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研究報告の概要

○慢性消耗病に感染したシカの唾液および血液中の感染性プリオン
シカ科動物における慢性消耗病(CWD)やその他のプリオン疾患の伝播には、体液中のプリオン潜在が重大な懸念事項である。この問題を解決するため、CWD非感染シカのコホートをCWD陽性のシカの唾液、血液または尿・糞便に曝露させた。その結果、CWDを伝播しうる感染性プリオンが唾液(経口経路)および血液(輸血経路)中に認められた。この結果から、CWDはシカ科の動物に容易に伝播すると言える。プリオンに感染した体液との接触には注意が必要である。

報告企業の意見	今後の対応
CWD感染シカの唾液および血液からCWD陰性シカに感染性プリオンが伝播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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赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

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

abnormal hyperphosphorylation of TDP-43 might play a role in FTLD-U pathogenesis. Because UBIs are defined by ubiquitin immunohistochemistry, we asked whether TDP-43 recovered from urea fractions of FTLD-U brains is ubiquitinated, and this was shown to be the case by immunoprecipitation studies using the rabbit polyclonal anti–TDP-43 followed by immunoblot analyses with both anti–TDP-43 and ubiquitin antibodies (Fig. 3D).

FTLD-U and ALS have been suggested to be part of a clinicopathological spectrum (*23*), sharing similar pathogenic mechanisms that affect different populations of CNS neurons. We examined classic ALS cases for the presence of TDP-43–positive UBIs (table S1, nos. 54 to 72). Although none of the inclusions typical of ALS were detected by mAbs 182 and 406, all UBIs (including skeinlike, round, and Lewy body–like inclusions) in motor neurons of ALS were robustly double-labeled by TDP-43 and ubiquitin antibodies (Fig. 4, A to F) and by single-label TDP-43 immunohistochemistry (Fig. 4, G to I). A significant number of ALS patients demonstrate UBIs in hippocampus and frontal and temporal cortex (*23*), which were also immunolabeled by TDP-43 (Fig. 4, J and K).

Immunoblots of urea fractions of spinal cord as well as frontal and temporal cortices of ALS cases demonstrated a disease-specific signature for TDP-43 similar to that described above for FTLD-U (Fig. 4L). Dephosphoryation of the urea fractions showed that the 45-kD band in ALS corresponds to pathologically hyperphosphorylated TDP-43 as in FTLD-U (Fig. 4M). However, because the presence of UBIs in ALS cases is more variable than their presence in FTLD-U, not all brain regions examined in all cases exhibited pathological TDP-43.

These studies identify TDP-43 as the major disease protein in the signature UBIs of FTLD-U and ALS. Although pathologically altered TDP-43 proteins were present in all sporadic and familial FTLD-U as well as ALS cases, there were subtle differences in these abnormal TDP-43 variants among the three FTLD-U subtypes, which may be the result of similar but not identical pathogenic mechanisms. The differential distribution of UBIs detected by ubiquitin antibodies in FTLD-U subtypes (*18*) supports this view.

TDP-43 is a ubiquitously expressed, highly conserved nuclear protein (*24*) that may be a transcription repressor and an activator of exon skipping (*21*, *25*, *26*) as well as a scaffold for nuclear bodies through interactions with survival motor neuron protein (*27*). TDP-43 is normally localized primarily to the nucleus, but our data indicate that, under pathological conditions in FTLD-U, TDP-43 is eliminated from nuclei of UBI-bearing neurons, a consequence of which may be a loss of TDP-43 nuclear functions. Moreover, nuclear UBIs are rare in sporadic FTLD-U because most pathological TDP-43 accumulates in neuronal cell bodies or their processes, and it is unclear whether physiological TDP-43 is present at significant quantities in the cytoplasm, axons, and dendrites of normal neurons. Lastly, both FTDP-17U pedigrees examined here contain *PGRN* gene mutations (*11*), but the relation between TDP-43 and *PGRN*, which encodes a secreted growth factor involved in the regulation of multiple processes in development, wound repair, and inflammation (*28*), remains unclear.

