Prions and Orthopedic Surgery

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Abstract

Prions are a novel class of infectious agents that cause subacute encephalopathy in man and animals as human Creutzfeldt-Jakob disease (CJD), sheep scrapie and bovine spongiform encephalopathy (BSE). Previously, prions were shown to be transmitted by neuro- and ophthalmosurgical measures and by application of brain-derived therapeutic hormones. Recently, prions have been detected in blood specimens of experimentally infected monkeys indicating a principal threat to transfusion medicine, furthermore in human or bovine materials used in reconstitutive surgery. In this article the risk of prion transmission from the surgeon to the patient or vice versa during (orthopedic) surgery is reevaluated including the issues of blood transfusion. This is accomplished based on recent epidemiologic findings and biometric calculations on the spread of prions in animals and humans as well as in terms of experimental data on artificially contaminated medical materials and devices. The overall risk of prion transmission in orthopedic surgery is considered very low if adequately prepared and sterilized materials and devices are used.

Infection 2003; 31: 163–171
DOI 10.1007/s15010-003-3108-3

Medical History of Creutzfeldt-Jakob Disease (CJD)

The first human prion disease was diagnosed and described in 1920 by the German neurologists *Hans Gerhard Creutzfeldt* (1885–1964) and *Alfons M. Jako*b (1884–1931). They considered it to be a rare, spontaneously developing encephalopathy with unknown etiology [1]. Later on, rare familial cases of similar brain diseases were described presenting with a distinct pathology (Table 1). However, in some cases of CJD, iatrogenic transmission via (micro)contamination with pathological brain tissue was discovered. CJD was now suspected to be a slow virus disease presenting with a typically subacute onset and an incubation period of years to decades (transmissible spongiform encephalopathy [TSE]). From 1959 to 1963, the American virologist *D. Carleton Gajdusek* investigated an outbreak of CJD-like illness (called "kuru") in a native tribe on New Guinea. By epidemiological analysis, he found out that CJD/kuru is an infectious disease and that the infectious agent is present in the brain. Oral uptake of brain, ritually prepared and eaten after the death of patients, transfers the infectious disease, which becomes apparent after years up to decades of latent infection. By inoculation of this brain material into monkeys, which developed a similar disease, *Gajdusek* could clearly demonstrate the infectivity. Histological analysis revealed a particular brain amyloidosis. Therefore *Gajdusek* termed the disease transmissible amyloidosis of the brain [2], as opposed to nontransmissible brain amyloidoses (e.g., Alzheimer's disease). For his work, *Gajdusek* was awarded the Nobel Prize in 1976. As early as 1966, the British veterinarian *Tikwar Alper* and her colleagues investigated the etiology of scrapie, a slow virus brain disease of sheep similar to CJD, but known for more than two centuries. They suggested from the anomalous resistance of the scrapie agent against both ionizing and ultraviolet (UV) irradiation, that the target size is too small for a viral genome [3].

Biology of Prion Infection and Disease

The American neurologist *Stanley B. Prusiner* purified infectious amyloid proteins from scrapie cases and inoculated these proteins into mice [4–6]. Infectivity could be destroyed by digestion with proteinase K. He therefore proposed the protein-only hypothesis of the infectious agent and defined the term prion [7]. Prion means proteinaceous infectious organism. Prions are derived from physiological, but nonessential glycoproteins found on the membrane of nearly all cells in the body of man and animals, particularly in the nervous and lymphoreticular systems. The normal prion protein (PrP) was well characterized by *Weissmann* and others (for details and overview [8–10]). The cellular gene locus of the PrP, which is a single-copy gene with a length of 750 base

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Received: June 24, 2002 • Revision accepted: December 23, 2002

