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SPONTANEOUSLY ARISING DISEASE

Increased Immunohistochemical Labelling for Prion Protein Occurs in Diverse Neurological Disorders of Sheep: Relevance for Normal Cellular PrP Function

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Summary

The classical prion diseases (e.g. scrapie of sheep and goats and bovine spongiform encephalopathy of cattle) are characterized by the accumulation of abnormal forms of the prion protein (PrP), usually recognized by their relative resistance to proteolysis compared with the physiological cellular forms of PrP. However, novel prion diseases have been detected in sheep, cattle and man, in which the abnormal PrP has less resistance to proteolysis than identified previously. These more subtle differences between abnormal and normal forms of PrP can be problematic in routine diagnostic tests and raise questions in respect of the range of PrP disorders. Abnormal accumulations of PrP in atypical and classical prion diseases can be recognized by immunohistochemistry. To determine whether altered PrP expression or trafficking might occur in nosological entities not previously connected with prion disease, the brains of sheep affected with diverse neurological conditions were examined for evidence of altered PrP labelling. Such altered immunolabelling was detected in association with either basic lesions or specific diseases. Some reactive glial cells and degenerate neurons found in several different recognized disorders and non-specific inflammatory processes were associated with abnormal PrP labelling, which was absent from brains of healthy, age-matched sheep. The results agree with previous indications that normal PrP function may be linked with the oxidative stress response, but the data also suggest that PrP functions are more extensive than simple protective responses against stress insults.

Keywords: prion disease; prion protein; scrapie; transmissible spongiform encephalopathy

Introduction

Scrapie of sheep and goats and bovine spongiform encephalopathy (BSE) of cattle are transmissible spongiform encephalopathies (TSEs) or prion diseases. These disorders are characterized histologically by vacuolation, neuronal loss and gliosis, and immunohistochemically by the accumulation of disease-associated prion protein (PrP^{d}) in the brain. This protein results from conversion of a host-encoded cell surface sialoglycoprotein known as cellular prion protein (PrP^{c}), the expression of which is essential for the replication of the infectious agent of TSEs (Bueler *et al.*, 1993). The prion hypothesis suggests that the infectious agent arises from conversion of PrP^{c} into abnormal and infectious isoforms (Prusiner, 1982). Abnormal infection-associated isoforms of prion protein (PrP) are characterized biochemically by an increase in the proportions of β-pleated sheet, decreased solubility and increased aggregation, which contributes to the diagnostically useful greater resistance to protease digestion (PrP^{res}; Caughey *et al.*, 2009). However, infectivity in brains of mice can occur in the absence of significant abnormal PrP^{d} or PrP^{res} accumulations (Manson *et al.*, 1999) and, conversely, spontaneous degeneration associated with accumulation of PrP^{d} can occur in the absence of infectivity (Chiesa *et al.*, 2003). In addition, there are human prion disorders

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that, although transmissible to mice, lack PrP^{res} (Piccardo *et al.*, 2001).

In some recently recognized TSEs of animals, such as Nor98 of sheep and the H and L types of BSE in cattle, vacuolation is an inconsistent feature of the pathology and the disease-associated PrP forms accumulating in the affected brains are only weakly protease resistant (Benestad *et al.*, 2003; Buschmann *et al.*, 2004; Jacobs *et al.*, 2007). Similarly, specific variants of human prion diseases have also been identified, in which the abnormal PrP accumulates in a protease sensitive form (Jansen *et al.*, 2010; Zou *et al.*, 2010). Mutant forms of PrP can also aggregate and accumulate in association with morphological change (Jeffrey *et al.*, 2009) in the absence of any detectable infectivity and minimal protease resistance (Chiesa *et al.*, 1998, 2003).

