includes HTLV-II, bovine leukemia virus (BLV), and simian T-cell leukemia virus (STLV) (Koralnik, 1996; Brady, 1996). Similar to other retroviruses, the HTLV-I proviral genome has the group antigen (*gag*), polymerase (*pol*), and envelope (*env*) genes flanked by long terminal repeat (LTR) sequence at both ends (Fig. 23.1) (Seiki *et al.*, 1983). The characteristic feature of this retroviral group is an additional proviral sequence of regulatory genes at the 3′-end of the proviral genome (Seiki *et al.*, 1983). This sequence, called *pX* (tax) in HTLV-I, contains four open reading frames and codes for three major regulatory proteins, p40tax, p27rex and p21rex (Koralnik, 1996).

The p40tax (Tax) protein has the capacity to transactivate viral transcription through indirect action upon the Tax-responsive element to the LTR as well as numerous cellular genes through several cellular transcription factors such as cyclic AMP response element binding protein (CREB), nuclear factor-κB (NF-κB), serum response factor (SRF), and basic helix– loop–helix proteins (bHLH) (Leung and Nabel, 1988; Zhao and Giam, 1991; Fujii *et al.*, 1992; Suzuki *et al.*, 1993; Uittenbogaard *et al.*, 1994). Transactivated cellular genes include cytokines, cytokine receptors, proto-oncogenes, and adhesion molecules such as interleukin-2 (IL-2), IL-3, IL-6, tumor necrosis factor-α (TNF-α), granulocyte/macrophage colony- stimulating factor (GM-CSF), transforming growth factor-β1 (TGF-β1), nerve growth factor-β (NGF-β), IL-2 receptor (IL-2R) α-chain, c-*fos*, c-*sis*, and parathyroid hormone related protein (Brady, 1996). In contrast, the expression of certain genes such as *lck*, and the β-polymerase gene which is involved in DNA repair, are down-regulated by the Tax protein (Lemasson *et al.*, 1997; Jeang *et al.*, 1990).

Although the function of the HTLV-I p21rex protein is still unclear, like the HTLV-I Tax protein, the p27rex (Rex) protein is essential for HTLV-I replication, but unlike Tax, Rex acts at a post-transcriptional level to regulate viral gene expression. Rex has been shown to increase the expression of unspliced mRNA coding for viral structure and enzymatic proteins, Gag and Pol, and singly spliced mRNA coding for Env protein, while Rex indirectly inhibits the expression of the doubly spliced mRNA coding for Tax and Rex (Seiki *et al.*, 1988; Hidaka *et al.*, 1988; Koralnik, 1996) (Fig. 23.1). The ultimate effect of Rex is therefore to regulate the levels of expression of genes encoding virion components, and thus determining whether infectious virions are produced (Koralnik, 1996).

Genetic variation of HTLV-I

HTLV-I can be classified phylogenetically into three major groups: Cosmopolitan, Central African, and Melanesian. Because different clinical outcomes are associated with an HTLV-I infection, it is important to clarify whether a specific variant of HTLV-I is associated with a particular HTLV-I-related disease. However, despite considerable studies, there is no significant indication that specific subtypes of HTLV-I are associated with particular pathologic consequences. For example, there are no consistent differences between subgroups from patients with ATL and those from patients with HAM/TSP. Recently, Furukawa *et al.* (2000) demonstrated that there was a four nucleotide substitution in the HTLV-I *tax* gene that was associated with a high risk of the development of HAM/TSP. Although it is still unclear how this HTLV-I *tax* variant is directly related to the pathogenesis of HAM/TSP, it is possible that a variation in HTLV-I *tax* gene alters host immune function, because HTLV-I Tax protein is a strong transactivator of numerous host genes including inflammatory cytokines, and is dominant epitope recognized by HTLV-I-specific CD8+ cytotoxic T lymphocytes (CTL) (Niewiesk *et al.*, 1995).

HTLV-I infectivity

Although extensive studies have been performed, the cellular receptor for HTLV-I has not been identified. *In vitro* infection experiments suggested that the HTLV-I receptor has a wide species and cell type distribution (Clapham *et al.*, 1983; Yamamoto *et al.*, 1984; Krichbaum-Stenger *et al.*, 1987). The gene encoding the receptor was mapped to chromosome 17 and further localized to 17q23.2–25.3 (Sommerfelt *et al.*, 1988; Gavalchin *et al.*, 1995), although later studies have questioned this assignment (Okuma *et al.*, 1999; Jassal *et al.*, 2001).

HTLV-I can infect a wide range of human and non-human cells *in vitro* (Trejo and Ratner, 2000); however, as in human immunodeficiency virus type1 (HIV-1) infection, the lymphatic organs are the major reservoir in the HTLV-I-infected individuals (Jacobson *et al.*, 1997; Kazanji *et al.*, 2000). HTLV-I has been thought to preferentially infect CD4+ T cells *in vivo* (Richardson *et al.*, 1990), and recent work using quantitative polymerase chain reaction (PCR) indicated that CD8+ T cells were also a significant *in vivo* cellular reservoir for HTLV-I (Nagai *et al.*, 2001*a*). There is some evidence that macrophages and dendritic cells also might be infected *in vivo* (Hoffman *et al.*, 1992; Koyanagi *et al.*, 1993; Ali *et al.*, 1993). Therefore, from 90–99% of the HTLV-I proviral DNA in peripheral blood from infected patients is found in CD4+ or CD8+ T cells (Richardson *et al.*, 1990; Hanon *et al.*, 2000; Nagai *et al.*, 2001*a*).

In addition to peripheral blood, there are several reports that have demonstrated viral localization in infiltrating inflammatory cells or resident cells within central nervous system (CNS) lesions (Hara *et al.*, 1994; Furukawa *et al.*, 1994; Lehky *et al.*, 1995; Moritoyo *et al.*, 1996). For example, in the affected lesions of the spinal cord, infiltrating CD4+ T cells have been shown by *in situ* PCR hybridization to have proviral DNA, and to express viral protein by *in situ* hybridization (Hara *et al.*, 1994; Moritoyo *et al.*, 1996). HTLV-I RNA has been reported to be localized in resident astrocyte populations in the affected spinal cord (Lehky *et al.*, 1995). Other potential cell types harboring HTLV-I proviral DNA in the CNS may include macrophages, microglial cells, oligodendrocytes, and neurons, however, HTLV-I proviral DNA or viral RNA and protein have not yet been conclusively demonstrated in these cell types in the CNS (Hara *et al.*, 1994; Kubota *et al.*, 1994; Lehky *et al.*, 1995; Moritoyo *et al.*, 1996).

T-cell immortalization and transformation by HTLV-I

One of the striking abilities of HTLV-I that appears to be associated with oncogenesis and immune abnormalities is immortalization and transformation of T cells. In contrast to the cytopathic effect of HIV-1 for its host CD4+ cell, HTLV-I infection is not directly cytotoxic. However, HTLV-I can activate and immortalize human T lymphocytes *in vitro*, resulting in oligoclonal or monoclonal expansion of HTLV-I-infected cells in the absence of exogenous IL-2 (Kimata and Ratner, 1991). While HTLV-I has been demonstrated to be oncogenic in humans, HTLV-I does not contain homologous sequences to any known proto-oncogenes in the viral construct. In addition, HTLV-I has been known to integrate randomly into the host genomic DNA, and there is no specific integration site where the virus can selectively up-regulate proto-oncogene expression by a *cis*-acting effect (Seiki *et al.*, 1983; Koralnik, 1996).

It has been shown that HTLV-I Tax is critical for transformation of T lymphocytes, because specific mutations of the HTLV-I *tax* gene eliminate the transforming potential of the virus (Grassmann *et al.*, 1992). HTLV-I Tax can transactivate IL-2 and IL-2R genes, the products of which have a pivotal role in T-cell proliferation and differentiation. This observation leads to the suggestion of an IL-2 autocrine model for T-cell transformation by HTLV-I (Brady, 1996; Arima *et al.*, 1986). However, while ATL cells expressed abundant IL-2R on their surface, the majority of ATL cells do not respond to IL-2 and produce undetectable levels of this cytokine, indicating that the IL-2 autocrine model does not solely account for leukemogenesis (Arya *et al.*, 1984; Kodaka *et al.*, 1989). Recently, it has been demonstrated that HTLV-I transformation is associated with constitutive activation of the Janus family of tyrosine kinases (JAK)/signal transducers and activators of transcription (STAT) pathway (Migone *et al.*, 1995).

Epidemiology of HTLV-I

HTLV-I is distributed worldwide, and 10–20 million people are infected (Poiesz *et al.*, 1980; de The and Bomford, 1993). There are small clusters of high prevalence, most notably in the southern region of Japan (Kyushu, Shikoku, Okinawa), the Caribbean (Jamaica, Trinidad, Martinique, Barbados, Haiti), the equatorial regions of Africa (Ivory Coast, Nigeria, Zaire, Kenya, Tanzania), South America (Brazil, Colombia), the Middle East (Iran), and Melanesia (Tajima and Hinuma, 1984; Levine *et al.*, 1988; Mueller, 1991; Kaplan and Khabbaz, 1993; Tajima *et al.*, 1994; Blattner and Gallo, 1994; Gessain, 1996). This highly restricted geographic seropreval-

ence is a remarkable feature of HTLV-I epidemiology. The seroprevalence rate in the endemic areas can exceed 30% and the majority of infected individuals are clinically asymptomatic (Gessain, 1996). In the general population of the USA, the seroprevalence of HTLV-I is as low as 0.025%, but it can be as high as 2.1% in the southeastern region of the country (Williams *et al.*, 1988; Khabbaz *et al.*, 1990). It is not surprising that the prevalence of HTLV-I is significantly higher in intravenous drug users and patients in clinics for sexually transmitted diseases (Khabbaz *et al.*, 1992).

Transmission of HTLV-I

Unlike HIV-1, there is little or no cell-free HTLV-I in the plasma, therefore HTLV-I requires cell-to-cell contact to infect other cells. *In vitro*, efficient infection can occur by co-cultivation with HTLV-I-infected cells (Yamamoto *et al.*, 1982; Hollsberg and Hafler, 1993), where cell-free infection is extremely inefficient. Indeed, epidemiological studies suggest that transmission of HTLV-I *in vivo* also requires infected cells (Okochi *et al.*, 1984; Lairmore *et al.*, 1989; Sandler *et al.*, 1991). There is no evidence for the transmission of HTLV-I by cell-free blood products including factor VIII (Okochi *et al.*, 1984; Lairmore *et al.*, 1989; Sandler *et al.*, 1991).

