Creutzfeldt-Jakob Disease[1]

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Synonyms: CJD, Jakob-Creutzfeldt Disease, corticostriatal-spinal degeneration, spastic pseudosclerosis, subacute spongiform encephalopathy, prion dementia, transmissible degenerative encephalopathy, infectious cerebral amyloidoses.

Background

Creutzfeldt-Jakob Disease (CJD) is a rare, incurable, fatal degenerative disease of the central nervous system. The majority of cases occur between the ages of 50 and 75 years, although patients as young as 10 years and as old as 80 years have been reported.[1,2] The disease is not geographically limited and has been reported from over 50 countries around the world. CJD affects both males and females equally. The annual incidence is about one case per million inhabitants in metropolitan areas where the disease is more likely to be diagnosed. Approximately 250 deaths occur annually in the United States due to CJD.[3]

Signs and Symptoms

CJD can have a very long incubation period—in excess of 35 years—without any symptoms.[2] After the onset of symptoms however, the disease progresses rapidly—the majority of victims (90%) die within one year, and most within a few months.[1]

Although CJD may show diverse symptoms, it usually begins with gradually progressive mental deterioration in the form of memory loss, mood changes and errors in judgment. Disturbances of stance, gait, motor control, visual disturbances, dizziness and vertigo may also be prominent in early stages of the disease. As the condition worsens, the individual becomes mute, stuporous, spastic and rigid.

In late stages of the disease CJD produces characteristic changes in an electroencephalogram (EEG)—a tracing of brain waves—which is useful in diagnosing the disease.[1] Microscopic examination of the brain shows a loss of neurons (nerve cells) causing the brain to have a holey or spongy appearance. Numerous other changes may also occur in the brain, including an increase in fibrous connective tissue (gliosis) and amyloid plaques ("starch-like" protein deposits).

Due to the holey appearance of the brain, CJD is grouped with several other similar diseases collectively known as *transmissible spongiform encephalopathies* or **TSE**s. *Encephalopathy* refers to a disease or dysfunction of the brain; *spongiform* refers to the holey or sponge-like appearance of the brain; and *transmissible* means that the disease can be communicated to another individual. Although CJD was first described in the 1920s, it was not considered communicable until 1966 when kuru (another human TSE) was found to be transmissible.[4]

Eight spongiform encephalopathies have been identified; four in humans and four in animals. They are, in humans: CJD, kuru, Gerstmann-Sträussler-Scheinker Disease (GSS) and fatal familial insomnia. In animals: scrapie in sheep and goats, bovine spongiform encephalopathy (BSE or "mad cow" disease) in cows, transmissible mink encephalopathy, and chronic wasting disease of elk and mule deer.[5] TSEs have also been communicated to other animals, primarily in zoos, through the use of protein food supplements produced from rendered offal (carcasses of dead animals and waste remaining from butchered animals) contaminated with TSE. It is believed that TSEs may exist in all species of mammals at very low levels.

[1] Treatment of Hemophilia Monograph Series, Number 12. World Federation of Hemophilia: 1998.

What Causes CJD?

The cause of CJD and the other spongiform encephalopathies has been a source of debate for several decades. Some are naturally transmissible, others inherited, and some have been shown to be both transmissible and inherited.[5] For many years it was assumed that the causative agent of the transmissible form of these diseases was an extremely small virus that was yet to be discovered (sometimes termed a "slow virus" because of its long incubation period). Even after decades of searching, however, researchers have been unable to isolate a virus from purified brain extracts capable of transmitting the disease.[5] (These experiments were usually performed with scrapie—a spongiform encephalopathy affecting sheep, which was easier to obtain and handle than CJD.) Even more puzzling, the researchers could find only a very small protein and no nucleic acids in the extracts. Nucleic acids, in the form of DNA or RNA, are compounds found in all living cells. Nucleic acids are necessary for the survival and reproduction of cells and the replication of viruses. The absence of nucleic acids suggested that CJD and the spongiform encephalopathies were not caused by a virus. Additional experiments demonstrated that scrapie extracts remained infectious even after being subjected to treatments known to inactivate viruses and destroy nucleic acids—further evidence that these diseases were not caused by a virus.[6]

Building on the work of others who speculated that the transmissible agent of TSE consisted only of protein, Stanley Prusiner, M.D.—a neurologist at the University of California, San Francisco, School of Medicine—coined the term ***prion*** (pronounced “preeon”) in 1982 to designate the *pro*teinaceous *in*fectious particle he believed responsible for the disease.[5, 7] Prusiner’s heretical hypothesis—that a protein could infect an organism and replicate without nucleic acids—was met with ridicule.[8,9] No known life form can replicate without nucleic acids—not even viruses. Prusiner’s hypothesis was met with skepticism not only because he was unable to explain how the prion protein (abbreviated PrP) could infect an organism and replicate without nucleic acids, but also because he could not explain how the PrP could cause CJD in three different ways: most cases of CJD arise spontaneously, without any apparent cause or source of infection; a few cases are caused by an infective agent; and an estimated 5 to 15% of the cases of CJD are inherited.[5,9]

