Journal of NeuroVirology (1996) 2, 60–69

Review

HAM/TSP and ATL: persistent paradoxes and new hypotheses

Xiao Xu, Olaf Heidenreich and Michael Nerenberg

Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10666 N. Torrey Pines Road, CAL-1, La Jolla, California 92037, USA

Keywords: TSP/HAM; HTLV-1; retrovirus; ATL; latency

Introduction

Greater than 15 years of study of the biology and pathogenesis of Human T-Lymphotropic Virus type 1 (HTLV-1), have highlighted several persistent puzzles. One of the most profound is the lack of explanation for the difference between the phenotypes of ATL and HAM/TSP.

In the vast majority of cases, infection leads to little or no apparent pathology. It is an unfortunate subgroup of patients (less than 5%) which develop the malignancy Adult T-cell leukemia/lymphoma (ATL) (Kaplan *et al*, 1990; Tajima and Kiroishi, 1985). These patients may exhibit an incubation period up to 40 years prior to development of disease, which is characteristically highly lethal. In another category of disease caused by the virus, HTLV-1-associated myelopathy also called tropical spastic paraparesis (HAM/TSP) (Gessain *et al*, 1985; Osame *et al*, 1986), patients develop chronic progressive spasticity, motor weakness and bladder and bowel dysfunction (Roman *et al*, 1987). These patients exhibit high levels of cellular immunity against the virus (Elovaara *et al*, 1993), and express higher levels of virus than ATL (Furukawa *et al*, 1995). This disease may occur after a very short incubation period.

How can (apparently) the same virus cause such different diseases? Clues may lie in the unusual biologic cycle.

Natural and unnatural means of transmission

Because free virus is very poorly infectious, transmission of infection generally requires a sizable innoculum of infected cells. Like other mammalian retroviruses, the virus may be transmitted vertically between mother and offspring by breast milk. However, studies indicate this to be a rather inefficient process, with greater than 60% of infected carrier mothers failing to transmit infection to their offspring (Maehama *et al*, 1992) and transmission to offspring generally requires greater than 7 months (Oki *et al*, 1992). Transmission associated with leukemia most frequently occurs through breast milk as this allows for the long incubation period required. Alternatively, infection at a young age may induce abnormalities in lymphoid development, such as was recently observed in HTLV-1 infection in a SCID-hu model (Feuer *et al*, 1995). This could favor late development of leukemia.

The other natural route of infection is through sexual intercourse. Epidemiologic studies suggest that such transmission is more commonly male to female (Stuver *et al*, 1993) with approximately half the wives converting 1–4 years after marriage (Take *et al*, 1993). Thus, the above two naturally occurring means of transmission need multiple exposures, suggesting low multiplicity of infectious units per contact.

In contrast, a much more rapid means of transmitting HTLV-1 infection is through inoculation of whole blood or blood cells. Infection may occur in up to 80% of patients given freshly collected (less than 6 days) infected red cell transfusions (Sullivan *et al*, 1991). In this later population, the incidence of HAM/TSP is much more common (Osame *et al*, 1990) and may occur with an incubation period as short as several months. This pattern of transmission could be consistent with an uncommon viral mutation giving rise to TSP/HAM, which might not be maintained under more selective conditions characterized by lower innocula. Such a mutation could be detrimental for the virus, either for transmission or immune surveillance against the virus. In the extreme case, the virus giving rise to HAM/TSP may be defective, requiring coinfection by a fully competent virus. For example, development of mouse MAIDS requires such an interaction.

Correspondence: M Nerenberg
Received 17 August 1995; revised 6 November 1995; accepted 18 December 1995

Viral replication and expression

Are HTLV-1 genes completely latent *in vivo* or are they still expressed to some extent during the long incubation period? Does HTLV-1 gene expression and viral load determine the site and character of disease? Unfortunately, these crucial questions still remain only partially answered. Results from limited studies utilize different methods and only partially agree. Early analyses by conventional Western and Nothern blotting failed to detect HTLV-1 gene expression in circulating ATL cells (Sugamura *et al*, 1984; Tochikura *et al*, 1985). Using the sensitive RT-PCR method, HTLV-1 tax/rex mRNA was barely detectable in ATL cells. From these early studies it was estimated that tax/rex mRNA was expressed in only about 1:1000 cells (Kinoshita *et al*, 1989), suggesting viral genes for the most part remain silent even in highly activated ATL cells. In contrast, Setoyama *et al.* (1994) used digoxigenin labelled probes for *in situ* analysis of tax/rex mRNA on purified PBLs from HTLV-1 infected patients. These authors claimed positive staining in seven out of 10 ATLL patients. The percentage of tax/rex mRNA positive cells seen in total PBLs was 1–5% in asymptomatic carriers (three cases), 10–50% in chronic ATL (two cases), and 50–100% in seven ATL patients (Setoyama *et al*, 1994). These studies were not quantitative. However, in contrast to the previous study, these results suggested a low but uniform level of virus expression in ATL which per infected cell, did not differ greatly from that seen in carriers. In a third study, Furukawa *et al* use PCR to perform more precise comparisons between copies of proviral DNA and levels of the viral gene expression in PBLs from patients with ATL or HAM/TSP and asymptomatic carriers. Tax RNA was expressed at up to 100× higher levels in HAM/TSP patients than in carriers or ATL patients. HAM/TSP patients were shown to have a higher pro-viral load (2–20 copies/100 PBL), while carriers demonstrated 0.4–8 copies/100 PBL (Kubota *et al*, 1993). Higher viral replication in HAM/TSP has been confirmed in two additional studies (Shinzato *et al*, 1993; Yoshida *et al*, 1989). When normalized to DNA content, ATL patients were found to express at least 50-fold lower levels of tax per infected cell than either carrier or HAM/TSP patients. Taken together, these data are most consistent with a uniformly lower level of expression of HTLV 1 in ATL cells, while carrier and HAM/TSP express approximately equal amounts of tax per cell. The overall higher level expression in HAM/TSP may result from a high viral load when compared to carriers.

