Review

HTLV-1 infection: what determines the risk of inflammatory disease?

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Human T-lymphotropic virus type 1 (HTLV-1) is an exogenous retrovirus that persists lifelong in the infected host. Infection has been linked to a spectrum of diverse diseases: adult T cell leukemia, encephalomyelopathy, and predisposition to opportunistic bacterial and helminth infections. Applications of new technologies and biological concepts to the field have provided new insights into viral persistence and pathogenesis in HTLV-1 infection. Here, we summarize the emerging concepts of dynamic HTLV-1–host interactions and propose that chronic interferon (IFN) production causes tissue damage in HTLV-1-associated inflammatory diseases.

HTLV-1 infection and associated diseases in humans

Since its discovery as the first human retrovirus in 1980, human T-lymphotropic virus type 1 (HTLV-1) has been linked to highly disparate types of disease in the human host: adult T cell leukemia/lymphoma (ATLL), a spectrum of chronic inflammatory diseases [1], and increased susceptibility to particular opportunistic bacterial and helminth infections.

The global burden of HTLV-1 infection is unknown: the widely cited estimate of 10 to 20 million infected people worldwide dates back to studies that are now more than 25 years old [2], and systematic epidemiological studies are lacking in many areas where HTLV-1 infection is endemic. However, in recent years the debilitating effects of HTLV-1-associated diseases have been increasingly recognized as an economic health burden in developing countries where endemic foci overlap with regions of low socioeconomic status [3]. The best recognized of the HTLV-1-induced inflammatory conditions is a chronic progressive encephalomyelopathy termed HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) [1,4].

It is not understood how the virus causes such diverse diseases. Because of the absence of viral particles in the plasma of infected individuals, HTLV-1 was long assumed to remain latent in the host during the chronic phase of infection [5–7]. However, the discovery of a strong antiviral immune response evidenced a highly dynamic interaction between viral expression in infected $CD4^+$ T cells *in vivo* and the host immune system [8]. In the early 1990s extensive sequencing efforts were made to link distinct strains of HTLV-1 to ATLL or HAM/TSP [9–12] but no definite viral genotype has been exclusively associated with the neurological disorder or the leukemia [13]. These findings led to the currently accepted hypothesis that the host immune response is the main determinant of the risk of disease in HTLV-1 infection.

To date the best predictor of HAM/TSP and ATLL is a high HTLV-1 proviral load [14,15], in other words, the percentage of peripheral blood mononuclear cells (PBMCs) that carry the provirus. However, a significant proportion of infected individuals who present with a high HTLV-1 proviral load never develop disease, suggesting that additional factors contribute to HTLV-1-associated pathologies. Investigations into the nature of these factors have been hampered by technical limitations to experimental work: (i) the absence of viral particles in plasma; (ii) the inability to purify live HTLV-1 infected cells directly from blood samples; (iii) the rapid spontaneous activation of viral protein expression in freshly isolated PBMCs, which causes strong changes in host gene expression; (iv) the inaccessibility of the central nervous system lesions in HAM/TSP; and (v) the lack of an animal model that mirrors the human diseases.

Recently the application of new technologies and microbiological concepts has overcome some of these limitations and has made it possible to identify factors that differ systematically between asymptomatic and diseased HTLV-1 carriers, independently of proviral load. In this review, we summarize these recent advances in the understanding of HTLV-1 pathogenesis and discuss questions that remain to be answered.

Genomic location of the HTLV-1 proviral integration site

HTLV-1 is transmitted between individuals via breast milk, semen, or blood [3]. The virus preferentially integrates into the genome of host T lymphocytes but initial infection is subclinical. In chronic HTLV-1 infection about 90–95% of the proviral load is carried by $CD4^+$ T cells and 5–10% by $CD8^+$ T cells [16,17]. The proviral load 'set point' remains relatively stable over time within one individual, but can vary ~1000-fold between infected individuals [18].

To persist within the host, HTLV-1 pursues two strategies: (i) clonal expansion, in other words, active promotion of mitotic proliferation of infected cells thus passing on the provirus to daughter cells, and (ii) infectious spread to uninfected cells via a cell-to-cell contact known as the virological synapse [19]. It remains difficult to quantify the relative contribution of each route to the maintenance of the proviral load; however, both are driven by the expression of viral proteins.

Recently, Meekings *et al.* demonstrated a link between spontaneous proviral expression, genomic location of the

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Box 1. HTLV-1 clonality [21]

- The total number of HTLV-1-infected clones in each individual frequently exceeds 10 000, whereas it was previously believed to lie between 10 and 100.
- The oligoclonality of the HTLV-1-infected population is not greater in HAM/TSP than in asymptomatic carriers, as previously believed; instead, the higher proviral load in HAM/TSP patients appears to be attributable to a larger number of infected T cell clones.
- The integration site of the provirus in the host genome is not random, but is biased towards transcriptionally active regions of DNA.
- In proviruses integrated inside host transcriptional units, orientation of the provirus in the same transcriptional sense as the host gene is associated with higher clonal abundance.

