Carol Shoshkes Reiss *Editor*

Neurotropic Viral Infections

Volume 2: Neurotropic Retroviruses, DNA Viruses, Immunity and Transmission

Second Edition

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Preface

This is one of two books that comprise a total of 29 cutting-edge review articles written by leaders in the basic, clinical, and translational fields working on viruses that infect the central nervous system (CNS). Book 1 highlights those pathogens with an RNA genome. Book 2 includes chapters on retroviruses, DNA viruses, prions, immunity, transmission, and beneficial uses of neurotropic viruses.

In this second volume, there are 15 chapters on neurotropic or neuroinvasive viruses that are human pathogens. Viruses capable of infecting the cells within the brain can be spread to people by many routes including ingestion (Chapter "Transmissible Spongiform Encephalopathies"), by the respiratory route (one example is JC virus or Varicella zoster virus, Chapters "Molecular Biology of JC Virus and the Human Demyelinating Disease, Progressive" and "The Pathogenesis of Varicella-Zoster Virus Neurotropism and Infection"), by insect (Chapter "Influences of Arthropod Vectors on Encephalitic Arboviruses") or animal (bats can transmit Rabies, Chapter "The Role of Bats as Reservoir Hosts of Emerging Viruses") bites, as sexually transmitted infections (HIV, for instance, Chapter "HIV"), or iatrogenically by transplantation of infected organs (Chapter "Transmission of Neurotropic Viruses by Transplantation").

Some viruses cause central nervous system (CNS) disease in a small subset of people infected, and this may be due to many factors including variants in host genes, underlying chronic health conditions, or mutations in the virus. To become successful pathogens, many neurotropic viruses have become masters of evasion of host innate (Chapter "Innate Immunity in Viral Encephalitis") or adaptive immune responses.

Viral infections can be prevented by avoiding exposure or by some excellent vaccines. The vaccine against varicella zoster virus can prevent the reactivation of the CNS infection in older individuals who had chicken pox as children (Chapter "The Pathogenesis of Varicella-Zoster Virus Neurotropism and Infection").

Attenuated neurotropic viruses have beneficial roles in vaccine carriers against other infections; the highly effective recombinant ebola glycoprotein vaccine is vectored by vesicular stomatitis virus, a virus related to rabies. Many tumors are susceptible to viral infections while neighboring normal cells are resistant; this has led

to the development of targeted virus infections as a treatment for cancer (Chapter "Viral Oncolysis of Glioblastoma"). Finally, viruses can also be used to deliver genes to correct defects (Chapter "Viral Gene Therapy for Central Nervous System Disorders").

This volume 2 is organized into two parts: (1) Retroviruses, DNA Viruses, and Prions and (2) Immunity, Diagnosis, and Beneficial Uses of Neurotropic Viruses.

Two retroviruses whose genomes are RNA but encode a reverse transcriptase which makes a DNA copy are described in Part 2; these are HIV (Chapter "HIV") and HTLV-1 (Chapter "The Pathogenesis of HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis"). These viruses are capable of both acute infection and latency, where the genome is in cells not actively making progeny. Latency can be transient or last for decades until conditions change. DNA viruses that undergo latency are described in the following chapters: JC (Chapter "Molecular Biology of JC Virus and the Human Demyelinating Disease, Progressive"), herpes simplex (Chapter "Herpes Simplex Viruses"), varicella zoster virus (Chapter "The Pathogenesis of Varicella-Zoster Virus Neurotropism and Infection"), and Epstein–Barr virus (Chapter "Virus-Induced Demyelination: The Case for Virus(es) in Multiple Sclerosis"). The final chapter in Part 2 is devoted to prionoses, that is, neurodegenerative diseases caused by infectious proteins.

The final Part of the book has chapters on the essential role of innate immune responses (Chapter "Innate Immunity in Viral Encephalitis") and on the impact of the interaction between the CNS and immune responses to viral infections (Chapter "Neuroendocrine-Immune Interactions in Neurotropic Viral Infections"). The chapter on Clinical Management of neurotropic viral infections (Chapter "Clinical Management of Viral Encephalitis") is followed by chapters on the roles of insects (Chapter "Influences of Arthropod Vectors on Encephalitic Arboviruses") and bats (Chapter "The Role of Bats as Reservoir Hosts of Emerging Viruses") in transmission of these infections. Iatrogenic transmission associated with transfusions and organ transplants is covered in Chapter "Transmission of Neurotropic Viruses by Transplantation". The last two chapters are devoted to viral oncolysis (Chapter "Viral Oncolysis of Glioblastoma") and to therapeutic transfer of genes using viruses (Chapter "Viral Gene Therapy for Central Nervous System Disorders").