Specific tropism within the CNS still remains controversial. Kubota *et al.* used quantitative PCR to correlate proviral copy number with lymphocytic infiltration (Kubota *et al*, 1993). Good correlation with $CD40^+$ T cell infiltration suggested lymphocytes were the major reservoir of HTLV-1 in the CNS. In contrast, Jacobsen and colleagues (Lekhy *et al*, 1995) have suggested that HTLV-1 may infect nonlymphocytic parenchymal cells of the brain. This was not confirmed in an additional *in situ* PCR study (Hara *et al*, 1994). Occasional infection of cells other than lymphocytes would not be surprising given the ability of this virus to infect many cell types *in vitro* (Hoxie *et al*, 1984; Ho *et al*, 1984; Nagy *et al*, 1983; Sinangil *et al*, 1985; Giraudon *et al*, 1995; Sakai *et al*, 1993; Koyanagi *et al*, 1993; Macatonia *et al*, 1992). Even if present, without evidence of expression in these cells *in vivo*, the role in pathogenesis of HAM/TSP remains unclear.

Human host factors

HTLV-1 specific cytotoxic T lymphocytes (CTLs) may play an important role in HTLV-1 mediated neuron disorders or other HTLV-1 related inflammatory reactions. Early reports indicated that HAM/TSP patients showed high levels of CTL activity against Tax protein, while asymptomatic carriers did not (Jacobson *et al*, 1990). HLA A2.1 in particular was strongly associated with presentation of an important Tax epitope (Utz *et al*, 1992). Initial studies also suggested a possible genetic predisposition to the development of HAM/TSP with HLA clustering possibly causing high and low responder phenotypers (Usuku *et al*, 1988). However, more recent data suggests little difference in the magnitude of CTL response between HAM/TSP patients and carriers. Induction rate of HTLV-1 specific CTLs was 86% in HAM/TSP patients or patient with other HTLV-1 mediated inflammatory disorders, 57% in asymptomatic carriers and 18% in ATL patients (Kannagi *et al*, 1994). Although Tax has an epitope strongly associated with HLA-A2, Tax specific CTLs were only found frequently in HLA-A2 positive HAM/TSP patients but not from other HLA-A2 positive HTLV-1 infected individuals such as ATL patients. Thus, there is no clear correlation of this genetic marker with development of HAM/TSP (Kannagi *et al*, 1994). Nevertheless, other genetically determined host factors may be responsible for development of HAM/TSP, which may be difficult to distinguish from viral genetics. One such example occurs in Tumaco, Colombia, where the incidence of HAM/TSP is greater than 100 times higher than that found in Japan despite a seroprevalence which is not higher (Trujillo *et al*, 1992). Other examples of familial clusterings of HAM/TSP have also been reported (Salazar-Grueso *et al*, 1990; Miyai *et al*, 1987).

Clues from other mammalian retroviruses

Genetic restriction of disease susceptibility has also been demonstrated for other mammalian retro-

viruses. For example, development of MAIDS is mouse MHC haplotype dependent (Makino *et al*, 1990; Hamelin-Bourassa *et al*, 1989). Further, this intense immune mediated disease occurs when a highly variant and defective virus is coinoculated with a fully competent retrovirus (Huang *et al*, 1991; Chattopadhyaya *et al*, 1991). In this case, development of disease is associated with a high innoculum of virus, such as might occur after blood transfusion or heavy contamination, a situation similar to what may occur in HAM/TSP. Nevertheless, there is no current evidence for association of a defective retrovirus in the genesis of HAM/TSP.

Extensive studies have been performed on families of murine leukemia viruses which cause variant tissue tropism and disease. In these cases disease outcomes may be heavily influenced by changes in envelope protein products or in the enhancer regions which affect tissue specific infection or expression (Oliff *et al*, 1984; Des Groseillers *et al*, 1985). For example, nondefective Friend murine leukemia virus (MuLV) causes erythroleukemia when injected into newborn mice, while Moloney MuLV causes T-cell lymphoma. Exchange of the Friend virus enhancer region (core site) within the LTR for the corresponding region in Moloney MuLV confers the ability to cause erythroid disease on Moloney MuLV (Lenz *et al*, 1984; Oliff *et al*, 1984; Des Groseillers *et al*, 1985). More refined studies demonstrated that such significant disease shifts in Moloney virus may result from single point mutations introduced into the core or its adjacent site (Speck *et al*, 1990). Specific LTR changes also contribute to neurotropism in Lake Casitas virus (Cas-Bv-E-MuLV) (see review in Jolicoeur *et al*, 1992).

