Journal of Immunological Methods 337 (2008) 106–120

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Characterisation of new monoclonal antibodies reacting with prions from both human and animal brain tissues

Henriette Cordes [a,*], Ann-Louise Bergström [a,b], Jakob Ohm [a], Henning Laursen [b], Peter M.H. Heegaard [a]

[a] National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, DK-1790 Copenhagen V, Denmark
[b] The Neuropathology Laboratory, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 7 November 2007
Received in revised form 30 June 2008
Accepted 1 July 2008
Available online 25 July 2008

Keywords:
Prion
Monoclonal antibodies
BSE
Creutzfeldt-Jakob Disease
Diagnostics

ABSTRACT

Post-mortem diagnosis of transmissible spongiform encephalopathies (prion diseases) is primarily based on the detection of a protease resistant, misfolded disease associated isoform (PrP^{Sc}) of the prion protein (PrP^{C}) on neuronal cells. These methods depend on antibodies directed against PrP^{C} and capable of reacting with PrP^{Sc} *in situ* (immunohistochemistry on nervous tissue sections) or with the unfolded form of the protein (western and paraffin embedded tissue (PET) blotting). Here, high-affinity monoclonal antibodies (mAbs 1.5D7, 1.6F4) were produced against synthetic PrP peptides in wild-type mice and used for western blotting and immunohistochemistry to detect several types of human prion-disease associated PrP^{Sc}, including sporadic Creutzfeldt-Jakob Disease (CJD) (subtypes MM1 and VV2), familial CJD and Gerstmann-Sträussler-Scheinker (GSS) disease PrP^{Sc} as well as PrP^{Sc} of bovine spongiform encephalopathy (bovine brain), scrapie (ovine brain) and experimental scrapie in hamster and in mice. The antibodies were also used for PET-blotting in which PrP^{Sc} blotted from brain tissue sections onto a nitrocellulose membrane is visualized with antibodies after protease and denaturant treatment allowing the detection of protease resistant PrP forms (PrP^{RES}) *in situ*.

Monoclonal antibodies 1.5D7 and 1.6F4 were raised against the reported epitope (PrP153–165) of the commercial antibody 6H4. While 1.5D7 and 1.6F4 were completely inhibitable by PrP153–165, 6H4 was not, indicating that the specificity of 6H4 is not defined completely by PrP153–165. The two antibodies performed similarly to 6H4 in western blotting with human samples, but showed less reactivity and enhanced background staining with animal samples in this method. In immunohistochemistry 1.5D7 and 1.6F4 performed better than 6H4 suggesting that the binding affinity of 1.5D7 and 1.6F4 with native (aggregated) PrP^{Sc} *in situ* was higher than that of 6H4. On the other hand in PET-blotting, 6H4 reached the same level of reactivity as 1.5D7 and 1.6F4. This shows that 6H4 needs denatured PrP^{RES} to reach maximal reactivity, confirming earlier results. As an exception, human PrP^{RES} still reacted relatively poorly with 6H4 in PET-blotting, while 1.5D7 and 1.6F4 reacted well with PrP^{RES} from most human CJD types.

Taken together this implies that the binding epitope of 1.5D7 and 1.6F4 is accessible in the aggregates of undenatured PrP^{Sc} (IHC) while the binding site of 6H4 is at least partly inaccessible. In techniques incorporating a denaturing and/or disaggregating step 6H4 showed good binding indicating increased accessibility of the binding site. An exception to this is human samples in PET-blotting suggesting that $huPrP^{RES}$ might not be as easily unfolded by denaturation as BSE and scrapie PrP^{RES}. Also of interest was the ability of 1.5D7 and 1.6F4 to

Abbreviations: BSE, Bovine spongiform encephalopathies; CJD, Creutzfeldt-Jakob Disease; fCJD, Familial CJD; sCJD, Sporadic CJD; GSS, Gerstmann-Sträussler-Scheinker disease; Ig, Immunoglobulin; IHC, Immunohistochemistry; mAb, Monoclonal antibody; PBS, Phosphate buffered saline; PET-blot, Paraffin embedded tissue blot; PLP, Paraformaldehyde-lysine-periodate; PMSF, Phenyl methyl sulfonyl fluoride; PrD, Prion-disease; PrP, Prion protein; PrP^{C}, Normally folded PrP; PrP^{Sc}, Misfolded PrP; PrP^{RES}, Protease resistant PrP; rPrP, Recombinant PrP; rboPrP, Recombinant bovine PrP; rhuPrP, Recombinant human PrP; rovPrP, Recombinant ovine PrP; TSE, Transmissible spongiform encephalopathy; WB, Western blotting.

* Corresponding author. Tel.: +45 72 34 60 00; fax: +45 72 34 60 01.
E-mail address: hcr@vet.dtu.dk (H. Cordes).