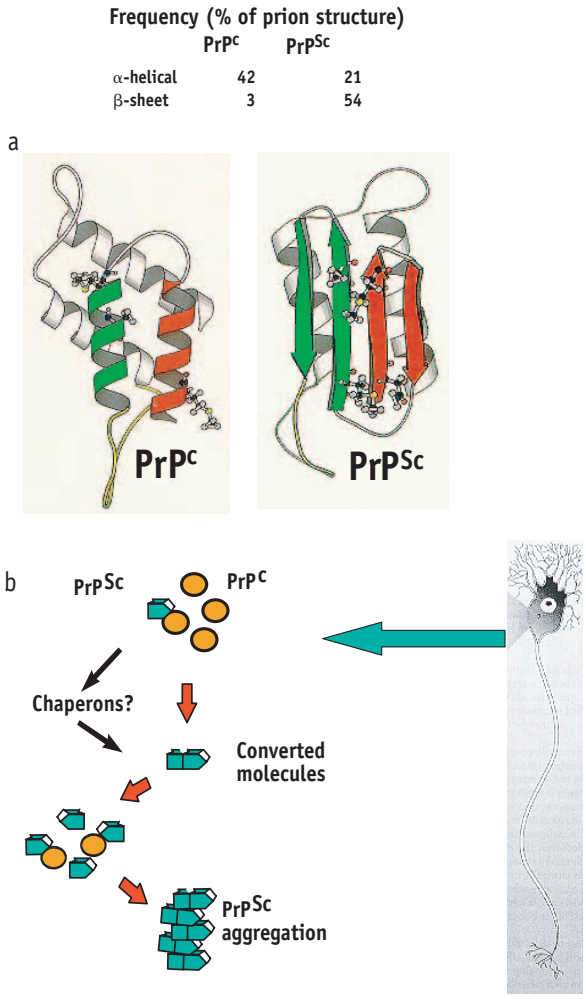

Figure 1a and 1b. Prion structure and replication. a) shows that the cellular prion protein (PrP^c) is dominated by an α-helical structure, while in the pathogenic PrP^{Sc} the β-sheet is disproportionate (modified from [13]). b) demonstrates the hypothesis that the conversion of PrP^c into PrP^{Sc} is related to the direct contact of the molecules and may be triggered by helper molecules (chaperons). As a result of this process, aggregates of the pathogenic and very stable PrP^{Sc} are generated [14].

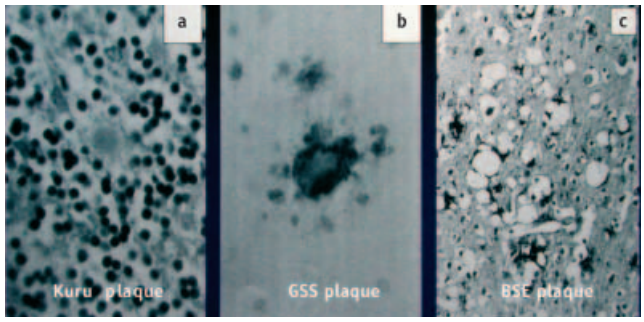

Figure 2a to 2c. Prion diseases in man and cattle: typical brain histology with prion-specific amyloid plaques. a) Kuru. b) Gerstmann-Sträussler-Scheinker (GSS) syndrome. c) Bovine spongiform encephalopathy (BSE). Note the distinct vacuolization in BSE [2].

pairs (bp), is located on the short arm of chromosome 20 [11]. A couple of point mutations were found to favor the development of CJD and scrapie. The best genetic linkage was identified in the rare Gerstmann-Sträussler-Scheinker (GSS) syndromes and cases of fatal familial insomnia (FFI), whereas no linkage was traced in kuru [12]. *Prusiner's* research tried to explain the twin nature of CJD as a degenerative (sporadic) encephalopathy and infectious "slow virus encephalitis": brain damage arises from conversion of physiological prion protein present in cells of the nervous and (to a lower degree) lymphoreticular cell system (PrP^c) to pathological prion protein (PrP^{CJD}, PrP^{Sc}). "Replication" and expansion of pathological prions result from "contamination" of normal (physiological) prion protein (PrP^c) with pathological prion ("biocrystallization"). One of the favorite hypotheses speculates that this might be initiated by somatic mutations in the PrP gene (inherited or acquired during the development of the central nervous system) and either limited to one host (sporadic disease), iatrogenically, or orally transmissible/transferred to the next host (infectious disease). The replication process develops in the cell. It is not known whether chaperons are involved, regulating the folding of newly synthesized proteins. The conversion to the pathological isoform (Figure 1) makes the prions resistant

Table 1
Prion diseases in man.

