Prion ; The Unconventional Slow Infectious Agent

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INTRODUCTION

Unconventional slow infectious agents cause among other things kuru, Creutzfeldt-Jacob disease (CJD), Gerstmann-Sträussler-Scheinker syndromes(GSS), and fatal familial insomnia (FFI) in humans, as well as scrapie in sheep and goats and similar spongiform encephalopathies of mink (transmissible mink encephalopathy ; TME), and cow (bovine spongiform encephalopathy ; BSE)(1~5). Approximately 45 years ago Bjorn Sigurdsson, an Icelandic virologist, who was studying a group of Icelandic sheep, proposed the concept of slow infections (6). The criteria he established were aimed at distinguishing this group of diseases from acute, subacute and chronic infections. Sigurdsson established the following four criteria : 1) A very long incubation period (months or years). 2) Long clinical course with fatal outcome. 3) Pathology in only one organ system. 4) Limitation of the infection to a single host species. Sigurdsson correctly predicted that the fourth point might have to be modified by further findings. For example, scrapie and those disease caused by similar agents (kuru, CJD, TME, BSE) can be transmitted experimentally to a wide variety of species (7~11). The term slow infections now refers principally to a small number of infectious diseases of the central nervous system (CNS) caused by conventional viruses or unconventional virus-like agents (Table 1). The structure of the agents in the latter group remains unknown and the nomenclature is yet to be decided (12~14).

ORIGIN OF THE PRION PROTEIN

In recent years, a key breakthrough in the study of these diseases was the discovery of abnormal fibrillar structures, scrapie associated fibrils (SAF), consistently present in cellular extracts from diseased brains caused by unconventional slow infectious agents but never in normal material or in preparations from other diseases (15~17).

The next advance was the identification of a 27-30 kDa protease resistant protein(PrP) in infectious fractions (18~20). This molecule was later named prion protein. Subsequent studies showed that PrP 27-30 is derived from larger protein of Mr 33-35 kDa. At the same time the brains of normal and unconventional slow agent-infected animals were found to express similar levels of PrP mRNA and a protease-sensitive prion protein designated PrP^{C}. PrP^{C} is protease-sensitive, whereas PrP 27-30 is the protease-resistant product of a 33-35 kDa disease-specific protein, designated PrP^{Sc}(21). PrP^{Sc} is

Table 1. Prion diseases in human and animals

Human
Sporadic prion disease
Creutzfeldt-Jakob disease (CJD)
Inherited prion diseases
Familial CJD
Gerstmann-Sträussler-Scheinker disease (GSS)
Fatal familial insomnia (FFI)
Infectious prion diseases
kuru
Iatrogenic CJD
Animals
Sporadic prion disease
Natural scrapie
Inherited prion disease
Natural scrapie
Infectious prion diseases
Bovine spongiform encephalopathy
Feline spongiform encephalopathy
Transmissible mink encephalopathy
Chronic wasting disease mule deer and elk
Experimental scrapie
Natural scrapie

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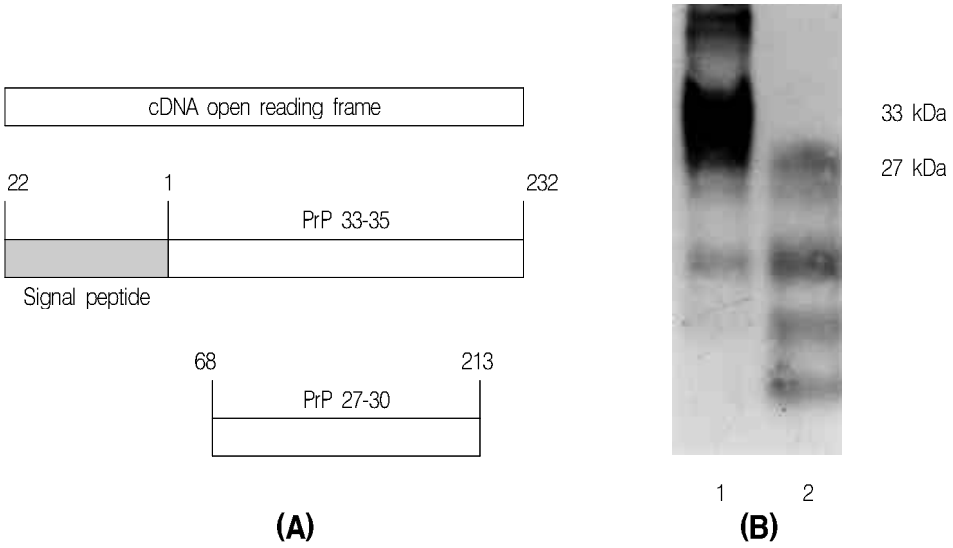

