SPECIAL ARTICLE

HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis

How Far have We Come?

O Morgan

Ladies and gentlemen

Distinguished lectures are neither commonplace nor rare. They are generally accorded to those, who during their lifetime, so contributed to our intellectual store, that we may fairly identify their enduring gifts as a legacy of ideas. And the purpose of these lectures at least in significant part, is to value that legacy, ensure that it is not wasted, and provide an environment in which, hopefully, it will attract some accretion.

I value greatly the honour of giving The Professor Eric Cruickshank Distinguished Lecture. Along with Spillane and Scott, Denny-Brown and Brain, Kinnear Wilson and Osuntokun, Eric Cruickshank was a pioneer in the study of the obscure paraplegias, many of which, at that time, were of nutritional origin.

Eric Cruickshank came to Mona in 1949 to be the foundation Professor of Medicine. His remarkable clinical skills combined with an unusual capacity for hard work and organization, enabled him to build an outstanding medical school, whose graduates were prepared for service in the region. He required his students, many of whom have gained prominence on the world stage, to examine patients early in their career, present case histories, review physical findings and discuss treatment with him. He insisted that they attend autopsies so as to be able to correlate the changes found there, with the signs and symptoms which preceded death. The physicians' profession was after all, acquaintance with disease in its many guises.

Cruickshank will, however, be best remembered for his description of the 'Jamaican neuropathy' which brought clarity to a particular group of tropical diseases of the nervous system, which, until then, had been misunderstood. Further studies of the disease using modern molecular biological techniques continue today in our Department of Medicine as well as in the most sophisticated laboratories of the world. His legacy of shaping our medical school with its commitment to excellence must be transmitted pure to future generations. Cruickshank died in London at the age of 92 years, a revered and highly respected clinician scientist.

To many, he remains the perfect clinical teacher, a profound thinker, and an ingenious artist. He commands, to our admiration, the art whose domain he enlarged, and whose practice he rendered more useful and more fertile.

Three broad questions have driven research on the subject of HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP):

* Why do some human T-lymphotropic virus type 1 (HTLV-1) infected individuals develop a consequent inflammatory disease such as HAM/TSP and is the difference in outcome of infection due to a variation in the host or in the virus?
* How is the inflammatory lesion in HAM/TSP initiated and maintained, and how can it be halted?
* What is the role of the immune response and other factors in initiating, controlling or limiting disease?

When Cruickshank studied the disease, the causative agent had not yet been identified. The eventual discovery of HTLV-1 and its association with human disease stimulated a new interest in tropical neurology. With the advent of amplified cell culture systems and other sophisticated laboratory techniques, we now have excellent diagnostic tests for the virus and are able to study virus-host interrelationships in considerable detail.

I have chosen as the subject for this lecture "HAM/TSP – How far have we come?" I will focus on the role of the virus in the aetiology of the disease and discuss the significance of certain cellular and molecular events which may be involved in the pathogenesis of the disease.

ORIGIN OF HTLV-1

The source of human retroviruses is not known. Primate retroviruses called simian T-lymphotropic virus (STLV) have been isolated from several primate species in Africa and Asia. These viruses share significant homology with HTLV-1 and raise the possibility of enzootic transmission to humans. How HTLV-1 has spread among various human populations is not known but it appears to represent an ancient virus that has followed the migrations of human populations.

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This was the third Eric Cruickshank Distinguished Lecture, delivered in July 2010 by Professor Owen St C Morgan, Emeritus Professor of Medicine and Neurology.

Retroviruses represent a group of RNA viruses that are capable of causing a variety of diseases including cancer, immune disorders and neurologic disease. Shortly after its discovery in 1978, HTLV-1 was recognized as the first retrovirus to be linked with human disease, notably, adult T-cell leukaemia (ATL) and HAM/TSP, chronic inflammatory arthropathy, HTLV-1 associated uveitis, infective dermatitis and Sjogren's syndrome.

