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Review

The immunodetection of the abnormal isoform of prion protein

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Summary

Transmissible spongiform encephalopathies such as scrapie in sheep and goats, Creutzfeldt-Jakob disease in humans and bovine spongiform encephalopathy in cattle, are neurodegenerative disorders. A proposed causative agent for these diseases is an infectious protein, the so called 'prion'. An abnormal isoform of prion protein (PrP^{Sc}) can be detected according to the prion propagation method used. As PrP^{Sc} appears to constitute the main, if not the only, infectious entity its detection for the diagnosis of prion diseases is important. Immunodetection methods for PrP^{Sc} analysis are popular tools for diagnosis and research studies. In this paper, a review of the present knowledge concerning immunodetection is presented and the enhancement of the immunoreactivity of antisera to mouse and hamster prion protein peptides using the techniques of Western blotting and immunohistochemistry is summarized.

Scrapie in sheep and goat, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease (CJD) in humans, are transmissible neurodegenerative disorders resulting in spongiform encephalopathy. It has been proposed that the infectious agent of these diseases is an infectious protein which has been designated by the term 'Prion' (Prusiner 1991). The diseases are characterized by a long incubation period in affected animals and the host shows no detectable immunological response. The emergence of BSE as a new disease of cattle and its potential to be a possible hazard to human health has raised many academic and practical questions. Evidence of the possible link between BSE in cattle and the recent human cases of new variant CJD (nvCJD), which have been mainly recognized in the United Kingdom (with one case in France), has added to the social confusion (Bruce *et al*. 1997, Hill *et al*. 1997). At present, there is no prevention or cure for these diseases. Therefore, the detection of BSE in cattle and its exclusion from the human food-chain are considered to be matters of the highest importance. Unfortunately, the nature of the infectious agent involved in prion diseases has still not been fully elucidated (Bolton & Bendheim 1991), but an abnormal isoform of prion protein (PrP^{Sc} in the case of scrapie), which is generated by post translational modification of cellular protein (PrP^{C}), has been found to accumulate in the brains of affected animals (Bolton *et al*. 1984), and to constitute the main, if not the only, infectious entity.

PrP^{C} is a sialoglycoprotein with a molecular mass of 33–35 kDa and is attached to the cell membrane by a glycosyl phosphatidyl inositol anchor (Stahl *et al*. 1987). Pan *et al*. (1993) analyzed the secondary structures of PrP^{C} and PrP^{Sc} by Fourier-transform infrared spectroscopy and their results showed that PrP^{Sc} had a high Beta pleated-sheet content (43%), in contrast to PrP^{C} which had very little (3%). This conformational transition is believed to be the molecular basis for the conversion of PrP^{C} and PrP^{Sc}, with the latter exhibiting a high tendency to aggregate into scrapie associated fibrils (Merz *et al*. 1981) and showing a partial resistance to proteinase K digestion. The protease-resistant core (PrP_{27-30}), has an apparent molecular weight of 27–30 kDa, and is the main component of PrP^{Sc} (Prusiner *et al*. 1984).

Although rodents have been extensively used for studies of prion disease infection, inter-species transmission of the diseases can be difficult resulting in either complete failure to transmit disease, or greatly extended incubation periods. These difficulties in inter-species transmissibility have led to the recognition of a species barrier effect (Kimberlin 1991). Experiments using mice devoid of a functional prion protein (*Prnp*) gene have clearly demonstrated that the host *Prnp* gene is essential for the propagation of infectious agents and pathogenesis (Büeler *et al*. 1993). Furthermore, the results of experiments using transgenic mice expressing exogenous *Prnp* genes have indicated that the species barrier effect is due to the structural differences between different host prion protein (PrP) (Prusiner *et al*. 1990). *Prnp* genes are highly conserved and show high homology among many species. The amino acid sequences of PrP are also conserved within many species. Only about 5% amino acid differences exist among the known sequences of PrP from mouse, hamster, sheep, and cattle (Basler *et al*. 1986, Locht *et al*. 1986, Oesch *et al*. 1985, Robakis *et al*. 1986, Westaway *et al*. 1987). To determine the conformational influence of species-specific amino acid substitutions, species-specific antibodies are required. Synthetic peptides corresponding to the species-specific sequences of PrP are considered useful immunogens

to generate such antibodies. Specific antibodies are useful tools not only for diagnosis of prion diseases, but also for conformational analysis of the prion protein.

