医薬品　研究報告 調査報告書

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販売名(企業名)	－					

研究報告の概要	使用上の注意記載状況・その他参考事項等
輸血による vCJD 伝播の予防を目的とした米国の現行規制では、英国に 3 ヵ月以上滞在した者、あるいは 1979 年以降に英国内で輸血を受けた者からの献血を停止することが定められている。 米国食品医薬局の伝達性海綿状脳症に関する諮問委員会(TSEAC)は、vCJD により死亡した献血者由来の血液を受血した 2 名の英国居住経験者に同疾患が伝播している可能性が高いことを考慮した結果、米国の現行規制を変更しない旨の決定を下した。 また、メリーランド大学ボルティモア校の Robert G. Rohwer らは、白血球除去を行っても高率で感染性が認められ、感染性が残留することから、白血球除去処理のみ実施しても、vCJD の輸血感染リスクを回避する有効な手段とはなり得ないと結論付けている。 CJD の血液感染リスクを評価するために 1995 年に開始された米国疾病予防管理センター(CDC)と米国赤十字社の共同研究で行われた遡及調査により、1995 年から 2004 年 6 月の間で、献血者 28 人および受血者 368 人が特定され、献血後に CJD 感染が認められた献血者由来の全血および血液成分を輸血された 342 人に関しては、いずれも CJD は認められなかった(残る受血者については調査中である)。 米国血液センター(ABC)および米国赤十字社によれば、現在の規則により約 400,000 人の献血者が停止措置を受けた。	

報告企業の意見	今後の対応
米国における vCJD 伝播防止対策に関する報告であり、現行の規制を当面変更しないこと、CJD 感染者からの受血例 342 例において感染例が認められていないこと等が述べられている。 日本においても、英国等 10 ヵ国に 1980 年以降 6 ヵ月以上滞在歴がある方、輸血歴のある方等からの献血は受け付けていない。 本報告による対応は必要ないと考える。	今後ともプリオン蛋白除去等の安全対策に関する情報収集に努めていく。

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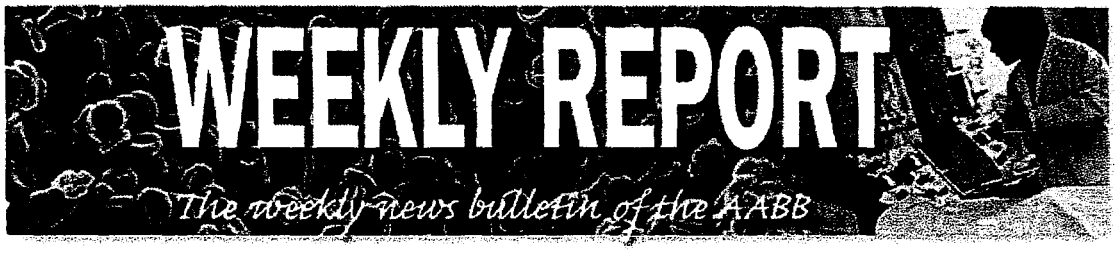

This is a combined issue of Weekly Report, *due to the Annual Meeting. The next issue will be posted online Nov. 5.*

TSEAC Leaves vCJD/CJD Screening Unchanged

Following careful deliberation of current scientific data, the FDA's Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) determined that measures currently in place in the United States to reduce the risk of transmitting CJD and vCJD by blood and blood products are adequate and, at least for the foreseeable future, justified. Variant Creutzfeldt-Jakob (vCJD) is the human form of bovine spongiform encephalitis (BSE), known as "Mad Cow Disease"; CJD, or sporadic CJD is a transfusion-transmitted disease that was identified in the 1920s.

To prevent transmitting vCJD through blood transfusions, current U.S. restrictions include deferring potential blood donors who lived more than three months in Great Britain or who received transfusions there after 1979.

The decision to leave the restrictions unchanged came after the TSEAC, the advisory committee to the Food and Drug Administration's Center for Biologics Evaluation and Research (CBER), considered evidence at its October 14 meeting in Silver Spring, Md., that two British residents had likely acquired vCJD from transfusions from blood donors who later died from the disease.

The TSEAC weighed the British evidence, but also considered evidence that the U.S. cattle population has not been infected with BSE, nor have there been any cases of transfusion-transmitted vCJD reported in the United States. This information came from updates provided at the meeting by the U.S. Department of Agriculture on the worldwide status of and the result of testing U.S. cattle under the BSE National Surveillance Plan, which showed no evidence of BSE in the U.S. There have been more than 188,900 cases of BSE reported around the world, 96 percent of which occurred in the United Kingdom. The overall prevalence of the disease decreased by approximately 35 percent in 2003, compared to 2002 data. Testing of U.S. cattle since June 1, 2004, has shown negative results on all 81,349 samples obtained.

Inside *Weekly* ...

At the TSEAC meeting Robert G. Will, MD, from the United Kingdom CJD Surveillance Unit, provided a comprehensive review of the two presumptive transfusion-transmitted vCJD cases that were reported in 2003 and early 2004 in Great Britain (The 2003 case had previously been reported to TSEAC.) Both patients received blood from donors subsequently diagnosed with vCJD. The 2004 case continues to be of interest since the patient died of other causes and did not exhibit any symptoms of neurological disease. Because the patient had been identified via lookback policies when the donor became ill, a post-mortem examination was performed and malformed infective prion proteins—believed to be the causative agent of vCJD and CJD—were found in the spleen and cervical lymph nodes. The one confirmed case of vCJD in the United States was a British citizen who health authorities suspect contracted the disease while living in Europe.

