Effect of intraventricular infusion of anti-prion protein monoclonal antibodies on disease progression in prion-infected mice

Chang-Hyun Song,¹ Hidefumi Furuoka,² Chan-Lan Kim,¹† Michiko Ogino,¹‡ Akio Suzuki,¹ Rie Hasebe¹ and Motohiro Horiuchi¹

Correspondence Motohiro Horiuchi horiuchi@vetmed.hokudai.ac.jp

¹Laboratory of Prion Diseases, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan
²Department of Pathobiological Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro 080-8555, Japan

Received 9 November 2007
Accepted 28 February 2008

It is well known that anti-prion protein (PrP) monoclonal antibodies (mAbs) inhibit abnormal isoform PrP (PrPSc) formation in cell culture. Additionally, passive immunization of anti-PrP mAbs protects the animals from prion infection via peripheral challenge when mAbs are administered simultaneously or soon after prion inoculation. Thus, anti-PrP mAbs are candidates for the treatment of prion diseases. However, the effects of mAbs on disease progression in the middle and late stages of the disease remain unclear. This study carried out intraventricular infusion of mAbs into prion-infected mice before and after clinical onset to assess their ability to delay disease progression. A 4-week infusion of anti-PrP mAbs initiated at 120 days post-inoculation (p.i.), which is just after clinical onset, reduced PrPSc levels to 70–80% of those found in mice treated with a negative-control mAb. Spongiform changes, microglial activation and astrogliosis in the hippocampus and thalamus appeared milder in mice treated with anti-PrP mAbs than in those treated with a negative-control mAb. Treatment with anti-PrP mAb prolonged the survival of mice infected with Chandler or Obihiro strain when infusion was initiated at 60 days p.i., at which point PrPSc is detectable in the brain. In contrast, infusion initiated after clinical onset prolonged the survival time by about 8% only in mice infected with the Chandler strain. Although the effects on survival varied for different prion strains, the anti-PrP mAb could partly prevent disease progression, even after clinical onset, suggesting immunotherapy as a candidate for treatment of prion diseases.

INTRODUCTION

Prion diseases, such as scrapie, bovine spongiform encephalopathy (BSE) and Creutzfeldt–Jakob disease (CJD), are fatal neurodegenerative disorders characterized by accumulation of a disease-specific, abnormal isoform of the prion protein (PrPSc) in the central nervous system (CNS), astroglisis, neuronal vacuolation and neuronal cell death. The appearance of BSE and variant CJD (vCJD), possibly linked to consumption of food derived from BSE-infected cattle, has increased awareness of prion diseases, but at present there is no effective treatment available for prion diseases. Given that transformation of a normal prion protein (PrPC) to PrPSc is a central event in the pathogenesis of prion disease, compounds and/or strategies that inhibit PrPSc formation are of therapeutic interest.

Many compounds or strategies have been reported to inhibit PrPSc formation, including polyanions, glycosaminoglycans, phosphorothioate oligonucleotides, tetrapyroles, polyene antibiotics, tricyclic compounds, PrP peptides, dominant-negative PrP, cysteine protease inhibitors, PrP immunization and small interfering RNAs (reviewed by Trevitt & Collinge, 2006). Most of these compounds and treatments antagonize PrPSc formation in cells persistently infected with prions. However, the anti-prion effects in vivo are not always consistent with those observed in vitro. Indeed, some of the compounds and treatments protect animals from experimental inoculation with prions or delay the onset of disease when administered before, simultaneously or soon after inoculation with prions via a peripheral route (Ehlers & Diringer, 1984; Farquhar & Dickinson, 1986; Ladogana et al., 1992; Priola
et al., 2000). In addition, only a few compounds, such as amphotericin B, its derivative, MS-8209 and pentosan polysulfate (PPS), can prolong survival of mice infected with prions even when administered in the middle or late stage of prion infection via intracerebral inoculation (Demainay et al., 1997; Doh-ura et al., 2004). Because intraventricular infusion of PPS at a late stage prolongs the incubation period of the disease in transgenic mice that overexpress PrP (Doh-ura et al., 2004), clinical trials using PPS to treat human prion diseases are moving forward (Todd et al., 2005). The current evidence suggests that PPS treatment of vCJD patients appears to have some beneficial effects, although the specificity of the effects still needs to be evaluated carefully (Rainov et al., 2007).