Diagnosis of prion diseases has been carried out by histopathological observation of spongiform degeneration and astrocytosis in the central nervous system (Marsh & Kimberlin 1975). Electron microscopical observations (Dawson *et al*. 1987, DeArmond *et al*. 1985, Gibson *et al*. 1987), immunochemical analysis (Farquhar *et al*. 1989, Serban *et al*. 1990) and immunohistochemical techniques (Haritani *et al*. 1994, Kitamoto *et al*. 1986, Roberts *et al*. 1986) for the detection of accumulated PrP^{Sc} have also been used as diagnostic techniques. Many antibodies against PrP have been generated for PrP detection techniques by using purified PrP^{Sc} and synthetic peptides as immunogens (Barry & Prusiner 1986, Barry *et al*. 1988, Bendheim *et al*. 1984, Bode *et al*. 1985, Diringer *et al*. 1984, Farquhar *et al*. 1989, Groschup and Pfaff 1993, Kascsak *et al*. 1986, 1987, Rubenstein *et al*. 1986, Shinagawa *et al*. 1986). Epitope analysis has shown that regions 109 (Met) and/or 112 (Met) are implicated in the species specificity of hamster PrP (Bolton *et al*. 1991, Rogers *et al*. 1991). However, epitope analysis of mouse PrP had not been so straightforward. There are 8 amino acid substitutions in the core region of PrP_{27-30}, between mouse and hamster PrP amino acid sequences. Seven peptides were designed to contain these substituted amino acids to obtain species specific antisera to mouse and hamster PrP^{Sc}, respectively (shown in Table 1). These peptides were synthesized with a multiple antigen peptide (MAP) system to obtain high immuno-response (Tam & Zavala 1989) and inoculated subcutaneously into rabbits with Freund's complete adjuvant and boosted with incomplete adjuvant. These antisera to each peptide were designed as antibody (Ab).Mo-I, Ab.Mo-III, Ab.Mo-IV, Ab.Mo-V, Ab.Mo-VI, Ab.Ha-l, and Ab.Ha-V, respectively (Yokoyama *et al*. 1995, 1996a).

Firstly, the immunoreactivity of obtained antisera was evaluated with Western blotting, and enzyme-linked immunosorbent assay (ELISA) using mice and hamster PrP^{Sc} (Table 1). Although all the antisera were reactive with each immunized peptide, there was a difference in immunoreactivity to PrP^{Sc} by ELISA and Western blotting. Abs.Mo-I, Mo-V, and Mo-VI reacted with mouse PrP^{Sc}, and Ab.Ha-I reacted with hamster PrP^{Sc} in ELISA. Abs.Mo-l and Mo-V reacted with mouse PrP^{Sc} but not with hamster PrP^{Sc}. Ab.Ha-I reacted strongly with hamster PrP^{Sc} and also showed weak cross-reactivity with mouse PrP^{Sc} in Western blotting. Pre-adsorption of Ab.Ha-I with Mo-I peptide, which substituted two amino acids, eliminated the reactivity with mouse PrP^{Sc}, but not with hamster PrP^{Sc}. Abs.Mo-IV, Mo-VI reacted with both mouse and hamster PrP^{Sc}. However, Abs.Mo-III, and Ha-V did not react neither mouse nor hamster PrP^{Sc}. These results suggest that sub-region I and V of mouse, and sub-region I of hamster PrP consist of species-specific epitopes, which are determined by the primary amino acid sequences.

The immunoreactivity of each antisera to PrP^{Sc} by immunocytochemical techniques has also been assessed. Retrospective examination of formalin-fixed paraffin embedded sections was used for this analysis. However, PrP^{Sc} does not react with antibodies immunocytochemically without pretreatment. It is known that in immunocytochemical staining of PrP^{Sc}, PrP^{CJD} (in the case of CJD), PrP^{BSE} (in the case of BSE), by antiserum is enhanced by pretreatment in sodium dodecyl sulphate (SDS) (Doi-Yi *et al*. 1991) formic acid (Kitamoto *et al*. 1987) or guanidine (Taraboulos *et al*. 1990), or by autoclave pretreatment (Kitamoto *et al*. 1991, Haritani *et al*. 1994). After the autoclave pretreatment, Abs.Mo-V, and VI recognized mouse PrP^{Sc} immunocytochemically. Ab.Mo-V recognized mouse PrP^{Sc}, but not hamster PrP^{Sc}; these results correlated well with the result of Western blotting. In contrast, Ab.Mo-VI recognized PrP^{Sc} in mouse, hamster, sheep,

Table 1. Summary of reactivities of anti-peptide antisera.

