医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 3. 30	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		AABB Weekly Report. 2010 March 19.	米国

研究報告の概要

○オランダのQ熱アウトブレイクに対する準備
オランダの血液銀行は、発生が予測される2010年のQ熱アウトブレイクに対する準備を行っている。疾病対策センターの声明によると、オランダでは、2009年11月25日時点で2,293名の症例(死亡例6例を含む)が確認されている。これらは2007年以降続いているQ熱のアウトブレイクの症例である(2007年190例、2008年1,000例)。同国で血液事業の業務を担っているSanquinは、Q熱流行期間に高リスク地域で採血された供血血液をスクリーニングするための核酸増幅検査をする予定である。Transfusion誌2009年8月のSupplementには、Q熱コクシエラのFact Sheetが掲載された。AABBの輸血伝播性疾患委員会は、Fact Sheetを更新するために、オランダのデータを使用する予定である。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-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

報告企業の意見	今後の対応
オランダの血液銀行は、2010年のQ熱アウトブレイクに対する準備を行っており、血液事業を行っているSanquinは、Q熱流行期間に高リスク地域で採血された供血血液をスクリーニングするための核酸増幅検査を行う予定であるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

CDC Notice Alerts Travelers to Possible Dengue Exposure

A recent advisory from the Centers for Disease Control and Prevention has alerted travelers to avoid infection with the dengue virus, the causative agent of Dengue fever as well as one of the most common causes of fever in visitors to tropical and subtropical regions of the world. Although currently not a major threat in the U.S. — except in the commonwealth of Puerto Rico, where the virus is endemic — it has been listed by AABB's Transfusion Transmitted Diseases Committee as one of the top agents of concern to the blood banking and transfusion medicine communities, along with Babesia and the agent responsible for variant Creutzfeldt-Jakob disease. The August 2009 supplement of Transfusion included a fact sheet on the pathogen.

Netherlands Prepares for Q Fever Outbreak

Blood banking officials in the Netherlands are making preparations for the expected 2010 outbreak of Q fever in that country. According to a statement from the Centers for Disease Control and Prevention, as of Nov. 25, 2,293 human cases had been confirmed in the Netherlands in 2009, including six deaths. These cases represent an ongoing outbreak of Q fever in the Netherlands since 2007; 190 cases were reported in 2007 and 1,000 cases in 2008. Sanquin, an organization that provides blood banking technical assistance in the country, will be implementing a nucleic acid amplification testing procedure to screen selected donations from high-risk regions for the duration of the current Q fever epidemic. The August 2009 supplement of Transfusion included a fact sheet on the pathogen involved in Q fever, Coxiella burnetii. AABB's Transfusion Transmitted Diseases Committee will use the data from the Netherlands to update the fact sheet.

Lawmakers, Health Experts Call for Increase in Funding for Hepatitis Efforts

The National Viral Hepatitis Roundtable on Tuesday held a press conference on Capitol Hill urging support of a bipartisan bill, HR 3974, that would direct $90 million to the Centers for Disease Control and Prevention to fund state-based screening, education and prevention efforts targeting chronic viral hepatitis. According to NVHR, an estimated 5 million U.S. residents are infected with viral hepatitis B or C, and the administration's current budget proposal for 2011 would underfund the Division of Viral Hepatitis. Among the lawmakers participating at the media briefing were U.S. Reps. Hank Johnson (D-Ga.), who recently underwent treatment for hepatitis C; Mike Honda (D-Calif.), lead sponsor of the bill; and Bill Cassidy (R-La.), a practicing hepatologist.

Task Force Looks to Improve Transparency Between FDA and Industry

The Transparency Task Force of the Food and Drug Administration is collecting information on ways in which transparency can be improved between the agency and industry. According to a March 12 Federal Register notice, FDA is seeking comments on how improvements can be made in areas such as training and education on the agency's regulatory and guidance-development processes as well as communication, particularly in times of crisis. FDA is making available on the U.S. government regulations e-portal transcripts and summaries of three public listening sessions it held for background information; visitors to the site should use the keywords "transparency task force 2010." Comments must be received by April 12 and can be submitted through this Web site.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 3. 16	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Centers for Disease Control and Prevention (CDC). MMWR Morb Mortal Wkly Rep. 2010 Mar 5;59(8):217-9.	公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			米国

研究報告の概要

○震災後のマラリア(ハイチ)
2010年1月12日、イスパニョーラ島に位置し、ドミニカ共和国と国境を接するハイチにおいて、M7.0の大地震が発生した。震源地は首都ポルトープランスから西に10マイルの地点であった。ハイチ政府によると、約20万人が亡くなり50万人が家を失った。ハイチでは、熱帯熱マラリアが流行しており、主要媒介蚊である*Anopheles albimanus*はしばしば屋外でヒトを刺すため、屋外生活者や一時避難所の収容者、何千名もの緊急対応スタッフは、マラリアへの感染リスクが高い。1月12日～2月25日の期間、CDCは、ハイチで感染した熱帯熱マラリアの検査確定症例の報告を受領した。患者は米国居住者である緊急対応スタッフ7名、ハイチの住民3名、米国人旅行者1名であった。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
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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

報告企業の意見	今後の対応
2010年1月12日に大地震が発生したハイチにおいて熱帯熱マラリアが流行しているとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の献血を一定期間延期している(1～3年の延期を行うとともに、帰国(入国)後マラリアを思わせる症状があった場合は、感染が否定されるまで、献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

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Morbidity and Mortality Weekly Report (*MMWR*)

Malaria Acquired in Haiti --- 2010

Weekly
March 5, 2010 / 59(08);217-219

On January 12, 2010, a 7.0 magnitude earthquake struck Haiti, which borders the Dominican Republic on the island of Hispaniola. The earthquake's epicenter was 10 miles west of the Haiti capital city of Port-au-Prince (estimated population: 2 million). According to the Haitian government, approximately 200,000 persons were killed, and 500,000 were left homeless (*1*). Malaria caused by *Plasmodium falciparum* infection is endemic in Haiti, and the principal mosquito vector is *Anopheles albimanus*, which frequently bites outdoors. Thus, displaced persons living outdoors or in temporary shelters and thousands of emergency responders in Haiti are at substantial risk for malaria. During January 12 -- February 25, CDC received reports of 11 laboratory-confirmed cases of *P. falciparum* malaria acquired in Haiti. Patients included seven U.S. residents who were emergency responders, three Haitian residents, and one U.S. traveler. This report summarizes the 11 cases and provides chemoprophylactic and additional preventive recommendations to minimize the risk for acquiring malaria for persons traveling to Haiti.

Of the seven emergency responders, six were U.S. military personnel. Among the six, four cases were uncomplicated and treated locally in Haiti. Two other patients were moderately to seriously ill and transferred to the United States for intensive care; one required intubation and mechanical ventilation for acute respiratory distress syndrome. All are expected to make a full recovery.

All six military personnel had been provided oral chemoprophylaxis with doxycycline before departure from the United States and personal protective equipment (e.g., insect repellent and insecticide-treated netting and uniforms) after arrival in Haiti. Of the 11 total patients, chemoprophylaxis was indicated for the seven emergency responders and the lone U.S. traveler. Six of these eight patients (including the two hospitalized military personnel) reported nonadherence to the recommended malaria medication regimen. Adherence status was unknown for the remaining two patients.

Three cases occurred in Haitian residents who traveled to the United States, including one Haitian adoptee. The number of U.S. malaria cases imported from Haiti likely is underestimated because typically not all cases are reported to CDC.

Reported by

K Mung, MD, B Renamy, MSc, Pan American Health Organization. JF Vely, MD, R Magloire MD, Ministry of Public Health and Population, Haiti. N Wells, MD, US Navy Medical Corps, J Ferguson, DO, US Army Medical Corps. D Townes, MD, M McMorrow, MD, K Tan, MD, B Divine, L Slutsker, MD, Malaria Br, Div of Parasitic Diseases, Center for Global Health, CDC.

Editorial Note

In 2008, a total of 1,298 cases of malaria in the United States were reported provisionally to CDC, and 527 (40.6%) were caused by *P. falciparum*; all but two of the malaria cases were imported (CDC, unpublished data, 2009). Most imported cases are in travelers returning to the United States from areas in Africa, Asia, and the Americas where malaria transmission is known to occur (2). Of the four *Plasmodium* species that routinely infect humans (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*), *P. falciparum* causes the most severe disease and highest mortality and is the predominant species in Haiti (3,4). Information regarding the incidence of malaria in Haiti is limited. Historically, malaria transmission peaks in Haiti after the two rainy seasons, with a primary peak during November--January and a secondary peak during May--June. Although each year Haiti reports approximately 30,000 confirmed cases of malaria to the Pan American Health Organization, as many as 200,000 cases might occur annually. One population-based survey in 2006 in the Artibonite Valley, located 75 miles north of Port-au-Prince, found an overall prevalence of *P. falciparum* infection of 3.1% (14.2% in febrile and 2.1% in nonfebrile persons) (4).