Canada Follows U.S. Restrictions

Changes in Canadian CJD/vCJD blood safety policies were explained by Peter Ganz, MD, of Health Canada. Beginning in June 1999, all red blood cells transfused in Canada have been leukoreduced (filtered). Donor deferral criteria for the most part have adhered to FDA recommendations; however, deferrals related to residence or travel in France are activated by a period of six months residence/ travel, rather than five years. When queried, Ganz stated that the decision was not based on scientific data, but on a previous decision to implement such a deferral if and when a country reported vCJD deaths. The more restrictive criteria are still in place because successful blood donor recruitment activities in Canada have made it unnecessary to relax the policy.

Leukoreduction and BSE Infectivity

Leukoreduction data from a study on how well leukoreduction removes BSE infectivity from blood were presented by Luisa Gregori and Robert G. Rohwer, PhD, of the V.A. Medical Center and University of Maryland–Baltimore. They discovered that a significant proportion of infectivity is found in plasma and remains in blood products after the leukoreduction process. Their conclusion was that leukoreduction alone is not an effective way to reduce the risk of transfusion transmission of vCJD.

CBER Donor Survey Reinforce Decision

Alan Williams, PhD, and Dorothy Scott, MD, from CBER's Office of Blood Research and Review, reviewed the deliberative process used by FDA to develop recommendations for donor deferral based on BSE exposure and to determine the current safeguards. In the winter of 1999, surveys on blood donor travel were mailed to approximately 19,000 eligible blood donors, with slightly more than 50 percent of the donors responding. The survey included questions on travel dates and residence in BSE-endemic countries, and FDA used these data as a surrogate to determine the donor's dietary exposure to BSE and the potential to transmit vCJD via blood. FDA developed the deferral criteria to effectively respond to the spread of vCJD in Europe and still maintain an optimal balance between vCJD risk reduction and the preservation of the blood supply in the U.S.

Lookback Study Finds No Infected Recipients

The American Red Cross presented results of a joint study with the Centers for Disease Control and Prevention (CDC) begun in 1995 to assess the risk of blood-borne transmission of CJD. Presented by ARC Biomedical Services' Peter Page, MD, the CJD Lookback Study identifies blood donors diagnosed with CJD and uses lookbacks to identify recipients of these donors' blood components. Each year since 1995, the names of these recipients are checked against the NDI*Plus* (the National Donor Index-Plus from the National Center for Health Statistics) for cause of death. As of June 2004, 28 donors and 368 recipients have been identified. No cases of CJD have occurred in 342 recipients of whole blood and blood components from donors who subsequently developed CJD. The remaining cases are under investigation. Long-term follow-up of the survivors will provide a more accurate estimate of the risk, if any, of transmission of CJD by blood components.

There was some question raised during public comment of relaxing current U.S. restrictions on donations, since they seem to be working. America's Blood Centers (ABC) and the American Red Cross estimate that current restrictions have deferred approximately 400,000 previous donors. During the open public hearing, G. Michael Fitzpatrick, PhD, ABC's Chief Policy Officer, suggested an "exit strategy" for the geographical residence/travel deferral that would set thresholds for ending the restrictions, which have significantly reduced the donor base. Other committee members disagreed with that strategy, calling it "premature" in light of recently discovered cases of BSE in Canada, Israel and Japan.

AABB Receives $2.4 Million CDC Grant to Reduce TT-HIV in Africa and South America

AABB has begun work on a CDC cooperative agreement to help reform national blood transfusion services (NBTS) programs in three African countries and one in South America with the objective of reducing transfusion-transmitted HIV. AABB personnel traveled to the small South American country of Guyana to study plans for expanding and improving that nation's National Blood Transfusion Services facilities and donor recruitment efforts.

AABB's $2.4 million is part of a $12 million grant, renewable for five years, from the President's Emergency Plan for AIDS Relief (PEPFAR). With the assistance of Emory University, Atlanta; Blood Source, Sacramento, Calif.; and the American Red Cross, the AABB will provide technical assistance to national blood transfusion services (NTBS) in three African countries—Kenya, South Africa, and Mozambique—as well as Guyana, just off the Caribbean on South America's northeast coast. The program is part of a PEPFAR effort to reduce transfusion-transmitted HIV (TT-HIV [human immunodeficiency virus]) and AIDS in 14 countries. Technical assistance agreements for Nigeria, Ethiopia, Tanzania, Uganda, Cote d'Ivoire, Zambia, Rwanda, Nambia, Botswana, and Haiti were awarded to other organizations. AABB will work cooperatively with CDC/HHS personnel and key leadership in the individual countries, whose governments each received separate PEPFAR grants as mirror funding for their part of the effort.

The PEPFAR grants are intended to effect significant change in the incidence of transfusion-transmitted HIV (TT-HIV [human immunodeficiency virus]) and AIDS, and reduce the massive personal, social, economic, and political toll that HIV has in these nations. The practical purpose, according to Jim Reilly, Chief Operating Officer on the project, is to help countries improve their ability to recruit blood donors and collect, test, and distribute blood, as well as improve transfusion practices. Some countries have well-organized, centralized systems and some very localized, hospital blood programs. All have high rates of transfusion-transmitted HIV, as well as HIV/AIDS.

