Dynamics of CD4+ T cells in HIV-1 Infection

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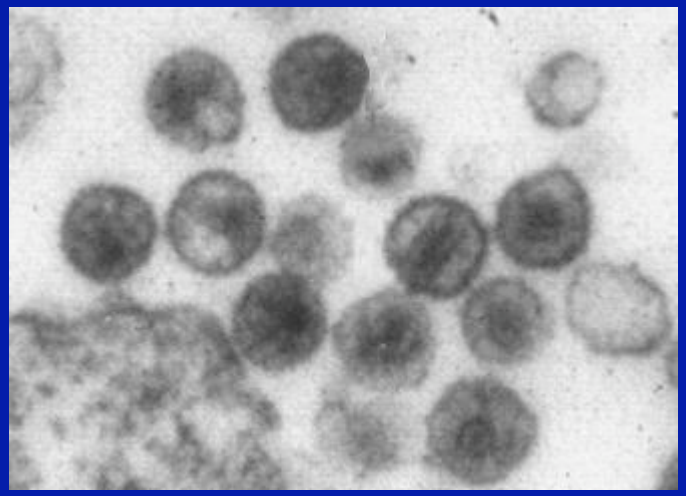
What is HIV infection?

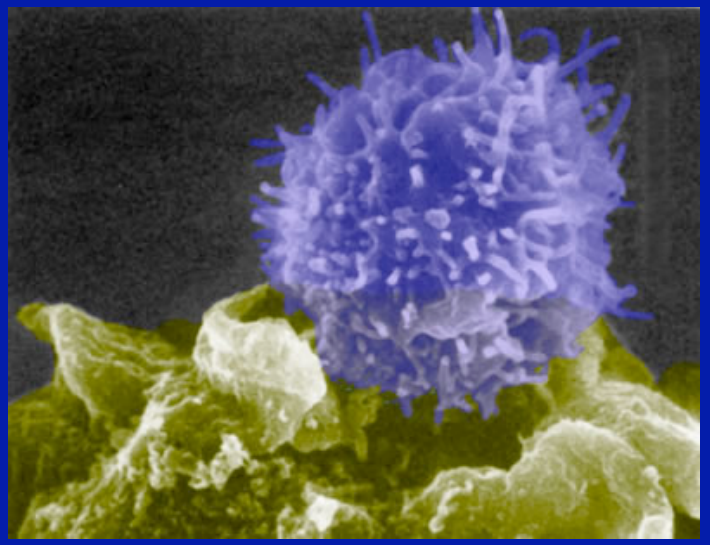
The virus



A retrovirus

Infects immune cells bearing: CD4 & CCR5/CXCR4

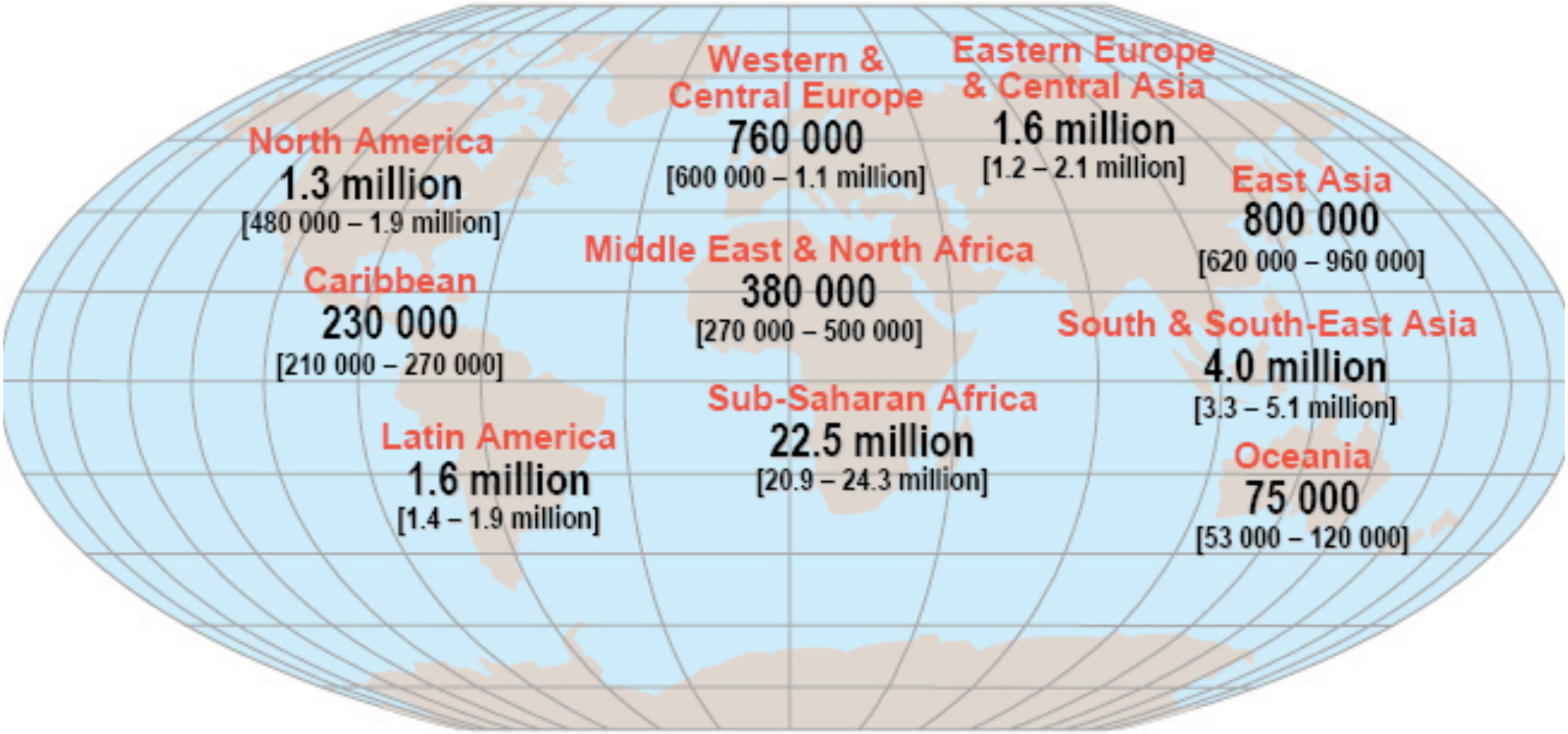
The host



CD4+ T-cells (or helper T cells)

Macrophages and dendritic cells

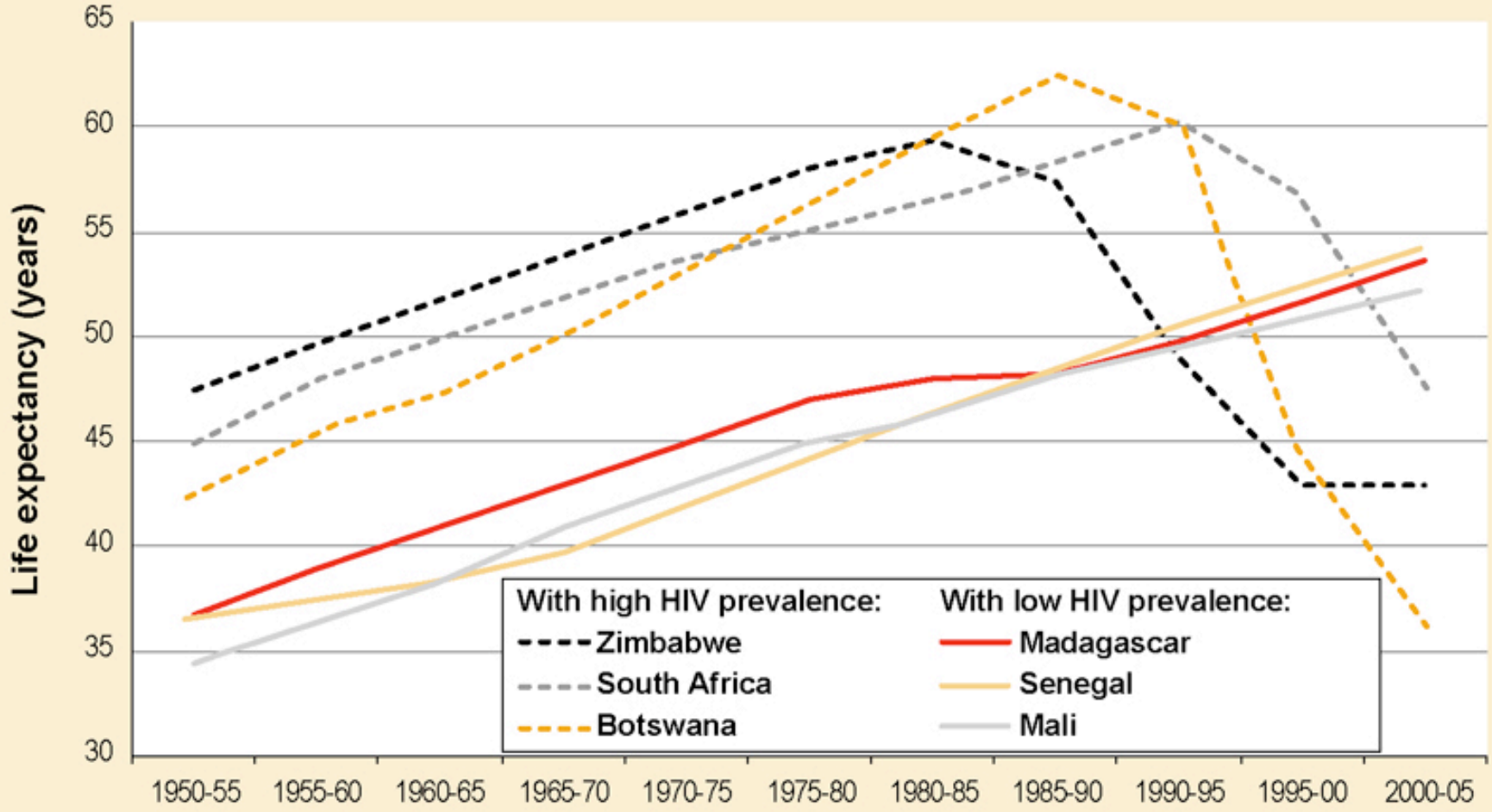
People living with HIV (2007)



Total: 33.2 (30.6 – 36.1) million

UNAIDS, Epi Update 2007

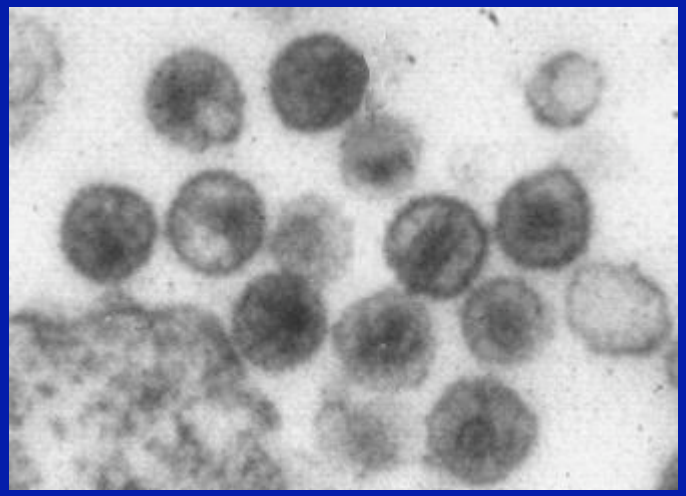
Changes in life expectancy in selected African countries with high and low HIV prevalence: 1950-2005



Source: UN Department of Economic and Social Affairs (2001) *World Population Prospects, the 2000 Revision*

What is HIV infection?