DISTRIBUTIION OF HTLV-1

HTLV-1 is widely distributed in the tropics and subtropics. Large endemic foci exist in the Caribbean, South Japan, Central and South Africa and South America, particularly Brazil but the virus is also present in Southern India, Northern Iran, the aboriginal populations of Northern Australia and many islands in the tropics. Within North America, the virus is found chiefly in certain immigrant groups and in intravenous drug users. In the endemic areas, the distribution of HTLV-1 is typically uneven; the sero-prevalence varies widely between 0.1% and 30% in adults often with greater differences between neighbouring areas. In contrast to HIV, the majority of infected individuals remain asymptomatic.

VIRAL TRANSMISSION

Of the many possible routes of virus transmission, mother to child through breast feeding, is the most predominant route. Transmission rates are 16% for children born to infected mothers, 27% for children nursed by infected mothers for more than three months and 5% for children nursed by infected mothers for less than three months. The infants seroconvert within 1–3 years of age.

Sexual transmission rates are 60% for male to female but only 0.4% for female to male transmission. Predisposing factors associated with sexual transmission include the presence of genital ulcers, high viral loads, and high antibody titres in the donor.

Among non-drug using sexual partners of intravenous drug users (IDUs), sexual transmission is a more common mode than parental transmission. Among IDUs, blood and blood products are the most significant source of infection. Unlike HIV-1, whole cell transfusion is required for transmission of the virus with a seroconversion rate of approximately 50%. The development of HAM/TSP has been noted as early as six months after transfusion of an individual with infected blood. Concerns about HTLV-1 transmission through blood components led to mandatory blood screening for HTLV-1 in Jamaica.

Cell-free infection with HTLV-1 is very inefficient; efficient transmission depends on cell-to-cell transfer through direct cell contact, polarization of the microtubules – organizing centre (MTOC) which is triggered by *tax*, and the formation of a virological synapse which allows the entry of viral particles, viral proteins and genomic RNA into fresh target cells.

DIAGNOSIS

The diagnosis of HTLV-1 infection is based on the detection of specific antibodies by particle agglutination or enzyme linked immunosorbent assay (ELISA) and subsequent confirmation by Western blot (WB) or polymerase chain reaction (PCR) assays. The most specific serological assay is the Western blot in which the antigen is a mixture of whole virus lysate with recombinant viral envelope proteins from HTLV-1. The identity of the virus can be confirmed by PCR which is especially useful in cases where the Western blot analysis is indeterminate.

VIROLOGY

HTLV-1 is a complex retrovirus with a single stranded positive RNA genome that expresses unique proteins with oncogenic potential. There are four known strains of HTLV-1 of which HTLV-1 and HTLV-2 are the most prevalent worldwide. HTLV-1 was originally identified in 1980 in a T-cell line derived from a patient with cutaneous lymphoma. The virus can infect T-cells, B-cells, monocytes, dendritic cells and endothelial cells, yet it can transform only primary T-cells.

HTLV-1 is an enveloped virus of about 100 nm in diameter. The inner membrane of the vircon envelope is lined by the viral matrix protein. This structure encloses the viral capsid, which carries two identical strands of genomic RNA as well as functional protease, integrase and reverse transcriptase enzymes. A newly synthesized viral particle attaches to the target cell receptor through the viral envelope and enters *via* fusion which is followed by uncoating of the capsid and release of its contents into the cell cytoplasm. The viral RNA is reverse transcribed into double stranded DNA by the RT transported to the nucleus and integrated into the host chromosome forming the provirus.

The provirus contains the promoter and enhancer elements for transcription initiation in the long terminal repeats (LTRs).

The complex retroviral genome encodes for the structural proteins:

Gag – group specific antigen, which functions as structural protein of the matrix, capsid and nucleocapsid;

Pol – encodes for several enzymes – reverse transcriptase RT involved in RNA to DNA transcription, endonuclease, protease and integrase for viral integration.

Env – encodes for major components of the viral coat – the surface glycoprotein and the transmembrane as well as the regulatory and accessory proteins.

The two regulatory genes *tax* and *rex* are encoded by open reading frames (ORF) IV and III respectively.

Tax is the transactivator gene, which increases the rate of viral LTR mediated transcription and modulates the transcription of numerous cellular genes involved in cell

proliferation and differentiation, cell cycle control and DNA repair.