The modes of transmission include perinatal transmission, sexual transmission, and transmission by blood, either from transfusion or by contaminated needles and syringes. Mother-to-child transmission occurs mainly via breast milk, and this is the major mode of transmission in endemic areas. As breast milk is known to contain maternal HTLV-I-infected T cells, approximately 10–30% of children fed breast milk from HTLV-I-infected mothers become infected with HTLV-I (Hino *et al.*, 1985, 1994; Ando *et al.*, 1987; Gessain, 1996). A preventive program in which HTLV-I-infected mothers were advised not to feed their children with breast milk led to a significant decrease of mother-to-child infection, from 20–30% to 3% in an endemic area of Japan (Hino *et al.*, 1994). A reduction in the duration of breast feeding to less than 6 months may also decrease the possibility of mother-to-child infection (Takahashi *et al.*, 1991). Other mechanisms of transmission, such as transplacental, have been less well documented.

Sexual transmission is greater from infected men to women than from infected women to men, probably through HTLV-I-infected T cells in semen (Murphy *et al.*, 1989; Gessain, 1996). Some studies estimate the transmission risk for HTLV-I seropositive husbands to wives as approximately 60% over 10 years, while 0.4% of husbands with HTLV-I seropositive wives can be infected in the same period (Kajiyama *et al.*, 1986). Transmission by breast milk generally requires breast feeding for more than 7 months, and wives of seropositive husbands reach 50% seroconversion after 1 to 4 years of marriage. These finding suggest inefficient transmission of HTLV-I and the need for multiple exposures (Xu *et al.*, 1996).

In contrast, transmission of HTLV-I after receiving a contaminated whole blood transfusion and transmission among drug abusers through shared needles is well documented. Transmission by blood appears very efficient, and 25% of the patients with HAM/TSP in Japan have had a transfusion history (Osame *et al.*, 1990*b*). It has been indicated that most patients seroconvert after receiving contaminated blood and that up to 20% of those receiving contaminated blood develop a myelopathy (Gessain and Gout, 1992). This is an extraordinary percentage and suggests that an infection by this route of inoculation (with greater dosage of virus or greater numbers of infected cells) imposes particular risk for developing myelopathy or that reinfection causes activation or induces an immune response related to the development of neurologic disease. The number of new HAM/TSP patients dramatically decreased after screening for HTLV-I in blood donors was launched in Japan (Osame *et al.*, 1986*a*, 1990*b*).

HTLV-I infection and the nervous system

Clinical features of HAM/TSP

Several neurological manifestations have been linked to HTLV-I infection, e.g. muscular atrophy, polymyositis, peripheral neuropathy, polyradiculopathy, cranial neuropathy, meningitis, encephalopathy (Osame, 1990); however, the most common neurological disorder associated with an HTLV-I infection is HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Gessain *et al.*, 1985; Osame *et al.*, 1986*b*).

HAM/TSP is characterized clinically by paraparesis associated with spasticity, hyper-reflexia, and Babinski's sign of the lower extremities (Osame *et al.*, 1987, 1990*a*; Osame and McArthur, 1990; McFarlin and Blattner, 1991). The main neurological symptoms of HAM/TSP include bladder dysfunction, paraesthesia, low lumber pain, and impairment of vibration sense (Osame, 1990). Less frequent neurological findings are ataxia, hand tremor, optic atrophy, deafness, nystagmus, and other cranial nerve deficits. Convulsions, cognitive impairment, and dementia are rare. Usually HAM/TSP progress slowly over many years, but rarely acute cases have been observed (Osame, 1990).

The age of onset is usually 35 to 45 years, but can be as early as 12 years of age (Osame *et al.*, 1990*b*; McFarlin and Blattner, 1991). HAM/TSP is three times more prevalent in women than men (Osame and McArthur, 1990; Osame *et al.*, 1990*b*). The incubation period from time of infection to onset of disease ranges typically from years to decades, but can be as short as 18 weeks following blood transfusion with HTLV-I-contaminated blood (Osame *et al.*, 1986*a*, 1987, 1990*b*; Gout *et al.*, 1990). A quarter of the HAM/TSP patients in Japan have a previous history of blood transfusion (Osame *et al.*, 1986*a*, 1990*b*). In these cases, progression of symptoms appears to be more rapid than in those patients with HAM/TSP who acquired HTLV-I through vertical transmission. Familial occurrence of HAM/TSP has been reported in a total of 44 cases in Japan and in occasional tropical cases (Osame *et al.*, 1990*b*).

It has been reported that several inflammatory diseases, such as Sjögren's syndrome (Vernant *et al.*, 1988), arthropathy (Kitajima *et al.*, 1989), alveolitis (Sugimoto *et al.*, 1987), uveitis (Sasaki *et al.*, 1989), and interstitial cystitis (Nomata *et al.*, 1992), occasionally occur in conjunction with HAM/TSP. In a clinical analysis of 213 cases of HAM/TSP patients in Japan, other organ diseases were frequently observed in HAM/TSP patients, including leukoencephalopathy (69%), abnormal chest X-ray (50%), Sjögren's syndrome (25%), and arthropathy (17%) (Nakagawa *et al.*, 1995).

Diagnostic investigations of HAM/TSP

As shown in Table 23.1, the diagnosis of HAM/TSP is based on clinical criteria in association with positive serum and cerebrospinal fluid (CSF) antibody titers to HTLV-I, as determined by enzyme-linked immunosorbent assay (ELISA), particle agglutination (PA) testing, Western blot (WB), and immunofluorescent assays (IFA) (Osame, 1990).

In HAM/TSP, anti-HTLV-I-specific antibodies (Osame *et al.*, 1987; Gessain *et al.*, 1988), HTLV-I proviral DNA (Puccioni-Sohler *et al.*, 1999), and HTLV-I Tax-expressing lymphocytes (Moritoyo *et al.*, 1999) can be detected both in peripheral blood and CSF. ATL-like cells make up approximately 1% of peripheral lymphocytes in about 50% of HAM/TSP patients (Osame *et al.*, 1987). Furthermore, there are several additional non-specific findings in CSF which demonstrate inflammatory changes, including mild lymphocytic pleocytosis, mild protein elevation, elevated IgG synthesis and IgG index (which indicates antibody production in the CSF), oligoclonal bands (Osame *et al.*, 1987; Ceroni *et al.*, 1988; Link *et al.*, 1989; Jacobson *et al.*, 1990*a*; Hollsberg and Hafler, 1993), and increased neopterin levels (Nomoto *et al.*, 1991). Neopterin is released by macrophages under stimulation by T lymphocytes, and measurement of neopterin in the CSF can be a useful marker for the differential diagnosis of HAM/TSP, specifically to rule out HAM/TSP in HTLV-I carriers suffering from other forms of chronic myelopathy, such as cervical spondylosis, spinal canal stenosis, or chronic multiple sclerosis (Nomoto *et al.*, 1991).

MRI of the spinal cord of HAM/TSP patients may show swelling or atrophy, and MRI of the brain shows periventricular white matter lesions in as many as 50% of patients (Mattson *et al.*, 1987; Cruickshank *et al.*, 1989; Godoy *et al.*, 1995; Nakagawa *et al.*, 1995). Electrophysiologic abnormalities are frequently seen in lower-limb somatosensory evoked potentials, peripheral nerve conductions, and visual and brainstem evoked potentials (Arimura *et al.*, 1987, 1989; Ludolph *et al.*, 1988; Cruickshank *et al.*, 1989).

Table 23.1 Diagnostic guidelines for human T-cell lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis

Clinical criteria	The florid picture of chronic spastic paraparesis is not always seen when the patient first comes to medical attention. A single symptom or physical sign may be the only evidence of early HAM/TSP
A. Age and sex incidence	Mostly sporadic and adult, but sometimes familial: occasionally seen in childhood: female patients predominant
B. Onset	Usually insidious, but may be sudden
C. Main neurologic manifestations	1. Chronic spastic paraparesis usually slowly but sometimes remains static after progression
	2. Weakness of the lower limb occurs and is more marked proximally
	3. Bladder disturbance is usually an early feature, constipation usually occurs later, and impotence or decreased libido is common
	4. Sensory symptoms, such as tingling, pins-and-needles sensation, and burning, are more prominent than objective physical signs
	5. Low lumber pain with radiation to the legs is common
	6. Vibration sense is frequently impaired: proprioception is less often affected
	7. Hyper-reflexia of the lower limbs, often with clonus and Babinbki's sign, occurs
	8. Hyper-reflexia of the upper limbs and positive Hoffmann's and Trömner's signs are frequent; weakness may be absent
	9. Exaggerated jaw jerk is seen in some patients
D. Less frequent neurologic findings	1. Cerebellar signs, optic atrophy, deafness, nystagmus and other cranial nerve deficits, hand tremor, and absent or depressed ankle jerk are found
	2. Convulsions, cognitive impairment, dementia, and impaired consciousness are rare
E. Other neurologic manifestations that may be associated with HAM/TSP	Muscular atrophy, fasciculations (rare): polymyositis, peripheral neuropathy, polyradiculopathy, cranial neuropathy, meningitis, and encephalopathy may be noted
F. Systemic non-neurologic manifestations that may be associated with HAM/TSP	Pulmonary alveolotis, uveitis, Sjögren's syndrome, arthropathy, vasculitis, ichthyosis, cryoglobulinemia, monoclonal gammopathy, and adult T-cell leukemia/lymphoma may be found
Laboratory diagnosis	A. HTLV-I antibodies or antigens are present in blood and CSF
	B. CSF may show mild lymphocytic pleocytosis
	C. Lobulated lymphocytes may be present in blood and/or CSF
	D. Mild to moderate increase of protein concentration may be present in CSF
	E. Viral isolation when possible from blood and/or CSF

In addition, electromyographic abnormalities of lower thoracic paraspinal muscles are frequently seen and help in the diagnosis of HAM/TSP (Arimura *et al.*, 1995).

Host genetic background in HAM/TSP

As mentioned, the majority (95%) of HTLV-I-infected individuals remain asymptomatic while fewer than 5% develop clinical disease. A number of possibilities have been proposed to explain these vastly different outcomes of an HTLV-I infection, including the suggestion that the genetic background of the host is important for development of HAM/TSP and ATL. Indeed, several studies have demonstrated an association between HTLV-I-related diseases and genetic background, specifically with HLA allele typing. In patients with HAM/TSP, HLA-typing studies have shown an increased frequency of HLA-DRB1*0101 (Sonoda *et al.*, 1996). In addition, there are a few studies which have demonstrated the association of HLA-A*201 in HAM/TSP patients. Previously, it was demonstrated that HLA-A*201 was associated with reduction in both the HTLV-I proviral load and the risk of development of neurologic disease (Jeffery *et al.*, 1999), although this report has been challenged by a more recent study that showed that there was no significant difference in the frequency of HLA-A*201 in the development of HAM/TSP (Yashiki *et al.*, 2001). Vine *et al.* (2002) showed polymorphisms in TNF-α-863A, SDF-1 and IL-15 that were associated with increased risk of HAM/TSP.

Neuropathology in HAM/TSP

Neuropathological findings in HAM/TSP have demonstrated that the affected site is predominantly the spinal cord, especially the thoracic region (Izumo *et al.*, 1989; Levin and Jacobson, 1997). Inflammatory changes are most pronounced in the affected lesions and include mononuclear cellular infiltration, destruction of myelin sheaths and axons, and gliosis (Iwasaki, 1990; Umehara *et al.*, 1993).

