

**Imperial College**  
**London**

# Lymphocyte kinetics in HTLV-1 infection

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# ***Human T-lymphotropic virus type 1 (HTLV-1)***

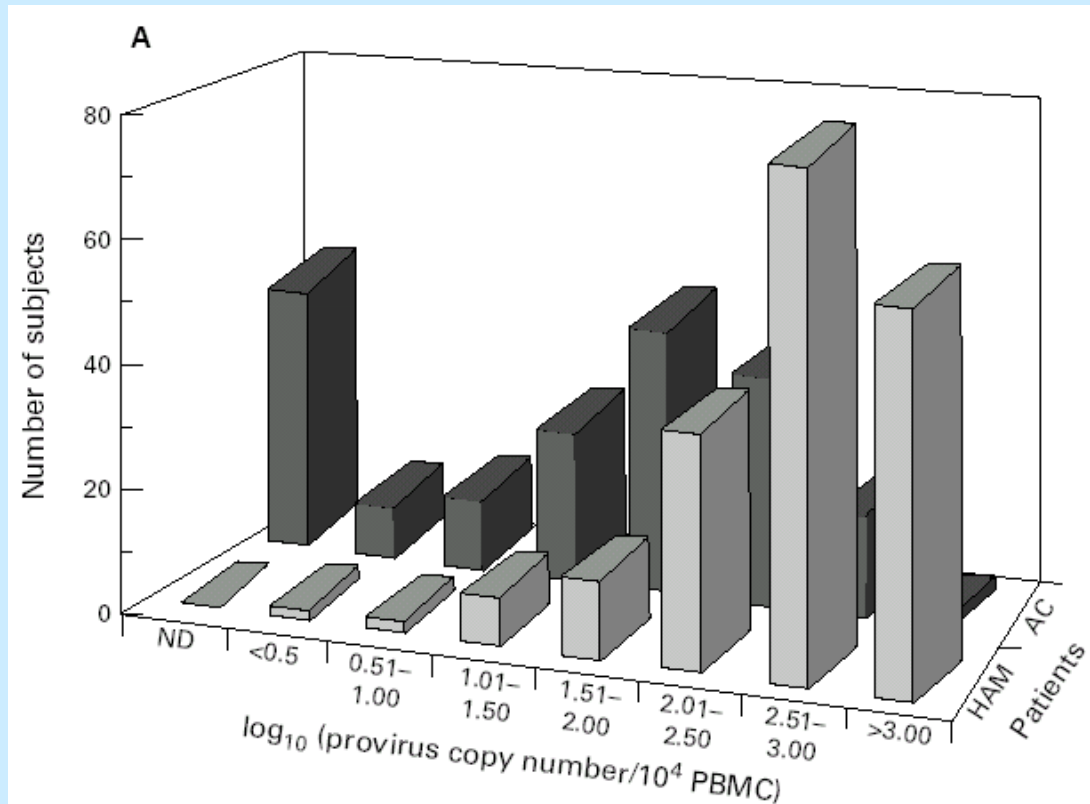
- infects 10-20 million people worldwide.
- endemic (1-20% of adults) in South America, Caribbean, Central Africa, southern Japan.
- 2-3% develop an aggressive T-cell leukaemia/lymphoma
- 2-3% develop a chronic inflammatory disease either of CNS, eyes, muscles, joints, lungs or skin.
- 95% remain healthy carriers of HTLV-1.

# ***HTLV-1 persistence and inflammatory disease***

Three main questions:

1. How does HTLV-1 persist?
2. How does it spread?
3. Why do some develop HAM/TSP, whereas most remain healthy carriers?

# *The proviral load of HTLV-1 is high and correlates with the risk of HAM/TSP*



median proviral load  
(copies/100 PBMCs)

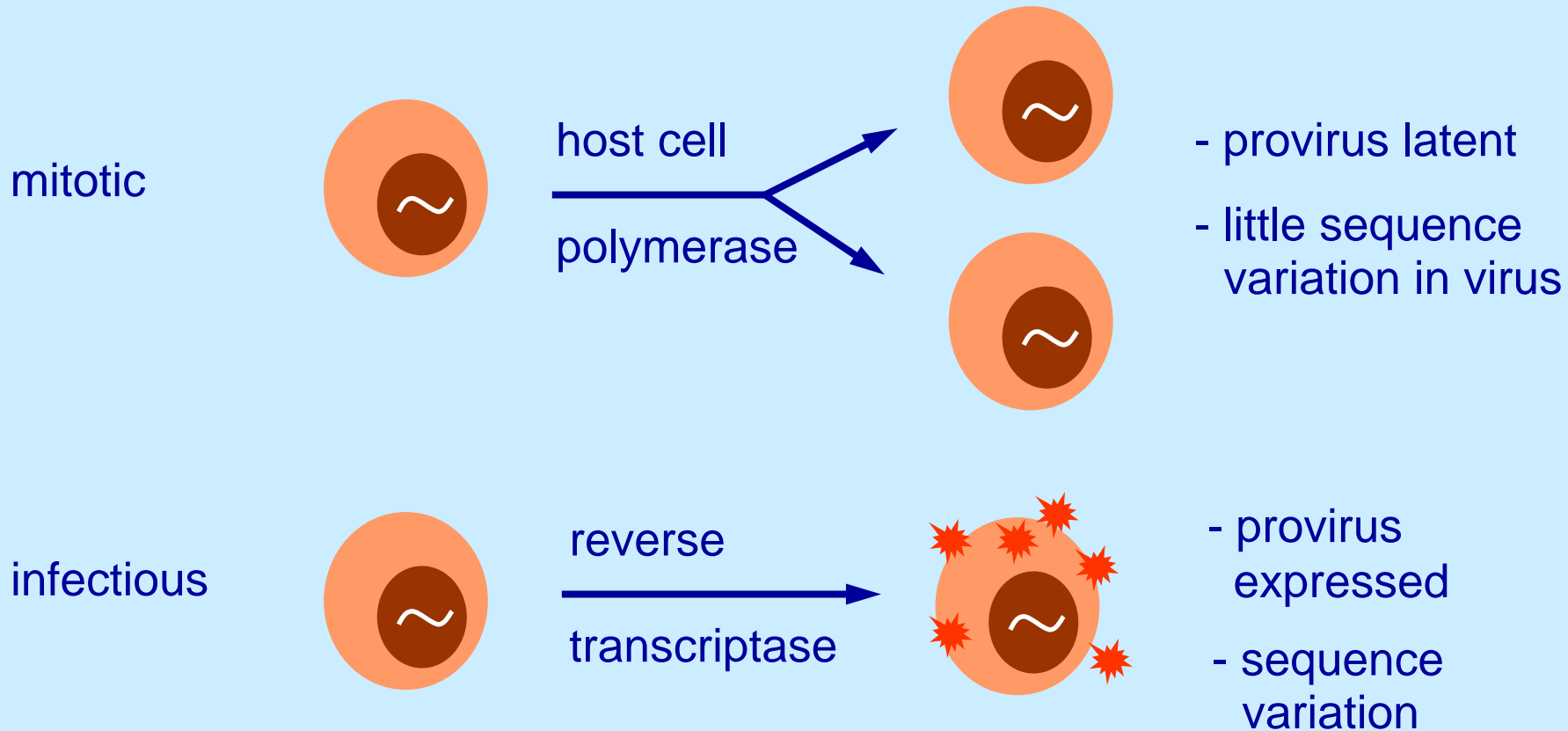
HAM/TSP: 5.4

asymptomatic: 0.34

Nagai et al 1998  
J. Neurovirol. **4**, 586

# How is the high proviral load maintained?

Retroviruses replicate by two routes:

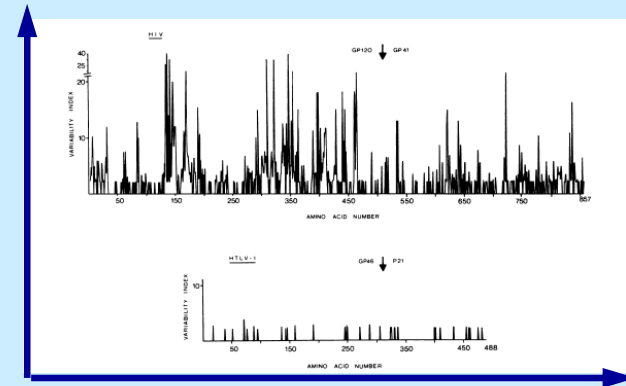


# *Evidence for latency of HTLV-1*

variability (Kabat-Wu)

Env

1. HTLV-1 varies little in sequence:



HIV-1

HTLV-1

amino acid position

Daenke et al. 1990: J. Virol. **64**, 1278

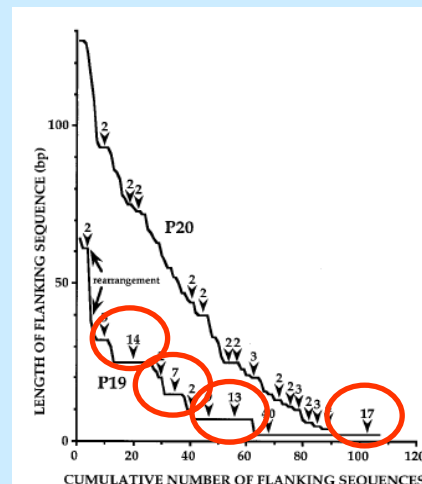
2. HTLV-1 mRNA and proteins are usually undetectable in PBMCs.
3. Virions are absent and plasma is non-infectious.

