panying neuronal loss and gliosis. Although these studies have reported increased levels of MI in sCJD patients, results from the present study demonstrated reduced NAA/Cr and Cho/Cr ratios but normal MI levels in the right frontal lobe. These results were assumed to reflect the severe spongiform change and neuronal loss as revealed by MRS.

SPECT imaging revealed marked cerebral blood flow reduction, predominantly in cerebral cortical regions corresponding to brain areas with high-intensity DWI signals. This was most likely due to severe neuronal loss and/or severe spongiform change. However, one study reported a preserved cerebral blood flow, or increased perfusion, and marked high-intensity DWI changes [17]. Autopsy findings revealed mild neuronal loss, which most likely was responsible for the preserved perfusion. Moreover, SPECT results revealed hypoperfusion due to spongiform change and mild neuronal loss.

CSF S100 protein levels are affected by astrocytic gliosis, and CSF PGE_2 levels are influenced by microglial activation. The present results clearly identified reduced levels of S100 in CSF and decreased PGE_2 titers. CSF t-tau protein concentration was increased due to neuronal loss, resulting in lower t-tau protein levels in CJD180 patients compared with classical CJD patients. One of the neuropathological features in sCJD patients is severe neuronal loss in the cerebral cortex. Accordingly, neuropathological findings and CSF analysis (t-tau protein) revealed that neuronal loss in CJD180 patients was less than in sCJD patients. Biochemical CSF markers do not necessarily reflect the clinical condition, but CSF biomarker levels can be used as a pathological index. CSF analysis from the present study demonstrated mild astrocytic gliosis or spongiform change in the CJD180 patients.

Biochemical CSF analysis and neuroimaging results suggested that the cause of abnormal DWI signals was spongiform change and neuronal loss. However, this is difficult to prove. Indeed, results demonstrated that neurons in CJD180 patients were better preserved than in sCJD patients, and the spongiform change in CJD180 patients was more moderate than in the 4 typical sCJD cases examined.

In conclusion, results from the present study suggested that high-intensity DWI signals in CJD180 patients were influenced by spongiform changes.

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CASE REPORT

Rheumatoid vasculitis of crural muscles confirmed by muscle biopsy in the absence of inflammatory myopathy: histologic and MRI study

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Abstract A 60-year-old man who had been diagnosed as rheumatoid arthritis admitted to our hospital by dysesthesia on his legs with edema. Nerve conduction velocity test led to diagnosis of mononeuritis multiplex. Magnetic resonance imaging (MRI) of lower legs showed high intensity in slow tau inversion recovery. Typical vasculitis with neutrophil-dominant cell infiltration was observed by muscle biopsy without inflammatory myopathy or fascitis. Diagnosis was made by rheumatoid vasculitis found in crural muscles. Intravenous cyclophosphamide with oral tacrolimus effectively improved dysesthesia with reduction of inflammatory response.

Keywords Rheumatoid vasculitis · Magnetic resonance imaging · Muscle biopsy · Cyclophosphamide

Abbreviations

IVCY	Intravenous cyclophosphamide
MRI	Magnetic resonance imaging
NCV	Nerve conduction velocity
PAN	Polyarteritis nodosa
RA	Rheumatoid arthritis
STIR	Slow tau inversion recovery

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease that affects multiple organs as well as synovial joints [1]. Extra-articular manifestations of RA contain cardiopulmonary abnormalities, hematological or neurological manifestations [2]. Although rheumatoid vasculitis represents various organ involvements such as pleuritis, ocular manifestations or neuropathies based on systemic vasculitis, the relationship between imaging abnormality and pathological characteristics of rheumatoid vasculitis is rarely reported. In the present case, we show typical vasculitis, comparing to magnetic resonance imaging findings.

Case report

A 60-year-old man had been diagnosed as RA according to a criteria determined by American college of rheumatology [3]. Although he was treated with salazosulfapyridine or methotrexate, he recently began to feel dysesthesia on his bilateral legs with edema. Since local heat and swelling of lower legs also appeared, oral prednisolone was increased

