Quantification of T-cell dynamics

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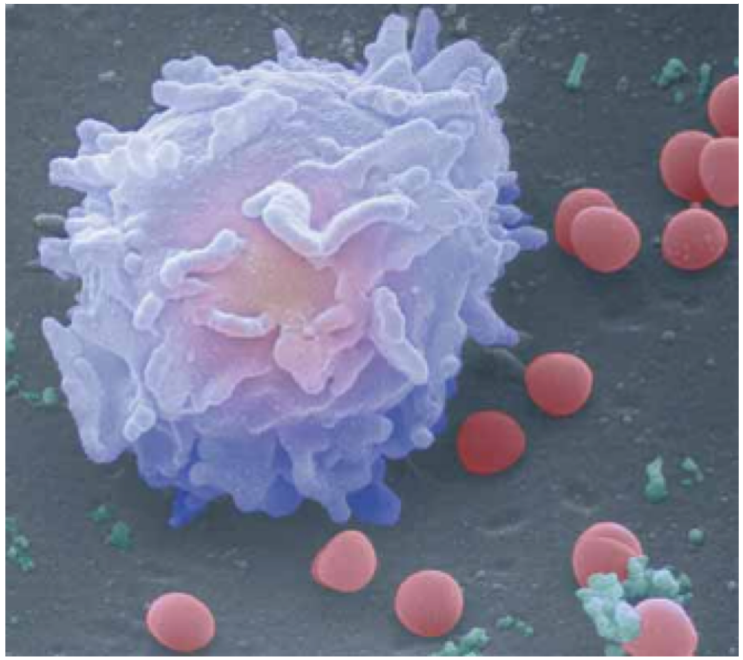
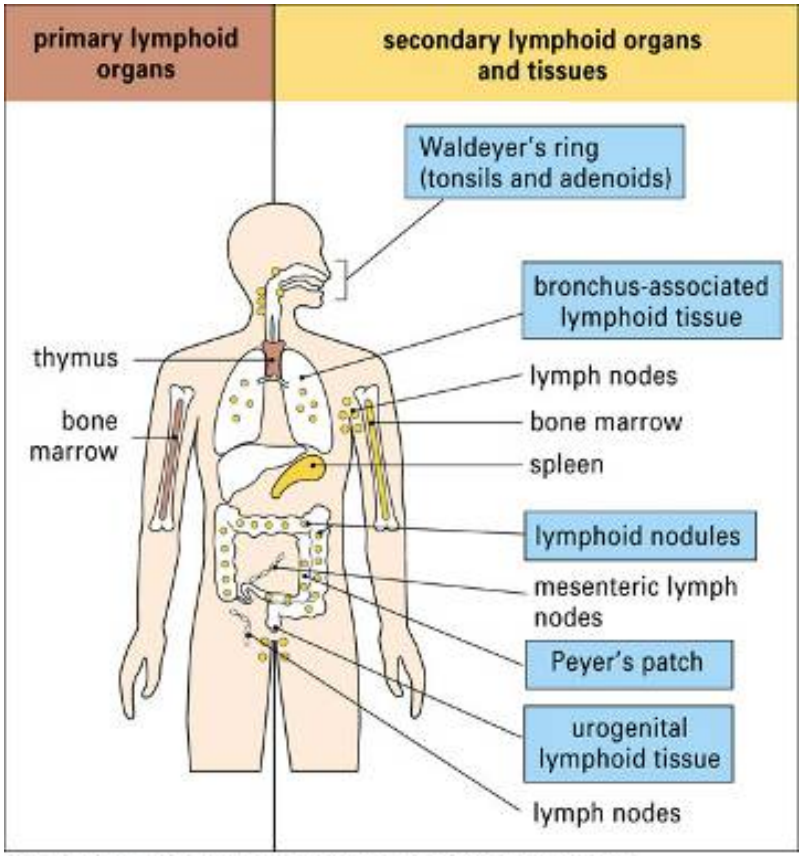
How long does a T cell live?

- Estimates vary widely
- How is T-cell turnover disturbed in HIV infection, leukemia, or after stem cell transplantation?



What is the difficulty?

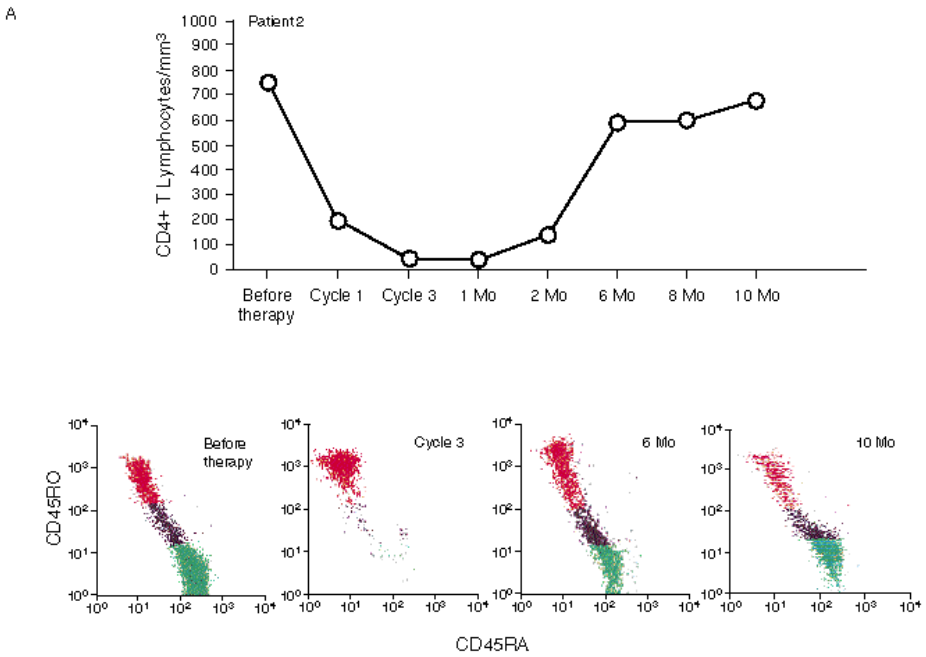


© **Fleshandbones.com** Roitt et al: Immunology 6E

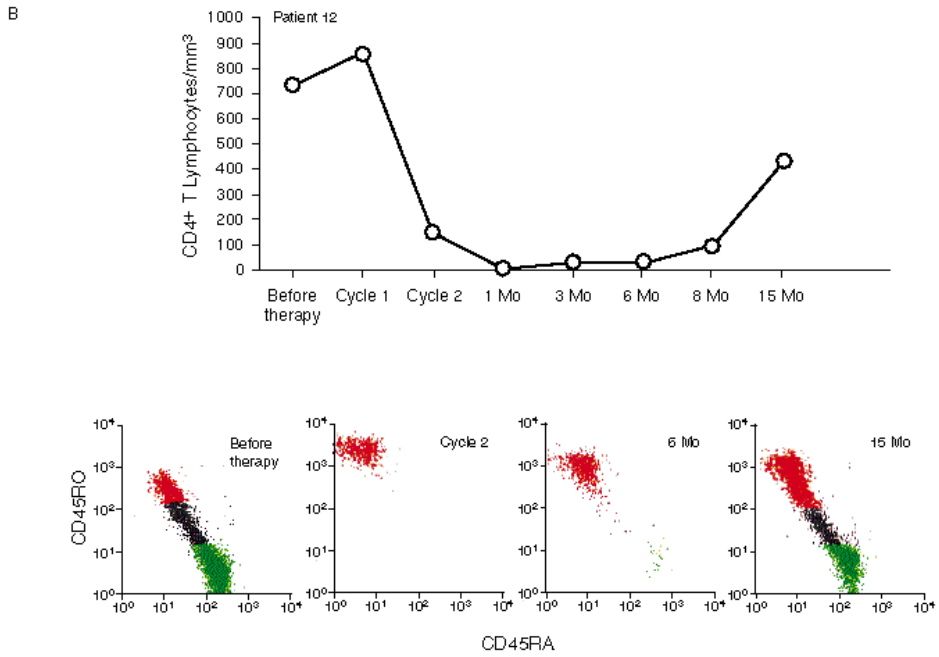
How to follow a lymphocyte from its birth to its death?

Extrapolation from mice to men

Experiments of nature (1): T-cell reconstitution after chemotherapy



3 years of age



23 years of age

Underestimate?: Cells may also die during reconstitution

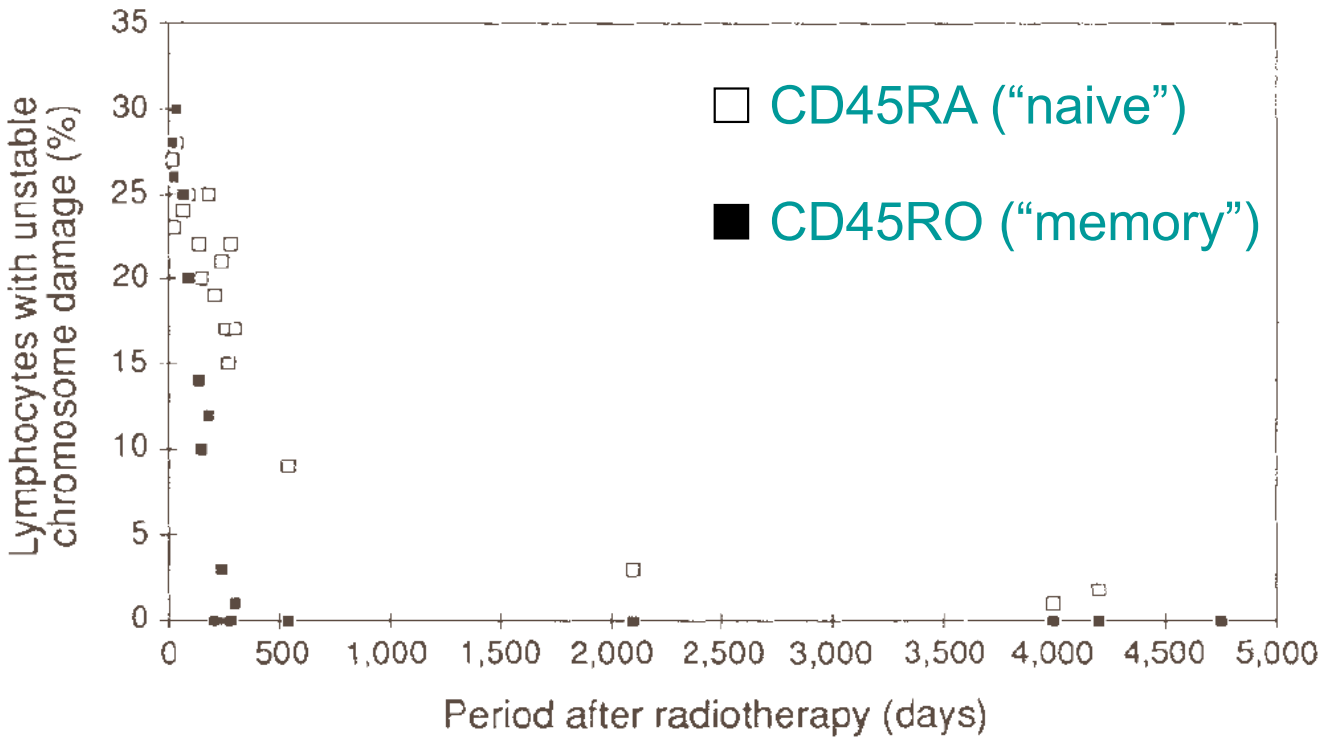
Overestimate?: Cells undergo little competition

Mackall et al. 1995

Experiments of nature (2): Loss of T cells with chromosome damage

Immunological memory resides in a population with rapid turnover:

Memory T cells have a shorter lifespan (~250 d) than naive T cells (~1000 d)



Michie et al.
Nature 1992

Caution: Cells have DNA damage, and cell numbers are low

Static versus dynamic markers of T-lymphocyte turnover

Static

- Ki67-expression

  (protein expressed in G_1,S,G_2,M phase)
- Annexin V staining

  (stains phosphatidylserine translocation)