Anti-PrP antibodies prevent direct interaction between PrPC and PrPSc in a cell-free conversion reaction (Kaneko et al., 1995; Horiuchi & Caughey, 1999). Subsequent reports have shown that anti-PrP antibodies prevent prion propagation in cells persistently infected with prion (Enari et al., 2001; Peretz et al., 2001; Gilch et al., 2003; Kim et al., 2004b; Perrier et al., 2004; Feraudet et al., 2005). The inhibitory effect of anti-PrP antibodies has also been demonstrated in vivo. Transgenic mice expressing monoclonal antibody (mAb) 6H4 were shown to be resistant to prion infection via the intraperitoneal route (Heppner et al., 2001). Moreover, active immunization with recombinant PrP, synthetic PrP peptide or a DNA vaccine has been shown to delay the onset of the disease in mice following peripheral prion infection, although immunization was a prerequisite to obtain the prophylactic effect (Sigurdsson et al., 2002; Schwarz et al., 2003; Goïni et al., 2005; Fernandez-Borges et al., 2006). Passive immunization with anti-PrP antibodies was found to be effective in preventing prion infection via the peripheral route if antibodies were administered shortly after prion inoculation, but was not following intracerebral prion infection or if administered on or after clinical onset following intraperitoneal prion infection (White et al., 2003). These results suggest that anti-PrP antibodies can protect against establishment of prion infection in peripheral tissues and thus may be useful for post-exposure prophylaxis. However, the therapeutic potential of anti-PrP antibodies, including whether or not anti-PrP antibodies antagonize prion propagation in the brain and can inhibit disease progression when applied after clinical onset, remains to be elucidated.

To evaluate the therapeutic effects of anti-PrP antibodies on prion diseases more precisely, we carried out intraventricular infusion of anti-PrP mAbs in mice that had been inoculated intracerebrally with prions. Here, we show that intraventricular infusion of anti-PrP mAbs reduced the level of accumulation of PrPSc and reduced spongiform changes and gliosis relative to negative controls. Furthermore, we observed prolongation of the incubation time in mice infected with the Chandler strain, even when infusion was initiated at the time of clinical onset of the disease.

**METHODS**

**Antibodies.** The following anti-PrP mAbs were used in this study: 106 (IgG2b), 110 (IgG2b), 31C6 (IgG1) and 44B1 (IgG2a). The mAbs 106, 110 and 31C6 recognized linear epitopes consisting of mouse PrP aa 88–90, 83–89 and 143–149, respectively, whereas mAb 44B1 recognized a discontinuous epitope within aa 155–231 (Kim et al., 2004a). Anti-feline parvovirus mAb P2-284 (IgG1) was used as a negative control (Horiuchi et al., 1997). The mAbs were dialysed for 3 days against PBS prior to intraventricular infusion. An Alexa Fluor 488 Protein Labelling kit (Molecular Probes) was used for fluorescent labelling of mAbs.

The following rabbit polyclonal antibodies were used as primary antibodies for immunohistochemistry: B103, which recognizes bovine PrP synthetic peptide aa 103–121 (Horiuchi et al., 1995), anti-glial fibrillary acidic protein (GFAP; Dako) to visualize astrocytes and anti-Iba1 (Wako) to visualize microglia.

**Mice and prion strains.** All procedures for animal experiments were carried out according to protocols approved by the Institutional Committee for Animal Experiments. Mouse-adapted scrapie strains Oibhiro and Chandler were used in this study. For intracerebral inoculation, 4-week-old female ICR mice were purchased from CLEA Japan. Twenty microlitres of 10% brain homogenate from mice infected with the Oibhiro or Chandler strain was injected into the left hemisphere. Twelve-week-old female ICR mice were used to determine the distribution of mAbs and to analyse neuronal toxicity by anti-PrP mAbs.

**Intraventricular infusion of mAbs using an osmotic pump.** Alzet Mini-Osmotic Pumps, models 2001, 2002 and 2004 (DURECT), were used in this study. Filling of the osmotic pumps with antibody solution was carried out according to the manufacturer’s instructions. The pre-filled pumps were then placed in PBS at 37°C for 24 h. Mice were fitted with a stainless steel cannula supplied with the Alzet Brain Infusion kit (DURECT) and positioned according to stereotaxic coordinates into the left lateral ventricle of the brain (bregma – caudal 1.0 mm, lateral 1.0 mm with a depth of 3 mm below the dura). The osmotic pumps were subsequently implanted subcutaneously into the back and connected to the fitted cannula. All surgical procedures were performed under anaesthesia by intramuscular injection of xylazine (10 mg kg–1) and ketamine (50 mg kg–1). After surgery, cefotaxime (Chugai) was administered subcutaneously (40 mg kg–1) and a gentamicin ointment (Scherings-Ploough) was pasted on the suture line for 3 days. All mice were housed individually during post-surgery observation periods. Mice that died within a few days of the operation were excluded from the statistical analysis.