Disease	Distribution	Notes/incidence
Kuru	Papua New Guinea	Vanishing
Creutzfeldt-Jakob disease (CJD)	Worldwide	1 : 1,000,000/year
Iatrogenic Creutzfeldt-Jakob disease (iCJD)	Worldwide	≥ 267 cases
Variant Creutzfeldt-Jakob disease (vCJD)	130 cases in UK, six in France, two in Ireland, one in Italy, one in Canada and one in Hong Kong[a]	
Gerstmann-Sträussler-Scheinker syndrome (GSS)	Worldwide – around 50 families affected	Familial – genetic
Fatal familial insomnia (FFI)	Italy, France, USA, Germany – around ten families affected	Familial – genetic

[a] as of February 3, 2003

to metabolic breakdown leading to aggregation, accumulation and deposition in the brain tissue, which histologically appears as amyloidosis (Figure 2). Although it was possible to convert recombinant PrP^c into a β-sheet-rich, partially protease-resistant structure by physicochemical procedures, it has not, so far, been possible to induce a transmissible prion disease with this material [15, 16].

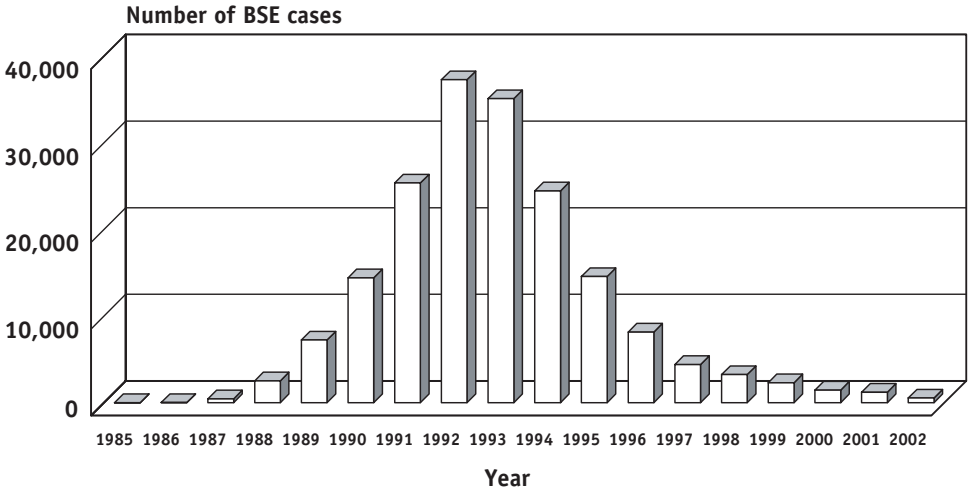

Figure 3. Development of the bovine spongiform encephalopathy (BSE) epizootic in the United Kingdom over the years. Total number of BSE cases 182,581, therefrom 534 in 2002 (as of June 30, 2002; Office International des Epizooties [OIE], http://www.oie.int/eng/info/en_esbmonde.htm).

Brain damage involves activation of microglial cells, synaptic damage, neuronal apoptosis and amyloid deposition. In full-blown cases, the brain tissue is more or less vacuolized and assumes the consistence of a sponge (spongiform encephalopathy). In strict opposition to conventional slow virus brain diseases, no signs of inflammation and no real immune reactions are visible. For his work, *Prusiner* was awarded the Nobel Prize in 1997. Subacute spongiform encephalopathy (SSE) was considered a highly interesting disease from the scientific point of view.

The advent of the huge bovine spongiform encephalopathy (BSE, "mad-cow disease") epizootic in British cattle herds since 1985 transformed it into an important public health problem (Figure 3). The origin of the BSE epizootic was the recycling of slaughter residues from cattle and other animal cadavers into a protein-rich supplement cattle feed. In 1981/82, sterilization procedures applied to this cadaver meal had been replaced to save costs. By this food recycling, an hitherto rare animal prion strain was accumulated in British cattle herds. After the etiology had been recognized and cadaver meal banned, the BSE epizootic has definitively been controlled. However, more than half a million infected cattle had entered the human food chain.