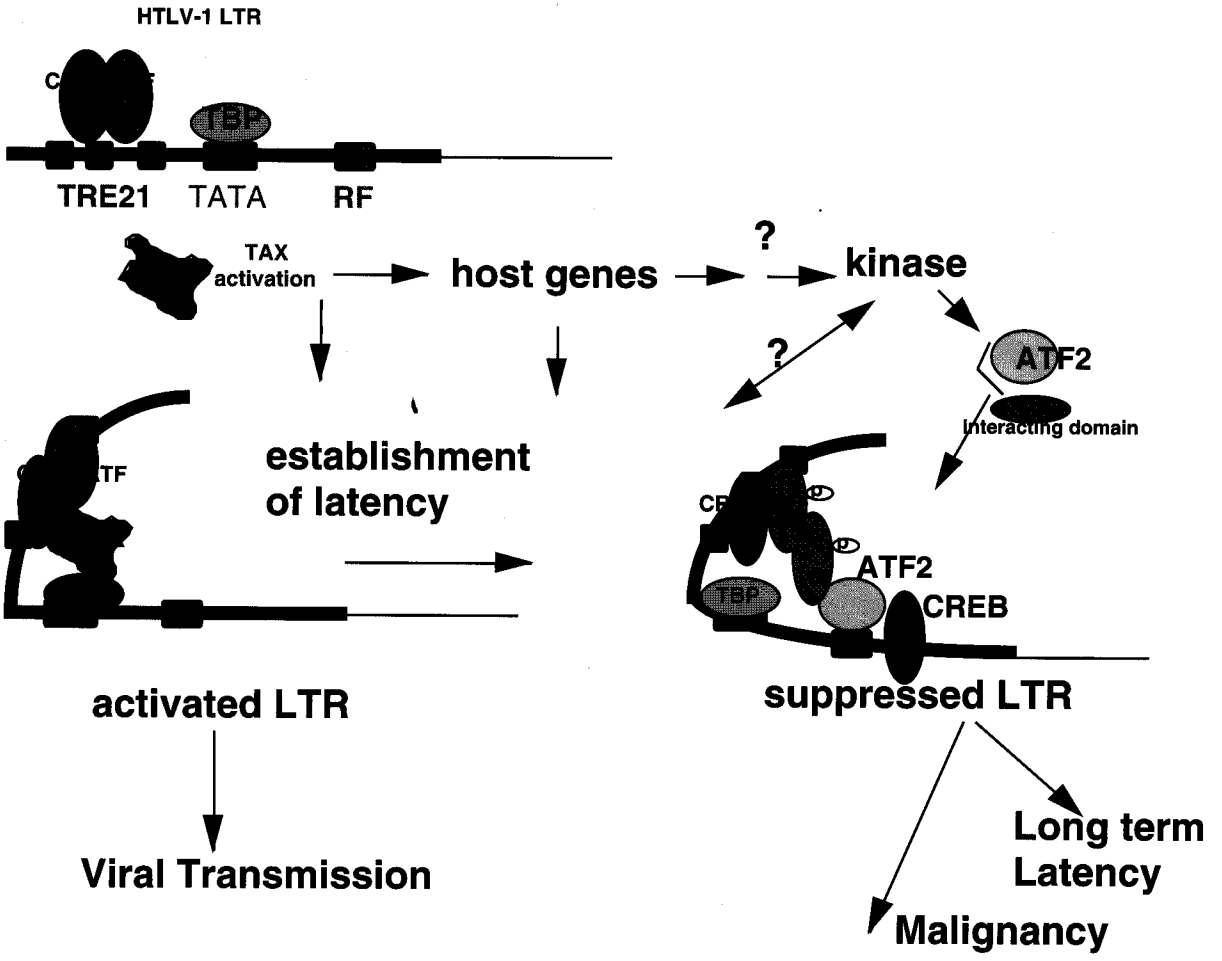

Figure 1 Model of regulation of HTLV-1 promoter. In the basal state, many CREB/ATF family members may bind to the 21 bp repeat enhancers found in the U3 region of the LTR. This may lead to low level transcription of the LTR which gives rise to the tax encoding transcripts. In the presence of tax, transcription from the LTR is greatly augmented, either by serving as a co-activator or by increasing promoter loading of bZip proteins. Up-modulation of the promoter would lead to production of viral structural products and spread from cell to cell. Later on, phosphorylated ATF2 binds either as a homodimer or heterodimer with CREB to the downstream R region and causes suppression of transcription. Such suppression may be seen in naturally occurring malignant cells as well as long term latency.

In contrast to these murine studies, it remains controversial whether the cellular site for replication in HAM/TSP patients is different from that of other patients (Lehky *et al*, 1995; Kubota *et al*, 1993; Hara *et al*, 1994).

Sequence variations within HTLV-1

By this point greater than 50 independent sequences of HTLV-1 isolates from around the world have been obtained and deposited in Genbank. Analysis reveals five major groups (Vidal *et al*, 1994): Cosmopolitan (C) subtype, widespread all over the world; Japanese (J) subtype; West African (WA) subtype; Central African (CA) subtype; and Melanesian (M) subtype. Based on the above information, many strains of HAM/TSP-associated HTLV-1 have been compared to their ATL causing counterparts within respective geographic areas. The LTR and envelope sequences have been most frequently compared. For the most part, these have failed to detect profound sequence differences (Mukhopadhyaya and Sadaie, 1993; Komurian *et al*, 1991; Ratner *et al*, 1991; Evangelista *et al*, 1990; Gonzalez-Dunia *et al*, 1992a,b; Saksena *et al*, 1992). Among HTLV-1 isolates from different geographic locations, TSP/HAM sequences usually demonstrate a higher incidence of variation. This variation may also frequently occur within a single HAM/TSP patient (Saito *et al*, 1995). This is consistent with a higher level of viral replication. However, no specific variations have been defined within the extensively characterized regulatory elements of the U3 region. The tax reponsive 21 base pair repeats in particular, have been noted to be highly conserved. Direct sequence comparisons between infected HAM/TSP patients and their infected asymptomatic spouses has also failed to reveal consistent differences (Nishimura, 1993). However, the significance of mutations adjacent to crucial regulating elements or in important coding regions may be overlooked unless epidemiologic data is carefully correlated with functional assays. Recently, such comparison was performed for the pX region. A partial correlation of HAM/TSP with tax coding sequence variants has been noted (Renjifo *et al*, 1995). These point mutations found in some HAM/TSP patients cause approximately 2–5 fold increase in transactivation in Jurkat cells when compared to ATL isolates. Such subtle differences could be important determinants of the higher tendency toward latency in ATL strains, as well as geographic clustering of HAM/TSP incidence. However, more recent analysis by Gessain and colleagues (Mahieux *et al*, 1995) suggests that these mutations in tax are restricted to the cosmopolitan subtype and do not correlate with specific tendency toward development of HAM/TSP. Further sequence analysis of additional HAM/TSP mutants will be required to determine whether such mutations correlate with disease.

Transcriptional regulation of the U3 region

The vast majority of footprinting and functional analyses of the HTLV-1 LTR have focused on the U3 region, as this is the region which most influences transcription in other retroviruses. Deletion and transient transfection analysis of HTLV-1-LTR-CAT expression vectors has shown that three 21 bp repeats in the HTLV-1 LTR are highly responsive to transactivation by Tax (Shimotohno *et al*, 1986; Paskalis *et al*, 1986; Brady *et al*, 1987). These 21 base elements are called TRE1. Site-directed mutagenesis of these 21 bp repeats abolishes responsiveness to Tax or cAMP, and has demonstrated a core sequence TGACGT to be essential. The first and fifth bases of this sequence appear to be the most crucial for transactivation (Giam and Xu, 1989). The TRE core binds CREB and ATF related factors including CREB, CREM, ATF-1 and ATF-2 (Giam and Xu, 1989, Yoshimura *et al*, 1990, Xu *et al*, 1990, Beimling and Moelling, 1992, Zhao and Giam, 1992, Suzuki *et al*, 1993, Franklin *et al*, 1993, Low *et al*, 1994). In the region of TRE1 just adjacent to the core, AP2 and Sp1 may bind (Muchardt *et al*, 1992, Nyborg *et al*, 1990a, Tillmann *et al*, 1994). In the regions between the TRE1 elements are additional elements which also respond to Tax. These are called TRE2. These appear to bind Ets related factors such as Ets1 and 2 and Elf-1 and other factors such as THP-1 and myb (Gitlin *et al*, 1991, Bosselut *et al*, 1990, 1992, Franklin *et al*, 1993, Clark *et al*, 1993, Tanimura *et al*, 1993).