**Stereotaxic injection of mAbs.** Mice were anaesthetized as described above and placed onto a stereotaxic apparatus (Narisnge). A linear scalp incision was made and the skull was exposed. Bilateral burr holes were drilled to accommodate stereotaxic placement into the left and right hippocampus (bregma – caudal 2.0 mm, lateral 2.1 mm). Using a Hamilton syringe with a 31-gauge needle, 2 μl mAbs (2 mg ml–1) were injected into the left and right hippocampus, respectively, at a depth of 2 mm below the dura. Injection was carried out over a period of 15 min.

**Western blotting.** Brains were sagittally hemi-sectioned and homogenized in 10% (w/v) TMS buffer [50 mM Tris/HCl (pH 7.5), 5 mM MgCl2, 5% glucose]. To detect PrPSc, 200 μl brain homogenate was mixed with an equal volume of a detergent buffer [8% Zwittergent 3-14, 1% Sarkosyl, 100 mM NaCl, 50 mM Tris/HCl (pH 7.5)] and treated with collagenase (0.5 mg ml–1) for 15 min at 37°C. The samples were then digested with proteinase K (PK; Roche).
at 20 μg ml⁻¹ for 30 min at 37 °C. After terminating PK activity by adding Pefabloc (Roche) at 2 mM, samples were treated with 40 μg DNase I ml⁻¹ for 5 min. A half volume of a mixture of 2-butanol and methanol (5:1) was added and the PrPSc was pelleted by centrifugation at 20,000 g for 10 min at 20 °C. The resulting pellet was dissolved in 1 x SDS sample buffer [62.5 mM Tris/HCl (pH 6.8), 5% glycerol, 3 mM EDTA, 4% β-mercaptoethanol, 0.04% bromophenol blue, 5% SDS, 4 M urea] by boiling for 5 min. SDS-PAGE and Western blotting were carried out as described elsewhere (Uryu et al., 2007).

**Histopathology and immunohistochemistry.** Mouse brains that had been infused with Alexa Fluor 488-conjugated mAbs were frozen in Tissue-Tek OCT compound (Sakura) and cryosections of 16–20 μm were prepared. The sections were dried and fixed with acetone for 10 min. Sections were mounted with Vectashield containing propidium iodine (PI; Vector Laboratories) and examined with a C1 laser confocal microscope. The presence of infused mAb was also confirmed by direct detection as follows. The sections were reacted with EnVision⁺ System-labelled polymer conjugated to horseradish peroxidase (HRP) (Dako) for 45 min at 37 °C with B103 at a dilution of 1:100, anti-GFAP at 1:5000 or anti-Iba1 at 1:100. After washing with PBS, the sections were reacted with EnVision⁺ System-labelled polymer–HRP for 45 min at 37 °C. The sections were then rinsed and developed with Simple Stain DAB, followed by counterstaining with Mayer’s haematoxylin (Wako).

Dissected mouse brains were fixed in 10% formalin and embedded in paraffin. Sections (4 μm) were deparaffinized, rehydrated and subjected to haematoxylin and eosin (H&E) staining or immunohistochemistry. Antigen retrieval for immunohistochemistry was performed by hydrolytic autoclaving at 135 °C for 20 min for detection of PrPSc and at 121 °C for 10 min for GFAP and Iba1 (Furuoka et al., 2005). The sections were treated with 3% H₂O₂ for 5 min, blocked with 10% normal goat serum for 30 min and then incubated for 45 min at 37 °C with B103 at a dilution of 1:100, anti-GFAP at 1:5000 or anti-Iba1 at 1:100. After washing with PBS, the sections were reacted with EnVision⁺ System-labelled polymer–HRP for 45 min at 37 °C. The sections were then rinsed and developed with Simple Stain DAB, followed by counterstaining with Mayer’s haematoxylin.

**Terminal uridine deoxynucleotide transferase dUTP nick end labeling (TUNEL) staining.** Neuronal cell death was examined using an In situ Cell Death Detection kit (Roche). Four micrometer sections of paraffin-embedded brain tissue were deparaffinized, rehydrated and incubated with 10 μg PK ml⁻¹ for 10 min at 37 °C. After washing with PBS, the sections were incubated with labelling mixture containing terminal deoxynucleotidyl transferase and digoxigenin-labelled dUTP-conjugated FITC for 60 min at 37 °C. The sections were counterstained with PI and examined with a C1 laser confocal microscope.

