別紙様式第2-1
番号 14

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2006年6月26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	①②ポリエチレングリコール処理人免疫グロブリン ③人免疫グロブリン		研究報告の公表状況	The Lancet 2006; 367(9528):2068-2074	公表国 パプアニューギニア	
販売名（企業名）	①献血ヴェノグロブリン-IH ヨシトミ（ベネシス） ②ヴェノグロブリン-IH（ベネシス） ③グロブリン-Wf（ベネシス）					

研究報告の概要

＜背景＞
クールー病は、ヒトの流行性プリオン病について重要な経験を提供している。その発生は、1950年代にパプアニューギニアでの感染ルート（部族内食人）の突然の中断の後、着実に減っていった。vCJDが発生し、英国において食事により広範囲にBSEプリオンへの曝露された後の感染率は不明であるために、クールー病が再び関心を呼ぶことになった。我々は、パプアニューギニアのクールー病患者における、潜伏期間、病因及び感染しやすさについての遺伝的要因について調査した。
＜方法＞
我々は、全ての疑わしい患者を調査するために、調査チームの規模を広げるとともに1996年にクールー病の監視を強化した。住民の居住歴と葬儀の宴での曝露歴の情報が、一連の神経学的検査とともに、可能な限り集められた。
＜結果＞
我々は、1996年7月から2004年6月までに11人のクールー病患者を確認したが、全員がSouth Foreに住んでいた。患者は全員、1950年代後半に食人習慣が中止される前に生れていた。最短の推定潜伏期間は、34年から41年の範囲であった。しかし、男性における潜伏期間は39年から56年の範囲と考えられたが、実際はこれより最長で7年長かった可能性がある。プリオン蛋白の分析によって、殆どのクールー病の患者は、潜伏期間の延長とプリオン病への抵抗性に関連づけられている多形コドン129がヘテロ接合体の遺伝子型であった。
＜解釈＞
ヒトのプリオンに感染した場合の潜伏期間は、50年を超える可能性がある。BSEプリオンにヒトが感染した場合、種を超えた場合の感染の特徴である「種の壁の効果」が、同じ種の中でのプリオンの感染と比較して、潜伏期間の平均値と範囲をさらに大きくするであろう。これらのデータは、vCJDの疫学モデルの作成の試みに影響を与えるはずである。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
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
パプアニューギニアのクールー病患者の研究から、ヒトのプリオンに感染した場合の潜伏期間は、50年を超える可能性があることを示唆した報告である。 これまで血漿分画製剤によってvCJDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、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。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	vCJDの疫学情報については、今後も注視することとする。

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Articles

Kuru in the 21st century—an acquired human prion disease with very long incubation periods

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Lancet 2006; 367: 2068–74

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Summary

Background Kuru provides the principal experience of epidemic human prion disease. Its incidence has steadily fallen after the abrupt cessation of its route of transmission (endocannibalism) in Papua New Guinea in the 1950s. The onset of variant Creutzfeldt-Jakob disease (vCJD), and the unknown prevalence of infection after the extensive dietary exposure to bovine spongiform encephalopathy (BSE) prions in the UK, has led to renewed interest in kuru. We investigated possible incubation periods, pathogenesis, and genetic susceptibility factors in kuru patients in Papua New Guinea.

Methods We strengthened active kuru surveillance in 1996 with an expanded field team to investigate all suspected patients. Detailed histories of residence and exposure to mortuary feasts were obtained together with serial neurological examination, if possible.

Findings We identified 11 patients with kuru from July, 1996, to June, 2004, all living in the South Fore. All patients were born before the cessation of cannibalism in the late 1950s. The minimum estimated incubation periods ranged from 34 to 41 years. However, likely incubation periods in men ranged from 39 to 56 years and could have been up to 7 years longer. *PRNP* analysis showed that most patients with kuru were heterozygous at polymorphic codon 129, a genotype associated with extended incubation periods and resistance to prion disease.

Interpretation Incubation periods of infection with human prions can exceed 50 years. In human infection with BSE prions, species-barrier effects, which are characteristic of cross-species transmission, would be expected to further increase the mean and range of incubation periods, compared with recycling of prions within species. These data should inform attempts to model variant CJD epidemiology.

Kuru is one of a group of closely related neurodegenerative conditions that affect both human beings and animals, known as the transmissible spongiform encephalopathies or prion diseases.[1] Prion diseases are associated with the accumulation in the brain of an abnormal, partly protease-resistant, isoform of a host-encoded glycoprotein known as prion protein (PrP). According to the protein-only hypothesis, an abnormal PrP isoform is the main, and possibly the only, constituent of the transmissible agent or prion.

The large-scale epidemic of bovine spongiform encephalopathy (BSE) in the UK led to fears of a serious threat to public health. Since 1996, cases of a novel human prion disease, variant Creutzfeldt-Jakob disease (vCJD), have been identified in the UK, and strain typing has confirmed that both vCJD and BSE are caused by the same prion strain.[1] Dietary exposure of the UK population to BSE prions has been widespread; the total cattle epidemic is thought to have affected 2 million cows.[2] Cattle BSE has also been reported in most EU states, Israel, Switzerland, Canada, the USA, and Japan. So far, about 160 vCJD patients have been identified in the UK, with cases also reported in France, Italy, Ireland, the Netherlands, Canada, Japan, and the USA. Predictions of the eventual size of a vCJD epidemic have varied widely, although some recent estimates, based on current cases of vCJD, suggest that the total epidemic may be relatively small.[3] However, key uncertainties, notably with respect to major genetic effects on the incubation period,[4] suggest the need for caution. Importantly, such models cannot estimate the number of infected individuals, which remains unknown, and concerns of secondary transmission have heightened.[5] These uncertainties, especially with the possibility of very long incubation periods of BSE in people, have renewed interest in kuru, which remains the only example of a major human epidemic.

Kuru reached epidemic proportions in a defined population of the Eastern Highlands of Papua New Guinea. Local oral history, taken when the disease was first studied by western medicine in the 1950s, dated the onset of the first cases to the 1920s. Kuru mainly affected the people of the Fore linguistic group and also their neighbours with whom they intermarried. The disease predominantly affected women and children (of both sexes), with only 2% of cases in adult men;[6] kuru also became the most common cause of death in women in affected villages. Kuru is a cerebellar syndrome with a characteristic and relentless progression through defined clinical stages, and is invariably fatal. Cognition is fairly preserved, and the disease is highly distinctive and is usually recognised easily by both the patients and their local community.[6]

These communities practised the consumption ritual of dead relatives as a mark of respect and mourning. Boys older than 6–8 years participated little in mortuary

feasting, which could explain the differential age and sex incidence. From the age of the youngest affected patient, the shortest incubation period is estimated to be 5 years, although this time could be shorter, since time of infection is usually unknown.