In the mid 1980’s, while searching for the elusive CJD viral nucleic acids, a gene for PrP was discovered both in infectious neurons and in normal cells.[5,10] Prusiner came to the conclusion that the transmissible “agent” of the spongiform encephalopathies was in fact a variation of a normal protein found predominately on the outer surface of neurons.[5] To distinguish between the different forms of the protein, the abbreviation for the normal prion protein was superscripted (or sometimes subscripted) PrP^{C} to designate the normal cellular version and PrP^{CJD} to represent the abnormal CJD prion.[10]

Chemically, the makeup of normal prion protein (PrP^{C}) and the disease-causing prion protein (PrP^{CJD}) are identical. Yet the chemical properties of the two proteins are markedly different. PrP^{C} dissolves in water and falls apart when attacked by cellular enzymes called proteases; whereas PrP^{CJD} is insoluble in water and resists cellular protease breakdown as well as viral inactivation procedures and many sterilization procedures. The basis for this radical difference in chemical properties lies in the three-dimensional arrangement, or *conformation*, of the amino acids that make up the two proteins.[5,11] The shape, or conformation, of PrP^{CJD} allows the amino acids to form close connections with each other, thereby stabilizing the protein and causing its unique chemical properties.[1]

It is believed that, in the brain, PrP^{CJD} acts as a template, or catalyst, causing the normal PrP^{C} to refold itself, changing its conformation to become the disease-causing PrP^{CJD}. Because PrP^{CJD} is insoluble and resists degradation by enzymes, the abnormal prion accumulates in the brain. This process is very slow at first, however, as more and more PrP^{CJD} accumulates, the process accelerates and snowballs. The accumulation of abnormal prions creates insoluble protein deposits in the brain called amyloid plaques. As the disease progresses neurons die, resulting in spongiform degeneration of the brain and, ultimately, death.

Exactly how TSEs cause the death of neurons is unclear and is still a matter of ongoing research.[11] Evidence obtained from studies of "knockout" mice in which the PrP gene was removed indicates that it is perhaps not the accumulation of PrP^{CJD}, *per se*, that causes the death of neurons, but rather the *lack of normal* PrP^{C} that results in the death of neurons. Although the exact function of PrP is still a mystery, these studies of PrP knockout mice suggest that PrP is necessary for long-term health of some types of neurons and may be involved in synaptic transmission (movement of nerve impulses between nerve cells) and the regulation of sleep.[12,13]

In light of new evidence gained through ongoing research into prion diseases, Prusiner, as well as several other research teams, proposed a revised theory explaining how the PrP^{CJD} causes Creutzfeldt-Jakob Disease.[5] One of the major arguments against the original prion theory, namely, how the prions could replicate without nucleic acids, was side-stepped because it became apparent that the prions were not “alive” and replicating like a virus but were merely abnormally-folded proteins which were capable of inducing a change in the shape of normal proteins already present in the brain. How the PrP^{CJD} is capable of causing the three forms of CJD is summarized as follows.

Approximately 10% of the cases of CJD are inherited. The inherited form of the disease, known as *familial CJD*, is caused by mutations to the prion protein gene, called PRNP. Mutations to the prion protein gene, located on the short arm of chromosome 20, results in additions, deletions or substitutions to one or more of the 253 amino acids which make up the prion protein. Individuals with inherited CJD have been found to have an altered amino acid at one or more positions on the PrP. It is believed these slight alterations to the prion protein make it prone to spontaneously changing its conformation to the abnormal PrP^{CJD}, causing the familial form of CJD.

Approximately 1% of the cases CJD are caused by infection, referred to as *acquired CJD*. In acquired CJD, PrP^{CJD} introduced into the body finds its way to the brain and, acting as a template, induces the normal PrP^{C}, through simple contact, to change its conformation and become the insoluble PrP^{CJD}.

Sporadic CJD, the most common form, accounts for almost 90% of the cases of CJD. In sporadic CJD there is no known source of infection and no mutations to the PrP gene associated with familial CJD. There is speculation that some cases of sporadic CJD are the result of a somatic mutation (mutation to an individual cell or cells, as opposed to inherited mutations, which affect all cells) in the prion protein gene, resulting in changes in the amino acids of the prion protein. These changes predispose the PrP^{C} to change its conformation and convert into PrP^{CJD}.

The sequence of amino acids in the PrP also plays a role in the susceptibility of an individual to infection by TSEs. Proteins may sometimes come in different forms with slightly different amino acid sequences, known as *polymorphisms*. In humans, three polymorphisms at position 129 of the PrP gene have been correlated with genetic susceptibility to prion diseases.[14] It is unknown what percentage of sporadic CJD cases are caused by spontaneous somatic mutations or lateral transmission (infection) by the PrP^{CJD}.[15]

Another challenge to the prion hypothesis of TSEs involves the presence of *strains*. TSEs such as bovine spongiform encephalopathy (a TSE of cows), scrapie (a TSE of sheep) and CJD have been found to occur in distinct strains that have different incubation periods, different symptoms and different neuropathology. Furthermore, these strains retain their unique characteristics even after experimental passage through a variety of intermediate species.[16,17] (The ability of strains to retain their characteristics has been used to trace the origin of some cases of CJD and to eliminate some possible sources of infection in some individuals who have contracted CJD.) Proponents of the viral hypothesis of TSEs argue that changes in PrP conformation alone cannot account for all of the strains of TSEs (some 20 strains of TSEs have been transmitted to mice[18]) and that some type of genetic material must be present to transmit the information needed to continue strain characteristics from one species to another. Proponents of the prion hypothesis of TSEs argue that PrP can assume several conformations and differences in amino acid sequence of the PrP and route of acquisition,

among other factors, can affect the presentation of the disease and strain characteristics. [19,20] The existence of multiple strains of TSE is still a point yet to be satisfactorily resolved and is an area of ongoing research.

