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研究報告の概要	使用上の注意記載状況・その他参考事項等
英国で白血球除去が導入される前に赤血球製剤の輸血により、変異型クロイツフェルト-ヤコブ病（vCJD）の伝播が3例報告されている。 げっ歯動物の伝染性海綿状脳症（TSE）の実験では、赤血球製剤の感染性は赤血球自体とは無関係であり、感染性は残留白血球や血漿中の他の成分と関連することが示された。 ハムスターの異常プリオンを添加した白血球除去ヒト赤血球の感染性は 0.01%まで除去されたと報告もあり、これは感染性が赤血球によるものではない、あるいは赤血球と感染物質が結合していてもその結合は緩く、ろ過プロセスによって除去されることを示している。 vCJD の原因物質がヒト赤血球と結合しないことを確認できたならば、血中の PRPsc の適切なスクリーニング検査がない現状においては、vCJD 発生国の輸血サービスは赤血球製剤を輸血する前に洗浄またはろ過処理して感染性除去を行うことが賢明である。	**重要な基本的注意** 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
英国における輸血による vCJD 感染例は白血球除去を行っていない赤血球製剤によるものであるが、赤血球自体には感染性はなく、ろ過により除去できるとの情報である。 現時点まで血漿分画製剤からの vCJD 伝播の報告はなく、血漿分画製剤の製造工程でプリオンが除去できるとの情報もある。	今後とも vCJD に関する安全性情報等に留意していく。

Prion protein and the red cell

David J. Anstee

Purpose of review
This review focuses on transfusion-transmission of variant Creutzfeldt-Jakob disease by red cell preparations.
Recent findings
Recently, three cases of probable transmission of variant Creutzfeldt-Jakob infectivity by transfusion of red cell preparations have been described in humans. Experiments on transmissible spongiform encephalopathies affecting rodents have led to the conclusion that infectivity in red cell preparations is not bound to the red cells themselves but contained within the suspending medium from which it can be removed by filtration.
Summary
Red cell preparations are the main transfusion product provided by blood services. If experiments demonstrating significant removal of rodent transmissible spongiform encephalopathy infections by filtration of red cell preparations are appreciable to variant Creutzfeldt-Jakob in humans then a method for rendering human red cell preparations safe for transfusion is provided.

Keywords
bovine spongiform encephalopathy, normal prion protein, abnormal (infectious) prion protein, transmissible spongiform encephalopathy, variant Creutzfeldt-Jakob disease

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Abbreviations

BSE	bovine spongiform encephalopathy
GPI	glycosylphosphatidylinositol
PRPc	normal prion protein
PRPsc	abnormal (infectious) prion protein
TSE	transmissible spongiform encephalopathy
vCJD	variant Creutzfeldt-Jakob disease

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Introduction

Variant Creutzfeld-Jakob Disease (vCJD) was described in the UK in 1996 [1]. The emergence of this novel form of CJD is most probably related to the ingestion of food products obtained from cattle with bovine spongiform encephalopathy (BSE) [2,3]. From the outset the possibility was considered that passage of infectivity from the gut to the brain in affected individuals could involve blood. Therefore, transfusion services were alerted to the potential for transmission of vCJD to a patient by transfusion of blood components from a donor in the preclinical stages of disease. Consequently, precautionary measures were taken in the UK to minimize this risk, in particular leucodepletion, sourcing plasma for fractionation from non-UK donor populations, sourcing fresh frozen plasma for children born after 1 January 1996 (and therefore not exposed through diet) from non-UK donor populations and deferral of blood donors who had themselves been transfused [4]. Sourcing sufficient red cell and platelet components outside the UK is not feasible. The first cases of probable transfusion–transmission emerged in late 2003 and three probable transmissions, all linked to red cell preparations transfused before the introduction of leukodepletion, are now recorded [5,6,7•,8••].*

The infectious agent

There is a large body of evidence suggesting the infectious agent causing BSE and vCJD is an abnormal conformer of the prion protein [9]. Recently, evidence has emerged suggesting that retroviral infection can increase the release of infectious prions from cells and may be an important cofactor in the spread of infection [10•].

