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Prospects for Preventative Vaccines Against Prion Diseases

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Abstract: Emergence of variant type of Creutzfeldt-Jakob disease (vCJD) in humans due to infection from bovine spongiform encephalopathy contaminated beef and recent reports of human-to-human transmission of vCJD via blood transfusion have raised great concern about an epidemic of vCJD. The disease is currently difficult to diagnose during preclinical stages and requires a very long incubation period for neurological symptoms to be evident. This therefore suggests that the disease is already latently spreading and that opportunity for infection is thus growing among human populations. Interestingly, passive immunization with antibodies against prion protein (PrP), a major component of the prion infectious agents, was shown to protect mice from infection, indicating the possibility of prion vaccines. However, PrP is a host protein therefore immune tolerance to PrP has hampered development of them. Here, the so far reported attempts to overcome the tolerance to elicit protective immunity to prions are briefly reviewed.

Keyword: Vaccine, prion, prion protein, prion disease, immune tolerance.

INTRODUCTION

Transmissible spongiform encephalopathies or prion diseases, including Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI) and kuru in humans and scrapie and bovine spongiform encephalopathy (BSE) in animals, are a group of devastating neurodegenerative disorders caused by unconventional agents, the so-called prions [1, 2]. These diseases are characterized by pathological hallmarks of neuronal cell loss, marked gliosis, spongiform degeneration and accumulation of the abnormally folded, amyloidogenic isoform of prion protein (PrP), designated PrP^{Sc}, in brains [3]. It is widely believed that prions consist of, if not entirely, PrP^{Sc} and propagate via conformational conversion of the normal cellular isoform of PrP (PrP^{C}) into PrP^{Sc} (Fig. **1**) [4]. Indeed, we and others showed that mice devoid of PrP^{C} (PrP-/-) neither accumulated PrP^{Sc} nor propagated prions in brains even after intracerebral inoculation of prions [5-8]. However, the exact nature of prions is still controversial.

Prion diseases progress very slowly, requiring a very long incubation period until neurological symptoms become evident [9]. However, once such abnormal symptoms are developed, the diseases become aggressive and affected patients usually die within a year [9]. Intriguingly, human prion diseases manifest sporadic, genetic, and infectious disorders (Table **1**) [9]. Most cases (85-90%) of the diseases are a sporadic type of CJD with unknown etiologies [3]. Familial CJD, GSS and FFI are inherited diseases associated with specific mutations of the PrP gene and about 10% of the cases belong to this type of disease [3]. The remaining cases, including those of iatrogenic CJD, variant CJD (vCJD) and kuru, are caused by infection [3, 10]. Human prion diseases are rare, with an incidence of about 1:1,000,000 worldwide [3]. However, recent reports indicate that vCJD might be transmissible between humans via blood transfusion [11, 12], giving rise to the possibility of a vCJD epidemic in human populations. Therefore development of therapeutic and/or preventative measures against the diseases is urgently awaited. Here, recent studies of vaccines for prion diseases are reviewed.

vCJD TRANSMISSION IN HUMAN POPULATIONS

Zoonotic Transmission

Epidemiological studies have shown that a species barrier for scrapie prions between sheep or goats and humans is substantially present [13, 14]. However, it is believed that BSE prions overcame the species barrier and are transmitted to humans via consumption of contaminated food [15, 16]. vCJD was first recognized as a new and distinctive disease in the United Kingdom (UK) in 1996 [17]. Since then, great public health concerns about an epidemic of vCJD via the zoonotic transmission of BSE to humans have been raised. However, to date, fortunately, only about 160 vCJD cases were reported in the UK despite more than 160,000 cases of BSE being reported in cattle [18]. This might indicate that BSE prions can overcome the species barrier between cattle and humans but the efficacy would be very low. In addition, BSE cases have dramatically decreased in the UK these days due to banned use of meat and bone meal ingredients in animal feed [18]. It is thus rational to consider that risk of the transmission of BSE to humans has been markedly reduced today. However, new cases of BSE are still reported in the UK and other countries, indicating that constant survey of the disease is still necessary.

Chronic wasting disease (CWD), another type of animal prion disease, is spreading within captive and free-ranging mule deer and elk populations in North America [19], giving rise to a health concern of whether or not CWD also could be

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Table 1. Human Prion Diseases

Etiologies	Diseases	Incidence (%)
Sporadic (unknown)	Creutzfeldt-Jakob disease (CJD)	85-90
Genetic (mutations in the PrP gene)	Familial CJD	~10
	Gerstmann-Sträussler-Scheinker syndrome (GSS)	
	Fatal familial insomnia (FFI)	
Infectious	Kuru •cannibalism	~5
	Iatrogenic •growth hormone therapy •cornea transplantation •dura mater transplantation •via neurosurgical instruments •via depth electrodes of electroencephalography	
	Variant CJD (vCJD) •highly suspected to be transmitted from bovine spongiform encephalopathy infected cattle	

transmissible to humans. However, epidemiological studies indicate that risk, if any, of CWD transmission to humans is low [20, 21]. In addition, it was recently reported that humanized mice, which express transgenic human PrP, were susceptible to human prions but not to elk CWD prions [22]. These results indicate the presence of a substantial species barrier for the transmission of CWD to humans from deer, as occurs for scrapie prions to humans. However, the possibility for transmission of CWD to humans cannot be completely excluded.

Human-to-Human Transmission

In contrast to the reduced risk of transmission of BSE to humans, opportunities for human-to-human transmission of vCJD via medical treatments or procedures are growing. First, in contrast to classical type CJD prions, vCJD prions can be spread via blood transfusion as they can circulate in blood in a considerably high titer. Unfortunately, three cases of vCJD considered to be transmitted via blood transfusion have been reported [11, 12, 23]. One case was diagnosed as vCJD by autopsy and the others developed the disease several years after blood transfusion from donors who eventually succumbed to vCJD. Second, vCJD prions appear to be more virulent than BSE prions in humans. Genetic polymorphism of methionine (M) or valine (V) at codon 129 of the PrP gene is known to be a major determinant of susceptibility to human prions [24-26]. MM is the most susceptible, MV intermediate, and VV protective. All cases of vCJD so far reported to be infected from BSE are MM homozygous [23, 27]. No MV or VV cases have been identified. However, one case of blood transfusion-related vCJD was heterozygous at codon 129 [12], suggesting that vCJD prions may be transmissible to humans carrying not only MM but also MV or VV genotypes. Consistent with this, studies using transgenic (tg) mice expressing human PrP with codon 129 MM, MV or VV genotypes showed that MM, MV and VV tg mice could be infected by vCJD prions [27]. Finally, since prion diseases require a very long incubation time and pre-symptomatic diagnosis is currently impossible, it is assumed that, among human populations, there might be a considerable number of individuals who are latently infected with and incubating prions without any clinical symptoms. Hilton *et al.* investigated surgically removed appendectomy or tonsillectomy specimens for accumulation of PrP^{Sc} by immunohistochemistry and showed that 3 out of 12,674 samples were positive for staining of PrP^{Sc}, although two specimens displayed a dissimilar staining pattern of PrP^{Sc} from that in vCJD [28]. This incidence seems much greater than that so far reported for conventional human prion diseases, suggesting that vCJD prions might be latently spreading among human populations and that latently infected people might become sources for secondary transmission of vCJD. It is thus important to detect these latently infected individuals as early as possible and take proper measures to prevent human-to-human transmission of the disease.

ANTI-PrP ANTIBODIES AND ANTI-PRION ACTIVITY

Prophylactic Immunization with Anti-PrP Antibodies

It was first reported that polyclonal antibodies to PrP, α-PrP27-30, when mixed with hamster-adapted scrapie prion rods in the form of detergent-lipid-protein complexes prior to inoculation, could reduce infectivity by a factor of 100 in hamsters [29]. These results suggested that anti-PrP antibodies might be effective against prion infection. Indeed, it was subsequently shown that transgenic mice expressing a 6H4 mouse anti-PrP monoclonal antibody were resistant to the disease even after intraperitoneal inoculation with mouse-adapted scrapie RML prions [30]. It was also demonstrated that passive immunization with anti-PrP antibodies could prevent prion infection in mice. White *et al.* intraperitoneally