The virus



A retrovirus

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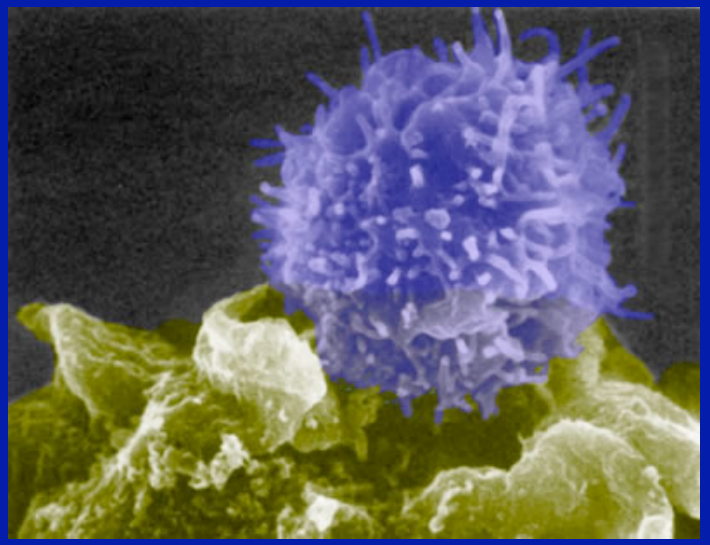
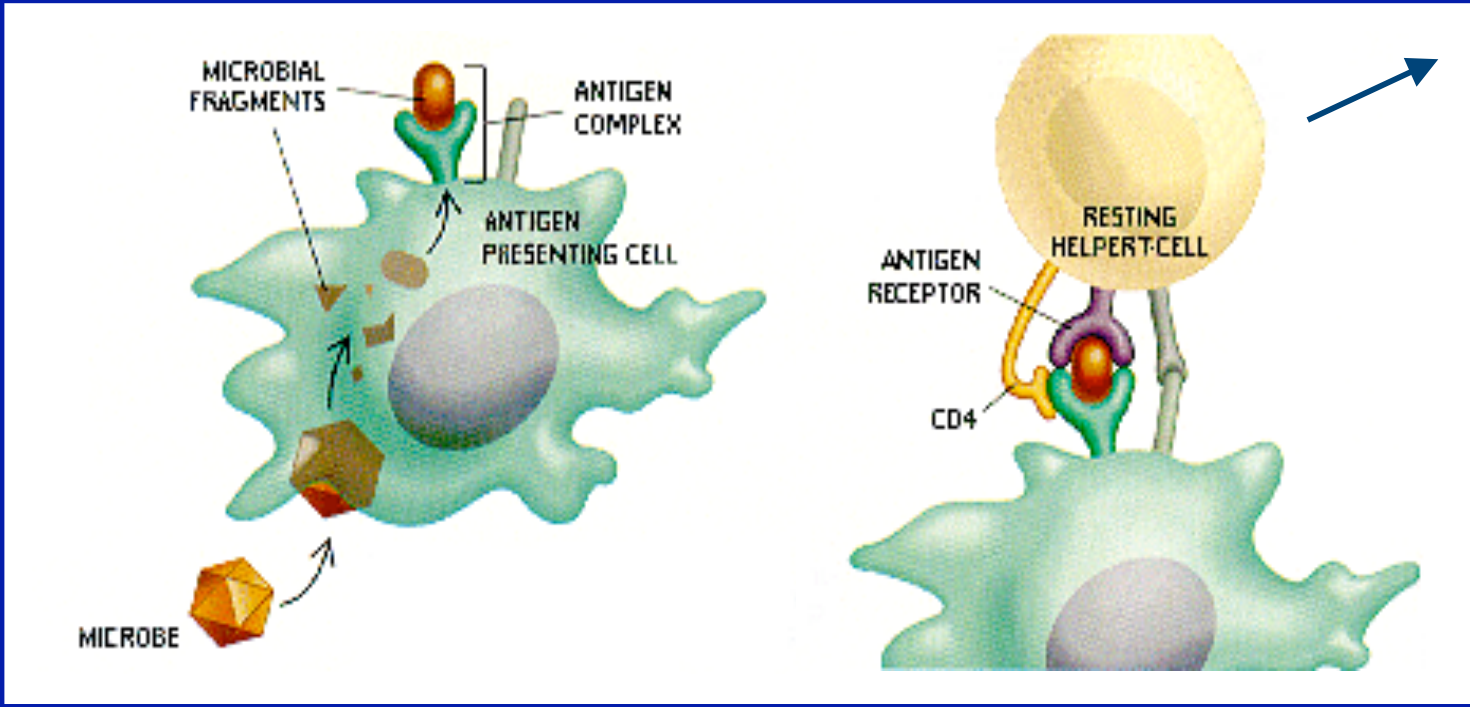


CD4+ T-cells (or helper T cells)

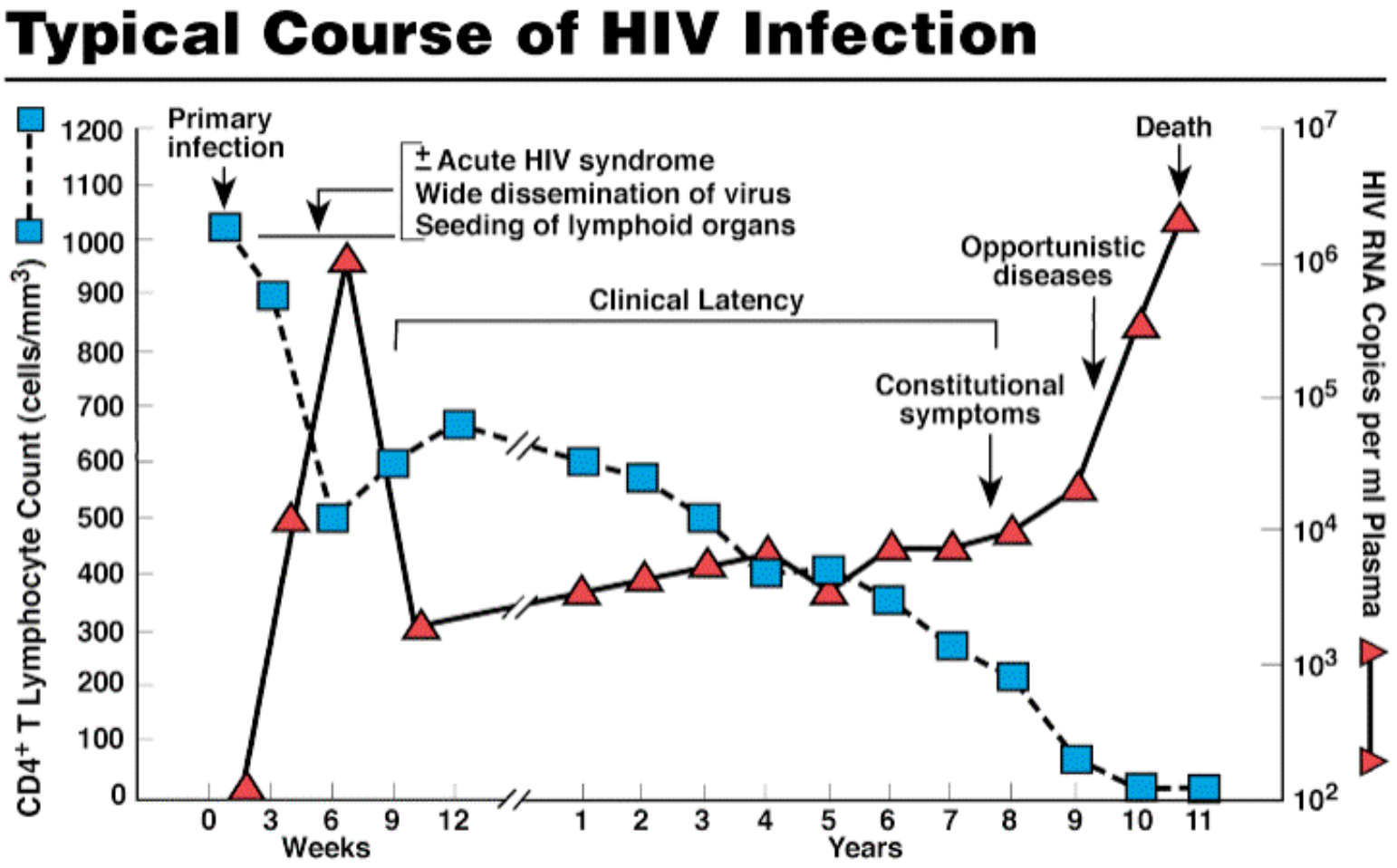
Macrophages and dendritic cells

CD4+ T-cell Function



CD8+ T cells
B cells

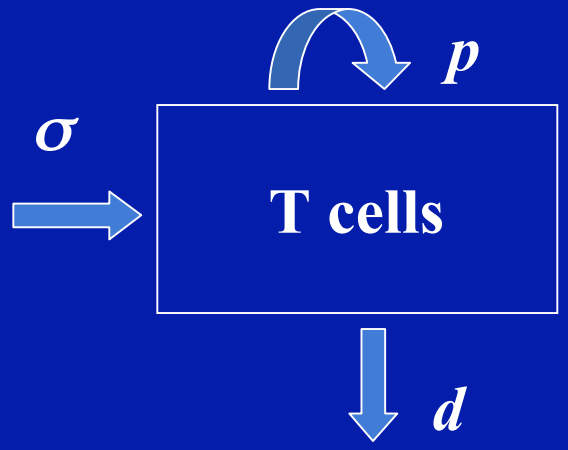
Clinical course of disease



Modified From: Fauci, A.S., et al, *Ann. Intern. Med.*, 124:654, 1996

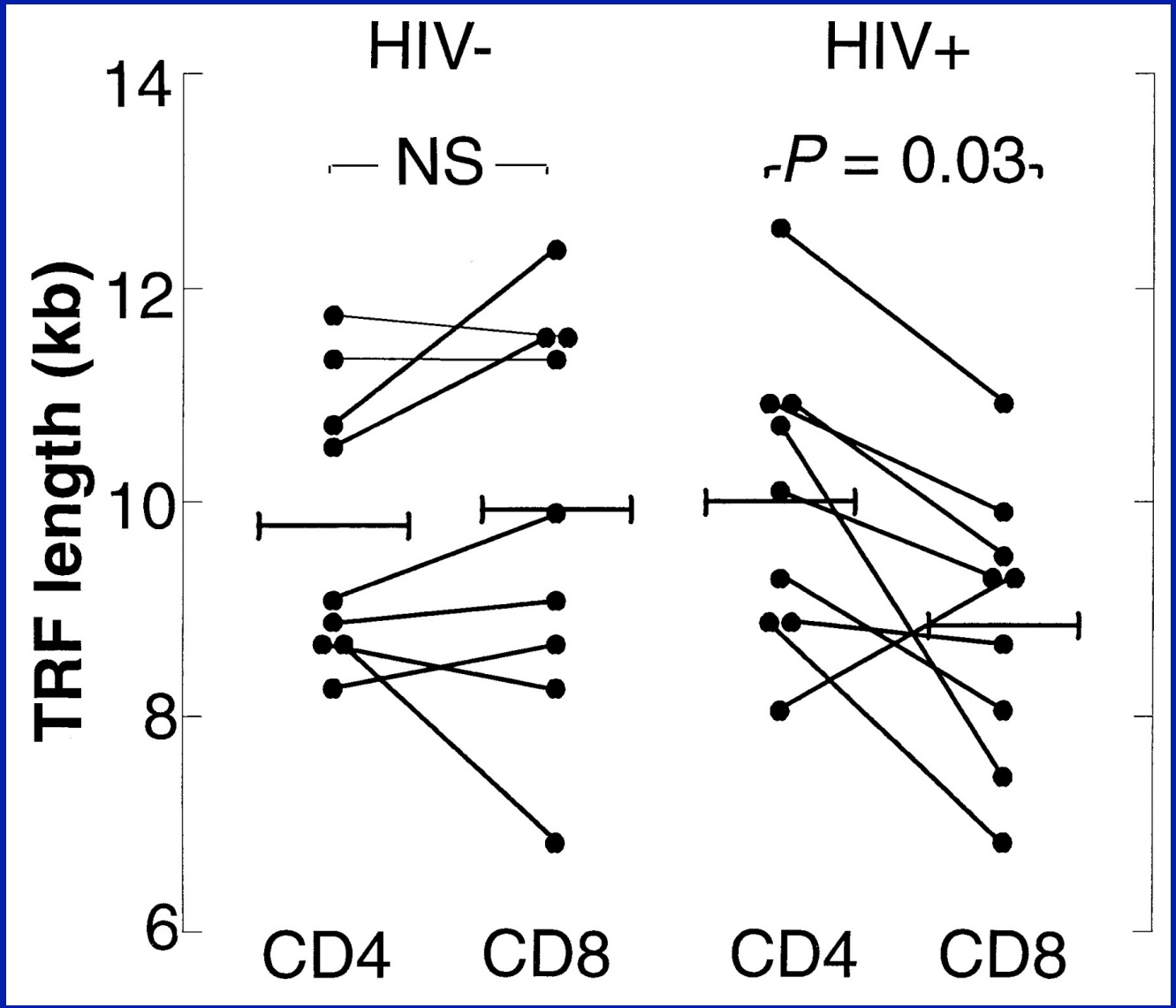
No treatment

T-cell dynamics



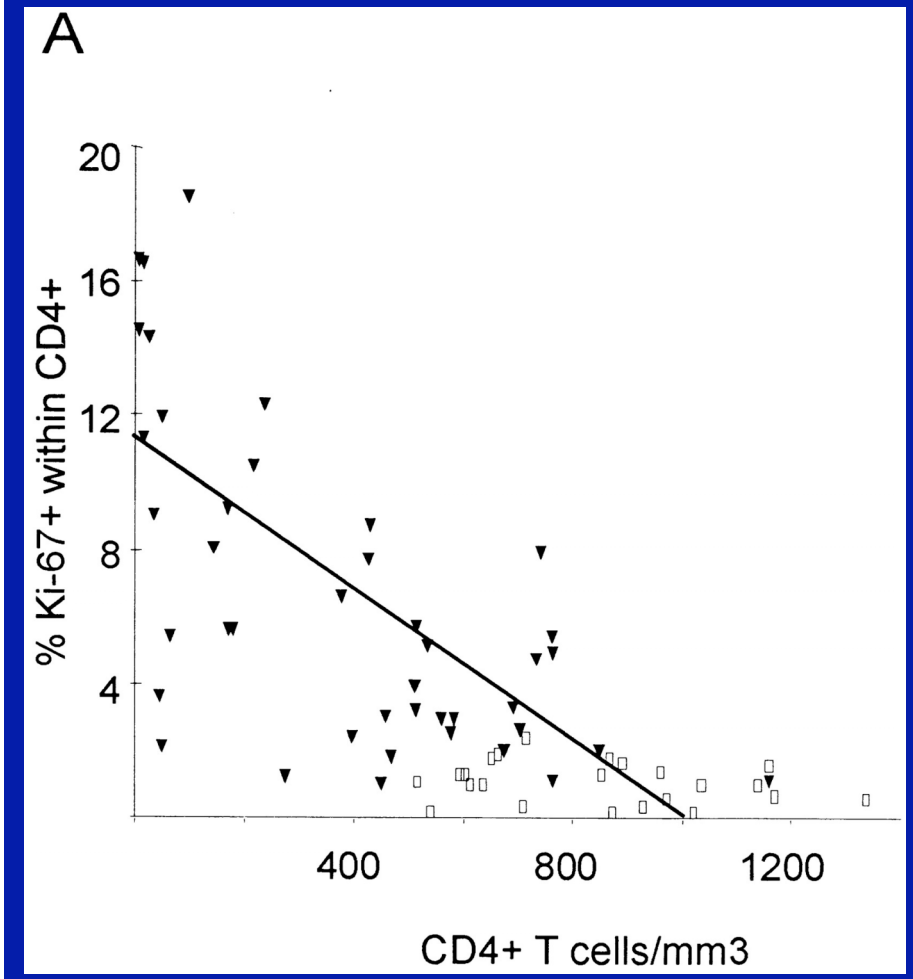
- Ki67
  - Sachsenberg, Hazenberg, Fleury
- BrdU
  - Mohri, Kovacs
- D-glucose
  - Hellerstein, Mohri

