

Minireview

Human T cell leukemia virus type I-induced disease: pathways to cancer and neurodegeneration

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Received 18 July 2002; returned to author for revision 3 September 2002; accepted 30 October 2002

Abstract

Retroviral infection is associated with a number of pathologic abnormalities, including a variety of cancers, immunologic diseases, and neurologic disorders. Shortly after its discovery in 1980, human T cell leukemia virus type I (HTLV-I) was found to be the etiologic agent of both adult T cell leukemia (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a neurologic disease characterized by demyelinating lesions in both the brain and the spinal cord. Approximately 5–10% of HTLV-I-infected individuals develop either ATL or HAM/TSP. Interestingly, the two diseases have vastly different pathologies and have rarely been found to occur within the same individual. While a number of host and viral factors including virus strain, viral load, and HLA haplotype have been hypothesized to influence disease outcome associated with HTLV-I infection, the relative contributions of such factors to disease pathogenesis have not been fully established. Recent research has suggested that the route of primary viral infection may dictate the course of disease pathogenesis associated with HTLV-I infection. Specifically, mucosal exposure to HTLV-I has been associated with cases of ATL, while primary viral infection based in the peripheral blood has been correlated with progression to HAM/TSP. However, the cellular and molecular mechanisms regulating disease progression resulting from primary viral invasion remain to be elucidated. Although a variety of factors likely influence these mechanisms, the differential immune response mounted by the host against the incoming virus initiated in either the peripheral blood or the mucosal compartments likely plays a key role in determining the outcome of HTLV-I infection. It has been proposed that the route of infection and size of the initial viral inoculum allows HTLV-I to infect different target cell populations, in turn influencing the breadth of the immune response mounted against HTLV-I and affecting disease pathogenesis. A model of HTLV-I-induced disease progression is presented, integrating information regarding the role of several host and viral factors in the genesis of both neoplasia and neurologic disease induced following HTLV-I infection, focusing specifically on differential viral invasion into the bone marrow (BM) and the influence of this event on the virus-specific CD8⁺ cytotoxic T lymphocyte (CTL) response that is initiated following HTLV-I infection.

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Introduction

An estimated 10–20 million people worldwide are infected with the human T cell leukemia virus type I (HTLV-I) (Edlich et al., 2000). Although the majority of seropositive individuals are considered to be asymptomatic carriers of the virus, 5–10% of infected individuals develop either adult T cell leukemia (ATL) or HTLV-I-

associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Cann, 1996; Uchiyama, 1997). Due to the fact that most infected individuals do not develop disease and HTLV-I infection causes two clinically distinct disease entities, two general questions arise. What factors contribute to whether an individual infected with HTLV-I develops ATL, HAM/TSP, or remains asymptomatic? What are the differences in the pathogenic processes that lead to the two diseases? This review will focus on the role of HTLV-I in the etiology of both neoplasia and neurologic disease, and will specifically address the cellular and molecular events following HTLV-I infection that result in differential disease pathogenesis.

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Viral genome and replication cycle

Mature HTLV-I virions are surrounded by a host-derived lipid envelope embedded with glycoprotein spikes encoded by the viral *env* gene, which encodes two protein components: a 21-kDa transmembrane protein (TM), and a 46 kDa surface glycoprotein (SU). Viral attachment and entry into susceptible cells involves the interaction between the surface glycoprotein gp46 and specific host cell surface receptor(s). Although the receptor for HTLV-I is expressed on a broad range of cell lines derived from multiple species (Trejo and Ratner, 2000), it has yet to be definitively identified. Specific cell surface coreceptors may also facilitate viral attachment and entry, although no such molecules have yet been identified. Following entry, reverse transcriptase (RT) present within the capsid initiates synthesis of viral DNA utilizing genomic RNA as a template (Gallo et al., 1981). The proviral DNA is then transported to the nucleus where it integrates into the host cell genome with the assistance of the viral integrase (IN) carried into the cell within the HTLV-I virion (Varmus, 1988). Following integration, the viral life cycle proceeds with genome replication, transcription of viral genes, translation of viral proteins, virion assembly, and viral release. All of these processes require the participation of cellular transcription, translation, and transport machinery, as well as a number of viral factors (Cullen, 1992; Tillmann et al., 1994; Tillmann and Wigdahl, 1994; Wessner et al., 1995, 1997; Wessner and Wigdahl, 1997).

The HTLV-I genome contains elements that are common to all known replication-competent retroviruses, as well as a region encoding gene products unique to HTLV-I. Common to all retroviruses are the group-specific antigen (*gag*), protease (*pro*)/polymerase (*pol*), and envelope (*env*) genes. Six functionally distinct proteins unique to HTLV-I are encoded within the pX region of the genome. The HTLV-I genome is flanked on either end with noncoding long terminal repeats (LTRs) composed of U3, R, and U5 regions. The viral promoter consisting of the U3 region of the 5' LTR plays a role in determining whether an infected cell is permissive for viral replication and gene expression. Several factors, either host-derived or viral-specific, may regulate gene expression from the integrated provirus and may potentially impact the course of disease progression during and after the primary infection. The viral protein Tax, encoded within the pX region, has been demonstrated to be a critical factor with respect to genomic activation and viral gene expression. Tax is a 40-kDa phosphoprotein essential for both viral replication and cellular transformation. Tax is a transcriptional transactivator and can dramatically increase viral gene expression through its interaction with cellular factors and the 5' LTR of the proviral genome.