Of interest is the observation that the neuropathology of HAM/TSP appears to change gradually during the progression of the disease. Initially, most of the infiltrating cells are CD4+ T lymphocytes, CD8+ T cells, or macrophages in the predominantly perivascular areas (Umehara *et al.*, 1993). Later in the disease, the number of CD8+ T cells in the affected lesions increases (Umehara *et al.*, 1993; Levin *et al.*, 1997). CD8+ cells, thought to represent functionally cytotoxic cells, are observed frequently in active chronic lesions and occasionally in inactive chronic lesions in HAM/TSP patients (Umehara *et al.*, 1994*b*). In addition to the detection of inflammatory cells in the affected lesions of HAM/TSP, the expression of pro-inflammatory cytokines (such as IL-1β, TNF-α, and IFN-γ) and adhesion molecules (Umehara *et al.*, 1996) were also detected in the spinal cord of HAM/TSP patients (Umehara *et al.*, 1994*a*). As the disease progresses to a more inactive chronic form, the number of inflammatory cells as well as the expression of inflammatory cytokines decreases.

To investigate the immunopathogenesis of HAM/TSP, extensive efforts have been made to localize HTLV-I in the CNS lesions of HAM/TSP patients and to determine which cells might serve as targets for inflammatory CD8+ cells in the CNS. HTLV-I *gag*, *pX*, and *pol* sequences have been reported to localize in the thoracic cord areas (Akizuki et al., 1989; Yoshioka *et al.*, 1993) and greater in areas of increased CD4+ cell infiltration. By using *in situ* hybridization, both HTLV-I proviral DNA and RNA were detected in many infiltrating CD4+ T lymphocytes (Hara *et al.*, 1994; Moritoyo *et al.*, 1996; Matsuoka *et al.*, 1998), and resident astrocytes also have been reported to be positive for HTLV-I RNA (Lehky *et al.*, 1995).

Immune responses and immune abnormalities in HAM/TSP

Although immune responses against virus are typically beneficial in eliminating infected cells and in recovery from viral infection, virus-specific immune response can also be immunopathologic and cause disease. A number of immunologic parameters have been described in HAM/TSP including HTLV-I proviral load, humoral and cellular immune response, and cytokine production. Based on a large body of evidence, it has been suggested that HTLV-I-specific immune responses and immune dysregulation contribute to the inflammatory process in the CNS of HAM/TSP patients.

Humoral immune response to HTLV-I

The humoral immune response in HTLV-I infection is mainly directed against products of HTLV-I envelope (*env*), core (*gag*), and *pX* genes (Constantine *et al.*, 1992). In general, HAM/TSP patients tend to have increased levels of HTLV-I-specific antibodies (Gessain and Gout, 1992), and antibodies against HTLV-I can be readily detected in serum (Chen *et al.*, 1989; Palker *et al.*, 1989; Ida *et al.*, 1991; Inoue *et al.*, 1992) and CSF (Gessain *et al.*, 1988; Ceroni *et al.*, 1988; McLean *et al.*, 1989; Link *et al.*, 1989; Jacobson *et al.*, 1990*a*; Nakamura *et al.*, 1991; Kitze *et al.*, 1995). Unlike other acute viral infections, seroconversion to HTLV-I does not mean that the virus has been eliminated. The first specific antibodies to appear after HTLV-I infection are directed against Gag protein and predominate in the first 2 months. Subsequently, anti-envelope antibodies appear, and finally about 50% of infected people produce detectable levels of antibodies to the Tax protein (Manns *et al.*, 1991).

An important question is the function of anti-HTLV-I antibodies in HAM/TSP. Although some peptide-specific antibodies have been shown to neutralize HTLV-I *in vitro* (Inoue *et al.*, 1992), high levels of these antibodies were found both in serum and CSF of many patients with HAM/TSP. The titer of anti-HTLV-I antibodies correlates with the provirus load and may reach very high levels. Some studies suggested that antibody titer (particularly to HTLV-I Tax) was proportional to the severity of disease (Osame *et al.*, 1987). Therefore, it is unlikely that these antibodies have a protective effect against HTLV-I-associated CNS disease, although they may be important to initially control HTLV-I infection after transmission by transfusion or in infancy (Hino *et al.*, 1985).

Cellular immune response to HTLV-I

HTLV-I-specific cytotoxic T lymphocytes (CTL) have been investigated in the pathogenesis of HAM/TSP. CTL have an important role in the normal immunologically mediated recovery from infectious disease through recognition and subsequent elimination of foreign antigens. Utilizing their antigen-specific T-cell receptors, CTL recognize foreign proteins as short peptide fragments in association with HLA. CD8+ CTL recognize foreign antigens in the context of HLA Class I molecules while CD4+ CTL recognize slightly larger peptide fragments in association with HLA Class II molecules. Both populations have been shown to be beneficial in eliminating infected cells and in recovery from viral infection. However, it has been suggested that virus-specific CTL could also be immunopathologic. Since a characteristic feature of HAM/TSP is an accumulation of inflammatory T lymphocytes in the affected lesion, and it has been known that immunomodulatory therapies such as prednisolone can be effective in reducing clinical symptoms of HAM/TSP, it is possible that T-cell antiviral immune responses are closely related to the immunopathogenesis of HAM/TSP.

To date there has been relatively little information on the CD4+ T-cell responses against HTLV-I in patients with HAM/TSP. The HLA-DRB1* 0101 haplotype (an HLA Class II allele) is related to a higher risk of HAM/TSP (Sonoda *et al.*, 1996; Jeffery *et al.*, 1999). Immunodominant epitopes in the HTLV-I *env* gp21 and gp46 were demonstrated from CD4+ T lymphocytes in patients with HAM/TSP (Jacobson *et al.*, 1991; Kitze *et al.*, 1998). These CD4+ HTLV-I-specific CTL could only be demonstrated after

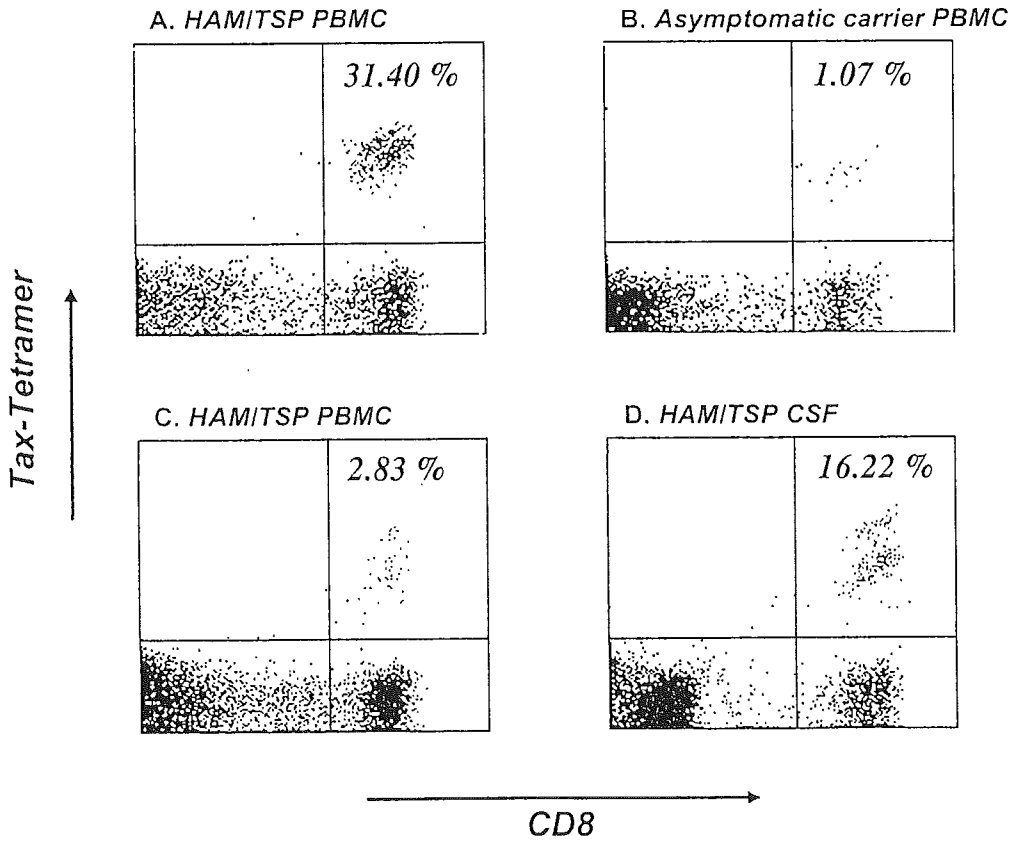

Fig. 23.2 Representative flow cytometry analysis of HTLV-I Tax 11–19-specific CD8+ T cells from peripheral blood mononuclear cells (PBMC) and cerebrospinal fluid (CSF) of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients stained with HLA-A*201/Tax 11–19 tetramers and anti-CD8 antibody. An extraordinarily high frequency of HTLV-I Tax 11–19-specific CD8+ T cells is detected in PBMC of a HLA-A*201 HAM/TSP patient (A) relative to HLA-A*201 asymptomatic carrier (B). An example of patient with fewer HTLV-I Tax 11–19-specific CD8+ T cells is demonstrated in (C). These cells are selectively expanded in the CSF (D).

repeated *in vitro* antigenic stimulation. In addition, HTLV-I-specific CD4+ CTL were demonstrated from patients with HAM/TSP as well as asymptomatic HTLV-I carriers (Jacobson *et al.*, 1991; Kannagi *et al.*, 1994). Recently, Goon *et al.* (2002) demonstrated that frequencies of HTLV-I Env- and Tax-specific CD4+ T cells were greater in HAM/TSP than in asymptomatic carriers, and that HTLV-I-specific CD4+ T cells in the patients with HAM/TSP were mainly of the T helper 1 (Th1) phenotype. Although the role of CD4+ T-cell responses in the HAM/TSP is not fully understood, CD4+ T cells might be important for inducing efficient HTLV-I humoral and cellular immune responses (Ahmed and Gray, 1996).