Numerous studies and many converging lines of evidence argue persuasively that prions represent an entirely new class of infectious pathogens and that prion diseases result from aberrations of protein conformation. Scientists also speculate that prions consisting of other proteins may play a part in more common neurodegenerative conditions, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Although the question of whether rogue prions are the sole cause of human TSEs is not yet settled, the "protein only" prion hypothesis has won over the majority of scientists working in this field. In contrast to the ridicule Dr. Prusiner received in the early 80s, the importance of his work on prions was acknowledged in 1997 when he was awarded the Nobel Prize in medicine for his work on prions.

How is CJD Transmitted?

How CJD is transmitted naturally is unknown.[1] Observations of other prion diseases indicate that they have a limited transferability.[15] It appears that CJD is communicated only through direct inoculation. Some prion diseases can be transmitted through the ingestion or handling of contaminated food. (Since the brain and nerves contain the greatest concentrations of prions and thus have a higher potential of transmitting a TSE, it is probably prudent behavior to avoid eating foods containing brain tissue.)

Prion diseases have also been known to spread from one species to another.[9,11] Researchers however, have found that it is often difficult for prions made by one species to cause disease in animals of another species, especially if the two species are not closely related. This phenomenon is referred to as the *species barrier*. Due to the species barrier, the amount of infective material (in the form of purified brain extracts) necessary to infect an animal of a different species with TSE may be 10 to 104 times that necessary to infect an animal of the same species.[21] It is believed that the species barrier is due to differences in the composition and structure of the prion protein between species. Once the species barrier has been overcome and infection occurs, however, subsequent inoculates of brain extracts made from infected animals are highly infectious to others of the same species.

Perhaps of more interest are cases of CJD transmitted though infection. Two routes of infection for CJD have been identified: one through food and the other via medical treatments and surgical procedures. Below is a review of some of the known humans TSEs and routes of infection.

Kuru, a CJD-like prion disease found primarily among the Fore tribal people in a remote area of central Papua New Guinea, was common in the 1950s and affected up to one percent of the population per year, causing approximately 200 deaths per year. The disease affected all ages beyond infants and toddlers. It was eventually determined that the disease was being transmitted through ritual cannibalism in which parts of the bodies of deceased kinsmen, including the brain, were cooked and eaten as a rite of mourning. (Cooking did not destroy the infectivity of the prions.) Since the elimination of cannibalism between 1958 and 1962, Kuru has all but disappeared among children, adolescents and young adults of the Fore.[22,23]

In England, an epidemic of **bovine spongiform encephalopathy** (BSE or "mad cow disease"), a TSE of cows, erupted in 1986. The epidemic has killed more than 170,000 cattle and, through culling of herds suspected of being infected with BSE, has resulted in the destruction of nearly 2 million animals.[10] The number of cases diagnosed peaked in early 1993 when almost 40,000 cows were diagnosed with BSE. Since 1993, the number of cases of BSE in the UK has decreased: in early 1998 there were only about 100 new BSE suspect cases a week, compared to over 1000 per week at the peak of the epidemic (*per* the UK Ministry of Agriculture, Fisheries and Food, or MAFF). This epidemic of BSE is believed to have been caused by cattle feed containing protein food supplements

contaminated by the processed remains of sheep infected with scrapie, a prion disease affecting sheep and goats. The UK government moved to control the epidemic by imposing a ban on the practice of feeding sheep meat and bone meal to cattle and bovine (cow) meat and bone meal to young calves in 1988. Over the past decade the UK and the European Commission (EC) have instituted several dozen additional rules and regulations designed to keep BSE out of the food chain for both animals and humans and to reduce other possible sources of exposure to BSE, such as additives in pharmaceutical products.

For a decade following the announcement of the BSE epidemic, MAFF, citing a lack of clear biological evidence that BSE could jump from cows to humans, continued to insist that British beef was safe and could be eaten with "confidence". In March of 1996 the UK Ministry of Health, based on evidence from the UK National Creutzfeldt-Jakob Disease Surveillance Unit and the conclusions of an independent committee of scientists known as the Spongiform Encephalopathy Advisory Committee (SEAC), announced that 10 cases of a previously unrecognized form of CJD had been identified and may be related to the BSE epidemic.[24] The new strain of CJD, dubbed "new variant CJD" or "nvCJD", had highly unusual symptoms and neuropathology, distinct from those of sporadic CJD. New variant CJD was occuring in much younger individuals (median age 28 years, as opposed to 63 years for sporadic CJD) and presenting symptoms consisted of behavioral changes and ataxia (inability to coordinate muscular movements) as opposed to dementia and myoclonus (violent muscle spasms) in sporadic CJD. New variant CJD had an insidious onset and prolonged course, as opposed to the rapidly progressive course of sporadic CJD (most survive only 2-6 months after onset of symptoms), also, individuals with nvCJD did not have the characteristic EEG usually found in sporadic CJD (30% of sporadic CJD cases and most pituitary hormone-related cases of CJD also do not develop the characteristic EEG appearance). At the time of the announcement of the 10 cases of nvCJD, the link between BSE and nvCJD was suspected, but unproven. In 1996 and 1997, several studies, including two in the October 2, 1997 issue of *Nature*, provided compelling evidence that the prion responsible for nvCJD was the same as that causing BSE.[16,25]

