Molecular biology and pathogenesis of the human T-cell leukaemia/lymphotropic virus Type-1 (HTLV-1)

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Received for publication 12 January 2001
Accepted for publication 30 January 2001

Summary. Retroviruses are associated with a variety of diseases, including immunological and neurological disorders, and various forms of cancer. In humans, the Human T-cell Leukaemia/Lymphotropic virus type 1 (HTLV-1), which belongs to the Oncovirus family, is the aetiological agent of two diverse diseases: Adult T-cell leukaemia/lymphoma (ATLL) (Poiesz *et al*. 1980; Hinuma *et al*. 1981; Yoshida *et al*. 1982), as well as the neurological disorder tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM) (Gessain *et al*. 1985; Rodgers-Johnson *et al*. 1985; Osame *et al*. 1986). HTLV-1 is the only human retrovirus known to be the aetiological agent of cancer.

A genetically related virus, HTLV-2, has been identified and isolated (Kalyanaraman *et al*. 1982). However, there has been no demonstration of a definitive aetiological role for HTLV-2 in human disease to date. Simian T-cell lymphotropic viruses types 1 and 2 (STLV-1 and -2) and bovine leukaemia virus (BLV) have also been classified in same group, Oncoviridae, based upon their similarities in genetic sequence and structure to HTLV-1 and -2 (Burny *et al*. 1988; Dekaban *et al*. 1995; Slattery *et al*. 1999). This article will focus on HTLV-1, reviewing its discovery, molecular biology, and its role in disease pathogenesis.

History

Epidemiological studies had suggested that a transmissible agent was involved in ATLL, as unusual clusters of ATLL were noted in some areas of Japan (Uchiyama *et al*. 1977). The use of T-cell growth factor, now designated Interleukin 2 (IL-2), enabled the culture of leukaemic T-cells (Morgan *et al*. 1976). This finding was significant, as it resulted in the detection and isolation of the first human retrovirus HTLV-1 from a T-lymphoblastoid cell line (HUT 102) established from a patient with a cutaneous T-cell lymphoma (Poiesz *et al*. 1980). Type C retroviral particles were detected in the culture supernatant by electron microscopy. At the same time, an independently isolated cell line (MT-1), derived from a patient with leukaemia, was shown to harbour a retrovirus and to produce antigens reactive against sera from ATLL patients (Hinuma *et al*. 1981). The viruses

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Table 1. Regulatory proteins encoded by the pX region of HTLV-1

Protein	Size	Localization	Function
$p12^{I}$	12 kDa	Endomembranes Golgi and endoplasmic reticulum	Required for viral replication and infectivity of primary lymphocytes *in vivo*; Binds to β and γ_c chains of the IL-2 receptor, the 16 kDa subunit of the vacuolar ATPase, and the MHC I heavy chain
$p13^{II}$	13 kDa	Mitochondria	Required for the maintenance of high viral loads *in vivo*; May interfere with mitochondrial function
$p30^{II}$	30 kDa	Nucleoli	Required for the maintenance of high viral loads *in vivo*; Modulate the transcription of cellular genes
Rex, $p27^{III}$	27 kDa	Nucleoli	Post-transcriptional regulator of viral gene expression
Rex, $p21^{III}$	21 kDa	Cytoplasm	Unknown
Tax, $p40^{IV}$	40 kDa	Nuclei	Transcriptional and post-translational regulator

from the two different cell lines were shown to be identical and thus, HTLV-1 was identified as the aetiological agent of ATLL (Yoshida *et al*. 1982).

The neurological disorder tropical spastic paraparesis or HTLV-1 associated myelopathy (TSP/HAM) is the other major disease associated with HTLV-1 infection. In 1985, sera from neurological patients with TSP, which is endemic in the West Indies, were also shown to have immune reactivity to HTLV-1 (Gessain *et al*. 1985). At the same time in Japan, HTLV-1 serum reactivity was found in non-ATLL patients. This condition was designated HAM (Osame *et al*. 1986).

HTLV-1 infection has also been associated with a number of other diseases, such as chronic arthropathy, uveitis, infective dermatitis, and polymyositis (Murphy *et al*. 1989; Blattner 1990; Yamaguchi & Takatsuki 1993). However the role of HTLV-1 in these disorders is still under investigation.

It is estimated that 10–20 million people world-wide are infected with HTLV-1, which is endemic to southern Japan, Africa, the Caribbean, and eastern parts of South America. HTLV and STLV appear to have originated from a common ancestor virus that may have been transmitted to humans by contact with nonhuman primates (Koralnik *et al*. 1994; Slattery *et al*. 1999). PCR amplification of HTLV-1 proviral sequences from Andean mummies indicate that HTLV-1 infection has existed in humans for a long period of time (Li *et al*. 1999).