Tax itself does not appear to directly bind to the LTR, and does not alter the footprinting patterns on the HTLV-1 LTR (Jeang *et al*, 1988, Nyborg *et al*, 1990, Nyborg and Dynan, 1990, Altman *et al*, 1988). Experiments with inhibitors have demonstrated that new protein synthesis is not necessary for tax to cause this transcriptional augmentation (Giam *et al*, 1986, Ruben *et al*, 1989). Tax has been demonstrated to increase loading of B ZIP family members in general onto DNA (Franklin *et al*, 1993, Wagner and Green, 1993, Armstrong *et al*, 1993, Baranger *et al*, 1995, Perini *et al*, 1995), and thus to potentiate their effects.

Potential downstream transcription targets

In contrast to the large body of work describing the importance of the U3 (enhancer) region of HTLV-1 in regulation, much less work has been performed analyzing the downstream R or U5 regions and little attention has been paid to naturally occurring

mutations in this region. Early studies showed the R region could dramatically modulate heterologous promoters such as SV40 (Takebe *et al*, 1988). Deletion analysis identified a functional element within the R region which mapped between +104 and +240 (Nakamura 1988, Gartenhaus *et al*, 1991, Seiki *et al*, 1990). In addition, a 45 bp element located near the R-U5 junction (+202−+246) was shown to strongly bind cellular proteins (Kashanchi *et al*, 1993). We have carefully mapped the R region, defining sequences which bind nuclear proteins and their potential effects on promoter regulation. Using the DNAse 1 protection assay, we identified a specific 18 bp (+210−+227) region. Further characterization of this sequence by methylation interference with binding revealed an 8 bp region of close protein contact as positive +220>+227. Single point mutations at +220 (C>T), +223 (A>G) or +225 (C>A) greatly diminished protein binding (Xu *et al*, 1996). Protein binding was highly dependent on phosphorylation. Binding could be increased by protein kinase A stimulation (Xu *et al*, 1994) or by MAP-like kinase activation. *In vitro* dephosphorylation greatly diminished binding to DNA. Antibodies to CREB or ATF2 identified these transcription factors as components of the complex. This was also confirmed by affinity purification using the target in the R region and by Southwestern analysis (Xu *et al*, 1996). Transfection analysis revealed that occupancy of the R but not U3 region was associated with negative regulation of the promoter. Such downstream binding occurred irrespective of tax expression. Further, a single point mutation at +223 (A>G) in the LTR, totally abolished the protein binding and suppression.

Interestingly, some of the transcription factors binding to the downstream R region sequences may also bind the upstream U3 region. How can the same proteins cause opposite effects when binding to different regions? Why does Tax only regulate upstream binding but not downstream binding?

Recent studies suggest that Tax activates promoters by direct interaction with nuclear proteins (in most cases these are bZIP transcription factors) which may also bind constitutively to the U3 target in the absence of tax. Tax may either further increase the binding affinity or serve as a coactivator. Indeed, both *in vivo* (Brown *et al*, 1994) and *in vitro* (Nyborg *et al*, 1990, Nyborg and Dynan, 1990) footprinting, reveal little difference in binding induced by tax. In contrast, protein binding to the R region is highly regulated. In unstimulated cells such as Balb/3T3 cells no binding was detected (Xu *et al*, 1994). Bacterial ATF2 only binds to the R region when phosphorylated. In addition, CREB homodimers by themselves show no binding to the RF element (Xu *et al*, 1994). Further, in contrast to CREB and ATF-1, tax does not appear to directly interact with the bZIP domain of ATF-2 (Bantignies *et al*, 1995). This highly restricted phenomenon may explain the reason that the R region binding is specifically regulated by phosphorylation modification rather than direct Tax interaction as it does on the U3 region.

Phosphorylation on the N-terminus of ATF2 by MAP kinase family members is required for the protein to bind to the target DNA sequence or to form homo- or hetero-dimers (Abdel-Hafiz *et al*, 1992, 1993). In addition to ERKs, c-jun N-terminal kinase (JNK) was recently characterized as a kinase specifically phosphorylating ATF2 (Gupta *et al*, 1995). Most recently, we have found that JNK was constitutively and selectively activated in HTLV-1 transformed human T cells (Xu *et al*, manuscript submitted 1995). This activation may greatly contribute to increases in specific ATF2 loading on the R region, leading to negative regulation of the promoter.

What could go wrong?

In the normal viral life cycle, a balance between activation and suppression may confer biologic advantages. Above all, it may allow a rapid mechanism for environmental adaptation, which may be particularly important for an obligate cell associated virus which has no extracellular life phase. Through tax, the virus would be able to rapidly up-modulate expression at the time of transmission of maternal cells into the fetal circulation. Through suppression, the virus could then rapidly re-establish latency and effectively evade immune response. In the vast majority of cases (>95%) this task is apparently accomplished without causing any disease in the infected host.

Disease may be a consequence of one or two unusual events. In leukemia, viral latency is rarely broken. However, cells take on a highly activated phenotype with expression of NF-κB and cytokines, especially TGF-β (Tendler *et al*, 1991). In primary isolates and in cell lines such as MT1 and MT4, this apparently occurs in the absence of tax or other viral protein expression. Such a 'hit and run' mechanism for virus transformation suggests that tax expression may be necessary for early stages but is not required to maintain this malignancy. Indeed, antisense studies from our lab suggest that Tax expression in tax transformed murine fibroblasts is not necessary to maintain the transformed state (Kitajima *et al*, 1992). Such pleiomorphic disregulation of cytokine expression may result from constitutive activation of far upstream signal transduction pathways such as recently demonstrated for the JAK kinases (Xu *et al*, 1995; Migone *et al*, 1995). The role of tax in malignancy may thus be one of initiating expansion of a cell population susceptible to further events. Indeed throughout the long period prior to devel-

opment of frank malignancy, infected cells undergo polyclonal followed by oligoclonal expansion, prior to monoclonal dominance (Kimata and Ratner 1991, Hahn *et al*, 1984).

The phenotype of HAM/TSP suggests higher overall expression of HTLV-1 proteins with a consequent brisk immune response. These cells apparently secrete a different set of inflammatory cytokines, most notably TNF-α, IL-1β and IFN-γ (Tendler *et al*, 1991). Therefore, HAM/TSP may result from a relative failure to establish long term latency. Association with higher than usual innocula, suggests selection of a rare mutation. To address this, we have analyzed sequences obtained from HAM/TSP patients. We focused on the recently characterized transcription factor binding site in the downstream R region (Xu *et al*, 1994). We have noted that greater than 69% of the HAM/TSP sequences available have variations in this site, the most common of which interfere with binding when analyzed by gel shift. Further, nonbinding variants show higher basal expression in transient assays (Xu *et al*, 1996).