The identification of TDP-43 as the major component of UBIs specific to sporadic and familial FTLD-U as well as sporadic ALS resolves a long-standing enigma concerning the nature of the ubiquitinated disease protein in these disorders. Thus, these diseases may represent a spectrum of disorders that share similar pathological mechanisms, culminating in the progressive degeneration of different selectively vulnerable neurons. These insights into the molecular pathology of FTLD-U and ALS can accelerate efforts to develop better therapies for these disorders.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/130/DC1
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Infectious Prions in the Saliva and Blood of Deer with Chronic Wasting Disease

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A critical concern in the transmission of prion diseases, including chronic wasting disease (CWD) of cervids, is the potential presence of prions in body fluids. To address this issue directly, we exposed cohorts of CWD-naïve deer to saliva, blood, or urine and feces from CWD-positive deer. We found infectious prions capable of transmitting CWD in saliva (by the oral route) and in blood (by transfusion). The results help to explain the facile transmission of CWD among cervids and prompt caution concerning contact with body fluids in prion infections.

The prion diseases, or transmissible spongiform encephalopathies (TSEs), are chronic, degenerative, neurological diseases with uniformly fatal outcomes. TSEs are characterized by the conversion of the normal cellular prion protein (PrP^{c}) to an aberrant insoluble partially protease-resistant isoform (PrP^{res}). CWD, a transmissible spongiform encephalopathy of cervids (deer, elk, and moose), was first observed in the 1960s in captive deer and free-ranging deer and elk in northeastern Colorado and southeastern

Wyoming (*1–4*). CWD has now been identified in 14 states in the United States and two Canadian provinces. Despite its facile transmission, the exact mode of CWD infection has not been determined. Indeed, surprisingly little is known about the transmission of naturally occurring TSEs. For example, scrapie in sheep has been recognized for centuries, yet the precise mode of natural transmission remains unclear (*5*, *6*).

To determine whether infectious prions capable of transmitting CWD are present in body fluids and excreta of CWD-infected deer (CWD+), we exposed four cohorts (numbered 1 to 4, n = 3 to 4 per cohort) of 6-month-old CWD-naïve hand-raised white-tailed deer (*Odocoileus virginianus*) fawns from Georgia, United States (Table 1) to blood, saliva, a combination of urine and feces, or brain from free-ranging or captive CWD+ mule deer (*Odocoileus hemionus*) from Colorado, United States (tables S1 and S2). A control cohort (cohort 5, n = 2) received matching inocula collected from confirmed CWD-negative white-tailed deer (*O. virginianus*) from Georgia, United States. Because polymorphism in the normal prion protein gene (PRNP) may influence CWD susceptibility or incubation time in white-tailed deer, PRNP codon 96 genotype for each deer was determined (table S2) (*7*).

The deer fawns were housed in separate isolation suites under strict isolation conditions to exclude adventitious sources of prion exposure [supporting online material (SOM) text], thus permitting conclusions based on only the point-source exposure. After inoculation, the deer were monitored for CWD infection by serial tonsil biopsy performed at 0, 3, 6, and 12 months postinoculation (pi), and at termination (18 to 22 months pi). Equal portions of tissue were collected and stored (–70°C or fixed in 10% formalin) at each serial collection time point (tonsil) and at study termination (palantine tonsil, brain, and retropharyngeal lymph nodes) for the detection of the protease-resistant abnormal prion protein associated with CWD (PrP^{CWD}) (*8*).

Serial tonsil biopsy of each recipient deer revealed that infectious CWD prions were present in saliva and blood from CWD+ donor deer (Table 2). As expected, PrP^{CWD} was demonstrated between 3 and 12 months pi in tonsil biopsies of all four animals inoculated either orally or intercranially with CWD+ brain (cohort 4). More notably, PrP^{CWD} was detected in tonsil biopsies of two of three deer each in both the saliva and blood cohorts (numbers 1 and 2) at 12 months pi. By contrast, deer in the urine and feces inoculation cohort 3 remained tonsil biopsy negative for PrP^{CWD} throughout the 18-month study. Animals in the negative control inoculation cohort 5 also remained tonsil biopsy negative throughout the study.

