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研究報告の概要

ヒトの牛海綿状脳症（BSE）への暴露は、変異型クロイツフェルト・ヤコブ病（vCJD）発症の原因となることが知られているが、経口での感染効率や、牛―ヒトの種間バリアが伝播に及ぼす影響などの情報が未解明であるため、大きな混乱が生じている。本研究ではヒト以外の霊長類を対象としてBSEの経口感染実験を行った。2匹のサルにBSEに感染した牛脳ホモジネートを経口で5g投与した。このうち1匹は暴露後60ヶ月後にvCJD様神経症状を呈したが、もう1匹は76ヶ月後も無症状であった。本研究で得られた知見から、食物を介してヒトがvCJDへ暴露される危険性があることが示唆され、現行の公衆衛生当局の対応によりヒトへのBSE感染を予防できるという認識を裏付けた。

報告企業の意見	今後の対応
BSEの霊長類への経口感染実験を行い、BSE感染牛の脳を経口で5g投与したサルがvCJD様の症状を示したとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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Expression of PrP^C on cellular components of sheep blood

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PrP^C, a glycosylphosphatidylinositol-linked glycoprotein, plays a central role in the pathogenesis of transmissible spongiform encephalopathies (TSEs), undergoing a conformational alteration to the disease-associated isoform, commonly designated PrP^{Sc}. PrP^C is expressed in many tissues other than the nervous system, although its precise function(s) remains unclear. It has previously been demonstrated that TSEs can be transmitted by blood transfusion in sheep. The aim of this work was to identify which components of blood carried the infection. As an initial step, the distribution of PrP^C on cellular components of sheep blood was examined to identify potential targets for infection. Cell-surface expression of PrP^C was found only on peripheral blood mononuclear cells (PBMCs); however, platelets also contained significant amounts of intracellular PrP^C. The level of PrP^C expressed on the cell surface of PBMCs was influenced by PrP genotype, with the highest levels found in scrapie-susceptible VRQ/VRQ sheep and the lowest levels in scrapie-resistant ARR/ARR sheep. In susceptible sheep, PrP^C was expressed at varying levels on all major subsets of PBMCs, with the highest levels on the $CD21^+$ subset of B cells, and PrP expression was upregulated dramatically on $CD21^+$ B cells in some scrapie-infected sheep.

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INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) or prion diseases are neurodegenerative diseases that occur in a variety of species, including sheep (scrapie), cattle (bovine spongiform encephalopathy, BSE) and humans (e.g. Creutzfeld–Jakob disease, CJD). A characteristic feature of these diseases is the accumulation of PrP^{Sc}, a post-translationally modified form of the host glycoprotein PrP^C, in the central nervous system (CNS). As PrP^{Sc} and infectivity co-purify (Bolton *et al.*, 1982), the presence of PrP^{Sc} is considered a marker for TSE infection. PrP^C is expressed in many different tissues, with the highest levels found in neurons of the CNS (Prusiner, 1998), but its function is still unclear. PrP-null mice are resistant to TSE infection, emphasizing the key role of PrP in the pathogenesis of these diseases.

Replication of TSE agents and deposition of PrP^{Sc} in lymphoreticular tissues precedes infection of the CNS in certain TSE diseases, e.g. scrapie, variant CJD (vCJD) and many experimental rodent TSE models (Eklund *et al.*, 1967; Hadlow *et al.*, 1982; Hill *et al.*, 1999). In lymphoid tissues, PrP^{Sc} deposits are localized to germinal centres, and follicular dendritic cells have been shown to play a key role in replication of infectivity (Bruce *et al.*, 2000). Infection of lymphoid tissues raises the possibility of haematogenous spread of infectivity, as lymphocytes recirculate between blood and secondary lymphoid organs. Although this route is not believed to be significant for neuroinvasion (Blättler *et al.*, 1997), it probably explains the widespread dissemination of infection in lymphoid tissues seen, for example, in clinical cases of scrapie. Until recently, it has proved difficult to show conclusively that blood from natural TSE cases (human or animal) contains infectivity or PrP^{Sc}, despite the fact that low levels of infectivity have been demonstrated in the blood of experimental rodent TSE models (Brown, 1995). However, it has now been shown that both natural scrapie and experimental BSE can be transmitted between sheep by transfusion of whole blood or buffy coat (Houston *et al.*, 2000; Hunter *et al.*, 2002). In addition, there has been a report of a vCJD case that may have resulted from an infected blood transfusion (Llewelyn *et al.*, 2004) and a more recent report of a pre-clinical vCJD case detected in a patient heterozygous for codon 129 of the prion-protein gene after blood transfusion (Peden *et al.*, 2004).