HTLV-1 has been shown to infect a wide variety of human and nonhuman cells *in vitro* (Clapham *et al*. 1984; Krichbaum-Stenger *et al*. 1987). However, CD4+ T-cells are the predominant target *in vivo*. The cellular receptor has yet to be identified, although the use of viral interference assays suggests that the receptor localizes to human chromosome 17 (Sommerfelt *et al*. 1988).

HTLV-1 is transmitted sexually or by blood, blood products, and breast milk and induces a lifelong chronic infection. Unlike HIV, HTLV-1 virions are poorly infectious *in vitro* and transmission of HTLV-1 occurs mainly through cell-to-cell contact. ATLL occurs in 1–2% of infected carriers generally 20–30 years after infection (Yamaguchi & Takatsuki 1993). Epidemiological studies have shown that ATLL develops mainly in individuals that were infected in infancy. This long latency period of ATLL suggests that the accumulation of genetic mutations, in addition to HTLV-1 infection, may be required for the induction of ATLL.

HTLV-1-associated diseases

ATLL is an aggressive lymphoproliferative disease whose clinical course can be classified into five different stages: asymptomatic, pre-Leukaemic, chronic/smouldering, lymphoma, and acute (Yamaguchi *et al*. 1983; Yamaguchi & Takatsuki 1993). The majority of HTLV-1 infected individuals are asymptomatic, but capable of transmitting the virus. Morphologically abnormal T-cells with highly lobulated or flower-shaped nuclei are pathognomonic of HTLV-1 infection (Takatsuki *et al*. 1985) and usually have a mature phenotype (CD2+, CD3+, CD4+, CD8-, CD25+, and HLA-DR +). Clonal populations of T-cells carry HTLV-1 proviral DNA, as detected by southern hybridization and of these, one T-cell clone may become malignant. Approximately one-half of preleukaemic individuals undergo a spontaneous regression, while some progress to chronic/smouldering ATLL. Skin lesions and marrow involvement are typically found in patients with smouldering ATLL, whereas patients with chronic ATLL usually have elevated numbers of circulating leukaemic cells. Patients with smouldering or chronic ATLL can progress into acute

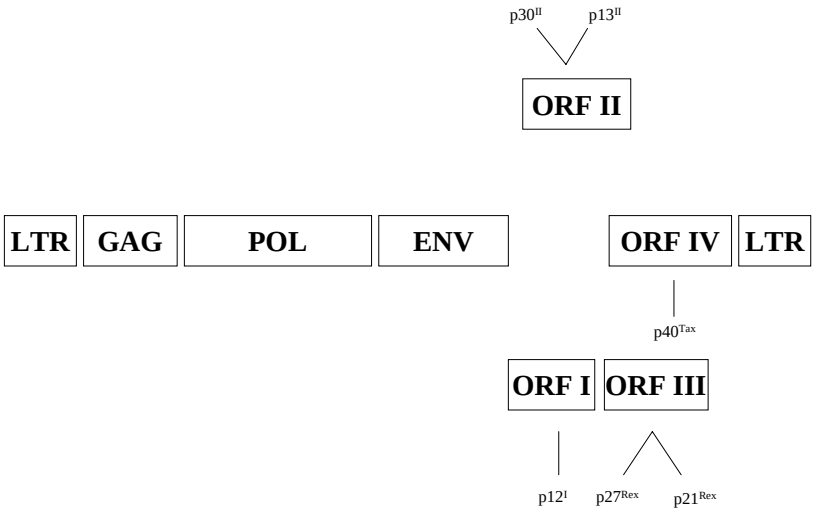

Figure 1. Schematic representation of the HTLV-1 genome.

ATLL, a very aggressive form of leukaemia, within a period of months. Acute ATLL is characterized clinically by hypercalcemia, elevated lactate dehydrogenase levels (LDH), skin lesions, lymphadenopathy, lymphomatous meningitis, lytic bone lesions, spleen or liver involvement, and immunodeficiency (Kondo *et al*. 1987; Murphy *et al*. 1989). There is a dominant clone of malignant cells, as demonstrated by a single rearrangement of the T-cell receptor gene (TCR), as well as one or two (rarely more) proviral copies in the neoplastic T-cells (Shimoyama *et al*. 1983).

Although HTLV-1 is the cause of ATLL, the various mechanisms by which leukaemogenesis occurs are not fully defined. The virus itself does not carry any host-derived oncogenes (Yamaguchi *et al*. 1986), nor does it activate a cellular oncogene upon proviral integration at a common site (Seiki *et al*. 1984). It has been suggested that HTLV-1 infection is only one step in a multistep process that has yet to be entirely elucidated.