Dynamic

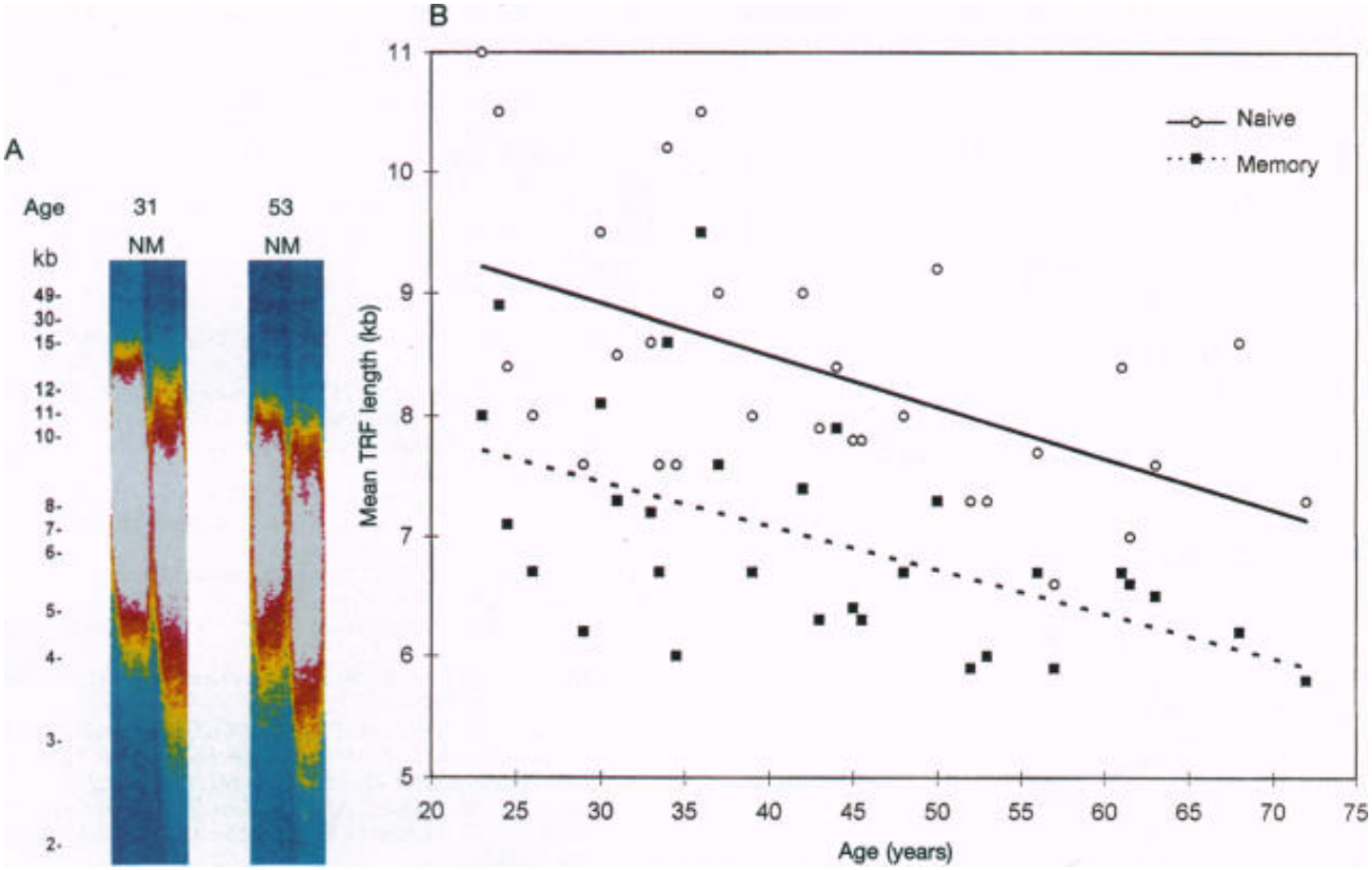
Natural markers:

- T-cell telomere lengths
- T-cell receptor excision circles

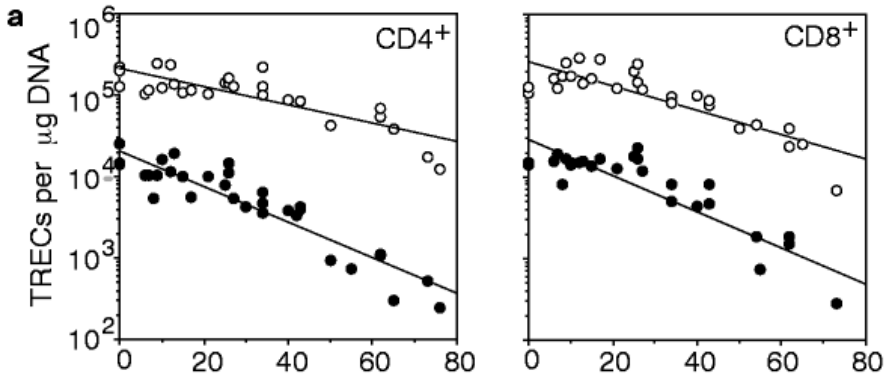
Labelling:

- (CFSE labelling)
- BrdU labelling
- Stable isotope labelling

Changes in telomere lengths are no direct measure of T-cell division

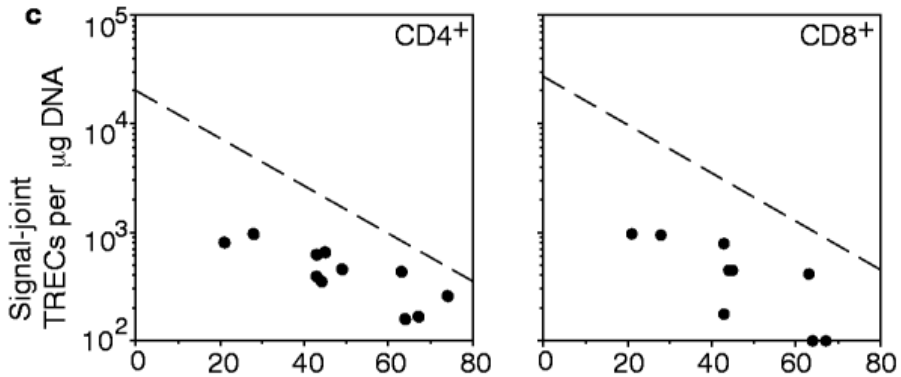


Weng et al. PNAS 1995

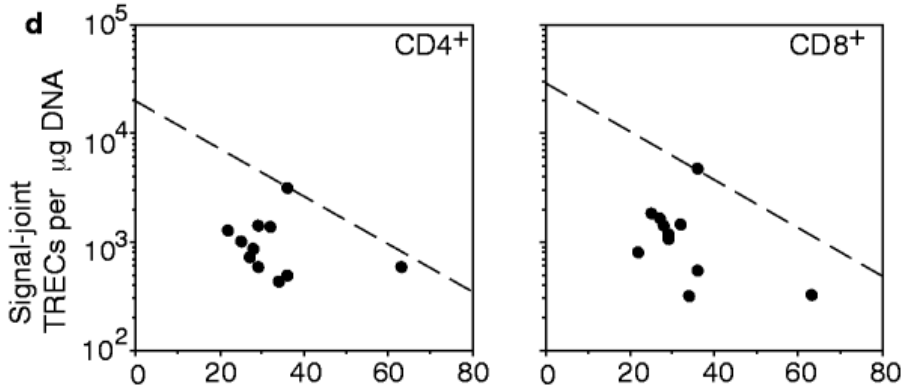


Healthy individuals

Changes in TRECs do not directly reflect thymus output



Thymectomized individuals



HIV-infected individuals

Douek et al. Nature 1998

Static versus dynamic markers of T-lymphocyte turnover

Static

- Ki67-expression

  (protein expressed in G_1,S,G_2,M phase)
- Annexin V staining

  (stains phosphatidylserine translocation)

Dynamic

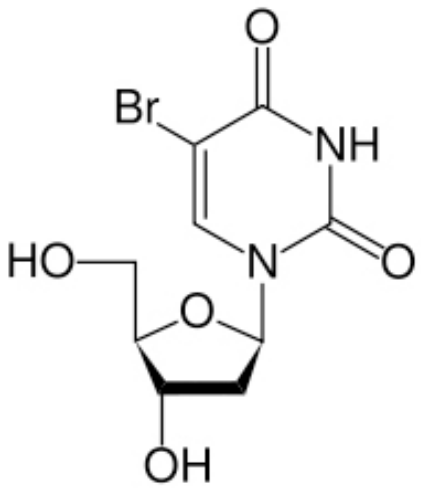
Natural markers:

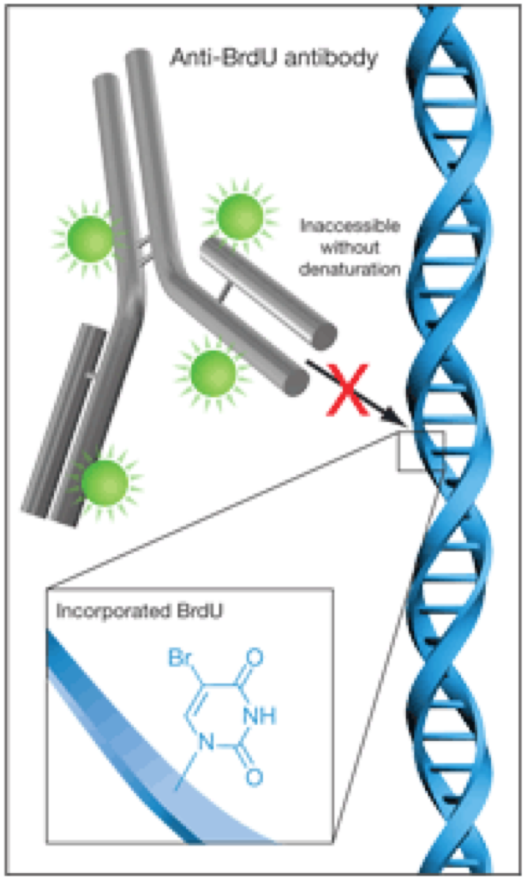
- T-cell telomere lengths
- T-cell receptor excision circles

Labelling:

- (CFSE labelling)
- BrdU labelling
- Stable isotope labelling

Dynamic markers (1)
BrdU Labelling



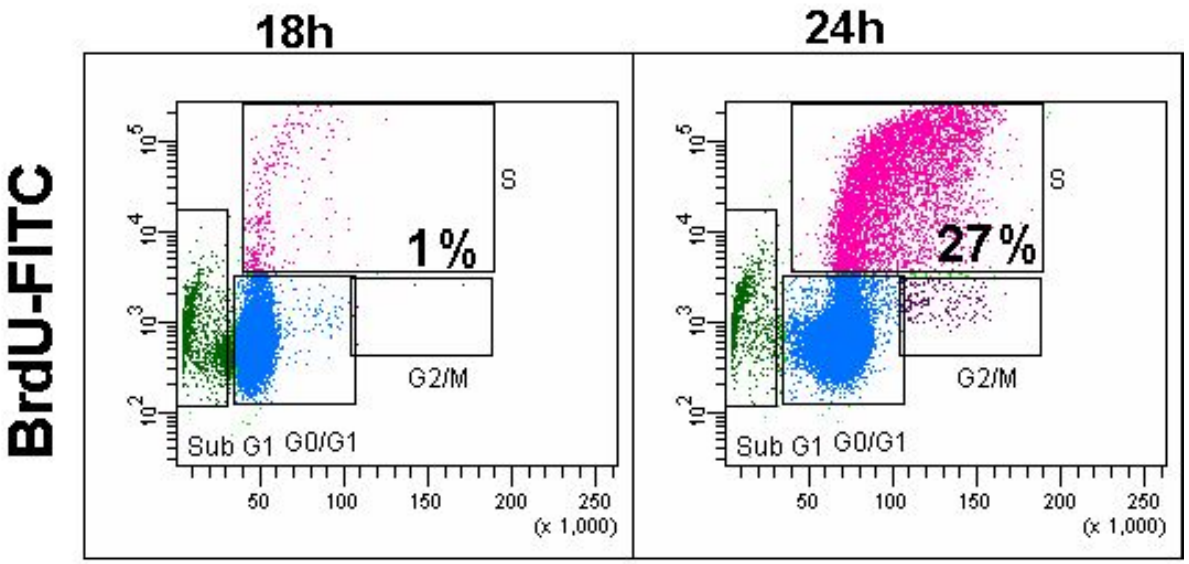


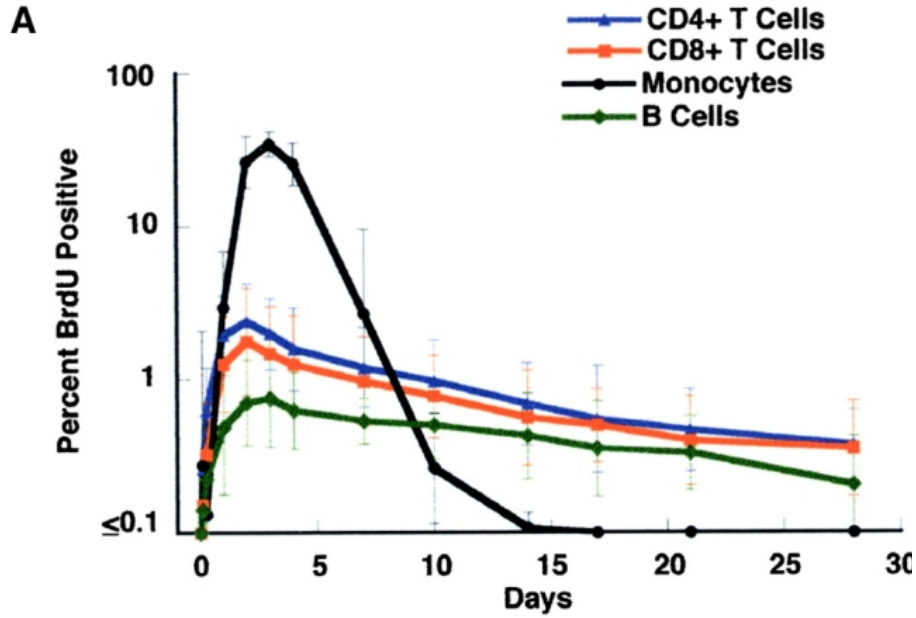
BrdU = 5-bromo-2’-deoxyuridine

Nucleoside analog

incorporated instead of thymidine

Determine percentage BrdU+ cells by FACS analysis





Kovacs et al. 2001

How to quantify leukocyte turnover?