Prompt diagnosis and treatment of malaria as well as chemoprophylaxis when appropriate are critical. Recommendations for antimalarials for treatment and prevention are based on information on parasite drug susceptibility for a specific geographic setting. In Haiti, the first-line treatment for malaria is chloroquine. No evidence exists of clinical failure of chloroquine treatment in persons with *P. falciparum* infection acquired in Hispaniola, nor has chloroquine prophylaxis failure been documented in travelers. However, one published study found five of 79 (6.3%) *P. falciparum* isolates collected in the Artibonite Valley in Haiti in 2006 and 2007 carried a mutation associated with parasite resistance to chloroquine (5). Although the findings do not serve as a basis for prophylaxis and treatment policy change, they do point out the need for heightened awareness of potential failure of chloroquine treatment or prophylaxis in persons in Haiti or returning from Haiti.

Persons traveling to Haiti should receive chemoprophylaxis with one of the following medications: atovaquone-proguanil, chloroquine, doxycycline, or mefloquine (6). If preventive medications are started <1 week before departure, or while already in Haiti, either atovaquone-proguanil or doxycycline are recommended. Use of weekly chloroquine requires receiving the initial dose 1 week before departure, and use of weekly mefloquine requires receiving the initial dose 2 weeks before departure. Mosquito avoidance measures should be taken, such as using mosquito repellent, wearing protective clothing, and sleeping under an insecticide-treated mosquito net. Chemoprophylaxis, although highly effective in preventing malaria, is not 100% effective. Therefore, if fever develops in persons taking chloroquine or other antimalarials for chemoprophylaxis, they still should be evaluated for malaria infection with a diagnostic test.

CDC currently recommends microscopic examination of blood smears for malaria diagnosis. Three negative malaria smears spaced 12--24 hours apart are needed to rule out malaria. However, microscopy capacity in Haiti is limited at this time. A diagnostic option frequently used in emergency settings in areas with high prevalence of malaria is a rapid diagnostic test based on antigen detection. However, if laboratory diagnosis of malaria is not possible, presumptive treatment based on clinical suspicion of malaria (e.g., unexplained fever) should be given. Rapid diagnostic tests for malaria can remain positive up to 3 weeks after treatment and should not be used to assess treatment failure in a patient with malaria.

Persons with laboratory-confirmed *P. falciparum* malaria acquired in Haiti and treated in the United States and emergency responders treated in the field should receive treatment according to CDC guidelines (7). Uncomplicated malaria can be treated with one of the following regimens: chloroquine, artemether-lumefantrine, atovaquone-proguanil, or the combination of quinine and doxycycline, tetracycline, or clindamycin. In patients with confirmed malaria who report adherence to chemoprophylaxis in Haiti, a change to a

different drug than that taken for chemoprophylaxis is recommended for treatment. Clinicians should consider switching patients with uncomplicated, laboratory-confirmed malaria from chloroquine treatment to other recommended drugs after any indication of poor response to chloroquine such as increasing parasite density 24 hours after starting treatment, persistent parasitemia 48 hours after starting treatment, or clinical deterioration. Severe malaria requires treatment with intravenous quinidine and one of the following: doxycycline, tetracycline, or clindamycin. Intravenous artesunate also is available from CDC for use in the United States as part of an investigational drug protocol. If treating severe malaria in a responder in the field, treatment should be initiated with available medications and consideration given to immediate medical evacuation.

In Haiti, residents with malaria should be treated in accordance with that country's national treatment guidelines. First-line treatment for uncomplicated malaria in Haiti is chloroquine. First-line treatment for severe malaria in Haiti is intravenous or intramuscular quinine.

CDC continues to monitor the malaria situation in Haiti, including any reports of possible chloroquine prophylaxis or treatment failures in those returning from Haiti. Medical providers should contact the CDC Malaria Branch clinician on call (770-488-7100) for clinical consultations and to discuss cases of apparent chloroquine treatment or prophylaxis failures and testing of parasites at CDC for resistance markers. Additional information on malaria is available at http://www.cdc.gov/malaria.

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What is already known on this topic?

Malaria caused by *Plasmodium falciparum* infection is endemic in Haiti, where the January 12 earthquake and resultant living conditions have placed many displaced residents and emergency responders at substantial risk for malaria.

What is added by this report?

This report summarizes 11 cases of malaria from Haiti reported to CDC and outlines

recommendations for appropriate malaria chemoprophylaxis for persons traveling to Haiti.

What are the implications for public health practice?

Adherence to preventive chemoprophylaxis recommendations and appropriate personal protective measures can lower malaria risk, and prompt diagnosis and treatment of malaria in travelers to Haiti and persons in Haiti can improve their outcomes.

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Content source: Centers for Disease Control and Prevention

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医薬品
医薬部外品　　研究報告　調査報告書
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識別番号・報告回数			報告日	第一報入手日 2010年4月12日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②③ポリエチレングリコール処理抗破傷風人免疫グロブリン ④⑤乾燥抗破傷風人免疫グロブリン		研究報告の公表状況	Neuropathology 2010; 30(2): 159-164	公表国 日本	
販売名 (企業名)	①テタノブリンIH静注250単位　(ベネシス) ②テタノブリンIH静注1500単位　(ベネシス) ③テタノブリン-IH　(ベネシス) ④テタノブリン筋注用250単位　(ベネシス) ⑤テタノブリン　(ベネシス)					

研究報告の概要

プリオン・タンパク質遺伝子（PRNP）のコドン180の位置の点変異によるCreutzfeldt-Jakob病の剖検症例についての報告である。
77歳の女性は不安定な歩行を呈し、痴呆および腕/体幹の失調が続いた。彼女は運動不能および18ヵ月間無言になり、発症から26ヵ月後に肺炎で死亡した。PRNP遺伝子解析によりコドン180の点変異が明らかになった。解剖により著しい海綿状態、神経細胞の喪失、および大脳皮質における神経膠星状細胞腫が明らかになった。軽度から中等度の海綿状態および神経細胞の喪失は大脳辺縁系と大脳基底核で観察された。海馬、脳幹または小脳での海綿状変化は見られなかった。多くの老人斑と神経細胞変化が発見され、それぞれブラークステージはステージCとⅣであった。
プリオン蛋白（PrP）の免疫染色では大脳皮質と特に海馬での粒状（シナプス型）と斑点状のPrP蓄積物が確認された。ほとんどの斑点状の蓄積はアミロイドβプラークと共存していたが、一部は分離していた。相対的に強固なPrP蓄積物とこの症例のアルツハイマー型病変の共存は顕著であった。アミロイドβプラークはPrP蓄積の促進因子として作用している可能性がある。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンIH静注250単位の記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)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
プリオン・タンパク質遺伝子（PRNP）のコドン180の位置の点変異によるCreutzfeldt-Jakob病の剖検症例で、PRNP遺伝子解析によりコドン180の点変異が明らかになったことについての報告である。 血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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Case Report

An autopsy case of Creutzfeldt-Jakob disease with a V180I mutation of the PrP gene and Alzheimer-type pathology

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We report an autopsy case of Creutzfeldt-Jakob disease with a codon 180 point mutation of the prion protein gene (PRNP). A 77-year-old woman developed gait instability, followed by dementia and limb/truncal ataxia. She became akinetic and mute 18 months and died of pneumonia 26 months after the disease onset. Analysis of the *PRNP* gene revealed a codon 180 point mutation. Post-mortem examination revealed marked spongiosis, neuronal loss, and astrocytic gliosis in the cerebral cortex. Mild to moderate spongiosis and neuronal loss were observed in the limbic cortex and basal ganglia. There was no spongiform change in the hippocampus, brain stem or cerebellum. Many senile plaques and neurofibrillary tangles were found, and the Braak stages were stage C and stage IV, respectively. Immunostaining for prion protein (PrP) revealed granular (synaptic-type) and patchy PrP deposition in the cerebral cortex and especially in the hippocampus. Most patchy PrP deposits were colocalized with amyloid β plaques, but some of them were isolated. The relatively strong PrP deposition and coexistence of Alzheimer-type pathology of this case are remarkable. We suppose that amyloid β plaques might act as a facilitating factor for PrP deposition.

Key words: Creutzfeldt-Jakob disease, histopathology, prion proteins, senile plaques, V180I mutation.