Rex acts post-transcriptionally, by preferentially binding, stabilizing and selectively exporting intron containing viral MRNAs from the nucleus to the cytoplasm.

The accessory genes p12/p8, p30/p13 seem to play a role in gene regulation and contribute to the productive infection of quiescent T-lymphocytes *in vitro.*

The minus strand of the proviral genome encodes several isoforms of the HTLV-1 basic leucine zipper protein (HBZ) which contributes to modulation of both viral and cellular gene transcription; HBZ also plays a crucial role in T-cell proliferation.

The two diseases most commonly associated with HTLV-1 infection are ATL and HAM/TSP.

ATL

There are five different clinical stages of ATL: asymptomatic, pre-leukaemic, chronic/smoldering ATL, lymphomatous type and acute ATL. The majority of HTLV-1 infected patients are asymptomatic carriers who do not show any clinical symptoms. Even in the absence of symptoms, these individuals are capable of transmitting the virus to others.

HAM/TSP

We continue to see large numbers of patients with HAM/TSP in the clinics at the University Hospital of the West Indies. It manifests as a progressive paraparesis but careful examination often reveals disease of the peripheral nerves and muscles as well as other organs.

The neurological symptoms usually begin in adult life, most frequently after the age of 30 years and have a striking female predominance.

In most cases, the onset is slowly progressive and consists of pain in the low back, thighs or the sciatic nerves, mimicking rheumatic disease. In other instances, urinary complaints may be the only symptoms for many months until motor disturbances or distal paraesthesias call attention to the need for a neurological examination. The diagnosis of HAM/TSP is rarely made at this stage except in endemic areas. After this initial progressive course, the pace of the illness becomes slower and the disease may even be arrested.

The degree of handicap in the chronic stage is highly variable. Some patients continue to work but others are confined to a wheelchair or are bedridden.

In the mildest cases, the only complaint is of leg stiffness which may interfere with walking and climbing stairs. In the more severe cases, there is a spastic gait. Examination then reveals signs of pyramidal tract involvement with hyperreflexia in all four limbs. The ankle jerks may, however, be normal, depressed or even absent suggesting peripheral nerve involvement. In most cases, bilateral Babinski sign is present. Strength is usually normal in the arms and decreased in the legs with weakness more marked proximally probably due to involvement of muscles. Sensory disturbances consist mainly of dysesthesias in the feet. The striking difference between mild sensory disturbances and the more obvious motor signs and symptoms is characteristic of the disease.

In some rare instances, the onset is rapidly progressive especially after transfusional contamination when complete paraplegia appears within weeks of seroconversion.

The coexistence of peripheral nerve involvement, dermatomyositis and polymyositis has been noted by many authors. These combinations are probably more frequent than suspected.

When the peripheral nerve involvement is particularly marked, it will alter the usual clinical picture since it may result in atrophy of the hand muscles giving the appearances of pseudo-ALS (amyotrophic lateral sclerosis). The common bladder disturbances, the rarity of bulbar involvement and the presence of minor sensory changes mitigate against the diagnosis of ALS.

Associated Systemic Symptoms

The existence of systemic symptoms in addition to the neurological syndrome is one of the striking features of HTLV-1 associated pathology.

Lymphocytic alveolitis is present in 80% of cases and is usually silent – abnormalities on chest X-Ray are rarely seen. The diagnosis is made by bronchiolar lavage which shows alveolar lymphocytosis.

Some patients complain of a sensation of ocular and/or oral dryness. The diagnosis of the sicca syndrome can always be confirmed by the Schirmer test or by biopsy of an accessory salivary gland. In addition to the sicca syndrome, uveitis is probably more common than suspected.

Rheumatological features are of two types – atypical lumbosacral back and root pain, arthritis and polyarthritis which may be difficult to differentiate from other inflammatory polyarthritides.

Two types of cutaneous manifestations which are different from ATL have been reported. The first consists of xerosis and hypohydrosis. The second, infective dermatitis, was reported in Jamaica, in children, where there is a prevalence of 100% seropositivity.