Deer cohorts 1 (blood), 2 (saliva), and 3 (urine and feces) were electively euthanized at 18 months pi to permit whole-body examination for PrP^{CWD}. The greatest scrutiny was directed toward those tissues previously established to have highest frequency of PrP^{CWD} deposition in infected deer and generally regarded as the most sensitive indicators of infection—medulla oblongata and other brainstem regions, tonsil, and retropharyngeal lymph node. We found unequivocal evidence of PrP^{CWD} in brain and lymphoid tissue of all six tonsil biopsy–positive deer in cohorts 1 (blood) and 2 (saliva), whereas all deer in cohorts 3 and 5 were negative for PrP^{CWD} in all tissues (Table 2 and Figs. 1 and 2).

The transmission of CWD by a single blood transfusion from two symptomatic and one asymptomatic CWD+ donor is important in at least three contexts: (i) It reinforces that no tissue from CWD-infected cervids can be considered free of prion infectivity; (ii) it poses the possibility of hematogenous spread of CWD, such as through insects; and (iii) it provides a basis for seeking in vitro assays sufficiently sensitive to demonstrate PrP^{CWD} or alternate prion protein conformers in blood—one of the grails of prion biology and epidemiology.

The identification of blood-borne prion transmission has been sought before with mixed results (*9–11*). Bovine spongiform encephalopathy and scrapie have been transmitted to naïve sheep through the transfer of 500 ml of blood or buffy coat white blood cells from infected sheep (*12*, *13*). In addition, limited but compelling evidence argues for the transmission of variant Creutzfeldt-Jakob disease (vCJD) through blood from asymptomatic donors (*14–16*). Even in sporadic CJD, PrP^{res} has been found in periph-

Table 1. CWD prion bioassay inoculation cohorts. Cohort 1 fawns received either a single intraperitoneal (IP) inoculation of 250 ml of frozen citrated blood (n = 2) or an intravenous (IV) transfusion with 250 ml fresh citrated whole blood (n = 1) each from a single CWD+ donor. Cohort 2 fawns received a total of 50 ml saliva, each from a different CWD+ donor, orally (PO) in three doses over a 3-day period. Cohort 3 fawns received a total of 50 ml urine and 50 g of feces PO, each from a different CWD+ donor, in divided doses over a 3- to 14-day period. As positive controls, cohort 4 fawns were inoculated with a 10% brain homogenate from a CWD+ donor deer through either a single intracranial (IC) injection of 1 g equivalent of brain (n = 2) or PO with a total of 10 g equivalents of brain (n = 2) divided over a 3-day period. Cohort 5 fawns (n = 2) were inoculated with equivalent amounts of each of the above materials from a single CWD-negative donor deer to serve as negative controls for the study.

Animal cohort	n	Inoculum	Route (n)	Amount	No. of inoculations
1	3	Blood	IV (1), IP (2)	250 ml	1
2	3	Saliva	PO (3)	50 ml	3
3	3	Urine and feces	PO (3)	50 ml + 50 g	3 to 14
4	4	Brain	IC (2), PO (2)	1 g (IC), 10 g (PO)	1 (IC), 3 (PO)
5	2	All of the above	PO (2)	All of the above	1 to 14

Table 2. PrP^{CWD} detection by longitudinal tonsil biopsy and necropsy of deer exposed to body fluids or excreta from CWD+ deer. PrP^{CWD} assay results for tonsil (T), brain (B) (medulla oblongata at obex), and retropharyngeal lymph node (RLN) are shown. The number of deer in which PrP^{CWD} was detected (*8*) is shown over the total number of deer in the cohort. One of the three original animals inoculated with urine and feces was euthanized prematurely 61 days pi due to a bacterial infection. The deer in cohorts 1, 2, and 3 were terminated at 18 months (mo.) pi. Two of the four cohort 4 deer were terminated at 20 and 21 months pi. The two cohort 5 deer were terminated at 22 months pi.

Animal cohort	Inoculum	Biopsy collection					
		3 mo. (T)	6 mo. (T)	12 mo. (T)	Termination		
					T	B	RLN
1	Blood	0/3	0/3	2/3	3/3	2/3	3/3
2	Saliva	0/3	0/3	2/3	3/3	2/3	3/3
3	Urine and feces	0/2	0/2	0/2	0/2	0/2	0/2
4	Brain	1/4	2/4	4/4	2/2	2/2	2/2
5	Negative samples	0/2	0/2	0/2	0/2	0/2	0/2

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eral organs of some patients (*17*). The present work helps establish that prion diseases can be transmitted through blood.

