Strategies for Diagnosis of HTLV-I and immune response to HTLV-1

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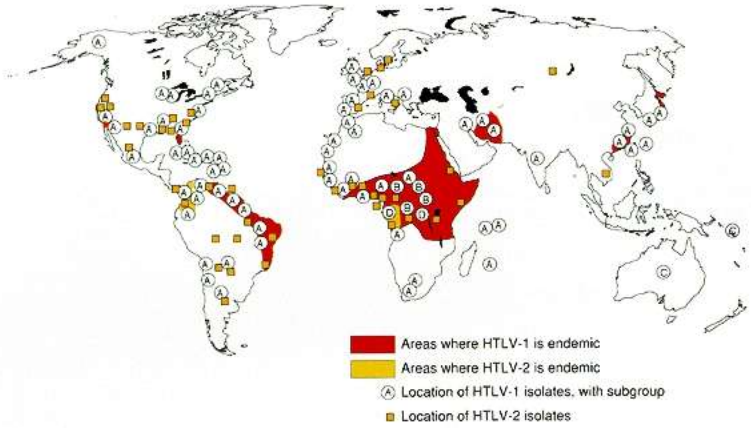
HTLV-I World-wide Distribution

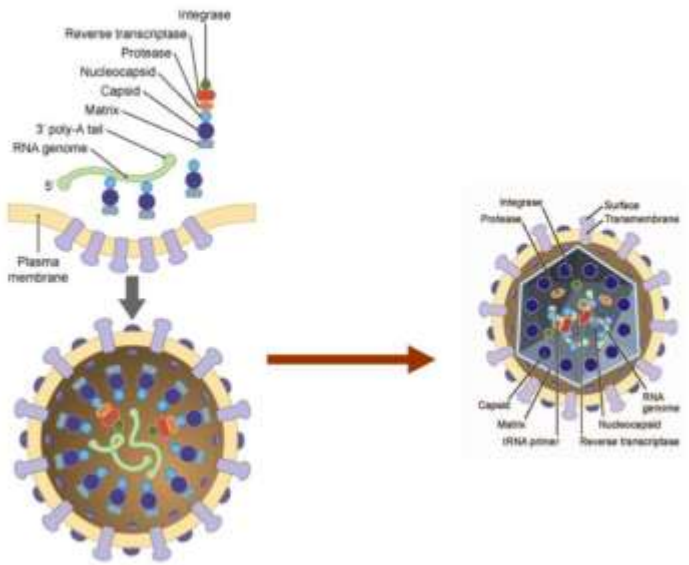


Genetic structure of HTLV-1

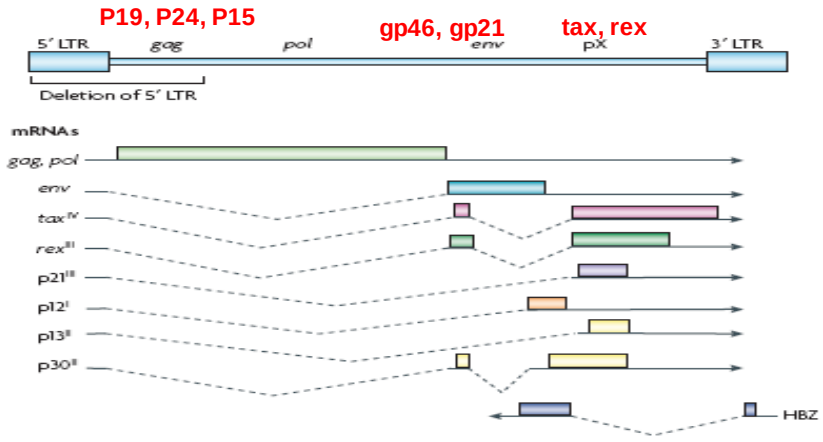
- **HTLV-I is classified as a complex retrovirus, in the genus Deltaretrovirus of the subfamily Orthoretrovirinae.**

- **The diploid plus-strand RNA genous is 9032bp. In addition to the gag, pol and env genes found in a typical exogenous retrovirus, HTLV-I encodes a number of small regulatory proteins, including Tax and Rex.**

Figure 2. HTLV-1 assembly and incorporation of viral components (left) and fully developed mature virion following budding from cell membrane (right).