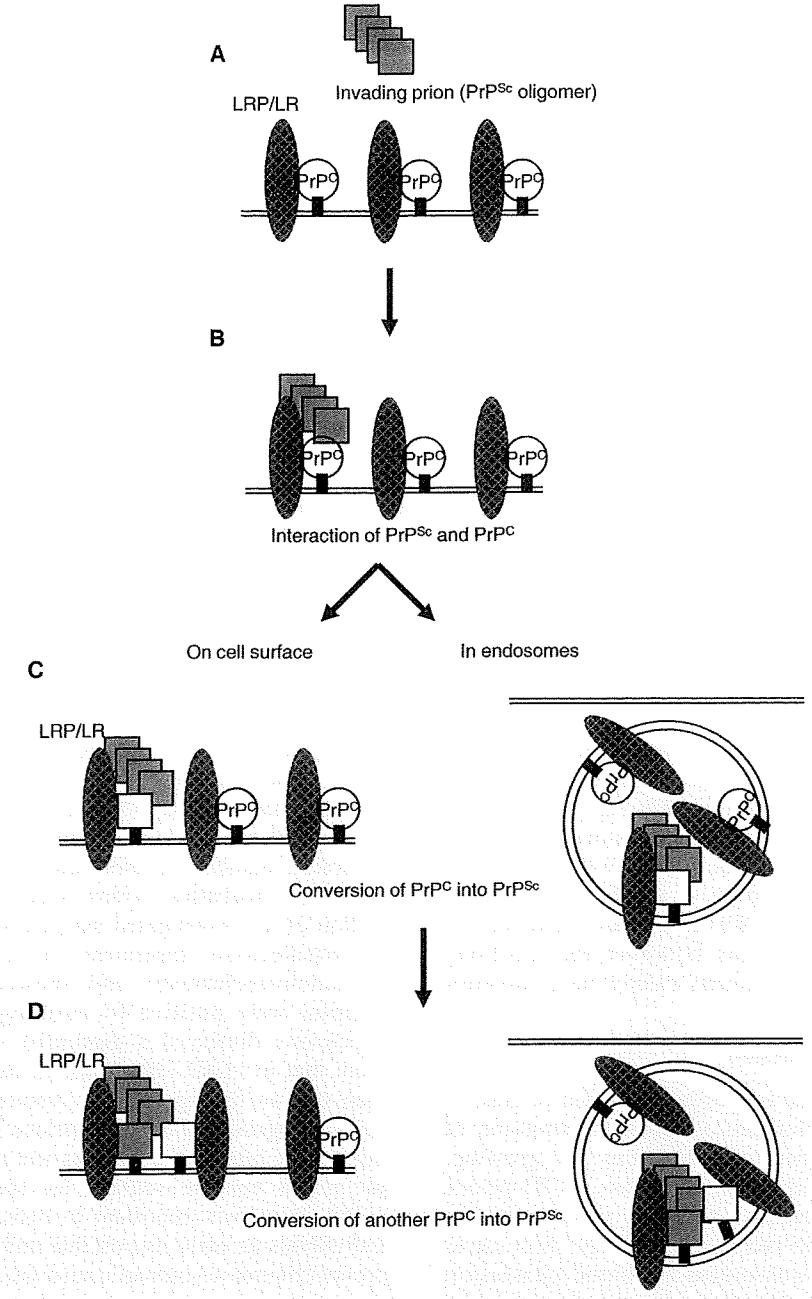

Figure 1. A model of prion replication or PrP^{Sc} generation. A prion, which is thought to consist of PrP^{Sc} oligomer, invades the CNS **(A)** and interacts with PrP^{C} expressed on the cell surface of neurons directly or indirectly via LRP/LR **(B)**. The interacting PrP^{C} then undergoes conformational transformation to PrP^{Sc} either on the cell surface or in endosomes **(C)**. The newly generated PrP^{Sc} in turn interacts with and converts another PrP^{C} into PrP^{Sc} **(D)**.

administrated a high dose (2 mg) of anti-PrP monoclonal antibodies, ICSM 18 or 35, into mice twice weekly from 7 or 30 days post-inoculation (dpi) of RML prions and showed that the treated mice could be protected from the disease over 500 dpi [31]. However, the intraperitoneally administered antibodies had no effects on prions directly inoculated into the brains of mice, probably due to the blood brain barrier, which prevents antibodies from accessing brains [31]. These results indicate that development of preventative vaccines against prion diseases may be possible, but might not be therapeutic.

Possible Mechanisms of Anti-Prion Activity by Anti-PrP Antibodies

It is postulated that the first step of prion infection or conformational conversion of PrP^{C} into PrP^{Sc} is interaction of invading PrP^{Sc} with PrP^{C} expressed on the cell surface. It is therefore conceivable that anti-PrP antibodies might interfere with this interaction and prevent the subsequent conversion of PrP, thereby exerting prophylactic effects on the diseases. Consistent with this idea, Kaneko *et al.* reported that anti-PrP monoclonal antibodies, 3F4 and 13A5, which rec-

ognize PrP residues 109-112 and an epitope formed by residues 138-165, respectively, prevented both binding of hamster PrP^C to PrP^{Sc} and subsequent conversion in a cell free conversion system [32]. Horiuchi and Caughey also reported that the polyclonal anti-peptide antibody α219-232 exhibited similar effects, prevented the binding of both molecules and subsequent conversion in the cell free system but α23-37 and α90-104 antibodies did not [33]. These results indicate that binding of PrP^C to PrP^{Sc} might be mediated via specific sites, including epitopes for 3F4, 13A5, and α219-232 antibodies. However, in contrast to Kaneko *et al.* Horiuchi and Caughey showed no inhibitory effects of 3F4 and α143-156 antibodies on the conversion [33]. This might be due to different experimental designs of the cell free conversion system used [32, 33]. The exact *in vivo* binding sites of PrP^C and PrP^{Sc} remain to be identified.

Interestingly, it was shown that antibodies that bind to PrP^C could clear both PrP^{Sc} and prions from persistently infected cultured cells, indicating that anti-PrP antibodies could inhibit *de novo* generation of PrP^{Sc} even in cells previously infected by prions [34, 35]. It is therefore conceivable that this anti-prion activity also might be associated with the prophylactic effects of anti-PrP antibodies. The exact mechanism of this antibody-mediated clearance of PrP^{Sc} or prions is not fully understood. Kim *et al.* showed that anti-PrP antibodies carrying anti-prion activity disturbed PrP^C internalization, suggesting that the antibody-induced impairment of subcellular localization of PrP^C might be involved in its anti-prion activity [36]. It is also conceivable that, as discussed above, anti-PrP antibodies might disturb the interaction of PrP^C and PrP^{Sc}. Moreover, Perrier *et al.* showed that anti-PrP antibodies accelerated degradation of PrP^C in cells [37]. It is therefore also possible that the reduced substrate of PrP^C might be responsible for inhibition of PrP^{Sc} formation.

Anti-Prion Activity by Anti-Laminin Receptor Antibodies

PrP^C interacts with the 37/67 kDa laminin receptor (LRP/LR) either directly or indirectly (Fig. **1**) [38]. Interestingly, Leucht *et al.* showed that the LRP/LR-specific antibody, W3, could reduce PrP^{Sc} levels in infected N2a cells [39]. It is also shown that single chain Fv (scFv) against LRP/LR, termed S18, successfully reduced PrP^{Sc} by approximately 40% in the spleens of mice infected with RML prions when intraperitoneally injected once a week for 8 weeks from 1 day prior to the infection [40]. These results indicate that LRP/LR might be a co-factor essential for PrP^{Sc} formation and that such co-factors might be molecular targets for preventative vaccines aginst prion diseases.

W3 was shown to compete with recombinant PrP for binding to LRP/LR expressed on the cell surface and reduce PrP^C levels in cells [39]. This therefore suggested that the disturbed interaction between PrP^C and LRP/LR by W3 might be responsible for the reduction of PrP^{Sc} formation. It is also possible that the W3-mediated dissociation between LRP/LR and PrP^C might destabilize PrP^C, or that LRP/LR-PrP^C-W3 complexes might stimulate internalization of PrP^C into lysosomes for degradation.

IMMUNE TOLERANCE AND PRION VACCINES

Immune Tolerance to PrP

PrP^C is a host protein expressed in various tissues, especially most abundantly in brains and therefore immunologically tolerated by the host [41]. Since PrP^{Sc} is a major or only component of prions and produced by conformational changes of PrP^C, prions are also immunologically tolerant in the host [4, 42]. Indeed, it is well-known that specific immune responses are not induced in prion diseases [43]. Thus, overcoming immune tolerance to PrP is a crucial first step for development of prion vaccines.

Heterologous PrPs Disrupt PrP Tolerance

Molecular mimicry between microbial and host antigens is a well-known hypothetical mechanism for triggering autoimmune diseases via production of auto-antibodies and/or auto-reactive T cells due to similar amino acid sequences shared between both antigens [44, 45]. We there-fore hypothesized that heterologous PrPs, which are derived from a different species to the host, could overcome the immune tolerance and elicit antibody responses against PrP [46], because they are similar but not identical to the host PrP in amino acid sequence [47]. Consistent with this idea, we showed that mouse recombinant PrP failed to induce antibody responses in BALB/c mice (Fig. **2A**). However, mice were highly responsive to heterologous sheep and bovine recombinant PrPs, producing high titers of antibodies against them (Fig. **2A**). In addition, most mice immunized with sheep or bovine recombinant PrP generated antibodies capable for reacting with mouse PrP in consi-derably high titers (Fig. **2B**), but no autoimmune-related symptoms such as arthritis or abnormal behavior were detected in immunized mice. These results indicate that heterologous PrPs are highly immunogenic and able to disrupt immune tolerance to PrP, inducing anti-PrP autoanti-bodies.

We then investigated prophylactic effects of immunization of mice with sheep or bovine PrP on infection with a mouse-adapted Fukuoka-1 prion [46]. Non-immunized BALB/c mice developed the disease at 291 ± 10 dpi (Fig. **2C**). However, BALB/c mice immunized with recombinant bovine PrP showed delayed onsets at 322 ± 15 dpi (Fig. **2C**). Recombinant sheep PrP exhibited variable effects against the prion in BALB/c mice (Fig. **2C**). About 70% of the immunized mice developed the disease with prolonged onsets (Fig. **2C**). It might be conceivable that mice producing higher titers of anti-PrP antibodies are more resistant to the disease than those with lower titers of the antibodies. However, we do not know whether or not incubation times in the mice were inversely correlated with titers of anti-PrP antibodies because each of the immunized animals was not independently identified in this study. Moreover, the exact reason why bovine and sheep recombinant PrPs exhibited different prophylactic effects against prion infection remains unknown. The amino acid sequence between bovine and sheep PrPs is highly homologous but some amino acids differ. It might thus be possible that these different amino acids between sheep and bovine PrPs could differentially stimulate immune responses in mice. Indeed, titers of anti-mouse PrP autoantibodies were more variable in mice immunized with sheep recombinant PrP than in mice given bovine PrP (Fig. **2B**).

Nevertheless, the results indicate that heterologous PrPs might be effective stimulator of protective immunity against prions.

