Review article

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Non-human primates in prion research

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Abstract

Prion diseases or transmissible spongiform encephalopathies are neurodegenerative disorders affecting a broad range of mammals including humans. Initially thought to be of viral origin, it became apparent that prion diseases are unique transmissible entities where a misfolded, highly stable conformer (PrP^{Sc}) of the host encoded prion protein (PrP^{C}) represents an essential component of infectious "prions". Prion diseases are mainly studied in rodents, yet several scientific breakthroughs in prion research can be attributed to prion research in primates.
In this review we summarize and discuss how studies in non-human primates have advanced our knowledge on transmissibility, pathophysiology and tissue tropism of prions. We discuss assets of non-human primate and rodent models of prion disease pointing out alternatives to experiments in primates.

Key words: *prions, neurodegeneration, PrP, primates.*

Introduction

Transmissible spongiform encephalopathies (TSEs) or prion diseases are transmissible, fatal neurodegenerative disorders that have been known for more than two centuries. In the last decades, the emergence of new prion diseases in animals (bovine spongiform encephalopathy, BSE) or humans (variant Creutzfeldt-Jakob disease, vCJD) brought this unique group of diseases into public focus and led to an unparalleled boost in scientific breakthroughs to unravel the basic mechanisms behind this fascinating class of diseases.

First to be considered of viral origin (slow virus disease), it became obvious that prion diseases are a prototype of cerebral proteinopathies [3,76]. This class of diseases is characterized by abnormal folding of an otherwise physiologically expressed protein into a misfolded conformer that is prone to aggregation and subsequently leads to cell dysfunction and death [76]. A key step in prion disease initiation is the conversion of the cellular prion protein (PrP^{C}) into PrP^{Sc}, which is partially resistant to proteolytic digestion and represents an essential part of prion infectivity [1,51,78]. The fact that transmission of prions, from affected to healthy organisms, occurs by imposing its aberrant conformation to the host protein via templated misfolding and the fact that transmission of prion diseases occurs under natural conditions, make this disease unique [39]. Besides other

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factors, the efficiency of transmission seems to be dependent on the route of transmission, the amount of infectious prions and the degree of amino acid homology of the incoming PrP^{Sc} with the host encoded PrP^{C} [82]. The latter may lead to a species barrier, where transmission cannot occur or the incubation time to clinical prion disease is considerably prolonged [49,80]. Nevertheless, interspecies transmission of prion diseases occurs not only between animals and humans but also between different animal species [9,85]. Although subtle alterations in amino acid homology lead to drastic changes in transmission of prions, major structural features of PrP^{C} are remarkably preserved among both, mammalian and non-mammalian PrP^{C} [16,64].

Human prion diseases

The first human prion disease was already described at the beginning of the twentieth century [22,53]. Creutzfeldt and Jakob published two papers describing a novel neurodegenerative disorder that was accordingly termed Creutzfeldt-Jakob disease (CJD). Now it is obvious that human prion diseases encompass a group of diseases with a wide phenotypical spectrum. Besides the most common form of CJD (sporadic, sCJD), there are genetic variants (genetic CJD) and acquired forms of the disease (variant and iatrogenic CJD) [7,32,42,52]. In genetic CJD, there are diseases with distinct clinicopathological profiles such as Gerstmann-Sträussler-Scheinker disease (GSS) or Fatal Familial Insomnia (FFI) [56]. Among the acquired forms of human prion diseases, two stand out: kuru, which was endemic in Papua New Guinea and where transmission occurred via ritualistic cannibalism [31, 62] and vCJD which is probably caused by transmission of BSE-prions to humans [5,50,90]. In both instances, incubation times from putative exposure towards prions to onset of the disease are exceedingly long possibly surpassing decades [20]. For vCJD, iatrogenic transmission from subclinically-infected vCJD carriers to humans by blood transfusion has occurred raising a question as to whether transfusion-associated vCJD may lead to a second wave of vCJD in the future [63,91].

Thus, there are at least three ways to initiate prion disease:

1) Transmission of infectious prions from an exogenous prion source. This is the case of acquired forms of human prion diseases [11,79].
2) Disease initiation associated with changes in the gene encoding PrP^{C}. This is the case of genetic CJD, which is inherited as an autosomal dominant trait. The spectrum of disease-causing genetic changes includes point mutations with amino acid changes, mutations introducing a stop codon and leading to a truncated protein or deletions as well as insertions of octapeptide motives within the octarepeat region of the prion gene [56]. It is currently debated how these changes lead to prion disease. Possibly, mutant PrP^{C} is less stable, thereby facilitating a cascade of events leading to misfolding of PrP^{C} and disease [77].
3) Spontaneous generation of PrP^{Sc}. Interestingly, the events underlying the most common form of human prion disease (sCJD) are not yet fully understood [19,42]. It was proposed that a rare stochastic event of misfolding of endogenous PrP^{C} to PrP^{Sc} lies at the basis of this disease [3,39].

Pathology of prion diseases is limited to the central nervous system (CNS) and characterized by neuronal loss, the typical name-giving spongiform lesions, astrogliosis and deposition of PrP^{Sc} [15]. The latter can differ in the glycotype ratio and core fragment size between different prion diseases [71]. Moreover, these different types of PrP^{Sc} show specific patterns of deposition and neuronal cell tropism, which are reflected by disease-specific lesion profiles that can be utilized for diagnostic purposes and to determine CJD subtypes [15,81].

In human prion diseases in general, but especially in vCJD, deposition of PrP^{Sc} in peripheral sites such as lymphoid and muscular compartments have been described [37,74].

Prion diseases in animals and transmission to humans

Non-human prion diseases include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, transmissible mink encephalopathy (TME) in minks and chronic wasting disease (CWD) in elk and deer.

Scrapie in sheep is the oldest known prion disease. It was already described as early as in 1732 and affects sheep, goats and mouflons. The transmissibility of scrapie was shown in 1939 [23]. Neuropathological findings include spongiform vacuolation, astrogliosis and the deposition of PrP^{Sc} in the CNS, but also in peripheral tissues such as the lym-

phoreticular system, the neuromuscular system and the digestive tract [46,72]. Moreover, secretions and excretions have also been found to contain prion infectivity and may account for horizontal transmission within flocks [66]. Similarities between the neuropathological and clinical presentation of scrapie and the human prion disease, kuru, were already noted in 1959 [44].

