Available online at www.sciencedirect.com
ScienceDirect
Biologicals 37 (2009) 210–215
IABS BIOLOGICALS
www.elsevier.com/locate/biologicals

Further characterisation of the prion protein molecular types detectable in the NIBSC Creutzfeldt–Jakob disease brain reference materials

Helen M. Yull, James W. Ironside, Mark W. Head*

National CJD Surveillance Unit, School of Molecular & Clinical Medicine (Pathology), University of Edinburgh, Edinburgh, United Kingdom

Received 17 November 2008; revised 29 December 2008; accepted 23 January 2009

Abstract

Sporadic and variant Creutzfeldt–Jakob disease brain reference materials available from the UK National Institute for Biological Standards and Control have been subjected to further characterisation by Western blot analysis, with particular reference to the co-occurrence of different abnormal disease-associated prion protein (PrP^{Sc}) types. The results confirm the presence of genuine type 1 and type 2 protease-resistant PrP (PrP^{res}) in each of the three sporadic Creutzfeldt–Jakob disease reagents, and provide evidence supporting the lower level presence of type 1 PrP^{res} in the variant Creutzfeldt–Jakob disease reagents. We conclude that these reagents provide a valuable resource for future research and development.

Keywords: Creutzfeldt–Jakob disease; Standards; Prion protein; Molecular typing; Co-occurrence

1. Introduction

1.1. Creutzfeldt–Jakob disease

Creutzfeldt–Jakob disease (CJD) is a rare fatal neurodegenerative condition. It is a member of the family of transmissible spongiform encephalopathies or prion diseases and it can occur in sporadic (sporadic CJD or sCJD), familial and acquired forms, including variant CJD [1]. Variant CJD (vCJD) is a new disease resulting from human infection by the bovine spongiform encephalopathy (BSE) agent. A series of key uncertainties are associated with vCJD, including the numbers of people infected with the BSE agent, the length of the incubation period and the potential for secondary transmission from these individuals (particularly by blood transfusion); these uncertainties have stimulated academic and commercial interest in developing methods to test for CJD [2]. To meet the need for standardised reagents for these purposes the National Institute for Biological Standards and Control (NIBSC) have prepared and made available a series of reference reagents prepared from autopsy human brain specimens [3].

1.2. The prion protein

The prion hypothesis proposes that the agent is composed largely or exclusively of a misfolded form (PrP^{Sc}) of a normal cellular glycoprotein (PrP^{C}) [1]. The presence of PrP^{Sc}, and by inference the presence and titre of the agent, is most commonly detected in the form of PrP^{res}, the protease-resistant core fragment of PrP^{Sc}, usually by Western blotting after digestion with proteinase K. Different human prion diseases are associated with forms of PrP^{res} that differ in the precise size of the protease-resistant core fragment and the proportion of the three possible glycoforms. The physico-chemical properties of PrP^{Sc} in combination with the patient's prion protein gene (*PRNP*) sequence, in particular the methionine (M)/valine (V) polymorphism at codon 129, are thought to play a major role in defining the prion disease phenotype. In sCJD this means either a type 1 (21 kDa nonglycosylated

* Corresponding author at: National CJD Surveillance Unit, Bryan Matthews Building, Western General Hospital, Edinburgh EH4 2XU, The United Kingdom. Tel: +44 131 537 2483; fax: +44 131 537 3056.
E-mail address: m.w.head@ed.ac.uk (M.W. Head).

1045-1056/09/$36.00
doi:10.1016/j.biologicals.2009.01.009

PrP^{res}) or type 2 (19 kDa nonglycosylated PrP^{res}) in association with MM, MV, or VV genotypes [4,5]. Clinical cases of vCJD have thus far only occurred in MM individuals, all of whom have a type 2 PrP^{res} that can be distinguished from the type 2 found in sCJD by the predominance of the diglycosylated form. This PrP^{res} type is termed type 2B to distinguish it from the usually monoglycosylated dominant type 1 and type 2A forms found in cases of sCJD [6,7].

1.3. NIBSC reference materials

Characterisation of the NIBSC reference materials by Western blotting showed, as expected, that PrP^{res} was undetectable in the control (non-CJD) brain specimen and that the vCJD reagent contained readily detectable type 2B PrP^{res} [8,9]. In contrast, the two cases selected as representative sCJD MM1 and MM2 subtypes, on the basis of routine sampling and analysis, were both found to contain roughly equal amounts of type 1 and type 2 PrP^{res} when the high volume reference material was prepared and analysed in a multiple centre study [8]. Subsequently a case of sCJD MV1 was included in the reference panel series and this reagent was also found to have doublet non-glycosylated PrP^{res} band [9], consistent with it too comprising a mixture of type 1 and type 2 PrP^{res}.

