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Susceptibility to Scrapie in Mice is Dependent on PrP<latex>$^{\text{C}}$</latex>

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Susceptibility to scrapie in mice is dependent on PrP^C

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SUMMARY

Mice devoid of functional PrP genes (*Prn-p*$^{o/o}$ mice) showed normal development and behaviour. When inoculated with mouse scrapie prions they remained free of scrapie symptoms for at least 18 months whereas wild-type controls all died within 6 months. No propagation of infectivity could be detected in the PrP null mice. Surprisingly, heterozygous *Prn-p*$^{o/+}$ mice also showed enhanced resistance to scrapie. After introduction of Syrian hamster PrP transgenes, *Prn-p*$^{o/o}$ mice became highly susceptible to hamster but not to mouse prions. These experiments show that PrP^C, possibly at close to normal levels, is required for the usual susceptibility to scrapie and that lack of homology between incoming prions and the host's PrP genes retards disease.

1. INTRODUCTION

The 'protein only' hypothesis as proposed by Prusiner suggests that the prion contains no nucleic acid and is identical with PrP^{Sc}, a modified form of PrP^C (Prusiner 1989). PrP^C is a normal host protein (Oesch *et al.* 1985; Basler *et al.* 1986) found predominantly on the outer surface of neurons. PrP^{Sc} is defined as a form of PrP^C that readily forms protease-resistant aggregates after treatment with detergents (Oesch *et al.* 1985; McKinley *et al.* 1991). Prusiner proposed that PrP^{Sc}, when introduced into a normal cell, causes the conversion of PrP^C or its precursor into PrP^{Sc}. The nature of the conversion is unknown and could be due to a post-translational chemical or conformational modification.

We have reported earlier that mice homozygous for disrupted *Prn-p* genes (*Prn-p*$^{o/o}$ mice) develop and reproduce normally and show no detectable physical or behavioural anomalies (Büeler *et al.* 1992). It thus became possible to study the response of *Prn-p*$^{o/o}$ mice to inoculation with scrapie prions, as well as of animals carrying a single *Prn-p* allele (*Prn-p*$^{o/+}$ mice) and of *Prn-p*$^{o/o}$ mice reconstituted with mouse or Syrian hamster PrP transgenes. If indeed PrP^{Sc} constitutes the scrapie agent, or is an essential component of it (Prusiner 1991), then mice devoid of PrP should be resistant to infection, developing neither symptoms of scrapie nor allowing propagation of the infectious agent. Conversely, if the animals succumb to the disease or propagate infectivity albeit without showing symptoms of neurological disease, the 'protein only' hypothesis would be falsified.

2. RESULTS

(*a*) *Challenge of* Prn-p$^{o/o}$ *and* Prn-p$^{o/+}$ *mice with scrapie prions*

Prn-p$^{o/o}$ and *Prn-p*$^{+/+}$ mice were inoculated intracerebrally with about 10^7 LD_{50} units of mouse-adapted prions. As a further control, Swiss CD-1 mice were inoculated similarly. CD-1 mice showed typical neurological symptoms at 140 ± 6 days and died at 153 ± 7 days. All *Prn-p*$^{+/+}$ mice with the C57BL-129/Sv background showed symptoms at 158 ± 11 days and died at 171 ± 11 days. In stark contrast, no *Prn-p*$^{o/o}$ mice succumbed to scrapie at the time of writing, more than 18 months after inoculation and 12 months after the last *Prn-p*$^{+/+}$ controls died (Büeler *et al.* 1993) (figure 1).

In a further experiment heterozygous *Prn-p*$^{o/+}$ mice were inoculated intracerebrally with mouse-adapted prions. They developed scrapie symptoms much later than the *Prn-p*$^{o/o}$ mice, namely 290 days after inoculation and died around 450 days after inoculation. Thus the average incubation time is about 130 days longer than in the case of the wild-type controls and survival after onset of symptoms is about 160 rather than 13 days (Büeler *et al.* 1993; H. Büeler & A. Sailer, unpublished results).

(*b*) *Lack of neuropathology in scrapie-infected* Prn-p$^{o/o}$ *mice*

Brain sections of *Prn-p*$^{o/o}$ and *Prn-p*$^{+/+}$ mice inoculated with normal or mouse prion-containing brain homogenate were stained with anti-GFAP antibodies or hematoxylin-eosin. *Prn-p*$^{+/+}$ mice 23–25 weeks after inoculation with mouse prions showed pronounced astrogliosis and vacuolation mainly in the thalamus, cortex and hippocampus. In addition, some of these mice displayed neuronal loss in the hippocampus and thalamus. In contrast, brains from *Prn-p*$^{o/o}$ animals 57 weeks after inoculation with mouse scrapie prions showed no scrapie-specific pathology and were indistinguishable from those 56 weeks after inoculation with normal brain homogenate (Büeler *et al.* 1993).