This second edition of *Neurotropic Viral Infections* builds upon the highly successful first edition published in 2008 by Cambridge University Press ISBN-13: 978-0521869645. I would like to acknowledge the generosity of Cambridge University Press in permitting us to move *Neurotropic Viral Infections* to Springer Scientific Publishers for the second edition. Arthur Smilios convinced me to undertake this volume. When he left Springer, Rita Beck ably succeeded him with the project. The book would not have reached the final stage without the fantastic production assistance by Portia Formento Wong.

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Part I
Retroviruses, DNA Viruses and Prions

The Pathogenesis of HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis

Aileen G. Rowan and Charles R.M. Bangham

Introduction

Human T lymphotropic virus 1 (HTLV-1) was discovered in 1980, when Robert Gallo and his colleagues observed production of retroviral particles by a cell line established from a patient with a T-cell lymphoma (Poiesz et al. 1980). Concurrently, two groups in Jamaica and Japan detected HTLV-1-specific antibodies in the cerebrospinal fluid (CSF) and serum of patients with a progressive myelopathy that was previously known as tropical spastic paraparesis (TSP) and named by the Japanese group HTLV-1-associated myelopathy (HAM) (Gessain et al. 1985; Osame et al. 1986). TSP and HAM were subsequently identified as the same condition, and the disease is now designated HAM/TSP. HAM/TSP is characterised by lesions in the spinal cord, resulting in a loss of control of motor functions below the waist, constipation, incontinence and neuropathic pain.

The primary target cell infected by HTLV-1 in vivo is the CD4+ T lymphocyte: HTLV-1 is not neurotropic in the strict sense, because it does not infect neurons. Instead, HTLV-1 reaches the CNS via migration of infected lymphocytes across the blood– brain barrier (BBB), and this process is thought to initiate HAM/TSP. The risk of developing HAM/TSP rises exponentially with increasing viral burden (Nagai et al. 1998), and whilst the disease is not directly life-threatening, it lowers life expectancy and causes significant morbidity (Olindo et al. 2006). Here, we discuss the recent developments in our understanding of the factors influencing HTLV-1 spread, immune control and the pathogenesis of the inflammatory disease.

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Molecular Virology of HTLV-1

HTLV-1 is a member of the *Deltaretrovirus* genus of the *Orthoviridae* subfamily (Poiesz et al. 1980). In addition to genes encoding the core protein (Gag), reverse transcriptase (Pol) and envelope proteins (Env) present in all replication-competent retroviruses, the HTLV-1 genome contains the pX region (Fig. 1a) (Coffin et al. 1997). This region encodes several nonstructural regulatory and accessory proteins,

