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PrP^{Sc} Binding Antibodies Are Potent Inhibitors of Prion Replication in Cell Lines*

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Conversion of the cellular α-helical prion protein (PrP^{C}) into a disease-associated isoform (PrP^{Sc}) is central to the pathogenesis of prion diseases. Molecules targeting either normal or disease-associated isoforms may be of therapeutic interest, and the antibodies binding PrP^{C} have been shown to inhibit prion accumulation *in vitro*. Here we investigate whether antibodies that additionally target disease-associated isoforms such as PrP^{Sc} inhibit prion replication in ovine PrP-inducible scrapie-infected Rov cells. We conclude from these experiments that antibodies exclusively binding PrP^{C} were relatively inefficient inhibitors of ScRov cell PrP^{Sc} accumulation compared with antibodies that additionally targeted disease-associated PrP isoforms. Although the mechanism by which these monoclonal antibodies inhibit prion replication is unclear, some of the data suggest that antibodies might actively increase PrP^{Sc} turnover. Thus antibodies that bind to both normal and disease-associated isoforms represent very promising anti-prion agents.

Prion diseases are fatal neurodegenerative disorders that include scrapie in sheep and goats, bovine spongiform encephalopathy in cattle, and Creutzfeldt-Jakob disease in humans. The recent emergence of a new human prion disease, variant Creutzfeldt-Jakob disease, almost certainly resulting from the human consumption of bovine spongiform encephalopathy-infected material (1) is a major public health and safety issue (2, 3). The infectious agent or prion is mainly composed of PrP^{Sc},[1] a detergent-insoluble and partially protease-resistant isoform of the host-encoded cellular prion protein, PrP^{C} (4). According to the protein-only hypothesis, in the course of prion infection, α-helical PrP^{C} is refolded without post-translational modification into β-sheet-rich PrP^{Sc}, initially in the presence of exogenous PrP^{Sc} and then by an autocatalytic process (5). Currently, no treatment of prion disease is effective once neurological illness has developed, but any molecule able to interact with either one or both isoforms could potentially delay or even cure the disease (6). Recent reports indicate that anti-PrP monoclonal antibodies (mAbs) efficiently inhibit PrP^{Sc} accumulation in ScN2a mouse neuroblastoma cells (7, 8) and in infected transgenic mice engineered to produce one of these mAbs, 6H4 (9). Their efficiency was related directly to their epitope and to their affinity for PrP^{C} (10, 11). We also have recently reported that mAbs effectively suppress systemic prion replication *in vivo* (12). In that study, two mAbs with differential affinity for normal and disease-associated isoforms of prion protein were used: ICSM 18, which almost exclusively binds to PrP^{C}, and ICSM 35, which efficiently binds to both normal and disease-associated isoforms. Both mAbs were efficient equally at delaying the onset of prion disease in the treated mice, but it was unclear whether the additional targeting of PrP^{Sc} by ICSM 35 played a role in controlling prion replication.

In this study, we have used a larger panel of mAbs raised in PrP^{C} null mice ($Prnp^{0/0}$) against the α and β isoforms of human recombinant PrP (13) to inhibit prion replication in scrapie-infected epithelial Rov (ScRov) cells (14). Rov cell PrP^{C} expression is inducible by doxycycline, thereby allowing the clearance of PrP^{Sc} to be studied in the absence of its PrP^{C} substrate. Here we show that mAbs that additionally target disease-associated isoforms of PrP block PrP^{Sc} accumulation much more efficiently than mAbs recognizing PrP^{C} alone. We also found that the inhibition by several mAbs was similar and even greater than turning off the production of the PrP^{C} substrate, suggesting antibody-mediated enhancement of the proteolysis of intracellular PrP^{Sc}.

EXPERIMENTAL PROCEDURES

Production of Monoclonal Antibodies—The panel of ICSM mAbs was produced as described previously (15). They were affinity-purified from hybridoma culture supernatant over the protein A or G matrix (Äkta Prime, Amersham Biosciences), filter-sterilized, and stored at 4 °C.

Treatment of ScRov Cells with the ICSM Antibodies—ScRov cells were grown in 24-well plates as described previously (14). Doxycycline (1 μg/ml) was present in the culture medium unless mentioned. A triplicate of ScRov cells was treated with the ICSM mAbs once a week just after splitting. The controls were either left untreated or treated with isotype control mAb (15, 16). In one experiment, the cells were exposed to similar concentrations of dextran sulfate 500 (DS500, Sigma). One quarter of the cells was passaged weekly, and the residual cells (typically $\sim 24 \times 10^4$ cells or 80 μg of proteins) were pelleted, lysed in lysis buffer (20 mM Tris-HCl, pH 7.5, 1% Nonidet P40, and 0.5% sodium deoxycholate), and stored at −80 °C for subsequent analysis. PrP^{Sc} was extracted from 40 μg of protein by 100 μg/ml proteinase K for 1 h at 37 °C. The protein was then denatured with 3 volumes of Lae-

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[1] The abbreviations used are: PrP^{Sc}, disease-associated isoform of prion protein; PrP^{C}, cellular prion protein; mAb, monoclonal antibody; DS500, dextran sulfate 500; PBS, phosphate-buffered saline.

mmli buffer (17) for 5 min at 100 °C before an additional concentration of the protein with cold acetone. Typically, 15 μg of proteins (*i.e.* the equivalent of 45 × 10^3 cells) were used for Western blots (see below).

