Review

HTLV-1: Persistence and pathogenesis

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Introduction

Human T lymphotropic virus type 1 (HTLV-1) is an exogenous retrovirus that establishes a persistent infection in humans. It infects 10–20 million people worldwide with endemic regions in Japan, equitorial Africa, the Caribbean and South America (Proietti et al., 2005). The virus is transmitted from mother to child, between sexual partners, by needle sharing and in contaminated blood products. HTLV-1 is a complex retrovirus. So, in addition to the standard repertoire of structural proteins and enzymes shared by all retroviridae (gag, pol, pro and env), the 3' region of the HTLV-1 genome (pX region) also encodes a number of accessory genes: tax, rex, p12, p21, p13, p30 and HBZ. HBZ, HTLV-1 basic leucine zipper factor, is the only transcript from the antisense strand of the viral genome (Gaudray et al., 2002).

Research into HTLV-1 is important for two main reasons. Firstly, HTLV-1 is the aetiological agent of a number of pathologies for which there is no cure and no effective treatment. Consequently, HTLV-1 is responsible for a significant burden of morbidity and mortality. Research into HTLV-1 pathogenesis aims
to alleviate this burden. Secondly, HTLV-1 infection is an excellent model of within-host pathogen dynamics and immune control. HTLV-1 succeeds in establishing a persistent infection, often with a high proviral load (it is not unusual for > 5% of PBMC to be infected) despite a large and chronically activated cellular immune response. Furthermore, there is considerable between-individual variation both in clinical status and set point proviral load. Work to understand how HTLV-1 persists and what underlies the inter-individual variation in outcome has led to fundamental advances in human immunology. Recently, both areas of research, pathogenesis and viral persistence, have started to converge with several lines of enquiry pointing to the central importance of the viral-encoded protein HBZ. Here we review recent progress in HTLV-1 pathogenesis and some of the lessons in immunology learnt from HTLV-1.

**HTLV-1 pathogenesis**

**HTLV-1 and disease**

HTLV-1 is causatively associated with a number of pathologies; the two most common are adult T-cell leukaemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). ATL is a spectrum of CD4+ T cell malignancies that is categorised into four types: acute, chronic, smouldering and lymphoma; the acute form is the most common and is rapidly fatal. HAM/TSP is a chronic progressive myelopathy characterised by spastic paraparesis. Only a minority of HTLV-1-infected individuals develop disease. Depending on ethnicity and gender, approximately 2–3% of infected individuals develop ATL and 0.25–4% develop HAM/TSP (Hisada et al., 2004; Osame et al., 1990; Tajima, 1990). Other associated diseases include HTLV-1-associated uveitis and infective dermatitis. The majority of infected individuals remain lifelong asymptomatic carriers (ACs).

The mechanisms by which HTLV-1 causes such diverse clinical diseases are not understood and it is also not known why disease typically occurs decades after initial infection and affects less than 10% of carriers. Understanding of HTLV-1-associated disease is further limited by the lack of suitable animal models and inaccessibility of tissue from the central nervous system of individuals. Since no viral genotype has been associated with any particular susceptibility of tissue from the central nervous system of individuals.

**Determinants of proviral load**

HTLV-1 proviral load (the number of integrated copies of HTLV-1 expressed as a proportion of PBMCs) remains approximately stable in one individual over years, but may vary ~1000-fold between individuals (Demontis et al., 2012; Matsuaki et al., 2001). A high proviral load is one of the best predictors of HAM/TSP and ATL, although many patients with a high load will remain lifelong asymptomatic carriers (Iwanaga et al., 2010; Nagai and Osame, 2003). However, there is little longitudinal data to confirm that a high proviral load is the cause of disease rather than the consequence (Kira et al., 1991; Taylor et al., 1999). Nevertheless, given the association between proviral load and disease a number of studies have attempted to elucidate the determinants of proviral load.

**Integration and clonal expansion**

Following T cell infection, HTLV-1 genomic RNA is reverse transcribed and integrated into the host cellular genomic DNA. Cellular proliferation results in large clonal expansions of infected

**HAM/TSP**

Immunostaining of post mortem biopsies shows that early in the disease process the leptomeninges, blood vessels and parenchyma are infiltrated with CD4+, CD8+, B lymphocytes and foamy macrophages, whereas later in the disease CD8+ lymphocytes predominate with subsequent progression to a relatively acellular, atrophic pattern with axonal and myelin degeneration (Iwasaki et al., 1992). HTLV-1-infected cells are present in the cerebrospinal fluid and are typically present at greater than twice their frequency in the peripheral blood (Hayashi et al., 2008; Kubota et al., 1994) reflecting either recruitment or expansion of HTLV-1-infected cells in the central nervous system. However, there is no evidence that HTLV-1 directly infects neuronal cells, astrocytes or microglia. HTLV-1-specific CD8+ lymphocytes that secrete the neurotoxic cytokines interferon γ (IFN-γ) and tumour necrosis factor α are present (Greten et al., 1998) and may be responsible for bystander damage (Hoger et al., 1997; Yamano et al., 2002).

