Antibody-based immunotherapeutic attempts in experimental animal models of prion diseases

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Abstract

Background: There has been a dramatic decrease in the risk of transmission of bovine spongiform encephalopathy to humans. In contrast, the risk of human-to-human transmission of variant Creutzfeldt-Jakob disease (vCJD) via medical treatments became potentially high since 4 vCJD cases were reported to be possibly transmitted via blood transfusion in the United Kingdom. However, no treatments are yet available for curing prion diseases. Objective: Conversion of the normal prion protein, PrPC, to the amyloidogenic PrP, PrPSc, plays a pivotal role in the pathogenesis. Recently, certain anti-PrP or anti-37/67-kDa laminin receptor (LRP/LR) antibodies were shown to have the potential to cure chronically infected cells, clearing PrPSc from the cells. This has raised the possibility of antibody based-immunotherapy for prion diseases. This article aims to introduce and discuss the recently published attempts of immunotherapy in prion diseases.

Methods: Bibliographic research was carried out using the PubMed database. Patent literature was searched using the UK Intellectual Property Office website. Results/Conclusion: No satisfying consequences in animals could be detected with anti-PrP antibodies directly infused into the brains of animals by the intraventricular route or by anti-PrP or anti-LRP/LR single chain fragment antibodies directly delivered into the brain by virus vector-mediated gene transfer. This is probably because such delivery systems failed to deliver the antibodies to the neurons relevant for the treatments.

Keywords: prion, prion protein, prion diseases, antibody, immunotherapy
1. Introduction

Prion diseases or transmissible spongiform encephalopathies are a group of devastating neurodegenerative disorders that include 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 [1, 2]. A sporadic type of CJD accounts for 85%-90% of most of prion diseases in human beings [2]. The etiology of sporadic CJD remains unknown [2]. Interestingly, approximately 10% of cases are inherited, including those of familial CJD, GSS, and FFI [2]. These inherited diseases are etiologically linked to specific mutations of \(Prnp\), the gene for prion protein (PrP) [2]. Other types of the diseases are caused by infectious events, including iatrogenic CJD, kuru, and variant CJD (vCJD) [2]. Most cases of the infectious type are iatrogenic CJDs [3-6]. Kuru is a disease that emerged due to ritualistic cannibalism in Papua New Guinea [7]. vCJD is thought to be transmitted from BSE-infected cattle via contaminated food [8, 9]. No effective therapy for these diseases has been developed yet.

The advent of vCJD has raised concerns of the possibility of a disease epidemic among the human population [10, 11]. However, only ~160 cases of vCJD have thus far been reported in England and a much lesser number of cases in other countries (http://www.cjd.ed.ac.uk/figures.htm). This low number of vCJD cases could be attributed to the inefficiency of oral transmission, the species barrier between cattle and humans, and the marked reduction in BSE cases due to the ban on using meat and bone meal ingredients in animal feed. However, new cases of BSE are still reported in the United Kingdom (UK) and other countries
(http://niah.naro.affrc.go.jp/disease/bse/count.html). Therefore, we still have to constantly survey the disease. Another animal prion disease, the chronic wasting disease, is spreading within mule deer and elk in North America [12], raising similar health concerns of the possibility of the disease being transmitted to humans, causing another type of vCJD.

Four cases of vCJD considered to be transmitted via blood transfusion have been reported in the UK [13-16], raising the more serious concern of human-to-human secondary transmission of vCJD via medical treatments or procedures. vCJD is more transmissible among human populations than is BSE from cattle to humans. In humans, codon 129 of Prnp is polymorphic, coding methionine (M) or valine (V), and is a major determinant of susceptibility to the disease: MM is the most susceptible; MV, intermediate; and VV, protective [17-19]. All cases of vCJD due to BSE, reported to date, have been found in MM individuals [20]. No MV or VV cases have been identified thus far [20]. However, one case of blood transfusion-related vCJD was heterozygous at codon 129 [14]. These results suggest that vCJD might be transmissible to humans with any genotypes of Prnp. Consistent with these results, it was shown that vCJD was transmitted to mice expressing human PrP with MM, MV, or VV [20]. Thus, it is believed that there might be a considerable number of individuals who are latently infected with vCJD without any clinical symptoms, and that these latently infected people might become the sources of secondary transmission of vCJD. Indeed, Hilton et al. reported a much greater incidence of the disease than that so far reported for conventional human prion diseases. They showed that 3 out of 12,674 surgically removed appendectomy or tonsillectomy specimens were positive for staining of PrPSc, although two specimens displayed a dissimilar staining pattern of PrPSc from
that in vCJD [21]. Therefore, the development of therapeutic and/or prophylactic measures for prion diseases is urgently awaited.

Recently, in addition to chemical compound-based conventional therapeutic approaches, a new approach of antibody-based immunotherapeutics is being attempted using experimental animal models of prion diseases. Here, we will introduce and discuss such immunotherapeutic attempts.