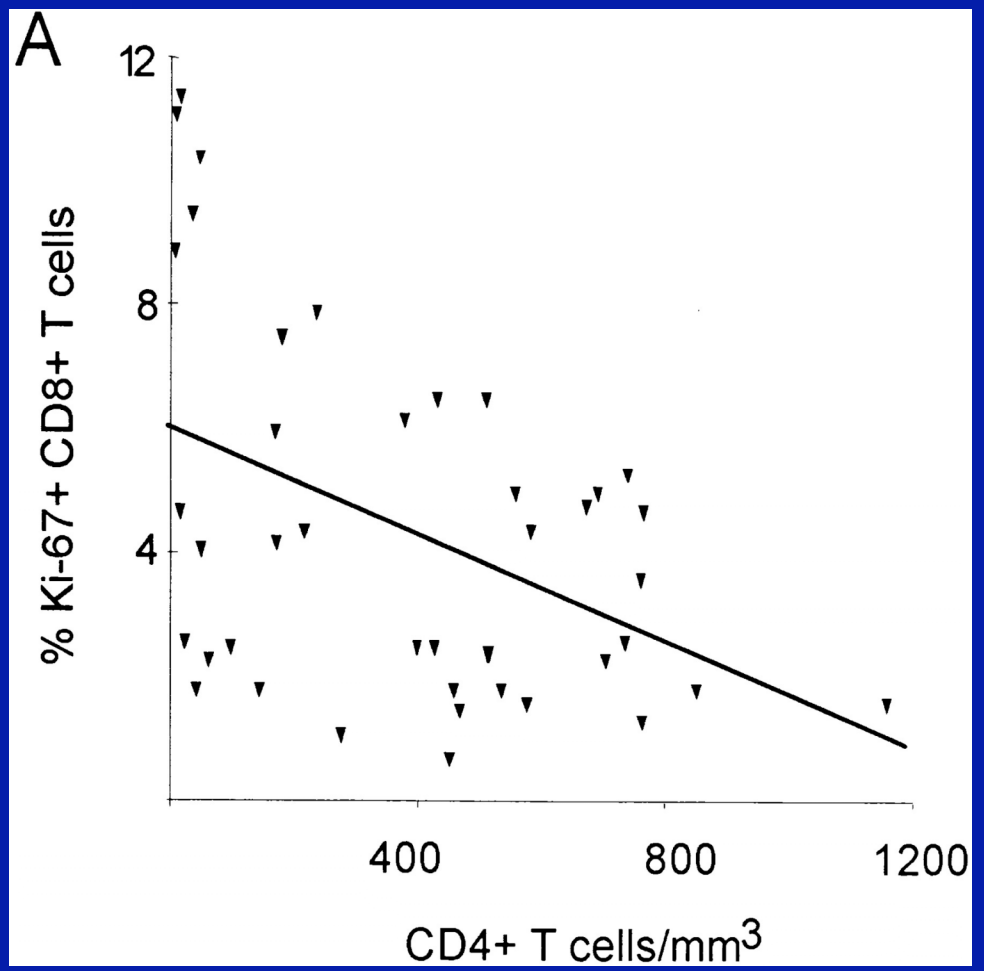
Telomere length



Wolthers *et al*, *Science* **274**: 1543 (1996)

Turnover by Ki67





Sachsenberg *et al*, *J Exp Med* **187**: 1295 (1998)

Labeling with deuterated glucose

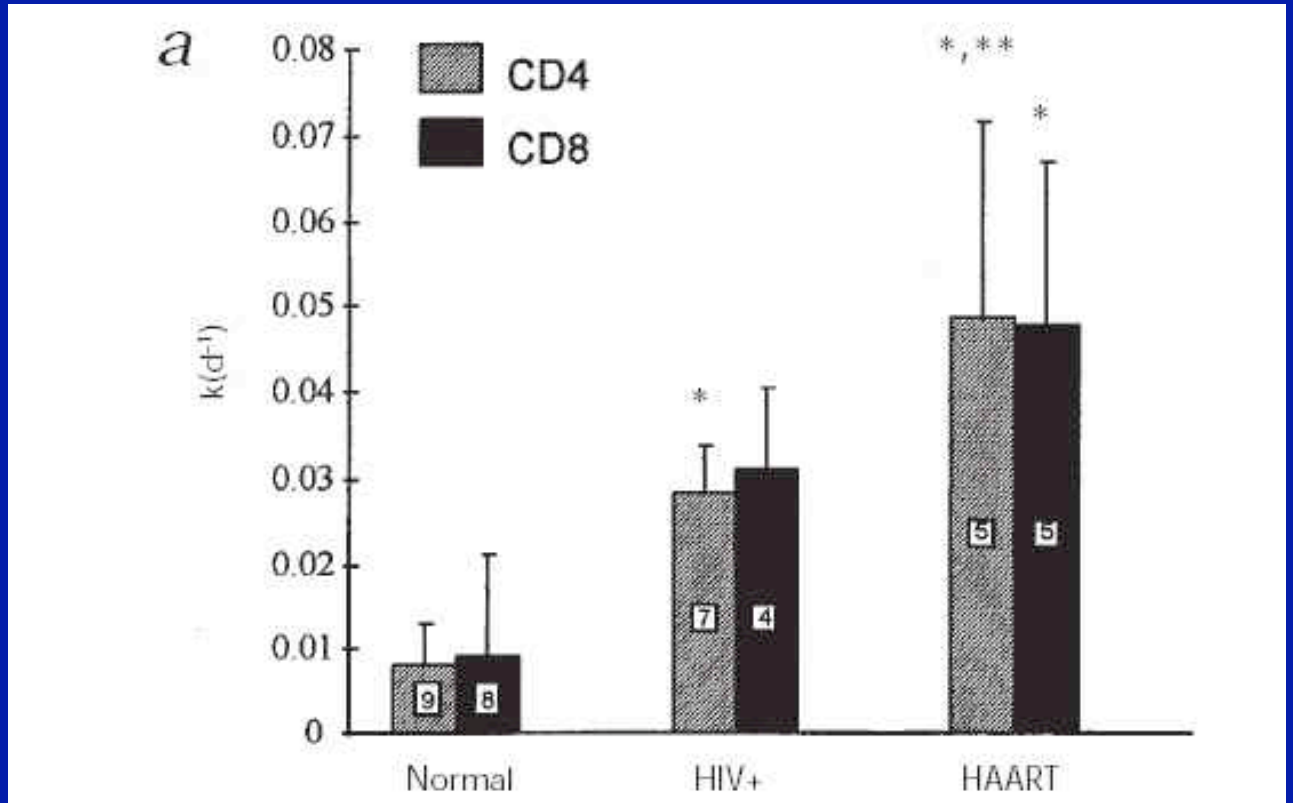
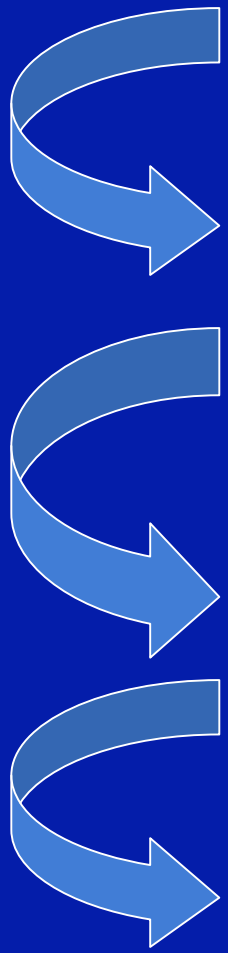


Fig. 3 ***a***, Values of k (per day) for blood $CD4^+$ and $CD8^+$ T cells in different groups. Numbers in bars represent number of subjects.

Hellerstein *et al*, *Nature Med*. **5** (1999)

Assessing T-cell dynamics



^{2}H Glucose administration - 7 days

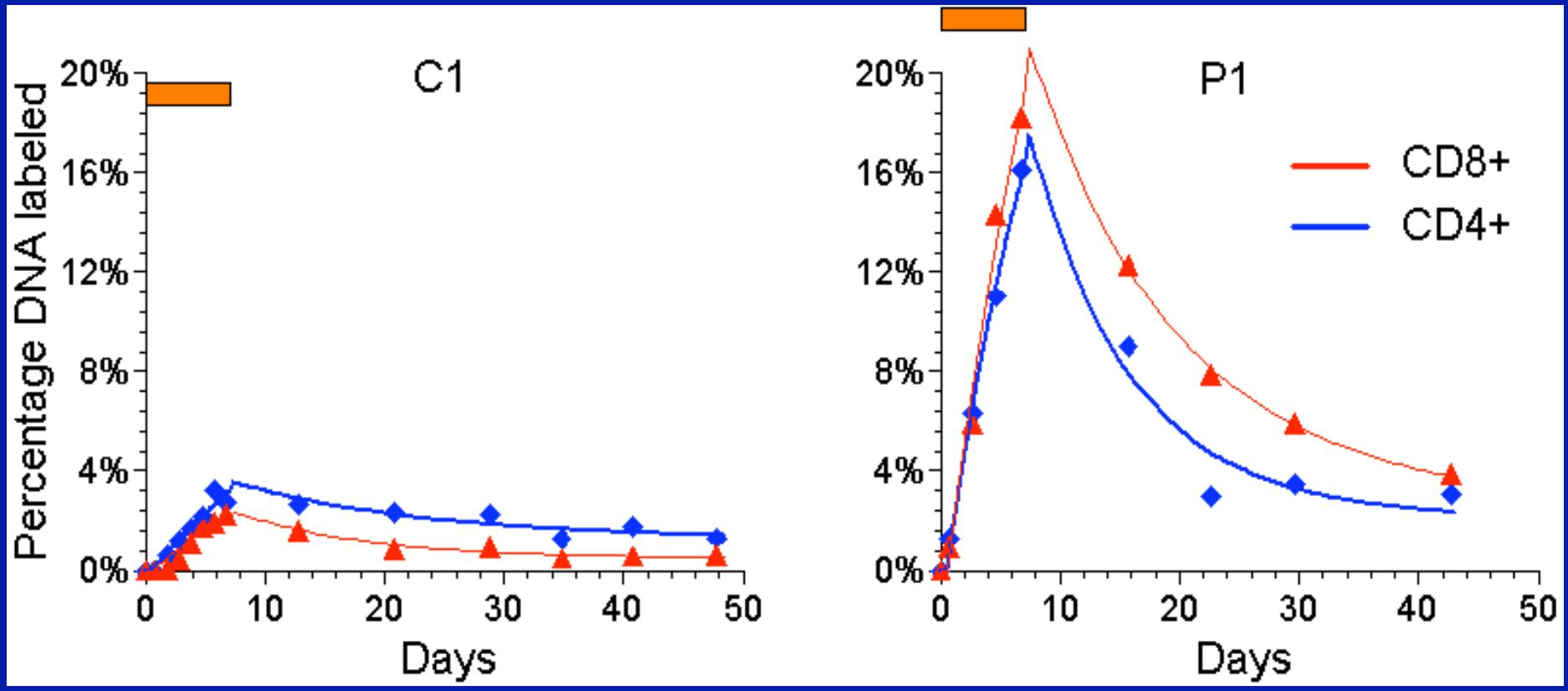
Blood sampling

- every 2 days during glucose infusion
- then every week for 5 - 7 weeks

Cell sorting (flow cytometry)

Cell lysis and DNA preparation for gas chromatography-mass spectrometry

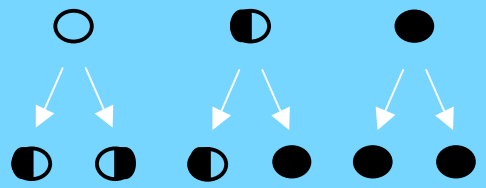
T-cell dynamics (D-glucose)



Mohri et al. *J. Exp. Med.* **194**: 1277 (2001)

Modelling T-cell dynamics

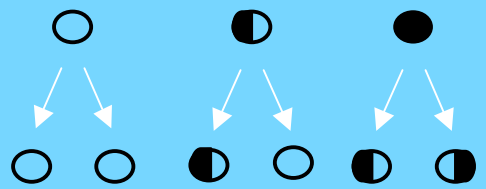
Labeling



$$U_A \rightarrow U_A + L_A$$

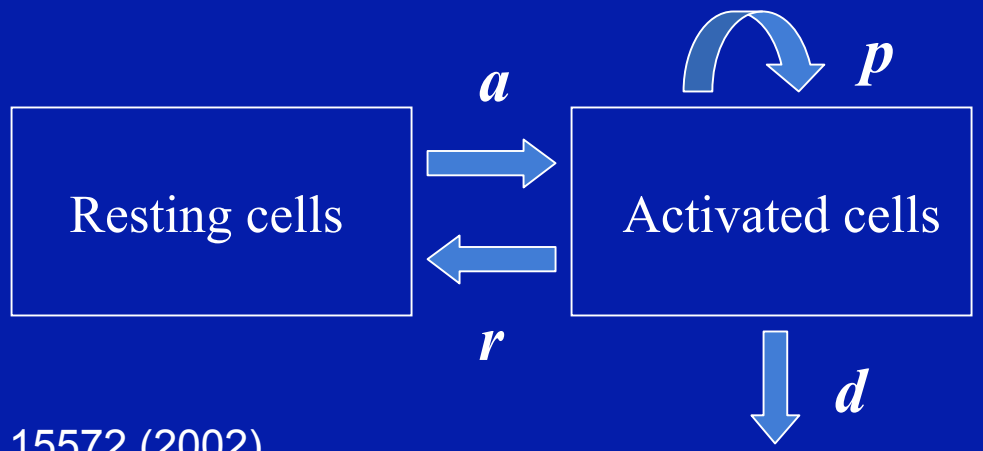
$$L_A \rightarrow L_A + L_A$$

De-labeling



$$U_A \rightarrow U_A + U_A$$

$$L_A \rightarrow L_A + U_A$$



Ribeiro et al. *PNAS* **99**: 15572 (2002)