# 'Standard model' of HTLV-1 persistence

HTLV-1 is maintained by passive proliferation of provirus-containing lymphocytes.

A fraction of cells express HTLV-1, but too few to allow the immune response to make an impact on proviral load.

*Supported* by observation of large clones of HTLV-1<sup>+</sup> lymphocytes *in vivo*:



Wattel et al. 1995:  
J. Virol. **69**, 2863

# ***What is wrong with the 'standard model'?***

There is a strong T-cell and antibody response to HTLV-1.

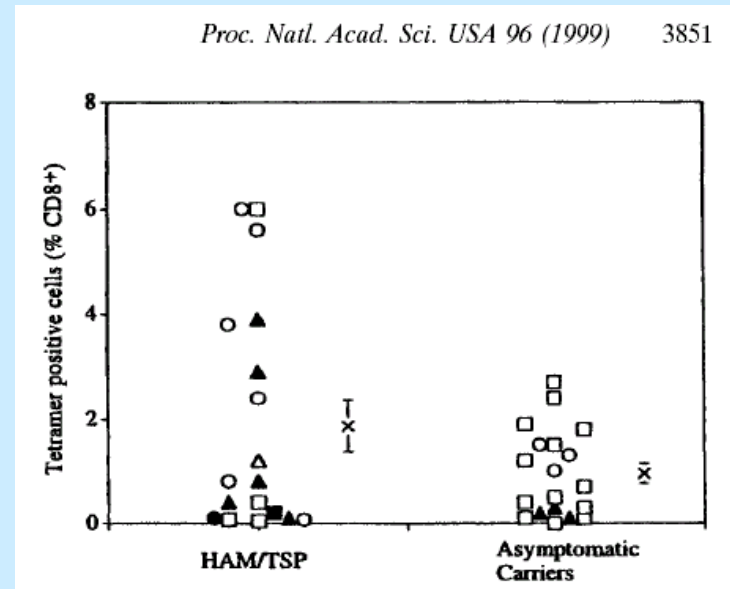
Anti-HTLV-1 cytotoxic T lymphocytes (CTLs) are chronically activated; virus-specific IgM is produced.

Passively proliferating HTLV-1<sup>+</sup> cells would be outgrown if *any* start to express HTLV-1: 1%/day → 40-fold drop in load over 1 year.

*- does the CTL response make any impact?*

# Anti-HTLV-1 CTLs are -

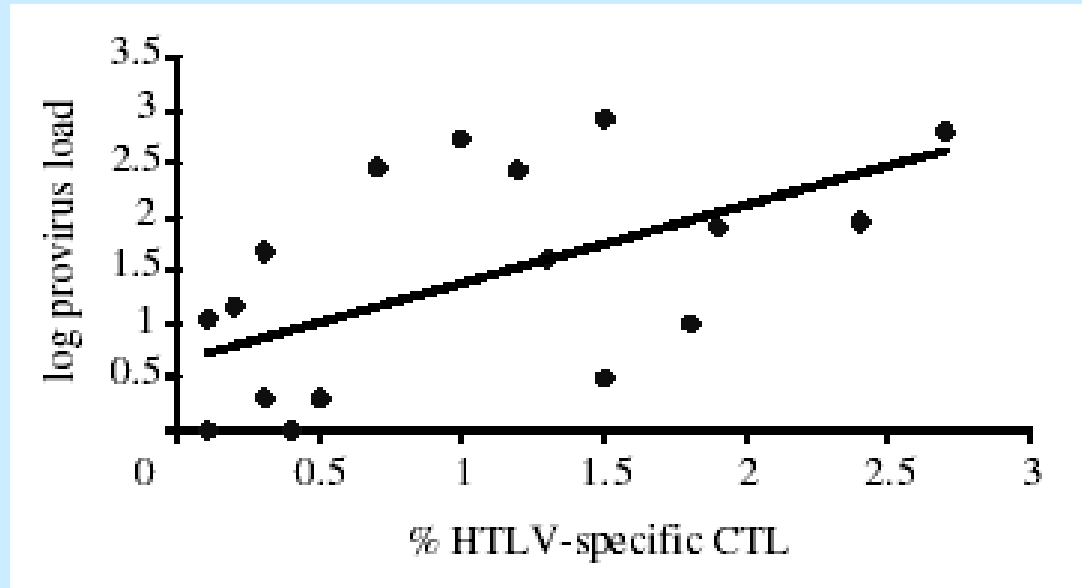
- abundant



- chronically activated
- chiefly directed against the HTLV-1 Tax protein

**but** CTL frequency, specificity and activation do not differ between HAM/TSP and asymptomatics

# *HTLV-1-specific CTL frequency is positively correlated with proviral load*



Kubota et al. 2000  
Wodarz et al. 2001

- so do CTLs determine load, or passively reflect the load?

# CTLs exert selection on HTLV-1 in vivo

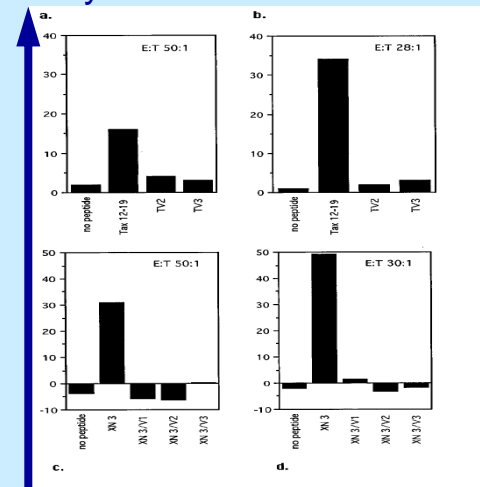
1. The *tax* gene is under positive selection in asymptomatic carriers but not in HAM/TSP patients:

$$D_N / D_S = \frac{\text{AC (N=4)}}{1.15} \quad \frac{\text{HAM (N=4)}}{0.42}$$

2. Naturally occurring Tax variants escape CTL recognition:

Niewiesk et al 1994: J. Virol. **68**, 6778  
Niewiesk et al 1995: J. Virol. **69**, 2649

% specific lysis

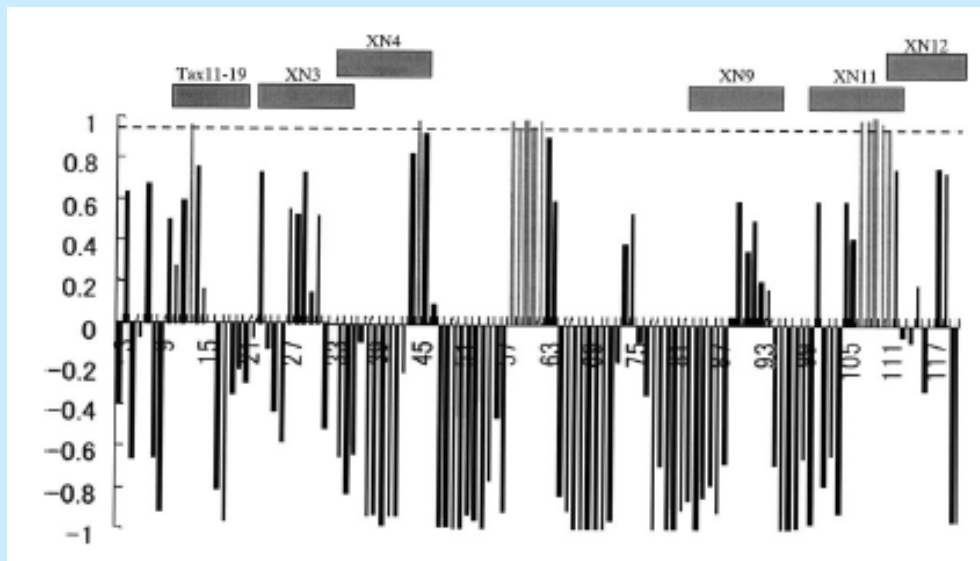


# Positive selection at individual Tax residues

## Genetic Stability of Human T Lymphotropic Virus Type I despite Antiviral Pressures by CTLs<sup>1</sup>

Ryuji Kubota,<sup>2\*</sup> Kousuke Hanada,<sup>§</sup> Yoshitaka Furukawa,<sup>†</sup> Kimiyoshi Arimura,<sup>‡</sup> Mitsuhiro Osame,<sup>‡</sup> Takashi Gojobori,<sup>§</sup> and Shuji Izumo<sup>\*</sup>

*The Journal of Immunology*, 2007, 178: 5966–5972.