Normal prion protein (PRPc) is a glycosylphosphatidylinositol (GPI)-linked protein expressed at cell surfaces of many tissues. It is a glycoprotein rich in α-helix. The function of PRPc is unclear, although recent studies suggest a role in self-renewal of haemopoietic progenitor cells [11•]. The infectious prion protein (PRPsc) is an abnormal conformer of PRPc in which the α-helical regions become predominantly β sheet. This change in secondary structure alters the properties of the protein so that PRPsc has a greater propensity to aggregate. Aggregates of PRPsc accumulate within cells in the brain of

* Since this manuscript was submitted for publication a fourth case of probable transfusion-transmission of vCJD by non-leukodepleted red cell preparations has been reported in the UK (http://www.hpa.org.uk) and a further study demonstrating removal of endogenous TSE infectivity from leukodepleted scrapie-infected hamster whole blood by filtration through prion-specific affinity resins has been published (Gregori L, Gurgel PV, Lathrop JT *et al.*, 2006 Lancet 368;2226–2230).

affected individuals creating the toxic environment that ultimately results in spongiform encephalopathy. Size fractionation suggests the most infectious prion particles comprise aggregates of 14–28 molecules [12]. Once a small amount of PRPsc is ingested it can associate with PRPc from the affected individual and convert this PRPc to PRPsc creating an autocatalytic effect, which greatly increases the amount of PRPsc in the affected individual. This autocatalytic effect has been reproduced *in vitro* in a hamster model [13].

Normal prion protein is essential for the disease process

PRPc must be available for prion disease to occur [14,15,16•]. Furthermore, mice engineered to translate PRPc without a GPI anchor accumulate PRPsc in the brain, blood and heart but do not develop clinical scrapie [17,18]. These results indicate that membrane tethering of PRPc is essential for disease progression. GPI-linked proteins frequently occupy lipid rafts in the plasma membrane. In cultured adult sensory neurones PRPc leaves lipid rafts to recycle between the cell surface and recycling endosomes in a time scale of minutes [19]. The mechanism whereby PRPc is converted to PRPsc is not fully understood but may occur at the cell surface when PRPc leaves its lipid raft prior to endocytosis [20]. After endocytosis, PRPc goes to recycling endosomes [21] while PRPsc trafficks to lysosomes [22]. Passage of PRPsc to lysosomes can be via multivesicular bodies from which small vesicles 40–100 nm in diameter (exosomes) rich in GPI-linked proteins bud off and are released from the cell. These exosomes can contain PRPsc and have the potential to transfer infectivity from one cell/tissue to another [23].

Transfer of infectious prion protein from gut to brain

After peripheral infection, PRPsc accumulates and replicates in the lymphoreticular system, particularly the spleen and lymph nodes, prior to neuroinvasion and disease. The process by which PRPsc travels from gut to the lymphoid organs may occur via the blood through bone marrow-derived dendritic cells, which pick up PRPsc in the gut and transport it directly to the lymphoid tissues [24]. In rodents, follicular dendritic cells (FDCs) found in the germinal centres of lymphoid organs are major sites of PRPsc accumulation and the rate of transfer of PRPsc from lymphoid tissue to sympathetic nerves is likely determined by the relative positioning of FDCs and sympathetic nerve endings [9,25].

