

Severe Demyelinating Myelopathy with Low Human T Cell Lymphotropic Virus Type 1 Expression after Transfusion in an Immunosuppressed Patient

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We describe an immunosuppressed patient who developed myelopathy after transfusion with human T cell lymphotropic virus type 1–infected blood products during cardiac transplantation; immunoglobulins and fibrinogen deposition indicated disruption of the blood-brain barrier. The low degree of inflammation and virus expression suggests that demyelination may have been caused by an antibody- and complement-mediated process and by an alteration of the spinal cord microenvironment with activation of microglial cells and astrocytes.

Human T cell leukemia virus type 1 (HTLV-1) causes a variety of diseases, including a neurologic syndrome called “tropical spastic paraparesis” and “HTLV-1–associated myelopathy” (TSP-HAM) [1, 2]. HTLV-1 neuropathogenesis is still not completely understood; viral and host factors, such as the immune response, are thought to be involved in myelin destruction. The low level of HTLV-1 expression *in vivo* suggests that tissue damage is not the result of a direct viral effect but, rather, that it depends on infiltrating cells present in the focal lesions. Anti-Tax–specific cytotoxic T lymphocytes have been implicated in the pathogenesis of TSP-HAM [3]. Demyelination and axonal loss observed in spinal cord lesions could also be the result of bystander damage due to the T cell activation and increased

expression of cytokines [4], or to an autoimmune response, as described for autoimmune reactivity associated with viral-specific IgG [5].

Antibodies and complement have been involved in the pathogenesis of another demyelinating disease, multiple sclerosis. Deposition of complement complexes on endothelial cells in the areas of active myelin destruction and colocalization of immunoglobulins and complement components have been observed, suggesting antibody- and complement-mediated demyelination [6, 7]. To gain new insights into the mechanism of demyelination in a severely immunosuppressed host, we investigated a case of TSP-HAM [8] with rapid development of myelopathy after HTLV-1 infection acquired during cardiac transplantation.

Patient. A 41-year-old white man, who was from an area where HTLV-1 was not endemic and who had no personal or family history of neurologic disease, developed a subacute myelopathy with progressive paraplegia after cardiac transplantation. He showed no sign of graft rejection due to severe immunosuppression [8]. He became infected with HTLV-1 via contaminated blood products and developed signs of TSP-HAM 5 months after transplantation. The patient was not HIV seropositive. Corticosteroid therapy transiently improved the clinical course. Chemotherapy was required for the treatment of a systemic Epstein-Barr virus (EBV) B cell lymphoma and was complicated by bone marrow aplasia and septic syndrome. The patient died 2 years after receiving the transplant.

Paraffin-embedded tissue samples from the spinal cord were stained with hematoxylin and eosin, or Luxol blue and Bodian's silver impregnation for the axons. Frozen sections were processed for immunohistochemistry and *in situ* hybridization (ISH).

Simple and double immunohistochemistry were performed by using the following primary antibodies: antibodies to lymphocyte subsets CD8⁺ and CD4⁺ (Leu-2a and NU-TH/1); Ki-M7 antibodies to the macrophage CD68⁺ marker; antigenic fibrillary acidic protein (GFAP) rabbit polyclonal antibodies; mouse antihuman C3 monoclonal antibody, rabbit antihuman C5 polyclonal antibodies, and mouse antihuman C5b9 monoclonal antibody; antihuman IgG; rabbit antihuman fibrinogen antibodies; and rabbit anti-Tax and TSP-HAM patient IgG. Secondary immunoglobulins were conjugated with horseradish peroxidase or alkaline phosphatase. Sections from the fibularis brevis muscle were examined by immunocytochemistry for CD4⁺, CD8⁺, and CD68⁺ cells.

Apoptosis (DNA fragmentation) was evaluated by use of TdT-mediated dUTP nick end labeling (TUNEL), according to

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the methods of Gavrieli et al. [9]. Dig-dUTP was incorporated by terminal deoxynucleotidyl transferase at 3'-OH ends of DNA breaks, detected by means of a sheep antidigoxigenin antibody coupled with alkaline phosphatase, and revealed with fast-red chromogen. As controls, we used tissue samples obtained from 2 patients who had neither HIV nor HTLV infection.

