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Invited Paper

Neuropathology of HAM/TSP; Subsets of Cellular Infiltrates and Detection of HTLV-I provirus in the spinal cord lesion

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Introduction

The retrovirus human T lymphotropic virus type I (HTLV-I) is the causative agent of adult T-cell leukemia/lymphoma(ATLL) and the chronic progressive spinal disease known as HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[1,2]. Several pathological studies of the autopsied patients with HAM/TSP have shown characteristic pathological changes of marked infiltration of lymphocytes and monocytes/macrophages and diffuse degeneration of spinal white matter, which suggest the inflammatory process is involved in the pathogenesis of this disease[3,4,5]. We review our recent studies focused on the pathogenic mechanism of inflammation and detection of HTLV-I infected cells in the spinal cord lesions[6,7,8,910].

Patients examined

We examined six autopsied patients with HAM/TSP. All patients had chronic progressive spastic paraparesis and bladder dysfunction, with or without mild sensory impairment in the legs, with the elevation of anti-HTLV-I antibodies in both serum and CSF. All patients were treated with steroids but the treatment was discontinued prior to death. CNS tissues from all patiens were fixed routinely in 4% paraformaldehyde in PBS or buffered formalin for four to seven days and embedded in paraffin. Frozen materials were also served for immunohistochemistry and PCR detection of HTLV-I provirus.

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Immunocytochemical analysis of cellular infiltrates[6] (Table 1)

Immunocytochemical staining of the spinal cord lesions was done using a panel of monoclonal antibodies reactive with T cells, T cell subsets, B cells, macrophages, natural killer cells, IL-2 receptor-positive cells, and HLA-ABC and HLA-DR. In the patients with shorter duration of illness, CD4+ cells, CD8+ cells and macrophages were evenly distributibuted in active inflammatory spinal cord lesions. On the other hand, the predominance of CD8+ cells over CD4+ cells in the inactive-chronic lesions of patients with longer duration of illness. Natural killer cells, IL2 receptor positive cells and B cells were only rarely present in both active and inactive chronic lesions. HLA-ABC was positive in the endothelial cells and infiltrating mononuclear cells, and HLA-DR was positive in endothelial cells, microglia, and infiltrating mononuclear cells. These findings suggested that immune responses in the spinal cord lesions of HAM/TSP gradually change along with the duration of illness.

Table 1. Quantitative analysis of infiltrating cells in the spinal cords

	Leu4	T4	T8	B	Mp	1L2-R	NK
Patient 1							
T(15)†	101.2±25.7	48.4±14.7	50.0±11.3	0.1±0.3	113.2±26.3	n.e.	n.e.
Patient 2							
T(30)	47.5±19.8	21.2±7.5	27.3±7.5	0.2±0.4	60.3±13.1	0.2±0.4	0
L(30)	28.1±10.9	4.4±2.4	23.5±6.3	0.2±0.4	66.9±24.1	0.1±0.3	0.1±0.3
S(30)	79.0±14.7	9.8±7.9	54.9±19.0	0.2±0.4	37.0±9.2	0.5±0.5	0.1±0.3
Patient 3							
T(25)	5.6±4.9	0.4±0.5	4.9±3.0	0.1±0.4	20.0±13.0	0	0
L(25)	4.8±4.8	0.6±1.1	4.7±3.7	0	34.3±4.8	0	2.0±0.6
Patient 4							
C(25)	54.3±10.9	3.8±2.1	44.9±0.1	0	56.4±8.5	0	0.2±0.4
T(25)	27.3±8.3	1.5±2.2	27.1±7.6	0	33.3±9.3	0	0
L(25)	23.9±8.6	1.0±1.4	21.6±8.1	0	30.3±9.8	0	0
Patient 5							
C(30)	20.6±6.0	1.3±1.1	20.3±9.0	0	16.9±7.9	0	0
T(20)	17.4±7.1	0.7±0.7	16.9±5.2	0.2±0.4	74.9±10.3	0	0
L(20)	25.8±11.5	0.7±0.6	21.0±5.4	0.2±0.4	68.1±14.6	0	0

IL-2R: IL-2 receptor; NK: natural killer cell; Mp: macrophage; C: cervical T: lower thoracic; L: lumbar spinal cord; n.e.: not examined.

*Numbers represent mean values ±1 SEM of the cell numbers in representative areas of 0.18mm^2 using an indexing grid and ×200 magnification.

†Numbers in parentheses mean numbers of counted fields.