Transmissible mink encephalopathy was first described in 1947 and is restricted to farmed animals [10,84]. Neuropathological findings include typical spongiform vacuolation, astrogliosis and detection of PrP^{Sc}. As in scrapie, widespread colonization of non-CNS compartments occurs [84].

Bovine spongiform encephalopathy or "mad cow disease" was first reported in the early 1980s in the UK and soon afterwards developed epidemic proportions [24,89]. A ban on feeding meat and bone meal to cattle resulted in a progressive decline in the number of diagnosed cases of BSE. Disease-associated PrP^{Sc} is mainly found in the CNS with a limited involvement of peripheral organs [6]. Beside the classical form of BSE, other atypical BSE-types, termed bovine amyloidotic spongiform encephalopathy (BASE)/L-BSE and H-BSE have been described [17].

Chronic wasting disease is a prion disease affecting captive and free-ranging cervids such as deer and elk, mainly in the US. The origin of CWD is still unknown [83]. CWD prions can be found in the CNS and other non-CNS compartments including bodily fluids or excretions such as saliva, feces, urine and blood [45]. This is of relevance since horizontal transmission seems to occur in CWD.

The first wave of prion research using non-human primates

The first experiments with prions involving primates were started after the description of kuru, which challenged the view on neurodegenerative diseases [31].

Kuru and sCJD can be transmitted to non-human primates

The transmissibility of human prion diseases to non-human primates was demonstrated in 1966 when Gajdusek *et al.* transmitted kuru to chimpanzees [27]. This was the first demonstration of the transmissibility of a neurodegenerative disorder affecting humans. One year later, it could be demonstrated that the incubation time of kuru shortened upon the second passage into chimpanzees hinting to adaptation [29]. In 1968, Gibbs *et al.* proved that intracerebral transmission to chimpanzees occurred in another human prion disease: CJD [36]. Incubation time to onset of disease was 13 months with the disease duration of only two months. The neuropathological analysis showed typical signs of prion disease with severe spongiosis. The two other chimpanzees which were caged together with the former ape were inoculated with pooled urine from kuru patients and brain homogenate from a patient with amyotrophic lateral sclerosis and failed to develop any neurological disease. When these primate studies were performed, the infectious entity was considered to be a slow virus as infectivity passed through small pore filters and transmitted easily with long incubation times. Efficient transmission of kuru and CJD to other primate species like marmoset monkeys, stump-tail macaques, gibbons, spider monkeys, sooty mangabey, pigtail macaques and others followed [25,26,28,30,68,75].

Genetic CJD can be transmitted to non-human primates

In 1980, the oral transmission to primates via consumption of PrP^{Sc} containing tissue was demonstrated for kuru, CJD and scrapie [33]. Interestingly, scrapie has never been reported to be transmitted to humans, although incidence of scrapie in certain regions is high and consumption of meat from diseased animals has certainly occurred.

In 1981, the transmission of GSS to non-human primates was successful, subsequently GSS was classified as a transmissible spongiform encephalopathy [68].

Altogether 1,914 primates were infected with a total of 440 cases of human prion diseases between 1963 and 1993 at the NIH [12]. The main findings of this and other transmission studies are summarized in Table I. Inoculations were performed intracerebrally in most cases with fresh-frozen brain homogenate, although other preparations (e.g. formalin-fixed brain) as well as other tissue were investigated, too. These include tissues from sCJD, gCJD, iCJD, FFI, GSS and kuru patients. From these cases, in 291 individuals, transmission occurred without doubt. Primates utilized in this study in-

Table I. Time line of prion transmission experiments to non-human primates

Year	Main finding	Reference
1966	Transmission of kuru to chimpanzees First transmission of a prion disease to non-human primates	[27]
1967	A second passage into chimpanzees with shortened incubation time of kuru hinting to adaptation	[29]
1968	Transmission of sCJD to chimpanzees by intracerebral inoculation	[36]
1972	Transmission of scrapie to cynomolgus monkeys	[35]
1972	Transmission of kuru to rhesus monkey with very long incubation time	[26]
1980	Oral transmission of kuru, sCJD and scrapie to squirrel monkeys	[33]
1981	Transmission of GSS to squirrel and spider monkeys	[69]
1994	Transmission of sCJD to a chimpanzee via brain electrodes used in a sCJD patient	[34]
1996	BSE is transmissible to cynomolgus macaques and presents like vCJD in humans	[59]
2001	A second passage of "primate" BSE shortened incubation time hinting to adaptation; *i.v.* inoculation is almost as efficient as *i.c.* infection	[60]
2001	vCJD transmit to primates; incubation time is shorter than with BSE	[60]
2004	Oral transmission of macaque adapted BSE to cynomolgus macaques	[48]
2004	Widespread detection of primate adapted BSE PrP^{Sc} in peripheral tissues	[48]
2005	Oral transmission of cow BSE to cynomolgus macaques	[58]
2005	Transmission of CWD to squirrel monkeys	[67]
2008	L-BSE is transmissible to cynomolgus macaques with shorter incubation time than classical BSE	[21,73]
2010	vCJD PrP^{Sc} in peripheral tissues can be detected at preclinical time points	[57]
2012	Oral transmission of L-BSE to mouse lemur	[70]

clude chimpanzees, the new world monkeys squirrel, spider and capuchin monkey and the old world monkeys African green, rhesus and cynomolgus monkey. Susceptibility was highest in chimpanzees, squirrel and spider monkeys. Experimental transmission rates were 100% for iCJD, 95% for kuru, 90% for sCJD and about 68% for the genetic forms (GSS, gCJD, FFI). Data on transmission of FFI were inconclusive. Transmission efficiencies have been shown to be influenced by a number of factors including the amount of disease-associated PrP^{Sc} contained in the tissue used for inoculation [38]. Many brain regions remain unaffected in FFI and diseased brain tissue contains relatively low amounts of PrP^{Sc}, which might explain failure to transmit FFI to primates [13]. However, the transmissibility of FFI and therefore its correct classification as a prion disease could be proven by the transmission of FFI to mice [86].