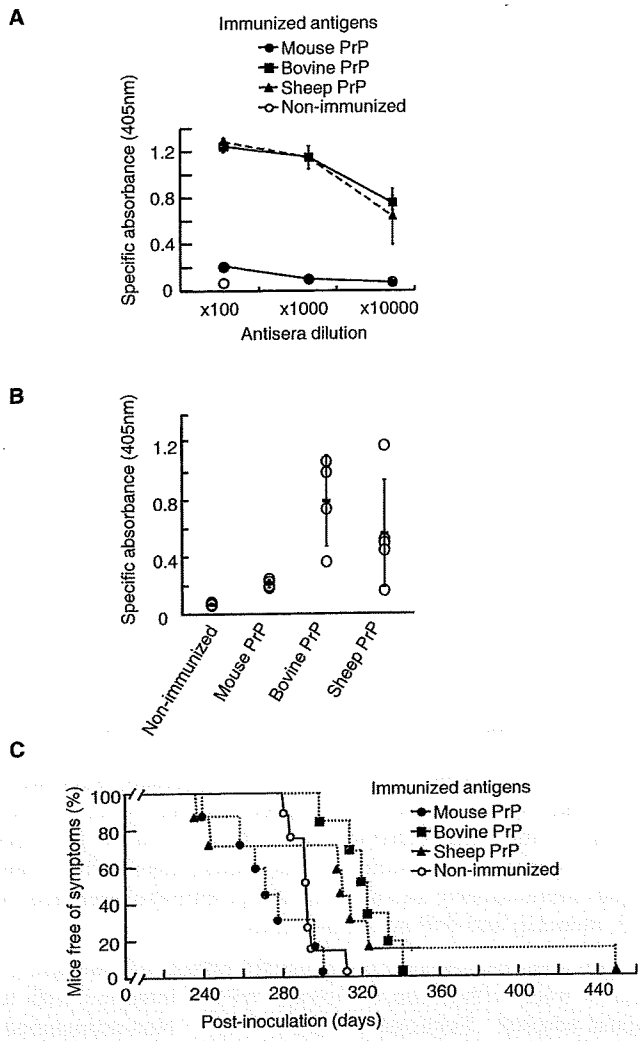

Figure 2. Immunization with heterologous PrPs delays the onset of disease in mice inoculated with a mouse-adapted Fukuoka-1 prion. BALB/c mice were intraperitoneally immunized with either purified recombinant mouse, bovine or sheep PrP 5 times at 2-week intervals and antiserum was collected from each of the immunized mice from each group. Specific anti-PrP IgG response against the respective immunizing antigens **(A)** or mouse recombinant PrP **(B)** was investigated by an enzyme-linked immunosorbent assay (ELISA). Mouse recombinant PrP elicited only very low antibody response. In contrast, higher response was detected in mice immunized with bovine and sheep PrPs. **(C)** Prophylactic effects of immunization with heterologous bovine and sheep PrPs against the Fukuoka-1 prion. Each immunized mouse was intraperitoneally inoculated with the prion. No effect was detected in mice immunized with mouse recombinant PrP (n=7). Instead, incubation times appeared shortened, compared with those of non-immunized mice (n=8). In contrast, mice immunized with bovine PrP (n=6) developed the disease with significantly delayed onset (p=0.0008, Log-rank test) and, except for two of the sheep PrP-immunized mice, the other five mice also developed the disease later than non-immunized mice. Reprinted in part from [46]. Copyright (2006), with permission from Elsevier.

Interestingly, in contrast to our results, Sigurdsson *et al.* reported that subcutaneous immunization of CD-1 mice with homologous recombinant mouse PrP could induce anti-PrP autoantibodies and slightly retarded onset of the disease following inoculation with a mouse-adapted 139A prion [48]. The immunized mice died at 189 ± 4 dpi while control mice died at 173 ± 2 dpi after intraperitoneal inoculation with a 10-fold dilution of infected brain homogenate [48]. The exact reason why Sigurdsson *et al.* successfully overcame immune tolerance to PrP in mice by immunization with homologous mouse recombinant PrP is unclear. They used 0.5 M urea solution, in which recombinant PrP was resolved, and mixed it with complete or incomplete Freund's adjuvant prior to immunization [48]. The potential immunomodulatory effects of hydroxyurea have been suggested [49, 50]. It may therefore be possible that urea modulates immune responses, thereby stimulating immunogenicity of mouse recombinant PrP in mice.

Immune Modulators Disrupt PrP Tolerance

Pathogenic organism-derived pathogen-associated molecular patterns, including unmethylated CpG oligodeoxynucleotides, are recognized by pattern recognition Toll-like receptors and strongly stimulate innate and ultimately acquired immune responses [51]. Interestingly, it was reported that administration of CpG alone could extend survival times in mice inoculated with the RML prions [52]. Mice were free of the disease more than 330 dpi when CpG was intraperitoneally administrated 7h post-inoculation and thereafter daily for 20 days, while the control mice developed the disease at 181 dpi [52]. It was therefore considered that CpG might induce protective immunity against prion diseases. However, it was shown that such repeated administration of CpG caused suppression of follicular dendritic cells (FDCs), which are essentially involved in induction of innate and acquired immune responses and are already known to be important for prion propagation before invading the CNS [53]. Thus, the prophylactic effects of CpG might be due to suppression of FDCs, caused by its repeated administration.

On the other hand, several studies indicated that, when co-administrated with PrP, CpG effectively disrupted immune tolerance to PrP. Rosset *et al.* first reported that subcutaneous co-administration of CpG with PrP peptides could break the immune tolerance to PrP in C57BL/6 mice, effectively eliciting antibodies against the peptides [54]. It was also reported that CpG stimulated a humoral immune response against nondenatured 139A scrapie prion-associated fibrils in mice, inducing significant anti-PrP autoantibody production [55]. CpG is thus a potent modulator for overcoming immune tolerance to PrP.

DNA vaccination has been shown to break immune tolerance to host proteins [56-58]. DNA vaccines stimulate immune responses via uptake by professional antigen-presenting cell (DCs), where a DNA-encoded antigen is expressed and presented for T cell recognition, and by non-DCs such as keratinocytes or myocytes, which express the encoded antigens and transfer them to DCs possibly as apoptotic vesicles [56]. It is also conceivable that DNAs themselves might modulate immune responses similarly to CpGs. Fernandez-Borges *et al.* showed that immunization with

plasmid pCMV-PrPLII, encoding mouse PrP fused to the lysosomal integral membrane protein type II lysosome-targeting signal, into the anterior tibial muscle of 129/ola mice stimulated production of anti-PrP autoantibodies and extended the disease incubation times by about 73 days in mice after inoculation of a mouse-adapted BSE_I prion [59]. However, pCMP-PrP, which encodes mouse PrP alone, neither stimulated antibody responses in 129/ola mice nor protected the mice from the disease [59]. This result was consistent with those of Nitschke *et al.* showing that pCG-PrP encoding mouse PrP alone failed to elicit anti-PrP autoantibodies and protect the immunized mice from RML prions [60]. Taken together, these results indicate that DNA vaccines encoding PrP alone might have difficulty disrupting tolerance to PrP. Thus, the encoded PrP should be modified to increase its immunogenicity.

Modifications of PrP Stimulate its Immunogenicity

Using the Multiple Antigen Peptide (MAP) method, Arbel *et al.* fused eight copies of a peptide corresponding to residues 144-153 (helix 1) of human PrP, which is distinguished from that of mice by one amino acid, and immunized BALB/c mice with the fused peptide [61]. The immunized mice produced high titers of IgG specific to the immunogen and the antisera could react with other heterologous PrPs, including bovine, sheep and mouse PrPs [61]. These results indicate that the MAP-mediated fusion of PrP peptides could break tolerance of PrP. However, the PrP peptide used in this immunization was different from that of mice by one amino acid [61]. It is therefore conceivable that, rather than fusion of multiple PrP peptides by MAP, antigenic heterogeneity of the peptide might contribute to the disruption of immune tolerance. On the other hand, Magri *et al.* showed that three different peptides corresponding to the hamster PrP residues 105-128, 119-146, and 142-179, each of which was conjugated with keyhole limpet hemocyanin alone, were immunogenic even in hamsters, inducing autoantibodies reactive with the corresponding free peptides and slightly prolonging survival times in the immunized hamsters after inoculation with the 263K prions [62].

Heat shock proteins (Hsps) have been reported to exert a strong adjuvant effect when coupled to an antigen [63, 64]. The antigens coupled to Hsps are captured by antigen-presenting cells via specific cellular surface receptor and displayed on cell surface MHC class I, stimulating immune responses [63, 64]. Koller *et al.* reported that PrP conjugated with DnaK, a member of the heat shock protein 70 family, successfully induced anti-PrP auto-antibodies in BALB/c mice [65].

Gilch *et al.* demonstrated that recombinant dimeric PrP, which consists of a tandem duplication of mouse PrP with a human or hamster-derived 3F4 epitope replaced at the corresponding region, was immunogenic when subcutaneously immunized, eliciting anti-PrP autoantibodies in C57BL/6 mice [66]. They also showed that monomeric PrP with the 3F4 epitope was similarly immunogenic in mice [66]. However, antibody repertoire induced by dimeric and monomeric PrPs were different [66]. Dimeric but not monomeric PrP could produce antibodies, which effectively inhibited PrP^{Sc} formation in persistently infected N2a cells [66]. These results provide the possibility that dimeric PrP might stimulate protective immunity against prions. Polymenidou *et al.* also showed that dimeric PrP was immunogenic in C57BL/6× 129Sv mice, inducing anti-PrP autoantibodies, but the immunization had no protective effects against intraperitoneally inoculated RML prions [67]. Dimeric PrP used by Gilch *et al.* was different from that used by Polymenidou *et al.* The former contains a human or hamster-derived 3F4 epitope [66]. It is therefore conceivable that dimeric PrP used by Gilch *et al.* might acquire heterologous PrP-like immunogenicities in part, resulting in production of anti-PrP autoantibodies with anti-prion activity. In contrast, the latter is a mouse PrP without the epitope and therefore might have failed to induce anti-PrP autoantibodies protective against the prion infection.

Enhanced Immunogenicity of PrP Expressed on Virus-Like Particles

Virus-like particles (VLPs) formed by self-assembly of virus-encoded capsid proteins are known to effectively stimulate immune responses [68]. Nikles *et al.* generated murine leukemia retrovirus-derived VLP displaying the C-terminal 111 amino acids (PrP111) fused to the transmembrane domain of platelet-derived growth factor receptor and subsequently immunized C57BL/6 mice with either PrP111-VLP or bacterially expressed recombinant PrP [69]. As a result, PrP111-VLP but not recombinant PrP could induce anti-PrP autoantibodies, which can recognize the native form of PrP^C expressed on the cell surface [69]. These results suggest that PrP111-VPL could be effective as an anti-prion vaccine. However, no experimental data for cell cultures or using animal models are available. Handisurya *et al.* also produced bovine papillomavirus type 1-derived VPL displaying the nine amino acid eitope DWEDRYYRE, of the mouse or rat PrP that was inserted into the L1 major capsid protein and used it to immunize rabbits and rats [70]. Sera of both species could react with the native form of mouse PrP^C [70]. However, the DWEDRYYRE-VPL was more immunogenic in rabbits than in rats [70]. Rabbit anti-PrP serum contained higher titers of antibodies than the rat anti-PrP serum and exhibited inhibition of *de novo* synthesis of PrP^{Sc} in infected N2a cells [70]. The corresponding amino acid sequence of rabbit PrP is different from the mouse or rat PrP peptide by one amino acid (DYEDRYYRE, the underline indicates a different amino acid) [70]. It is therefore conceivable that the augmented immune response in rabbit against DWEDRYYRE-VPL might be due to antigenic heterogeneity of the displayed peptide.