Since 1996, 13 additional cases of nvCJD have been positively identified (almost all in the UK), raising the total to 23. New variant CJD is causing widespread concern in the UK. The question that has crossed the minds of consumers of UK beef is—are these 23 cases of nvCJD the vanguard of an epidemic or just isolated incidents? There is no way to predict how many people will eventually develop nvCJD as a result of eating BSE-contaminated beef or receiving blood transfusions from nvCJD-infected individuals. All 23 confirmed cases of nvCJD are homozygous for methionine (have 2 copies of the same amino acid) at position 129 of the prion. Only 38% of the total population carry this homozygosity—a finding that will possibly restrict the population at risk. Only time will tell whether the BSE epidemic will initiate a corresponding epidemic of nvCJD in the United Kingdom.[26]

In addition to the ingestion of food, CJD has also been accidentally transmitted through medical procedures, termed *iatrogenic* CJD. CJD has been transmitted through corneal implant surgery (replacement of the transparent membrane on the front of the eye), pericardium homografts (repair of the membrane around the heart), brain electrodes, neurosurgical instruments, dura mater homografts (repair of the membrane around the brain) and the administration of hormones derived from human pituitary glands.[2]

One of the most dramatic examples of iatrogenic CJD involves the use of **human pituitary growth hormone**. Human growth hormone (hPG) was given to children of short stature to help increase their growth rate. The hormone was administered through a series of intramuscular injections, usually three times daily, over a period of several years. Prior to 1985, human growth hormone was derived from batches of 5,000 to 20,000 pituitary glands (a gland located at the base of the brain) harvested from cadavers. After harvesting, the pituitary glands were processed and then subjected to harsh chemical procedures to extract the growth hormone. The hormone extract was then purified

by passing it through two different filter media and finally, an ion-exchange chromatography column (a step introduced in 1977), which reduced contaminants to undetectable levels.[27,28]

The hormone extraction procedures, known to inactivate bacterial and viral pathogens, and the chromatographic purification steps, both proved to be ineffective at preventing the transmission of PrP^{CJD}. To date, more than 75 people have developed CJD as a result of exposure to human pituitary growth hormone (the youngest patient was 10 years old).[2,11,29] Approximately 10,000 people received human growth hormone from cadaveric pituitaries before most countries ceased its use when a synthetic hormone became available in 1985.[3] Because of the long incubation period of CJD, it is expected that more cases of the disease will surface among recipients of human pituitary growth hormone in the next few decades.[2]

Studies of individuals who have contracted CJD from human pituitary growth hormone have found that the total time of exposure to hormone therapy is the most important determinant of risk of contracting CJD. This indicates that incubation periods are related to the dose of infectivity. Several of these patients also have similar variations of the prion gene which may confer an increased susceptibility to CJD.[2]

Another case of iatrogenic CJD transmission through pituitary hormones occurred in Australia between 1967 and 1985. During this time the Australian Human Pituitary Hormone Program (AHPHP), run under the auspices of the Australian Commonwealth Department of Health, administered **pituitary gonadotrophin** (hPG) to over 1,500 women as a treatment for infertility. Pituitary gonadotrophin treatment for infertility has resulted in the transmission of 5 confirmed cases and 1 suspected case of CJD. The latest case, provisionally diagnosed in 1997 (positive diagnosis cannot be made until death), had only two courses of hPG in 1970 and, at 27 years, will have one of the longest known incubation periods for iatrogenic CJD.[30,31,32]

Contamination of lots of pituitary hormones with PrP^{CJD} appears not to have been a rare event. Retrospective studies indicate a high likelihood that a single batch of 5,000 to 20,000 pituitary glands would include at least one gland from a patient dying of CJD.[2] Contamination of pituitary hormones has also been shown clearly to be a random event, with multiple lots of contaminated hormone prepared in several different countries.

At least 61 cases of CJD have been transmitted through **dura mater homografts**. The dura mater is a tough, fibrous sheath covering the spinal cord and brain. A *homograft* is a body part harvested from another individual for use in a surgical procedure. Dura mater are harvested from cadavers for use in neurosurgical procedures. In 1977, a non-governmental surveillance group for CJD in Japan reported on 43 cases of CJD contracted through dura mater homografts. Of these cases, 41 received dura mater grafts produced between 1979-1989 from the same processor. The brand of dura mater these individuals received was not treated with sodium hydroxide (NaOH), a compound known to reduce the infectivity of PrP. Twenty-one additional cases of CJD in other countries have also been reported in patients who received the same brand of graft during the same time period. Approximately 100,000 individuals received this brand of graft between 1983 and 1987. The Centers for Disease Control and Prevention (CDC) commented on this report stating that "an international outbreak of CJD associated with a single brand of dura mater grafts is larger than previously recognized and that recipients of contaminated grafts may remain at risk for CJD at least 16 years following receipt of grafts." The CDC also cautioned that sodium hydroxide (NaOH) treatment may not inactivate all the PrP and surgeons should be aware of the possibly inherent risk of dura mater grafts and consider the use of alternatives.[33]

How Will This Disease Affect The Hemophilia Community?