Within the framework of the NIH study, a series of 1,113 human cases of neurological disorders and syndromes (besides human prion diseases, other disorders such as Alzheimer's disease, multiple sclerosis, schizophrenia, amyotrophic lateral sclerosis, epilepsy, and others) were inoculated into 3,418 primates to investigate which of these disorders have transmission potential [12]. Only prion diseases have shown to be transmissible. After inoculation of tissue samples from more than 600 patients with non-spongiform disorders, 9 cases of non-prion neurological diseases also produced spongiform degeneration upon inoculation in 9 out of 1,504 animals. None of these cases could be confirmed by subsequent multiple reinoculations of the human tissue in

additional animals [12,14,43]. This needs to be taken into account when concepts of transmissibility of other neurodegenerative diseases are currently being discussed [54].

Determination of prion infectivity in peripheral human tissues

Within the NIH study, the distribution of prion-infectivity in prion-diseased humans was also assessed by bioassay into primates. Infectivity was, as expected, found in the brain, spinal cord and eye. In all other tissues tested, infectivity was only irregularly detectable in the lung, liver, kidney, spleen and lymph nodes after inoculation into mainly squirrel monkeys [12]. In contrast, no infectivity could be detected in peripheral nerves, intestine, bone marrow, whole blood, leukocytes, serum, thyroid gland, adrenal gland, heart, skeletal muscle, adipose tissue, gingiva, prostate, testis and placenta/amnion. Diverse human body fluids and secretions were also tested by bioassay in non-human primates. These included tears, nasal mucus, saliva, sputum, urine, feces, semen, vaginal secretion and milk. None of these samples contained detectable levels of infectivity in non-human primate bioassay.

In contrast to the body fluids, it could be shown in 1994 that stereotactic electrodes used for recordings of electrical activity in the cerebral cortex of a patient with progressive dementia, easily transmitted a prion disease when implanted in the cortex of a chimpanzee [34]. Remarkably, this transmission occurred two years after the last use in humans and after repeated cleanings and sterilization procedures. This finding highlights the extreme resistance of prion-infectivity especially on metal surfaces and underscores the possibility of accidentally transmitting human prion diseases by contaminated metal instruments [61].

The first wave of prion research using non-human primates: conclusions

The main findings of this first wave of prion research using non-human primates can be summarized as follows:

1) Human prion diseases can be transmitted experimentally to a variety of non-human primates.
2) Efficiency of transmission depends on the primate species (host) as well as the origin of inoculated material (donor) with iCJD being most and familial cases being least efficient.
3) Efficiency also depends on the inoculation route with highest transmission rates after intracerebral inoculation.
4) The same material injected into multiple primates leads to a range of incubation times.
5) Characteristics of the original disease are preserved upon transmission (e.g. lesion profile).
6) Incubation time to clinical disease is shortened upon secondary transmission (= adaptation).

The second wave of prion research using non-human primates

The development of BSE into an epidemic and the putative transmission of BSE to humans giving rise to a novel human prion disease (vCJD) initiated a second round of experiments with prions involving primates [24,89].

BSE and non-human primates

The main focus of the initial experiments using primates was to address the zoonotic potential and risk of transmissibility of BSE and vCJD. Since the use of chimpanzees for these types of studies was practically banned and the use of other non-human primates drastically reduced due to ethical considerations, animal numbers were usually much smaller in these studies [88]. Instead of chimpanzees, cynomolgus and rhesus macaques were used in most of the studies. Cynomolgus and rhesus macaques are ideally suited since these species are evolutionary very close to humans, have a high degree of amino acid homology in PrP^{C} and are also polymorphic on codon 129 of the gene encoding PrP, an important genetic determinant of susceptibility towards prion diseases in humans [41].

Thus, cynomolgus macaques were used to investigate the transmissibility of BSE. In 1996, it became obvious that BSE may be transmitted by intracerebral inoculation [59]. Moreover, the clinical presentation and the pathological signature in primates with typical so called “florid” plaques in brain tissue was identical to that of humans with vCJD and differed from that seen after transmission of sCJD. In addition, the PrP^{Sc}-type indicative of the predominant species of infectious prions present also supported the link between BSE and vCJD, since PrP^{Sc}-types between BSE-diseased cattle, vCJD and BSE-inoculated primates were identical and differed significantly from sCJD in humans and primates (see Fig. 1).

