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Review

Mediators of central nervous system damage during the progression of human T-cell leukemia type I–associated myelopathy/tropical spastic paraparesis

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Human T-cell leukemia virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) represents one of the most devastating diseases associated with HTLV-I infection. Despite the delineation of clinical features associated with this neurologic disease, more progress needs to be made with respect to understanding the molecular mechanisms relating to the genesis of HAM/TSP. Several factors have been hypothesized to contribute to whether an HTLV-I–infected individual remains asymptomatic, develops adult T-cell leukemia (ATL), or progresses to HAM/TSP. Among the most intriguing of these factors is the immune response mounted by the host against HTLV-I. Several cell populations are crucial with respect to generating an efficient immune response against the virus. This includes $CD4^+$ T cells, $CD8^+$ T cells, dendritic cells (DCs), monocytes/macrophages, and HTLV-I–infected cells that interact with immune cells to stimulate their effector functions. Although all of these cell types likely play important roles in the etiology of HAM/TSP, this review focuses specifically on the potential function of the $CD8^+$ T-cell population during the progression of HTLV-I–induced neurologic disease. The immune response in HAM/TSP patients may transition from a beneficial response aimed at controlling the viral infection, to a detrimental response that ultimately participates in mediating the pathology observed in HAM/TSP. In this respect, the generation of a hyperactive $CD8^+$ cytotoxic T lymphocyte (CTL) response primarily targeting the HTLV-I Tax protein likely plays a key role in the genesis of pathologic abnormalities associated with HAM/TSP. The efficiency and activity of Tax-specific $CD8^+$ CTLs may be regulated at a number of levels, and deregulation of Tax-specific CTL activation may contribute to HAM/TSP. This review focuses on potential mechanisms of central nervous system (CNS) damage associated with the genesis of HAM/TSP following HTLV-I infection, focusing on the role of the Tax-specific CTL compartment. *Journal of NeuroVirology* (2003) **9,** 522–529.

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HTLV-I infection

Retroviruses represent a group of RNA viruses that are capable of causing numerous diseases. Among this group of viruses, the human T-cell leukemia virus type I (HTLV-I) has been associated with a spectrum of pathologic abnormalities. HTLV-I is the etiologic agent of both adult T-cell leukemia (ATL) and a progressive neurologic disease designated HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP). Cases of ATL or HAM/TSP only occur in approximately 5% to 10% of infected individuals, whereas the remainder maintain an asymptomatic state. A vast minority of HTLV-I–infected individuals develop HAM/TSP, a chronic, progressive demyelinating disease predominantly affecting

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the spinal cord and brain. Clinical features associated with HAM/TSP include weakness and stiffness in the lower extremities, persistent lower back pain, urinary incontinence, thoracic myelopathy, and spastic paraparesis or paraplegia. Multiple white matter lesions are apparent in the spinal cord and brain, involving perivascular demyelination and axonal degeneration. Despite the delineation of clinical characteristics associated with HTLV-I–induced neurologic disease, the molecular mechanisms involved in the genesis of HAM/TSP following HTLV-I infection remain to be more clearly defined. Several host and viral factors have been proposed to play a role in determining whether an infected individual progresses to overt neurologic disease. One of the most intriguing factors contributing to disease pathogenesis associated with HTLV-I infection is the immune response mounted by the host against the virus. The immune response to HTLV-I initially serves to control the infection. However, there may be a transition from an immune response that serves a beneficial function, to one whose activity ultimately becomes detrimental.

$CD4^+$ T cells represent the primary target for HTLV-I infection *in vivo* (Richardson *et al*, 1990). However, it is becoming increasingly clear that other cellular compartments, including $CD8^+$ T cells, may become infected with HTLV-I and serve as virus reservoirs (Nagai *et al*, 2001). Recent studies indicate that in HAM/TSP patients, but not ATL patients or asymptomatic carriers, there is a latent infection of the bone marrow (BM) in which resident BM cells harbor proviral DNA, but express little or no viral RNA or protein (Levin *et al*, 1997a). Furthermore, $CD34^+$ progenitor cells infected with HTLV-I *in vitro* have been shown to maintain the proviral genome throughout the process of differentiation (Feuer *et al*, 1996). As previously reviewed (Grant *et al*, 2002), egress of HTLV-I–infected BM progenitor cells could carry HTLV-I infection into the periphery, and perhaps into the central nervous system (CNS). Although HTLV-I infection appears to be silent in resident BM cells, differentiation of progenitor cells is likely accompanied by alterations in the transcription factor milieu within the differentiating cells. Changes in the abundance or activation of transcription factors could result in viral gene expression within cells derived from HTLV-I–infected BM progenitor cells. This is an interesting area of investigation and additional studies are clearly warranted.

Studies have demonstrated that during the course of natural HTLV-I infection, a significant proportion of $CD8^+$ T cells carry proviral DNA capable of directing the synthesis of viral proteins (Hanon *et al*, 2000b; Nagai *et al*, 2001). Relevant to HTLV-I–induced neurologic dysfunction and CNS destruction, HTLV-I has been shown to infect astrocytes, microglia, and monocytes/macrophages (Hoffman *et al*, 1992; Kasai *et al*, 1999; Watabe *et al*, 1989; Yamada *et al*, 1991). Any or all of these cell types may be capable of productive virus replication under selected physiologic conditions, and each cell type may play a role in mediating the pathology observed in cases of HAM/TSP, either directly or indirectly. However, in order for HTLV-I–infected cells to be involved in neuropathology, they must first enter the CNS by traversing the blood-brain barrier (BBB). Additionally, CNS-resident cells are capable of being infected by HTLV-I. It remains possible, although not proven, that CNS-resident cells may potentially be infected as progenitor cells in the BM. However, it is also possible that these cells become infected in the CNS through direct contact with HTLV-I–infected T cells. Because viral infection is thought to require cell-cell contact, presumably HTLV-I–infected T lymphocytes must enter the CNS and come into contact with CNS-resident cells, thereby leading to infection of selected CNS cell populations.