Model equations

$$\frac{dR}{dt} = -aR + rA$$

$$\frac{dA}{dt} = (p - d)A + aR - rA$$

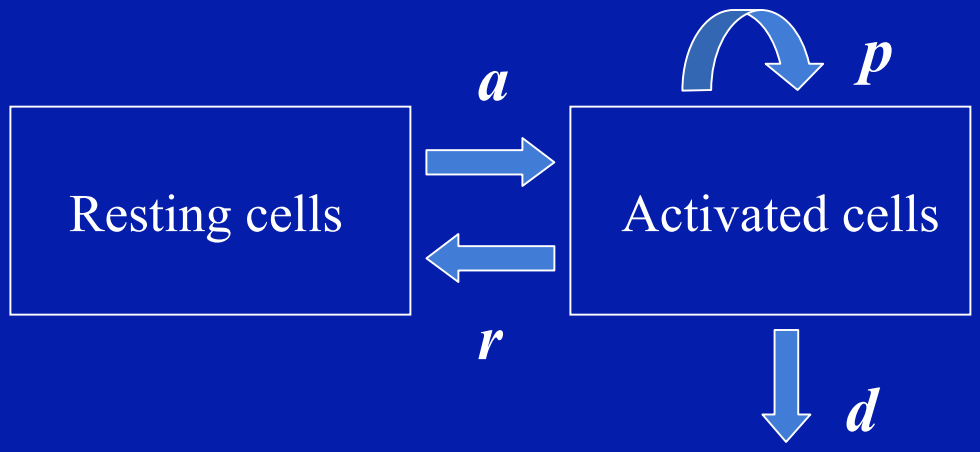
Labeling

$$\frac{dU_R}{dt} = -aU_R + rU_A$$

$$\frac{dU_A}{dt} = -(d + r)U_A + aU_R$$

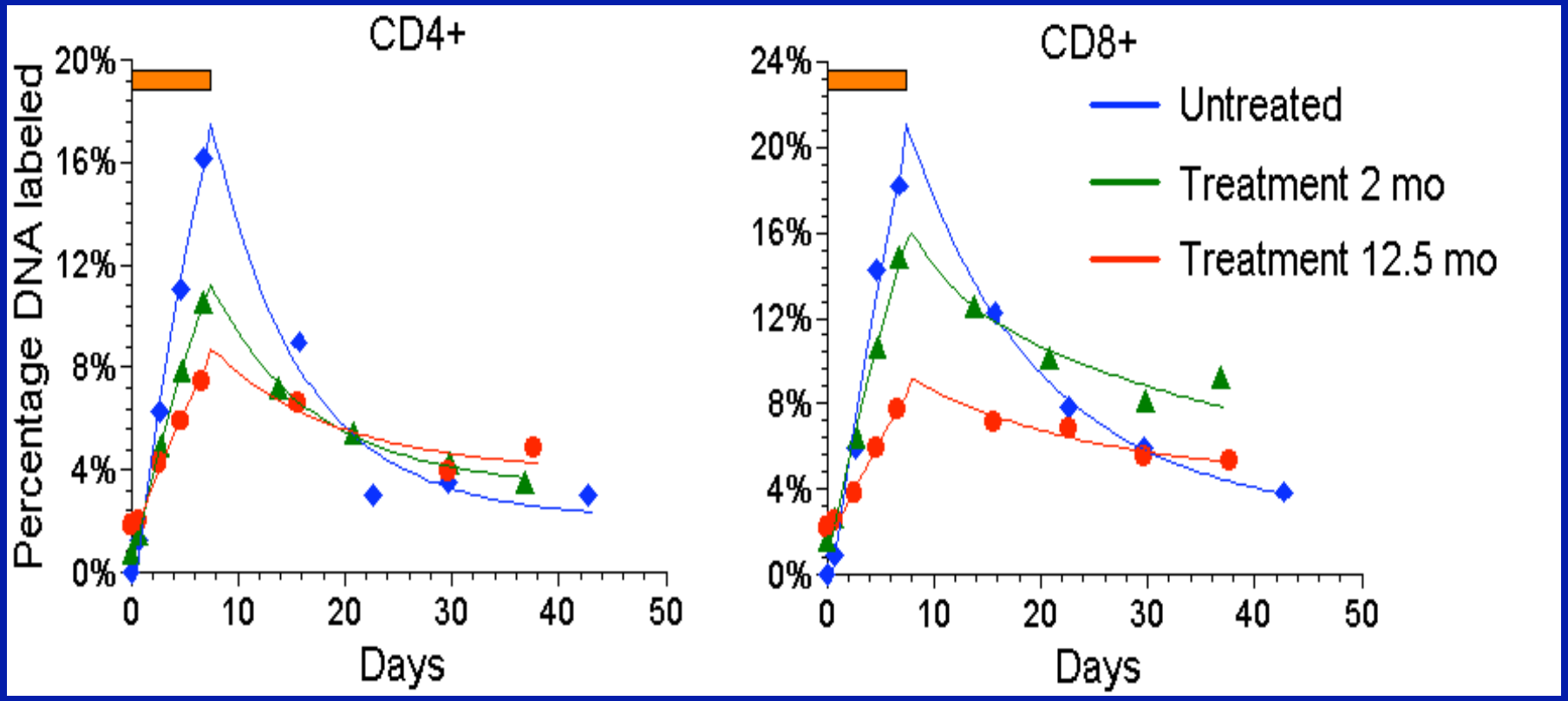
$$\frac{dL_R}{dt} = -aL_R + rL_A$$

$$\frac{dL_A}{dt} = (p - d)L_A + pU_A - rL_A + aL_R$$



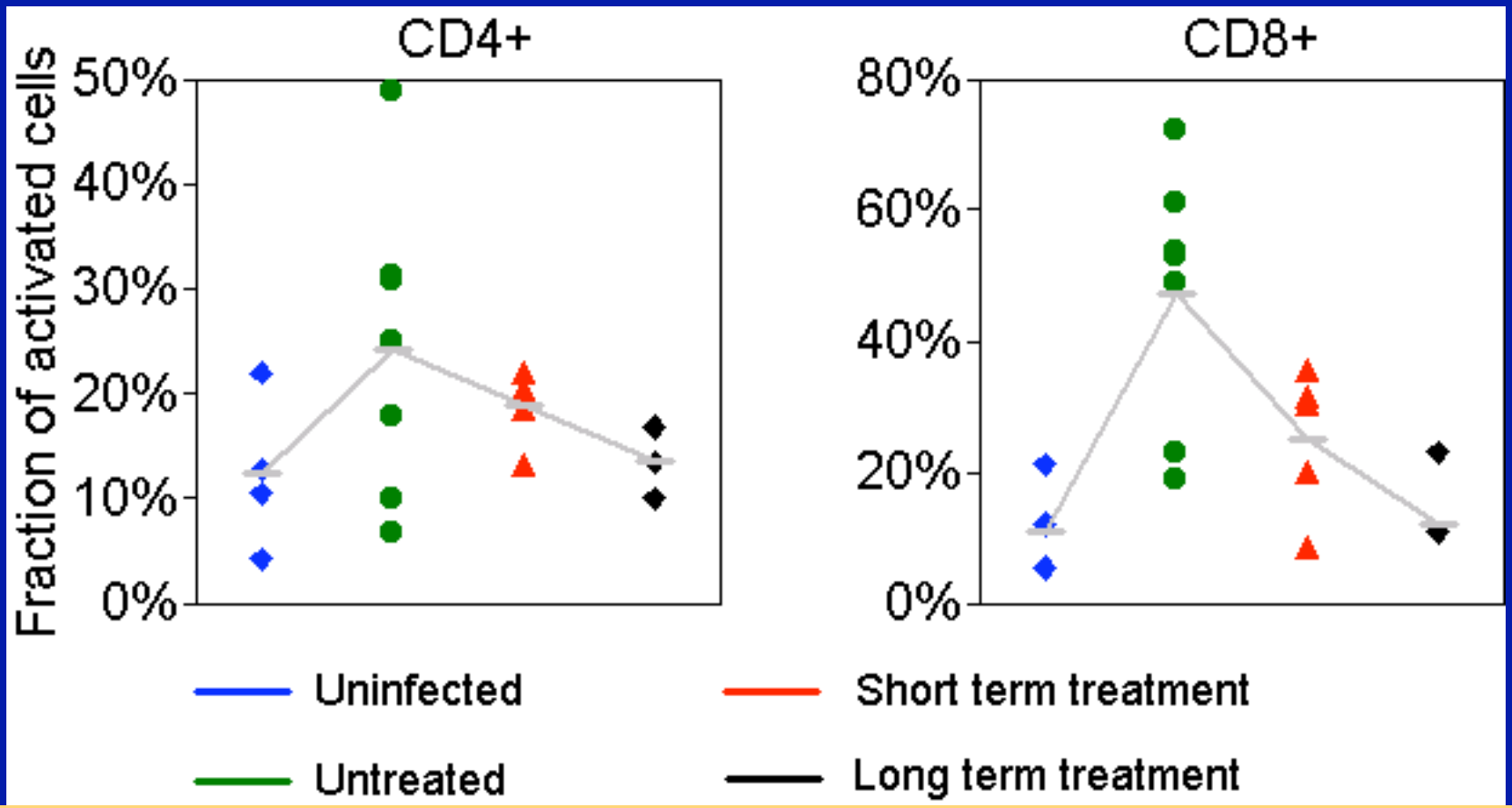
$f_A = a/(a+r)$

Results: untreated *vs.* treated



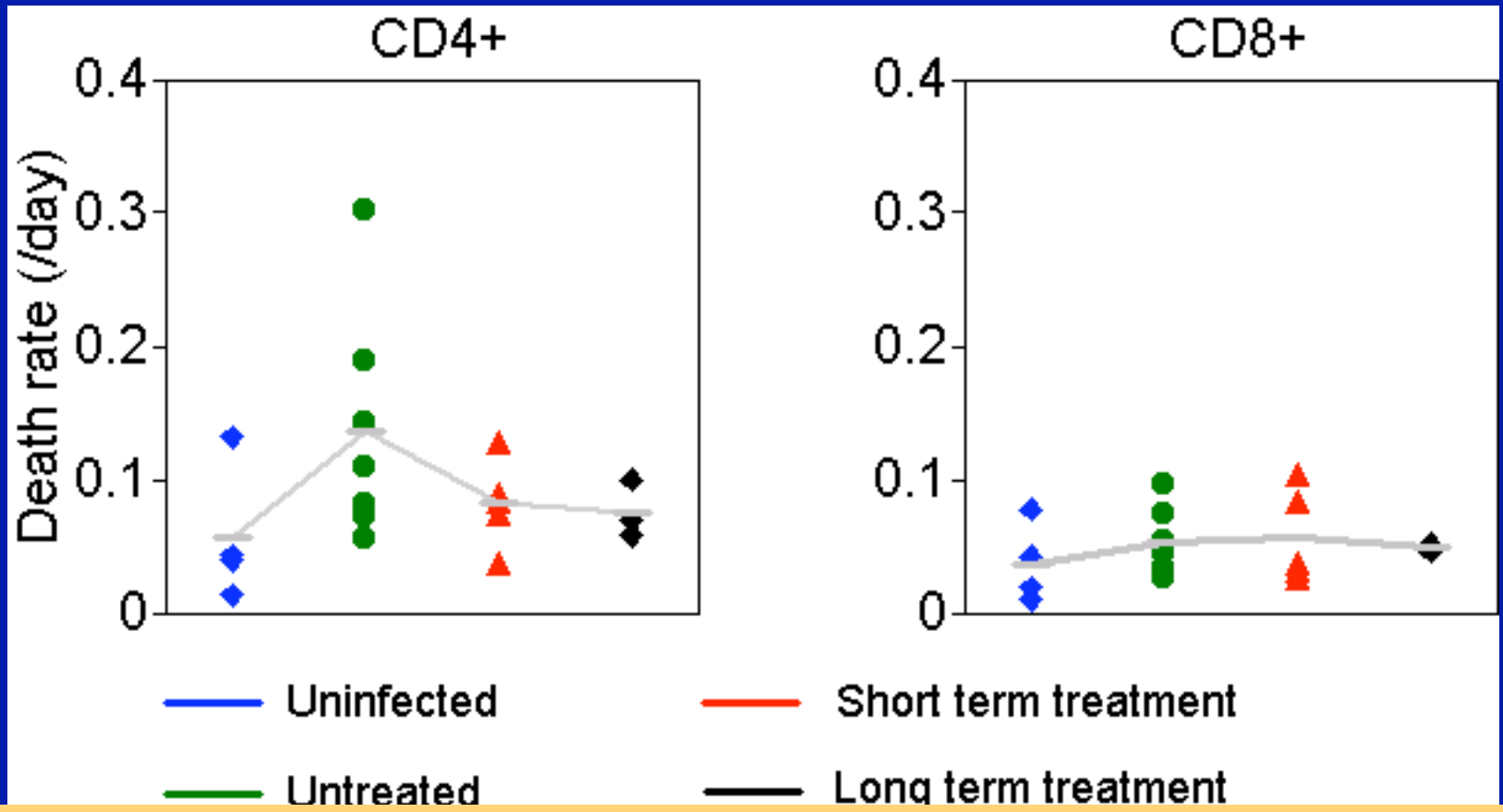
The model is appropriate to fit the data. The data demonstrate increased turnover in HIV infection.