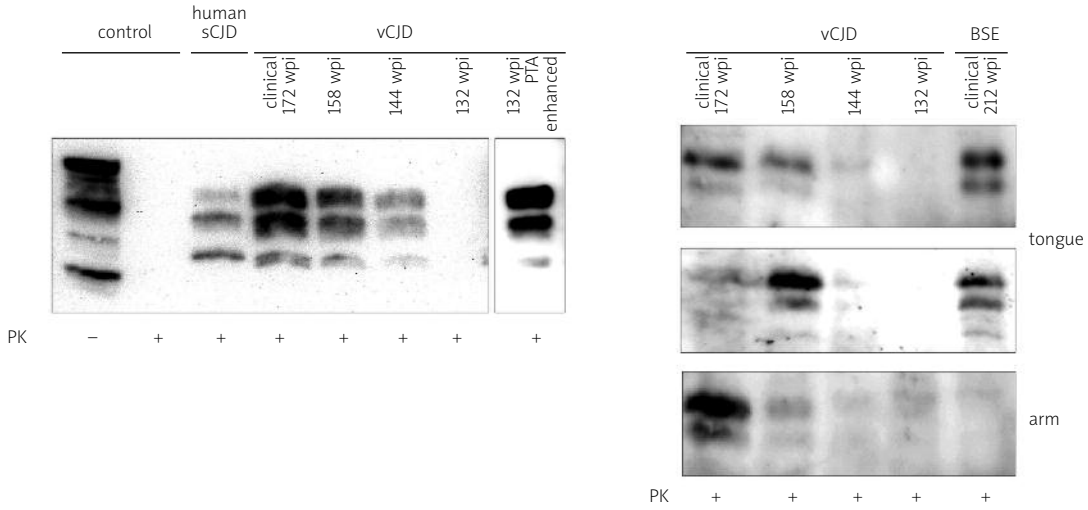

Fig. 1. PrP^{Sc} pattern is indicative of the prion strain, plateaus in preclinical brain tissue and show a specific deposition pattern. **A**) Western Blot analysis for PrP^{Sc} of vCJD infected primates (cerebellum) in comparison to human sCJD (frontal cortex). The typical PK-digestion pattern for the two different prion isolates can be clearly distinguished with typical glycotype ratios of three different PrP-bands and typical mobility of the lowest unglycosylated band. PrP^{Sc} could be demonstrated in the brains of preclinical vCJD and prion-diseased vCJD. In the vCJD cohort, PrP^{Sc} is detectable (using NaPTA enhancement of 25 mg of brain tissue) in subclinical state 40 weeks before the onset of symptoms. Thus, PrP^{Sc} plateaus in the brain before the onset of clinical symptoms. As a control, brain tissue of an uninfected control animal was also digested with PK. **B**) In PTA-enhanced Western blotting, PrP^{Sc} could be detected in peripheral muscle tissues of vCJD- and BSE-infected primates. In tongue tissue, PrP^{Sc} can be detected in vCJD and BSE infected primates. In vCJD, PrP^{Sc} can be detected already about 30 weeks before the onset of clinical symptoms. In contrast to tongue tissue, PrP^{Sc} in arm muscle can be detected only in vCJD infected primates and not in BSE inoculated animals. NaPTAs were done with 50 mg tissue per lane.

Five years later, it could be shown that secondary transmission of macaque adapted BSE leads to a shortening in incubation time, as seen for other transmission studies [60]. In addition, the successful transmission of vCJD with a comparable short incubation time in primates suggested that BSE easily adapted to primates with enhanced virulence. In this study, it could also be shown that primate adapted BSE transmit via intravenous inoculation with incubation times only slightly higher than in i.c. inoculations [60]. These experiments showed that BSE can be easily transmitted by the intravenous route from primate to primate and suggested that secondary i.v. transmission of vCJD in humans may occur. In 2004 and 2005, the capability of BSE to be orally transmitted to primates via consumption of prion-containing tissue was proven [48, 58]. However, incubation time to clinical disease was considerably longer than with i.c. or i.v. inoculation.

The discovery of L-BSE/BASE and H-BSE gave rise to the question of transmissibility to humans. This was first evaluated in mice transgenic for human prion protein [8,55]. These mice were susceptible towards BASE much more efficiently than classical BSE. However, BSE-H failed to transmit in these experiments [8]. The transmission of BASE via intracerebral injection to cynomolgus macaques was demonstrated in 2008 and 2011 [21,73]. Moreover, the oral transmission of BASE was demonstrated only recently in another primate model (mouse lemur) [70].

PrP^{Sc} in peripheral tissue of non-human primates

Improvements in the detection of minute amounts of PrP^{Sc} by selective precipitation (NaPTA) or highly sensitive ELISA led to the demonstration of PrP^{Sc} in peripheral tissues [37]. Subsequently, these methods were used to assess the degree of periph-

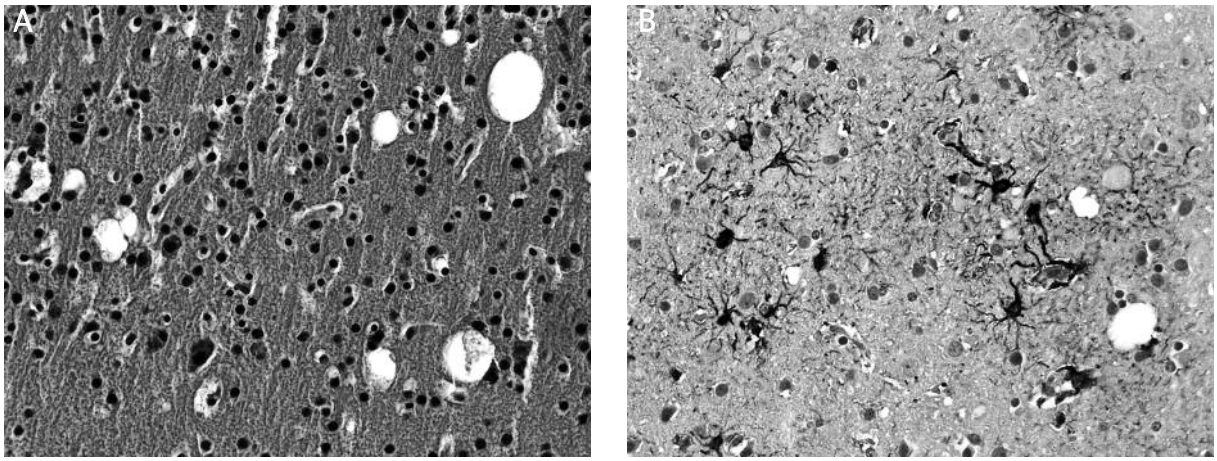

Fig. 2. Occipital cortex of a clinically prion diseased, vCJD inoculated primate. **A**) Lesion pattern in the brain of non-human primates are highly comparable to that of humans. Pathognomonic spongiosis can be seen in deeper cortical layers (HE, scale bar 20 µm). **B**) The astrocyte to neuron ratio differs remarkably between rodents and humans. Therefore, astrocytic changes might be different in the diseased brain. Here, pronounced astrogliosis is observed in the vicinity of spongiotic lesions in the brain of a clinically prion diseased primate (immunohistochemical staining for glial fibrillary acidic protein).

eral deposition of PrP^{Sc} in non-human primates challenged with various human prion-isolates [47]. Interestingly, spreading of PrP^{Sc} to the periphery seems to be prion strain and host dependant, since vCJD could be detected in tongue and skeletal muscle in a primate model of human prion diseases [57] (see Fig. 1). In this model, very small amounts of aberrantly folded PrP^{Sc} could also be detected in heart tissue. In contrast, BSE-infected monkeys repeatedly failed to show any detectable PrP^{Sc} deposition in heart tissue and the ultrastructural changes in the brain were minimal (Fig. 3). This is in line with data from mice inoculated with mouse-adapted BSE, where no heart-associated PrP^{Sc} could be found in preclinical or clinical stages [18]. PrP^{Sc} found in skeletal muscle mainly associated to lymphatic tissues and only at later time points to neuromuscular junctions and small nerves in this model.

