

Presymptomatic Detection of Prions in Blood

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

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

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

from the onset of infection to the appearance of clinical symptoms (8–10).

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

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

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

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

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

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an interesting trend (Fig. 2). A first peak of PrP^{Sc} detection was observed early during the presymptomatic phase, between 20 and 60 days after inoculation. The peripheral administration of prions is known to result in an early phase of replication in lymphoid tissues and the spleen, before any infectious material reaches the brain (17, 18). Indeed, little or no infectivity can be detected in the brain of animals peripherally inoculated during the first half of the incubation period (19). Thus, it is likely that the source of PrP^{Sc} in blood during the early presymptomatic phase is the spleen and other lymphoid organs. The quantity of PrP^{Sc} in blood goes down after this initial phase and actually disappears 80 days after

inoculation (Table 1 and Fig. 2). The rise of PrP^{Sc} in blood during the early presymptomatic phase appears to coincide with the time of its exponential replication in lymphoid organs, whereas the reduction of PrP^{Sc} in blood occurs when infectivity in peripheral tissues has reached a plateau and is migrating from the periphery to the brain (17, 18). Although the explanation for these results is unknown, it is possible that the proportion of circulating lymphocytes carrying PrP^{Sc} is much higher during the exponential phase of peripheral replication than during the stationary phase. At the symptomatic period, PrP^{Sc} can again be detected in the blood of most of the animals (Fig. 2). It has been reported that large

quantities of PrP^{Sc} appear in the brain only a few weeks before the onset of clinical signs (19, 20). Thus, PrP^{Sc} in blood samples at the symptomatic stage is likely to have come from brain leakage. It is known that at the time of symptomatic disease, TSE-affected individuals have extensive brain degeneration in the form of massive neuronal death, synaptic alterations, and brain inflammation (21). These abnormalities probably cause a disruption of the blood/brain barrier resulting in the leakage of cerebral proteins to the blood (22), in particular PrP^{Sc}, which by this time is highly abundant in the brain.

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

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

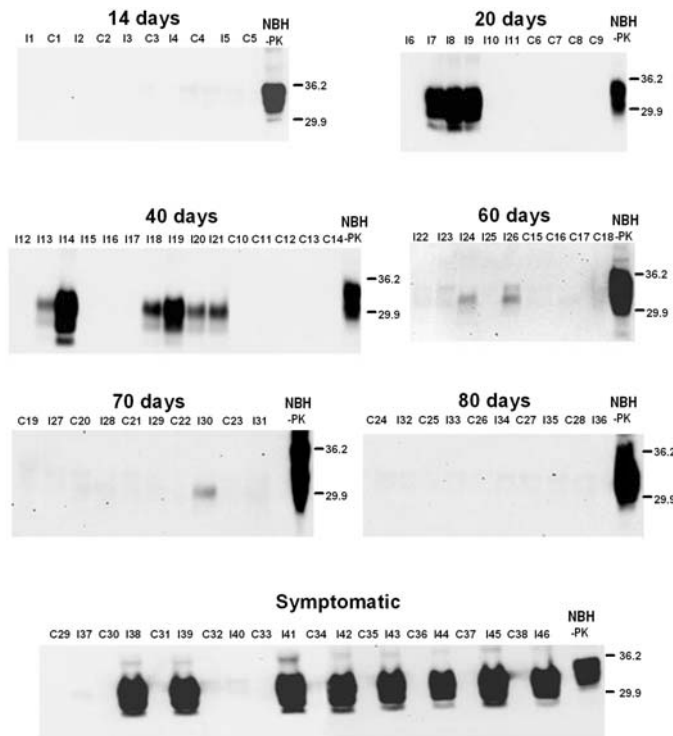


Table 1. Number of animals used and results obtained regarding the presymptomatic detection of PrP^{Sc} in the blood.

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

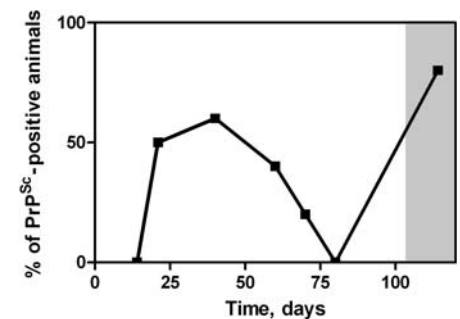


Fig. 2. Proportion of animals whose blood was PrP^{Sc} positive at different times during the incubation period. The percentage of samples scoring positive for PrP^{Sc} in blood is represented versus the time after inoculation at which samples were taken. Two phases of PrP^{Sc} detectability were observed: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

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

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Materials and Methods
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