UNAIDS (the Joint United Nations Programme on HIV/AIDS) estimates 40 million individuals in sub-Saharan Africa are infected with HIV (about 75 percent of the world's total), and that 2.5 million of those are children. There were 5 million new HIV infections in that region in 2003. Although most cases of HIV/AIDS are transmitted through exchange of bodily fluids, between 5 and 10 percent of global HIV infections are caused by transfusion of unsafe blood and blood products, according to World Health Organization estimates. In fact, WHO estimates that TT-HIV incidence in Africa is much greater, considering that HIV-positive rates exceed 20 percent in some regions, few hospitals regularly test blood for HIV, and less than one-third of the countries have policies, procedures, or transfusion guidelines to limit TT-HIV.

According to Reilly, many of these countries do not have successful, sustained donor programs based on voluntary, non-remunerated donors, due to a variety of cultural, religious, and economic barriers.

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識別番号・報告回数			報告日	第一報入手日 2004年11月15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	人ハプトグロビン	研究報告の公表状況	Science/ Sciencexpress 11 November 2004	公表国 イギリス
販売名 (企業名)	ハプトグロビン注－ヨシトミ(ベネシス)			

研究報告の概要

プリオン株はそれぞれ生化学的に特有の形態の疾患関連プリオンタンパク質(PrP^{Sc})を伴っている。クロイツフェルト・ヤコブ病(CJD)の特有な表現型を有する患者から得た脳組織中には4種類のPrP^{Sc}のタイプがあることが観察されている：タイプ1−3は古典的(孤発性または医原性)CJDに認められ、タイプ4はvCJD患者に認められている。ヒトPrPの残基129の位置での多型性(メチオニン(M)またはバリン(V)がコードされている)はヒトのプリオン病への遺伝性の罹患しやすさに大きな影響を与えており、これらのヒトPrP^{Sc}タイプの増殖にきわめて重要な影響があるものと考えられる。現在までのところ、タイプ1とタイプ4のPrP^{Sc}は129がメチオニンの同型接合体を有するヒトにのみ認められており、タイプ3のPrP^{Sc}はほぼ例外なく少なくとも1つのバリンアレルを有するヒトに見られ、タイプ2のPrP^{Sc}はコドン129の全ての遺伝子型に共通に観察されている。
129がメチオニンのヒトPrPを発現しているトランスジェニックマウス(129MM Tg35、および129MM TG45マウス)にBSEプリオンおよびvCJDプリオンをチャレンジすると、PrPのflorid plaqueに富んだvCJDの神経病理学的な特徴を伴うタイプ4のPrP^{Sc}の忠実な増殖がもたらされた。しかし、129がバリンのヒトPrPを発現しているトランスジェニックマウス(129VV Tg152マウス)へのvCJDプリオンの初回チャレンジでは、感染率は50%以下であり、感染に対して伝播のバリアーがかなりの程度存在することを示していた。さらに、vCJDを接種された129VV Tg152マウスはタイプ4のPrP^{Sc}でなくタイプ5のPrP^{Sc}を増殖したが、このタイプ5のPrP^{Sc}はタイプ4のPrP^{Sc}で見られるものと同じジグリコシル化された糖鎖型が主体であるが、プロテイナーゼKの消化産物はタイプ4の消化産物より分子量が大きいことで区別され、このものはヒトのタイプ2のPrP^{Sc}で見られたものと非常に類似している。
ある動物種から別の動物種へのプリオンの一次伝播には種間または伝播のバリアーがあるが、一次伝播後、第2の動物種の体内でそのプリオンが新しい宿主に適応するので、第2代目およびそれ以降の継代をその第2の動物種で行うとバリアーはかなりの程度または完全に失われている。BSEを接種され臨床症状の出ている129VV Tg152マウス4匹の脳から得た接種物は、別の129VV Tg152マウスに対して臨床症状の出現するプリオン感染または無症状のプリオン感染を伝播させなかった。しかし、BSEを接種された129VV Tg152マウスの体内で作られたプリオン株は、野生型のFVBマウスに対して感染性を有していたが、別の129VV Tg152マウスに対しては感染性がなかった。129MM Tg35マウスでのBSEプリオンの第2代目の継代では、一次伝播は増殖後の株のタイプの分岐をもたらし、タイプ2またはタイプ4のPrP^{Sc}のいずれかを産生し、神経病理学的にはヒト孤発性CJDまたはvCJDとそれぞれ一致していた。これらの知見は129VV Tg152マウスでは同じBSE接種物の第2代目の継代でプリオン伝播が全く見られないこととは全く異なっており、このことはヒトPrP V129はBSEプリオン株の増殖を著しく制限するとの解釈を裏付けるものである。
この結論はさらに、vCJDプリオンの第2代目の継代のパラメーターの研究で補強された。BSEプリオンの第2代の継代で見られたとおり、臨床症状はFVBマウスでのみ認められ、129VV Tg152マウスのレシピエントでは認められなかった。臨床症状の出現している、タイプ5 PrP^{Sc}陽性の初代継代のvCJDを接種された129VV Tg152マウスの脳を接種物としてFVBマウスに接種すると、FVBマウスのそれ以降の継代でプリオン病の臨床症状が出現し、PrP^{Sc}の蓄積が認められたが、129VV Tg152に接種すると11匹中7匹では不顕性の感染(PrP^{Sc}の蓄積を伴う)のみが認められた。タイプ5のPrP^{Sc}は129VV Tg152マウス中で第2代目の継代で忠実に増殖した。
動物モデルを外挿することには注意を払う必要はあるが、ここで示したような、分子的および病理学的表現型の再現が可能であるような場合においても、我々の知見は、ヒトBSEプリオン一次感染、および医原性の経路によるvCJDプリオンによる二次感染は、疾病の表現型が一つに限定されない可能性を示している。これらのデータは、血液のレシピエント(PRNPコドン129 MVヘテロ接合体を有するヒトを含む)に対してvCJDプリオンの医原性の伝播が起こった可能性があるとの最近の知見と考え合わせると、ヒトプリオン病の患者全てをPrP^{Sc}のタイプによって分類することの必要性を再度強調するものである。そのサーベイランスによって、BSE曝露のパターンやvCJDプリオンの医原性ソースに関連した新規のPrP^{Sc}タイプや特定のPrP^{Sc}サブタイプの相対的頻度の何らかの変化を早期に発見できるであろう。