**RESULTS**

**Distribution of mAb in brain following intraventricular infusion.**

We first examined the distribution of mAb infused into the left lateral ventricle of mouse brain. Alexa Fluor 488-conjugated mAb 31C6 (anti-PrP mAb) or P2-284 (negative-control mAb) was infused into the left lateral ventricle and the distribution of mAb was examined at 7, 14, 24 and 34 days after the initiation of infusion. To examine the distribution of the mAbs, brain cryosections at the levels indicated in Fig. 1(a) were prepared. Fig. 1(c) shows the detection of Alexa Fluor 488-conjugated mAb 31C6 in the hippocampus. Fluorescence was detected over the hippocampus (up to 14 days). Although the area of distribution gradually narrowed thereafter, mAbs were still detectable in the hippocampus at 20 days after the termination of infusion (i.e. at 34 days). In contrast, a very low-level fluorescent signal was detected in the hippocampus of a mouse infused with Alexa Fluor 488-conjugated mAb P2-284, even at 7 days after the initiation of infusion. These results suggested that the longer duration of mAb 31C6 in the hippocampus compared with the control mAb was due to binding of mAb 31C6 to PrPC.

Fig. 1(d) summarizes the distribution of the anti-PrP mAb. The mAb was well distributed to areas surrounding the lateral and dorsal third ventricles, hippocampus and thalamus. The mAb was also detected in areas close to the ventral third ventricles. In addition, mAb was detected in regions of the medulla oblongata that face the fourth ventricle and the subarachnoid space, suggesting that the mAb infused into the lateral ventricle was distributed to many parts of brain, presumably via the flow of cerebrospinal fluid. Although the mAb was distributed to parts of the brain parenchyma, distribution of mAb into the cortex and cerebellum appeared to be less efficient. When observing sections under a microscope, we noticed that the mAb-infused hemisphere showed higher fluorescence intensity than that observed in the contralateral side (data not shown), suggesting that the distribution of mAb was not symmetrical. This tendency was confirmed by direct detection of Alexa Fluor 488-conjugated mAb 31C6 (Fig. 1b).

**Effects of anti-PrP mAbs on PrPSc accumulation in the brain.**

Mice inoculated with Obihiro or Chandler strain reach the terminal stage of the disease at around 150 days post-inoculation (p.i.). Early clinical signs such as ataxia of hind limbs and changes in pelage and posture appear at around 120 days p.i. To evaluate the therapeutic potential of anti-PrP mAbs in a late stage of the disease, infusion of mAbs was started at 120 days p.i. and accumulation of PrPSc and neurohistopathological lesions were analysed.

Fig. 2(a) shows PrPSc accumulation in the brains of mice infected with the Obihiro strain at 30 days post-infusion (150 days p.i.). The mean PrPSc levels in mice treated with mAbs 110, 31C6 and 44B1 were 78, 69 and 77%, respectively, compared with the control (mAb P2-284; n=2). To determine whether the relative reduction in PrPSc levels was caused by acceleration of PrPSc degradation or deceleration of PrPSc accumulation, we analysed the kinetics of PrPSc accumulation during the period from 127 to 150 days p.i. (Fig. 2b). There was no difference in PrPSc levels in mice treated with anti-PrP mAbs compared with those treated with the negative-control mAb at 127 days p.i. (7 days after the initiation of infusion). However, PrPSc levels increased 2.3-fold in mice treated with the control
mAb over the period 127–150 days p.i., whereas PrPSc levels increased only 1.6-, 1.3- and 1.3-fold in mice treated with mAbs 110, 31C6 and 44B1, respectively. These results indicated that anti-PrP mAbs can reduce the rate of PrPSc accumulation in the brain, even when treatment is initiated at a late stage of the disease.

**Fig. 1.** Distribution of mAbs following intraventricular infusion. Alexa Fluor 488-conjugated mAb 31C6 or P2-284 was infused into the left lateral ventricle using an Alzet Mini-Osmotic Pump model 2002 (mAb concentration 0.5 mg ml⁻¹, pumping rate 0.5 μl h⁻¹, duration 14 days, volume 200 μl). (a) Levels of coronal section examined. Cryosections at the indicated levels were prepared. (b) Detection of mAbs by direct staining. A frozen section at level b was prepared from the brain of a mouse sacrificed at 5 days after starting infusion and the distribution of mAb was visualized by direct staining. Bar, 500 μm. (c) Detection of mAbs in the hippocampus. mAbs conjugated with Alexa Fluor 488 were analysed by laser confocal microscopy. Bars, 200 μm. (d) Distribution of mAb after infusion. The distribution of mAb at 7, 14, 24 and 34 days after starting infusion (green) was superimposed on the images taken from Paxinos & Franklin (2001).
Effects of anti-PrP mAbs on neurodegeneration

Next, we investigated the effects of mAbs on neurodegeneration. To do this, the contralateral hemispheres of brains used in Fig. 2(a) (at 150 days p.i.) were examined histopathologically. Although mAbs were more readily detected in the infused side than in the contralateral side (Fig. 1), spongiform changes in the hippocampus and thalamus of mice treated with mAbs 110, 31C6 and 44B1 were nevertheless milder than those treated with the negative-control mAb (Fig. 2c).