Immunoprecipitation—Scrapie-infected and -uninfected Rov cells were washed three times in cold PBS and scraped in cold lysis buffer. A mixture of protease inhibitors (Roche Applied Science) in addition to 5 mM phenylmethylsulfonyl fluoride was added prior to (Rov cells) or after proteinase K treatment (ScRov cells) at 50 μg/ml for 1 h at 37 °C. Lysates then were incubated with 10 μg/ml purified mAbs in lysis buffer for 2 h at 4 °C on a rotator. Negative controls omitted the capture mAb or used the relevant isotype control (15, 16). The immune complexes were adsorbed overnight onto protein G-agarose beads (Roche Applied Science) at 4 °C on a rotator. The beads were washed 4–5 times according to the manufacturer's instructions. They were re-suspended in Laemmli buffer (17), heated at 100 °C for 5 min, and pelleted at 12,000 × *g* to detach/denature the bound protein. The supernatant was analyzed by Western blot (see below).

For live cell immunoprecipitation, Rov cells were incubated overnight with 10 μg/ml ICSM mAbs in the culture medium. The protocol then was similar with the exception that the lysates were incubated directly with protein G-agarose beads. The bound fraction was compared with the total levels of PrP^{C}, which corresponds to the unbound fraction when immunoprecipitation is performed with the relevant isotype controls. This fraction was precipitated by cold acetone, re-suspended in Laemmli buffer, and analyzed by Western blot.

Immunofluorescence—Rov and ScRov cells were incubated overnight on slides with 10 μg/ml ICSM mAbs. Controls were left untreated or treated with isotype control (15, 16). Cells were washed twice with cold PBS and fixed in 4% paraformaldehyde for 30 min. After three washes in PBS, cells were permeabilized with 0.5% Triton X-100 for 5 min. After several washes, the cells were incubated for 1 h with a 1/400 dilution of an isothiocyanate-conjugated anti-mouse IgG mAb (P.A.R.I.S, Paris, France) in 5% milk in PBS. After three washes in PBS, slides were mounted in antifading solution (Dabko) and kept in the dark at 4 °C until microscopic analysis with the Nikon fluorescent microscope.

Western Blotting—Samples were run on 12% polyacrylamide Criterion gels (Bio-Rad) or 12% NuPAGE gels (Invitrogen), electrotransferred onto polyvinylidene difluoride membranes (Millipore), and immunoblotted with 0.1–0.2 μg/ml of the biotinylated anti-PrP antibody, ICSM 18. Immunoreactivity was visualized with an enhanced chemiluminescence kit on autoradiographic films (ECL+, Amersham Biosciences). Densitometric analyses of the films were performed with the program NIH Image (Waine Rasband, National Institutes of Health) as described previously (18). The amount of PrP^{Sc} was estimated by comparison with a dilution scale of sheep scrapie PrP^{Sc} prepared in similar conditions and at the same time.

RESULTS

Monoclonal Antibodies Raised against β-PrP Inhibit More Efficiently PrP^{Sc} Accumulation in ScRov Cells—In initial studies, we assessed how efficiently ICSM 4, 17, 18, and 19 raised against α-PrP and ICSM 35 raised against β-PrP inhibited prion replication (Table I). ICSM 18 and ICSM 17 recognize residues 146–159 and 140–159 of murine PrP. ICSM 35 recognizes residues 96 and 109 on the N-terminal region of PrP$^{27–30}$. The epitopes of ICSM 4 and 19 are not definable with overlapping synthetic peptides and may be conformation-dependent (Table I) (15). Triplicate cultures of ScRov cells were treated with mAb concentrations ranging from 10 ng/ml to 10 μg/ml. The treatment was renewed once a week when the cells were split, as we identified by ELISA that the concentration in the culture supernatant fell only by 50% over each treatment period. Cells were collected each week, and the level of PrP^{Sc} was assessed by Western blot. ScRov cells also were treated with various concentrations of the anti-prion drug DS500, known to potently inhibit prion replication in other scrapie-infected cell lines (19). As expected, DS500 rapidly inhibited PrP^{Sc} accumulation in a dose-dependent manner with a 50% inhibitory concentration (IC_{50}) of 110 ng/ml (0.22 nM) after 3 weeks treatment (Fig. 1, *a* and *b*). When compared with DS500, mAb ICSM 35 (raised against β-PrP) was a more potent inhibitor (IC_{50}, 4 ng/ml or 0.03 nM) (Fig. 1, *a* and *b*). Among the mAbs raised against α-PrP, ICSM 19 and ICSM 18 were ~100 and 1500 times less efficient (IC_{50} of 360 ng/ml (3.30 nM) and 5 μg/ml (45.5 nM), respectively) (Fig. 1*a*). 1 μg/ml ICSM 19 strongly inhibited PrP^{Sc} accumulation after 6 weeks of treatment, whereas ICSM 18 at this dose could not prevent PrP^{Sc} accumulation (Fig. 1*b*). ICSM 4 and 17 were unable to inhibit PrP^{Sc} accumulation, even after prolonged treatment with 10 μg/ml (Table I). None of the mAbs modified PrP^{C} expression regardless of the dose or length of treatment used (data not shown), indicating that they did not exhibit any toxicity for the cells. After 6 weeks treatment with 1 μg/ml DS500, ICSM 35, or ICSM 19, PrP^{Sc} levels were lowered 100–1000-fold (Fig. 1*b*). Interestingly, when the inhibitor was washed off, PrP^{Sc} re-accumulated (Fig. 1, *b* and *c*). Cells treated with ICSM 19, and DS500 reached control values 6 weeks after the treatment was stopped. At this point, those treated with ICSM 35 still accumulated ~5 times less PrP^{Sc} than the controls (Fig. 1, *b* and *c*). This confirmed that ICSM 35 was a more potent inhibitor than ICSM 19 and DS500.

