Molecular Neurobiology

ISSN0893-7648/94/8(2–3): 139–145/$5.40

The Presence of HTLV-I Proviral DNA in the Central Nervous System of Patients with HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis

Jun-ichi Kira

Department of Neurology, Neurological Institute, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

Abstract

Human T-lymphotropic virus type 1 (HTLV-I) is a pathogenic retrovirus associated with a chronic progressive myelopathy, termed HTLV-I-associated myelopathy (HAM)/tropical spastic paraparesis (TSP), as well as adult T-cell leukemia (ATL). A chronic inflammatory process has been implicated in HAM/TSP by a pathological study, but the exact mechanism still remains unknown. To understand better the complex mechanism of disease induction by HTLV-I, I studied the spreading pattern of HTLV-I in both peripheral blood mononuclear cells (PBMNCs) and central nervous system (CNS) tissues in patients with HAM/TSP using a quantitative polymerase chain reaction (PCR) method. My results indicated the primary event to be the efficient replication of HTLV-I in vivo, whereas HTLV-I is likely to be present in the constituent cells of the CNS in addition to the infiltrating mononuclear cells.

Index Entries: Human T-lymphotropic virus type 1 (HTLV-I); HTLV-I-associated myelopathy; tropical spastic paraparesis; polymerase chain reaction; central nervous system.

Introduction

HTLV-I is an etiologic agent of HAM/TSP *(1,2)*, and its mechanism is presently undergoing intensive research. Pathological studies on the CNS tissue of HAM/TSP patients has demonstrated perivascular lymphocyte infiltration as well as the loss of myelin and axon *(3)*. Thus, it appears that a chronic inflammatory process is operative. However, the exact mechanism of CNS damage remains unclear. The following mechanisms are thought to be possible:

1. The presence of an immunologic aberrance induced by the HTLV-I gene products may cause an autoimmune response against some CNS antigens;
2. The existence of cytotoxic T-lymphocytes (CTL) specific for HTLV-I may destroy the constituent cells of the CNS infected with HTLV-I; or

3. HTLV-I invading into the CNS may directly exert a destructive effect on the CNS cells.

The immunologic abnormalities frequently found in HAM/TSP patients, i.e., spontaneous lymphoproliferation *(4)*, increased activated T-lymphocytes in both the peripheral blood and cerebrospinal fluid (CSF) *(5)*, high levels of interleukin 2 (IL-2) and IL-6 in the sera and CSF *(6,7)*, and the presence of oligoclonal bands in the CSF *(2)*, lend support to the autoimmune hypothesis. However, these features are not specific for HAM/TSP alone and some of them are also present asymptomatic carriers to a lesser extent.

On the other hand, HTLV-I can also infect the wide spectrum of target cells in vitro, including microglia, oligodendrocytes, and astrocytes *(8,9)*. Therefore, a high level of CTL against HTLV-I in CSF *(10,11)* may be directed to these CNS cells infected with HTLV-I. Alternatively, HTLV-I may directly destroy these CNS cells in vivo, as observed in the human immunodeficiency virus (HIV) *(12)*.

In either case, it is of critical importance to determine whether or not the HTLV-I genome is present in the constituent cells of the CNS. Up to now, neither conventional immunohistochemical methods nor *in situ* hybridization unambiguously showed the presence of HTLV-I antigens or genome in the HAM/TSP CNS. This indicates that the amount of the HTLV-I genome in HAM/TSP CNS, if any, is extremely low. Therefore, I used a quantitative PCR method to evaluate the amount of HTLV-I proviral DNA present in the CNS tissue as well as in the PBMNCs of HAM/TSP patients *(13,14)*. The following sections summarize the results of my quantitative PCR studies in order to provide new insights into the mechanism of HAM/TSP.

Analysis of HTLV-I Proviral DNA Amounts in Peripheral Blood Mononuclear Cells

Using the quantitative PCR method, I compared the amounts of HTLV-I proviral DNA in the PBMNCs from patients with HAM/TSP, HTLV-I carriers without HAM/TSP, and HTLV-I seronegative control subjects *(13)*. All subjects were residents of the southwestern part of Japan, which is an endemic area for HTLV-I infection *(15)*. At the same time, antibody titers to recombinant HTLV-I $p40^{tax}$ protein and the gag–env hybrid protein in the sera were measured by an enzyme-linked immunosorbent assay (ELISA) *(16)*. The following results were obtained.