MUCOSAL VACCINES AGAINST PRION DISEASES

Mucosal Vaccines

Mucosal vaccines are able to prime a full range of local and systemic immune responses by inducing not only secretory IgA at mucosal surfaces but also serum IgG [71]. Hence, mucosal vaccines are effective against infectious diseases caused by mucosally and non-mucosally invasive pathogenic organisms. Indeed, protective efficacy of mucosal vaccines to non-mucosal pathogens such as arthropod vector-borne pathogens has been demonstrated [72-74]. Mucosal vaccines are needle-free, non-invasive, and painless

[71]. They are also safer than conventional injected vaccines by reducing the risk of infection from blood-borne pathogens, and may be cost-effective because their administration does not require highly trained personnel. Therefore, mucosal vaccines might be favorable over conventional parenteral vaccines.

Bacterial Toxins Stimulate Mucosal Immunogenicity of PrP

Bacterial toxins, such as cholera toxin (CT) or heat-labile enterotoxin (LT) of *Escherichia coli*, are the most powerful mucosal adjuvants [75, 76]. Intranasal or oral delivery of antigens admixed with these toxins elicits very strong humoral and cellular immune responses [75, 76]. The mechanism underlying such effective immunomodulating activity of these toxins is not fully elucidated however. CT and LT belong to the AB_5-type enterotoxin family, with the A subunit possessing toxic ADP-ribosyltransferase activity and the B subunit forming a non-toxic pentamer with binding affinity for receptors located on the eukaryotic cell surface [75, 76]. The immunomodulating activity of these toxins is strongly associated with their binding affinity for cell surface receptors such as G_{M1}-ganglioside on most nucleated cells including DCs [77, 78].

Bade *et al.* intranasally or intragastrically immunized BALB/c mice with recombinant mouse PrP90-231 with CT and showed that the intranasal but not intragastric immunization could stimulate IgG and IgA antibody responses against the antigen and slightly protected mice from the disease after inoculation with 139A mouse prion [79]. The immunized mice survived 266.0 dpi while non-immunized mice died at 257.5 dpi [79]. These results indicate that co-immunization with bacterial toxins are able to enhance the mucosal immunogenicity of PrP effectively but not to levels high enough to elicit protective immunity against prions.

We investigated the effects of LTB fusion on the mucosal immunogenicity of PrP in mice by generating LTB-moPrP120-231 and LTB-boPrP132-242 fusion proteins, in which the C-terminal residues 120-231 and 132-242 of mouse and bovine PrPs, respectively, were fused to the C-terminus of LTB with the hinge sequence Gly-Pro-Gly-Pro (Fig. **3A**) [80]. In contrast to the results of Bade *et al.* co-immunization of non-fused moPrP120-231 with recombinant mutant non-toxic LT into nasal cavities of BALB/c mice induced no IgG antibody response (Fig. **3B**) [80]. However, LTB-moPrP120-231 fusion protein elicited slightly but significantly higher antibody responses in mice (Fig. **3B**) [80], indicating that fusion with LTB could enhance the mucosal immunogenicity of PrP. We also immunized mice with LTB-boPrP132-242 fusion protein as well as non-fusion boPrP132-242. BoPrP132-242 itself moderately elicited IgG antibody response in mice probably due to its antigenic heterogeneity (Fig. **3C**) [80]. However, no IgA response could be detected in the mice (Fig. **3C**) [80]. In contrast, the mucosal immunogenicity of LTB-boPrP132-242 was markedly enhanced in mice, producing much higher titers of anti-boPrP IgG in serum and anti-boPrP IgA in serum (Fig. **3C**) [80]. The specific IgA was also abundantly secreted in the intestines [80]. These results indicate that fusion with LTB could markedly augment the mucosal immunogenicity of bovine PrP in mice. However, the efficiency of LTB-boPrP132-242 to induce antibodies crossreactive with mouse PrP was very low (Fig. **3B**) [80].

Attenuated *Salmonella* Vector Expressing PrP Elicits Protective Mucosal Immunity Against Prions

Live-attenuated pathogenic *Salmonella*, Bacillus Calmette-Guérin and *Bordetella* as well as commensal lactobacilli or certain streptococci and staphylococci are efficient mucosal delivery vectors of antigens [81]. Goñi *et al.* produced an attenuated *Salmonella typhimurium* LVR01 LPS vaccine strain expressing mouse PrP fused with non-toxic fragment C of tetanus toxin and orally immunized CD-1 mice with it [82]. The orally immunized mice elicited higher IgG and IgA antibody responses against PrP [82]. Interestingly, about 30% of the immunized mice were alive without any clinical signs up to 500 days post oral-infection with 139A mouse prion [82]. More interestingly, the authors subsequently showed that all of the mice producing high titers of anti-PrP IgG and IgA antibodies were completely resistant to the prion, being free of any disease-specific symptoms up to at least 400 days post oral-infection [83]. Neither specific pathological changes nor accumulation of PrP^{Sc} could be detected in their brains [83]. In contrast, no significant extension of the survival times could be observed in mice producing lower titers of anti-PrP antibodies [83]. These results indicate that the *Salmonella* delivery system for PrP could stimulate protective immunity against the disease.

Salmonella is commensal enteric bacteria. It is therefore possible that PrP expressed by the vaccinated *Salmonella* might be produced continuously in a large amount in the gut for a considerably long period, thus efficiently taken into the epithelium and eliciting high immune responses in the immunized mice. In this system, PrP is expressed from the attenuated *Salmonella* vaccine as a fusion protein with the non-toxic fragment C of tetanus toxin, which is a highly immunogenic molecule [83]. It is thus alternatively possible that fusion with fragment C might increase the mucosal immunogenicity of PrP. Development of a more effective system, which is capable of eliciting powerful immune responses against PrP more consistently in immunized individuals, is awaited with great anticipation.

PROSPECTS FOR PRION VACCINES

I have mainly focused on the attempts so far reported of how immune tolerance to PrP can be overcome and elicit protective immune responses against prion diseases. Immunization of heterologous PrPs and modified PrPs, including dimeric PrP or VLP-displaying PrPs, co-immunization with immune modulators such as CpG, and *Salmonella* vector-mediated delivery of PrP were effective, stimulating immune responses against PrP and producing anti-PrP autoantibodies in mice. These results thus may indicate that, if more efficient ways to disrupt tolerance to PrP are developed, more effective prion vaccines could be possible. In contrast, since the reported vaccines showed only inadequate disruption of the tolerance, resulting in marginal or partial effects on prion infection in animal models, it may be alternatively suggested that PrP-targeting prion vaccines might be difficult to develop. In addition, in the case of Alzheimer's disease vac-

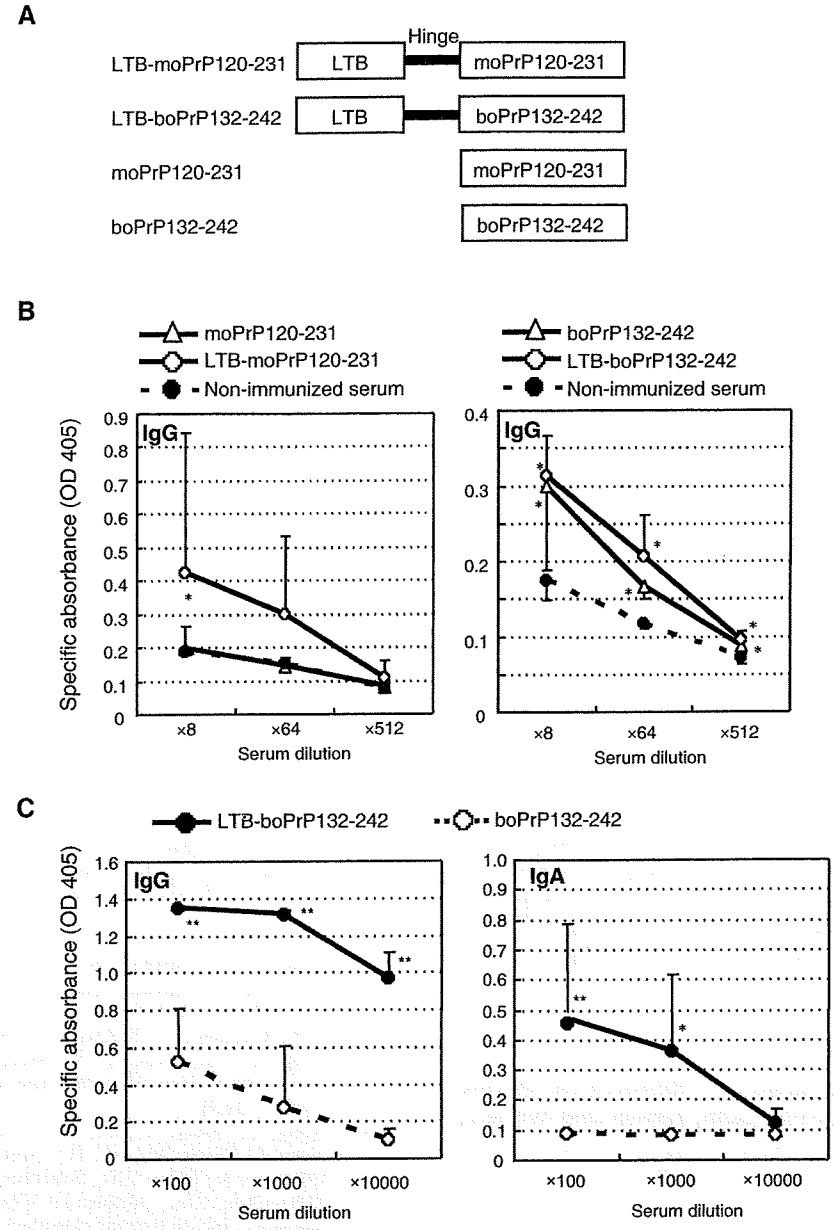

Figure 3. Enhancement of mucosal immunogenicity of PrPs following fusion with LTB. **(A)** Structure of moPrP120-231 and boPrP132-242 immunogens fused with or without LTB. **(B)** Anti-moPrP IgG autoantibodies in the immunized mice. The antisera were collected from 4-5 mice from each group after 6 intranasal immunizations at 2-week intervals and subjected to ELISA against moPrP. **(C)** Specific IgG and IgA antibody responses against boPrP were much stronger in mice intranasally immunized with LTB-boPrP132-242 than in those given boPrP132-242 three times at 2-week intervals. Data were analyzed using the Mann-Whitney U-test. Data were represented by mean±SD. *, $p<0.05$; **, $p<0.01$. Reprinted in part from [80]. Copyright (2006), with permission from Elsevier.

cines using the Aβ amyloid peptide derived from the host molecule amyloid precursor protein as an antigen, no adverse effects were reported in immunized mice but severe encephalitis was observed in immunized people [84, 85]. Thus, PrP-targeting vaccines might also have potential adverse effects, including autoimmune-related pathologies, due to the resulting autoantibodies against PrP^C in humans although no abnormal symptoms have been reported in mice vaccinated with PrPs or passively immunized with anti-PrP antibodies [31, 46, 48]. Moreover, it was reported that neurons markedly succumbed to apoptosis in mice when anti-PrP antibodies were directly administrated into the hippocampus of mice [86]. Therefore, rather than vaccines potentially stimulating antibody response against PrP^C, other types of prion vaccines might be favorable.