Simultaneous isolation of DNA and RNA from the same sample was performed by using TRI-Reagent (Euromedex). A *tax* proviral DNA fragment of 340 bp was amplified by PCR. To detect the expression of spliced HTLV-1 *tax* mRNA, cDNA (217 bp) was synthesized as described elsewhere [10]. *tax* DNA or cDNA were detected by Southern blot hybridization with a ³²P-specific oligonucleotide probe located inside the amplified fragment. To check for the integrity of mRNA and the efficacy of reverse-transcriptase PCR (RT-PCR), the glyceraldehyde phosphate dehydrogenase was also amplified in each cDNA sample. Samples in which this housekeeping mRNA could not be amplified were discarded. ISH was performed on serial sections of frozen tissues by means of ³³P antisense and sense riboprobes corresponding to the complete *tax* mRNA, as described elsewhere [11].

Results. The spinal cord showed severe atrophy of the thoracic cord, with involvement of gray and, especially, white matter. Extensive active demyelination lesions and axonal loss were detected throughout the spinal cord via Luxol blue stain and Bodian's silver impregnation (figure 1). Numerous foamy cells were detected in actively demyelinating lesions. Goll and Burdach tracts were particularly damaged in the cervical cord. Pyramidal tracts were severely affected in the thoracic cord. Demyelination was detected even in the absence of massive inflammation. Rare CD4⁺ (figure 2A) and CD8⁺ lymphocytes (data not shown) were detected in the damaged areas. Conversely, increased CD68⁺ expression was detected in large perivascular macrophages and disseminated parenchymal microglial cells (figure 2C). Analysis of several spinal cord sections revealed extensive astrogliosis with increased number of GFAP-immunoreactive cells (figure 2B). Immunoreactivity for complement components (C3, C5, C5b9) was detected in active demyelination lesions (figure 2C, 2D). A faint signal for C5 was detected in the controls, corresponding to postmortem perivascular leakage. Cell death with margination of condensed chromatin and nuclear fragmentation was detected in the areas of active myelin breakdown (figure 2F).

Immunoglobulins from a case of TSP-HAM, as well as rabbit antibodies directed against Tax protein, demonstrated extensive neuronal staining (figure 2G), confirming that Tax-specific antibodies react with a neuronal autoantigen [5]. Negative controls were performed by use of a non-HTLV-1 human serum sample as well as mouse anti-HSV1 and anti-EBV serum samples. No staining was observed with these reagents.

Results of the analysis of a sample obtained from the fibularis brevis muscle revealed large irregularity in the fiber diameter and the presence of atrophic and necrotic fibers. Randomly distributed atrophic myofibers indicate a nonneurogenic pathogenesis. Occasional CD4⁺ cells, CD8⁺ lymphocytes, and numerous CD68⁺ macrophages were observed (figure 2H). HTLV-1 proviral DNA integration and *tax* mRNA expression were found in different fragments of the spinal cord, as indicated by PCR and RT-PCR (figure 3A).

To investigate virus expression within the lesions, ISH was performed on serial frozen sections. An antisense *tax* probe was chosen because all types of HTLV-1 RNAs, genomic as well as single or double spliced, hybridize to this region of the viral genome. This antisense probe was tested on an infected MT2 cell line as a positive control (data not shown). Rare infected cells located in the white matter of the thoracic cord with a specific HTLV-1 *tax* mRNA signal were detected in several frozen sections. Some of them showed a simultaneous signal for GFAP antigen and viral mRNA detection (figure 3B), suggesting that astrocytes could contain *tax* mRNA, as has been reported elsewhere [12]. In adjacent sections, no signal was detected in ISH performed with a sense *tax* probe. No viral mRNA or antigens were observed in the muscle sections.

Human fibrinogen and IgG, 2 serum proteins rarely found in normal CNS parenchyma, were sought in spinal cord sections obtained from control patients and patients with TSP-HAM. Extensive immunoreactivity in the white and gray matter, with positive neuronal and glial signals, was observed only in the patient with TSP-HAM by use of antihuman fibrinogen antibodies (figure 2E). In addition, diffuse extracellular staining was present uniformly throughout such sections. Accumulation of fibrinogen and IgG with immune-reactive neurons and astrocytes indicates blood-brain barrier alteration in this patients with TSP-HAM. In control tissues, faint serum protein immunoreactivity was restricted to intravascular and perivascular areas due to postmortem leakage.