2. Therapeutic targets for prion diseases

The causative agents of prion diseases, the so-called prions, are thought to be composed of the abnormally folded, amyloidogenic isoform of PrP, termed PrP<sup>Sc</sup> [22]. PrP<sup>Sc</sup> is generated via the conformational conversion of the normal cellular isoform of PrP, PrP<sup>C</sup>, a membrane glycoprotein anchored to the cell surface via a glycosylphosphatidylinositol (GPI) moiety and expressed in various tissues, with the highest expression in the brain, particularly in neurons [22, 23].

Accumulating lines of evidence indicate that the conformational conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> plays a pivotal role in the pathogenesis of prion diseases. Indeed, along with other researchers’ studies, we have shown that PrP<sup>C</sup>-deficient (Prnp<sup>0/0</sup>) mice, in which the conversion never occurs due to lack of PrP<sup>C</sup>, were resistant to the diseases even after inoculation with mouse-adapted prions [24-27]. Moreover, it was reported that the removal of PrP<sup>C</sup> specifically from the infected neurons rescued mice from the disease [28, 29], indicating that neurons undertaking the conversion may undergo degeneration. Therefore, inhibition of the conversion in the brain, particularly in neurons, may be therapeutic for prion diseases.
Yokoyama et al. carried out histoblot analysis of scrapie prion-infected mouse and hamster brains and showed that immunoreactive signals against PrP\textsuperscript{C} were decreased in the affected regions whereas those against PrP\textsuperscript{Sc} were increased [30]: this suggested that constitutive conversion might lead to a decrease in PrP\textsuperscript{C} in the brains and that the resultant functional impairment of PrP\textsuperscript{C} might be involved in the pathogenesis. Indeed, Prnp\textsuperscript{0/0} mice spontaneously developed abnormal phenotypes, some of which are often observed in prion diseases, including behavioral alterations in circadian activity and sleep, and demyelinated axons in the spinal cord and peripheral nerves [31-34]. It is therefore possible that such abnormalities in prion diseases might be attributable to the functional loss of PrP\textsuperscript{C}. Thus, approaches to enhance the function of PrP\textsuperscript{C} might be alternatively therapeutic against prion diseases. However, the physiological function of PrP\textsuperscript{C} remains unknown. In contrast to the reduction in PrP\textsuperscript{C}, constitutive conversion causes the accumulation of PrP\textsuperscript{Sc} in the brain. It is therefore suggested that PrP\textsuperscript{Sc} might be a toxic neurodegenerative molecule. Indeed, the accumulation of PrP\textsuperscript{Sc} is well correlated to pathological changes, including gliosis, spongiform changes, and neuronal cell death [2]. Moreover, an amyloidogenic PrP peptide, PrP106-126, or purified PrP\textsuperscript{Sc} was shown to be toxic to cultured cells, inducing apoptotic cell death [35-37]. Therefore, approaches that lead to the protection of neurons from PrP\textsuperscript{Sc} neurotoxicity might also be therapeutic.

3. Anti-prion chemical compounds

A large number of chemical compounds were screened for anti-prion activity or activity that reduces the total amount of PrP\textsuperscript{Sc} in chronically infected cultured cells, such as scrapie
prion-infected mouse neuroblastoma N2a cells [38, 39]. As a result, many compounds have been isolated as therapeutic candidates for prion diseases (Table 1). However, the chemical characteristics of these compounds are diverse and, for most of them, the exact mechanism of the anti-prion activity remains unknown.

The compounds that could reduce PrP<sup>Sc</sup> levels in infected cells were then tested for their therapeutic usefulness in prion-infected animals. These compounds showed prophylactic effects on the disease, prolonging incubation times or rescuing the animals from the disease when administered to animals before or immediately after prion inoculation [38, 39]. However, no curable effects of these compounds could be detected [38, 39]. Prolongation of incubation times became marginal and no animals were rescued from the disease when the compounds were administered at an advanced stage or in the clinical phase of the disease. This therapeutic ineffectiveness of the compounds is probably because the compounds fail to efficiently reach the therapeutically relevant brain regions due to their inability to cross the blood-brain barrier (BBB) or their inadequate spreading within the brain parenchyma even after direct administration into the brain. It is also conceivable that the compounds might be less effective against prions in the brain at an advanced clinical stage of the disease. Recently, De Luigi <i>et al.</i> reported that even a single intracerebroventricular infusion of liposome-entrapped doxycycline and minocycline, termed LipoDoxycycline and LipoMinocycline, respectively, into hamsters at an advanced stage of prion disease could significantly extend incubation times by 10 and 14 days, respectively [40]. It is thus interesting to investigate whether or not continuous or multiple infusions of the compounds could be more effective and cure animals of prion diseases.
Only a few compounds have been clinically tested against prion diseases so far. However, no patients have been reported to be cured from the diseases by treatment with these compounds, although some reports have shown that clinical symptoms, such as decreased cognitive activities, appeared to be slightly improved. Pentosan polysulfate (PPS) is a polyanionic compound exhibiting marked anti-prion activity in prion-infected cells by blocking the binding of the 37/67-kDa laminin receptor (LRP/LR), a possible prion receptor, to PrP$^{\text{Sc}}$ on the cell surface [41], and was directly injected into the brain ventricle of patients with various types of prion diseases [42, 43]. Bone et al. showed that the mean survival of all treated patients was longer than the reported values for non-treated patients [43]. However, due to lack of proper controls, the therapeutic benefits of PPS remain to be proven. Quinacrine, an anti-malarial agent, has anti-prion activity. A large-scale randomized controlled clinical trial of quinacrine is being undertaken in the UK as the PRION-1 study (http://www.ctu.mrc.ac.uk/studies/cjd.asp). Nakajima et al. reported transient and modest improvement in mood or cognitive function by treatment of 3 patients with quinacrine [44]. Flupirtine is a centrally acting, non-opiate analgesic compound as also an anti-apoptotic agent. A double-blind, placebo-controlled study in 28 CJD patients by Otto et al. showed that flupirtine improved patients’ scores on several different dementia tests as compared to placebo [45]. These treatments were clinically unsuccessful probably because of the same reasons for which the compounds were ineffective in animal models. Thus, appropriate chemical modifications that enable the compounds to efficiently cross the BBB would be required for the compounds to be more effective. In addition, the compounds were administered to clinically advanced patients due to the lack of effective procedures that can diagnose presymptomatic
individuals, which might have reduced the anti-prion activity of the compounds. Therefore, the development of more sensitive diagnostic techniques for the detection of preclinical patients is essential.