1.4. Mixed PrP types

The co-occurrence of types 1 and 2 in cases of sCJD is now a well recognised phenomenon [5,10,11,12] and several independent studies have each concluded that when an extensive brain sampling protocol is employed 20–50% of sCJD cases can be seen to contain both type 1 and type 2 PrP^{res} [7,10,13,14,15]. We and others have investigated this co-occurrence phenomenon by using antibodies developed specifically to bind to epitopes present on the N-terminally digested type 1 PrP^{res} that are absent from the more extensively N-terminally truncated type 2 PrP^{res} [16,17]. These studies independently concluded that low levels of type 1 PrP^{res} are detectable in all cases of sCJD classified as type 2 by conventional means and in all analysed cases of vCJD [16,17]. Given the controversial nature of this observation [18] we have conducted a detailed investigation into the PrP^{res} types present in the full series of NIBSC CJD brain reference materials.

2. Materials and methods

2.1. NIBSC reference materials

The NIBSC human brain reference reagents were obtained from the CJD Resource Centre in the form of 10% (w/v) homogenates in 0.25 M sucrose: sCJD preparation 1, MM (NHBX0/0001, designation Red); sCJD preparation 2, MM, (NHBX0/0002, designation Green); vCJD, MM, (NHBY0/0003, designation Blue); CJD control (NHBZ0/0005, designation Clear); sCJD, MV (NHBX0/0004, designation Yellow); vCJD, MM (NHBY0/0014, designation White). The red, green, blue and clear reagents have WHO Reference Reagent Status. The yellow and white reagents do not.

2.2. Western blotting

Entire aliquots of the reference reagents were brought to a final concentration of 0.5% Nonidet P40, 0.5% sodium deoxycholate, 10 mM Tris pH 7.2 by addition of a ten times concentrate, homogenised and briefly cleared by low speed centrifugation. Proteinase K digestion and Western blot analysis were exactly as described previously [17] including the use of the 3F4 monoclonal antibody that detects both type 1 and type 2 PrP^{res} (epitope $_{109}MKHM_{112}$ of the human PrP sequence) and the 12B2 monoclonal antibody that does not detect type 2 PrP^{res} ($_{98}WGQGG_{93}$ of the human PrP sequence). Molecular weight markers and our own in-house sCJD MM type 1 standard (referred to as the type 1 standard) were run on every Western blot as described previously [17] as mobility markers to distinguish between type 1 and type 2 PrP^{res}. Multiple exposures were made of each Western blot.

2.3. Scanning, densitometry and presentation

Suitable exposures were scanned using a Bio-Rad GS-800 densitometer and Western blot image figures constructed in Adobe Photoshop. Densitometry was performed using Quantity One software (Bio-Rad Laboratories) and graphic representations of the time-course and concentration dependence of PrP digestion were constructed in Microsoft Excel all as previously described [7,17]. The densitometric values at time zero (time-course study) and 5 μg/ml PK (titration study) were designated as 100% and the remaining signals for 3F4 or 12B2 antibodies calculated accordingly. The 12B2 detectable percentage remaining was then expressed as a percentage of the 3F4 detectable percentage remaining.

3. Results

3.1. Initial characterisation

Western blot analysis confirmed the absence of detectable PrP^{res} in the non-CJD (Clear) sample when monoclonal antibodies 3F4 or 12B2 were used (Fig. 1). 3F4 detected a band in the CJD (Blue, White, Red, Green and Yellow) samples that migrated ahead of the type 1 standard sample, was ~19 kDa and is therefore type 2 PrP^{res}. The loading and resolution of the sCJD samples (Red, Green, Yellow) on this Western blot precluded clear resolution of type 1 (~21 kDa) PrP^{res} by 3F4. However, when a duplicate blot was screened with the monoclonal antibody 12B2 that specifically detects type 1 PrP^{res} in proteinase K treated material, a higher molecular weight band at ~21 kDa was seen to be present in all CJD samples, of a similar mobility to that detected in the type 1 standard sample. The level of PrP^{res} detected in the sCJD reference materials (Red, Green, Yellow) was similar to that of the type 1 standard, whereas the level of PrP^{res} detected in the vCJD reference materials (Blue, White) was seen to be