HISTOPATHOLOGIC LESIONS IN THE CNS

HAM/TSP affects the spinal cord, especially the thoracic cord, where there is degeneration of the lateral corticospinal, spinocerebellar and spinothalamic tracts of the lateral funiculi. Characteristic of these lesions are the perivascular and parenchymal infiltrates mainly with lymphocytes and foamy macrophages, astrocytic proliferation and fibrillary gliosis.

There is widespread destruction of myelin and loss of axons especially of the corticospinal tracts, findings consistent with the clinical picture.

B-amyloid precursor protein, a marker of early axonal damage in HAM/TSP lesions, is more intensely expressed in areas of active inflammatory lesions than in those of inactive-

chronic lesions. The proximity to areas of inflammation with activated macrophage/microglia, suggests that axonal damage is closely associated with inflammation in active-chronic lesions.

The following observations concerning T cell subsets and cytokine expressions have also been made. The active-chronic lesions of the parenchyma, with both inflammatory and degenerative lesions in the parenchyma of the lateral funiculi, occur in patients whose illness is of short duration (2.4 – 4 years). There is an even distribution of $CD4^+$, $CD8^+$ T cells and macrophages within these lesions and it can be shown by immunohistochemistry that inflammatory cytokines, including TNFα, IL-1β, and IFN-γ are expressed on perivascular infiltrating macrophages, astrocytes and microglia.

In contrast, the spinal cord with disease of eight to ten years duration demonstrate the inactive-chronic lesions, expressed as monotonous degeneration of the lateral funiculi with few inflammatory cells in the subarachnoid and perivascular spaces.

Many chronically activated haematogenous macrophages and microglia are to be found in the active-chronic lesions. Monocyte/macrophage recruitment and activation are downgraded during the course of the disease.

$CD8^+$ CTLs IN HAM/TSP

To confirm the existence of $CD8^+$ cytotoxic T lymphocytes (CTLs) in the central nervous system (CNS) of HAM/TSP patients, the distribution of TIA-1 cells was analysed. TIA-1 (T cell intracellular antigen 1) is a monoclonal antibody that recognizes a 15 KDa granule associated protein contained in CTLs.

In active chronic lesions, many TIA-1^+ cells were distributed throughout the parenchyma and perivascular cuffs and 80% of these TIA-1^+ cells also expressed CD8. In contrast, TIA-1 cells were scarcely observed in inactive chronic lesions, even though $CD8^+$ cells predominated in the parenchyma and perivascular cuffs.

The number of TIA-1^+ cells correlated with the HTLV-1 proviral DNA *in situ*. The protein TIA-1 has also been associated with the induction of apoptosis in target cells. In active inflammatory lesions, cells undergoing apoptosis were found, most of them being identified as helper inducer CD45 RO+ T lymphocytes. Infiltrating CD8 CTLs appeared to correlate with the presence of apoptotic $CD4^+$ cells in inflammatory lesions.

These studies support the view that HTLV-1 infected $CD4^+$ T cells enter the CNS and may drive local expansions of virus specific $CD8^+$ CTLs. These virus specific T cells may then directly lyse virus infected cells, and release a cascade of cytokines and chemokines that result in pathologic changes. Clearly, it is important to define the target of the CTLs in the CNS.

These findings suggest that a T-cell mediated inflammatory process targeting the HTLV-1 infected T-cells is a major event in the pathogenisis of HAM/TSP.

HOST AND VIRAL FACTORS CONTRIBUTING TO HTLV-1 ASSOCIATED DISEASE PATHOGENESIS

The prevalence of developing HAM/TSP among infected individuals is between 0.1% and 3%. Risk factors associated with the disease may be viral, host related or both.

Risk factors associated with the disease may be the viral-size of inoculum, proviral load, virus strain, host related-HLA haplotype, immune response, or both.

THE PROVIRAL LOAD

The proviral load (PVL) of HTLV-1 is unusually high compared with other retroviral infections; a typically healthy carrier of HTLV-1 carries the provirus in about 0.1% – 1% of the peripheral blood mononuclear cells (PBMC). However, the PVL is even higher in the chronic inflammatory disease such as TSP. The PVL remains stable over many years but the prevalence of HAM/TSP rises sharply once the PVL exceeds 1% of the PBMCs.