What effect, if any, CJD will have on the hemophilia community and other users of blood fractions remains unknown. The US Food and Drug Administration (FDA) states that “there has never been a reported case of transmission of CJD by blood or plasma products.”[15] In spite of these

reassurances from the FDA, there remain concerns in the hemophilia community regarding the possible risk of contracting CJD from clotting factor concentrates (both porcine and those with human-derived components). These concerns revolve around four facts:

(1) PrP^{CJD} has been found in blood. Although the highest concentrations of the CJD prion protein are found in the brains and nerve tissue of affected individuals, the protein has also been found, at much lower concentrations, in many other organs. The CJD prion protein has been found in lymph nodes, tonsils, liver, kidney, spleen, lung, cornea, cerebral spinal fluid (CSF), urine and blood of infected animals and patients with the disorder. [22,34,35] CJD prions have been found in both cellular components of blood and non-cellular products as well. Animal studies have demonstrated that CJD prions are present in the blood of infected individuals in the early stages of the disease.[21,36] Recent evidence indicates that a type of white blood cell known as a *lymphocyte* may harbor much of the PrP^{CJD} infectivity of blood and may play an important role in the transport of PrP^{CJD} to the brain and lymph tissues.[37]

Whether CJD can be transmitted through blood products is a topic of heated debate. The debate revolves around two opposing viewpoints: one viewpoint contends that CJD cannot be transmitted through blood products (or, at least, not without great difficulty) because there is no epidemiological evidence to support transmission of CJD through blood products (i.e., we have been unable to detect an increase in CJD in users of blood products). The other viewpoint is that blood products can transmit CJD because blood is known to carry PrP^{CJD} and has been shown to transmit CJD through direct inoculation of infected blood into the brain of an animal.

At least two research teams have been successful in transmitting CJD from human blood and urine to rodents through intracerebral injection.[35,38] However, another retrospective ("look-back") study of recipients of blood transfusions from a blood donor who later died of CJD found no cases of CJD in the recipients, one of which has survived 23 years without symptoms.[39] Retrospective studies of mortality data conducted by the US CDC and the Canadian Laboratory Centre for Disease Control have found no evidence of nvCJD and no significant increase in deaths due to CJD.[40,41] Authors of these studies caution that the results of these studies do not prove that CJD is not transmitted by blood or blood products. The results of these studies are inconclusive because the sample sizes are small, CJD is often misdiagnosed (one study found 13% of patients diagnosed with Alzheimer disease actually had CJD[42]), and death certificates are often incomplete.

The variable and often long incubation time of CJD, accompanied by its rarity, makes it difficult to draw a definitive conclusion regarding the apparent lack of transmissibility of CJD through blood transfusions. It is especially difficult to draw conclusions from retrospective data from studies not specifically designed to look for CJD. As MJ Rees wrote: "Absence of evidence is not the same as evidence of absence".

(2) PrP^{CJD} is not affected by viral inactivation procedures used on blood products. The CJD prion protein (PrP^{CJD}) is extremely stable and remains infective after passing through viral inactivation processes used on blood products. PrP^{CJD} also remains infective after being subjected to other procedures, including treatment with 70% alcohol, formaldehyde, 10% hydrogen peroxide, ionizing radiation (gamma radiation), ultraviolet light, ultrasonic energy, heating to 80°C, lyophilization (freeze drying), years in cold storage (-70ºC), ethylenediaminetetraacetic acid (EDTA), beta-propriolactone, as well as nucleases and many proteases.[2,22] Sterilization procedures capable of inactivating PrP^{CJD}, such as prolonged autoclaving or exposure to strong solutions of sodium hydroxide or sodium hypochlorite, cannot be used on blood products such as clotting factor concentrates because they also destroy useful proteins, including clotting factor.

(3) PrP^{CJD} cannot easily be detected. There is currently no blood test able to identify individuals with CJD, and none expected in the near future. This is due to the fact that CJD does not elicit an immune response from the body, a response used by many blood tests to indicate the presence of a pathogen.[5] In addition, gene amplification techniques such as polymerase chain reaction (PCR), used to detect miniscule amounts of viral

nucleic acids in the blood (and thus, viral infections), cannot be used to detect PrP^{CJD}, as prions have no nucleic acid.

(4) Only minute quantities of PrP^{CJD} are necessary for infection. PrP^{CJD} is infective even at extremely low concentrations, as evidenced by the transmission of CJD through cadaveric human growth hormone purified with chromatography.[28] It is likely that even monoclonal antibody purification, used in the production of most clotting factor concentrates, would be ineffective at removing all the potentially infectious PrP^{CJD}.