In a recent study Tatternsuch et al. compared blood transcriptional profiles of asymptomatic carriers, patients with HAM/TSP and seronegative control subjects. The authors found that at a given proviral load, the presence of disease was associated with overexpression of a distinct subset of IFN-stimulated genes. This signature was absent in healthy HTLV-1 carriers and correlated positively with the clinical severity of HAM/TSP (Tatternsuch et al., 2012). However it remains unclear whether chronic overexpression of IFN-stimulated genes contributes to the tissue damage seen in HAM/TSP or is merely a consequence of the inflammatory condition.
cells with a single proviral integration site in the cellular genome (Wattel et al., 1996). Recently Gillet et al. used high throughput sequencing to investigate clone distribution and abundance as a function of proviral integration site (Gillet et al., 2011). The principal findings were that (i) in vitro integration was biased towards transcriptionally active regions of the genome but that these sites were counterselected in vivo, (ii) the increase in proviral load seen in patients with HAM/TSP (compared with asymptomatic carriers) is due to the presence of an increased number of clones, (iii) the largest clones were associated with proximity to CpG islands, host genes and activating epigenetic marks and this distribution differed between asymptomatic carriers, HAM/TSP and ATL, (iv) among established abundant T cell clones, integration of the provirus in the same transcriptional orientation as the nearby host gene also drives selective expansion.

CD8+ T cell lysis
A method to quantify the rate at which naturally, endogenously infected cells are cleared by autologous CD8+ cytotoxic T-lymphocytes (CTL) ex vivo, has been developed (Asquith et al., 2005). This ex vivo lysis assay was used to quantify the relationship between the strength of the CTL response and HTLV-1 proviral load. Correlation of the rate of CTL lysis with HTLV-1 proviral load showed a significant inverse relationship (Asquith et al., 2005; Kattan et al., 2009). Thus, individuals with a high rate of clearance of infected cells tended to have a low proviral load. This correlation was found both in ACs and in HAM/TSP patients. A negative correlation does not necessarily imply that the rate of CTL lysis determines proviral load; the opposite could also be true. However, the efficient, perforin-dependent reduction in Tax+ cells by CTL ex vivo provides a plausible mechanism by which CTL killing rate could determine proviral load. This analysis showed that a large proportion of the variation between individuals in HTLV-1 proviral load can be explained by the rate at which CTLs kill infected cells. In both the ACs and the HAM/TSP patients 40–50% of the inter-individual variation in proviral load could be accounted for by variation in antiviral efficacy, thereby being the largest single predictor of inter-individual variation in HTLV-1 proviral load (Asquith et al., 2005).

The immune response to HTLV-1
The observation that the HTLV-1 sequence is conserved (Gessain et al., 1992) and the finding of large clones of infected cells (Wattel et al., 1996) suggests that HTLV-1 replicates predominantly by driving mitosis of infected cells rather than by de novo infection of previously uninfected target cells. Additionally, HTLV-1 is largely cell-associated and produces few virions (Derse et al., 2001). Thus, although the HTLV-1-specific antibody titre is high, particularly in HAM/TSP (Enose-Akahata et al., 2012; Manns et al., 1999), antibody surveillance is thought to be largely ineffective (Dumenil, 2011).

Similarly, there is little evidence that natural killer (NK) cells play an important role in controlling HTLV-1 infection. CD56+ and CD3− NK cells have no detectable lytic activity against HTLV-1-infected target cells. This is true whether the targets are naturally infected CD4+ cells isolated from PBMC of HTLV-1-infected subjects or when the targets are produced in vitro by infection of primary CD4+ T cells (Banerjee et al., 2007; Hanon et al., 2000). NK cells may have little effect on HTLV-1-infected cells because, unlike many viruses, HTLV-1 does not appear to cause significant down-modulation of inhibitory NK ligands or upregulation of activating ligands. In particular, surface MHC class I expression is not significantly downregulated in primary HTLV-1-infected cells (Yoshida et al., 2000) although the HTLV-1 accessory protein p17 has been shown to cause some down-modulation on T-cell lines (Johnson et al., 2001). Additionally, ligands for the NK cell activating receptors, NCR and NKG2D are not expressed by HTLV-1-infected cells (Banerjee et al., 2007); all these factors are likely to reduce NK cell recognition of HTLV-1-infected cells. Furthermore, in contrast to human immunodeficiency virus (HIV-1) and hepatitis C virus (HCV) (Khakoo et al., 2004; Martin et al., 2002; Martin et al., 2007), there is no evidence from immunogenetic studies that particular NK receptor genes are associated with the outcome of HTLV-1 infection (O'Connor et al., 2012; Seich et al, Basatena et al., 2011). Again, these observations argue against a major role for NK cells in HTLV-I infection.