Genetic susceptibility is important in both the sporadic and acquired forms of human prion disease; human PrP has a common polymorphism, with either methionine or valine present at residue 129. About 38% of Europeans are homozygous for the more frequent methionine allele, 51% are heterozygous, and 11% homozygous for valine. Most sporadic CJD occurs in individuals homozygous for this polymorphism.[7] This susceptibility factor is also relevant in the acquired forms of CJD, most strikingly in vCJD; all clinical cases studied so far have been homozygous for codon 129 methionine of the PrP gene *PRNP*.[5] The *PRNP* codon 129 genotype has shown a pronounced effect on kuru incubation periods and susceptibility, and most elderly survivors of the kuru epidemic are heterozygotes.[8,9] The clear survival advantage for codon 129 heterozygotes provides a powerful basis for selection pressure in the Fore. However, an analysis of worldwide haplotype diversity and allele frequency of coding and non-coding polymorphisms of *PRNP* suggests that balancing selection at this locus (in which there is more variation than expected because of heterozygote advantage) is much older and more geographically widespread. Evidence for balancing selection has been shown in only a few human genes. With biochemical and physical evidence of cannibalism on five continents, one explanation is that cannibalism resulted in several prion disease epidemics in human prehistory, thus imposing balancing selection on *PRNP*.[9]

Kuru was extensively studied at its peak in the late 1950s and early 1960s and monitoring was continued through the Papua New Guinea Institute of Medical Research. We strengthened active kuru surveillance in 1996 and aimed to study all patients with kuru until the end of the epidemic. Here, we aimed to determine the maximum period possible for incubation in human prion infection and to investigate genetic factors in recent patients with kuru. The abrupt interruption of transmission of kuru, after the effective prohibition of cannibalism by Australian authorities in the mid-1950s, allows a unique opportunity to investigate the incubation period of infection as a key variable in human prion disease. Our findings of detailed clinical features and mortuary feast practices will be reported elsewhere.

Methods

Research ethics

Our study was approved by the Papua New Guinea Medical Research Advisory Committee and by the local research ethics committees of St Mary's Hospital and National Hospital for Neurology and Neurosurgery, in London, UK. The full participation in the project from the communities, which was critical with respect to the ethics and operation of the study, was established and maintained through discussions with village leaders, communities, families, and individuals, and the field studies followed the principles and practice of the Papua New Guinea Institute of Medical Research.

Kuru surveillance and clinical studies

A field base and laboratory was established in Waisa in the South Fore. A team of local kuru reporters communicated details of any suspected case to the field base. 50 suspect cases investigated during this period proved not to have kuru. The field team consisted of staff from the UK Medical Research Council (MRC), the Papua New Guinea Institute of Medical Research, and local communities, who undertook regular field patrols throughout the kuru-

	Sex	Year of birth	Onset	Age at onset (years)	Age at death (years)	*PRNP* 129 genotype	Minimum incubation period (years)*	Likely incubation period (years)†
PKW	F	1946	August, 1995	49	50	Heterozygous	35	..
YAK	M	1948	November, 1994	46	48	Heterozygous	34	39
MWK	M	1933	April, 1996	63	64	Methionine homozygous	36	56
AKA	M	1949	November, 1996	47	49	Heterozygous	36	40
AYA	M	1936	November, 1998	62	63	n/a	38	55
TAM	F	1945	March, 1999	54	55	Valine homozygous	39	..
AYY	M	1940	June, 1998	58	60	Heterozygous	38	51
WKW	M	1943	January, 1999	56	57	Heterozygous	39	49
MAA	F	1944	April, 1999	55	57	Heterozygous	39	..
INO	F	1942	January, 2000	58	59	Heterozygous	40	..
KAW	M	1943	October, 2001	58	60	Heterozygous	41	51

Patients' initials are based on name and coded. *Calculated conservatively as the number of years between 1960 and onset of kuru (assuming latest possible exposure at mortuary feast in 1959). This period would be an underestimate (in some cases of many years) of the actual incubation period (measured from the date that infection was actually acquired). The maximum incubation period possible (in the event of neonatal infection) would be the same as age at onset. †Since male individuals were unlikely to be infected after age 6–8 years, the likely minimum incubation period can be calculated as the number of years from age 7 years to disease onset, which is also a conservative estimate, since actual infection could have taken place (up to 7 years) earlier. F=female. M=male. n/a=not available

Table 1: Estimation of kuru incubation periods in 11 patients identified in current study

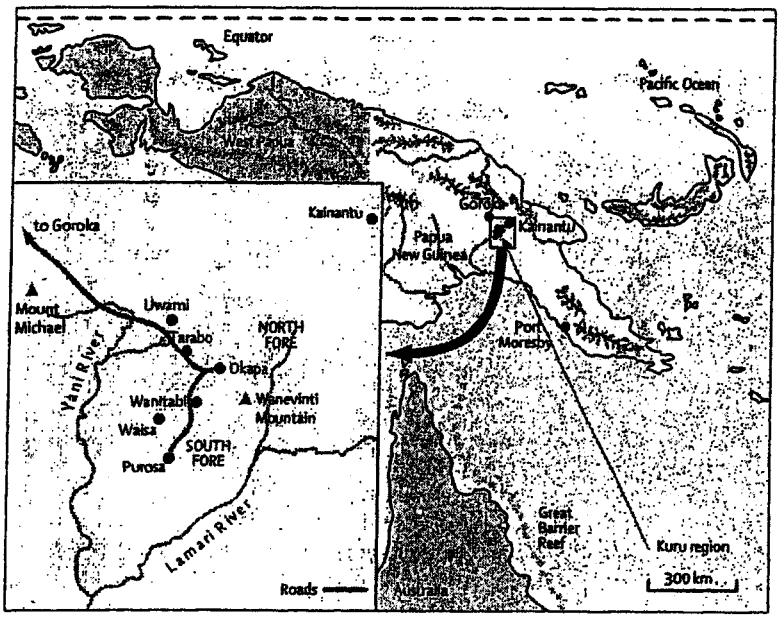

Figure 1: Area and Fore groups historically affected by kuru in the Eastern Highlands Province of Papua New Guinea

affected area, which included the North and South Fore, Keiagana, Kanite, and Gimi linguistic groups. The fieldworkers had to work under very difficult conditions during the surveillance, and heavy-vehicle rescue equipment and a six-man team were needed at times to proceed with the project. Security issues intermittently affected our ability to travel to and from the field site. Regular field neurological examinations were done when possible and recorded by video and photography. Case histories and patterns of exposure to mortuary feasts were also documented.

Molecular genetic studies

We extracted genomic DNA from venous blood to determine the complete coding sequence of *PRNP* (PrP gene).[10] Papua New Guinean control genotype data were obtained by restriction endonuclease digestion of PCR amplicons (for comparison with *APOE* [apolipoprotein E] and *PRND* [Doppel, a prion-protein-like protein] genes), and allelic discrimination with the ABI SDS7000 sequence detection system (for comparison with codon 129 polymorphisms and haplotypes of *PRNP*). We identified HLA-DQB1 alleles by automated fluorescent sequencing of PCR amplicons using the Amersham MEGAbace DNA analysis system (Amersham, UK).

