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Species-specificity of a Panel of Prion Protein Antibodies for the Immunohistochemical Study of Animal and Human Prion Diseases

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Summary

Monoclonal antibodies to the prion protein (PrP) have been of critical importance in the neuropathological characterization of PrP-related disease in men and animals. To determine the influence of species-specific amino-acid substitutions recognized by monoclonal antibodies, and to investigate the immunohistochemical reactivity of the latter, analyses were carried out on brain sections of cattle with bovine spongiform encephalopathy, sheep with scrapie, mice infected with scrapie, and human beings with Creutzfeldt-Jakob disease (CJD) or Gerstmann-Sträussler-Sheinker disease (GSS). Immunoreactivity varied between the antibodies, probably as the result of differences in the amino-acid sequence of the prion protein in the various species. Some monoclonal antibodies against mouse recombinant PrP gave strong signals with bovine, ovine and human PrP^{Sc}, in addition to murine PrP^{Sc}, even though the amino-acid sequences determined by the antibody epitope are not fully identical with the amino-acid sequences proper to the species. On the other hand, in certain regions of the PrP sequence, when the species-specificity of the antibodies is defined by one amino-acid substitution, the antibodies revealed no reactivity with other animal species. In the region corresponding to positions 134–159 of murine PrP, immunohistochemical reactivity or species-specificity recognized by the antibodies may be determined by one amino acid corresponding to position 144 of murine PrP. Not all epitopes recognized by a monoclonal antibody play an important role in antigen–antibody reactions in immunohistochemistry. The presence of the core epitope is therefore vital in understanding antibody binding ability.

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Introduction

Sheep and goat scrapie, bovine spongiform encephalopathy (BSE), and Creutzfeldt-Jakob disease (CJD) (sporadic, iatrogenic, familial and variant forms), Gerstmann-Sträussler-Sheinker disease or syndrome (GSS) and Kuru disease in man are all referred to as prion diseases. A common feature of these diseases is the accumulation of abnormal proteinase-resistant prion protein (PrP^{Sc}), an isoform of the cellular proteinase-sensitive prion protein (PrP^{C}), which occurs as a result of post-translational modification leading to increases in the population of β-sheet conformation in the brain (Prusiner, 1998).

Monoclonal antibodies raised against the prion protein (PrP) have been of critical importance in the neuropathological characterization of PrP-related disease in man and animals (Bodemer, 1999). Numerous monoclonal antibodies (mAbs) for detecting prion proteins in tissue sections have been developed and characterized

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in terms of species-specificity and epitope (Kascsak *et al.*, 1987; Bolton *et al.*, 1991; Piccardo *et al.*, 1998; Zanusso *et al.*, 1998; Van Everbroeck *et al.*, 1999a; Privat *et al.*, 2000). For example, mAb 3F4, whose epitope is mapped between amino acids 109 and 112 of the human prion protein, has been widely used in immunohistochemistry and immunoblotting experiments in human prion disease (Kascsak *et al.*, 1987; Zanusso *et al.*, 1998). This antibody detects the prion protein in man and the hamster but not in the mouse, cow, sheep, Capuchin monkey or squirrel (Zanusso *et al.*, 1998). On the other hand, mAb F89/160.1.5, raised against a synthetic peptide and representing residues 146 to 159 of the bovine prion protein, reacts with the prion protein in human, ovine and bovine tissue (O'Rourke *et al.*, 1998; Van Everbroeck *et al.*, 1999a). Although the prion protein is highly conserved, there is some sequence divergence among species. Possibly a single amino-acid substitution affects epitope recognition by mAb 3F4, in the case of mAb F89/160.1.5 it is possible that the amino-acid sequence recognized is based on the epitope conserved in man and ruminant species.

To determine the effects of species-specific amino-acid substitutions, immunohistochemical analysis with a panel of monoclonal antibodies was undertaken on sections of brain tissue from BSE-infected cattle, scrapie-infected sheep, scrapie-infected mice, and human CJD and GSS cases.

Materials and Methods

Samples

Immunohistochemical analysis was carried out on the following brain tissues, cut coronally at the appropriate level: hippocampus and thalamus from two ICR mice inoculated intracerebrally with the Obihiro strain of scrapie (Shinagawa *et al.*, 1985) and from two negative control mice; medulla oblongata at the level of the obex and spinal cord from three scrapie-affected and two negative control sheep and from three BSE-affected and two control cattle. The disease status of the cattle and sheep was established by histological, immunohistochemical, and Western blot methodology. These samples were fixed in 15% formalin for 48–72 h and embedded in paraffin wax by conventional methods. Tissue blocks containing BSE-affected tissue were treated with 98% formic acid for 1 h to reduce the risk of prion infectivity.

In addition, human post-mortem brain samples were obtained from one patient with sporadic CJD (sCJD) (63-year-old male; codon 129M/M, codon 219E/E) and one with GSS (57-year-old male with PrP P105L mutation; codon 129Val/Val, codon 219E/E). These samples had been fixed in 15% formalin, after which the fixed blocks were immersed in 98% formic acid for 1 h and embedded in paraffin wax. A few tissue sections, including those of the cerebral cortex, prepared from these blocks, were submitted to our laboratory.

Immunohistochemistry (IHC)

Serial tissue sections (4 µm) were placed on silane-coated glass slides (Muto Purechemicals, Tokyo, Japan). After dewaxing, endogenous peroxidases were blocked by incubation in 3% H_2O_2 for 5 min. Six different pretreatment procedures were used, as follows. (1) Pretreatment "FA" (98% formic acid for 5 min). (2) Pretreatment "121DWHA" (hydrated autoclaving at 121 °C, 2 atmospheres [atm] for 20 min in distilled water). (3) Pretreatment "121DWHA/FA" (121DWHA and 98% formic acid for 5 min). (4) Pretreatment "121DWHA/PK" (121DWHA and Proteinase K [0.4 mg/ml; DAKO, California, USA] treatment for 1 min. (5) Pretreatment "135DWHA" (hydrated autoclaving at 135 °C, 3 atm for 20 min in distilled water). (6) Pretreatment "135DWHA/FA" (135DWHA and 98% formic acid for 5 min). The last two methods (5 and 6) were improved hydrated autoclaving methods designed to retrieve PrP^{Sc} immunoreactivity and to be more sensitive than the previous three methods (2–4) for the antibodies reacting with linear epitopes (Furuoka *et al.*, 2004). Because of the limited number of slide sections, sCJD and GSS cases were pretreated by only one, or at most two, of the six methods. After pretreatment, tissue sections were incubated with 10% normal goat or normal horse serum (Nichirei, Tokyo, Japan) for 30 min. The horseradish peroxidase-labelled polymer method (Envision+ kit; DAKO) was used to "visualize" positive antibody binding. The 13 primary antibodies (12 monoclonal and one polyclonal) used are listed in Table 1. Sections were exposed to primary antibodies for 1 h at room temperature. As negative controls, further sections were exposed to each primary antibody without any of the pretreatments. The sections were then incubated with the second antibody for 30 min at room temperature. Positive immunoreactive binding signals were detected with diaminobenzidine (Simple stain DAB; Nichirei). Sections were counterstained with Mayer's haematoxylin. The intensity of specific labelling was scored as follows: 3+, strong; 2+, moderate; +, weak; –, nil.

Results

Histopathology and Immunohistochemistry

The typical lesions and patterns described previously in each species (Fraser and Dickinson, 1968; Wells *et al.*, 1992; Privat *et al.*, 2000; Ryder *et al.*, 2001) were seen. In scrapie-infected mice, neuropil vacuolation was associated with astrogliosis, and microglial proliferation

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