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INTRODUCTION

Creutzfeldt-Jakob disease (CJD) is a fatal neurodegenerative disease affecting humans and a wide variety of animals. Most cases are sporadic with an unknown mode of transmission; 10–15% of cases are inherited, and a small number have been caused by medical procedures.[1] The incidence of CJD is estimated to be stable at between 0.5 and 1.5 cases per million people per year.[1–3] The typical clinical picture of sporadic CJD is a rapidly progressive cognitive decline with ataxia and myoclonus, associated with periodic synchronous discharges (PSD) on electroencephalogram (EEG), and positive CSF 14-3-3 protein test.[2,3] The disease is neuropathologically characterized by spongiform degeneration, neuronal loss, gliosis, and the presence of altered forms of prion protein (scrapie form of prion protein, PrP^{Sc}).[3]

Some cases of familial CJD are actually sporadic cases with no relevant family history due to incomplete genetic penetrance and the misdiagnosis of other affected family members. The clinical features depend on the genetic mutations. However, most patients demonstrate PSD on EEG, an accepted diagnostic marker for CJD.[1,4] CJD with a causative point mutation of valine to isoleucine at codon 180 (V180I) of the prion protein gene (*PRNP*) is a type of familial CJD with no relevant family history.[4–12] In case reports, the clinical features of CJD with V180I were different from those of sporadic CJD (sCJD). CJD with V180I is a late-onset disease, and the symptoms never start with visual or cerebellar involvement.[4,5] The patients show slower progression of the disease compared with sCJD.[4] They never show PSD on EEG.[4] MRI demonstrates

remarkable high-intensity areas with swelling in the cerebral cortex except for the medial occipital and cerebellar cortices.[4] Therefore, the premortem clinical diagnosis is sometimes difficult, and the cases are misdiagnosed as neurodegenerative disorders with dementia.

As stated above, the clinical symptoms of CJD with V180I have previously been described in detail, but reports of autopsied cases of CJD with V180I have been limited. In this report, we document a rare autopsy case with coexisting CJD with V180I and Alzheimer disease (AD) pathology.

CASE REPORT

Clinical course

The patient was a Japanese woman who was 79 years old at the time of death. She had neither a family history of neurological disease nor dementing disorder anamnesis.

She developed gait instability in May 2004 at the age of 77. Four months later, she showed agraphia and right-left disorientation. Seven months after the onset, she could neither stand nor walk, even with help. She also showed dressing apraxia and acalculia. At admission, 8 months after the disease onset, she was obviously demented. The scores of the Mini Mental State Examination and Hasegawa Dementia Scale Revised were 14/30 and 8/30, respectively. Neurological examination revealed limb and truncal ataxia. Ideomotor apraxia and constructional disturbance were obvious, and urinary incontinence was observed. However, she showed neither myoclonus nor tremor. Deep tendon reflex was within normal limits without pathological reflex, and she showed no rigidity.

An EEG showed no PSD. Head MRI revealed diffuse cerebral cortical atrophy. Diffusion-weighted imaging showed high intensity, wide-ranging cortical lesions in the temporal, frontal and parietal lobes. A suspiciously slight elevation of 14-3-3 protein in her CSF was observed. Analysis of the *PRNP* revealed a point mutation of valine to isoleucine at codon 180, methionine/methionine homozygosity (Met/Met) at codon 129, and glutamate/glutamate homozygosity (Glu/Glu) at codon 219.

Her cognitive deterioration worsened gradually, and she developed akinetic mutism 18 months after the onset. She died of pneumonia 26 months after the disease onset. The autopsy was limited to the brain, and frozen tissue was not taken.

Neuropathological findings

The fixed brain weighed 950 g. Meninges and vessels were normal. Macroscopic examination revealed diffuse moderate cerebral atrophy. Basal ganglia, brain stem, and cerebellum were not atrophic. No pallor was noted in the substantia nigra and locus ceruleus.

The brain was fixed in 10% buffered formalin. Tissue blocks were taken from the mid-frontal and orbitofrontal areas; superior, middle and inferior temporal, inferior parietal and occipital cortices; anterior cingulate; amygdala; hippocampus; striatum; thalamus; midbrain; pons; medulla; and cerebellum. Multiple paraffin-embedded tissue blocks were prepared, and 7-mm-thick sections were cut. These sections were stained with HE, KB, thenamine silver and modified Gallyas-Braak methods.

The following primary antibodies and dilutions were used: anti-prion protein (3F4; mouse monoclonal; against prion protein amino acids 108–111; 1 : 30; Dako, Glostrup, Denmark), anti-amyloid β42 (rabbit polyclonal; against amyloid β C-terminal; 1 : 100; Immuno-Biological Laboratories, Gunma, Japan), anti-phosphorylated tau (AT8; mouse monoclonal; 1 : 1000; Innogenetics, Ghent, Belgium).

For amyloid β and phosphorylated tau immunohistochemistry, deparaffinized sections were incubated with 1% H_2O_2 in methanol for 30 min to eliminate endogenous peroxidase activity in the tissue and treated with formic acid (99%, 5 min; Sigma, St. Louis, MO, USA) to retrieve immunogenicity (for phosphorylated tau immunostaining, the treatment with formic acid was not performed). After blocking with 10% normal serum, sections were incubated overnight at 4°C with the primary antibody. The sections were washed in PBS and incubated with a biotinylated secondary antibody, followed by avidin-biotinylated horseradish peroxidase complex (ABC Elite kit, Vector, Burlingame, CA, USA). The reaction was visualized with 0.2% 3'3-diaminobenzidine (DAB) in 50 mmol TRIS-HCL buffer, pH 7.4, containing 0.003% H_2O_2. Counterstaining was carried out with hematoxylin. For prion protein (PrP) immunohistochemistry, sections were boiled in 35% HCl for 2 min before the treatment with formic acid.

For double staining with amyloid β and PrP, the primary antibody labeling in the first cycle was detected in the same way as single staining to yield a brown precipitate. Then, the primary antibody in the second cycle was detected in the same way as single staining except that the DAB reaction was intensified with nickel ammonium sulfate to yield a dark gray precipitate.

Microscopic examination revealed marked spongiosis, neuronal loss, and astrocytic gliosis in the neocortex of the frontal, temporal and parietal lobes (Fig. 1A). Neuronal loss with neuropil rarefaction of all cortical layers was also observed broadly in the affected cortex, especially severe in the temporal and frontal lobes (Fig. 1B). Neuronal loss and rarefaction were mild to moderate in the occipital cortex. Spongiosis, gliosis and neuronal loss were moderate in the entorhinal cortex and putamen (Fig. 1C), and mild in