4. Antibodies cure chronically infected cultured cells

4.1 Anti-PrP antibodies

Peretz et al. first reported that recombinant PrP-specific Fab fragments cured chronically infected N2a cells [46]. They added PrP-specific Fab fragments, termed D13, D18, R1, R2, E123, E149, and R72, to chronically infected N2a cell cultures for 7 days. The anti-prion activity of the Fab fragments was then assessed by calculating the values of 50%-inhibitory concentration ($IC_{50}$), the concentration necessary for halving PrP$_{Sc}$ levels. Fabs D13 and D18 were most effective with the $IC_{50}$ being 0.6 µg/ml (12 nM) and 0.45 µg/ml (9 nM), respectively. Fabs R1 and R2 were slightly less efficient, with the $IC_{50}$ being 2.5 µg/ml (50 nM) and 2.0 µg/ml (40 nM), respectively. Prion infectivity was concomitantly reduced in these cells by over three orders of magnitude. Fabs D12, D18, R1, and R2 recognize residues 95-103, 132-156, 220-231, and 225-231, respectively, indicating that anti-prion activity might be independently mediated via broadly located multiple sites of PrP. In contrast, no reduction in PrP$_{Sc}$ levels and prion infectivity were detected in the cells treated with Fabs E123, E149, and R72, which bind to residues 29-37, 72-86, and 151-162, respectively. Enari et al. reported similar results [47]: they added an anti-PrP monoclonal antibody (mAb), termed 6H4 which binds to residues 144-152 to their newly established infected N2a/Bos2 cells and showed that the antibody reduced PrP$_{Sc}$ levels in the cells in a dose-dependent
manner.

4.2 Possible anti-prion mechanisms of anti-PrP antibodies

The first step of the conversion is an interaction between PrP\textsuperscript{C} and PrP\textsuperscript{Sc} probably on the cell surface, particularly on lipid rafts, and/or along the endocytotic pathway to late endosomes/lysosomes [48]. It is therefore envisaged that anti-prion antibodies might interfere with the interaction. It has been reported that 3F4 and 13A5 mAbs, which recognize residues 109-112 and 138-165, respectively, and the polyclonal antibody against residues 219-232 disturbed the interaction and subsequently inhibited the conversion in a cell-free system [49, 50]. Another possibility is that anti-PrP antibodies might reduce PrP\textsuperscript{Sc} levels in infected cells by altering the subcellular localization of PrP\textsuperscript{C}. Kim et al. showed that 31C6, 110, 44B1, and 72 anti-PrP mAbs with anti-prion activity disturbed PrP\textsuperscript{C} internalization [51]. The 31C6 and 110 mAbs react with residues 143-149 and the PHGGGWG sequence at residues 59-65 and 83-89 in the octapeptide repeat region, respectively, and 44B1 and 72 mAbs recognize discontinuous epitopes [51]. Perrier et al. showed that the anti-PrP mAbs SAF34 and SAF61, which react with the octapeptide repeat region and residues 144-152, respectively, accelerated the degradation of PrP\textsuperscript{C} in cells [52], suggesting another possibility that anti-PrP antibodies might inhibit PrP\textsuperscript{Sc} formation by reducing PrP\textsuperscript{C}. It is further possible that anti-PrP antibodies might interfere with the interaction of the so-called cofactor(s), which is postulated to play an important role in the conversion, with either PrP\textsuperscript{C} or PrP\textsuperscript{Sc}, or both.
4.3 Anti-LRP/LR antibody

It was shown that LRP/LR interacts with PrP\(^C\) directly between LRP/LR residues 161-179 and PrP residues 144-179, and indirectly between LRP/LR residues 101-160 or 180-285 and PrP residues 53-93 via a heparan sulfate chain of proteoglycan [53, 54]. Subsequently, LRP/LR was also demonstrated to act as the cell-surface receptor for PrP\(^C\) [55] and PrP27-30, the proteinase K-resistant core of PrP\(^S\) [41]. These indicate that LRP/LR might be involved in PrP\(^S\) formation. Indeed, Leucht et al. reported that the LRP/LR-specific polyclonal antibody, termed W3, competed with recombinant PrP for binding to the LRP/LR expressed on the cell surface, reduced PrP\(^S\) levels in infected N2a cells, and finally cured the cells [56]. This result indicates that W3 might inhibit PrP\(^S\) formation by disturbing the interaction between PrP\(^C\) and LRP/LR. W3 was also shown to reduce PrP\(^C\) levels in cells [56]. It is thus alternatively 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 the internalization of PrP\(^C\) into lysosomes for degradation, resulting in inhibition of PrP\(^S\) formation. Thus, LRP/LR might be a therapeutic target of prion diseases [57-60].