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The total number of cases of kuru from 1957 to 2004 exceeded 2700, with more than 200 dying every year in the late 1950s. This number fell to about six a year in the early 1990s and between one and two a year during the study; since July, 1996, we identified 11 kuru patients up to the end of June, 2004.

Table 1 shows the ages at onset of all 11 patients in the study. Age might not be accurately known or reported by individuals in these communities, but can be reliably and accurately estimated by reference to family relationships and clearly defined recent historical events.[11] Figures 1 and 2 show the area and populations historically affected by kuru. All patients identified in the current study were from the South Fore, now that kuru has disappeared from the North Fore and adjacent linguistic groups (figure 2).

On the basis of a conjunction of experimental, epidemiological, and human behavioural evidence,[12] we can assume that all transmission through the traditional mortuary practice had ceased by 1960. The practice had been important to the Fore people as a way of respecting their dead relatives, but it was rigorously forbidden by Australian Government officers in one of their first acts of administrative control after making contact with the people, when the Okapa patrol post was established in 1954. Public consumption of dead relatives ceased almost immediately and compliance was ensured by the police force responsible for the subdistrict. By 1956, endocannibalism was effectively gone. Surreptitious eating of dead relatives had been reported in remote communities for some years afterwards, but by the end of the 1950s the practice had ended. Epidemiological surveillance for kuru began in 1957 and has been continued ever since. Because of the wide geographical extent of the families participating in a feast, secret feasting with entire families taking part would not have gone undetected. The communities of the North Fore, who had been the first of the Fore people to lose their traditional practices in the wake of Australian administrative control, ended their mortuary feasting at the beginning of the decade or earlier; kuru is no longer present in this area. The latest year of birth recorded for any patient with kuru is 1959; only nine patients are recorded as being born since 1956. These individuals were mostly young patients and their ages at onset could have been underestimated by a few years. Therefore, for practical purposes, we can assume that all transmission through the traditional mortuary practices had ceased by 1960.

Therefore, we could define the minimum incubation period as the time between 1960 and the date of onset of kuru (table 1). For patients born in earlier decades, the actual incubation could be much longer; however, since the infecting event can never be known, all individuals alive during the period when consumption of dead relatives' bodies was universally practised must be regarded as being at risk.

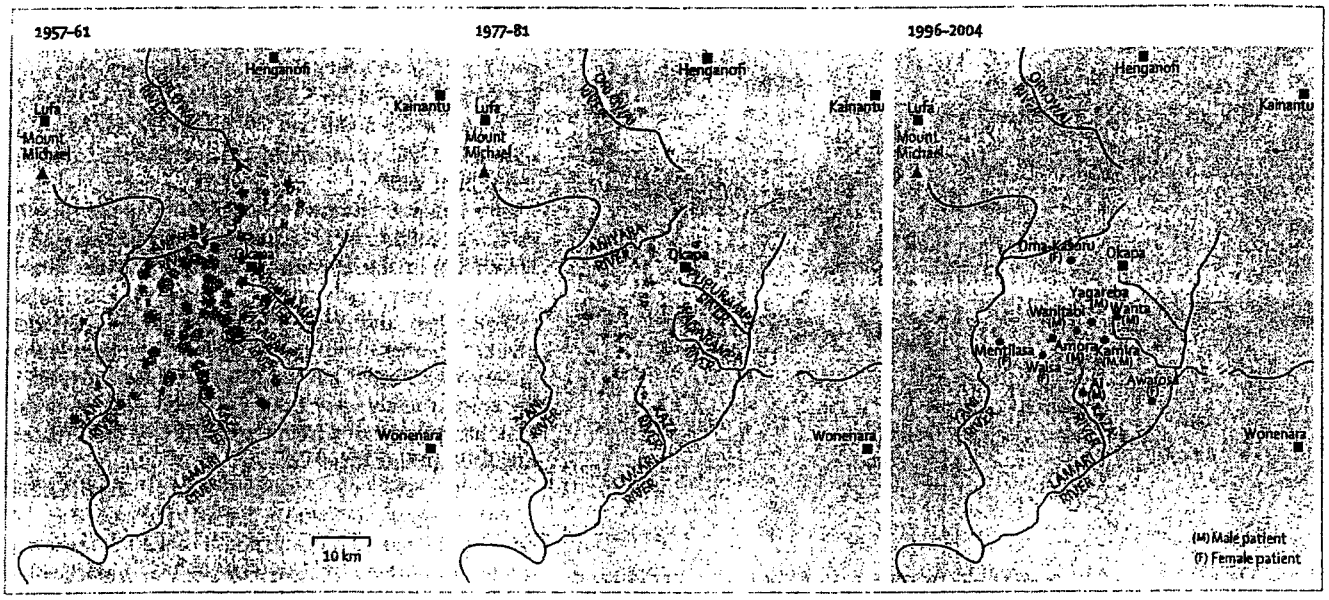

Figure 2: **Individual patients with kuru recorded during the 5 years immediately after cessation of endocannibalism (1957–61), after 20 years (1977–81), and in the current study (1996–2004)**
Patients plotted by village of residence. Black squares indicate towns.

After the age of 6–8 years, boys were taken from their mothers and brought up in the men's house. From this point on, they were exposed only to the same risk as adult men, who participated little in feasts and did not eat the brain, by far the most infectious organ in kuru. This practice can explain why adult men in 1957–58 contributed only 2% to the total number of kuru cases; and from what we now know about the incubation period, most would have been from transmissions in their childhood. Therefore, we can estimate that the likely incubation period of the male patients identified in this study began at about age 7 years, lasting up to the age of onset (table 1). These projections are less robust than the minimum estimates, but nevertheless are potentially real and should not be ignored in any consideration of the incubation period of human prion diseases.

	PRNP haplotype	*APOE*	*PRND* 174 genotype	*HLA-DQB1*
PKW	AF	E2,E3	MT	*050301/*0602
YAK	AB	E2,E3	MT	*0401/NR
MWK	FF	E3,E3	TT	*050301/*050201
AKA	AF	E4,E4	MT	*050301/*030101
AYA	n/a	n/a	n/a	n/a
TAM	AA	E3,E3	TT	*0602/*0602
AYY	AF	E3,E3	TT	*050301/*0602
WKW	AF	E3,E3	TT	*0602/*0602
MAA	AF	E2,E3	TT	*050301/*0602
INO	AF	E2,E4	TT	*050301/*0602
KAW	AF1 or A1F	E3,E4	TT	*050301/*0602

Patient's initials are based on name and coded. NR=allele not recognised. n/a=not available. MT=methionine-threonine heterozygous. *APOE*, *PRND* 174, and *HLA-DQB1* are genetic loci implicated in human prion disease susceptibility.