1. The amounts of HTLV-I proviral DNA in the PBMNCs were 10- to 100-fold higher in the HAM/TSP patients than in the carriers without HAM/TSP and without any inflammatory diseases (Fig. 1).
2. The patients with an early onset (15–39 yr, mean = 32.8 yr, eight females and four males), as compared to those with a late onset of the disease (44–61 yr, mean = 53.5 yr, six females), had significantly higher amounts of HTLV-I proviral DNA, particularly in the early stages of the disease (Fig. 2).
3. The carriers without HAM/TSP, but with other inflammatory diseases, i.e., polymyositis, optic neuritis, or bronchoalveolitis, also had a high level of HTLV-I proviral DNA (Fig. 1).
4. The HTLV-I proviral DNA amounts correlated significantly with both the IgG and IgA antibody titers to the recombinant HTLV-I proteins. The HAM/TSP patients had significantly higher titers of the IgG and IgA antibodies to the HTLV-I proteins than did the HTLV-I carriers without HAM/TSP.
5. HAM/TSP patients, having both a high HTLV-I proviral DNA load and high titers of the IgG and IgA antibodies, frequently demonstrated IgM antibodies to the HTLV-I proteins. These patients with IgM antibodies had a tendency to deteriorate more frequently based on both clinical evaluation and magnetic resonance imaging (MRI) of the brain *(16,17)*. The IgM antibodies to the HTLV-I proteins were detected more frequently in the HAM/TSP patients than in the carriers without HAM/TSP (40 vs 6%).

These results strongly suggest that the large increase in the HTLV-I proviral DNA is associated with the development of HAM/TSP. I and others estimated that approx 5–50% of the PBMNCs harbor the HTLV-I genome in HAM/TSP *(13,18)*. The provirus was shown to be integrated polyclonally into host DNA in PBMNCs *(19)*. The vigorous replication of HTLV-I may well explain the extremely high titer antibodies as well as the strong CTL against the virus in HAM/TSP patients. Moreover, the spreading of HTLV-I can induce extensive alteration in T-cell function, since the viral tax protein enhances the expression of specific cellular genes, such as IL-2, IL-2 receptor, and c-*fos* protooncogene *(20,21)*. Therefore, such a large proviral DNA load is likely to predispose to inflammatory conditions through the widespread T-cell activation. Alternatively, it is possible that the activation