5. Immunotherapeutic attempts in experimental animal models of prion diseases

5.1 Direct infusion of anti-PrP antibodies into the brain

In a previous study, we produced anti-PrP mAbs, termed 3S9 and 2H9 that recognize residues 141-161 and 151-221, respectively, and showed that both mAbs have anti-prion activity, reducing PrP\(^S\) levels in infected N2a cells [61]. The IC\(_{50}\) value of 3S9 mAb was 0.6 nM, which was over
10 times stronger than that (8.4 nM) of 2H9 mAb. Hence, we were interested in studying the immunotherapeutic potential of 3S9 mAb against prion diseases. Antibodies are macromolecules and are, therefore, unable to pass the BBB. White et al. demonstrated that prophylactic intraperitoneal administration of two anti-PrP mAbs, ICSM 18 and 35 [62], could protect mice from the peripheral infection of RML prions but had no effects on prions directly introduced into the brains of mice [63]. This could be due to the incapability of the antibodies to cross the BBB. Therefore, we directly administered 3S9 mAb into the right ventricle of mice that had been intracerebrally inoculated with a mouse-adapted Fukuoka-1 prion (SS and DI, unpublished data). The antibody was continuously delivered into the ventricle using the ALLZET mini-osmotic pump model 2004 (DURECT Corporation) at a flow rate of 0.25 ± 0.05 µg/h for 28 days from 8 or 13 weeks post inoculation (p.i.), which corresponds to the middle or late disease stage, respectively. Mice infused with control IgG antibody at 8 or 13 weeks p.i. developed the disease at 117.8 ± 9.9 and 121.2 ± 2.5 days p.i. and eventually died at 122.5 ± 6.1 and 124.0 ± 3.7 days p.i., respectively (Table 2). Unexpectedly, no significant extension in incubation times and survival times could be detected in mice treated with 3S9 mAb. The mice treated at 8 or 13 weeks p.i. succumbed to the disease at 127.0 ± 9.0 (P = 0.158, Logrank test) and 125.0 ± 4.7 days p.i. (P = 0.851) and died at 130.0 ± 11.7 (P = 0.272) and 127.2 ± 5.8 days p.i. (P = 0.942), respectively (Table 2). In contrast, PrP<sub>Sc</sub> levels were reduced in the brains of mice treated with 3S9 mAb at 8 weeks p.i. to half of those in the control mice. However, no decrease in PrP<sub>Sc</sub> levels could be observed in mice treated at 13 weeks p.i.

On the other hand, Song et al. reported that 31C6 mAb carrying anti-prion activity (0.7
nM IC\textsubscript{50}) could marginally but significantly prolong survival times in mice that had been intracerebrally inoculated with Chandler prion [64]. The dose of the mAb used was twice (0.5 µg/h) that of 3S9 mAb (0.25 µg/h) used by us. The mAb was infused for 28 days into the left ventricle of the mice at 60 (corresponding to the early stage), 90 (the middle stage), or 120 days p.i. (the late stage). The treated mice survived the disease by approximately 10 days more than did the control mice, regardless of the time points of treatment. Moreover, milder pathologies including PrP\textsuperscript{Sc} accumulation, gliosis, and vacuolation were consistently observed in the brains of the treated mice. Interestingly, 31C6 mAb was less effective against a different Obihiro prion. Mice inoculated with an Obihiro prion could survive the disease by approximately 10 days more than the control mice, only when the treatment was started at 60 days p.i., but not at 90 and 120 days p.i. These results suggest that the anti-prion effect of anti-PrP antibodies may differ for each of the prion strains.

5.2 Virus vector-mediated gene delivery of anti-PrP scFv antibodies

The direct intraventricular infusion of anti-prion antibodies presented only slight therapeutic benefits against prion disease in mice. Due to their higher molecular weight, the intraventricularly injected antibodies are unable to infiltrate regions where neurons mediating vital functions are infected by prions at concentrations that are sufficiently high for the antibodies to exert therapeutic effects. Indeed, Lefebvre-Roque \textit{et al.} observed that, compared to whole IgGs, lower molecular weight F(\text{ab‘})\textsubscript{2} fragments were more widely distributed at higher concentrations in the brain of mice when intraventricularly injected [65]. Therefore, a reduction in the molecular size of
anti-PrP antibodies without reducing their anti-prion activity might be helpful in overcoming this problem [66]. It has already been shown that fragmented anti-PrP antibodies, such as Fab and scFv antibodies, remain active against PrPSc formation in infected cells [67, 68]. However, Fab and scFv antibodies have the disadvantage of a short half-life, compared to full-length antibodies. Therefore, such fragmented anti-PrP antibodies require to be modified to overcome this problem.