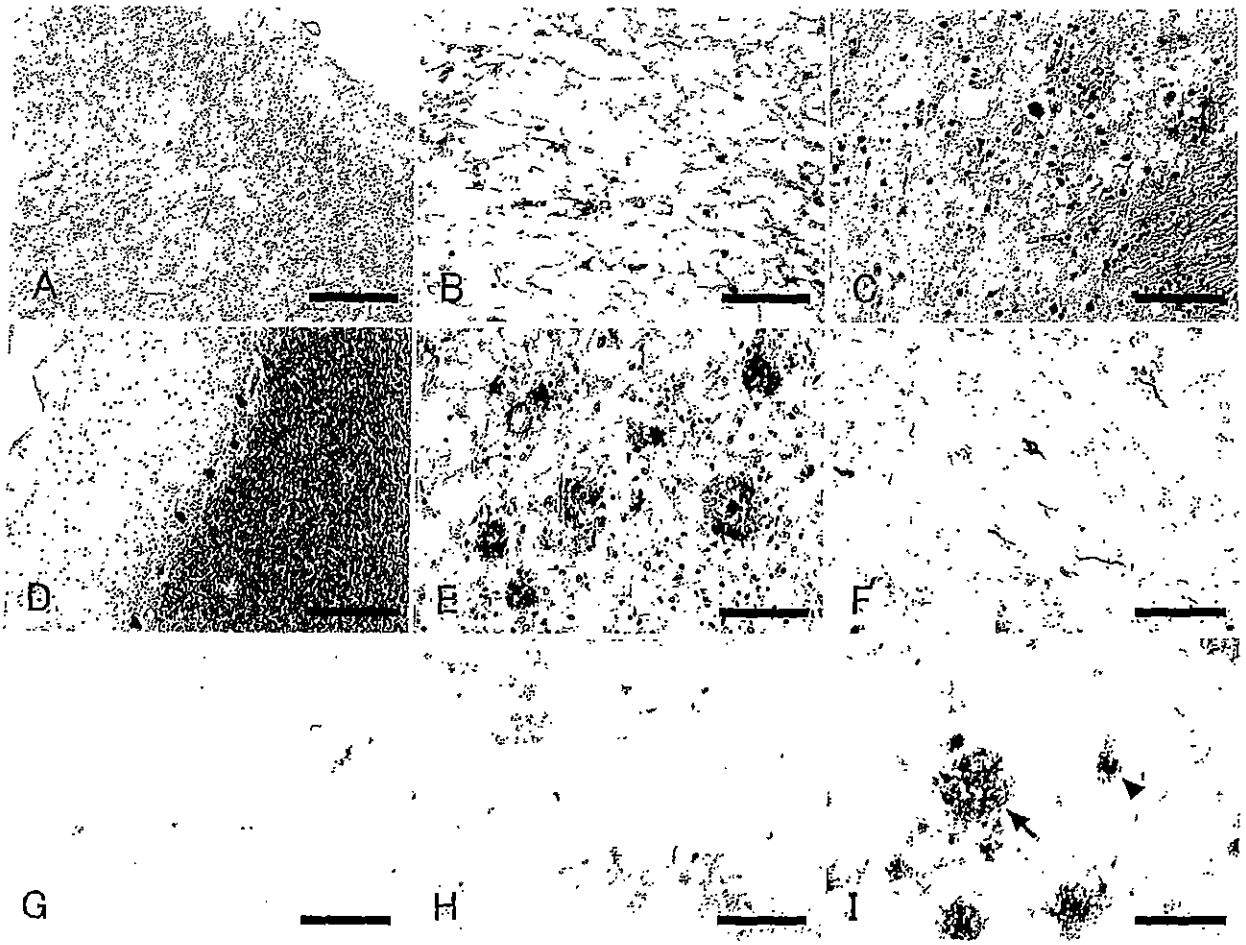

Fig. 1 Microscopic appearance of representative lesions. (A) The cerebral neocortex shows widespread status spongiosus. Temporal lobe. HE. (B) Severe neuronal loss and neuropil rarefaction of all cortical layers are observed. Temporal lobe. HE. (C) The putamen shows moderate spongiform degeneration. HE. (D) The cerebellum shows no spongiosis, neuronal loss, or astrocytic gliosis. HE. (E) Many senile plaques are found in all isocortical areas. Parietal lobe. Methenamine silver stain. (F) The CA4 of the hippocampus shows AT8-positive NFTs and neuropil threads. Phosphorylated tau immunostaining. (G) The cerebral neocortex shows a mild synaptic-type PrP deposition and sparse patchy deposition. Temporal lobe. PrP immunostaining. (H) The hippocampus shows a relatively strong PrP immunoreactivity and many patchy PrP deposits. PrP immunostaining. (I) Most patchy PrP deposits are colocalized with amyloid β plaques (arrow), but some of them are isolated (arrowhead). Hippocampus. PrP (dark gray) and amyloid β (brown) immunostaining. Scale bar = (A) 500 μm, (B, C, E–I) 100 μm, (D) 200 μm.

the caudate nucleus, globus pallidus, medial thalamic nucleus, and amygdala. In the cerebral white matter, mild to moderate spongiosis and gliosis were observed. There was no spongiform degeneration in the hippocampus, brain stem or cerebellum (Fig. 1D).

In addition to the spongiform changes, many senile plaques (SPs) were found in all isocortical areas (Fig. 1E), compatible with stage C of Braak's classification.[13] A modified Gallyas-Braak stain demonstrated a moderate number of neurofibrillary tangles (NFTs) and neuropil threads in the CA1–4 of the hippocampus and parahippocampal gyrus, consistent with stage IV of Braak's classification.[13] Phosphorylated tau immunostaining revealed the AT8-positive neurofibrillary pathology, compatible with stage IV of Braak's staging.[14] (Fig. 1F). Amyloid angiopathy was not detected by amyloid β immunostaining. No vascular lesions were observed. Lewy bodies were not identified by HE staining.

Immunohistochemical analysis with a monoclonal antibody to PrP demonstrated a mild fine granular (synaptic-type) deposition[15] and sparse patchy deposition in the cerebral cortex (Fig. 1G). The immunoreactivity of PrP was weak in the severely damaged cortex with neuropil rarefaction, but in the preserved area of the neocortex, for

Table 1 Autopsied cases of Creutzfeldt-Jakob disease with V180I mutation

	Matsumura *et al.*[7]	Iwasaki *et al.*[10]	Suzuki *et al.*[12]	Present case
Clinical features				
Sex	Female	Male	Male	Female
Age at onset (years)	78	78	79	77
Age at death (years)	79	80	80	79
Duration (months)	12	21	13	26
Onset symptoms	Tremor	Motor aphasia	Delusion	Gait disturbance
Dementia	+	+	+	+
Myoclonus	+	+	−	−
PSD	−	−	−	−
CSF 14-3-3	n.d.	n.d.	+	+−
Codon 129 in PRNP	Met/Val	Met/Val	Met/Val	Met/Met
Histopathological features				
Brain weight (g)	1220	1060	1180	950
Rarefaction in cerebral cortex	−	−	−	+
Spongiform change				
Cerebral cortex	+	+	+	+
Caudate	+	+	n.d.	+
Putamen	+	+	n.d.	+
Thalamus	+	+	n.d.	+
Brain stem	−	n.d.	n.d.	−
Cerebellum	−	−	−	−
Spinal cord	n.d.	n.d.	n.d.	Not examined
PrP staining	+−	+−	+−	+
	Synaptic	Synaptic	Synaptic	Patchy, synaptic
Senile Plaques	+−	−	Braak stage B	Braak stage C
Neurofibrillary changes	−	+−	+−	Braak stage IV

PRNP, prion protein gene; PrP, prion protein; PSD, periodic synchronous discharges; +, present; +−, suspicious or slight; −, absent; n.d., not described; Met/Val, methionine/valine heterozygosity; Met/Met, methionine/methionine homozygosity.

example, in the occipital cortex, the immunoreactivity was comparatively strong. Particularly in the hippocampus, diffuse synaptic-type deposition and a large number of patchy PrP deposits were detected (Fig. 1H). Most patchy PrP deposits were colocalized with amyloid β plaques, but some of them were isolated (Fig. 1I). No PrP deposition was observed in the basal nucleus, cerebral white matter, brain stem, or cerebellum. Plaque-type and perivacuolar-type PrP depositions[15] were not found.

DISCUSSION

The diagnosis of CJD in this case was established by the analysis of the PRNP of V180I, diffuse spongiosis in the cerebral cortex, and PrP immunostaining. As far as we know, there have been four detailed reports, including ours, of autopsied CJD case with V180I mutation (Table 1),[7,10,12] and this is the first detailed report of an autopsied CJD case with a V180I mutation and Met/Met at codon 129.

Brain weights of the four cases ranged from 950 g to 1220 g, and inversely correlated with disease duration (950 g, 26 months; 1060 g, 21 months; 1180 g, 13 months; 1220 g, 12 months) (Table 1). In all four cases, diffuse spongiosis in the cerebral cortex and basal ganglia was noted without obvious lesions in the cerebellum and brain stem. However, severe neuronal loss with neuropil rarefaction (so-called status spongiosus) in the cerebral cortex was found only in our case. The long survival time might have affected the appearance of the rarefaction.

The other major pathological characteristics of our patient were the presence of Alzheimer-type pathology, that is, NFTs consistent with stage IV of Braak's classification, as well as SPs compatible with stage C according to Braak's classification. The coexistence of Alzheimer pathology and prion pathology is uncommon.[16–24] However, there are few reports of the frequency of AD pathology combined with CJD. In 1998, Hainfellner *et al.* investigated Alzheimer-type pathology according to Consortium to Establish a Registry for Alzheimer Disease (CERAD) criteria[25] in the neocortices of 110 neuropathologically proven CJD patients and noted that Alzheimer-type pathology compatible with definite and probable AD according to CERAD criteria occurred in 12 (10.9%) of the CJD patients.[22] However, in the 12 cases of CJD with definite or probable AD according to CERAD criteria, they found a moderate frequency of NFTs in only one patient, who died at 82 after a clinical duration of 3 months.[22] In 1994, Brown *et al.* presented a synopsis of the clinical, neuropathological, and biological details of a National Institutes of Health series of 189 CJD cases autopsied during the past 30 years, but only four of them were found to exhibit evidence of AD with senile plaques and numerous NFTs in the hippocampus and cerebral cortex.[26] From a review of the published literature, Tsuchiya

et al. found that the coexistence of pathological features of CJD and AD in the same patient occurs in a very small number of patients, and that there are two forms of coexistence of CJD and AD in the same patient.[23] The first form is AD cases developing CJD in the late stage of AD.[18,20,21] The second form is sporadic CJD cases having AD pathological features without any clinical features typical of AD.[16,17,19,23,24] Our case corresponds to the second form.