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。

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報告企業の意見	今後の対応	
BSE 由来プリオンでのヒトへの 1 次及び 2 次感染は、プリオンソース及びレシピエントの遺伝型の如何により、孤発性 CJD 様表現型もしくは vCJD に加えてさらに新規の表現型をもたらす可能性があり、また、ヒトプリオン病の患者全てを PrP^{Sc} のタイプに分類したサーベランスを行なうことによって、BSE 曝露パターンや vCJD プリオンの医原性ソースに関連した新規の PrP^{Sc} タイプや特定の PrP^{Sc} サブタイプの相対的頻度の何らかの変化を早期に発見できるであろうとする報告である。 現時点で vCJD が報告されているのは英国と、英国滞在歴のない vCJD 患者についてはフランス、イタリア、アイルランドのみである。なお、これまで血漿分画製剤からの vCJD 伝播は報告されていない。しかしながら、万一 vCJD 感染者の血液が原料に混入した場合には、血漿分画製剤の製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ないため、弊社においても血漿分画製剤の製造工程における TSE の感染性の低減に関する検証実験を行っている。	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。	

Sciencexpress Report

Human Prion Protein V129 Prevents Expression of Variant CJD Phenotype

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Variant CJD (vCJD) is a unique and highly distinctive clinicopathological and molecular phenotype of human prion disease associated with infection with BSE-like prions. Here we found that generation of this phenotype in transgenic mice required expression of human PrP 129 methionine. Expression of human PrP 129 valine resulted in a distinct phenotype and, remarkably, persistence of a barrier to transmission of BSE-derived prions on sub-passage. Polymorphic residue 129 of human prion protein dictated propagation of distinct prion strains following BSE prion infection. Thus primary and secondary human infection with BSE-derived prions may result in sporadic CJD-like or novel phenotypes in addition to vCJD, depending on the genotype of the prion source and the recipient.

Distinct prion strains are associated with biochemically distinct forms of disease-related prion protein (PrP^{Sc}). Four PrP^{Sc} types have been observed in brain tissue from patients with distinct Creutzfeldt-Jakob disease (CJD) phenotypes: types 1-3 in classical (sporadic or iatrogenic) CJD, and type 4 in vCJD (*1–3*). Polymorphism at residue 129 of human PrP (where either methionine (M) or valine (V) can be encoded) powerfully affects genetic susceptibility to human prion diseases (*4–7*) and appears to critically influence the propagation of these human PrP^{Sc} types. So far, types 1 and 4 PrP^{Sc} have been found only in humans homozygous for 129 methionine, type 3 PrP^{Sc} is seen almost exclusively in individuals with at least one valine allele, while type 2 PrP^{Sc} has been commonly observed in all codon 129 genotypes (*1–3*). Bovine spongiform encephalopathy (BSE) and vCJD prion infection in transgenic mice expressing human PrP, but not mouse PrP (*1, 8–10*), indicates that codon 129 polymorphism determines the ability of human PrP to form type 4 PrP^{Sc} and generate the neuropathological phenotype of vCJD.

Challenge of transgenic mice expressing human PrP 129 methionine (129MM Tg35 and 129MM Tg45 mice) with BSE and vCJD prions (*11*) resulted in faithful propagation of type 4 PrP^{Sc} (*10*) (Figs.1 and 2) accompanied by the key neuropathological hallmark of vCJD the presence of abundant florid PrP plaques (*10*). However, transgenic mice expressing human PrP 129 valine (129VV Tg152 mice) responded quite differently. Although these 129VV Tg152 mice lack a transmission barrier to classical forms of CJD, regardless of the codon 129 genotype of the inoculum (*1, 8, 9*), primary challenge with vCJD prions was characterised by a substantial transmission barrier to infection (only ~50% of inoculated mice were infected, compared to 100% of 129MM Tg35 and 129MM Tg45 mice) (Fig. 1, table S1). In addition, rather than type 4 PrP^{Sc}, vCJD-inoculated 129VV Tg152 mice propagated type 5 PrP^{Sc} (*9*), which shares the same predominance of the diglycosylated glycoform seen in type 4 PrP^{Sc} but is distinguished by proteinase K digestion products of greater molecular mass (Fig. 2A), which closely resemble those seen in human type 2 PrP^{Sc} (*9*). Type 5 PrP^{Sc} is associated with very weak diffuse PrP deposition in the brain (*9*) which contrasts markedly with the florid PrP plaques associated with the propagation of type 4 PrP^{Sc} in humans (*12*) or transgenic mice (*10*). Similar diffuse deposition of PrP is also observed in clinically-affected BSE-inoculated 129VV Tg152 mice, however type 5 PrP^{Sc} is undetectable in brain homogenate (*9*).