Discussion. The patient that we describe presented with clinical myelopathy with an unusually short incubation after transfusion of HTLV-1-contaminated blood [8]. The short latency period could have been caused by the immunosuppression or a high viral inoculum. In addition, the brief duration of the disease (18 months) allowed the observation of active demyelination process at the time of autopsy; generally, the TSP-HAM tissues are obtained after a long clinical course, when the early disease events may be masked by extensive astrogliosis. Another unusual feature of this case is the restricted T cell infiltration in the spinal cord. Our study indicates that HTLV-1-linked demyelination can occur in the absence of a high perivascular inflammation.

It has been shown that the mechanisms of myelin damage in

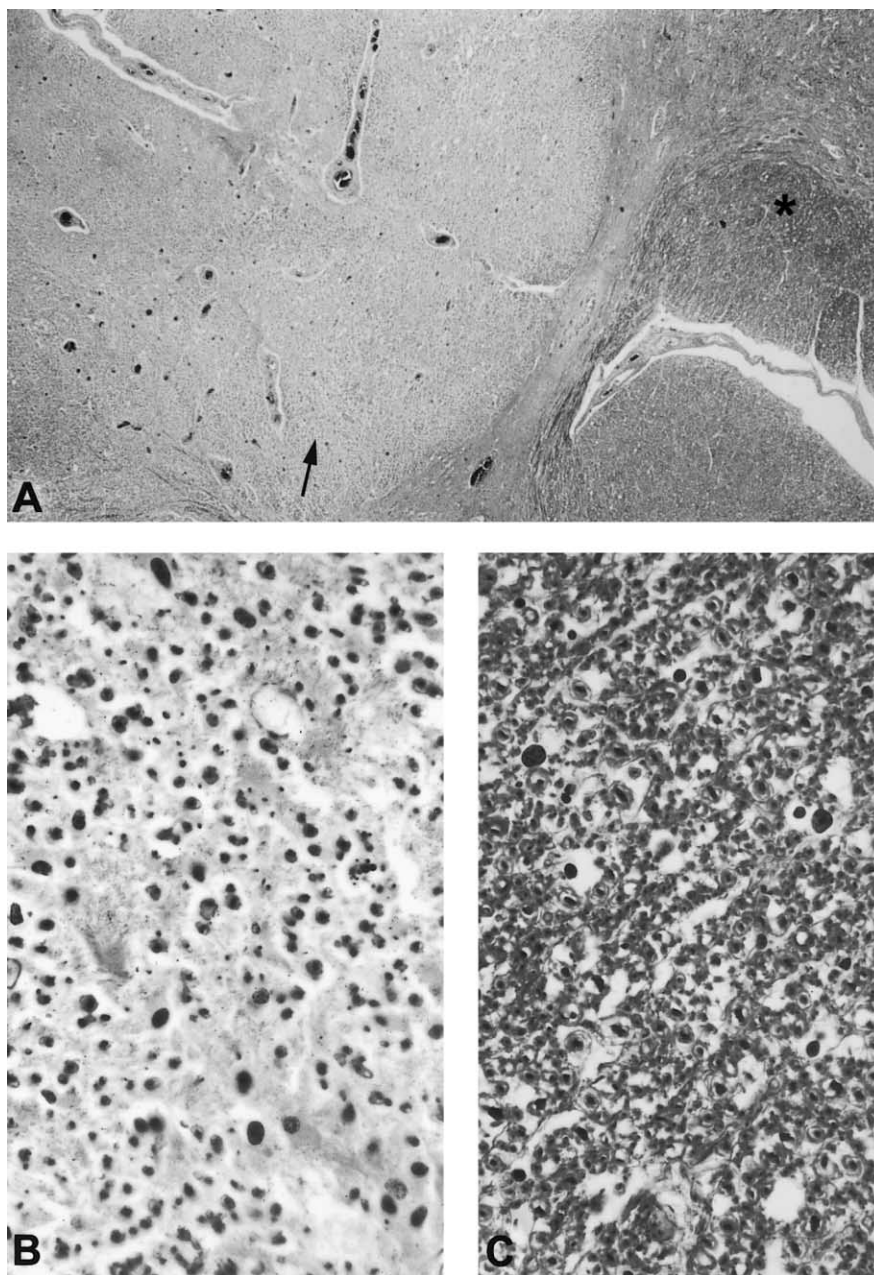


Figure 1. White matter changes in the thoracic spinal cord observed after Luxol blue staining associated with Bodian's silver impregnation. *A*, Histologic examination of transversal sections at low magnification ($\times 120$) showing the presence of a colorless demyelinated area (*arrow*), close to a normal myelin zone (*star*). *B* and *C*, Same areas at higher magnification ($\times 400$). (A full-color version is available in the on-line edition of this report.)