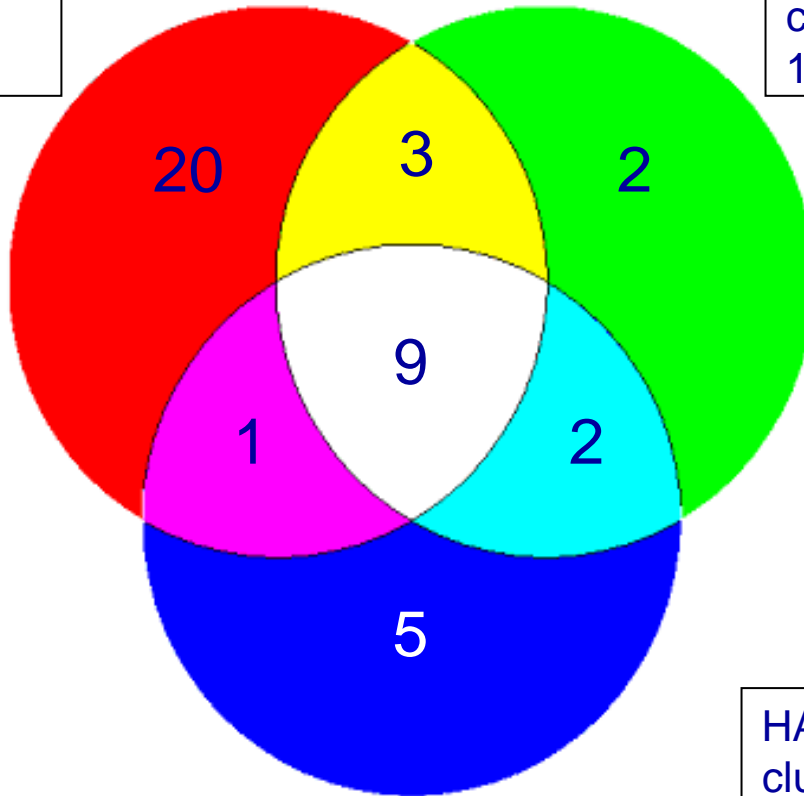


HLA-A2-  
restricted CTL  
epitopes

Positive  
selection at  
individual  
residues

# 9 genes were overexpressed in CD8<sup>+</sup> cells from individuals with a low HTLV-1 proviral load

AC1 CD8L  
cluster:  
33 genes



AC2 CD8L  
cluster:  
16 genes

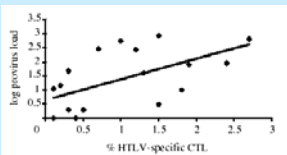
HAM CD8L  
cluster:  
17 genes

**A**  
 1 (AJ001687): NKG2D  
 2 (M57888): Granzyme B  
 3 (U20350): CX3CR1  
 4 (M30894): TCR-gamma  
 5 (M12824): CD8 A  
 6 (AF031824): Leukocystatin  
 7 (M18737): Granzyme A  
 8 (M85276): Granulysin (NKG5)  
 9 (S69115): NKG7

**B**  
 1 (X57352): IFITM3 (I-8U)

**C**  
 1 (U26174): Granzyme K  
 2 (M28393): Perforin

**D**  
 1 (M17016): Granzyme B precursor  
 2 (M1121): RANTES (1)  
 3 (M1121): RANTES (2)



# ***Protective role of HLA class 1 indicates that CTLs limit HTLV-1 expression in vivo***

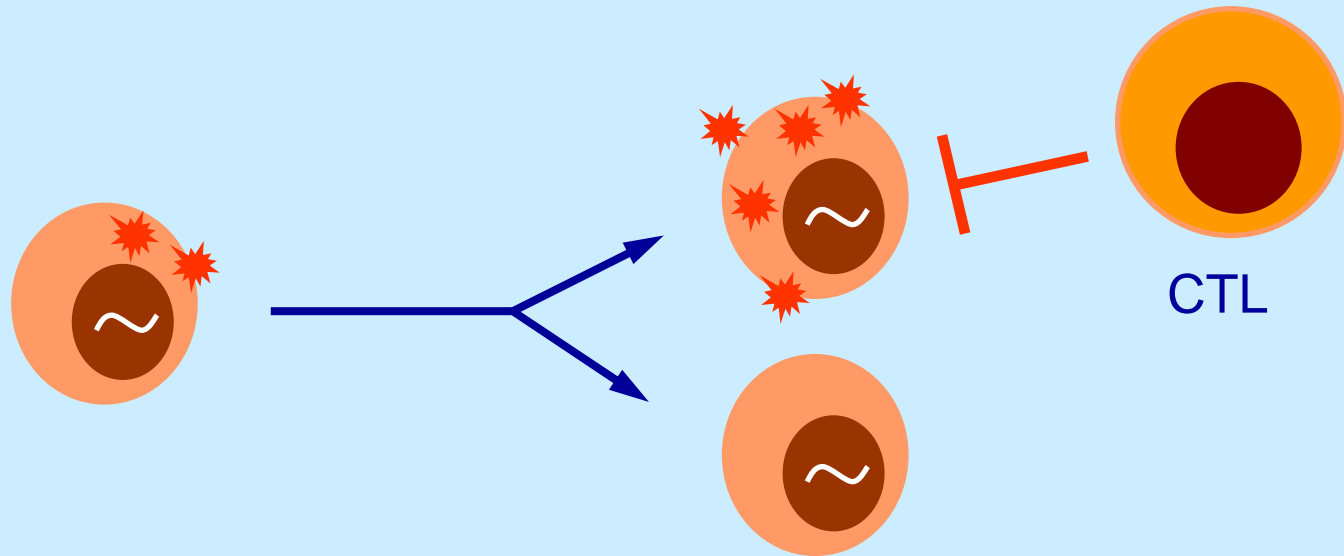
## **1. Possession of *either HLA-A\*02 or HLA-Cw\*08* :**

- reduced proviral load by 3-fold
- halved the odds of HAM/TSP

*HLA-A2 and HLA-Cw8 prevent 36% of potential HAM/TSP cases.*

## **2. HLA class 1 heterozygosity was associated with a lower proviral load.**

# *Alternative scheme of HTLV-1 persistence*



Spontaneously expressed Tax protein drives proliferation of provirus<sup>+</sup> cells.

CTLs kill virus-expressing cells.

A fraction of daughter cells survive by shutting down expression (how?)

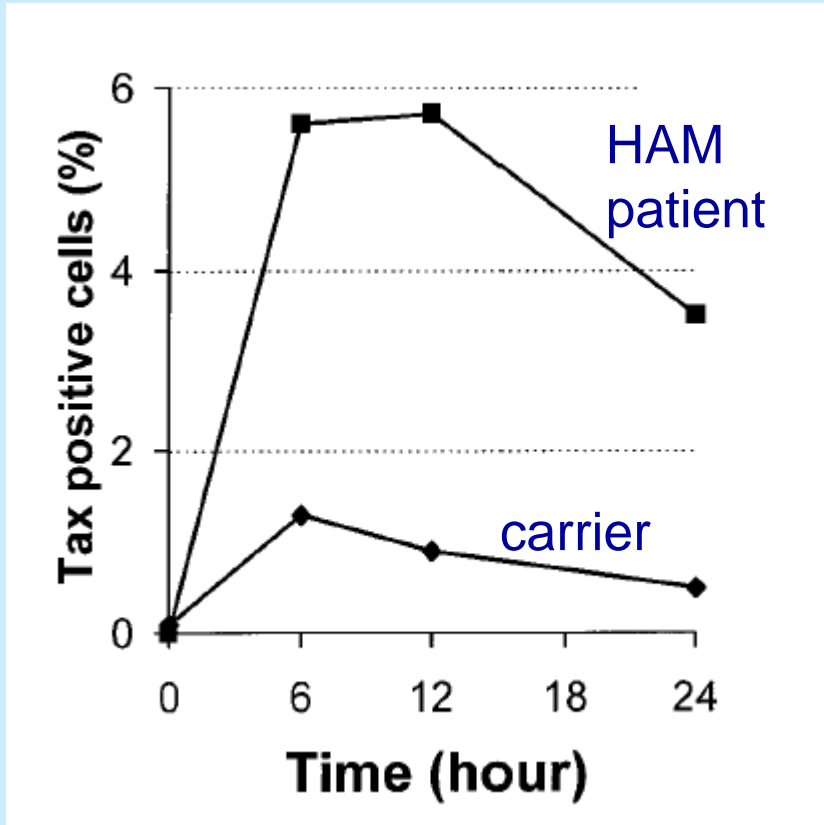
Proviral load is determined by an equilibrium between virus and CTLs : the chief determinant of load is the rate – the ‘efficiency’ – of CTL killing.

