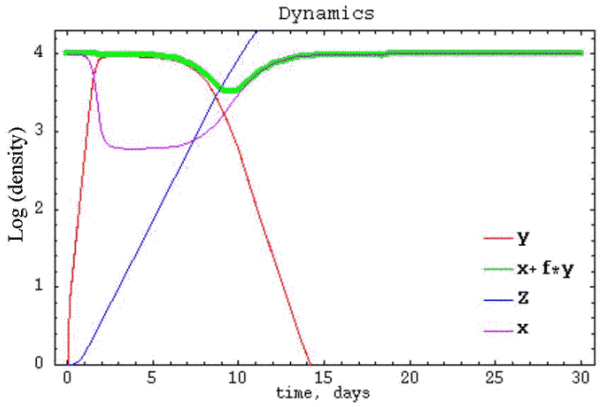
uninfected cells $\frac{dX}{dt} = d(X_0 - X) - \beta XV$

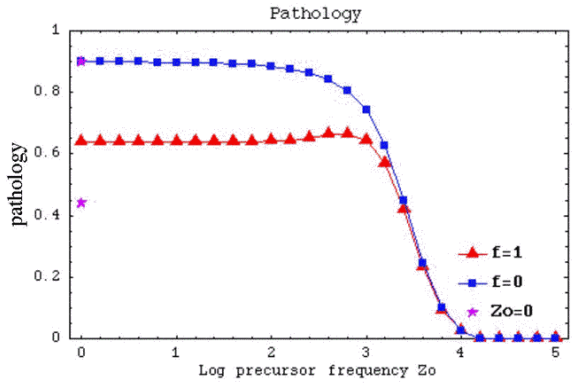
infected cells $\frac{dY}{dt} = \beta XV - \alpha Y - hYZ$

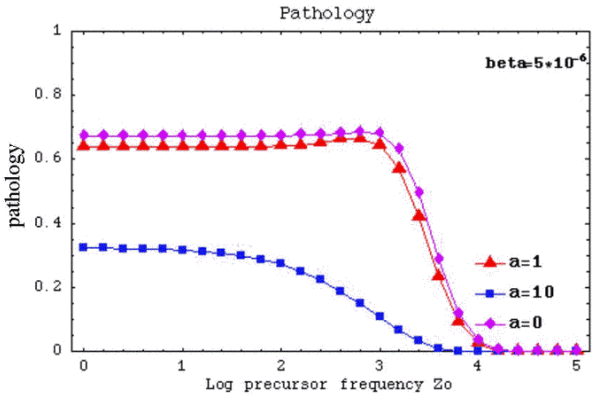
free virus $\frac{dV}{dt} = k_0 Y - cV$

CD8 response $\frac{dZ}{dt} = \frac{sYZ}{k+Y}$

pathology $p = \frac{X_0 - (X + fY)}{X_0}$ $0 < f < 1$







Tentative conclusions for immunopathology

1. We are only just beginning to model immunopathology during acute infections.
2. In general an increased precursor frequency of CD8 cells leads to decreased immunopathology.
   Memory is related to protection.
3. We still have to incorporate other immune responses (antibodies etc)…..

Summary

1. We have a basic quantitative model for immune memory

2. Memory cell turnover is stochastic independent of antigenic specificity.

3. Memory is related to protection
   We need to model specific examples where it is not.

How fast do CTL kill infected cells in vivo?

1. We have a basic quantitative model for immune memory

2. Memory cell turnover is stochastic independent of antigenic specificity.

3. Memory is related to protection
   We need to model specific examples where it is not.