Intuition vs modelling

During label administration:

Production (at rate p):

U -> 2L

L -> 2L

Death removes cells at rate d

U+L=1

After stop of label:

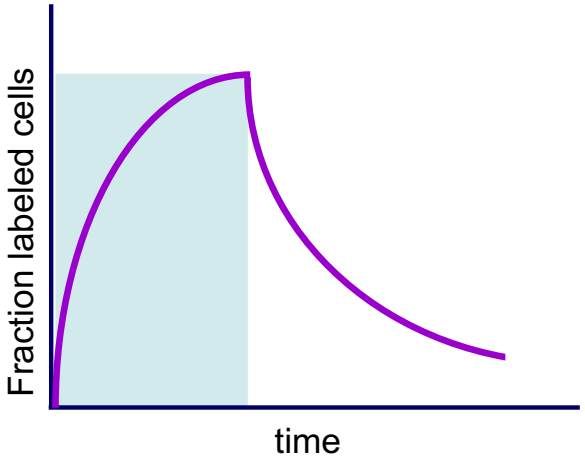
Production (at rate p):

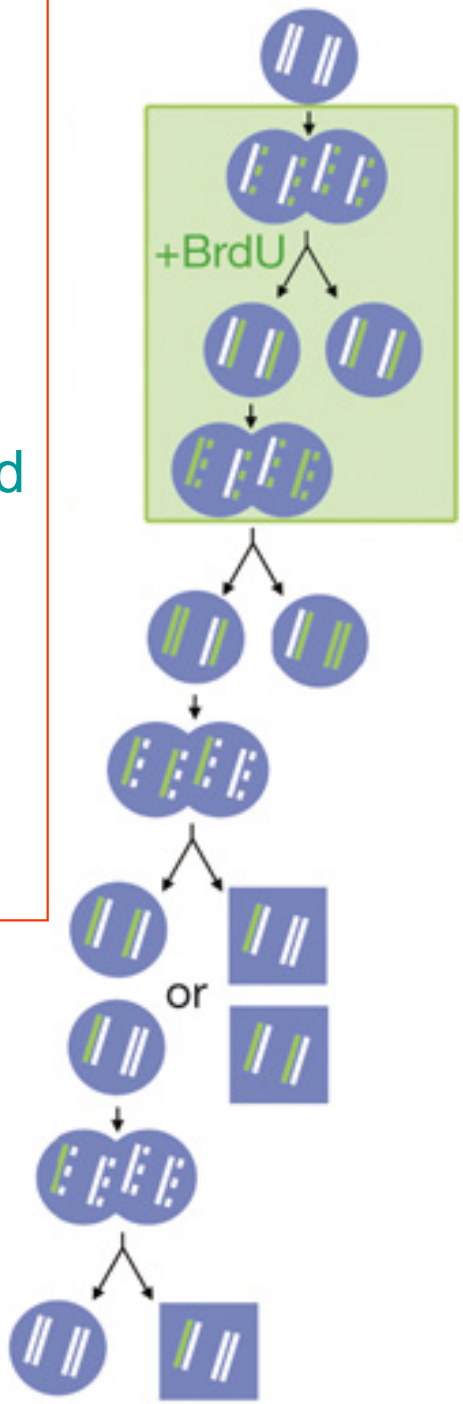
L -> 2L

U -> 2U

Death removes cells at rate d

U+L=1





Model for BrdU labelling

$$\frac{\mathrm{d}L}{\mathrm{d}t} = s + 2pU + pL - dL$$

$$\frac{\mathrm{d}U}{\mathrm{d}t} = -pU - dU$$

up-labelling:

determined by *p+d*

L=1-U

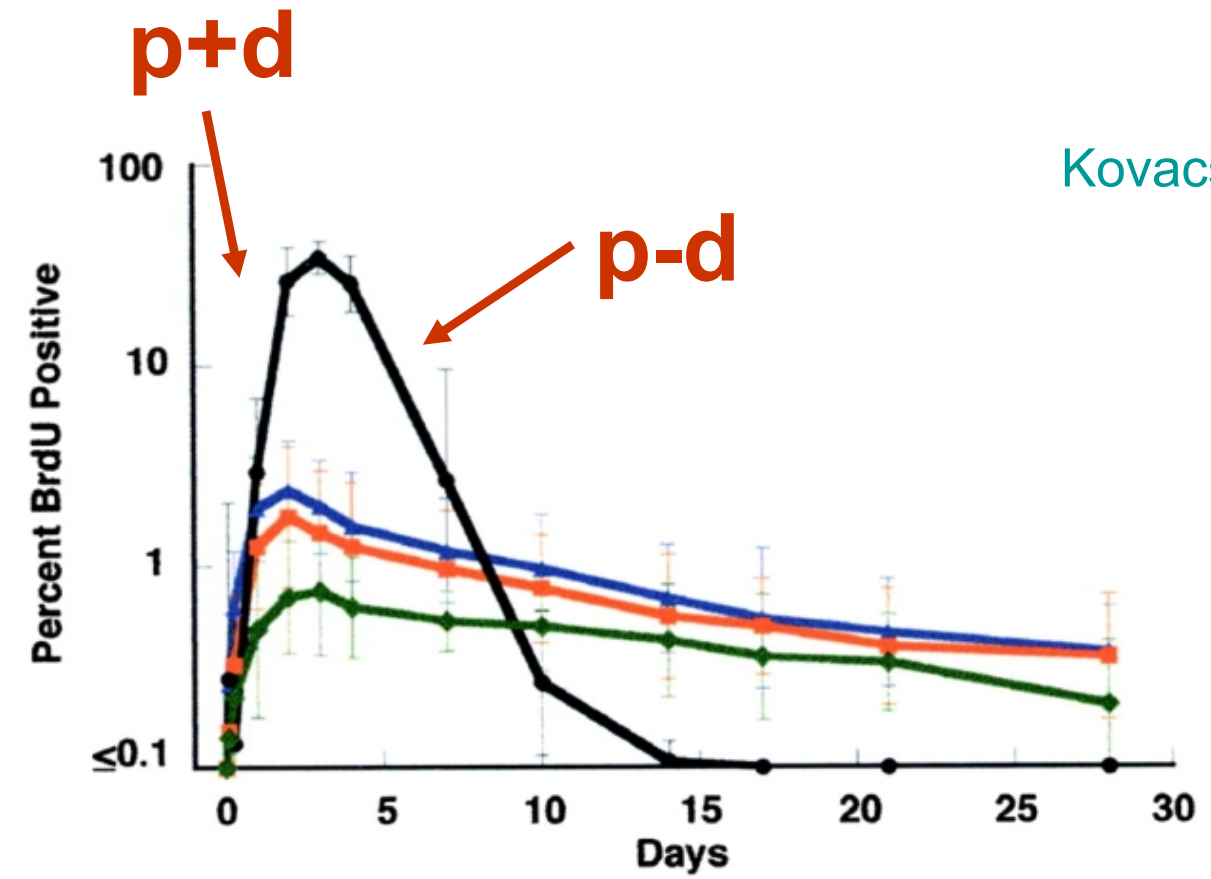
$$\frac{\mathrm{d}U}{\mathrm{d}t} = s + pU - dU$$

$$\frac{\mathrm{d}L}{\mathrm{d}t} = pL - dL$$

down-labelling

determined by *p-d*

Expected changes in the percentage of BrdU+ cells

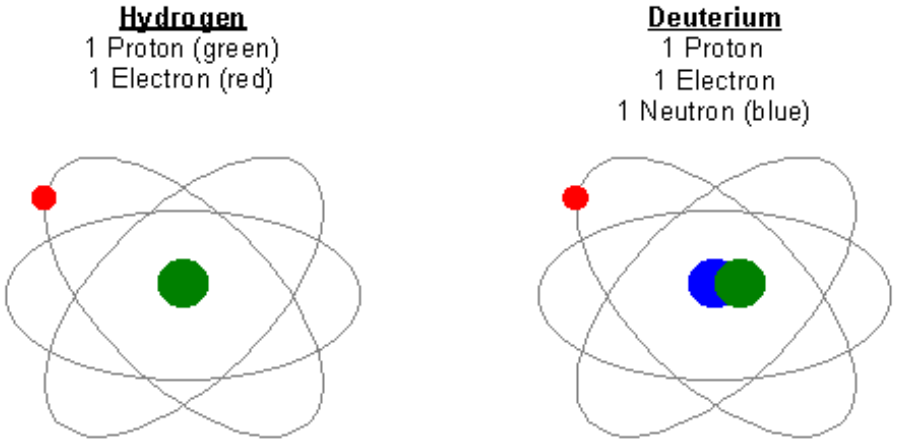


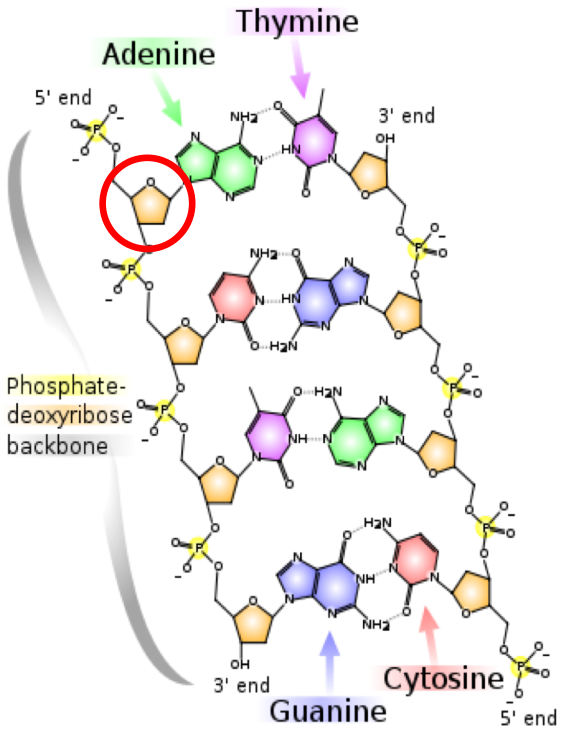
Kovacs et al. 2001

See today's exercise

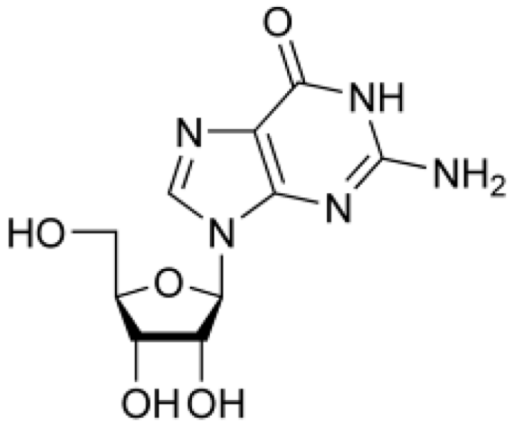
But… possible toxicity, almost only done in mice, only short-term labelling

Dynamic markers (2)
Stable isotope labelling





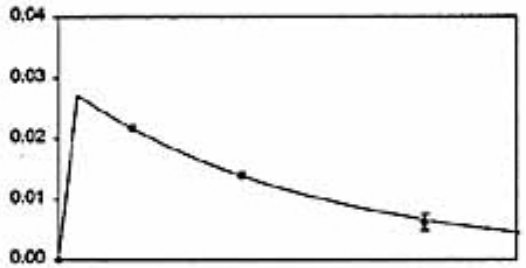
- **Deuterium (^{2}H)** as ^2H_2O or ^{2}H-glucose
- **Non-toxic and no interference with cell dynamics**
- *de novo* nucleotide synthesis pathway
  - ➢ ^{2}H is incorporated into DNA of newly dividing cells



- **Fraction of labeled DNA** is measured by GC-MS

Stable isotope labelling

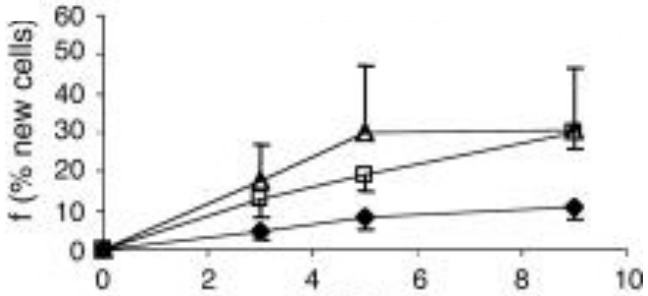
$^2H_2$-glucose



Short-term

Hellerstein et al. 1999, 2003
McCune et al. 2000
Mohri et al. 2001
Ribeiro et al. 2002
Macallan et al. 2003, 2004
Wallace et al. 2004
...