Infectious prion protein in blood

The foregoing discussion describes a process whereby infectivity (PRPsc) in the gut passes via the blood to the spleen and lymphoid cells and thence to the brain by way of antigen-presenting cells capable of taking up and replicating PRPsc. If the antigen-presenting cells come into contact with other blood cells whilst in transit from gut to lymphoid tissues or process PRPsc in a manner which results in the generation of exosomes containing PRPsc it is possible infectivity could transfer to other cells in blood. The cycle of PRPsc replication could continue within those other blood cells that have the necessary intracellular organelles, and those cells without the necessary machinery for recycling PRPsc like red cells may act as passive carriers of infectivity. There is considerable evidence for the occurrence of exosomes in human blood [26] and that they can derive from platelets [27] and reticulocytes [28] as well as from circulating dendritic cells [29]. Furthermore, transfer of GPI-linked proteins CD55 and CD59 from transfused red cells to the red cells of a patient with paroxysmal nocturnal hemoglobinuria has been demonstrated *in vivo* [30]. In this context it is interesting to note that exosomes containing HIV-1 released from immature dendritic cells were found to be 10 times more infective of CD4+ T cells than cell-free virus particles [31].

Exosomes do not provide the only hydrophobic environment in plasma. Recently, evidence has been presented [32•] showing that brain-derived PRPsc binds with high affinity to apolipoprotein B, the major component of very low density and low-density lipoproteins (VLDL and LDL) in plasma.

Infectivity in red cell preparations used for transfusion

There is persuasive evidence [8••] that transfusion of red cell preparations from donors who subsequently developed vCJD has transmitted the disease to three recipients. In each of these cases, the transfusions took place before leucodepletion of red cell preparations was introduced in the UK. Leucodepletion of 450 ml whole blood collected from scrapie-infected hamsters removed 42% of the total infectivity [33]. Whether or not a similar reduction in infectivity is achieved by leucodepletion of human blood is unknown. More relevant is whether or not leucodepletion of human blood is sufficient to prevent transfusion–transmission of vCJD. The follow-up of recipients of leucodepleted red cell preparations from donors who subsequently developed vCJD will provide information of relevance to this question [8••]. The leucodepletion process itself does not appear to result in increased numbers of leucocyte microvesicles that may carry infectivity [34] but would probably not remove exosomes. Given the uncertainty concerning the effectiveness of leucodepletion in removing infectivity from human blood, attention has turned to the possibility of employing filters, which selectively remove PRPsc. Sowemimo-Coker *et al.* [35] filtered 300 ml red cells from 500 ml anticoagulated whole blood collected from scrapie-infected hamsters. They report transmission of

disease to six of 43 hamsters receiving unfiltered red cells but none of 35 hamsters given filtered cells. Gregori *et al.* [36•] report removal of all but 0.01% infectivity from leucodepleted human red cells spiked with scrapie from hamster brain. These studies indicate that infectivity is not intrinsic to red cells or that if infectivity is associated with red cells it is loosely bound and removed by the filtration process. These data, if transferable to the human situation, provide a means of securing the safety of red cell transfusions in countries where the population has been exposed to BSE. Neither study, however, precisely mimics the human situation and so it is necessary to consider the suitability of hamster scrapie as a model for BSE and the similarity between the blood cells of hamsters and humans.*

Of hamsters and men

As it is extremely difficult to design experiments that directly address the biology of vCJD in human blood, most of the data available relate to animal red cells and transmission of scrapie rather than BSE. Whole blood transfusions between sheep have demonstrated transmission of BSE but these experiments have not yet been extended to transfusion of the individual components of blood [37].

As described above, available evidence suggests that prion disease cannot develop in the absence of PRPc. It is therefore reasonable to ask what is the distribution of PRPc in human blood cells and how does it compare with PRPc distribution in blood cells of animals used for investigation of blood-borne TSE infectivity, since differences in PRPc expression may occur and be relevant to disease progression. Holada and Vostal [38] report flow cytometric experiments demonstrating low levels of PRPc on human red cells and absence of PRPc from hamster red cells. Experiments of this type, which utilize a single monoclonal antibody to PRPc, may give erroneous information if the relevant PRPc epitope is not accessible on the cell type examined because of differences in posttranslational modifications like glycosylation [39]. If hamster red cells differ from human red cells in lacking PRPc expression, however, are hamsters a relevant model with which to study the infectivity of human red cells?