Table 2: **Genetic analysis in kuru patients identified in current study**

DNA was available for genetic analysis from ten of 11 patients with kuru (table 2). Eight patients were heterozygous at the polymorphic residue 129 of *PRNP*. In 140 unrelated healthy individuals from the South Fore, allele frequencies at the *PRNP* residue 129 were 48% methionine and 52% valine. Although most of the kuru cases with long incubation periods were heterozygous (as expected), this distribution did not differ from the current frequencies of the control population (χ^2 test, p=0·22). The *PRNP* B haplotype has been associated with susceptibility to sporadic CJD in the UK,[18] and *PRNP* haplotyping was also done in these patients and in the healthy Fore population (table 2). Although the analysis had a small sample size, *PRNP* haplotypes of kuru patients with long incubation periods did not differ significantly from the healthy South Fore population (χ^2 test, p=0·85). For other genetic loci implicated in human prion disease susceptibility (*PRND*,[13–15] *APOE*,[16,17] and *HLA*;[18,19] table 2), these alleles for kuru patients with long incubation periods also were not significantly different from the healthy population in the South Fore.

Discussion

The early clinical, epidemiological, and anthropological study of kuru; the recognition of its neuropathological, and then causal parallels to ovine scrapie;[10] and then crucially, the experimental transmission of the disease

to primates,[21] originated the concept of the human transmissible spongiform encephalopathies, which was followed in turn by the eventual unifying concept of the mammalian prion diseases. However, in addition to the central historical importance of kuru, study of the end-stage of this epidemic offers a unique opportunity to study the variables of a near-complete epidemic of human prion disease. In particular, recognition of the incubation periods possible after natural prion infection in people is important in providing an insight (from actual case histories rather than from mathematical models) into the probable span of the vCJD epidemic in the UK. Although estimation of kuru incubation periods early in the epidemic was difficult, and the timing of the actual infecting event for an individual can rarely be determined, the abrupt and permanent interruption of the source of infection, endocannibalism, in the late 1950s, has progressively allowed recognition of an enormous span of possible incubation periods, at its shortest extreme bracketed by the rare onset of disease in children as young as 5 years and extending up to (and perhaps beyond) the incubations covering more than half a century, as we describe here.

In our field studies, we have interviewed many individuals who participated in traditional mortuary feasting or who described the participation of family members from the preceding generation. These detailed descriptions will be published elsewhere but have reaffirmed the oral histories of endocannibalism in the Fore recorded previously[12,22–24] and that this practice ceased abruptly at the time of Australian administrative control over the kuru areas. Although isolated events might have occurred for a few years after this prohibition, we are confident that new exposures of individuals to kuru at mortuary feasts would not have occurred after 1960. Not only have no cases of kuru been recorded in people born after 1959 (and only nine were recorded in those born after 1956); but also all the 11 last recorded cases of kuru that we report here were born before 1950. If any source of infection remained, whether from surreptitious cannibalism, possible ground contamination with human prions at sites where food was prepared, or other lateral routes, we would expect individuals born after this period to have kuru—especially since children are thought to have had shorter incubation periods than adults. However, no such cases have been observed.

Additionally, although a fraction of hamster-adapted scrapie prions have been shown to survive in soil for at least 3 years,[25] the mortuary feast practices (during which the entire body would be consumed) were undertaken so that any substantial contamination of soil would not have occurred, and traditional bamboo knives and leaf plates were burned after the feast. Furthermore, no clusters of kuru cases, as seen earlier in the epidemic,[26] have been recorded for many years. We have also reviewed the assertion that maternal transmission of kuru did not occur, and saw no evidence for maternal transmission from kuru archives, interviews of colleagues who have practised medicine in the Fore, or local oral history. Again, any possible vertical route of kuru transmission would have resulted in the presence of kuru in children born after 1960, especially since kuru was common in women of childbearing age; no such cases have occurred.

With respect to extrapolation of incubation periods of BSE prion infection in people, we should recognise that the kuru epidemic arose from intraspecies recycling of infectious prions. However, transmission of prions between different mammalian species is associated with a species barrier, which is better described as a transmission barrier, because of the importance of within-species prion strain type, in addition to species-specific differences in its determination.[27] The biological effects of such a barrier are: extended mean incubation period; increased spread of incubation periods in individual animals; and reduced attack rate (in which only a fraction of inoculated animals will succumb), by comparison with the 100% mortality generally associated with within-species inoculation with high-titre infectivity. Incubation periods approaching the natural lifespan of the inoculated species are often seen in such primary cross-species transmissions of prions. Second and subsequent passage of prions within the new species is always associated with adaptation involving a considerable shortening of the mean and spread of incubation periods and high or total lethality to high-titre inocula. Thus, estimation of the range of possible incubation periods in human BSE infection needs superimposition of the effect of a transmission barrier onto these findings of natural human incubation periods.

The mean incubation period for kuru has been estimated to be around 12 years,[27] with a similar estimate in iatrogenic CJD associated with the use of human-cadaver-derived pituitary growth hormone.[28] As shown here, maximum incubation periods in kuru can exceed 50 years. The transmission barrier of BSE between cattle and human beings is unknown and cannot be directly measured. However, the cattle-to-mouse barrier for BSE has been well characterised experimentally by comparative endpoint titration. BSE prions transmit readily to laboratory mice, including after oral dosing.[29] The murine LD_{50} (lethal dose causing 50% mortality) in C57Bl/6 mice is about 500-fold higher than that in cattle;[30] this barrier also results in a three-fold to four-fold increase in mean incubation period.[27] Mean incubation periods of human BSE infection of 30 years or more should therefore be regarded as possible, if not probable,[27] with the longest incubation periods approaching (and perhaps exceeding) the typical human lifespan. The shortest incubation periods in kuru were estimated from the age of the youngest patients—suggesting that the shortest incubation period was

4–5 years. Similarly in vCJD, although the total clinical caseload so far has been small, the youngest onsets of vCJD have been at age 12 years or above, providing an early estimate of a minimum incubation period.