On immunohistochemical examination, the pattern of PrP staining in our case is different from other reported cases (Table 1). In the three other cases, the staining pattern was diffuse fine granular PrP deposition (synaptic type) only, and immunoreactivity was very weak. Moreover, PrP deposition was localized to the medial temporal cortex. In our case, diffuse synaptic-type PrP deposition was relatively strong and not localized to the medial temporal cortex. Moreover, patchy PrP deposition was observed, mainly colocalized with senile plaques, but some were isolated. It was reported that amyloid β protein and PrP compound plaques were found in 11/12 CJD patients with concomitant Alzheimer-type pathology.[22] In most compound plaques, PrP accumulates at the periphery of amyloid β plaques.[22] Therefore, it was supposed that pre-existing amyloid β plaques might act in CJD as a micro-environmental factor influencing PrP morphogenesis by fostering the aggregation of one amyloidogenic protein onto a core composed of the other.[22] In our case also, patchy PrP deposition was observed mainly at the periphery of amyloid β plaques, and pre-existing amyloid β plaques might have acted as a facilitating factor for patchy PrP deposition. In another report, patchy deposition of normal PrP (the normal cellular form of prion protein, PrP^{C}) was found in neuritic plaques.[27] Anti-prion protein antibody (3F4) recognizes both PrP^{C} and PrP^{Sc},[27] so we cannot exclude the possibility that the patchy PrP deposition consisted of PrP^{C}. However, the analysis of the PRNP gene of V180I, diffuse spongiosis in the cerebral cortex, and synaptic-type PrP immunostaining in the cerebral cortex support the diagnosis of CJD. Moreover, some patchy PrP deposition isolated from amyloid plaques was present in our case. Therefore, we suppose that the patchy PrP deposition in amyloid β plaques in our case consisted of PrP^{Sc}.

Limitations of this study

First, frozen tissue of this case was not taken. Therefore, we could not perform Western blot analysis and determine the type of accumulated PrP. Second, in our case, truncal and limb ataxia was described, but we did not find obvious pathological correlates in the cerebellum and brain stem. We can propose two possibilities. One is that the pathological lesion was present in the cerebellum, but we did not find it. The other is that ataxia in this patient was not induced by a cerebellar lesion, but rather lesions outside the cerebellum, for example, in the cortex or spinal cord. For example, ataxic symptoms of Gerstmann-Sträussler-Scheinker disease (P102L) were explained partly by spinal cord lesions.[28,29] The spinal cord was not examined in our case as well as previous autopsied cases (Table 1). We cannot determine conclusively the neural substrate of the ataxia at present. Nevertheless, the relatively strong PrP deposition in the cerebral cortex and hippocampus and coexistence of Alzheimer-type pathology in this case are remarkable.

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研究報告の概要

生物学的な液体からの伝染性プリオンの除去は、通常、ハムスターまたはマウスの大脳内接種に基づくバイオアッセイによって定量化される。これらの試験は、遅くて、扱いにくく、不正確で、非常に高価である。
本研究において、我々が新しく非常に感度が高い細胞培養の方法（プリオン除去プロトタイプフィルターの性能評価）について記載する。生食/アデニン/グルコース/マンニトール中の1～2日経ったABO適合性人赤血球（RBC）5単位は、AABB公認の血液銀行から入手した。
Rocky Mountain Laboratory株のスクレイピー感染したマウス脳ホモジネートはプールされたRBCに加えられた。
プールされたRBCを300mLずつに分け、各々に標準的な白血球除去フィルター又は4つのプロトタイプのプリオン除去フィルターでろ過した。ろ過前後のサンプルのプリオン感染性レベルは、標準スクレイピー細胞分析(SSCA)に基づく細胞培養により測定された。
その結果、10層では異型の感染性が残っていることを示し、22層のプリオン除去フィルターはSSCAの検出限界以下（2.0$\log_{10}LD_{50}$/mL）までプリオン感染性を除去した。
これらの結果は、プリオン除去フィルターのスクリーニングにおける高感度細胞培養に基いた感染性試験の有用性を示す。
この種のin vitroでの感染性測定の使用は、輸血を介するvCJD伝播リスクを減らす手段のスクリーニングと発見の手助けとなるであろう。

報告企業の意見	今後の対応
プリオン除去フィルターのスクリーニングにおける高感度細胞培養に基づいた感染性試験の有用性についての報告である。 血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)略
1)略
2)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。

Evaluation of removal of prion infectivity from red blood cells with prion reduction filters using a new rapid and highly sensitive cell culture–based infectivity assay

Samuel O. Sowemimo-Coker, Cheryl A. Demczyk, Fabiola Andrade, and Christopher A. Baker

BACKGROUND: The clearance of infectious prions from biologic fluids is usually quantified by bioassays based on intracerebral inoculation of hamsters or mice; these tests are slow, cumbersome, imprecise, and very expensive. In the present study we describe the use of a new and highly sensitive cell culture–based infectivity assay to evaluate the performance of several prion removal prototype filters.

STUDY DESIGN AND METHODS: Five units of 1- to 2-day-old ABO-compatible human red blood cells (RBCs) in saline-adenine-glucose-mannitol were obtained from an AABB-accredited blood bank. The 5 units were combined to create a homogenous pool. Scrapie-infected mouse brain homogenate of a Rocky Mountain Laboratory strain was added to the pooled RBCs. The pooled RBCs were divided into 300-mL aliquots, which were filtered with either standard leukoreduction filter or four prototypes of prion reduction filter. The levels of prion infectivity in the pre- and postfiltration samples were measured with a cell culture–based standard scrapie cell assay (SSCA).

RESULTS: All the 22-layer prion reduction filters removed prion infectivity below the limit of detection of the SSCA (reduction in prion infectivity $\geq$2.0 $\log^{10}LD^{50}$/mL) while the 10-layer variant showed some residual infectivity.

CONCLUSIONS: These results demonstrate the utility of a highly sensitive cell culture–based infectivity assay for screening prion reduction filters. The use of this type of in vitro infectivity assay will substantially help expedite the screening and discovery of devices aimed at reducing the risk of variant Creutzfeldt-Jakob disease transmission through blood transfusion.

Prion diseases or transmissible spongiform encephalopathies are fatal neurodegenerative diseases that affect both humans and animals. Creutzfeldt-Jakob disease (CJD) is the most common form of human transmissible spongiform encephalopathy, and although usually sporadic, it has been transmitted from person to person through medical instruments and transplant of tissues or organs,[1,2] but so far as is known, not through the administration of blood or blood products.[3-5]

A variant form of CJD appeared in the United Kingdom in the mid-1990s as a result of the consumption of tissue or meat products from cattle infected with bovine spongiform encephalopathy.[6] To date, there have been 217 confirmed cases worldwide with the vast majority (170) in the United Kingdom.[7,8] Recent animal data[9-11] together with four reported cases of probable transmission of vCJD in humans from transfused blood components[12,13] have raised concerns about the transmission of the causative agent by this means.

Because there are no diagnostic tests with which to identify preclinical infection, precautionary measures have been introduced in many countries to reduce the risk of disease transmission through blood or blood products, including donor deferral and the implementation of a

ABBREVIATIONS: CDI = conformational-dependent immunoassay; LAPRF = Leukotrap affinity prion reduction filter; PrP = prion protein; RML = Rocky Mountain Laboratory; SSCA = standard scrapie cell assay; vCJD = variant Creutzfeldt-Jakob disease.

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universal leukoreduction strategy in the United Kingdom.[14,15] In the absence of a preclinical screening test, removal of the infectious agent by processing is the only means by which risk to recipients of blood from donors with inapparent vCJD infections can be reduced. Therefore, several filtration devices are being developed for the removal of infectious prions from blood and blood products.[16-19] However, methods for accurate detection and quantification of prion infectivity are essential for a successful identification and development of an effective prion decontamination device.

According to the "protein-only" hypothesis, prion diseases are caused by an abnormal protease-resistant, aggregated form of prion protein (PrP) designated as PrP^{Sc},[20] which accumulates in the brain. Therefore, PrP^{Sc} has been used as a surrogate marker of infection for prion disease. However, it has been shown that prion infectivity may accumulate in the absence of detectable levels of this marker, and the level of PrP^{Sc} by some in vitro methods does not necessarily correlate with infectivity.[21-23] The current methods for screening prion reduction devices include in vitro assays such as enzyme-linked immunosorbent assay (ELISA), Western blot, and a variation of ELISA called conformational-dependent immunoassay (CDI) that detect the surrogate marker of infectivity, PrP^{Sc}[24-28] and an in vivo infectivity bioassay. The in vivo infectivity assay is an animal bioassay based on either intracerebral inoculation of hundreds of animals with test samples and determining the time for the appearance of clinical symptoms of prion disease (incubation time method)[29] or by injecting serial dilutions of the test sample and determining the dilution at which 50% of the animals acquire scrapie infection (endpoint titration).[30] These bioassays of infectivity are very slow and cumbersome, involve the use of hundreds of hamsters, and are extremely expensive with a typical endogenous infectivity study costing as much as $250,000 to $500,000 for a single study with a duration of 500 to 600 days. However, the long duration, complexity, and cost of in vivo infectivity studies have prompted many investigators to seek precise and reliable alternatives.[31-34] The development of cell-based assays may greatly accelerate the direct measurement of prion infectivity rather than the inferred infectivity data obtained with measurement of the surrogate marker of infectivity. This cell culture–based infectivity assay may also permit more prototypes and prion reduction filters to be tested in a timely and cost-effective manner for development and continuous quality improvement efforts.