Crossing the blood-brain barrier

HTLV-I–infected cells have been found to be present in both the peripheral blood and cerebrospinal fluid (CSF) of patients with HAM/TSP. Infiltrating $CD4^+$ and $CD8^+$ T lymphocytes and activated macrophages have been identified in the CSF and in white matter lesions of HAM/TSP patients, suggesting a pathogenic role for these cells in the development of neurologic disease resulting from HTLV-I infection. HTLV-I–infected $CD4^+$ and $CD8^+$ T lymphocytes must traverse the BBB and enter the CNS to mediate HTLV-I–specific immune responses and cause CNS damage resulting from such a response. Analysis of HTLV-I–infected $CD4^+$ T cells present in HAM/TSP patients demonstrated an oligoclonal proliferation of HTLV-I–infected $CD4^+$ T cells in both the blood and CSF. Furthermore, clonal populations of HTLV-I–infected $CD4^+$ T cells with HTLV-I proviral DNA sharing the same flanking cellular sequences (and therefore derived from a single HTLV-I–infected progenitor) were found in both the CSF and peripheral blood (Cavrois *et al*, 2000). These results strongly suggest that entry of HTLV-I into the CNS is primarily mediated by invasion of clonal populations of HTLV-I–infected $CD4^+$ T cells across the BBB *in vivo*, although the possibility of viral invasion by other means, including infection of choroids plexus cells, as recently proposed (Coscoy *et al*, 1997), cannot be eliminated. Although immune cell populations, including $CD4^+$ T cells, dendritic cells (DCs), and monocytes/macrophages certainly contribute to the etiology of HAM/TSP, $CD8^+$ T lymphocytes also likely play an important role in neurodegeneration resulting from HTLV-I infection. This is reflected by the fact that there is an accumulation of HTLV-I–specific $CD8^+$ T cells in the CSF as compared to the peripheral blood (Greten *et al*, 1998). Immunocytochemical analyses of spinal cords from HAM/TSP patients have demonstrated that many activated $CD8^+$ cytotoxic T lymphocytes (CTLs) are present in active

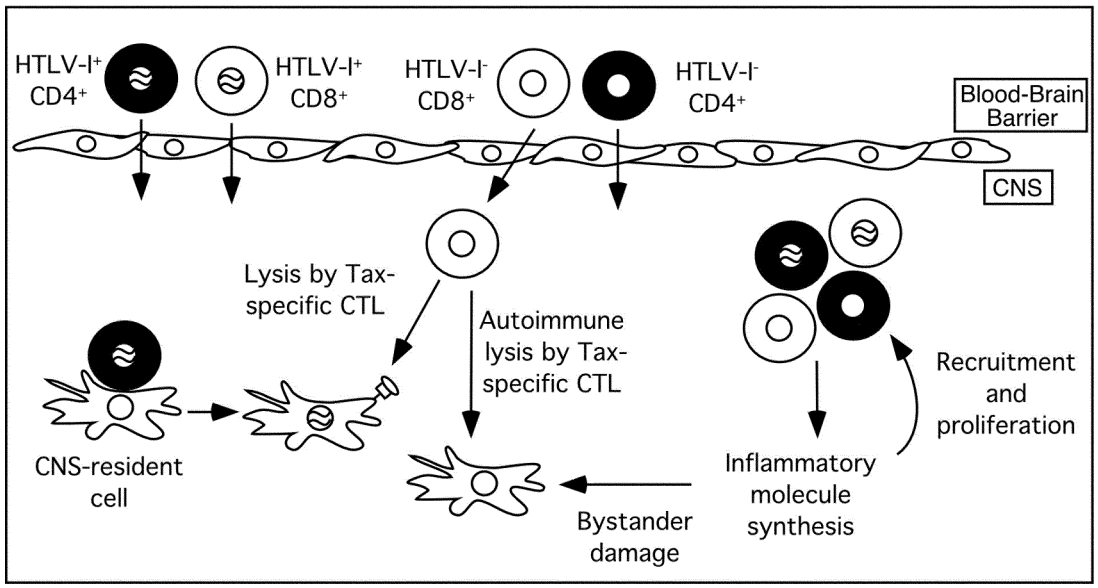

Figure 1 Modeling mechanisms of CNS damage. HTLV-I–infected $CD4^+$ and $CD8^+$ T lymphocytes likely synthesize cytokines that facilitate BBB breakdown, as well as cell adhesion molecules to facilitate passage through the BBB and entry into the CNS. Breakdown of the BBB likely enhances the passage of HTLV-I–negative lymphocytes as well. Infected lymphocytes may come into contact with CNS-resident cells, resulting in infection of cells critical to brain function. Tax-specific $CD8^+$ CTLs may directly target CNS-resident HTLV-I–infected cells for lysis. Alternatively, immune cells recruited to the CNS through the expression of cytokines such as IL-16 and MIP-1α may synthesize a number of inflammatory cytokines that are toxic to CNS-resident cells, resulting in the death of HTLV-I–negative cells. Finally, an immune response generated against Tax may be cross-reactive to cellular proteins, thereby targeting HTLV-I–negative cells for cell death by molecular mimickry.

inflammatory lesions of HAM/TSP patients (Levin *et al*, 1997b).