The presence of infectious CWD prions in saliva may explain the facile transmission of CWD. Cervid-to-cervid interactions (SOM text), especially in high density and captive situations, would be expected to facilitate salivary cross-contact (*11*, *18*, *19*). Salivary dissemination of prions may not be limited to CWD. Protease-resistant prion protein has been demonstrated in the oral mucosa, taste buds, lingual epithelium, vomeronasal organ, and olfactory mucosa of hamsters infected with transmissible mink encephalopathy (*19*) and ferrets infected with CWD (*20*). Although no instance of CWD transmission to humans has been detected, the present results emphasize the prudence of using impervious gloves during contact with saliva or blood of cervids that may be CWD-infected.

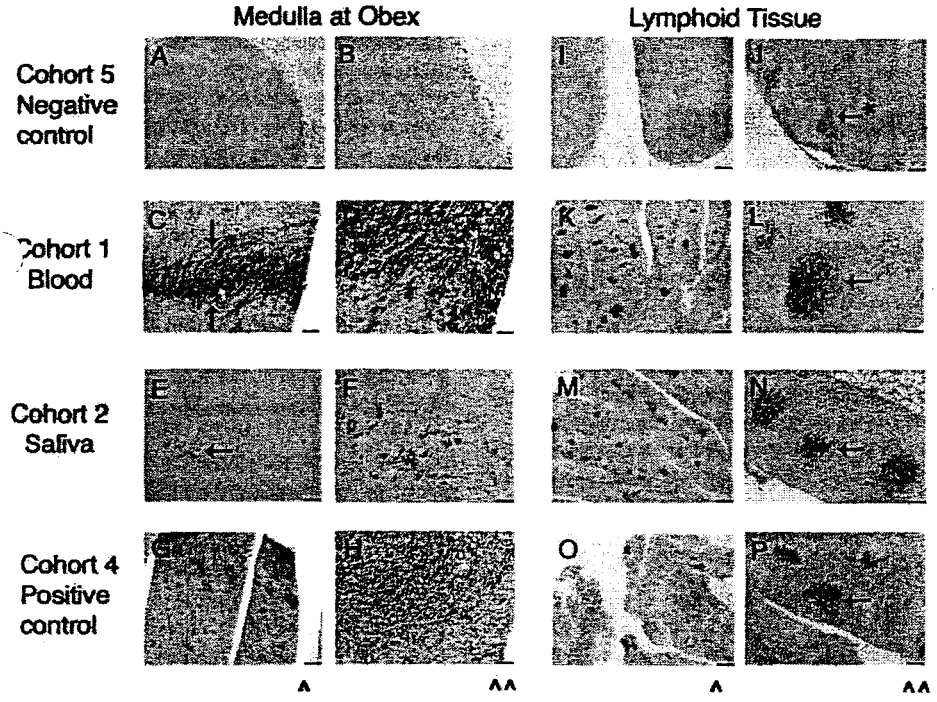

Fig. 1. PrP^{CWD} demonstrated by immunohistochemistry in tonsil, brain (medulla oblongata at obex), and retropharyngeal lymph node of deer receiving saliva or blood from CWD-infected donors. CWD immunohistochemistry is shown in the medulla at obex (**A** to **H**) and either tonsil or retropharyngeal lymph node (**I** to **P**) (*8*). Arrows indicate PrP^{CWD} staining (red) within brain and lymphoid follicles. Arrow with asterisk indicates lymphoid follicle negative for PrP^{CWD}. ^, scale bar = 50 μm; ^^, scale bar = 110 μm.