PrP^C and PrP^{Sc} have different structures. PrP^C contains higher α-helix and much less β-sheet contents [87]. In contrast, PrP^{Sc} is formed by higher contents of β-sheet strands [87]. It is therefore conceivable that PrP^{Sc}-specific epitopes or conformation could be potential targets for prion vaccines not to stimulate production of anti-PrP^C autoantibodies. Cashman and colleagues reported that a PrP-derived Tyr-

Tyr-Arg peptide conjugated with keyhole limpet hemocyanin could efficiently elicit antibodies specifically reactive with PrP^{Sc} but not with PrP^{C} in immunized animals [88]. It is suggested that the epitope is normally buried in PrP^{C} but becomes exposed on the outside of PrP^{Sc} due to structural alteration of PrP^{C} into PrP^{Sc} [88]. Thus, these results indicate that PrP^{Sc}-specific vaccines might be possible using PrP^{Sc}-specific epitopes or conformation. However, it remains to be investigated whether or not the Tyr-Tyr-Arg vaccine is effective against prion infection.

It is already known that, when two different prion strains infect a single host, one strain interferes with the other. Dickinson *et al.* first reported that mice intracerebrally inoculated with the long-incubation-period scrapie 22C prion prior to intracrebral superinfection with the short-incubation-period scrapie 22A prion developed the disease later than control mice inoculated with the 22A prion alone [89]. Similar interference could be subsequently detected between other strains. Manuelidis showed that intracerebral inoculation with low infectious doses of a sporadic CJD-derived SY prion interfered with superinfection of more virulent GSS-derived Fukuoka-1 prion in CD-1 mice [90]. It was further shown that the long-incubation-period transmissible mink encephalopathy (TME) DY prion prevented superinfection of short-incubation-period HY TME prion in hamsters [91]. Recently, it was demonstrated that this unique interference could be observed in cultured mouse hypothalamic GT1-7 cells between SY CJD prion and Chandler or 22L scrapie prion, and 22L and Fukuoka-1 prions, clearly indicating that this interference does not require any immune responses [92]. Therefore, understanding of the mechanism of prion interference might open a new avenue for a novel type of prion vaccine.

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プリオン病と治療戦略の最近の動向

A Systematic Review of the Therapeutics for Prion Diseases

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Abstract

Prion diseases are fatal infectious neurodegenerative disorders; examples include the Creutzfeldt-Jakob disease affecting humans and bovine spongiform encephalopathy in cattle. The causative agents of these diseases—the prions—are thought to consist of the pathogenic isoform of the prion protein PrP^{Sc}, which is produced by the conformational conversion of the normal isoform PrP^{C}. Many lines of evidence indicate that the constitutive conversion of PrP^{C} to PrP^{Sc}, resulting in a marked accumulation of PrP^{Sc} in the brain, is a central event in the pathogenesis of prion diseases. A large number of compounds have been identified as anti-prion agents and capable of reducing the PrP^{Sc} levels in infected cells. Some of these compounds have been found to be partially effective in infected animals, thus resulting in the prolongation of the incubation or survival times and a few of these compounds were or are under clinical trials. However, none of these compounds have proven to be therapeutically effective against this group of diseases. This is probably because (1) these compounds fail to cross the blood-brain barrier and (2) their effectiveness is reduced because they are administered only to patients with clinically advanced disease owing to a lack of diagnostic indicators for presymptomatic individuals. In this communication, we systematically list these anti-prion compounds and summarize their effectiveness and possible mechanisms of action.

Key words：prion disease, prion, prion protein, therapy, neurodegeneration

はじめに

プリオン病は，“プリオン”の脳内増殖により起こるヒトおよび動物の神経変性疾患である[1]。動物プリオン病のプロトタイプであるヒツジのスクレーピー（scrapie）はヒトに感染しないことがこれまでの疫学的調査から示唆され，動物からヒトへの感染にはいわゆる“種の壁”といわれる大きなバリアーが存在すると考えられてきた[2]。しかし，英国で大量に発生した牛海綿状脳症（bovine spongiform encephalopathy：BSE，または狂牛病）がヒトに感染し，新型クロイツフェルト・ヤコブ病（new variant Creutzfeldt-Jakob disease：nvCJD）を引き起こすという事実は[3,4]，あるタイプの動物プリオン病は種の壁を乗り越えてヒトに感染する可能性を示した。また英国では，nvCJDに感染しているヒトの血液を輸血されたヒトが後にnvCJDを発病した例が4例報告され，nvCJDが血液を介しヒトに2次感染する可能性も示された[5]。しかし，プリオン病に対する有効な治療法は開発されていない。

本稿では，プリオンおよびプリオン病について概説するとともに，プリオン病の治療開発の現状について紹介する。

Ⅰ．プリオン蛋白質とプリオン

1．プリオン蛋白質：2つの構造アイソフォーム

プリオン蛋白質（prion protein：PrP）には，アミノ酸構造はまったく同じであるにもかかわらず，構造が異なる2つの構造アイソフォームが存在する[6]。正常プリ

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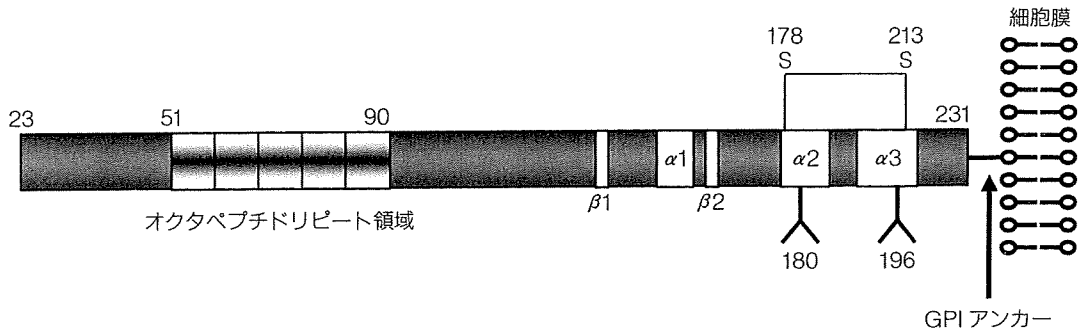

Fig. 1　PrP^{C} の蛋白構造

マウス PrP^{C} は 254 個のアミノ酸からなる前駆体蛋白として翻訳された後，シグナルペプチドのN末 22 個のアミノ酸と GPI アンカーシグナルのC末 23 個のアミノ酸は小胞体内で切断され，アミノ酸 23〜231 からなる成熟したマウス PrP^{C} が GPI アンカーを介して細胞膜表面に発現する。成熟 PrP^{C} のN末領域はランダムコイル構造で，PrP に特有な 8 個のアミノ酸〔P（H/Q）GG（G/−）WGQ〕が 5 回繰り返したオクタペプチドリピート領域が存在する。一方C末領域は，2 つの短い β シート構造と 3 つの α ヘリックス構造を有し球状構造を形成している。2 番目と 3 番目の α ヘリックスはジスルフィド結合で連結する。また，N型糖鎖結合が 2 カ所に存在する。β1（アミノ酸 127〜130）と β2（アミノ酸 160〜163）は β シート構造領域を，α1（アミノ酸 143〜153），α2（アミノ酸 178〜192）と α3（アミノ酸 199〜216）は α ヘリックス構造領域を示している。数字はアミノ酸番号を示す。

Table 1　PrP^{C} と PrP^{Sc} の蛋白質構造および生化学的特質の違い

	蛋白質構造		生化学的特質	
PrP	α ヘリックス	β シート	溶解性	プロテイナーゼK抵抗性
PrP^{C}	42%	3%	易溶性	感受性
PrP^{Sc}	30%	43%	難容性	抵抗性

オン蛋白質（cellular PrP：PrP^{C}）と異常プリオン蛋白質（scrapie PrP：PrP^{Sc}）である[6]。PrP^{C} は，グリコシルフォスファチジルイノシトール（glycosylphosphatidylinositol：GPI）を介して細胞膜表面に結合する糖蛋白質である[6]。ランダム構造のN末領域と，3 つの α ヘリックスと 2 つの β シートからなる球状構造のC末領域とからなる（Fig. 1）[7]。N末領域には，グリシン（G）に富んだ 8 個のアミノ酸が 5 回繰り返したオクタペプチドリピート（octapeptide repeat：OR）領域が存在する（Fig. 1）[6]。この領域は PrP に特異的な配列であるが，その機能は十分に解明されていない。

PrP^{C} は多くの正常組織に発現するが，中枢神経，特に神経細胞に最も強く発現する[8]。一方，PrP^{Sc} はプリオン病に特異的に検出される蛋白質で，感染脳内に過剰に蓄積する[8]。PrP^{C} が何らかの原因で構造変化をきたし産生される。PrP^{C} は α ヘリックス構造に富み，β シート構造が少ない（α ヘリックス 42%，β シート 3%，Table 1）[9]。一方 PrP^{Sc} では，β シート構造が著明に増加している（α ヘリックス 30%，β シート 43%，Table 1）[9]。したがって，β シート構造への変化が PrP^{C} から PrP^{Sc} への変化に重要であると考えられている。

PrP^{C} と PrP^{Sc} は生化学的特質も異なる。PrP^{C} は易溶性で，蛋白質分解酵素であるプロテイナーゼKにて完全に分解される[8]。しかし，PrP^{Sc} は難容性で凝集体を形成しやすく，プロテイナーゼKに対して抵抗性で消化されにくい（Table 1）[8]。このプロテイナーゼKに対する感受性の違いを利用して PrP^{Sc} が検出され，プリオン病の診断が行われている。