By biochemical and biological experiments (in mice) it was proven that the BSE prion was transferred to felines

Table 2
Emergence of vCJD: main differences from "classic" (sporadic) CJD.

	vCJD	Sporadic CJD
Median age (range) at onset (years)	26 (12–74)	65 (55–70)
Typical median age (range) at death (years)	29	65 (55–70)
Median duration (range) (months)	13 (6–39)	4 (1–74)
Typical presentation	Psychiatric/behavioral ± sensory symptoms	Progressive dementia, ataxia (occasionally other neurologic features)
EEG	Normal, becoming nonspecifically abnormal	Typical periodic discharges develop in many cases
CSF	14-3-3 positive (≤ 50% of cases)	14-3-3 positive (majority of cases)
MRI	"Pulvinar sign" present in the majority of cases	"Pulvinar sign" not seen
PrP genotype at codon 129	Methionine-homozygote	Mainly homozygote (val/val or met/met)
PrP^{Sc} type (Western blot)	Type 4	Type 1 or 2 (some type 3)
Tonsil biopsy	Positive	Negative

CSF: cerebrospinal fluid; EEG: electroencephalography; MRI: magnetic resonance imaging; PrP: prion protein; PrP^{Sc}: pathological prion protein (scrapie)

Table 3
Cases of iatrogenic CJD transmission (as of July 2000) [17].

Mode of transmission	No. of cases	Agent entry into brain	Mean incubation period (range)	Country of occurrence
Gonadotropin	4	Hematogenous	13 years (12–16)	Australia
Growth hormone	139	Hematogenous	12 years (5–30)	France, UK, USA
Corneal transplant	3	Optic nerve	16, 18, 320 months	Germany, Japan, USA
Stereotactic EEG	2	Intracerebral	16, 20 months	Switzerland
Neurosurgery (without dura graft)	5	Intracerebral	19 months (12–28)	France, UK
Dura mater graft	114	Cerebral surface	6 years (1.5–18)	Worldwide, mostly in Japan

EEG: electroencephalography

fed with cattle cadavers in zoological gardens. In 1995, the first case of transmission to man was discovered. Since then, 139 people have fallen ill from this new variant of CJD (vCJD), mainly in Great Britain, but some also in other countries (six cases in France, two in Ireland, one in Hong Kong, one in Canada and one in Italy [as of November 4, 2002]; Tables 1 and 2).

Iatrogenic CJD (iCJD) Transmission

As mentioned above, to date about 267 cases of iCJD transmission have been described, mainly subsequent to medical or surgical measures (Table 3). Over 11,700 patients in the USA, the United Kingdom, France and New Zealand with a diagnosis of growth retardation were injected with growth hormone prepared from corpses, among whom there was at least one CJD patient. After a median of 12 years (range 5–30 years), 137 of them have fallen ill with CJD and died (USA 23, United Kingdom 35, New Zealand five, France 74). The frequency of growth hormone-related iCJD cases in the different countries varied between 0.3% (USA) and 4.4% (France) [17]. At present, it cannot been assessed how many additional iCJD cases will develop. Concerning surgery, iCJD transmission via dura mater graft transplantation is important. Worldwide, 114 cases are described. Graft placement (supratentorial, infratentorial and cervical or peripheral vein embolization) seems to have little or no effect on the clinical iCJD presentation [17]. The details are compiled in table 3.

Dura mater grafts have also been prepared from calf material, raising the question: **is there a vCJD risk by xenotransplantation of (bovine) material?**

Bovine materials currently available for use in reconstructive, orbital and cranial surgery include:
- bovine bone anophthalmic implants,
- bovine pericardium,
- bovine amniotic membrane,
- bovine scleral wraps (e.g. dura mater),
- bovine cartilage.

The risk of xenografting bovine tissues to humans is currently unknown. Therefore, the WHO stated in a memorandum in 1997 [18]: "The ideal situation would be to avoid the use of bovine materials in the manufacture of medicinal products and the use of materials from other animal species in which TSEs naturally occur." If the use of bovine material is unavoidable, the WHO recommends:
- careful selection of source material from BSE-free herds,
- reducing exposure to neural tissue in the collection process,
- introduction of processing procedures to reduce prion exposure (e.g., soaking in 1 mol/l NaOH for 1 h).