One of the most striking features of the cellular immune responses in HAM/TSP patients is the extraordinarily increased numbers of HTLV-I-specific CD8+ T cells in peripheral blood mononuclear cells (PBMC) and CSF (Jacobson *et al.*, 1990*b*; Elovaara *et al.*, 1993; Kubota *et al.*, 1998; Nagai *et al.*, 2001*c*; Yamano *et al.*, 2002). Although HTLV-I-specific CD8+ cells are seen in PBMC of asymptomatic carriers (Parker *et al.*, 1992; Yamano *et al.*, 2002), the magnitude and frequency of these responses are higher in patients with neurologic disease (Elovaara *et al.*, 1993; Yamano *et al.*, 2002). Several experimental studies have generated strongly corroborative evidence that there is an intense CD8+ cytotoxic T-cell response against the HTLV-I Tax protein in HAM/TSP (Jacobson *et al.*, 1990*b*; Kannagi *et al.*, 1992; Daenke *et al.*, 1996). Specifically, the HTLV-I Tax 11–19 peptide (LLFGYPVYV) was defined as an immunodominant epitope by HLA-A2-restricted CD8+ CTL (Koenig *et al.*, 1993; Parker *et al.*, 1994). Recently, HTLV-I Tax peptide-loaded HLA-A2 (*0201) dimers and tetramers were developed and used to demonstrate HTLV-I Tax-specific HLA-A2-restricted CD8+ cells (Greten *et al.*, 1998; Bieganowska *et al.*, 1999). Using these techniques, HTLV-I Tax 11–19-specific CD8+ cells from PBMC of HLA-A2 HAM/TSP patients were found to represent an extraordinarily high proportion of the total CD8+ population (as high as 31% of CD8+ cells in some HAM/TSP patients) (Fig. 23.2) (Greten *et al.*, 1998; Nagai *et al.*, 2001*b*; Yamano *et al.*, 2002). In addition, the high frequency of HTLV-I-specific CD8+ cells in HAM/TSP patients correlates with the production of several cytokines such as IFN-γ, TNF-α, and IL-2 (Kubota *et al.*, 1998).

Immune dysfunction and immunological abnormalities in HAM/TSP

Abnormalities in cellular immune responses of HAM/TSP patients have also been identified. Natural killer cells tend to be diminished in both number and activity in HAM/TSP (Kitajima *et al.*, 1988). In particular, the phenomenon of spontaneous lymphoproliferation, defined as the ability of PBMC to proliferate *ex vivo* in the absence of antigenic stimulation or IL-2, has been well described in HAM/TSP PBMC, in HTLV-I asymptomatic carriers, and in HTLV-II-infected persons (Itoyama *et al.*, 1988; Kramer *et al.*, 1989). However, the magnitude of the response is typically higher in HAM/TSP patients (Wiktor *et al.*, 1991). The spontaneous lymphoproliferation of HTLV-I-infected PBMC is thought to consist of two components; proliferation of HTLV-I-infected CD4+ cells and expansion of CD8+ cells (Ijichi *et al.*, 1989; Eiraku *et al.*, 1992; Nagai *et al.*, 1995; Machigashira *et al.*, 1997). Ijichi *et al.* (1989, 1993) proposed that this *in vitro* spontaneous lymphoproliferation may epitomize the immunological events occurring in the CNS of HAM/TSP patients, where high inducibility of viral antigens and increased CD8+ cell response may induce severe inflammation.

Expression of cytokine and immune-mediated molecules in HAM/TSP

Elevated levels of several pro-inflammatory cytokines, chemokines, matrix metalloproteinase (MMP) which is implicated in extracellular matrix degradation, and adhesion molecules have been demonstrated in serum, CSF, and spinal cord lesions of HAM/TSP patients (Kuroda and Matsui, 1993; Nakamura *et al.*, 1993; Umehara *et al.*, 1994*a*). Patients have elevated levels of cytokines such as IFN–γ, TNF-α, and IL-6, in serum and CSF (Nishimoto *et al.*, 1990; Hollsberg and Hafler, 1993; Kuroda and Matsui, 1993; Nakamura *et al.*, 1993; Furuya *et al.*, 1999). IL-12 (p70 heterodimer) is also elevated in serum (Furuya *et al.*, 1999). Moreover, mRNA expression of IL-1β, IL-2, TNF-α, and IFN-γ has been shown to be up-regulated in the PBMC of patients with HAM/TSP (Tendler *et al.*, 1990, 1991). The levels of MMP-9 and tissue inhibitors of metalloproteinases (TIMP)-3 in CSF of HAM/TSP patients were higher than that of HTLV-I carriers without neurological symptoms (Lezin *et al.*, 2000). HAM/TSP patients have increased levels of soluble vascular cell adhesion molecule-1 (VCAM-1) in sera and CSF (Matsuda *et al.*, 1995). In addition to the detection of these molecules from serum and CSF of patients with HAM/TSP, immunohistochemistry showed localized expression of pro-inflammatory cytokines such as IL-1β, TNF-α, and IFN-γ in the perivascular infiltrating cells (Umehara *et al.*, 1994*a*). MMP-2, MMP-9, and very late antigen-4 (VLA-4) were also detected in infiltrating cells (Umehara *et al.*, 1998), and the expression of VCAM-1 was demonstrated on endothelium cells in the spinal cord lesions of HAM/TSP (Umehara *et al.*, 1996). Moreover, lymphocyte function-associated antigen-1 (LFA-1), Mac-1, and monocyte chemoattractant protein-1 (MCP-1) were up-regulated in CNS lesions (Umehara *et al.*, 1996).

It has been reported that CD4+ T cells from patients with HAM/TSP can spontaneously express pro-inflammatory cytokines such as IFN-γ, TNF-α, and GM-CSF (Nishiura *et al.*, 1996). In addition, these pro-inflammatory cytokines secreted by HTLV-I-infected CD4+ T cells can induce production of MMP-2, -3, and -9 and TIMP-1, -2, and -3 in human astrocytes *in vitro* (Giraudon *et al.*, 2000). Moreover, it has been demonstrated that HTLV-I Tax 11–19-specific CD8+ CTL clones from HLA-A2 HAM/TSP patients can secrete pro-inflammatory cytokines, chemokines, and matrix metalloproteinase upon recognition of the HTLV-I Tax 11–19 peptide, including IFN-γ, TNF-α, macrophage inflammatory protein (MIP)-1α, IL-1β, IL-2, IL-6, and MMP-9 (Biddison *et al.*, 1997; Kubota *et al.*, 1998). This IFN-γ production was observed with co-cultivation with autologous CD4 cells or HTLV-I Tax 11–19 peptide-pulsed HLA-A2-matched cells, and suppressed by anti-HLA Class I antibodies. Similar to the increased frequency of HTLV-I-specific CTL in HAM/TSP (Jacobson *et al.*, 1990*b*; Kannagi *et al.*, 1991; Elovaara *et al.*, 1993; Koenig *et al.*, 1993), CD8+ T cells of HAM/TSP patients also have a potential to produce IFN-γ and TNF-α at a high frequency in comparison with asymptomatic carriers or healthy controls.

The recently described cytokine IL-15 has also been reported to be increased in PBMC from HAM/TSP patients (Azimi *et al.*, 2000), and may function to maintain virus-specific CD8+ T cells *in vivo* (Ku *et al.*, 2000; Azimi *et al.*, 2001).

Autoimmune responses

Although an autoimmune hypothesis had been proposed in the pathogenesis of HAM/TSP (Hollsberg and Hafler, 1993; Oger and Dekaban, 1995), to date there are only a few immunological studies which suggest that autoimmune responses may play a role in the pathogenesis of HAM/TSP. Levin *et al.* (1998) previously showed that IgG antibody from patients with HAM/TSP specifically labeled neurons in uninfected CNS, but not cells in the other organs. Antibodies to a neuronal antigen could be demonstrated in all tested patients with HAM/TSP by Western blot, but not in HTLV-I-uninfected controls. Interestingly, by Western blotting, HTLV-I Tax-specific antibodies labeled a neuronal protein with the same molecular weight to the IgG antibody from patients with HAM/TSP, suggesting that antibodies to the viral Tax protein bound to the same neuronal antigen as patient antibodies. Most recently, they identified a neuronal protein, hnRNP-A1, as a target antigen recognized by IgG antibody from patients with HAM/TSP as well as Tax-specific antibody. In addition (Levin *et al.*, 2002) they demonstrated that affinity-purified antibodies from patients and Tax antibodies inhibit neuronal function, indicating that the observed cross-reactivity with a neuronal protein may be relevant in the pathogenesis of the HTLV-I-associated neurological disease.

Hara *et al.* (1994) reported that the lymphocytes in the spinal cord lesions of HAM/TSP patients have a unique CDR3 motif, which has been demonstrated in brain lesions of multiple sclerosis and experimental autoimmune encephalomyelitis by the analysis of T-cell receptor Vβ genes, suggesting that a T-cell clone recognizing autoantigens was activated by HTLV-I and that this clone was related to the pathogenesis of HAM/TSP.

Migration of HTLV-I-infected cells into the CNS

HAM/TSP is an inflammatory disease of the spinal cord where inflammatory cells, predominantly T cells (both CD4+ and CD8+), infiltrate perivascular area (Umehara *et al.*, 1993). To understand the pathogenesis of HAM/TSP, it is important to know the process of migration of inflammatory cells from the periphery into CNS lesions.

In PBMC of patients with HAM/TSP, HTLV-I has been shown to preferentially infect CD4+ and CD8+ T cells *in vivo* (Richardson *et al.*, 1990; Nagai *et al.*, 2001*a*). Since it was demonstrated that HTLV-I-infected lymphocytes shared the same HTLV-I integration site of cellular DNA in both the peripheral blood and CSF from a HAM/TSP patient, this suggested that HTLV-I-infected cells migrate from peripheral blood to CNS *in vivo* (Cavrois *et al.*, 2000). Although the mechanism of migration of HTLV-I-infected cells to the CNS is still unclear, several possibilities have been demonstrated. In patients with HAM/TSP, CD4+ T cells (particularly HTLV-I-infected CD4+ cells) have been shown to increase adherent activity to endothelial cells and transmigrating activity through basement membranes (Ichinose *et al.*, 1992, 1994; Furuya *et al.*, 1997). In addition, it has been observed that CD4+ cells of patients with HAM/TSP have splicing variants of CD44 (v6 variants). CD44 is known as a multifunctional cell adhesion molecule as well as a lymphocyte homing receptor. Interestingly, these splicing variants of CD44 were highly detected in PBMC (especially CD4+ cells) from HAM/TSP and in some of HTLV-I-infected cells by *in situ* PCR (Matsuoka *et al.*, 2000).

As mentioned, it has been known that the number of CD8+ T cells in the affected lesions increases later in disease and infiltration of CD8+ cells is observed frequently in active chronic lesions of HAM/TSP patients (Umehara *et al.*, 1993, 1994*b*; Levin *et al.*, 1997). Indeed, it has been demonstrated that the frequency of HTLV-I-specific CD8+ T cells in CSF is much higher than in PBMC of HAM/TSP patients (Fig. 23.2) (Elovaara *et al.*, 1993; Greten *et al.*, 1998), and that this accumulation was HTLV-I-specific (Nagai *et al.*, 2001*c*). These results suggest that HTLV-I-specific CD8+ T cells may preferentially migrate into the CSF from peripheral blood and/or these cells may selectively expand in this compartment. The increased expression of T-cell activation markers has demonstrated in HTLV-I Tax-specific CD8+ T cells in the PBMC of HAM/TSP patients, suggesting that these cells may have selective advantage to migrate into the CNS of HAM/TSP patients (Nagai *et al.*, 2001*b*,*c*).

