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:

- **mitotic**
  - provirus latent
  - little sequence variation in virus
  - host cell polymerase

- **infectious**
  - provirus expressed
  - sequence variation
  - reverse transcriptase
Evidence for latency of HTLV-1

1. HTLV-1 varies little in sequence:

2. HTLV-1 mRNA and proteins are usually undetectable in PBMCs.

3. Virions are absent and plasma is non-infectious.

Daenke et al. 1990: J. Virol. 64, 1278
‘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+ 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+ cells would be outgrown if any start to express HTLV-1: 1%/day $\rightarrow$ 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

Bangham 2000 Curr Opin Immunol 12, 397
HTLV-1-specific CTL frequency is positively correlated with proviral load

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

Kubota et al. 2000
Wodarz et al. 2001
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:

\[
\frac{D_N}{D_S} = 1.15 \quad 0.42
\]

2. Naturally occurring Tax variants escape CTL recognition:

Niewiesk et al 1994: J. Virol. 68, 6778
Niewiesk et al 1995: J. Virol. 69, 2649
Positive selection at individual Tax residues

Genetic Stability of Human T Lymphotrophic Virus Type I
despite Antiviral Pressures by CTLs

Ryuji Kubota, Kousuke Hanada, Yoshitaka Furukawa, Kimiyoshi Arimura,
Mitsuhiro Osame, Takashi Gojobori, and Shuji Izumo


HLA-A2-restricted CTL epitopes

Positive selection at individual residues
9 genes were overexpressed in CD8+ 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)

Vine, Heaps et al., 2004: J Immunol 173, 5121
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

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

Spontaneously expressed Tax protein drives proliferation of provirus$^+$ 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.


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

\[ \frac{dy}{dt} = c - \varepsilon y z \]

- \( y \) = 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

ε 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

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

Asquith, Mosley et al. 2005: J Gen Virol 86, 1515
**Impact of CTL activity in HTLV-1 infection**

1. The rate of CD8$^+$ 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$^+$ cell kills
   ~5 HTLV-1-infected cells/day.
   turnover rate of Tax$^+$ cells of ~7% per day.
   i.e. total of ~2 x 10$^9$ infected CD4$^+$ cells killed/day.
Measurement of lymphocyte turnover rates in vivo

- infuse $^{2}$H-labelled glucose (5%) i.v. overnight

- carbon ring is incorporated into newly synthesized nucleosides $\rightarrow$ genomic DNA of newly divided cells

- decay of $^{2}$H/$^{1}$H in DNA $\rightarrow$ direct estimate of $t_{1/2}$ of specific (sorted) lymphocyte subsets

Macallan et al (1998) PNAS 95, 708
$^2$H-glucose kinetics in vivo:
CD45RO$^+$ cells turn over faster than CD45RA$^+$

Asquith et al. 2006: submitted
Prediction 2a: mean proliferation rate of CD4⁺ T cells in HAM/TSP > asymptomatic carriers

Asquith et al. 2007
PNAS 104, 8035-8040

p = 0.01
(Mann-Whitney, 2-tailed)
In vivo turnover rate of CD4⁺CD45RO⁺ cells correlates with Tax expression ex vivo

![Graph showing correlation between CD4⁺CD45RO⁺ proliferation rate in vivo and Tax expression ex vivo.](https://example.com/graph.png)

\[ P = 0.016 \] (Spearman rank correlation)

Asquith et al. 2007
PNAS 104, 8035-8040
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

\[ y = 0.86x + 3.08 \]

\[ R^2 = 0.71 \]

\[ y = 0.61x + 0.56 \]

\[ R^2 = 0.82 \]

Asquith et al. 2005: Retrovirology 2, 75
Prediction 4: advantage conferred by Tax expression falls as CTL lysis rate increases

Asquith et al. 2005: Retrovirology 2, 75
Quantification of HTLV-1 infection dynamics in vivo

resting HTLV-1+ cell

HTLV-1-expressing cell

0.05 – 5% per day

1d

(30d)

cytokines (II-2, IL-15) antigen

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\textsuperscript{II}, 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

HTLV-1 integration frequency in vivo correlates with gene density

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

HTLV-1 proviral integration in vivo predominates:

- near CpG islands

- and is associated with

- Tax expression

- and with HAM-TSP

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

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<tr>
<th>HTLV-1 proviral expression</th>
<th>CTL lysis</th>
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<td>fast</td>
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<td>slow</td>
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<th>HAM</th>
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<td>moderate load</td>
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Conclusion: persistence of HTLV-1 in vivo

- Proviral expression
- Integration into transcriptionally active units
- Histone deacetylation
- p12\text{I}
- CD4\textsuperscript{+}FoxP3\textsuperscript{+} cells
- CTLs
- Infectious spread (viral synapse)
- T-cell proliferation
- p30, Rex, HBZ