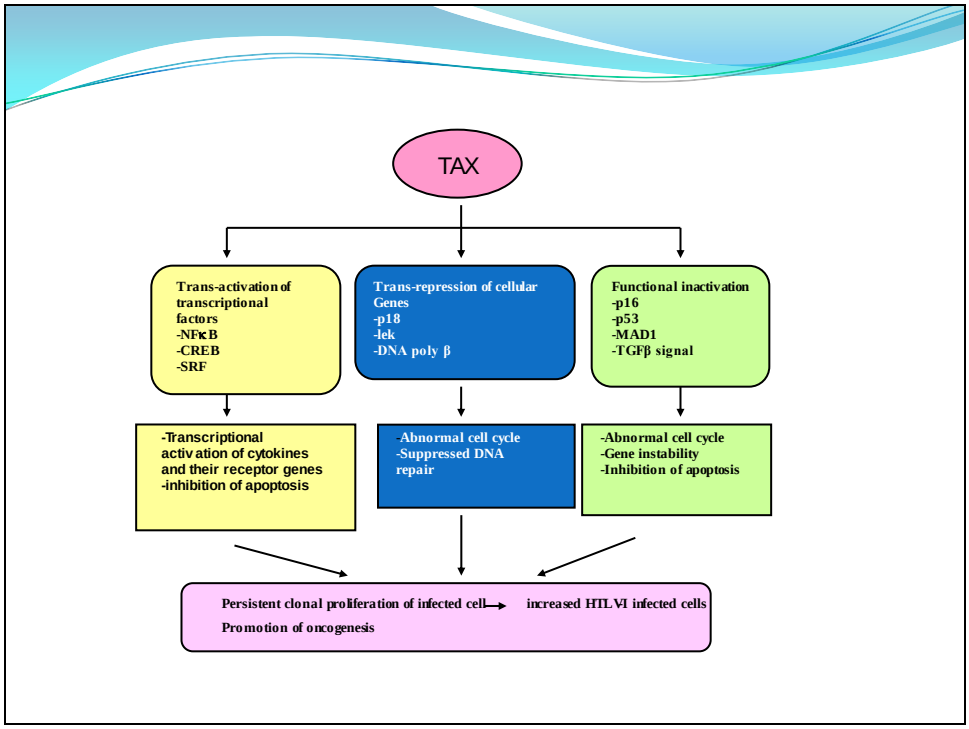


Genomic structure of HTLV-I and its products



Tax and Rex

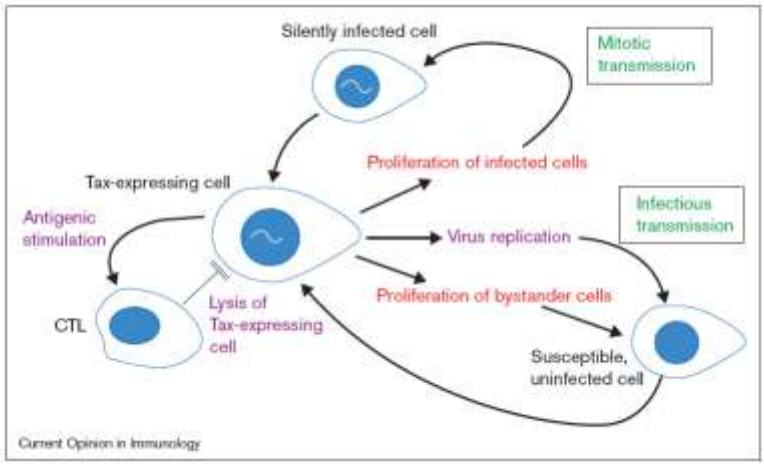
- Tax activates transcription of the HTLV-I provirus
- Rex regulates the intracellular transport of unsplised and singly spliced HTLV-I mRNA



Infectivity of HTLV-I

- CD4+D45RO+ T cells
- CD8+ T cells
- DC cells
- Monocytes
- Macrophages
- B cells
- NK cells
- Glial cells
- Endothelial cells

Spread of HTLV-I



HTLV-1 diagnosis

The diagnosis of HTLV-I infection is based on the detection specific antibodies by screening tests such as enzyme linked immunosorbent assay (ELISA) or particle agglutination.