In this study, we describe the use of a highly sensitive cell culture–based infectivity assay in experiments using infectious prions from the mouse-adapted Rocky Mountain Laboratory (RML) scrapie strain to 1) evaluate the effectiveness of several prototypes of new white blood cell (WBC)-prion reduction filters in removing prion infectivity from 300-mL units of RBCs, 2) determine whether our standard leukoreduction filter could remove prion infectivity from RBCs, and 3) determine whether the assay is sensitive enough to detect differences in prion clearance between our 10- and 22-layer variants of our WBC-prion reduction filters.

MATERIALS AND METHODS

Five units of 1- to 2-day-old ABO-compatible nonleukoreduced RBCs in saline-adenine-glucose-mannitol were purchased directly from AABB-accredited blood banks. All 5 units were transferred into a 2-L blood bag to create a homogenous pool. Approximately 10.5 mL of infectious prions from 10% (wt/vol) brain homogenate from RML scrapie strain were added to 1570 mL of the pooled RBCs (final dilution of approx. 1:151). The infectious prions were mixed with the RBCs by end-over-end rotation (approx. 30 rotations) of the blood bag to ensure homogenous dispersion of the infectious prions into the RBCs. A total of 20 mL of the contaminated RBCs was removed for measurement of prefiltration level of infectivity. The remaining pool of contaminated RBCs was divided into 300-mL aliquots. One unit of 300 mL of nonleukoreduced RBCs was filtered at room temperature (22 ± 2°C) without prion leukoreduction step using filter B-1570AK. Four of the 300-mL units of RBCs were filtered at room temperature with a standard leukoreduction filter (BPF4, Pall Medical, Port Washington, NY) according to the manufacturer's instructions for use. After the leukoreduction step, a 20-mL aliquot was taken from each unit for analysis of infectivity in the leukoreduced RBCs. The residual volume from each 300-mL leukoreduced RBC unit was filtered again at room temperature (22 ± 2°C) at a filtration height of 30 inches using one of the following prototypes of prion reduction filters:

1. Leukotrap affinity prion reduction filter (LAPRF; Pall Medical)—contained 10 layers of PRM3 prion removal material.[18]
2. Prion filter B-1451AQ—contained 22 layers of PRM3 prion removal material.[17]
3. Prion filter B-1570AI—contained 22 layers of PRM6 prion removal material.
4. Prion filter B-1570AK—contained 22 layers of PRM7 prion removal material.

The levels of infectivity in the pre- and postfiltration samples were measured with a cell culture–based infectivity assay called standard scrapie cell assay (SSCA). For the SSCA, 20-mL aliquots of the pre- and postfiltration samples were centrifuged at 2500 × *g* for 5 minutes at room temperature (Sorvall RC3C, Kendro Laboratory Products, Asheville, NC), and the supernatants were assayed for infectivity.

SSCA and quality control

The SSCA is based on the isolation of a cell line (Cath-a–differentiated cells, CAD5; Scripps Infectology Laboratory, Jupiter, FL) that is highly susceptible to RML scrapie strain and on a method for identifying individual prion-infected cells and quantifying them with an automated counting equipment. This study was performed as previously described.[31,32] Briefly, 5000 CAD5 cells in reduced serum medium (OPti-MEM, Invitrogen, Carlsbad, CA) and 9.1% bovine growth serum (Hyclone Laboratories, Logan, UT), 10,000 units/mL penicillin G, and 100 µg/mL streptomycin G (Hyclone) were dispensed into tissue culture plates and allowed to attach to the plates overnight. After the overnight incubation, the cells were exposed to serial dilutions (1:5, 1:10, and 1:30) of RML and test samples for 4 days. After the 4-day incubation period, the cells were split 1:10 for a total of three times. After the third split, 20,000 cells of each sample were filtered onto membranes of a 96-well plate (AcroRead, Pall Life Sciences, Ann Arbor, MI). The cells were lysed and then treated with proteinase K to eliminate normal-PrP^{C}. PrP^{Sc}-positive cells were identified by an ELISA using an anti-PrP monoclonal antibody (MoAb) D18[31,32] and alkaline phosphatase–linked anti-IgG antiserum (Southern Biotechnology Associates, Birmingham, AL). The PrP^{Sc}-positive cells were counted using an automated imaging system (Zeiss KS enzyme-linked immunospot [ELISPOT] system, Stemi 2000-C stereomicroscope equipped with a Hitachi HV-C20A color camera and a KL 1500 LCD scanner and Wellscan software from Imaging Associates, Bicester, Oxfordshire, UK). The settings on the imaging system were optimized to give a maximal ratio of counts for PrP^{Sc}-positive samples relative to negative control samples. The data are expressed as the total number of infected cells or spots per 20,000 CAD5 cells. Background readings were determined by averaging the number of spots observed in uninfected CAD5 cells across multiple culture plates. In parallel, CAD5 cells were also exposed to serial dilutions of standard RML-infected brain homogenates with a starting titer of $10^{8.75}$ LD_{50} units per gram of mouse brain.

Spiking study to determine inhibitory effects of test samples on SSCA

To confirm that any observed reductions in infectivity were not due to components in the test samples that were inhibitory to the SSCA, aliquots of postfiltration samples at different dilutions (1:5, 1:10, 1:30, and 1:90) were mixed with a predefined amount of infectious RML prions. In this test, 1 mL of test sample was added to 10 µL of $10^{8.75}$ LD_{50} infectious RML. Approximately 0.145 mL of the test sample-RML solution was placed into the tissue culture wells containing 5000 CAD5 cells and the level of infectivity at the different dilutions of test samples was determined.

Endogenous infectivity studies with 10- and 22-layer prion reduction filters

In the endogenous infectivity studies,[17,18] units of whole blood were obtained from 500 scrapie-infected hamsters into anticoagulants and then processed into RBCs according to standard procedure.[17,18] The RBCs were resuspended in additive solutions and then filtered with prion reduction filters. Aliquots of the pre- and postfiltration samples were injected intracranially into healthy normal hamsters. The animals were monitored and maintained for 300 days; those that developed clinical symptoms of scrapie were killed and the brain tested for the presence of PrP^{sc} by Western blot assay using 3F4 MoAb.[17,18]

Statistical analysis

The differences in reduction in prion infectivity between pre- and postfiltration samples with the different prototypes were analyzed using a Wilcoxon paired test with probability level of less than 0.05 being considered significant while Kaplan-Meier statistic was used to analyze the survival data of hamsters that developed scrapie infection (GraphPad Intuitive Software for Sciences, San Diego, CA).

RESULTS

SSCA and quality control

The resulting spots minus background counts of the serial dilutions of the RML-brain homogenate were plotted versus the LD_{50} and fitted with an exponential association function to allow for conversion of spots into LD_{50} in subsequent analyses as shown in Fig. 1. Note that the spot number corresponding to the highest concentration of RML was artifactually low because the individual spots were no longer resolved by the imaging system used. The limit of detection is the lowest reference point on the curve, and it corresponded to a value of approximately 50 LD_{50} units.

Determination of the inhibitory effects of postfiltration samples on prion infectivity

When the postfiltration samples were diluted at 1:30 and 1:90 before incubation with the CAD5 cells, there were no inhibitory effects of the samples on prion infectivity (Fig. 2). For example, the observed infectivity level per 20,000 cells was 1017 ± 97.8 spots per well in the control RML sample, and when the postfiltration samples from the prion reduction filters tested were diluted 1:30 and then mixed with the defined amount of the RML sample, there was no significant change in the observed prion infectivity in any of the postfiltration samples tested at this dilution when compared to the control undiluted RML sample ($p > 0.05$; Fig. 2). However, at 1:5 and 1:10

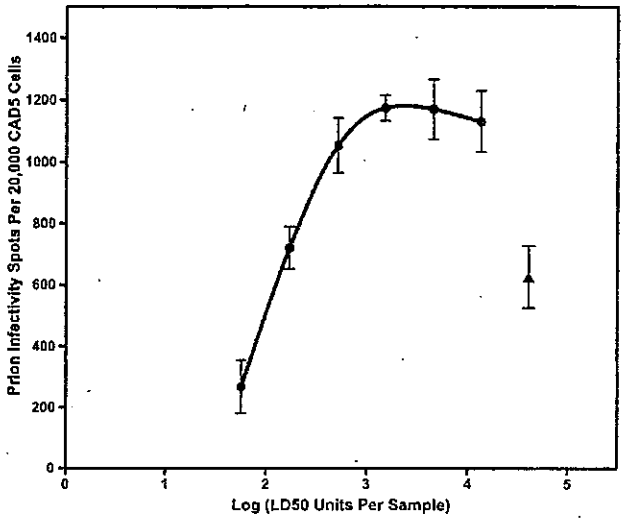

Fig. 1. RML standard curve (●). SSCA plot of the dose-response relationship between the various dilutions of RML brain homogenate expressed as LD_{50} units per dilution and the number of infectivity spots per 20,000 inoculated. At very high concentration of infectious prions (greater than 10,000 LD_{50} units) the response becomes nonlinear because the image analyzer could not adequately resolve the infectivity spots at this level. (▲) Saturated point.