2．プリオン

「プリオンは PrP^{Sc} のみから構成されている」とするプリオン仮説または蛋白質唯一仮説（protein-only hypothesis）が，広く受け入れられている[10]。実際，精製プリオンの分画には PrP^{Sc} 以外の蛋白質やプリオン特異的核酸は検出されていない[11]。また，PrP 遺伝子欠損（PrP−/−）マウスはプリオンを脳内に接種されてもプリオンに感染せず，プリオン病を発症しない[12-15]。さらに最近，試験管内で作製された PrP^{Sc} 様の PrP が感染性を有し，マウスにプリオン病を起こすことが報告されている[16]。

またこの仮説によると，プリオンは体内に侵入するとその構成成分である PrP^{Sc} が細胞表面の PrP^{C} に結合し，PrP^{C} の高次構造を PrP^{Sc} へと変化させ，PrP^{C} から新たな PrP^{Sc} が産生されると考えられている[10]。新たに産生された PrP^{Sc} は，次の PrP^{C} に作用し PrP^{Sc} へと変換させる。こうして，PrP^{Sc} が次から次へと産生され，プリオンは増殖するとされている（Fig. 2）[10]。

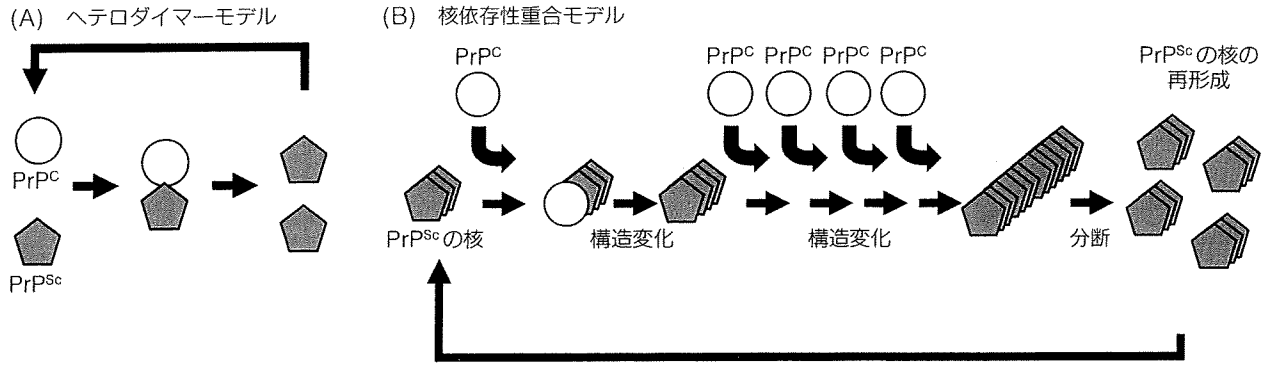

Fig. 2 プリオン複製モデル

A：1分子の PrP^{Sc} が1分子の PrP^{C} とヘテロダイマーを形成し，何らかの機構を通して PrP^{C} が構造変化を起こし，PrP^{Sc} へと変換する。新しく産生された PrP^{Sc} は，再び1分子の PrP^{C} を PrP^{Sc} へと変換させる。このようにして PrP^{Sc} が産生され，プリオンは複製する。B：PrP^{Sc} は複数個重合し，核(seed)を形成する。PrP^{C} は，この核に順次重合することにより，構造変化を起こし PrP^{Sc} へと変換する。こうしてできた長い PrP^{Sc} のポリマーは分断され，新たな PrP^{Sc} の核が形成され，プリオンの増殖が起こる。

Table 2 成因別によるヒトプリオン病の分類

分類	成因（頻度）	疾患名
孤発性プリオン病	不明 (85～90%)	孤発性クロイツフェルト・ヤコブ病
遺伝性プリオン病	PrP 遺伝子変異 (5～15%)	家族性クロイツフェルト・ヤコブ病 ゲルストマン・ストロイスラー・シャインカー症候群 致死性家族性不眠症
感染性プリオン病	プリオン感染	医原性プリオン病（脳下垂体ホルモン投与，角膜移植，脳硬膜移植，深部脳波電極の挿入，輸血） 新型クロイツフェルト・ヤコブ病（BSE からの感染） クールー（食人慣習による感染）

では，プリオンは PrP^{Sc} からどのようにできているのだろうか。ヘテロダイマーモデルでは，プリオンは1分子の PrP^{Sc} からできていると考えられている（Fig. 2 A）[17]。一方，複数の PrP^{Sc} が重合し，感染性のプリオンを構築しているという考えも提唱されている（核依存性重合モデル，Fig. 2 B）[18]。Silveira らは，14～28個の PrP^{Sc} が重合した高分子を含む分画が，5個以下の PrP^{Sc} が重合した分子を含む分画よりも感染性が高いことを示し，後者のモデルを支持した[19]。

II. プリオン病

1. ヒトプリオン病

ヒトプリオン病は，中年以降に発病する，有病率が人口100万人対1という稀な疾患である[20]。その大部分(85～90%)を原因不明の孤発性クロイツフェルト・ヤコブ病（sporadic CJD）が占めている（Table 2）[20]。初発症状として，歩行障害，性格変化，認知障害などが認められる。進行は早く，多くは2年以内に死亡する。病変は中枢神経内に限局している。病理学的所見として，多数の空胞が脳全体に認められる[20]。また，神経細胞変性死やグリオーシス（活性化したアストロサイトやミクログリアの増生）が認められる[20]。

ヒトプリオン病の5～15%は PrP 遺伝子に変異を有し，常染色体優性遺伝型式を示す遺伝性プリオン病である（Table 2）[20]。変異の位置により，病型が異なる（Fig. 3 A-C）。遺伝性プリオン病として，家族性クロイツフェルト・ヤコブ病（familial CJD），ゲルストマン・ストロイスラー・シャインカー症候群（Gerstmann-Sträussler-Scheinker syndrome：GSS），致死性家族性不眠症（fatal familial insomnia：FFI）が知られている（Table 2）。GSS は慢性の小脳失調が続いた後に，認知障害が現れる疾患である。FFI は不眠，自律神経障害，運動機能障害などをきたす。

残りのわずか数パーセントが，感染が原因であると特定できる感染性プリオン病である[20]。この中には，BSE の感染による nvCJD や，パプアニューギニアのフォア族による食人慣習により感染したクールーがある。また，

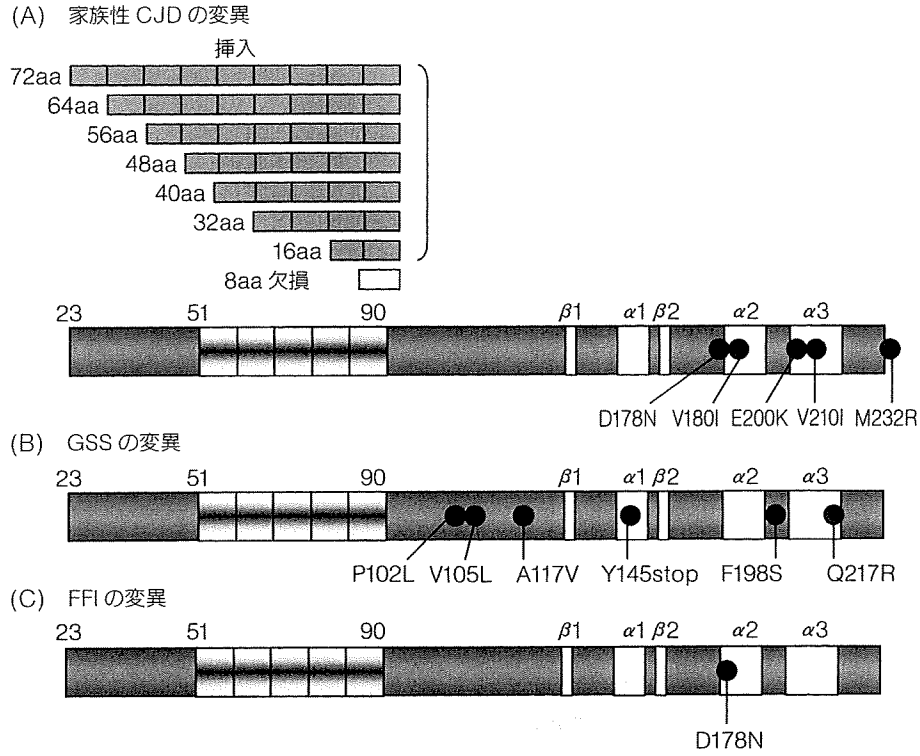

Fig. 3　遺伝性プリオン病の PrP 遺伝子変異

A：家族性 CJD の PrP 遺伝子変異には，オクタペプチドリピート領域にそれぞれ 2，4，5，6，7，8，9 個のオクタペプチドに相当する 16，32，40，48，56，64，72 個のアミノ酸の挿入や，1 個のオクタペプチドに相当する 8 個のアミノ酸の欠損，および C 末領域のアミノ酸変異が報告されている。B：GSS の PrP 遺伝子変異によるアミノ酸置換。C：FFI の PrP 遺伝子変異によるアミノ酸置換。黒丸は変異の場所を，番号はスタートコドンからのアミノ酸番号を，大文字のアルファベットはそれぞれのアミノ酸を表している。

医療行為を介して感染した医原性プリオン病も知られている。プリオンに汚染した脳下垂体ホルモンの投与，角膜の移植，深部脳波電極の挿入，そして脳硬膜の移植による医原性プリオン病が報告されている。わが国でも，硬膜移植による CJD が報告されている。また，nvCJD が輸血を介して感染したケースも報告されている（Table 2）[5]。

2．BSE と“種の壁”

プリオンの感染には，動物種を超えた感染は起こりにくいという“種の壁”が存在する[21]。例えばハムスターで継代されたプリオンは，ハムスターには容易に感染するのに対し，マウスには非常に感染しにくい。しかし，継代を重ねると種の壁は消失し，潜伏期は短くなる。