In contrast there is compelling evidence that the CD8+ cytotoxic T lymphocyte (CTL) response is an important determinant of the outcome of HTLV-I infection (Bangham, 2009). Perhaps the most convincing is the strong association between certain human leucocyte antigen (HLA) class I alleles and the outcome of infection (see Section “Determinants of protective immunity” below).

Determinants of protective immunity
A number of associations between particular HLA class I alleles and proviral load and clinical outcome have been identified. In a Japanese cohort, possession of HLA-A*02 and C*08 was associated with a significant decrease in the odds of HAM/TSP and a significant decrease in proviral load amongst asymptomatic carriers of the virus. In contrast, HLA-B*54 was detrimental. Carriers of B*54 were significantly more likely to have HAM/TSP and, amongst individuals with HAM/TSP, B*54 was associated with a significant increase in proviral load (Jeffery et al., 2000; Jeffery et al., 1999).

In HIV-1 infection, protective HLA class I alleles tend to be those that bind epitopes in more conserved regions of the genome. More conserved regions are presumably those that need to be maintained to preserve viral function. Epitopes in conserved regions are likely to reduce the chance of viral escape from the CTL response and may result in a heavier fitness cost when escape does occur (Borghans et al., 2007; Kiepiela et al., 2007). In contrast to HIV-1, HTLV-I is highly genetically stable (Kubota et al., 2007) and, whilst viral variants that can escape CTLs do occur (Niewiesk et al., 1995), there is little evidence that these variants have a net selective advantage in asymptomatic carriers or HAM/TSP patients (Gessain et al., 1992) though a number of studies have highlighted the importance of viral mutants in ATL (Furukawa et al., 2001; Furukawa et al., 2006; Koiwa et al., 2002; Takeda et al., 2004). Certainly, the phenomenon of viral escape from CTL, which is prevalent in HIV-1 infection (Goonetilleke et al., 2009), is not a defining feature of HTLV-I infection in the absence of malignancy. HLA class I-associated protection from HAM/TSP is therefore unlikely to be correlated with targeting invariant regions of the viral genome. Studying the mechanisms underlying HLA class I-associated protection in HTLV-I infection thus provides additional information about the determinants of protective immunity.

Using a combination of epitope prediction (MacNamara et al., 2009) and ex vivo cellular work it has been shown that the protective HLA class I alleles HLA-A*02 and C*08 bind epitopes from the viral protein HBZ significantly more strongly than the detrimental molecule HLA-B*54 (MacNamara et al., 2010). In a cohort of 432 HTLV-I-infected individuals from Kagoshima in Japan, carriage of HLA class I molecules which bind HBZ epitopes

1 NK receptor genes (with or without their ligand) have no effect of the outcome of HTLV-I infection. This is not to be confused with NK receptor genes in the context of particular protective or detrimental HLA class I molecule where a clear effect on outcome is seen. See Section “Innate receptors enhance the HLA class I-restricted response”.

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strongly was significantly associated with asymptomatic status. This was true for both HLA-A and HLA-B molecules (HLA-C was not studied). Importantly, these associations held even after carriers of HLA-A*02, Cw*08 and Bw*54 were excluded from the cohort, demonstrating that the protective effect of binding HBZ was common to many HLA alleles and was not just a feature of a few particular alleles. Additionally, in both asymptomatic carriers and HAM/TSP patients, carriage of HLA alleles which could bind epitopes from HBZ strongly was associated with a significant reduction in proviral load (MacNamara et al., 2010).