If hamster scrapie strain 237K PRPsc does not bind to human red cells does this necessarily mean that BSE/vCJD PRPsc does not bind either? Nishina *et al.* [40•] reported that diglycosylated hamster brain PRPc is required for the amplification of hamster PRPsc strain 237 *in vitro* whereas unglycosylated mouse brain PRPc is required for the amplification of RML PRPsc, a clear indication that different sources of PRPsc have different requirements for glycosylation of PRPc. Earlier work [41] also demonstrated that the glycosylation profile of PRPc can influence the amount of PRPsc bound.

The same protein can have different glycosylation profiles in different tissues from the same animal [42,43]. Clearly, such tissue-specific differences in glycosylation of PRPc could result in tissue-specific differences in binding and replication of PRPsc and account for heterogeneity of PRPc isoforms observed in different regions of mouse brain and for different patterns of PRPsc deposition by different PRPsc strains [44].

These considerations lead to the conclusion that expression of PRPc on a given cell or tissue is not, of itself, an indication of susceptibility to PRPsc binding. Consequently, the glycosylation profile of PRPc on red cells may influence the ability of different strains of PRPsc to bind to red cells and may account for the lack of PRPsc binding observed in animal experiments described above. The same reasoning applies to the interpretation of animal experiments examining the infectivity of blood platelets. Hamster platelets lack PRPc whereas human platelets express PRPc at high levels [45]. Platelets were found to lack infectivity in the blood of hamsters infected with hamster scrapie [46]. The glycosylation profile of the complement regulatory protein CD59 on human red cells and platelets has been determined in detail. The protein on both cell types is extensively glycosylated but the glycosylation profiles of the protein on the two cell types are distinct [43]. If PRPc on red cells and platelets is glycosylated in a similar manner this may account for absence of PRPsc binding because large N-linked oligosaccharides at Asn181 and Asn197 could shield large parts of the surface of the prion protein and sterically hinder protein–protein interactions [47]. This could also explain why murine red cells which express PRPc [38] lacked infectivity when derived from animals infected with mouse-adapted vCJD [48]. Nevertheless, it would be prudent to investigate binding of BSE PRPsc to human red cells and platelets before assuming that these cells do not carry vCJD infectivity, since the glycosylation profile of a protein can differ between species [49,50].

Human red cell PRPc and hamster brain PRPc may differ in the structure of the GPI anchor. On human red cells, GPI-linked proteins CD59 and acetylcholinesterase are unusual in that the GPI anchor is palmitoylated in a way that renders it resistant to phospholipase C [43,51]. If as Rudd *et al.* [43] point out this is likely to be a feature of all GPI-linked proteins on the red cell, then red cell PRPc would have the same anchor. The GPI anchor found on PRPc from Syrian hamster brain is not palmitoylated in

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this way [47]. This difference may influence the location of PRPc in lipid rafts and thence accessibility to PRPsc [20,52].

Finally, there is also the possibility that human red cells could bind PRPsc independently of PRPc. PRPsc binds with high affinity to plasma lipoproteins [32•]. Plasma LDLs have been reported to bind red cells, albeit with low affinity [53].

Conclusion

Recent reports show there is a high probability that human red cell preparations have transmitted vCJD. Experiments carried out with rodent TSEs indicate that infectivity in red cell preparations is not associated with the red cells themselves but with other constituents of the product such as residual leukocytes and plasma. Lack of intrinsic red cell infectivity may result from posttranslational modifications of the structure of red cell PRPc which prevent PRPsc binding. If it can be shown that the causative agent of vCJD fails to bind human red cells and in the absence of a suitable screening test for PRPsc in blood, it may be prudent for blood services in countries where vCJD occurs to consider processing red cell preparations by washing or filtration to remove fluid phase infectivity prior to transfusion.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 291).

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