Fig. 1. (A) Structure of the prion protein (PrP). (B) Western blotting analysis of PrP^{Sc} purified from animal infected with scrapie (263K strain). Molecular weight of protease treated PrP^{Sc} (lane 2) was diminished compared with non-proteinase treated PrP^{Sc} (lane 1)

encoded by a host chromosomal gene (short arm of chromosome 20 in humans) and identical sequences were deduced from genomic clones derived from DNA of uninfected persons(22). Most prion diseases are associated with the accumulation of PrP^{Sc} in the brain. The presence of PrP^{Sc} in astrocytes is one of the important marker for diagnosis of prion diseases(23). However, in rare patients(and animals) which appear to lack PrP^{Sc} but express mutant form of PrP, at least in part, be caused by abnormal metabolism of mutant PrP. As the molecular characteristics of PrP in neurodegenerative diseases have been studied in greater detail, the definition of human prion disease has evolved to include any neurodegenerative condition in which a pathogenic form of PrP—PrP^{Sc} or mutant PrP—is detected(Fig. 1)(24).

INHERITED HUMAN PRION DISEASES

The human prion diseases, kuru, CJD, and GSS, have infectious, inherited, and sporadic forms. Infectious forms of prion diseases result from the horizontal transmission of the infectious agent, as occurs in iatrogenic CJD (corneal transplantation, human pituitary growth hormone, dura mater grafts) (25). Inherited forms, GSS, FFI and familial CJD, comprise approximately 10% of all cases of prion diseases. A mutation in the PrP gene has been found in all reported kindreds with inherited human prion disease (26).

Since 1989, molecular genetic analyses of kindreds around the world have revealed at least 15 pathogenic PrP mutations: 10 point mutations and 5 insertional mutations. Seven PrP missense mutations have been identified in patients with GSS, three PrP missense mutations in patients with CJD, and a set of five insertional mutations in the octapeptide coding repeats of PrP (Table 2).

Recently we have studied the two cases of Korean patients who had symptoms of CJD. Both cases are showed histopathologically diffuse spongiform encephalopathy in the brain and the abnormal isoform of prion protein (PrP^{Sc}) in astrocytes were detected. These results made us conclude that they are CJD patients (27). To investigate whether these two patients are familial CJD or not, the prion protein coding genes (PRNP) were sequenced and their restriction fragment length polymorphisms (RFLP) were analyzed. We could not find any

Table 2. Inherited prion diseases of human

Name of inherited prion diseases	Site of missense mutation
Gerstmann-Sträussler-Scheinker disease	Pro 102 → Leu 102
	Pro 105 → Leu 105
	Ala 117 → Val 117
	Tyr 145 → Stop
	Val 180 → Ile 180
	Phe 198 → Ser 198
	Gln 217 → Arg 217
Familial Creutzfeldt-Jakob disease	Asp 178 → Asn 178
	Glu 200 → Lys 200
	Val 210 → Ile 210
	octarepeat insert
Fatal familial insomnia	Asp 178 → Asn 178

missense mutation at the codon 178, 180, 200, 210 or 232. These two patients are thought to be sporadic cases CJD (unpublished data). Familial CJD patients have not been found in Korea, yet.

PATHOGENESIS OF PRION DISEASES

Most of our understanding of the pathogenesis of the prion diseases comes from studies of experimental scrapie. Scrapie occurs naturally in sheep and goats and it can be experimentally transmitted from animal to animal by inoculation of brain or spleen homogenate prepared from sick animals. The agent can also be passaged in a number of experimental hosts, including mice, hamsters, rats and gerbils(28～30). The routes used in the experimental passage of the agent from animal to animal include intracerebral, intraperitoneal, intravenous, subcutaneous and oral. The shortest incubation periods are found following intracerebral inoculation.