To further evaluate the molecular and neuropathological phenotype of vCJD-or BSE-inoculated 129VV Tg152 mice, we performed a second passage in the same breed of mice. Primary transmission of prions from one species to another is associated with a species or transmission barrier that is largely or completely abrogated on second and subsequent passage in the second species as the prions adapt to the new host. Second passage then resembles within-species transmission with a high (typically 100%) attack rate and

much shortened and more consistent incubation period. Remarkably, however, such adaptation did not occur on second passage of BSE or vCJD prions in 129VV Tg152 mice. Brain inocula derived from four clinically-affected BSE-inoculated 129VV Tg152 mice failed to transmit clinical disease or asymptomatic prion infection to further 129VV Tg152 mice (Figs. 1 and 3, table S2). However, two of these inocula produced clinical prion disease (Fig. 3, table S2) with abundant PrP^{Sc} accumulation (fig. S1) on inoculation of wild type FVB mice with incubation periods that are not compatible with persistence of the original BSE inoculum (supporting online text). The prion strain generated in BSE-inoculated 129VV Tg152 mice was thus infectious in wild type FVB mice, but not in further 129VV Tg152 mice.

V129 is unique to human PrP and the failure of BSE prions to adapt in 129VV Tg152 mice on second passage contrasts sharply with the marked adaptation of BSE prions in FVB mice (Fig. 3, table S2) or in other murine (*13*) or primate (*14*) hosts that encode methionine at the corresponding position of PrP. BSE prions also efficiently adapt on second passage in 129MM Tg35 transgenic mice. Primary transmission of BSE prions in 129MM Tg35 mice resulted in bifurcation of propagated strain type, producing either type 2 or 4 PrP^{Sc} (Figs. 1 and 2) and neuropathology consistent with human sporadic CJD or vCJD, respectively (*10*). These distinct molecular and neuropathological phenotypes consistently 'breed true' with very high efficiency on second passage in further 129MM Tg35 transgenic mice (*15*). These findings contrast sharply with the complete lack of prion transmission on second passage of the same BSE inocula in 129VV Tg152 mice, supporting the interpretation that human PrP V129 severely restricts propagation of the BSE prion strain.

This conclusion was further reinforced by study of the parameters of second passage of vCJD prions. As seen with second passage of BSE prions, clinical disease was observed only in FVB and not in 129VV Tg152 recipients. Brain inocula from clinically affected, type 5 PrP^{Sc} positive, primary vCJD-inoculated 129VV Tg152 mice produced clinical prion disease (Fig. 3, table S2) and PrP^{Sc} accumulation (fig. S1) on sub-passage in FVB mice, but produced only sub-clinical infection (with PrP^{Sc} accumulation) in 7/11 inoculated 129VV Tg152 mice (Figs. 1 and 3). Notably, in four of these, high sensitivity methods (*16*) were required to detect PrP^{Sc} in brain homogenate (table S2). Type 5 PrP^{Sc} was faithfully propagated on second passage in 129VV Tg152 mice (Fig. 2A). In the three mice containing the highest levels of type 5 PrP^{Sc}, extensive spongiosis was also observed (Fig. 4) and in one of these, in contrast to the pathology seen on first passage, numerous PrP plaques were seen (Fig. 4). Type 4 PrP^{Sc} is invariably associated with prominent florid plaques in the cortex of human vCJD brain (*12*) and in vCJD- or BSE-prion inoculated 129MM Tg35 and Tg45 transgenic mice (*10*), whereas plaques associated with type 5 PrP^{Sc} were restricted to the corpus callosum and had a non-florid morphology (Fig. 4). The lack of adaptation of vCJD prions on second passage in 129VV Tg152 mice contrasted sharply with the behaviour of vCJD prions in wild type FVB mice, where typical adaptation was observed on second passage with 100% clinical prion disease with abundant PrP^{Sc} accumulation (Fig. S1) at markedly reduced incubation periods (Fig. 3, table S2).