Nowak and Bangham 1996: **Science** 272, 74-79; Bangham and Osame 2005: **Oncogene** 24, 6035–6046.

## ***Predictions of model 2***

1. Proviral load is determined by a) CTL efficiency &  
b) rate of Tax expression.
2. Mean lymphocyte turnover rate a) is abnormally high in HTLV-1 infection, especially in HAM/TSP patients, and  
b) correlates with [Tax].
3. Proviral load correlates with Tax protein expression.
4. The advantage to the virus conferred by Tax expression diminishes as the CTL lysis rate increases.

# Quantification of anti-viral CTL efficiency



$$\frac{dy}{dt} = c - \varepsilon yz$$

$y$  = freq. of infected cells

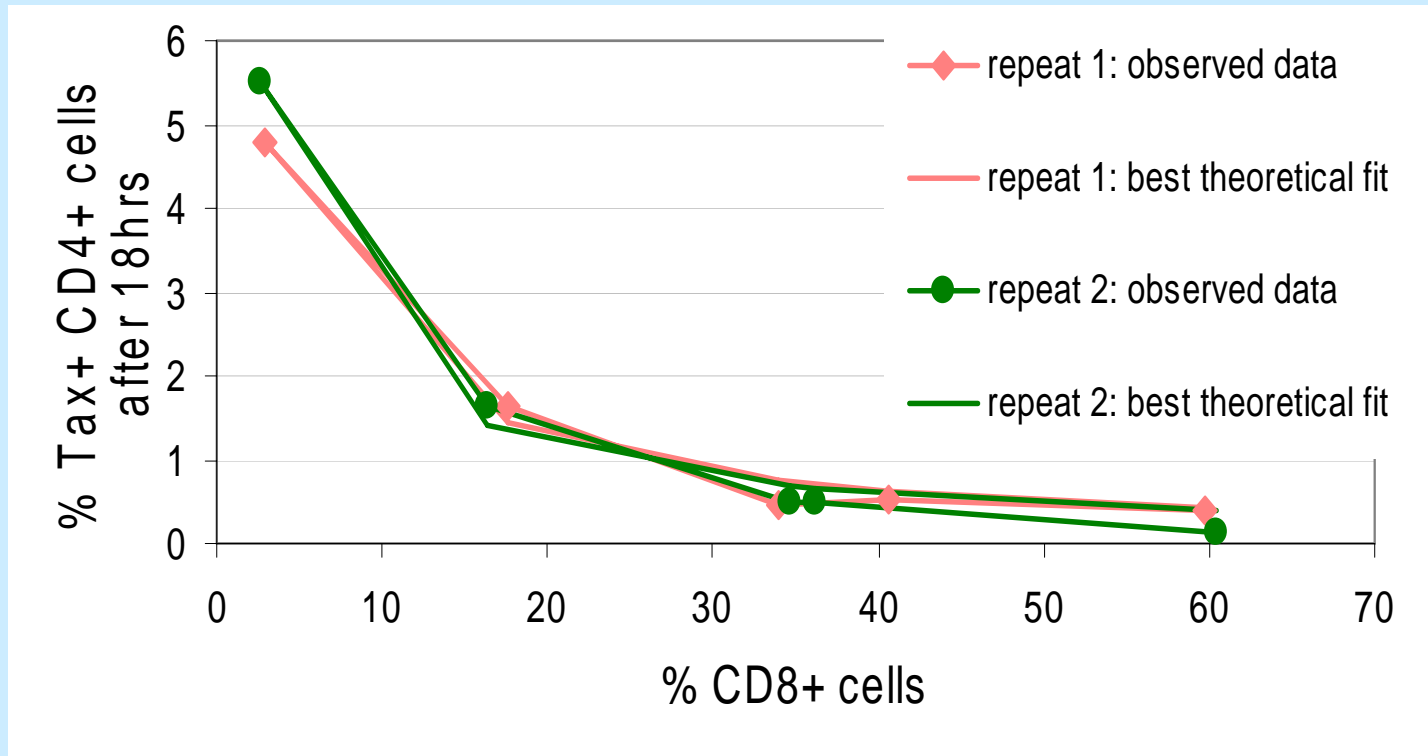
$z$  = freq. of CTLs

$\varepsilon$  = lysis rate constant - 'CTL efficiency'

$c$  = constant

Asquith, Mosley et al., 2005:  
J Gen Virol **86**, 1515

# Quantification of anti-viral CTL efficiency



$\epsilon$  is reduced:

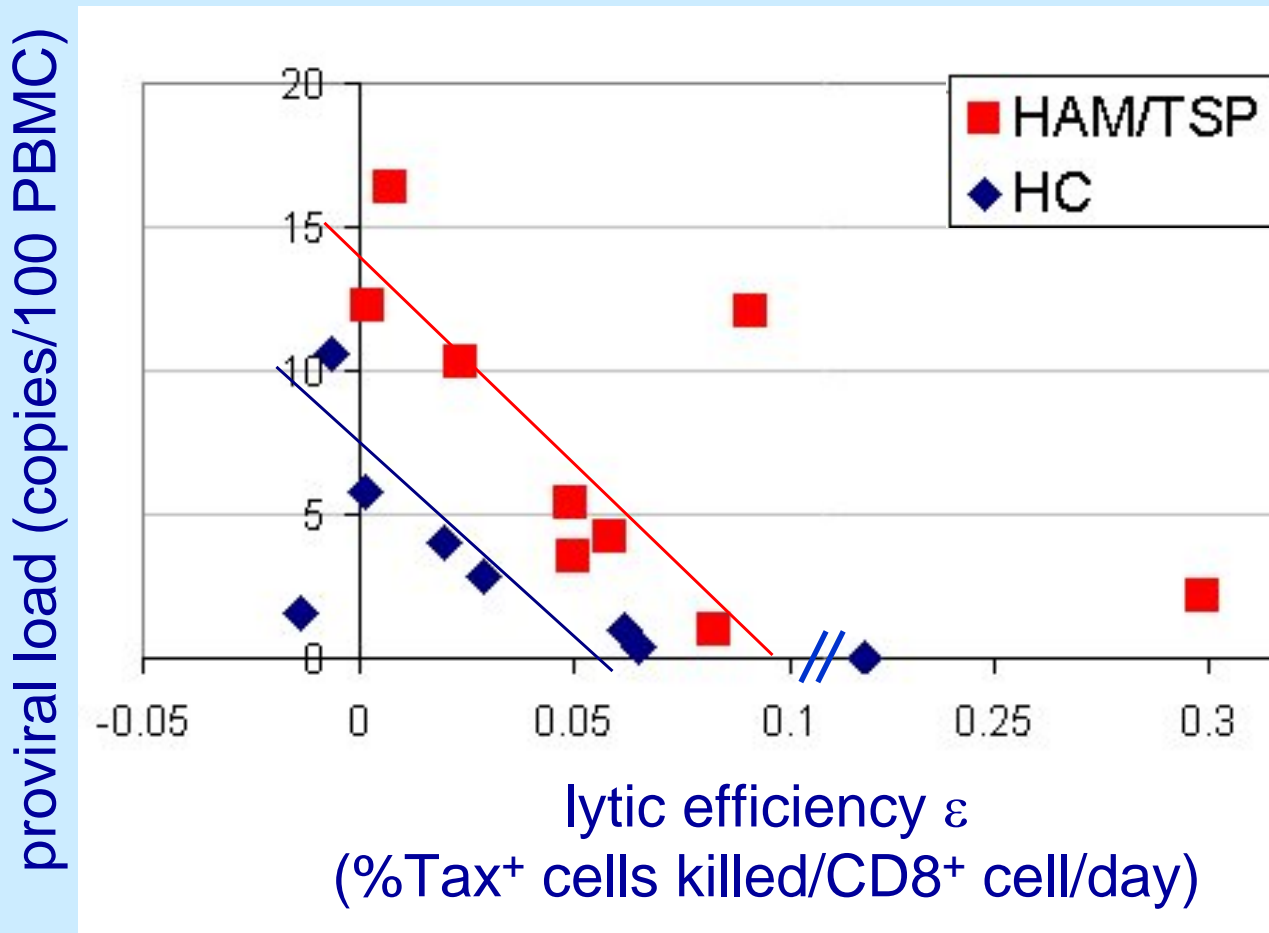
80% by block of perforin

29% by partial HLA mismatch

100% by full HLA mismatch

Asquith, Mosley et al., 2005  
J Gen Virol **86**, 1515

# Prediction 1: CTL lytic efficiency determines HTLV-1 proviral load in vivo



Asquith, Mosley et al.  
2005: J Gen Virol **86**,  
1515

**Conclusion:** 30% to 50% of observed variation in HTLV-1 proviral load is accounted for by variation in  $\epsilon$ .

# ***Impact of CTL activity in HTLV-1 infection***

1. The rate of CD8<sup>+</sup> cell-mediated lysis is an important determinant - perhaps the largest single determinant - of variation in HTLV-1 load between individuals.
2. In a typical infected individual, each CD8<sup>+</sup> cell kills  
~5 HTLV-1-infected cells/day.  
➔ turnover rate of Tax<sup>+</sup> cells of ~7% per day.  
i.e. total of ~2 x 10<sup>9</sup> infected CD4<sup>+</sup> cells killed/day.