(*c*) *Titration of scrapie infectivity in brains and spleens of* Prn-p$^{+/+}$ *and* Prn-p$^{o/o}$ *mice*

At 4 days, 2, 8, 12, 20 and 23–25 weeks after inoculation infectivity was determined in brains and

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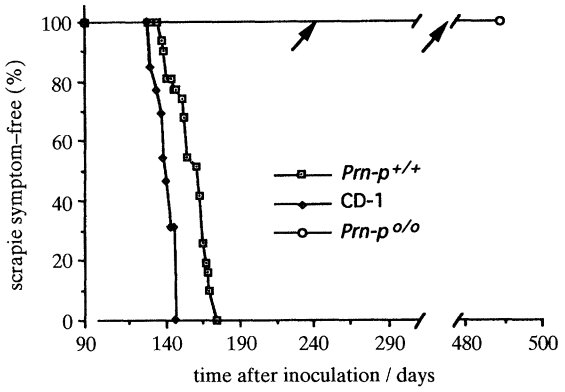

Figure 1. Scrapie resistance of mice with disrupted PrP genes *Prn-p$^{o/o}$* and *Prn-p$^{+/+}$* mice remaining symptoms-free at different times after inoculation with mouse scrapie prions. Thirty-one *Prn-p$^{+/+}$* and 25 *Prn-p$^{o/o}$* animals were inoculated with mouse prions and kept under observation. Arrows: five mice were killed at various times; none had scrapie symptoms. Modified from Büeler *et al.* (1993).

spleens from four replicate mice. Aliquots were heated at 80°C to inactivate conventional infectious agents and inoculated into indicator mice. The appearance of scrapie symptoms and death were recorded. The resulting prion titers in brain and spleen of wild-type and *Prn-p$^{o/o}$* mice are shown in table 1.

Spleen homogenates from inoculated *Prn-p$^{+/+}$* mice gave titers of about 5.7 log LD$_{50}$ units ml^{-1} at 4 days after infection and increased slightly thereafter, whereas in the case of *Prn-p$^{o/o}$* mice the titer was around 2.3 log LD$_{50}$ units ml^{-1} at 4 days after inoculation, and no infectivity (less than 1.5 log LD$_{50}$ units ml^{-1}) was detected at later times (table 1). The low titer at day 4 is presumably due to residual inoculum. The fact that after 4 days the infectivity titer in spleen is barely detectable in *Prn-p$^{o/o}$* animals while it is high in *Prn-p$^{+/+}$* mice suggests that in wild-type animals substantial prion propagation has occurred within this organ; alternatively, in *Prn-p$^{o/o}$* animals (conjectural) transport of prions from brain to spleen may be severely impaired or degradation in the spleen may be accelerated.

As shown in table 1, brain homogenates prepared from *Prn-p$^{+/+}$* mice showed an increase in titer from less than 1.5 log LD$_{50}$ units ml^{-1} at day 4 to 5.4 log LD$_{50}$ units ml^{-1} at 8 weeks and 8.1 log LD$_{50}$ units ml^{-1} at 23–25 weeks. No transmission was noted for 1:10 diluted brain homogenates up to 25 weeks after inoculation, the latest time point taken. From our results we conclude that if infectious agent is propagated in brain of *Prn-p$^{o/o}$* mice this occurs at a level five orders of magnitude lower than in the wild-type controls.

(*d*) *Complementation of* Prn-p$^{o/o}$ *mice with mouse or Syrian hamster* PrP *genes*

We introduced mouse PrP transgenes into *Prn-p$^{o/o}$* mice and inoculated them with mouse prions. The mice showed scrapie symptoms after 60 ± 10 days and died within about 3 days (M. Fischer & C. Weissmann, unpublished observations). The unusually short incubation time and rapid disease progression is likely due to the high expression level of the multiple transgenes (Scott *et al.* 1989). This experiment shows that susceptibility to mouse scrapie infection can be restored by the introduction of cloned mouse PrP genes and opens the way to the functional analysis of the PrP gene by reverse genetics.

Syrian hamster PrP genes were introduced into *Prn-p$^{o/o}$* mice by mating them with tg(SHaPrP)81 mice homozygous for the transgene-containing locus SHaPrP (Scott *et al.* 1989; Prusiner *et al.* 1990). *Prn-p$^{o/o}$*/SHaPrP$^{o/+}$ and *Prn-p$^{o/+}$*/SHaPrP$^{o/+}$ mice were inoculated with the Sc237 isolate of Syrian hamster prions or with the Chandler isolate of mouse scrapie prions (RML). The *Prn-p$^{o/o}$*/SHaPrP$^{o/+}$ mice inoculated with hamster prions showed neurological symptoms after 56 ± 3 days and died after 59 ± 5 days while the values for the *Prn-p$^{o/+}$*/SHaPrP$^{o/+}$ mice were 67 ± 4 and 71 ± 6 days, respectively (figure 2). In contrast, none of the non-transgenic *Prn-p$^{o/o}$* and *Prn-p$^{o/+}$* mice came down with scrapie by 430 days after inoculation with hamster prions (figure 2).