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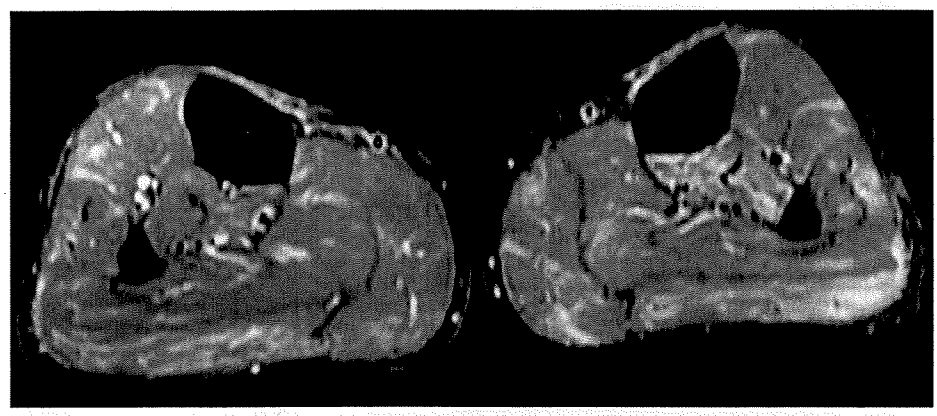

Fig. 1 Magnetic resonance imaging of the left lower leg. Slow tau inversion recovery (STIR) image shows patchy high intensity of gastrocnemius muscle or tibialis posterior muscle in the patient

to 20 mg/dl with discontinuation of disease modifying antirheumatic drugs. In February 2009, he was admitted to our hospital due to these persistent symptoms. He felt dysesthesia, especially on his left legs with edema and erythema, although no skin ulcer was observed.

Laboratory findings showed a hemoglobin level of 14.0 g/dl, total leukocyte count of 14,400/mm^3 and a platelet count of 37.2×10^4/mm^3. Although transaminases, renal function, creatinine kinase and aldolase levels were within normal limit, C-reactive protein was elevated to 6.99 mg/dl with accelerated erythrocyte sediment rate (99.2 mm/h, normal range <15). Although serum IgG and IgA were within normal limits, both rheumatoid factor (76.7 IU/ml, normal range <14) and anti-cyclic citrullinated peptide antibody (>100 U/ml, normal range <4.5) were positive without anti-SS-A or anti-SS-B antibody. On admission, he showed moderate disease activity of 3.82 points by disease activity score (DAS) 28-ESR. Antineutrophil cytoplasm antibodies (ANCAs), cryoglobulins, antiphospholipid antibodies, hepatitis B antigen, and angiotensin converting enzyme were negative. Radiographically, X-ray of his both hands represented bone erosion and joints narrowing as Steinblocker stage III. Since he had sensory disturbance of bilateral lower extremities, nerve conduction velocity (NCV) test was performed. The results showed a decrement of amplitude on the left leg with diagnosis of mononeuritis multiplex. Magnetic resonance imaging (MRI) of lower legs showed high intensity in slow tau inversion recovery (STIR) (Fig. 1). Because myositis, myofascitis or edema was suspected from the MRI findings, muscle biopsy of the lesion was performed, resulting in typical vasculitis with neutrophil-dominant cell infiltration (Fig. 2) without inflammatory myopathy, fascitis or sarcoidosis. Diagnosis was made by rheumatoid vasculitis found in the crural muscles. Since inflammatory myopathy was not found in the muscle biopsy specimens, STIR high lesion was considered to be edematous change induced by vasculitis. This may be consistent with clinical manifestation of absent muscle weakness. Intravenous cyclophosphamide

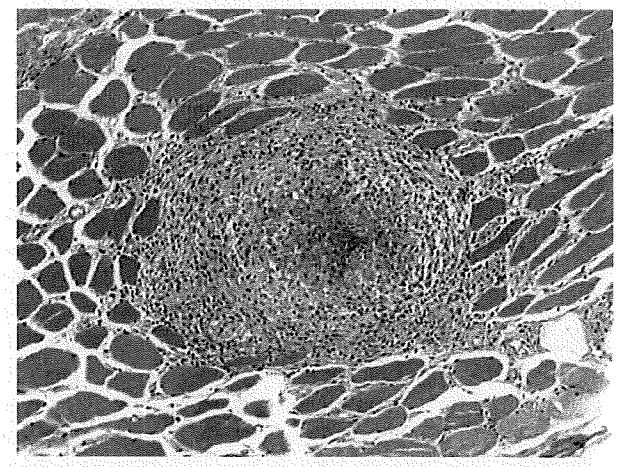

Fig. 2 Typical vasculitis in the biopsy specimen. Around small vessel in muscle of the left lower leg, typical vasculitis with neutrophil-dominant cell infiltration was accompanied by leukocytoclastic vasculitis. Neither myositis nor myofascitis was observed. (original magnification, ×100)

(IVCY) was monthly introduced twice with oral 3 mg of tacrolimus. These therapies were effective, showing obvious improvement of dysesthesia with reduction of CRP from 6.99 to 1.88 mg/dl; he was discharged.