In addition to regulating gene expression from the integrated viral LTR, Tax interacts with cellular transcription factors and signaling molecules to enhance or repress cellular gene expression (Grant et al., 2002; Harhaj et al., 1999;

Ng et al., 2001; Wigdahl and Brady, 1996). Aberrant regulation of cellular genes by Tax is thought to contribute to HTLV-I-induced disease pathogenesis. Tax affects the expression of several factors that may contribute to the shift from productive viral replication to cellular transformation observed in ATL. Tax increases the expression of several cytokines and receptors involved in T cell growth and proliferation (Azimi et al., 1998; Ballard et al., 1988; Crenon et al., 1993; Good et al., 1996; Mariner et al., 2001). Additionally, Tax increases the expression of several transcription factors and proto-oncogenes, as well as inhibitors of nucleotide excision repair and apoptosis (Kao and Marriott, 1999; Laherty et al., 1993). Tax has also been shown to repress the expression of cellular genes involved in DNA repair and apoptosis (Brauweiler et al., 1997; Uittenbogaard et al., 1994, 1995). The combined increase in growth-inducing factors and decrease in repair and apoptotic factors in HTLV-I-infected cells by Tax provides an environment that may facilitate T cell transformation and the genesis of ATL. Tax and/or other viral factors may play a similar role in the genesis of other HTLV-I-associated diseases.

Clinical and molecular features of HTLV-I-induced disease pathogenesis

Although HTLV-I infection has been associated with a number of pathologic abnormalities, the most well-characterized diseases caused by HTLV-I are ATL and HAM/TSP (Gessain et al., 1985; Hinuma et al., 1981; Osame et al., 1986; Poiesz et al., 1980; Yoshida et al., 1984). ATL is an aggressive lymphoproliferative disease that develops in approximately 1–5% of seropositive individuals. The leukemic cells in ATL are almost exclusively CD4⁺ T cells, likely reflecting the fact that HTLV-I displays an *in vivo* cellular tropism for this cell population within the peripheral blood. In this regard, it has been demonstrated that 90–99% of HTLV-I DNA segregates with CD4⁺ T cells within the peripheral blood of ATL patients infected with the virus (Richardson et al., 1990). Cases of ATL involving CD4/CD8 double-positive leukemic cells have been described (Ciminale et al., 2000; Ohata et al., 1999). However, while clonal expansion of CD4⁺ T cells has been associated with the development of ATL, clonal expansion of the CD8⁺ T cell population has been found to occur independently of disease association (Eiraku et al., 1998).

The mechanisms by which leukemogenesis is initiated following HTLV-I infection remain under intense scrutiny. Of particular interest to the process of carcinogenesis is the viral protein Tax, which is strongly linked to the genesis of ATL (Franchini, 1995; Uchiyama, 1997) (Fig. 1). Evidence has suggested that Tax induces the expression of cellular genes critical for T cell growth and proliferation, including granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor-alpha (TNF- α), interleukin 15 (IL-15), interleukin 2 (IL-2), and interleukin 2 receptor-

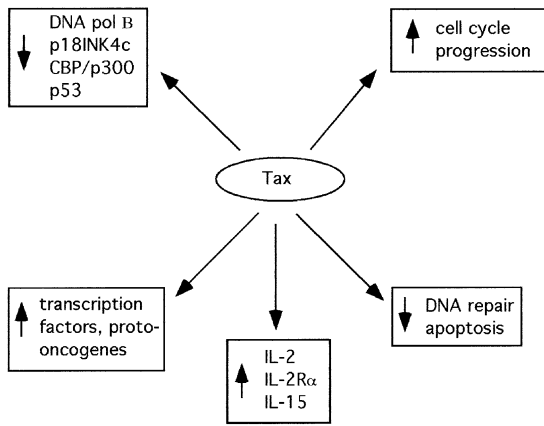


Fig. 1. Mechanisms of HTLV-I-induced cellular transformation. The HTLV-I Tax protein has been demonstrated to be the oncogenic protein of the virus. Tax may contribute to the process of carcinogenesis by a variety of mechanisms, including upregulating the expression of cellular genes involved in T cell growth and proliferation, including IL-2, IL-2R α , and IL-15. Tax is also known to increase the expression of cellular transcription factors, protooncogenes, and inhibitors of nucleotide excision repair and apoptosis. Tax also functions to repress the expression of transcription factors including p18INK4c and p53, while also inhibiting tumor suppressor protein including p16INK4a, p15INK4b, and hDlg to allow for cell-cycle progression. Finally, Tax represses the expression of cellular genes involved in DNA repair and apoptosis. These factors likely cooperate to enhance the process of cellular transformation resulting in ATL.

alpha (IL-2R α) (Azimi et al., 1998; Ballard et al., 1988; Crenon et al., 1993; Franchini, 1995; Good et al., 1996; Mariner et al., 2001; Uchiyama, 1997). Although Tax induces the expression of IL-2, neoplastic T cells from ATL patients eventually lose their dependence on this cytokine and, as a result, are able to proliferate continuously in its absence (Popovic et al., 1984). It has therefore been suggested that Tax contributes to the initiation of the transformation process and once this process is complete, Tax is no longer required to maintain a state of IL-2 independence. Although Tax is essential to the process of leukemogenesis (Ross et al., 1996) and can immortalize T cells in vitro (Akagi et al., 1995; Akagi and Shimotohno, 1993; Grassmann et al., 1992), its presence is necessary but not sufficient to induce T cell oncogenesis in vivo when the tax gene is expressed under the direction of the HTLV-I LTR. Transgenic mice expressing the tax gene driven by the HTLV-I LTR develop neurofibrosarcomas, but fail to develop leukemias or lymphomas (Nerenberg et al., 1987; Tanaka et al., 1990). Mice expressing the tax gene under the direction of the CD3-epsilon promoter-enhancer sequence develop mesenchymal tumors at wound sites, along with salivary and mammary adenomas (Hall et al., 1998), while those expressing the tax gene under the control of the granzyme B promoter develop large granular lymphocytic leukemia (Grossman et al., 1995). These results suggest that in the context of the HTLV-I promoter, the presence of an additional viral element, possibly encoded within the pX region, may be required to facilitate the process of T cell transfor-

mation and the resulting leukemogenesis observed in ATL (Fig. 2A).