The positive results should be confirmed by Western blot (WB) or polymerase chain reaction (PCR).

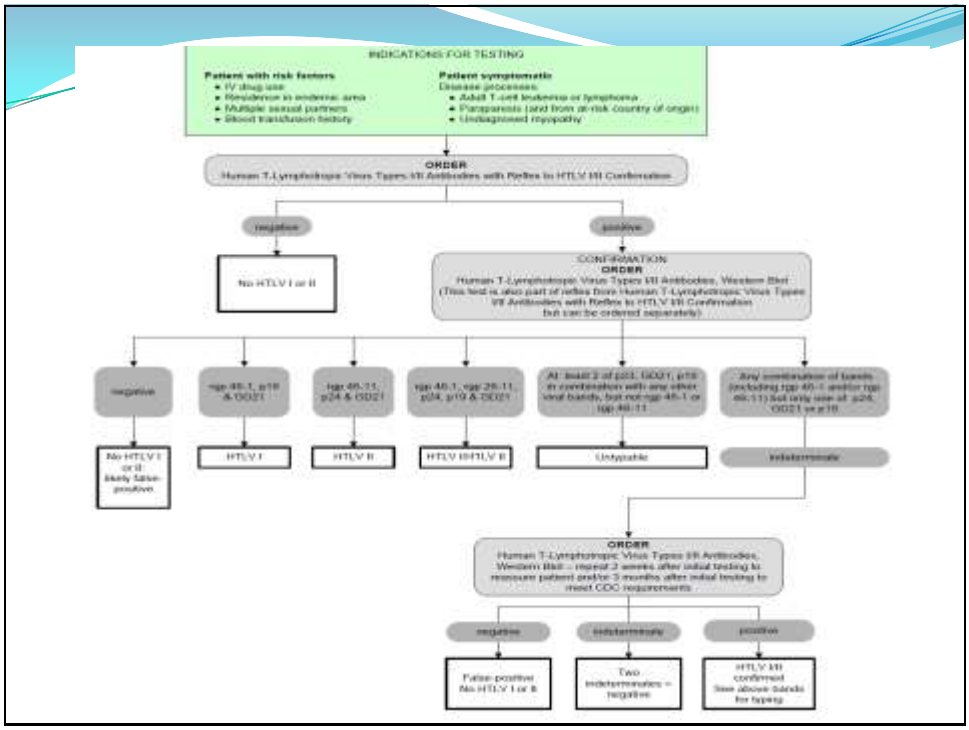
In Western blot, the reactivity of the test is examined against the products of HTLV-I such as gag (p19 or p24) and env (gp21 or gp46) gene products

After infection with HTLV-I, antibodies to core, envelope and tax protein in serum appeared.

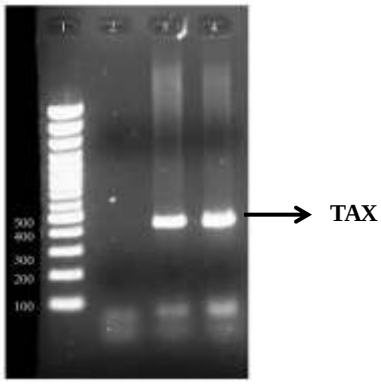
Within 30 to 60 days after primary HTLV-I infection, antibody to gag proteins predominant with anti-p24 generally appearing before anti-p19 antibodies.

Antibody to p-21 envelope protein frequently appears before gp46 antibodies.

Anti tax antibodies are the latest antibodies to appear in the time course of seroconversion



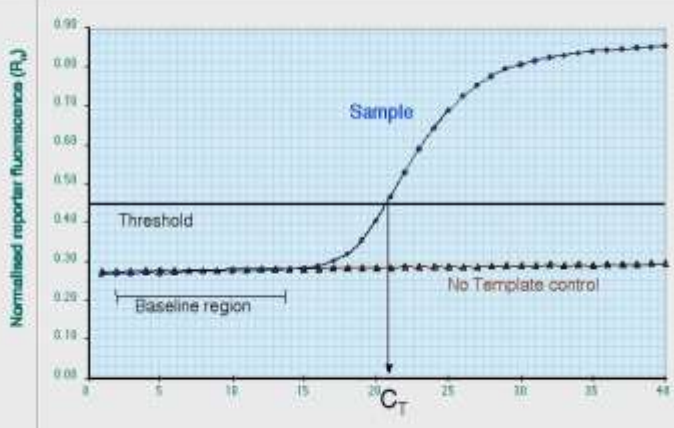
HTLV-I PCR



Proviral load

- The provirus load of HTLV-I usually reaches a stable equilibrium "set point" that fluctuates in most cases by no more than 2- to 4-fold over a period of years
- High proviral load in Japanese population (median 5% PBMCs in HAM/TSP and 0.3% in carriers
- In contrast with HIV sequence variation is very limited in HTLV-I.