In animal studies, two factors must be considered when discussing the transmission of CJD: one is the level of infectivity of the study tissue (titer of infectivity), the other is the route of administration (efficiency of infective route).[36] Human pituitary hormones have a high level of infectivity (high titer of infectivity) due to the close proximity of the pituitary gland to the brain. Blood, as compared to pituitary hormones or brain tissue, has low levels of infectivity (low titer of infectivity). Aside from the titer of infectivity, there are some disturbing similarities between growth hormones and blood products such as clotting factors: both are purified and both are administered via a route that is considered to have a low efficiency of infectivity—yet the hormones remained infectious and capable of transmitting CJD.

Although many consider it likely, it is unknown whether CJD can be transmitted by clotting factor. If clotting factor does transmit CJD, there are several possible scenarios. The worst-case scenario would involve many people with hemophilia succumbing to CJD, much like the AIDS epidemic created by HIV-contaminated clotting factor produced prior to 1985. Another scenario is that so little PrP^{CJD} remains in the final product and the route of administration is so inefficient at transmitting CJD, that it cannot transmit the disease. A third possibility is that the incubation period will be so long, because of the minute quantities of PrP^{CJD} in the final product and the inefficient route of administration, that many individuals may be infected with CJD, yet most would die in old age from causes other than CJD. It is also possible that only those with a genetic susceptibility to the disease, (such as those having homozygous methionine at position 129 of the prion, occuring in approximately 38% of the population) could be affected by PrP^{CJD}.

(5) Regular consumers of blood products may have already been exposed to CJD. Evidence presented before the US House of Representatives Committee on Government Reform and Oversight, Subcommittee on Human Resources, on July 31, 1997, indicates that individuals who are regular consumers of blood products, such as people with hemophilia who use plasma-derived clotting concentrates and those who receive immune globulins, have been regularly exposed to plasma pools of over 100,000 donors. When this total is combined with plasma pools used to produce additives to clotting factors, such as albumin, the total may climb to as high as 400,000 donors. Thus, users of clotting factor and other blood products have, hypothetically, a high probability of being exposed to PrP^{CJD} from a CJD-infected plasma donor. The International Plasma Products Industry Association (IPPIA) has voluntarily agreed to begin implementation of limiting plasma pool sizes to 60,000 donors for the major product lines, including Factor VIII, Factor IX, albumin and intravenous immune globulin (IVIG) on January 1, 1998.

Reducing the Risk

Currently, the risk of contracting CJD from blood products is theoretical and is not supported by epidemiological data. However, an accurate assessment of the risk of contracting CJD through blood products cannot be made with current data due to the rarity, highly variable presentation and the often very long incubation period of the disease. Erring on the side of safety, and assuming that blood products such as clotting factors and IVIG can transmit CJD, how can the risk of contracting CJD from these blood products be reduced or eliminated? At present, our options are limited. The following are actions that may lessen the risk of CJD and some directions of future research into CJD that may decrease the risk of contracting this rare disease.

(1) Reduce plasma pool sizes. Reductions in plasma pool sizes would reduce the potential exposure to PrP^{CJD} as well as reduce the amount of product withdrawn from the market due to later discovery of evidence of CJD-risk factors in

plasma donors. The recent voluntary reduction in plasma pool size the IPPIA is a step in this direction. However, reduction in pool sizes is generally not supported by the IPPIA. In a letter to the US FDA, after reviewing the Blood Products Advisory Committee (BPAC) proposals regarding plasma pool reduction, the IPPIA stated that no safety benefits would result from pool size reduction and that pool size reduction would result in significant product supply reductions, as well as significant time and cost increases involved for remodeling manufacturing facilities to accommodate smaller production scale equipment.[43] The IPPIA and FDA position on plasma pool size (that reductions in pool size would provide no safety benefits) has been strongly challenged, both publicly and privately, by the US National Hemophilia Foundation (NHF).

(2) Use products that are not derived from blood. Clotting factor concentrates made with recombinant DNA technology do not use blood as a source for the clotting factor. Using a recombinant factor product reduces your exposure to blood-borne pathogens, including PrP^{CJD}. Unfortunately, all recombinant Factor VIII products currently licensed in the US use large amounts of albumin, a blood protein, as a stabilizer for the Factor VIII molecule. Clinical trials of several albumin-free and reduced-albumin Factor VIII products are currently underway. A recombinant, albumin-free Factor IX product, Benefix™, was licensed for marketing by the US FDA in February 1997.

In the UK, due to concerns over nvCJD, the Haemophilia Centre Director's Organization executive committee has recommended that all people with hemophilia in the UK use only recombinant factor products, or, if these are not available, plasma derived products produced from plasma pools outside of the UK (where, presumably, the risk of nvCJD is less).

Concerns over nvCJD ("mad cow" disease) have also prompted calls for the removal of bovine (cow) proteins from pharmaceuticals. In 1997, the European Commission considered a ban on the inclusion of bovine material in pharmaceuticals (including tallow—used to make gelatin, found in almost 85% of all pharmaceuticals) (97/534/EC). After protests from leading pharmaceutical companies that no substitutes were available on short notice, the decision on the ban was put on hold. The removal of all bovine (cow) proteins (as well as human proteins) from clotting factor concentrates may be difficult to attain—several compounds used in the processing of factor concentrates are often derived from cattle. Some brands of recombinant Factor VIII products are produced by cell lines grown in calf serum. Tween, used in the viral inactivation treatment of some brands of factor may be derived from cows. The monoclonal antibodies used to remove clotting factors from growth media and subsequent purification steps are frequently grown with the aid of small amounts of transferrin, derived from human blood plasma. Individuals who have central lines or ports often flush them with heparin, another compound often derived from cows. (The UK has recently switched from bovine (cow) mucosa to porcine (pig) mucosa for the production of heparin.) As can be seen by these few examples, removing all bovine compounds and human plasma-derived compounds from factor concentrates and other pharmaceuticals will not be a simple task. Removal of these compounds may involve the destruction of some industries and creation of new industries that supply these compounds, as well as re-tooling of manufacturing processes and subsequent re-licensing of factor production facilities.