Dissemination of HTLV-I to the CSF is a hallmark of HAM/TSP. This is reflected by the large proportion of $CD4^+$ and $CD8^+$ T cells and activated macrophages in the CSF and spinal cord lesions of HAM/TSP patients. Despite this fact, the mechanism by which HTLV-I crosses the BBB to enter the CNS remains to be more clearly defined. However, HTLV-I infection is known to increase the expression of several molecules capable of decreasing the integrity of the BBB, thereby leading to an increased penetration of infected cells into the CNS. This includes several cell adhesion molecules as well as a number of inflammatory cytokines (Umehara *et al*, 1996; Watanabe *et al*, 1995). Furthermore, HTLV-I–transformed $CD4^+$ T cells have recently been demonstrated to establish functional gap junctions with endothelial cells through the production of vascular endothelial growth factor (VEGF) (El-Sabban *et al*, 2002). Preferential recruitment of immune cells to the CNS of HTLV-I–infected individuals indicates the potential pathologic role of the host immune response in the genesis of HAM/TSP.

Damage mediated by infiltrating immune cells

Several mechanisms of CNS damage can be hypothesized based on experimental observations that HTLV-I–infected lymphocytes infiltrate the CNS, and CNS-resident cells are susceptible to viral infection. Damage to CNS tissue likely involves the combined activity of several immune cell populations, including $CD4^+$ T cells, $CD8^+$ T cells, DCs, and monocytes/macrophages. Focusing specifically on the $CD8^+$ T-cell compartment, possible mechanisms involved in CNS damage during the progression of HAM/TSP include (1) direct killing of HTLV-I–infected resident CNS cell populations by infiltrating Tax-specific $CD8^+$ CTLs; (2) bystander damage through the synthesis and secretion of toxic inflammatory cytokines; and (3) autoimmunity due to molecular mimickry (Figure 1).

Direct killing of HTLV-I–infected CNS-resident cells by Tax-specific CTLs

HAM/TSP is characterized by highly active and efficient cell-mediated and humoral immune responses, largely targeting the Tax protein (Jacobson *et al*, 1990; Kira *et al*, 1992). Although other HTLV-I proteins may be targets of virus-specific CTLs, the magnitude of CTL activity against Tax is much higher than that against other viral antigens. Immunohistochemical staining has demonstrated the presence of infiltrating $CD8^+$ T cells in spinal cord lesions of HAM/TSP patients (Levin *et al*, 1997b). Although it is likely that Tax-specific $CD8^+$ CTLs function to control HTLV-I infection through lysis of infected cells, it remains

possible that these CTLs are also involved in tissue damage observed in HAM/TSP patients. Inflammatory T cells in affected spinal cord areas have a predominantly $CD8^+$ T-cell phenotype and this cell population has been shown to increase in number as the disease progresses (Kubota *et al*, 1994). The $CD8^+$ response likely occurs in response to viral replication within HTLV-I–infected $CD4^+$ T cells in spinal cord lesions. Furthermore, a high frequency of virus-specific $CD8^+$ CTLs restricted to immunodominant epitopes of the Tax protein have been demonstrated to be present in the CSF of HAM/TSP patients (Jacobson *et al*, 1992). The CTL response is persistently activated and directed against Tax (Parker *et al*, 1992), suggesting that viral replication occurs within HTLV-I–infected cells with continuous expression of HTLV-I genes, particularly Tax. Although the primary cell type carrying HTLV-I provirus within the CNS is likely the $CD4^+$ T lymphocyte, HTLV-I is capable of infecting astrocytes, microglia, and monocytes/macrophages (Hoffman *et al*, 1992; Kasai *et al*, 1999; Watabe *et al*, 1989; Yamada *et al*, 1991). Although it remains possible that resident CNS cells could be infected by cell-cell contact with infiltrating HTLV-I–infected $CD4^+$ T cells, it is also possible that these cells are infected as progenitor cells long before they localize to the CNS. As previously described, HTLV-I has been shown to establish a latent infection in the BM of HAM/TSP patients (Levin *et al*, 1997a), in which proviral DNA is maintained in the absence of viral gene expression. This result has suggested the possibility that $CD34^+$ progenitor cells may become infected with the virus. Egress of these cells from the BM and their concomitant differentiation may result in the presence of a population of HTLV-I–infected cells comprised of $CD4^+$ T cells, $CD8^+$ T cells, DCs, and cells of the monocyte/macrophage lineage. Expression of viral proteins in conjunction with the expression of HLA molecules in HTLV-I–infected CNS-resident cells could lead to a potent cell-mediated immune response. Synthesis of Tax within infected CNS-resident cells likely results in the targeted killing of these cells by infiltrating Tax-specific $CD8^+$ CTLs, which target cells displaying immunodominant epitopes of Tax in the context of major histocompatability complex (MHC) class I molecules on the surface of infected cells. HTLV-I–specific CTLs are known to kill cells expressing HTLV-I antigens directly by a perforin-dependent mechanism (Hanon *et al*, 2000a), as well as indirectly through the production of large amounts of inflammatory molecules involved in tissue destruction.

