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?

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)

Caution: Cells have DNA damage, and cell numbers are low
Static versus dynamic markers of T-lymphocyte turnover

**Static**
- Ki67-expression
  (protein expressed in G₁,S,G₂,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
Changes in TRECs do not directly reflect thymus output

Healthy individuals

Thymectomized individuals

HIV-infected individuals

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:
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Dynamic markers (1)
BrdU Labelling

BrdU = 5-bromo-2’-deoxyuridine
Nucleoside analog
incorporated instead of thymidine
Determine percentage BrdU+ cells by FACS analysis

How to quantify leukocyte turnover?
Intuition vs modelling

Kovacs et al. 2001
During label administration:

Production (at rate p):
U $\rightarrow$ 2L
L $\rightarrow$ 2L

Death removes cells at rate d

U + L = 1

After stop of label:

Production (at rate p):
L $\rightarrow$ 2L
U $\rightarrow$ 2U

Death removes cells at rate d

U + L = 1
Model for BrdU labelling

\[
\frac{dL}{dt} = s + 2pU + pL - dL
\]

up-labelling: determined by \( p+d \)

\[
\frac{dU}{dt} = -pU - dU
\]

\[
\frac{dL}{dt} = pL - dL
\]

down-labelling
determined by \( p-d \)

\[L=1-U\]
Expected changes in the percentage of BrdU+ cells

See today’s exercise

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

Kovacs et al. 2001
Dynamic markers (2)
Stable isotope labelling

- **Deuterium** ($^2$H) as $^2$H$_2$O 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

$^2\text{H}_2\text{-glucose}$

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{graph1.png}
\caption{Graph showing short-term administration of $^2\text{H}_2\text{-glucose}$.
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
...}
\end{figure}

$^2\text{H}_2\text{O}$

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{graph2.png}
\caption{Graph showing long-term administration of $^2\text{H}_2\text{O}$.
Hellerstein et al. 2003
Vrisekoop et al. 2008
Den Braber et al. 2012
Vrisekoop et al. 2015
...}
\end{figure}
During label administration:

Production (at rate $p$):

$\begin{align*}
L &\rightarrow 2L \\
U &\rightarrow U + L
\end{align*}$

you gain $L$

Death removes $L$ at rate $d$

\[
\frac{dL}{dt} = p(L+U) - dL
\]

After stop of label:

Death removes $L$ at rate $d$

\[
\frac{dL}{dt} = -dL
\]
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

\(^2\text{H}_2\text{O}\) labeling does not distinguish between production in thymus and periphery!
Paradox: $d$ is typically larger than $p$

Typically: $p < d$

Conclusion: $d$ is no good measure of average turnover rate. It represents the turnover of *labelled* cells.

Asquith et al. 2002
Short labelling periods give higher estimate of $d$

Asquith et al. 2002
Average turnover ($p$) needs data during up-labeling period

D-glucose

Advantages of heavy water: long-term labeling possible
many data points during labeling

D2O

Label enrichment

Time after start of labeling (in days)
BrdU

Potentially toxic

Measures labeled cells

Up: $p+d$  \hspace{1cm} Down: $p-d$

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
- Thymic output declines with age

Steinmann et al. 1985

Vrisekoop et al. 2008
“Mouse immunology consensus”

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

Influence on intuition in human immunology:

e.g. Loss of CD4 T cells in HIV due to loss of thymic output?
D2O labeling experiment

- 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

Lifespan

|   | 6.0 | 9.4 | 0.6 | 1.0 year |

Vrisekoop et al.
PNAS 2008
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
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

0.9% of thymocytes per day

Den Braber et al. Immunity 2012
Contribution of thymic output and T-cell proliferation in mice

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

Total Naive T-cell production
Naive CD4 $\rightarrow$ $0.28 \times 10^6$ / day
Naive CD8 $\rightarrow$ $0.13 \times 10^6$ /day

Thymectomy

Thymic output:
CD4 thymic emigrants $\rightarrow$ $0.35 \times 10^6$ / day
CD8 thymic emigrants $\rightarrow$ $0.12 \times 10^6$ / day

Thymic output

Peripheral proliferation

Heavy water
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)
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:
\[
\frac{dN}{dt} = \sigma(t) + pN - dN
\]

TRECs:
\[
\frac{dT}{dt} = c\sigma(t) - dT
\]

TREC content:
\[
\frac{dA}{dt} = \frac{\sigma(t)(c - A)}{N} - pA
\]

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

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
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