TSP/HAM is a chronic demyelinating disease affecting women more often than men, usually beginning in adulthood (Gessain *et al*. 1985; Rodgers-Johnson *et al*. 1985; Osame *et al*. 1986). In contrast to ATLL, TSP/HAM can develop in some patients within years of HTLV-1 infection, which is often a result of a blood transfusion. TSP/HAM is characterized by weakness and spasticity of the extremities, mild peripheral sensory loss, and hyperreflexia. There are lesions in the white matter of the spinal cord with demyelination and axonal changes. The majority of TSP/HAM patients are seropositive for anti-HTLV-1 antibodies (Osame *et al*. 1987). As with ATLL, morphologically atypical lymphocytes can be seen in the peripheral blood; distinct from ATLL, polyclonal integration of proviral DNA is common. In a few cases however, ATLL and TSP/HAM have been known to occur in the same patient. HTLV-1 DNA can be found in blood and CSF lymphocytes (Yoshida *et al*. 1987; Bhagavati *et al*. 1988). Characterization of viral isolates have not demonstrated any obvious differences, biological or genetic, from those isolated from ATLL patients and the disease mechanism underlying TSP/HAM has yet to be fully determined. Some evidence suggests that central nervous system (CNS) damage may be a direct result of infected cells being recognized and lysed by the host's immune system. HTLV-1 can infect neuronal cells *in vitro* (Lehky *et al*. 1995), as well as *in vivo* (Yoshida *et al*. 1987; Bhagavati *et al*. 1988) and high levels of HTLV-1 specific cytotoxic T-lymphocytes (CTLs) have been detected in TSP/HAM patients and may contribute to the neurological damage (Greten *et al*. 1998; Jacobson *et al*. 1990; Usuku *et al*. 1991). Alternatively, damage may result indirectly due to autoimmune or cytokine-mediated mechanisms. TNF-α, GM-CSF, IFN-γ, and IL-1 levels found in TSP/HAM patients are increased vs. levels in asymptomatic carriers (Watanabe *et al*. 1995). Certain HLA haplotypes have been associated with the development of TSP/HAM, which is not the case for ATL (Usuku *et al*. 1991; Jeffery *et al*. 1999).

Animal models

HTLV-1 can infect animals, such as rabbits, rats, and monkeys (Akagi *et al*. 1985; Nakamura *et al*. 1987; Oka *et al*. 1992; Taguchi *et al*. 1993). Rats infected with HTLV-1 were shown to develop a chronic progressive myeloneuropathy with similarities to HAM (Kushida *et al*. 1994; Kasai *et al*. 1999). Several animal models for ATLL have been described in rats and rabbits (Ohashi

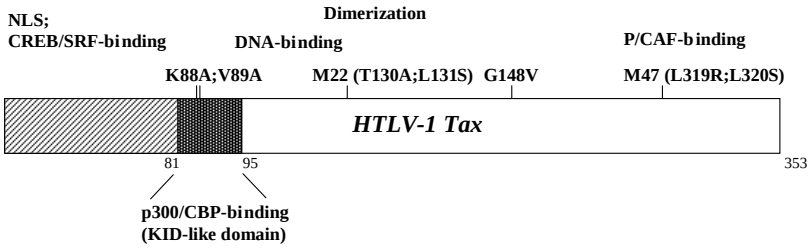

Figure 2. Schematic representation of the domain organization of the HTLV-1 trans-activator. The nuclear localization sequence (NLS) as well as the CREB/SRF-binding domains are located at the N-terminus of the protein. A KID-like sequence which recruits p300/CBP spans residues 81–95; a DNA-binding domain that has been shown to contact the minor-groove of the G/C-rich flanking sequences of the U3 21 bp-repeat elements is found immediately adjacent to the co-activator-binding region. The homodimerization domain overlaps the M22 mutation. A trans-activation domain overlaps the M47 mutation and is responsible for the recruitment of the co-adaptor/histone acetyltransferase, P/CAF.

et al. 1999; Simpson *et al*. 1996). However, while leukaemic cells can be transplanted in these models, leukaemia of rat or rabbit origin does not develop in any of these animals. Ongoing studies using transgenic animals are currently examining the effect of single or multiple viral genes, particularly the viral transactivator Tax, on leukaemogenesis. Tax has been shown to play an important role in the induction of tumours in transgenic mice (Hinrichs *et al*. 1987; Grossman *et al*. 1995; Yamada *et al*. 1995).

Genetic organization

The HTLV-1 carries a single-stranded RNA genome of approximately 9 kb, which encodes the structural and enzymatic proteins, gag, env, and pol, similar to other retroviruses (Figure 1) (Franchini 1995). However, the HTLV-1 genome contains a unique region at the 3′ end, designated the pX region, which encodes regulatory proteins, such as Tax and Rex and additional proteins whose functions are now being investigated (Table 1).