In addition, the development of systems for the efficient delivery of the fragmented antibodies into the brain, particularly to neurons relevant for therapy, would be very important to increase the therapeutic benefits of the antibodies in prion diseases.

Recently, the anti-PrP potential of anti-PrP scFv fragments was investigated in mice using a virus vector-mediated brain delivery system [69]. Several serotypes of recombinant adeno-associated vector (rAAV) are useful as gene delivery vehicles to treat neurological disorders due to their efficient gene transduction into neurons and their safety profiles [70-72]. However, there exist several potential limitations for using the vectors. The first is the efficiency of gene delivery to target cells. Serotype 2 of rAAV (rAAV2) is the most commonly studied vector. rAAV2 preferentially transduces neurons in various regions of the brain, including the hippocampus, substantia nigra, and cerebellum, and in spinal cord. The transduction efficiency varies from one region to another. rAAV1 and rAAV5 have higher transduced distribution and number of neurons than does rAAV2. rAAV1 transduces neurons, and glial and ependymal cells: and rAAV5, neurons and astrocytes. In contrast, rAAV4 almost exclusively transduces ependymal cells. Prions affect neurons throughout the brain. Therefore, rAAV1 and rAAV5 may be more effective as therapeutic gene delivery vectors in prion diseases than would be rAAV2. However, it
may be difficult for even rAAV1 and rAAV5 vectors to extend to regions remote from the injected site. Therefore, multiple injections or further development of the vectors to transduce all of the target cells would be required. The second limitation is the presence of preexisting or newly induced anti-AAV neutralizing antibodies. No report has shown that the preexisting antibodies influence the transduction efficiency of rAAV. In contrast, the repeated administration of rAAV into the airway surface has been shown to produce an increased titer of the antibodies and reduced the transduction efficiency [73, 74]. However, it was reported that repeated injection of rAAV vectors into the rat or mouse brain was possible [75, 76]. The third limitation is the duration of the expression of a delivered gene. The stable expression of an rAAV2-delivered gene was observed at least up to 9 months in the rat brain [71].

Wuertz et al. generated an rAAV2 vector encoding anti-PrP scFv fragments, termed scFv 3:3, scFv 6:4, scFv 6:6, and scFv D18 [69]. Each rAAV2 scFv vector with $9 \times 10^9$ expression units was bilaterally injected into the thalamus and striatum of mice; and 1 month later, the mice were intraperitoneally inoculated with RML prions [69]. Mice treated with the control vector developed the disease at $199 \pm 1$ days p.i. No significant prolongation in incubation times could be detected in mice injected with rAAV scFv 6:4 and 6:6. However, mice injected with rAAV scFvs 3:3 and D18 showed significantly extended incubation times of $222 \pm 13$ and $250 \pm 8$ days p.i., respectively. Reduced accumulation of PrPSc was also observed in the brains of mice injected with rAAV scFvs D18. These different anti-prion activities of the rAAV scFvs were well correlated to their binding affinity to recombinant PrP. These results indicate that anti-PrP scFv fragments transduced by the rAAV2 vector-mediated gene transfer were effective in inhibiting
PrP$_{Sc}$ formation in the brains. However, regions expressing scFvs appeared restricted to sites where the rAAV2 scFvs were injected. Thus, the incomplete prophylactic prevention of the disease by rAAV2 vector-mediated gene transfer of the anti-PrP scFv fragments might be attributable to this limited regional expression of the scFvs in the brain. Campana et al. produced another lentivirus vector for scFv D18 and tested it for its anti-prion activity in prion-infected N2a cells [68]. Results showed that the lentivirus could transduce scFv D18 in the cells more efficiently than could rAAV2 [68]. However, as in the case of the rAAV2-mediated gene transfer, the transduction efficiency of anti-PrP scFv fragments in the brain might be low even with lentivirus-mediated gene transfer.

