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Prion Protein Immunohistochemistry in Creutzfeldt-Jakob Disease*

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Creutzfeldt-Jakob disease is a transmissible spongiform encephalopathy characterized clinically by dementia, myoclonus and, in some cases, periodic triphasic EEG-patterns. Neuropathologically the main features are spongiform change, astrocytosis, neuronal cell loss and, in a small percent of cases, amyloid plaques. Prion protein immunohistochemistry is used for definitive diagnosis of these diseases. In our study we present different immunostaining patterns in light microscopy using anti prion protein, and with immunogold labelling for ultrastructural localization of prion protein. Our results demonstrate the clinicopathological heterogeneity of Creutzfeldt-Jakob disease and reveal the role of the endosomal-lysosomal system in the pathogenesis. (Pathology Oncology Research Vol 3, No 3, 193–197, 1997)

Key words: Creutzfeldt-Jakob disease, prion protein, immunohistochemistry

Introduction

Creutzfeldt-Jakob disease (CJD) is a transmissible spongiform encephalopathy (TSE), also referred to as a prion disease, since an abnormal protease-resistant isoform (PrP^{res}) of the host-encoded prion protein (PrP) accumulates predominantly in the nervous system in these diseases.[14]

The human prion protein is a 253 amino acid sialoglycoprotein encoded by a cellular gene mapped to chromosome 20.[15] Its function is still to be determined, although some data provide evidence of it having a role in the circadian rhythm and sleep, in the normal synaptic function and muscle physiology.[8] Human prion diseases can be acquired (eg. kuru and iatrogenic CJD), idiopathic (eg. sporadic CJD), and inherited (eg. familial CJD, Fatal Familial Insomnia /FFI/, Gerstmann–Straussler–Scheinker syndrome /GSS/, and atypical prion dementia).[15]

The classical presentation of CJD is characterized clinically by progressive dementia, myoclonus and in some cases periodic sharp wave complexes with triphasic components on the EEG.[15] Neuropathologically the main features are spongiosity predominantly in the cortex, astrocytosis, neuronal cell loss and, in a small percent of cases, amyloid plaques.[9,12] Immunohistochemistry with anti-prion protein antibody reveal distinct staining patterns, and give the opportunity for definitive diagnosis.[1,2]

In this study our aim was to demonstrate the different prion protein immunostaining patterns in Creutzfeldt-Jakob disease.

Materials and Methods

Case selection and preparation of brain tissue

Four cases were selected from the brain bank of the Hungarian National Institute for Psychiatry and Neurology (Dr. Katalin Majtényi), and one from the CJD Surveillance Unit in Edinburgh, U.K. (courtesy of Dr. James W Ironside).

Light microscopic immunohistochemistry

After autopsy, blocks were fixed by immersion in 10% formalin. Blocks from the frontal and occipital cortex, basal ganglia, and cerebellum were decontaminated