Similar to the genesis of ATL, approximately 1–5% of HTLV-I-infected individuals develop HAM/TSP following HTLV-I infection (Hollberg and Hafler, 1993). However, in contrast to the carcinogenic process associated with ATL, HAM/TSP is a progressive neurodegenerative disease that primarily affects the spinal cord and brain. Magnetic resonance imaging (MRI) reveals the presence of multiple white matter lesions in both the spinal cord and the brain involving perivascular demyelination and axonal degeneration (Cruikshank et al., 1989; Godoy et al., 1995; Levin and Jacobson, 1997; Mattson et al., 1987; Nakagawa et al., 1995). Recent studies have indicated that an inflammatory immune response directed against HTLV-I within the CNS may contribute to the observed pathology. Infiltrating CD4⁺ T cells, CD8⁺ T cells, and activated macrophages have been shown to be present within white matter lesions of HAM/TSP patients (Jacobson et al., 1988; Kubota et al., 1994; Levin and Jacobson, 1997; Moritoyo et al., 1996; Umehara et al., 1993). Activated lymphocytes have also been identified in the cerebrospinal fluid (CSF) of these individuals (Jacobson et al., 1992; Levin and Jacobson, 1997), indicating a possible pathogenic role for activated lymphocytes and macrophages in the genesis of HTLV-I-associated neurologic disease. Recently, cytotoxic T lymphocytes (CTLs)

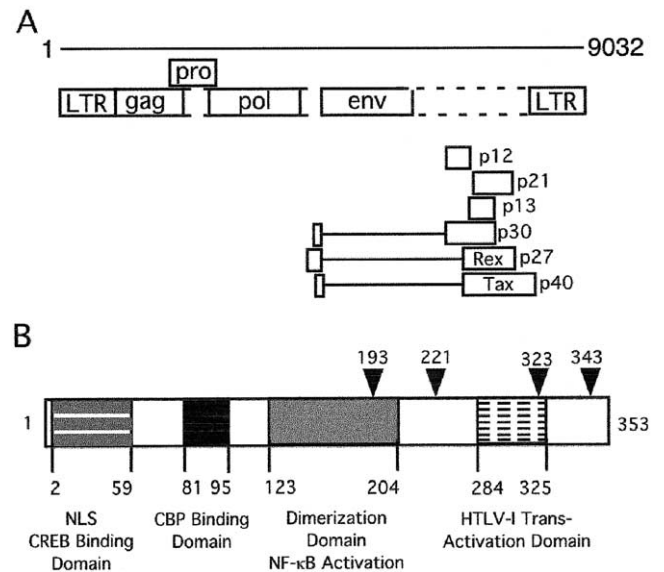


Fig. 2. Structural features of HTLV-I. (A) Proteins encoded by the HTLV-I genome include those common to all known retroviruses, as well as those encoded by a unique pX region at the 3' end of the genome. Several of these proteins may be critical to disease pathogenesis associated with HTLV-I infection. (B) The immunodominant Tax protein is known to be critical to the process of CD4⁺ T cell transformation resulting from HTLV-I infection. However, the role of this protein in the genesis of HAM/TSP is not completely clear. Variation within the sequence of Tax may be a contributing factor. Shown are nonsynonymous substitutions found in a patient that did not progress to HAM/TSP (Accession No. AB 038239).

present within the CSF of HAM/TSP patients have been demonstrated to differ from those found within the periphery by virtue of the fact that those found in the CSF have a high specificity for the HTLV-I Tax protein (Kubota et al., 2002). Targeted lysis of HTLV-I-infected cells within the CNS may contribute to disease pathogenesis associated with HTLV-I-induced neurologic disease. Recent studies have also demonstrated the presence of high levels of proinflammatory cytokines in both the peripheral blood and the CSF of HAM/TSP patients, including interferon gamma (IFN- γ), TNF- α , IL-1, and IL-6 (Kuroda and Matsui, 1993; Kuroda et al., 1993; Nakamura et al., 1993; Nishimoto et al., 1990). Similarly, increased expression of IL-1 β , TNF- α , and IFN- γ has been demonstrated in the CSF of HAM/TSP patients and further substantiates the importance of proinflammatory cytokines with respect to the neuropathogenesis of HTLV-I infection (Umehara et al., 1994).

ATL and HAM/TSP clearly have vastly different pathologies; ATL is characterized by T cell oncogenesis, whereas the genesis of HAM/TSP involves cellular destruction and inflammation. It is therefore not surprising that ATL and HAM/TSP have seldom been found to occur within the same individual, although the combined probabilities of acquiring both diseases simultaneously necessarily dictates a low frequency for the occurrence of ATL and HAM/TSP within the same individual. What factors determine whether an infected individual remains asymptomatic or progresses to either ATL or HAM/TSP? What role do viral factors play in contributing to the inflammatory immune response directed against HTLV-I in HAM/TSP patients? While Tax is known to function as an oncoprotein to facilitate T cell transformation, the molecular mechanisms concerning possible viral contributions to the genesis of HAM/TSP are not known. Additionally, a variety of host factors likely play a significant role in the onset of neurologic disease.