Immunohistochemical examination also revealed that anti-PrP mAbs affected the progression of neuropathological lesions in mice infected with the Obihiro strain (Fig. 3). Consistent with the reduction in PrPSc levels by anti-PrP mAbs (Fig. 2a), PrPSc deposition in the hippocampus and thalamus of mice infused with mAbs 110 and 31C6 was milder than in the negative control. In addition, astrogliaosis (as evaluated by GFAP staining) appeared to be reduced in mice treated with anti-PrP mAbs compared with the negative control. Microglial activation in the

---

**Fig. 2.** Effects of anti-PrP mAbs on PrPSc accumulation and spongiform changes in mice infected with the Obihiro strain. mAbs were infused into the left lateral ventricle of mice inoculated with the Obihiro strain at 120 days p.i. using an Alzet Mini-Osmotic Pump model 2004 (mAb concentration 2 mg ml$^{-1}$, pumping rate 0.25 ml h$^{-1}$, duration 28 days, volume 200 µl). Mouse brains were cut sagittally along the midline. The left hemisphere (mAb-infused side) was used for the detection of PrPSc by Western blotting, whereas the right hemisphere (non-infused side) was fixed with 10% formalin for paraffin sections. (a) Accumulation of PrPSc at 150 days p.i. Samples from individual mice (50 µg brain equivalent) were loaded in each lane and the intensities of PrPSc bands were quantified. The mean intensity for mice treated with the negative-control mAb (P2-284) was designated 100% and the graph shows relative PrPSc levels for mice treated with anti-PrP mAbs. (b) Kinetics of PrPSc accumulation. Mice were sacrificed at 127 and 150 days p.i. and the left hemisphere (mAb-infused side) was used for Western blotting. Samples from individual mice (50 µg brain equivalent) were loaded in each lane and chemiluminescence intensities were quantified. The graph on the right shows the mean level of PrPSc at 150 days p.i. compared with the level at 127 days p.i. Samples at 150 days p.i. were the same as those in (a). (c) Spongiform changes at 150 days p.i. Paraffin sections were prepared from the contralateral hemisphere of the brain described in (a) and stained with H&E.
hippocampus (as detected with anti-Iba1 antibodies) was also reduced in the presence of anti-PrP mAbs; in contrast, the effect was marginal in the thalamus.

To investigate the effects of anti-PrP mAbs on different prion strains, we carried out the same experiment using mice infected with the Chandler strain. Similar to what was observed for mice infected with the Obihiro strain, in mice infected with the Chandler strain, mAb 31C6 reduced spongiform changes and PrPSc deposition in the hippocampus and thalamus compared with the negative-control mAb (Fig. 4). However, the effect of anti-PrP mAb on gliosis appeared to differ for the two different prion strains. Anti-PrP mAbs apparently reduced astrogliosis in the hippocampus and thalamus of mice infected with the Obihiro strain (Fig. 3), but only a slight reduction in astrogliosis was observed for mice infected with the Chandler strain (Fig. 4). Moreover, although microglial activation in the thalamus of mice infected with the Obihiro strain was slightly reduced by treatment with anti-PrP mAbs, it was obviously reduced relative to controls by treatment with mAb 31C6 in mice infected with the Chandler strain (Fig. 4). However, microglial activation as a whole appeared to be moderate in mice infected with the Chandler strain compared with the Obihiro strain; thus, the difference observed could be due to a difference in the level of activation of the microglia between mice infected with the two prion strains.

Prolongation of survival time

To determine whether treatment with anti-PrP mAbs can prolong survival of prion-infected mice when administered at different stages in progression of the disease, infusion was started at a middle stage of infection (60 or 90 days p.i.) and after clinical onset (120 days p.i.). In mice infected with the Obihiro strain, infusion of mAb 31C6 initiated at 60 days p.i. prolonged survival by about 11 days compared with the negative control; however, no effect was observed when infusions were initiated at 90 or 120 days p.i. (Fig. 5 and Table 1). In contrast, for mice infected with the Chandler strain, prolongation of survival was observed in all three groups: infusion initiated at 60, 90 or 120 days p.i. prolonged survival by approximately 10, 13 or 12 days, respectively. Brain sections (H&E stained) of all mice were examined for possible causes of death other than prion disease. Of 96 mice tested, two had severe abscesses around the infused area and thus were excluded from the experimental group.