(3) Screen blood donors for CJD-related risks. Removal of potentially CJD-contaminated plasma from the plasma pool will reduce the theoretical risk of transmitting CJD. Although identifying risk factors for CJD in potential blood donors will reduce the amount of PrP^{CJD} in the plasma pool, it will not eliminate the hypothetical risk of transmitting CJD. Those who are infected with CJD and are preclinical (showing no symptoms), and have no CJD risk factors (that would result in their blood being deferred), may have donated blood for decades before showing signs of the disease. Without a blood test specific for CJD, it is impossible to detect these CJD carriers.

The US FDA currently recommends that blood from donors with CJD risk factors, including those who received cadaveric human growth hormones, dura mater grafts, corneal implants and family members who have died from

the disease, be deferred. The FDA also recommends that manufacturers withdraw and destroy injectable blood products made from plasma of donors who later develop the disease or reveal CJD-risk factors. Similar recommendations are in force in Canada and Europe. The World Health Organization also endorsed similar recommendations in a statement in March 1997.

It is important that consumers of blood products and informed and objective persons maintain a voice on advisory panels to organizations such as the FDA and the CDC. The cautious path being followed by regulatory agencies at present is by no means assured. The FDA is under constant pressure to reverse its withdrawal recommendations on the grounds we are wasting hundreds of millions of dollars of product in protecting against the theoretical transmission of a disease for which there is no epidemiological evidence of transmission through blood or blood products. At a Blood Products Advisory Committee meeting in October 1997, the American Red Cross reported that since the autumn of 1994, $120 million in products had been returned by customers, taken off its own shelves, and otherwise destroyed due to the discovery of 14 donors who had CJD, and of others who had received growth hormone or brain grafts.

The Medical and Scientific Advisory Council (MASAC) of the US National Hemophilia Foundation has voiced the concerns of people with hemophilia and provided the FDA with a contrasting viewpoint. The NHF has been instrumental in the formation of some FDA policies to help protect the consumer.

(4) Development of a blood test. There is an urgent need of a blood test for CJD. Such a test could identify preclinical carriers of CJD, aid in the screening of blood donors and help in answering the question of whether or not CJD can be transmitted through blood.

At present, the only way to positively diagnose CJD is through a careful examination of the brain at death. (Brain biopsies on living patients are widely considered unethical and may miss the disease.) As was mentioned earlier, the development of a blood test for CJD is extraordinarily difficult because PrP^{CJD} does not elicit an immune response and does not contain nucleic acids. Although research in this area has accelerated considerably since the BSE epidemic in the UK, we are likely still several years away from a useful blood test for CJD.

In 1996, a test for a protein in cerebrospinal fluid called "14-3-3" was developed that was hoped would be a marker for TSEs.[44,45] After eliminating other possible causes of dementia, the test was reported to be very accurate in diagnosing CJD. The test, however, is not CJD-specific and is not a marker of PrP. This test has had some disappointing results, especially in diagnosing nvCJD.

Currently, hopes are high that two new tests—one, a monoclonal antibody specific for PrP^{CJD} called "15B3", and another using "RNA aptamers"—will be the keys to the development of a diagnostic assay for the detection of TSEs.[46,47] Both of these tests are capable of recognizing the disease-specific forms of prion proteins (while ignoring the normal form) and should make it possible to test samples from living people and animals. It remains to be seen whether these tests will be capable of detecting minute amounts of PrP^{CJD} in blood and it may be years before a commercial test using these techniques is available on the market.

Recently developed strains of *transgenic* mice—mice in which the normal mouse prion gene has been removed and replaced with the human prion gene—will also accelerate the testing of the transmission of human TSEs. Such mice are susceptible to some strains of human TSEs and develop the disease in less than a year—a much shorter time than similar tests in animals which do not express human prions.

(5) Studies of CJD transmission: Studies specifically designed to detect possible transmission of CJD are necessary. Such studies are either planned or currently underway in Canada, the United Kingdom and the United States.

In the US, the CDC, at the request of the US Congress, is working with hemophilia treatment centers (HTCs) to study whether CJD can be spread through blood or blood products. The CDC CJD surveillance program involves donation of the brain after death so that testing for CJD can be performed. To date, the CDC has analyzed 24

brains in its retrospective study and 6 brains in the prospective study—all with no evidence of CJD.