TABLE I
Inhibition of ScRov PrP^{Sc} accumulation by ICSM monoclonal antibodies

−, no inhibition; +, <10× decrease of PrP^{Sc} levels; ++, ~10× decrease; +++++, >500× decrease; ++++++, >1000× decrease.

ICSM	Immunogen[a]	Epitope[b]	Immunoreactivity[d]		Inhibition[e]
			PrP[c]	PrP^{Sc}	
			% ± S.D.		
4	α	C	37 ± 8	0	−
6	α	C	27 ± 14	0	−
7	α	C	26 ± 17	0	−
17	α	140–159	26 ± 11	0	−
18	α	146–159	183 ± 8	4 ± 1	+
19	α	C	91 ± 26	0	++
41	α	C	162 ± 44	4	−
44	α	C	58 ± 37	3	−
35	β	96–109	100	100	+++++
37	β	96–109	109 ± 8	190 ± 7	++++++
42	β	96–109	94 ± 22	90 ± 4	+++++

[a] α and β refer to human recombinant α- and β-PrP$^{91–231}$.
[b] Residue numbering refers to mouse prion protein sequence.
[c] C, conformational epitope.
[d] Estimated by at least three independent immunoprecipitation experiments and compared with the affinity obtained with ICSM 35.
[e] Observed at the 10 μg/ml dose after 3 weeks of treatment.

PrP^{C} expression in Rov cells is controlled by doxycycline (14), allowing a comparison of the efficiency of antibody-mediated PrP^{Sc} clearance to turning off the PrP^{C} promoter, the latter representing the maximal inhibition achievable by pure PrP^{C} targeting. ScRov cells were treated with ICSM 19 (raised against α-PrP), ICSM 35 (raised against β-PrP), or control mAbs (each at 10 μg/ml) in the presence or absence of doxycycline. With doxycycline removed from the culture medium, the PrP^{Sc} half-life was 3 ± 0.2 days (Fig. 2). This was similar to treatment with ICSM 35 (Fig. 2, PrP^{Sc} half-life of 3.5 ± 0.5 days). Interestingly, both mechanisms of inhibition were synergistic, because ICSM 35 added to the cells in which doxycycline has just been removed induced an even faster clearance of PrP^{Sc} (Fig. 2, half-life 2.1 ± 0.3 day; $p < 0.05$; Mann-Whitney *U* test). In contrast, inhibition by ICSM 19 was much slower than turning off the PrP^{C} substrate (PrP^{Sc} half-life 16 ± 4.4 days) (Fig. 2).

Two other mAbs raised against β-PrP were similarly potent. In fact PrP^{Sc} clearance with ICSM 37 was significantly faster than with ICSM 35 and even faster than turning off the PrP^{C} promoter (Fig. 3; $p < 0.05$; Mann-Whitney *U* test). Inhibition induced by ICSM 42 was similar to ICSM 35 (Table I and data not shown). In contrast, other mAbs raised against α-PrP (ICSM 6, 7, 41, and 44) failed to reduce PrP^{Sc} levels even after 3 weeks treatment with 10 μg/ml (Table I).