Both BSE and vCJD prions failed to propagate efficiently on either primary or, remarkably, on second passage, in 129VV Tg152 mice in sharp contrast to 129MM Tg mice or wild type animals, and where detectable, infection was associated with a distinct PrP^{Sc} type and pathological phenotype. Thus human PrP V129 appears not to be a compatible substrate for propagation of the prion strain seen in vCJD. This interpretation was supported by the transmission properties of 129VV Tg152-passaged vCJD prions in 129MM Tg35 mice. Here 14/15 129MM Tg35 mice inoculated with isolates containing type 5 PrP^{Sc} showed PrP^{Sc} accumulation (Fig. 1, Table S3), typically to much higher levels than seen in 129VV Tg152 mice receiving the same inocula. However, the PrP^{Sc} seen was not of the type 5 pattern but instead these transmissions mirrored the behaviour of BSE prions in 129MM Tg35 mice (*10*), where, instead, either type 4 or type 2 PrP^{Sc} were seen (Figs. 1 and 2, B-D). Thus human PrP^{Sc} types 4 and 5 are restricted to propagating in mice expressing human PrP M129 or V129, respectively.

Neuropathologically, type 4 PrP^{Sc} was associated with relatively little spongiosis and abundant florid plaques (Fig. 4) typical of vCJD in humans (*12*), whereas type 2 PrP^{Sc} was associated with much higher levels of vacuolation in many areas of the brain, accompanied by generally diffuse PrP deposition and occasional small, non-florid plaques (Fig. 4) that closely resembled human sporadic CJD with type 2 PrP^{Sc} *PRNP* 129MM (*3*). Clinical prion disease was observed in all 129MM Tg35 mice propagating type 2 PrP^{Sc}, whereas mice propagating type 4 PrP^{Sc} were sub-clinically infected (table S3).

In conclusion, we have demonstrated that BSE and vCJD prion infection in transgenic mice can result in the propagation of distinct molecular and neuropathological phenotypes dependent upon host PrP residue 129 and possibly other, as yet unidentified, disease modifying loci (*10*). These data predict a critical role for *PRNP* codon 129 in governing the thermodynamic permissibility of human PrP^{Sc} conformation that can be interpreted within a conformational selection model of prion transmission barriers (*17–19*) (supporting online text) and suggest that there is no overlapping preferred conformation for V129 and M129 human PrP that can be generated as a result of exposure to the

vCJD/BSE prion strain. Biophysical measurements suggest that this powerful effect of residue 129 on prion strain selection is likely to be mediated via its effect on the conformation of PrP^{Sc} or its precursors or on the kinetics of their formation, as it has no measurable effect on the folding, dynamics or stability of PrP^{C} (*20*).

While caution must be exercised extrapolating from animal models, even where, as here, faithful recapitulation of molecular and pathological phenotypes is possible, our findings argue that primary human BSE prion infection, and secondary infection with vCJD prions by iatrogenic routes, may not be restricted to a single disease phenotype. These data, together with the recent recognition of probable iatrogenic transmission of vCJD prions to recipients of blood (*21*, *22*), including a *PRNP* codon 129 MV heterozygote individual (*22*), reiterate the need to stratify all human prion disease patients by PrP^{Sc} type. This surveillance will facilitate rapid recognition of novel PrP^{Sc} types and any change in relative frequencies of particular PrP^{Sc} sub-types in relation to either BSE exposure patterns or iatrogenic sources of vCJD prions.

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Supporting Online Material

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Materials and Methods
SOM Text
Fig. S1
Tables S1 to S3
References and Notes

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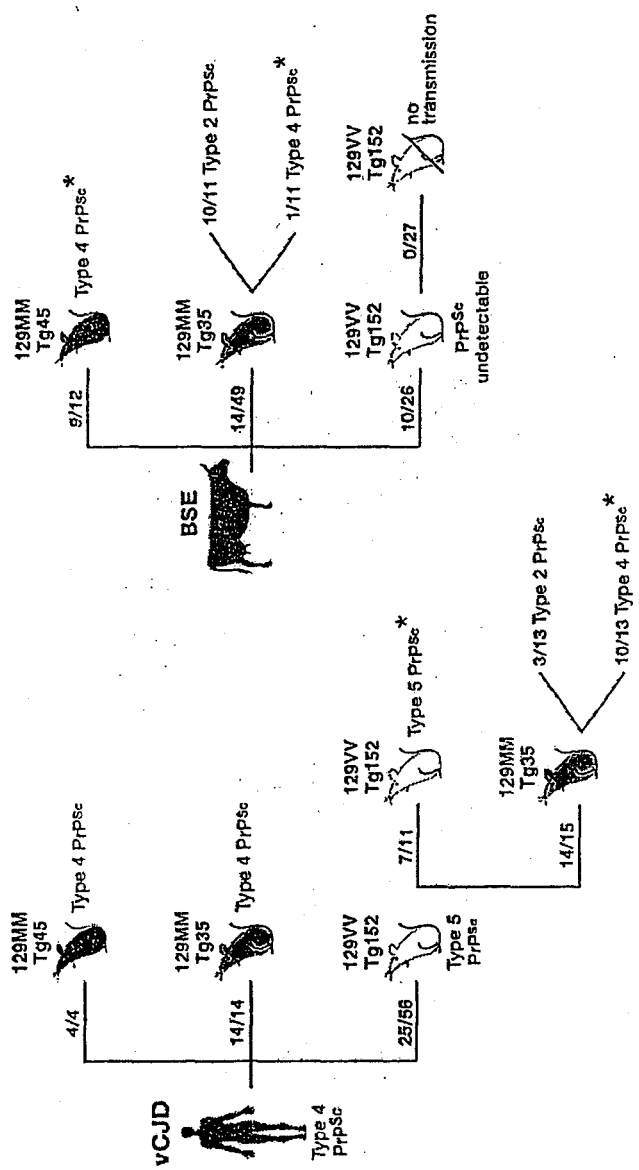
Fig. 1. Summary of transmissions of vCJD and BSE prions to transgenic mice. The total number of prion-affected mice (both clinical and sub-clinical) is reported for each inoculated group; 129MM Tg45 mice (black), 129MM Tg35 mice (grey), 129VV Tg152 mice (white). Animals were scored by clinical signs, immuno-blotting and/or immunohistochemistry. Primary transmission data have been published previously (*9*, *10*). In transmissions that result in bifurcation of propagated PrP^{Sc} type, the number of type 2 or type 4 PrP^{Sc} positive samples is reported as a proportion of the total number of samples examined by immuno-blotting. * denotes the occurrence of sub-clinical prion infection only.