HTLV-1 long-terminal repeat (LTR)

The HTLV-1 long-terminal repeat (LTR) located at the 5′ and 3′ ends of the viral genome, contains the viral promoter and other regulatory elements and is divided into U3, R, and U5 regions. The U3 region contains elements that control proviral transcription and mRNA termination and polyadenylation signals. There are three imperfect 21-base pair nucleotide repeats, designated Tax responsive elements (TRE), that are necessary for transcriptional activation by the Tax protein. Similar to other retroviruses, the full length mRNA encodes the gag protein (p55), which is then cleaved by the viral protease to yield the matrix (MA, p19), capsid (CA, p24), and nucleocapsid (NC, p15) proteins. The protease is encoded by a reading frame that spans the 3′ end of gag and the 5′ end of pol and results from ribosomal frameshifting. In addition, the full length mRNA also encodes the pol protein, which is synthesized by ribosomal frameshifting. A single spliced mRNA encodes the env protein and a double spliced mRNA encodes the Tax and Rex regulatory proteins. In

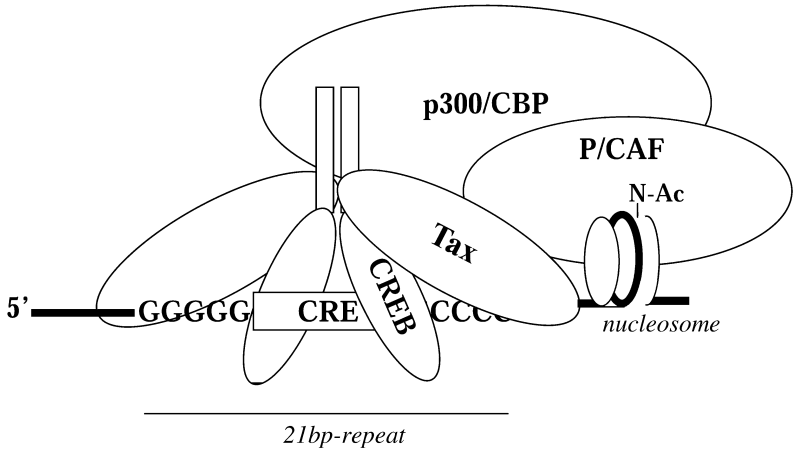

Figure 3. Diagram of the CREB-dimer bound to the core CRE of the HTLV-1 21 bp-repeat. Tax interacts with CREB as a dimer and independently recruits the cellular coactivators, p300/CBP and P/CAF, which activate transcription by histone-acetylation/chromatin-remodeling.

addition, several alternatively spliced products are derived from open reading frames (ORFs) I, II, III, IV of the pX region. mRNAs encoding these proteins have been detected in HTLV-1 infected cells *in vitro* and in *ex vivo* samples isolated from asymptomatic carriers, ATLL, and TSP/HAM patients (Ciminale *et al*. 1992; Koralnik *et al*. 1992a, b).

Tax

The HTLV-1 trans-activator, Tax, is a 40-kDa protein comprised of 353 amino acid residues that drives viral gene expression from three repetitive 21 bp-enhancer elements located within the U3 region of the long-terminal repeat (LTR) (Beimling & Moelling 1992; Zhao & Giam 1992; Paca-Uccaralertkun *et al*. 1994). The domain organization of Tax is depicted schematically in Fig. 2. An atypical nuclear localization sequence spans the first 48 amino acids and an amino-terminal domain that interacts with the cellular transcription factors, cyclic AMP-responsive element-binding protein (CREB) and serum-response factor (SRF or $p67^{SRF}$) overlaps this region (Adya & Giam 1995; Smith & Greene 1992; Suzuki *et al*. 1993; Goren *et al*. 1995; Tian *et al*. 1995). Interactions with these factors are responsible for HTLV-1 LTR-trans-activation, as well as the activation of certain proliferative genes during cellular transformation. The dimerization domain spans the M22 (T130A; L131S) mutation and the transcriptional coactivators, p300/CREB-binding protein (p300/CBP) and the p300/CBP-associated factor (P/CAF), bind two distinct regions in the amino-terminus and carboxyl-terminus of Tax, respectively (Figure 3) (Harrod *et al*. 1998; Smith & Greene 1990; Tie *et al*. 1996; Jiang *et al*. 1999; Harrod *et al*. 2000). Importantly, a stretch of amino acids located downstream from the p300/CBP-binding domain that interacts with the G/C-rich flanking sequences in the HTLV-1 21 bp-repeats was identified through photochemical cross-linking and endopeptidase cleavage experiments (Kimzey & Dynan 1998; Lenzmeier *et al*. 1998; Kimzey & Dynan 1999).

