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EXPERIMENTALLY INDUCED DISEASE

Influence of Breed and Genotype on the Onset and Distribution of Infectivity and Disease-associated Prion Protein in Sheep Following Oral Infection with the Bovine Spongiform Encephalopathy Agent

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Summary

The onset and distribution of infectivity and disease-specific prion protein (PrP^d) accumulation was studied in Romney and Suffolk sheep of the ARQ/ARQ, ARQ/ARR and ARR/ARR prion protein gene (*Prnp*) genotypes (where A stands for alanine, R for arginine and Q for glutamine at codons 136, 154 and 171 of PrP), following experimental oral infection with cattle-derived bovine spongiform encephalopathy (BSE) agent. Groups of sheep were killed at regular intervals and a wide range of tissues taken for mouse bioassay or immunohistochemistry (IHC), or both. Bioassay results for infectivity were mostly coincident with those of PrP^d detection by IHC both in terms of tissues and time post infection. Neither PrP^d nor infectivity was detected in any tissues of BSE-dosed ARQ/ARR or ARR/ARR sheep or of undosed controls. Moreover, four ARQ/ARQ Suffolk sheep, which were methionine (M)/threonine heterozygous at codon 112 of the *Prnp* gene, did not show any biological or immunohistochemical evidence of infection, while those homozygous for methionine (MARQ/MARQ) did. In MARQ/MARQ sheep of both breeds, initial PrP^d accumulation was identified in lymphoreticular system (LRS) tissues followed by the central nervous system (CNS) and enteric nervous system (ENS) and finally by the autonomic nervous system and peripheral nervous system and other organs. Detection of infectivity closely mimicked this sequence. No PrP^d was observed in the ENS prior to its accumulation in the CNS, suggesting that ENS involvement occurred simultaneously to that of, or followed centrifugal spread from, the CNS. The distribution of PrP^d within the ENS further suggested a progressive spread from the ileal plexus to other ENS segments via neuronal connections of the gut wall. Differences between the two breeds were noted in terms of involvement of LRS and ENS tissues, with Romney sheep showing a more delayed and less consistent PrP^d accumulation than Suffolk sheep in such tissues. Whether this accounted for the slight delay (~5 months) in the appearance of clinical signs in Romney sheep is debatable since by the last scheduled kill before animals reached clinical end point, both breeds showed widespread accumulation and similar magnitudes of PrP^d accumulation in the brain.

Keywords: bovine spongiform encephalopathy; pathogenesis; *Prnp* genotype; sheep

Introduction

Transmissible spongiform encephalopathies (TSEs) are a group of neurodegenerative disorders that includes, amongst others, bovine spongiform encephalopathy (BSE), scrapie of sheep and goats, chronic

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wasting disease (CWD) of cervids and human variant Creutzfeldt–Jakob disease (vCJD). These diseases are progressive and invariably fatal and are often acquired following oral exposure to infectivity, as is believed to be the case for BSE and vCJD (Wilesmith and Wells, 1991; Will *et al*., 1996). TSEs can be naturally, experimentally or iatrogenically transmitted to several mammalian species and are characterized by the accumulation of abnormal isoforms of a host-encoded cell-surface glycoprotein called prion protein (PrP^{c}). These disease-associated isoforms (PrP^{d}) can accumulate in the central nervous system (CNS), in the peripheral nervous system (PNS), in the lymphoreticular system (LRS) and in some other organs. Thus, detection of PrP^{d} (or its protease resistant form, PrP^{res}) by a variety of laboratory methods constitutes the basis for the detection of infected animals and TSE diagnosis.

