Fast and ultrasensitive method for quantitating prion infectivity titre

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Bioassay by end-point dilution has been used for decades for routine determination of prion infectivity titre. Here we show that the new protein misfolding cyclic amplification with beads (PMCAb) technique can be used to estimate titres of the infection-specific forms of the prion protein with a higher level of precision and in 3–6 days as opposed to 2 years, when compared with the bioassay. For two hamster strains, 263K and SSLOW, the median reactive doses determined by PMCAb (PMCAb\textsubscript{50}) were found to be $10^{1.8}$ and $10^{2.2}$ per gram of brain tissue, which are 160- and 4,000-fold higher than the corresponding median infectious dose (ID\textsubscript{50}) values measured by bioassay. The $10^{-2}$- to $10^{-3}$-fold differences between ID\textsubscript{50} and PMCAb\textsubscript{50} values could be due to a large excess of PMCAb-reactive prion protein seeds with little or no infectivity. Alternatively, the differences between ID\textsubscript{50} and PMCAb\textsubscript{50} could be due to higher rate of clearance of infection-specific prion protein seeds in animals versus PMCAb reactions. A well-calibrated PMCAb reaction can be an efficient and cost-effective method for the estimation of infection-specific prion protein titre.

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A pathognomonic hallmark of the prion diseases is the accumulation of the misfolded isoform of the prion protein (PrPSc). The traditional method for obtaining a quantitative estimate of prion infectivity is end-point dilution titration in animals. A suspension of the tissue or fluid of interest is diluted in tenfold serial steps, and then each dilution is inoculated into a group of animals. A dilution at which only a fraction of the inoculated animals develops clinical signs of disease or shows positive evidence of PrPSc on immunoassay is called a ‘limiting dilution’. At limiting dilution there are only one or a few infectious doses per inoculation volume. End-point dilution titres are typically expressed as the median infective dose (ID₅₀); the reciprocal of the dilution required to infect only 50% of the animals inoculated as determined by interpolation or other statistical methods. While the end-point bioassay has been the principal method for determining prion infectivity, the assay is extremely long, expensive and laborious. Moreover, the bioassay works optimally only for prion strains with incubation times well within the lifespan of the host.

Alternatives to end-point titration are biochemical or immunochromic assays that assess either the presence, mass or concentration of PrPSc (refs 1–4). However, establishing accurate quantitative relationships between PrPSc concentration and prion titre has proven to be difficult because of the size heterogeneity of prion particles and uncertainty over whether all prion particles are equally infectious5–7. Moreover, the size distribution and physical properties of prion particles appear to vary with agent strain and host species8. In 2003, Weissmann and co-workers8 introduced a scrapie cell assay that quantitatively estimates prion infectivity titres within a much shorter time frame than animal bioassay. The scrapie cell assay was shown to be capable of detecting prions in a dilution as low as 10¹⁰-fold of scrapie-infected brain material9. Moreover, in recent studies, the scrapie cell assay was adapted for detecting prions from various species10.

Protein misfolding cyclic amplification (PMCA) propagates PrPSc and infectivity in vitro11–14. The sensitivity of the PMCA reaction to detect prion particles exceeds that of the bioassay15,16. While PMCA has been proven for detecting and amplifying prions from a broad range of species including human, cow, sheep, cervids, mouse, hamster and others16–20, highly robust PMCA amplification has been limited to mostly rodent and rodent-adapted strains. Nevertheless, the efficiency of prion replication in PMCA was shown to mimic cross-species transmission barriers21–23 or genetic susceptibility of sheep to scrapie that occurs due to prion protein polymorphisms24. The improvements in the PMCA assay found in PMCA with beads (PMCAb) have resulted in a much faster, more robust, sensitive and cost-efficient way of measuring PrPSc compared with either PMCA or bioassay25,26.

To illustrate the advantages of PMCAb-based end-point titration, we assessed the relative concentrations of PrPSc in brain material of two rodent strains, 263K and SSLOW, which display very short or long incubation periods, respectively (Fig. 1a,b), to the level detectable by western blot. An increase in the number of PMCAb rounds did not increase the percentage of positive reactions for the most highly diluted samples illustrating that the limiting dilution was reached (Fig. 1c).