patients with multiple sclerosis may be different in distinct subgroups of patients. Demyelination may proceed in some cases by the deposition of immunoglobulins and complement; other cases suggest that macrophages and endogenous glia (i.e., microglia, astrocytes) play a role as sources of injury mediators [6].

As generally reported for patients with TSP-HAM [11], virus expression in the present case seemed to be low, with the weak *tax* signal detected by ISH indicating the absence of a massive

virus replication. Nevertheless, the direct involvement of viral proteins early in the process cannot be formally excluded. Low level of Tax protein expression could be the initiating event of the myelin damage, particularly if some of the HTLV-1-infected cells are astrocytes. HTLV-1 infection of astrocytes with Tax expression is associated with activation of glial cells. This activation could be responsible of the continuous secretion of neurotoxic mediators, such as TNF- α , IL-1 α , MMP2, and

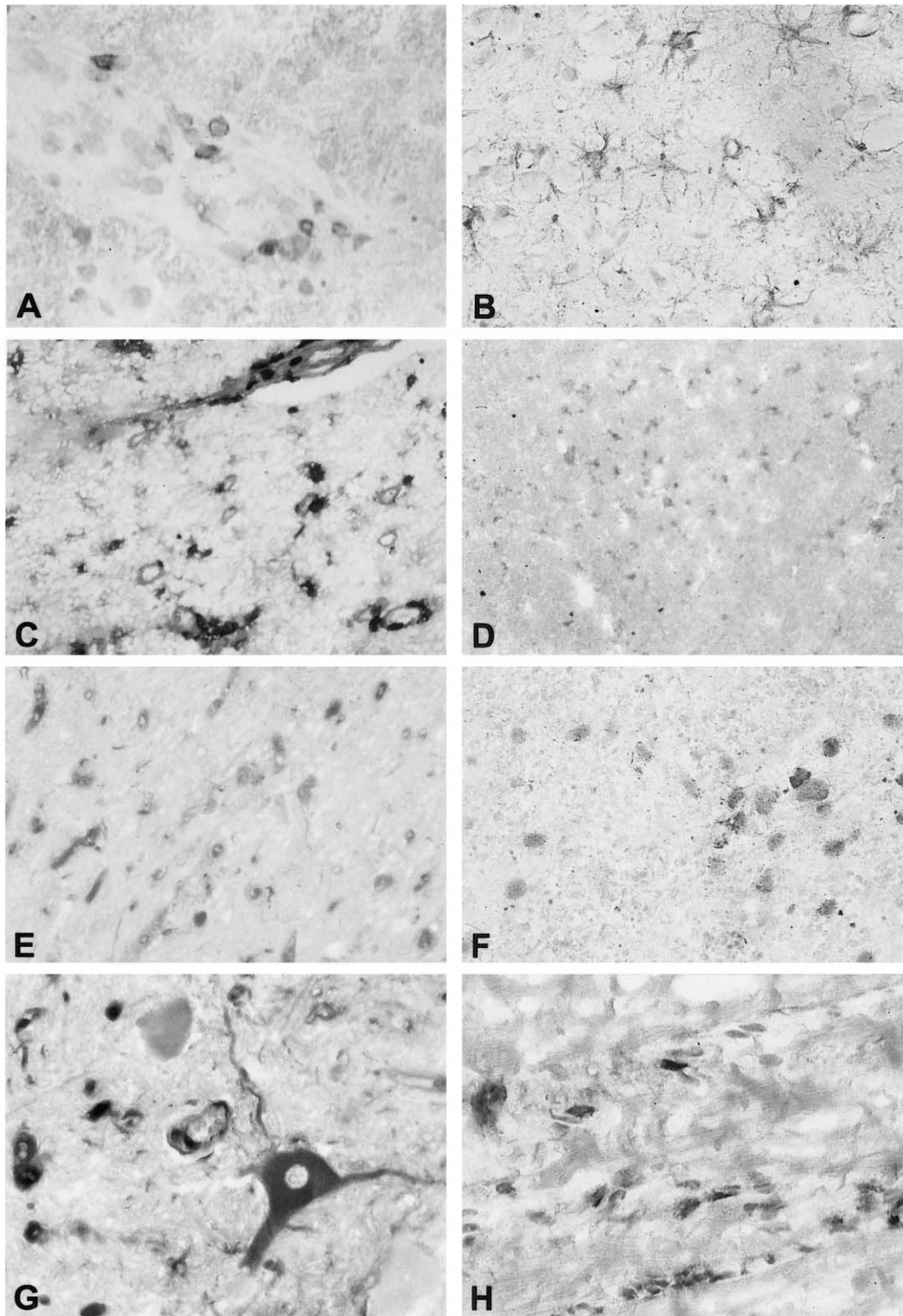


Figure 2. Characterization of inflammatory cells and local activation in thoracic cord by simple and double immunocytochemistry. *A*, T cell subset detection with NU-TH/1 (anti-CD4⁺) primary antibodies. *B*, Astrocytosis in white matter attested by anti-anti-glial fibrillary acidic protein immunostaining. Antirabbit secondary antibodies were conjugated with horseradish peroxidase (HRP). *C*, CD68⁺ detection with KI-M7 primary antibody and alkaline phosphatase (AP)-conjugated secondary antibodies (*blue staining*). High expression with significant increased density of the macrophages/microglial cells was observed. *D*, C5b9 complex detection realized by use of AP conjugate and fast-red chromogen. *E*, Human fibrinogen detection by HRP and diaminobenzidine (DAB) staining. *F*, Numerous cells located at the area of active myelin breakdown, exhibiting increased apoptotic process by use of the TdT-mediated dUTP nick end labeling (TUNEL) method. *G*, Neuron-specific immunoreactivity with anti-Tax rabbit polyclonal antibodies. *H*, CD68⁺ macrophage detection in sections of fibularis brevis muscle. Slides were counterstained with Harris hematoxylin and were mounted in Eukitt or gelatin. Original magnification, $\times 400$. (A full-color version is available in the on-line edition of this report.)