Tax interacts with CREB and the coactivators p300/CBP on three 21 bp-repeats in the HTLV-1 LTR and is reported to stabilize the formation of CREB/ATF-dimers bound to DNA (Giebler *et al*. 1997; Zhao & Giam 1992; Perini *et al*. 1995; Harrod *et al*. 1998). Each 21 bp-repeat element contains a core cyclic AMP-responsive element (CRE) that is flanked by 5′ and 3′ G/C-rich sequences that contact amino acid residues in Tax (Lenzmeier *et al*. 1998; Paca-Uccaralertkun *et al*. 1994). Binding of Tax to p300/CBP occurs independent of CREB Ser-133 phosphorylation and facilitates constitutive viral gene expression in the absence of cellular signalling; however, CREB-phosphorylation enhances the ability of Tax to trans-activate cellular CREs (Kwok *et al*. 1996). Interestingly, the kinase-inducible domain (KID)-like domain in Tax (spanning residues 81–95) that recognizes the hydrophobic kinase-inducible exchange (KIX) region in p300/CBP was found to bear significant similarity to amino acids comprising the KID surrounding Ser-133 in CREB that undergoes Ca^{2+}-dependent phosphorylation (Parker *et al*. 1996; Harrod *et al*. 1998; Yan *et al*. 1998). This domain in Tax likely mediates many of the interactions through which the viral trans-activator pleiotropically dysregulates the expression of numerous cellular genes. Indeed, competition for utilizing a limiting nuclear pool of p300/CBP may provide a basis for Tax-dependent inactivation/repression of certain transcription factors, including p53 and c-Myb (Ariumi *et al*. 2000; Colgin & Nyborg 1998; Van Orden *et al*. 1999). Most recently, the KID-like domain in Tax was shown to mediate Tax-associated cell-death or apoptosis through 'squelching' of the nuclear coactivator p300 (Nicot & Harrod, 2000). This observation suggests that aberrant coactivator usage might pose an early barrier to neoplastic transformation that must be selectively overcome for the establishment of malignancy. The carboxyl-terminus of Tax, proximal to the M47 (L319R; L320S) mutation, was shown to interact with P/CAF independent of p300/CBP-binding – providing further complexity to the molecular mechanisms underlying dysregulated gene expression by HTLV-1 (Smith & Greene 1990; Jiang *et al*. 1999; Harrod *et al*. 2000). The p300/CBP-binding defective Tax mutants, K88A and V89A, retain their abilities to bind P/CAF and significantly trans-activate an NF-κB-dependent promoter (Nicot & Harrod, 2000). Finally, coexpression of P/CAF increased Tax-dependent trans-activation from the HTLV-1 21 bp-repeats and prevented trans-repression by the adenoviral E1A 12S protein which inhibits the histone acetyl-transferase (HAT) activities of both p300/CBP and P/CAF (Chakravarti *et al*. 1999; Harrod *et al*. 2000).

Rex

Rex is a 27-kDa phosphoprotein encoded by ORF III that localizes to the nucleolus of infected cells (Nagashima *et al*. 1986; Inoue *et al*. 1991). Rex, p27, plays an essential role in viral replication and the regulation of viral structural genes, by functioning as a post-transcriptional regulator that increases the expression of singly spliced and unspliced viral mRNAs (env, gag, and pol, respectively) (Hidaka *et al*. 1988). As a result, Rex,

whose phosphorylation state appears to be important for its function (Adachi *et al*. 1992), increases the amount of incompletely spliced viral mRNA in the cytoplasm of infected cells at the expense of Tax/Rex mRNA (Inoue *et al*. 1986; Hidaka *et al*. 1988). HIV-1 encodes a protein, Rev, with similar functions.

Rex is a site specific RNA binding protein that binds to a cis-acting Rex responsive element (RxRE), a highly stable stem-loop structure located within the R region of the viral LTR (Ballaun *et al*. 1991; Yoshida *et al*. 1987; Seiki *et al*. 1988; Hanly *et al*. 1989; Bar-Shira *et al*. 1991; Unge *et al*. 1991). Rex-mediated regulation is required to balance the spliced and unspliced mRNAs necessary for the production of infectious virus. The exact mechanism(s) of Rex regulation is not known, but it is suggested that this interaction may promote transport of mRNAs from the nucleus to the cytoplasm (Hanly *et al*. 1989; Inoue *et al*. 1991). In addition, Rex may inhibit splicing and degradation of viral mRNAs (Grone *et al*. 1996).

ORF III also encodes a smaller alternatively spliced protein, p21, whose exact function is unknown and is not discussed (Kiyokawa *et al*. 1985; Orita *et al*. 1991).

$p13^{II}$ and $p30^{II}$

$p13^{II}$ and $p30^{II}$ proteins, encoded by the ORF-II reading frame, are now the subject of intensive studies. Proteins encoded by the ORF-II reading frame are not essential for viral replication *in vitro* or T-cell immortalization *in vitro* (Derse *et al*. 1997; Robek *et al*. 1998). However, ORF II is important for viral infectivity *in vitro*, suggesting their importance (Bartoe *et al*. 2000).