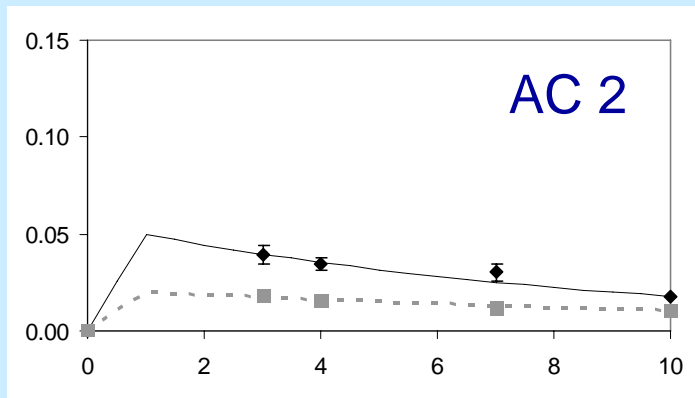
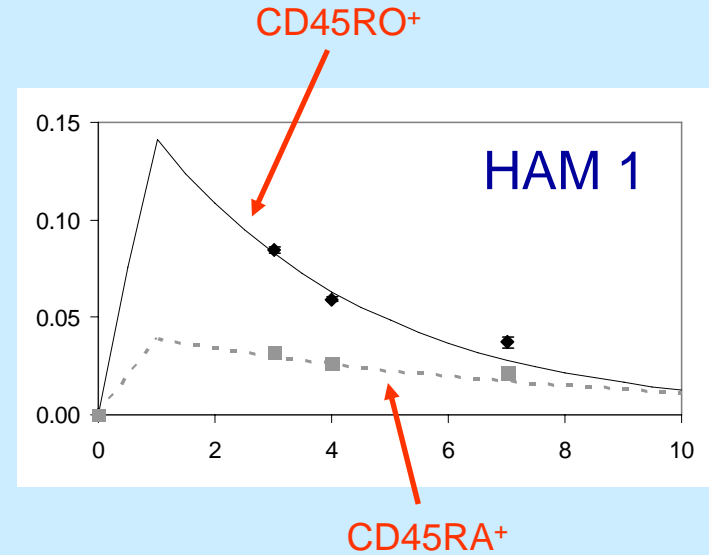
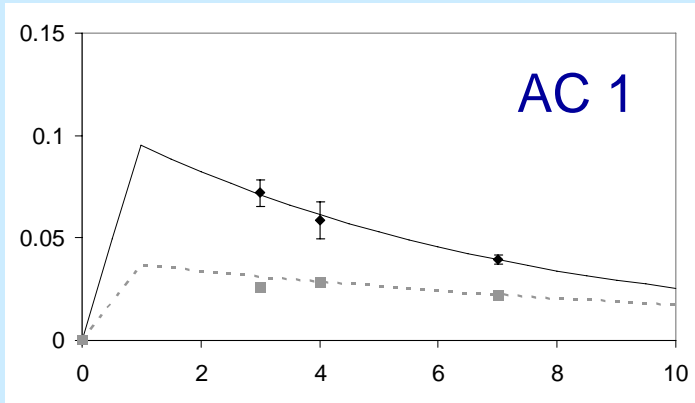
# *Measurement of lymphocyte turnover rates in vivo*

- infuse  $^2\text{H}$ -labelled glucose (5% ) i.v. overnight
- carbon ring is incorporated into newly synthesized nucleosides  $\longrightarrow$  genomic DNA of newly divided cells
- decay of  $^2\text{H}/^1\text{H}$  in DNA  $\longrightarrow$  direct estimate of  $t_{1/2}$  of specific (sorted) lymphocyte subsets

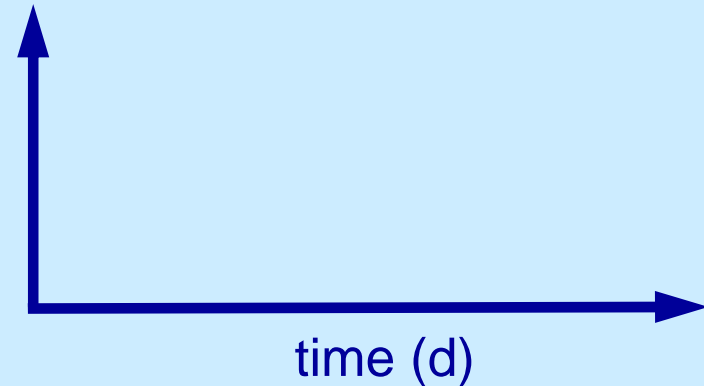
Macallan et al (1998) PNAS **95**, 708

Asquith et al (2002) Trends Immunol. **23**, 596

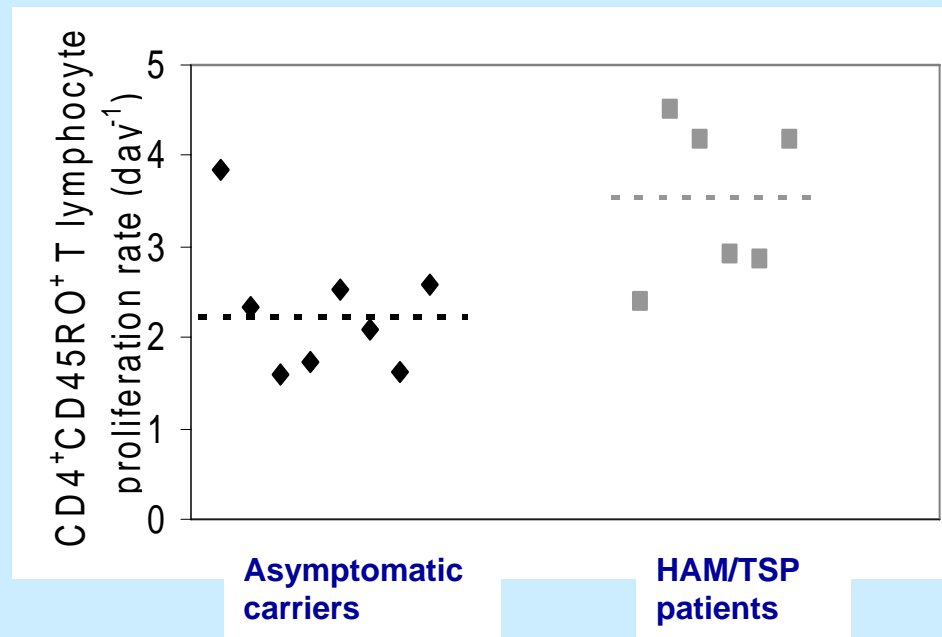
# $^2\text{H}$ -glucose kinetics in vivo: $\text{CD45RO}^+$ cells turn over faster than $\text{CD45RA}^+$



$^2\text{H}$  incorporation

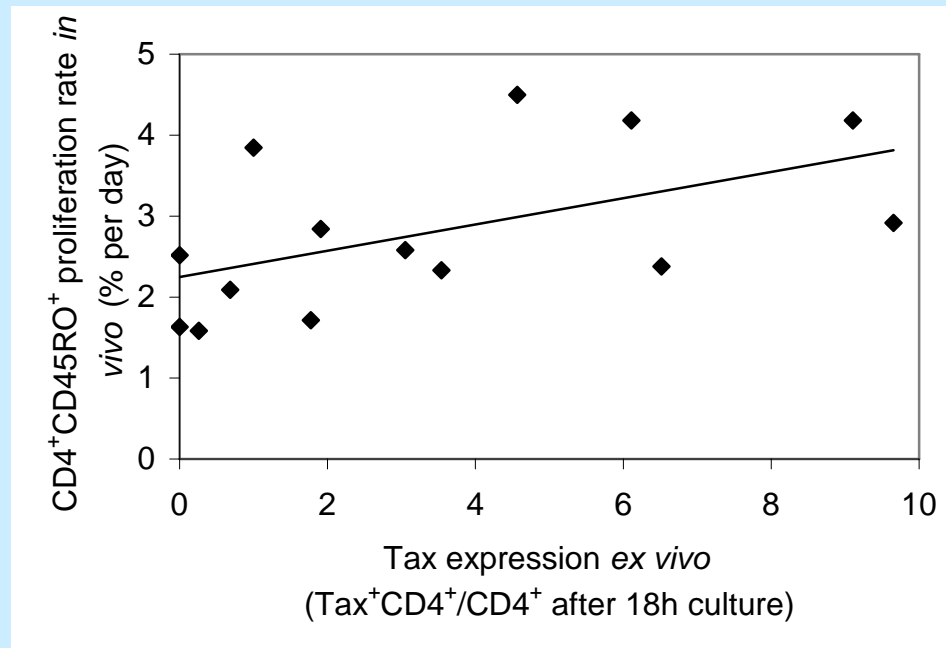


# Prediction 2a: mean proliferation rate of CD4<sup>+</sup> T cells in HAM/TSP > asymptomatic carriers



p = 0.01  
(Mann-Whitney,  
2-tailed)