5.3 Toxic anti-PrP antibodies

In contrast to the beneficial effects of anti-PrP antibodies, neurotoxic effects were reported with some anti-prion mAbs or Fab fragments. Solforosi et al. showed that anti-PrP D13 and P mAbs each recognizing epitopes within the residues 95-105 of PrP were toxic, causing neuronal cell death in normal mice when directly injected into the hippocampus or the cerebellar cortex [77]. In contrast, another anti-PrP D18 mAb against the residues 133-157 did not manifest any neurotoxicity [77]. Lefebvre-Roque et al. also reported that anti-prion 4H11 mAb or its F(ab’)$_2$ fragment induced extensive neuronal cell death and marked gliosis over the brain when administered daily for 2 weeks into the lateral ventricle of Tg20 mice that had been infected with the 6PB1 mouse-adapted BSE prion by the intraperitoneal route [65]. The treatment was initiated in the early stage of neuroinvasion (85 days p.i.) [65]. The neuronal loss was observed in regions
close to the injected lateral ventricle as well as in the occipital cortex, the hippocampus, the thalamus, and the striatum. No significant difference in the survival times could be detected between treated and untreated groups. Mice treated with 4H11 mAb or its F(ab’)2 fragment died at 140 ± 8 and 143 ± 14 days p.i. against 143 ± 10 and 144 ± 11 days p.i. for the control IgG and F(ab’)2 fragment, respectively. However, we detected no neuronal loss in the brains of mice inoculated with 3S9 mAb (SS and DI, unpublished data). No neurotoxicity was also reported in mice injected with 31C6 mAbs [64]. The neurotoxic 4H11, D13, and P mAbs bind to epitopes located within the N-terminal part of PrP. 4H11 mAb binds to the OR region (residues 51-90), and D13 and P mAbs recognize epitopes within residues 95-105. In contrast, nontoxic 3S9 and 31C6 mAbs bind to epitopes within residues 141-161 of the C-terminal part of PrP. Thus, the binding of anti-PrP antibodies to certain regions within the N-terminal part, such as the OR region or residues 95-105, might elicit a neurotoxic signal by either activating or adversely preventing the physiological function of PrP^C in neurons.

5.4 Anti-LRP/LR antibody

To investigate the prophylactic anti-prion activity of the polyclonal anti-LRP/LR antibody W3 in vivo, W3 was peripherally injected into mice via the intraperitoneal route once a week over a period of 12 weeks [78]. The mice were intraperitoneally inoculated with RML prions 1 week after the first W3 injection. No significant prolongation in incubation times or survival was detected in the treated mice, probably due to the inability of the antibody to cross the BBB. However, PrP^Sc levels were reduced by 17% in the brains and by 66% in the spleens of the mice,
compared to those in control mice, indicating that W3 could interfere with peripheral but not the neuronal formation of PrP\textsuperscript{Sc} [78]. Similar results were reported with anti-LRP/LR scFv fragments, termed S18 [79, 80]. S18 scFv reduced PrP\textsuperscript{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. These results clearly indicate that anti-LRP/LR antibody or scFv is active \textit{in vivo} and is able to reduce PrP\textsuperscript{Sc} levels.

Zuber \textit{et al}. used rAAV2 as a vector for anti-LRP/LR scFvs to be transduced into the brain [81]. They generated rAAV2 encoding two different scFvs, S18 and N3, and injected $5 \times 10^9$ particles of each rAAV2 scFv into the hippocampus of mice that were inoculated with RML prions in the same area 2 weeks after rAAV2 scFv injection [81]. Thereafter, anti-LRP/LR scFvs were produced in the mouse brains. However, in contrast to the results of Wuertzer \textit{et al}. [69], which showed that rAAV2 encoding anti-PrP scFvs 3:3 or D18 fragment was partially prophylactic, no prolongation in incubation times or survival was observed in mice that received rAAV2 scFv-S9 or N3. The authors suggested that the rAAVs might not have reached all the relevant brain cells that were infected by prions. Alternatively, anti-LRP/LR scFvs might be less effective in preventing PrP\textsuperscript{Sc} formation than are anti-PrP scFvs. Interestingly, the injected rAAV2s appeared to cross the BBB and also reach the spleen, where scFvs S18 and N3 were expressed and PrP\textsuperscript{Sc} levels were reduced by approximately 32% and 60%, respectively [81].

5.5 PrP-Fc\textsubscript{2}

Meier \textit{et al}. reported that wild-type mice transgenically expressing PrP-Fc\textsubscript{2}, a dimeric fusion
protein of PrP\textsuperscript{C} linked to the N-terminus of IgG Fc, showed marked resistant to RML prions, developing the disease with significantly prolonged incubation times and accumulating much less PrP\textsuperscript{Sc} in the brains as compared to control non-transgenic mice [82]. PrP-Fc\textsubscript{2} had a potential to bind to PrP\textsuperscript{Sc} via PrP\textsuperscript{C}, but the PrP\textsuperscript{C} part could not be converted to PrP\textsuperscript{Sc} probably due to fusion with Fc. The authors therefore suggested that PrP-Fc\textsubscript{2} might inhibit PrP\textsuperscript{Sc} formation by a dominant negative mechanism, in which PrP-Fc\textsubscript{2} disturbs the binding of native PrP\textsuperscript{C} to PrP\textsuperscript{Sc} by intercalation between the two molecules, resulting in the prevention of PrP\textsuperscript{Sc} formation [82]. The same group further investigated the prophylactic activity of lentivirus vector-transduced PrP-Fc\textsubscript{2} against RML prions [83]. The PrP-Fc\textsubscript{2} lentivirus was injected into the hippocampus. When the mice were intracerebrally inoculated with RML prions 20 days after the virus injection with 3 × 10\textsuperscript{8} infectious units, the mice developed the disease with prolonged incubation times by 36 days as compared to the control mice, which developed the disease at 175 ± 5 days p.i. Importantly, this prophylactic effect of lentivirus vector-transduced PrP-Fc\textsubscript{2} further increased when the virus vector was transduced into the brain with higher infectious units [83]. When mice were treated with 1.5 × 10\textsuperscript{9} infectious units of the virus vector, the incubation times were further prolonged by 72 days (treated vs. control mice, 247 ± 8 vs. 175 ± 5 days p.i.). However, when the virus (1.5 × 10\textsuperscript{9} infectious units) was injected 30 days after prion inoculation, the post-exposure prophylactic effect of PrP-Fc\textsubscript{2} was reduced in the mice, but the incubation times remained significantly extended by 25 days. No curative effect of the lentivirus vector-transduced PrP-Fc\textsubscript{2} was observed in mice when the vector was injected at 121 days p.i. (the late stage of disease) [83]. The treated and control mice succumbed to the disease at 197 ± 9 and 197 ± 17 days p.i., respectively.
5.6 Potential unfavorable responses in the immunotherapy