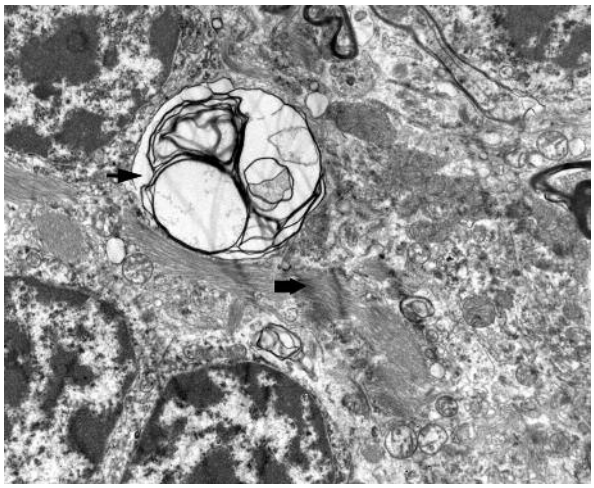

Fig. 3. Ultrastructural picture of the BSE-inoculated monkey. A vacuole is labelled with an arrow and a astrocytic process with a thick arrow.

Preclinical distribution and deposition of PrP^{Sc}

Data about the preclinical phase of prion diseases are difficult to assess in humans, thus small laboratory rodents have been used to address these questions instead [2]. In these experiments, it could be shown that PrP^{Sc} plateaus during the development of clinical symptoms [40]. Moreover, it could be shown that peripheral deposition of PrP^{Sc} occurs before the onset of clinical symptoms [65]. Interestingly, the degree of peripheral deposition is highly dependant on the host and prion strain combination. In hamsters, the deposition of PrP^{Sc} in the periphery is higher than in mice even with the use of transgenic approaches [18,87]. Recently, we could demonstrate that for PrP^{Sc} amounts and plateaus in preclinical prion disease in primates infected with vCJD [57] (see Fig. 1A). In these experiments, we were also able to assess the preclinical deposition of

PrP^{Sc} in muscle tissue. We could show that peripheral deposition is prion strain dependant also in non-human primates with most abundant prion-colonisation of the periphery after inoculation with vCJD. This study furthermore showed that muscular PrP^{Sc} occurs long before the onset of clinical symptoms and, as in sCJD, positively correlates with long disease duration [37].

The second wave of prion research using non-human primates: conclusions

The main focus of the second round of transmission studies to non-human primates was to assess the zoonotic potential of the novel and emerging prion diseases BSE and vCJD [48,58]. For a summary of the main transmission studies see Table I. Due to novel sensitive methods to detect PrP^{Sc} it was also possible to investigate the spread of PrP^{Sc} from or to peripheral tissues [57]. Moreover, the preclinical deposition of PrP^{Sc} in brain and peripheral tissue was assessed for the first time.

Conclusions

Prion diseases are intensively studied neurodegenerative disorders. A variety of different animal models and *ex vivo* models exists for this prototype of cerebral proteinopathy. Non-human primate models reproduce main characteristics of human prion diseases and have proven valuable to study their pathophysiology [48,57,58,60]. Although the idea of a simple protein as a transmissible entity that is able to impose its conformation to an otherwise normally folded protein was quite revolutionary [1,78], the prion concept has spread to other diseases such as Alzheimer's disease. On the other hand, it has to be said that these diseases lack the natural transmissibility of prion diseases although transmission can be achieved in experimental conditions [4].

Although ethical obstacles to perform primate studies are high, and usage of primates in biomedical research should be considered very carefully, some aspects of primate models are superior to studies in small laboratory animals. In prion disease-research, primate studies have laid the ground for a wealth of studies using genetically modified mice and cell-based methods. Thus, primate studies have contributed to the commonly heard notion that prion research is now the driving force of biomedical research in the field of neurodegenerative diseases.

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References

1. Aguzzi A. Understanding the diversity of prions. Nat Cell Biol 2004; 6: 290-292.
2. Aguzzi A, Klein MA, Montrasio F, Pekarik V, Brandner S, Furukawa H, Kaser P, Rockl C, Glatzel M. Prions: pathogenesis and reverse genetics. Ann N Y Acad Sci 2000; 920: 140-157.
3. Aguzzi A, O'Connor T. Protein aggregation diseases: pathogenicity and therapeutic perspectives. Nat Rev Drug Discov 2010; 9: 237-248.
4. Aguzzi A, Rajendran L. The transcellular spread of cytosolic amyloids, prions, and prionoids. Neuron 2009; 64: 783-790.
5. Armstrong RA. Dispersion of prion protein deposits around blood vessels in variant Creutzfeldt-Jakob disease. Folia Neuropathol 2010; 48: 150-158.
6. Balkema-Buschmann A, Eiden M, Hoffmann C, Kaatz M, Ziegler U, Keller M, Groschup MH. BSE infectivity in the absence of detectable PrP(Sc) accumulation in the tongue and nasal mucosa of terminally diseased cattle. J Gen Virol 2011; 92: 467-476.
7. Belay ED. Transmissible spongiform encephalopathies in humans. Annu Rev Microbiol 1999; 53: 283-314.
8. Beringue V, Herzog L, Reine F, Le Dur A, Casalone C, Vilotte JL, Laude H. Transmission of atypical bovine prions to mice transgenic for human prion protein. Emerg Infect Dis 2008; 14: 1898-1901.
9. Beringue V, Vilotte JL, Laude H. Prion agent diversity and species barrier. Vet Res 2008; 39: 47.
10. Bessen RA, Marsh RF. Identification of two biologically distinct strains of transmissible mink encephalopathy in hamsters. J Gen Virol 1992; 73: 329-334.
11. Brandner S, Isenmann S, Raeber A, Fischer M, Sailer A, Kobayashi Y, Marino S, Weissmann C, Aguzzi A. Normal host prion protein necessary for scrapie-induced neurotoxicity. Nature 1996; 379: 339-343.
12. Brown P, Gibbs CJ, Jr., Rodgers-Johnson P, Asher DM, Sulima MP, Bacote A, Goldfarb LG, Gajdusek DC. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. Ann Neurol 1994; 35: 513-529.
13. Brown P, Kenney K, Little B, Ironside J, Will R, Cervenakova L, Bjork RJ, San Martin RA, Safar J, Roos R, Haltia M, Gibbs CJ, Gajdusek DC. Intracerebral distribution of infectious amyloid protein in spongiform encephalopathy. Ann Neurol 1995; 38: 245-253.
14. Brown P, Salazar AM, Gibbs CJ, Jr., Gajdusek DC. Alzheimer's disease and transmissible virus dementia (Creutzfeldt-Jakob disease). Ann N Y Acad Sci 1982; 396: 131-143.
15. Cali I, Castellani R, Yuan J, Al-Shekhlee A, Cohen ML, Xiao X, Moleres FJ, Parchi P, Zou WQ, Gambetti P. Classification of spo-