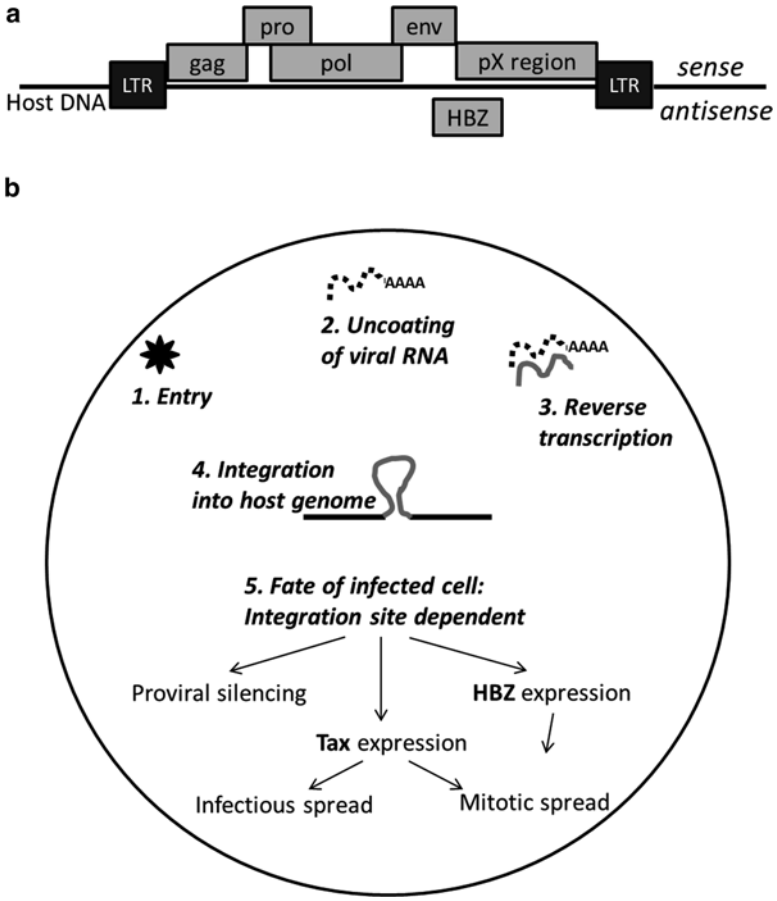

Fig. 1 Genome and life cycle of HTLV-1. (**a**) Schematic of the HTLV-1 genome. The pX region encodes several multiply spliced open reading frames which orchestrate viral persistence and spread (see Coffin et al. 1997 for further details). The antisense strand encodes HBZ, which is expressed at a steady rate in vivo. (**b**) Life cycle of HTLV-1 from the point of viral entry. The proviral genomic integration site influences the mode of viral gene expression and thus the fate of each clone generated by de novo infection. Tax expression initiates infectious spread; however production of viral proteins exposes the infected cell to immune surveillance. Cells which only express the weakly immunogenic HBZ undergo significantly reduced immune surveillance

which orchestrate viral transcription, spread and persistence (Nicot et al. 2005; Matsuoka and Jeang 2007), with the use of multiple splicing, alternative reading frames and antisense strand-encoded genes allowing compression of a remarkable amount of information into the 9 kb genome.

Although HTLV-1 is a blood-borne retrovirus, viral particles are virtually undetectable in the serum. Instead, HTLV-1 persists in vivo mainly in the form of the DNA provirus, stably integrated into T lymphocyte DNA. Contact between an infected lymphocyte and an uninfected 'target' cell triggers directional transport of viral components towards the intimate cell-to-cell contact known as the 'virological synapse' (Igakura et al. 2003; Majorovits et al. 2008; Nejmeddine et al. 2009), where viral particles are assembled and immediately fuse with the plasma membrane of the target cell. In order to produce infectious virus, the potent viral transcriptional transactivator protein Tax activates the promoter in the long terminal repeat region (LTR), following which viral transcripts are transported to the cytoplasm by a second viral protein (Rex) translated from the same *tax*/*rex* mRNA. Tax promiscuously binds cellular proteins (Grassmann et al. 2005) inducing many changes in the cellular phenotype, altering, expression of T-cell activation markers, cytokine receptors, e.g. CD25, (Inoue et al. 1986; Cross et al. 1987), adhesion markers, e.g. ICAM-1, (Fukudome et al. 1992) and dysregulating signalling pathways and cell cycle control (Yoshida 2001; Matsuoka and Jeang 2007). The antisense-encoded viral protein HTLV-1 b-ZIP factor (HBZ) tempers many of the actions of Tax (Matsuoka and Yasunaga 2013): Tax and HBZ exert opposing effects on the nuclear factor kappa beta (NFkB), cAMP response element-binding protein (CREB), transforming growth factor-β (TGF-β), activator protein 1 (AP-1) and WNT signalling pathways (Matsuoka and Yasunaga 2013).

HTLV-1 can also spread by inducing proliferation of the infected host cell, without the need to produce infectious particles, a process which may be called mitotic spread (Fig. 1b). $CD4^+$ T cells lend themselves perfectly to this purpose, being highly adapted to proliferate and establish long-lived clonal populations. Both Tax (Grassmann et al. 1989) and HBZ (Satou et al. 2006) promote proliferation and immortalisation of T cells, and the balance between Tax and HBZ expression appears to be of critical importance in viral persistence. Several lines of evidence indicate that Tax is frequently silenced (Koiwa et al. 2002; Taniguchi et al. 2005) and even deleted in chronic infection (Tamiya et al. 1996; Furukawa et al. 2001, 2006; Miyazaki et al. 2007). Silencing of Tax diminishes the production of virus particles, but also reduces the exposure of that infected T-cell clone to CTL- and antibody-mediated immune selection. In contrast to Tax, the region encoding HBZ is maintained intact and is constitutively transcribed (Satou et al. 2006). Untranslated HBZ mRNA also promotes cellular proliferation (Satou et al. 2006) and, unlike other viral genes, HBZ mRNA can be readily detected in all carriers in vivo (Saito et al. 2009), providing an elegant mechanism by which the virus cannot only survive but proliferate with minimal exposure to immune surveillance.

Viral Transmission and HTLV-1-Related Diseases

Transmission of the virus requires prolonged close contact between individuals: thus pockets of high prevalence are typically restricted to particular ethnic groups or communities, sometimes living in otherwise low prevalence areas (Proietti et al. 2005; Gessain and Cassar 2012). Infection typically occurs early in life through breastfeeding or later through sexual contact. Other routes include exposure to infected blood or transplanted organs. Infectious virus is almost entirely cell associated: plasma is not infectious, and leukodepletion of blood products greatly reduces the probability of infection by transfusion (Pennington et al. 2002; Hewitt et al. 2013). The current best estimate of the minimum number of infected individuals worldwide is 5–10 million people (Gessain and Cassar 2012), and as 90–95 % remain lifelong asymptomatic carriers of the virus, infection is often detected by chance or by screening of relatives of infected individuals. Whilst HAM/TSP is the best characterised and most common inflammatory disease associated with HTLV-1, a range of other conditions are frequently observed in carriers, including uveitis, polymyositis, pulmonary disease and infective dermatitis (Martin et al. 2014). HTLV-1 also is the etiological agent of adult T-cell leukaemia (ATL), an aggressive malignancy of mature $CD4^+$ T cells (Matsuoka and Jeang 2007).

Symptoms, Diagnosis and Prognosis of HAM/TSP

Patients with HAM/TSP experience spasticity in the lower portion of their bodies, manifesting as stiff, weak and heavy legs (Gessain et al. 1985; Osame et al. 1986). This is frequently accompanied by neuropathic pain, sensory disturbances, backache, constipation and incontinence (Martin et al. 2014). A recently proposed revision of the World Health Organization diagnostic criteria (Osame et al. 1990) outlines three levels of confidence of HAM/TSP diagnosis: definite, probable and possible (De Castro-Costa et al. 2006). HTLV-1 seropositivity is required in all cases, as is exclusion of other conditions which present in a similar manner, such as tumours of the spinal cord. Confidence in the accuracy of diagnosis is strengthened by detection of a high proviral load in peripheral blood, in conjunction with inflammatory markers in the CNS (Martin et al. 2014). The proviral load in the CNS does not correlate with that in the peripheral blood (Puccioni-Sohler et al. 1999; Nagai et al. 2001); however, a high ratio of CNS PVL/peripheral blood PVL appears to be a hallmark of HAM/TSP (Lezin et al. 2005).

The rate of onset and severity of symptoms can vary widely between patients, and timing of a fixed-distance (e.g. 10 m) walk has emerged as a useful objective method to monitor progression (Martin et al. 2010). A significant proportion of individuals diagnosed are slow progressors or non-progressors (41 % of patients with a history of disease >10 years) (Matsuzaki et al. 2001). In the remainder, the symptoms intensify, and a decade after diagnosis, the patient is likely to require walking assistance. After a further decade, he/she typically needs a wheelchair or becomes bedbound (Martin et al. 2010; Olindo et al. 2006).

Histopathological Observations

Autopsies of individuals with confirmed HAM/TSP show focal lesions in the mid- to lower thoracic regions of the spinal cord consisting of inflammatory mononuclear cell infiltrates (Montgomery et al. 1964; Iwasaki 1993). New lesions (in patients with a shorter history of symptoms) show perivascular infiltration, beginning with $CD4^+$ and $CD8^+$ lymphocytes (Iwasaki et al. 1992). The proviral load in the infiltrating cells is high and correlates with the frequency of infiltrating $CD4^+$ cells (Kubota et al. 1994). $CD8^+$ cells predominate in older lesions, which progressively become atrophic and acellular. Advances in imaging technology have revealed that small lesions are also present in the brain (Aye et al. 2000); however, their significance in disease is not yet understood.

Steps in the Development and Progression of HAM/TSP

After many years of asymptomatic carriage of HTLV-1, a minority develop HAM/TSP. Prior to the onset of symptoms, these individuals typically have a high viral load, indicating that the balance between viral spread and immune control favours the virus. Despite a clear and strong association between HAM/TSP and a high proviral load, not all carriers with a high proviral load develop disease. Here, we outline the known risk factors for HAM/TSP and summarise current theories as to how HTLV-1 reaches the CNS and causes irreversible damage.

How Is the Proviral Load Maintained in Chronic Infection?

Soon after initial exposure to the virus, a 'set point' viral load is established in the host. This set point is remarkably stable over the lifetime of the host, but varies by more than 1000-fold between individuals (Demontis et al. 2013). We can distinguish between infectious and mitotic viral spread by analysis of viral integration sites: infectious spread results in viral integration into a novel unique position in the host DNA, whereas mitotic spread generates two daughter cells which both carry the provirus integrated at the same genomic position. Recent advances in high-throughput sequencing technology have allowed precise mapping and quantification of large numbers of viral integration sites in HTLV-1-infected donors (Gillet et al. 2011). This previously unparalleled depth of information allows us to analyse the clonal structure of infected cells, estimate the total numbers of infected clones and individual infected cells in the host and quantify the contribution of infectious and mitotic spread to maintaining the PVL. Using mathematical modelling we estimate that there are on average 2.9×10^4 unique infected clones in the peripheral blood of patients with HAM/TSP (Laydon et al. 2014). Whilst HTLV-1 displays a preference for

integrating near certain genomic features (Melamed et al. 2013), there are no 'hotspots', and integration is sufficiently diverse to distinguish clones resulting from individual infection events.

The low degree of sequence variation within HTLV-1 had previously pointed towards clonal proliferation as the major mechanism by which the virus maintains the proviral load. The results of high-throughput sequencing are consistent with this conclusion: longitudinal analysis reveals that clones of infected cells which share a genomic integration site persist for long periods (>10 years) (Gillet et al. 2011). These clones are not static, as HTLV-1-infected cells both proliferate and die faster than uninfected cells in vivo, maintaining the system in dynamic equilibrium (Asquith et al. 2007). These observations do not exclude the possibility that infectious propagation continues throughout the course of infection; the conclusion is rather that new clones of infected cells do not frequently become established.

The clonal structure is complex: the relative contribution to the PVL made by the largest clones varies widely between individuals (Gillet et al. 2011). A useful metric of clonality—the Gini index—reveals that the clone frequency distribution of infected cells in patients with HAM/TSP is indistinguishable from that in asymptomatic carriers of the virus (Gillet et al. 2011). These data rule out the role of uncontrolled expansion of a large pathogenic clone in the pathogenesis of HAM/TSP. In fact, in comparison with ACs, patients with HAM/TSP had a significantly greater number of low abundance clones detectable in their peripheral blood (Niederer et al. 2014; Laydon et al. 2014), perhaps indicating a higher rate of infectious viral spread.

Who Is at Risk of Developing HAM/TSP?

Central to the prediction of the risk of developing HAM/TSP is the proviral load (Fig. 2). When the load is greater than 1 % of PBMCs infected, the risk increases exponentially (Nagai et al. 1998; Jeffery et al. 1999), and the great majority of patients with HAM/TSP have a viral load which exceeds this threshold (Nagai et al. 1998). A high viral load is necessary but not sufficient to induce HAM/TSP: many individuals who have a PVL > 1 % do not develop HAM/TSP. HAM/TSP is three to four times more common in females than males (Maloney et al. 1998; Lima et al. 2005), with a peak in the age at disease onset between 40 and 50 years, typically decades after infection with the virus. The lifetime risk of HAM/TSP also varies between populations, from 0.25 % of HTLV-1-infected individuals in Japan (Kaplan et al. 1990) to 2–4 % in Afro-Caribbeans (Murphy et al. 1997; Maloney et al. 1998; Orland et al. 2003). The risk of HAM/TSP is slightly greater in individuals which carry the Cosmopolitan (subtype A) strain of the virus, in comparison with the Japanese subtype B strain (Furukawa et al. 2000).

Despite geographic separation, HTLV-1 genomic sequences are remarkably highly conserved (Daenke et al. 1990; Komurian et al. 1991; Vandamme et al. 1994; Pecon Slattery et al. 1999). Thus, host factors must have a significant influence on the diverse outcomes of infection that are observed. In particular, several lines of

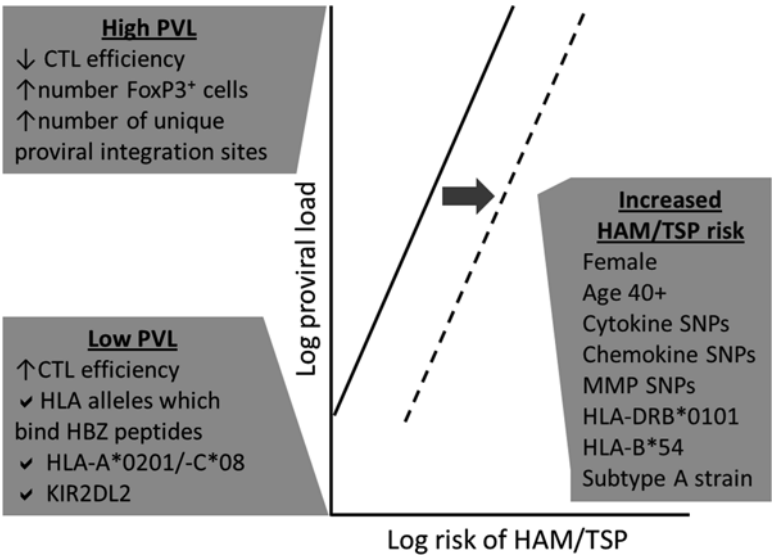

Fig. 2 Summary of factors determining risk of developing HAM/TSP. Risk of HAM/TSP rises exponentially with proviral load: typical immunological and virological features of individuals with low and high proviral loads are summarised. Host factors which can modify the risk of developing HAM/TSP are also outlined. *PVL* proviral load, *CTL* cytotoxic T lymphocyte, *HLA* human leukocyte antigen, *KIR* killer cell immunoglobulin-like receptor, *SNP* single nucleotide polymorphism, *MMP* matrix metalloproteinase

evidence highlight the importance of the heterogeneity of the host immune response both in the pathogenesis of HAM/TSP and in protection from the disease (Fig. 2). Firstly, single nucleotide polymorphisms in certain cytokine and chemokine genes have been associated with development of HAM/TSP: possession of the tumour necrosis factor (TNF)-863A allele and a promoter polymorphism in the matrix metalloproteinase 9 (MMP9) increases the risk of disease (Vine et al. 2002; Kodama et al. 2004), whereas the presence of the interleukin (IL)-10 −592A, stromal cell-derived factor-1 (SDF)-1 +801A and IL-15 +191C alleles confers protection (Vine et al. 2002; Sabouri et al. 2004). Secondly, possession of certain human leukocyte antigens (HLA) is protective and associated with a low viral load (Jeffery et al. 1999), emphasising the importance of an individual's ability to mount an efficient cytotoxic lymphocyte (CTL) response.

Contribution of the CTL Response to the Control of Viral Spread

The first substantial evidence of the critical role of the CTL response in controlling infection was the observation that possession of HLA-A*02 was protective (Jeffery et al. 1999). HLA-A*02 and C*08 were underrepresented in donors with HAM/TSP

in a cohort of 201 ACs and 232 unrelated patients with HAM/TSP from Kagoshima, Japan. A highly immunodominant peptide epitope in Tax (residues 11–19) binds to HLA-A*0201 with extremely high affinity (Kannagi et al. 1992), and CTL specific for $Tax_{11–19}$ are found at frequencies of up to 10 % circulating $CD8^+$ T cells in chronically infected donors (Kannagi et al. 1991; Nagai et al. 2001). This epitope in particular is subject to selection in vivo, and CTL escape mutations are more frequently observed in ACs than in patients with HAM/TSP, indicating that the selection pressure—the CTL efficiency or 'quality'—is greater in ACs (Niewiesk et al. 1994, 1995). These observations are supported by the data from an ex vivo assay of autologous CTL killing: the efficiency of lysis of naturally infected Tax-expressing cells negatively correlates with proviral load (Asquith et al. 2005), and CTL efficiency is closely correlated to the functional avidity of Tax-specific $CD8^+$ cells (Kattan et al. 2009). Certain HLA alleles can exacerbate the disease: HLA-DRB1*0101 and HLA-B*54 each increase the risk of HAM/TSP. The mechanism by which this may be effected is less clear; however, HLA-DRB1*0101 increased the probability of HAM/TSP development only in the absence of HLA-A*02, suggesting that efficient viral control can reduce the influence of disease susceptibility alleles.

Quantifying protective effects mediated by HLA molecules can be frustrated by their diversity, which necessitates large studies to quantify the influence of rare alleles. MacNamara et al. took an alternative approach: by ranking the predicted HLA-binding affinity of peptides from the HTLV-1 proteome, they tested whether an individual's predicted ability to present epitopes from each protein was associated with efficient control of the virus (MacNamara et al. 2010). Unexpectedly, in the Kagoshima cohort, the ability to present peptides from the regulatory protein HBZ was significantly associated with lower proviral load and a reduced probability of HAM/TSP. HBZ is very poorly immunogenic for CTL: the mean binding affinity of HLA alleles to HBZ peptides is significantly lower than their binding affinity to Tax peptides (MacNamara et al. 2010). This observation is mirrored by the low frequency of individuals (approx. 25 %) in which HBZ-specific CTL responses can be detected in the circulation (MacNamara et al. 2010; Hilburn et al. 2011), and in a given individual, the frequency of HBZ-specific $CD8^+$ T cells is usually significantly lower than the frequency of Tax-specific $CD8^+$ cells (MacNamara et al. 2010; Hilburn et al. 2011). Thus, low-frequency CTL responses to certain subdominant antigens can make an important contribution to the control of the proviral load and the risk of inflammatory disease.

Further analysis of the Kagoshima cohort revealed a surprising novel factor associated with efficient CTL control: the natural killer (NK) cell receptor known as killer cell Ig-like receptor (KIR)2DL2 potentiates the effect of possession of protective HLA class 1 alleles (Seich alBasatena et al. 2011). When expressed on NK cells, the main role of KIR molecules is to survey the HLA class 1 expression of potential target cells in order to detect any changes associated with infection or malignancy. In the case of HTLV-1 infection, it is not NK cells but rather KIR-expressing $CD8^+$ T cells that are most likely to be responsible for viral control, because the protective effect of high-affinity HBZ-binding class 1 alleles was lost in the absence of KIR2DL2. The mechanism by which KIR2DL2 enhances class 1 MHC-associated protection is not known; it is possible that expression of inhibitory KIR molecules by T cells extends the lifespan of the T cell by modulating its activa-

tion and thus reducing the probability of activation-induced cell death in chronic infection. However, KIR gene enhancement is not always necessary for efficient CTL control: the protective effect of HLA A*02 was observed both in the presence and absence of KIR2DL2 (Seich alBasatena et al. 2011).