Remaining questions

Naturally occurring mutations in tax or in the R region could explain higher viral expression in HAM/TSP after high innocula. However, neither set of mutations fully explain all of the cases. For example, there are some Japanese HAM/TSP isolates which fail to show mutations in either pX or R region. Furthermore, a higher level of replication in HAM/TSP may lead to more mutations which have no relationship to disease (Saito *et al*, 1995). Also, development and maintenance of antibodies against HTLV-1 in ATL patients suggests that at least some level of expression of the virus must be maintained throughout the long incubation period. Thus, there must be periodic escape from suppression in ATL patients. The anatomic sites for this, and the natural stimuli remain to be determined. Further, there must be other mechanisms or mutations at sites other than tax and the R region which allow escape from suppression in some HAM/TSP cases. Based on our current knowledge of pathogenesis of other viral and genetic diseases, it would certainly be surprising if such multigenetic etiology did not exist!

The newly available clones of infectious HTLV-1 (Derse *et al*, 1995, Kimata *et al*, 1994, Zhao *et al*, 1995) as well as rabbit (Zhao *et al*, 1993) and rat (Ishiguro *et al*, 1992) models should allow testing of each of these hypotheses.

Acknowledgements

We would like to thank V Moreau for help with preparation of this manuscript. These studies were supported by NIH grants CA50234 and NS12428 (to M Nerenberg); by fellowship DK07022 to X Xu; and a fellowship from the DFG to O Heidenreich.

References

Abdel-Hafiz HA, Heasley LE *et al* (1992). Activating transcription factor-2 DNA-binding activity is stimulated by phosphorylation catalyzed by p42 and p54 microtubule-associated protein kinases. *Molec Endocrin* **6:** 2079–2089.

Abdel-Hafiz HA, Chen CY *et al* (1993). Structural determinants outside of the leucine zipper influence the interactions of CREB and ATF-2: interaction of CREB with ATF-2 blocks Ela-ATF-2 complex formation. *Oncogene* **8:** 1161–1171.

Altman R, Harrich D *et al* (1988). Human T-cell leukemia virus types I and II exhibit different DNase I protection patterns. *J Virol* **62:** 1339–1346.

Armstrong AP, Franklin AA *et al* (1993). Pleiotropic effect of the human transcription fractors. *Proc Natl Acad Sci USA* **90:** 7303–7307.

Bantignies F, Rousset R *et al* (1995). Characterization of the cellular proteins permitting transactivation of the HTLV-I promoter by Tax using a genetic approach in yeast. *AIDS Res Hum Retrov* **10:** 216.

Baranger AM, Palmer CR *et al* (1995). Mechanism of DNA-binding enhancement by the human T-cell leukemia virus transactivator Tax. *Nature* **376:** 606–608.

Beimling P, Moelling K (1992). Direct interaction of CREB protein with 21 bp Tax-response elements of HTLV-ILTR. *Oncogene* **7:** 257–262.

Bosselut R, Duvall J *et al* (1990). The product of the c-Ets 1 proto-oncogene and the related Ets2 protein act as transcriptional activators of the long terminal repeat of the human T-cell leukemia virus HTLV-I. *EMBO J* **9:** 3137–3144.

Bosselut R, Lim F *et al* (1992). Myb protein binds to multiple sites in the human T-cell lymphotropic virus long terminal repeat of human T-cell leukemia virus 1 long terminal repeats and transactivates LTR-mediated expression. *J Virol* **186:** 764–769.

Brady J, Jeang KT *et al* (1987). Identification of p40x-responsive regulatory sequences within the human T-cell leukemia virus type I long terminal repeat. *J Virol* **67:** 2175–2181.

Brown DA, Xu X *et al* (1994). Genomic footprinting of the HTLV-I LTR in a transgenic mouse tumorigenic model. *Transgenics* **1:** 377–393.

Chattopadhayay SK, Sengupta DN *et al* (1991). Characteristics and contributions of defective, ecotropic, and mink cell focus-inducing viruses involved in a retrovirus-induced immunodeficiency syndrome of mice. *J Virol* **65:** 4232–4241.

Clark NM, Smith MJ *et al* (1993). Activation of the human T-cell leukemia virus type I enhancer is mediated by binding sites for Elf-1 and the pets factor. *J Virol* **67(9):** 5522–5528.

Derse D, Mikovits J *et al* (1995). Virions released from cells transfected with a molecular clone of human T-cell leukemia virus type I gave rise to primary and secondary infections of T cells. *J Virol* **69:** 1907–1912.

Des Groseillers L, Rassart E *et al* (1985). Retrovirus-induced spongiform encephalopathy: the 3′-long terminal repeat-containing viral sequences influence the incidence of the disease and the specificity of the neurological syndrome. *Proc Natl Acad Sci USA* **82:** 8818–8822.

Elovaara I, Koenig S *et al* (1993). High human T cell lymphotropic virus type 1 (HTLV-1)-specific precursor cytotoxic T lymphocyte frequencies in patients with HTLV-1-associated neurologic disease. *J Exp Med* **177:** 1567–1573.

Evangelista A, Maroushek S *et al* (1990). Nucleotide sequence analysis of a provirus derived from an individual with tropical spastic paraparesis. *Microbial Pathogenesis* **8:** 259–278.

Feuer G, Fraser JK *et al* (1995). HTLV-1 infection of human hematopoietic progenitor cells: effects on differentiation in vitro and on thymopoiesis *in vivo*. Abstract in The Second West Coast Retrovirus Meeting. p48.

Franklin AA, Kubik MF *et al* (1993). Transactivation by the human T-cell leukemia virus tax protein is mediated through enhanced binding of activating transcription factor 2 (ATF-2) response and cAMP element-binding protein (CREB). *J Biol Chem* **268:** 21225–21231.

Furukawa Y, Osame M *et al* (1995). Human T-cell leukemia virus type-1 (HTLV-1) tax is expressed at the same level in infected cells of HTLV-1-associated myelopathy of tropical spastic paraparesis patients as in asymptomatic carriers but at a lower level in adult T-cell leukemia cells. *Blood* **85:** 1865–1879.

Gartenhaus RB, Wong-Staal F *et al* (1991). The promoter of human T-cell leukemia virus type-I is repressed by the immediate-early gene region of human cytomegalovirus in primary blood. *Blood* **78:** 2956–2961.