proviral integration site, and the presence of disease [20]. Although HTLV-1 expression is usually undetectable in freshly isolated lymphocytes from an infected individual, a proportion of HTLV-1-infected cells spontaneously express the provirus after short-term (4–24 h) incubation *in vitro*. In individuals with a high frequency of spontaneous proviral expression *in vitro*, HTLV-1 was frequently found integrated in transcriptionally active units of the genome. Subsequent high-throughput sequencing revealed that the size of an HTLV-1 clone increased with its proximity to CpG islands, host genes, and activating epigenetic marks [21] (Box 1). Importantly, the distribution of viral integration sites within the host genome differed systematically between asymptomatic carriers and patients with HAM/TSP or ATLL. In healthy carriers the provirus was predominantly integrated in transcriptionally silenced parts of the genome, whereas integration into transcriptionally active units and subsequently increased expression of the provirus predisposed individuals to disease. However, this distribution of genomic integration sites *in vivo* represents the outcome of many years of selection in which proviral expression that drives viral spread is counterbalanced by the host immune response, chiefly by the cytotoxic T-lymphocyte (CTL) response to the virus.

Quality of the CTL response

The CTL response mounted against HTLV-1 is typically strong: chronically activated HTLV-1-specific $CD8^+$ T cells are highly abundant in HTLV-1 carriers and are able to kill HTLV-1-infected target cells directly *ex vivo* – in other words, without *in vitro* restimulation [22–25]; however, they fail to eradicate the virus *in vivo*. Emerging evidence now favors the conclusion that CTL 'quality', and not quantity, is crucial to the outcome of HTLV-1 infection [26–32]; CTL quality may be defined as the per cell efficiency of CTL function.

Two HTLV-1 proteins are of particular importance in the CTL-mediated immune response. First, the pleiotropic viral transactivator Tax which, in complex with the transcriptional coactivator complex CBP/p300, drives transcription of the provirus from the 5′ long terminal repeat. Second, the regulatory protein HBZ, which is encoded on the negative strand of the provirus, is thought to be required for persistence in the host. Two recent studies demonstrated differences between HTLV-1 carriers in the ability of their CTLs to recognize and respond to different HTLV-1 antigens. The immunodominant antigen recognized by HTLV-1-specific $CD8^+$ T cells – that is, the antigen that elicits the highest frequency of $CD8^+$ T cells – is Tax. However, using experimentally derived data on peptide-binding motifs, MacNamara *et al.* demonstrated that individuals whose HLA alleles strongly bind to peptides from a subdominant antigen, the viral protein HBZ, have lower HTLV-1 proviral loads and are more likely to be asymptomatic [33]. In a second study, Kattan *et al.* demonstrated that CTLs from asymptomatic carriers secreted interferon-γ (IFN-γ) when stimulated with a low concentration of HTLV-1 antigens, whereas CTLs from most patients with HAM/TSP required high antigen concentrations to do so [34]. Thus, a high-quality CTL response to HTLV-1 is characterized by sensitive recognition of HBZ and sustained CTL activity even at low antigen concentrations.

A recent key discovery by Seich al Basatena *et al.* adds a further layer of complexity to CTL responses in chronic viral infections [35]. These authors demonstrated that possession of the inhibitory killer cell immunoglobulin-like receptor (KIR) KIR2DL2 enhances known protective or detrimental effects of HLA-class 1 genotypes in both HTLV-1 infection and hepatitis C virus (HCV) infection [35]. Expression of inhibitory KIRs on $CD8^+$ T cells has been linked to elevated levels of the anti-apoptotic protein Bcl-2, thus increasing their resistance to activation-induced cell death during chronic antigen exposure [36,37]. The authors postulate that activated $CD8^+$ T cells that express KIR2DL2 are protected from clonal exhaustion and accumulate during the course of HTLV-1 infection [35]; thus, depending on whether the KIR2DL2-expressing $CD8^+$ T cell is restricted by a protective or detrimental HLA class 1 molecule, the respective protective or detrimental effect will be enhanced. Although the biological mechanism of KIR2DL2-mediated enhancement of class 1 major histocompatibility complex (MHC)-associated immune responses remains to be tested, the observed association between the KIR and HLA genotypes increases our understanding of the differences in CTL quality between HTLV-1 carriers that appear to account for the variation in proviral load and risk of disease. The five known correlates of CTL quality in HTLV-1 infection are summarized in Figure 1 (left-hand side).

High frequencies of regulatory T cells

Regulatory $CD4^+$ T cells are characterized by the expression of the transcription factor FoxP3 and play a crucial role in normal physiology by inhibiting antigen-specific T cells and thereby suppressing excessive immune responses. Two recent studies by Toulza *et al.* reported a higher abundance of $CD4^+FoxP3^+$ T cells in peripheral blood from patients with HAM/TSP and ATLL than in asymptomatic carriers with similar proviral loads [38,39]. The authors subsequently demonstrated that HTLV-1-infected cells secrete the chemokine CCL22, which induces and maintains high frequencies of $FoxP3^+$ T cells [40]. However, it remains to be established whether the increase in circulating $FoxP3^+$ T cells is a result of proliferation, or mobilization from tissues, or both. In peripheral blood, a high frequency of these HTLV-1-negative $CD4^+FoxP3^+$ T cells was associated with low CTL