Because PrP\textsuperscript{C} is a host-encoded glycoprotein expressed in various normal tissues, no immune responses are evoked against PrP\textsuperscript{C} under physiological conditions as well as PrP\textsuperscript{Sc} in prion diseases. LRP/LR is also a host-encoded protein. Therefore, exogenously injected antibodies against PrP and LRP/LR might cause autoimmune reactions \textit{in vivo} although no abnormal symptoms have been reported in mice peripherally administered with anti-PrP or anti-LRP/LR W3 antibodies [63, 78]. Moreover, the injected antibodies might generate detrimental signals upon binding to PrP\textsuperscript{C} or LRP/LR. Indeed, as described above, certain anti-PrP mAbs elicited neurotoxic signals by cross-linking of PrP\textsuperscript{C} when injected into the brain [65, 77]. Thus, the possibility that these unfavorable effects might be induced by antibody-based immunotherapy for prion diseases should be carefully considered.

6. Expert opinion

No complete cure of prion diseases in animals has been obtained with anti-PrP antibodies directly infused into the brain by the intraventricular route or by anti-PrP and anti-LRP/LR scFvs and PrP-Fc\textsubscript{2} directly delivered into the brain by virus vector-mediated gene transfer. This is probably because the antibodies could not infiltrate into the brain due to their higher molecular weight and because the virus vectors infected only those neurons that were located at or surrounding sites where the vectors were injected, resulting in the inability of the antibodies to reach and cure the infected neurons that would be essential and are relevant for effective treatments. Therefore,
delivery systems that are more efficient need to be developed for administering anti-PrP or anti-LRP/LR antibodies, scFv fragments, and PrP-Fc2 into the brain for greater therapeutic efficacy against prion diseases. In addition, the development of more sensitive preclinical diagnostic techniques is essential.

**Declaration of interest**

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**Bibliography**

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

   • Describes probable transmission of BSE to humans.
   • Describes probable transmission of BSE to humans.


- Describes the first case of possible human-to-human transmission of vCJD via blood transfusion.


- Describes possible transmission of vCJD to human carrying a M/V 129 codon via blood transfusion.


- Describes a possibility of a large number of people subclinically infected by vCJD.


Sakaguchi S, Katamine S, Shigematsu K et al. Accumulation of proteinase K-resistant prion protein (PrP) is restricted by the expression level of normal PrP in mice.


** Systematically reviews anti-prion molecules. **


** Systematically reviews anti-prion molecules. **


**The first paper describing a possibility of antibody-based immunotherapy against prion diseases.**


**The first paper describing interaction between LRP/LR and PrPC.**


**Demonstrates LRP/LR as the receptor for PrPC.**


57. Ludewigs H, Zuber C, Vana K et al. Therapeutic approaches for prion disorders. Expert


**Describes the first successful passive immunization against prion disease.**


74. Halbert CL, Rutledge EA, Allen JM, Russell DW, Miller AD. Repeat transduction in the


80. Weiss Stefan (DE); Little Melvyn (DE); Knackmuss Stefan (DE); Rey Clemence (DE); Roettgen Peter (DE); Buettner Claudia (DE); Reusch Uwe (DE). WO2005035580 (2005).