The distribution of infectivity in the blood of scrapie-infected sheep is not known, but experiments in rodent models have shown that the highest levels are found in buffy coats (which contains leukocytes and platelets), followed by plasma (Brown *et al.*, 1998, 1999). In hamster scrapie, very little infectivity is found in association with purified platelets (Holada *et al.*, 2002). Attempts have been made to detect PrP^{Sc} in blood by methods such as immunocapillary electrophoresis (Schmerr *et al.*, 1999), immunocytochemistry (Herrmann *et al.*, 2002) and Western blotting

(Wadsworth *et al.*, 2001), but the results so far have been negative or inconclusive. The distribution of PrP^C in different blood components may help to define potential targets for infection, although cautious interpretation of the results is required, because there is not a precise correlation between infection of tissues and their expression of PrP^C. Interestingly, comparative studies of healthy animals of different species (mouse, hamster, human, sheep and cattle) have revealed marked differences in the distribution and expression levels of PrP^C on blood cells (Barclay *et al.*, 2002; Holada & Vostal, 2000). In sheep, cell-surface expression of PrP^C was confined to peripheral blood mononuclear cells (PBMCs) and the distribution of PrP mRNA followed the same pattern (Herrmann *et al.*, 2001). In humans, the highest levels of PrP^C expression were found on platelets and PBMCs, and much lower levels were demonstrated on red cells and neutrophils (Barclay *et al.*, 1999, 2002). Human platelets also appear to contain PrP^C in intracellular granules and may be responsible for release of soluble PrP^C into plasma (Perini *et al.*, 1996). The function of PrP^C in blood-associated cells is unknown, although experiments with PBMCs from humans and PrP-null mice have suggested that it may play a role in lymphocyte activation and proliferation (Cashman *et al.*, 1990; Mabbott *et al.*, 1997).

In the absence of specific methods to distinguish PrP^{Sc} from PrP^C in blood, changes in the distribution or expression levels of PrP on blood cells during scrapie infection may provide indirect evidence of cell types transporting, or infected with, the scrapie agent. Here, we have described experiments that have characterized in greater detail the expression of PrP in sheep-blood components, including subsets of PBMCs, from uninfected and scrapie-infected sheep. In uninfected sheep, we showed that platelets contained significant amounts of intracellular PrP^C and that the level of PrP^C expressed on PBMCs differed between scrapie-susceptible and -resistant sheep. We also demonstrated for the first time that PrP^C expression appears to be greatly upregulated on the $CD21^+$ subset of B cells in some scrapie-infected sheep.

METHODS

Sheep. The sheep used in these experiments originated from the Department for Environment, Food and Rural Affairs (UK) scrapie-free flock, which was established in 1998 from sheep imported from New Zealand and has been maintained in strict isolation from UK livestock. Male and female Cheviot and Poll Dorset sheep between 1 and 3 years old were used. PrP-gene polymorphisms at codons 136 (A/V), 154 (R/H) and 171 (R/Q) were confirmed for each animal by automated DNA sequencing of the PCR-amplified coding region. In one experiment, groups of VRQ/VRQ and VRQ/ARQ Poll Dorset sheep were infected experimentally with scrapie by subcutaneous inoculation of 2 ml 10% SSBP/1 brain homogenate, as described previously (Houston *et al.*, 2002). These sheep were housed throughout the experiment until clinical signs of scrapie developed and were then euthanized in accordance with UK Home Office guidelines.

Isolation of different cellular components from sheep blood. Blood samples were obtained by jugular venipuncture, using 3·8% (w/v) sodium citrate solution (Sigma) as anticoagulant. Cellular components of sheep blood were separated by density-gradient centrifugation on Histopaque-1083 (1·083 g ml^{-1}; Sigma) or Polymorphprep (Nycomed), with slight variations in the methods depending on the application.