Furthermore, prion disease in mice follows a well-defined course with a highly distinctive and repeatable incubation time for a specific prion strain in a defined inbred mouse line. In addition to the PrP gene, a few additional genetic loci with a major effect on incubation period have been mapped.[4,11,12] Human homologues of such loci could be important in human susceptibility to prion disease, both after accidental human prion exposure and after exposure to the BSE agent. By definition, patients identified so far with vCJD are those with the shortest incubation periods for BSE. These patients could have received an especially high dose of BSE prions. However, no unusual history of dietary, occupational, or other exposure to BSE has been reported from case-control studies. Because of the powerful genetic effects on incubation period in laboratory animals, vCJD patients identified could represent a distinct genetic subpopulation with unusually short incubation periods to BSE prions, with vCJD so far occurring predominantly in those individuals with short incubation time alleles at these multiple genetic loci, in addition to having the homozygous *PRNP* genotype of codon 129 methionine. Therefore, a human BSE epidemic may be multiphasic, and recent estimates of the size of the vCJD epidemic based on uniform genetic susceptibility could be substantial underestimations.[11,14] Genes implicated in species-barrier effects, which would further increase both the mean and range of human BSE incubation periods, are also probably relevant. In this context, a human epidemic will be difficult to accurately model until such modifier loci are identified and their gene frequencies in the population can be measured.[4]

Heterozygosity at *PRNP* codon 129 is a major determinant of susceptibility to and incubation time of human prion diseases.[5,7,9,15] As expected, most of these recent kuru cases with extended incubation periods (eight of ten) were heterozygotes. We have reported previously that most elderly survivors of exposure to traditional mortuary feasts are heterozygous.[9] Although the study included a small number of patients with kuru with long incubation periods, we saw no evidence of association with *PRNP* haplotype,[10] HLA-DQ7,[16] *APOE*,[16] or *PRND* alleles.[13]

Contributors

J Whitfield led the field patrol team throughout the study and investigated all suspect cases; E McKintosh provided assistance during this time. J Beck and S Mead undertook the molecular genetic studies. J Collinge, M P Alpers, E McKintosh, and D J Thomas did field neurological examinations. J Collinge and M P Alpers supervised the study and drafted the manuscript. All authors contributed to and approved the final version of the manuscript.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This study would not have been possible without the generous support of the Fore communities and the assistance of many people in the UK and Papua New Guinea. We thank our team of local kuru reporters including Auyana Winagaiya, Anua Senagaiya, Igana Aresagu, Kabina Yaraki, Anderson Puwa, David Pako, Henry Pako, Pibi Auyana, Jolam Ove, Jack Kosinto, Dasta Hutu, Oma Artimo, James Kisava, Sena Anua, David Ikabala, and Amalasa Pallar; and James Uphill, Mark Poulter, Tracy Campbell, Gary Adamson, and Huda Al-Doujaily for assistance with genetic analysis. We also thank John Millan, Peter Lantos, Chris Foster, Jonathan Boreham, Mike Devine, Anthony Jackson, Edward Lagan, and Lionel Astwood; John Reeder, Charles Mgone, and other staff at the Papua New Guinea Institute of Medical Research; Mark and Deborah Brandt of Open Bible Mission; and the British High Commission in Port Moresby for their help. This study was initially funded by a Wellcome Trust Principal Research Fellowship in the Clinical Sciences to JC, and since 2001 by the Medical Research Council. EM is a Wellcome Trust Clinical Training Fellow.

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別紙様式第2-1

No. 16

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	機構処理欄
				2006. 7. 7	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Saa P, Castilla J, Soto C. Science. 2006 Jul 7;313(5783):92-4.	公表国 米国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			

研究報告の概要

○血中におけるプリオンの発症前検出

プリオンは伝達性海綿状脳症の病因となるタンパク様の感染性物質であると考えられている。PrP^{Sc}は、疾患の唯一の確認された代理マーカーであり、その検出は感染の拡大を最小限に抑えるために不可欠である。スクレイピーに感染したハムスターの血中から、疾患の発症前の期間の大半で、生化学的にPrP^{Sc}を検出した。潜伏期間の早期には、PrP^{Sc}はプリオンの末梢複製によって産生される傾向が強かった。一方、発症期には血液中のPrP^{Sc}は脳から漏出していた。感染したものの臨床症状を呈していない動物の血液中のプリオンの生化学的検出が可能になることで、TSEの早期の非侵襲的診断が見込まれる。

報告企業の意見	今後の対応
スクレイピーに感染したハムスターの血中から、疾患の発症前の期間の大半で、生化学的にPrP^{Sc}を検出したとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「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

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MedDRA/J Ver.9.0J

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26. We thank D. Lane for critical reading of the manuscript. This work was supported in part by European Union grant QLK2-CT-2000-00543.

Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5783/89/DC1
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27 March 2006; accepted 1 June 2006
10.1126/science.1127912

Presymptomatic Detection of Prions in Blood

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Prions are thought to be the proteinaceous infectious agents responsible for transmissible spongiform encephalopathies (TSEs). PrP^{Sc}, the main component of the infectious agent, is also the only validated surrogate marker for the disease, and its sensitive detection is critical for minimizing the spread of the disease. We detected PrP^{Sc} biochemically in the blood of hamsters infected with scrapie during most of the presymptomatic phase of the disease. At early stages of the incubation period, PrP^{Sc} detected in blood was likely to be from the peripheral replication of prions, whereas at the symptomatic phase, PrP^{Sc} in blood was more likely to have leaked from the brain. The ability to detect prions biochemically in the blood of infected but not clinically sick animals offers a great promise for the noninvasive early diagnosis of TSEs.

Prion diseases, also called transmissible spongiform encephalopathies (TSEs), are a group of fatal and infectious neurodegenerative diseases, including Creutzfeldt-Jakob disease (CJD) in humans and bovine spongiform encephalopathy (BSE), scrapie, and chronic wasting disease (CWD) in animals. Prions are composed mainly or exclusively of the misfolded prion protein (PrP^{Sc}) (*1*), which replicates in the body, transforming the normal prion protein (PrP^{C}) into more of the misfolded isoform.

Although prion diseases are rare in humans, the established link between a new variant form of CJD (vCJD) and BSE (*2–4*) has raised concern about a potential epidemic in the human population. Over the past few years, BSE has become a substantial health problem affecting many countries (*5*), and it seems now apparent that vCJD can be iatrogenically transmitted from human to human by blood transfusion (*6*, *7*). Exacerbating this state of affairs is the lack of a reliable test to identify individuals incubating the disease during the long and silent period from the onset of infection to the appearance of clinical symptoms (*8–10*).

PrP^{Sc} is not only the main component of the infectious agent and the most likely cause of the disease, but it is also the only validated surrogate marker for TSEs (*9*). However, PrP^{Sc} concentration is high enough for routine biochemical detection only in the brain and some lymphoid tissues at a time close to the symptomatic stage of the disease (*9*). The development of highly sensitive presymptomatic assays for the biochemical detection of PrP^{Sc} is critical for minimizing the spread of the disease (*9*). One important aim in prion diagnosis is the noninvasive and presymptomatic biochemical detection of PrP^{Sc} in biological fluids, particularly using blood, a fluid known to contain infectivity even before the onset of clinical signs (*6*, *11*, *12*).

PrP^{Sc} has been detected in the blood of sick animals by means of the protein misfolding cyclic amplification (PMCA) technology (*13*). PMCA produces accelerated prion replication, which dramatically amplifies the quantity of PrP^{Sc} present in a sample (*14*, *15*). In a cyclical process, large quantities of PrP^{C} are converted into the misfolded form triggered by the presence of minute and otherwise undetectable amounts of PrP^{Sc}. The method is highly specific for the detection of PrP^{Sc} and leads to a several-million-fold increase in sensitivity as compared to that of standard Western blot assays (*13*).

In order to evaluate the application of PMCA for the detection of prions in blood during the presymptomatic phase, 46 hamsters were inoculated intraperitoneally with 10% brain homogenate of the 263K scrapie strain, and 38 control animals were injected with phosphate-buffered saline (PBS). At different times during the incubation period, groups of animals were killed, blood was collected, and the buffy coat fraction was separated (*13*). Samples of the buffy coat were resuspended directly on healthy hamster brain homogenate and subjected to 144 PMCA cycles. Three different aliquots were tested from each sample. To refresh the substrate, after a round of PMCA cycling, samples were diluted 10-fold into normal brain homogenate, followed by another round of 144 PMCA cycles. This procedure was repeated seven times, because according to our results, this enables the detection of 20 to 50 molecules of monomeric hamster PrP, which seems to correspond to a single unit of infectious oligomeric PrP^{Sc} (*16*).

The first group of hamsters was killed 2 weeks after intraperitoneal inoculation. None of the five infected or control animals showed any detectable quantity of PrP^{Sc} in their blood (Fig. 1 and Table 1). Thus, the PrP^{Sc} present in the inoculum disappeared to undetectable levels during the first few days after inoculation. PrP^{Sc} was, however, readily detectable in blood 1 week later (20 days after inoculation) in 50% of the animals infected but in none of the controls (Fig. 1 and Table 1). The highest percentage of positive animals during the presymptomatic phase was observed 40 days after intraperitoneal inoculation, in which the sensitivity of PrP^{Sc} detection was 60%. After 60 days, the detection of PrP^{Sc} in blood became harder. Indeed, only one out of five animals scored positive at 70 days, whereas none of the five infected hamsters had detectable PrP^{Sc} in their blood 80 days after inoculation (Table 1). At the symptomatic stage, which in this experiment was at 114.2 ± 5.6 days, 80% of animals had PrP^{Sc} in their blood (Fig. 1). We never detected a false positive result in any of the 38 control samples analyzed (Table 1).

The distribution of PrP^{Sc} detection at different times of the incubation period showed

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an interesting trend (Fig. 2). A first peak of PrP^{Sc} detection was observed early during the presymptomatic phase, between 20 and 60 days after inoculation. The peripheral administration of prions is known to result in an early phase of replication in lymphoid tissues and the spleen, before any infectious material reaches the brain (*17*, *18*). Indeed, little or no infectivity can be detected in the brain of animals peripherally inoculated during the first half of the incubation period (*19*). Thus, it is likely that the source of PrP^{Sc} in blood during the early presymptomatic phase is the spleen and other lymphoid organs. The quantity of PrP^{Sc} in blood goes down after this initial phase and actually disappears 80 days after inoculation (Table 1 and Fig. 2). The rise of PrP^{Sc} in blood during the early presymptomatic phase appears to coincide with the time of its exponential replication in lymphoid organs, whereas the reduction of PrP^{Sc} in blood occurs when infectivity in peripheral tissues has reached a plateau and is migrating from the periphery to the brain (*17*, *18*). Although the explanation for these results in unknown, it is possible that the proportion of circulating lymphocytes carrying PrP^{Sc} is much higher during the exponential phase of peripheral replication than during the stationary phase. At the symptomatic period, PrP^{Sc} can again be detected in the blood of most of the animals (Fig. 2). It has been reported that large quantities of PrP^{Sc} appear in the brain only a few weeks before the onset of clinical signs (*19*, *20*). Thus, PrP^{Sc} in blood samples at the symptomatic stage is likely to have come from brain leakage. It is known that at the time of symptomatic disease, TSE-affected individuals have extensive brain degeneration in the form of massive neuronal death, synaptic alterations, and brain inflammation (*21*). These abnormalities probably cause a disruption of the blood/brain barrier resulting in the leakage of cerebral proteins to the blood (*22*), in particular PrP^{Sc}, which by this time is highly abundant in the brain.

Infectivity studies have shown that the blood carries prions in both the symptomatic and presymptomatic stages of the disease in animals (*11*, *23*, *24*). Upon experimental BSE infection of sheep, infectivity can be transmitted by blood transfusion from asymptomatic infected animals (*25*), indicating that the infectious agent is present in blood during the incubation period. Recently, three cases of vCJD have been associated with blood transfusion from asymptomatic donors who subsequently died from vCJD (*6*, *7*). The alarmingly high proportion of cases transmitted by blood transfusion suggests that prions exist in relatively elevated quantities in the blood of individuals silently incubating vCJD. Based on studies with animal models, it is believed that all of the human population may be susceptible to vCJD infection (*26*), although clinical cases have so far occurred only in methionine homozygotes at codon 129 in the human prion protein gene. Because the incubation period may be several decades, it is currently unknown how many people may be in an asymptomatic phase of

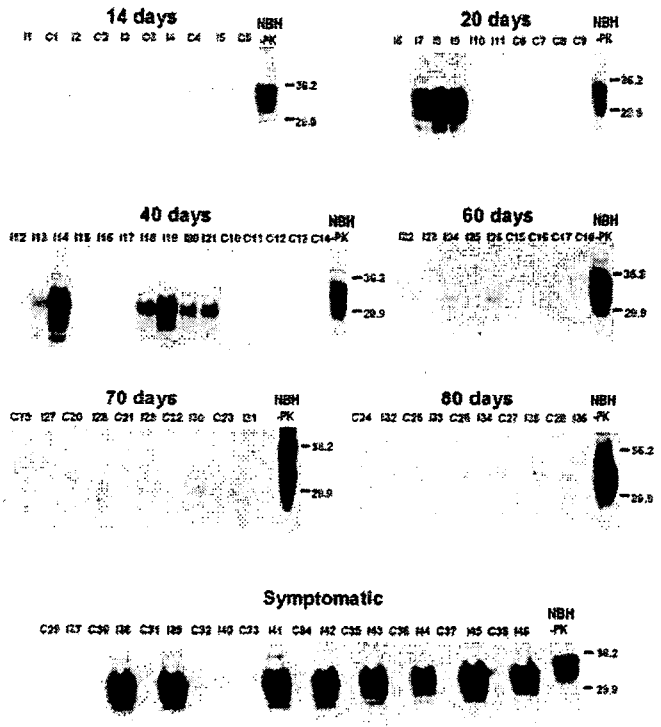

Fig. 1. PrP^{Sc} detection in the blood of scrapie-infected hamsters by PMCA. Blood samples of groups of scrapie-inoculated and control animals were taken at different times during the incubation period. Three milliliters of blood were separated in three aliquots of 1 ml each to prepare the buffy coat (*13*). Samples were subjected to 144 cycles of PMCA. Ten microliters of the sample from this first round of amplification were diluted into 90 μl of normal brain homogenate, and a new round of 144 PMCA cycles was performed. This process was repeated a total of seven times. Each panel represents the results obtained in the seventh round of PMCA with the samples from each group of animals, which are representative of the three independent aliquots taken from each animal. Ix, samples from hamsters infected with 263K scrapie; Cx, samples from control animals injected with PBS. All samples were treated with proteinase K (PK) before electrophoresis, except for the normal brain homogenate (NBH), in which no PK treatment (–PK) is indicated.