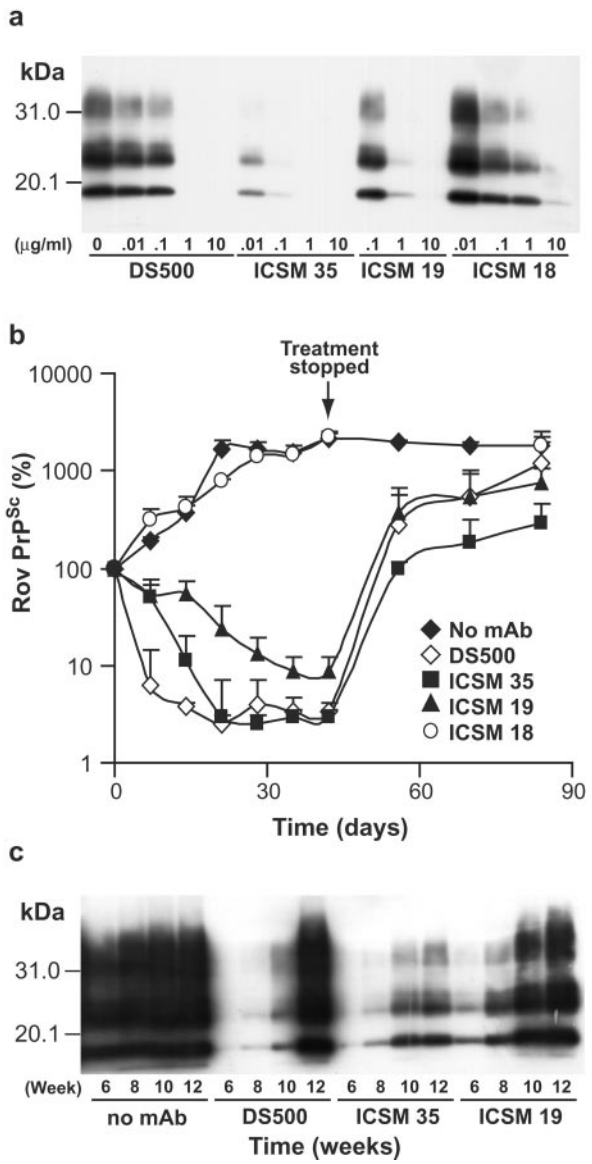

FIG. 1. **Inhibition of PrP^{Sc} formation in ScRov cells induced by antibodies raised against α- and β-PrP (ICSM mAbs).** *a*, dose-dependent inhibition of PrP^{Sc} accumulation in ScRov cells induced by the ICSM mAbs. PrP^{Sc} Levels were analyzed by immunoblot after 3 weeks of treatment with the mAbs or DS500. Controls were left untreated. *b*, time course of ICSM mAb-mediated clearance of PrP^{Sc}. Cells were treated in triplicate for 6 weeks at the dose of 1 μg/ml or were left untreated. Measurements were performed in triplicate, and values represent its mean ± S.D. The treatment was lifted after 6 weeks to study the re-emergence of PrP^{Sc}. *c*, the rapidity of PrP^{Sc} reappearance is a function of the treatment performed. Levels of the protein were analyzed by immunoblot after 6 weeks of treatment with ICSM 35 and ICSM 19 and DS500. The treatment was then lifted, and PrP^{Sc} production was assessed at several time points. This was compared with untreated cells.

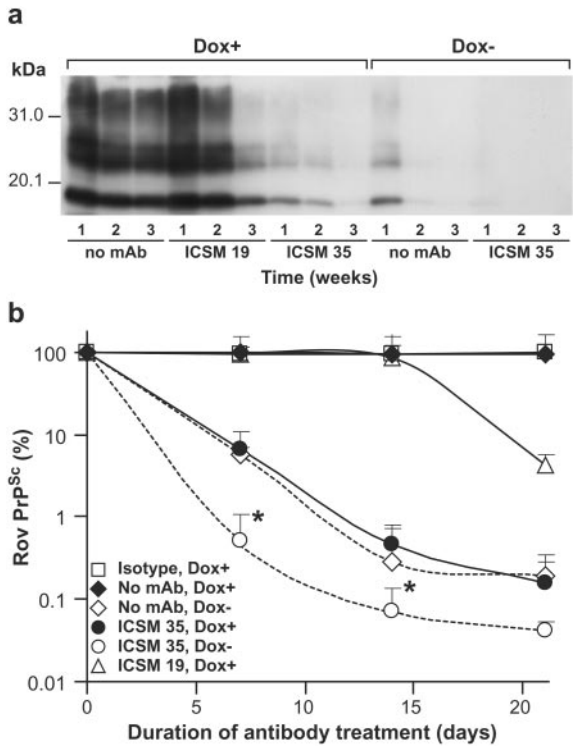

FIG. 2. **ICSM 35 curtails PrP^{Sc} accumulation as efficiently as turning off PrP^{C} production in ScRov cells.** *a*, PrP^{Sc} levels were determined by immunoblotting after 10 μg/ml treatment with ICSM 19 or ICSM 35. Antibody effects were compared with the removal of doxycycline (*Dox*), which silenced PrP^{C} expression in the culture. Controls were left untreated. *b*, densitometric quantification of PrP^{Sc} accumulation from the immunoblots. Values are given as a percentage of PrP^{Sc} intensity in the absence of antibody treatment or treatment with isotype control. Measurements were performed in triplicate, and values represent its mean ± S.D. *, the difference in PrP^{Sc} accumulation between ICSM mAb-treated cells and the untreated cells, both in the absence of doxycycline, was statistically significant ($p < 0.05$; Mann-Whitney *U* test).