0022-1759/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jim.2008.07.004

discriminate between two allelic variants of PrP CJD^{Sc} (VV vs. MM) in immunohistochemistry as opposed to the normally used antibody 3F4.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs) are characterised by the deposition of insoluble, misfolded protein, astrocytosis, neuronal cell death and spongiform changes in the brain. Well-known TSEs include bovine spongiform encephalopathy (BSE), scrapie in sheep and Creutzfeldt-Jakob Disease (CJD) in humans. The deposited protein is known as the prion protein (PrP) which, in its misfolded β-sheet rich conformation (PrP^{Sc}) forms highly ordered, insoluble and highly protease resistant aggregates (the so-called prions). This is the only known specific molecular marker of the prion diseases and can also transmit disease (Prusiner, 1982). The normally folded prion protein (PrP^{C}) is a constitutively expressed, normal neuronal membrane protein (Oesch et al., 1985). The tertiary structure of PrP^{C} molecules from a number of species has been determined experimentally (Lysek et al., 2005) and they all conform to the same overall organization: N-terminal half of the molecule is unordered and the C-terminal half has an ordered globular structure with the following sequence of secondary structure elements: β1α1β2α2α3. The structure of PrP^{Sc} has not been determined experimentally, although the available evidence and modelling studies suggest that the C-terminal part of PrP is folded into a tightly packed highly protease resistant domain enriched in β-structure while parts of the N-terminal half of the molecule is still protease sensitive.

Most established diagnostic methods for detection of prion diseases are based on post-mortem detection of PrP^{Sc} in brain tissue (Kubler et al., 2003). In these methods the crucial point is to discriminate between PrP^{Sc} and PrP^{C}. In western blotting (WB) methods proteinase K (PK) treatment and denaturation make the protease resistant core of PrP^{Sc} (PrP27–30 or PrP^{RES}) in its unfolded form available for detection with PrP-specific antibodies. In immunohistochemistry (IHC), PrP^{Sc} (and PrP^{C}) is detected using limited or no protease treatment and mild denaturation only (typically formic acid) (Kitamoto et al., 1987), PrP^{Sc} being readily identified by its characteristic deposition patterns as revealed by antibody. The paraffin embedded tissue (PET)-blot technique combines WB and IHC (Schulz-Schaeffer et al., 2000). After attachment of paraffin embedded tissue sections to a nitrocellulose membrane, the brain tissue is extensively PK digested and denatured leaving only membrane-bound PK resistant proteins which are then visualized by antibodies on the membrane, still in their original position in the tissue section. Ritchie et al. (2004) have published a comparison of various antibodies in IHC and PET-blotting (including 6H4 and 3F4) showing that there are differences between the performance of different antibodies in these techniques.

Evidently, antibodies are of pivotal importance for the detection of PrP^{Sc} by these methods. However, neither PrP^{C} nor PrP^{Sc} are very immunogenic in the species normally used to prepare antibodies (rabbits, rats and mice) (Kascsak et al., 1987) and not even in chickens (Groschup et al., 1997) as the PrP sequence is highly homologous between species (van Rheede et al., 2003). Even so, very useful antibodies have been produced by relatively simple methods, including mAbs 3F4 (Kascsak et al., 1987) that was obtained after immunization of mice with semi-purified hamster PrP^{Sc} and 6H4 (Korth et al., 1997, 1999) prepared by immunization of PrP$^{0/0}$ mice (*PRNP* knock-out mice) with recombinant, reduced bovine PrP. These two antibodies have been used extensively for detection of PrP^{Sc} and PrP^{RES}. 3F4 has a high selectivity for hamster and human PrP and has been shown to have a four amino acid minimal epitope (109–112 in hamster PrP) (Bolton et al., 1991). 6H4 is more broadly reacting between species and a minimal epitope consisting of boPrP155–163 (bovine sequence numbers are used throughout unless indicated otherwise) has been suggested for this antibody (Korth et al., 1997, 1999) although there is some evidence that boPrP153–165 might be more representative (Holada and Vostal, 2000).

In the present work, a simple immunization approach was used to prepare monoclonal antibodies with known, pre-determined binding epitopes by immunization of wild-type mice with carrier-protein coupled peptides mixed with conventional adjuvant (Freund's adjuvant). Synthetic peptides corresponding to different regions of the globular part of PrP were used, including PrP153–165 (bovine numbering, see Table 1). This epitope is situated around α1 and comprises the

Table 1
Sequences of immunizing peptides and epitopes

Species					3F4-epitope							Immunizing peptide (boPrP153–165)																
														6H4-epitope														
Bovine		.	.	.	M	K	H	V	.	.	.	**G**	**S**	**D**	**Y**	**E**	**D**	**R**	**Y**	**Y**	**R**	**E**	**N**	**M**	.	.	.	
Ovine		.	.	.	M	K	H	V	.	.	.	G	N	D	Y	E	D	R	Y	Y	R	E	N	M	.	.	.	
Mouse	N	.	.	.	L	K	H	V	.	.	.	G	N	D	W	E	D	R	Y	Y	R	E	N	M	.	.	.	C
Hamster		.	.	.	M	K	H	M	.	.	.	G	N	D	W	E	D	R	Y	Y	R	E	N	M	.	.	.	
Human		.	.	.	M	K	H	M	.	.	.	G	S	D	Y	E	D	R	Y	Y	R	E	N	M	.	.	.	
Human (GSS)		.	.	.	M	K	H	M	.	.	.	G	S	D	Y	E	D	R	Y	Y	R	E	N	M	.	.	.	

Sequence of the bovine PrP peptide used for immunizations is shown in bold. Corresponding sequences for sheep, mouse, hamster and human are listed below with species differences underlined. The minimal epitope for mAb 6H4 is shown by shading (Korth et al., 1997).

Download English Version:

https://daneshyari.com/en/article/2088898

Download Persian Version:

https://daneshyari.com/article/2088898

Daneshyari.com