Gessain A, Barin F *et al* (1985). Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet* **2:** 407–409.

Giam GZ, Nerenberg M *et al* (1986). Expression of the complete human T-cell leukemia virus type I pX coding sequence as a functional protein in *Escherichia coli*. *Proc Natl Acad Sci (USA)* **83(19):** 7192–7196.

Giam CZ, Xu YL (1989). HTLV-I tax gene product activates transcription via pre-existing cellular factors and cAMP responsive element. *J Biol Chem* **264(26):** 15236–15241.

Giraudon P, Bernard A, Malcus C, Dufay N, Desgranges C, Belin MF (1995). Retroviral infection (HTLV-I) induces cytokine-regulated immunomodulation and cytotoxicity of medulloblastoma cells. *Journal of Neuropathology & Experimental Neurology* **54(2):** 165–174.

Gitlin SD, Bosselut R *et al* (1991). Sequence-specific interaction of the Ets1 protein with the long terminal repeat of the human T-lymphotropic virus type I. *Virol* **65:** 5513–5523.

Gonzalez-Dunia D, Chirinian-Syan S *et al* (1992a). Functional analysis of two long terminal repeats from the HTLV-1 retroviruses. *Gene* **161:** 151–158.

Gonzalez-Dunia D, Grimber G *et al* (1992b). Tissue expression pattern directed in transgenic mice by the LTR of an HTLV-1 provirus isolated from a case of tropical spastic paraparesis. *Virol* **187:** 705–710.

Gupta S, Campbell D *et al* (1995). Transcription factor ATF-2 regulation by the JNK signal transduction pathway. *Science* **267:** 389–393.

Hahn B, Gallo RC *et al* (1984). Clonal selection of human T-cell leukemia virus-infected cells in vivo and in vitro. *Mol Biol Med* **2(1):** 29–36.

Hamelin-Bourassa D, Skamene E *et al* (1989). Susceptibility to a mouse acquired immunodeficiency syndrome is influenced by the H-2. *Immunogenetics* **30(4):** 266–272.

Hara H, Morita M *et al* (1994). Detection of human T lymphotropic virus type 1 (HTLV-1) proviral DNA and analysis of T-cell receptor Vβ CDR3 sequences in spinal cord lesions of HTLV-1 associated myelopathy/tropical spastic paraparesis. *J Exp Med* **180:** 381–839.

Ho D, Rota T *et al* (1984). Infection of human endothelial cells by human T lymphotropic virus type 1. *Proc Natl Acad Sci USA* **81:** 7588–7590.

Hoxie JA, Matthews DM *et al* (1984). Infection of human endothelial cells by human T-cell leukemia virus type I. *Proc Natl Acad Sci USA* **81(23):** 7591–7595.

Huang M, Simard C *et al* (1991). The majority of cells infected with the defective murine AIDS virus belong to the B-cell lineage. *J Virol* **65(12):** 6562–6571.

Ishiguro N, Abe M *et al* (1992). A rat model of human T lymphocyte virus type I (HTLV-I) infection. 1. Humoral antibody response, provirus integration, and HTLV-I-associated myelopathy/tropical spastic paraparesis-like myelopathy in seronegative HTLV-I carrier rats. *J Exp Med* **176(4):** 981–989.

Jacobson S, Shida H *et al* (1990). Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature* **348(6298):** 245–248.

Jeang KT, Boros I *et al* (1988). Characterization of cellular factors that interact with the human T-cell leukemia virus type I p40x-responsive 21-base-pair sequence. *J Virol* **62(12):** 4499–4509.

Jolicoeur P, Gravel C *et al* (1992). Pathogenesis of murine spongiform myeloencephalopathy induced by a murine retrovirus. In *Molecular Neurovirology*. Roos, RP (ed) Humana Press, pp 199–244.

Kannagi M, Matsushita S *et al* (1994). Cytotoxic T cells response and expression of the target antigen in HTLV-I infection. *Leukemia* **8:** S54–59.

Kaplan JE, Osame M *et al* (1990). The risk of development of HTLV-I-associated myelopathy/tropical spastic paraparesis among persons infected with HTLV-I. *J Aquir Immune Defic Syndr* **3:** 1096–1101.

Kashanchi F, Duvall JF *et al* (1993). Sequences downstream of the RNA initiation site regulate human T-cell lymphotropic virus type I basal gene expression. *J Virol* **67:** 2894–2902.

Kimata JT, Ratner L (1991). Temporal regulation of viral and cellular gene expression during human T-lymphotropic virus type 1 mediated lymphocyte immortalization. *Virol* **65:** 4398–4401.

Kimata JT, Wong FH *et al* (1994). Construction and characterization of infectious human T-cell leukemia virus type 1 molecular clones. *J Virol* **204(2):** 656–664.

Kinoshita T, Shimoyama M *et al* (1989). Detection of mRNA for the tax/rex1 gene of human T-cell leukemia virus type I in fresh peripheral blood mononuclear cells of adult T-cell leukemia patients and viral carriers by using the polymerase chain reaction. *Proc Natl Acad Sci USA* **86:** 5620–5624.

Kitajima I, Shinohara T *et al* (1992). Ablation of transplanted HTLV-I tax mediated tumors in mice by antisense inhibition of NF-κB. *Science* **258:** 1792–1795.

Komurian F, Pelloquin F *et al* (1991). *In vitro* genomic variability of human T-cell leukemia virus type 1 depends more on geography than upon pathologies. *J Virol* **65:** 3770–3778.

Koyanagi Y, Itoyama Y *et al* (1993). *In vivo* infection of human T-cell leukemia virus type I in non-T cells. *Virol* **196(1):** 25–33.

Kubota R, Fujiyoshi T *et al* (1993). Fluctuation of HTLV-I proviral DNA in peripheral blood mononuclear cells of HTLV-I associated myelopathy. *J Neuroimmunol* **42:** 147–154.

Lehky TJ, Fox CH *et al* (1995). Detection of human T-lymphotropic virus type I (HTLV-I) tax RNA in the central nervous system of HTLV-I-associated myelopathy/tropical spastic paraparesis patients by *in situ* hybridization. *Ann Neurol* **37:** 167–175.

Lenz J, Celander D *et al* (1984). Determination of the leukemogenicity of a murine retrovirus by sequences within the long terminal repeat. *Nature* **308:** 467–470.