Prn-p$^{o/o}$/SHaPr$^{o/+}$ mice inoculated with mouse prions showed scrapie symptoms at 303 ± 19 days and *Prn-p$^{o/+}$*/SHaPr$^{o/+}$ mice inoculated with mouse prions all fell ill at 255 ± 28 days after inoculation (Büeler *et al.* 1993, unpublished data). It seems that hamster PrP^C is able to interact with mouse prions, albeit with low efficiency, as might be expected from the fact that

Table 1. *Prion titers in brain and spleen of* Prn-p$^{+/+}$ *and* Prn-p$^{o/o}$ *mice*

(The titers are from table 3, Büeler *et al.* (1993). Samples were heated at 80°C for 20 min in the presence of 0.1% sarkosyl prior to assaying. The values with standard errors were obtained from end point dilutions, the others by incubation time assays.)

	log LD$_{50}$ units ml^{-1}			
	brain		spleen	
time after inoculation	Prp$^{+/+}$	PrP$^{o/o}$	PrP$^{+/+}$	PrP$^{o/o}$
4 days	< 1.5	2.0	5.7 ± 0.9	2.3
2 weeks	< 1.5	< 1.5	6.2 ± 0.8	< 1.5
8 weeks	5.4	< 1.5	6.9 ± 1.0	< 1.5
12 weeks	6.8	< 1.5	5.9 ± 0.6	< 1.5
20 weeks	8.6	< 1.5	6.9 ± 0.6	< 1.5
23/25 weeks	8.1 ± 0.8	< 1.5	n.d.	< 1.5

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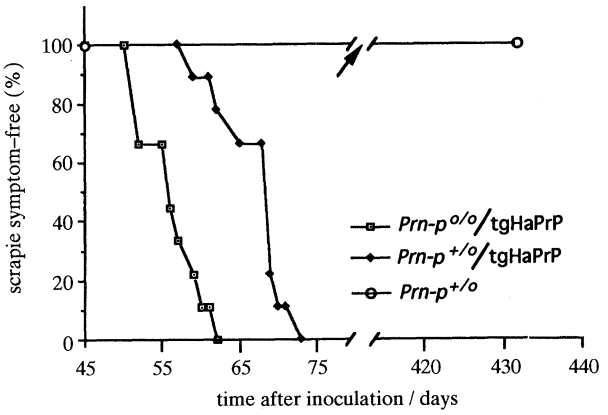

Figure 2. *Prn-p*$^{o/o}$ and *Prn-p*$^{o/+}$ mice with hamster PrP transgenes remaining symptom-free at different times after inoculation with hamster scrapie prions. Groups of 9–11 mice of each genotype were inoculated with the Sc237 isolate of hamster prions. Arrow: one animal died spontaneously without scrapie symptoms and one was killed because of a tumor. Modified from Büeler *et al.* (1993).

hamsters inoculated with mouse prions ultimately also succumb to scrapie disease (Scott *et al.* 1989).

3. DISCUSSION

Our results show that mice devoid of PrP are completely protected against scrapie disease, at least up to 18 months after inoculation. Moreover, even heterozygous *Prn-p*$^{o/+}$ mice are partially protected, inasmuch as they showed signs of scrapie only about 300 days after inoculation while all *Prn-p*$^{+/+}$ controls died within about 180 days. Disease progression in *Prn-p*$^{o/+}$ mice is very much slower than in *Prn-p*$^{+/+}$ mice. No propagation of infectious agent was detected in *Prn-p*$^{o/o}$ mice (Büeler *et al.* 1993).

We conclude that development of scrapie symptoms and neuropathology is strictly dependent on the presence of PrP^C and that incubation time and disease progression is inversely related to the level of PrP^C. The finding that *Prn-p*$^{o/o}$ mice carrying hamster PrP genes become very susceptible to hamster-derived prions (56 days incubation time) but show long incubation times (not less than 271 days) with mouse-derived prions demonstrates the requirement of a homotypic relationship between incoming prion and resident PrP protein for prion propagation and development of pathology, as foreshadowed by the results of Prusiner *et al.* (1990).

We failed to detect anti-PrP antibodies in scrapie-inoculated *Prn-p*$^{o/o}$ mice. Moreover, *Prn-p*$^{o/+}$ mice, which should be as tolerant toward PrP as *Prn-p*$^{+/+}$ mice, show prolonged incubation times, suggesting that resistance to scrapie of *Prn-p*$^{o/o}$ mice is not due to an immune response (Büeler *et al.* 1993).

The findings described in this paper are in accordance with the 'protein only' hypothesis and together with the large body of evidence amassed by Prusiner and his colleagues (Weissmann 1991; Prusiner 1992*a*,*b*) provide strong support for this model.

Because it is possible to generate normal mice which are resistant to scrapie by disrupting their PrP genes, it should, in principle, be possible to breed sheep or cattle resistant to this disease, either by PrP gene disruption or by the introduction of transgenes expressing PrP antisense RNA. Moreover, the fact that *Prn-p*$^{o/+}$ heterozygous mice show much longer scrapie incubation times than their wild-type counterparts argues that disease progression may be rate-limited by the PrP^C concentration. A practical implication of this conclusion is that a moderate reduction of PrP^C synthesis, such as might eventually be achieved by antisense oligonucleotide therapy, could substantially mitigate disease progression in incipient cases of spongiform encephalopathies.

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