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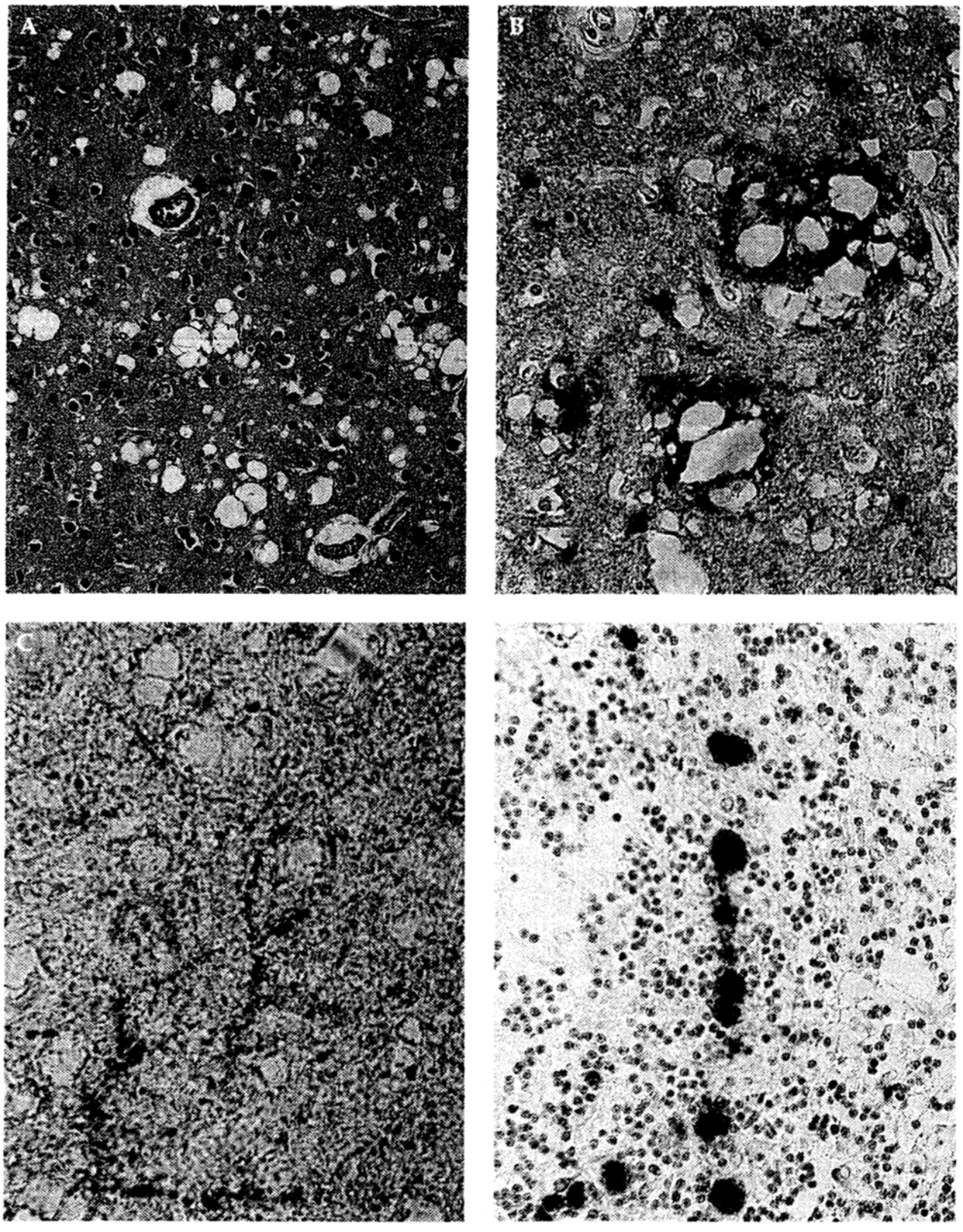

Figure 1. *Light microscopy of CJD brain tissue. Routine H-E staining showing spongiform change throughout the cortex (A). Prion protein immunohistochemistry reveals perivacuolar granular positivity (B), 'dot-like' diffuse and perineuronal staining (C), and so-called 'kuru-type' plaques (D). (A:x125; B,C,D:x250)*

in 96% formic acid for 1 hour, prior to embedding in paraffin wax. From each block 5 μm serial sections were floated on Vectabond-coated slides. The dewaxed sections were taken to water and immersed in 3% hydrogen peroxide in methanol for 30 min. After microwave and pretreatment with 96% formic acid for 5 min and 3M guanidine-thyocyanate for 2h, the sections were incubated in appropriate blocking serum followed by primary antisera (KG9-monoclonal, 1:200 dilution, provided by C. Birkett – Compton, UK, and 3F4-monoclonal, 1:2000 dilution, source: R. Kascsak – New York, USA). The immunolabelling in sections were visualised using avidin-biotin-peroxidase complex and diaminobenzidine (DAB) in a routine manner. After counterstaining with hematoxylin, sections were dehydrated in xylene and mounted in Pertex.

Immunolabelling for electron microscopy

Brain specimens obtained from the frontal cortex were fixed in a mixture of 3% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 1% sucrose and 2 mM $CaCl_2$ (pH7.4). The samples were postfixed in 0.5% osmium, block-stained with 2.5% uranyl acetate, dehydrated in a graded series of ethanol and embedded in araldite (TAAB Laboratories, Reading, U.K.)