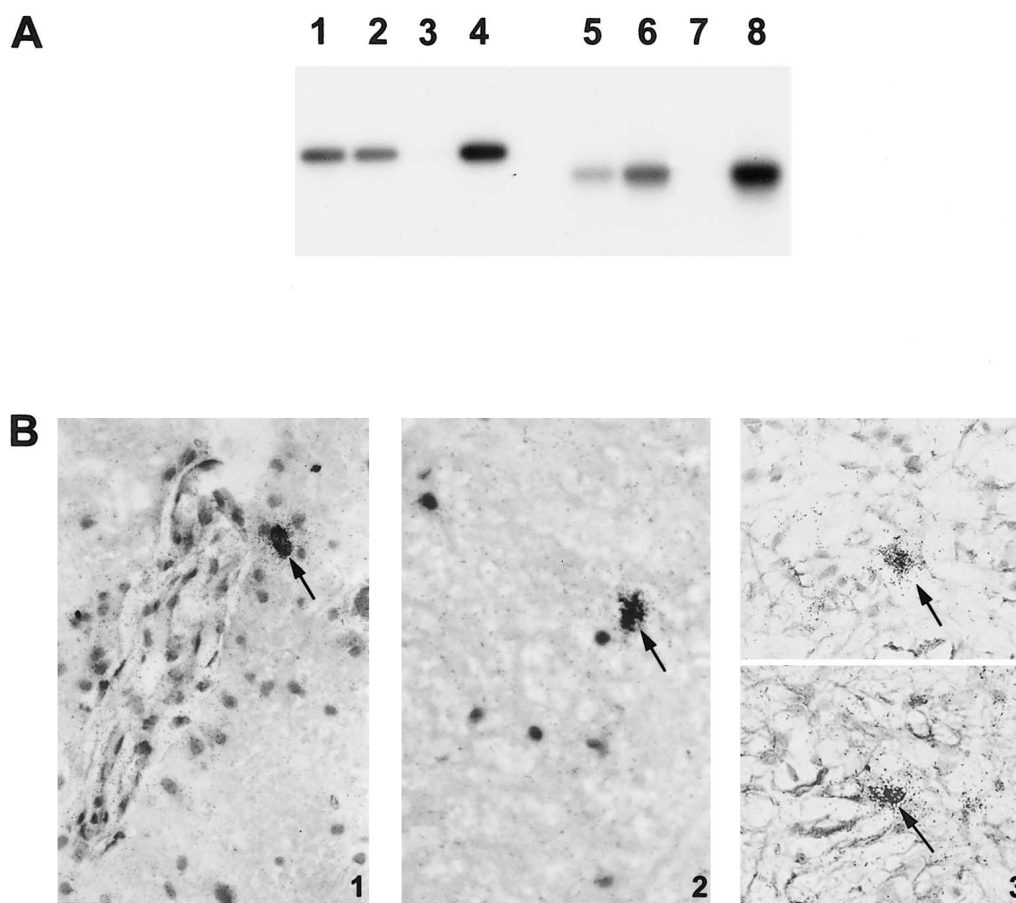


Figure 3. Human T cell leukemia virus type 1 (HTLV-1) integration and expression in the spinal cord. *A*, PCR and reverse-transcriptase PCR (RT-PCR) products, obtained from DNA and total RNA extracted from spinal cord, detected by Southern blot hybridization with a ^{32}P -specific *tax* oligonucleotide internal probe. *Lanes 1 and 2*, PCR amplification obtained with DNA from the cervical and thoracic spinal cord, from CEM cells as a negative control (*lane 3*) and from MT2 cells as a positive control (*lane 4*). *Lanes 5 and 6*, RT-PCR amplification obtained with *tax* cDNA from the cervical and thoracic spinal cord, and negative (*lane 7*) and positive (*lane 8*) controls. *B*, Detection of HTLV-1 mRNA expression by in situ hybridization with a ^{32}P *tax* antisense riboprobes on frozen sections from thoracic cord (panels 1–3). 3, Simultaneous detection of the antigliar fibrillary acidic protein antigen and *tax* mRNA. Exposure time was 21 days. Original magnification, $\times 400$. (A full-color version is available in the on-line edition of this report.)

MMP9. Our data are in agreement with results obtained by Szymocha et al. [13] regarding the effects of HTLV-1 on astrocytes infected in vitro. On the other hand, viral proteins, such as Tax, may induce apoptosis and cause the demyelination process.

In this case, despite the efficacy of the immunosuppressive treatment, as indicated by the absence of graft rejection and the development of an EBV lymphoma, anti-HTLV-1 antibodies were detected in both blood and CSF [8]. Furthermore, immunosuppression might preferentially limit the