The $p30^{II}$ protein, encoded by a doubly spliced pX-Tax-ORF II mRNA, localizes to the nucleoli (Koralnik *et al*. 1993). As $p30^{II}$ contains serine-rich domains with distant homologies with several transcriptional activators: Oct-1, Oct-2, Pit-1, Engrailed, and POU-M1, it is suggested that it may modulate transcription of cellular genes (Ciminale *et al*. 1992).

The $p13^{II}$ protein is encoded by a singly spliced ORF II mRNA and localizes to mitochondria *in vitro* (Koralnik *et al*. 1993). As a result of $p13^{II}$ expression, there is an alteration of the mitochondrial structure and a disruption of the inner membrane potential, which could be relevant for HTLV-1 replication or pathogenicity (Ciminale *et al*. 1999). African swine fever virus has been shown to induce a redistribution of mitochondria to perinuclear viral assembly sites, resulting in mitochondria with enhanced respiratory functions that could provide energy for viral morphogenesis (Rojo *et al*. 1998).

$p12^{I}$

ORF I encodes a 12-kDa protein ($p12^{I}$). While $p12^{I}$ expression has been difficult to demonstrate in HTLV-1-infected cells, indirect evidence suggests its importance. The spliced mRNA encoding $p12^{I}$ has been detected in *in vitro* and *ex vivo* HTLV-1-infected T-cells and macrophages (Koralnik *et al*. 1992a, b). Recently, sera from rabbits experimentally infected with HTLV-1, as well as sera from humans infected with HTLV-1, has been shown to recognize the ORF-1 product (Dekaban *et al*. 2000). Moroever, a CTL response to ORF-1 products can be detected in HTLV-1-infected individuals (Pique *et al*. 2000). Importantly, while $p12^{I}$ does not appear necessary for HTLV-1 replication *in vitro* (Derse *et al*. 1997; Robek *et al*. 1998), the ablation of the acceptor splice site for the $p12^{I}$ mRNA results in impairment of viral infectivity *in vitro* (Collins *et al*. 1998).

$p12^{I}$ exhibits weak oncogenic activity, shares amino-acid similarities with the bovine papillomavirus (BPV) type 1 E5 oncoprotein (Franchini *et al*. 1993), and binds to the IL-2-receptor (IL-2R) β and γ_c chains (Mulloy *et al*. 1996). Recent work has demonstrated that while IL-2-mediated proliferation and Jak/Stat activation in HTLV- 1 immortalized T-cells appears independent of ORF-1 expression (Collins *et al*. 1999), $p12^{I}$ is necessary for the infection of primary lymphocytes *in vitro* (Albrecht *et al*. 2000). This suggests a role for $p12^{I}$ in the activation of host cells in the early stages of infection where interaction of $p12^{I}$ with components of cell signalling pathways, such as the IL-2-receptor β and γ_c chains, may contribute to host cell activation, thus resulting in an increased rate of viral infection.

Two natural variants of the $p12^{I}$ protein have been identified: one carries a Lysine at position 88 and is commonly found in HTLV-1 strains from TSP-HAM patients; the second carries an Arginine at position 88 and is found in HTLV-1 strains from all ATLL patients and healthy carriers studied (Trovato *et al*. 1999). The $p12^{I}R88$ protein has a much greater stability compared to the $p12^{I}K88$ protein, which is ubiquitinated and rapidly degraded by the proteasome (Trovato *et al*. 1999), suggesting that this sequence variation might have a functional relevance.

Interestingly, the HTLV-1 $p12^{I}$ and Nef proteins of HIV/SIV appear to share common features. Nef is dispensable *in vitro*, but is required for *in vivo* replication and pathogenicity (Kestler *et al*. 1991), as is $p12^{I}$ (Collins *et al*. 1998). *In vitro*, $p12^{I}$ has been shown to bind the 16- kDa subunit of the vacuolar ATPase (Franchini *et al*. 1993) and Nef to bind to the catalytic subunit of the same enzyme (Lu *et al*. 1998).

Recently, $p12^I$ has been shown to interfere with the assembly of the MHC I heavy chain and β_2-microglobulin complex and its trafficking to the cell membrane (Johnson et al. Submitted). It may do so by taking advantage of a pathway termed ERAD (ER-associated degradation pathway), whose purpose is to remove misfolded, inappropriately glycosylated or improperly assembled proteins from the ER (Bonifacino & Klausner 1994). Several viruses have evolved mechanisms to escape immune recognition by affecting the expression of MHC I on the cell surface (Ploegh 1998; Tortorella *et al*. 2000), including Nef, which has been shown to affect MHC I levels at the cell surface (Schwartz *et al*. 1996). The two natural alleles of $p12^I$, one of which ($p12^IK88$) is ubiquitinated, may differ in their ability to affect antigen presentation and therefore modulate the host-specific immune response. In this regard, the finding that the ubiquitinated form of $p12^I$ ($p12^IK88$) is commonly found in TSP-HAM (Trovato *et al*. 1999), an immune-mediated disease (Osame *et al*. 1986; Jacobson *et al*. 1988), indicates that dissecting the functional consequence of the two natural alleles of $p12^I$ may further our understanding of HTLV-1 pathogenesis.