Changes in body weight were consistent with prolonged survival times. For experimental groups in which survival was prolonged by infusion with mAb 31C6, the decrease in body weight observed in control groups was delayed by about 1 or 2 weeks (Fig. 6). In contrast, no difference was observed for mice infected with the Obihiro strain when mAb infusion was started at 90 or 120 days p.i.
Neuronal toxicity of mAbs

As it has been reported that an anti-PrP mAb recognizing aa 95–105 of murine PrP can induce apoptosis in hippocampal neurons (Solforosi et al., 2004), we assessed the neurotoxicity of the mAbs used in this study. First, mAbs were infused into the lateral ventricle for 7 days using an Alzet Mini-Osmotic Pump model 2001 (mAb concentration 1 mg ml$^{-1}$, pumping rate 1 μl h$^{-1}$, duration 7 days, volume 200 μl); however, no difference was observed between mice treated with anti-PrP mAbs and those treated with the negative-control mAb (data not shown). To assess neurotoxicity directly, we next stereotaxically injected anti-PrP mAbs and the control mAb into the left and right hippocampus, respectively. The distribution of mAb in the hippocampus was confirmed by injecting Alexa Fluor 488-conjugated mAb 31C6 (Fig. 7a). Although mAb was well-distributed throughout the entire hippocampus on the injected side, TUNEL-positive cells were only detected in a limited area of the pyramidal layer and this was observed even in the right side (the side injected with the control mAb P2-284). Indeed, the TUNEL-positive cells were close to the injection site, suggesting that the TUNEL-positive cells resulted from the trauma of mAb injection. It was interesting that mAbs 106 and 110 recognizing the region adjacent to aa 95–105 did not induce apparent neuronal death.

DISCUSSION

In this study, we investigated the effects of anti-PrP mAbs on progression of prion disease, focusing on treatment during late stages of infection. We showed that anti-PrP mAbs antagonized PrP$^{Sc}$ formation in the brain when intraventricular administration was initiated at the time of clinical onset (Figs 2 and 3). The effect of anti-PrP mAbs appeared to be mainly due to deceleration of PrP$^{Sc}$ formation rather than active degradation of PrP$^{Sc}$. Several reports have suggested that binding of anti-PrP antibodies to the first α-helical domain of PrP$^{C}$ (aa 143–155), which is proposed to be important for the PrP$^{C}$–PrP$^{Sc}$ interaction (Morrissey & Shakhnovich, 1999; Speare et al., 2003), prevents PrP$^{Sc}$ formation by inhibiting the direct inter-
action between PrPc and PrPSc (Enari et al., 2001; Peretz et al., 2001). It has also been suggested that a perturbation of the usual PrPc trafficking by binding of the antibody to PrPc on the cell surface, e.g. sequestration of PrPc on the cell membrane, may be one of the mechanisms of inhibition (Kim et al., 2004b; Feraudet et al., 2005). Consistent with our previous observations (Kim et al., 2004b), mAbs directed against the C-terminal domain (mAb 44B1) and the octapeptide repeat in the N-terminal region (mAb 110), as well as one directed against the first α-helix (mAb 31C6), antagonized PrPSc formation in the mouse brain (Fig. 2). Indeed, anti-PrP mAb infused into the lateral ventricle was still detectable in the hippocampus at 20 days after the termination of infusion, whereas only a low level of the negative-control mAb was detected in the same region, even during infusion (Fig. 1). These results suggest that the antibody–PrPc complex remains in the brain parenchyma and therefore that sequestration of PrPc by the antibody is implicated in the inhibition of PrPSc formation in vivo.