HTLV-I proviral load and mRNA expression in HAM/TSP

As noted, HTLV-I-specific immune responses and immune abnormalities are significantly related to the pathogenesis of HAM/TSP, specifically with regard to viral antigen-specific CD8+ T-cell responses. An important question is how HTLV-I-specific immune responses are continuously stimulated in HAM/TSP patients. There is considerable evidence to suggest that a high HTLV-I proviral load in patients with HAM/TSP may drive increased HTLV-I-specific immune responses (Fig. 23.3). In PBMC of HAM/TSP patients, it has been demonstrated that HTLV-I proviral loads are significantly higher than in asymptomatic HTLV-I carriers (Yoshida *et al.*, 1989; Gessain *et al.*, 1990*b*; Kira *et al.*, 1991; Kubota *et al.*, 1993). More recently, using newly established real-time quantitative PCR techniques, it has been reported that the level of HTLV-I proviral DNA in the PBMC of HAM/TSP patients is approximately 16-fold greater than in asymptomatic carriers (Nagai *et al.*, 1998). For example, HAM/TSP patients had usually 2–20 copies per 100 isolated PBMC (and certain HAM/TSP patients had as many as 60 copies of HTLV-I proviral DNA per 100 isolated PBMC), while the median value of HTLV-I proviral load in asymptomatic carriers was around 0.1–1 copy per 100 isolated PBMC. Moreover, analysis of HTLV-I proviral loads from lymphocytes of the CSF of HAM/TSP patients also demonstrated high levels of HTLV-I *tax* DNA (Nagai *et al.*, 2001*c*). Importantly, HTLV-I proviral levels were even higher compared with PBMC proviral loads. In HLA A201 HAM/TSP patients, the increased HTLV-I proviral DNA loads in CSF were proportional to the frequency of HTLV-I Tax 11–19-specific CD8+ T cells. These observations support the hypothesis that the HTLV-I proviral load may drive the increased HTLV-I-specific immune responses that have been suggested to be immunopathogenic in HAM/TSP (Fig. 23.3). Interestingly, the HTLV-I proviral loads of HTLV-I asymptomatic carriers in the families of HAM/TSP patients were higher than those of unrelated asymptomatic carriers, suggesting that genetic factors may also influence HTLV-I proviral levels (Nagai *et al.*, 1998, 2001*c*).

Paradoxically, even though a high proviral DNA load is characteristic of HAM/TSP patients, the expression of HTLV-I in PBMC appears to be low (Shimoyama *et al.*, 1983; Sugamura *et al.*, 1984; Tochikura *et al.*, 1985; Kinoshita *et al.*, 1989; Gessain *et al.*, 1990*a*). These observations have led a number of investigators to consider that HTLV-I may be latent in peripheral blood. Recently, using a newly established real-time quantitative RT-

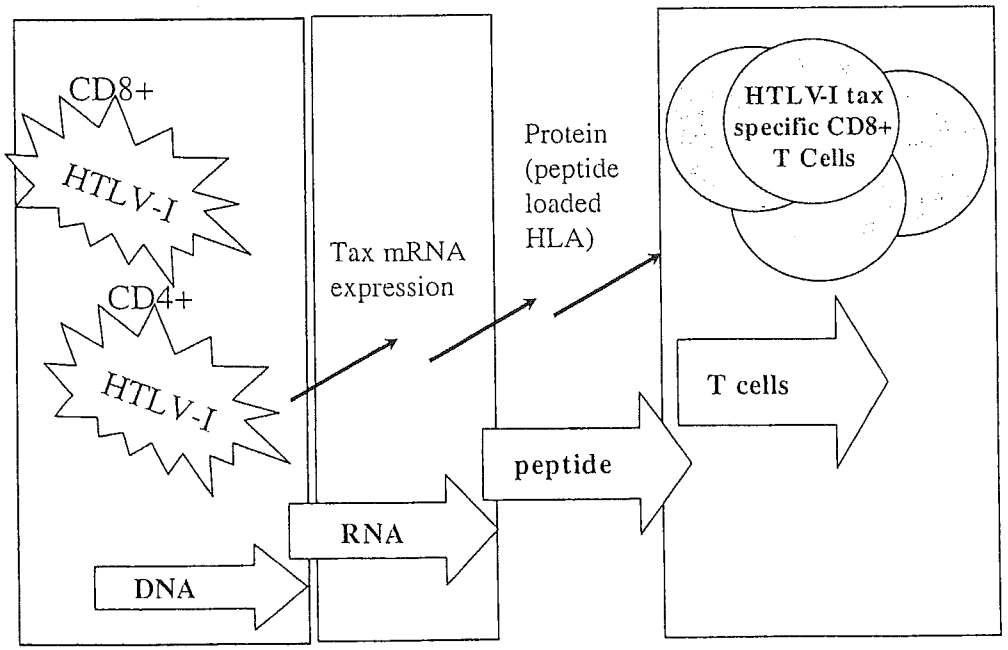

Fig. 23.3 Scheme of induction of HTLV-I-specific CD8+ T-cell responses associated with immunopathogenesis of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). High levels of HTLV-I proviral load are observed in the patients with HAM/TSP, which is directly proportional to increased mRNA levels for HTLV-I tax. Elevated levels of HTLV-I tax mRNA lead to increased expression of HTLV-I protein that can be processed into immunodominant peptides. HTLV-I peptides (e.g. Tax 11–19). strongly bind to HLA-A*201 molecules and can stimulate virus-specific CD8+ T-cell responses (which are detected by HLA-A*201/Tax 11–19-specific tetramers). These antigen-specific responses are expanded in the cerebrospinal fluid of HAM/TSP patients and may contribute to disease progression.

PCR technique, it was demonstrated that HTLV-I mRNA load in PBMC of HAM/TSP patients appeared to be significantly higher than that in asymptomatic HTLV-I carriers (0.004–0.4% of PBMC were expressing HTLV-I mRNA in HAM/TSP patients) (Yamano *et al.*, 2002). This is consistent with a previous study that detected HTLV-I Tax protein in PBMC and CSF cells of HAM/TSP patients by use of laser scanning cytometry (0.02–0.54% of PBMC and 0.04–1.16% of CSF cells were positive for the HTLV-I Tax protein) (Moritoyo *et al.*, 1999). HTLV-I Tax protein expression in CSF cells was higher than that in PBMC and was more frequent in HAM/TSP patients with a shorter duration of illness. This low number of HTLV-I mRNA and Tax protein-expressing PBMC compared with the high HTLV-I proviral DNA load suggests that the majority of HTLV-I-infected cells are latent in peripheral blood, although this amount may be sufficient to continuously activate the immune system *in vivo*.

Animal models for HAM/TSP

The development of an animal model for HTLV-I infection would be extremely useful for investigating mechanisms of immunopathogenesis in HTLV-I-associated diseases. To date, several animal models, including HTLV-I infectious models and experimental transgenic models of HTLV-I genes, have been generated.

It has been known that HTLV-I can be successfully transmitted to rats and to rabbits by inoculation of HTLV-I-infected cells. Recently, the development of a chronic myelopathy similar to HAM/TSP has been reported in one specific strain of rats inoculated with an HTLV-I-infected cell line (Ishiguro *et al.*, 1992). This HTLV-I-infected rat model develops paraparesis of the hind limbs after an incubation period of 15 months. In the spinal cord of infected rats, the expression of HTLV-I *tax* mRNA was observed (Tomaru *et al.*, 1996), and moreover, it was demonstrated that some resident glial cells were expressing Tax protein in the CNS (Jiang *et al.*, 2000). An increased level of neurotoxic cytokines such as TNF-α was also detected in the CSF of infected rats (Tomaru *et al.*, 1996). Interestingly, the pathologic analysis shows demyelination centered in the thoracic cord with massive infiltration of activated macrophages/microglias and apoptosis of resident glial cells such as oligodendrocytes, while lymphocytic infiltration was not observed (Ohya *et al.*, 1997). Although the mechanism of demyelination in HTLV-I-infected rats is still unclear, it has been suggested that the increased sensitivity to inflammatory cytokines such as TNF-α in glial cells, which may be derived from infection with HTLV-I, was related to the development of myelopathy in this rat model (Jiang *et al.*, 2000). In addition, a rabbit model has also been used to examine HTLV-I infection, replication, cellular transformation, transmission, induced immune responses, disease manifestations, vaccine candidates, and therapeutic approaches (Ratner, 1996).

Recently, a number of transgenic models containing portions of the HTLV-I genome have been generated; however, these animals did not develop a neurologic disease like HAM/TSP but thy did manifest a number of symptoms. For example, mice carrying the HTLV-I genome or HTLV-I Tax developed chronic arthritis resembling rheumatoid arthritis (Iwakura *et al.*, 1991). LTR-*env-pX* transgenic rats have been shown to develop a wide spectrum of collagen vascular and autoimmune diseases (Yamazaki *et al.*, 1997).

Model of immunopathogenesis in HAM/TSP

The results discussed above demonstrate that virus–host immunological interactions play a pivotal role in HAM/TSP. Based on extensive experimentation, a number of possible models for the pathogenesis of this disorder have been proposed. Three major immunopathogenic models have been considered:

1. Cytotoxic model: inflammatory HTLV-I-infected T cells in the CNS or CNS resident glial cells may be presenting HTLV-I antigens and become direct targets for lysis by HTLV-I-specific CTL (Jacobson *et al.*, 1990*b*).
2. Autoaggressive bystander model: HTLV-I virus-specific cellular infiltrates that have migrated into the CNS that recognize HTLV-I-infected cells may result in the release of cytokines and chemokines which may cause damage to nearby cells such as glial and neural cells (Ijichi *et al.*, 1993).
3. Autoimmune model: activated HTLV-I-specific T cells cross-react with self antigens in the CNS (Hollsberg and Hafler, 1993) and/or virus specific immune responses lead to epitope spreading and recognition of multiple CNS antigens (Hafler, 1999).

Although it has not been proven which, if any, of these mechanisms predominate in HAM/TSP, or if one or more of these mechanisms act in concert, the large body of evidence summarized in this chapter suggests that HTLV-I-specific CD8+ T cells contribute to the inflammatory process in CNS lesions of HAM/TSP regardless of which mechanism is involved. HTLV-I-specific CD8+ T cells may kill HTLV-I antigen(s)-expressing target cells directly by a perforin-dependent mechanism (Hanon *et al.*, 2000) as well as by the production of a large amount of matrix metalloproteinases (MMP-9), chemoattractants (MIP-1α and -1β) and pro-inflammatory cytokines (TNF-α and IFN-γ) which can induce HLA expression in neuronal cells and damage CNS tissue (Biddison *et al.*, 1997; Greten *et al.*, 1998; Kubota *et al.*, 1998). High levels of HTLV-I proviral loads were observed in both CD4+ and CD8+ T-cell populations in HAM/TSP patients, and it is possible to propose that these high viral loads can drive increased levels of HTLV-I mRNA and HTLV-I protein expressions. As shown in Fig. 23.3, processing and presentation of HTLV-I-specific peptides leads to activation and expansion of antigen-specific T-cell responses. The hypothesis that HTLV-I-specific CD8+ T cells play a role in the development of HAM/TSP is supported by localization of these T cells in the CNS. Inflammatory CD8+ cells have been found in the spinal cord lesions of HAM/TSP patients (Jacobson *et al.*, 1992) and tend to increase with disease progression. Activated T cells have been reported in the CSF of HAM/TSP patients, usually of the CD8+, CD11a+, CD45RO+, CD28– phenotype (Elovaara *et al.*, 1995).