Nunery [19] compiled the results of research on prion distribution in organs and tissues of cattle suffering from BSE and classified categories of BSE infectivity in bovine tissues and body fluid:
- **category I (high infectivity):** brain, spinal cord (eyes);
- **category II (medium infectivity):** spleen, tonsil, lymph nodes, ileum, proximal colon, cerebrospinal fluid (CSF), pituitary gland, adrenal gland (dura mater, pineal gland, placenta, distal colon);
- **category III (low infectivity):** peripheral nerves, nasal mucosa, thymus, bone marrow, liver, lung, pancreas;
- **category IV (no detectable infectivity):** skeletal muscle, heart, mammary gland, milk, blood clot, serum, feces, kidney, thyroid, salivary gland, saliva, ovary, uterus, testis, fetal tissue (colostrum, bile, bone, cartilaginous tissue, connective tissue, hair, skin, urine).

Wenz et al. [20] analyzed the risk of transmitting BSE through bone grafts derived from bovine bone in the model of a defined manufacturing process. Applying adequate measures for the elimination or sterilization of putatively contaminating infectious agents, they drew the conclusion: "Theoretical and experimental data indicate that the use of bone substitutes of bovine origin does not carry a risk of transmitting BSE to patients." This statement is considered valid also for the production of other bovine materials for reconstructive surgery as listed above.

Is there a risk of iatrogenic acquisition of prion infection during surgery? Newer data showed that a history of surgery was significantly associated with the risk of sporadic CJD [21]. These results might support the hypothesis that cases of sporadic CJD may result from hitherto unrecognized surgical contamination events [21].

Table 4
Infectivity of tissues and organs of CJD and vCJD patients.

	CJD patients	vCJD patients
Infectivity detectable	CSF, brain, spinal cord, eyes, small intestine, colon, dura mater, pineal gland, pituitary gland, adrenal gland, ischiadic nerve, liver, lung, pancreas, thymus	CSF, brain, spinal cord, eyes, small intestine, colon, dura mater, pineal gland, pituitary gland, spleen, lymph nodes, lymphatic tissue (e.g. tonsils, appendix)
No infectivity detectable	Feces, serum, thyroid, heart, milk, skin, skeletal muscle, saliva, sputum, testis, prostate, urine, semen, vaginal secretion, amnion, tears, kidney, mammary gland, ovary, salivary gland, uterus, fetal tissue, bile, bones, cartilaginous tissue	Feces, serum, thyroid, heart, milk, skin, skeletal muscle, saliva, sputum, testis, prostate, urine, semen, vaginal secretion, amnion, tears, kidney, mammary gland, ovary, salivary gland, uterus, fetal tissue, bile, bones, cartilaginous tissue, placenta, nasal mucosa, bone marrow
No infectivity detectable in mouse and primate models, respectively, but in other animal models	No infectivity detectable in primates (but some evidence in mouse models) Blood, leukocytes, placenta, nasal mucosa, bone marrow	No infectivity detectable (but in primates and sheep some evidence) Blood, leukocytes

CSF: cerebrospinal fluid

Scott et al. [22] investigated this problem and published a paper entitled "Temporal bone dissection: a possible route for prion transmission?". In their investigations they found that mastoid surgery and temporal bone dissection with a hand drill lead to a wide scattering of bone and soft tissue and create a significant cloud of tissue and bone "dust." Even when macroscopic neural structures such as the facial nerve are carefully avoided, neural tissue is present in this bone "dust." One of the possible microbiological hazards is aerosol inoculation of the surgeon's conjunctiva with infected material. Is there a risk of prion transmission if neural tissue is present in the tissue "dust"? To estimate this, it is necessary to determine the level of significance of this risk for this type of surgery. There may also be implications for other specialities such as orthopedics and neurosurgery where power tools are used as well.

How big is the risk to encounter a CJD or vCJD patient or a still asymptomatic prion carrier? Is it possible to assess the spread of CJD or vCJD? Conventional CJD is extremely rare (Table 1), with the exception of iatrogenic "clusters." Focused on vCJD and according to the latest biometric calculations, one has to take into account statistically the emergence of one vCJD case for every 100–1,000 BSE cases. Other calculations estimate that in countries with low BSE incidence there will be one vCJD case per 1,000 vCJD cases in the United Kingdom. Of course a much higher number of still healthy prion carriers might be alive. However, it is commonly accepted that those people harbor only a much smaller infectious dose than symptomatic CJD patients. Regarding transmission of prions by surgery, it is helpful to estimate the infectivity of tissues and organs of vCJD patients (Table 4).