In our experience, PMCAb displays a high level of selectivity for infection-specific forms of PrP. To date, we have detected no false-positive amplifications in the routine control reactions that are included with every PMCAb titration experiment. These controls consist of six serial rounds of 48 cycles of PMCAb of unseeded substrate only. Other controls include six rounds of serial PMCAb of 10% normal BHs from 700-day-old hamsters (Fig. 2), and PMCAb amplifications of hamster substrate seeded with full-length recombinant hamster PrP amyloid fibrils prepared under a variety of previously described experimental conditions and protocols26–30.

No positive signals were detected in any of the controls (Fig. 2).

End-point dilution titration in animals. In parallel to PMCAb titration, the infectivity titres of brains from 263K- and SSLOW-infected animals were measured using end-point dilution bioassay. Animals were considered infected if they develop symptomatic disease or if their brains contained PrPSc, as judged by western blot even without symptomatic disease. The fractions of animals infected at each dilution are presented in Table 1.
For each dilution, the fraction of animals infected or the fraction of PMCAb reactions with a positive signal on western blot was plotted against the logarithm of dilution for both 263K and SSLOW (Fig. 3). At limiting dilution, PrPSc particles must necessarily assort randomly into the reaction aliquots, and their distribution is described by the Poisson equation. While there was a good fit to the Poisson equation by the animal infectivity data for both 263K and SSLOW, the PMCAb titration curves for both strains showed a more gradual slope than predicted by the Poisson equation (Fig. 3a). This is consistent with increasing reaction efficiency at higher dilutions. One possibility is that dilution results in a concentration-dependent dissociation of aggregates, thereby releasing and increasing the concentration of PMCAb reactive centres.

Alternatively, dilution might diminish the effects of an inhibitor. As the effect is at very high dilutions, the inhibitor alternative seems less likely. An arbitrary sigmoidal function and nonlinear regression analysis were used to calculate ID50 or PMCAb50 values from bioassay- or PMCAb-based end-point curves, respectively (Fig. 3b,c, Table 2). (Note that the Poisson equation is a special instance of the more generalized sigmoidal function used.) The infectivity titres determined in this way were almost identical to those determined by the Poisson equation or by more traditional application of the Reed and Muench and Spearman and Karber methods (Table 2). Analogous to the bioassay, a PMCAb50 is the reciprocal of the concentration at which only 50% of the PMCAb reactions were positive. As judged

**Analysis of prion titre.** For each dilution, the fraction of animals infected or the fraction of PMCAb reactions with a positive signal on western blot was plotted against the logarithm of dilution for both 263K and SSLOW (Fig. 3). At limiting dilution, PrPSc particles must necessarily assort randomly into the reaction aliquots, and their distribution is described by the Poisson equation. While there was a good fit to the Poisson equation by the animal infectivity data for both 263K and SSLOW, the PMCAb titration curves for both strains showed a more gradual slope than predicted by the Poisson equation (Fig. 3a). This is consistent with increasing reaction efficiency at higher dilutions. One possibility is that dilution results in a concentration-dependent dissociation of aggregates, thereby releasing and increasing the concentration of PMCAb reactive centres.
from ID$_{50}$ and PMCA$_{50}$ values, PMCAb was more sensitive than bioassay by ~4,000-fold for SSLOW and ~160-fold in the case of 263K. Importantly, PMCAb titration was completed in a few days, whereas the bioassay required nearly 2 years.

### Discussion

We show here that PMCAb, when well calibrated against bioassay, can be used to obtain an estimate of PrP$^{Sc}$ titre in only 3–6 days, ~100 times faster than the bioassay. At the same time, PMCAb offers ethical advantages of reducing the need for animal use. The precision of the measurements in PMCAb is limited only by the number of replicates performed. The new PMCAb-based assay can be used as a fast, efficient and ultrasensitive method for determining PrP$^{Sc}$ titre and is uniquely beneficial for samples that have extremely low levels of infectivity and for determining infectivity concentrations for prion strains with long incubation times. On the other hand, while PMCA has been used for amplification of PrP$^{Sc}$ from a wide range of species,$^{16,18–25}$ the most robust amplification was achieved, so far, for a limited number of rodent or rodent-adapted strains. Further technical improvements are needed for PMCA to be used as a platform for quantitative estimates of PrP$^{Sc}$ from a broad range of species.