Intraventricular infusion of anti-PrP mAbs at a late stage of infection (initiated at 120 days p.i.) reduced levels not only of PrPSc accumulation but also of microglial activation, astrogliosis and spongiform changes. Comparison of neurohistopathological changes observed at 127 versus 150 days p.i. in mice treated with anti-PrP mAbs and the negative-control mAb revealed an apparent reduction in gliosis observed at 150 days p.i., which may be due to a slowdown in the progression of gliosis (data not shown). Although the severity of microglial activation and astrogliosis differed in animals infected with the two different prion strains, the reduction observed was in accordance with the mAb distribution, and the levels of microglial activation and astrogliosis in the hippocampus and thalamus of mice infused with anti-PrP mAbs appeared

Table 1. Effect of intraventricular infusion of anti-PrP mAbs on survival of mice infected with the Obihiro or Chandler strain

<table>
<thead>
<tr>
<th>Initiation of mAb infusion (days p.i.)</th>
<th>Survival time of mice [mean ± sd (days)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obihiro strain</td>
</tr>
<tr>
<td></td>
<td>P2-284 (n)</td>
</tr>
<tr>
<td></td>
<td>P2-284 (n)</td>
</tr>
<tr>
<td>60</td>
<td>153.4 ± 9.0 (8)</td>
</tr>
<tr>
<td>90</td>
<td>152.8 ± 4.3 (6)</td>
</tr>
<tr>
<td>120</td>
<td>154.4 ± 9.4 (8)</td>
</tr>
<tr>
<td></td>
<td>150.7 ± 10.2 (7)</td>
</tr>
<tr>
<td></td>
<td>151.7 ± 9.8 (10)</td>
</tr>
<tr>
<td></td>
<td>152.9 ± 7.4 (7)</td>
</tr>
</tbody>
</table>

*Statistically significant difference based on Student’s t-test (P<0.05).
to be milder than those observed in mice infused with the negative-control mAb. In contrast, no apparent reduction in microglial activation or astrogliosis by anti-PrP mAb was observed in the lateral cortex, where delivery of mAbs appeared to be inefficient (data not shown). It has been reported that the process of conversion of PrPC to PrPSc on neurons rather than extracellular deposition of PrPSc is involved in neuronal degeneration (Brandner et al., 1996; Mallucci et al., 2003; Chesebro et al., 2005). Although the mechanism by which PrPSc formation provokes microglial activation and astrogliosis remains to be elucidated, these results imply that arresting conversion of PrPC to PrPSc via mAb infusion may contribute, at least to some extent, to the observed reduction in microglial activation and astrogliosis.

Previous results have shown that passive immunization with anti-PrP mAb via intraperitoneal injection does not have a protective effect after invasion of prion into the CNS or inoculation of prion via the intracerebral route (White et al., 2003). In contrast, intraventricular infusion of anti-PrP mAb prolonged survival when mAb was infused at the time that PrPSc became detectable in the CNS (e.g. at 60 days p.i.). The difference seems to be explained by inadequate transfer of anti-PrP mAbs into the CNS across the blood–brain barrier when mAbs are administered peripherally. Several compounds, including amphotericin B, PPS, porphyrin derivatives and GN8, have been reported to prolong the survival of mice infected with prion when administered at the middle or late stage of infection, but the animals were still prior to clinical onset (Demaimay et al., 1997; Doh-ura et al., 2004; Kocisko et al., 2006; Kuwata et al., 2007). To the best of our knowledge, there has been only one report of a treatment that prolongs survival of animals already in the clinical phase. Specifically, intraventricular administration of MS-8209 was shown to prolong survival of mice infected with the C506M3 strain when treatment was carried out at the time of the appearance of neurological symptoms (Demaimay et al., 1997). Human prion diseases are usually detected after clinical onset and thus the availability of treatments that are effective even after symptoms have begun to appear is highly important. Therefore, it is of interest that intraventricular infusion of anti-PrP mAb was effective for prolongation of survival, even when treatment was initiated after the appearance of early clinical signs (at 120 days p.i.) in mice infected with the Chandler strain. Although in this study mAb infusion for 14 days of duration achieved only 8% prolongation, the result should encourage further trials with mAbs that may be useful in the development of therapeutic treatment for prion diseases. For instance, continuing treatment over a longer duration, which may keep the effective concentration of mAbs in the brain higher over a longer period of time, may improve the effect on survival. In addition, anti-PrP mAb prolonged survival, despite the fact that the infused mAb was not evenly distributed in the brain but rather was primarily restricted to the hippocampus and thalamus. Thus, it is conceivable that improved delivery of mAb might enhance the effects of anti-PrP mAb on survival. Fab and single-chain antibody fragments have also been shown to inhibit PrPSc formation (Peretz et al., 2001; Donofrio et al., 2005), and the smaller size of these fragments may be beneficial for efficient delivery in tissues.