Discussion

A variety of extra-articular manifestations were found in RA. Although Turesson et al. [4] reviewed extra-articular manifestations in RA, peripheral neuropathy is one of these clinical entities. Gorson [5] showed that vasculitis was seen in so-called vasculitis syndromes such as polyarteritis nodosa (PAN) or secondary process of other connective tissue diseases including RA. From the diagnostic approach of rheumatic vasculitis, NCV study or subsequent muscle biopsy seem to be crucial to confirm vasculitis. Especially, biopsy specimen directly gives us available information to detect vasculitis.

In our case, positive MRI findings ended up with neutrophil-infiltration dominant vasculitis. Gallien et al. [6] previously demonstrated positive MRI findings in T2 weighed and STIR in PAN patients. Although vasculitis was restricted in limbs in their cases, the positive MRI findings were considered to be increased muscle fluid content. As they showed, the MRI findings are observed in both edema and myopathies. Since myopathy was absent in our case, edema-like change as a result of vasculitis might exist.

In summary, we show positive MRI findings and subsequent pathological confirmation of typical vasculitis without myositis or fascitis. Since our case responded to IVCY therapy based on existence of vasculitis, MRI is beneficial before performing muscle biopsy in case of rheumatoid vasculitis with peripheral neuropathy.

Conflict of interest statement The authors declare no conflict of interest.

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ACTA NEUROLOGICA
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Anti-cyclic citrullinated peptide antibody (anti-CCP antibody) is present in the sera of patients with dementia of Alzheimer's type in Asian

Satoh K, Kawakami A, Shirabe S, Tamai M, Sato A, Tsujihata M, Nagasato K, Eguchi K. Anti-cyclic citrullinated peptide antibody (anti-CCP antibody) is present in the sera of patients with dementia of Alzheimer's type in Asian.
Acta Neurol Scand. 2010: 121: 338–341.

Background – In the hippocampi of Alzheimer's disease (AD) patients, aberrant expression of citrullinated proteins and peptidylarginase 2 (PADI2) has been identified. We explored the functional roles of these proteins by means of detection of serum anti-cyclic citrullinated peptide antibody (anti-CCP antibody) in patients with dementia of Alzheimer's type (DAT). *Methods* – Sera were obtained from 42 patients with DAT, 30 patients with other neurological disorders and 42 healthy controls. Gender ratio and age were comparable among the three groups. The level of anti-CCP antibody in sera was examined by ELISA. *Findings* – Anti-CCP antibody was not found in the 30 patients with other neurological disorders, and only one of the 42 healthy controls (2.4%) was positive. However, surprisingly, anti-CCP antibody was clearly detected in eight of the 42 DAT patients. *Interpretation* – Anti-CCP antibody appears to be a simple and early serologic biomarker for DAT among dementia patients. Additionally, our data imply that citrullinated proteins accumulated in the astrocytes of AD patients acquire neo-antigenicity, inducing anti-CCP antibody production.

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Key words: anti-cyclic citrullinated peptide antibody, dementia of Alzheimer's type

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Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease, leading to progressive dementia. Recent promising studies have revealed that functional imaging with positron emission tomography (PET) and magnetic resonance imaging (MRI) can detect, even in the early stages, the pathological lesions underlying AD. Also, cerebrospinal fluid markers, including tau, phospholated tau and A beta 1–42, have emerged as important biological markers that could provide an early diagnosis of AD; however, serum markers for AD have not been identified.

In this regard, Ishigami et al. (1) have elegantly demonstrated the accumulation of citrullinated proteins in the hippocampi of AD. The enzyme peptidylarginase 2 (PADI2), which can catalyze the post-translational process in which arginine residues undergo modification into citrulline residues, is co-localized with citrullinated proteins in AD patients (1). Considering the acceptable hypothesis that an excess of citrullinated proteins *in situ* leads to the presence of anti-cyclic citrullinated peptide antibody (anti-CCP antibody) in serum (1, 2), we examined seropositivity for anti-CCP antibody in patients with dementia of Alzheimer's type (DAT) in the present study, revealing that anti-CCP antibody is specific for DAT, as compared with other types of neurological disease. Many papers were described as some relationship between rheumatoid arthritis (RA) and DAT, but they

were not able to identify some relationship between RA and DAT. Our data showed much more relationship between RA and DAT.

Patients and methods

Patients

Forty-two patients were typical cases of DAT in terms of time course, MR image findings, and abnormal lesions on SPECT, were selected from among 200 DAT patients who fulfilled the diagnostic criteria of DSM-IV and the criteria of NINCDS established by the NINCDS-ADRDA Work Group. The male:female ratio and the average age of DAT patients were equal to those of standard DAT studies. Sera from 42 DAT patients were collected within 3 years of disease onset (Tables 1 and 2).