This work suggests that HLA class I binding of epitopes from HBZ is associated with good control of HTLV-1 and reduced risk of HAM/TSP. This raises the question why is binding of HBZ epitopes particularly protective? We hypothesise that an HTLV-1-infected cell which represses expression of all viral genes except HBZ has a survival advantage because it can escape from the host immune response against these proteins but maintains the ability to proliferate at an elevated rate (see Section "Persistence in the face of a strong immune response"). This dual action – reduction of HTLV-1 expression (and subsequent protection from immune surveillance), and enhancement of infected cell proliferation – probably confers a survival advantage on HBZ-expressing cells. That HBZ-expressing cells have a survival advantage is consistent with the observations that HBZ enhances persistence in HTLV-1 inoculated rabbits (Li et al., 2009). We suggest that if HBZ-specific CD8+ T cells are weak or absent then infected cells that express HBZ but not other viral proteins will evade immune surveillance and proliferate rapidly, leading to an increase in proviral load. HBZ-specific CD8+ T cells would then play an important role in preventing this proliferation of provirus-positive cells and blocking this strategy of persistence. We hypothesise that the requirement for HBZ expression is one of the few weak points in HTLV-1's lifecycle and thus HBZ-specific CD8+ T cells are unusually protective.

**Immunodominance**

Initially, it was postulated (Jeffery et al., 1999) that HLA-A*02 was protective because it bound an epitope (Tax 11–19) from the dominant CTL target antigen Tax with unusually high avidity and restricted high frequency HTLV-1-specific CD8+ T cell responses (Jacobson et al., 1990). More generally, Tax-specific CD8+ T were often considered as the best candidate for ‘efficient’ or ‘protective’ CD8+ T cells because Tax is the immunodominant antigen (Goon et al., 2004).2 Our finding that binding of HBZ peptides rather than Tax peptides is protective challenges the assumption that immunodominant responses are necessarily protective. Interestingly, in contrast to Tax, HBZ is very poorly immunogenic. We found that the frequency of HBZ-specific CD8+ T cells is significantly lower than the frequency of Tax-specific CD8+ T cells in the same person and, consistent with this, that the predicted binding affinity of HLA molecules to HBZ peptides is significantly weaker than that of Tax peptides (Hilburn et al., 2011; MacNamara et al., 2010). Although we would predict that, given the central importance of HBZ in maintaining HTLV-1 persistence, HTLV-1 would have evolved to minimise HBZ immunogenicity, it is nevertheless striking that these low frequency T cell responses are so important.

More generally, it was possible to rank all the HTLV-1 proteins by whether binding their peptides was associated with a reduced risk of HAM/TSP (Table 1). This list could be viewed as the “rank order of targets for a vaccine designed to reduce HAM/TSP risk.”

### Table 1

<table>
<thead>
<tr>
<th>Rank order of protein targets associated with reducing HAM/TSP risk</th>
<th>Proximal load</th>
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<tr>
<td>HBZ</td>
<td>Gag</td>
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<tr>
<td>Pol</td>
<td>HBZ</td>
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<tr>
<td>Gag</td>
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<td>Tax</td>
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### Fig. 1

HILA class I binding of peptides from different HTLV-1 proteins has a differentiated impact on both proviral load and HAM/TSP risk. Left hand column: The HTLV-1 proteins were ranked according to whether they targeting them was associated with asymptomatic status. This list could be viewed as the “rank order of targets for a vaccine designed to reduce HAM/TSP risk.” Right hand column: The HTLV-1 proteins were ranked according to whether targeting them was associated with reduced proviral load. This list could be viewed as the “rank order of targets for a vaccine designed to reduce proviral load.” List reproduced from (MacNamara et al., 2010).

Additionally, it was possible to rank all the HTLV-1 proteins by whether binding their peptides was associated with a reduced proviral load. This list could be viewed as the “rank order of targets for a vaccine designed to reduce proviral load.” These two lists are strongly positively correlated (Rs=0.86, P=0.0005, Spearman’s rank correlation). That is, proteins whose peptides are bound by asymptomatic carriers (right hand side of the graph) are, independently, those associated with a lower proviral load when bound (top of the graph); (MacNamara et al., 2010).

1. HLA class I binding of peptides from different proteins has a differential impact on both proviral load and HAM/TSP risk. So far

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2 The immunodominant antigen can be defined in different ways: the antigen that is most often recognised, the antigen eliciting the largest response (e.g. by IFNγ ELISpot), or the antigen eliciting the largest response after correction for different protein lengths. Tax is immunodominant by all three measures.
we have investigated why targeting one extreme example (HBZ) may be beneficial. Much is still to be understood, such as why targeting say Gag is better than targeting Pol or why targeting p12 is apparently so ineffective (or perhaps actively detrimental?)