In recent studies, Soto and co-workers,$^{16}$ estimated the amount of PrP$^{Sc}$ based on the number of PMCA rounds necessary to amplify prions to a detectable level. While PMCAb and PMCA methods have similar sensitivities for detection of 263K, PMCA requires 18 replicates performed. The new PMCAb-based assay can be used as a fast, efficient and ultrasensitive method for determining PrP$^{Sc}$ titre to prion infectivity. A PMCAb-active particle can infect an animal at a ratio of 160 to 10$^{10}$-fold greater sensitivity of the PMCAb assay.

Strain 263K (short incubation, short clinical duration) and SSLOW (very long incubation and clinical duration$^{31}$) represent the two extremes of prion hamster disease. Nevertheless, the concentration of PMCAb-reactive particles was similar in brains of animals infected with 263K or SSLOW (Table 2). No PMCAb-reactive particles were found in brains from aged animals, confirming that these particles are disease-specific. In contrast to having similar PMCA$_{50}$ values, the infectivity titre was ~100-fold lower for SSLOW than for 263K (Table 2). The PMCA$_{50}$/ID$_{50}$ ratio was 160 and 4,000 particles for 263K and SSLOW, respectively (Table 2). If the hypothesis that PMCAb amplifies infectious and non-infectious prion protein seeds is correct, then PMCA$_{50}$/ID$_{50}$ value reflects a strain-specific ratio of non-infectious, disease-associated PMCAb-reactive particles per infectious PrP$^{Sc}$ seeds. Alternatively, the difference in the PMCA$_{50}$/ID$_{50}$ ratio could also reflect strain-specific differences in the amount of PMCAb-active particles required to infect an animal. The difference in efficiency of infection between the two strains could be attributed to several factors. First, the differences could be due to the differences in strain-specific rates of PrP$^{Sc}$ clearance on inoculation. Second, the PrP$^{Sc}$ species that is the most reactive in PMCAb might be not the one that is the most toxic. In fact, previous studies pointed to an uncoupling of prion infectious titre and neurotoxicity$^{40}$ while PMCAb presumably counts PrP$^{Sc}$ particles, the read-out parameter for bioassay is the presence of clinical or subclinical disease, which results from an accumulation of neurotoxic PrP species. Therefore, if infectious and neurotoxic PrP species are two different entities, the differences in PMCA$_{50}$/ID$_{50}$ value could reflect a complex strain-specific relationship between a PMCAb-active PrP$^{Sc}$ species and a neurotoxic species.

### Table 2 | ID$_{50}$ values measured by end-point bioassay or PMCAb.

| Strain  | ID$_{50}$ (Bioassay$^{a}$) | ID$_{50}$ (PMCAb$^{b}$) | Ratio PMCAb$^{b}$/ID$_{50}$ |
|---------|---------------------------|-------------------------|-----------------------------|
| 263K    | 10$^{10.5}$               | 10$^{12.8}$             | 160                         |
| SSLOW   | 10$^{8.7}$               | 10$^{12.2}$             | 4000                         |

$^{a}$The ID$_{50}$ values were estimated using the Poisson equation.

$^{b}$The ID$_{50}$ values were estimated using the sigmoidal equation.

Klingeborn et al.$^{36}$ showed that PrP$^{Sc}$ generated in PMCA had considerably lower infectivity titre than that of brain-derived PrP$^{Sc}$ (ref. 36). This work suggested that two competitive PMCA pathways that amplify infectious PrP$^{Sc}$ and non-infectious particles exist. In contrast, the work by Shikiya and Bartz$^{37}$ reported that PMCA-generated PrP$^{Sc}$ had a titre similar to that of brain-derived PrP$^{Sc}$. Further studies are needed to clarify these contradictory results.