As patients with other neurological disorders, 11 patients with Creutzfeldt–Jakob disease (CJD), nine patients with Parkinson's disease (PD), four patients with dementia with Lewy bodies (DLB), four patients with cerebrovascular dementia (CVD), one patient with corticobasal degeneration (CBD) and one patient with progressive supranuclear palsy (PSP), were included in the present study. All patients met the neurological disorder diagnostic criteria established by the Ministry of Health, Welfare and Labour of Japan, or standard criteria. As a control, sera from 30 healthy subjects were also collected. And 10 patients without 42 DAT patients, 30 non-DAT patients and 42 healthy subjects, were mild cognitive impairments (MCI). All samples were collected after obtaining informed consent, and the protocol was approved by the Institutional Review Board of Nagasaki University. As shown in Table 1, gender ratio and age were comparable among the three groups, as calculated by chi-square test. No cases had RA.

Table 1 Patient distribution

Dementia of Alzheimer's type (DAT)	42 cases
Healthy subjects	42 cases
Non-DAT	30 cases
Creutzfeldt–Jakob disease	11 cases
Cerebrovascular disorders;	4 cases
Dementia with Lewy bodies	4 cases
MCI: Mild cognitive impairment	10 cases
Parkinson's disease	8 cases
Corticobasal degeneration	1 case
Progressive supranuclear palsy	2 cases

Measurement of anti-CCP antibody

The levels of anti-CCP antibody were measured with an enzyme-linked immunosorbent assay (ELISA) system using the DIASTAT anti-CCP antibody kit (AXIS-Shield Dundee Co., Dundee, UK), with a cut-off value of 4.5 U/ml according to results described by Suzuki et al. (3).

Statistical analysis

The statistical significance among three groups was examined by Chi-square test, and *P*-values less than 0.05 were considered to represent significance.

Results

Table 3 shows the levels of anti-CCP antibody positivity in the three subject groups. Anti-CCP antibody was found in eight out of 42 DAT patients (19.1%), but not detected in any of the 30 patients with other neurological disorders. Only one healthy control subject was positive for anti-CCP antibody, the titre of which was low compared with those in DAT patients (data not shown). A Chi-square test identified significant anti-CCP antibody seropositivity in DAT patients compared with the other two subject groups ($P = 0.0032$).

In the group of patients with MCI, the male:female ratio was 2:3, and the average age was 72.9 ± 2.2 years. After 3 years, three out of 10

Table 2 Profile of patient characteristics

	Patients with dementia of Alzheimer's type	Patients with other neurological disorders	Healthy subjects	*P*-value
Total number (cases)	$n = 42$	$n = 30$	$n = 42$	
Age (years)	72.4 ± 8.7	73.3 ± 10.1	72.4 ± 8.7	n.p.
Age range (years)	52–86	42–96	52–86	
Proportion of females (%)	61.90%	56.67%	61.90%	n.p.
Male:female ratio	16:26	13:17	16:26	

Other neurological disorders ($n = 30$) includes 11 patients with Creutzfeldt–Jakob disease (CJD), nine patients with Parkinson's disease (PD), four patients with dementia with Lewy bodies (DLB), four patients with cerebrovascular dementia (CVD), one patient with corticobasal degeneration (CBD) and one patient with progressive supranuclear palsy (PSP). No significant difference in gender ratio or age was detected among three groups, as calculated by chi-square test. n.p., profile in patients' characteristics.

Table 3 Anti-CCP antibody is predominantly found in patients with dementia of Alzheimer's type (DAT), as compared with patients with other neurological disorders and healthy subjects

	Patients with 'dementia of Alzheimer's type (n = 42)	Patients with other neurological disorders	Healthy subjects (n = 42)	P value
% Positive anti-CCP antibody	19.10%	0%	2.40%	0.0032

Anti-CCP antibody was found in eight out of 42 DAT patients, whereas no patients with other neurological disorders and one out of 42 healthy subjects, were positive. A significantly high level of anti-CCP antibody-positivity in DAT patients was determined by chi-square test.

Table 4 Summary of the clinical profiles of the DAT patients positive for anti-CCP antibody

Age (years)	Sex	Diagnosis	Duration from disease onset (months)	MMSE	Titre of anti-CCP antibody (U/ml)
67	F	DAT	17	13	8.1
79	F	DAT	20	9	14
70	M	DAT	16	12	28.8
55	F	DAT	26	8	15.2
74	F	DAT	37	4	55
73	M	DAT	36	6	55
58	F	DAT	8	12	9.3
71	M	DAT	10	11	11.5

MCI patients developed DAT and a further three were classified as 'AD convert', on of which was positive for anti-CCP antibody (5.0 U/ml) (Table 4) (3).