Fig. 2. Molecular strain typing of vCJD and BSE prion transmissions in transgenic mice. (A-D) Immuno-blots of proteinase-K treated brain homogenates from variant and sporadic CJD (*PRNP* 129 MM genotype) and transgenic mice were analysed by enhanced chemiluminescence with either anti-PrP monoclonal antibody 3F4 (A) or biotinylated anti-PrP monoclonal antibody ICSM 35 (B-D). The identity of the brain sample is designated above each lane with the type of PrP^{Sc} present in the sample designated below. ‡ denotes

transmissions that result in the propagation of either type 2 or type 4 PrP^{Sc}.

Fig. 3. Summary of transmissions of vCJD and BSE prions to transgenic and wild type FVB mice. The total number of prion-affected mice (both clinical and sub-clinical) is reported for each inoculated group; 129VV Tg152 mice (white), wild type FVB mice (grey). Animals were scored by clinical signs, immuno-blotting and/or immunohistochemistry. Data are derived from tables S1 and S2. * denotes the occurrence of sub-clinical prion infection only.

Fig. 4. Neuropathological analysis of transgenic mouse brain. Primary transmission of vCJD prions in 129VV Tg152 mice produces type 5 PrP^{Sc} that is maintained after secondary passage in 129VV Tg152 mice but induces propagation of either type 2 or type 4 PrP^{Sc} after passage in 129MM Tg35 mice. Immunohistochemistry (PrP) shows abnormal PrP immunoreactivity, including PrP-positive plaques, stained with anti-PrP monoclonal antibody 3F4. Haematoxylin- and eosin-stained sections (H&E) show spongiform neurodegeneration (left, corpus callosum; middle and right, parietal cortex). Scale bar: 100 μm. Lower panels show the regional distribution of abnormal PrP deposition. Green boxes in the sketches denote the area from which PrP stained sections are derived.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4/4