Monoclonal Antibodies Raised against β-PrP-immunoprecipitated ScRov Cell PrP^{Sc}—One possible explanation for the differences observed in efficacy between the mAbs tested was that they differentially recognized normal and disease-associated isoforms of PrP. Therefore, we immunoprecipitated PrP^{C} and the protease-resistant core of PrP^{Sc} (PrP$^{27–30}$) from Rov and ScRov cells lysates. All of the mAbs reacted with PrP^{C}, ICSM 18 exhibiting about twice the affinity of any mAbs raised against β-PrP (Table I and Fig. 4*a*, *left panel*). In stark contrast, only mAbs raised against β-PrP reacted strongly with PrP$^{27–30}$, ICSM 37 immunoprecipitating 2-fold more PrP^{Sc} than ICSM 35 and 42 at an equivalent concentration (Table I and Fig. 4*a*, *right panel*).

We then pulsed uninfected Rov cells with ICSM 18 or ICSM 19 (10 μg/ml overnight) and immunoprecipitated the PrP^{C}-bound fraction after thoroughly washing the cells. Interestingly, ICSM 18 and ICSM 19 bound 62 ± 3 and 75 ± 10% PrP^{C} molecules, whereas ICSM 35 and 37 bound, respectively, only 14 ± 3 and 33 ± 4% (Fig. 4, *b* and *c*). Similar experiments on ScRov cells were inconclusive, as we frequently observed nonspecific binding of PrP^{Sc} to the beads used to bring down the antigen/antibody complexes (data not shown). However ICSM 35 and 37 do bind PrP^{Sc} in living cells. Using immunofluorescence microscopy, we found dotlike intracellular staining in ScRov cells (as indicated by an *arrow*) but not in Rov cells (Fig. 4*d*). This was similar to the staining of fixed ScRov cells with these mAbs after guanidium thiocyanate denaturation,[2] a treatment known to increase specifically PrP^{Sc} immunoreactivity (20, 21). In contrast, we were unable to observe any differences in the binding of ICSM 18 and 19 between Rov and ScRov cells (Fig. 4*d*). Overall, these experiments indicate that mAbs raised against β-PrP bind endogenous intracellular PrP^{Sc}.

DISCUSSION

Given that the transformation of normal cellular prion protein is central to the pathogenesis of prion disease, it is not surprising that most of the available therapeutic strategies

[2] V. Beringue and F. Archer, unpublished data.

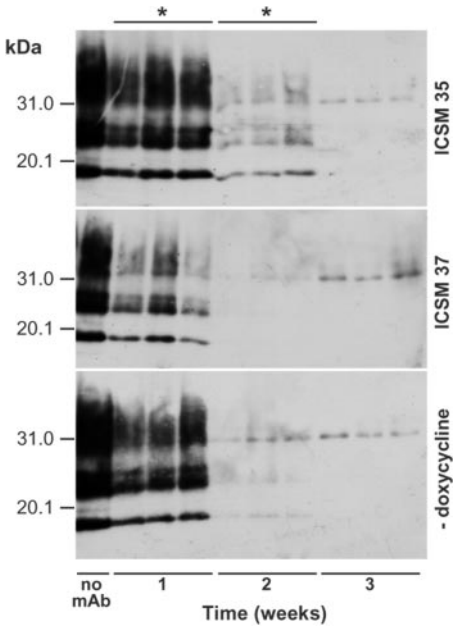

FIG. 3. **ICSM 37 is the most potent inhibitor of PrP^{Sc} accumulation cells and accelerates its proteolysis.** Triplicates of ScRov cells were treated with 10 μg/ml ICSM 35 or 37 for 3 weeks. Their effects were compared with the removal of doxycycline, which silenced PrP^{C} expression in the culture. Controls were left untreated. The band at 31 kDa represents cross-reaction of the secondary reagent with proteinase K. This experiment is representative of three independent experiments. *, PrP^{Sc} inhibition with ICSM 37 was significantly faster than with ICSM 35 or turning off the PrP^{C} promoter ($p < 0.05$; Mann-Whitney *U* test).