Discussion

Ishigami et al. revealed aberrant expression of citrullinated proteins and PADI2, especially in astrocytes, in the hippocampi of Alzheimer's disease patients, which was the first direct evidence of citrullination in a human neurological disorder. Anticipation of protein citrullination *in situ* may drive the production of anti-CCP antibody, which has been widely observed in RA patients (2, 3). Based on the above findings, we examined the presence of anti-CCP antibody in the sera of DAT patients.

We did not fully screen, at present, the seropositivity of anti-CCP antibody in neurodegenerative diseases with dementia; however, anti-CCP antibody was not found in patients with CJD, PD, DLB, CVD or PSP. The antibody was detected in DAT patients, but the frequency of seropositivity was relatively low compared with that of patients with RA. Recent investigations have found that the protein citrullination process is driven by PADIs, at least five human isoforms of which have been cloned (4). The importance of PADI4 in anti-CCP antibody production in RA patients is strongly suggested (1–3, 5, 6); however, PADI2 is suggested to be important in Alzheimer's disease (7). The anti-CCP antibody ELISA kit contains varying kinds of artificially engineered citrullinated peptides; thus, the kit screens not a uniform but a heterogeneous human IgG, which binds to citrullinated peptides. Additionally, enzymatic properties differ between PADI2 and PADI4 (8), suggesting that the citrullinated molecules detected in the sera of DAT and RA patients are different, leading to the difference in seropositivity for anti-CCP antibody between DAT and RA patients. In RA, an association of HLA-DRB1 shared epitope (SE) carriership with anti-CCP antibody is determined, thus, HLA-DR typing of DAT is also warranted (9).

There is an ongoing research theme investigating what citrullinated molecules lead to the production of anti-CCP antibody in human diseases. Mastronardi et al. have very recently shown that tumour necrosis factor α induces translocation of PADI4 from the cytoplasm to the nucleus, which induces nuclear histone citrullination in multiple sclerosis brain, leading to destabilisation of myelin basic protein (10). Nevertheless, anti-CCP antibody was not detected in sera from 16 multiple sclerosis patients in our study (data not shown). In RA, some candidate citrullinated molecules, such as fibronectin, collagen type I and vimentin, are targeted by serum anti-CCP antibody (11–13). Thus, the findings of the present study may contribute to the discrimination of DAT from the pool of dementia; however, further proteomic examination is necessary to clarify the molecular mechanism underlying anti-CCP antibody production in DAT patients.

Bodil Roth et al. (14) were examined the frequency of seropositivity of anti-CCP antibody serum in the neurologic disease in the European. And in the study, 2–3% of AD or multiple sclerosis was positive with anti-CCP antibody. Our data identified that anti-CCP antibody was clearly detected in eight of the 42 DAT patients (19%), but anti-CCP antibody was not found in the 30 patients with other neurological disorders.

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Expert Opinion

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

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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 through blood transfusion in the UK. However, no treatments are yet available for curing prion diseases. *Objective*: Conversion of the normal prion protein, PrP^{C}, to the amyloidogenic PrP, PrP^{Sc}, 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 PrP^{Sc} 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: antibody, immunotherapy, prion, prion diseases, prion protein

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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 prion diseases in human beings [2]. The etiology of sporadic CJD remains unknown [2]. Interestingly, ~ 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 through contaminated food [8,9]. No effective therapy for these diseases has been developed yet.

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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 [12]. 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 UK and other countries [13]. 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 [14], raising similar health concerns about the possibility of the disease being transmitted to humans, causing another type of vCJD.