cellular response, might account for the unusual lack of lymphocytic infiltration, and might give prominence to the immunoglobulin reactivity in this case. This is not applicable to the majority of patients with TSP-HAM, who are immunocompetent. It has been suggested that neuronal molecular mimicry, mediated by the anti-Tax IgG autoimmune reactivity, could play a role in the pathogenesis of TSP-HAM [5]. Our data confirm that the

neurons in the spinal cord sections we studied reacted strongly with TSP-HAM serum, as well as with anti-Tax rabbit antibodies. A compromised barrier may allow the entry of harmful serum constituents, such as autoreactive anti-Tax IgG. High albumin and IgG indexes of the CSF [8], as well as the fibrinogen detection, suggest an injury of the blood-brain barrier. Activation of perivascular macrophages with elevated cytokine expression could account for the blood-brain barrier dysfunction. IL-1 β and TNF- α , which are both increased by Tax [14, 15], could augment the blood-brain barrier permeability by enhancing cerebral endothelial pinocytosis and by altering endothelial tight junctions [16, 17].

It is generally accepted that complement contributes to the tissue damage underlying demyelination in the peripheral nervous system and the CNS [18, 19]. Complement activation in the CSF and brain has been reported in patients with multiple sclerosis [6, 7]. In the case described here, activated comple-

ment constituents were detected, suggesting complement mediated cell lysis. Increased barrier permeability is probably the priming event in active myelin breakdown, as described in patients with AIDS dementia [20].