129MM Tg45
Type 4 PrPSc
14/14
129MM Tg35
Type 4 PrPSc
25/56
129VV Tg152
Type 5 PrPSc
BSE
9/12
129MM Tg45
Type 4 PrPSc*
14/49
129MM Tg35
10/11 Type 2 PrPSc
1/11 Type 4 PrPSc*
10/26
129VV Tg152
PrPSc undetectable
0/27
129VV Tg152
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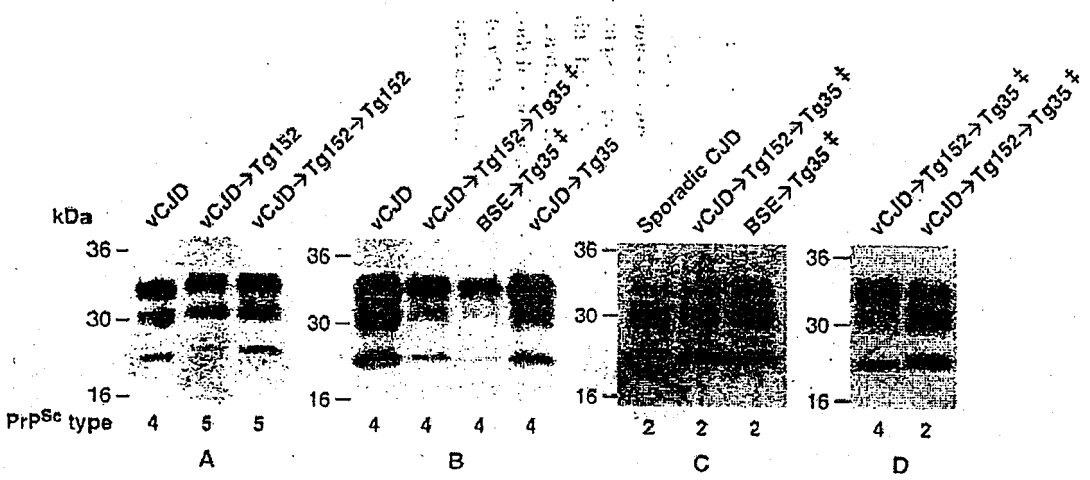
10/13 Type 4 PrPSc*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vCJD→Tg152→Tg152
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30
16
PrPSc type 4 5 5
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vCJD
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BSE→Tg35+
vCJD→Tg35
36
30
16
4 4 4 4
B
Sporadic CJD
vCJD→Tg152→Tg35+
BSE→Tg35+
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30
16
2 2 2
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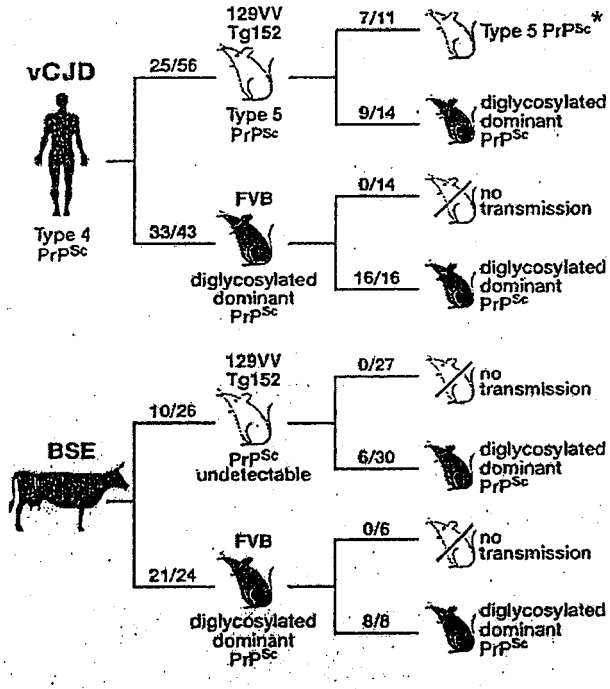
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PrPSc
25/56
129VV
Tg152
Type 5
PrPSc
7/11
Type 5 PrPSc*
9/14
diglycosylated
dominant
PrPSc
33/43
FVB
diglycosylated
dominant
PrPSc
0/14
no
transmission
16/16
diglycosylated
dominant
PrPSc
BSE
10/26
129VV
Tg152
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undetectable
0/27
no
transmission
6/30
diglycosylated
dominant
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0/6
no
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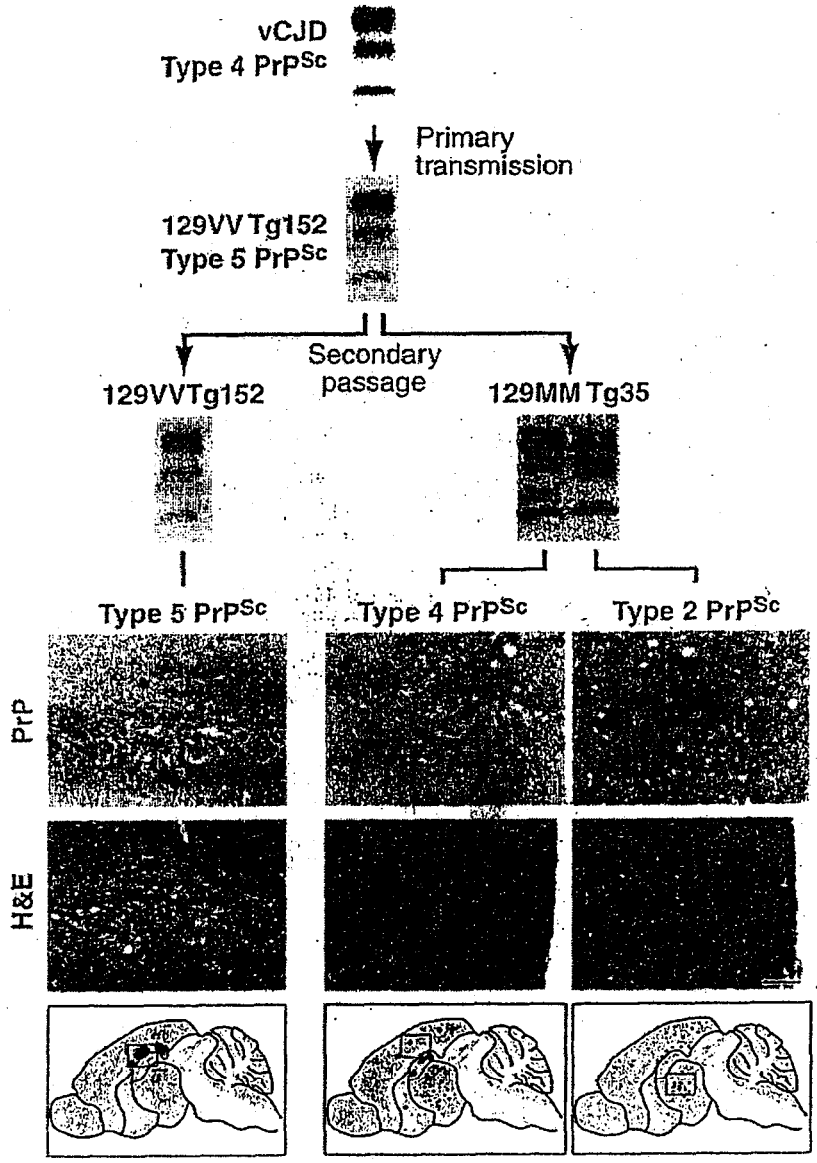
vCJD
Type 4 PrPSc
Primary transmission
129VV Tg152
Type 5 PrPSc
Secondary passage
129VVTg152
129MM Tg35
Type 5 PrPSc
Type 4 PrPSc
Type 2 PrPSc
PrP
H&E