Neuropathological studies of the spinal cord in early stage HTLV-I-associated myelopathy (HAM)

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Abstract
Necropsy findings for a patient with HTLV-I-associated myelopathy (HAM) of 9 months clinical duration are reported. Loss of myelin sheaths and axons together with perivascular lymphocytic infiltration was seen in the lateral and posterior columns of the spinal cord from the cervical to the lumbar region where vascular changes caused by the splitting of myelin sheaths were prominent. Immunohistochemical analyses revealed CD8+ cytotoxic T cell infiltration predominated in the absence of HTLV-I core protein antigen bearing cells in the brain and spinal cord. Myelin sheath damage and predominant CD8+ cytotoxic T cell infiltration are thought to be the main neuropathological findings in the spinal cord in early stage HAM.

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Although pathological findings of Human T lymphotropic virus type I (HTLV-I) associated myelopathy/tropical spastic paraparesis (HAM/TSP) have been reported, neuropathological findings for the early stage of HAM/TSP are lacking. Moreover, immunohistochemical investigations of frozen sections are few, and the question of what kind of lymphocyte is involved and whether HTLV-I viral antigen is present in the nerve tissue has not been answered. We investigated the spinal cord of a patient with HAM/TSP of nine months clinical duration.

Case report
A 69 year old Japanese woman, who had no history of transfusion, suffered progressive gait disturbance which started in April 1989. By August of that year she was unable to walk without assistance. On admission in August neurological examination revealed a spastic paraparesis. The deep tendon reflexes in the legs were hyperactive with extensor plantar responses and sustained ankle clonus. A slight decrease in vibratory sensation was noted below the knees. Residual urine was more than 300 ml but there was no dysuria. The MRI of cervical and thoracic spinal cords revealed no abnormalities. The spinal fluid showed mild pleocytosis (25/3/mm³) and elevation of protein (68 mg/dl) and IgG (30·7 mg/dl). The three oligoclonal IgG bands were present. HTLV-I antibody titres were 1:5, 120 in the serum and 1:256 in the CSF by the FA method. On clinical diagnosis of HAM/TSP she was given 60 mg of prednisolone daily for 2 months but there was no improvement in her spastic paraparesis and urinary incontinence. The dose of prednisolone was reduced to 30 mg daily thereafter. In December 1989, she died of respiratory failure brought on by interstitial pneumonitis of unknown etiology. Her total clinical course was nine months.

Pathology
Gross and histological findings
The brain weighed 1350 g. Its gross appearance and the coronal sections were unremarkable. The spinal cord appeared to be atrophic dorso-laterally at the lower thoracic level with mild pial thickening. The anterior and posterior roots seemed to be intact. Perivascular as well as leptomeningeal lymphocytic infiltrations were noted in the midbrain, pons, thalamus and cerebral white matter. Scattered parenchymal lymphocytic infiltration was seen in the thalamus although the nerve cells were well preserved. In the spinal cord, the main neuropathological findings were the loss of myelin and axons with perivascular lymphocytic infiltration. Losses were greatest in the lateral columns, less so in the posterior columns with relatively preserved anterior columns (fig 1). The most severe changes were in the lower thoracic cord, the severity lessening in the rostro-caudal direction. The severely damaged cord showed vacuolar changes in the white matter, in particular in the marginal zone of the lateral columns, but with less severe changes in the anteromedial region of the posterior columns (fig 2A). These lesions present from the middle cervical to the lower lumbar region, indicating that they were not due to Wallerian degeneration. Semi-thin Epon sections of the lower thoracic cord disclosed myelinated fibres with split myelin sheaths and preserved axons (fig 2B). Myelin sheath damage predominated. Lymphocytic infiltration was prominent in the perivascular cuffs but less so in the...
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pressive, we stained the serial sections with Leu2, Leu7 and Leu 15 monoclonal antibodies. Leu2+ cells were not stained by Leu7 or Leu15, indicative of their cytotoxicity. No Leu14+ (B cells) cells were found in the parenchyma and perivascular cuffs. The vascular endothelium, macrophages, microglia and some lymphocytes were HLA-DR positive. No HLA-ABC, class I antigen-bearing cells were present in the nerve tissue or infiltrating cells, except in the vascular endothelium. Macrophages, stained with LeuM5 were aggregated in the vicinity of the perivascular areas and parenchyma in the severely damaged regions, particularly in the lateral columns.

Use of frozen sections treated with three different monoclonal antibodies against HTLV-I core protein (GIN7 and FR19 (Fujirevio) against HTLV-I core protein p19 and p28 and MAB811 (Chemicon) against core protein p19) showed no viral antigens in the nerve tissue although a few intravascularly localised lymphocytes were positively stained with GIN7.