The precursor frequency of HTLV-I-specific CTL from CSF lymphocytes is extraordinarily high (Elovaara *et al.*, 1993). HTLV-I-specific CD8+ T cells could recognize HTLV-I antigen-expressing target cells in the CNS and induce large amounts of pro-inflammatory cytokines and chemokines that can induce HLA expression in resident cells in the CNS and damage CNS tissue (Lehky *et al.*, 1994). As the HTLV-I proviral load in CSF of HAM/TSP patients was more than two times higher than in PBMC (Nagai *et al.*, 2001*c*), this suggests that HTLV-I-infected lymphocytes may preferentially migrate into the CSF from peripheral blood, or that HTLV-I-infected lymphocytes may selectively expand in this compartment. Indeed, the exaggerated transmigrating activity of HTLV-I-infected cells to the CNS tissue has been reported (Cavrois *et al.*, 2000; Romero *et al.*, 2000; Matsuoka *et al.*, 2000; Hanon *et al.*, 2001), and expansion of HTLV-I-infected T-cell clones has also been observed (Furukawa *et al.*, 1992; Wattel *et al.*, 1995; Cavrois *et al.*, 1998; Eiraku *et al.*, 1998). In addition, HTLV-I genomic sequences, RNA, and the HTLV-I proteins have been shown to localize in the spinal cord lesions. Therefore, all requirements for CTL recognition including viral antigen and HLA Class I expression are present in the HAM/TSP lesions, lending support to the argument that HTLV-I-specific CD8+ CTL may be immunopathogenic in this disease.

A model for HTLV-I immunopathogenesis is represented in Fig. 23.4 that encompasses many aspects of HTLV-I T-cell tropism and host immune responses to the virus. In the HAM/TSP lesions, HTLV-I-infected CD4+ and CD8+ T cells can cross the blood–brain barrier. In addition, activated CD8+ antigen-specific cells may also migrate into the CNS. These cells may recognize productively infected cells and respond by either direct lysis of the infected cells (which may also be HTLV-I-infected resident CNS cells such as glial cells) or through the release of chemokines and cytokines. These molecules can act to recruit and expand additional inflammatory cells and have been shown to be toxic to resident CNS cells. Intensive studies regarding the interaction between HTLV-I-specific CD8+ T cells and HTLV-I-infected cells will clarify the pathogenesis of HAM/TSP. This understanding will allow for directed immunotherapeutic strategies for the treatment of this disease.

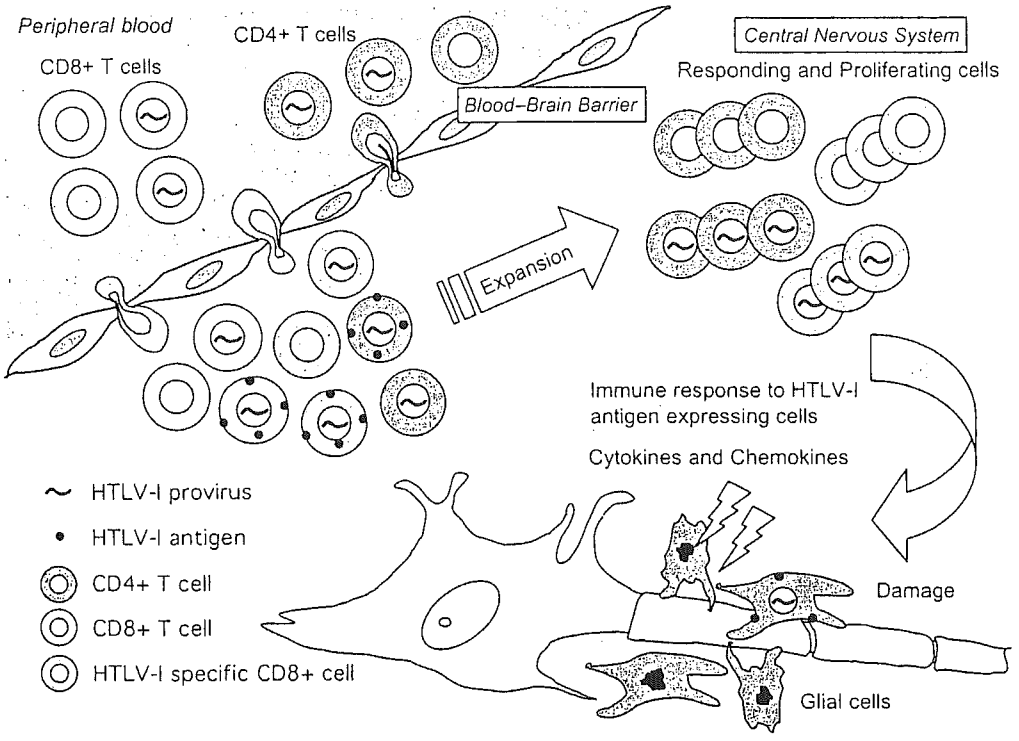

Fig. 23.4 Immunopathogenic model of HAM/TSP. Activated T cells, which may consist of HTLV-I-infected CD4+, CD8+, or antigen-specific T cells, migrate across the blood–brain barrier from the peripheral blood to the CNS. As a portion of the HTLV-I-infected cells such as infected inflammatory cells and possible resident CNS cells express HTLV-I antigens, T-cell immune responses recognize and attack these HTLV-I antigen-expressing cells. Recognition by HTLV-I-specific CD8+ cytotoxic T lymphocytes can result in lysis of infected target cells and/or the release of a cascade of inflammatory cytokines and chemokines in the CNS. HTLV-I-infected CD4+ and CD8+ T cells may also produce these immunomodulatory molecules. Cytokines such as IL-2 and IL-15 may help bystander T cells to expand, whereas IFN-γ and TNF-α may damage resident CNS cells such as glia and neurons.

Treatment

Based on a number of investigative studies on the immunopathogenesis of HAM/TSP, it has been suggested that reduction of the virus and control of excessive immune responses to the virus are important for therapeutic approaches. Although an ideal therapeutic strategy for HAM/TSP patients has still not been established, there are several reports regarding treatment focusing on both antiviral and immunomodulatory action.

For the reduction of virus in the patients with HAM/TSP, anti-IL-2R therapy has been undertaken to reduce IL-2R-expressing HTLV-I-infected cells, and the reduction of HTLV-I proviral load in PBMC has been demonstrated without exacerbation of disease (Lehky *et al.*, 1998). Treatment with antiretroviral reagents (reverse transcriptase inhibitors) such as zidovudine and lamivudine is also under investigation, and regimens including these antiretroviral reagents have been reported to decrease HTLV-I proviral load in HTLV-I-infected subjects (Sheremata *et al.*, 1993; Taylor *et al.*, 1999). A preliminary trial with lamivudine has shown a decrease in both HTLV-I proviral load and HTLV-I-specific CTL responses in some HAM/TSP patients (Wodarz *et al.*, 1999); however, an improvement in neurological symptoms was not observed. Further clinical trials are necessary to develop treatments for HAM/TSP. Since clonal expansion of HTLV-I-infected cells seems to be a major pathway for virus propagation rather than viral replication through reverse transcriptase enzymes (Wattel *et al.*, 1996), it is suggested that therapeutic approaches to prevent clonal expansion of infected cells may be effective in reducing virus load.

Besides antiviral therapies, immunomodulatory therapies such as prednisolone, IFN-α methylprednisolone, and azathioprine, have been reported as possible treatments for HAM/TSP (Izumo *et al.*, 1996; Nakagawa *et al.*, 1996). Since activated immune responses to the virus are significantly observed in spinal cord lesions of HAM/TSP patients it has been suggested that immunomodulatory therapies to control harmful immune reaction against the virus may be effective for treatment. Among these therapies, it has been demonstrated that predonisolone and IFN-α may be effective in improving the clinical symptoms of HAM/TSP. For example, oral prednisolone has been reported to show a beneficial effect in 69.5% of patients with HAM/TSP, especially in patients with a short duration of illness in which active inflammation presumably occurs in the CNS (Nakagawa *et al.*, 1996). The treatment with IFN-α shows effective improvement in 61.5% of patients (Izumo *et al.*, 1996). Azathioprine (22.2%), methylprednisolone (30%), and plasmapheresis and lymphocytopheresis (43.8%) also showed partial effectiveness for patients with HAM/TSP (Nakagawa *et al.*, 1996).

Conclusion

The large body of evidence summarized in this chapter suggests that HTLV-I-specific immune responses contribute to the inflammatory process in CNS lesions of HAM/TSP. Immunological abnormalities induced by a high HTLV-I proviral load may be involved in the neuropathologic events of this disease. In this chapter, we have emphasized the role of HTLV-I-specific CD8+ cells in the development of HAM/TSP, but many questions remain. Intensive studies regarding the interaction between HTLV-I-specific T cells and HTLV-I-infected cells will clarify the pathogenesis of HAM/TSP. This understanding will allow for directed immunotherapeutic strategies for the treatment of this chronic progressive neurologic disease similar to treatments currently being evaluated in multiple sclerosis (Bielekova and Martin, 1999). These experimental therapeutic strategies include inhibition of T-cell activation, altered peptide ligand therapies, and transmigration through the blood–brain barrier.