Low KG, Chu HM *et al* (1994). Novel interactions between human T-cell leukemia virus type I Tax and activating transcription factor 3 at a cyclic AMP-responsive element. *Mol Cell Biol* **14(7):** 4958–4974.

Macatonia SE, Cruickshank JK *et al* (1992). Dendritic cells from patients with tropical spastic paraparesis are infected with HTLV-1 and stimulate autologous lymphocyte proliferation. *AIDS Res Hum Retrov* **8(9):** 1699–1706.

Maehama T, Nakayama M *et al* (1992). Studies on factor affecting mother to child HTLV-I transmission. *Acta Obstetrica et Gynaecologica Japonica* **44:** 215–222.

Mahieux R, de The G *et al* (1995). The tax mutation at nucleotide 7959 of human T-cell leukemia virus type 1 (HTLV-1) is not associated with tropical spastic paraparesis/HTLV-1-associated myelopathy but is linked to the cosmopolitan molecular genotype [letter]. *J Virol* **69(9):** 5925–5927.

Makino M, Morse HC III *et al* (1990). H-2-associated and background genes influence the development of a murine retrovirus-induced immunodeficiency syndrome. *J Immunol* **144(11):** 4347–4355.

Migone TS, Lin JX *et al* (1995). Constitutively activated Jak-STAT pathway in T cells transformed with HTLV-I. *Science* **269:** 79–81.

Miyai I, Saida T *et al* (1987). Familial cases of HTLV-I-associated myelopathy. *Ann Neurol* **22(5):** 601–605.

Muchardt C, Seeler J-S *et al* (1992). Regulation of HTLV-I gene expression by tax and AP-2. *The New Biologist* **4:** 541–550.

Mukhopadhyaya R, Sadaie M (1993). Nucleotide sequence analysis of HTLV-1 isolated from cerebrospinal fluid of a patient with TSP/HAM: comparison to other HTLV-1 isolates. *AIDS Res Hum Retrov* **9:** 109–114.

Nagy K, Clapham P *et al* (1983). Human T cell leukemia virus type 1: Induction of syncytia and inhibition by patients' sera. *Int J Cancer* **32:** 321–328.

Nakamura M, Ohtani K *et al* (1989). Functional mapping of the activity of the R region in the human T-cell leukemia virus type I long terminal repeat to increase gene expression. *Virus Genes* **2:** 147–155.

Nishimura M, McFarlin DE *et al* (1993). Sequence comparisons of HTLV-I from HAM/TSP patients and their asymptomatic spouses. *Neurol* **43:** 2621–2624.

Nyborg JK, Matthews MA *et al* (1990). Interaction of host cell proteins with the human T-cell leukemia virus type I transcriptional control region. II. A comprehensive map of protein-binding sites facilitates construction of a simple chimeric promoter responsive to the viral tax2 gene product. *J Biol Chem* **265(14):** 8237–8242.

Nyborg JK, Dynan WS (1990). Interaction of cellular proteins with the human T-cell leukemia virus type I transcriptional control region. Purification of cellular proteins that bind the 21-base pair repeat elements. *J Biol Chem* **265:** 8230–8236.

Oki T, Yoshinaga M *et al* (1992). A sero-epidemiological study of mother-to-child transmission of HTLV-I in southern Kyushu. *Asia-Oceania J Obstet Gyne* **18:** 371–377.

Oliff A, Signorelli K *et al* (1984). The envelope gene and long terminal repeat sequences contribute to the pathogenic phenotype of helper-independent Friend virus. *J Virol* **51:** 788–794.

Osame M, Usuku K *et al* (1986). HTLV-I associated myelopathy: a new clinical entity. *Lancet* **1:** 1031–1032.

Osame M, Janssen R *et al* (1990). Nationwide survey of HTLV-1 associated myelopathy in Japan association with blood transfusion. *Ann Neurol* **28:** 50–56.

Paskalis H, Felber BK *et al* (1986). Cis-acting sequences responsible for the transcriptional activation of human T-cell leukemia virus type I constitute a conditional enhancer. *Proc Natl Acad Sci (USA)* **83:** 6558–6562.

Perini G, Wagner S *et al* (1995). Recognition of bZIP proteins by the human T-cell leukemia virus transactivator Tax. *Nature* **376** 602–605.

Ratner L, Philpott T *et al* (1991). Nucleotide sequence analysis of human T-lymphotropic virus type 1 of diverse geographical origins. *AIDS Res Hum Retrov* **7:** 923–941.

Renjifo B, Borrero I *et al* (1995). Tax mutations associated with tropical spastic paraparesis/human T-cell leukemia virus type 1-associated myelopathy. *J Virol* **69:** 2611–2616.

Roman GC, Spencer PS *et al* (1987). Tropical spastic paraparesis in the Seychelles Islands: a clinical and case-control neuroepidemiolgic study. *Neurol* **37:** 1323–1328.

Ruben SM, Perkins A *et al* (1989). Activation of NF-kappa B by the HTLV-I trans-activator protein Tax requires an additional factor present in lymphoid cells. *New Biologist* **1(3):** 275–284.

Saito M, Furukawa Y *et al* (1995). Frequent mutation in pX region of HTLV-1 is observed in HAM/TSP patients, but is not specifically associated with the central nervous system lesions. *J Neurovirol* **1:** 286–294.

Sakai M, Eguchi K *et al* (1993). Infection of human synovial cells by human T cell lymphotropic virus type I. Proliferation and granulocyte/macrophage colony-stimulating factor production by synovial cells. *J Clin Invest* **92(4):** 1957–1966.

Saksena N, Sherman MP *et al* (1992). LTR sequence and phylogenetic analyses of a newly discovered variant of HTLV-I isolated from the Hagahai of Papua New Guinea. *Virol* **189:** 1–9.

Salazar-Grueso EF, Holzer TJ *et al* (1990). Familial spastic paraparesis syndrome associated with HTLV-1 infection. *New Engl J Med* **323(11):** 732–737.

Seiki M, Hikikoshi A *et al* (1990). The U5 sequence is a cis-acting repressive element for genomic RNA expression of human T cell leukemia virus type 1. *Virol* **176:** 81–86.

Setoyama M, Fujiyoshi T *et al* (1994). HTLV-1 messenger RNA is expressed *in vivo* in adult T cell leukemia/lymphoma patients: an *in situ* hybridization study. *J Int Cancer* **57:** 760–764.