Immunogold labelling for PrP was performed on 80–100 nm ultrathin sections with a three step biotin anti-biotin-gold conjugate method, as previously described.[10] The partially rehydrated sections were incubated with proteinase K for 1 h at room temperature, and treated with the guanidine enhancement method[16] prior to immunostaining for PrP^{res} Sections were counterstained with uranyl acetate and lead citrate and investigated with a Philips 410 microscope.

Results

Clinically the patients presented with decline in cognitive functions, and later developed gait disturbances and myoclonus; the EEG showed periodic sharp wave complexes. Routine histopathology revealed spongiform changes throughout the cortex. (*Fig. 1A*)

We observed large confluent vacuoles only, but other type of vacuolation has also been described elsewhere. This latter is called microcystic vacuolation, since small, discrete holes are scattered in the neuropil.[9] In all cases, intense astrocytosis was seen using GFAP (glial fibrillar acidic protein) immunohistochemistry; the astrocytes showed both hyperplasia and hypertrophy. (The application of Gallyas glia impregnation is equally informative.[12])

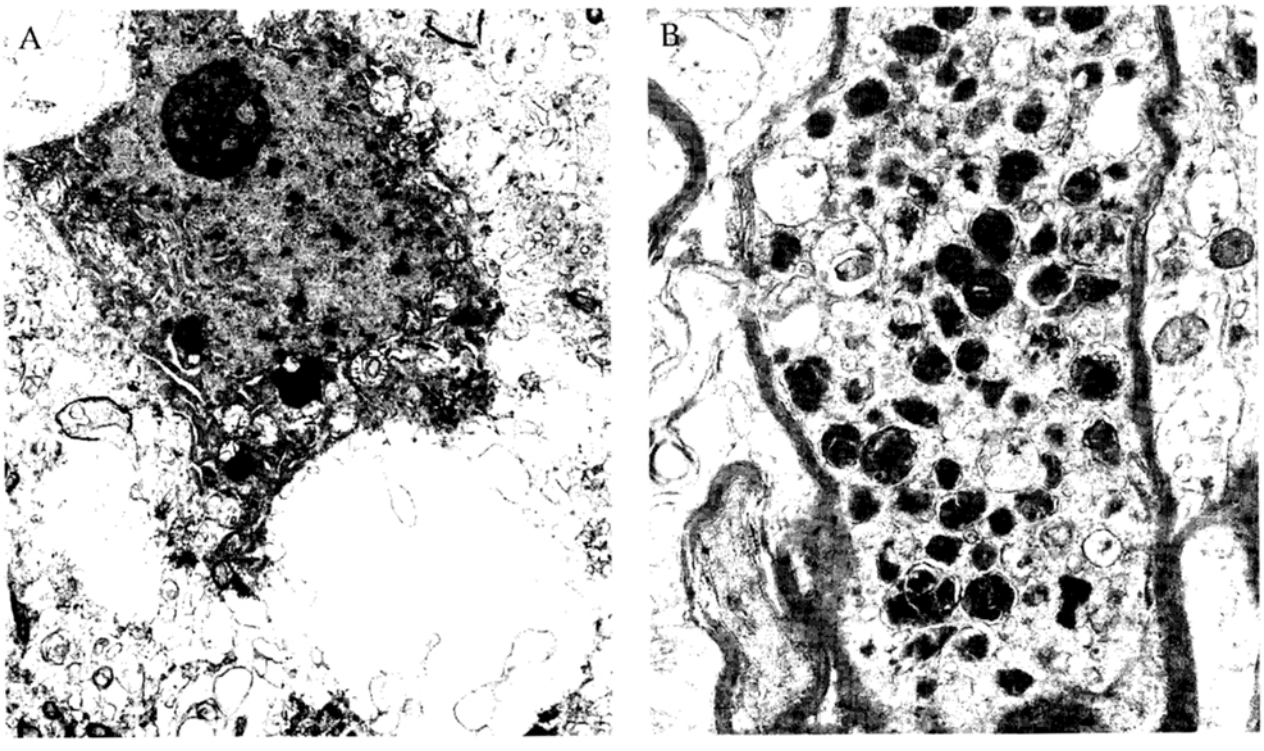

Figure 2. *Electron micrographs representing pathological changes caused by prion infection. Several spongiform vacuoles (A) and dystrophic neurites containing multilamellar, multivesicular dense bodies and abnormal mitochondria (B) are visible in the cerebral cortex of CJD brain. (A: x16,200, B: x32,000)*