Bystander damage

Dysregulation of cytokine production through uncontrolled expression may result in a variety of autoimmune disorders. HTLV-I–positive infiltrating lymphocytes may mediate damage in the CNS through the production of neurotoxic mediators, including inflammatory cytokines. Tax-specific $CD8^+$ CTL clones have been demonstrated to secrete a variety of proinflammatory factors. Among these are interferon (IFN)-γ, tumor necrosis factor (TNF)-α, monocyte inflammatory protein (MIP)-1α, MIP-1β, interleukin (IL)-16, and matrix metalloproteinase (MMP)-9 (Biddison *et al*, 1997). TNF-α can cause cytotoxic damage to endothelial cells, thus decreasing the integrity of the BBB. This cytokine can also directly injure oligodendrocytes (Selmaj *et al*, 1991). MIP-1α is a chemoattractant and pro-adhesive molecule with respect to $CD8^+$ T cells, and therefore may play a role in attracting more immune cells to sites of inflammation. IL-16 is a chemoattractant for $CD4^+$ T cells, which secrete IL-2, a growth factor required for the proliferation of $CD8^+$ T cells. Production of IL-16 may actually play a pathogenic role by inducing the recruitment of HTLV-I–infected $CD4^+$ T cells into the CNS (Biddison *et al*, 1997). Synthesis of proinflammatory cytokines, chemokines, and other inflammatory mediators clearly contributes to the pathogenesis of HAM/TSP through breakdown of the BBB and migration of both HTLV-I–infected cells and uninfected immune cells into the CNS. However, viral proteins synthesized by infected cells may also contribute to the cellular destruction characteristic of the disease. The viral Tax protein is implicated in this process. In addition to being the immunodominant protein of the virus, Tax is a potent transcriptional activator capable of *trans*-activating both viral and cellular genes. Relevant to CNS disease, Tax is known to *trans*-activate many host genes. This includes genes encoding cytokines and MMPs in lymphocytes and astrocytes, which have been hypothesized to directly damage myelin and axons (Cabre *et al*, 2000). Prolonged synthesis of the viral Tax protein in brain astrocytes has also been demonstrated to be associated with the synthesis and secretion of inflammatory mediators. Recent studies by Alefantis *et al* have suggested that elevated levels of cytoplasmic Tax may be associated with secretion of Tax from infected astrocytes (Alefantis *et al*, in press, 2003). Specifically, prolonged expression of Tax in HTLV-I–infected astrocytes is associated with the secretion of IL-1α, IL-6, TNF-α, and MMPs (Szymocha *et al*, 2000a, 2000b). Local production of these factors by lymphocytes and astrocytes, as well as production of IFN-γ by infiltrating $CD8^+$ T cells, likely leads to an environment that facilitates neuronal damage and death. Studies have demonstrated that HTLV-I–infected cell lines abnormally express several cytokines, including lymphotoxin, IL-2, IL-6, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IFN-γ. Examination of cytokine expression in HAM/TSP patients has revealed that these patients express elevated levels of TNF-α and IL-1β (Tendler *et al*, 1991). Uncontrolled production of a toxic combination of inflammatory factors may cause the death of uninfected cells, thereby contributing to the death of resident CNS cell populations observed in HAM/TSP.

Molecular mimickry

Molecular mimickry refers to the generation of an immune response to an environmental agent that cross-reacts with a host antigen, resulting in autoimmune damage. HAM/TSP is characterized by a hyperstimulated immune response, including a $CD8^+$ CTL response specific for the N-terminus of Tax and an antibody response specific for the C-terminus of Tax. Studies have indicated that HAM/TSP patients also develop antibodies to neurons (Levin *et al*, 1998). These antibodies were subsequently found to react with heterogeneous ribonuclear protein-A1 (hnRNP-A1), a CNS autoantigen. Interestingly, these antibodies also cross-reacted with HTLV-I Tax, and the distribution of hnRNP-A1 in the CNS corresponded to structures affected in HAM/TSP (Levin *et al*, 2002). Additional studies in a rabbit model of HTLV-I infection indicate that the sera of HTLV-I–infected rabbits contains antibodies against the cellular proteins keratin and thyroglobulin. These antibodies were absent in uninfected rabbits, or those infected with HTLV-I–negative lymphocytes. Additionally, no anti-keratin or anti-thyroglobulin was found in the sera of infected rabbits (Mahana *et al*, 2000). These results suggest that HTLV-I infection may stimulate the production of antibodies that cross-react with cellular proteins. The production of antiviral antibodies that cross-react with cellular proteins sets the stage for an autoimmune response in which HTLV-I–negative cells in the CNS could be targeted for destruction.

Deregulation of the $CD8^+$ T-cell response

There are clearly several mechanisms by which the immune response mounted by the host against HTLV-I may have a detrimental impact in terms of the progression of neurologic disease. Although the induction of an efficient immune response is necessary to control HTLV-I infection, an overstimulated immune response may become autoimmune in nature and destroy the population it was meant to protect. Delineating the mechanisms involved in harnessing the immune response to HTLV-I will likely prove to be valuable in terms of defining options for the prevention or treatment of HAM/TSP. To this end, understanding the mechanisms underlying the induction of the potent HTLV-I–specific $CD8^+$ T-cell response in HAM/TSP patients will provide important clues concerning methods to control this ultimately destructive immune response.