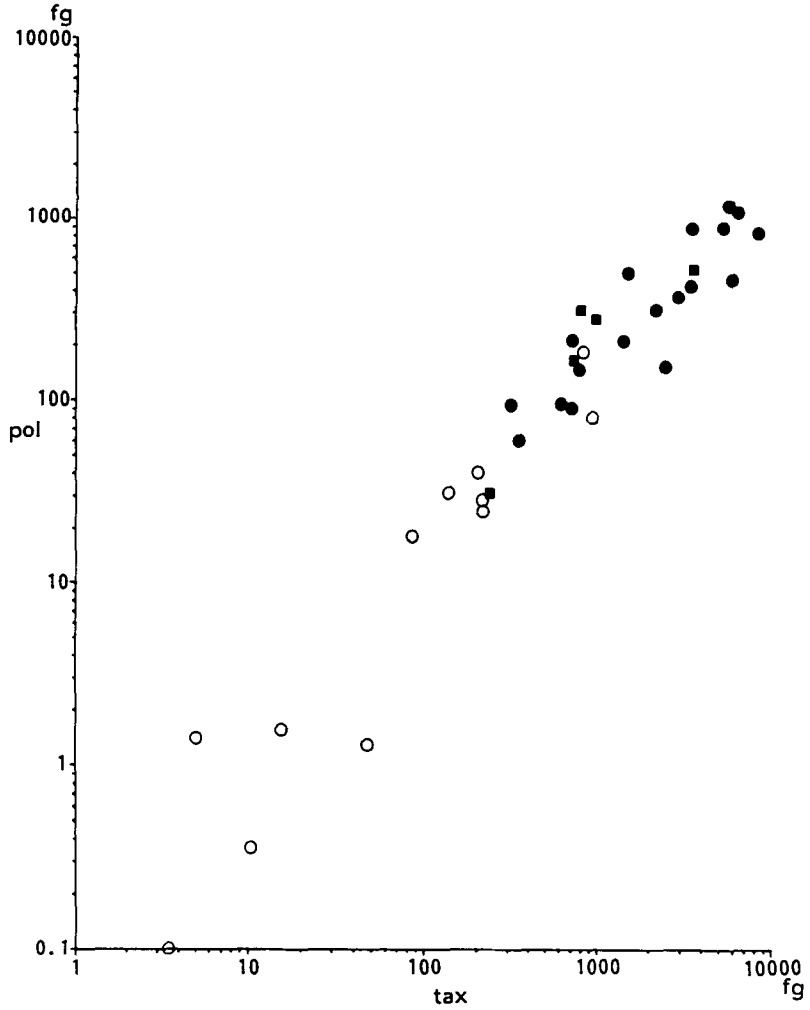

Fig. 1. The amount of HTLV-I tax (pX) and pol DNA present in 1 μg of sample DNA, as referred to plasmid DNA (logarithmic scale). Black circles = patients with HAM/TSP; black squares = HTLV-I carriers without HAM/TSP and with inflammatory diseases; white circles = carriers without HAM/TSP and without inflammation. Reproduced from ref. *13* with permission.

of T-cells and other cells under these inflammatory conditions enhance the replication of HTLV-I, either directly or indirectly through cytokine action *(22,23)*.

In asymptomatic carriers, about 10–30% were assumed to have relatively high amounts of HTLV-I proviral DNA, and some immunologic dysfunctions *(13,24)*. The efficient replication of HTLV-I is thus probably the primary event, and such a population of asymptomatic carriers may represent a group at high risk for HAM/TSP and other HTLV-I-associated inflammatory diseases. Finally, the large increase of HTLV-I-infected cells in the PBMNCs perhaps increases the probability for such cells to enter into and make contact with the constituent cells of the CNS.

Analysis of HTLV-I Proviral DNA Amounts in the Central Nervous System Tissue

I investigated the presence and amount of HTLV-I proviral DNA in the CNS tissue of HAM/TSP patients by a combined quantitative PCR and histological study *(14)*. Cellular DNA was extracted from the formalin-fixed CNS tissue from six patients with HAM/TSP, one patient with ATL and a marked CNS infiltration of leukemic cells, and nine control subjects with other neurological diseases. The presence and amounts of chromosomal DNA available for PCR were first evaluated by the β-globin-specific PCR (Fig. 3 and Table 1). HTLV-I pX and env,