Host and viral factors contributing to HTLV-I-associated disease pathogenesis

An ever-increasing body of knowledge concerning the role of Tax in the genesis of ATL continues to accumulate. Furthermore, several studies have examined the contributions of a number of other viral-related factors including the size of the initial viral inoculum and the overall proviral DNA load during the course of disease in the differential disease pathogenesis associated with HTLV-I infection. A number of investigators have also focused on the contribution of host factors and virus–host interactions to the pathological processes resulting from HTLV-I infection. This includes virus strain, HLA haplotype (Jeffery et al., 1999, 2000; Yashiki et al., 2001), route of infection (Kannagi et al., 2000a,b; Yashiki et al., 2001), and the immune response to HTLV-I (Bangham, 2000a,b; Jeffery et al., 2000).

Virus strain

A number of studies have focused on identifying strains of HTLV-I that are either neuropathogenic or leukemogenic, without conclusive results (Bangham, 2000a; Daenke et al., 1990; Kinoshita et al., 1991; Renjifo et al., 1995). Analyses of proviruses found in the CNS of HAM/TSP patients demonstrated the frequent existence of defective proviruses lacking a portion of the tax gene, suggesting a role for Tax-defective mutants in the genesis of HAM/TSP (Kira et al., 1994). Further studies have demonstrated the existence of escape mutants of Tax during the development of ATL in some patients. Such mutants were found to have a premature stop codon in the 5' half of the tax gene, leading to a protein product with a reduced ability to transactivate the viral enhancer, while other patients had large deletions within the tax gene (Furukawa et al., 2001). Phylogenetic analysis has revealed two tax gene subgroups, with one subgroup displaying a significantly higher incidence in HAM/TSP patients as compared to ATL patients or asymptomatic carriers (Furukawa et al., 2000). An additional study examining the *tax* sequence from monozygotic twins and their infected mother and brother who both suffered from HAM/TSP indicated that three of the infected individuals, including the twin with HAM/TSP, shared a consensus *tax* sequence. However, the *tax* sequence derived from the asymptomatic twin differed at five nucleotide positions, including four substitutions that resulted in changes in the amino acids incorporated into the corresponding protein (Nakane et al., 2000) (Fig. 2B). These results indicate that small nucleotide changes in the tax gene may impact the clinical outcome of HTLV-I infection.

In addition to Tax, other viral proteins may also be involved in the pathogenesis resulting from HTLV-I infection. Based on its interaction with the free immature form of the MHC class I heavy chain molecule (Johnson et al., 2001), p12^I may facilitate HTLV-I replication and evade the host immune response by preventing presentation of viral proteins in the context of MHC class I molecules at the surface of HTLV-I-infected cells by downregulating the cell surface expression of MHC class I molecules. Interestingly, two naturally occurring variants of p12^I have been described, one carrying a lysine at amino acid 88 (K88; predominantly found in HAM/TSP patients), and the other carrying an arginine at amino acid 88 (R88; predominantly found in ATL patients and asymptomatic carriers) (Trovato et al., 1999). Sequence variation within the p12^I protein may be important to its function, since the R88 protein was found to have a much greater stability than the K88 protein, which is ubiquitinated and rapidly degraded by the proteasome (Trovato et al., 1999). The reduced stability of p12^I in HAM/TSP patients as a result of sequence variation may facilitate the generation of a viral-specific CTL response, since degradation of p12^I would alleviate the reduction of MHC class I molecules at the cell surface. These studies indicate that although neuropathogenic or leukemogenic

strains of HTLV-I have not been identified to date, a small number of alterations within the nucleotide sequence of viral genes may result in the production of viral factors that differentially affect the immune response.

HLA haplotype

Specific HLA alleles have been linked to protection from developing HAM/TSP, whereas other HLA alleles have been correlated with an increased risk of developing ATL (Jeffery et al., 1999, 2000; Yashiki et al., 2001). Recent studies indicate that individuals possessing MHC class I molecules that predispose to ATL (HLA-A*26, HLA-B*4002, HLA-B*4006, and HLA-B*4801) have binding peptide anchor motifs that are largely incapable of recognizing 69 different HTLV-I Tax peptides. These individuals are therefore not able to generate an efficient population of Tax-specific CTLs (Yashiki et al., 2001), the generation of which is a hallmark of HAM/TSP. The absence of Tax-specific CTLs results in the severely diminished killing of HTLV-I-infected CD4⁺ T cells, thus allowing for their survival and eventual transformation. In contrast, MHC class I molecules that may predispose HTLV-I-infected individuals to HAM/TSP likely have strong anchor motifs for binding Tax peptides, and therefore these patients mount a strong CTL response against this immunodominant protein. This allows for efficient cytolysis of HTLV-I-infected cells, including CD4⁺ T cells, resulting in the elimination of cells that have the potential to become leukemic. However, a population of efficient Tax-specific CTLs could lead to the targeted lysis of many other cells infected with HTLV-I, including resident CNS cell populations, thus facilitating the pathologic process observed in HAM/TSP. These observations reflect the fact that HTLV-I-specific CTL activity is extremely high in HAM/TSP, but low in ATL patients (Jacobson et al., 1990; Kannagi et al., 1984; Parker et al., 1992). Studies have indicated that the HLA-A*02 allele is protective with respect to HAM/TSP (Jeffery et al., 1999). It has been suggested that a strong antiviral CTL response is initiated by individuals with this HLA type by virtue of its high binding affinity to the Tax (residues 11–19) peptide, resulting in targeted killing of HTLV-I-infected cells and a subsequent low proviral DNA load, thus leading to protection from HAM/TSP. However, a conflicting report has demonstrated that the frequency of the HLA-A*02 allele in HAM/TSP patients is not significantly different from the frequency of this allele found in asymptomatic carriers of the virus (Yashiki et al., 2001). Additionally, ATL patients with this particular allele have been identified. Sequence analysis has indicated that these patients possess variants of Tax that have either a stop codon in the 5' half of the tax gene or an amino acid change in the critical Tax epitope that leads to evasion of the immune response in HLA-A*02 ATL patients (Furukawa et al., 2001). The importance of sequence variation in viral genes is again apparent, and the interplay between HTLV-I genomic variability and the host

immune response to the virus appears to play an important role in the genesis of HTLV-I-induced disease.