HTLV-I infection elicits a vigorous cell-mediated immune response directed towards viral antigens, particularly the Tax protein (Jacobson *et al*, 1990). CTL responses are present within ATL patients, HAM/TSP patients, and asymptomatic carriers. However, there are quantitative differences between the magnitude of the CTL response relative to the disease state of the patient. Whereas ATL is characterized by a poor CTL response to Tax, HAM/TSP is associated with the presence of a large number of Tax-specific CTLs (Yamano *et al*, 2002). Although high levels of circulating Tax-specific CTLs observed in HAM/TSP patients likely reflect the high proviral load typically observed in these patients, it is possible that these CTLs are deregulated in function. This is indicated by the fact that despite the presence of a large number of Tax-specific CTLs that would presumably serve to clear HTLV-I–infected cells, the proviral DNA load remains elevated in HAM/TSP patients throughout the course of disease. The inability of Tax-specific CTLs to clear the virus from the CNS indicates that perhaps the function of the CTLs in the CNS has been altered or become deregulated. The molecular mechanisms responsible for the deregulated CTL responses in both ATL and HAM/TSP are currently unknown but recent studies have provided important clues.

Antigen-mediated priming or activation of naïve $CD8^+$ T cells by professional antigen-presenting cells (APCs) such as DCs, leads to differentiation into CTLs (Guermonprez *et al*, 2002). CTL priming is tightly regulated, and recognition of antigen is not a sufficient signal for activation. $CD8^+$ T cells generally require a second signal, or costimulatory signal, which can be provided by $CD4^+$ T cells. Activated $CD4^+$ T cells express costimulatory molecules, most notably CD40L, that interact with and "license" the APCs to efficiently activate a $CD8^+$ T cell with the same antigenic specificity (Bennett *et al*, 1998; Ridge *et al*, 1998; Schoenberger *et al*, 1998). These observations provide support for a "three cell model" of CTL activation involving $CD4^+$ T cells, DCs, and $CD8^+$ T cells (Figure 2). First, a DC encounters the antigen in a tissue or the peripheral blood, and then migrates to the draining lymph node where the antigen is processed and presented on the cell surface by MHC molecules. A $CD4^+$ T cell recognizes the antigen, becomes activated, and expresses a costimulatory molecule such as CD40L, which engages CD40 on the DC and induces functional maturation. The mature DC up-regulates the expression of MHC and costimulatory molecules and is then competent to activate a $CD8^+$ T cell with the same antigenic specificity as the initial $CD4^+$ T cell.

HTLV-I infection can potentially deregulate CTL priming by disrupting the interaction of each of the requisite cells in the "three cell model." Indeed, HTLV-I infection is not limited to $CD4^+$ T cells, but also infects $CD8^+$ T cells (Hanon *et al*, 2000b; Nagai *et al*, 2001) and DCs *in vitro* (Ali *et al*, 1993). These cell populations may also be infected as progenitor cells in the BM. Migration of HTLV-I–infected progenitor cells from the BM to the periphery and their concurrent differentiation may result in the continuous generation of a reservoir of HTLV-I–infected immune cells. Based on the potential infection of $CD8^+$ T cells and DCs, there are several potential mechanisms that the virus may employ to deregulate CTL priming. HTLV-I may potentially influence CTL function by infection of HTLV-I–specific $CD8^+$

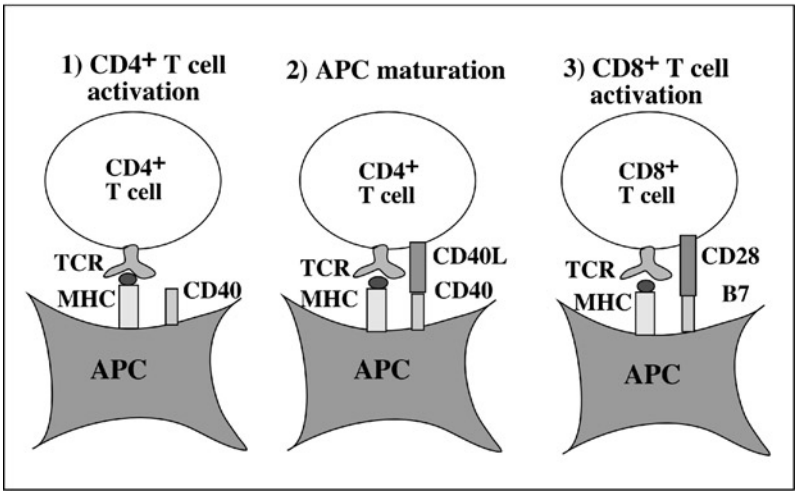

Figure 2 The three-cell model of $CD8^+$ T-cell activation. An antigen-presenting cell requires a signal from $CD4^+$ T cells prior to the priming of $CD8^+$ T cells. A $CD4^+$ T cell first recognizes antigen presented by the APC (1), and is then activated and expresses CD40L, which binds to CD40 expressed on the APC (2). The CD40 signal induces functional maturation of the APC, including increased expression of costimulatory B7 molecules, which then facilitates efficient priming of the $CD8^+$ T cell (3).