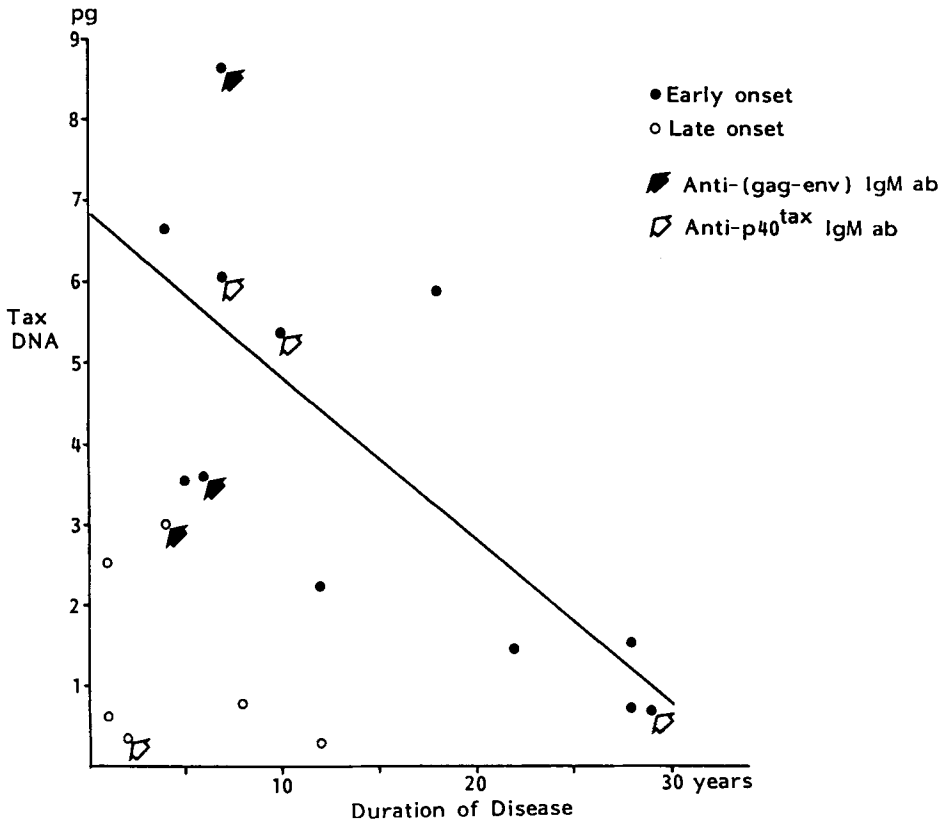

Fig. 2. Relationship between pX DNA amounts (ordinate) and the duration of illness (abscissa). Black circles = HAM/TSP patients with an early onset; white circles = patients with a late onset. Arrows indicate the patients with IgM antibodies to the recombinant HTLV-I proteins. Reproduced from ref. *13* with some modifications.

but not pol DNA were detected in five of six patients with HAM/TSP. The ATL sample was positive for pX, env, and pol by PCR. None of the control samples were consistently positive for HTLV-I by PCR, but all showed positive bands on the β-globin PCR (Table 1).

In order to compare the amounts of HTLV-I proviral DNA precisely, the amounts of pX DNA were adjusted by the amounts of β-globin DNA present in 1 μg of extracted DNA. The extent of perivascular cell infiltration in the several paraffin sections adjacent to those used for DNA extraction was evaluated semiquantitatively. There was no correlation between the amounts of HTLV-I proviral DNA and the extent of mononuclear cell infiltration in the adjacent sections (Table 2). Some HAM/TSP samples that had practically no perivascular cell infiltration, also had abundant HTLV-I proviral DNA in the lesion. The amount of HTLV-I proviral DNA was greater in the HAM/TSP samples than in the ATL sample, although the extent of perivascular cell infiltration was far less in the HAM/TSP samples than in the ATL sample.

These results suggest that the constituent cells of the CNS in addition to infiltrating mononuclear cells harbor the HTLV-I genome in the HAM/TSP CNS. HTLV-I can infect the constituent cells of the CNS in vitro, such as microglial cells, oligodendroglia, and astroglia *(8,9)*. Koyanagi et al. *(25)* demonstrated the infection of HTLV-I in the monocytes of patients with HAM/TSP in vivo, and suggested that tissue macrophages may comprise a virus reservoir in vivo. Finally, by using an *in situ* hybridization technique Kuroda et al. *(26)* recently reported that the HTLV-I genome was found in the microglia- and astroglia-like cells in the autopsied CNS tissue of a patient with HAM/TSP. The HTLV-I genome detected in the cerebrum may thus contribute to the development of multiple white matter lesions seen in more than two-thirds of all HAM/TSP patients *(7)*.

Together with the existence of a high level of HTLV-I-specific CTL in the CSF of HAM/TSP patients *(11)*, these observations imply the destruction of HTLV-I-infected CNS cells by the CTL-mediated mechanism in HAM/TSP. The presence