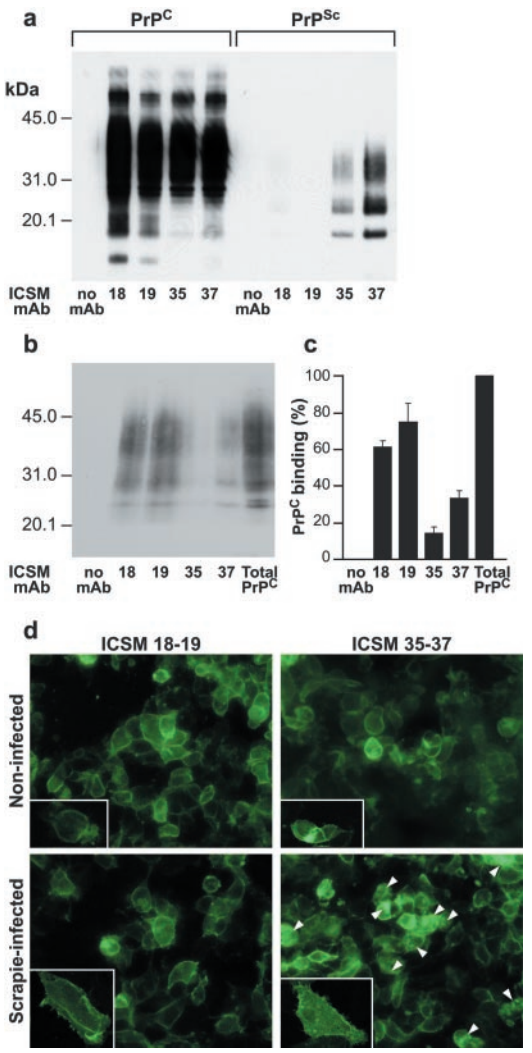

FIG. 4. **Reactivity of the ICSM mAbs for Rov cells PrP^{C} and PrP^{Sc}.** *a*, the ICSM mAb immunoreactivity for native PrP^{C} and protease-resistant PrP^{Sc} (PrP$^{27–30}$) was assessed by immunoprecipitation from cell lysates of Rov and ScRov cells, respectively. Controls (no mAb) omitting the precipitating mAb or using an isotypic mAb were negative. *b* and *c*, immunoreactivity for endogenous PrP^{C} was assessed by immunoprecipitation on live cells. Controls using an isotypic mAb (*no mAb*) were negative. The amount immunoprecipitated by the mAbs were compared with the levels of PrP^{C} present in the culture (*Total PrP^{C}*). Values represent the mean ± S.D. of three independent experiments. *d*, the binding abilities of the mAbs also were tested by immunofluorescence on live Rov and ScRov cells. In the ScRov cells, only ICSM 35 and 37 bound PrP^{Sc}, as detected by a dotlike intracellular staining (indicated by *arrows*). (×40).

target either normal or disease-related isoforms. However, success has been limited when translating methods that were shown to be effective *in vitro* to animal models and patients (22). Studies in neuroblastoma cells clearly indicate that targeting PrP^{C} either by cleaving it from the cell surface with phosphatidylinositol-specific phospholipase C or stabilizing it with monoclonal antibodies inhibits prion replication very efficiently (7, 8, 23). In our system, turning off the ovine PrP promoter completely abrogates prion replication (this study and Ref. 14), analogous to the situation in PrP null mice that do not support prion replication (24). However, not all PrP^{C}-binding antibodies have inhibitory effects (Table I). Peretz *et al.* (8) elegantly show that artificially engineered Fabs were most potent when they targeted helix 1, a region to which ICSM 18 and mAb 6H4 bind (10, 15). Fabs binding the 90–109 region also inhibited prion replication (8), although interestingly, these artificially engineered antibodies do not recognize native PrP^{Sc} (11), unlike our mAbs raised against β-PrP in which the 90–109 region is immunodominant (this study).[3] It is of some concern for the general applicability of cell lines as systems for screening anti-prion agents that prion replication in ScRov cells was inhibited inefficiently by mAbs binding helix 1 such as ICSM 18 that clearly have a very high affinity for PrP^{C} and very efficiently inhibit the replication of mouse prions in ScN2a[3] cells and in mice (12).

Although targeting PrP^{C} exclusively is clearly an effective strategy in some cell lines, failure to inhibit infectious prions may allow the conversion to recur once the PrP^{C}-binding inhibitor is removed. Thus infection is merely suppressed and not eradicated. Previous work indicates that ScN2a cells are curable with anti-PrP^{C} antibodies (7, 8), but clearly this does not apply to all cells capable of supporting prion replication as we show here. Perhaps this is mainly due to the level of PrP^{C}. It is worth noting that the clone successfully treated by mAb 6H4 expressed very low levels of PrP^{C} (7) compared with Rov cells that express similar levels of PrP^{C} to those found in sheep brain (14). One might anticipate that differential sensitivity to anti-prion agents may exist similarly *in vivo*, and continuous suppression with high concentrations of inhibitor may be required given that PrP^{C} is widely expressed in variable amounts and rapidly turned over at the cell surface (25, 26). Such a strategy may be employed with caution during the neuroinvasion course of the disease, because the administration of high doses of mAbs with high affinity for PrP^{C} within the central nervous system may trigger neuronal apoptosis *in vivo* (27).

An alternative strategy is to target both PrP^{C} and PrP^{Sc}, thereby blocking the incorporation of PrP^{C} into propagating prions and additionally capping the infectious template. In

[3] V. Beringue, G. Mallinson, M. Kaisar, M. Tayebi, and S. Hawke, unpublished observations.

these experiments, it was striking that the ability of each mAb to suppress prion replication correlated so well with its affinity for PrP^{Sc} but not for PrP^{C} (Table I). Therefore it seems reasonable to suggest that PrP^{Sc} binding plays the major effector role, but differences between species and/or strains cannot be excluded despite helix I being highly conserved between species (28). The most potent mAbs bound significantly less PrP^{C} than ISCM 18, and immunofluorescence confirmed that the mAbs raised against β-PrP bound disease-associated prion protein. In fact these studies correlated very well with the indirect immunoprecipitation. Thus mAbs exhibiting high affinity for PrP^{Sc} by immunoprecipitation stained intracellular organelles and inhibited prion replication very efficiently. Clearly, unless mAbs completely specific for PrP^{Sc} are used, it will not be possible to conclusively prove that PrP^{Sc} binding is crucial. It is possible, for example, that ovine PrP^{C} is stabilized most efficiently by interactions at the 90–109 region. In any case, PrP^{C} binding may be necessary for the mAbs to be internalized and/or presented to intracellular PrP^{Sc}. Currently, we are attempting to characterize mAbs that bind PrP^{Sc} exclusively and mAbs that bind to the N-terminal portion of PrP$^{27–30}$ but that have low affinity for PrP^{Sc} (such as mAb 3F4) (29). However, taken together, we suggest that additional targeting of PrP^{Sc} may improve the efficacy of anti-prion agents.