What is still missing, is a sensitive and reliable *in vivo* diagnostic test similar to the identification of human immunodeficiency virus (HIV) carriers (Table 5).

vCJD/CJD: a Risk Factor in Blood Transfusion and Iatrogenic Blood Contamination?

To date, not a single case of CJD has been traced to a blood donation. Neither epidemiological data (from hemophiliac patients) nor *in vivo* animal studies gave evidence of a hematogenous CJD-prion transport (or the presence of CJD prions in blood cells). But some of the attempts to detect infectivity in the blood of humans and animals affected with CJD (or BSE) have been inconclusive and are therefore controversially discussed. The inconclusiveness of the *in vivo* animal-model data was mostly related to the small volumes of blood that can be injected by the intracerebral route and the limitations of cross-species bioassays.

In relation to vCJD, the situation is somewhat different. In humans, vCJD prion was found extracerebrally in tonsil, appendix, spleen and lymph nodes, but not in the bone marrow (Table 4) which produces the blood cells. No

Table 5
Diagnostic values of different methods for diagnosing suspected CJD cases [23].

Analysis of CSF	Sensitivity (%)	Specificity (%)
14-3-3	94	93
Tau (> 1,400 pg/ml)	93	91
S 100 (≥ 4.2 ng/ml)	84	91
NSE (> 35 ng/ml)	81	92
Nuclear resonance scanning	67	92
EEG	66	74
PrP^{Sc}	20	100

CSF: cerebrospinal fluid; EEG: electroencephalography; NSE: neuron-specific enolase; PrP^{Sc}: pathological prion protein (scrapie)

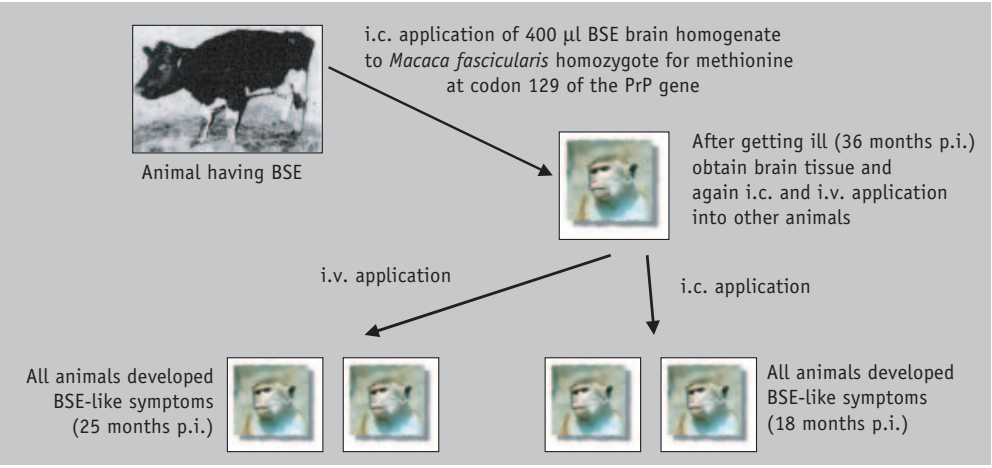

Figure 4. Prion infectivity of blood? Experimental approach: after intracerebral (i.c.) inoculation of bovine spongiform encephalopathy (BSE) homogenate into a monkey and development of clinical symptoms (36 months p.i.), brain tissue was applied into new monkeys by intravenous (i.v.) or intracerebral (i.c.) route. Both groups of animals also developed BSE-like symptoms, after different time periods. In conclusion: a) hematogenous prion transport is possible, and b) after adaptation to the new host, a reduction of the incubation period is seen [24].

direct vCJD-blood infectivity could be shown so far. In opposition to this data, experimental research with monkeys has shown that for BSE – as the animal counterpart to vCJD – a hematogenous transport is possible [24] (Figure 4). Furthermore, data from sheep experimentally infected with BSE (or natural scrapie) support the finding that prions are transmissible by blood transfusion [25, 26]. In the sheep study, positive transmissions occurred with blood taken at preclinical and clinical stages of infection [26]. These results are of concern for the public health system [27], particularly with regard to the fact that human platelets contain a significant concentration of the PrP^c, and therefore are susceptible to prion infection [28].