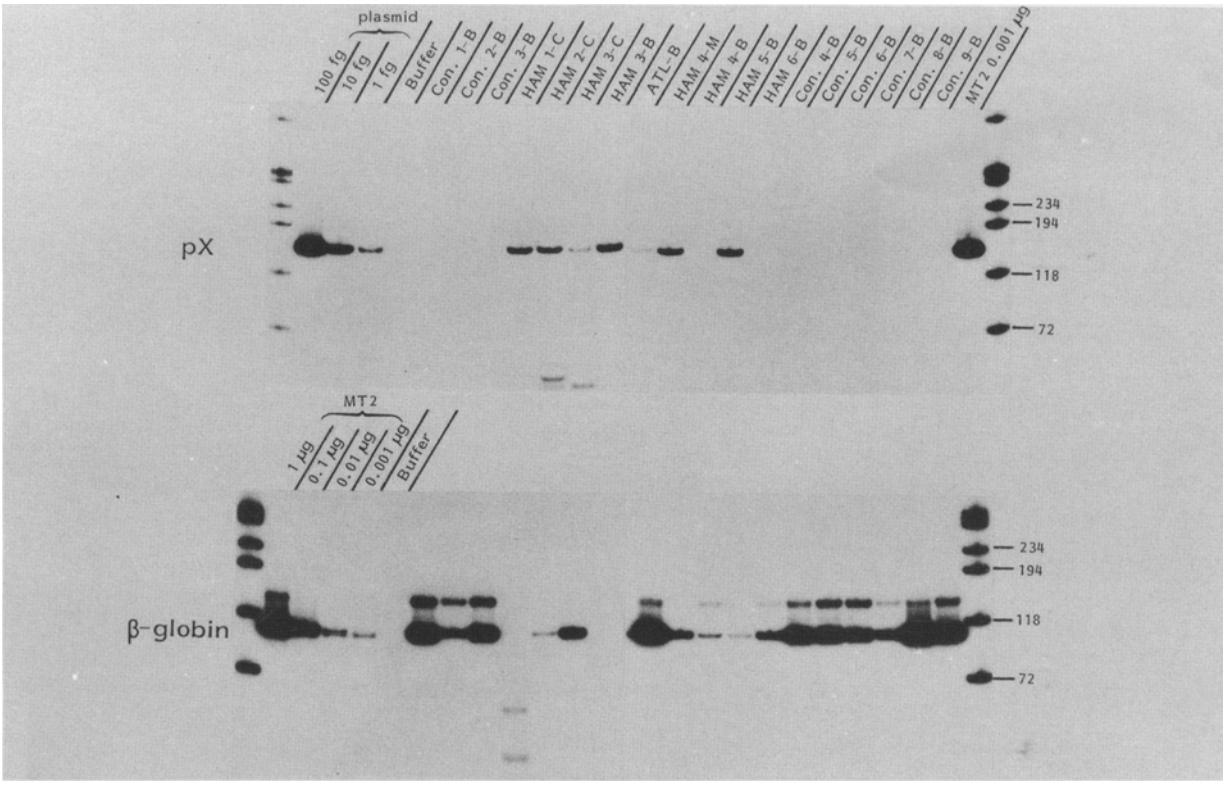

Fig. 3. A PCR analysis of the pX (upper panel) and β-globin (lower panel) DNA. The same sample runs in the corresponding lane in the upper and lower panels. C = spinal cord; M = medulla; B = brain (cerebrum). Reproduced from ref. *14* with permission.

Table 1
Detection of HTLV-I Proviral DNA and β-Globin DNA

	HAM/TSP								
Samples	1-C[a]	2-C	3-C	3-B	4-M	4-B	5-B	6-B	ATL	Control
pX	4/4[b]	3/3	3/3	4/4	4/4	0/4	4/4	1/4	4/4	3/9 (4/36)
env	2/3	1/3	2/4	2/4	2/4	0/4	4/4	1/4	2/4	0/9 (0/36)
pol	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	3/3	0/9 (0/27)
β-globin	1/2	2/2	2/2	1/2	2/2	2/2	2/2	2/2	2/2	9/9 (18/18)

[a]C = spinal cord, M = medulla, B = brain (cerebrum).

[b]Number positive/number tested. Samples that were constantly positive in the PCR and positive at least one occasion in env PCR were considered positive for HTLV-I genome. Thus, all HAM/TSP samples, except for 4-B and 6-B, were considered positive for HTLV-I genome. Reproduced from ref. *14* with permission.

of CD8+ T-cells in HAM/TSP CNS *(27)* and the induction of a major histocompatibility complex class I by the HTLV-I pX gene products on glial cells *(28)* further support the CTL hypothesis.