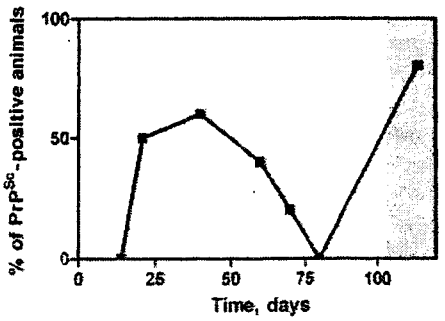

Fig. 2. Proportion of animals whose blood was PrP^{Sc} positive at different times during the incubation period. The percentage of samples scoring positive for PrP^{Sc} in blood is represented versus the time after inoculation at which samples were taken. Two phases of PrP^{Sc} detectability were observed: an early stage during the incubation period, which probably corresponds to the time during which peripheral prion replication in lymphoid tissues is occurring, and a second phase at the symptomatic stage, in which the brain contains extensive quantities of PrP^{Sc}. The vertical gray section indicates the symptomatic phase.

Table 1. Number of animals used and results obtained regarding the presymptomatic detection of PrP^{Sc} in the blood.

Time (days)	Controls (positives/total)	Infected (positives/total)	Sensitivity/ specificity
14	0/5	0/5	0%/100%
20	0/4	3/6	50%/100%
40	0/5	6/10	60%/100%
60	0/4	2/5	40%/100%
70	0/5	1/5	20%/100%
80	0/5	0/5	0%/100%
Symptomatic phase	0/10	8/10	80%/100%

vCJD infection. In addition, it is possible that some infected patients may never develop clinical symptoms but will remain asymptomatic carriers who can potentially transmit the disease to other individuals (*26*, *27*). In the absence of screening tests and effective therapies to treat this disease, a formidable worldwide public health challenge lies ahead to prevent further infections, assess infection rates, and treat infected patients. The ability to detect PrP^{Sc}, the major component of infectious prions, biochemically in the blood of infected but asymptomatic experimental animals will hopefully lead to the development of tests for human blood. Indeed, although technically more challenging, the PMCA technology has been adapted to amplify prions of human origin (*20*). The ability to accurately detect PrP^{Sc} in the presymptomatic stages of vCJD would potentially help to reduce the risk that many more people will be infected by this fatal and terrible disease.

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28. This research was supported in part by NIH grants AG0224642 and N5049173.

Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5783/92/DC1
Materials and Methods
References

21 April 2006; accepted 5 June 2006
10.1126/science.1129051

Prion-Induced Amyloid Heart Disease with High Blood Infectivity in Transgenic Mice

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We investigated extraneural manifestations in scrapie-infected transgenic mice expressing prion protein lacking the glycophosphatydylinositol membrane anchor. In the brain, blood, and heart, both abnormal protease-resistant prion protein (PrPres) and prion infectivity were readily detected by immunoblot and by inoculation into nontransgenic recipients. The titer of infectious scrapie in blood plasma exceeded 10^7 50% infectious doses per milliliter. The hearts of these transgenic mice contained PrPres-positive amyloid deposits that led to myocardial stiffness and cardiac disease.

In humans and animals, transmissible spongiform encephalopathies (TSEs), or prion diseases, cause neurodegeneration and death following ingestion or experimental inoculation of infected material. Prion diseases are characterized by the conversion of the normal protease-sensitive host prion protein (PrPsen) to a disease-associated protease-resistant form (PrPres). Although prion disease damages the central nervous system (CNS), infectivity and PrPres can be detected within peripheral tissues, including lymphoid organs in humans, sheep, and deer (*1*, *2*), as well as skeletal muscle (*3*), kidney, and pancreas (*4*) of some transgenic rodent models. Despite the toxic effect on the CNS, few if any histopathological changes have been observed at peripheral sites.

Transmission of TSE disease to humans has resulted from cannibalism, contaminated surgical instrumentation, and tainted growth hormone (*5*–*7*). A human disease termed variant Creutzfeldt-Jakob disease (vCJD) has occurred more recently, apparently through the ingestion of bovine spongiform encephalopathy (BSE)–infected cattle products (*8*). Recent evidence suggests that transmission of vCJD between humans may occur through blood transfusion (*9*, *10*), and this conclusion is supported by experimental transmission of BSE between sheep via blood transfusion (*11*). TSE infectivity has been demonstrated in blood by intracerebral-inoculation in mouse, mink, hamster, and goat models (*7*, *12*–*20*). However, infectivity in such cases is low, $\leq 10^2$ 50% infectious doses (ID_{50}) per ml of blood compared to 10^6 to 10^{10} ID_{50}/g in the brain.

Normal prion protein, PrPsen, is expressed primarily as a membrane-bound, glycophosphatydylinositol (GPI)–anchored protein. The role of cellular PrP membrane anchoring in prion disease has been studied in transgenic mice expressing GPI-negative anchorless PrP, which is secreted from cells (*21*). Intracerebral inoculation of these GPI-negative anchorless PrP transgenic (tg) mice with murine scrapie results in scrapie replication and deposition of PrPres within the brain. Although wild-type (WT) mice infected with scrapie usually develop a nonamyloid form of PrPres, in these tg mice the PrPres is primarily in the form of amyloid plaques (*21*). At the same time, these mice do not manifest the clinical and pathologic alterations normally associated with prion disease, thus demonstrating a separation between PrPres amyloid accumulation and clinical CNS disease (*21*). In the brain of these infected tg mice, PrPres was located primarily within and around endothelial cells (*21*) (Fig. 1A), leading to the hypothesis that anchorless PrPres may be secreted in the blood. Here we examined this possibility.

To determine whether PrPres and/or scrapie infectivity was present in blood, four infected tg mice were bled between 450 and 512 days postinfection (dpi) with the RML strain of scrapie. Inoculation of a 1:500 dilution of blood from all four mice induced scrapie in WT (C57BL/6) recipients in ~145 days. In addition, blood of two mice analyzed by serial dilution titration gave titers of $\geq 1.6 \times 10^7$ and $\geq 1.6 \times 10^5$ ID_{50}/ml blood (Table 1).