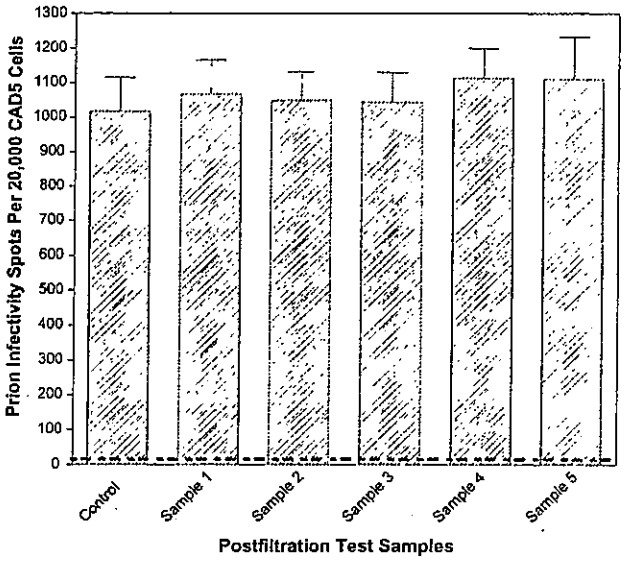

Fig. 2. Determination of the inhibitory effects of postfiltration RBC samples on the development of infectivity in the SSCA. Supernatants from RBCs were mixed with known concentrations of RML brain homogenate. The dotted line toward the bottom of the graph represents the background level for prion infectivity per 20,000 of uninfected CAD5 cells. Each bar represents the mean ± SD of six replicates.

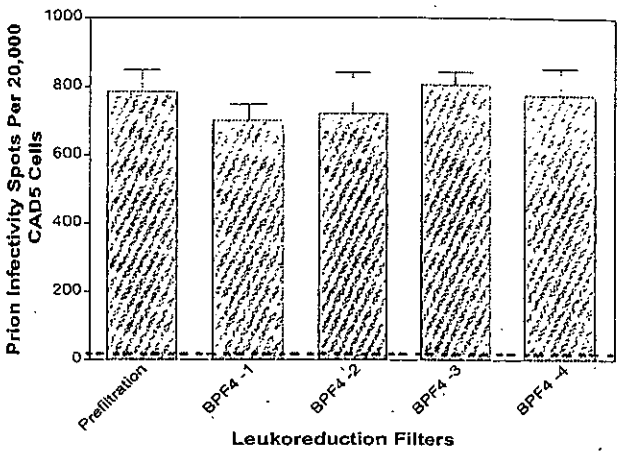

Fig. 3. Effects of leukoreduction step on prion infectivity in units of RBCs. Each bar represents the mean ± SD of six replicates. The levels of infectivity in the RBC were measured before and after filtration with standard leukoreduction filter, BPF4. Both pre- and postfiltration RBC samples produced similar levels of infectivity. The dotted line toward the bottom of the graph represents the background level for prion infectivity per 20,000 of uninfected CAD5 cells.

dilutions, some of the postfiltration samples showed significant inhibition of prion infectivity (data not shown). Therefore, based on these results, all the pre- and postfiltration test samples were evaluated and compared at 1:30 dilutions where there was no inhibition of prion infectivity.

Effects of leukoreduction on prion infectivity in RBCs

The levels of PrP^{Sc} infectivity in the prefiltration sample and after filtration of RBCs with a standard leukoreduction filter, BPF4 showed only a slight reduction in infectivity in two of the leukoreduced samples tested (Fig. 3). The line on the graph indicates the background level for uninfected CAD5 cells. Note that all the signals were significantly higher than the background for both pre- and postfiltration samples. On average, leukoreduction with BPF4 reduced PrP^{Sc} infectivity level from 787 ± 63 infected cells per 20,000 to 752 ± 48 infected cells per 20,000 CAD5 cells, a reduction of only 4.5%, which was not significant ($p > 0.05$).

Removal of prion infectivity from RBC unit with different prototypes of prion reduction filter

When full units of prion-contaminated leukoreduced and nonleukoreduced RBCs were filtered with the prion reduction filters, the levels of infectivity in the samples were significantly reduced with all the filters tested

($p < 0.05$; Fig. 4). All the 22-layer variants reduced prion infectivity below the limit of detection of the SSCA, and the values were not distinguishable from values that were obtained from background counts of uninfected CAD5 cells ($p < 0.05$; Fig. 4). In contrast to the 22-layer variants, the residual level of infectivity in the 10-layer variant, LAPRF[18] was above the limit of detection of the SSCA (Fig. 4) indicating some amount of residual infectivity in the filtered RBCs.

Removal of endogenous infectivity from RBCs with 10- and 22-layer variants

The endogenous infectivity studies[17,18] were designed to evaluate the effectiveness of the 10-[18] and 22-layer[17] variants of the prion reduction filters in removing prion infectivity endogenously produced in RBCs of scrapie-infected hamsters. In these studies, RBCs from scrapie-infected hamsters were filtered with leukoreduction filters containing 10 and 22 layers of prion removal materials. The pre- and postfiltration RBCs were injected intracranially into normal hamsters and the animals were monitored for 300 days for signs of scrapie infection. The results showed that the 22-layer variant prevented the transmission of scrapie infectivity into healthy animals while 6 of 46 control animals developed scrapie infection (Table 1). At the end of the 200-day incubation period, none of the animals that received LAPRF-filtered RBCs developed scrapie (0/413; Fig. 5); however, by the end of the 300-day incubation period three animals of 413 had developed scrapie infection indicating the presence of residual infectivity in the 10× filtered group, while 7 of 183 control animals that received unfiltered RBCs developed scrapie (Table 1). The median onset of scrapie infection in the control group was 130 days compared to 230 days in the 10× LAPRF-filtered group. This difference in the onset of scrapie infection is highly significant ($p = 0.0085$; Fig. 5).

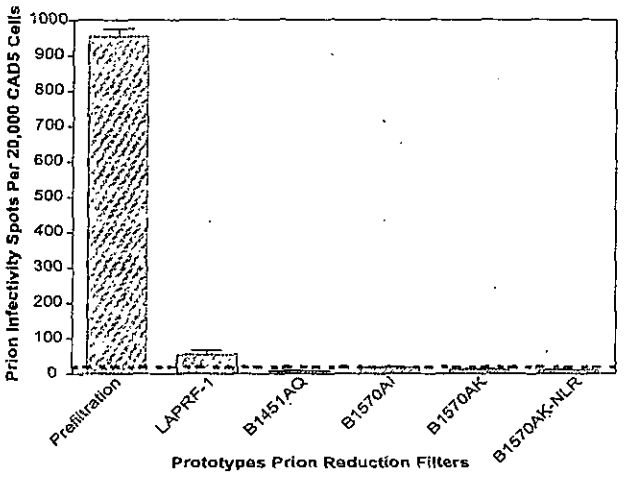

Fig. 4. Reduction in prion infectivity in full units (300 mL) of RBCs using prototypes of prion reduction filters containing 10 and 20 layers of prion-binding surface chemistries. Each bar represents mean ± SD of six replicates. NLR = nonleukoreduced RBCs. LAPRF-1 contained 10 layers of prion removal filters; B145AQ, B1570AI, and B1570AK contained 22 layers of prion removal filters. The dotted line toward the bottom of the graph represents the background level for of prion infectivity per 20,000 of uninfected CAD5 cells.

DISCUSSION

The recent reports of four probable cases of vCJD transmission by blood transfusion support the idea that the causative agent of vCJD can be transmitted to recipients of blood components.[12,13] Currently, there is no antemortem screening test that can identify potential blood donors who may be carrying the causative agent of vCJD. Therefore, several devices are being developed for the removal of causative agents from blood and blood components.