T cells (Hanon *et al*, 2000b). However, little is known about the functional consequences of HTLV-I infection of $CD8^+$ T cells. Functional abnormalities have been described in DCs from both ATL and HAM/TSP patients. DCs from ATL patients are relatively poor stimulators of CTL priming (Makino *et al*, 2000), whereas DCs from HAM/TSP patients are potent activators of CTLs (Makino *et al*, 1999). Deregulation of DC function may occur as a result of infection of DCs by HTLV-I, or more likely is due to interaction with HTLV-I–infected $CD4^+$ T cells. We have previously demonstrated that HTLV-I infection alters gene expression profiles in $CD4^+$ T cells, and the expression of costimulatory molecules such as CD40L are deregulated (Harhaj *et al*, 1999). CD40L expression is down-regulated upon HTLV-I–mediated T cell transformation, and HTLV-I–transformed cell lines lack CD40L expression (Harhaj and Wigdahl, unpublished observations). Interestingly, $CD4^+$ T cells from ATL patients lack CD40L expression, but $CD4^+$ T cells from HAM/TSP patients overexpress CD40L (Makino *et al*, 2001). Lack of CD40L expression on $CD4^+$ T cells from ATL patients impairs CTL priming in response to HTLV-I antigens, but can be rescued by soluble CD40L (Makino *et al*, 2001). However, it is currently unknown if CD40L overexpression in HAM/TSP patients contributes to the inflammatory response observed in the CNS.

Functional interactions between $CD4^+$ T cells, $CD8^+$ T cells, and DCs in the activation of Tax-specific CTLs may potentially be altered due to infection of any or all of these cell populations. Clearly, this is an area that warrants further investigation. It would be of interest to delineate the degree to which the increase in Tax-specific CTLs (both in number and in function) parallels an increase in proviral DNA load in the CNS. A correlation has been made between HTLV-I mRNA, proviral DNA load, virus-specific $CD8^+$ T cells, and disease severity in HAM/TSP (Selmaj *et al*, 1991). It was also recently demonstrated that the frequency of HTLV-I–specific $CD8^+$ T cells in the peripheral blood correlates with the proviral load, suggesting that Tax-specific $CD8^+$ T cells proliferate to control viral replication (Kubota *et al*, 2003). However, both of these studies dealt with CTLs found in the peripheral blood rather than the CNS. There may be functional differences in T-cell responses between the two compartments. It is known that $CD8^+$ T cells accumulate in the CSF of HAM/TSP patients, but the degree to which this reflects proviral DNA load has not been conclusively determined. Further questions include whether the CTLs found in the CNS differ functionally from those found in the peripheral blood. This is only beginning to be examined (Kubota *et al*, 2002). For example, CTLs found in the CNS may be more or less efficient at killing HTLV-I–infected cells. Additionally, it would be of interest to determine the precise specificity of Tax-specific CTLs in the CNS. As previously described, studies have indicated that HTLV-I infection may stimulate the production of antiviral antibodies that cross-react with cellular proteins. It is possible, although not proven, that CTLs within the CNS may have a degenerate specificity, recognizing both viral and cellular antigens.

In conjunction with other cell populations of the immune system, the Tax-specific $CD8^+$ CTL response clearly impacts disease progression resulting from HTLV-I infection. Whether Tax-specific $CD8^+$ CTLs function to control HTLV-I infection and replication in the CNS, or serve to mediate tissue damage, necessitates further investigation. It is most probable that the initial antiviral function of Tax-specific $CD8^+$ CTLs in the CNS serves to control viral replication through the destruction of HTLV-I–infected $CD4^+$

T cells. Although evidence is largely circumstantial at this point, there appears to be a transition from normal immune surveillance to a hyperinflammatory state in HAM/TSP patients. In this case, Tax-specific $CD8^+$ CTLs shift from having a beneficial function to becoming detrimental, in the sense that these CTLs may be involved in mediating tissue damage observed in HAM/TSP. What are the factors that contribute to crossing the threshold between the beneficialand detrimental functions of Tax-specific $CD8^+$ CTLs? Clearly, this is an area that requires further investigation. Clarification of the differences in the immune response between asymptomatic carriers, ATL patients, and HAM/TSP patients, as well as the mechanisms underlying the different responses will allow for the delineation of therapeutic strategies aimed at harnessing the power of this immune response. Modulating the HTLV-I–specific immune response will be highly beneficial in terms of prevention of HTLV-I–induced disease.

References

Alefantis T, Barmak K, Harhaj E, Grant C, Wigdahl B (2003). Characterization of a nuclear export signal within the human T cell leukemia virus type I transactivator protein Tax. In press.

Ali A, Patterson S, Cruickshank K, Rudge P, Dalgleish AG, Knight SC (1993). Dendritic cells infected in vitro with human T cell leukaemia/lymphoma virus type-1 (HTLV-1); enhanced lymphocytic proliferation and tropical spastic paraparesis. *Clin Exp Immunol* **94:** 32–37.

Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JF, Heath WR (1998). Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* **393:** 478–480.

Biddison WE, Kubota R, Kawanishi T, Taub DD, Cruikshank WW, Center DM, Connor EW, Utz U, Jacobson S (1997). Human T cell leukemia virus type I (HTLV-I)-specific CD8+ CTL clones from patients with HTLV-I-associated neurologic disease secrete proinflammatory cytokines, chemokines, and matrix metalloproteinase. *J Immunol* **159:** 2018–2025.

Cabre P, Smadja D, Cabie A, Newton CR (2000). HTLV-1 and HIV infections of the central nervous system in tropical areas. *J Neurol Neurosurg Psychiatry* **68:** 550–557.

Cavrois M, Gessain A, Gout O, Wain-Hobson S, Wattel E (2000). Common human T cell leukemia virus type 1 (HTLV-1) integration sites in cerebrospinal fluid and blood lymphocytes of patients with HTLV-1-associated myelopathy/tropical spastic paraparesis indicate that HTLV-1 crosses the blood-brain barrier via clonal HTLV-1-infected cells. *J Infect Dis* **182:** 1044–1050.