This association between HAM/TSP and a high PVL of HTLV-1 has been recognized for many years and it is now believed that healthy carriers of HTLV-1 with a high PVL are at risk of progression to disease.

VIRUS STRAIN

Studies have sought to identify strains of HTLV-1 that are either leukaemogenic or neuropathogenic, but with inconclusive results.

Analysis of proviruses found in the CNS of HAM/TSP patients demonstrate 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. Such mutants are 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 have large deletions within the *tax* gene.

Phylogenetic analysis has revealed two *tax* gene subgroups with one subgroup displaying a slightly higher incidence in HAM/TSP as compared to ATL or asymptomatic carriers.

Other studies examining the *tax* sequence from monozygotic twins and their infected mother and brother who had HAM/TSP, indicated that three of the infected adults including the twin with HAM/TSP shared a consensus *tax* sequence. However, the *tax* sequence derived from the asymptomatic twin differed at 5 nucleotide positions including 4 substitutions that resulted in changes in the amino-acids incorporated into the corresponding protein. It can therefore be surmised that small nucleotide changes in the *tax* gene may impact on the clinical outcome.

In addition to *tax*, other viral proteins may be involved in the pathogenesis resulting from HTLV-1 infection. Accessory protein p12 may facilitate HTLV-1 replication and evade the host immune response by preventing presentation of viral proteins in the context of MCH Class 1 molecules.

Interestingly, two naturally occurring mutants of p12 have been described. Sequence variation within the p12 protein may be important to its function. Finally, the reduced stability of p12 in HAM/TSP patients as a result of sequence variation may facilitate the generation of a viral specific CTL response.

Therefore, it appears that 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

Host genetic factors, the most well studied being human leucocyte antigen (HLA) class 1 haplotype are believed to make a critical difference in determining whether an individual will develop an effective immune response to HTLV-1 infection. The effectiveness of the HTLV-1 specific immune response especially the $CD8^+$ CTL response has been shown to be key to controlling the proviral DNA load.

The expression of HLA-A*0201, HLA CW* 08 alleles is associated with a lower proviral DNA load, and a lower risk of developing HAM/TSP, whereas expression of HLA-B5401 was associated with higher proviral DNA load and an increased risk of developing HAM/TSP.

Immunodominant *tax* peptides, such as *tax* 11 – 19, are believed to make strong, stable interactions with the peptide-binding groove of HLA-A* 0201 and HLA CW *08 molecules. Stable interactions facilitate the expression of peptide MHC class 1 molecules on the surface of an infected cell and recognition by antigen-specific $CD8^+$ CTLs.

The stronger the interaction between peptide and MHC molecule, the longer the peptide will remain bound in the peptide binding groove; this will allow for efficient immune surveillance and targeting of the HTLV-1 infected cells. This results in a more efficient cytolysis of HTLV-1 infected cells including $CD4^+$ T cells and the elimination of cells that are potentially leukaemic. However, a population of highly efficient *tax* specific CTLs could lead to targeted lysis of many other cells infected with HTLV-1, usually the resident cell populations, thus facilitating the pathological process seen in HAM/TSP.

Other HLA class 1 alleles have been shown to predispose HTLV-1 infected patients to develop ATL including HLA–A* 2b, HLA–B 4002, HLA–B4006 and HLA–B4801 alleles. The MHC class 1 alleles do not form stable/strong complexes with immunodominant *tax* peptides and thus are not able to stimulate strong *tax*-specific $CD8^+$ CTL responses.

These observations suggest that the relative affinity of the immunodominant epitopes of viral proteins for HLA peptide binding groove acts as a critical determinant with respect to controlling the proviral DNA load, and viral gene expression.

A common theme, therefore, emerges between HAM/TSP and ATL concerning the efficiency of the immune response with respect to control of the proviral DNA load.

THE IMMUNE RESPONSE IN HAM/TSP

Further support for the role of immune mechanisms, both cellular and humoral, in the pathogenesis of the disease is provided by the immunological profile of HAM/TSP patients.