How could the mAbs interact with PrP^{Sc}? This may be direct if the PrP^{C} conversion occurred at the cell surface (23). We have shown by immunofluorescence that our mAbs are internalized in contrast with recent studies in human cells (30). If conversion occurred in endosomes, the antibodies may be internalized via PrP^{C} and then bind to PrP^{Sc} whether or not they were released from PrP^{C}. We did not find by immunofluorescence Fc receptors at the Rov cell surface, making Fc-mediated internalization of the mAbs unlikely (data not shown).

Finally, the Rov cell system allowed a comparison of the kinetics of PrP^{Sc} inhibition to be studied in the absence of the PrP^{C} substrate. Again, supporting a direct role for PrP^{Sc} binding, we found that several mAbs inhibited as rapidly as repressing PrP^{C} expression and that one mAb, ICSM 37, was even more rapid, suggesting that it enhanced the breakdown of PrP^{Sc}. Interestingly, PrP^{Sc} was not released from the cells into the supernatant (data not shown). Perhaps mAb binding facilitates the intracellular clearance of PrP^{Sc}. Pulse-chase experiments are planned to study this intriguing phenomenon in greater detail.

How does the work described here apply to our recent *in vivo* data (12)? We have recently shown that both ICSM 18 and 35 effectively inhibit prion replication *in vivo*. In these experiments, it was not determined by which mechanism prion replication was inhibited or whether the infection was eradicated or merely suppressed. The fact that spleen PrP^{Sc} levels were lowered more efficiently with ICSM 18 compared with ICSM 35 and that the former mAb binds to native mouse PrP^{Sc} weakly suggests that targeting PrP^{C} was the predominant inhibitory mechanism. Yet ICSM 35 was equally efficient at delaying the onset of clinical scrapie, and it is possible that the dose response curves for clinical effectiveness may not mirror those for PrP^{Sc} levels in the spleen. Isotype differences may also be relevant. Unfortunately, the short half-life of antibody fragments precludes a direct comparison of the variable regions without elaborate genetic engineering.

Two reports using ScN2a cells have suggested that prion-infected cultures could be cured after long term treatment with antibodies (7, 8). However, the ScN2a cells have levels of infectivity that are much lower than the ScRov cells (31) and therefore may be easier to cure. We showed in one of our experiments that mAb treatment of ScRov cells resulted in a 100–1000-fold decrease in PrP^{Sc} levels (Fig. 1). However, replication re-started when the treatment was stopped, an effect noted with both antibodies and DS500. This finding indicates that a cellular reservoir of PrP^{Sc} remained untouched by the antibodies or DS500 providing a template for conversion to recur. It is tempting to speculate that aggregates of PrP^{Sc} may be involved.

Several sites of post-exposure therapeutic intervention could be envisaged in prion diseases. One comprises the central nervous system, and *in vitro* investigations in ScN2a cells have shown that mAbs might also in theory prevent PrP^{Sc} accumulation in neurons (7, 8). Our study has shown that mAbs also were able to prevent PrP^{Sc} production in an epithelial cell line. These cells may play an important role in the spread of the infection in periphery (32). In addition, the engineering of transgenic mice to produce anti-prion mAbs (9) or passive immunization (12) have shown that they could prevent the generation of infectivity in the spleen when these mice were infected with scrapie. Therefore, antibodies may target different pathways of prion pathogenesis encompassing the transit from the site of infection, its early propagation in periphery and more lately in the central nervous system, and thus be an efficient therapy regardless of the incubation stage in the infected individuals.