Four cases of vCJD considered to be transmitted through blood transfusion have been reported in the UK [15-18], raising the more serious concern of human-to-human secondary transmission of vCJD through 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 [19-21]. All cases of vCJD owing to BSE, reported so far, have been found in MM individuals [22]. No MV or VV cases have been identified so far [22]. However, one case of blood transfusion related vCJD was heterozygous at codon 129 [16]. 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 [22]. 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 reported so far for conventional human prion diseases. They showed that 3 out of 12,674 surgically removed appendectomy or tonsillectomy specimens were positive for staining of PrP^{Sc}, although two specimens displayed a dissimilar staining pattern of PrP^{Sc} from that in vCJD [23]. 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^{Sc} [24]. This is generated via the conformational conversion of the normal cellular isoform of PrP, PrP^{C}, a membrane glycoprotein anchored to the cell surface by means of a glycosylphosphatidylinositol (GPI) moiety and expressed in various tissues, with the highest expression in the brain, particularly in neurons [24,25]. Accumulating lines of evidence indicate that the conformational conversion of PrP^{C} into PrP^{Sc} plays a pivotal role in the pathogenesis of prion diseases. Indeed, along with other researchers' studies, we have shown that PrP^{C}-deficient ($Prnp^{0/0}$) mice, in which the conversion never occurs owing to lack of PrP^{C}, were resistant to the diseases even after inoculation with mouse-adapted prions [26-29]. Moreover, it was reported that the removal of PrP^{C} specifically from the infected neurons rescued mice from the disease [30,31], 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^{C} were decreased in the affected regions whereas those against PrP^{Sc} were increased [32]: this suggested that constitutive conversion might lead to a decrease in PrP^{C} in the brains and that the resultant functional impairment of PrP^{C} might be involved in the pathogenesis. Indeed, $Prnp^{0/0}$ 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 [33-36]. Therefore, it is possible that such abnormalities in prion diseases might be attributable to the functional loss of PrP^{C}. Thus, approaches to enhance the function of PrP^{C} might be alternatively therapeutic against prion diseases. However, the physiological function of PrP^{C} remains unknown. In contrast to the reduction in PrP^{C}, constitutive conversion causes the accumulation of PrP^{Sc} in the brain. Hence, it is suggested that PrP^{Sc} might be a toxic neurodegenerative molecule. Indeed, the accumulation of PrP^{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^{Sc} was shown to be toxic to cultured cells, inducing apoptotic cell death [37-39]. Therefore, approaches that lead to the protection of neurons from PrP^{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^{Sc} in chronically infected cultured cells, such as scrapie prion-infected mouse neuroblastoma N2a cells [40,41]. 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.

Table 1. Therapeutic chemical agents [40,41].

Class	Chemical agents	Possible mechanisms
Polyanionic compounds	Heteropolyanion-23, carrageenan, dextran sulfate, pentosan polysulfate, 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^C and/or PrP^{Sc} Disturb subcellular localization of PrP^C and/or PrP^{Sc}
Polycationic compounds	Polyamidoamide branched polymer, polypropyleneimine branched polymer, polyethyleneimine branched polymer, cationic phosphorus-containing dendrimers, cationic lipopolyamine, cationic polysaccharides	Accelerate PrP^{Sc} degradation in lysosomes
Amyloid-binding compounds	Congo red, Trypan blue, Evans blue, Sirius red F3B, Primuline, Thioflavin-S, BF-168, cpd-B	Overstablize PrP^{Sc} conformation Compete with endogenous GAGs for the interaction with PrP^C and/or PrP^{Sc}
Tetracyclic compounds	Anthracycline 4′-iodo-4′-deoxyrubicin, tetracycline, doxycycline	Destabilize PrP^{Sc} conformation
Tetrapyrrolic compounds	Porphyrins, phthalocyanins	Change PrP^{Sc} conformation
Cholesterol metabolism-related compounds	Polyene antibiotics (amphotericin B, MS-8209), statins (lovastatin, squalestatin, 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, bebeerine, tetrandrine, amodiaquine, tiotixene, prochlorperazine, thioridazine, trifluoperazine	Bind to PrP^{Sc} fibrils and inhibit *de novo* synthesis of PrP^{Sc}
Lysosomal cystein protease inhibitors	E-64, E-64d, leupeptin	Unknown
Cell signaling inhibitors	Tyrosine kinase inhibitor (imatinib mesilate) Phopholipase A2 inhibitors (cytidine-5-diphosphocholine, bromoenol lactone, aristolochic acid, arachidonyl trifluoromethyl ketone) Mitogen-activated protein kinase kinase (MEK) 1/2 inhibitors	Stimulate PrP^{Sc} degradation by inhibiting c-Abl kinase Inhibit PrP^{Sc} formation by altering cell membrane integrity Stimulate PrP^{Sc} degradation by inhibiting MEK1/2 activity
Polyphenols	Tannic acid, katacine, bisepigallocatechin digallate	Unknown
Anti-histamines	Astemizole, terfenadine	Unknown
Steroid-type compounds	Budesonide, clomifene, chrysanthellin A	Unknown
Others	2-Pyrrolidin-1-yl-*N*-[4-[4-(2-pyrrolidin-1-yl-acetylamino)-benzyl]-phenyl]-acetamidee	Stabilize PrP^C formation
	Kastellpaolitines	Unknown
	N′-Benzylidene-benzohydrazides	Inhibit interaction between PrP^C and PrP^{Sc}
	Pridine dicarbonitrile compounds	Inhibit PrP^{Sc} formation by mimicking the residues of the dominant negative PrP^C mutants

GAG: Glycosaminoglycans; LRP/LR: Laminin receptor.

