Pathologic Prion Protein Spreading in the Peripheral Nervous System of a Patient With Sporadic Creutzfeldt-Jakob Disease

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Background: Involvement of the peripheral nervous system in the pathogenesis of prion diseases is becoming increasingly evident. However, pathologic protease-resistant prion protein deposition in the peripheral nerves of patients with Creutzfeldt-Jakob disease has never been demonstrated, to our knowledge.

Objective: To determine whether mutated prion protein accumulation could be shown in the peripheral nervous system of patients with sporadic Creutzfeldt-Jakob disease.

Design: Autopsy study.

Patients: Three patients with sporadic Creutzfeldt-Jakob disease.

Interventions: Study of the brain, spinal cord, and sciatic and superficial peroneal nerves by immunohistochemistry and Western blot analysis.

Main Outcome Measure: Demonstration of protease-resistant prion protein accumulation.

Results: In all cases, protease-resistant prion protein accumulation was found in the brain and posterior horns of the spinal cord. In 1 case, protease-resistant prion protein deposits were also evidenced in the dorsal root ganglia and the superficial peroneal nerve.

Conclusions: Protease-resistant prion protein may be found in the peripheral nervous system of some patients with sporadic Creutzfeldt-Jakob disease. However, a larger series is required to assess the incidence of peripheral nervous system involvement and to discuss the diagnostic usefulness of peripheral nerve biopsy in sporadic Creutzfeldt-Jakob disease.

Arch Neurol. 2004;61:747-750

CREUTZFELDT-JAKOB DISEASE (CJD) is a neurodegenerative disorder in which host cellular prion protein (PrPc) is conformationally changed into protease-resistant isoform (PrPsc).1 Neuropathologic features are mainly severe neuronal loss and reactive astrogliosis with spongiform change in the cortical and subcortical gray matter. Among the different types of human CJD, sporadic cases account for about 85%.2 Clinical symptoms of peripheral nerve damage have been reported in some patients with either sporadic3,4 or hereditary5,6 CJD. Immunohistochemistry showed prion protein (PrP) accumulation involving the posterior roots in experimental scrapie7 and in one patient with sporadic CJD.8 However, pathologic PrPsc deposition in the peripheral nervous system (PNS) of humans has never been confirmed by Western blot analysis, a method that is essential to distinguish PrPsc from PrPc. The aim of this study was to determine whether mutated PrP accumulation could be demonstrated in the PNS of patients with sporadic CJD.

METHODS

CASE MATERIAL

Three autopsy cases of sporadic CJD were studied. Three cases without neurologic disease were taken as negative controls. Clinical and histologic data concerning the sporadic CJD cases are summarized in Table 1.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

Histologic studies and immunohistochemistry were performed on 10% formalin-fixed, paraffin-embedded specimens from frontal, occipital, and cerebellar cortex; spinal cord; dorsal root ganglia; and sciatic and superficial peroneal nerves. Hematoxylin-eosin staining was performed to evaluate spongiosis and neuronal loss. Astrocytic gliosis was studied by anti-gliial fibrillary acidic protein immunohisto-
chemistry. Prion protein labeling was carried out with 3F4 and 12F10 monoclonal antibodies (1:2000) after protease K digestion (20°C, 10 minutes) and guanidine thiocyanate treatment (4M, 4°C, 2 hours); counterstaining was done with Mayer hemalum. To avoid misinterpretation, controls for each anatomic site were performed.

WESTERN BLOT ANALYSIS

Frozen specimens of frontal, occipital, and cerebellar cortex, as well as spinal cord and dorsal root ganglia, were homogenized at 10% (wt/vol) in a 5% glucose solution. Peripheral nerve homogenates were obtained by impact grinding method in a liquid nitrogen bath resulting in a fine powder of tissue. Homogenates were forced through a 0.5-mm-diameter needle before protease K treatment. Protease K digestion and Western blot process were performed as previously described.

RESULTS

Histopathologic examination of the 3 sporadic CJD cases showed marked spongiosis in the brain, whereas the spinal cord, dorsal root ganglia, and peripheral nerves appeared unmodified. In the 3 cases, neuronal loss was marked in the cerebral cortex, mild in the cerebellum, and absent in the spinal cord and the dorsal root ganglia. Gliosis was marked in the cerebral cortex, mild in the cerebellum and the spinal cord, and absent in the dorsal root ganglia.

Results of PrP immunohistochemistry and Western blot analyses are summarized in Table 2. In the 3 sporadic CJD cases, PrP immunohistochemistry showed granular deposits in the cerebral cortex as well as in the molecular and granular layers of the cerebellum. In the 3 cases, a fine granular staining was found in the spinal cord gray matter from cervical to lumbar level. This PrP accumulation was more marked in the posterior horns (Figure 1A). In the 3 control cases, specimens from the same anatomic sites exhibited no PrP labeling. In case 1, examination of the dorsal root ganglia showed positive PrP immunostaining, distinct from lipofuscin accumulation, along nerve fibers (Figure 1B). Prion protein–positive linear deposits were also present along nerve fibers in the posterior roots and the superficial peroneal nerve (Figure 1C). On transverse sections, these PrP deposits appeared to be likely located in the Schwann cells of both myelinated and unmyelinated fibers (Figure 1C, inset). In this case, no PrP labeling was found on the sciatic nerve.

The PrP immunohistochemistry was negative in the PNS specimens from control cases (Figure 1D), confirming that the PrP labeling observed in case 1 was not a background staining.