Fig. 6. Changes in body weight. After the initiation of mAb infusion, mice were weighed weekly up to the terminal stage of the disease. The groups of mice used in Fig. 5 were used to monitor body weight. ○, Mice treated with mAb P2-284; ■, mice treated with mAb 31C6.
Although mice infected with either the Obihiro or Chandler strain succumbed to the disease at around 150 days p.i., the effect of mAb infusion on survival differed when administered to mice challenged with different prion strains. For example, mAb infusion of mice infected with the Chandler strain was effective when the mAb was administered at any of the three time points tested (60, 90 and 120 days p.i.), whereas no prolongation was observed in mice infected with the Obihiro strain when mAb infusion was initiated at 90 or 120 days p.i. (Fig. 6). At present, we do not have a precise explanation for this but can speculate on what might explain the difference. First, microglial activation in mice infected with the Obihiro strain was more severe than that in mice infected with the Chandler strain (Figs 3 and 4). Similar to the mouse model for Alzheimer’s disease (El Khoury et al., 2007), microglial recruitment is expected to have a protective role in prion disease; however, activated microglia also have neurotoxic effects via production of cytokines, chemokines and reactive oxygen species (Bate et al., 2001, 2002; Marella et al., 2005). Thus, even when mitigated by anti-PrP mAb, the severe microglial activation in mice infected with Obihiro strain may be sufficient for progression of the disease. Secondly, differences in the distribution of PrPSc may account for the differences in the effect of anti-PrP mAb in one prion strain versus the other. For instance, PrPSc accumulation in the hypothalamus of mice infected with the Obihiro strain was more severe than that in mice infected with the Chandler strain (data not shown). Moreover, PrPSc formation in the hypothalamus might not be inhibited efficiently due to the uneven distribution of mAb in the hypothalamus (Fig. 1). These results may explain in part the lack of prolongation in the Obihiro strain-infected mice infused with mAb at 90 or 120 days p.i.

Immunotherapy has been of interest in the treatment of Alzheimer’s disease; however, the fact that immunization of β-amyloid peptide caused meningoencephalitis in some patients in clinical trials warns of the adverse effects of autoimmune reactions in vivo (Check, 2002; Nicoll et al., 2003; Orgogozo et al., 2003). Additionally, cross-linking of PrPSc by an anti-PrP mAb that recognizes a specific epitope (aa 95–105) provoked degeneration of hippocampal and cerebellar neurons (Solforosi et al., 2004). These adverse effects of antibodies on the CNS have prompted extreme

---

**Fig. 7.** Neuronal toxicity of anti-PrP mAbs. Anti-PrP mAbs (106, 110, 31C6 or 44B1) and the negative-control mAb P2-284 were injected into the left and right hippocampus, respectively, using stereotaxic apparatus. Seventy-two hours after injection, brains were obtained and fixed in 10% formalin. Paraffin sections were subjected to TUNEL and H&E staining. (a) Distribution of mAb. Alexa Fluor 488-conjugated mAb 31C6 was injected into the hippocampus and the distribution was analysed by confocal laser microscopy. (b) Positive control. Coronal sections were pre-treated with DNase I and then stained using the TUNEL procedure. The pyramidal cell layers of the hippocampus are shown. (c) TUNEL- and H&E-stained samples. Low-magnification (top panels) and high-magnification micrographs (middle panels) of TUNEL-stained samples and corresponding H&E-stained samples (bottom panels) are shown. The mAbs injected are indicated above the panels. Three to six mice were examined for each mAb. Bars, 200 μm.
caution in the use of anti-PrP antibodies, in particular, their introduction into the CNS. However, passive immunization is less likely to initiate an autoimmune reaction (Schenk, 2002; Sadowski & Wisniewski, 2004). In this study, we observed no antibody-induced inflammation by intraventricular infusion. In addition, neuronal death in the hippocampus was not observed, even though mAbs 106 and 110, which recognize the region adjacent to aa 95–105, were injected directly into the hippocampus. Although the potential adverse effects, especially an inflammatory response, should be examined carefully, the fact that anti-PrP mAbs interfered with disease progression, even when administered after clinical onset, is particularly encouraging. Although the effect of anti-PrP mAb differed in prion strains, this implies that the immunotherapy might be effective in certain types of human prion disease, if not all. Therefore, the results of this study should promote further efforts to improve the effect of anti-PrP mAbs, such as the form of the antibody, the route of administration and an efficient way of delivering the antibodies.

ACKNOWLEDGEMENTS

This work was supported by the Regional New Consortium R&D Projects from the Ministry of Economy, Trade and Industry, a grant from The 21st Century COE Program (A-1) and a Grant-in-Aid for Science Research (A) (grant no. 18208026) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. This work was also supported by a grant from the Ministry of Health, Labour and Welfare of Japan (grant no. 17270701). This work was also partly supported by a grant for Strategic Cooperation to Control Emerging and Re-emerging Infections and the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases, from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES