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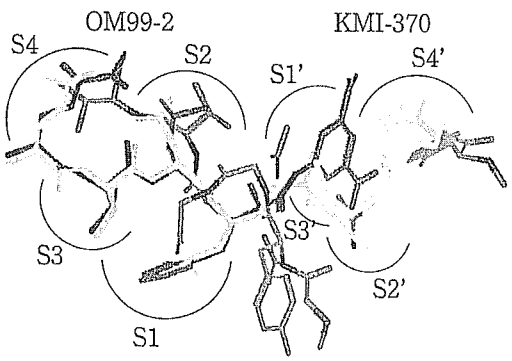
アルツハイマー病：治療の可能性を探る

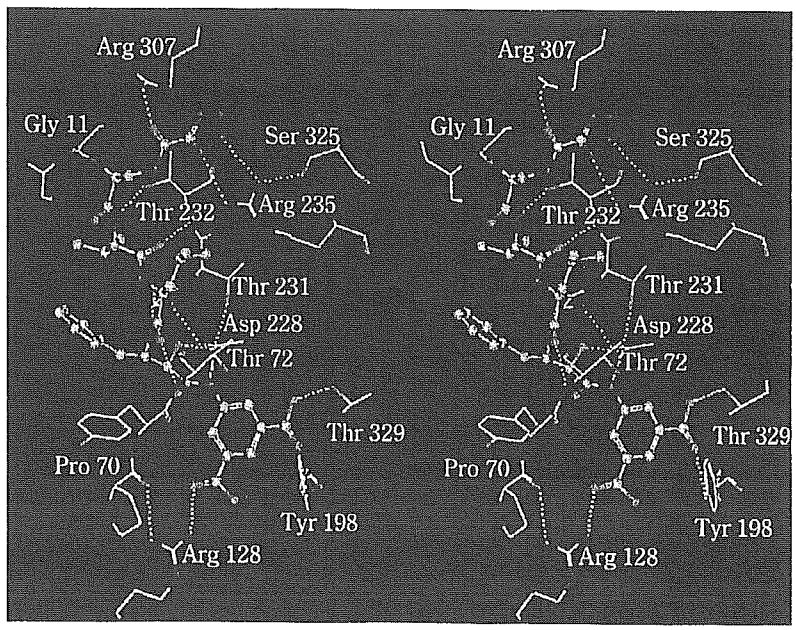
アミロイド生成阻害薬開発の展望

京都薬科大学創薬科学
フロンティア研究センター教授
木曽　良明



BACE1：緑 / 茶(flap)
OM99-2：黄、KMI-370：青

OM99-2　vs　KMI-370 (p. 171、図 14 参照)



BACE1 と結合した KMI-429 の分子モデル (p. 172、図 17 参照)

主　催：平成17年度文部科学省科学研究費補助金
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東京大学大学院医学系研究科教授　井原　康夫

後　援：福岡市教育委員会／NPO法人脳の世紀推進会議／福岡痴呆を考える会／筑紫痴呆セミナー
北九州高次脳機能研究会／七隈アルツハイマー病研究会／ニューロサイエンス
大牟田臨床痴呆研究会／大牟田神経アーベント／エーザイ株式会社／ファイザー株式会社

アミロイド生成阻害薬開発の展望

木曽　良明
京都薬科大学創薬科学フロンティア研究センター教授

私は、先月、プラハでチャールズ大学附属病院に3日間入院しました。日本に帰ってすぐ、MRIなどの検査をうけて、患者さんの立場がよくわかるようになりました。検査の結果、異常がなかったのでお医者さんは薬はいらないというのですが，症状は完全に治まっていないので、薬が欲しくなります。

私は現在、日本ペプチド学会の会長も務めさせていただいており、本日の1時から国際会議場で開催されている日本ペプチド学会主催の市民フォーラム「生命を守り、健康をつくるアミノ酸・ペプチド」で講演してきました。今晩からは、アジア太平洋国際ペプチドシンポジウムも始まります。そのためにも何か薬が欲しいのです。名医といわれている先生方は、薬はできるだけ使わないようにといいます。お医者さんは健康な方が多く、患者さんの立場が少しわからないのかもしれません。

図1　地球誕生

生物分子の誕生

そこで、最初に薬の話をしたいと思います。アミロイドβタンパク質（Aβ）がアルツハイマー病発症の主原因であることから、その生成を阻害すればよいと単純に推察され、現在、β-セクレターゼを阻害する物質と、γ-セクレターゼを阻害する物質が検討されています。また、Aβの凝集を阻害する物質、ワクチンも考えられます。私は現在、β-セクレターゼに焦点をあてていますが、その原理的に優れている理由をお話しします。

地球が誕生したのは46億年前といわれています（図1）。太古の大陸は現在とはまったく違います。その時代にはアミノ酸もタンパク質も存在しません。地球誕生から数億年で、現在のウイルスや動物、植物、人が使っている生体分子が誕生しました。分子の進化からみると、タンパク質・核酸のような高度な生

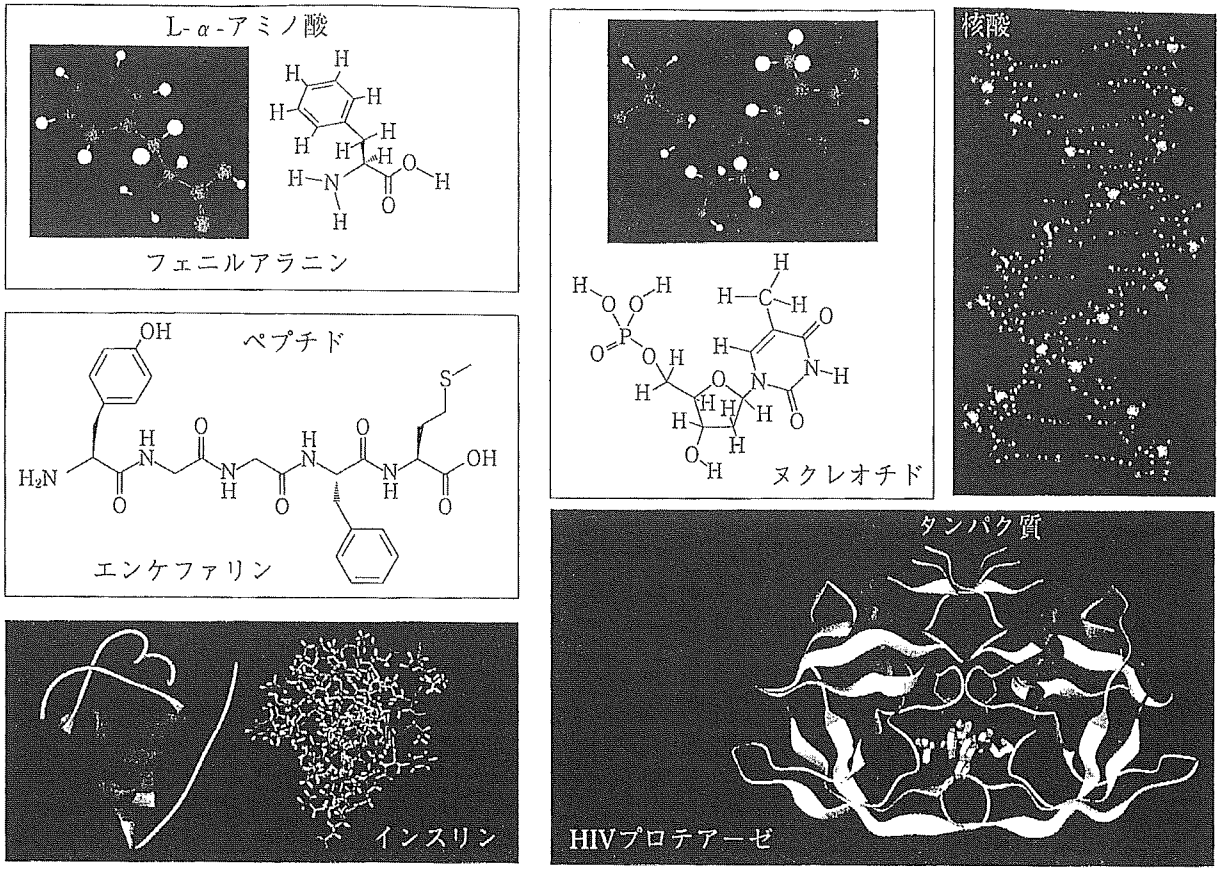

図2　生物分子の誕生

物分子が非常に早くにできたと思われています。

図2にL-α-アミノ酸のひとつであるフェニルアラニン構造を示しますが、それら20種類のアミノ酸は4種類ほどの塩基を用いている核酸からなる遺伝子にコードされています。また、脳神経系に重要なドパミンやアドレナリンなどの原料になるアミノ酸がペプチド結合して、エンケファリンやエンドルフィンという脳内にある麻薬物質が生成されます。マラソン選手などはジョギングすると気持ちよくなるようですが、エンドルフィンが分泌されるため、非常に気分がよくなるわけです。また、心頭滅却して脳内麻薬物質が産生されると痛みも止まりますし、火のなかでも熱くなく、疲れもないといわれています。

血糖値に関係するインスリンは、アミノ酸がさらに多く結合したものです。また、複雑なタンパク質の代表例として、エイズウイルス(HIV)が産生するタンパク質分解酵素であるHIVプロテアーゼがあり、これは2量体です。今日の話の中心は、HIVプロテアーゼと親戚の物質です。

世界の伝承薬物とドラッグの発見・開発

このようなアミノ酸の情報を保持しているDNAやRNAという核酸が、地球誕生から数億年で誕生したことは非常に驚くべきことです。その物質が現在も使われているのには意味があります。私は、タンパク質が進化の最終物質ではないかと考えています。というのは、アミノ酸をつないでいるペプチド結合は非常に安定で、水素結合をつくることもできます。それによってさまざまな構造をとることができ、また側鎖の官能基によって機能を発揮できるようになります。ウイルスもこのように複雑な酵素を使っています(図3)。

サルも同じです。ウイルスが高度な酵素を使うように、サルも高度な植物(薬草)を使っているといわれています。霊長類は病気の状

態では、通常とは異なる植物（薬草）を食べます。人はもちろんのことです。人のあるところ文明があり、文明のあるところには薬が存在します（図4）。

ちなみに、この6月、文部科学省21世紀COEプログラムのヒヤリングで、私の哲学をお話しする機会をいただき、そのとき使ったのが図4です。

現在は、ポストゲノム時代です。ゲノム塩基配列を決定することによって遺伝子の塩基配列を推測し、タンパク質のアミノ酸配列の推測、それからさらに、機能タンパク質や酵素の発見、受容体・トランスポータの発見などを経て、最後に薬剤の発見・開発につながっていきます（図5）。アセチルコリンエステラーゼという酵素は、活性部位にセリンがあり、この酵素を阻害する物質を見つけたことが、塩酸ドネペジルという薬剤の発見・開発につながったわけです。