2. We have established a ranked list of protein targets of the most efficient CD8+ T cell response through to the least efficient response. Identification of protective epitopes immediately suggests a practical approach to measure and enhance, via vaccination, the efficiency of an individual's anti-viral response.

3. Finally, the observation that was made for HBZ-specific responses (that immunogenicity is not correlated with protection) is extended and supported by this work. Even if we exclude HBZ, there is no correlation between the magnitude of a response (after correction for protein length) and protection from HAM/TSP ($Rs = -0.20$, $p = 0.64$) or between magnitude and reduction in viral load ($Rs = -0.57$, $p = 0.14$) (Goon et al., 2004; Hilburn et al., 2011; MacNamara et al., 2010). Conclusions are unchanged if we do not correct for protein length ($Rs = 0.33$, $p = 0.42$). $Rs = -0.14$, $p = 0.74$) or if we define the immunodominance hierarchy by the frequency of recognition ($Rs = 0.22$, $p = 0.61$). $Rs = -0.24$, $p = 0.57$).

“Innate” receptors enhance the HLA class I-restricted response

Immunogenetics studies of HTLV-1 infection have revealed another determinant of protective immunity: killer immunoglobulin-like receptor (KIR) genotype. The KIRs are a family of receptors that are expressed on NK cells and subsets of T cells, particularly effector memory CD8+ T cells (Anfossi et al., 2001). The KIR family contains both inhibitory and activating receptors. The majority of the inhibitory KIR (KIR2DL1, KIR2DL2/3, KIR3DL1 and KIR3DL2) as well as an activating KIR (KIR2DS1) bind allotypes of HLA class I molecules. The ligands of most of the activating KIR are still unknown. An individual's KIR functionality is determined by three factors: the number of KIR genes that individual possesses (individuals typically carry between 7 and 14 KIR genes with varying numbers of inhibitory and activating KIR), which alleles of these polymorphic genes are present, and which HLA ligands (if any) of the KIRs are present (Khakoo and Carrington, 2006).

It has been shown that a particular inhibitory KIR, namely KIR2DL2, is associated with an enhancement of HLA class I-mediated immunity in HTLV-I infection (Seich al Basatena et al., 2011). Briefly, it was found that HLA class I associations were stronger in the presence of KIR2DL2 than in the absence. KIR2DL2-associated enhancement was seen for both protective and detrimental HLA alleles, for multiple HLA-A, B and C molecules and for two independent metrics (proviral load and protection from HAM/TSP). Strikingly, exactly the same behaviour (KIR2DL2 enhancement of HLA class I associations) was observed in infection with an unrelated virus, namely hepatitis C virus (HCV). The odds of making this observation by chance were less than $10^{-11}$. Several associations between KIR and HLA pairs have been reported in the literature (Khakoo et al., 2004; Martin et al., 2002; Martin et al., 2007); in every case the KIR:HLA pair was a KIR with its known or putative ligand and the underlying mechanism was attributed to NK cells (Fig. 2a). In contrast, the observed KIR2DL2-associated enhancement of HLA is not readily explained by NK cells. There are four reasons for this:

1. When KIR2DL2 is present with the genes encoding its ligands, but in the absence of a particular protective or detrimental HLA class I molecule (i.e. the parallel of the previous studies) there is no effect on clinical outcome or viral burden in HTLV-I or HCV infection.
2. The majority of the HLA class I molecules whose effects are enhanced do not appear to bind KIR2DL2 (with the possible exception of C08).
3. KIR2DL2 enhances both protective and detrimental HLA class I associations, a property that is difficult to reconcile with an NK-mediated effect.
4. The protective effect of binding HBZ peptides was enhanced. Although NK cells display some peptide specificity, such marked protein specificity is more reminiscent of T cells.

We thus hypothesise that the effector cells that are enhanced by the presence of KIR2DL2 are CD8+ T cells. This study has two important implications for cellular immunology, not only in HTLV-1 infection but more generally. Firstly, it provides a plausible explanation for the incomplete penetrance of protective and detrimental HLA class I traits. Although certain HLA