Fraction of activated cells



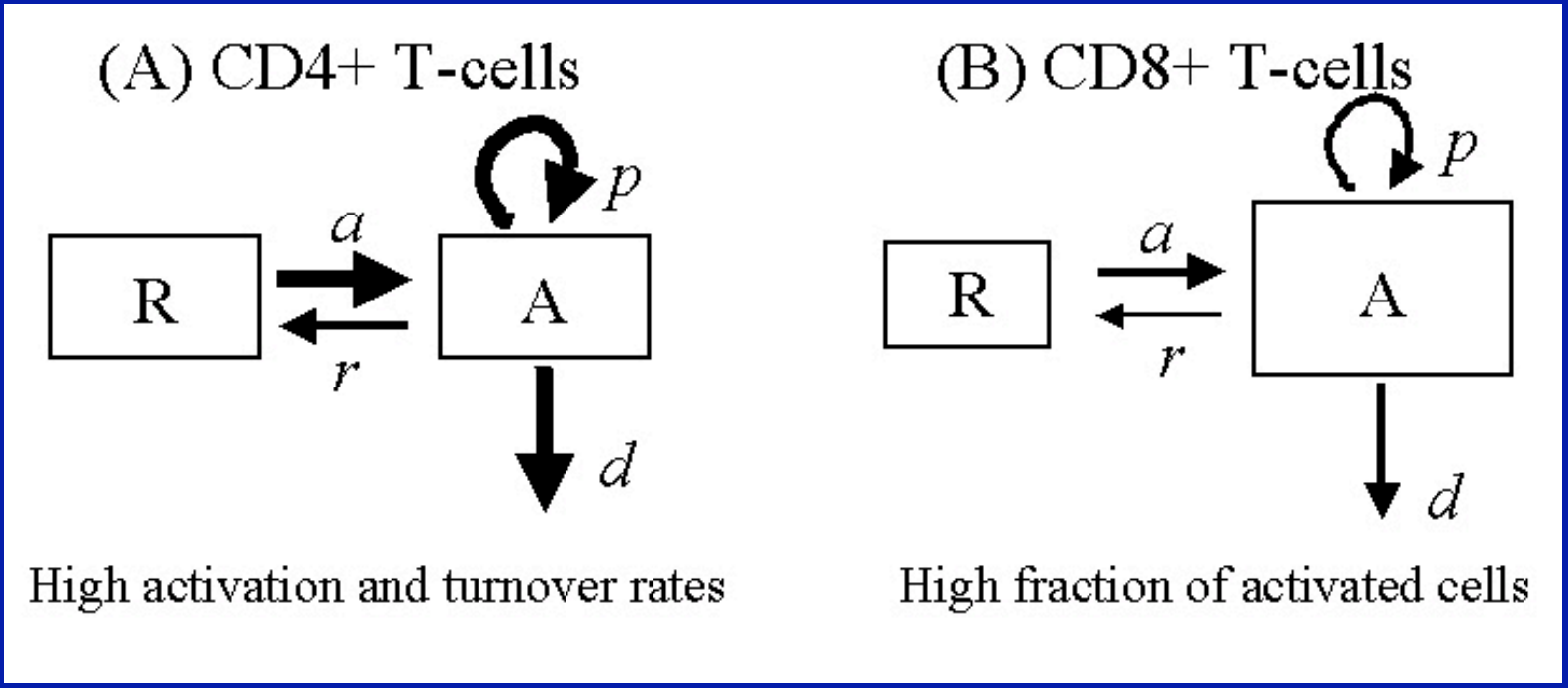
The fraction of activated cells is significantly increased in the CD8+ population of infected individuals, but not in the CD4+ population.

Death rate of activated cells



There is a trend for increased death rate in the CD4+ activated cell population, but no difference in death rates for activated CD8+ cells.

Interpreting the results

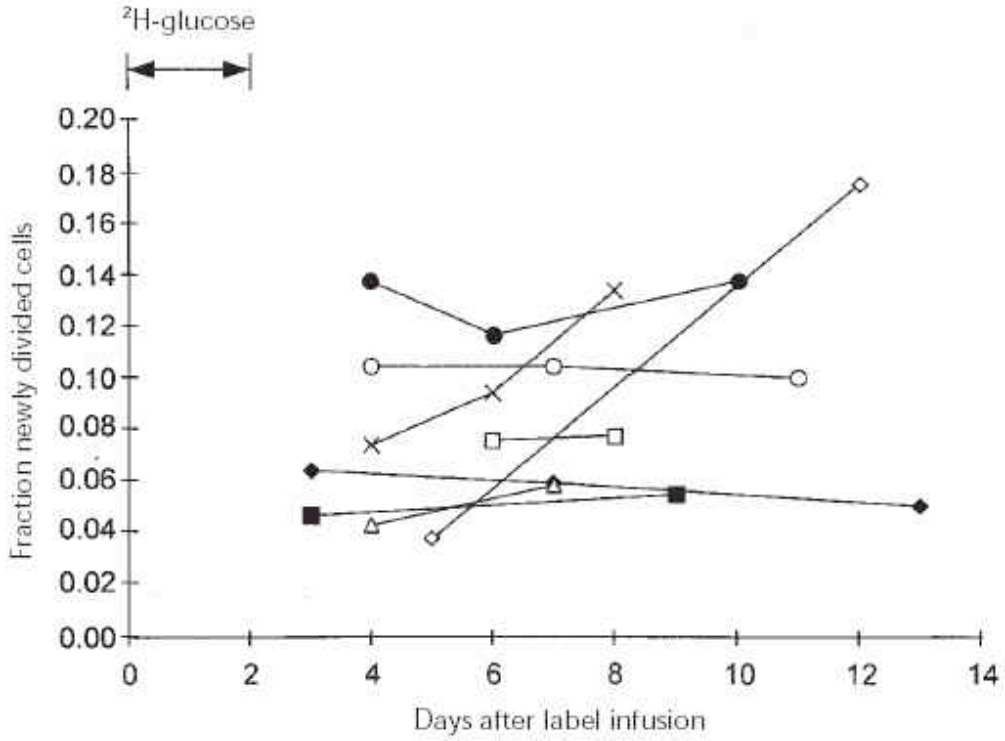


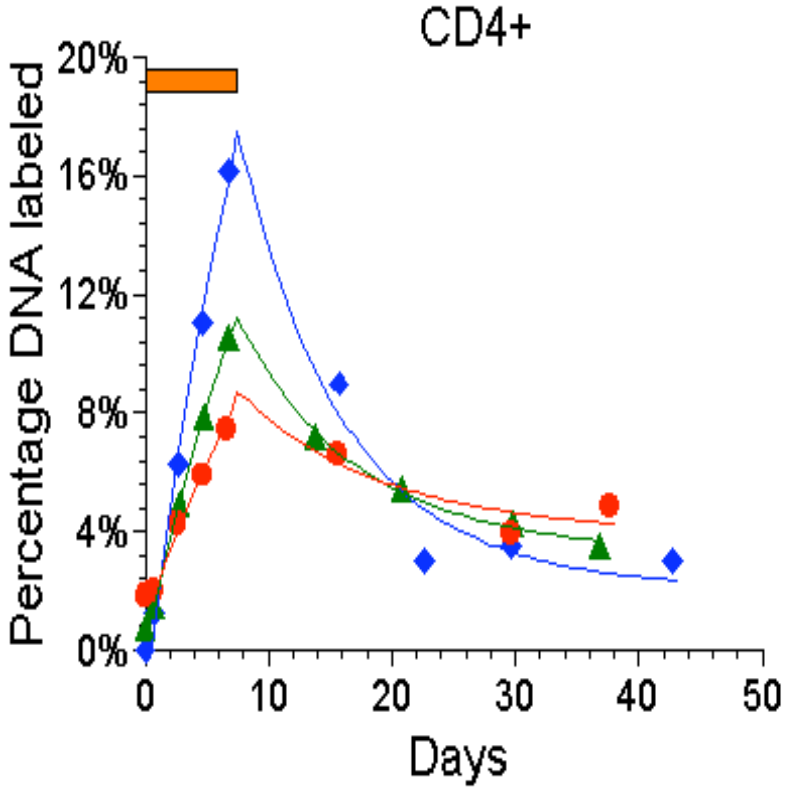
Explaining conflicting results

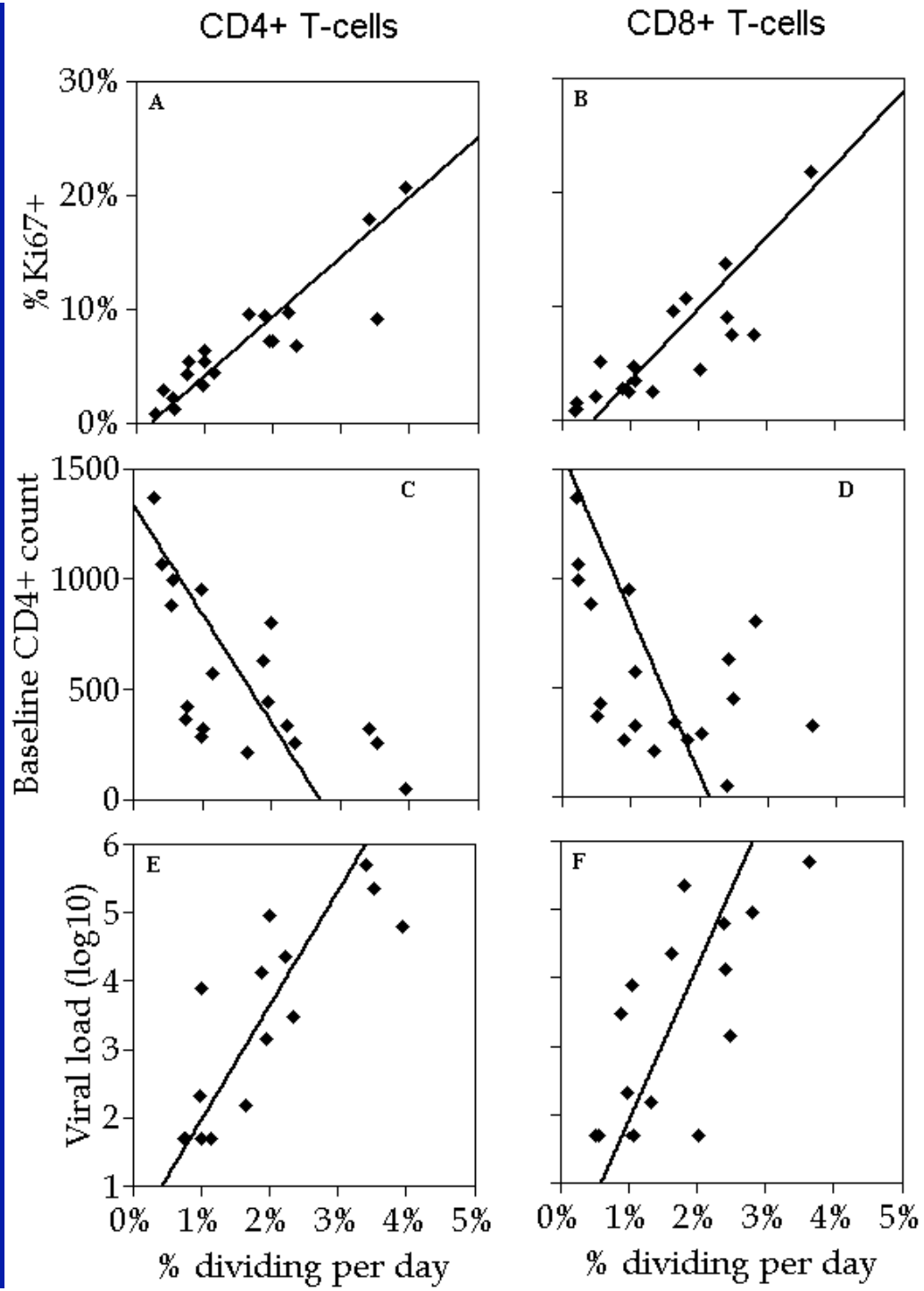
- The length of telomeres
  - Wolthers et al, “T cell telomere length in HIV-1 infection: no evidence for increased CD4+ T cell turnover”, *Science* **274**: 1543 (1996)
  - Wolthers et al., AIDS Res Hum Ret 15: 1053 (1999)

- Early HAART turnover data
  - Hellerstein, Nature Medicine (1999)

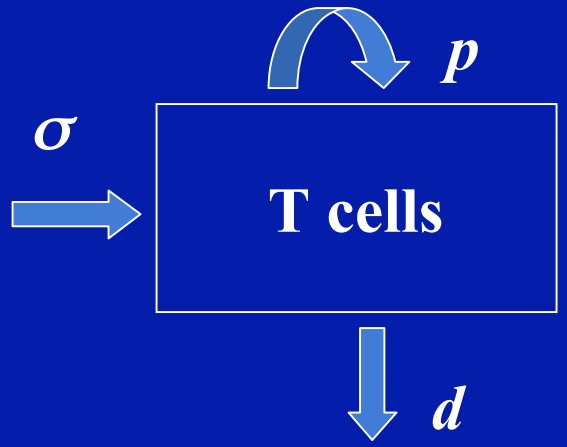
D-glucose labeling revisited





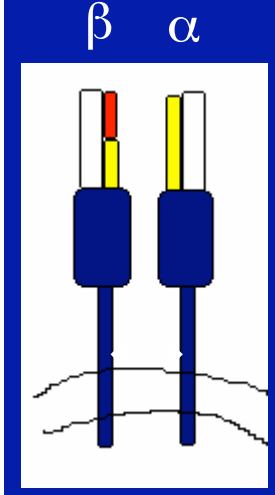

CD4+ T-cells
CD8+ T-cells
A
B
C
D
E
F
30%
20%
10%
0%
% Ki67+
1500
1000
500
0
Baseline CD4+ count
6
5
4
3
2
1
Viral load (log10)
0% 1% 2% 3% 4% 5%
% dividing per day
0% 1% 2% 3% 4% 5%
% dividing per day

Thymic contribution



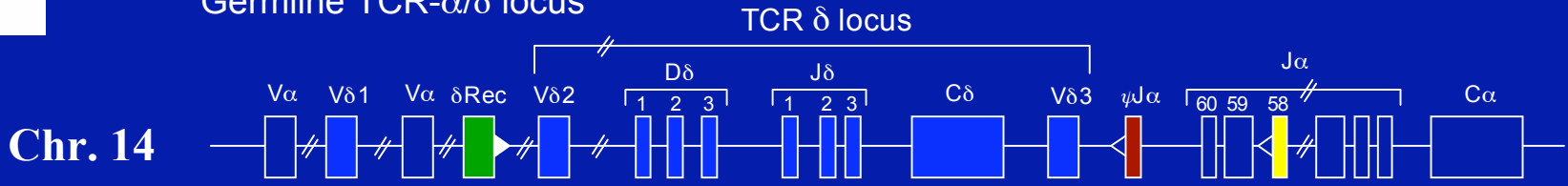
Quantify the role of the thymus in peripheral T cell homeostasis by assessing the impact of thymectomy on α TREC in the periphery of macaques.