Route of primary HTLV-I infection

Viral transmission is thought to require the transfer of HTLV-I-infected T lymphocytes. It has been hypothesized that the initial route of viral infection contributes significantly to the pathogenesis associated with HTLV-I infection (Kannagi et al., 2000a,b; Osame et al., 1990). Specifically, exposure to HTLV-I through the peripheral blood has been correlated with cases of HAM/TSP (Osame et al., 1990), whereas mucosal exposure to the virus is thought to favor the genesis of ATL (Kannagi et al., 2000a,b). When inoculated with HTLV-I-producing MT-2 cells either orally or intravenously, immune-competent rats become persistently infected (Kannagi et al., 2000a,b). However, none of the orally but all of the intravenously-inoculated rats produced significant levels of anti-HTLV-I antibodies and generated a proliferative T cell response against the virus (Kannagi et al., 2000a,b). This coincides with an earlier study demonstrating that oral inoculation of adult rats with HTLV-I induces a state of immune unresponsiveness despite the establishment of a persistent HTLV-I infection (Kato et al., 1998). In general, these results indicate that mucosal (oral) exposure to the virus, rather than bloodborne exposure, leads to the absence of both humoral and cellular immune responses directed against HTLV-I. Such immune unresponsiveness during the primary infection may affect the course of disease pathogenesis associated with HTLV-I infection by impacting the ability of the host to mount an effective immune response against the virus. The lack of a strong T cell response against the virus likely results in the absence of cytolytic activity specific for HTLV-I-infected cells, including CD4⁺ T cells. This could result in the survival and eventual transformation of this infected cell population, eventually leading to leukemogenesis associated with ATL. Conversely, the induction of a strong antiviral immune response, as observed in intravenously-inoculated rats, could lead to the targeted destruction of most cells infected with HTLV-I, including CD4⁺ T cells and cells within the CNS which may themselves become infected by HTLV-I. The end result of this process would be consistent with cellular destruction and inflammation characteristic of HTLV-I-induced neurodegenerative disease.

Immune response to HTLV-I and proviral DNA load

Many of the host and viral factors described above directly impact the host immune response to HTLV-I. HAM/TSP patients have high levels of anti-HTLV-I antibodies in both the serum and the CSF (Gessain et al., 1988; Osame et al., 1986), and the frequency of Tax-specific precursor CTLs was found to be at least 40-

280-fold higher in HAM/TSP patients than in asymptomatic carriers of HTLV-I (Elovaara et al., 1993). Consistent with this finding, HAM/TSP patients exhibit a marked increase in the virus-specific CD8⁺ CTL response, with the majority of antiviral CTLs specific for Tax (Jacobson et al., 1990). Additionally, several studies have demonstrated that patients with HTLV-I-induced neurologic disease display a significant increase in the presence of Tax-specific memory and effector CD8⁺ T cells. Previous studies have reported the presence of high levels of circulating CD8⁺ CTLs (up to 1 in 500) specific for the pX region of the HTLV-I genome (Jacobson et al., 1990, 1992). Significantly, these CTLs were absent in patients without neurologic disease (Jacobson et al., 1990). Similarly, studies have demonstrated that HTLV-I infection induces a greater expansion of CD8⁺ T cells in patients with HAM/TSP as compared to asymptomatic carriers of the virus (Sakai et al., 2001). Interestingly, no correlation was found between an expanded CD8⁺ T cell population and HTLV-I proviral DNA load. Activated Tax-specific CD8⁺ CTLs have been demonstrated to be present in the peripheral blood and accumulate in the CSF of HAM/TSP patients (Greten et al., 1998). Simultaneous comparison of the peripheral blood and CSF from one HAM/TSP patient demonstrated a 2.5-fold higher level of Tax-specific CTLs in the CSF, suggesting that Tax-specific CTLs may play a role in mediating the cellular destruction and inflammation within the CNS. Additionally, longevity of Tax-specific CTL expression demonstrated that these cells were observed consistently over a 9-year time course in one patient at least 19 years following the onset of disease symptoms (Greten et al., 1998). Therefore, activated Tax-specific CTLs are clearly present during the progression of HAM/TSP and their accumulation in the CSF may facilitate the pathogenic role of this cell population in the genesis of HTLV-I-induced neurologic disease. Consistent with the concept that Tax-specific CTLs contribute to the pathogenesis associated with HAM/TSP, immunocytochemical analyses of spinal cords from HAM/TSP patients demonstrated that many activated CTLs are present in active inflammatory lesions (Levin et al., 1997b). The CTLs could function in a number of ways. If HTLV-I infects cells of the CNS, Tax-specific CTLs could directly kill infected cells expressing the Tax protein. Neuronal cells could be killed in a bystander mechanism where the expression of toxic inflammatory cytokines induces apoptosis of uninfected neighboring cells. Alternatively, Tax-specific CTLs could kill resident CNS cell populations expressing a cross-reactive cellular determinant. Clearly, the development of a strong cell-mediated anti-Tax immune response may be a contributing factor in the development of HTLV-I-associated neurologic disease.