Alternatively, the differences between ID$_{50}$ and PMCA$_{50}$ values might reflect substantial differences in obstacles to successfully initiate prion infection in an animal versus PMCAb reaction. It has been reported that prions inoculated into animals are subject to intensive proteolytic degradation and clearance.$^{38}$ In contrast, the PMCAb amplification is conducted in the presence of protease inhibitors to prevent degradation of the normal prion protein substrate for conversion. It is conceivable that this environment also preserves a greater fraction of infectious PMCAb-active particles, thereby accounting for the ~10$^9$- to 10$^3$-fold greater sensitivity of the PMCAb assay.

In recent studies, Soto and co-workers$^{16}$ estimated the amount of PrP$^{Sc}$ based on the number of PMCA rounds necessary to amplify prions to a detectable level. While PMCAb and PMCA methods have similar sensitivities for detection of 263K, PMCA requires 18 days to reach sensitivity comparable with that achieved by PMCAb in 3 days. Furthermore, PMCA did not work well for amplification of SSLOW PrP$^{Sc}$ (ref. 29). PMCAb is also far less sensitive to inhibitors and small variations in sonication conditions that have plagued conventional PMCA.

It is of interest to consider the molecular relationship of PMCAb titre to prion infectivity. A PMCA$_{50}$ titre represents the number of PMCAb-active, PrP$^{Sc}$-particles capable of initiating PMCAb amplification per gram of material sampled. Both PMCAb amplification products and PrP$^{Sc}$ are heterogeneous in at least size, and perhaps structure, and exactly which structures represent infectivity is not yet clear. Furthermore, contradictory data exist regarding the specific infectivity of PMCAb-generated versus that of brain-derived PrP$^{Sc}$ (refs 36,37).

Close comparison of the two sets of data, obtained from the end-point dilution titration on animals and PMCAb, can be used to establish a quantitative relationship. The ratio of PMCA$_{50}$/ID$_{50}$ is the number of PMCAb-active particles that corresponds to one prion infectious dose or unit. The PMCA$_{50}$/ID$_{50}$ ratio was 160 and 4,000 particles per one infectious dose for 263K and SSLOW, respectively (Table 2). There are several ways to explain the 10$^{4}$- to 10$^{6}$-fold excess in the number of PMCAb-active particles over the number of the infectious doses. The large PMCA$_{50}$/ID$_{50}$ ratio could be interpreted as only a few or one out of each 160 or 4,000 PrP$^{Sc}$ particles being truly infectious, whereas the majority of particles while detectable and amplifiable by PMCAb have little or no infectivity. Consistent with this interpretation, recent studies reported a dissociation between PMCA seeding ability and biological infectivity for PrP$^{Sc}$ seeds produced from PrP constructs with deletions of polybasic domains.$^{38}$ Furthermore, the studies by...
Further comparison of end-point titration data from bioassay and PMCAb will test whether PMCAb/ID₅₀ ratio represents an inherent strain-specific property. Fortunately, uncertainties as to the relationship of PMCAb-active particles to infectivity do not diminish the power or usefulness of PMCAb as a quantitative assay. PMCAb can be used as is, as a relative indicator of infectivity titre between two similar samples. With careful calibration and sample control, it can be used to estimate PrPSc infectivity titre itself.

Methods

End-point titration bioassay. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of the University of Maryland, Baltimore (assurance number: A32000-01; permit number: 0309001).

Ten percent scrapie BH was prepared in PBS, pH 7.4, by sonication and serially diluted up to 10⁻¹₀-fold in PBS, as previously described. SLOW-inoculated animals from the second passage of SLOW were used for both bioassay and PMCAb. Before inoculation, samples were dispersed by 90% of maximum power ultrasonication in PBS. Each hamster received 50-µl inoculum intracerebrally under general anaesthesia (2 LPM O₂/A MAC isoflurane). The inoculated animals were observed closely for up to 660 days post-inoculation or until they developed clinical signs of prion disease. For SLOW-inoculated animals, the clinical signs were observed as early as 318±10 days post-inoculation for the 10⁻⁰-fold dilution of brain material or as late as 560±10 days for the 10⁻⁵-fold dilution of brain material. Only one out of eight animals inoculated with the 10⁻⁰-fold dilution showed clinical signs, whereas no clinical signs were observed for dilutions 10⁻⁵-fold and higher. Affected animals died or were euthanized and their disease status was confirmed by western blot analysis of their brains. At the end of the incubation (660 days post-inoculation) all remaining animals were euthanized, and all brains were assessed for the presence of PrPSc by western blot. Brain materials that contained PrPSc but had not yet developed symptomatic disease were considered infected.