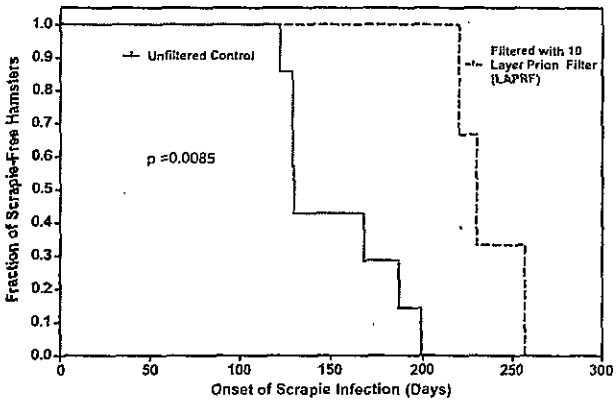

Fig. 5. Comparison of the onset of scrapie infection in normal hamsters after intracranial injection of unfiltered and filtered (10-layer prion filter) RBCs from scrapie-infected hamsters. The Kaplan-Meier statistic was used to analyze the survival data of hamsters that developed scrapie infection.

TABLE 1. Removal of endogenous infectivity from units of RBC using 10- and 22-layer variants of the prion-reduction filters[17,18]

	Unfiltered RBCs		Filtered RBCs	
Filter type	Number of animals injected	Number of animals infected	Number of animals injected	Number of animals infected
10 layer	187	7	413	3
22 layer	43	6	35	0

Most of these devices for prion clearance are evaluated using either in vitro or in vivo bioassays. These in vitro methods for evaluating prion clearance include Western blot assay, CDI, and an ELISA all of which depend on the detection of PrP^{Sc} as the surrogate marker for prion infection.[25-27] Although these in vitro methods are very simple and rapid and provide useful tools for screening various devices or blood processing conditions for prion clearance, the data derived do not necessarily correlate with prion infectivity.[21-23] However, more recently, to take advantage of the positive attributes of these in vitro assays several modifications and new assays have been developed, and the data published with the CDI showed some correlations with infectivity in different animal models.[35-39] In contrast to other in vitro assays, the SSCA directly measures prion infectivity in CAD5 cell lines that are highly susceptible to RML strains of infectious prions. In the SSCA, the CAD5 cells are exposed to samples containing infectious prions and then propagated for three passages during which the PrP^{Sc} particles are diluted out and infection spreads through the growing cell population, thus increasing the proportion of infected CAD5 cells.[31,32] The infected cells are identified and then counted using automated imaging equipment. The SSCA from start to finish takes 14 days; it is as sensitive as the mouse bioassay, 10 times faster and at least 2 orders of magnitude less expensive as the standard in vivo bioassay. Most importantly, previous study showed significant correlation between SSCA infectivity and standard in vivo infectivity bioassay.[31] Therefore, the SSCA by directly measuring prion infectivity provides another useful tool that complements other in vitro bioassays for rapid screening of prion clearance from blood products.

While the in vivo infectivity bioassay has been the assay of choice, the extended incubation period, complexity, and cost make this approach impractical for the routine screening of medical devices that are being developed for preventing the transmission of vCJD. The SSCA can replace some of the expensive and time-consuming in vivo infectivity bioassays that are needed in the initial screening of devices and thus reduce the overall cost and number of animals that are needed for evaluating the effectiveness of new devices for prion clearance.

In this study, we used the SSCA to study the prion clearance properties of standard leukoreduction filters and several prototypes of prion reduction filters containing 10 and 22 layers of prion removal materials. The results of our SSCA showed that standard leukoreduction filter did not remove infectivity from RBCs. In this study, the exogenously added infectious PrP^{Sc} were not associated with WBCs and, thus, were not removed with BPF4. This failure of standard leukoreduction filter to remove WBC-free infectious prions was to be expected since BPF4 was designed for specific removal of WBCs and not for the removal of soluble or non-WBC–associated infectious PrP^{Sc}. This result is in agreement with previous reports that showed that standard leukoreduction filter was effective in removing WBC-associated infectivity and not effective against non-WBC–associated prion infectivity.[16-18] On the other hand, all the prototypes of the prion reduction filters significantly reduced prion infectivity to different degrees dependent on the number of layers of prion reduction filtration materials used (Fig. 4). Prion infectivity was reduced below the level of detection of the SSCA with 22 layers of prion-reducing materials. These data suggest that the 22-layer variant of our prion-reducing filter should remove at least 2.0 log of prion infectivity from RBCs, which is more than the theoretical 0.85 log of clearance required to prevent the transmission of prion infectivity in RBCs, thus providing a large margin of safety for the removal of prion infectivity in RBCs. The fact that the 22-layer variant reduced prion infectivity below the limit of detection of the SSCA is very interesting, because in an endogenous infectivity study in which scrapie-infected RBCs were filtered with a similar 22-layer variant, none of the animals that received the filtered RBCs developed scrapie at the end of the 300-day postinoculation period.[17] In contrast to the 22-layer variant, the SSCA results showed that the 10-layer variant significantly reduced, but did not abolish infectivity in the RBCs. The detection of some residual infectivity in the RBCs by the SSCA after filtration with the 10-layer variant is consistent with the data from an endogenous infectivity in which three of 413 animals that received LAPRF-filtered RBCs developed scrapie infection.[18] The median age of onset of scrapie in the control animal group was 130 days compared to 230 days in the LAPRF group, which is also consistent with the SSCA data that showed significant reduction in prion infectivity with the 10-layer variant. Therefore, it is very important that methods for screening potential prion removal chemistries or ligands include an infectivity assay at the very early stage of the screening process to complement other in vitro assays for monitoring prion clearance.

Data from animal models show that the concentration of pathogenic PrP in blood at approximately 10 ID/mL during the clinical phase of the disease is believed to be several orders of magnitude lower than what is present in the brain (approx. 10^9 ID/mL).[40-42] Therefore, in human whole blood unit of 500 mL, the concentration of infectious prions is 5000 ID/unit (3.7 log ID/unit), which corresponds to 7200 ID_{50}/unit (3.86 log ID_{50}).[19,43] The expected infectivity in a 350-mL unit of RBC containing 20% plasma is approximately 700 ID (2.85 log ID/unit). Although it is highly unlikely that blood will be donated during the clinical phase of the disease when the infectious titer is at the highest, the SSCA may be useful in detecting the prion infectivity in these units of infected whole blood and RBCs. However, in the preclinical stage of prion disease the concentration of pathogenic prion is at

least 2 orders of magnitude lower than the concentration in the clinical phase.[40-42] Therefore, if the expected prion infectivity in a unit of 350 mL of RBCs is approximately 2 ID/mL,[19] the concentration of pathogenic PrP in the RBCs during the preclinical stage of the disease may be approximately 0.02 ID/mL, which corresponds to approximately 7 ID in a unit of RBCs. During this period when there are no clinical symptoms of the disease, the carrier may still be able to donate blood. A recent study showed that blood components are infective during the preclinical stage and have been shown to transmit infectivity to normal animal recipients of the blood components.[44] It is interesting to note that preliminary data (approx. 900 days posttransfusion) show that none of the leukoreduced blood components have given rise to positive transmission of prion disease,[44] which suggests that leukoreduction alone may be effective in reducing the risk of transmission of vCJD during the preclinical stage of the disease by removing a substantial amount of the WBC-associated infectivity. Therefore, since the SSCA showed that both the 10- and 22-layer variants removed a much higher level of infectivity from RBCs than would be expected in the preclinical stage of vCJD, both filters with their additional WBC-reducing properties[17,18] may be effective in reducing the risk of transmission of pathogenic PrP during transfusion of RBCs from a blood donor in the preclinical phase of the disease.

It is very important that methods for screening potential prion removal chemistries or ligands include an infectivity assay at the very early stage of the screening process to complement other in vitro assays for monitoring prion clearance. The current SSCA is very simple, cost-effective, accurate, reproducible, precise, and rapid, such that useful data can be obtained within 14 days compared to 300 to 600 days with the traditional bioassay using hamsters. Most importantly, SSCA has been shown to correlate with in vivo infectivity bioassay,[31] and the present SSCA data with the 10- and 22-layer prion reduction filters also agreed with the results of our endogenous infectivity studies using these two types of filters.[17,18] Although for the final release of any prion reduction device it may still be necessary to conduct a limited endogenous infectivity bioassay, the use of SSCA should help improve and greatly expedite the process for screening and developing new devices for prion clearance. This will help improve the safety of the blood supply by identifying devices that would help reduce, and perhaps even eliminate, the risk of transmission of human vCJD and other forms of human prion disease through blood transfusion.

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CONFLICT OF INTEREST

There are no conflicts of interests associated with any of the authors.

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