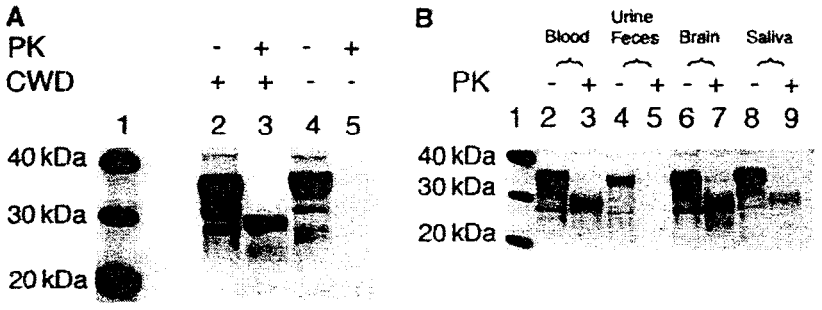

Fig. 2. Immunoblot demonstration of PrP^{CWD} in brain (medulla) of white-tailed deer. (**A**) PrP^{CWD} detection in positive and negative control deer (*8*). Lane 3 demonstrates the expected molecular weight shift upon partial proteinase K (PK) digestion of PrP^{CWD} in CWD+ deer, whereas lane 5 shows the complete digestion of PrP^{C} in CWD-negative deer. Molecular weight markers are indicated in lane 1. (**B**) Assay for PrP^{CWD} in medulla at obex homogenates for deer inoculated with blood, urine and feces, brain, and saliva, with and without PK digestion (*8*). Molecular weight markers are indicated in lane 1. Lanes 3, 7, and 9 demonstrate the detection of PrP^{CWD}, whereas lane 5 demonstrates the lack of PrP^{CWD}.

Environmental contamination by excreta from infected cervids has traditionally seemed the most plausible explanation for the dissemination of CWD (*21*). However, we could not detect PrP^{CWD} in cohort 3 deer inoculated repeatedly with urine and feces from CWD+ deer and examined up to 18 months pi (Table 2). There are several reasons to view this negative finding cautiously, including small sample size, elective preclinical termination, and potential variation in individual susceptibility that may be associated with the 96 G/S polymorphism in the PRNP gene (*7*, *22*). Although no genotype of white-tailed deer is resistant to CWD infection, PRNP genotypes S/S or G/S at codon 96 appear to have reduced susceptibility manifest by longer survival (*7*). Both deer in cohort 3 (urine and feces) were subsequently shown to be of the PRNP 96 G/S genotype. Thus, it is possible, although we think unlikely, that these deer had a prolonged incubation period (>18 months pi) before the amplification of PrP^{CWD} became detectable in tissues. Recent studies have shown that PrP^{res} is poorly preserved after incubation with intestinal or fecal content (*23*, *24*). Further research using cervid and surrogate cervid PrP transgenic mice (*25*) are indicated to continue to address the presence of infectious CWD prions in excreta of CWD+ deer and to provide a more substantial basis for reconsideration of the assumption that excreta are the chief vehicle for CWD dissemination and transmission.

The results reported here provide a plausible basis for the efficient transmission of CWD in nature. We demonstrate that blood and saliva in particular are able to transmit CWD to naïve deer and produce incubation periods consistent with those observed in naturally acquired infections (*3*, *26*). The time from exposure to first detection of PrP^{CWD} by tonsil biopsy was variable—as short as 3 months but as long as 18 months (likely underestimates due to sampling frequency). The results also reinforce a cautious view of the exposure risk presented by body fluids, excreta, and all tissues from CWD+ cervids.

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Modulation of Cell Adhesion and Motility in the Immune System by Myo1f

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Although class I myosins are known to play a wide range of roles, the physiological function of long-tailed class I myosins in vertebrates remains elusive. We demonstrated that one of these proteins, Myo1f, is expressed predominantly in the mammalian immune system. Cells from Myo1f-deficient mice exhibited abnormally increased adhesion and reduced motility, resulting from augmented exocytosis of β2 integrin–containing granules. Also, the cortical actin that co-localizes with Myo1f was reduced in Myo1f-deficient cells. In vivo, Myo1f-deficient mice showed increased susceptibility to infection by *Listeria monocytogenes* and an impaired neutrophil response. Thus, Myo1f directs immune cell motility and innate host defense against infection.

In both mouse and human genomes, 16 genes encode conventional class II muscle and nonmuscle myosins, with 25 "unconventional" myosin genes encoding 11 other classes (*1*). Natural mutations of various myosin genes result in an array of genetic disorders, including cardiomyopathies, deafness, blindness, glomerular nephritis, and neuropathies (*2*, *3*). The class I myosins are the largest group of unconventional myosins and are evolutionarily ancient, existing in a wide range of species from yeast to vertebrates (*1*, *4*). Mice and humans have a total of eight class I myosin heavy-chain genes, six of which encode short-tailed forms (Myo1a, b, c, d, g, and h) and two of which encode long-tailed (amoeboid) forms (Myo1e and f) (*1*). All class I myosins consist of an N-terminal motor domain, light-chain–binding IQ motifs, and a basic tail homology 1 (TH1) domain thought to affect interactions with membranes (*2*). The long-tailed class I myosins have an additional proline-rich TH2 domain and a TH3 domain containing a single Src homology 3 (SH3) domain (*2*).