^2H_2O



Long-term administration

Hellerstein et al. 2003
Vrisekoop et al. 2008
Den Braber et al. 2012
Vrisekoop et al. 2015
....

During label administration:

Production (at rate p):

L -> 2L

U -> U+L

you gain L

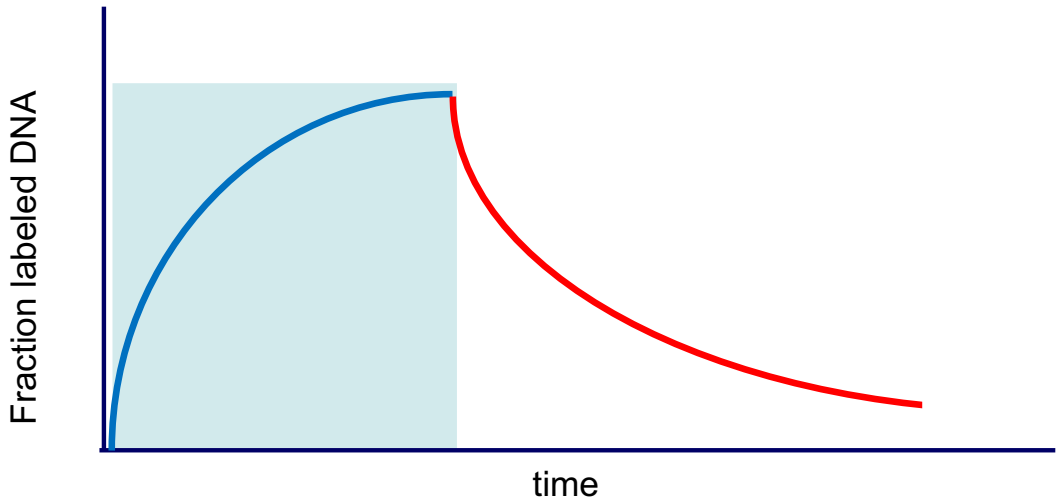
Death removes L at rate d

$$dL/dt = p(L+U) - dL$$

After stop of label:

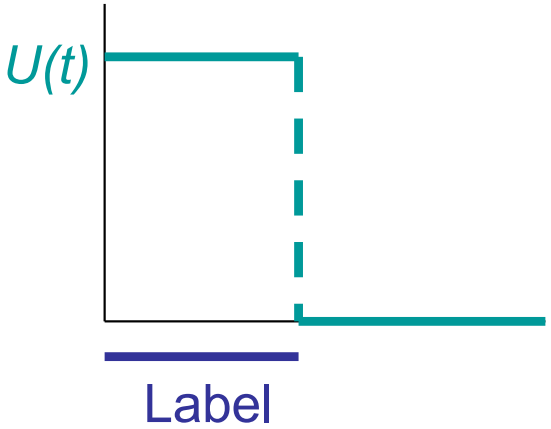
Death removes L at rate d

$$dL/dt = -dL$$



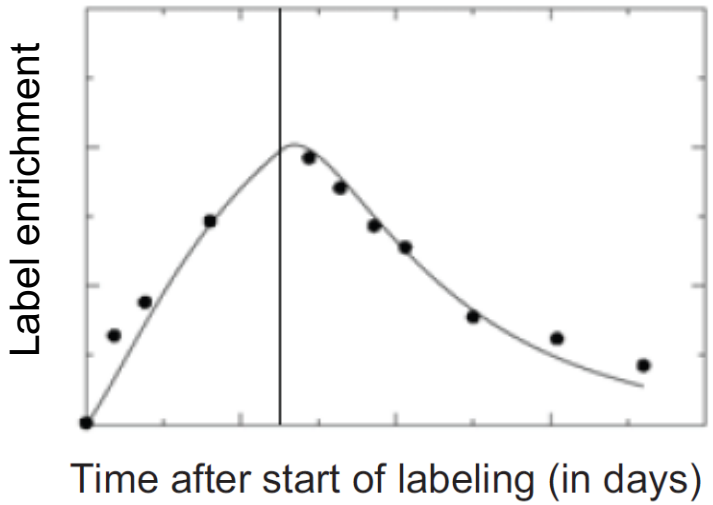
Enrichment in DNA of cells

Fraction of labeled DNA



$$\frac{dL}{dt} = p - dL$$

$$\frac{dL}{dt} = -dL$$

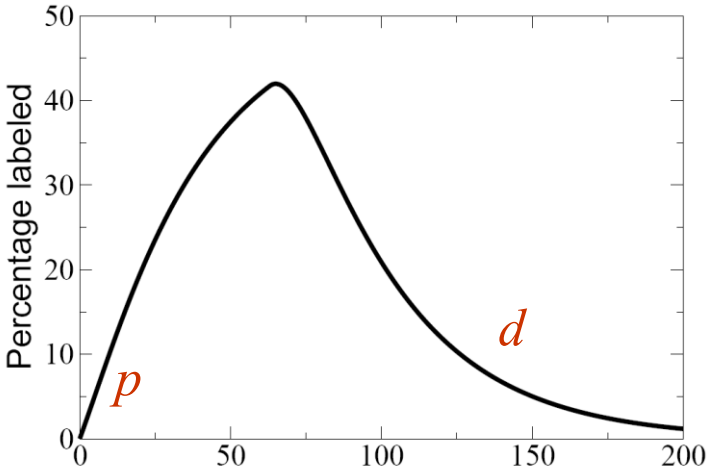


Yields two parameters: *p* and *d*

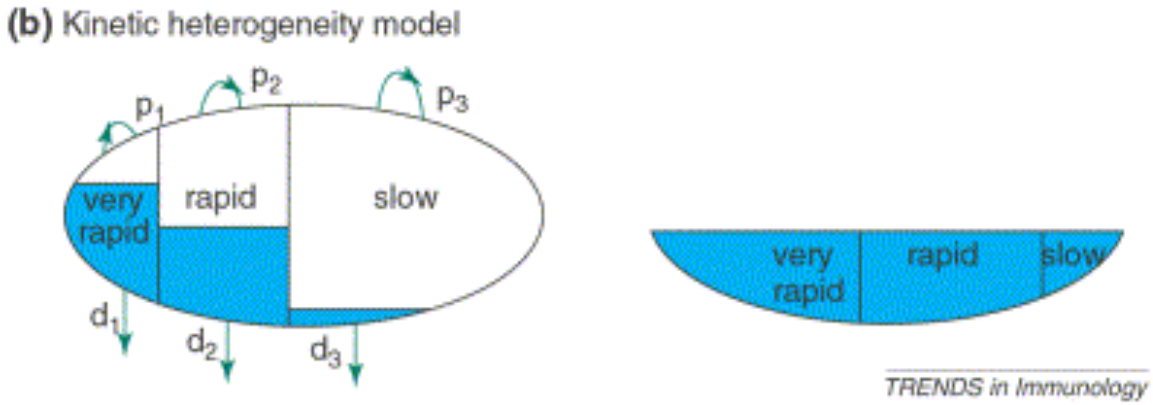
p from upslope, *d* from up- and downslope

^2H_2O labeling does not distinguish between production in thymus and periphery!