After injection by non-neural peripheral routes, pathogenesis necessarily involves the lymphoreticular system (LRS) such as spleen and lymph nodes before the CNS. The importance of spleen in mouse scrapie was demonstrated by splenectomy or genetic asplenia, which lengthened the incubation period of intraperitoneal injected scrapie (31～33). The intracerebral route of injection is used usually for bioassay of infectivity because it is the most efficient and sensitive. However, this route bypasses the need for the extraneural events of pathogenesis. For example, splenectomy prior to CNS injection had no effect on incubation

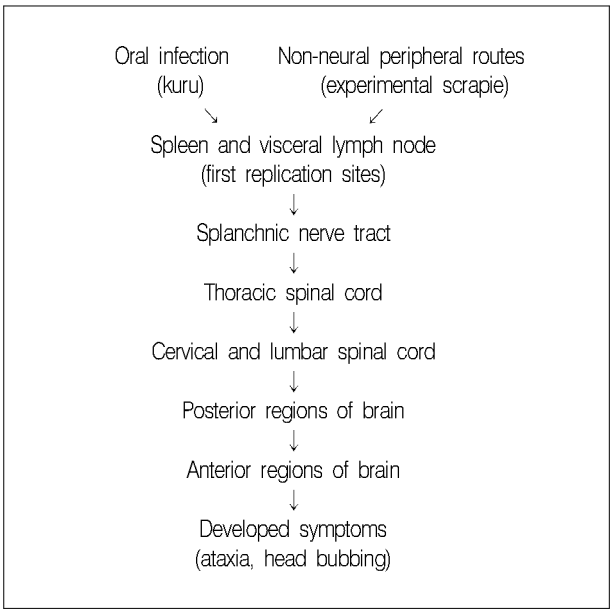

Fig. 2. Pathogenesis of prion diseases

The concept that neural spread plays a role in the phase of pathogenesis in which infectivity moves from the LRS organs to the brain is supported by a series of experiments done by Kimberlin and colleagues. In these experiments, after peripheral injection and the appearance of infectivity in spleen, the first area of the CNS that showed infectivity was the thoracic region of the spinal cord, which is where the splanchnic nerve enters the cord in mice(34～36). From there, infectivity spread to the lumbar and cervical regions of the spinal cord. Infectivity then spread to the brain, and was found first in posterior regions and subsequently appeared in anterior regions (Fig. 2).

In order to assess the role of neural spread of scrapie agent within the brain, a series of experiments were initiated in which the cerebellum was bisected just prior to stereotaxic injection in the cerebellum. Differences in the results seen between mice injected stereotaxically in intact and bisected cerebella with the 22L scrapie strain all point to reduced spread of agent in bisected animals. The key findings include : 1) longer incubation periods and survival times in mice injected in bisected cerebella ; 2) a delay in occurrence of both pathological changes and high levels of infectivity in non-injected side of bisected cerebella compared to the occurrence after injection in intact cerebellum ; 3) lower titers in the four brain regions tested in mice injected in bisected cerebella. The results in this study suggest that the infectious agent spreads along intact nerve cell tract, probably by axonal transport(37).

GENETIC CONTROL OF INFECTIOUS AGENT-HOST GENOTYPE INTERACTIONS

The precision of unconventional slow infectious agent-host genotype interactions has been important for researchers in that it has provided the opportunity to establish reliable details about disease pathogenesis. Recently, a series of parameters have been shown to be controlled by both host genotype and strains of unconventional slow infectious agent. These parameters include incubation period, the distribution and intensity of vacuolation, the production of amyloid plaques, body weight gains and altered glucose tolerance during the preclinical phase of disease.

(1) Incubation period.

Many experimental mouse models have been obtained with different ranges of incubation period. These models vary in the strain of scrapie agent and the *Sinc* genotype of mouse. *Sinc* is the major mouse gene controlling

incubation period. For example, s7s7 mouse genotype have shorter incubation periods for the ME7 and 139A scrapie strains, whereas p7p7 mouse strains have longer incubation periods for the ME7 or 139A scrapie strains. However, other scrapie strains, such as 22A and 87V, have an opposite pattern with a longer incubation period in s7s7 mice than in p7p7 mouse genotypes. With regard to incubation periods in s7p7 hybrid mice, some scrapie strains(e.g. ME7, 22L) had values that were between those for the two parent mouse genotypes, whereas the incubation period for other scrapie strains (e.g. 139A, 87V) were longer than the value for either parental mouse genotype (Table 3) (38, 39).