Immune Response	Serum	CSF
High HTLV-1 antibody titres	x	x
Spontaneous lymphoproliferation	x	–
High HTLV-1 proviral DNA load	x	–
Increased cytokine production	x	x
Increased levels of VCAM-1	–	x
Increased activated T cells	x	–

* Antibodies against the *gag* (p24) protein are the first to appear after infection and predominate in the first 2 months, followed by anti-*Env* antibodies. About 50% of individuals subsequently produce antibodies to the *tax* protein. However, it is unclear whether the serum antibody titre correlates significantly to the protection from or the pathogenesis of HTLV-1 associated disease or to controlling the equilibrium virus load.
* Spontaneous lymphoproliferation occurs in all HTLV-1 infected patients but the magnitude is most pronounced in HAM/TSP. This lymphoproliferation is thought to consist of the proliferation of $CD4^+$ cells and the expansion of $CD8^+$ T cells, based on the demonstration of an increase in virus expressing cells, concomitant with an increase in the percentage of $CD8^+$ T cells.
* Dysregulation of immune responses, such as elevated cytokine expression and production, has been demonstrated in the PB and CSF of HAM/TSP patients. Increased levels of IFN-gamma, TNF alpha and IL6 as well as the pro-inflammatory cytokines induced by HTLV-1 *tax* have been incriminated in HAM/TSP pathogenesis.

HTLV-1 *tax* seems to affect the ability of astrocytes to manage steady state levels of glutamate which in turn would affect neuronal and oligodendrocyte function.

THE HELPER T CELL RESPONSE

The $CD4^+$ T cells are the main subset of cells infected with HTLV-1 *in vivo.* $CD4^+$ T cells in HAM/TSP patients have a more Th-1-like phenotype as characterized by upregulated secretion of pro-inflammatory cytokines such as IFN-γ and TNFα and downregulated levels of Th2 cytokines such as IL4.

$CD4^+$ T cells have increased adhesion activity to endothelial cells and transmigrating activity through the basement membrane that allow migration of infected $CD4^+$T

cells into the CNS. Entry into the CNS by HTLV-1 $CD4^+$ T cells that have increased production of TNFα – a neurotoxic cytokine – may be responsible for the initiation of the inflammatory process in HAM/TSP.

$CD8^+$ T CELL RESPONSE TO THE VIRUS

The main features of the CD8 T cell response are:

* the high frequency of the HTLV-1 specific CD8 T cells
* the ability of the circulating $CD8^+$ T cells (up to 10%) to recognize just one epitope of the virus
* chronic activation.

HAM/TSP PATIENTS:

* have higher frequencies of *tax* specific precursor T cell, at least a 100-fold higher than is found in asymptomatic carriers of the virus
* exhibit a marked increase in virus specific $CD8^+$ T cells response with the antiviral CTLs specific for *tax*
* display a significant increase in the presence of *tax* specific memory and effector $CD8^+$ T cells;
* and high levels of circulating CD8 T cells specific for the PX region of the HTLV-1 genome.

These cells are absent in patients without neurological disease.

Activated *tax*-specific CTLs have been found in the peripheral blood and accumulate in the CSF of HAM/TSP. Longevity of the *tax*-specific CTL expression was observed consistently over a 9-year time course in one patient at least 19 years after the onset of disease. 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 HAM/TSP.

HTLV-1 INDUCED DISEASE PROGRESSION

The events leading to disease following exposure to the virus seem to evolve in three stages – host infection, clinical latency, CNS invasion.

THE ROUTE OF INFECTION

There is an interesting connection between the route of infection and the initial host immune response. It is a widely held view that the primary route of infection permits the virus access to different cellular compartments of the host and exposes particular subsets of target cells to it. HTLV-1 infection of the peripheral blood leads to viral invasion of the $CD4^+$ and $CD8^+$ T cell populations as well as cells of the monocyte/macrophage lineage. T cell migration to the peripheral tissues is limited in the absence of inflammation. Consequently, greater levels of these cells are to be found in the peripheral blood than in the peripheral tissues.

In contrast, professional antigen presenting cells (APCs) – dendritic cells – and monocytes/macrophages which represent only a small percentage of PBMCs in the peripheral blood, are found in large numbers in the peripheral tissues and mucosal membranes.