In all sporadic CJD cases, Western blot analysis demonstrated that the PrP immunoreactivity observed on the brain and spinal cord paraffin sections was related to protease K–resistant PrP (PrPsc) accumulation (Table 2 and Figure 2). Brain and spinal cord specimens from control cases contained physiologic protease K–sensitive PrP (PrPc) (Table 2). In case 1, Western blot analysis confirmed that the PrP immunostaining observed in the spinal ganglia and the superficial peroneal nerve corresponded to PrPsc deposits (Figure 3), whereas PNS specimens from cases 2 and 3, as well as controls, contained PrPc (Figure 3). In accordance with immunohistochemistry findings, PrPc was detected in the sciatic nerve in case 1. For all the patients with sporadic CJD and in all tested areas, the electrophoretic mobility and the ratio of the 3 glycosylated PrPsc isoforms were consistent with PrPsc type 1 according to classification by Parchi et al.

COMMENT

Involvement of both central nervous system and PNS in the pathogenesis of transmissible spongiform encephalopathy suggests a common mechanism of PrP accumulation.
lopathies is becoming increasingly evident. Deposits of PrP have been shown, by immunohistochemistry, in the spinal cord from patients with either sporadic or growth hormone–associated CJD. Such PrP immunolabeling was also seen in 2 cases of Gerstmann-Straussler-Scheinker (GSS) syndrome associated with a missense mutation (proline to leucine) at codon 102 of the PrP gene and in new-variant CJD. Deposits of PrP in the posterior roots were reported in only 1 patient with GSS syndrome and 1 with CJD. A recent study demonstrated, by immunohistochemistry and Western blot analysis, PrPsc deposits in the olfactory cilia and central olfactory pathway in 9 patients with sporadic CJD. Several experimental studies have also demonstrated involvement of the PNS in the pathogenesis of prion diseases. Tissue-specific expression of PrPc in transgenic mice showed that PrPc-positive peripheral nerves are necessary and sufficient for successful infection of the brain after oral or intraperitoneal infection with scrapie. Glatzel and Aguzzi demonstrated that transgenic mice over-expressing PrPc undergo rapid PrPsc neuroinvasion on intraneurinoculation of prions. A transgenic mouse and

Figure 1. A-C, Prion protein immunostaining in patient 1. A, Spinal cord showing fine granular deposits in the posterior horn (immunoperoxidase, original magnification ×20). B, Dorsal root ganglion exhibiting linear prion protein deposits along nerve fibers (lipofuscin deposits are visible in some neuron cytoplasms) (immunoperoxidase, original magnification ×200). C, Superficial peroneal nerve demonstrating prion protein linear deposits along nerve fibers. Inset, Transverse section showing deposits likely located in the Schwann cells of myelinated and unmyelinated fibers (immunoperoxidase, original magnification ×200 [inset, ×400]). D, Absence of prion protein immunolabeling in the superficial peroneal nerve from a control subject (immunoperoxidase, original magnification ×200).

Figure 2. Western blot analysis of prion protein in homogenates of frontal (F), temporal (T), occipital cortex (O), cerebellum (C), spinal cord (S), and dorsal root ganglia (G) from patient 1. Type 1 (T1) and type 2 (T2) sporadic Creutzfeldt-Jakob disease were used as positive controls. In all tested areas, type 1 protease-resistant prion protein was found. MW indicates molecular weight.
culture cell study established that PrPc could be expressed by Schwann cells in vivo and that PrPSc could be replicated in Schwann cell line.17

In our study, we found evidence, for the first time in the literature to our knowledge, of PrPsc deposits in the dorsal root ganglia and the superficial peroneal nerve from 1 of 3 patients with sporadic CJD. Immunohistochemistry permitted us to localize PrPsc accumulation likely to Schwann cells of both myelinated and unmyelinated nerve fibers. A suggested explanation for the discrepancy between the consistent immunolabeling observed in experimental models and the less frequent positivity in human prion disease is centrifugal (experimental scrapie) vs centripetal (sporadic and genetic human prion diseases) spreading of PrPsc, resulting in different patterns and amounts of PrPsc accumulation in the PNS. Thus, the absence of PrPsc detection in the sciatic nerve of patient 1 and in all PNS specimens from patients 2 and 3 might be related to the very small amounts of PrPsc accumulated in these tissues and to a lack of sensitivity of our methods. The use of protein misfolding cyclic amplification procedure18 in Western blot analysis of PNS specimens might be helpful in such cases. Although our series is not large enough to draw firm conclusions, the specificity of PrPsc testing in the PNS appears to be fair, because our 3 control cases showed negative results. Nevertheless, the sensitivity of our techniques needs to be improved, because 1 positive CJD case of 3 is still insufficient to suggest peripheral nerve biopsy as a useful tool in the diagnosis of CJD.

Accepted for publication December 3, 2003.

Author contributions: Study concept and design (Drs Favereaux, Perret-Liaudet, Petry, and A. Vital); acquisition of data (Drs Favereaux, Quadrio, Perret-Liaudet, Anne, Laplanche, and A. Vital); analysis and interpretation of data (Drs Favereaux, Quadrio, C. Vital, Perret-Liaudet, and A. Vital); drafting of the manuscript (Drs Favereaux and A. Vital); critical revision of the manuscript for important intellectual content (Drs Quadrio, C. Vital, Perret-Liaudet, Anne, Laplanche, Petry, and A. Vital); obtained funding (Dr Petry); administrative, technical, and material support (Drs C. Vital, Perret-Liaudet, Laplanche, Petry, and A. Vital); study supervision (Dr A. Vital).

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REFERENCES