T-cell Receptor Excision Circles (TREC)

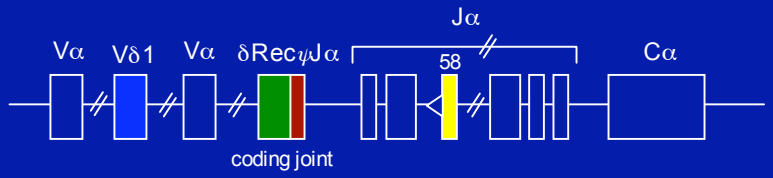


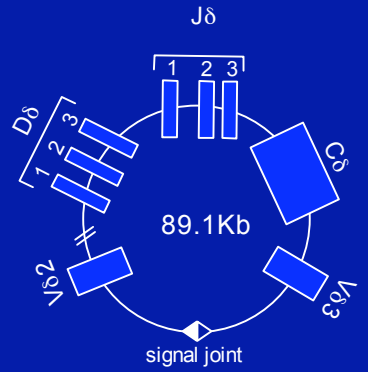
Variable
Diversity
Joining
Constant

Germline TCR-α/δ locus



δRec- ψJα rearrangement





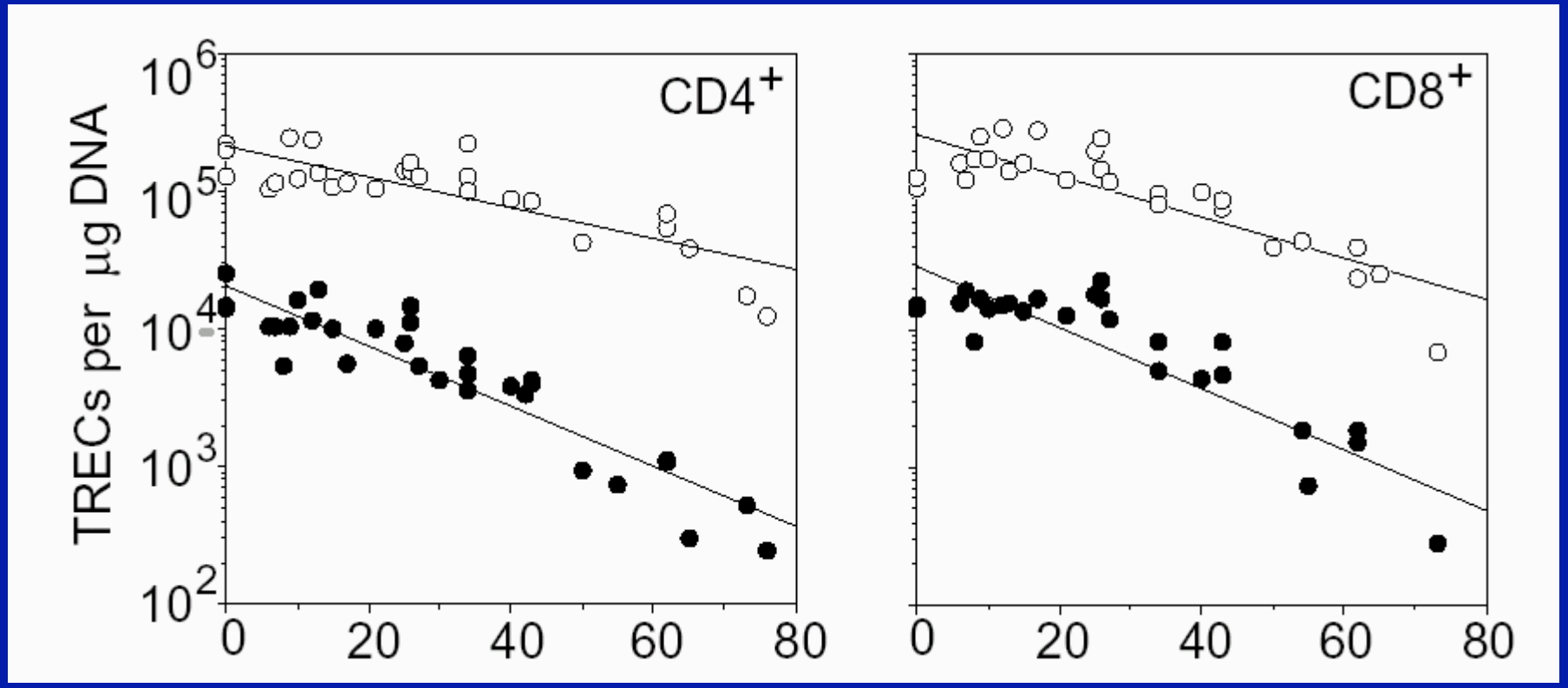
α 1 TREC

Douek *et al.*, Nature 1998; Zhang *et al.*, J Exp Med 1999
Dion *et al.*, Immunity 2004

Properties of (these) TREC

- Stable, *i.e.* do not degrade (Livak, *Mol Cel Biol* 1996, Kong, *PNAS* 1999)
- Do not divide (Douek, *Nature* 1998)
- Thymic origin (Douek, *Nature* 1998, Kong *PNAS* 1999, Guy-Grand, *J Exp Med* 2003)
- Identical in 70% of $\alpha\beta$ T-cells (Verschuren, *J Immunol* 1997)
- Kong *et al.* showed that in chicken they mark RTE (similar to chT1+ T-cells)

Decline of TREC with age

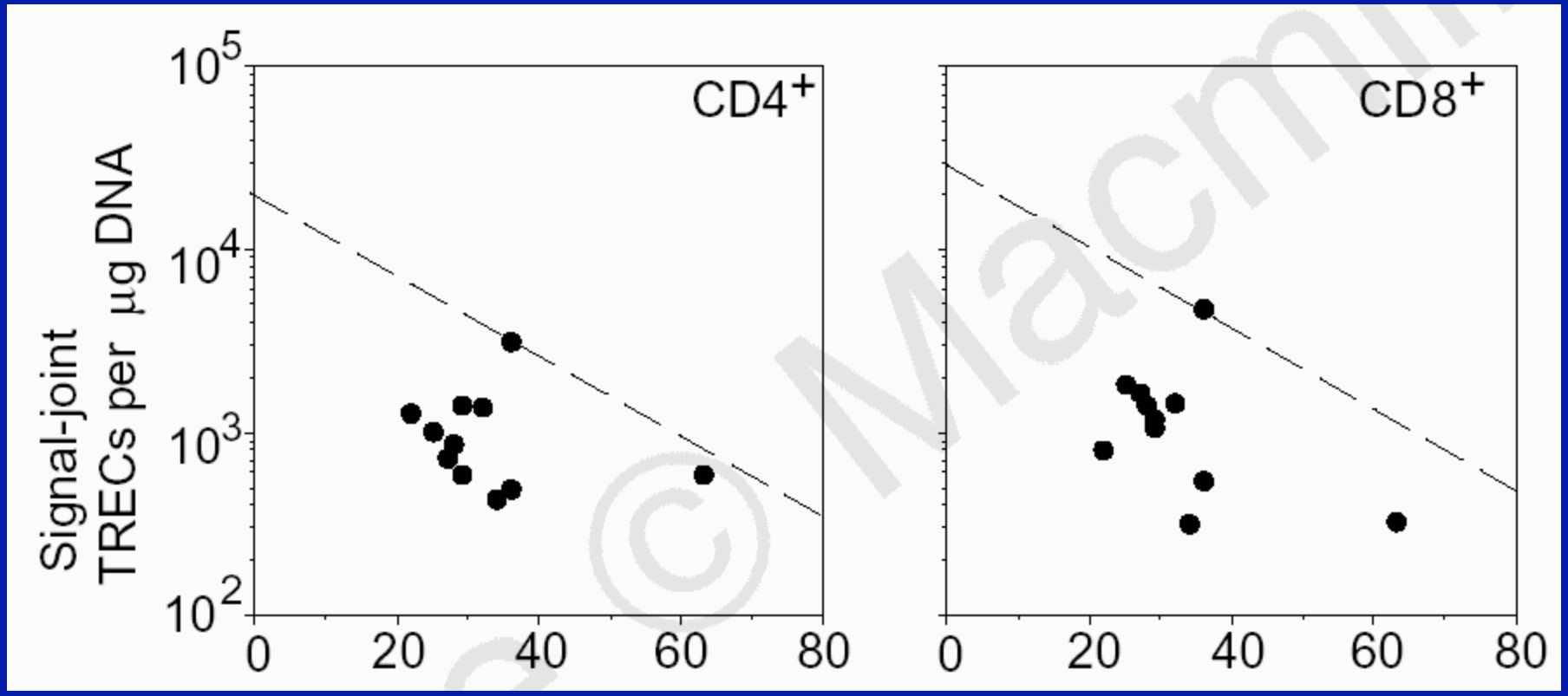


○ Coding joint (cjTREC)

● Signal joint (sjTREC)

Douek et al., *Nature* 1998

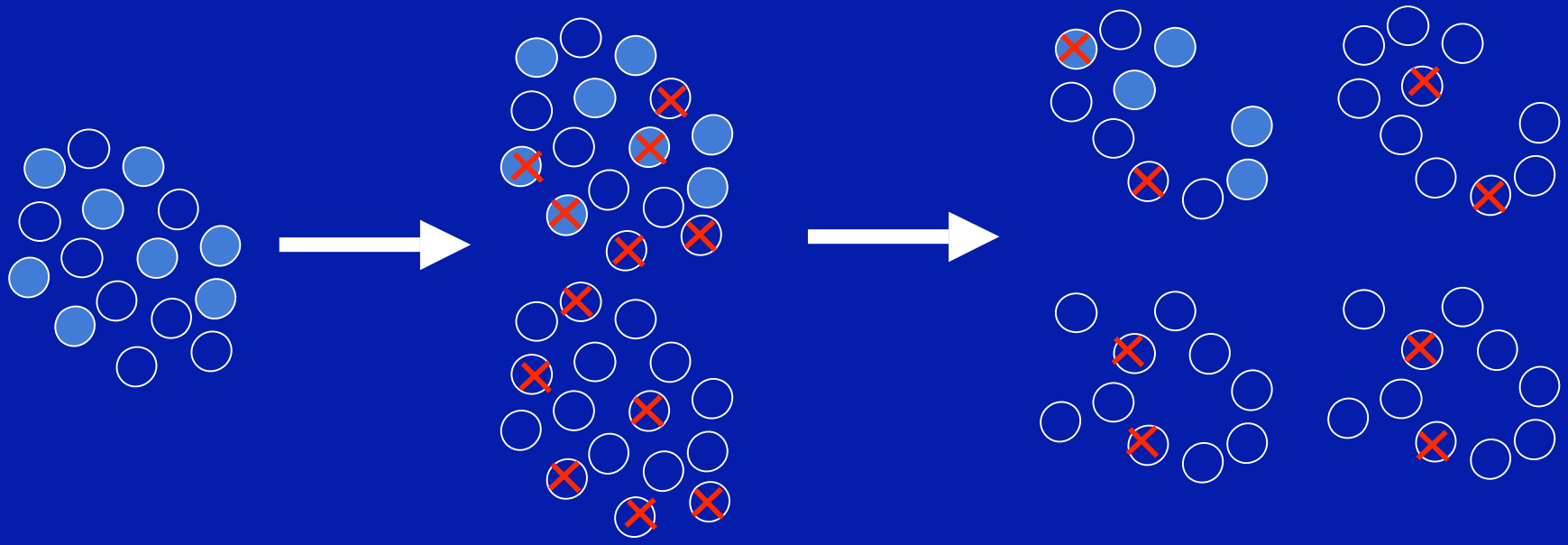
Reduced TREC in HIV infection



- Signal joint (sjTREC)

Douek et al., *Nature* 1998

TREC Dynamics



Input from thymus:

Cells – changes TREC/ml

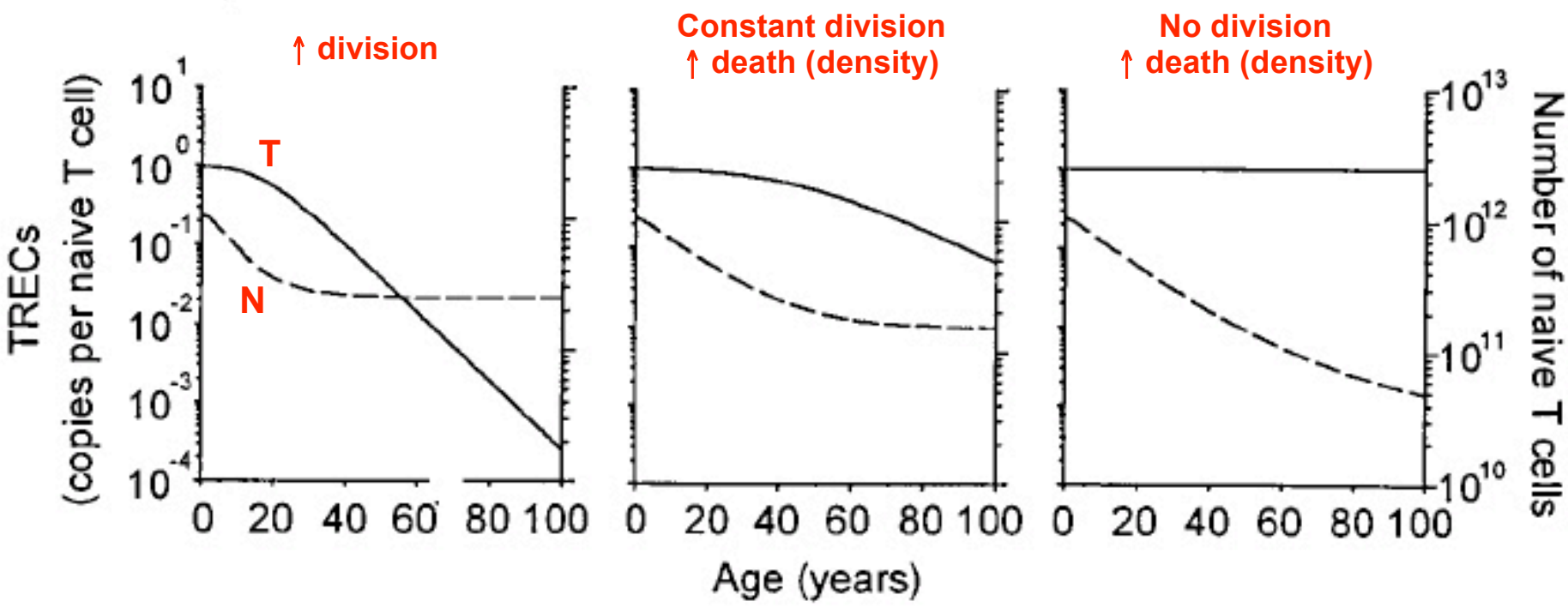
% TREC+ – changes TREC/10^6 cells

In the periphery:

TREC/10^6 cell – decrease by proliferation

TREC/ml – decrease by death of TREC+ cells

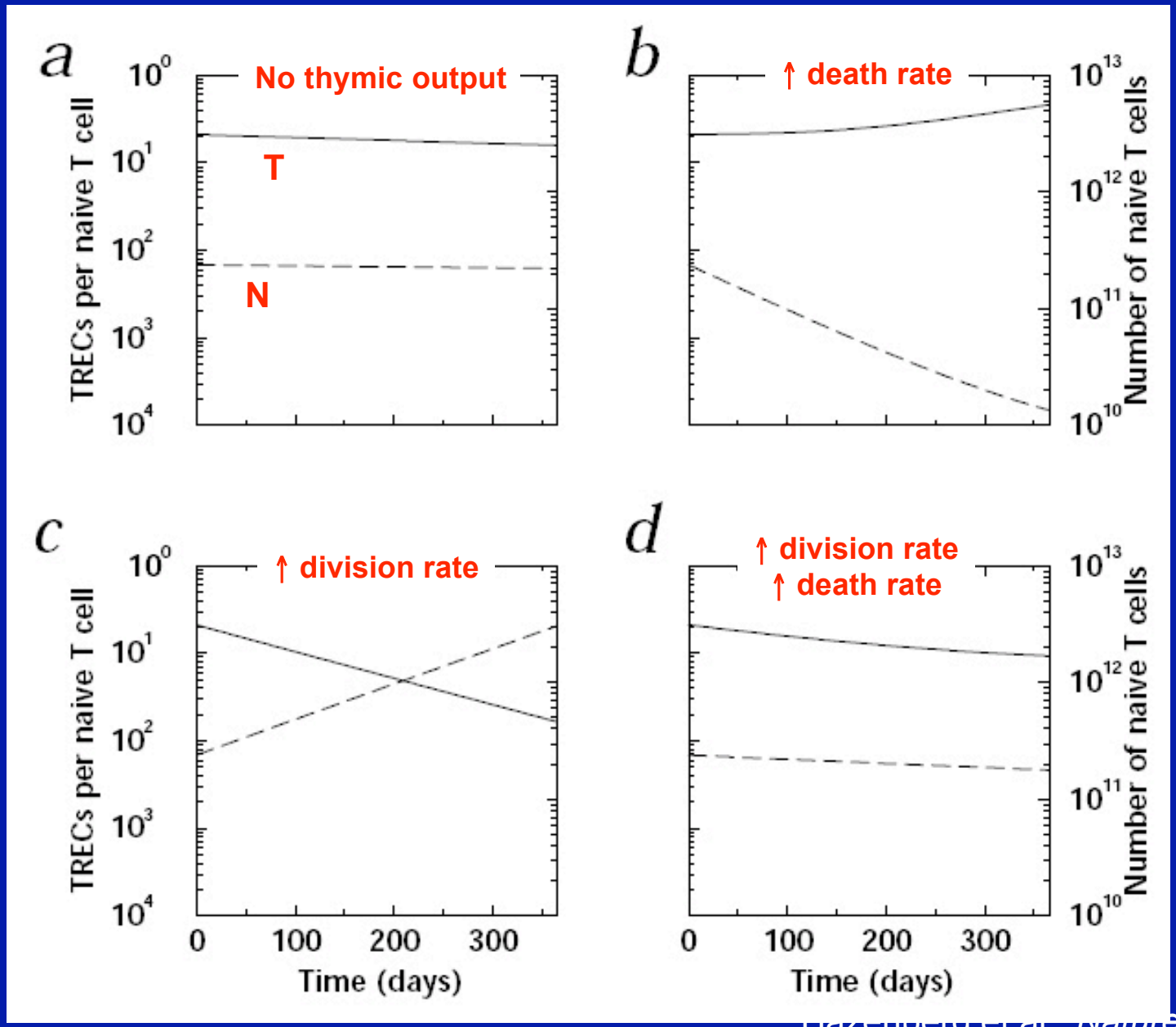
Model of TREC and ageing



Thymic output decays exponentially

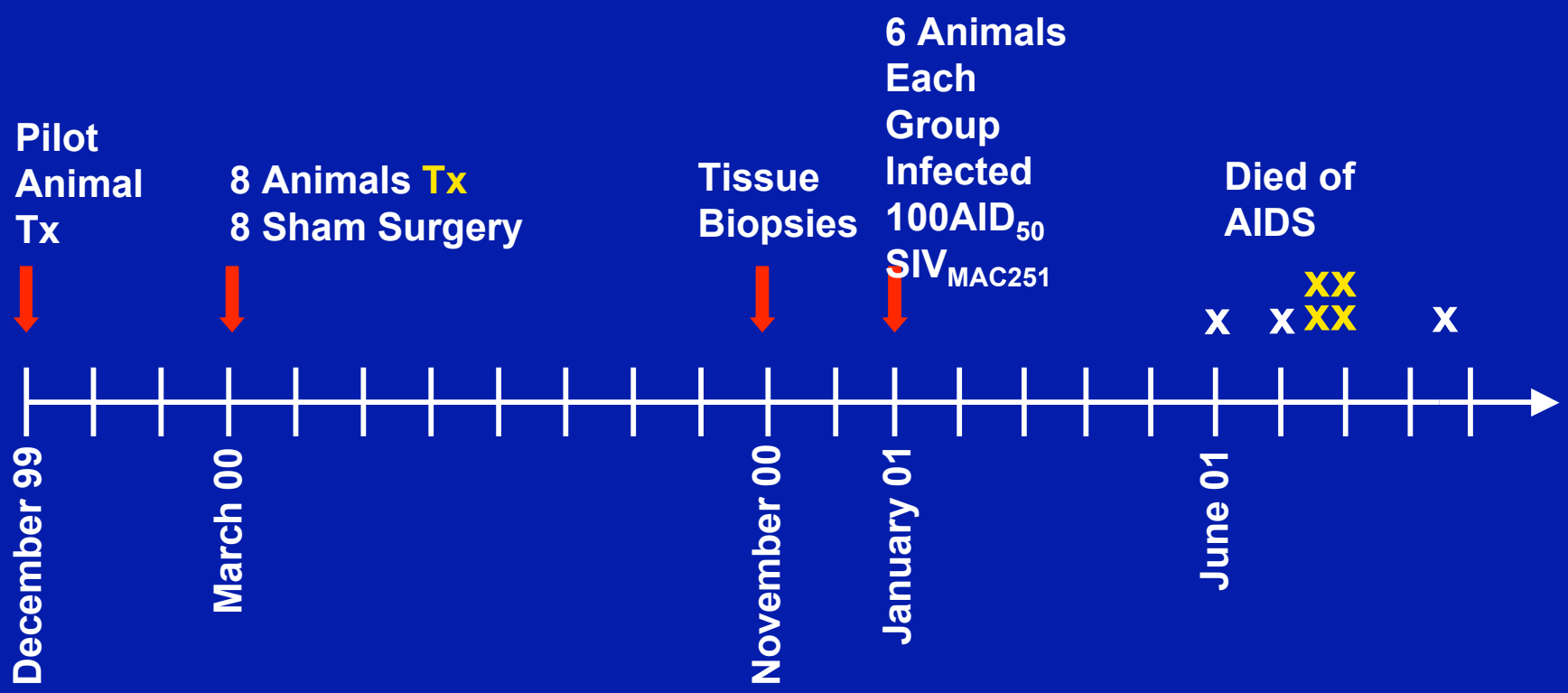
Hazenberg et al., *Nature Med* 2000

Model of TREC and HIV infection



Hazenberg et al., *Nature Med* 2000

Experimental timeline

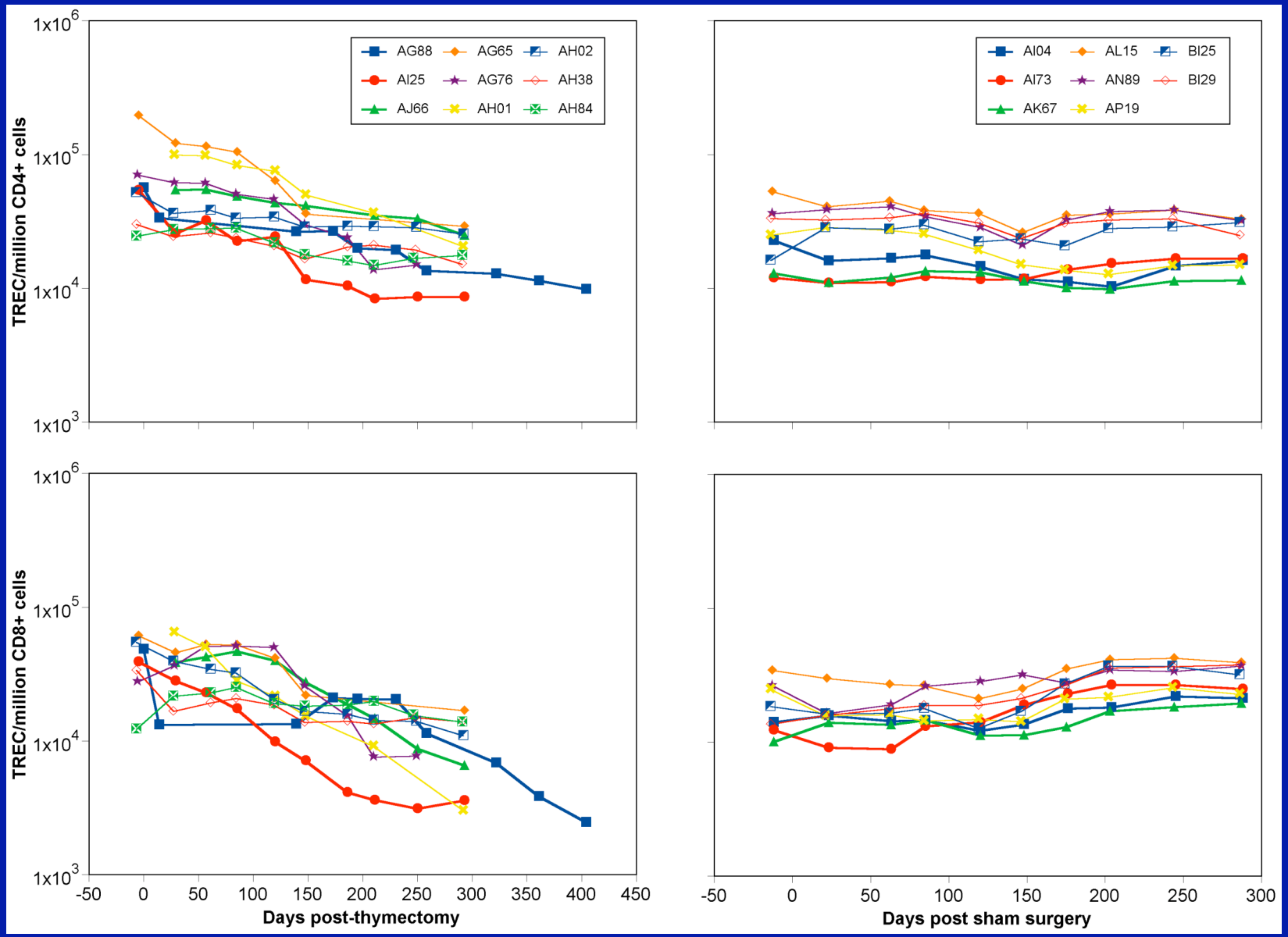


Brief experimental protocols

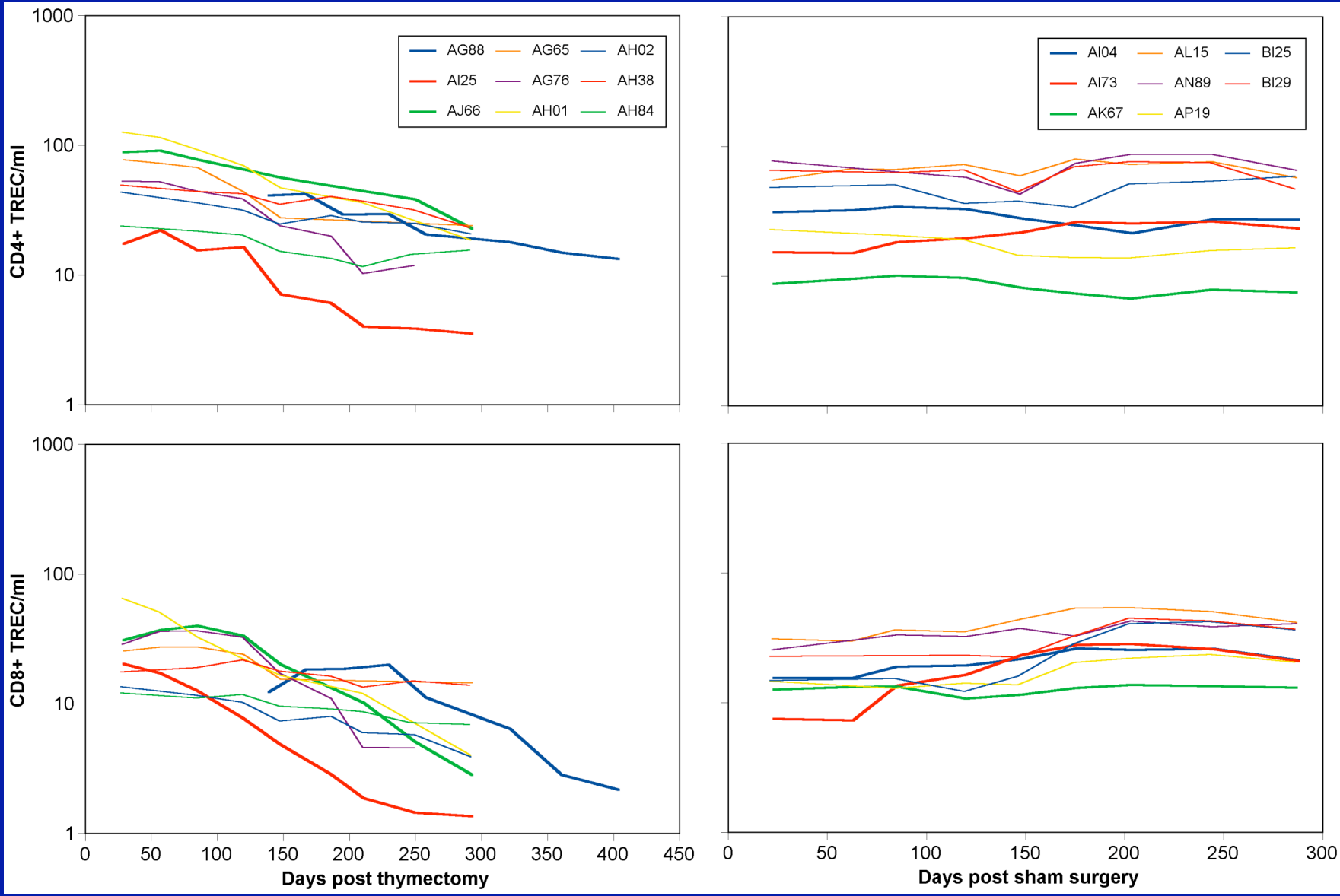
- Ventral sternotomy. Removal of the largest part of the thymus. Dissection completed by removing small remnants of fat and thymus in piecemeal fashion.
- Sham animals underwent the same surgery without removal of the thymus.

- Four-colour flow cytometry for cell counting
  - CD3+, CD4+, CD8+, CD20+, CD45RA+
- TREC by real-time PCR with molecular beacons, normalized by real-time PCR of CCR5 (2 copies)

TREC/10^6 cell



TREC per ml


CD4+ TREC/ml
CD8+ TREC/ml
1000
100
10
1
AG88
AG65
AH02
AI25
AG76
AH38
AJ66
AH01
AH84
AI04
AL15
BI25
AI73
AN89
BI29
AK67
AP19
0
50
100
150
200
250
300
350
400
450
Days post thymectomy
Days post sham surgery

General linear model to calculate slopes

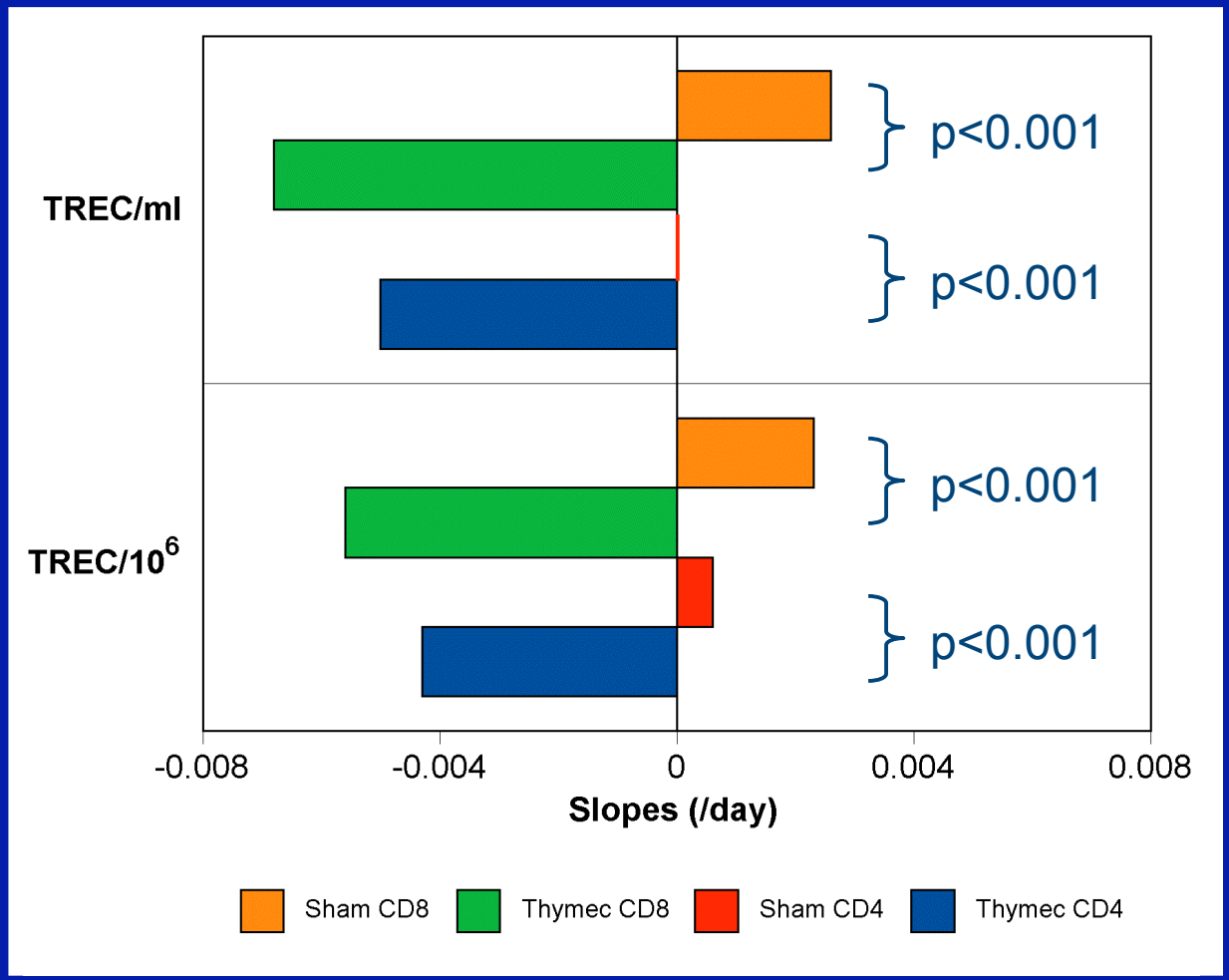
- Assumes linear changes (of the natural logs)
- Estimates the slopes of the population, taking into account the variation in the data
- Allows for a random effect for macaques
- Proper comparison between sham and Tx slopes

Is this significant?

$$\ln y_i(t) = \alpha + \beta t + (a_i + b_i t) + \begin{pmatrix} \alpha_1 \\ \alpha_2 \end{pmatrix} + \begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix} t + \varepsilon_i$$

Is this significant?

α TREC decay slopes after surgery



What does all this mean?

Model to estimate thymic source

We assume that all other cell processes (proliferation, activation,…) do not affect TREC, and *d* is the average

$$\frac{dC}{dt} = \alpha\sigma - dC \Rightarrow \frac{d\ln C}{dt} = \alpha\frac{\sigma}{C} - d$$

In thymectomized animals, the slope of ln C is -*d*

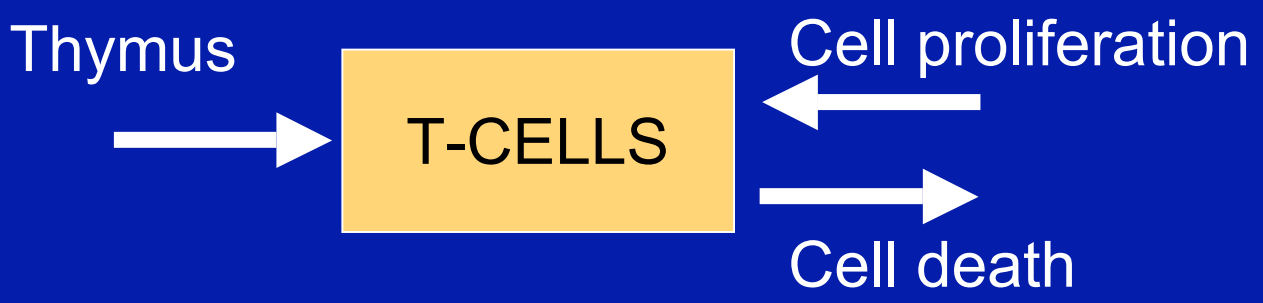
Estimates of thymic output

Before thymectomy, if TREC/ml and TREC/cell are in equilibrium, since slopes not significant in sham surgery:

$$\alpha\sigma = dC \Leftrightarrow \alpha\frac{\sigma}{T} = d\frac{C}{T} \text{ and } \frac{\sigma}{T} = \frac{d}{\alpha}C_T$$

	CD4	CD8
d (day^{-1})	0.005	0.007
α	0.11	0.11
C_T	0.070	0.033
σ/T (day^{-1})	0.32%	0.21%

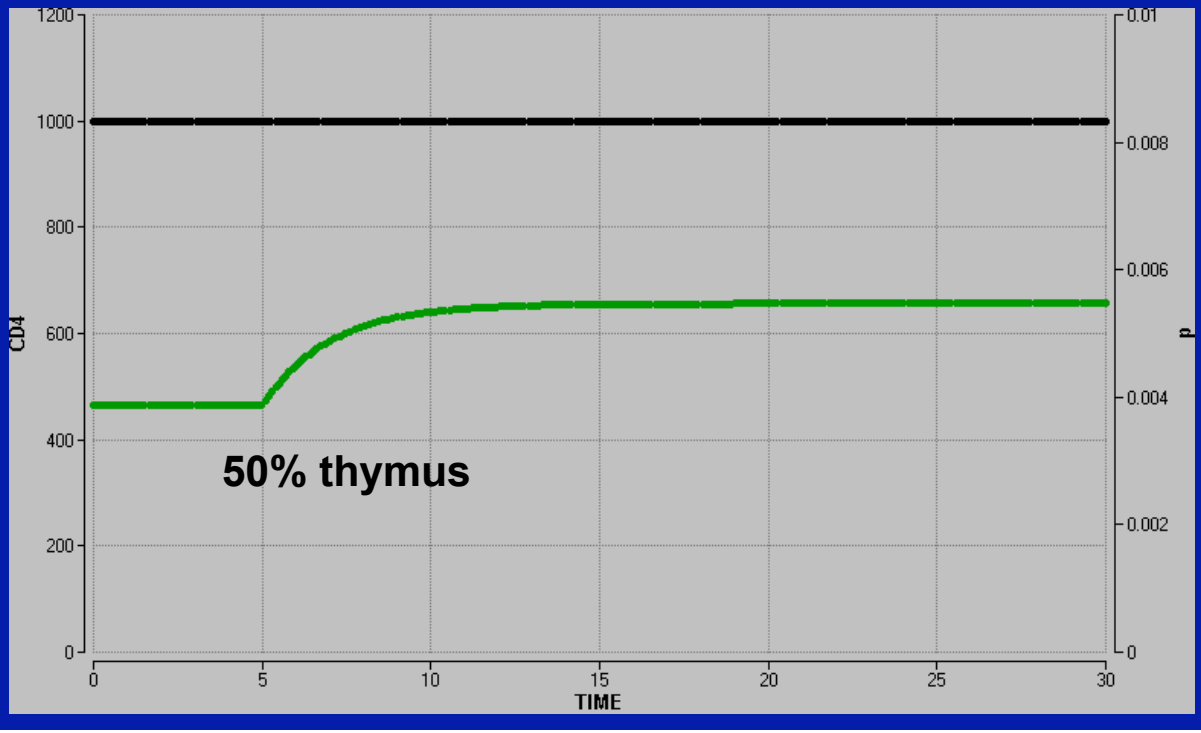
How “large” is the thymic output?



If T_{eq}=1000 cells/μl, death=0.007 day^{-1}

Proliferation (/day)

0.0039



Proliferation (/day)

0.0055

So what?

- Immune activation of CD4 and CD8
  - Activation, death and proliferation rates elevated “by HIV”
- But, CD4 are dying faster than CD8, thus decline
- Thymus, may have a contribution, but peripheral increase of proliferation should be enough to keep numbers (what about repertoire and recovery?)
  - Indeed in this model, SIV outcome is no worse

Conclusions

- USED FOR:
  - Generating hypotheses,
  - Estimation of parameters,
  - Interpretation of data,
  - Definition of quantities to assay,
- Not always possible, depends on data
- Better when there is cooperation from start

"… if at one time, we knew the positions and speeds of all the particles in the universe, then we could calculate their behavior at any other time, in the past or future."

Pierre Simon, Marquis de Laplace (1749-1827)