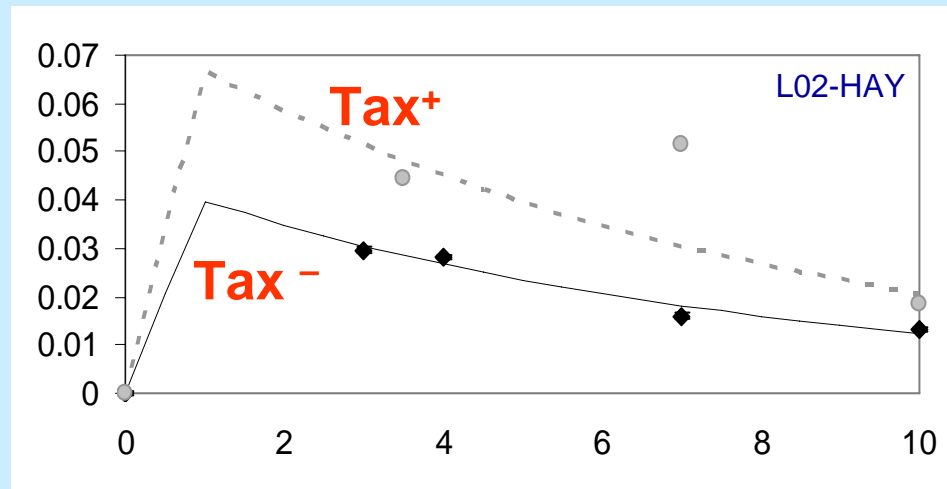
# *In vivo* turnover rate of CD4<sup>+</sup>CD45RO<sup>+</sup> cells correlates with Tax expression ex vivo



P = 0.016  
(Spearman rank correlation)

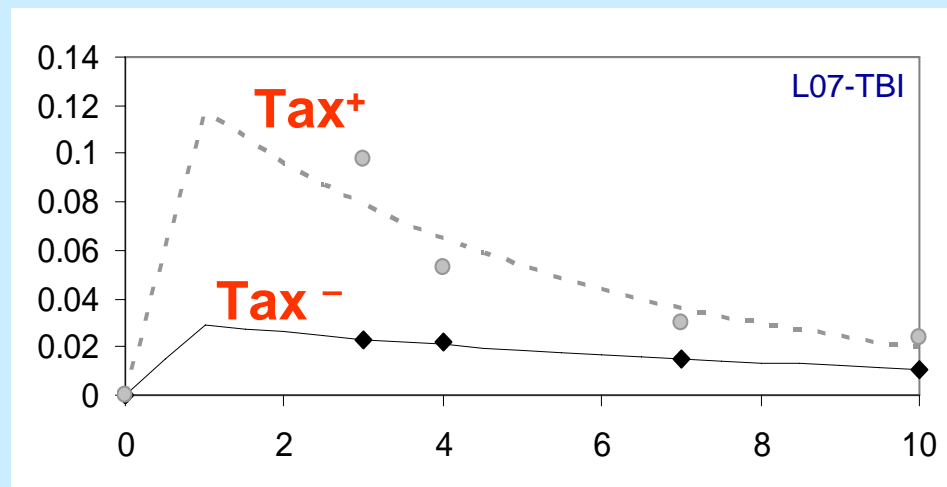
# Prediction 2b:

## Tax-expressing cells turn over faster than Tax - cells in vivo



turnover rate:

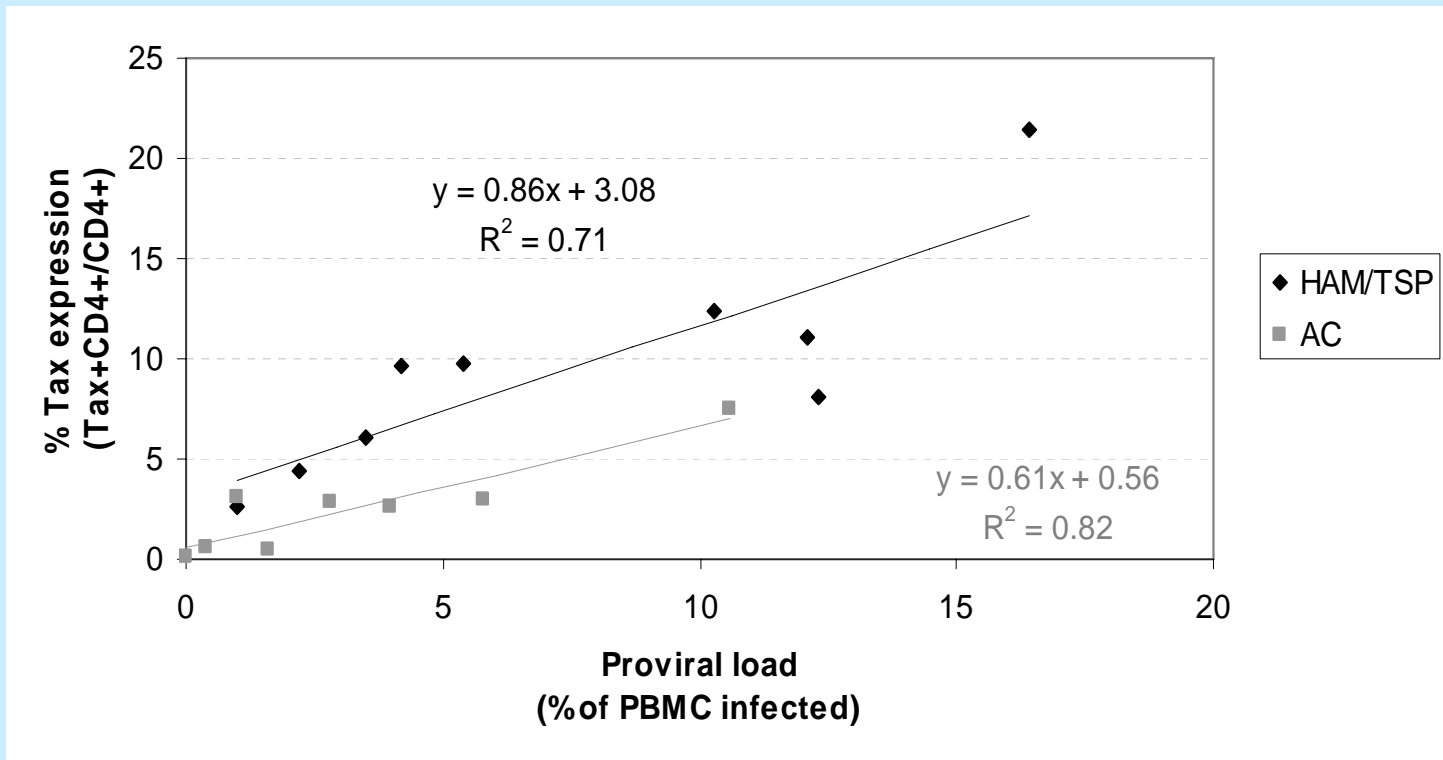
7% per day



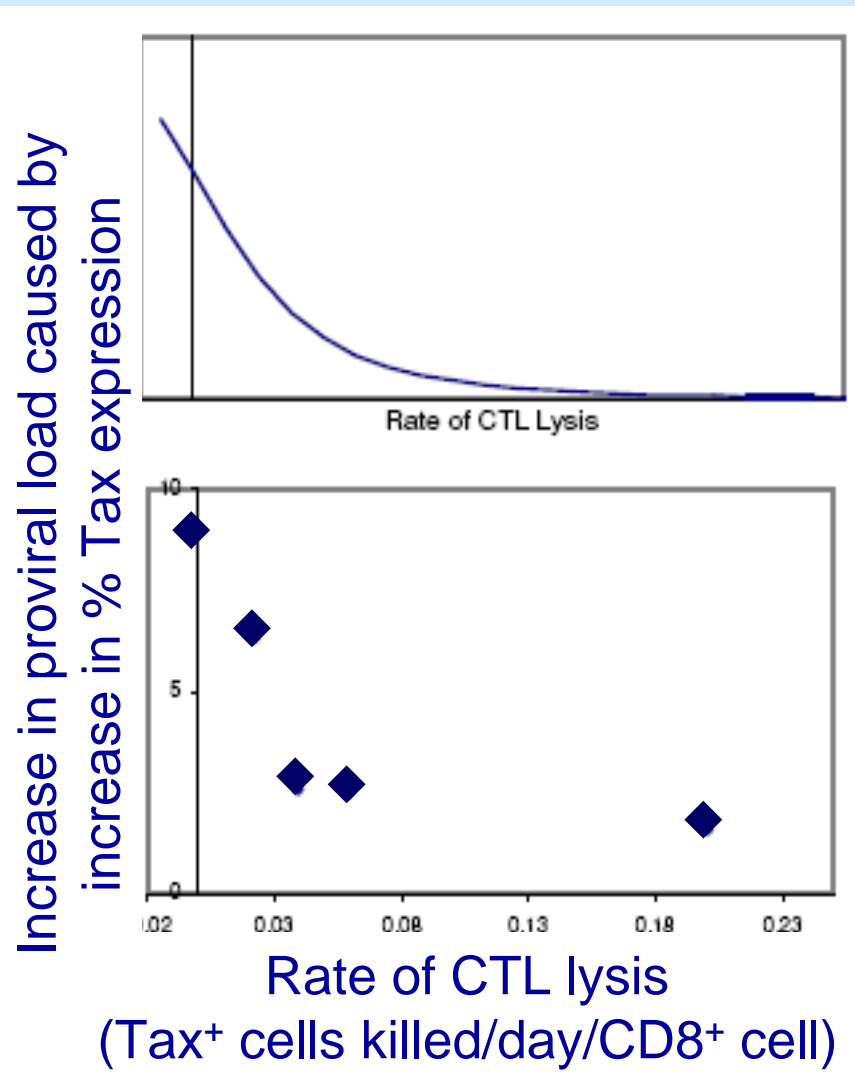
13% per day

Asquith et al. 2007  
PNAS **104**, 8035-8040

# Prediction 3: proviral load correlates with Tax expression



# ***Prediction 4: advantage conferred by Tax expression falls as CTL lysis rate increases***

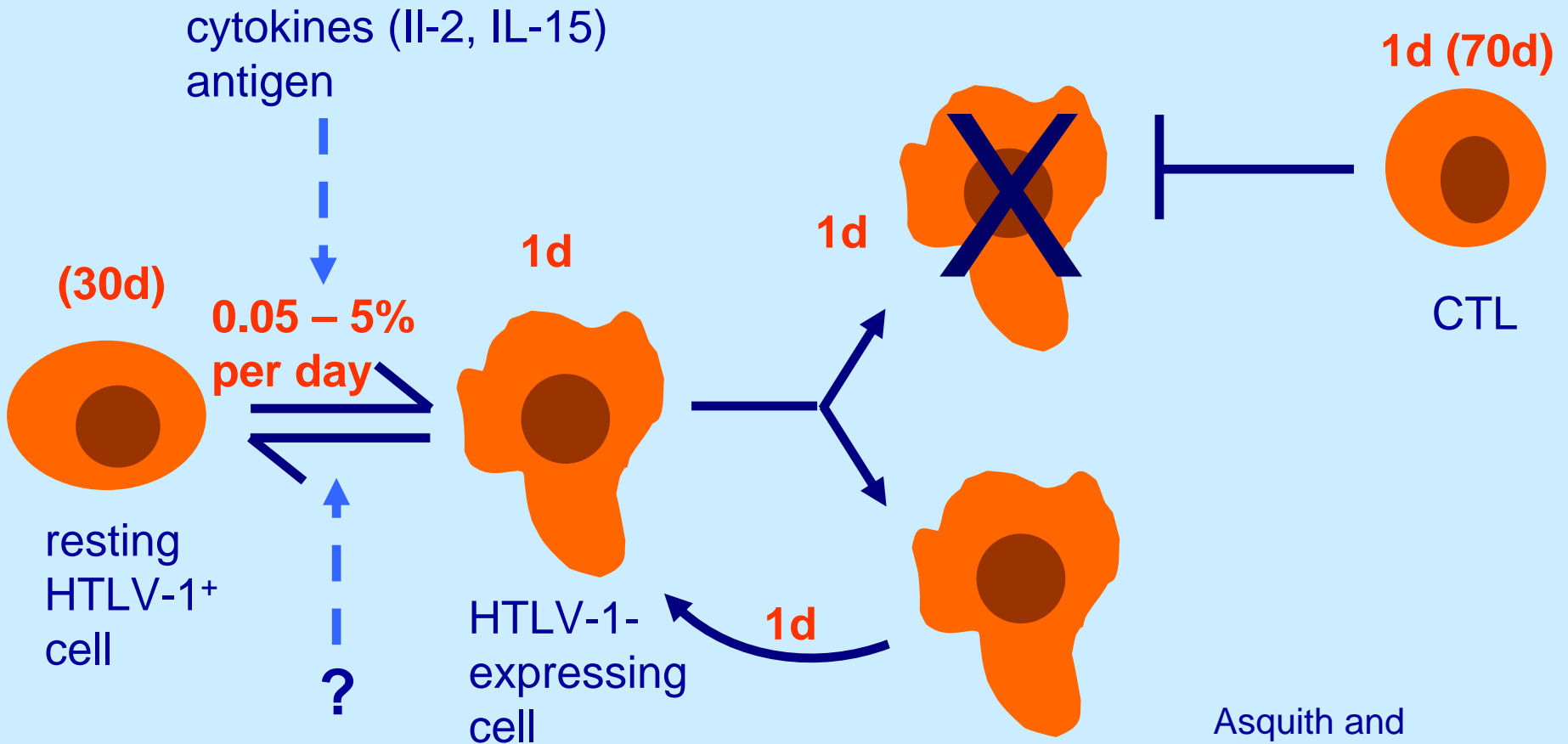


model prediction

experimental  
results

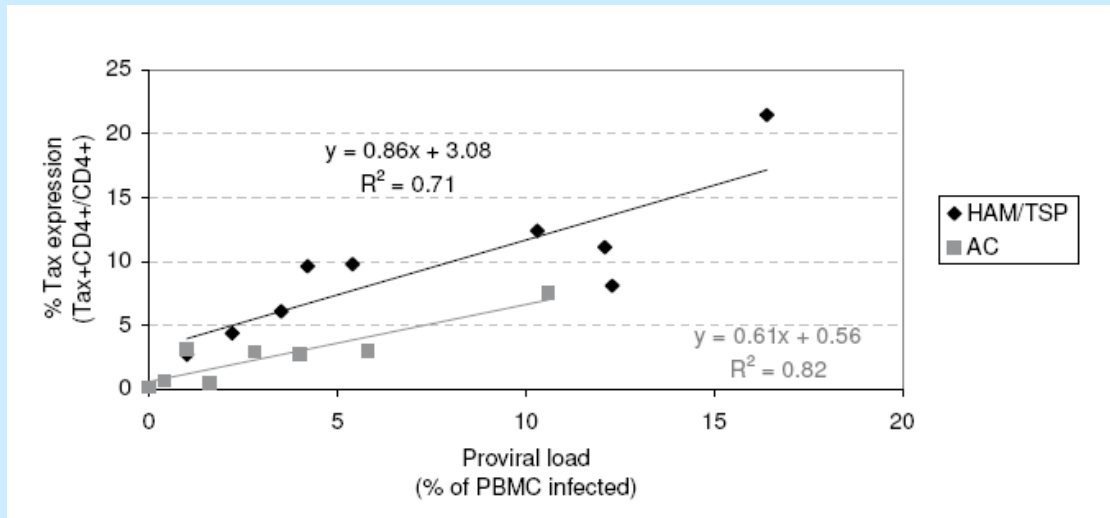
Asquith et al. 2005: Retrovirology 2, 75

