How does HTLV-1 cause malignant and inflammatory diseases?

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Infects 10-20 million. Transmitted by sexual contact, breastfeeding and blood transfusion

>90% remain asymptomatic:

- 1-5% develop adult T-cell leukaemia/lymphoma (ATL)
- 4% develop a chronic inflammatory disease

Unlike HIV-1, no satisfactory treatment exists for the malignant or inflammatory diseases

Human T-lymphotropic virus type 1 (HTLV-1)
Adult T-cell leukaemia (ATL)

Frequent cutaneous involvement

Therapies now under trial:
- AZT + IFN-α (As₂O₃)
- anti-CCR4
- HSCT
HTLV-I-induced inflammatory diseases

- spasticity/weakness of the legs
- hyperreflexia
- bladder dysfunction
- lumbar pain
- constipation
- impotence

**also:**
- hepatitis
- myositis
- arthritis
- uveitis
- thyroiditis
- infective dermatitis
- bronchiectasis
- alveolitis

HTLV-1-associated myelopathy/
tropical spastic paraparesis
(HAM/TSP)
Infective dermatitis

Scaly-erythematous lesions with crusts in the scalp, ear, eyebrows, nose, and perioral area. Crusting of the anterior nares.
Two main questions in HTLV-1 infection

– what determines the risk of inflammatory diseases (HAM/TSP*) and malignant diseases (ATL)?

– how does HTLV-1 persist in the host?

* HAM/TSP = HTLV-1-associated myelopathy/tropical spastic paraparesis
The proviral load of HTLV-1 correlates with the risk of leukaemia & inflammatory diseases.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Proviral Load (copies/100 PBMCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAM/TSP</td>
<td>5.4</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0.34</td>
</tr>
<tr>
<td>(HIV: &lt;0.1%)</td>
<td></td>
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</table>

Jeffery et al, 1999, PNAS

What limits the proviral load?
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.

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

CTL quality determines proviral load and disease risk

High CTL quality (→ low viral load & disease risk):

- **HLA-A*02**
- **HLA-C*08**
- **KIR2DL2**

Host genotype

CTL

HTLV-1+

FoxP3+

- high antigen avidity
- high granzyme mRNA
- HBZ-specific

References:

1. Jeffery et al 1999, PNAS
6. Toulza et al 2008, Blood
Which cells carry HTLV-I in vivo?

1. HTLV-1 infects both CD4$^+$ and CD8$^+$ T cells and induces CD8$^+$ T-cell fratricide

   - Hanon et al. 2000: Immunity

2. HTLV-1-specific T-cells are preferentially infected:
   - and CD8$^+$: Hanon et al. 2000: Immunity

   antigen recognition appears to promote cell-cell spread of HTLV-1

Hypothesis:

HTLV-1 spreads from cell to cell via the immunological synapse
HTLV-1 spread is triggered by cell-cell contact – the virological synapse

Gag protein complexes (red) polarize to the cell-cell contact area - which contains organized adhesion domains (green)

Gag is then transferred with the HTLV-1 genome to the target cell

Igakura et al 2003: Science 299, 1713
How is the high proviral load maintained?

Retroviruses replicate by two routes:

**Infectious route**
- provirus expressed
- sequence variation

**Mitotic route**
- provirus latent
- little sequence variation in virus

Directional cell-cell spread via virological synapse is triggered by cell contact

Genomic Southern blot
- oligoclonal
- polyclonal
- HAM
- AC
- HAM
**Is HTLV-1 latent in vivo?**

Virions, viral mRNA and proteins usually undetectable in fresh PBMCs

HTLV-1 varies little in sequence, implying that RT contributes little

Typically 1 to 10 clones of HTLV-1\(^+\) T cells are detected on genomic southern blot:

- oligoclonal proliferation maintains the high load, especially in HAM/TSP
- *but* the chronically activated CTL response implies that the provirus is expressed in vivo
  - what regulates proviral latency/expression, and so clonality?
Hypothesis

Genomic integration site regulates switch between proviral latency ↔ expression and so determines each T-cell clone’s

• abundance
• pathogenicity (inflammatory potential)
• risk of malignant transformation ➔ leukaemia
High-throughput mapping and quantification of proviral integration sites

random DNA shearing by sonication

HTLV-1

integration site

host

shear site

same integration site 4 distinct shear sites = 4 different cells in clone

• integration site identifies the HTLV-1+ T-cell clone
• amplicon length (shear site) identifies an individual cell of that clone
∴ clone abundance can be quantified

Gillet et al. 2011, Blood
Proviral integration: quantification in one subject (1 µg DNA)

- 3 major clones represent 92.5% of all infected cells
  - 04_2896318
  - 17_22083293
  - 07_137243904

- 106 other clones represent 7.5% of all infected cells
1. How many provirus copies/cell?