Persistent Inflammation and Inefficient Immune Responses in Established HAM/TSP

Because HAM/TSP is uncommon and its onset is unpredictable, there have been few opportunities to study HTLV-1-specific immune responses in individuals before they develop the disease. Instead, most published studies compare steady-state immune responses in ACs and patients with HAM/TSP. A major confounding factor in the study of immune responses in chronic infection is the fact that high levels of antigenic stimulation increase the frequency and activation state of antigen-specific cells. For example, the frequency of HTLV-1-specific CTLs is partly the cause and partly the effect of the efficiency of control of HTLV-1 proviral load. As the average proviral load in patients with HAM/TSP is significantly higher than in ACs, careful analysis must be performed to test whether any differences observed are correlated with antigen load rather than with disease status per se.

In HTLV-1-infected individuals, Tax is consistently strongly immunodominant for CTLs (Goon et al. 2004). Antigen-specific $CD8^+$ T cells are present in patients with HAM/TSP in equal or greater abundance than in ACs (Jacobson et al. 1990; Kannagi et al. 1991). The presence of more HTLV-specific CTLs does not translate into efficient viral control: HTLV-1-specific $CD8^+$ T cells from patients with HAM/TSP are less efficient at killing naturally infected cells (Asquith et al. 2005). In fact, incomplete clearance of infected cells may contribute to the generalised inflammatory symptoms observed in HAM/TSP: this hypothesis is supported by the fact that antigen-specific $CD8^+$ T cells express maturation markers which are consistent with recent or chronic activation ($CD27^+CD28^-$) (Nagai et al. 2001).

Whilst less abundant than HTLV-1-specific $CD8^+$ cells, up to 25-fold greater frequencies of HTLV-1-specific $CD4^+$ T cells are observed in patients with HAM/TSP compared with ACs (Goon et al. 2002). Abnormally high frequencies of $CD4^+CD25^+CCR4^+$ cells of undefined specificity are also observed in patients with HAM/TSP and asymptomatic carriers (Yamano et al. 2005). Their frequency correlates closely with proviral load, and they carry the majority of the proviral load (Yamano et al. 2009). In uninfected individuals, $CD4^+CD25^{high}$ cells have a regulatory phenotype; however, this association is less clear in HTLV-1 infection. Several recent papers have reported that $CD4^+CD25^+$ cells from HTLV-1-infected individuals fail to suppress T-cell proliferation in a classical regulatory T-cell functional assay (Yamano et al. 2005; Araya et al. 2014). Stable expression of the transcription factor FoxP3 is necessary but not sufficient for a cell to exert regulatory activity: in HTLV-1 infection, FoxP3 expression is variable (Toulza et al. 2008; Satou et al. 2012), and HBZ can transiently induce inflammatory $FoxP3^+$ cells in mice (Yamamoto-Taguchi et al. 2013). Thus, $CD4^+CD25^+FoxP3^{+/-}$ cells should not be

considered as having regulatory function in the context of HTLV-1 infection. It remains technically challenging to separate pure populations of infected $CD4^+$ $CD25^+$ cells from uninfected $CD4^+CD25^+$ cells in order to directly test their suppressive capacity; however, the frequency of Tax^-FoxP3^+ cells has a strong negative correlation with the CTL efficiency (Toulza et al. 2008).

There appears to be dysregulation of natural killer (NK) cell homeostasis in individuals with a high viral load of HTLV-1, in whom the frequency and activity of both NK cells and NKT cells are significantly reduced (Saito et al. 2003; Azakami et al. 2009). Both the cause and consequence of this NK(T) impairment are unknown: there is no evidence for control of HTLV-1 by NK cells, and infected cells express high levels of MHC class 1 (Rowan et al. 2014). Anti-HTLV-1 antibody responses are present in high titre in most infected individuals, particularly in patients with HAM/TSP (Nagai et al. 1998), and there is evidence for both oligoclonal IgG bands in CSF and intrathecal antibody synthesis (Ceroni et al. 1988; Gessain et al. 1988). However, the ability of an antibody response to control HTLV-1 infection is unproven. Transfer of infectious particles across an enclosed virological synapse suggests that HTLV-1 has in the past evolved to escape antibody-mediated blocking of de novo infection. Both high-titre antibodies and high frequencies of inefficient CTLs and HTLV-specific $CD4^+$ cells may contribute to the inflammation observed in chronic infection.