The immune response generated against the incoming virus has direct implications with respect to the proviral DNA load. Approximately 1–5% of peripheral blood mono-

nuclear cells (PBMCs) of asymptomatic carriers contain integrated proviral DNA (Bangham et al., 1999). Although proviral DNA load is increased in both HAM/TSP and ATL patients as compared to asymptomatic carriers of the virus (Matsuzaki et al., 2001), patients with HAM/TSP display a significant increase (10- to 100-fold) in proviral DNA within PBMCs (Kira et al., 1991), with the incidence of HAM/TSP rising dramatically as the proviral DNA load exceeds 1% of PBMCs (Bangham, 2000a; Nagai et al., 1998). However, a high proviral DNA load itself is not sufficient to cause HAM/TSP, as a small number of patients progress to HAM/TSP despite having a low proviral DNA load (Nagai et al., 1998). It is likely that there is an interplay between a requirement for a high proviral DNA load and host factors including the immune response, which is clearly influenced by HLA haplotype. Perhaps an individual's immune response to the virus plays a role in determining the proviral DNA load, and therefore, their risk of developing HTLV-I-associated neurologic disease.

Models of HTLV-I-induced disease progression: from primary infection to progressive disease

The connection between the route of infection and the initial host immune response to HTLV-I remains an intriguing area of investigation. Cellular and molecular determinants associated with viral infection that influence the immune response mounted against the virus during primary infection remain to be clearly defined. The initial route of infection is expected to permit the virus access to unique cellular compartments of the host, thereby leading to viral invasion of different subsets of target cells. HTLV-I infection via the peripheral blood likely leads to viral invasion of the CD4⁺ and CD8⁺ T cell populations, as well as cells of the monocyte/macrophage lineage (Knight et al., 1993; Macatonia et al., 1992; Minato et al., 1988; Richardson et al., 1990). In the absence of inflammation, T cell migration into peripheral tissues is limited. As a result, significantly greater levels of CD4⁺ and CD8⁺ T cells are found in the peripheral blood as compared to peripheral tissues. In contrast, professional antigen-presenting cells (APCs) such as dendritic cells (DCs) and monocytes/macrophages represent only approximately 2% of PBMCs present in the peripheral blood. However, a large number of these cells are present in the peripheral tissue and mucosal membranes as compared to CD4⁺ and CD8⁺ T lymphocytes. In addition to CD4⁺ T cells, both monocytes/macrophages and DCs are also susceptible to HTLV-I infection (Knight et al., 1993; Macatonia et al., 1992; Minato et al., 1988; Richardson et al., 1990). Due to the relative proportions of T cells and APCs in the peripheral blood and at mucosal surfaces, viral entry via the peripheral blood likely leads to infection of CD4⁺ T cells and, to a lesser extent, CD8⁺ T cells. Alternatively, HTLV-I entry at mucosal surfaces likely leads to infection of target cells including APCs, with a relatively small num-

ber of T cells being infected. Within the context of primary viral invasion in the peripheral blood, infection of CD4⁺ T cells likely leads to enhanced virus production, thus leading to amplification of peripheral blood virus and enhanced infection of lymphoid DCs. However, based on available information concerning the interaction of HTLV-I with the DC compartment both *in vitro* and *in vivo*, viral infection of DCs is only modestly productive, with small numbers of cells becoming infected and producing small amounts of viral proteins and infectious virus. Infection of DCs in conjunction with helper T cells likely leads to a cell-mediated immune response and an antibody response aimed at controlling the viral infection. The process of normal immune surveillance controls the primary infection by killing infected cells, ultimately abrogating productive viral replication in either the mucosa or the peripheral blood.

After resolution of primary HTLV-I infection, a long period of clinical latency ensues. During clinical latency a number of HTLV-I-associated pathogenic processes may occur which set the stage for the development of HAM/TSP. *In situ* PCR analyses have demonstrated that the BM of HAM/TSP patients contains high levels of proviral DNA. These cells are essentially negative for viral RNA and protein, suggesting that there is an extensive latent infection in the BM of HAM/TSP patients (Jacobson et al., 1997; Levin and Jacobson, 1997; Levin et al., 1997a). This latently infected cell population likely includes CD34⁺ progenitor cells. Additional analyses have demonstrated that peripheral blood and BM CD34⁺ progenitor cells are susceptible to HTLV-I infection, and the proviral genome is maintained throughout the process of differentiation into multiple hematopoietic lineages, including cells which ultimately differentiate into cells of the monocytic lineage present in the peripheral blood, lymph nodes, and brain (Feuer et al., 1996). As previously described, it is hypothesized that peripheral blood exposure to the virus leads to infection of predominantly CD4⁺ T cells. This cell population routinely trafficks to the BM as part of normal immune surveillance. We propose that normal trafficking of HTLV-I-infected CD4⁺ T cells into the BM over a prolonged period of time leads to progressive and extensive HTLV-I invasion of the hematopoietic stem cell system, including infection of resident BM CD34⁺ cells. Although CD34⁺ progenitor cells are capable of harboring the integrated provirus, there is little or no viral gene expression in these cells, as evidenced by the lack of viral RNA or protein. However, these cells are capable of differentiating into multiple cell lineages that may promote cell-type-specific synthesis of viral proteins from the integrated provirus. We hypothesize that differentiation of HTLV-I-infected CD34⁺ progenitor cells into infected APCs, such as DCs and monocytes/macrophages, leads to activation of the HTLV-I LTR mediated by changes in the transcription factor milieu induced by the process of cellular differentiation. Changes in gene expression leading to differentiation of CD34⁺ progenitor cells into DCs and monocytes/macro-