radic Creutzfeldt-Jakob disease revisited. Brain 2006; 129: 2266-2277.
16. Calzolai L, Lysek DA, Perez DR, Guntert P, Wuthrich K. Prion protein NMR structures of chickens, turtles, and frogs. Proc Natl Acad Sci U S A 2005; 102: 651-655.
17. Capobianco R, Casalone C, Suardi S, Mangieri M, Miccolo C, Limido L, Catania M, Rossi G, Di Fede G, Giaccone G, Bruzzone MG, Minati L, Corona C, Acutis P, Gelmetti D, Lombardi G, Groschup MH, Buschmann A, Zanusso G, Monaco S, Caramelli M, Tagliavini F. Conversion of the BASE prion strain into the BSE strain: the origin of BSE? PLoS Pathog 2007; 3: e31.
18. Cardone F, Thomzig A, Schulz-Schaeffer W, Valanzano A, Sbriccoli M, Abdel-Haq H, Graziano S, Pritzkow S, Puopolo M, Brown P, Beekes M, Pocchiari M. PrPTSE in muscle-associated lymphatic tissue during the preclinical stage of mice infected orally with bovine spongiform encephalopathy. J Gen Virol 2009; 90: 2563-2568.
19. Collinge J. Prion diseases of humans and animals: their causes and molecular basis. Annu Rev Neurosci 2001; 24: 519-550.
20. Collinge J, Whitfield J, McKintosh E, Beck J, Mead S, Thomas DJ, Alpers MP. Kuru in the 21st century--an acquired human prion disease with very long incubation periods. Lancet 2006; 367: 2068-2074.
21. Comoy EE, Casalone C, Lescoutra-Etchegaray N, Zanusso G, Freire S, Marce D, Auvre F, Ruchoux MM, Ferrari S, Monaco S, Sales N, Caramelli M, Leboulch P, Brown P, Lasmezas CI, Deslys JP. Atypical BSE (BASE) transmitted from asymptomatic aging cattle to a primate. PLoS One 2008; 3: e3017.
22. Creutzfeldt HG. Über eine eigenartige herdförmige Erkrankung des Zentralnervensystems. Z ges Neurol Psychiatr 1920; 57: 1-19.
23. Cuille J, Chelle PL. Experimental transmission of trembling to the goat. C R Seances Acad Sci 1939; 208: 1058-1160.
24. Ducrot C, Arnold M, de Koeijer A, Heim D, Calavas D. Review on the epidemiology and dynamics of BSE epidemics. Vet Res 2008; 39: 15.
25. Espana C, Gajdusek DC, Gibbs CJ, Jr., Osburn BI, Gribble DH, Cardinet GH, Chanock RM. Transmission of Creutzfeldt-Jakob disease to the stumptail macaque (Macaca arctoides). Proc Soc Exp Biol Med 1975; 149: 723-724.
26. Gajdusek DC, Gibbs CJ. Transmission of kuru from man to rhesus monkey (Macaca mulatta) 8 and one-half years after inoculation. Nature 1972; 240: 351.
27. Gajdusek DC, Gibbs CJ, Alpers M. Experimental transmission of a Kuru-like syndrome to chimpanzees. Nature 1966; 209: 794-796.
28. Gajdusek DC, Gibbs CJ, Jr. Transmission of two subacute spongiform encephalopathies of man (Kuru and Creutzfeldt-Jakob disease) to new world monkeys. Nature 1971; 230: 588-591.
29. Gajdusek DC, Gibbs CJ, Jr., Alpers M. Transmission and passage of experimenal "kuru" to chimpanzees. Science 1967; 155: 212-214.
30. Gajdusek DC, Gibbs CJ, Jr., Asher DM, David E. Transmission of experimental kuru to the spider monkey (Ateles geoffreyi). Science 1968; 162: 693-694.
31. Gajdusek DC, Reid LH. Studies on kuru. IV. The kuru pattern in Moke, a representative Fore village. Am J Trop Med Hyg 1961; 10: 628-638.
32. Geissen M, Krasemann S, Matschke J, Glatzel M. Understanding the natural variability of prion diseases. Vaccine 2007; 25: 5631-5636.
33. Gibbs CJ, Jr., Amyx HL, Bacote A, Masters CL, Gajdusek DC. Oral transmission of kuru, Creutzfeldt-Jakob disease, and scrapie to nonhuman primates. J Infect Dis 1980; 142: 205-208.
34. Gibbs CJ, Jr., Asher DM, Kobrine A, Amyx HL, Sulima MP, Gajdusek DC. Transmission of Creutzfeldt-Jakob disease to a chimpanzee by electrodes contaminated during neurosurgery. J Neurol Neurosurg Psychiatry 1994; 57: 757-758.
35. Gibbs CJ, Jr., Gajdusek DC. Transmission of scrapie to the cynomolgus monkey (Macaca fascicularis). Nature 1972; 236: 73-74.
36. Gibbs CJ, Jr., Gajdusek DC, Asher DM, Alpers MP, Beck E, Daniel PM, Matthews WB. Creutzfeldt-Jakob disease (spongiform encephalopathy): transmission to the chimpanzee. Science 1968; 161: 388-389.
37. Glatzel M, Abela E, Maissen M, Aguzzi A. Extraneural pathologic prion protein in sporadic Creutzfeldt-Jakob disease. N Engl J Med 2003; 349: 1812-1820.
38. Glatzel M, Aguzzi A. PrP(C) expression in the peripheral nervous system is a determinant of prion neuroinvasion. J Gen Virol 2000; 81: 2813-2821.
39. Glatzel M, Aguzzi A. The shifting biology of prions. Brain Res Brain Res Rev 2001; 36: 241-248.
40. Glatzel M, Klein MA, Brandner S, Aguzzi A. Prions: from neurografts to neuroinvasion. Arch Virol Suppl 2000; 16: 3-12.
41. Glatzel M, Pekarik V, Luhrs T, Dittami J, Aguzzi A. Analysis of the prion protein in primates reveals a new polymorphism in codon 226 (Y226F). Biol Chem 2002; 383: 1021-1025.
42. Glatzel M, Rogivue C, Ghani A, Streffer JR, Amsler L, Aguzzi A. Incidence of Creutzfeldt-Jakob disease in Switzerland. Lancet 2002; 360: 139-141.
43. Goudsmit J, Morrow CH, Asher DM, Yanagihara RT, Masters CL, Gibbs CJ, Jr., Gajdusek DC. Evidence for and against the transmissibility of Alzheimer disease. Neurology 1980; 30: 945-950.
44. Hadlow WJ. Scrapie and kuru. Lancet 1959; 2: 289-290.
45. Haley NJ, Seelig DM, Zabel MD, Telling GC, Hoover EA. Detection of CWD prions in urine and saliva of deer by transgenic mouse bioassay. PLoS One 2009; 4: e4848.
46. Heggebo R, Press CM, Gunnes G, Gonzalez L, Jeffrey M. Distribution and accumulation of PrP in gut-associated and peripheral lymphoid tissue of scrapie-affected Suffolk sheep. J Gen Virol 2002; 83: 479-489.
47. Herzog C, Riviere J, Lescoutra-Etchegaray N, Charbonnier A, Leblanc V, Sales N, Deslys JP, Lasmezas CI. PrPTSE distribution in a primate model of variant, sporadic, and iatrogenic Creutzfeldt-Jakob disease. J Virol 2005; 79: 14339-14345.
48. Herzog C, Sales N, Etchegaray N, Charbonnier A, Freire S, Dormont D, Deslys JP, Lasmezas CI. Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. Lancet 2004; 363: 422-428.
49. Hill AF, Collinge J. Prion strains and species barriers. Contrib Microbiol 2004; 11: 33-49.
50. Hill AF, Desbruslais M, Joiner S, Sidle KC, Gowland I, Collinge J, Doey LJ, Lantos P. The same prion strain causes vCJD and BSE [letter]. Nature 1997; 389: 448-450.