Apoptosis of T lymphocytes in the spinal cord lesion[8)]

We applied a monoclonal antibody TIA-1, a maker of cytotoxic T lymphocytes, to detect possible effector cells of the inflammation in HAM/TSP. Many TIA-1+, CD8+ cells were distributed in the above mentioned active inflammatory lesions, however, few cells were positive in the inactive chronic lesions. The protein TIA-1 has been associated with induction of apoptosis in the target cell. With careful observation we found cells undergoing apoptosis, most of them identified as helper-inducer CD45RO+ T lymphocytes which has been known as in vivo target cells of HTLV-I. These findings suggested that CTL-induced apoptosis of T lymphocytes may be one of the possible mechanisms which eliminate HTLV-I-infected cells from the central nervous system.

Detection of HTLV-I provirus in the spinal cord lesions[9)]

It is crucially important whether there are HTLV-I infected cells in the inflamed spinal cord lesions. In order to evaluate the amount of HTLV-I proviral DNA, a quantitative method utilizing polymerase chain reaction was employed in the affected spinal cords which were freshly frozen and stored in liquid nitrogen. The presence of HTLV-I pX and pol sequences in the CNS tissues were demonstrated in all patients examined. The proviral DNA amounts quantified in the thoracic cord were 0.002 to 2 copies per 100 tissue cells, and those in the peripheral blood mononuclear cells(PBMC) were 2 to 8 copies per 100 PBMC. The amount of integrated HTLV-I in the thoracic cord tended to decrease with the disease duration and this decline was paralleled with the alteration of CD4+ T cell numbers. These findings suggest that preferential virus reservoir may be infiltrating CD4+ T lymphocytes in the spinal cord lesions of patients with HAM/TSP, and HTLV-I infection in CNS compartment of the patients is declining with the disease duration.

In situ PCR detection of infected cells[10)]

A knoweledge of which cell types hardor this virus in infected tissues is clearly important for understanding the pathogenesis of this HTLV-I-associated disease. We applied *in situ* polymerase chain reaction to determine which cells harbor the HTLV-I provirus *in vivo* in autopsied spinal lesions of HAM/TSP patients.

Paraffin sections were cut and picked up aminosylane coated glass slides. After permiabilization with Tween 20 and digestion with proteinase K, the slides were applied for hot start PCR on the heating block of a thermal-cycler (Zymoreactor II ; Atto, Tokyo, Japan) using a nested primer set consisted of six primers spanning nucleotides 6856-7965 of the HTLV-I pX[11)].

Table 2. Case description of study patients and result of IS-PCR for HTLV-I proviral DNA

Patient	Sex	Age (yr)	Duration of illness (yr)	Degree of disability[a]	Anti-HTLV-IAb[b] serum	Anti-HTLV-IAb[b] CSF	No. of total cells (cells/mm^2)	No. of OPD4 positive cells (cells/mm^2)	No. of IS-PCR positive cells (cells/mm^2)
1	M	77	2.5	6	×2048	×16	2130	325	19
2	F	71	4.5	7	×16382	×1024	1044	52	7
3	F	52	8	4	×8192	×128	1130	11	<1[d]
4	M	73	10	4	×32768	×1024	795	14	<1
5[c]	F	47	20	5	×8192	×256	746	17	<1
6	M	67	15	7	(++)	(++)	816	8	<1

a : The degree of motor disability was evaluated based on Osame's score.
b : Anti-HTLV-I antibodies were titrated by the particle agglutination method and in patient 6 evaluated by fluorescence antibody method (in parentheses).
c : Patient 5 also sufferd with ATLL.
d : Total number of IS-PCR positive cells were ranged 1 to 10 on three slides (represented by "<1")

After 40 cycles of denaturation at 95°C for 1.5 min, annealing at 45°C for 2 min and polymerization at 72°C for 2 min, the amplified HTLV-I DNA was detected with an intermal oligonucleotide probe (SK45) end-labeled with digoxigenin.

The signal of amplified proviral HTLV-I DNA was localized to the nuclei of infiltrating mononuclear cells especially in patients with a shorter duration of illness. The number of positive cells was correlated with the number of OPD4 positive cells. We did not detect conspicuous viral DNA in the endothelial cells or the cells of CNS origin such as astrocytes or neurons. These results suggest that infiltraing mononuclear cells are major reservoir for the HTLV-I provirus in HAM/TSP.

Pathogenesis of HAM/TSP

HAM/TSP is now a well-defined clinicopathological entity in which the virus infection and the host immune responses are involved in the pathogenesis. Our recent series of studies suggested that T cell mediated chronic inflammatory process targeting the HTLV-I infected T cells is the initial pathogenic mechanism of HAM/TSP. Anatomically determined hemodynamic conditions may contribute to the localization of infected T cells and forming of main lesions in the middle to lower thoracic spinal cord. Destruction of myelin and axons may occur in the presence of cytokines such as tumor necrosis factor or γ interferon as a bystander damage[7,12].

Key word: Autopsy, HAM/TSP, HTLV-I, *in situ* polymerase chain reaction, neuropathology, T cell subset

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