The Role of the Internal Prion Transport: the Gut, Cellular Blood Fraction, and Others – Hints from Different Animal Models

In 2001, *Aguzzi* performed numerous investigations to elucidate the way in which prions taken up with contaminated food reach the brain from the gut. As displayed in figure 5, the presumable carriers are macrophages or follicular dendritic cells, possibly under the influence of a lymphotoxin produced and secreted by B-lymphocytes [29].

In addition, different studies in animals could show that the scrapie-prion infectivity in the blood of infected rodents is low, and that the infectivity is prevailingly detectable in the cellular fractions compared to only trace amounts in plasma and plasma fractions, suggesting a prevailing association with blood platelets [30, 31]. Infectivity rose sharply at the onset of clinical signs, and plasma infectivity was not eliminated by white cell reduction filtration [31]. Contrary to the data mentioned before, newer data show that only about 10% of the scrapie infectivity in hamster blood is associated with platelets, while a larger proportion of the total infectivity was recovered from the mononuclear leukocyte fraction [32].

Also in scrapie-spiked human blood, most of the infectivity was associated with cellular blood components; the smaller amount was present in plasma, suggesting a potential but minimal risk of prion transmission by human plasma-protein concentrates [30].

Taking this knowledge from the animal research and transferring it to the human blood donation service, the depletion of leukocytes from blood transfusion units [27], as stipulated in Germany by law since October 2001, might be useful to reduce also this risk of infection. But until now it has not been possible to definitely exclude the presence of the agent in blood or blood cells.

Table 6
Measures and efficacy to inactivate prions (adapted from [33, 34]).

No detectable infectivity	Significant titer reduction
Sodium hypochlorite (16,500 ppm available chlorine) for 30 min	1 or 2 M sodium hydroxide
Autoclaving at 121 °C after 1 M sodium hydroxide treatment	Sodium dichloroisocyanurate (16,500 ppm available chlorine)
Autoclaving at 121°C in 1 M sodium hydroxide	Chaotropes (e.g. guanidine thiocyanate)
Boiling in 1 M sodium hydroxide	95% formic acid
Autoclaving for 18 min at 134–138 °C	Hot 1 M hydrochloric acid
2.5% bleaching for 1 h	Autoclaving for 1 h at 132 °C
	Autoclaving at 121 °C in 5% sodium dodecyl sulphate
	Dry heat at > 200 °C