The class I myosins in *Dictyostelium* and yeast are involved in migration, phagocytosis, endocytosis, and actin remodeling (*5*, *6*). Short-tailed class I myosins in vertebrates are involved in more specialized functions, such as the adaptation of hair cells in the ear (*7*) and the transport of vesicles and organelles (*8*, *9*), as well as the structural maintenance of the enterocyte microvilli (*10*). However, the function of long-tailed class I myosins in vertebrates is poorly characterized (*11*–*14*).

Myo1f was first identified in our screen for differentially expressed genes in subsets of murine lymphocytes. In contrast to previous data suggesting the widespread expression of Myo1f in tissues (*15*), our results, which we obtained using specific probes, showed that Myo1f is selectively expressed in the spleen, mesenteric lymph nodes, thymus, and lung (Fig. 1A). By comparison, specific detection of Myo1e showed a predominant expression pattern in the spleen and mesenteric lymph nodes and moderate expression in the lung, small intestine, and large intestine (Fig. 1B). Within the lymphoid tissues, natural killer (NK) cells, macrophages, and dendritic cells were found to express considerable levels of both Myo1f and Myo1e; neutrophils and B cells showed selective expression of Myo1f and Myo1e, respectively (Fig. 1, C and D).

To determine the function of Myo1f in the vertebrate immune system, we generated Myo1f gene–deficient mice. We focused on neutrophils because Myo1f was detected exclusively in neutrophils (Fig. 1, C and D). Immunoglobulin G (IgG)–mediated phagocytosis was similar between wild-type and knockout (KO) neutrophils (Fig. 1E). To evaluate the degree of pathogen killing that follows phagocytosis, we measured the production of reactive oxygen species. Again, no considerable difference was detected between wild-type and KO neutrophils (Fig. 1F). Thus, Myo1f is dispensable for both the phagocytosis of bacteria and their destruction.

Integrin-mediated adhesion to the vascular endothelium is crucial in the process of neutrophil migration to infected tissue, and the dominant integrins involved in this process belong to the β2 integrin (CD18) family (*16*). Myo1f-deficient neutrophils exhibited strong adhesion to integrin ligands, including the intercellular adhesion molecule–1 (ICAM-1) (CD54) and fibronectin (Fig. 2, A and B). Activation of neutrophils by the proinflammatory cytokine tumor necrosis factor–α did not compensate for this difference, suggesting that increased adhesion did not result from changes in the activation status of Myo1f-deficient cells. Experiments with a blocking antibody showed that most of the adhesion was mediated by β2 integrin (Fig. 2, A and B). In addition, Myo1f affected only integrin-mediated adhesion, not integrin-independent adhesion to polylysine-coated substrate (Fig. 2C) (*17*). Spreading of Myo1f-deficient neutrophils on ICAM-1 was also increased as compared to that of wild-type neutrophils (Fig. 2D). Increased spreading was not due to a loss of cortical tension (fig. S2), which acts to maintain the round shape of the cells (Fig. 2E). In contrast, myosin I double mutants in *Dictyostelium* exhibit abnormalities

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研究報告の概要

○PRDT TSE親和性リガンドを用いた血中の内在性TSE感染性の除去及びMacoPharma P-Capt™ Filterへのリガンドの組み込み

背景:供血後にvCJDで死亡した供血者の血液を輸血されたことが判明し、疾患が検知可能となるまで生存した受血者19名のうち、vCJD輸血感染症例は3例で感染率は15%以上である。外科的に除去した組織の調査では、英国のvCJD潜伏症例は最低でも4000例と見積もられ、うち平均7%が受血者である。

目的:プリオンタンパク質およびTSE感染性に高親和性のリガンドを開発し、血液製剤によるTSE感染症伝播リスクを軽減するため、RBCやその他血液成分からTSE感染性を除去する器具に当該リガンドを組み入れること。