Paradox: *d* is typically larger than *p*



Typically: $p < d$

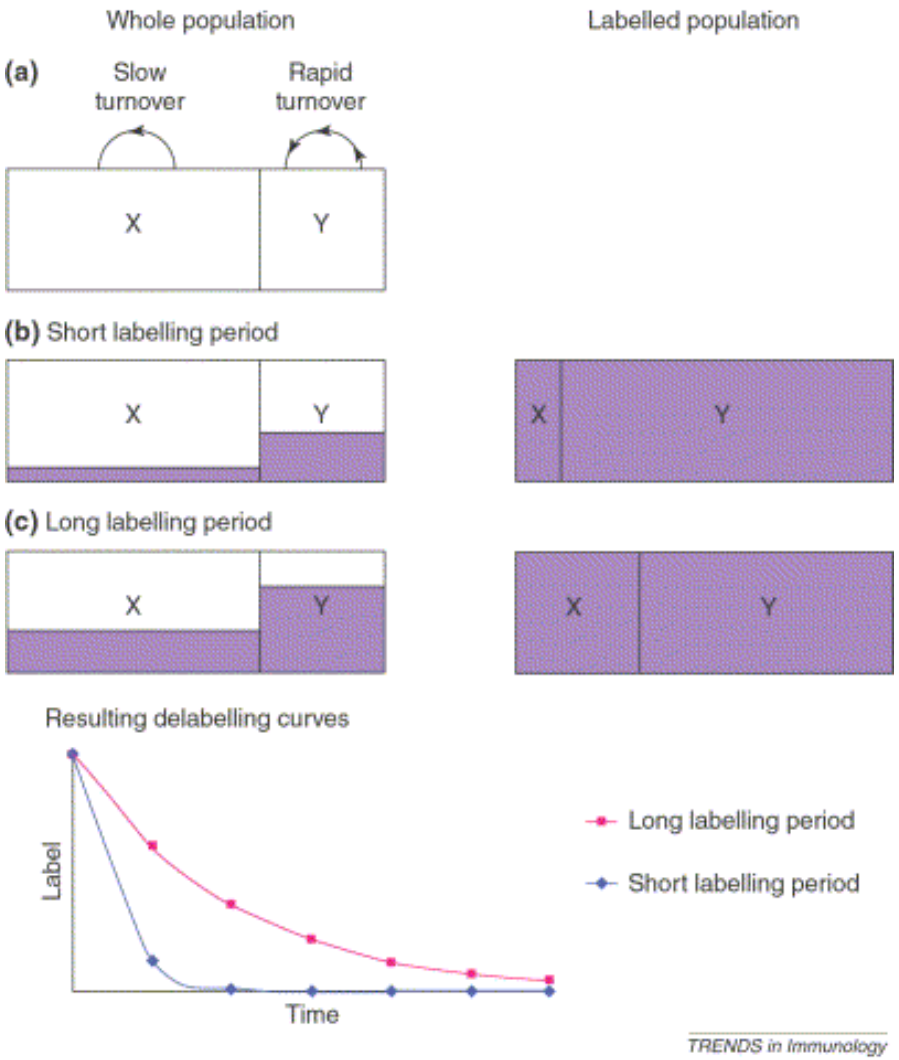


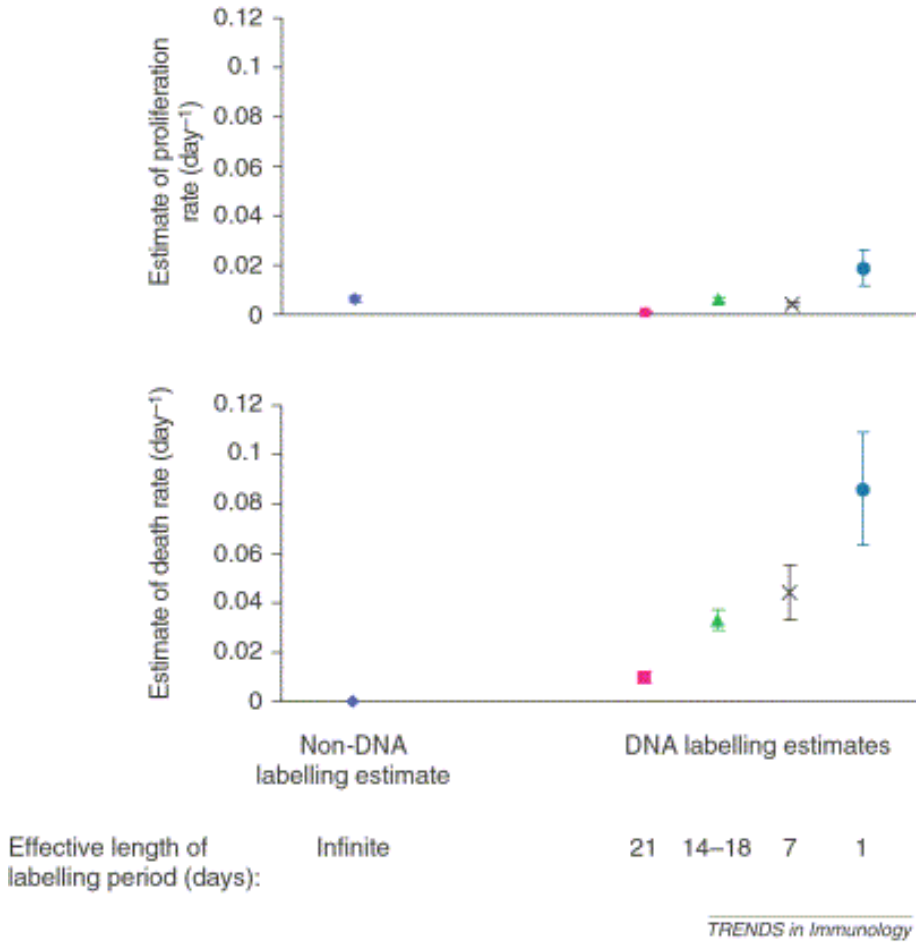
Asquith et al. 2002

Conclusion: *d* is <u>no good measure</u> of average turnover rate

It represents the turnover of <u>*labelled*</u> cells

Short labelling periods give higher estimate of *d*

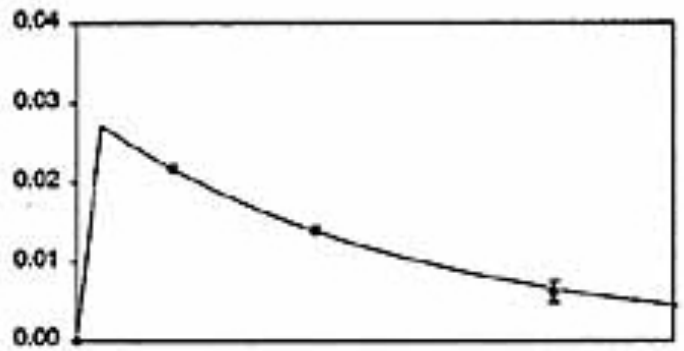




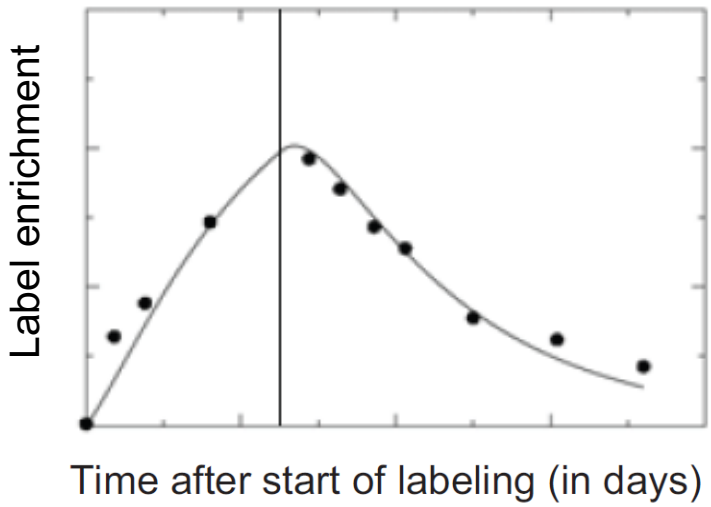
Asquith et al. 2002

Average turnover (*p*) needs data during up-labeling period

D-glucose



D2O



Advantages of heavy water: long-term labeling possible

many data points during labeling

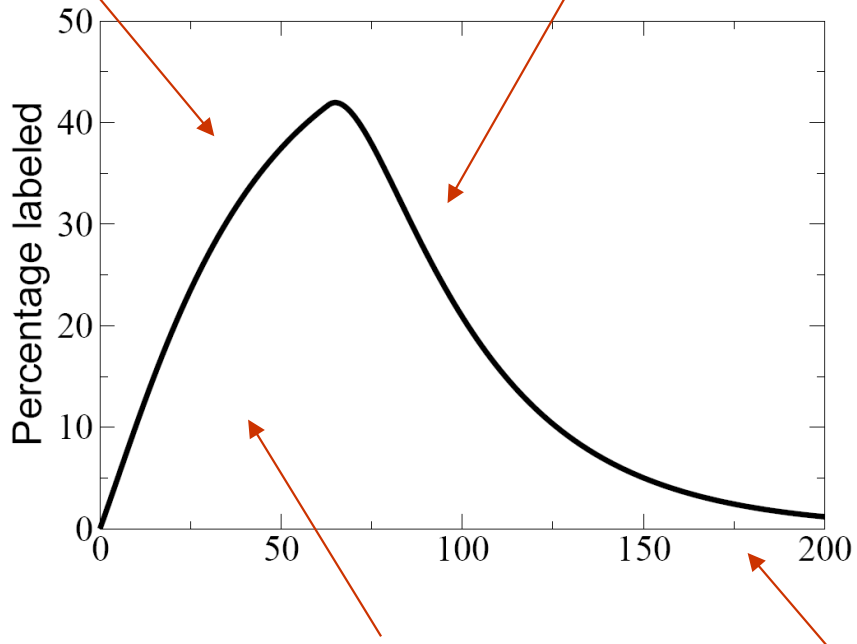
BrdU

Potentially toxic

Measures labeled cells

Up: $p+d$

Down: p-d



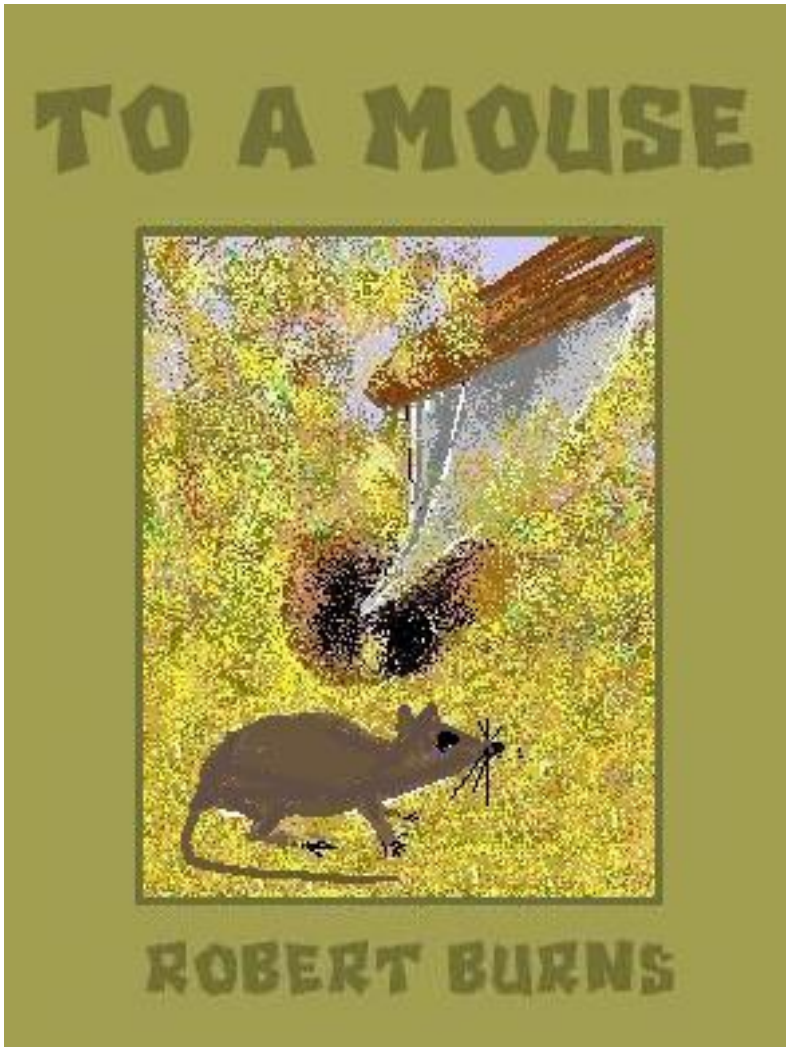
Up: p (and d)

Down: -d

Deuterium labeling

Non-toxic (non-radioactive)

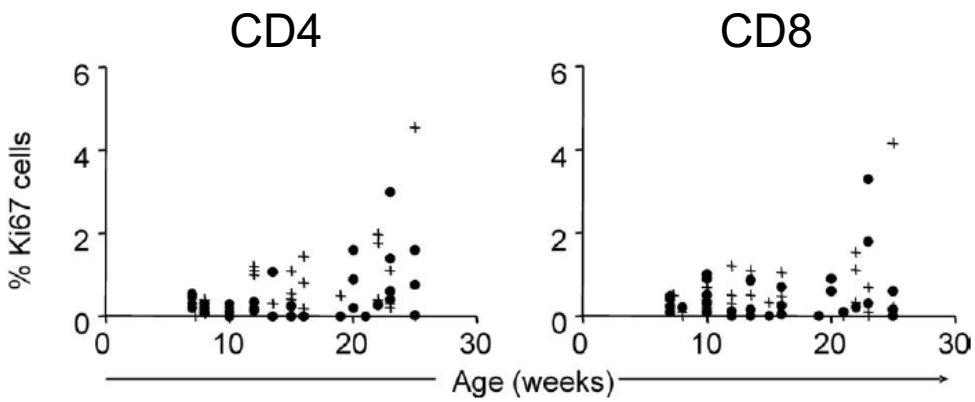
Measures labeled DNA strands



NaiveT-cell dynamics
of mice and men

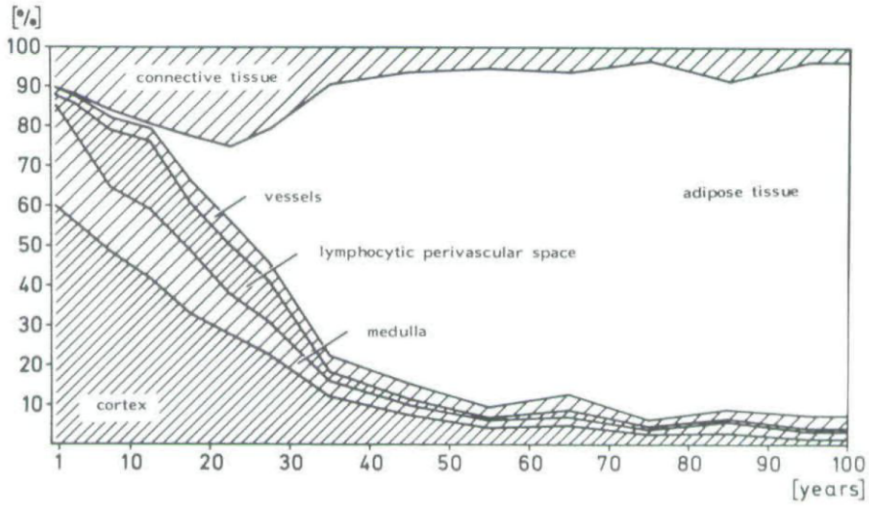
Similarities mouse and human T-cell dynamics

- Small fraction of naive T cells proliferate



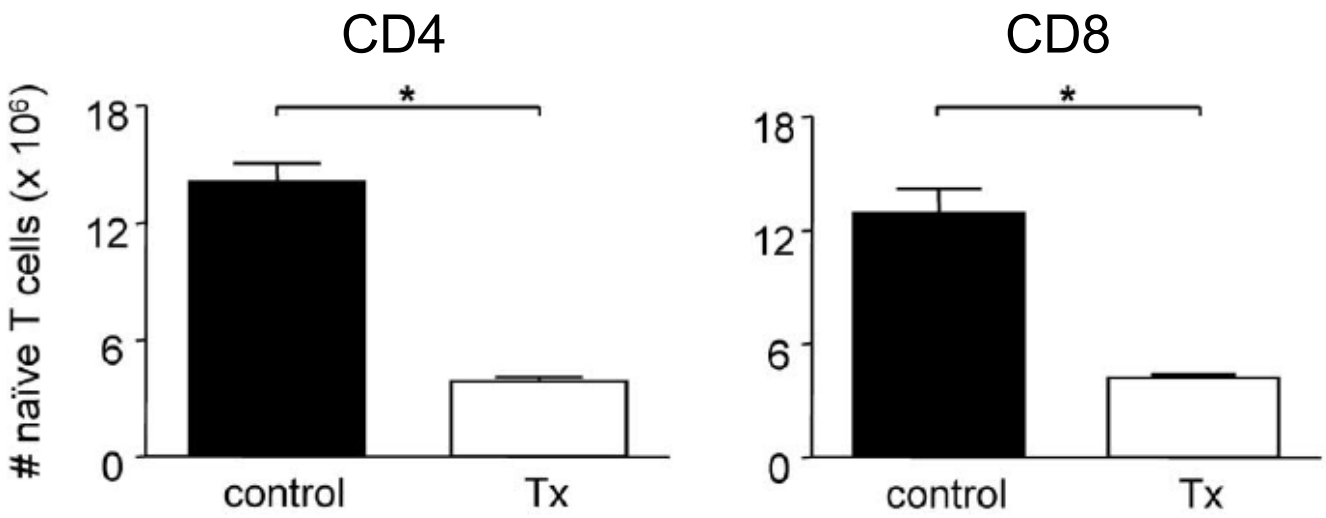
Vrisekoop et al. 2008

- Thymic output declines with age



Steinmann et al. 1985

“Mouse immunology consensus”



Vrisekoop et al. 2008

- Naive T-cell pool is very dynamic
- New naive T cells come from the thymus

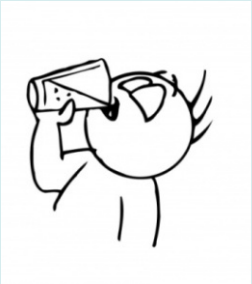
Quantify

Influence on intuition in human immunology:

e.g. Loss of CD4 T cells in HIV due to loss of thymic output?