The compounds that could reduce PrP^{Sc} 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 them before or immediately after prion inoculation [40,41]. However, no curable effects of these compounds could be detected [40,41]. 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 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 *et al.* 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 [42]. 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, there have been no reports of patients being 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^{Sc} on the cell surface [43], and was directly injected into the brain ventricle of patients with various types of prion diseases [44,45]. Bone *et al.* showed that the mean survival of all treated patients was longer than the reported values for non-treated patients [45]. However, owing to the 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 [46]. Nakajima *et al.* reported transient and modest improvement in mood or cognitive function by treatment of 3 patients with quinacrine [47]. 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 [48]. 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 owing 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 [49]. 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} values 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} values 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 [50]: they added an anti-PrP mAb, termed 6H4, which binds 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^{C} and PrP^{Sc} probably on the cell surface, particularly on lipid rafts and/or along the endocytotic pathway to late endosomes/lysosomes [51]. Therefore, it is 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 [52,53]. Another possibility is that anti-PrP antibodies might reduce PrP^{Sc} levels in infected cells by altering the subcellular localization of PrP^{C}. Kim *et al.* showed that 31C6, 110, 44B1 and 72 anti-PrP mAbs with anti-prion activity disturbed PrP^{C} internalization [54]. 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 [54]. 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^C in cells [55], suggesting another possibility that anti-PrP antibodies might inhibit PrP^{Sc} formation by reducing PrP^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^C or PrP^{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 by means of a heparan sulfate chain of proteoglycan [56,57]. Subsequently, LRP/LR was also demonstrated to act as the cell-surface receptor for PrP^C [58] and PrP27-30, the proteinase K-resistant core of PrP^{Sc} [43]. These indicate that LRP/LR might be involved in PrP^{Sc} 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^{Sc} levels in infected N2a cells and finally cured the cells [59]. This result indicates that W3 might inhibit PrP^{Sc} formation by disturbing the interaction between PrP^C and LRP/LR. W3 was also shown to reduce PrP^C levels in cells [59]. 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^{Sc} formation. Thus, LRP/LR might be a therapeutic target of prion diseases [60-63].

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^{Sc} levels in infected N2a cells [64]. 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 [65], could protect mice from the peripheral infection of RML prions but had no effects on prions directly introduced into the brains of mice [66]. This could be owing 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 ($p = 0.158$, Log-rank 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^{Sc} 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^{Sc} 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_{50}) could marginally but significantly prolong survival times in mice that had been intracerebrally inoculated with Chandler prion [67]. 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 ~ 10 days more than did the control mice, regardless of the time points of treatment. Moreover, milder pathologies including PrP^{Sc} accumulation, gliosis and vaccuolation 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 ~ 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. Owing 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 *et al.* observed that, compared to whole IgGs, lower molecular weight $F(ab')_2$ fragments were more widely distributed at higher concentrations in the brain of mice when intraventricularly injected [68]. Therefore, a reduction in the molecular size of anti-PrP antibodies without reducing their anti-prion

Table 2. No therapeutic effects of intraventricularly administrated 3S9 mAb in Fukuoka-1 prion-infected mice.

Treatment time	Antibodies	*N*	Incubation times (mean ± SD, days)	*P* values (Log-rank test)	Survival times (mean ± SD, days)	*P* values (Log-rank test)
8 Weeks p.i.	Control IgG	6	117.8 ± 9.9	0.158	122.5 ± 6.1	0.273
	3S9	5	127.0 ± 9.0		130.0 ± 11.7	
13 Weeks p.i.	Control IgG	5	121.2 ± 2.5	0.851	124.0 ± 3.7	0.942
	3S9	6	125.0 ± 4.7		127.2 ± 5.8	

p.i.: Post-inoculation.

activity might be helpful in overcoming this problem [69]. It has already been shown that fragmented anti-PrP antibodies, such as Fab and scFv antibodies, remain active against PrP^{Sc} formation in infected cells [70,71]. 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 [72]. Several serotypes of recombinant adeno-associated vector (rAAV) are useful as gene delivery vehicles to treat neurological disorders owing to their efficient gene transduction into neurons and their safety profiles [73-75]. 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. It 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. rAAVs 1 and 5 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 [76,77]. However, it was reported that repeated injection of rAAV vectors into the rat or mouse brain was possible [78,79]. The third limitation is the duration of the expression of a delivered gene. The stable expression of an rAAV2-delivered gene was observed up to ≥ 9 months in the rat brain [74].