Discussion

The review of ten necropsy cases of HAM/TSP by Iwasaki indicates a correlation between histological changes and the length of this illness before death. In five cases with a clinical duration of more than four years, the spinal cord showed monotonous chronic changes with marked glio-mesenchymal tissue reactions and little cellular infiltration. In the four cases with a clinical duration up to three years, both parenchymal tissue damage and cellular infiltration were prominent, loss of myelin sheaths and axons being associated with perivascular lymphocytic infiltration. The case of HAM/TSP of 10 months clinical duration, in which lymphocytic infiltration was slight in the immunodeficient state, does not appear to be typical of the neuropathological findings for HAM/TSP. As the shortest clinical duration in previously necropsied serologically-proved HAM/TSP cases was two years and two months, early histopathological findings for the disease for up to one year are lacking; whether the myelin sheaths or axons are the tissues primarily affected in HAM/TSP has yet to be determined.

Vacuolar changes of the white matter have been reported rarely in HAM/TSP. Clinicopathological characteristics of HAM/TSP patients with vacuolar changes are noted. Clinically, as vacuolar changes were noted in the white matter of the HAM/TSP patient whose illness lasted two years and two months and for TSP that showed a relatively short clinical course, the vacuolar changes in HAM/TSP appear to be localised only in the early stage of the disease and to disappear as the disease progresses. Pathologically, the spinal cords with vacuolar changes show less cellular infiltration than those without vacuolar changes. Semi-thin Epon sections of the lower thoracic cord showed split myelin sheaths with relatively well preserved axons,

parenchyma and leptomeninges. In the areas showing severe tissue damage, marked lipiddaden macrophages were present and vessels with hyaline thickening of walls were increased. The pia mater was thickened and hemosiderin granules were deposited.

Immunohistochemistry

The frozen sections of the lower and middle thoracic spinal cord were stained immunohistochemically by the avidin-biotin complex (ABC) technique. Many Leu2 positive CD8+ (cytotoxic/suppressor) cells were present in the perivascular cuffs as well as in the parenchymal regions of the severely damaged spinal cord (fig 3A). Leu3 positive CD4+ (helper/inducer) lymphocytes were also present in the same distribution; whereas the number of antigen positive cells was less than that of CD8+ (fig 3B). To determine whether the CD8+ lymphocytes were cytotoxic or sup-

Figure 1 Myelin sheath loss in the lateral and posterior columns of the cervical (A), mid-thoracic (B) and lumbar cord (C). Vacuolar changes of the white matter are seen in the cervical and mid-thoracic cord. Kluver-Barrera. × 12.
indicative of primary involvement of the myelin sheath during the early stage of HAM/TSP. Demyelination and remyelination also have been reported in electron-microscopic investigations of HAM/TSP. An immunohistochemical investigation showed the absence of HTLV-I antigen-bearing cells in the nerve tissue. Results of earlier studies concerning the existence of viral antigen in the nerve tissue of HAM/TSP patients are controversial. Several reports, however, are based on immunohistochemical and electron-microscopic studies of the absence of viral antigen in nerve tissue, whereas only two reports show the presence of viral antigen. Results of studies concerning the existence of proviral sequences in HAM/TSP spinal cords using the PCR (polymerase chain reaction) method are also controversial. Although Bhigjee et al.19 and Kira et al.30 found the proviral DNA in the spinal cords from HAM/TSP patients, Ohara et al.31 obtained negative results from 3 HAM/TSP spinal cords (including ours). From the PCR study, Kira et al.30 quantitated much larger volume of cord tissue than Ohara et al.31. An in situ hybridisation study was unsuccessful in spite of the detection of proviral DNA by PCR. From the results of these PCR studies, it is concluded that viral replication in HAM/TSP spinal cords is rare, if it exists at all.

What lymphocyte population infiltrates the nerve tissue of HAM/TSP has yet to be determined. Izumo et al.12 studied two cases of HAM and found both CD8+ and CD4+ T cells in the nerve tissue. In their case of two years and six months clinical duration, both CD8+ cells and CD4+ cells had infiltrated the tissue; whereas, CD8+ T cells predominated in the case of nine years duration. They suggested a correlation between the type of lymphocyte population and the duration of the clinical course of HAM. In TSP cases of one year duration and of more than six years duration, predominant CD8+ T cell infiltration has been shown.49 Our study also showed CD8+ T cells in the perivascular cuffs and parenchyma in a HAM case of 9 months duration. The finding of predominant CD8+ cytotoxic T cell infiltration in the absence of viral antigen strongly suggests that the cytotoxic T cells that infiltrate nerve tissue have an important function in the pathology of HAM/TSP. Lymphocytes from HAM/TSP patients show several immunological abnormalities; increased numbers of activated lymphocytes in the peripheral blood as well as spontaneous proliferative responses of cultured peripheral blood lymphocytes.13-15

In conclusion, the predominance of cytotoxic T cell infiltration and the involvement of myelin sheaths are thought to be related to the pathogenesis of HAM/TSP.

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2 Furuzono H, Nakazato O, Geoto, Akizuki S, Okajima T. An autopsy case of HTLV-I associated myelopathy...