D2O labeling experiment

D2O labeling



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Blood/urine withdrawals during and after label administration

↓

GC-MS

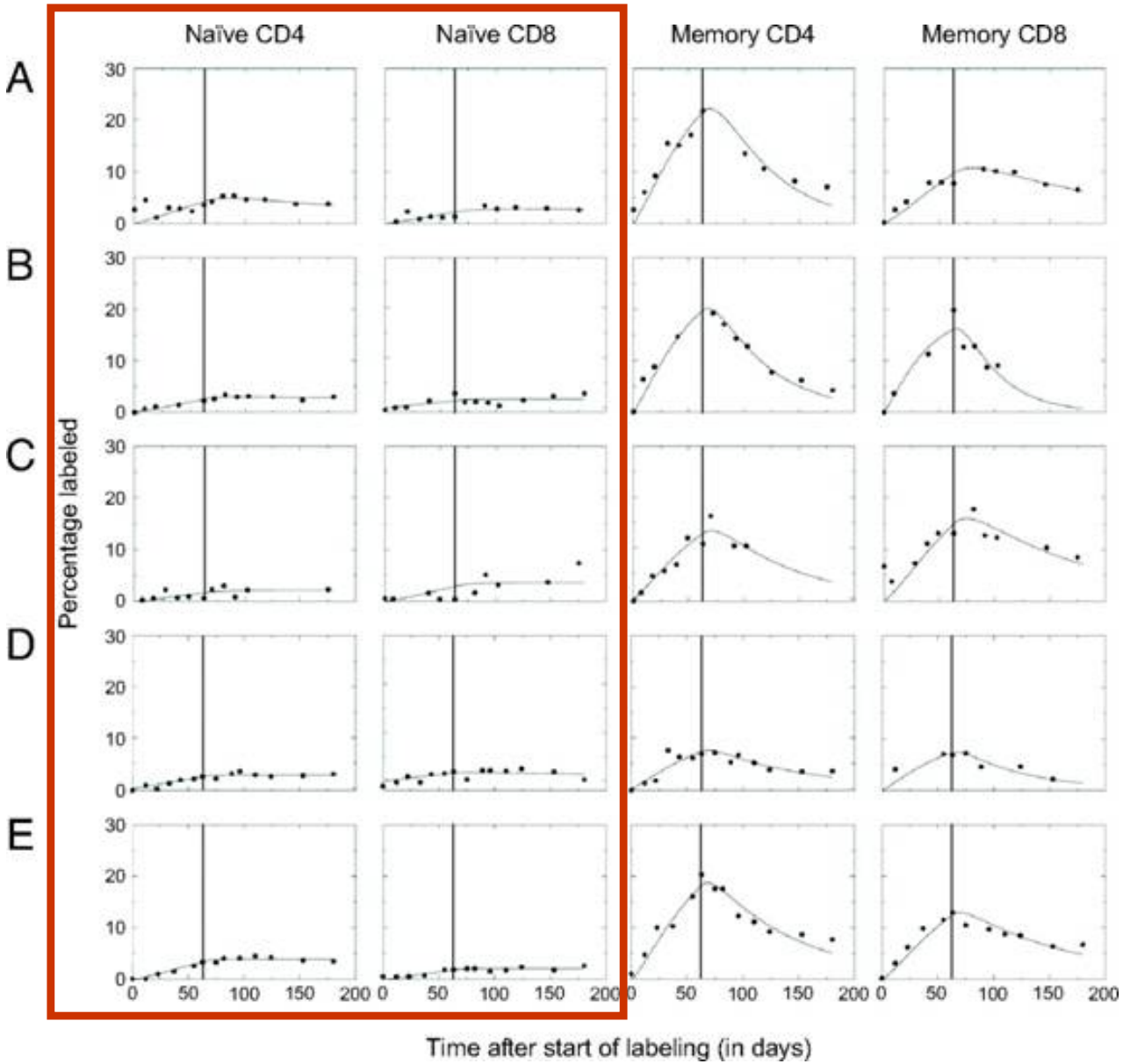
↓

Mathematical modeling

- Subjects drink $^{2}H_{2}O$ for 9 weeks
- Label enrichment in DNA of studied populations are followed during and after label administration

➢ *follow-up of 25 weeks in total*

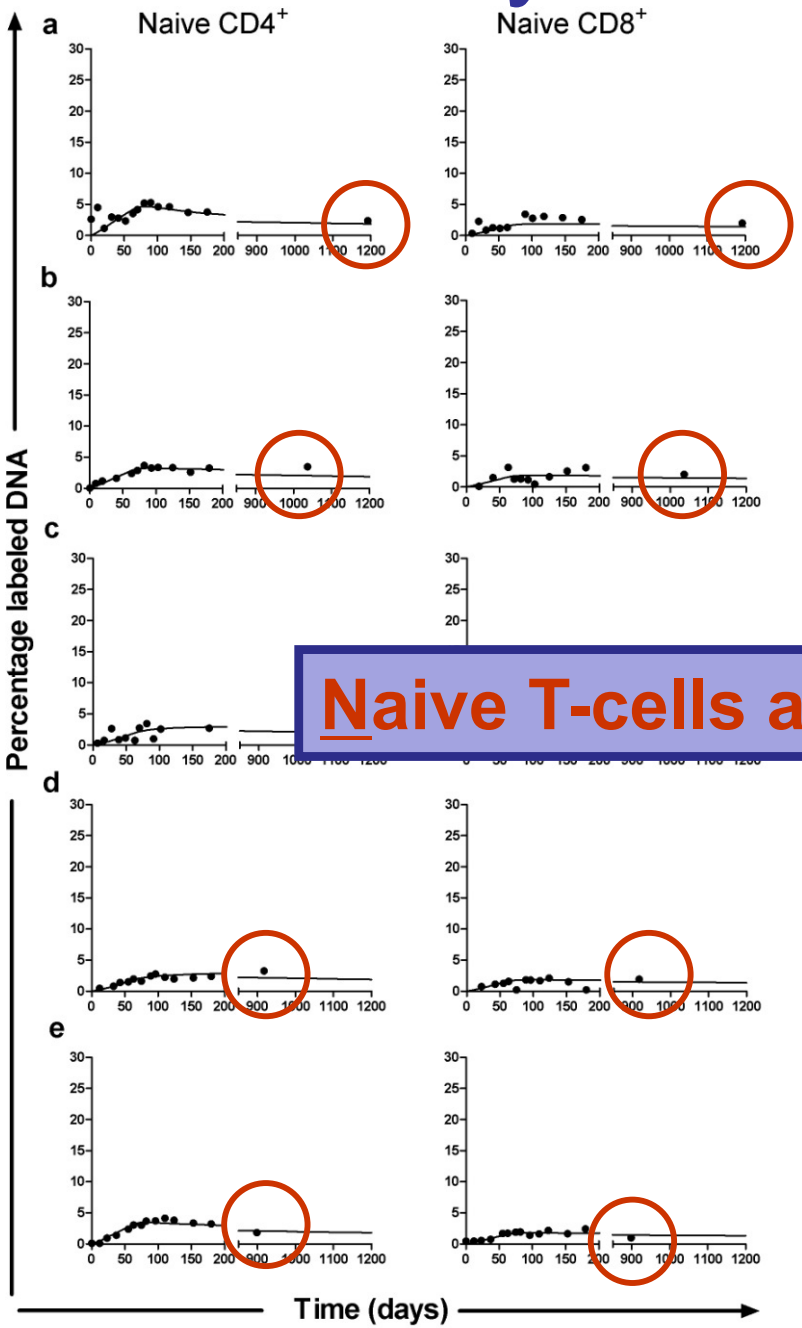
T-cell turnover in young healthy men



Vrisekoop et al.
PNAS 2008

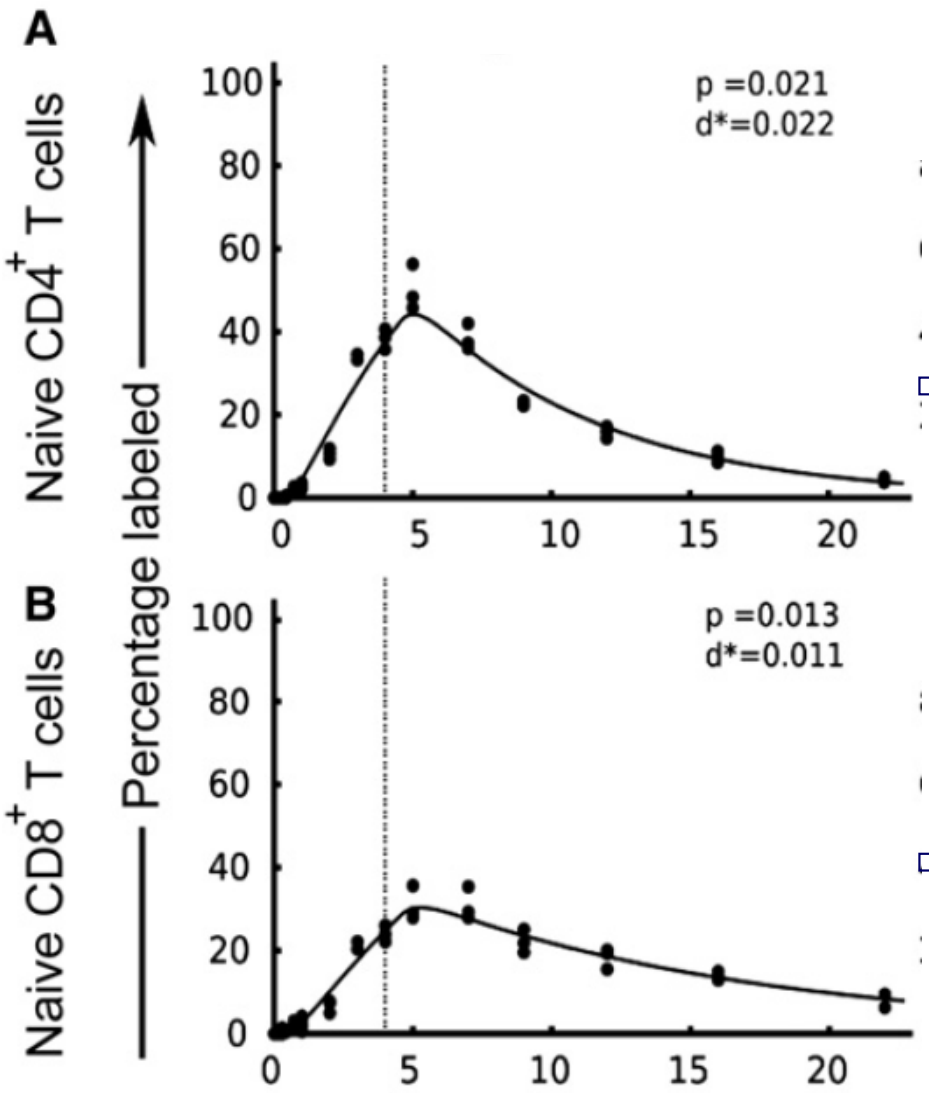
Lifespan 6.0 9.4 0.6 1.0 year

Labeled naive T cells present 3 years after stop of label



Naive T-cells are very long-lived in humans

In mice, naive T cells live much shorter



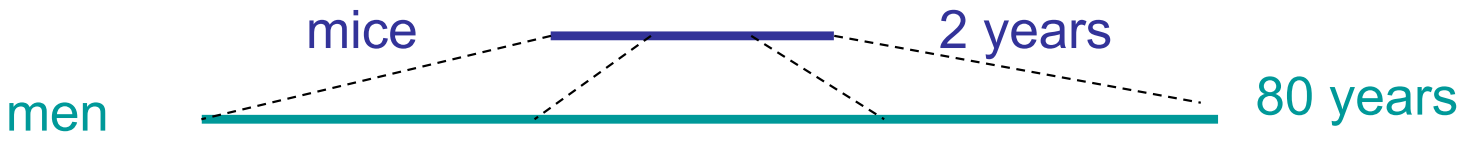
Expected lifespan:

Naive CD4: **6 weeks**

Naive CD8: **11 weeks**

Den Braber et al. Immunity 2012

Scaling from mice to men...