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REFERENCES

1. Collinge, J. (1999) *Lancet* **354,** 317–323
2. Ghani, A. C., Ferguson, N. M., Donnelly, C. A., and Anderson, R. M. (2000) *Nature* **406,** 583–584
3. Hill, A. F., Joiner, S., Linehan, J., Desbruslais, M., Lantos, P., and Collinge, J. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 10248–10253
4. Prusiner, S. B. (1982) *Science* **216,** 136–144
5. Clarke, A. R., Jackson, G. S., and Collinge, J. (2001) *Philos. Trans. R. Soc. Lond-Biol. Sci.* **356,** 185–195
6. Mallucci, G., Dickinson, A., Linehan, J., Klohn, P. C., Brandner, S., and Collinge, J. (2003) *Science* **302,** 871–874
7. Enari, M., Flechsig, E., and Weissmann, C. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 9295–9299
8. Peretz, D., Williamson, R. A., Kaneko, K., Vergara, J., Leclerc, E., Schmitt-Ulms, G., Mehlhorn, I. R., Legname, G., Wormald, M. R., Rudd, P. M., Dwek, R. A., Burton, D. R., and Prusiner, S. B. (2001) *Nature* **412,** 739–743
9. Heppner, F. L., Musahl, C., Arrighi, I., Klein, M. A., Rulicke, T., Oesch, B., Zinkernagel, R. M., Kalinke, U., and Aguzzi, A. (2001) *Science* **294,** 178–182
10. Korth, C., Stierli, B., Streit, P., Moser, M., Schaller, O., Fischer, R., SchulzSchaeffer, W., Kretzschmar, H., Raeber, A., Braun, U., Ehrensperger, F., Hornemann, S., Glockshuber, R., Riek, R., Billeter, M., Wuthrich, K., and Oesch, B. (1997) *Nature* **390,** 74–77
11. Williamson, R. A., Peretz, D., Pinilla, C., Ball, H., Bastidas, R. B., Rozenshteyn, R., Houghten, R. A., Prusiner, S. B., and Burton, D. R. (1998) *J. Virol.* **72,** 9413–9418
12. White, A. R., Enever, P., Tayebi, M., Mushens, R., Linehan, J., Brandner, S., Anstee, D., Collinge, J., and Hawke, S. (2003) *Nature* **422,** 80–83
13. Jackson, G. S., Hosszu, L. L., Power, A., Hill, A. F., Kenney, J., Saibil, H., Craven, C. J., Waltho, J. P., Clarke, A. R., and Collinge, J. (1999) *Science* **283,** 1935–1937
14. Vilette, D., Andreoletti, O., Archer, F., Madelaine, M. F., Vilotte, J. L., Lehmann, S., and Laude, H. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 4055–4059
15. Beringue, V., Mallinson, G., Kaisar, M., Tayebi, M., Sattar, Z., Jackson, G., Anstee, D., Collinge, J., and Hawke, S. (2003) *Brain* **126,** 2065–2073
16. Avent, N., Judson, P. A., Parsons, S. F., Mallinson, G., Anstee, D. J., Tanner, M. J., Evans, P. R., Hodges, E., Maciver, A. G., and Holmes, C. (1988) *Biochem. J.* **251,** 499–505
17. Laemmli, U. K. (1970) *Nature* **227,** 680–685
18. Beringue, V., Adjou, K. T., Lamoury, F., Maignien, T., Deslys, J. P., Race, R., and Dormont, D. (2000) *J. Virol.* **74,** 5432–5440
19. Caughey, B., and Raymond, G. J. (1993) *J. Virol.* **67,** 643–650
20. Taraboulos, A., Serban, D., and Prusiner, S. B. (1990) *J. Cell Biol.* **110,** 2117–2132
21. Archer, F., Bachelin, C., Andreoletti, O., Besnard, N., Perrot, G., Langevin, C., Le Dur, A., Vilette, D., Baron-Van Evercooren, A., Vilotte, J. L., and Laude, H. (2004) *J. Virol.* **78,** 482–490
22. Brown, P. (2002) *Neurology* **58,** 1720–1725
23. Caughey, B., and Raymond, G. J. (1991) *J. Biol. Chem.* **266,** 18217–18223
24. Büeler, H., Aguzzi, A., Sailer, A., Greiner, R. A., Autenried, P., Aguet, M., and Weissmann, C. (1993) *Cell* **73,** 1339–1347
25. Bendheim, P. E., Brown, H. R., Rudelli, R. D., Scala, L. J., Goller, N. L., Wen, G. Y., Kascsak, R. J., Cashman, N. R., and Bolton, D. C. (1992) *Neurology* **42,** 149–156
26. Taraboulos, A., Raeber, A. J., Borchelt, D. R., Serban, D., and Prusiner, S. B.

(1992) *Mol. Biol. Cell* **3,** 851–863
27. Solforosi, L., Criado, J. R., McGavern, D. B., Wirz, S., Sanchez-Alavez, M., Sugama, S., DeGiorgio, L. A., Volpe, B. T., Wiseman, E., Abalos, G., Masliah, E., Gilden, D., Oldstone, M. B., Conti, B., and Williamson, R. A. (2004) *Science* **303,** 1514–1516
28. Oesch, B., Westaway, D., and Prusiner, S. B. (1991) *Curr. Top. Microbiol. Immunol.* **172,** 109–124
29. Kascsak, R. J., Rubenstein, R., Merz, P. A., Tonna-DeMasi, M., Fersko, R., Carp, R. I., Wisniewski, H. M., and Diringer, H. (1987) *J. Virol.* **61,** 3688–3693
30. Paitel, E., Alves Da Costa, C., Vilette, D., Grassi, J., and Checler, F. (2002) *J. Neurochem.* **83,** 1208–1214
31. Sabuncu, E., Petit, S., Le Dur, A., Lan Lai, T., Vilotte, J. L., Laude, H., and Vilette, D. (2003) *J. Virol.* **77,** 2696–2700
32. Heppner, F. L., Christ, A. D., Klein, M. A., Prinz, M., Fried, M., Kraehenbuhl, J. P., and Aguzzi, A. (2001) *Nat. Med.* **7,** 976–977