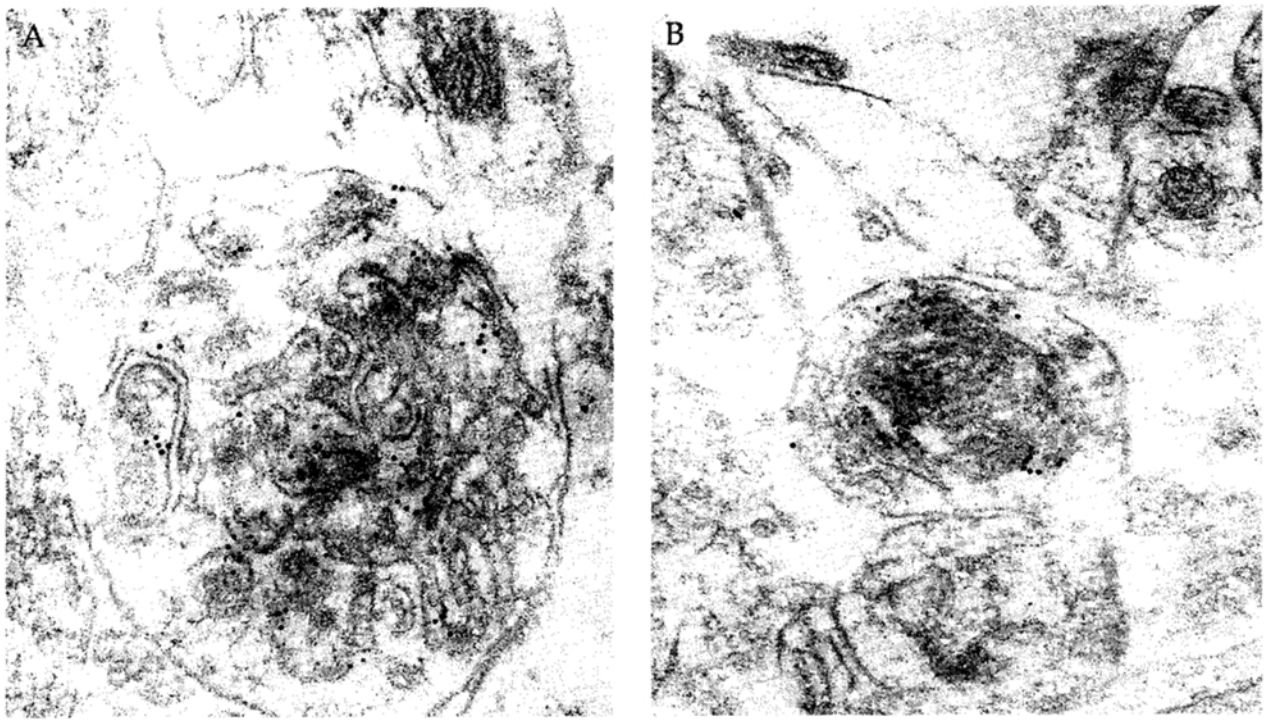

***Figure** 3. Immunogold electron micrographs demonstrating the ultrastructural localization of prion protein in CJD brain. Accumulation of gold particles specific for PrPres can be seen in tubulo-vesicular endosomal structures (A) and lysosome-related dense bodies (B) within neuronal processes. (A,B: x72,000)*

Patterns of light microscopic immunostaining for PrP

Perivacuolar staining – Granular positivity can be seen around and among the confluent vacuoles (*Fig. 1B*).

Diffuse/synaptic labelling – Generalised staining pattern in the neuropil. Small dot-like deposits are visible (*Fig. 1C*).

Neuronal staining – Pericellular punctate or granular positivity can be observed around unstained neuronal perikarya. Occasionally adjacent linear granular deposits are visible along what is thought to be cell processes (*Fig. 1C*). The diffuse/synaptic and the neuronal staining are usually presented together.

Immunoreactive plaques – So called "kuru-type" plaques can be seen in cerebellar cortex. These plaques comprised a homogenous center with a fibrillary structure radiating away from the center, and surrounded by a pale peripheral region or "halo" (*Fig. 1D*).