Table 3. The effects of strain of agent and *Sinc* genotype on incubation period

Mouse genotype	Strain of scrapie agent	
	ME7	87V
VM *(Sinc p7p7)*	326 ± 4*	285 ± 3
F1 *(Sinc s7p7)*	230 ± 3	562 ± 10
C57BL *(Sinc s7s7)*	155 ± 2	450 ± 5

* Number of days(mean ± standard error)

An another marker related to incubation period was defined several years ago in a study by Kim and colleagues. Using stereotaxic injection of five brain regions (cerebral cortex, caudate nucleus, thalamus, substantia nigra and cerebellum), incubation period values were compared for the ME7, 22L and 139A strains. For ME7 the thalamus and cerebellum yielded the shortest incubation periods; for 22L the incubation period for cerebellar injected mice was shortest, for 139A the cerebral cortex had the longest incubation period with the other four regions yielding similar, shorter incubation periods. These findings supported the concept of clinical target areas for these 3 strains and showed that there were differences in this marker for these strains(40). The concept of clinical target areas arouse form the observation that the duration of replication in the brain is always longer after intracerebral injection than after nonneural, peripheral injection (41, 42).

(2) Distribution and intensity of vacuolation.

The pathological lesions of unconventional slow infectious diseases are located primarily in the CNS, and the most characteristic histopathological change in human and animals terminally infected with unconventional slow infectious agent is vacuolation (43). The intensity of vacuolation in various regions of brain is also under genetic control of both infectious agent and host genotype. In previous studies we showed that the groups of mice with three genotypes of *Sinc* (s7s7, p7p7 and their F1 cross, s7p7) were injected with 22L scrapie strain into the cerebral cortex, thalamus or cerebellum. After cerebellar injection vacuolization was limited to the cerebellum, medullar and mesencephalon in all three host genotypes. However, the location of vacuoles within the cerebellum differed depending upon the host genotype. Vacuolization developed almost exclusively in grey matter in s7s7 mice, mainly in white matter in p7p7 mice, and in both grey and white matter in F1 mice. These results demonstrate that the selective vulnerability of the cerebellum to induction of clinical disease by 22L does not depend on host genotype, but host genotype does affect lesion distribution within the cerebellum (44, 45).

(3) Amyloid plaque production

The development of amyloid plaque in the unconventional slow infectious disease forms a link with an important human CNS disease, Alzheimer's disease. In recent years a number of parallels have been recognized between Alzheimer's disease and prion diseases (46). The most important similarity is the occurrence of amyloid plaques in the CNS in both diseases. Cerebral amyloid plaques were first described in scrapie in the naturally occurring disease in sheep. In experimental murine scrapie, amyloid plaques were first reported by Fraser and Bruce (47) and have been investigated extensively by Wisniewski and his colleagues (48). The occurrence of amyloid plaques in various scrapie strain-mouse strain combinations ranges from a high frequency in some combinations to complete absence in others. In general, amyloid plaques tend to be more abundant in combinations with comparatively long incubation periods (over 300 days in the plaque models). Amyloid plaque production is much more active toward the end of the incubation period. The histopathological changes in scrapie and the other unconventional slow infections and in Alzheimer's disease are characterized by a regional distribution of lesions. With regard to amyloid deposits, this suggests that the pathogenesis of plaque is a function of either the vulnerability of cells in particular areas or a selective access of the causative agent to different brain areas. The current studies are the first to us the stereotaxic method of injection of different brain areas with a plaque-forming scrapie strain-mouse strain combination, 87V scrapie strain in IM mice. In this combination, stereotaxic injection of the cerebellum yielded shorter incubation periods than injection of cerebral cortex, caudate nucleus, substantia nigra, or thalamus. Comparison of histopathological changes in 87V-IM mice to those obtained in other scrapie strain-mouse strain combinations also demonstrates selective vulnerability of cells in different regions