Mice live 80 weeks

naive CD4 T cells 6 weeks, CD8 11 weeks

Humans live 80 years

naive CD4 T cells 6 years, CD8 10 years

“Mouse immunology consensus”

Naive T-cell pool is highly dynamic ✗

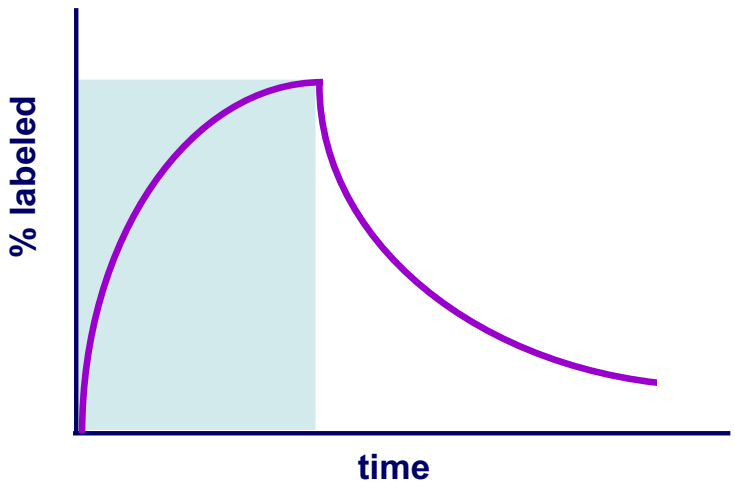
New naive T cells come from the thymus

© Fiep Westendorp

What is the contribution of thymic output?

Naive T-cell production = Thymic output + T-cell proliferation

Heavy Water



Thymectomy

ATx or ShamTx at
7 weeks of age

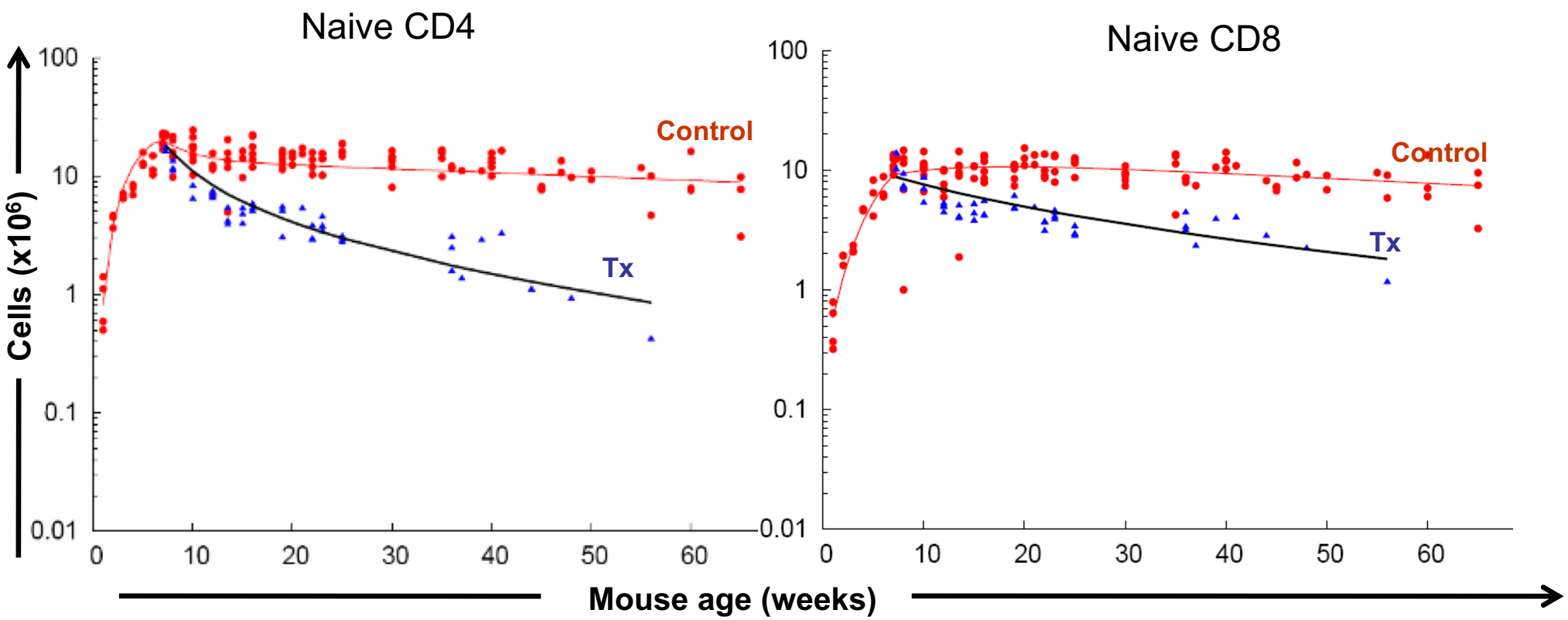
↓

Count cell numbers in spleen, PLNs and thymus

↓

Mathematical modelling

Naive T-cell dynamics after thymectomy



Den Braber et al. Immunity 2012

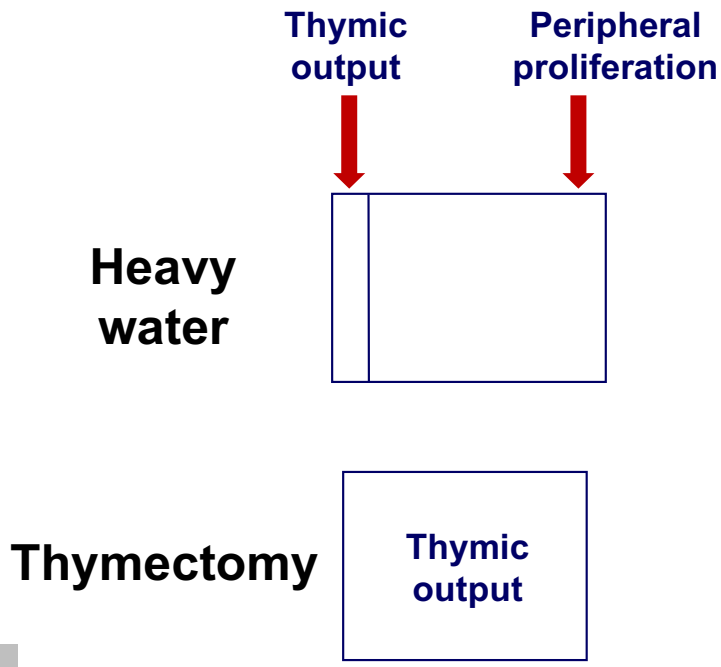
0.9% of thymocytes per day

Contribution of thymic output and T-cell proliferation in mice

Total Naive T-cell production

Naive CD4 → 0.28 10^6 / day

Naive CD8 → 0.13 10^6 /day



Thymic output:

CD4 thymic emigrants → 0.35 10^6 / day

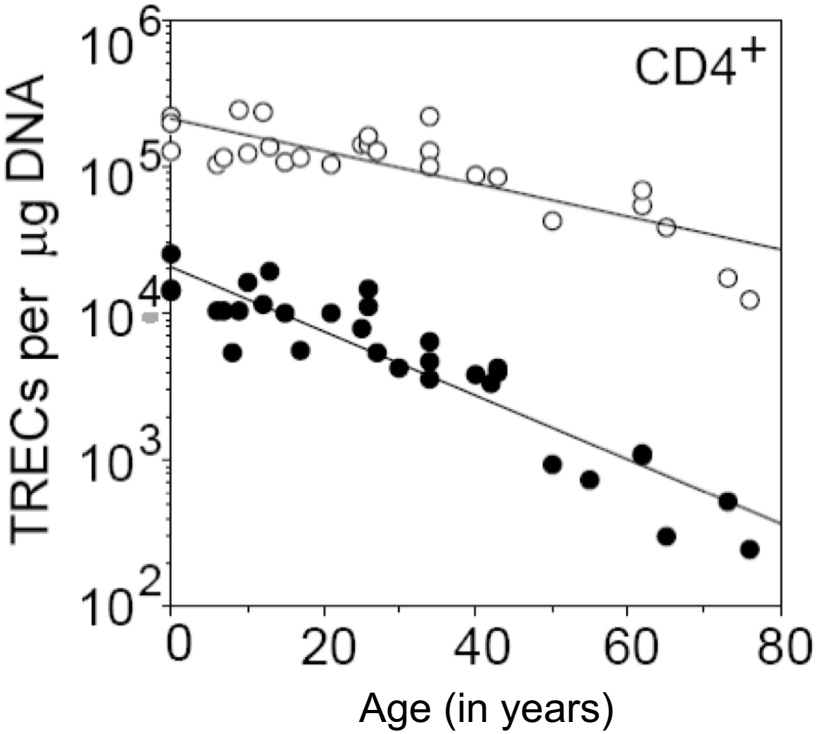
CD8 thymic emigrants → 0.12 10^6 / day

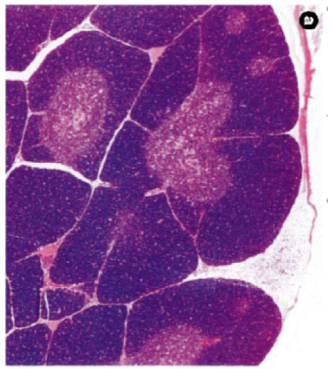
Naive T-cell production in mice almost completely due to thymic output!

This is completely different in humans!

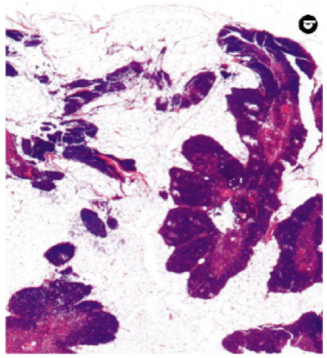
Evidence for naive T-cell proliferation in men…

Evidence for naive T-cell proliferation from T-cell receptor excision circles (TRECs)