方法:Pathogen Removal and Diagnostics Technologies(PRDT)は、全血、RBCまたは血漿存在下の脳由来プリオンタンパク質およびTSE感染性と高親和性のリガンドを得るため数百万の化合物のスクリーニングを行った。血中の内在性TSE感染性の除去をテストするため、スクレイピー感染ハムスターから4時間以内に血液~500 mlsを採取し、商用フィルターで白血球除去を行い被験樹脂を通過させた。全血、白血球除去血液および樹脂通過血液を分注し、限界希釈法で力価測定を行った。ハムスター100匹に各サンプルの分注50μg(合計5mL)を接種した。ハムスターの自然死が起り始めた540日で実験を終了し、プリオンアミロイドの証拠がないか各動物の脳を調べた。

結果:もっとも優れた樹脂は、脳由来プリオンタンパク質でスパイクしたRBC 10'ID/mlの力価を、4 log(10)ID(50)以上減少させ、血液やRBCに関して非常に低濃度の感染性に対して求められる性能を大きく上回った。脳由来物スパイクの妥当性が不確実であることから、血中に内在する感染源の除去能についても評価した。樹脂処理した血液を接種したハムスター100匹のうち、感染したハムスターはいなかった。当該樹脂は、TSE血液感染性(> 1.2 log(10)ID)を除去した。この樹脂は広範囲の血液適合性検査にパスした。これは、MacoPharmaにより白血球除去赤血球からTSE感染性を除去する独立型のP-Capt™ filterに取り入れられている。

結論:PRDTの樹脂は、高濃度のTSE感染性を吸着し、RBC存在下でも効果が高く、白血球除去血液中の低濃度内在性感染性を検出限界まで除去する。P-Capt™ filterとして実現した当該樹脂は、血液および血液製剤によるvCJD伝播のリスクを有意に減少させるはずである。

報告企業の意見	今後の対応
PRDT TSE親和性リガンドならびにリガンドを組み入れたMacoPharma P-Capt™ Filterを用いて血中に存在する内在性TSE感染性を除去できたとの報告である。	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

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

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

to the whole blood and RCC filtrations, including losses in tubes and bags by transfer, of about 20 g Hb. The CompoSafe Pr filtration causes an additional loss compared to whole blood filtration of about 7 g Hb. The filtration over the CompoSafe Pr filter induced a slight increase of hemolysis (about 0.03% increase), but this did not result in a more rapid increase during subsequent storage: after 42 days 0.26% hemolysis for group I and 0.16% for group II were detected. For group I at day 42 1.3 % of the red cells were positive for AnnexinV (representing PS-exposure), whereas for group II this was 0.9 % (comparable to standard leukodepleted RCC in SAGM). The amount of ATP was predicted to be above 2.7µmol/g Hb at day 35, with about 15 mM glucose remaining at day 42.

Conclusion: Despite the double filtration step in the total procedure (one to leukoreduce the whole blood and one to remove prions from RCC), the remaining RCC met the European requirements for Hb content. The amount of Hb in the final RCC is similar to that in leukodepleted RCC prepared from whole blood after buffy coat depletion. The in vitro quality after 42 days was similar to those of standard leukodepleted RCC, prepared from whole blood after buffy coat depletion.

P-091
DETECTION OF INFECTIOUS PRION IN BLOOD BY PROTEIN MISFOLDING CYCLIC AMPLIFICATION TECHNOLOGY (PMCA)

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Background: Since the publication of three cases of probable transmission to British patients of the variant of Creutzfeldt-Jacob Disease (vCJD) by blood transfusion, it is likely that infectious prion protein (PrPsc) is present in human blood. Studies on rodent indicate that the estimated sensitivity level of assays needs to reach a minimum of 1 femtomolar (0.1pg/ml or 10IU/ml) in order to detect PrPsc in the blood of patients in the pre-clinical phase of the disease. No test actually available does reach this level of detection and the development of highly sensitive assay for detection of PrPsc in blood is essential to evaluate secondary transmission of the disease by blood transfusion.

Aim: We have chosen to amplify the PrPsc by saPMCA (serial automated Protein Misfolding Cyclic Amplification) prior to the detection of very low levels of infectious prion present in the blood.