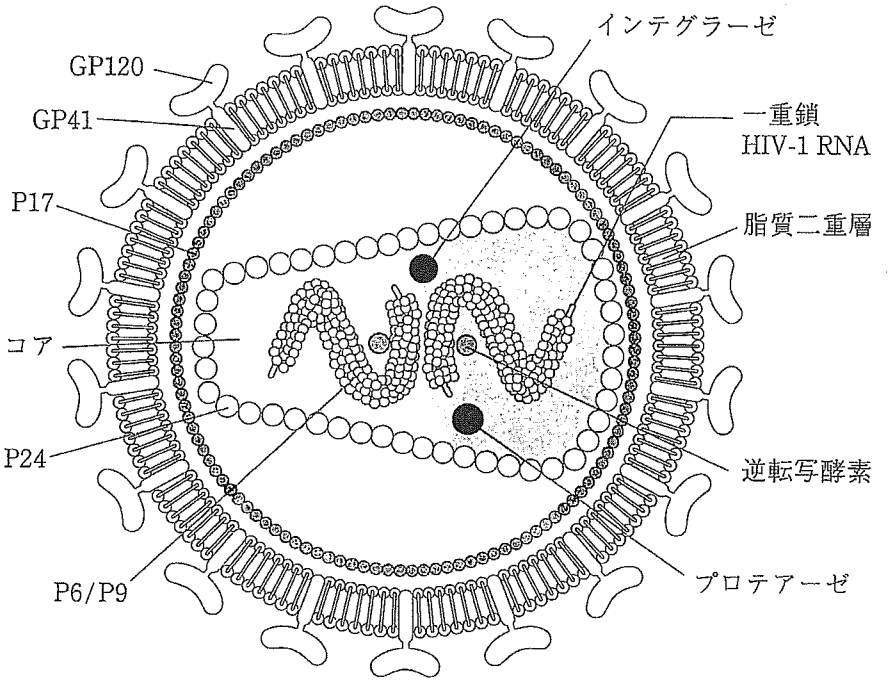

図3　エイズウイルス（HIV）の構造

四大文明	古代エジプト文明（ファラオ天然薬物）……エーベルス・パピルス メソポタミア文明……ハムラビ法典（医療の報酬、罰則） インダス文明……アーユル（生命、長寿）・ヴェーダ（知識） 中国文明……黄帝内経、傷寒論　　漢方医学（日本）

図4　世界の伝統薬物と伝承薬物

タンパク質分解酵素

アセチルコリンエステラーゼは、プロテアーゼと似ています。プロテアーゼもペプチド結合を切断するだけでなくエステル結合を切断します。プロテアーゼは、日本語では「タンパク質分解酵素」といわれていますが、これには4種類あります。

第1は、アスパラギン酸プロテアーゼです（表1）。ペプシンはもっとも基本的な胃の消化酵素で、酸性で働きます。第2は、トリプシンに代表されるセリ

ンプロテアーゼで、アセチルコリンエステラーゼとよく似ています。第3はシステインプロテアーゼ、第4は金属プロテアーゼです。

アスパラギン酸プロテアーゼ阻害剤であるHIVプロテアーゼ阻害剤は、エイズ研究において歴史に残る薬になりました。ちなみに、HIVプロテアーゼ阻害剤の研究は、酵素反応機構から論理的にドラッグデザインを行うというストーリーが非常にきれいであったためほんとうですかという話は多かったのですが、理詰めの研究が重要であるという一例です。

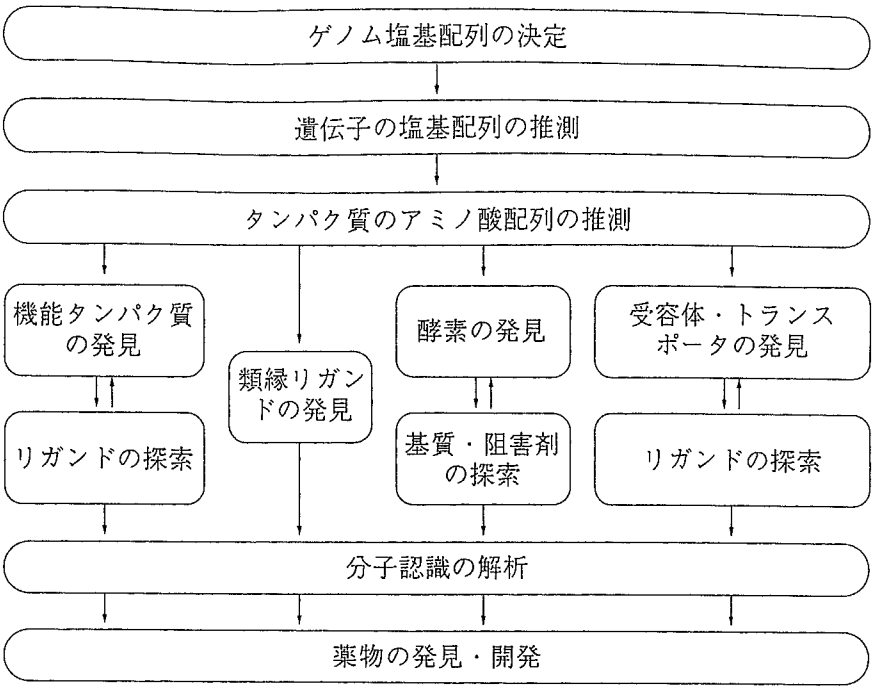

図5　薬物の開発の流れ

マラリアにもアスパラギン酸プロテアーゼであるプラスメプシンの阻害が有望です。アルツハイマー病のβ-セクレターゼが、同じ仲間であることが1999年に発見されました。それは、研究者にとって魅力でもあるし、治療にも使える可能性があることから研究がさかんになりました。

γ-セクレターゼは、非常に難しい複合体タンパク質です。表1で、？マークをつけているのは、まったく新規なもので、少し難しい話になるためです。

表1　アスパラギン酸プロテアーゼ

① レニン
② HIVプロテアーゼ（エイズ）
③ プラスメプシン（マラリア）
④ β-セクレターゼ（アルツハイマー病）
⑤ γ-セクレターゼ？（アルツハイマー病）

アミロイド前駆体タンパク質のプロセシング

アルツハイマー病は、脳内に、Aβからなるアミロイド線維が沈着してできる老人斑が広範に認められる神経変性疾患で、進行性の痴呆を特徴としています。Aβは、アミノ酸残基700個からなる1回膜貫通型のアミロイド前駆体タンパク質（APP）が、2種類のプロテアーゼで切断されることにより産生されます。AβはAPPの細胞外領域から膜貫通領域にかけて含まれ、まずβ-セクレターゼによりAβのアミノ末端（N末端）部分が切断され、次にγ-セクレターゼによってAβのカルボキシル末端（C末端）部分が切断されます（図6）。

Aβは40番目のアミノ酸残基Valで終わるAβ40と、さらに2個のアミノ酸残基（Ile-Ala）が伸びたAβ42があり、Aβ42はAβ40より凝集性が高くなっています。凝集したAβ42はAβ40を巻き込んでアミロイド線維を形成することから、Aβ42の蓄積がアルツハイマー病の発症に強くかかわっている可能性があります。

また、β-セクレターゼと異なる部位でAPPを切断し、Aβの産生を抑制する酵素として

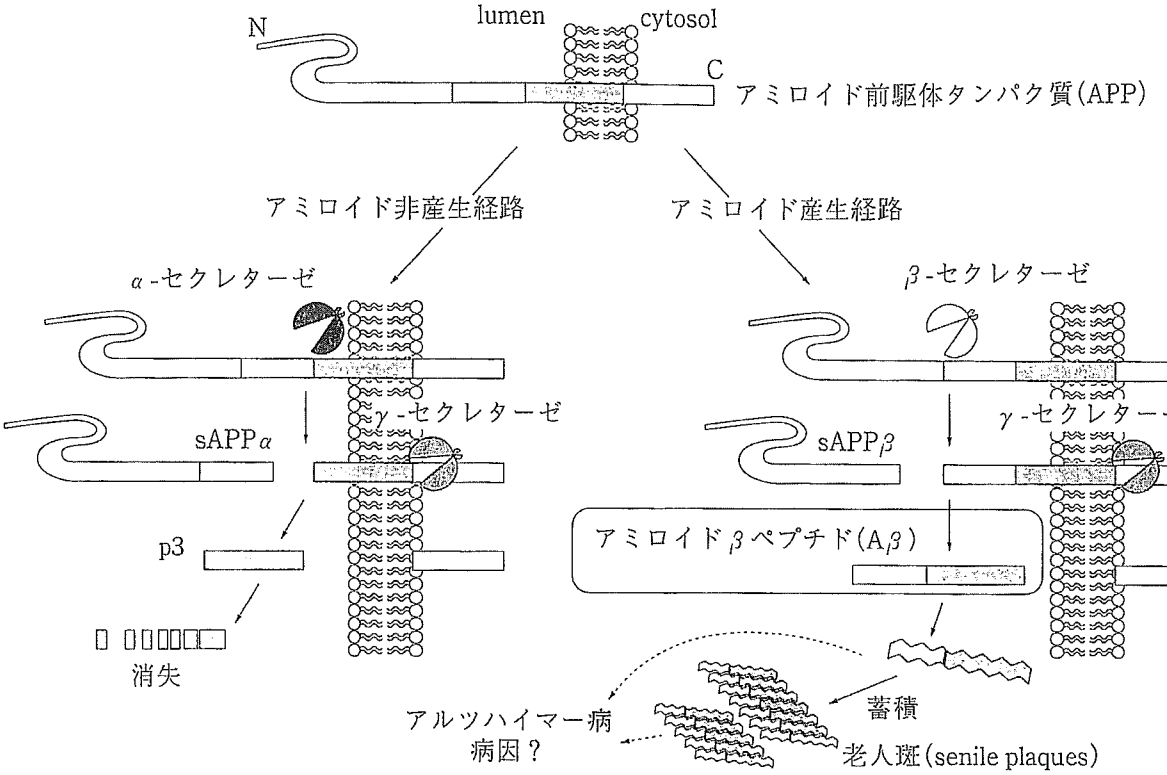

図6　アミロイド前駆体タンパク質(APP)のプロセシング

α-セクレターゼがあります(図6)。

ちなみに、今年のノーベル賞もタンパク質分解に関する研究が受賞しました。タンパク質の切断は、簡単にいうと加水分解です。有機化学の研究者や学生さんからみると簡単すぎて、そんな古いことには興味を示しませんが、タンパク質分解は生命にとって基本的なことです。

Aβ42はβシート構造をとって凝集しますが、βシートの構造が変化することで起こる病気が多くあります。たとえば、プリオンタンパク質もそうであり、HIVプロテアーゼもβシートで2量体になります。そのため、これを阻害することは、当然チャレンジングでありますが、メカニズム的には非常に困難です。私どもの研究室でも2量体化阻害剤について、外国人ポスドクが面白いためすぐやって論文をだしましたが、あまり活性も強くないし実用性もなさそうです。βシートの凝集を止めるものにトレハロースなどがありますが、選択性の問題や活性の問題で、現在のレベルでは道が遠いと私は理解しています。

APP切断部位のアミノ酸配列

APPが切断される部位のアミノ酸配列を図7に示します。βサイトは、普通の人ではメチオニンとアスパラギン酸の間で切断されます。私どもの長い研究からみると、これは非常に珍しい例です。切断部位は専門用語でP1-P1'と表示されていますが、P1'にアスパラギン酸が位置してその前で切断されることは非常に珍しいことです。

スウェーデンの家族性アルツハイマー病の発症家系の人は、このP1がロイシン、その前がアスパラギンになっているため、約100倍、切断されやすくなります。このため、Aβが産生しやすくなり発病しやすくなります。非常に論理的でわかりやすいので、私どもはすぐに研究を進めました。シンプルであることは科学の原点です。

そこで、スウェーデン型のアスパラギン、ロイシンの部分に注目しています。また、私