![Fig. 2. Proposed mechanism to explain inhibitory KIR enhancement of HLA class I associations. KIR–HLA associations are typically attributed to direct NK action. (a). However, Seich al Basatena's observations do not appear to be compatible with direct NK-killing for four reasons: firstly KIR2DL2 (alone or with its C1 ligands) has no impact on viral load or clinical status in either HTLV-1 or HCV; secondly most of the HLA molecules which were enhanced do not bind KIR2DL2, thirdly both protective and detrimental HLA associations were enhanced and finally the protective effect of binding HBZ was enhanced, NK cells display some peptide specificity but such marked protein specificity is more reminiscent of T cells (Seich al Basatena et al., 2011). Instead we hypothesise that the downstream effectors are CD8+ T cells; (b) We postulate that KIR2DL2 enhances CD8+ T cell responses by increasing the survival of memory CD8+ T cells. Either because (i) inhibitory KIRs on CD8+ T cells are associated with reduced activation induced cell death and protection from exhaustion (Gati et al., 2003; Ugolini et al., 2001; Young et al., 2001) or inhibitory KIRs on NK cells reduce NK-killing of activated CD8+ T cells (Corboni et al., 2007; Soderquest et al., 2011). Image reproduced from (Elemans et al., 2012) based on an image in (Seich al Basatena et al., 2011).]
class I alleles are significantly associated with protection or susceptibility there are numerous examples of individuals who are not consistent with the general trend. For example, in HIV-1 infection, although elite controllers are heavily enriched for HLA-B*57, the phenomenon of BS7+ individuals who progress to AIDS at normal rates is well described (Chen et al., 2012). Similarly, in HTLV-1 infection, although HLA-C*08 is protective (associated with reduced risk of HAM/TSP and reduced proviral load in asymptomatic carriers) it is not associated with a reduced proviral load amongst HAM/TSP patients (Jeffery et al., 1999). And HLA-B*54, which is detrimental (associated with increased risk of HAM/TSP and increased proviral load in HAM/TSP patients), is not associated with increased proviral load in asymptomatic carriers (Jeffery et al., 2000). If an additional gene (KIR2DL2) needs to be present for an HLA class I molecule to be effective then this could explain why the protective or detrimental effects of particular HLA alleles are not always manifest. Secondly, the suggestion that KIR2DL2 is associated with an enhancement of CD8+ T cell responses points to a novel role for KIR in adaptive immunity. We have hypothesised that KIR2DL2 enhances adaptive immunity by increasing the survival of memory CD8+ T cells. Interestingly, studies in ankylosing spondylitis have also implicated inhibitory KIR in the survival and aberrant expansion of autoreactive T cells (Bowness et al., 2011).

There are at least two mechanisms by which inhibitory KIRs could increase CD8+ T cell survival (Fig. 2b): via their expression on CD8+ T cells (Fig. 2b (i)) or NK cells (Fig. 2b (ii)). It is known that KIRs are expressed on CD8+ γδ T cells (Fig. 2b (i)) with an effector memory phenotype (CD28-CD45RA-CD45RO+CCR7-) (Anfossi et al., 2001) and have been reported in chronic infections including HTLV-1, HCV, HBV, CMV and HIV-1 (Alter et al., 2008; Bieganowska et al., 1999; Bonorino et al., 2007; Poon et al., 2005; van der Veken et al., 2009). In mice, inhibitory NK receptors (Ly49 receptors, the functional homologue of human KIRs) increase CD8+ T cell survival (Roger et al., 2001). Similarly, in KIR-transgenic mice, ligation of inhibitory KIRs increases the activation threshold of CD8+ T cells and reduces activation induced cell death (Anfossi et al., 2001; Ugolini et al., 2001; Ugolini and Vivier, 2000). Consistent with this, inhibitory KIRs on human T cells are associated with higher levels of the survival molecule Bcl-2 and lower levels of cell death (Young et al., 2001). Alternatively (Fig. 2b (ii)), it is known that NK cells help down-modulate the acute response by killing activated T cells and that this killing is reduced by inhibitory receptors expressed on NK cells (Cerboni et al., 2007; Soderquest et al., 2011). In both these scenarios, CD8+ T cells would survive longer in the presence of inhibitory KIRs and thus the protective and detrimental HLA class I associations would be enhanced. Interestingly, this may explain an early finding that the vivo survival of memory T cells required MHC class I molecules but not the restricting allele (Tanchot et al., 1997). At the time this result was difficult to understand as it was assumed that the survival signal came from the T cell receptor (TCR); but it can be explained by the requirement for MHC to bind the inhibitory NK receptor rather than the TCR. The NK cell-dependent pathway (depicted in Fig. 2b (ii)) could also proceed via a “third-party” cell e.g. dendritic cells (DCs) or CD4+ T cells. Cross-talk between NK cells and DCs influences adaptive immunity (Rautel, 2004). However, this interaction is not thought to be influenced by KIR (Della Chiesa et al., 2003; Vitale et al., 2005). Interestingly, control of antiviral CD8+ T cell frequency via NK cell-mediated modulation of CD4+ T cell help has recently been described in LCMV-infected mice (Waggoner et al., 2011).