End-point titration using PMCAb. Healthy hamsters were euthanized and immediately perfused with PBS, pH 7.4, supplemented with 5 mM EDTA. Brains were dissected, and 10% BH (wt/vol) was prepared using ice-cold conversion buffer and glass/Teflon tissue grinders cooled on ice and attached to a constant torque homogenizer (Heidolph, RZR20). The brains were ground at low speed until homogenization was complete, and then five additional strokes completed the homogenization. The composition of conversion buffer was as previously described. Ca²⁺- and Mg²⁺-free and Mg²⁺-free PBS, pH 7.4, supplemented with 0.15 M NaCl, 1% Triton X-100, a complete set of protease inhibitors cocktail (Roche, cat. no. 1836145) per 50 ml of conversion buffer. The resulting 10% normal BH (NBH) in conversion buffer was used as the substrate in PMCA reactions. To prepare seeds, scrapie-infected brains were homogenized as for inoculation (above), and 100 µl aliquots were sonicated in MISONIX S-4000 microplate horn (Misonix) horn for 30 s at 50% power before serial dilution from 10⁻³ to 10⁻¹₄-fold in conversion buffer. Then, 10 µl of each dilution were used to seed 90 µl of NBH for PMCAb. Teflon beads (2.38 mm diameter, McMaster-Carr, Los Angeles, CA, USA) were placed into the 0.2 ml tubes first, and then NBH and seeds were added. Samples in 0.2 ml thin-wall PCR tubes (Fisher, cat. no. 14230205) were placed in a floating rack inside a Misonix S-4000 microplate cup horn filled with 350 ml water. Two coils of rubber tubing attached to a circulating water bath were installed for maintaining 37°C inside the sonicator chamber. The standard sonication program consisted of 30 s sonication pulses delivered at 50% efficiency applied every 30 min during a 24-h period. For the probability that a given aliquot received no active particle, ID₅₀ was calculated according to the equation:

\[
F = (100\% \exp (A + B \times x)) / (1 + \exp (-A + B \times x))
\]

where \( F \) is percent of PMCAb reactions or infected animals, \( x \) is logarithm of the limiting dilution fold, \( A \) and \( B \) are two fitting parameters that define the position of a limiting dilution transition on the x axis and the slope of the transition, respectively. ID₅₀ and PMCAb were calculated according to the equation:

\[
\text{PMCAb or ID}_{50} = A / B
\]

Proteinase K assay. To analyse the PMCAb end-point titration reactions, 10 µl of each sample was supplemented with 5 µl SDS and 5 µl proteinase K (PK), to a final concentration of SDS and PK of 0.25% and 1 µg/ml, respectively, followed by incubation at 37°C for 1 h. The digestion was terminated by addition of SDS sample buffer and boiling for 10 min. Samples were loaded onto NuPAGE 12% BisTris gels, transferred to polyvinylidene difluoride membrane, and stained with 3F4 antibody. To analyse scrapie BHs, an aliquot of 10% BH was mixed with an equal volume of 4% sarcosyl in PBS, supplemented with 50 mM Tris, pH 7.5, and digested with 20 µg/ml PK for 30 min at 37°C with 1,000 r.p.m. shaking (Eppendorf Thermomixer). The reaction was stopped by SDS sample buffer. Samples were loaded for 10 min and loaded onto NuPAGE 12% BisTris gels. After transfer to polyvinylidene difluoride membrane, PrP was detected with 3F4 antibody.

Analysis using sigmoidal equation. ID₅₀ and PMCAb values were determined by regression analysis in Sigma Plot using nonlinear least squares fitting of both sets of data to the sigmoidal equation.
21. Ayers, J. L. et al. The strain-encoded relationship between PrP replication, stability and processing in neurons is predictive of the incubation period of disease. *PLOS Pathog.* 7, e1001317 (2011).