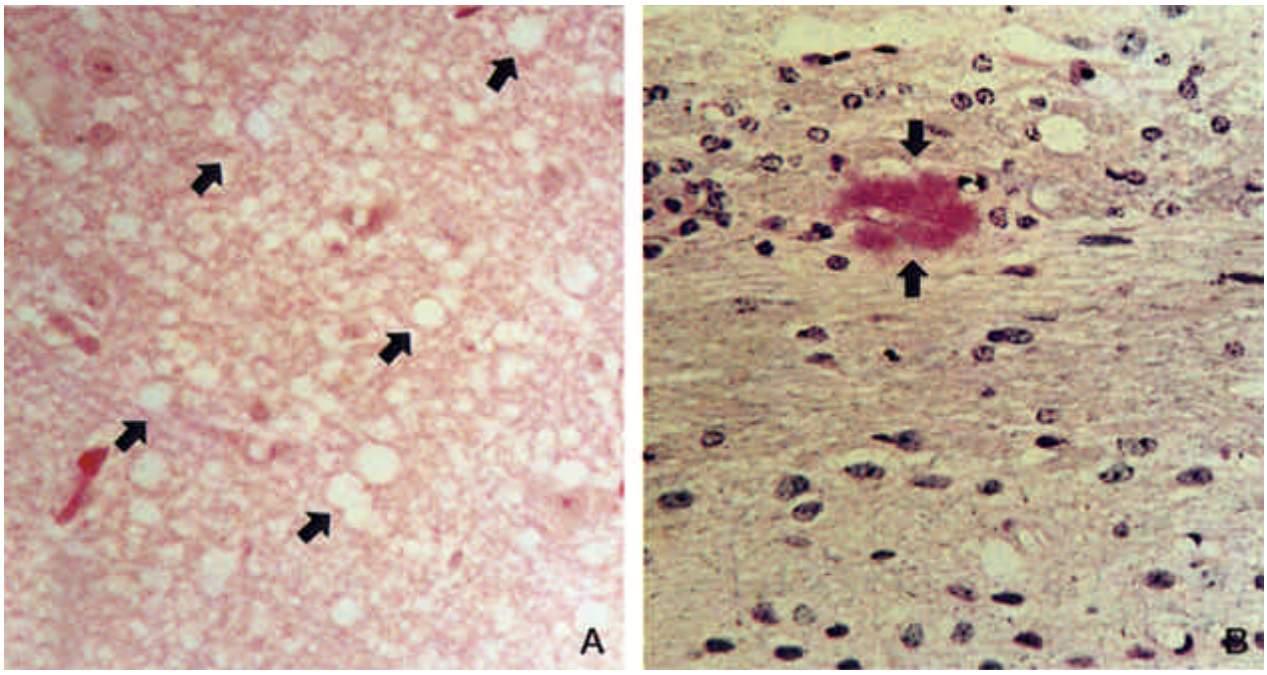

Fig. 3. Vacuolation(spongiform encephalopathy) in the hippocampus(arrow)(A) and amyloid plaque in the corpus callosum(arrow)(B) of the IM mice infected with 87V scrapie agent. Stained with Hematoxylin and eosin(A) and Bodian plus periodic acid- Schiff(B). ×400.

to the action of different scrapie strains. For example, in the 87V-IM combination, both vacuolation and plaque formation were seen in mid-brain and forebrain regions, whereas neither lesion was seen in cerebellum even after intracerebellar injection (Fig. 3) (49, 50).

(4) Body weight changes and altered glucose tolerance

For many combinations of scrapie strain and mouse strain, total body weight during the preclinical phase of disease was similar to the average weight for controls. For some combinations there was a significant increase in weight(compared to controls) during the latter part of the preclinical phase. The above studies were done by routine intracerebral injection (51, 52).