Child

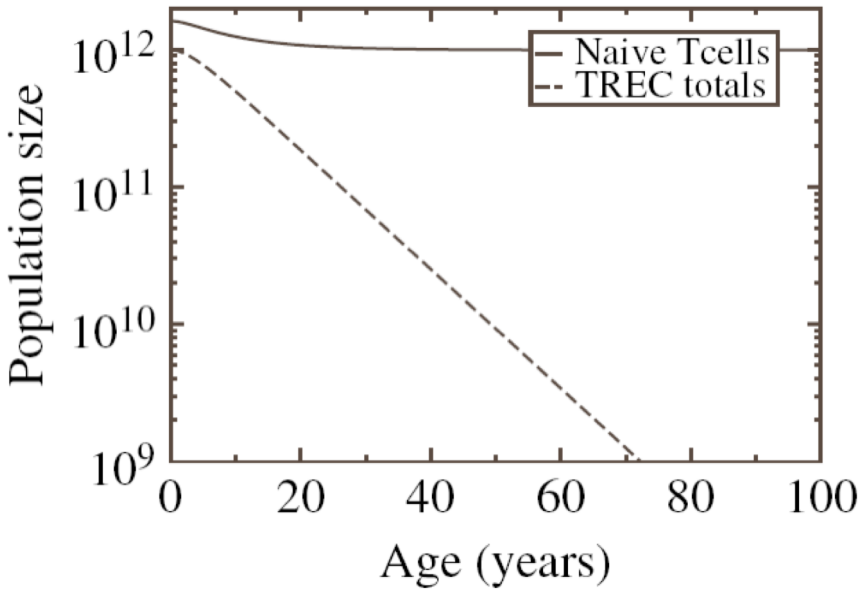


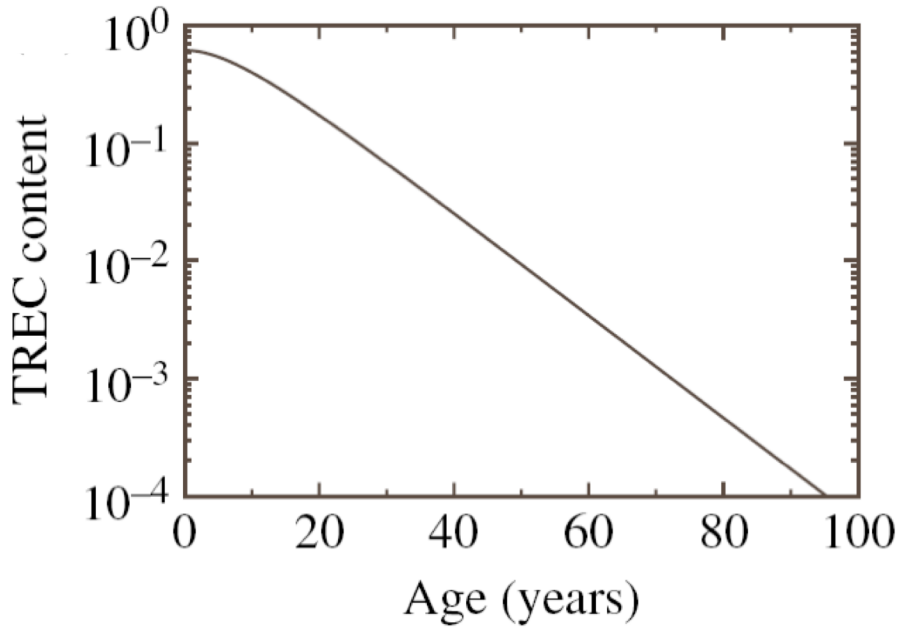
Adult

Douek et al. Nature 1998

Rodewald Nature 1998

If naive T-cells proliferate homeostatically, TREC contents do decline





Naive TREC decline in humans suggests that naive T-cell proliferation contributes to the naive T-cell pool in humans

Hazenberg et al. 2000, Dutilh et al. 2003

Which part of T-cell production comes from the thymus?

T cells:

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \sigma(t) + pN - dN$$

TRECs:

$$\frac{\mathrm{d}T}{\mathrm{d}t} = c\sigma(t) - dT$$

TREC content:

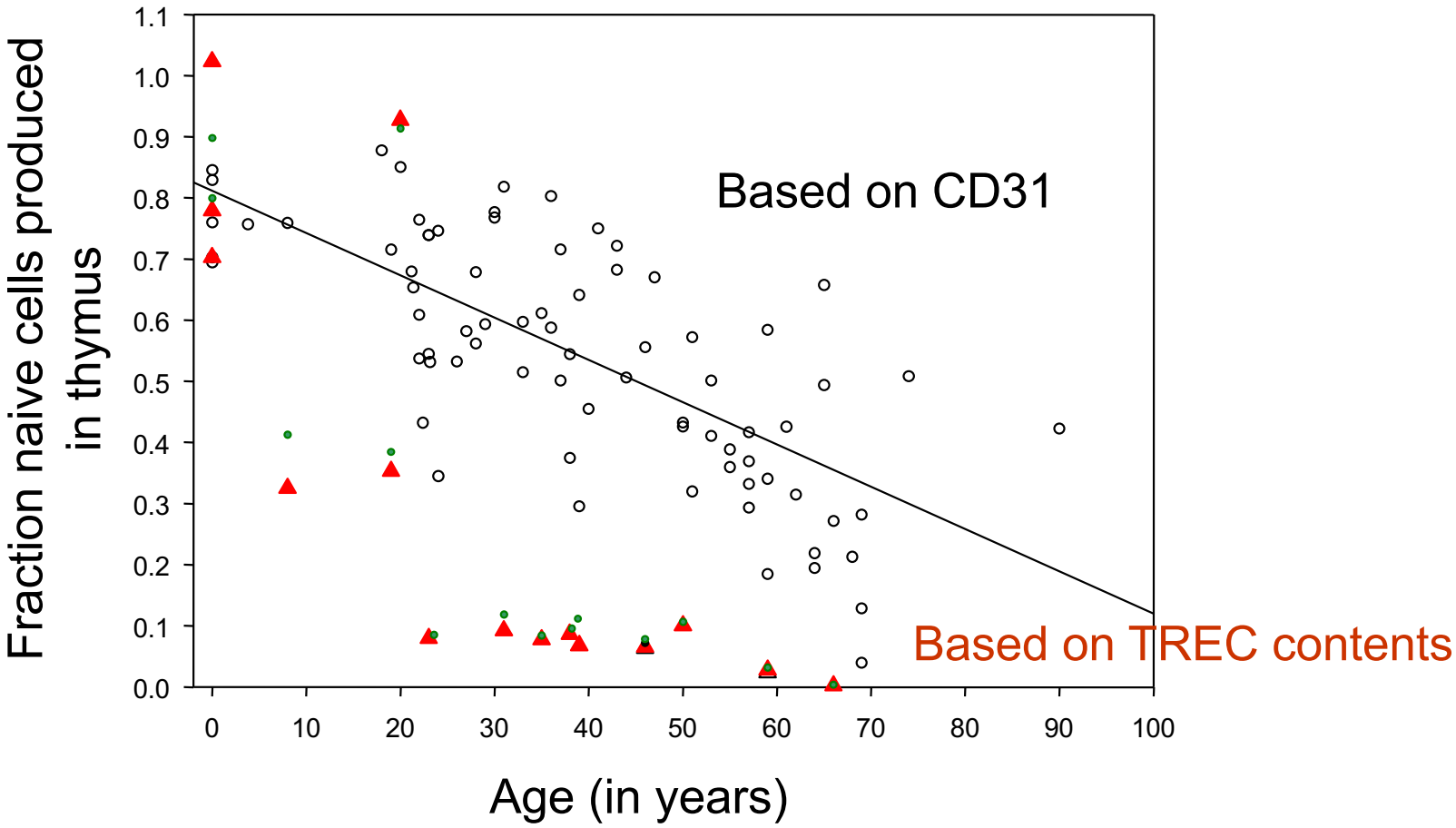
$$\frac{\mathrm{d}A}{\mathrm{d}t} = \frac{\sigma(t)(c - A)}{N} - pA$$

$$A/c = \frac{\sigma(t)}{pN + \sigma(t)}$$

c: TREC content of single-positive (SP) thymocytes

Adapted from Hazenberg et al. 2000

In human adults <10% of naive T cells is produced by the thymus



“Mouse immunology consensus”

- Naive T-cell pool is highly dynamic ✗
- New naive T cells come from the thymus ✗

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Naive T-cell maintenance: a mouse-man divide

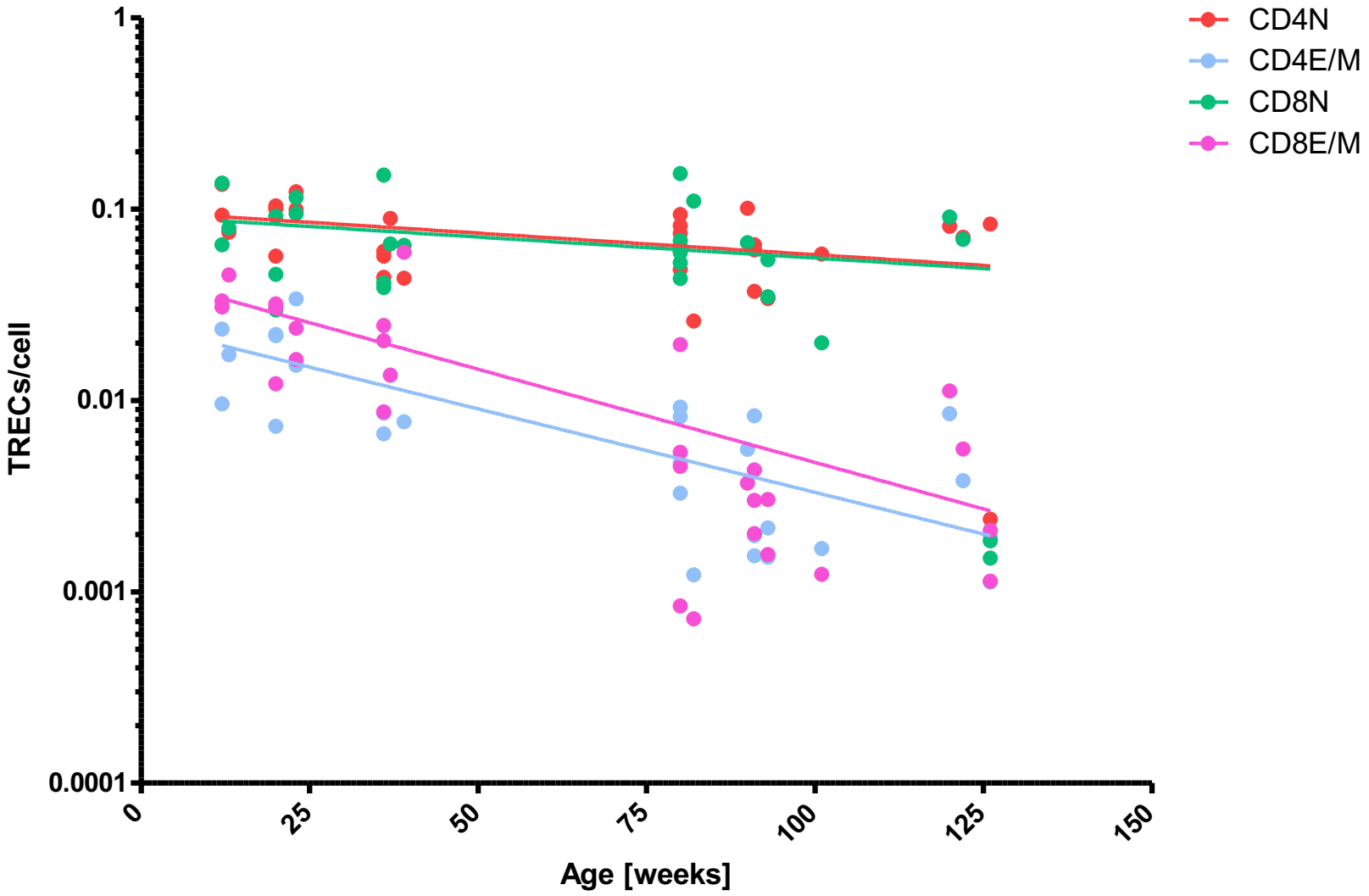
Naive T cells in humans are long-lived;

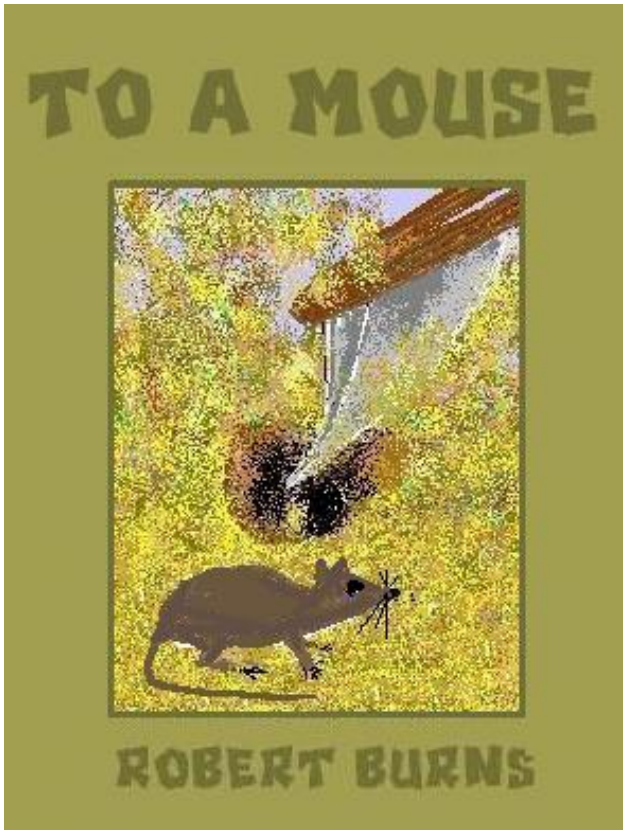
Thymus in human adults plays “little role”

Prediction:

without proliferation there should be no TREC dilution in mice…

And indeed... no TREC decline in naive T cells from mice





"The best-laid schemes o' mice an' men,
Gang aft agley"

The most carefully prepared plans
may go wrong

Maintenance of Peripheral Naive T Cells
Is Sustained by Thymus Output
in Mice but Not Humans

Den Braber et al.

Immunity, 2012