Antibody	Peptide		ELISA[1]		WB[2]			IHC[3]	
	Amino acid sequences	Residues	Peptide	PrP^{Sc}	Mo[4]	Ha[5]	Enh[6]	Mo	Ha
Ab.Mo-I	KPSKPKTNLKHVAGAA7	100–115	64,000	100	+	–	–	–	–
Ab.Ha-I	KPSKPKTNMKHMAGAA	101–116	512,000	400	(+)[8]	+	–	–	–
Ab.Mo-III	RENMYRYPNQ	150–159	4,000	<50	–	–	–	–	–
Ab.Mo-IV	VDQYSNQNNF	165–174	32,000	<50	+	+	–	–	–
Ab-Mo-V	ETDVKMMERV	199–208	256,000	1,600	+	–	+	+	–
Ab.Ha-V	ETDIKIMERV	200–209	256,000	<50	–	–	–	–	–
Ab.Mo-VI	CVTQYQKESQAYYD	213–226	1,024,000	1,600	+	+	+	+	+

[1]ELISA analysis to homologous peptide and mouse or hamster PrP^{Sc} (A415 > 0.1).
[2]Western blot analysis to purified mouse and hamster PrP^{Sc}.
[3]Immunohistochemical analysis to scrapie affected mouse brain.
[4]Mo = Mouse PrP^{Sc}.
[5]Ha = hamster PrP^{Sc}.
[6]Enhancement of immunoreactivity with autoclave pretreatment in Western blotting.
[7]Substituted amino acids between mouse and hamster PrP are underlined.
[8]Immunoreactivity was eliminated with adsorption of Mo-I peptide.

and PrP^{BSE} of cattle. This cross-reactivity was also confirmed with Western blotting analysis. However the other antisera (Abs.Mo-I, Mo-III, Mo-IV, Ha-I, and Ha-V) did not react with PrP^{Sc} immunohistochemically.

In terms of a pre-clinical diagnosis from non-neural tissues in sheep scrapie by identification of PrP^{Sc}, detection of the abnormal protein in the lymphoid tissue, placenta or tonsils has been proposed (Ikegami *et al*. 1991, Schreuder *et al*. 1996). However, as the rate of PrP^{Sc} accumulation in the affected animals is very slow it would be desirable to achieve higher sensitivity in the present immunodetection systems. It has been reported that the immunoreactivity of PrP^{Sc} prepared from affected brain homogenate samples is greatly enhanced by exposure to guanidine hydrochloride (Gdn-HCl) in dot blot analysis (Serban *et al*. 1990). To enhance the sensitivity, hydrated pretreatment, which is the same procedure used in immunohistochemistry was examined prior to Western blotting analysis. Although, the mechanism is obscure, the immunoreactivity of Abs.Mo-V, and Mo-VI to PrP^{Sc} was enhanced in Western flotting by autoclave pretreatment. However, for Abs.Mo-I, and Mo-IV there was no enhancement (Yokoyama *et al*. 1996b). These results agreed with the immunoreactivity results obtained by immunohistochemistry. The epitopes located close to the C-terminal end of PrP^{Sc} were enhanced with regard to immunoreactivity in Western blotting analysis and immunohistochemistry using the autoclave pretreatment. We also confirmed that the immunoreactivity of PrP^{C} was enhanced with this treatment (data unpublished).

It is, therefore, apparent that autoclave pretreatment enhances the immunoreactivity of both PrP^{C} and PrP^{Sc} for Western blotting analysis. However, although PrP^{Sc} detection could be enhanced for immunohistochemistry using the pretreatment it could not aid in the detection of PrP^{C} by this technique. This may have been simply due to the low expression of the protein. Although this is convenient for the diagnosis of prion diseases, for the study of the pathogenesis of disease group it would be beneficial to detect not only PrP^{Sc}, but also PrP^{C}. Further studies are required to analyze the dynamics of PrP^{Sc} and PrP^{C} *in situ*, according to the prion propagation. It is clear that specific antibodies are invaluable tools for the immunodetection of PrP^{Sc} for diagnostic purposes as well as for pathogenesis studies on prion diseases. If adequate pretreatment of each antibody could be used to enhance it's immunoreactivity, this could provide increased sensitivity of immunodetection of PrP^{Sc} and improve the potential for preclinical diagnostic techniques.

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