HAM/TSP and ATL: persistent paradoxes and new hypotheses

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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 inoculum 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 inocula. 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.
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 Northern 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 (Kinoshiota 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 ATL 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 times 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 (Shinmato 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 phenotypes (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-Gruese 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 inoculum 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 Groselliers 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 Groselliers 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 Castitas 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 responsive 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 TGA CGT 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, Beitinl 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, Tanamura 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−+264) 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 I 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 co-activator. 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-xB 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-\( \alpha \), IL-1\( \beta \) and IFN-\( \gamma \) (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.

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References


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