Polymorphprep, a 1·11 g ml^{-1} gradient containing sodium diatrizoate (13·8%) and dextran 500 (8·0%), is designed for use on human blood and separates polymorphonuclear cells (granulocytes) from PBMCs, with each cell type appearing as a distinct band at different levels of the gradient. In brief, 25 ml blood was layered undiluted on to Polymorphprep gradients and centrifuged at 500 *g* for 25 min, according to the manufacturer's instructions. All steps were performed at ambient temperature unless otherwise stated. PBMCs were harvested from the upper band and granulocytes from the lower band and diluted in PBS (pH 7·4) containing 9 mM EDTA (PBS/EDTA). Pelleted erythrocytes were also collected and diluted with PBS/EDTA. To remove residual PBMCs, the granulocytes were layered over Histopaque-1083, centrifuged at 1000 *g* for 25 min and the pelleted cells were washed twice by centrifugation at 300 *g* for 15 min, first in PBS/EDTA and then in PBS alone. PBMCs were washed twice as described for the granulocytes. After the first wash, the supernatant was collected and centrifuged at 7800 *g* for 5 min to isolate platelets. Platelets were then washed twice in PBS/EDTA at 7800 *g* for 5 min.

For some experiments, PBMCs, platelets and granulocytes were isolated by using Histopaque-1083. Briefly, blood was diluted 1:1 with PBS, layered over Histopaque-1083 and centrifuged at 1000 *g* for 30 min. PBMCs at the gradient interface were collected and washed three times by centrifugation in PBS, as above. To isolate platelets for permeabilization, harvested PBMCs were washed in platelet buffer [PBS containing 9 mM EDTA, 10 ng prostacyclin ml^{-1} (Sigma), 1% (w/v) BSA and 0·1% (w/v) sodium azide] for 10 min at 400 *g*. The supernatant from this wash was collected and washed twice in platelet buffer at 1000 *g* for 10 min. Granulocytes that passed through the Histopaque gradient were isolated by lysing the erythrocytes with ammonium chloride lysis buffer [155 mM NH_4Cl, 10 mM $KHCO_3$, 0·1 mM EDTA (pH 7·2)]. Cells were incubated in the buffer for 10 min at ambient temperature and then centrifuged at 300 *g* for 10 min at 4 °C, followed by two washes at 300 *g* for 5 min at 4 °C, first in PBS/EDTA, then in PBS alone.

Preparation of erythrocyte membranes ('ghosts'). Erythrocyte-membrane 'ghosts' were prepared by lysing the cells pelleted from the Polymorphprep gradient in 5 mM sodium dihydrogen orthophosphate (pH 8·0) containing 1 mM dithiothreitol. Briefly, 50 ml ice-cold lysis buffer was added to 1 ml packed erythrocytes on ice, vortexed and left on ice for 5 min. Cells were centrifuged at 300 *g* for 10 min at 4 °C to remove non-lysed cells and contaminating leukocytes. Membranes were pelleted by centrifuging the supernatant at 20 000 *g* for 40 min at 4 °C and washed twice with cold lysis buffer and once with cold PBS.

Monoclonal antibodies (mAbs). The mAbs used for identification of PrP^C and for phenotyping subsets of PBMCs, along with their isotypes and epitope specificity, are listed in Table 1. FH11 (TSE Resource Centre, IAH, Compton, UK) was raised against full-length, *Escherichia coli*-expressed, recombinant bovine PrP, 4F2 was a gift from Andreas Stuke (Deutsches Primatenzentrum, Göttingen, Germany) and 8G8 and 6H4 were purchased from Spi-Bio and Prionics, respectively. Dr C. J. Howard and the Monoclonal Antibody Production Section, IAH, Compton, UK, supplied CC21, CC32, CC125, CC-G33, IL-A51 and 86D. TD14 came from Professor J. Hopkins, University of Edinburgh, UK, and Du2-104 from Dr W. Hein, Basel Institute for Immunology, Switzerland. Antibodies 44-97 and 25-69 were supplied by Dr E. Meeusen, University of Melbourne, Australia, and CAPP2A was purchased from VMRD.