However, in the rat model of HAM/TSP, spinal cord damage developed without any lymphocytic infiltration in the lesion *(2)*. Moreover, I observed practically no lymphocytic infiltration in some spinal cord lesions in HAM/TSP *(14)*. Therefore, whether the CTL constitutes the essential part of the pathogenesis of HAM/TSP or not is still uncertain. In the case of human immunodeficiency virus, low copy numbers of the virus infected to macrophage/microglia can initiate the cascade of diffuse immune activation in the brain through the action of tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and

Table 2
Quantitation of HTLV-I Proviral DNA and Histological Findings

	HAM/TSP								
Sample	1-C	2-C	3-C	3-B	4-M	4-B	5-B	6-B	ATL
pX (fg)	2.5[a]	8	3	8	2	0	1	0	<1
β-globin (μg MT2 DNA)	<<0.001	0.001	0.03	<<0.001	0.05	0.03	<0.001	0.02	0.05
pX (fg)/1 μg of β-globin	<2500[b]	8000	100	>8000	40	0	>1000	0	<20
Cell infiltration									
small vessels	0	3.3[c]	0	0	0.6	2.5	0	5	8
large vessels	0	7[c]	0	0	3.6	4	1	8	17
Gliosis	++[d]	+++	++	+	++	+	+	+	nd

[a]Calculated DNA amounts equivalent to either plasmid or MT2 DNA present in 1 μg of extracted DNA. Means of three or four experiments are shown in the pX PCR, and means of two experiments in the β-globin PCR. The mean ± SD of all control samples was 0.023 ± 0.001 in the β-globin DNA.

[b]pX DNA amounts/1 μg of β-globin DNA, normalized to MT2 β-globin DNA.

[c]Predominantly macrophage infiltration in this patent.

[d] + = mild; ++ = moderate; +++ = marked. Reproduced from ref. *14* with permission.

arachidonic metabolites *(30)*. An infection of microglial cells with HTLV-I was shown to enhance the production of IL-6 and TNFα. TNFα is well known to induce demyelination *(31)*. Therefore, indirect neurotoxicity through microglial activation by HTLV-I in vivo may also play a role.

Future Directions

It is of critical importance to clarify the molecular mechanism of the extreme increase of HTLV-I proviral DNA load in HAM/TSP patients in vivo and the relationship between the increase of the HTLV-I proviral DNA load and the development of HAM/TSP. Therefore, a long-term followup study of asymptomatic carriers who have relatively high amounts of HTLV-I proviral DNA load and some immunologic dysfunction may provide important clues to these questions.

Several lines of evidence suggest that HTLV-I exists in vivo in a mixture of microvariants that have minor nucleotide sequence changes, like HIV *(32,33)*. Until now, no neurotropic or neuropathogenic variants have been found. However, one recent report showed that the viral long-terminal repeats isolated from the CSF lymphocytes of a HAM/TSP patient directed the expression of a reporter gene primarily in the CNS in transgenic mice containing the viral gene *(34)*. Thus, minor variations in the nucleotide sequence could play a role in the neurotropism and pathogenesis of HTLV-I. The biological function of such HTLV-I microvariants, therefore, still need to be determined in future studies.

Acknowledgment

I thank Ikuo Goto for critical comments on the manuscript.

References

1. Gessain A., Barin F., Vernant J. C., Gout O., Maurs L., Calender A., and de The G. C. (1985) *Lancet* **2,** 407–410.
2. Osame M., Matsumoto M., Usuku K., Izumo S., Ijichi N., Amitani H., Tara M., and Igata A. (1987) *Ann. Neurol.* **26,** 117–122.
3. Iwasaki Y. (1990) *J. Neurol. Sci.* **96,** 103–123.
4. Itoyama Y., Minato S., Kira J., Goto I., Sato H., Okochi K., and Yamamoto N. (1988) *Neurology* **38,** 1302–1307.
5. Itoyama Y., Kira J., Fujii N., Goto I., and Yamamoto N. (1989) *Ann. Neurol.* **26,** 257–262.
6. Nishimoto N., Yoshizaki K., Eiraku N., Machigashira K., Tagoh H., Ogata A., Kuritani T., Osame M., and Kishimoto T. (1990) *J. Neurol. Sci.* **97,** 183–193.
7. Tendler C. L., Greenberg S. J., Blattner W. A., Manns A., Murphy E., Fleisher T., Hanchard B., Morgan O., Burton J. D., Nelson D. L., and Waldmann T. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5218–5222.
8. Akagi T., Hoshida Y., Yoshino T., Teramoto N., Kondo E., Hayashi K., and Takahashi, K. (1992) *Acta Neuropathol.* **84,** 147–152.
9. Hoffman P. M., Dhib-Jalbut S., Mikovits J. A., Robbins D. S., Wolf A. L., Bergey G. K., Lohrey N. C., Weislow O. S., and Ruscetti F. W. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11,784–11,788.
10. Jacobson S., Shida H., McFarlin D. E., Fauci A. S., and Koenig S. (1990) *Nature* **348,** 245–248.
11. Jacobson S., McFarlin D. E., Robinson S., Voskuhl R., Martin R., Brewah A., Newell A. J., and Koenig S. (1992) *Ann. Neurol.* **32,** 651–657.