Gene expression analysis has revealed that a specific subset of genes which are induced by type 1 and type 2 interferons are reproducibly upregulated in patients with HAM/TSP, but not in ACs (Tattermusch et al. 2012). The identity of the interferon (or interferons) responsible for this 'interferon signature' is unknown, but this observation provides direct evidence of a chronic inflammatory response in vivo that is specific to HAM/TSP patients. Whilst interferons are potent antiviral effector molecules, they fail to suppress HTLV-1 gene expression from integrated proviruses in naturally infected cells (Kinpara et al. 2009; Tattermusch et al. 2012). Inappropriate expression of type 1 interferon can also be detrimental in the chronic phase of infection with HIV or SIV. Type 1 interferon activity is essential for viral control in early infection; however, administration of interferons during established chronic infection is associated with $CD4^+$ T-cell depletion and reduced responsiveness to IFN, presumably by inducing tolerance to IFN signalling by persistent stimulation (Sandler et al. 2014). Thus, interferon production can be both beneficial and detrimental in chronic retroviral infection, and downstream effectors associated with pathogenic outcomes might present useful drug targets.

How Does HTLV-1 Establish CNS Lesions?

To access the CNS, HTLV-1 must first cross the blood–brain barrier, which consists of a highly selective boundary which protects the immune privileged CNS (Ballabh et al. 2004). Endothelial cells form relatively impermeable tight junctions that exclude most of the components of blood, and astrocyte end-feet and the parenchymal

basement membrane together form a secondary barrier known as the glia limitans. The region between the endothelial basement membrane and the glia limitans is the perivascular space: in healthy individuals, leukocytes are confined to this region (Engelhardt and Coisne 2011). Central memory $CD4^+$ lymphocytes reach the perivascular space by two known mechanisms: by directly traversing the endothelial barrier and by migrating across the choroid plexus into the CSF in a CCR6-dependent manner (Reboldi et al. 2009). CCR6 expression is restricted to certain subsets of $CD4^+$ T cells in humans, namely, IL-17-producing T_H17 cells and IFN-γ-producing T_H1 cells. In healthy individuals, central memory $CD4^+$ cells are the most abundant population in CSF, followed by central memory $CD8^+$ lymphocytes (De Graaf et al. 2011). As a significant proportion of the proviral load is carried in central memory $CD4^+$ T cells (Hanon et al. 2001), it is reasonable to suggest that steady-state migration is the initial step in colonisation of the CNS by HTLV-1. Indeed, HTLV-1-infected cells can be recovered from the CNS of asymptomatic carriers (Lezin et al. 2005).

The proviral load in the CNS is consistently greater than in the peripheral blood in patients with HAM/TSP. It remains to be tested whether this is a direct result of enrichment of memory cells in the CNS or whether it reflects preferential migration of infected cells to this niche. The observed lack of expression of a well characterised mitosis marker, Ki67, by HTLV-1-infected cells in spinal cord sections suggests that minimal cellular proliferation occurs in this compartment (Matsuura et al. 2015). HTLV-1 expression in infected lymphocytes directly upregulates an array of molecules associated with cellular adhesion (Fukudome et al. 1992) and migration (Valentin et al. 1997; Kress et al. 2011); thus HTLV-1-expressing cells are primed to migrate to inflamed tissue. Regions of slow blood flow in the mid- to lower thoracic region of the spinal cord and brain are enriched in lesions (Izumo 2010), where reduced shear forces presumably also increase the likelihood of rolling and sticking of activated lymphocytes to the endothelium, promoting enhanced lymphocyte extravasation.

Whilst inflammatory lesions in HAM/TSP are most commonly detected in the perivascular space, there is evidence that infected lymphocytes can enter the CNS by degrading the glia limitans, forming lesions in the parenchyma (Aye et al. 2000). Experimental autoimmune encephalomyelitis (EAE) models reveal that MMP expression is essential for this process (Agrawal et al. 2006). Tax protein can activate expression of MMP7 (Nakachi et al. 2011) and MMP9 (Mori et al. 2002; Kodama et al. 2004), and MMP2 and MMP9 are detected in the CSF of patients with HAM/TSP (Umehara et al. 1998). Interestingly, in MOG-induced EAE, penetration of the glia limitans is associated with the onset of symptoms, rather than perivascular accumulation of inflammatory cells (Agrawal et al. 2006; Toft-Hansen et al. 2006). Once the glia limitans has been penetrated, the question arises as to whether HTLV-1 can infect the parenchymal tissue. In vitro, HTLV-1 can infect glial cells and oligodendrocytes (Watabe et al. 1989), but in situ PCR analysis of ex vivo tissue demonstrated viral sequences only in $CD4^+$ lymphocytes and astrocytes (Kubota et al. 1994; Lehky et al. 1995). Thus, direct infection of neurons with HTLV-1 is unlikely to explain the neurodegeneration observed in patients with HAM/TSP.

Viral gene expression (specifically Tax) has been detected in HAM/TSP lesions (Lehky et al. 1995; Moritoyo et al. 1996), and HTLV-1-specific cells dominate infiltrating