It has been suggested that $p12^I$ plays an important role early during HTLV-1 infection and in this respect, $p12^I$ expression might enable the virus to establish infection in the host. The ability of $p12^I$ to target the MHC I heavy chain for degradation may decrease the density of MHC I complexes presenting viral peptides on the cell surface and protect infected cells from lysis by cytotoxic lymphocytes (CTLs). In addition, the down-regulation of MHC I complexes containing Tax peptides would allow Tax-expressing cells to survive; Tax, a protein that plays a major role in HTLV-1 pathogenesis, is extremely immunogenic (Jacobson *et al*. 1990). Further, the interference of augmentation of the IL-2R-signalling pathway(s) by $p12^I$ may contribute to proliferation and activation of HTLV-1 infected T-cells, thus contributing to a persistant viral infection.

Pathogenic mechanisms

HTLV-1 has been shown to induce T-cell activation and proliferation. Activated and dividing T-cells have been shown to have an increased susceptibility to HTLV-1 infection, in comparison with quiescent T-cells (Merl *et al*. 1984). Thus, T-cell activation may be necessary to enable the virus to establish infection after entry. Spontaneous T-cell proliferation has been observed in cultures (Gazzolo & Duc Dodon 1987; Tendler *et al*. 1990) or in T-cell colony-forming cells derived from PBMCs of healthy carriers and TSP/HAM patients (Lunardi-Iskandar *et al*. 1993). T-cells are stimulated by cell contact to divide without the requirement of accessory cells. This activation is mediated by CD2/Lymphocyte function associated molecule-3 (LFA-3) and LFA-1/intracellular adhesion molecule, as well as IL-2/IL-2R. Further, *ex vivo* derived HTLV-1-infected T-cell clones have been shown to spontaneously proliferate up to two weeks after stimulation without exogenous IL-2 (Wucherpfennig *et al*. 1992). This proliferative capability is independent of the IL-2/IL-2R pathway. In addition, HTLV-1 virions have been shown to be mitogenic for quiescent human T-cells (Gazzolo & Duc Dodon 1987). As heat-inactivated virions also possess this mitogenic ability, it is suggested that the interaction of the virion with a specific cell surface receptor(s) causes this activation. CD2 has been suggested to be involved in this virion-induced cellular activation (Dodon *et al*. 1989), although CD2 has been ruled out as a principle receptor for HTLV-1, as CD2-negative cells are susceptible to HTLV-1 infection. However, it is possible that T-cell membrane contamination of the virion preparation could be responsible for this effect (Wucherpfennig *et al*. 1992; Kimata *et al*. 1993).

HTLV-1 has been shown to immortalize primary human peripheral blood T-cells *in vitro* and these cells become IL-2 independent after long-term culture. At this time, the T-cells express high levels of the IL-2 receptor α chain (IL-2Rα), characteristic of ATLL cells, as well as HTLV-1 transformed cells *in vitro*. In addition, IL-2 independence correlates with the constitutive activation of Jak/Stat pathways (Migone *et al*. 1995; Xu *et al*. 1995; Mulloy *et al*. 1998b), as well as a decreased expression of the src homology 2 (SH2)-containing tyrosine phosphatase 1 (SHP-1) protein that functions to regulate signalling from several haematopoietic surface receptors (Leonard & O'Shea 1998).

The proliferation of *ex vivo* derived ATLL cells was found to be associated with the constitutive activation of Jak/Stat proteins, which may contribute to neoplastic growth (Takemoto *et al*. 1997). Indeed, the constitutive activation of Jak and/or Stat proteins has been correlated with cell transformation in other transformation models: Abelson murine leukaemia virus (Danial *et al*. 1995), Epstein-Barr virus (Weber-Nordt *et al*. 1996), and spleen focus-forming virus (Ohashi *et al*. 1995). Jak/Stat proteins were not found to be constitutively activated in T-cell lines infected with HTLV-2 or STLV-2 which suggests that these phylogenetically related viruses may have different mechanisms of pathogenesis from HTLV-1 (Mulloy *et al*. 1998a).

While HTLV-1 transforms human primary T-cells *in*

vitro and *in vivo*, leukaemia develops in only a small number of infected individuals after an extended latency period. This suggests that a multistep oncogenic process may take place during this time, resulting in cellular proliferation and the accumulation of genetic mutations (Franchini 1995); several lines of evidence suggests that Tax plays a crucial role in this oncogenic process.