Ultrastructural findings

A great number of membrane-bound spongiform vacuoles containing membrane whirls and amorphous material are visible in the cerebral cortex (*Fig. 2A*). In some cortical neurons increased amount of lipofuscin granules and a few giant autophagic vacuoles can be detected. Dystrophic neurites filled with electron-dense bodies, multivesicular and multilamellar bodies and abnormal mitochondria are also observed (*Fig. 2B*).

Immunogold electronmicroscopy reveals the ultrastructural distribution of PrP in CJD-brain samples. Clusters of PrP-specific gold particles can be seen predominantly at and/or adjacent to the membranes surrounding spongiform lesions and in some endosome/lysosome related tubulovesicular structures and multivesicular dense bodies in cell processes.(*Fig. 3A,B*)

Discussion

In this study we presented four types of prion protein immunostaining pattern in CJD. Prion protein immunostaining is essential for definitive diagnosis as recently published diagnostic criteria advise.[3] Current findings suggest that a disorder of the normal cellular prion protein PrP^{c}("c" for cellular) is central in the etiology and pathogenesis of human and animal transmissible spongiform encephalopathies. PrP^{C} is a 33–35 kDa cell surface protein which is completely degraded by proteolysis in normal cells. In prion diseases an abnormal 27–30 kDa protease resistant form of prion protein accumulates (PrP^{res}, "res" for resistant) and possibly induces pathologic changes.[7] Formation of PrP^{res} is probably a multistep process. Firstly, a conformational change of alpha-helices to beta-pleated sheets is thought to happen (the exact mechanism

and the cellular site of this phenomenon is still debated). After this, the beta-pleated form undergoes a limited proteolysis, and accummulates in the acidic endosomal-lysosomal compartment.[4,10,14]

The different immunostaining patterns may represent the diverse pathological aspects of prion diseases, and give clues for the understanding of pathogenesis. Not only do the different clinical syndromes have various immunostaining patterns (as GSS, FFI, etc.), but Creutzfeldt-Jakob disease itself shows heterogeneity.[7,11] One reason for this variability must be a Methionine(M)-Valine(V) polymorphism at the 129 codon in the prion protein gene. The normal population in the United Kingdom has a frequency of 37% MM, 51%MV and 12%VV. Analysis of sporadic CJD cases showed 64.5% MM, 11% MV, 24.5% VV in the U.K., whilst in iatrogenic (growth hormone recipients) CJD significant association with valine homozigosity was revealed.[5,18] Studies on the molecular basis of phenotypic variability in CJD showed that four distinct bands of protease resistant PrP could be distinguished on Western blots of different CJD cases. According to these findings the creation of four different clinicopathological groups were recommended.[6,13] (In our cases genetical analysis could not be performed.)

The exact correlation between the different clinicopathological groups of CJD and the perivacuolar, diffuse/synaptic and neuronal immunostaining patterns of PrP is still to be determined. However, PrP^{res}-positive plaques are the major distinctive features for GSS and the iatrogenic and familiar forms of CJD, and can be observed in 15% of sporadic CJD cases.

Whereas the multicentric plaques are specific for GSS, in the CJD cases the "kuru"-type plaques are characteristic.[7] Finally, the recently described new variant CJD found in the U.K. and France differs from the "classical" sporadic CJD both clinically and neuropathologically. So-called "florid" plaques – plaques surrounded by spongiform vacuoles – are the specific hallmarks of these cases.[17]

The fine ultrastructural analysis of CJD brain tissue sections suggests that beside spongiform vacuolation, endosome-lysosome related multivesicular bodies and tubulo-vesicular structures are present in abnormally high numbers in neuronal cell processes. Light microscopic immunohistochemistry shows punctate and granular accummulations of PrP^{res} in these samples ("pericellular" staining pattern). Immunogold electron microscopy reveals that most of these PrP-positive structures correspond to the endosome-related tubular and multivesicular bodies. These observations are consistent with the previous findings that the endosomal-lysosomal system may play an important role in the processing and/or the pathogenesis of PrP^{res} in prion diseases.[2,4,10]

It is important to mention that one of the samples investigated by us was embedded in paraffin more than 30 years ago. The fact that the light microscopic immunostaining for PrP was successful in this sample gives the chance for retrospective confirmation of probable CJD cases through the immunohistochemical analysis of archive blocks.

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