51. Horiuchi M, Caughey B. Specific binding of normal prion protein to the scrapie form via a localized domain initiates its conversion to the protease-resistant state. Embo J 1999; 18: 3193-3203.
52. Ironside JW. Prion diseases in man. J Pathol 1998; 186: 227-234.
53. Jakob A. Über eigenartige Erkrankungen des Zentralnervensystems mit bemerkenswertem anatomischem Befunde. (Spastische Pseudosklerose-Encephalomyelopathie mit disseminierten Degenerationsherden). Z ges Neurol Psychiatr 1921; 64: 147-228.
54. Jucker M, Walker LC. Pathogenic protein seeding in alzheimer disease and other neurodegenerative disorders. Ann Neurol 2011; 70: 532-540.
55. Kong Q, Zheng M, Casalone C, Qing L, Huang S, Chakraborty B, Wang P, Chen F, Cali I, Corona C, Martucci F, Iulini B, Acutis P, Wang L, Liang J, Wang M, Li X, Monaco S, Zanusso G, Zou WQ, Caramelli M, Gambetti P. Evaluation of the human transmission risk of an atypical bovine spongiform encephalopathy prion strain. J Virol 2008; 82: 3697-3701.
56. Kovacs GG, Puopolo M, Ladogana A, Pocchiari M, Budka H, van Duijn C, Collins SJ, Boyd A, Giulivi A, Coulthart M, Delasnerie-Laupretre N, Brandel JP, Zerr I, Kretzschmar HA, de Pedro-Cuesta J, Calero-Lara M, Glatzel M, Aguzzi A, Bishop M, Knight R, Belay G, Will R, Mitrova E. Genetic prion disease: the EUROCJD experience. Hum Genet 2005; 118: 166-174.
57. Krasemann S, Neumann M, Geissen M, Bodemer W, Kaup FJ, Schulz-Schaeffer W, Morel N, Aguzzi A, Glatzel M. Preclinical deposition of pathological prion protein in muscle of experimentally infected primates. PLoS ONE 2010; 5: e13906.
58. Lasmezas CI, Comoy E, Hawkins S, Herzog C, Mouthon F, Konold T, Auvre F, Correia E, Lescoutra-Etchegaray N, Sales N, Wells G, Brown P, Deslys JP. Risk of oral infection with bovine spongiform encephalopathy agent in primates. Lancet 2005; 365: 781-783.
59. Lasmezas CI, Deslys JP, Demaimay R, Adjou KT, Lamoury F, Dormont D, Robain O, Ironside J, Hauw JJ. Bse Transmission to Macaques. Nature 1996; 381: 743-744.
60. Lasmezas CI, Fournier JG, Nouvel V, Boe H, Marce D, Lamoury F, Kopp N, Hauw JJ, Ironside J, Bruce M, Dormont D, Deslys JP. Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications for human health. Proc Natl Acad Sci U S A 2001; 98: 4142-4147.
61. Lemmer K, Mielke M, Pauli G, Beekes M. Decontamination of surgical instruments from prion proteins: in vitro studies on the detachment, destabilization and degradation of PrPSc bound to steel surfaces. J Gen Virol 2004; 85: 3805-3816.
62. Liberski PP. Kuru and D. Carleton Gajdusek: a close encounter. Folia Neuropathol 2009; 47: 114-137.
63. Llewelyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, Mackenzie J, Will RG. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 2004; 363: 417-421.
64. Lysek DA, Schorn C, Nivon LG, Esteve-Moya V, Christen B, Calzolai L, von Schroetter C, Fiorito F, Herrmann T, Guntert P, Wuthrich K. Prion protein NMR structures of cats, dogs, pigs, and sheep. Proc Natl Acad Sci U S A 2005; 102: 640-645.
65. Mabbott NA, Bruce ME. Prion disease: bridging the spleen-nerve gap. Nat Med 2003; 9: 1463-1464.
66. Maddison BC, Rees HC, Baker CA, Taema M, Bellworthy SJ, Thorne L, Terry LA, Gough KC. Prions are secreted into the oral cavity in sheep with preclinical scrapie. J Infect Dis 2010; 201: 1672-1676.
67. Marsh RF, Kincaid AE, Bessen RA, Bartz JC. Interspecies transmission of chronic wasting disease prions to squirrel monkeys (Saimiri sciureus). J Virol 2005; 79: 13794-13796.
68. Masters CL, Alpers MP, Gajdusek DC, Gibbs CJ, Jr., Kakulas BA. Experimental kuru in the gibbon and sooty mangabey and Creutzfeldt-Jakob disease in the pigtailed macaque. With a summary of the host range of the subacute spongiform virus encephalopathies. J Med Primatol 1976; 5: 205-209.
69. Masters CL, Gajdusek DC, Gibbs CJ. Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Straussler syndrome with an analysis of the various forms of amyloid plaque deposition in the virus-induced spongiform encephalopathies. Brain 1981; 104: 559-588.
70. Mestre-Frances N, Nicot S, Rouland S, Biacabe AG, Quadrio I, Perret-Liaudet A, Baron T, Verdier JM. Oral Transmission of L-type Bovine Spongiform Encephalopathy in Primate Model. Emerg Infect Dis 2012; 18: 142-145.
71. Notari S, Capellari S, Giese A, Westner I, Baruzzi A, Ghetti B, Gambetti P, Kretzschmar HA, Parchi P. Effects of different experimental conditions on the PrPSc core generated by protease digestion: Implications for strain typing and molecular classification of CJD. J Biol Chem 2004; 279: 16797-16804.
72. O'Rourke KI, Zhuang D, Truscott TC, Yan H, Schneider DA. Sparse PrP(Sc) accumulation in the placentas of goats with naturally acquired scrapie. BMC Vet Res 2011; 7: 7.
73. Ono F, Tase N, Kurosawa A, Hiyaoka A, Ohyama A, Tezuka Y, Wada N, Sato Y, Tobiume M, Hagiwara K, Yamakawa Y, Terao K, Sata T. Atypical L-type bovine spongiform encephalopathy (L-BSE) transmission to cynomolgus macaques, a non-human primate. Jpn J Infect Dis 2011; 64: 81-84.
74. Peden AH, Ritchie DL, Head MW, Ironside JW. Detection and localization of PrPSc in the skeletal muscle of patients with variant, iatrogenic, and sporadic forms of Creutzfeldt-Jakob disease. Am J Pathol 2006; 168: 927-935.
75. Peterson DA, Wolfe LG, Deinhardt F, Gajdusek DC, Gibbs CJ, Jr. Transmission of kuru and Creutzfeldt-Jakob disease to marmoset monkeys. Intervirology 1974; 2: 14-19.
76. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982; 216: 136-144.
77. Prusiner SB. Inherited prion diseases. Proc Natl Acad Sci U S A 1994; 91: 4611-4614.
78. Prusiner SB. Prions. Proc Natl Acad Sci U S A 1998; 95: 13363-13383.
79. Prusiner SB, Scott MR. Genetics of prions. Annu Rev Genet 1997; 31: 139-175.
80. Sandberg MK, Al-Doujaily H, Sigurdson CJ, Glatzel M, O'Malley C, Powell C, Asante EA, Linehan JM, Brandner S, Wadsworth JD, Collinge J. Chronic wasting disease prions are not transmissible to transgenic mice overexpressing human prion protein. J Gen Virol 2010; 91: 2651-2657.
81. Schoch G, Seeger H, Bogousslavsky J, Tolnay M, Janzer RC, Aguzzi A, Glatzel M. Analysis of prion strains by PrPSc profiling in sporadic Creutzfeldt-Jakob disease. PLoS Med 2006; 3: e14.