BSE はスクレーピーと異なり，種の壁を乗り越えてヒトに感染し，nvCJD を引き起こしたと考えられている。おそらく，BSE プリオン，つまりウシ PrP^{Sc} がヒト PrP^{C} に親和性を示しヒト PrP^{C} を PrP^{Sc} に変化させることができ，種の壁を乗り越えたと考えられる（Fig. 4 A）。一方，ヒツジ PrP^{Sc} はヒト PrP^{C} に親和性がないために，種の壁を乗り越えられないと考えられる（Fig. 4 B）。しかし実際は，ウシからウシの BSE プリオンの感染と比べるとウシからヒトへの感染効率は非常に低く，実質的な種の壁が存在したと考えられる。なぜなら，英国では，これまでに約 20 万頭弱の牛が BSE に感染したと報告されているにもかかわらず，約 160 ケースの nvCJD が報告されているだけである（http://www.cjd.ed.ac.uk/figures.htm/）。したがって，ウシ PrP^{Sc} とヒト PrP^{C} の親和性は弱いと考えられる。

BSE が種の壁を乗り越えて最も問題となるのは，種の壁を乗り越えて増殖したプリオンにはもはや種の壁は存在しないことである。つまり，nvCJD プリオンはヒトに容易に感染するということである。BSE 感染時のプリオンはウシ PrP^{Sc} であったが，新たに産生されたプリオンはヒト PrP^{Sc} であるため，ヒト PrP^{C} に強い親和性を持つようになるためである（Fig. 4 C）。実際，英国では，nvCJD に感染しているヒトの血液を輸血されたヒトが，後に nvCJD を発病した例が 4 例報告されている[5]。

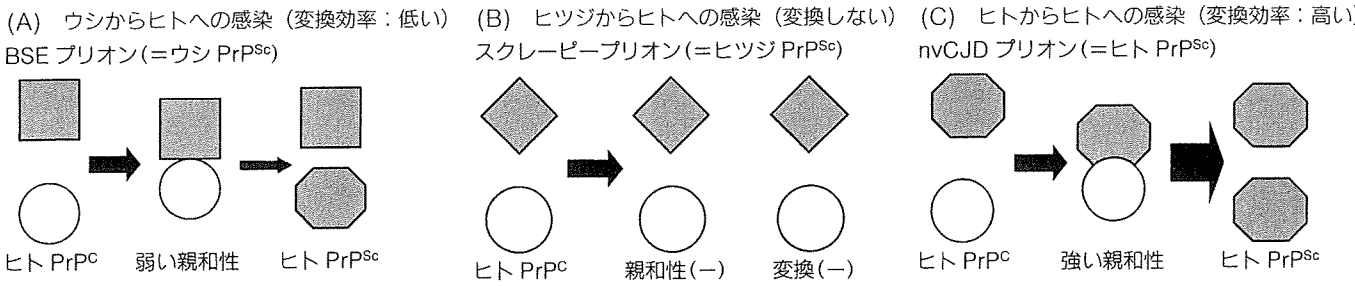

Fig. 4　PrP^{Sc} の産生メカニズム（ヘテロダイマーモデルによる）と種の壁

A：BSE プリオン，つまりウシ PrP^{Sc} はヒト PrP^{C} に低いながらも親和性を有し，ヒト PrP^{C} を PrP^{Sc} に変化させる。こうして，BSE プリオンは種の壁を乗り越え，ヒトに感染する。B：スクレーピープリオンつまりヒツジ PrP^{Sc} は，ヒト PrP^{C} に親和性がないために種の壁を乗り越えられず，ヒトに感染しない。C：BSE プリオンの感染で産生された nvCJD プリオンはヒト PrP^{Sc} であるため，ヒト PrP^{C} に強い親和性を持ち，ヒトに容易に感染する。

3．感染性プリオン病の拡大の危険性

感染性プリオン病の最も多いケースが医療行為を介した医原性プリオン病である。この主な理由は，①プリオン病が発症までに非常に長い潜伏期を有するために，プリオン病に感染しているが発病していないキャリアーが存在すること，②現時点ではプリオン病の早期診断，特に発症前診断ができないために，キャリアーの診断ができないことが挙げられる。このために，キャリアーから精製された脳下垂体ホルモンの投与，キャリアーから摘出された角膜や脳硬膜の移植，キャリアーに使用された深部脳波電極の挿入等の医療行為により感染したケースが報告されている[20]。また最近は，医療の高度化に伴い，臓器移植などが盛んに行われている。いまだ臓器移植によるプリオン病の感染は報告されていないが，注意が必要であると考えられる。さらに，血液による新たな感染性プリオン病の出現は，これまで以上のプリオン感染の広がりの危険性を示唆している。北米では，多数の野生および家畜のシカにプリオン病である慢性消耗性疾患（chronic wasting disease：CWD）が発生し，狂牛病と同様にヒトに感染するのか，また新型のプリオンが出現するのか，大きな問題となっている[22]。

III．標的治療戦略

プリオン病の治療ターゲットはプリオンである。いかにプリオンの産生を抑制するかが，治療のポイントとなる。プリオンは，PrP^{C} が PrP^{Sc} へ変換することにより産生される。したがって，PrP^{Sc} の産生過程のいずれかのステップを阻害し，PrP^{Sc} の産生を抑制することが，プリオン病の治療戦略となる。

PrP^{C} が PrP^{Sc} へ変化するには，PrP^{Sc} が PrP^{C} に結合することが必須である。PrP^{Sc} は細胞膜の脂質ラフトから早期エンドソームまでのエンドサイトーシス小胞に最も多く検出されることから，これらの部位で両者は結合し，PrP^{C} が PrP^{Sc} へ変換していると考えられている[23]。また，PrP と結合するグリコサミノグリカンや 36/67 kDa ラミニン受容体などの PrP 以外の分子も，この変換に重要な役割を担っていることが示唆されている[24]。

これまでに，プリオン感染細胞において多くの薬剤が PrP^{Sc} の産生を抑制することが報告されている[25,26]。しかし，PrP^{C} から PrP^{Sc} への変換のメカニズムが十分に解明されていないため，多くの薬剤が PrP^{Sc} 産生のどのステップに作用しているのか不明である。以下には，これまでに報告された抗プリオン薬剤を，それぞれの特質に応じて分類し，それぞれの抗プリオン効果について紹介する。

1．遺伝子サイレンシング療法

PrP−/−マウスは，PrP^{C} を完全に欠損するため，プリオンを接種されても PrP^{Sc} が産生されず，プリオン病にならない[12-15]。したがって，PrP^{C} の発現を抑制することは，プリオン病の治療となる。実際，コンディショナルノックアウトマウスを用いた実験において PrP 遺伝子をプリオン感染中期に排除すると，マウスはプリオン病にならないことが報告されている[27]。しかし，現状では生体内で遺伝子をノックアウトすることは困難である。

Small interfering RNA（siRNA）はターゲット遺伝子の mRNA を特異的に切断し，蛋白質の発現を抑制する。したがって，PrP 遺伝子をターゲットとした siRNA はプリオン病に有効であると考えられる。実際，感染細胞に PrP 特異的 siRNA を導入すると，PrP^{C} の産生が低下し，PrP^{Sc} の産生も抑制されることが報告されている[28]。Pfeifer らは，PrP 特異的 short hairpin RNA（shRNA）を産生するレンチウイルスベクターをマウス

脳内に接種した結果，ウイルス接種部位とその周辺に限局して PrP^C の発現が低下することを報告した[29]。しかしプリオン病では，脳の広い範囲がプリオンに感染し障害を受ける。したがって，レンチウイルスベクターを用いたsiRNAによるプリオン病の治療には限界があると考えられる。これに関しては，今後より有効なデリバリーシステムの開発が望まれる。

また，PrP−/−マウスが学習・記憶，日内周期，睡眠などに異常をきたすことや[30]，末梢神経および脊髄に脱髄を起こすことから[30]，PrP^C の発現を抑制する治療法の開発においては，これらの副作用を十分に考慮する必要がある。

2．抗体療法

抗PrPモノクロナル抗体（anti-PrP mAb）がプリオン感染細胞の PrP^{Sc} 産生を抑制することが報告され，プリオン病の抗体治療の可能性が示された[31,32]。抑制のメカニズムは不明である。抗体が PrP^C と PrP^{Sc} との結合を阻害するために，PrP^{Sc} の産生が抑制されるのかもしれない。また，抗体が PrP^C のエンドサイトーシスを抑制することも報告され[33]，PrP^{Sc} の産生サイトの1つであるライソゾームに PrP^C が移動できないため，PrP^{Sc} の産生が抑制される可能性も考えられる。さらに，抗体により PrP^C の分解が促進されることが報告されており[34]，PrP^C が少なくなるために PrP^{Sc} の産生が抑制される可能性も考えられる。

Songらは31C6 anti-PrP mAbをプリオン感染後期のマウスの脳室内に直接に28日間持続注入した結果，生存期間がわずかに約10日延長することを報告した[35]。一方われわれは，3S9 anti-PrP mAbをプリオン感染マウスの脳内に持続注入すると，潜伏期および生存期間が延長する傾向にあるが，有意差を持って延長しないことを見出した（未発表）。抗体は高分子であるため，anti-PrP mAbを脳室内に直接に注入しても治療のターゲットとなる領域まで浸透できず，治療効果を十分に発揮できなかった可能性が考えられる。このことから，分子量のより小さいFabフラグメントやscFv抗体がより高い治療効果を発揮する可能性が示唆される。実際，Lefebvre-Roqueらは，脳室内に注入した $F(ab')_2$ フラグメントが脳の広範囲な領域に浸透することを報告している[36]。

しかし，anti-PrP mAbによっては，神経細胞死をきたすことが報告されている。Solforosiらは，D13やP anti-PrP mAbsをマウス脳内に注入すると神経細胞死が起こることを報告した[37]。また，Lefebvre-Roqueらは，4H11 anti-PrP mAbやその $F(ab')_2$ フラグメントが同様に神経細胞死を起こすことを報告した[36]。おそらく，anti-PrP mAbの結合部位の違いによって抗プリオン効果を発揮するのか，神経細胞死を誘導するのかが決定されると考えられる。以上から，プリオン病の抗体治療に当たっては，抗体の選択に注意が必要である。

3．PrP-Fc_2

PrP^C をIgG抗体のFc部分に融合させた PrP-Fc_2（2分子の PrP^C を有する）が，抗プリオン効果を有することが報告された[38]。Meierらは，この分子を発現するトランスジェニックマウスを作製しプリオンを感染させた結果，内因性の PrP^C が PrP^{Sc} へ変換するのが阻害され，潜伏期が著明に延長することを報告した[38]。しかしGenoudらは，PrP-Fc_2 を発現するレンチウイルスを作成し感染マウスの脳内に注入した結果，潜伏期が延長するが，注入時期がプリオン感染の早期でないと効果が低いことを報告している[39]。