**Persistence in the face of a strong immune response**

HTLV-1-specific CTL in the peripheral blood of asymptomatic carriers and HAM/TSP patients are frequent, chronically activated and have immediate effector function ex vivo (Hanon et al., 2000; Jacobson et al., 1990). Yet as far as we know HTLV-1 is rarely, if ever, cleared (there are few documented case of HTLV-1 seroconversion in the absence of infected cells). Furthermore, HTLV-1 often persists at a high level: in a Japanese cohort the mean HTLV-1 proviral copy number was 798 per 10^4 PBMC in HAM/TSP patients; and 120 per 10^4 PBMC in asymptomatic carriers (Nagai et al., 1998). How HTLV-1 persists so successfully in the face of an apparently strong CTL response is a subject of great interest. The answer appears to be two-fold. Firstly, HTLV-1 appears to have evolved to minimise the immunogenicity of infected cells. Secondly, even when this mechanism is subverted and immunogenicity is increased, CTL killing appears to be less effective than was previously thought.

**Immunogenicity of HTLV-1-infected cells**

The immunogenicity of HTLV-1-infected cells is reduced by the inhibition of the transcription of viral genes from the positive strand (i.e. all genes with the exception of HBZ). This inhibition is achieved via multiple mechanisms:

(i) HBZ interacts with cellular factors to suppress Tax-mediated transactivation through the 5' LTR and thereby inhibits expression of HTLV-1 genes on the positive strand (Babos et al., 2003a; Gaudray et al., 2002).

(ii) The HTLV-1 provirus acquires mutations which prevent expression of viral proteins. In both ATL and asymptomatic carriers, APOBEC3G-generated nonsense mutations, deletions and insertions which result in loss or truncation of the viral protein are frequent in all viral proteins except HBZ (Fan et al., 2010). Abortive genetic changes may be less frequent in HBZ either because they are generated less frequently in HBZ and/or because they are selected against. It has been suggested that APOBEC3G generates less nonsense mutations in HBZ because HBZ has less APOBEC3G target sequences than any other HTLV-1 gene (Fan et al., 2010).

(iii) Additionally, ATL cells often have a hypermethylated or deleted 5' LTR but an intact functional 3' LTR (Satou et al., 2006). This will prevent transcription of genes on the forward strand but not HBZ which is transcribed from the 3' LTR.

Thus, via multiple mechanisms, the expression of all viral proteins except HBZ is repressed. Additionally, there is evidence that HBZ may directly reduce the immunogenicity of infected cells. It has been shown in HBZ-transgenic CD4+ T cells that an anti-inflammatory phenotype is induced with inhibition of IFN-γ production (Sugata et al., 2012), enhanced sensitivity to TGF-β, and enhanced expression of the regulatory transcription factor FoxP3 (Zhao et al., 2011). This may also facilitate immune evasion. Simultaneously, HBZ induces cellular proliferation. The exact molecular mechanism by which HBZ induces cellular proliferation is unknown. But it is known that HBZ can modulate cellular transcription and signalling pathways, interacting with p300 via its N-terminal activating domain (Clerc et al., 2008), and c-Jun, JunB, CREB and CREB-2 via its leucine zipper domain (Babos et al., 2003b; Gaudray et al., 2002; Hinvin et al., 2007; Thebault et al., 2004). Interestingly, HBZ RNA alone is sufficient to promote the proliferation of infected T-lymphocytes (Satou et al., 2006), HTLV-1 replicates within the host mainly by mitosis of infected cells rather than production of virions. Therefore, HTLV-1 does not need to express all the viral genes to replicate. By suppressing expression of the genes on the positive strand the immunogenicity of infected cells is reduced. By retaining HBZ expression, HTLV-1-infected cells are driven to proliferate and proviral load is increased.
The importance of HBZ in viral persistence has been shown in a rabbit model. Molecular clones of HTLV-1 which were deleted for HBZ could efficiently infect rabbit T cells in vitro and in vivo, however viral loads of HBZ-deleted viruses were consistently significantly lower than wild type HTLV-1, both in early and late infection (Arnold et al., 2006). Thus, while not essential for infection, HBZ contributes to viral persistence.

So the data are consistent with a picture in which expression of all viral genes except HBZ is repressed thereby helping to evade immune surveillance. Expression of HBZ needs to be retained in order to drive infected cell replication. In this picture HBZ expression would be the one weak point which exposed infected cells to the CTL response and would therefore be a crucial target for an effective CTL response. This would explain the finding that HTLV-1-infected individuals with HLA class I molecules which bind HBZ peptides with high affinity have reduced proviral load and reduced HAM/TSP risk (see section “Determinants of protective immunity” and (MacNamara et al., 2010)).