Amplification plot features



Does HTLV-I cause any disease?

- **Adult T-cell Leukaemia/Lymphoma (ATLL)**
- **HTLV-I-associated myelopathy (HAM)/Tropical spasticparaparesis.**

- **Other HTLV-I-associated diseases**. HTLV-I can also cause inflammation of the eye (uveitis), joints (arthritis), muscles (myositis), lung (alveolitis) and skin (dermatitis). These conditions are even less common than ATLL and HAM and the skin condition is usually only seen in tropical climates.
- **HTLV-I and other infections**

  Strongyloidiasis: A warm infection acquired in the tropics can, after lying dormant for years, cause a serious illness in HTLV-I carriers.

Can HTLV-I infection be treated?

- At present there is no treatment to cure (eradicate) the infection. Since 95% of all infected persons go through life without developing any HTLV-I-associated diseases any such treatment would have to be not only effective but also very safe.

Is everyone infected with HTLV-I at equal risk of developing an HTLV-I-associated disease?

- **ATL** is unlikely to develop following infection acquired in adult-life. This means that avoiding infection of babies by avoidance of **breast-feeding** is very important for the prevention of ATLL in the next generation.

- **HAM/TSP** seems to be less common among Japanese with HTLV-I infection than among other populations and evidence is emerging that the immune system is important in controlling infection. HTLV-I asymptomatic carriers with less virus in the blood are less likely to develop HAM

What is HAM/TSP?

- is an inflammation of the spinal cord seen in some people infected with the HTLV-I.
- Most of the research into the cause of HAM/TSP suggests that the disease is caused by the immune system trying to clear the HTLV-I infection.

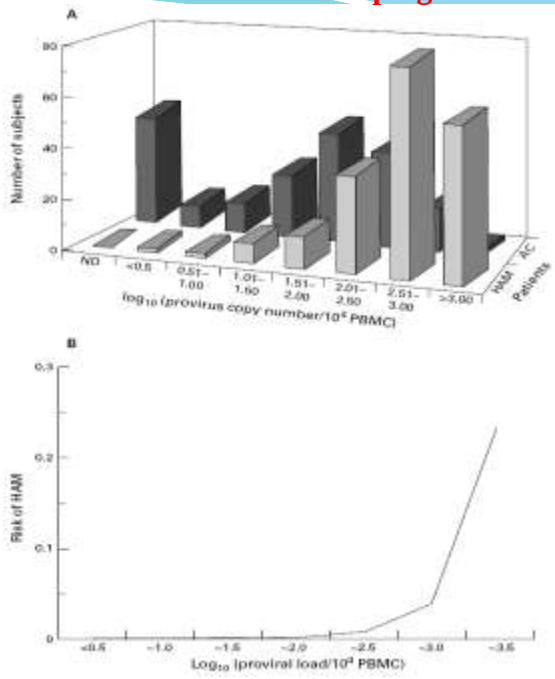
Who is likely to be affected?

- **Gender** - women infected with HTLV-I are more likely to develop HAM/TSP than men (3 women to every 2 men).
- **Time or route of infection** - initial infection with HTLV-I in adult life is a risk factor. The amount of virus in the blood (this is known as viral load) - the risk increases if more than 1 lymphocyte per 100 in the blood is infected with HTLV-I.
- **Immune system genetics** - certain HLA types (these are also called Tissue types and are like blood types on white blood cells rather than on red blood cells) seem to increase protection against HAM/TSP whereas others may increase susceptibility.