In addition to prion diseases, some studies have suggested that there may be alterations of PrP^c expression in other human neuropsychiatric or neurodegenerative disorders. Neuronal intra-somatic and intra-axonal PrP^c is detected in association with neurodegenerative changes in cerebral infarcts and in cases of perinatal hypoxia of infants (McLennan *et al.*, 2004), while regional variations in the expression of neuronal PrP^c are also reported in Alzheimer's disease (Rezaie *et al.*, 2005). In Alzheimer's disease, punctate PrP^c labelling is also found in neuritic plaques, and is thought to correspond to dystrophic neuritis, while presumably extracellular PrP^c deposits are present in diffuse plaques (Ferrer *et al.*, 2001). In cattle, abnormal immunohistochemical PrP labelling, possibly associated with a subtly altered protease sensitivity, is found in idiopathic brainstem neuronal chromatolysis (Jeffrey *et al.*, 2008), a condition of unknown aetiology. Neuronal PrP^c also appears to be altered in psychiatric conditions, as a decrease of PrP^c-expressing white matter glial cells has been reported in bipolar disorder, major depression and schizophrenia (Weis *et al.*, 2008).

One additional problem for the interpretation of these findings is that it is not possible to be certain of the molecular and biochemical properties of the PrP detected by immunohistochemistry (IHC), particularly with regard to its aggregation state or infectious potential. In some cases PrP^c was distinguished from PrP^d by the use of denaturing techniques (Voigtlander *et al.*, 2001), but in other studies no treatments were applied for such discrimination. A recent study indicated that denaturing by autoclaving in citric acid does not eliminate all PrP^c labelling (González *et al.*, 2005b), at least for some antibody/tissue combinations. Therefore, the relevance and interpretation of PrP immunolabelling is becoming more difficult to evaluate, particularly in a diagnostic context.

The aim of this study was to aid case definition of atypical and classical prion diseases of sheep by characterizing and comparing the alterations of PrP immunolabelling patterns in a range of neurodegenerative and inflammatory disorders. In this study we use the term 'PrP' to mean any immunohistochemical reactivity found in association with neurodegenerative changes that, when applying the same PrP antibodies and IHC methods, was not present in the brains of healthy control sheep.

Materials and Methods

Brains from 50 sheep were selected from archival material of cases that, as judged by the submitting veterinarian, had a clinical neurological presentation. Those cases were grouped into four broad categories: inflammatory, necrotizing neurodegenerative, non-necrotizing neurodegenerative and miscellaneous. The latter included cases of neurological disease for which no morphological or aetiological diagnosis could be established, and those in which the primary diagnosis was unrelated to any brain pathology (Table 1). All cases had been investigated previously by microscopical examination of haematoxylin and eosin (HE)-stained sections of brain and, where appropriate, by special stains such as silver impregnation methods. Specific aetiological diagnosis in most cases was supported by appropriate laboratory techniques in regional laboratories of the Animal Health and Veterinary Laboratories Agency.

From all 50 brains, tissue sections of cerebrum and cerebellum, and additional brain areas where lesions were most prominent (for example the brainstem in encephalic listeriosis), were labelled for PrP by routine methods (González *et al.*, 2005a). Briefly, antigen retrieval included immersion of tissue sections in 98% formic acid for 5 min and autoclaving in 0.2% citrate buffer for 5 min at 121°C. After two blocking steps (to quench endogenous peroxidase activity and to block non-specific binding to tissue antigens), incubation with the primary antibody was carried out overnight at 4°C. Subsequent steps were performed using a commercial immunoperoxidase technique (Vector-elite ABC kit; Vector Laboratories, Peterborough, UK), after which sections were immersed in 0.5% copper sulphate to enhance the colour reaction and were finally counterstained with Mayer's haematoxylin. Three PrP antibodies were used: R145, F99 and 12B2, which recognize ^{223}RESQA^{227} ^{220}QYQRES^{225} and ^{93}WGQGG^{97} of the ovine PrP sequence, respectively.

Five sheep terminally ill with scrapie were included as positive controls for the PrP^d IHC (for illustrations of patterns of PrP^d labelling in scrapie-affected sheep see González *et al.*, 2002). The background non-specific