Wuertzer *et al.* generated an rAAV2 vector encoding anti-PrP scFv fragments, termed scFv 3:3, scFv 6:4, scFv 6:6 and scFv D18 [72]. Each rAAV2 scFv vector with 9×10^9 expression units was bilaterally injected into the thalamus and striatum of mice, and 1 month later, the mice were intraperitoneally inoculated with RML prions [72]. Mice treated with the control vector developed the disease at 199 ± 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 ± 13 and 250 ± 8 days p.i., respectively. Reduced accumulation of PrP^{Sc} 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 [71]. Results showed that the lentivirus could transduce scFv D18 in the cells more efficiently than could rAAV2 [71]. 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 [80]. In contrast, another anti-PrP D18 mAb against the residues 133 – 157 did not manifest any neurotoxicity [80]. 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 [68]. The treatment was initiated in the early stage of neuroinvasion (85 days p.i.) [68]. 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 [67]. 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 [81]. 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 owing 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^{Sc} [81]. Similar results were reported with anti-LRP/LR scFv fragments, termed S18 [82,83]. S18 scFv reduced PrP^{Sc} by ~ 40% in the spleens of mice infected with RML prions when intraperitoneally injected once a week for 8 weeks from 1 day before the infection. These results clearly indicate that anti-LRP/LR antibody or scFv is active *in vivo* and is able to reduce PrP^{Sc} levels.

Zuber *et al.* used rAAV2 as a vector for anti-LRP/LR scFvs to be transduced into the brain [84]. They generated rAAV2 encoding two different scFvs, S18 and N3, and injected 5×10^9 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 [84]. Thereafter, anti-LRP/LR scFvs were produced in the mouse brains. However, in contrast to the results of Wuertzer *et al.* [72], 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^{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^{Sc} levels were reduced by ~ 32 and 60%, respectively [84].

5.5 PrP-Fc_2

Meier *et al.* reported that wild-type mice transgenically expressing PrP-Fc_2, a dimeric fusion protein of PrP^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^{Sc} in the brains as compared to control non-transgenic mice [85]. PrP-Fc_2 had a potential to bind to PrP^{Sc} via PrP^C, but the PrP^C part could not be converted to PrP^{Sc} probably owing to fusion with Fc. The authors, therefore, suggested that PrP-Fc_2 might inhibit PrP^{Sc} formation by a dominant negative mechanism, in which PrP-Fc_2 disturbs the binding of native PrP^C to PrP^{Sc} by intercalation between the two molecules, resulting in the prevention of PrP^{Sc} formation [85]. The same group further investigated the prophylactic activity of lentivirus vector-transduced PrP-Fc_2 against RML prions [86]. The PrP-Fc_2 lentivirus was injected into the hippocampus. When the mice were intracerebrally inoculated with RML prions 20 days after the virus injection with 3×10^8 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_2 further increased when the virus vector was transduced into the brain with higher infectious units [86]. When mice were treated with 1.5×10^9 infectious units of the virus vector, the incubation times were further prolonged by 72 days (treated versus control mice, 247 ± 8 versus 175 ± 5 days p.i.). However, when the virus (1.5×10^9 infectious units) was injected 30 days after prion inoculation, the post-exposure prophylactic effect of PrP-Fc_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_2 was observed in mice when the vector was injected at 121 days p.i. (the late stage of disease) [86]. 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^C is a host-encoded glycoprotein expressed in various normal tissues, no immune responses are evoked against PrP^C under physiological conditions as well as PrP^{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 *in vivo* although no abnormal symptoms have been reported in mice peripherally administered with anti-PrP or anti-LRP/LR W3 antibodies [66,81]. Moreover, the injected antibodies might generate detrimental signals on binding to PrP^C or LRP/LR. Indeed, as described earlier, certain anti-PrP mAbs elicited neurotoxic signals by cross-linking of PrP^C when injected into the brain [68,80]. Thus, the possibility that these unfavorable effects might be induced by antibody-based immunotherapy for prion diseases should be carefully considered.

6. Conclusion

Recent studies using experimental animal models have reinforced the possibility of antibody-based immunotherapeutic approaches against prion diseases. However, several problems were also delineated for the approaches to be in practice. The problem to be primarily overcome is insufficiency of antibody delivery into the brain regions that are relevant to the therapy for prion diseases. Unfavorable consequences might also occur owing to the antibodies used in the therapy. Finally, the immunotherapeutic effect was much less in clinically advanced animals than in presymptomatic animals. This indicates the importance of preclinical treatment of the diseases and development of sensitive diagnostic measures that enable the identification of preclinical patients. In conclusion, overcoming these problems is essential for the immunotherapy against prion diseases.

7. 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 $PrP\text{-}Fc_2$ directly delivered into the brain by virus vector-mediated gene transfer. This is probably because the antibodies could not infiltrate into the brain owing to their higher molecular weight and because the virus vectors infected only those neurons that were located at 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 $PrP\text{-}Fc_2$ 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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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.