phages may lead to alterations in the abundance or activity of cellular transcription factors capable of initiating gene expression from the LTR of the integrated provirus. In support of this hypothesis, we have demonstrated that *in vitro* differentiation of a monocytic cell line induces an increase in key cellular transcription factors such as the Sp1/Sp3 activator/repressor ratio relevant to activation of a retroviral LTR (McAllister et al., 2000). In addition, we have found that HTLV-I-infected G-CSF-mobilized peripheral blood CD34⁺ progenitor cells express tax mRNA when the cells are induced to differentiate toward either DC or macrophage phenotypes. Furthermore, differences exist in the tax mRNA/DNA ratio between cell types, indicating that differences in cellular differentiation contribute to viral gene expression (Grant and Wigdahl, unpublished observations). This is likely due to key alterations in the abundance of cellular transcription factors during the process of differentiation. In the context of HTLV-I infection, changes in transcription factor expression could lead to a regulated low level of basal transcription and Tax-mediated viral LTR transactivation. This would then allow for synthesis of viral proteins and subsequent antigen presentation of peptides derived from these proteins, including the immunodominant Tax protein. This process would serve to induce the differentiation of CD8⁺ T cells into Tax-specific effector CTLs. During the early phase of asymptomatic infection, this process would still be a part of normal immune surveillance, in which small numbers of HTLV-I-infected APCs stimulate the viral-specific cytotoxic activity of the immune system. However, the shift from normal immune surveillance to hyper-neuroinflammatory disease would occur as a result of a large, latently infected population of CD34⁺ progenitor cells in the BM that are capable of continuous egress from this location to the periphery concomitant with differentiation into infected APCs. This infected APC population is therefore continuously generated, despite the presence of Tax-specific CTLs (Fig. 3A). Newly generated APCs can continuously present viral peptides to CD8⁺ T cells, leading to an overproduction of Tax-specific CTLs. The proportion of CTLs specific for Tax is very high in HAM/TSP patients; the frequency of Tax-reactive CD8⁺ CTLs is 10-fold lower in asymptomatic carriers of the virus as compared to HAM/TSP patients (Elovaara et al., 1993).

How are so many CD8⁺ T cells stimulated to become activated CTLs specific for Tax? APCs such as DCs, macrophages, and B cells are capable of processing antigens from extracellular sources for presentation via MHC class I molecules in a pathway referred to as cross-presentation (Heath and Carbone, 2001). A recent study demonstrated that antigens derived from incoming HIV-1 virions are processed for CTL presentation in APCs, thus indicating that viral proteins are able to be processed for presentation by MHC class I molecules (Buseyne et al., 2001). Alternatively, APCs that are infected by a retrovirus may express viral genes under the direction of the viral LTR. Intracellular viral peptides