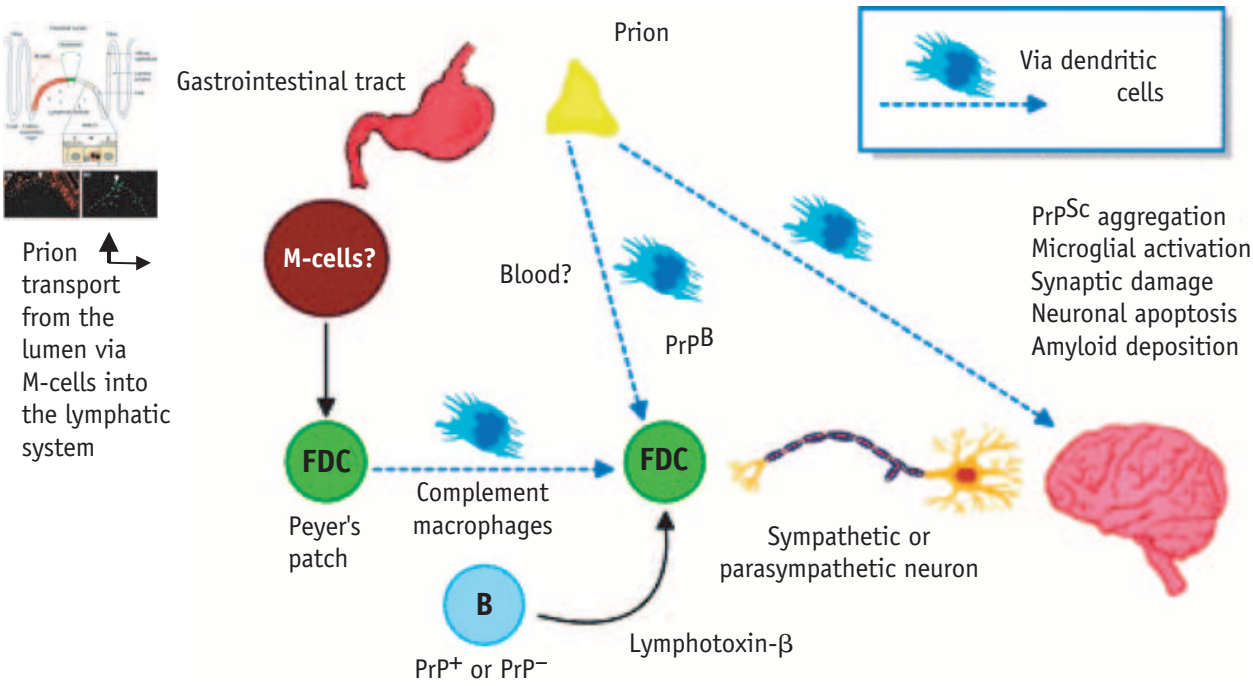

Figure 5. Hypothesis of prion transport from the gastrointestinal tract to the brain: after oral ingestion of prion-contaminated food and transport from the lumen (possible via M-cells) into the lymphatic system, the prions are presumably carried to macrophages or follicular dendritic cells (FDC) to the brain. Neuroinvasion, however, is possible in the absence of FDC, suggesting that other cell types in the periphery also can amplify and transport prions. A direct hematogenous prion transport is controversially discussed, but some current data support this hypothesis [29].

WHO measures to avoid vCJD/CJD transmission via blood transfusion recommend the exclusion of blood donors

- who were treated with pituitary hormones (e.g., growth hormones) of human origin,
- with CJD cases in the family and
- who received a dura mater or cornea transplant.

In Germany, the USA, Canada, Australia, New Zealand, Switzerland and Japan, the exclusion of blood donors is additionally recommended, if they lived – taken altogether – for over 6 months in the United Kingdom between 1980 and 1996.

Prevention of Prion Infection

In principle, three methods are possible: anamnestic evaluation (see above guidelines for blood transfusion), laboratory tests, and sterilization measures. Table 5 lists the assays currently used. To date, we have only *in vivo* surrogate markers with limited sensitivity and specificity. The direct test on prions based on the immunoblot technique is only applicable post mortem, when sufficient brain material can be taken.

Table 6 compiles some of the methods that are completely or partially efficacious to inactivate prions. They were evaluated in experimental animal models, mainly using mice. Insufficient or ineffective is treatment with aldehydes, organic solvents, hydrogen peroxide, phenolic disinfectants, chlorine dioxide, iodine and iodates, peracetic acid, proteolytic enzymes, microwave irradiation, UV irradiation or autoclaving after aldehyde, alcohol or dry-heat treatment [33–36]. For more information see, e.g. [5, 6, 33, 34, 36, 37]. Remarkably, there are a lot of sterilization measures widely used in medical care to inactivate the common infectious agents but not sufficient to inactivate prions. In the case of a particular risk, a hygiene specialist has to be consulted.

For risk assessment it has to be taken into account that in orthopedic surgery not only blood might be a source of contamination; even the contact of surgical instruments with potentially prion-containing lymphoid organs might represent a risk. In order to minimize this risk associated with reused, potentially contaminated instruments, the WHO recommends to immerse them in 1 N NaOH for 1 h (25 °C), followed by cleaning and autoclaving at 134 °C for 1 h [18], but other procedures have also proven efficacious [33, 34, 36]. In experimental studies it could be shown that treatment of prion-coated steel wires, mimicking contaminated surgical instruments with formaldehyde (10%, 1 h, 25 °C), is insufficient for sterilization, whereas treatment with sodium hydroxide (1 M, 1 h, 25 °C), guanidinium thiocyanate (4 M, 16 h, 25 °C), or autoclaving at 121 °C for 20 min is efficacious [35, 38, 39].

For a short while, only disposable instruments were used for tonsillectomy in the United Kingdom. After the

deaths of two patients following tonsillectomy with disposable instruments, this regulation was revoked in May 2002.

Conclusion

The risk of iatrogenic prion infection during orthopedic surgery cannot be excluded; it is, however, estimated to be rather small. In case of particular concern, there are several measures to avoid or minimize the risk of acquiring prion infection.

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