12. Brenneman D. E., Westbrook G. L., Fitzgerald S. P., Ennist D. L., Elkins K. L., Ruff M. R., and Pert C. B. (1988) *Nature* **335,** 639–642.
13. Kira J., Koyanagi Y., Yamada T., Itoyama Y., Goto I., Yamamoto N., Sasaki H., and Sakaki Y. (1991) *Ann. Neurol.* **29,** 194–201.
14. Kira J., Itoyama Y., Koyanagi Y., Tateishi J., Kishikawa M., Akizuki S., Kobayashi I., Toki N., Sueishi K., Sato H., Sakaki Y., Yamamoto N., and Goto I. (1992) *Ann. Neurol.* **31,** 39–45.
15. Hinuma Y., Nagata K., Hanaoka M., Nakai M., Matsumoto T., Kinoshita K., Shirakawa S., and Miyoshi I. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6476–6480.
16. Kira J., Nakamura M., Sawada T., Koyanagi Y., Ohori N., Itoyama Y., Yamamoto N., Sakaki Y., and Goto, I. (1992) *J. Neurol. Sci.* **107,** 98–104.
17. Kira J., Fujihara K., Itoyama Y., Goto I., and Hasuo K. (1991) *J. Neurol. Sci.* **106,** 41–49.
18. Gessain A., Saal F., Gout O., Daniel M.-T., Flandrin G., de The G., Peries J., and Sigaux, F. (1990) *Blood* **75,** 428–433.
19. Greenberg S. J., Jacobson S., Waldmann T. A., and McFarlin D. E. (1989) *J. Infect. Dis.* **159,** 741–744.
20. Wano Y., Feinberg M., Hoshing J. B., Bogerd H., and Greene W. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9733–9737.
21. Nagata K., Ohtani K., Nakamura M., and Sugamura K. (1989) *J. Virol.* **63,** 3220–3226.
22. Zagury D., Bernard J., and Leonard R. (1986) *Science* **231,** 850–853.
23. Matsuyama T., Hamamoto Y., and Kobayashi S., (1988) *Med. Microbiol. Immunol.* **177,** 181–187.
24. Tachibana N., Okayama A., Ishihara S., Shioiri S., Murai K., Tsuda K., Goya N., Matsuo Y., Essex M., Stuver S., and Mueller N. (1992) *Int. J. Cancer* **51,** 593–595.
25. Koyanagi Y., Itoyama Y., Nakamura N., Takamatsu K., Kira J., Iwamasa T., Goto I., and Yamamoto N. (1993) *Virology* **196,** 25–33.
26. Kuroda Y., Matsui M., Kikuchi M., Kurohara K., Takashima H., Endo C., and Kakigi R. (1993) *Neurology* **43 (Suppl.),** 371.
27. Moore G. R. W., Traugott U., Scheinberg L. C., and Raine C. S. (1989) *Ann. Neurol.* **26,** 523–530.
28. Sawada M., Suzumura A., Yoshida M., and Marunouchi T. (1990) *J. Virol.* **64,** 4002–4006.
29. Ishiguro N., Abe M., Seto K., Sakurai H., Ikeda H., Wakisaka A., Togashi T., Tateno M., and Yoshiki T. (1992) *J. Exp. Med.* **76,** 981–989.
30. Epstein L. G. and Gendelman H. E. (1993) *Ann. Neurol.* **33,** 429–436.
31. Selmaj K. W. and Raine C. S. (1988) *Ann. Neurol.* **23,** 339–346.
32. Daenke S., Nightingale S., Cruickshank J. K., and Bangham C. R. M. (1990) *J. Virol.* **64,** 1278–1282.
33. Gessain A., Gallo R. C., and Franchini G. (1992) *J. Virol.* **66,** 2288–2295.
34. Gonzalez-Dunia D., Grimber G., Briand P., Brahic M., and Ozden S. (1992) *Virology* **187,** 705–710.