Differences between carriers and HAM/TSP

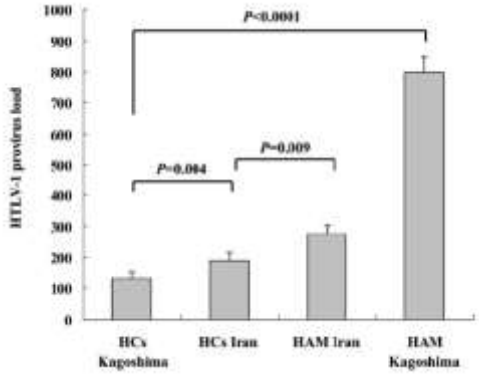
- High proviral load in HAM/TSP patients (about 10% of PBMC) compare to carriers (0.1-1% of PBMC)
- High titer to HTLV in HAM/TSP
- Spontaneous lymphoproliferation
- High frequency of HLA class I restricted CTL (Tax 11-19)

The effects of Proviral load on disease progression



Differences in viral and host genetic risk factors for development of human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis between Iranian and Japanese HTLV-1-infected individuals

Journal of General Virology (2005), 86, 773–781



The Immne response to HTLV-I

- **Humoral immuneresponse**

Anti gag Ab (anti p24), anti p19 Ab, Anti p21 Ab, anti gp 46Ab(env proteins), anti Tax Ab

- **The helper T cell response**

The high frequency of HTLV-I-specific CD4+ T cell (median frequency is 25 times greater in HAM/TSP compared with carriers)

- **CD8+ T cell response to HTLV-I**

Role of HTLV-I-specific CTL

- HTLV-I-specific CD8+ T cells recognize a nine –AA peptide of the Tax protein (Tax 11-19) in association with HLA-A2 allele
- High frequency of HTLV-I-specific CD8+ T cells and their state of chronic activation. These cells play a major part in determining the provirus load at equilibrium
- HTLV-I proviral load directly correlated with the frequency of these cells in HAM/TSP
- Existence and accumulation of HTLV-I-specific CTLs in the CNS
- Activation of CTL in the CNS by viral Ag

- HTLV-I-specific CTL consist of a heterogenous population with migratory capacity expressing CXCR3, IL-8R (CXCR1 & CXCR2), CCR5 and IL-2Rβ
- Increased proportion of CD8 cells produce IFN-γ
- Elevated levels of Inflammatory cytokines in CSF and serum (IFN- γ, TNF-α, IL-6)
- Production of MIP-1α , MIP-1β and MMP-9 by HTLV-I-specific CTL

Advantages of CTL response

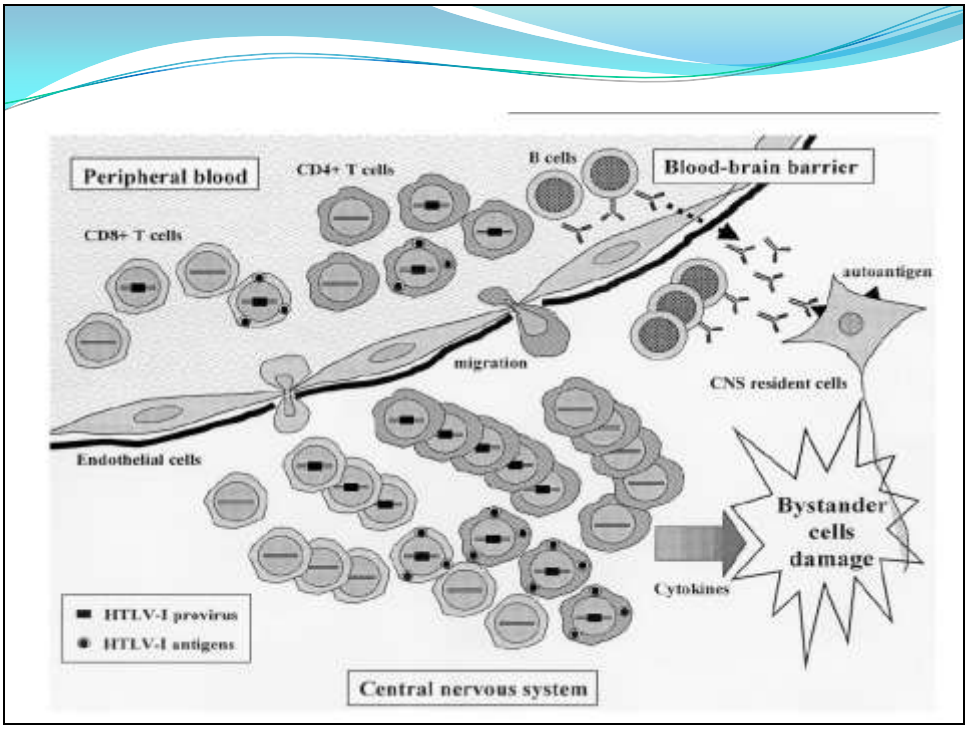
- Association between low proviral load and HLA-A2
- More nucleotide and amino acid sequence variation in Tax in HC compared with HAM/TSP
- Mathematical model