Coscoy L, Gonzalez-Dunia D, Brahic M, Ozden S (1997). Infection of choroid plexus cells by human T cell leukaemia virus type I. *J Gen Virol* **78(Pt 1):** 143–146.

El-Sabban ME, Merhi RA, Haidar HA, Arnulf B, Khoury H, Basbous J, Nijmeh J, de The H, Hermine O, Bazarbachi A (2002). Human T-cell lymphotropic virus type 1-transformed cells induce angiogenesis and establish functional gap junctions with endothelial cells. *Blood* **99:** 3383–3389.

Feuer G, Fraser JK, Zack JA, Lee F, Feuer R, Chen IS (1996). Human T-cell leukemia virus infection of human hematopoietic progenitor cells: maintenance of virus infection during differentiation in vitro and in vivo. *J Virol* **70:** 4038–4044.

Grant C, Barmak K, Alefantis T, Yao J, Jacobson S, Wigdahl B (2002). Human T cell leukemia virus type I and neurologic disease: events in bone marrow, peripheral blood, and central nervous system during normal immune surveillance and neuroinflammation. *J Cell Physiol* **190:** 133–159.

Greten TF, Slansky JE, Kubota R, Soldan SS, Jaffee EM, Leist TP, Pardoll DM, Jacobson S, Schneck JP (1998). Direct visualization of antigen-specific T cells: HTLV-1 Tax11-19-specific CD8(+) T cells are activated in peripheral blood and accumulate in cerebrospinal fluid from HAM/TSP patients. *Proc Natl Acad Sci U S A* **95:** 7568–7573.

Guermonprez P, Valladeau J, Zitvogel L, Thery C, Amigorena S (2002). Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol* **20:** 621–667.

Hanon E, Hall S, Taylor GP, Saito M, Davis R, Tanaka Y, Usuku K, Osame M, Weber JN, Bangham CR (2000a). Abundant tax protein expression in CD4+ T cells infected with human T-cell lymphotropic virus type I (HTLV-I) is prevented by cytotoxic T lymphocytes. *Blood* **95:** 1386–1392.

Hanon E, Stinchcombe JC, Saito M, Asquith BE, Taylor GP, Tanaka Y, Weber JN, Griffiths GM, Bangham CR (2000b). Fratricide among CD8(+) T lymphocytes naturally infected with human T cell lymphotropic virus type I. *Immunity* **13:** 657–664.

Harhaj EW, Good L, Xiao G, Sun SC (1999). Gene expression profiles in HTLV-I-immortalized T cells: deregulated expression of genes involved in apoptosis regulation. *Oncogene* **18:** 1341–1349.

Hoffman PM, Dhib-Jalbut S, Mikovits JA, Robbins DS, Wolf AL, Bergey GK, Lohrey NC, Weislow OS, Ruscetti FW (1992). Human T-cell leukemia virus type I infection of monocytes and microglial cells in primary human cultures. *Proc Natl Acad Sci U S A* **89:** 11784–11788.

Jacobson S, McFarlin DE, Robinson S, Voskuhl R, Martin R, Brewah A, Newell AJ, Koenig S (1992). HTLV-I-specific cytotoxic T lymphocytes in the cerebrospinal fluid of patients with HTLV-I-associated neurological disease. *Ann Neurol* **32:** 651–657.

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

Kasai T, Ikeda H, Tomaru U, Yamashita I, Ohya O, Morita K, Wakisaka A, Matsuoka E, Moritoyo T, Hashimoto K, Higuchi I, Izumo S, Osame M, Yoshiki T (1999). A rat model of human T lymphocyte virus type I (HTLV-I) infection: in situ detection of HTLV-I provirus DNA in microglia/macrophages in affected spinal cords of rats with HTLV-I-induced chronic progressive myeloneuropathy. *Acta Neuropathol (Berl)* **97:** 107–112.

Kira J, Nakamura M, Sawada T, Koyanagi Y, Ohori N, Itoyama Y, Yamamoto N, Sakaki Y, Goto I (1992). Antibody titers to HTLV-I-p40tax protein and gag-env

hybrid protein in HTLV-I-associated myelopathy/tropical spastic paraparesis: correlation with increased HTLV-I proviral DNA load. *J Neurol Sci* **107:** 98–104.

Kubota R, Furukawa Y, Izumo S, Usuku K, Osame M (2003). Degenerate specificity of HTLV-I-specific CD8+ T cells during viral replication in patients with HTLV-I-associated myelopathy (HAM/TSP). *Blood* **101:** 3074–3081.

Kubota R, Soldan SS, Martin R, Jacobson S (2002). Selected cytotoxic T lymphocytes with high specificity for HTLV-I in cerebrospinal fluid from a HAM/TSP patient. *J NeuroVirol* **8:** 53–57.

Kubota R, Umehara F, Izumo S, Ijichi S, Matsumuro K, Yashiki S, Fujiyoshi T, Sonoda S, Osame M (1994). HTLV-I proviral DNA amount correlates with infiltrating CD4+ lymphocytes in the spinal cord from patients with HTLV-I-associated myelopathy. *J Neuroimmunol* **53:** 23–29.

Levin MC, Krichavsky M, Berk J, Foley S, Rosenfeld M, Dalmau J, Chang G, Posner JB, Jacobson S (1998). Neuronal molecular mimicry in immune-mediated neurologic disease. *Ann Neurol* **44:** 87–98.