Tax-expressing transgenic mice develop tumours and Tax-transforms rat fibroblasts in combination with a constitutively active, mutant form of the ras oncogene (Nerenberg *et al*. 1987; Pozzatti *et al*. 1990; Yamaoka *et al*. 1996). Most recently, Tax has been demonstrated to cause a $CD8^+$ leukaemia-like condition in transgenic mice when expressed from a construct driven by the T-cell specific, granzyme-B promoter (Grossman *et al*. 1995). Neoplastic transformation caused by the HTLV-1 Tax requires activation of the NF-κB and SRF transcription pathways (Smith & Greene 1990; Yamaoka *et al*. 1996; Akagi *et al*. 1997). Tax activates a number of cellular genes, including IL-2, IL-2Rα, GM-CSF, and PTHRP (Dittmer *et al*. 1997; Ruben *et al*. 1988; Wano *et al*. 1988; Doi *et al*. 1989; Nimer *et al*. 1989). However, the expression of certain cellular genes can be inhibited by Tax; the β-polymerase gene is trans-repressed by Tax through interactions with beta-helix-loop-helix (bHLH)-factors (Jeang *et al*. 1990; Uittenbogaard *et al*. 1994). The Tax protein interacts with amino acids in the bZip domain of the IκK-γ subunit of the IκB-kinase signalling complex to specifically activate/phosphorylate its IκK-β subunit (Chu *et al*. 1999; Harhaj & Sun 1999; Jin *et al*. 1999). Indeed, flat revertants of rat fibroblasts transformed by Tax contained mutations in the NF-κB-essential modulator (NEMO, the rodent homologue of hIκK-γ) and were trans-complemented by a NEMO-expressing cDNA clone (Yamaoka *et al*. 1998). Human T-cell leukaemia virus, type-1, transformed cells exhibit constitutive NF-κB trans-activation and phosphorylation/degradation of cytoplasmic IκB-α/β (Good *et al*. 1996). Trans-activation of the adult T-cell leukaemia-derived factor (ADF) by Tax is also believed to play an essential role in activating NF-κB-dependent transcription by stabilizing the redox state of RelA (p65) (Tagaya *et al*. 1989; Okamoto *et al*. 1992). Interestingly, Rosin *et al*. (Rosin *et al*. 1998) have reported that a S258A Tax-mutant, defective for activation of the NF-κB pathway while retaining its ability to activate CREB-dependent transcription, immortalized primary lymphocytes *in vitro* when expressed from a herpes saimiri-based vector (Rosin *et al*. 1998).

The HTLV-1 trans-activator, Tax, has been demonstrated to aberrantly affect a number of cell-cycle regulatory molecules. The amino-terminus of Tax has also been shown to stabilize and inactivate the tumour suppressor p53 in HTLV-1 transformed cell-lines possibly by inducing hyperphosphorylation of p53 (Cereseto *et al*. 1996; Mulloy *et al*. 1998b; Pise-Masison *et al*. 1998a; Pise-Masison *et al*. 1998b; Takemoto *et al*. 2000). Hyperphosphorylation of the p110 retinoblastoma protein (Rb) is associated with Tax expression; Tax also induces phosphorylation of cyclin D1-cdk4/6 and cyclin D3, potentially contributing to G1/S-phase progression in ATLL cells despite elevated levels of $p21^{Waf/Cip1}$ (Cereseto *et al*. 1996; Neuveut *et al*. 1998; Schmitt *et al*. 1998). Further, the Tax protein is reported to inactivate the cdk-inhibitor, $p16^{Ink4a}$, as well as the human mitotic arrest-deficiency-1 (MAD-1) protein that regulates the anaphase-promoting complex (APC) and segregation of metaphase chromosomes during mitosis (Suzuki *et al*. 1996; Jin *et al*. 1998). Inhibition of MAD-1 by Tax has been proposed to promote the multinucleation that is frequently observed in Reed-Sternberg-like HTLV-1-transformed cells due to the formation of micronuclei, improper chromosomal alignment, or mitotic spindle-damage (Jin *et al*. 1998; Majone *et al*. 1993). Thus, Tax acts to promote the acquisition of genetic mutations, inhibits tumour suppressor and cdk-inhibitor functions, and drives viral gene expression and leukaemic proliferation through interactions with the cellular transcriptional machinery.

Conclusion

HTLV-1 infects approximately 10–20 million people worldwide. While only a relatively small percentage of infected individuals develop disease, knowledge of HTLV-1 pathogenic mechanisms has significantly contributed to the general understanding of cellular transformation.

Acknowledgements

The authors would like to thank Drs M.G. Ferrari and R. Fukumoto for the critical reading of this manuscript. We also thank Steven Snodgrass for editorial assistance.

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