In conclusion, these observations suggest that, despite low virus expression and little lymphocytic infiltration, demyelination may occur by several overlapping mechanisms, including deposition of antibodies and complement after blood-brain barrier injury; HTLV-1-dependent molecular mimicry due to the anti-Tax IgG reactivity to neuronal antigens; and predominance of activated macrophages/microglia and astrocytes in demyelinated areas.

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References

- Gessain A, Barin E, Vernant JC, et al. Antibodies to human T-lymphotropic virus type 1 in patients with tropical spastic paraparesis. *Lancet* **1985**;2(8452):407–10.
- Osame M, Usuku K, Izumo S, et al. HTLV-1 associated myelopathy, a new clinical entity. *Lancet* **1986**;1(8488):1031–2.
- Jacobson S, Shida H, McFarlin DE, et al. Circulating CD8⁺ cytotoxic T lymphocytes specific for HTLV-1 pX in patients with HTLV-1 associated neurological disease. *Nature* **1990**;348:245–8.
- Umehara F, Izumo S, Ronquillo AT, et al. Cytokine expression in the spinal cord lesions in HTLV-1 associated myelopathy. *J Neuropathol Exp Neurol* **1994**;53:72–7.
- Levin MC, Krichavsky M, Berk J, et al. Neuronal molecular mimicry in immune-mediated neurologic disease. *Ann Neurol* **1998**;44:87–98.
- Lucchinetti C, Brück W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* **2000**;47:707–17.
- Storch MK, Piddlesden S, Haltia M, Iivanainen M, Morgan P, Lassmann H. Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination. *Ann Neurol* **1998**;43:465–71.
- Gout O, Baulac M, Gessain A, et al. Rapid development of myelopathy after HTLV-1 infection acquired by transfusion during cardiac transplantation. *N Engl J Med* **1990**;322:383–8.
- Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol* **1992**;119:493–501.
- Tangy F, Ossondo M, Vernant JC, et al. HTLV-1 expression in salivary glands of infected patients. *J Infect Dis* **1999**;179:497–502.
- Tangy F, Vernant JC, Coscoy L, et al. A search for HTLV-1 in the lesions of patients with TSP and polymyositis. *Ann Neurol* **1995**;38:454–60.
- Lehki T, Fox CH, Koenig S, et al. Detection of HTLV-1 Tax RNA in the CNS of HAM/TSP patients by ISH. *Ann Neurol* **1995**;37:167–75.
- Szymocha R, Brisson C, Bernard A, et al. Long-term effects of HTLV-1 on brain astrocytes: sustained expression of Tax-1 associated with synthesis of inflammatory mediators. *J Neurovirol* **2000**;6:350–7.
- Dhib-Jalbut S, Hoffman PM, Yamabe T, et al. Extracellular human T-cell lymphotropic virus type I Tax protein induces cytokine production in adult human microglial cells. *Ann Neurol* **1994**;36:787–90.
- Nakamura S, Nagano I, Yoshioka M, et al. Detection of tumor necrosis factor-alpha-positive cells in CSF of patients with HTLV-1 associated myelopathy. *J Neuroimmunol* **1993**;42:223–6.
- Quagliarello VJ, Wispelwey B, Long WJ Jr, Scheld WM. Recombinant human interleukin-1 induces meningitis and blood-brain barrier injury in the rat: characterisation and comparison with tumor necrosis factor. *J Clin Invest* **1991**;87:1360–6.
- Ramilo O, Saez-Llorens X, Mertsola J, et al. Tumor necrosis factor alpha/cachectin and interleukin 1 beta initiate meningeal inflammation. *J Exp Med* **1990**;172:497–507.
- Morgan BP, Gasque P. Expression of complement in the brain: role in health and disease. *Immunol Today* **1996**;17:461–6.
- Morgan BP, Gasque P, Sanghrao S, Piddlesden S. The role of complement in disorders of the nervous system. *Immunopharmacology* **1997**;38:43–50.
- Power C, Kong P-A, Crawford TO, et al. Cerebral white matter changes in acquired immunodeficiency syndrome dementia: alteration of the blood-brain barrier. *Ann Neurol* **1993**;34:339–50.