Due to the relative proportions of T cells and APCs in the peripheral blood and at mucosal surfaces, viral entry by the PB largely leads to infection of $CD4^+$ and $CD8^+$ T cells; alternatively HTLV-1 entry at mucosal sites leads primarily to infection of target cells, including APCs.

Infection of $CD4^+$ T cells is associated with enhanced production amplification of peripheral blood virus and enhanced infection of lymphoid DCs. This contrasts with the interaction of HTLV-1 with the DC compartment – where viral infection of DC is only modestly productive, smaller numbers of cells becoming infected and smaller amounts of viral proteins and infectious virus being produced. Infection of DCs in conjunction with helper T cells likely lead 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 viral infection by killing infected cells and abrogating productive viral replication in both the mucosae and the peripheral blood.

EVENTS IN THE BONE MARROW – CLINICAL LATENCY

Following resolution/partial resolution of primary HTLV-1 infection, infected $CD4^+$ T cells routinely traffic to the bone marrow (BM). This normal trafficking over a prolonged period leads to a progressive and extensive invasion of the haemopoietic stem cell system including the $CD34^+$ progenitor cells. A long period of clinical latency ensues when a number of HTLV-1 pathogenic mechanisms occur and set the stages and development of HAM/TSP. The BM of HAM/TSP contains high levels of proviral DNA; cells are essentially negative for viral RNA and protein confirming the extensive latent infection of the BM and its $CD34^+$ progenitor cells.

The proviral genome is maintained throughout the process of differentiation into its multiple cell lineages. There is no viral gene expression in these cells, but they can differentiate into multiple cell lineages that promote cell-type specific synthesis of viral proteins from the integrated provirus.

Differentiation leads to activation of the HTLV-1 LTRs mediated by changes in the transcription factor *milieu* induced by the process of cell expression. Changes in gene expression lead to differentiation of $CD34^+$PG cells into DCs and monocytes/macrophages and to alterations in abundance or activity of cellular transcription factors capable of initiating gene expression from the LTRs. Changes in transcriptor factor expression in turn lead to a regulated low level of basal transcription and *tax* mediated viral LTR transactivation.

The stage is now set for synthesis of viral proteins and subsequent antigen presentation of peptides derived from these proteins including the immunodominant *tax* protein.

This process also serves to induce the differentiation of $CD8^+$ T cells into *tax* specific effector CTLs.

In the early phase of asymptomatic infection, this process would still be part of the normal immune surveillance in which small numbers of HTLV-1 infected APC 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 $CD34^+$PGC in the BM which is constantly being generated despite the presence of *tax* specific CTLs. Newly generated APC can continuously present viral peptides to $CD8^+$ T cells leading to an over-production of *tax* specific CTLs. The proportion of CTLs specific for *tax* is very high in HAM/TSP patients and about 10 fold lower in asymptomatic carriers of the virus.

In the next phase, members of the infected progenitor cells (PGC) population seed the PB with APCs capable of continuously stimulating the activation of CD8+ T cells to differentiate into *tax* specific CTL. The resulting generation of a highly expanded, highly efficient population of *tax* specific CTLs migrate to the CNS and mediate damage to it.

T CELL MIGRATION AND ACCUMULATION IN THE CNS

If cell migration is to result in disease, barriers to entry into the CNS must be overcome. Leucocyte adhesion molecules play an important role in the pathogenesis of many inflammatory diseases including HAM/TSP.

The spinal cord lesions of HAM/TSP have greater vascular cell adhesion molecule-1 (VCAM-1) expression on their endothelium compared with the spinal cord of controls. Infiltrating mononuclear cells especially perivascular lesions express very late antigen-4 (VLA) and monocyte chemo-attractant protein-1 (MCP-1) are also upregulated on perivascular infiltrating cells and vascular endothelium in active chronic inflammatory lesions.

These findings suggest that VLA-4/VCAM-1 interaction and MCP-1 are associated with the massive infiltration of lymphocytes observed in the spinal cord.

After transendothelial migration, T cells/macrophages then encounter the extracellular matrix (ECM) and must pass through the basement membrane before migrating into the interstitial matrix. Proteolytic disruption of ECM by matrix metalloproteinases (MMPs) is a key process for the damage to the blood brain barrier (BBB).