<table>
<thead>
<tr>
<th>Class</th>
<th>Chemical agents</th>
<th>Possible mechanisms</th>
</tr>
</thead>
</table>
| Polyanionic compounds | Heteropolyanion-23, Carrageenan, Dextran sulfate, Pentosan polysulfate, Glycosaminoglycans (GaGs), Heparan sulfate mimetics, Single-stranded phosphorothioate oligonucleotides, RNA aptamers, Suramin | • Compete with endogenous GAGs or LRP/LR, cofactors important for the conversion, for the interaction with PrP<sup>C</sup> and/or PrP<sup>Sc</sup>  
• Disturb subcellular localization of PrP<sup>C</sup> and/or PrP<sup>Sc</sup> |
| Polycationic compounds| Polyamidoamide branched polymer, Polypropyleneimine branched polymer, Polyethyleneimine branched polymer, Cationic phosphorus-containing dendrimers, Cationic lipopolyamine, Cationic polysaccharides | • Accelerate PrP<sup>Sc</sup> degradation in lysosomes |
| Amyloid-binding compounds | Congo red, Trypan blue, Evans blue, Sirius red F3B, Primuline, Thioflavin-S, BF-168, cpd-B | • Overstabilize PrP<sup>Sc</sup> conformation  
• Compete with endogenous GAG for the interaction with PrP<sup>C</sup> and/or PrP<sup>Sc</sup> |
<p>| Tetracyclic compounds | Anthracycline 4′-iodo-4′-deoxyrubicin, Tetracycline, Doxycycline | • Destabilize PrP&lt;sup&gt;Sc&lt;/sup&gt; conformation |
| Tetrapyrrolic compounds | Porphyrins, phthalocyanins | • Change PrP&lt;sup&gt;Sc&lt;/sup&gt; conformation |
| Cholesterol metabolism-related compounds | Polypeptide antibiotics (Amphotericin B, MS-8209), Statins (Lovastatin, Squalstatin, Simvastatin) | • Change the properties of lipid raft, a possible site of the conversion, by binding to cholesterol or inhibiting cholesterol synthesis |
| Tricyclic and related compounds | Quinacrine, Tilorone, Chloroquine, Suramin, Chlorpromazine, Bis-acridine, Quinoline, 2,2′-Bisquinolin, Mefloquine, Beberine, Tetrandrine, Amodiaquine, Tiotixene, Prochlorperazine, Thioridazine | • Bind to PrP&lt;sup&gt;Sc&lt;/sup&gt; fibrils and inhibit de novo synthesis of PrP&lt;sup&gt;Sc&lt;/sup&gt; |</p>
<table>
<thead>
<tr>
<th>Category</th>
<th>Example Compounds</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trifluoperazine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysosomal cystein protease inhibitors</td>
<td>E-64, E-64d, Leupeptin</td>
<td>• Unknown</td>
</tr>
<tr>
<td>Cell signaling inhibitors</td>
<td>Tyrosine kinase inhibitor (imatinib mesilate)</td>
<td>• Stimulate PrP&lt;sup&gt;Sc&lt;/sup&gt; degradation by inhibiting c-Able kinase</td>
</tr>
<tr>
<td></td>
<td>Phospholipase A2 inhibitors</td>
<td>• Inhibit PrP&lt;sup&gt;Sc&lt;/sup&gt; formation by altering cell membrane integrity</td>
</tr>
<tr>
<td></td>
<td>(Cytidine-5-diphosphocholine, Bromoenol lactone, Aristolochic acid, Arachidonyl trichloromethyl ketone)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mitogen-activated protein kinase kinase (MEK) 1/2 inhibitors</td>
<td>• Stimulate PrP&lt;sup&gt;Sc&lt;/sup&gt; degradation by inhibiting MEK1/2 activity</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>Tannic acid, Katacine, Bisepigallocatechin digallate</td>
<td>• Unknown</td>
</tr>
<tr>
<td>Anti-histamines</td>
<td>Astemizole, Terfenadine</td>
<td>• Unknown</td>
</tr>
<tr>
<td>Steroid-type compounds</td>
<td>Budesonide, Clomifene, Chrysanthellin A</td>
<td>• Unknown</td>
</tr>
<tr>
<td>Others</td>
<td>2-Pyrrolidin-1-yl-N-[4-(2-pyrrolidin-1-yl-acetylamino)-benzyl]-phenylacetamidee</td>
<td>• Stabilize PrP&lt;sup&gt;C&lt;/sup&gt; formation</td>
</tr>
<tr>
<td>Kastellpaolitines</td>
<td></td>
<td>• Unknown</td>
</tr>
<tr>
<td>N′-Benzyldiene-benzoehydrazides</td>
<td></td>
<td>• Inhibit interaction between PrP&lt;sup&gt;C&lt;/sup&gt; and PrP&lt;sup&gt;Sc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pridine dicarbonitrile compounds</td>
<td></td>
<td>• Inhibit PrP&lt;sup&gt;Sc&lt;/sup&gt; formation by mimicking the residues of the dominant negative PrP&lt;sup&gt;C&lt;/sup&gt; mutants</td>
</tr>
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</table>
Table 2: No therapeutic effects of intraventricularly administrated-3S9 mAb in Fukuoka-1 prion-infected mice

<table>
<thead>
<tr>
<th>Treatment time</th>
<th>Antibodies</th>
<th>N</th>
<th>Incubation times (Mean ± SD, days)</th>
<th>P values</th>
<th>Survival times (Mean ± SD, days)</th>
<th>P values</th>
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</thead>
<tbody>
<tr>
<td>8 weeks p.i.</td>
<td>Control IgG</td>
<td>6</td>
<td>117.8 ± 9.9</td>
<td>0.158</td>
<td>122.5 ± 6.1</td>
<td>0.273</td>
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<tr>
<td></td>
<td>3S9</td>
<td>5</td>
<td>127.0 ± 9.0</td>
<td></td>
<td>130.0 ± 11.7</td>
<td></td>
</tr>
<tr>
<td>13 weeks p.i.</td>
<td>Control IgG</td>
<td>5</td>
<td>121.2 ± 2.5</td>
<td>0.851</td>
<td>124.0 ± 3.7</td>
<td>0.942</td>
</tr>
<tr>
<td></td>
<td>3S9</td>
<td>6</td>
<td>125.0 ± 4.7</td>
<td></td>
<td>127.2 ± 5.8</td>
<td></td>
</tr>
</tbody>
</table>