# Quantification of HTLV-1 infection dynamics *in vivo*



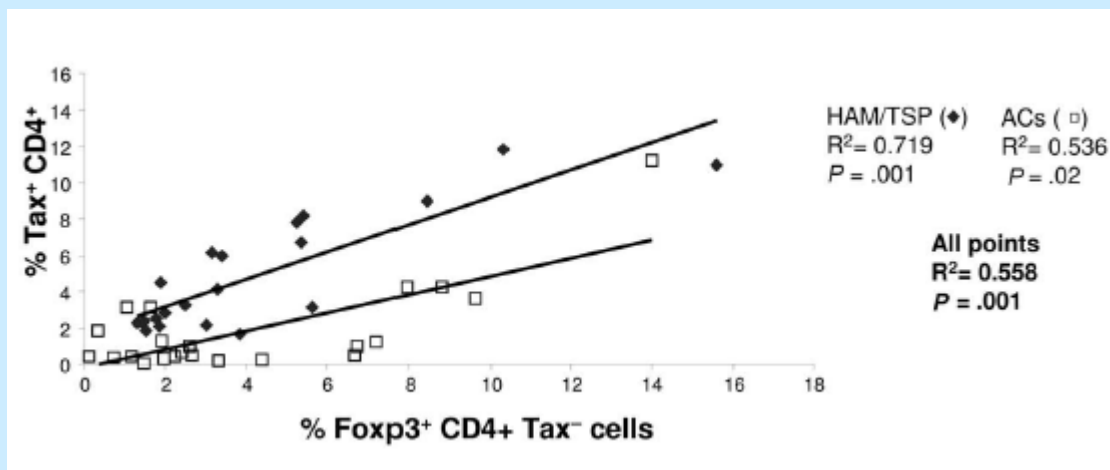
Asquith and  
Bangham 2008:  
Trends Immunol.  
29, 4-11

# Tax expression in HAM/TSP > carriers at a given proviral load



Asquith et al.  
2005:  
Retrovirology  
2, 75-83

- and a given  
FoxP3+  
frequency



Toulza et al.  
2008: Blood  
111, 5047-5053

# ***What determines the rate of HTLV-1 proviral expression in vivo?***

- strain (sequence) of virus? No.
- proportion of defective proviruses? Unknown.
- T cell activation (ag, cytokines)?  
Unlikely to explain observed between-individual variation.
- HTLV-1 regulatory products (p30<sup>II</sup>, Rex, HBZ)  
Limit existing proviral expression, but do not control onset.
- *epigenetic changes?*
- *genomic integration site?*

# *Is HTLV-1 integration random?*

Linker-mediated PCR

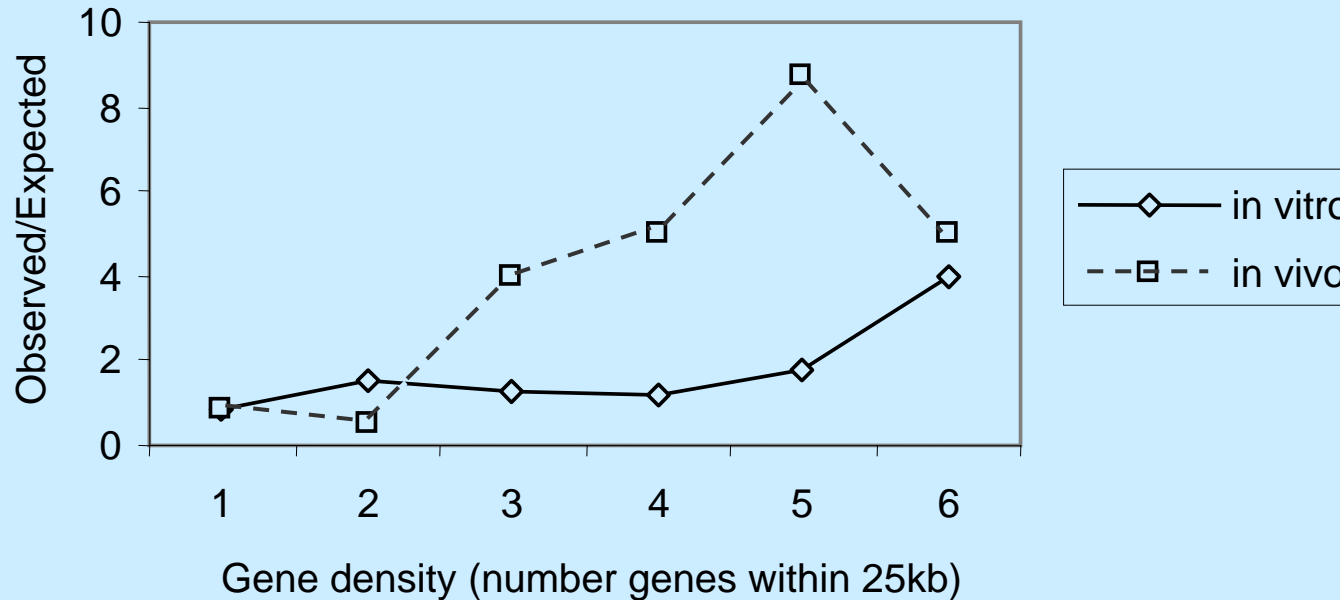
PBMCs taken from 10 HAM patients + 10 ACs

311 genomic integration sites mapped

Observed integration sites compared with random NlaIII sites in genome

Kiran Meekings, 2008: PLoS Pathogens **4(3)**: e1000027 .  
Statistical analysis carried out in collaboration with Rick Bushman,  
Jeremy Leipzig, Chuck Berry

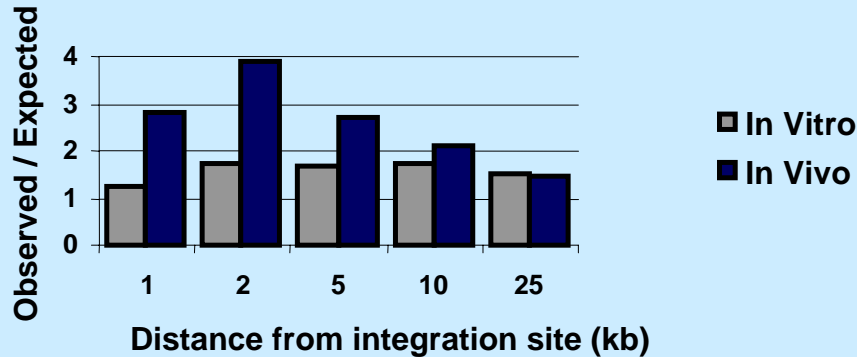
# *HTLV-1 integration frequency in vivo correlates with gene density*



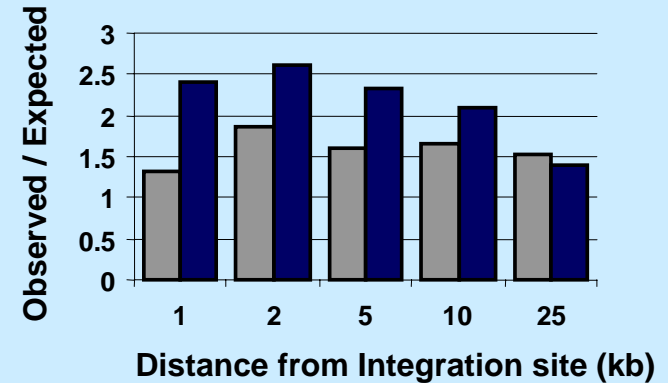
$p = 1.8 \times 10^{-5}$  (logistic regression)

Meekings et al. 2008:  
PLoS Pathogens  
**4(3):** e1000027

# HTLV-1 proviral integration in vivo predominates:

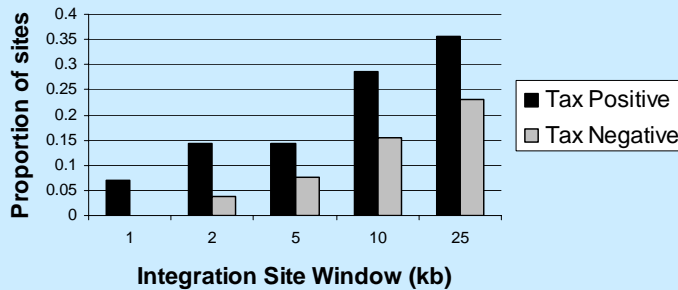


- near CpG islands

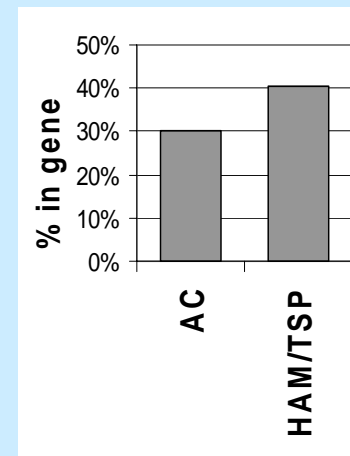


- near transcription start sites

- and is associated with



- Tax expression



- and with HAM-TSP

Meekings et al.  
2008: PLoS  
Pathogens  
4(3): e1000027

***Rate of proviral expression & rate of CTL lysis determine HTLV-1 load and risk of HAM/TSP***

<b>HTLV-1 proviral expression</b>	<b>fast</b>	HAM moderate load	HAM high load
	<b>slow</b>	AC low load	AC moderate load
		<b>fast</b>	<b>slow</b>
		<b>CTL lysis</b>	

# Conclusion: persistence of HTLV-1 in vivo