Method

- CD4^+ HTLV-1^+ T-cell clones (N = 28) isolated by limiting dilution
- Integrase inhibitor (raltegravir) added, to minimize secondary infection
- HTLV-1 proviral integration sites mapped & quantified

Results

- 99.9% of proviruses map to same genomic location
- 0.1% of proviruses in new sites – probably secondary infection in vitro

Conclusion: Most HTLV-1-infected cells carry a single provirus in vivo

Cook et al. 2012, Blood
2. How many HTLV-1\(^+\) clones in one host?

Previous estimates:

\~ 100 in a typical AC or HAM patient

\~ 1 in a patient with ATLL

High-throughput mapping: 200 to 5500 clones in 10\(\mu\)g DNA
How many HTLV-1+ T-cell clones in one host?

Number of clones

observed (in ≤10µg DNA) 200 to 3500

estimated total in circulation (DivE) 10^3 to 10^6 median ~ 10,000

Quantification of clonality

Clonal distribution - Patient 1

Number of cells seen in each clone

Number of cells

Clones in descending order of size

Gini (oligoclonality) index = A/(A+B)

Oligoclonality index

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Oligoclonality index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal</td>
<td>1.0</td>
</tr>
<tr>
<td>Polyclonal</td>
<td>0.0</td>
</tr>
</tbody>
</table>
3. Does HTLV-1 clonality correlate with disease?

<table>
<thead>
<tr>
<th></th>
<th>Asymptomatic carrier</th>
<th>HAM/TSP</th>
<th>Smouldering leukemia</th>
<th>Lymphoma (lymph node)</th>
<th>Chronic leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td># clones*</td>
<td>471</td>
<td>1442</td>
<td>234</td>
<td>5</td>
<td>269</td>
</tr>
<tr>
<td>Oligoclonality index</td>
<td>0.48</td>
<td>0.43</td>
<td>0.79</td>
<td>0.85</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Gillet et al. 2011, Blood
HTLV-1 oligoclonality rises progressively in vivo

Gillet et al. 2011, Blood
Proviral load – and so the disease risk – is correlated with total number of clones - but not with oligoclonality

P < 0.001 (HAM/TSP) (Spearman)

Conclusion:
oligoclonal proliferation per se is independent of disease risk

Gillet et al. 2011, Blood
Cook et al 2014, Blood
Rapid emergence of acute ATLL (patient TDC)

Feb 2009

PBMCs

0.04%

Relative abundance of putative malignant clone (red)

Bangham et al 2013
## Conclusions: clonality

<table>
<thead>
<tr>
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<th>Previous belief</th>
<th>New conclusion</th>
</tr>
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<tbody>
<tr>
<td><strong>Total clone number</strong></td>
<td>~100</td>
<td>$10^4$ to $10^5$</td>
</tr>
<tr>
<td><strong>HAM/TSP</strong></td>
<td>associated with oligoclonal proliferation</td>
<td>associated with greater <em>number</em> of clones</td>
</tr>
<tr>
<td><strong>Targeting of integration</strong></td>
<td>random</td>
<td>targets specific transcription factor binding sites</td>
</tr>
<tr>
<td><strong>Proviral copy no.</strong></td>
<td>multiple</td>
<td>one copy/cell</td>
</tr>
<tr>
<td><strong>Oligoclonal proliferation</strong></td>
<td>contributes to persistence and to HAM/TSP and ATL</td>
<td>no pathogenetic significance</td>
</tr>
</tbody>
</table>
How does HTLV-1 cause inflammatory disease?

1. HTLV-1 infection is associated with upregulation of p53 signalling

2. HAMTSP is associated with upregulation of Interferon-stimulated genes - but multiple sclerosis is not

Tattermusch et al 2012 PLoS Pathogens
How does HTLV-1 cause leukaemia?
Low-quality CTL response to HTLV-1

Unfavourable HLA & KIR genotype

FoxP3+CD4+

HTLV-1 infection in infancy = 1st hit

Co-infection with Strongyloides stercoralis

Large number of HTLV-1+ clones (high proviral load)

Clone-specific factors

Each clone has its own pattern and intensity of expression of HBZ and tax genes:

- CREB
- AP-1
- Classical NFκB
- TGFβ
- Telomerase
- Wnt

→ proliferation
→ survival

• genetic instability
• ? insertional mutagenesis

→ 2nd, 3rd, … hits

Anti-Tax CTLs

→ Loss of Tax expression

→ ATL

Host-specific factors

Bangham and Ratner 2015