Disadvantages of CTL response

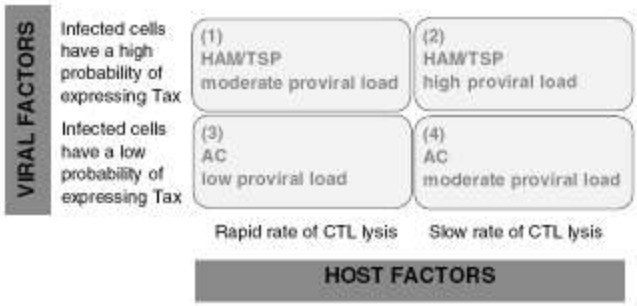
- Expression of viral protein in the CNS
- High proportion of IFN-γ and TNF-α producing CD8 T cells and the increased level of proinflammatory cytokines in CSF

The role of HTLV-I in development of HAM/TSP

- Neurological damage in the CNS by specific CD8 CTL
- Autoaggressive bystander model
- Autoimmune model



Both sides of the host-virus interaction determine the outcome of HTLV-I infection



Effect of class I HLA on outcome of HTLV-I infection

Table 1. Effect of class I HLA alleles

Class I HLA alleles *HLA-A*02* and *HLA-Cw*08* reduce both the risk of the inflammatory disease HAM/TSP and the provirus load of HTLV-1 in Kagoshima, Japan. Data taken from Jeffery *et al.* (1999, 2000).

	Reduction of provirus load in asymptomatic HTLV-1 carriers		Risk of HAM/TSP	
Genotype	Provirus load* (*N*)	*P*†	Odds ratio	*P*‡
*HLA-A*02*$^+$	16·8 (100)	0·014	0·43	<0·0001
*HLA-A*02*$^-$	50·1 (101)	0·046	0·42	0·002
*HLA-Cw*08*$^+$	12·0 (43)			
*HLA-Cw*08*$^-$	45·7 (159)			

*Median proviral copy number per 10^4 PBMCs.
†Mann–Whitney two-tailed test (uncorrected).
‡χ^2 with Yates' correction.

HLA-DRB1 phenotype frequencies in healthy controls, HTLV-I carriers and HAM/TSP patients

HLA-DRB1	Controls n=72	HAM/TSP n=36	HTLV-I Carriers n=34
*01	8 (11.1%)	7 (19.4%)[a]	1 (2.9%)[a]
*15	13 (18.1%)	6 (13.9%)	6 (17.6%)
*16	6 (8.3%)	4 (11.1%)	1 (2.9%)
*03	12 (16.7%)	2 (5.6%)	6 (17.6%)
*04	19 (26.4%)	10 (27.8%)	7 (20.6%)
*11	20 (27.8%)	11 (30.6%)	10 (29.4)
*12	3 (4.2%)	0 (0%)	0 (0%)
*13	23 (31.9%)	13 (36.1%)	11 (32.3%)
*14	5 (6.9%)	1 (2.8%)[b]	6 (17.6%)[b]
*07	11 (15.3%)	8 (22.2%)	5 (14.7%)
*08	1 (1.4%)	2 (5.6%)	2 (5.9%)
*09	4 (5.6%)	0 (0%)	2 (5.9%)
*10	3 (4.2%)	3 (8.3%)	2 (5.9%)
*11/13	2 (2.8%)	0 (0%)	2 (2.9%)
*11/14	3 (4.2%)	1 (2.8%)	0 (0%)
*13/14	0 (0%)	0 (0%)	0 (0%)

Rafatpanah et al., 2007 , IJI