In the current studies, scrapie strains were injected stereotaxically in the cortex or ventromedial nucleus of the hypothalamus of two mouse strains. In SJL mice injected with the 22L scrapie strain, both the cortex and hypothalamus injection groups developed preclinical weight increases to a similar extent. In the SJL-ME7 combination, both groups became obese, but a higher degree of obesity developed in the hypothalamus injection group than in the cortex group. For the C57BL-ME7 combination, the increase in weight was seen only in the group injected in the hypothalamus. These results suggest that scrapie-induced obesity is dependent on an effect on the hypothalamus (53). Food consumption was monitored in groups of SJL mice injected in the hypothalamus with normal mouse brain, 139A and ME7. There was an increase in food consumption (compared to the normal brain injection group) for both the 139A and ME7 groups, although only the ME7 group showed an increase in weight. In a scrapie strain-mouse strain combination that showed an increase in body weight, the adrenal gland was the only organ that showed a significant increase in weight. In a subsequent study, adrenalectomy prevented the increase in total body weight in two strains of mice injected with the ME7 scrapie strain(54). Aberrant glucose metabolism was seen in some of the scrapie mice that showed the increase in body weight. The development of aberrant glucose tolerance essentially paralleled the increase in body weight. The changes in glucose metabolism were seen after intracerebral, intraperitoneal and stereotaxic hypothalamic injection. Using scrapie sheep isolates that have extremely long incubation periods in C57BL mice, there were marked increases in body weight beginning a short time after the middle of the incubation period (55, 56). The weight of these mice attained average levels that were 1.7 to 2.1 times greater than that seen for mice injected with normal mouse brain. These mice were hyperglycemic and showed aberrant glucose tolerance during the preclinical phase of scrapie provides an interesting model system for the study of these conditions in humans.

THE HYPOTHESES

The facts that the slow infectious agent, prion, has the unconventional nature led us to postulate three possible hypotheses. The first is a virus theory that prion is one of virus types. The virus has very unusual biochemical and biophysical characteristics. This theory is not likely to be perfect, because the resistance of inactivation of the virus cannot be explained. The infectious agents are hard to be inactivated by the conventional ways (57, 58). Secondly, the prion protein theory that the agent is composed exclusively of the host-coded prion protein (PrP^{C}) and becomes protease resistant (PrP^{Sc}) after infection. The term prion has been derived from the words proteinaceous infectious particle. Non-host protein components have not been observed in the infectious agent, nor have nucleic acid materials. It is mysterious how these prion proteins multiplicate themselves without nucleic acid (59). The last is the virino theory in which there is a host-coded protein that protects the infectious agents specific low molecular nucleic acid(RNA or DNA) which postulated to be a similar to a viroid (14). In the virus and virino theories, genetic characteristics of infectious agent strains would be a result of changes at the nucleic acid molecular level. The prion protein theory cannot explain the presence of multiple genetic strains of infectious agents. In addition, the propagational mechanism of the prion is not possible to be established with only infectious protein molecules and without any genetic materials (Table 4).

Among these three hypotheses, the virino hypothesis is more favorable than the others. The virino hypothesis would explain a number of the unusual characteristics associated with infectivity and strain specificity. The host-originated proteins would explain the absence of an immunological response in infected hosts and the difficult of isolation of the infectious agent from infected materials. Furthermore, the small size of the nucleic acids would resist to irradiation and retain their infectivities. This lower molecular weight of the nucleic acid materials could also make it difficult to isolate and detect them in partially purified preparations.

CONCLUDING REMARKS

For years, researchers have been fascinated and frustrated with the puzzling nature of the unconventional slow infectious agent. However, it is worthy of noticing the essential points in this review.

(1) Strains of unconventional slow infectious agent exist.
(2) They play an important role in agent-host interactions.
(3) The differences of specific agent strains cannot be explained by the host-coded PrP molecule (prion protein theory).
(4) There must be a small nucleic acid (DNA or RNA) which serves as the information molecule in unconventional slow infectious agents.

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Table 4. Current hypotheses concerning the nature of the unconventional slow infectious agent

Hypothesis	Nature of agent	Mode of replication	Reference
Prion	Protein only	Reverse translation, protein-directed protein synthesis or induction of host transcription	Prusiner *et al* (1994)
Virino	Protein+nucleic acid : host-coded protein with small regulatory nucleic acid	Nucleic acid replicated by enzymes with virino nucleic acid as the template	Hope (1994) Carp (1994)
Virus	Protein+nucleic acid : protein coded by virus-specific nucleic acid	As per standard animal virus	Diringer *et al* (1994) Manuelidis (1994)

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