82. Scott MR, Peretz D, Nguyen HO, Dearmond SJ, Prusiner SB. Transmission barriers for bovine, ovine, and human prions in transgenic mice. J Virol 2005; 79: 5259-5271.
83. Sigurdson CJ, Mathiason CK, Perrott MR, Eliason GA, Spraker TR, Glatzel M, Manco G, Bartz JC, Miller MW, Hoover EA. Experimental chronic wasting disease (CWD) in the ferret. J Comp Pathol 2008; 138: 189-196.
84. Sigurdson CJ, Miller MW. Other animal prion diseases. Br Med Bull 2003; 66: 199-212.
85. Sigurdson CJ, Nilsson KP, Hornemann S, Manco G, Fernandez-Borges N, Schwarz P, Castilla J, Wuthrich K, Aguzzi A. A molecular switch controls interspecies prion disease transmission in mice. J Clin Invest 2010; 120: 2590-2599.
86. Tateishi J, Brown P, Kitamoto T, Hoque ZM, Roos R, Wollman R, Cervenakova L, Gajdusek DC. First experimental transmission of fatal familial insomnia. Nature 1995; 376: 434-435.
87. Thomzig A, Cardone F, Kruger D, Pocchiari M, Brown P, Beekes M. Pathological prion protein in muscles of hamsters and mice infected with rodent-adapted BSE or vCJD. J Gen Virol 2006; 87: 251-254.
88. Wadman M. Animal rights: Chimpanzee research on trial. Nature 2011; 474: 268-271.
89. Wells GA, Scott AC, Johnson CT, Gunning RF, Hancock RD, Jeffrey M, Dawson M, Bradley R. A novel progressive spongiform encephalopathy in cattle. VetRec 1987; 121: 419-420.
90. Will RG, Ironside JW, Zeidler M., Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 1996; 347: 921-925.
91. Wroe SJ, Pal S, Siddique D, Hyare H, Macfarlane R, Joiner S, Linehan JM, Brandner S, Wadsworth JD, Hewitt P, Collinge J. Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. Lancet 2006; 368: 2061-2067.