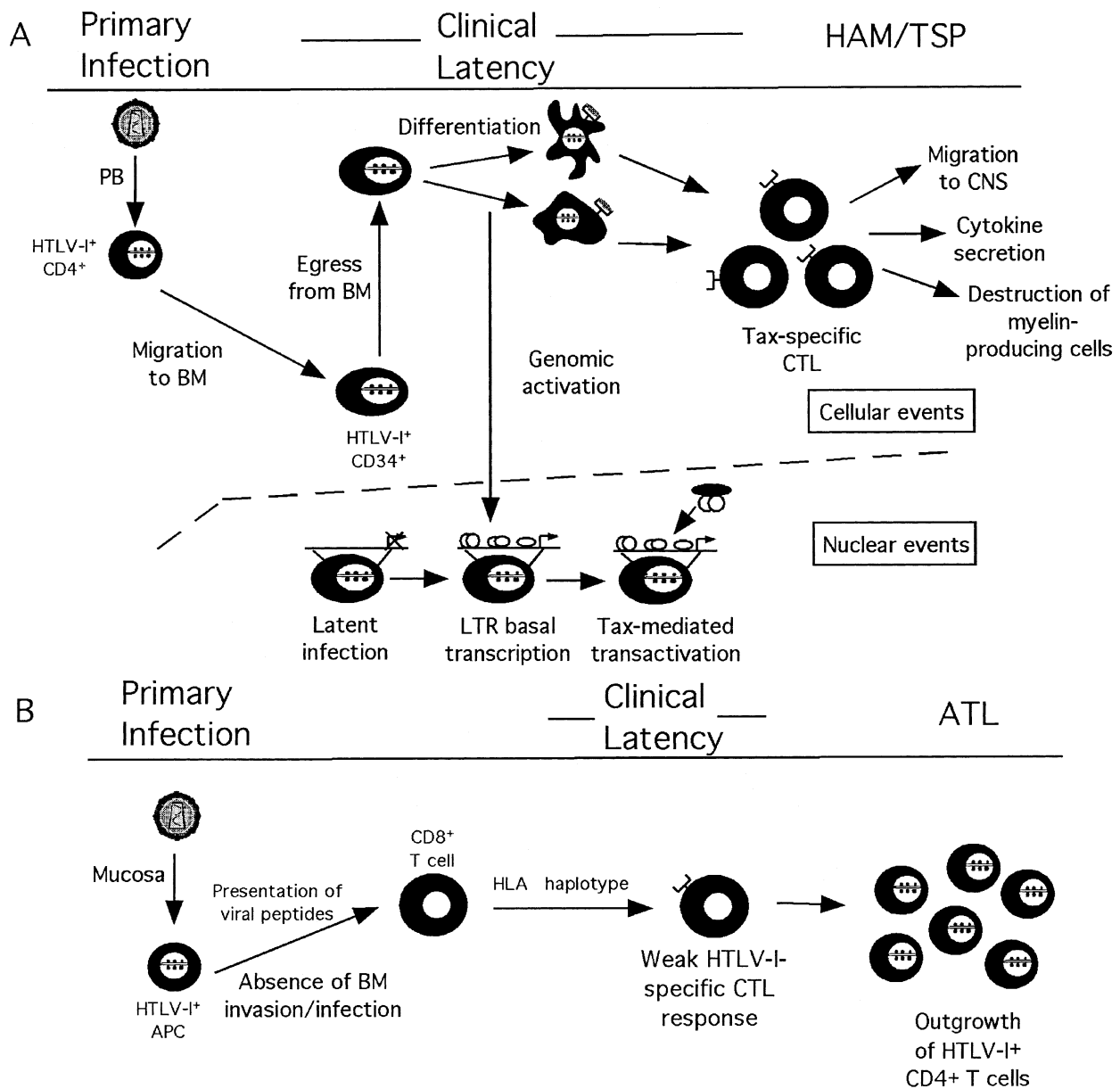


Fig. 3. Models of disease progression: pathways to HAM/TSP and ATL. (A) Viral invasion via the peripheral blood likely leads to invasion primarily of the CD4⁺ T cell population, which migrates to the BM as a part of normal immune surveillance. This results in the infection of bone marrow (BM)-resident CD34⁺ progenitor cells, in which HTLV-I maintains a latent infection. Following egress from the BM and subsequent cellular differentiation, the transcription factor milieu within the cells changes, allowing for LTR basal transcription and Tax-mediated transactivation. Differentiation of infected progenitor cells into infected APCs such as monocytes and dendritic cells could result in the overstimulation of the Tax-specific CTL compartment, a characteristic of HAM/TSP, since a population of infected APCs could be continuously generated from infected progenitor cells. (B) HTLV-I invasion via mucosal surfaces likely leads to the infection of an alternative population of target cells, such as APCs, which do not migrate to the BM. Depending on several host or viral factors including sequence variation within the tax gene and HLA haplotype, a weak HTLV-I-specific immune response may be initiated, allowing for the outgrowth of HTLV-I-infected cells, including CD4⁺ T cells, which may subsequently become leukemogenic, consistent with the genesis of ATL.

may then be processed and presented directly to T cells in the context of MHC class I molecules. Since HTLV-I is able to infect DCs (Knight et al., 1993), this cell population may provide a reservoir of Tax-producing APCs, resulting in the stimulation of a large number of CD8⁺ T cells. An infected DC or monocyte/macrophage population that is continuously generated from an infected pro-

genitor population could seed the peripheral blood with APCs capable of continuously stimulating the activation of CD8⁺ T cells to differentiate into effector Tax-specific CTLs. The resulting generation of a highly expanded, highly efficient population of Tax-specific CTLs could migrate to the CNS and mediate damage to the brain and spinal cord by the targeted lysis of HTLV-I-infected

cells, or by killing uninfected cells through a bystander mechanism. Finally, uninfected cells could be killed if they express a cross-reactive determinant recognized by Tax-specific CTLs. Overstimulation of the Tax-specific CTL compartment could ultimately lead to a shift from normal immune surveillance to a neuroinflammatory disease that centers on the overproduction of Tax-specific CD8⁺ CTLs.

Studies have indicated that the BM of HAM/TSP patients is extensively infected by HTLV-I (Jacobson et al., 1997; Levin and Jacobson, 1997; Levin et al., 1997a). In contrast, such an infection is largely absent in those individuals that either progress to ATL or remain asymptomatic carriers of the virus. The absence of an extensive BM infection likely corresponds to the low-level Tax-specific CD8⁺ CTL response observed in these individuals. A highly expanded and effective population of CTLs directed at viral epitopes is a characteristic observed in patients with neurologic disease, but not those with neoplasia or asymptomatic patients (Jacobson et al., 1990; Kannagi et al., 1984; Parker et al., 1992). Rather, ATL patients appear to have a decreased ability to mount a CTL response against the virus. Several factors likely contribute to this phenomenon, and these factors likely synergize to create an environment in which normal immune surveillance that perhaps would otherwise maintain an asymptomatic state by the targeted killing of HTLV-I-infected cells is absent, thus allowing for the outgrowth and eventual transformation of CD4⁺ T cells (Fig. 3B). Since a cell-mediated immune response could not be initiated in individuals with certain HLA alleles due to low binding affinity for Tax peptides, this would effectively prevent a state of hyperinflammatory disease characterized by the expansion of the Tax-specific CTL population present in HAM/TSP patients.

ATL and HAM/TSP represent the two most devastating diseases associated with HTLV-I infection, and neither disease can be effectively prevented or treated. This points to the importance of developing novel therapeutic strategies aimed at treating these disparate diseases, or potentially preventing the pathologic events that occur prior to clinical diagnosis of disease onset. While the clinical symptoms of ATL and HAM/TSP are defined, the cellular and molecular events following HTLV-I infection that lead to differential disease pathogenesis are not. Great strides have been made with respect to defining and understanding host and viral factors involved in the genesis of ATL and HAM/TSP. However, the continued dissection of these determinants, as well as elucidation of the events following HTLV-I infection that contribute to disease pathogenesis, will remain a critical goal with regard to the development of therapeutic strategies to reduce morbidity and mortality resulting from HTLV-I infection.

Acknowledgments

This work was supported by Public Health Service Grant grant (CA54559) awarded to B.W.

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