People with hemophilia are believed to be at a greater risk of contracting CJD than the general population because the clotting factor they infuse is manufactured from the plasma of tens to hundreds of thousands of donors. In addition, approximately 25% of death in patients with hemophilia involves central nervous system (CNS) symptoms (possibly due to CNS bleeding and HIV infections).[48] Therefore, in this population with CNS symptoms, CJD could conceivably go undiagnosed. It may also be argued that reaching conclusions from a study of people with hemophilia may be difficult—the hypothetically minute quantities of PrP^{CJD} transmitted through clotting factor may result in a prolonged incubation period for CJD, and many with hemophilia die at a relatively young age as a result of bleeds and HIV/AIDS and hepatitis infections. These individuals may die before displaying any symptoms of the disease.

(6) Treatment: CJD has been an untreatable and invariably fatal disease that takes decades to develop. This slow onset may offer a window of opportunity to develop a therapy to benefit those exposed to PrP^{CJD} and who may be incubating the disease.

Anti-sense prion gene therapy can block production of the prion protein, and presumably, halt the progression of the disease. Unfortunately, the normal function of prions is not known. If the prion has an important function, as some studies have shown, serious but subtle side effects of this therapy can be anticipated over the long term—effects that might be difficult to observe beforehand in animals.

A preferable and less risky treatment would be to chemically inactivate the CJD prion, leaving the normal prions intact and allowing the normal prions to return to normal levels through new protein synthesis. The new 15B3 monoclonal antibody and CJD-specific RNA aptamers are both capable of binding specifically to CJD prions and may prove to be useful not only in blood tests but as treatments as well.

Conclusion

The use of any blood product carries a small risk. At present, most researchers believe the risk of contracting CJD from blood products, if any, is very small. The exact risk of contracting CJD from pooled plasma products has not been quantified, and an accurate risk assessment may not be available for several years or even several decades.

Until the question of whether blood and blood products can transmit CJD is answered conclusively, we must err on the side of caution and continue to withdraw blood products suspected of being contaminated with CJD prions.

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Revised February, 1998. Paul Clement

Glossary

Albumin: A common protein found in animal tissue, blood, milk, egg, muscle and in plant tissue. Used as a stabilizer for clotting factor.

Amino acids: A group of 20 different organic compounds which, combined in various sequences, are the building blocks of proteins.

Ataxia: Partial or total inability to coordinate muscular movements.

BSE: Bovine Spongiform Encephalopathy or "mad cow" disease. A TSE of cows.

Chromatography: A procedure used to separate substances from complex mixtures.

Chromosome: A structure that carries genes in the cell nucleus.

CJD: Creutzfeldt-Jakob Disease.

Conformation: The shape or three-dimensional arrangement of the atoms that make up a molecule.

Creutzfeldt-Jakob Disease (CJD): A fatal degenerative disease of the central nervous system.

DNA: Deoxyribonucleic acid, the structure in the chromosomes that carries genetic information.

Dura mater: A tough, fibrous sheath covering the spinal cord and the brain.

Encephalopathy: Disease of the brain.

Epidemiology: The science which investigates the causes and control of epidemic diseases.

Gene: A hereditary unit that occupies a specific location on a chromosome, determines a particular characteristic in an organism, and can undergo mutation. Composed of DNA.

Homograft: A graft of tissue taken from another person. In the case of dura mater and pericardium homografts, the tissue is removed from cadavers.

Iatrogenic: Inadvertently caused (as in the transmission of a disease) by a physician or by treatments.

IVIG: Intravenous Immune Globulins.

IPPIA: **I**nternational **P**lasma **P**roducts **I**ndustry Association—and international trade association representing commercial producers of plasma-based therapies.

MAFF: Abbreviation for the UK Ministry of Agriculture, Fisheries and Food.

Myoclonus: Violent muscle spasms.

Nucleic acid: Compounds which make up DNA and RNA and code for genetic information.

nvCJD: New variant Creutzfeldt-Jakob disease. A strain of CJD, primarily found in the UK, believed to have been transmitted through the ingestion of BSE contaminated beef.

Plasma: The clear yellowish fluid portion of the blood, lymph, or intramuscular fluid in which cells are suspended.

Prion: A term coined from the words *pro*teinaceous *in*fectious to describe a protein capable of causing a disease without having DNA or RNA.

PRNP: Abbreviation for the prion protein gene.

Protein: An important class of compounds found in living organisms. Composed of amino acids.

PrP: Abbreviation for prion protein.

PrP^{C}: Normal cellular form of the prion protein.

PrP^{CJD}: Insoluble form of the prion protein, capable of causing CJD.

Recombinant: Short for *Recombinant DNA technology* in which the inherited characteristics of an organism are changed by altering its genetic material, such as inserting the gene for factor VIII into a Chinese hamster ovary cell to cause it to produce clotting factor.

Scrapie: A prion disease (TSE) affecting sheep and goats.

SEAC: Abbreviation for **S**pongiform **E**ncephalopathy **A**dvisory **C**ommittee. An independent committee of scientists in the UK who provide advice to MAFF.

Synaptic: Having to do with the synapse—the junction between two neurons, where an impulse is transmitted from one nerve to another.

TSE: Transmissible spongiform encephalopathy: a generic term for a variety of prion diseases.

RNA: Ribonucleic acid, similar function to DNA but often directs synthesis of proteins.

Virus: An agent with nucleic acids, much smaller than bacteria, that can reproduce itself within living cells, causing a wide range of diseases.

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