Collagen and decorin immune-reactivity on the basement membrane of the CNS parenchymal vessels is disrupted in areas where inflammatory mononuclear cells infiltrate inactive chronic lesions. In these lesions, MMP-2 (gelatinase A) was immunostained mainly on the surface of foamy macrophages and lymphocytes whereas MMP-9 (gelatinase B) expression was positive in the intravascular and perivascular mononuclear cells but not on foamy macrophages.

Inactive-chronic lesions contain much smaller numbers of MMP-2 positive or MMP-9 positive mononuclear cells than active-chronic lesions.

Production levels of MMP-2 and MMP-9 of sera and CSF are higher in patients with HAM/TSP than those in non-inflammatory neurologic conditions used as controls.

The requirements for migration and transit into the CNS, having been satisfied, the offending $CD8^+$ T cells are now able to inflict damage on the resident cell population of the CNS.

DAMAGE TO CNS TISSUE

It is widely assumed that the immune response caused the inflammatory tissue damage seen in HAM/TSP because the disease is accompanied by high titres or HTLV-1 antibody and high frequencies of activated T-cells. Also the tissue damage in the CNS is associated with dense infiltrates of mononuclear cells.

There are three possible ways in which tissue damages could occur.

DIRECT TOXICITY HYPOTHESIS

In the direct toxicity hypothesis, it is presumed that HTLV-1 infects glial cells *in vivo,* which then present HTLV-1 antigens on the cell surface. Circulating $CD8^+$ cytotoxic T cells specific for an HTLV-1 antigen, cross the blood brain barrier, encounter the infected cells and release cytokines which kill them. This possibility appears to be excluded by the observation that there is little or no HTLV-1 infection of resident cells in the central nervous system of HAM/TSP patients.

BYSTANDER THEORY (THE MOST LIKELY)

HTLV-1 infected $CD4^+$ and anti-HTLV-1 specific $CD8^+$ lymphocytes chronically activated by the *tax* protein, acting as an antigen and/or mitogen, migrate across the BBB and meet in the CNS.

They mediate CNS damage through the production of neurotoxic mediators including inflammatory cytokines. *Tax* specific $CD8^+$ CTLs have been demonstrated to secrete a variety of pro-inflammatory cytokines including IFNγ, TNFα, MIP1α, MIP1β, MMP-9 and IL16.

TNFα: can cause cytotoxic damage to endothelial cells that decrease the integrity of the BBB.

IL16: is a chemo-attractant for $CD4^+$ T cells which secrete IL-2, a growth factor required for proliferation of $CD8^+$ T cells. It therefore plays an important pathogenic role by inducing the recruitment of HTLV-1 infected $CD4^+$ T cells into the CNS.

The uncontrolled production of a toxic combination of inflammatory factors causes death of uninfected cells resident in the CNS.

AUTOIMMUNE THEORY

Molecular mimicry refers to the generation of an immune response to an environmental agent that cross-reacts with a host antigen resulting in autoimmune damage.

This hypothesis presumes that a glial cell self-antigen is similar to a viral antigen. $CD4^+$ helper cells encounter this viral antigen in the periphery and upon crossing the blood brain barrier, mistake the glial cell for an infected cell and trigger autoimmune activity with the death of the glial cell.

Mr Chairman, ladies and gentlemen – to summarize, the chain of events leading to disease would appear to be as follows:

* Inefficient CTL response to HTLV-1
* High proviral load and high level of *tax* expression
* High frequency of *tax* activated $CD4^+$ T-Cells
* Frequent migration of $CD4^+$ T-Cells to the CNS
* Release of IFN-γ, TNF and metalloproteinases by antigen-stimulated T-cells
* Tissue damage

Even today, after sustained research, there is still no effective treatment for HAM/TSP. Great strides have been made in creating animal models and they should provide a better understanding of ongoing events in the disease. An important challenge will be to identify critical points at which the disease may be aborted. "How far have we come?" was the question posed at the start of the presentation. The answer – quite far, but still not far enough!

I wish to thank the many persons who assisted with the preparation of this paper and you the audience for your kind attention. References are available on request.