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別紙様式第2-1

No. 15

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	機構処理欄
				2006. 5. 11	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Guardian Unlimited, The Guardian. 2006 May 2.	公表国 英国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			

研究報告の概要

○英国の血漿分画製剤が14カ国でvCJDのリスクを引き起こす可能性
英国政府は、1990年代に輸出された英国産の血漿分画製剤によって患者にvCJDのリスクがあると14カ国に警告した。
2003年12月に輸血によるvCJD感染が英国で発生し、血液製剤による感染伝播に対する安全対策が実施された。当局は、国営企業Bio Products Laboratory(BPL)から海外に輸出された製剤を再調査する必要に迫られた。
当局は、vCJDの潜在的リスクは「非常に不確実」であるとしながらも、ブラジルとトルコの保健省に対して予防措置を取るよう勧告した。この措置には患者の追跡調査、供血や臓器・組織提供をしないよう求めることが含まれる。また、患者は医師や歯科医師に治療を受ける際に申し出るよう求められる。
英国内では、危険のある人は最大で6000人と考えられる。問題は、血漿分画製剤が何千もの供血血液を処理して製造されることである。vCJDを発症した9人の供血者のたった23供血から懸念が生じた。
BPLを所管するNHSの血液・移植部門は「これまで血漿分画製剤が関係したvCJD症例はない。英国の血漿由来の製剤の使用は1999年に終了した。しかし、供血経験のある人がvCJDと診断されたため、終了以前に製剤を投与された患者にリスクがある可能性が出てきた。2004年以降、1980年以降に輸血を受けた人は供血不可としている」と話している。
ブルネイ、UAE、インド、ヨルダン、オマーン、シンガポールはブラジルやトルコに比べて危険性は低いものの、予防措置をとる必要がある。ベルギー、モロッコ、エジプト、フランス、オランダ、イスラエルには危険性の少ない製剤が輸出されており、製造の最終工程はこれらの国で行われたため、各国での分析を続けるよう勧告された。フランス政府は、同国から他の10カ国に再輸出された製剤には危険はないとしている。

報告企業の意見	今後の対応
英国政府は、英国産の汚染された血漿分画製剤によって患者にvCJDのリスクがあると14カ国に警告したとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「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

British blood products may pose vCJD risk in 14 countries

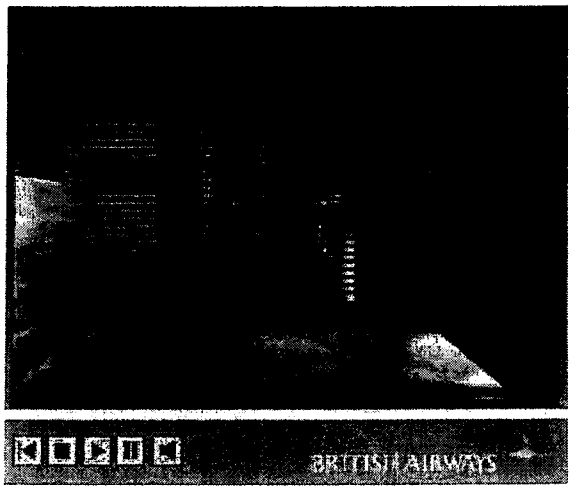
- UK issues warning on 'mad cow disease'
- Documents show Brazil and Turkey are high on list

James Meikle and Rob Evans
Tuesday May 2, 2006
The Guardian

The government has been forced to warn 14 countries that patients are in danger of developing the human form of mad cow disease as a result of contaminated British blood products sold abroad.

Documents released under the Freedom of Information Act show that patients in Brazil and Turkey are most at risk from the products, although it is too early to know how many, if any, foreign patients may develop the incurable variant CJD, as it takes many years to appear. The Turkish authorities said they had traced patients at risk and were closely monitoring them, while Brazil would not comment.

Article continues



The contaminated blood products were exported in the 1990s by the British government to treat conditions such as haemophilia, severe burns and immune deficiency. At the time the government considered there was no risk.

Twenty-eight people abroad have already developed vCJD by eating cattle meat from Britain infected with BSE. However, the

dangers of another route of transmission are now becoming more evident. Scientists are worried about a "second wave" of casualties caused by blood donated by people infected but not yet displaying symptoms of the disease.

The risk of passing on the disease in this way was considered only theoretical until December 2003, when it emerged that a patient in Britain had been infected through a blood transfusion, leading to new safety measures. Another two cases have since been identified. Health authorities then had to re-examine blood products sent abroad by the state-owned company Bio Products Laboratory (BPL).

The documents show that, following the rethink, the Health Protection Agency was concerned "about the potential infectivity of blood". Believing the potential risk of vCJD to be "very uncertain", the agency advised the Brazilian and Turkish health ministries to take precautions to reduce the possibility of spreading vCJD as "sufficient quantities" of the "at-risk" products had been exported.

These measures included tracking down patients and telling them not to donate blood, organs or tissues. Patients are also told to inform doctors and dentists if they need any treatment.

In Britain, up to 6,000 people were considered to be at risk. The problems stem from the way blood products are made, from processing thousands of separate donations. The concerns arise from just 23 donations made by nine people who went on to develop vCJD, showing how minute amounts may be infectious.

The NHS Blood and Transplant Authority, which is responsible for BPL, said: "So far no vCJD cases have been linked to plasma products ... The use of products derived from British blood plasma was ended in 1999 as a precautionary safety measure because of what were then regarded as only theoretical risks. But cases where patients might have been put at risk before that date have since come to light as further cases of vCJD have been diagnosed in people who were blood donors. Since 2004, no one who received a blood transfusion after 1980 has been allowed to donate blood themselves."

The Health Protection Agency decided that patients in six countries - Brunei, UAE , India, Jordan, Oman and Singapore - had been put in less jeopardy than those in Brazil and Turkey, but might need to take precautions. Less dangerous batches were imported by Belgium, Morocco and Egypt. France, Holland and Israel were advised to carry out their own assessments, as manufacture of the blood products was completed in their countries. The French government concluded that there was no danger from the products, which were re-exported to 10 unnamed countries.

The Guardian has previously reported that patients worldwide may have been exposed to vCJD, but the documents detail for the first time the countries, the amounts and the risk assessments. British authorities cannot say how many patients abroad may now be in danger.

There have been 161 cases of vCJD in Britain. There are 15 cases in France, four in Ireland, two in the US, and one each in Canada, Italy, Japan, the Netherlands, Portugal, Saudi Arabia and Spain.

Some of these victims are known to have caught vCJD by eating infected beef in Britain. Most others live in countries that

have also had outbreaks of BSE that may well have originated from Britain.

Graham Steel, whose brother Richard died from vCJD, drew parallels to the spread of BSE. "[It is] eerily reminiscent of the 1980s when 'theoretically' infectious meat and bonemeal was exported by the UK around Europe and beyond despite the fact that the risks of spreading diseases were known about in 1972-73. A total recall was deemed too expensive."

Special reports
BSE and CJD
What's wrong with our food?

Explained
The threat to humans from BSE

Cartoon
Steve Bell on the BSE report

Useful links
Official BSE inquiry
Human BSE Foundation
CJD Support Network
British Medical Journal CJD page
Department of Health CJD/BSE page
Department for Environment, Food and Rural Affairs BSE page
The UK CJD disease surveillance unit
Food Standards Agency BSE page

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