22. Murayama, Y. et al. Sulfated dextrins enhance *in vitro* amplification of bovine spongiform encephalopathy PrP(Sc) and enable ultra sensitive detection of bovine PrP(Sc). *Plos ONE* 5, e13152 (2010).

23. Tattum, M. H., Jones, S., Pal, S., Collinge, J. & Jackson, G. S. Discrimination between prion-infected and normal blood samples by protein misfolding cyclic amplification. *Transfusion* 50, 2619–2627 (2010).

24. Rubenstein, R. et al. Prion disease detection, PMCA kinetics, and IgG in urine from sheep naturally/experimentally infected with scrapie and deer with preclinical/clinical chronic wasting disease. *J. Virol.* 85, 9031–9038 (2011).

25. Nishina, K. et al. The stoichiometry of host PrPC glycoforms modulates the efficiency of PrPSc formation *in vitro*. *Biochemistry* 45, 14129–14139 (2006).

26. Gonzalez-Montalban, N., Makarava, N., Savtchenko, R. & Baskakov, I. V. Relationship between conformational stability and amplification efficiency of prions. *Biochemistry* 50, 7933–7940 (2011).

27. Castilla, J. et al. Crossing the species barrier by PrPSc replication *in vitro* generates unique infectious prions. *Cell* 134, 757–768 (2008).

28. Green, K. M. et al. Accelerated high fidelity prion amplification within and across prion species barriers. *PLOs Pathog.* 4, e1000139 (2008).

29. Gonzalez-Montalban, N. et al. Highly efficient protein misfolding cyclic amplification. *PLoS Pathog.* 7, e1001277 (2011).

30. Prizkow, S. et al. Quantitative detection and biological propagation of scrapie seeding activity in *in vitro* facilitate use of prions as model pathogens for disinfection. *Plos ONE* 6, e20384 (2011).

31. Makarava, N. et al. Recombinant PrP protein induces a new transmissible prion disease in wild type animals. *Acta Neuropathol.* 119, 177–187 (2010).

32. Bocharova, O. V., Breydo, L., Parfenov, A. S., Salnikov, V. V. & Baskakov, I. V. In *vitro* conversion of full length mammalian prion protein produces amyloid form with physical property of PrPSc. *J. Mol. Biol.* 346, 645–659 (2005).

33. Bocharova, O. V. et al. Annealing PrP amyloid fibrils at high temperature results in extension of a proteinase K resistant core. *J. Biol. Chem.* 281, 2373–2379 (2006).

34. Sun, Y. et al. Conformational stability of PrP amyloid fibrils controls their smallest possible fragment size. *J. Mol. Biol.* 376, 1155–1167 (2008).

35. Makarava, N. & Baskakov, I. V. The same primary structure of the prion protein yields two distinct self-propagating states. *J. Biol. Chem.* 283, 15988–15996 (2008).

36. Klingeborn, M., Race, B., Meade-White, K. D. & Chesebro, B. Lower specific infectivity of protease-resistant prion protein generated in cell-free reactions. *Proc. Acad. Natl Sci. USA* 108, 1244–1253 (2011).

37. Shikiya, A. S. & Bartz, J. C. *In vitro* generation of high titer prions. *J. Virol.* 85, 13439–13442 (2011).

38. Miller, M. B., Geoghegan, J. C. & Supattapone, S. Dissociation of infectivity from seeding ability in prions with alternate docking mechanism. *PLoS Pathog.* 7, e1002128 (2011).

39. Safar, J. et al. Prion clearance in bigenic mice. *J. Gen. Virol.* 86, 2913–2923 (2005).

40. Sandberg, M. K., Al-Doujaily, H., Sharps, B., Clarke, A. R. & Collinge, J. Prion propagation and toxicity in *vitro* occur in two distinct mechanistic phases. *Nature* 470, 540–542 (2011).

41. Gregori, L. et al. Reduction of transmissible spongiform encephalopathy infectivity from human red blood cells with prion protein affinity ligands. *Transfusion* 46, 1152–1161 (2006).

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