Studies of naturally occurring scrapie in sheep (Andreoletti *et al*., 2000; Van Keulen *et al*., 2000) and goats (González *et al*., 2010) and of experimental sheep scrapie (González *et al*., 2014a,b) and BSE in sheep (Van Keulen *et al*., 2008b) and cattle (Terry *et al*., 2003) unanimously identify the lymphoid tissue of the pharynx and/or distal ileum as the first sites of PrP^{d} accumulation after oral exposure. The mechanism by which the infectious agent reaches the brain from those peripheral sites (neuroinvasion) has not been fully elucidated and two routes, neural and haematogenous, have been proposed (reviewed by Sisó *et al*., 2010). Briefly, and as far as small ruminant TSEs is concerned, evidence in support of the neural route includes (1) the finding of nerve endings in the Peyer's patch (PP) lymphoid follicles in close proximity to follicular dendritic cells (FDCs) and tingible body macrophages (TBMs), both of which accumulate PrP^{d} (Jeffrey *et al*., 2000; Heggebo *et al*., 2002), (2) the detection of PrP^{d} in the ENS at early time points after infection (Andreoletti *et al*., 2000; Van Keulen *et al*., 2000; Ersdal *et al*., 2003) and (3) the identification of the dorsal motor nucleus of the vagus (DMNV) and the intermediolateral column of the thoracic spinal cord (IMLC) as the first points of PrP^{d} accumulation in the CNS (Van Keulen *et al*., 2000, 2002, 2008a). On the other hand, evidence in support of the haematogenous route and/or in opposition to the neural route is substantiated by findings such as (1) innervation of PP lymphoid follicles appears to be the exception rather than the norm (McGovern *et al*., 2009), (2) accumulation of PrP^{d} in the ENS and in the thoracic spinal cord is an inconsistent event at early stages of infection, only becoming evident once neuroinvasion has occurred or does not occur at all (Sisó *et al*., 2009a; González *et al*., 2014a,b), (3) the infectious agent circulates in peripheral blood at subclinical stages of infection in titres high enough to reproduce infection by blood transfusion (Houston *et al*., 2008; Andreoletti *et al*., 2012) and (4) PrP^{d} accumulates at early stages of infection in the circumventricular organs (CVOs) of the brain, where the blood–brain barrier is highly diminished (Sisó *et al*., 2009a; González *et al*., 2014a,b).

Another point of some controversy in the pathogenesis of small ruminant TSEs concerns the role of the LRS. While in some cases early replication and widespread dissemination of PrP^{d} in lymphoid tissues appears to correlate with early neuroinvasion and short incubation periods (González *et al*., 2014a,b) accumulation of PrP^{d} in the brain and clinical disease can occur with negligible and late involvement of LRS tissues (González *et al*., 2014a) or even in the absence of detectable PrP^{d} in such tissues (Andreoletti *et al*., 2000; Jeffrey *et al*., 2002; Van Keulen *et al*., 2002). These studies indicate that such discrepancy may be due to polymorphisms in the prion protein gene (*Prnp*) of the host.

A preliminary report on the transmission of BSE showed that *Prnp* ARQ/ARQ Romney sheep (where ARQ is a three letter code indicating alanine, arginine and glutamine at codons 136, 154 and 171 of PrP, respectively) were susceptible to infection via the oral route (Jeffrey *et al*., 2001). Initial sites of PrP^{d} accumulation varied and neuroinvasion occurred occasionally in the absence of peripheral PrP^{d} accumulation. The present report provides a final, more detailed analysis of (1) the relationship between susceptibility and *Prnp* genotype based on information unavailable at the time of that preliminary report and including sheep of ARQ/ARR and ARR/ARR genotypes, (2) immunohistochemical examinations of a much wider range of tissues, (3) time-course infection in a group of Suffolk sheep of the same three different *Prnp* genotypes and (4) the use of mouse bioassay to correlate PrP^{d} accumulation and infectivity. The overall aim of this report is to show the sequence of dissemination of PrP^{d} and infectivity in different tissue compartments following oral challenge of Suffolk and Romney sheep with cattle BSE.

Materials and Methods

Ethical Approval

All animal experiments were approved by the local ethics committee of the Animal Health and Veterinary Laboratories Agency and carried out at the institute in accordance with the Animals (Scientific Procedures) Act 1986 under Home Office license