Shimotohno K, Takano M *et al* (1986). Requirement of multiple copies of a 21-nucleotide sequence in the U3 regions of human T-cell leukemia virus type I and type II long terminal repeats for trans-acting activation of transcription. *Proc Natl Acad Sci (USA)* **83(21):** 8112–8116.

Shinzato O, Kamihira S *et al* (1993). Relationship between the anti-HTLV-1 antibody level, the number of abnormal lymphocytes and the viral genome dose in HTLV-1-infected individuals. *Int J Cancer* **54:** 208–212.

Sinangil F, Harada S *et al* (1985). Host cell range of adult T cell leukemia virus. I. Viral infectivity and binding to various cells as detected by flow cytometry. *Int J Cancer* **36:** 191–198.

Speck NA, Renjifo B *et al* (1990). Mutation of the core or adjacent LVb elements of the Moloney murine leukemia virus enhancer alters disease specificity. *Genes Dev* **4:** 543–550.

Sugamura K, Fujii M *et al* (1984). Cell surface phenotypes and expression of viral antigens of various human cell lines carrying human T-cell leukemia virus. *Int J Cancer* **34(2):** 221–228.

Sullivan MT, Williams AE *et al* (1991). Transmission of human T-lymphotropic virus types I and II by blood transfusion. A retrospective study of recipients of blood components (1983 through 1988). *Arch Intern Med* **151(10):** 2043–2048.

Stuver SO, Tachibana N *et al* (1993). Heterosexual transmission of human T cell leukemia/lymphoma virus type I among married couples in southwestern Japan: an initial report from the Miyazaki Cohort Study. *J Infect Dis* **167(1):** 57–65.

Suzuki T, Fujisawa JI *et al* (1993). The trans-activator tax of human T-cell leukemia virus type 1 (HTLV-1) interacts with cAMP-responsive element (CRE) binding and CRE modulator proteins that bind to the 21-base-pair enhancer of HTLV-1. *Proc Natl Acad Sci USA* **90(2):** 610–614.

Tajima K, Kiroishi T (1985). Estimation of rate of incidence of ATL among ATLV (HTLV-I) carriers in Kyushu, Japan. *Jap J Clin Oncol* **15:** 423–430.

Take H, Umemoto M *et al* (1993). Transmission routes of HTLV-I: an analysis of 66 families. *Japanese J Cancer Rsch* **84(12):** 1265–1267.

Takebe Y, Seiki M *et al* (1988). SRα promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol Cell Biol* **8:** 466–472.

Tanimura A, Teshima H *et al* (1993). A new regulatory element that augments the Tax-dependent enhancer of human T-cell leukemia virus type 1 and cloning of cDNAs encoding its binding proteins. *J Virol* **67(9):** 5375–5382.

Tendler CL, Greenberg SJ *et al* (1991). Cytokine induction in HTLV-I associated myelopathy and adult T-cell leukemia: Alternate molecular mechanisms underlying retroviral pathogenesis. *J Cell Biochem* **46:** 302–311.

Tillmann M, Wessner R *et al* (1994). Identification of human T-cell lymphotropic virus type I 21-base-pair repeat-specific and glial cell-specific DNA-protein complexes. *J Virol* **68(7):** 4597–4608.

Tochikura T, Iwahashi M *et al* (1985). Effect of human serum anti-HTLV antibodies on viral antigen induction *in vitro* cultured peripheral lymphocytes from adult T-cell leukemia patients and healthy virus carriers. *Int J Cancer* **36(1):** 1–7.

Trujillo JM, Cincha M *et al* (1992). Seroprevalence and cofactors of HTLV-I infection in Tumaoc, Colombia. *AIDS Res Hum Retrov* **8:** 651–657.

Usuku K, Sonada S *et al* (1988). Immune responsiveness against HTLV-I in HTLV-1-associated myelopathy: comparison with adult T-cell leukemia/lymphoma. *Ann Neurol* **23S:** 143–150.

Utz U, Koenig S *et al* (1992). Presentation of three different viral peptides, HTLV-I tax, HCMV gB, and influenza virus M1 is determined by common structural features of the HLA-A2.1 molecule. *J Immunol* **149:** 214–221.

Vidal AU, Gessain A *et al* (1994). Phylogenetic classification of human T cell leukemia/lymphoma virus type I genotypes in five major molecular and geographical subtypes. *J Gen Virol* **75 (Pt 12):** 3655–3666.

Wagner S, Green MR (1993). HTLV-1 Tax protein stimulation of DNA binding by bZIP proteins enhancing dimerization. *Science* **265:** 395–399.

Xu YL, Adya N *et al* (1990). Cellular factors involved in transcription and Tax-mediated trans-activation directed by the TGACGT motifs in human T-cell leukemia virus type I promoter. *J Biol Chem* **265(33):** 20285–20292.

Xu X, Brown DA *et al* (1994). Transcriptional suppression of the human T-cell leukemia virus type 1 long terminal repeat occurs by an unconventional interaction of a CREB factor with the R region. *Mol Cell Biol* **14:** 5371–5383.

Xu X, Kang SH *et al* (1995). Constitutive activation of different Jak tyrosine kinases in Human T-cell leukemia virus type 1 (HTLV-1) tax protein or virus transformed cells. *J Clin Invest* **96:** 1548–1555.

Xu X, Kang SH, *et al* (1996). Sequence requirements of ATF_2 and CREB binding to the Human T-cell leukemia virus type 1 (HTLV-1) LTR R region. *Virology* 1996 (in press).

Yoshida M, Osame M *et al* (1989). Increased replication of HTLV-1 in HTLV-1-associated-myelopathy. *Ann Neurol* **26:** 331–335.

Yoshimura T, Fujisawa J *et al* (1990). Multiple cDNA clones encoding nuclear proteins that bind to the tax-dependent enhancer of HTLV-1: all contain a leucine zipper structure and basic amino acid domain. *EMBO J* **9(8):** 2537–2542.

Zhao LJ, Giam CZ (1992). Human T-cell lymphotropic virus type I (HTLV-I) transcriptional activator, Tax, enhances CREB binding to HTLV-I 21-base-pair repeats by protein-protein interaction. *Proc Natl Acad Sci USA* **89(15):** 7070–7074.

Zhao TM, Robinson MA *et al* (1993). Variation in HTLV-I sequences from rabbit cell lines with diverse *in vivo* effects. *Virol* **195:** 271–274.

Zhao TM, Robinson MA *et al* (1995). Characterization of an infectious molecular clone of human T-cell leukemia virus type I. *J Virol* **69(4):** 2024–2034.