Levin MC, Krichavsky M, Fox RJ, Lehky T, Jacobson S, Fox C, Kleghorn F, White J, Young N, Edwards RJ, Jack NE, Bartholomew C (1997a). Extensive latent retroviral infection in bone marrow of patients with HTLV-I-associated neurologic disease. *Blood* **89:** 346–348.

Levin MC, Lee SM, Kalume F, Morcos Y, Dohan FC Jr, Hasty KA, Callaway JC, Zunt J, Desiderio D, Stuart JM (2002). Autoimmunity due to molecular mimicry as a cause of neurological disease. *Nat Med* **8:** 509–513.

Levin MC, Lehky TJ, Flerlage AN, Katz D, Kingma DW, Jaffe ES, Heiss JD, Patronas N, McFarland HF, Jacobson S (1997b). Immunologic analysis of a spinal cord-biopsy specimen from a patient with human T-cell lymphotropic virus type I-associated neurologic disease. *N Engl J Med* **336:** 839–845.

Mahana W, Samaan A, Zhao TM, Kindt TJ, Simpson RM (2000). Evidence for humoral and cellular reactivity against keratin and thyroglobulin in HTLV-I infected rabbits. *Autoimmunity* **32:** 57–65.

Makino M, Shimokubo S, Wakamatsu SI, Izumo S, Baba M (1999). The role of human T-lymphotropic virus type 1 (HTLV-1)-infected dendritic cells in the development of HTLV-1-associated myelopathy/tropical spastic paraparesis. *J Virol* **73:** 4575–4581.

Makino M, Utsunomiya A, Maeda Y, Shimokubo S, Izumo S, Baba M (2001). Association of CD40 ligand expression on HTLV-I-infected T cells and maturation of dendritic cells. *Scand J Immunol* **54:** 574–581.

Makino M, Wakamatsu S, Shimokubo S, Arima N, Baba M (2000). Production of functionally deficient dendritic cells from HTLV-I-infected monocytes: implications for the dendritic cell defect in adult T cell leukemia. *Virology* **274:** 140–148.

Nagai M, Brennan MB, Sakai JA, Mora CA, Jacobson S (2001). CD8(+) T cells are an in vivo reservoir for human T-cell lymphotropic virus type I. *Blood* **98:** 1858–1861.

Parker CE, Daenke S, Nightingale S, Bangham CR (1992). Activated, HTLV-1-specific cytotoxic T-lymphocytes are found in healthy seropositives as well as in patients with tropical spastic paraparesis. *Virology* **188:** 628–636.

Richardson JH, Edwards AJ, Cruickshank JK, Rudge P, Dalgleish AG (1990). In vivo cellular tropism of human T-cell leukemia virus type 1. *J Virol* **64:** 5682–5687.

Ridge JP, Di Rosa F, Matzinger P (1998). A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature* **393:** 474–478.

Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ (1998). T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* **393:** 480–483.

Selmaj K, Raine CS, Farooq M, Norton WT, Brosnan CF (1991). Cytokine cytotoxicity against oligodendrocytes. Apoptosis induced by lymphotoxin. *J Immunol* **147:** 1522–1529.

Szymocha R, Akaoka H, Brisson C, Beurton-Marduel P, Chalon A, Bernard A, Didier-Bazes M, Belin MF, Giraudon P (2000a). Astrocytic alterations induced by HTLV type 1-infected T lymphocytes: a role for Tax-1 and tumor necrosis factor alpha. *AIDS Res Hum Retroviruses* **16:** 1723–1729.

Szymocha R, Brisson C, Bernard A, Akaoka H, Belin MF, Giraudon P (2000b). Long-term effects of HTLV-1 on brain astrocytes: sustained expression of Tax-1 associated with synthesis of inflammatory mediators. *J NeuroVirol* **6:** 350–357.

Tendler CL, Greenberg SJ, Burton JD, Danielpour D, Kim SJ, Blattner WA, Manns A, Waldmann TA (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.

Umehara F, Izumo S, Takeya M, Takahashi K, Sato E, Osame M (1996). Expression of adhesion molecules and monocyte chemoattractant protein-1 (MCP-1) in the spinal cord lesions in HTLV-I-associated myelopathy. *Acta Neuropathol (Berl)* **91:** 343–350.

Watabe K, Saida T, Kim SU (1989). Human and simian glial cells infected by human T-lymphotropic virus type I in culture. *J Neuropathol Exp Neurol* **48:** 610–619.

Watanabe H, Nakamura T, Nagasato K, Shirabe S, Ohishi K, Ichinose K, Nishiura Y, Chiyoda S, Tsujihata M, Nagataki S (1995). Exaggerated messenger RNA expression of inflammatory cytokines in human T-cell lymphotropic virus type I-associated myelopathy. *Arch Neurol* **52:** 276–280.

Yamada M, Watabe K, Saida T, Kim SU (1991). Increased susceptibility of human fetal astrocytes to human T-lymphotropic virus type I in culture. *J Neuropathol Exp Neurol* **50:** 97–107.

Yamano Y, Nagai M, Brennan M, Mora CA, Soldan SS, Tomaru U, Takenouchi N, Izumo S, Osame M, Jacobson S (2002). Correlation of human T-cell lymphotropic virus type 1 (HTLV-1) mRNA with proviral DNA load, virus-specific CD8(+) T cells, and disease severity in HTLV-1-associated myelopathy (HAM/TSP). *Blood* **99:** 88–94.