PrP-Fc_2 は PrP^C の部分で PrP^{Sc} と結合するが，Fcが融合しているために構造変化が起こりにくく，PrP^{Sc} へと変換できない[38]。したがって，PrP-Fc_2 は内因性の PrP^C と PrP^{Sc} を競合し，PrP^{Sc} の産生を抑制すると考えられる。

4．ポリアニオン化合物

最初に抗プリオン活性が報告された薬剤が，ポリアニオン化合物であるヘテロポリアニオン-23である[40]。その後，多くのポリアニオン化合物に，抗プリオン活性が検出されている。これらの中には，カラギーナン，硫酸デキストラン，ペントサン・ポリサルフェート，グリコサミノグリカン，ヘパラン硫酸，スラミン，および1本鎖ホスホロチオエートオリゴヌクレオチドやRNAアプタマーなどの核酸が知られている[25,26]。これらの薬剤は，PrP^{Sc} の産生に関与すると考えられているグリコサミノグリカンと類似の構造を有することから，内因性のグリコサミノグリカンと競合し，PrP^{Sc} の産生を抑制すると考えられている。スラミンは，PrP^C に構造の変化をもたらしたり，小胞体／ゴルジ装置内に PrP^C をとどめることにより，PrP^C の細胞膜表面への輸送を阻害することが報告されている[41]。したがって，ポリアニオン化合物の抗プリオン効果のメカニズムの1つとして，PrP^C の構造を変化させ PrP^{Sc} へ変換できなくさせたり，PrP^C の細胞内局在を変化させることにより PrP^{Sc} への変換を抑制することが考えられる。

5．ポリカチオン化合物

抗プリオン活性を示すポリカチオン化合物として，ポリアミドアミドやポリプロピレンイミンおよびポリエチレンイミンの分枝ポリマー，リン陽イオン含有デンドリマー，さらに陽イオン性多糖類などが報告されている[25,26]。PrP^{Sc}の産生抑制メカニズムは不明であるが，ポリプロピレンイミンをPrP^{Sc}と試験管内で反応させると，PrP^{Sc}のプロテイナーゼK抵抗性が低下し分解されることが報告されている[42]。このことから，これらの化合物はPrP^{Sc}の構造を変化させ，細胞内での分解を促進させることにより，PrP^{Sc}の産生を抑制する可能性が指摘されている。

6．アミロイド結合化合物

PrP^{Sc}はアミロイド凝集体を形成する。そこで，アミロイド結合化合物であるコンゴレッドの抗プリオン活性が感染細胞を用いて調べた結果，PrP^{Sc}の産生が抑制されることが判明した[43]。その後，トリパンブルー，エバンスブルー，シリウスレッドF3B，ピリムリン，チオフラビンSなどのアミロイド結合化合物も抗プリオン活性を有することが報告された[25,26]。コンゴレッドをPrP^{Sc}アミロイド凝集体と反応させると，PrP^{Sc}のプロテイナーゼK抵抗性がより高まることが示された[44]。したがって，これらの化合物はPrP^{Sc}の構造を過剰に安定化させることにより，プリオンとしての機能を失活させ，PrP^{Sc}の産生を抑制するのではないかと考えられる。

Ishikawaらは，血液脳関門を通過するコンゴレッド類似の化合物BF-168を作製し，プリオン感染マウスに静脈内投与することにより，わずかであるが潜伏期が延長することを報告した[45]。また同グループは経口投与可能な化合物cpd-Bの合成にも成功し，プリオン感染直後から発症までの期間を通して投与することにより，プリオン病の発症が有意に遅延することも報告している[46]。

7．四環系化合物とテトラサイクリン系抗生物質

四環系の抗生物質であるテトラサイクリンやドキシサイクリン，また抗癌剤として使用されている四環系化合物ドキシルビシンやその誘導体アントラサイクリン4'-イオド-4'-ドキシルビシンが，抗プリオン活性を有することが報告された[25,26]。これらの化合物は，コンゴレッドと類似した構造を有し，PrP^{Sc}のアミロイド凝集体と結合する。しかし，コンゴレッドと異なり，これらの化合物はPrP^{Sc}のプロテイナーゼK抵抗性を低下させる[47]。以上から，これらの化合物はPrP^{Sc}の構造を不安定化させ分解されやすくし，抗プリオン活性を発揮すると考えられる。

8．抗マラリア製剤

抗マラリア薬として使用されているキナクリンやメフロキンが，抗プリオン活性を有していることが報告された[25,26]。これらの化合物は，PrP^{Sc}の産生サイトの1つであるライソゾームをターゲットとすることが知られている。詳細な抗プリオン活性のメカニズムは不明である。

9．コレステロール代謝関連薬剤

抗真菌薬であるアンフォテリシンBやコレステロールの合成阻害剤であるスタチン類が抗プリオン活性を有し，感染細胞のPrP^{Sc}産生を抑制することが報告されている[25,26]。アンフォテリシンBはコレステロールと結合し，細胞膜，特にコレステロールを豊富に含んでいる脂質ラフトの性状に変化をもたらす可能性が指摘されている。また，スタチン類は脂質ラフトの構成に重要なコレステロールの産生を阻害することにより，脂質ラフトの性状を変える。脂質ラフトは，PrP^{Sc}の産生サイトの1つである。したがって，これらの薬剤は脂質ラフトの性状を変化させることにより，抗プリオン活性を発揮すると考えられている。

10．テトラピロール系化合物

ポルフィリンやフタロシアニンはテトラピロール系の化合物で，ヘムとクロロフィルを有する。Caugheyらは，これらの化合物が抗プリオン活性を有し，感染細胞のPrP^{Sc}産生を抑制することを報告した[48]。ポルフィリンやフタロシアニンは蛋白質に結合し，その構造に変化をもたらすことが知られており，これらの化合物はPrP^{Sc}に結合してPrP^{Sc}の構造を変化させ，プリオン感染性を低下させる可能性が示唆されている。

11．シグナル伝達阻害剤

Ertmerらは，慢性骨髄性白血病の治療薬として使用されているc-Abl蛋白質キナーゼ阻害剤のイマチニブが抗プリオン活性を有し，感染細胞のPrP^{Sc}産生を抑制することを報告した[49]。またNordstromらは，mitogen-activated protein kinase kinase（MEK）1/2の阻害剤が，同様に感染細胞のPrP^{Sc}産生を抑制することを報告した[50]。これらの阻害剤で処理された感染細胞では，PrP^{Sc}の分解が促進していることが報告されている[49,50]。したがって，これらの阻害剤はPrP^{Sc}の分解経路を活性化し，抗プリオン活性を発揮している可能性が考えられる。

12. PrP^C 構造安定化剤

PrP^{Sc} が産生されるためには，PrP^C が PrP^{Sc} に変換されなければならい。したがって，PrP^C はある程度の構造不安定を有している必要がある。実際，Kuwata らは，PrP^C のあるアミノ酸残基群が構造上不安定で揺らいでいることを報告している[51]。また彼らは，これらの残基群をブリッジして安定化させるような化合物をコンピュータシミュレーションにより検索した結果，GN8 を見出し，GN8 が感染細胞の PrP^{Sc} を低下させ，またプリオン感染マウスに投与するとわずかであるが生存期間が延長するとしている[51]。

Ⅳ. 臨床トライアル

1. ペントサン・ポリサルフェート

Doh-ura らは，ペントサン・ポリサルフェートをプリオン感染マウスの脳室内に持続注入し，その治療効果を検討した。その結果，感染後期から注入を開始しても，潜伏期が有意に延長することを見出した[52]。ペントサン・ポリサルフェートは，抗血液凝固剤や抗炎症剤としてヒトに使用されており，危険性がある程度予測できるという利点を有していた。そこで，英国やわが国で，プリオン病の患者へのペントサン・ポリサルフェート脳室内持続投与が開始され，その治療効果が検討された。その結果，ペントサン・ポリサルフェートを投与された患者は，これまでに報告されているプリオン病患者より長い有病気間の後に死亡したか，または生存中であるという報告がある[53]。しかし残念ながら，未治療のコントロール患者群を設定した盲検試験でないために，その信憑性に問題が残っている。また，ペントサン・ポリサルフェートを投与された患者のほとんどに硬膜下水腫が認められることや[53]，脳出血を起こす危険性も考えられことから[53]，十分な管理の下に投与が行われる必要がある。

2. キナクリン

キナクリンは既に抗マラリア薬として使用されている薬剤である。Nakajima らは，4 人のプリオン病患者にキナクリンを経口投与した結果，治療を受けたすべての患者において一過性の認知機能の改善が認められたことを報告している。しかし，生存期間に有意な延長は認められていない[54]。英国では，現在，キナクリンのプリオン病に対する大規模な無作為化対照試験（PRION-1 study, www.ctu.mrc.ac.uk/studies/cjd.asp）が行われている。キナクリンを投与された患者では，皮膚の黄染や肝障害等の副作用が報告されている[54]。

3. フルピルチン

フルピルチンは非オピオイド性中枢性鎮痛薬である。またこの薬物は，抗アポトーシス分子 Bcl-2 のレベルや抗酸化物質グルタチオンの細胞内レベルを上昇させ，抗アポトーシス薬として作用することも知られている[55]。Perovic らは，フルピルチンが神経毒性ペプチド PrP106-126 の神経障害を抑制することを報告し，フルピルチンがプリオン病に有効である可能性を示した[56]。その後，Otto らは 28 名の患者に対しフルピルチンの二重盲検試験を行い，フルピルチンは生存期間を延長させないが，認知機能を有意に改善することを報告した[57]。

おわりに

プリオン感染細胞を用いて，これまでに多くのプリオン病の治療薬の候補が見出されてきた。しかし，臨床トライアルに供された薬剤はほんのわずかである。その理由の 1 つとして，候補薬の毒性の評価が定かでないことが挙げられる。そのほか，これらの候補薬が血液脳関門を容易に通過できないために，治療効果が見込めないこと，プリオン病の発症前診断が不可能であるために，病状がかなり進行した患者を対象としてトライアルを行わなければならず，治療効果が期待できないことも挙げられる。実際，動物実験でも，感染後期にこれらの薬剤を投与しても，治療効果が認められていない。このなかで，血液脳関門を通過できる抗プリオン薬の開発，および高感度の診断法の開発が急務であると考えられる。

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