Slow clearance of Tax-expressing cells in vivo

Given the number of mechanisms to reduce the expression of virus proteins one might predict that if these mechanisms were overcome then virus-infected cells would be rapidly killed by CTL. This appears not to be the case. In vitro and in BLV-infected sheep in vivo, sodium valproate, a histone deacetylase inhibitor, activates the expression of Tax and p19 core protein (other viral proteins were not studied) (Achachi et al., 2005; Lezin et al., 2007; Olindo et al., 2009). When given orally to HAM/TSP patients, sodium valproate caused a transient reduction in proviral load in all 19 subjects studied (Lezin et al., 2007; Olindo et al., 2011) consistent with the activation of viral expression in infected cells and subsequent destruction by the host immune system. However, the rate of clearance of infected cells was unexpectedly slow. By fitting a mathematical model to the data from valproate-treated HAM/TSP patients the in vivo rate of CTL lysis of viral-protein expressing cells has been estimated at 0.03–0.13 d−1 (Eleman et al., 2012). This means that, if infected cells were not replaced, then it would take between 5 and 23 days for half of the infected cells to be killed. This is considerably slower than ex vivo estimates of the rate of CTL lysis, which are approximately 1 d−1. This translates into a half life of approximately 1 day for an HTLV-1-infected CD4+ T cell ex vivo (Asquith et al., 2005). Discrepancies between in vitro and in vivo estimates are not uncommon and are probably attributable to factors such as the time CTL need to find their target which will be very different in vivo and in vitro. Interestingly, the rate of CTL killing of HTLV-1-infected cells in vivo is of the same order of magnitude as in vivo estimates for HIV-1 (Asquith et al., 2006) and SIV (Asquith and McLean, 2007; Elemans et al., 2011a; Elemans et al., 2011b; Mandl et al., 2007) obtained using independent methods.

Concluding remarks

We conclude by outlining some of the major questions arising from the work reviewed here.

Is HBZ a viable target for HTLV-1 vaccine design?

The central importance of HBZ in viral persistence makes HBZ a rational target for vaccine design. However, HBZ contains few peptides that are predicted to bind HLA class I molecules well (MacNamara et al., 2010): a feature that may help explain its poor immunogenicity. A lack of potential CTL epitopes is concerning. Nevertheless, the fact that between-individual differences in HBZ binding have such a large impact on clinical outcome is exciting. Possession of one or more alleles that can bind HBZ peptides was associated with the prevention of 48% of cases of HAM/TSP in a study cohort (MacNamara et al., 2010). This suggests that vaccines to boost HBZ-specific immunity could result in a large reduction HAM/TSP risk, at least in individuals with HLA class I molecules that permit HBZ binding.

Why is CTL killing low in vivo?

To date, the only intervention that has resulted in a convincing reduction in HTLV-1 proviral load is treatment with sodium valproate. Sodium valproate has an excellent safety profile and treatment caused a reproducible reduction in proviral load (Lezin et al., 2007; Olindo et al., 2011). However, the CTL killing of infected cells in vivo was slow and proviral load was not suppressed in the long term (Elemans et al., 2012; Olindo et al., 2011). Mouse studies and ex vivo work with HTLV-1-specific CTL indicate that CTL killing has the potential to be remarkably effective both in vivo and ex vivo (Asquith et al., 2005; Barber et al., 2003; Regoes et al., 2007). Understanding why CTL killing is low in vivo in HTLV-1 infection, even after viral gene expression has been activated, may be key to achieving a more rapid and sustained reduction in proviral load following treatment with sodium valproate.

How do KIRs affect the outcome of HTLV-1 infection?

KIR genotype has a striking impact on the outcome of HTLV-1 infection: KIR2DL2 is associated with a 6-fold increase in the odds of remaining asymptomatic in the context of the protective HLA molecule C*08 and a 12-fold increase in the risk of developing HAM/TSP in the context of the detrimental molecule B*54 (Seich Basatena et al., 2011). Understanding how KIR2DL2 interacts with the adaptive response to determine clinical outcome may suggest new pathways to reduce HAM/TSP risk. The observation that KIR2DL2 is also associated with the enhancement of HLA class I associations in HCV infections suggests that KIR2DL2 enhancement of the HLA class I-restricted response may be a general mechanism and thus of interest and importance for the wider immunology research community.

References


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