Objective: to develop a screening test for PrPsc in human blood components. The first phase consists in the manual reproduction and optimization of the PMCA technology described by C. Soto on hamster brain, in order to adapt the method on an automated sonicator (Misonix S-3000), allowing the detection of PrPsc in blood.

Methods: PMCA allows accelerated production of infectious prions by successive incubation and sonication steps. During incubation, aggregates of PrPsc proteins are produced from low quantity of PrPsc. This template initiates transconformation of the normal prion protein (PrPc) into PrPsc. Sonication of the aggregates leads to numerous small amplification units, each one allowing new conversions. Amplified PrPsc is then detected by Western Blot.

Results: After optimizations, manual PMCA prior to detection by Western blot permits to amplify by 3log10 folds the initial input of PrPsc present in hamster brain or in leukocytes. The saPMCA has then been optimized for high-efficiency amplification of PrPsc. The performances of the Misonix S-3000 have been evaluated on hamster brain which has confirmed the high throughput of the assay. The saPMCA method is reproducible and specific. Adaptation of this technology for PrPsc detection in blood is in progress.

Conclusion: The saPMCA prior to detection by Western blot appears to be a powerful approach for early diagnosis of prion diseases. The development of this technology as a non invasive screening test for PrPsc in human blood donation will allow to evaluate the potential spread of the prion by blood transfusion.

P-092
REMOVAL OF THE ENDOGENOUS TSE INFECTIVITY PRESENT IN BLOOD USING PRDT TSE AFFINITY LIGANDS AND INTEGRATION OF THE LIGANDS INTO THE MACOPHARMA P-CAPT(TM) FILTER

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Background: To date, there have been three transfusion transmitted cases of vCJD among 19 recipients that are known to have received blood from persons that later died of vCJD and lived long enough themselves to have developed detectable disease. This is a transmission rate of ~15%. If any of the other 16 recipients were infected it will be even higher (www.cjd.ed.ac.uk/TMER). A survey of surgically removed tissues concluded that the number of incubating cases of vCJD in the UK was at least 4000 (Hilton et al. 2004, J. Pathol. 203:733-9). 7% of this group would on average be blood donors.

Aim: To develop ligands with strong affinity for the prion protein and TSE infectivity and incorporate them into devices to remove TSE infectivity from RBCs and other blood components to reduce the risk of transmission of TSE infections from blood products.

Methods: Pathogen Removal and Diagnostics Technologies, PRDT, screened millions of compounds to obtain high affinity ligands that strongly bind brain-derived prion protein and TSE infectivity in the presence of whole blood, RBC or plasma. To test for removal of the endogenous TSE infectivity that is present in blood, ~500 mls of blood was collected in < 4 hours from scrapie infected hamsters, leukoreduced by a commercial filter, and passed through the test resin. Aliquots of whole blood, leukoreduced blood and resin flow-throughs were titered by the limiting dilution method. A total of 5 mls of each sample was inoculated in 50 □l aliquots into 100 hamsters. The experiment was terminated at 540 days when hamsters begin to die of natural causes and the brain of every animal was checked for evidence of prion amyloid.

Results: The best resins reduced the titer of a brain-derived spike into RBC of 10(7) ID/ml by over 4 log(10)ID(50), far exceeding the capacity needed for the very low concentrations of infectivity associated with blood or RBC. To address the uncertainty about the relevance of brain-derived spikes, the lead resin was also assessed for its ability to remove the infectivity endogenously present in blood. There were no infections among the 100 hamsters inoculated with the resin treated blood. The resin removed > 1.2 log(10)ID of relevant TSE blood infectivity. This resin has also passed a broad range of hemocompatibility tests. It has now been incorporated by MacoPharma into the P-Capt™ filter, a stand alone device for removing TSE infectivity from leukoreduced red blood cells.

Summary: PRDT's lead resin adsorbs high concentrations (10(7) ID50/ml) of brain derived TSE infectivity with high efficiency (4 log(10)ID50) even in the presence of RBC, and removes the low concentrations (10 ID/ml) of endogenous infectivity that is present in leukoreduced blood to the limit of detection (>1.2 log(10)ID). Implemented as the Macopharma P-Capt(TM) filter it should significantly reduce the risk of transmission of vCJD by blood and blood products.