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Normal host prion protein (PrP<sup>C</sup>) is required for scrapie spread within the central nervous system

(intraocular inoculation/neurografts)

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Contributed by Charles Weissmann, August 19, 1996

ABSTRACT Mice devoid of PrP<sup>C</sup> (Prnp<sup>−/−</sup>) are resistant to scrapie and do not allow propagation of the infectious agent (prion). PrP<sup>C</sup>-expressing neuroectodermal tissue grafted into Prnp<sup>−/−</sup> brains but not the surrounding tissue consistently exhibits scrapie-specific pathology and allows prion replication after inoculation. Scrapie prions administered intraocularly into wild-type mice spread efficiently to the central nervous system within 16 weeks. To determine whether PrP<sup>C</sup> is required for scrapie spread, we inoculated prions intraocularly into Prnp<sup>−/−</sup> mice containing a PrP-overexpressing neurograft. Neither encephalopathy nor protease-resistant PrP (PrP<sup>Sc</sup>) were detected in the grafts for up to 66 weeks. Because grafted PrP-expressing cells elicited an immune response that might have interfered with prion spread, we generated Prnp<sup>−/−</sup> mice immunotolerant to PrP and engrafted them with PrP-producing neuroectodermal tissue. Again, intraocular inoculation did not lead to disease in the PrP-producing graft. These results demonstrate that PrP is necessary for prion spread along neural pathways.

The infectious agent causing transmissible spongiform encephalopathies such as scrapie, bovine spongiform encephalopathy, and experimental scrapie of mice and hamsters seems to be devoid of informational nucleic acids (1–3) and may consist entirely of protein (4). A large body of evidence suggests that the prion is a modified form of the cellular protein PrP<sup>C</sup> (5–7), perhaps identical to the protease-resistant isofrom termed PrP<sup>Sc</sup> (but see ref. 8).

Intracerebral inoculation of tissue homogenate into suitable recipients is the most effective method for transmission of spongiform encephalopathies and frequently allows the species barrier to be circumvented, albeit with reduced efficacy. However, spongiform encephalopathies have also been transmitted by feeding (9) as well as by intravenous, intraperitoneal (10), and intramuscular injection (11). Prion diseases can also be initiated from the eye by conjunctival instillation (12), corneal grafts (13), and intraocular injection (14). The latter method has proved particularly useful to study neural spread of the agent, because the retina is a part of the central nervous system (CNS) and intraocular injection does not disrupt the blood–brain barrier or any other aspect of brain physiology. The assumption that spread of prions occurs axonally rests mainly on the demonstration of diachronic spongiform changes along the retinal pathway after intraocular infection (14).

It has been repeatedly shown that expression of PrP<sup>C</sup> is required for prion replication (15–18) and also for neurodegenerative changes to occur (19). We now set out to investigate whether spread of prions within the CNS is also dependent on PrP<sup>C</sup>. For the reasons mentioned above, the visual pathway lends itself ideally to the study of this question. We transplanted embryonic neuroectoderm derived from midgestation tg20 embryos overexpressing PrP<sup>C</sup> (20) into the caudoputamen of Prnp<sup>−/−</sup> mice that are not susceptible to scrapie (15, 16, 21). Such grafts grow and differentiate into neural, glial, and endothelial components in a ratio similar to that observed in adult brains (22). Intracerebral inoculation of scrapie prions was shown earlier to invariably produce transmissible spongiform encephalopathy in the graft after 70 days but not in the surrounding Prnp<sup>−/−</sup> tissue (19); therefore, neural grafts are sensitive indicators of the presence of prion infectivity in the brain of an otherwise scrapie-resistant host. The dependence of prion spread on the presence of PrP<sup>C</sup> was then studied by inoculating prions into the eye of a Prnp<sup>−/−</sup> mouse carrying a tg20 graft (Fig. 1a).

EXPERIMENTAL PROCEDURES

Grafting Procedure. The neuroectodermal anlage of tg20 embryos (day 12.5 post conceptionem) was transplanted to the caudoputamen of adult Prnp<sup>−/−</sup> or tg33 mice where it fully differentiates into normal neuronal and glial components and is integrated into the host brain (22). The tg20 mouse line from which grafts were derived carries multiple copies of a genomic Prnp transgene and develops scrapie 63 ± 2 days after intracerebral inoculation with 7 log ID<sub>50</sub> (in a total of 30 μl) of Rocky Mountain Laboratory prions (20). tg33 mice overexpress PrP on T cells and are scrapie-resistant (unpublished results).

Inoculation. A 10% homogenate of pooled RML-infected mouse brains (5 μl; 6.2 log ID<sub>50</sub> units) was injected into the eyeball of anesthetized mice 1, 25, 42, or 78 days after transplantation. Early intraocular inoculation, 24 h after grafting, was performed to minimize the probability of a neutralizing immune response to the PrP-expressing graft which might reduce infectivity. The later time points were chosen to ensure reconstitution of the blood–brain barrier, thereby minimizing nonspecific leakage of infectivity to the indicator tissue. The blood–brain barrier is largely reconstituted 60 days after grafting, as shown by gadolinium-enhanced magnetic resonance imaging (22).

Analysis of the Grafts. Mice were killed while under deep anesthesia. For paraffin histology, whole mouse brains were fixed for at least 24 h in 4% paraformaldehyde in PBS, immersed for 1 h in 98% formic acid to reduce prion infectivity (23), postfixed for 72 h in 4% paraformaldehyde/PBS, and processed for paraffin embedding. Hematoxylin and eosin staining and immunohistochemistry for GFAP (anti-GFAP antiserum, 1:300; Dako), synaptophysin (SY antiserum 1:40; Boehringer Mannheim), and the monoclonal antibody 6F11 (anti-PrP antiserum, 1:300; Dako) were used to detect PrP and PrP<sup>Sc</sup>. For the reasons mentioned above, the visual pathway lends itself ideally to the study of this question. We transplanted embryonic neuroectoderm derived from midgestation tg20 embryos overexpressing PrP<sup>C</sup> (20) into the caudoputamen of Prnp<sup>−/−</sup> mice that are not susceptible to scrapie (15, 16, 21). Such grafts grow and differentiate into neural, glial, and endothelial components in a ratio similar to that observed in adult brains (22). Intracerebral inoculation of scrapie prions was shown earlier to invariably produce transmissible spongiform encephalopathy in the graft after 70 days but not in the surrounding Prnp<sup>−/−</sup> tissue (19); therefore, neural grafts are sensitive indicators of the presence of prion infectivity in the brain of an otherwise scrapie-resistant host. The dependence of prion spread on the presence of PrP<sup>C</sup> was then studied by inoculating prions into the eye of a Prnp<sup>−/−</sup> mouse carrying a tg20 graft (Fig. 1a).

Abbreviations: CNS, central nervous system; GFAP, glial fibrillary acid protein; LGN, lateral geniculate nucleus.

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RESULTS

In tg20 mice, unilateral intraocular inoculation led to progressive appearance of scrapie pathology along the optic nerve and optic tract to the contralateral superior colliculus and lateral geniculate nucleus (Fig. 2 b–d) and was followed by generalized encephalopathy and death after 74–112 days. In agreement with earlier studies (14, 26), these results suggest that the infectious agent travels along fiber tracts of the CNS to the retina, the optic nerve and the retinotectal projection. After inoculation, Prnp<sup>o/o</sup> mice grafted with tg20 tissue were killed after 222–467 days. By this time, all intracerebrally infected grafts (n = 17) developed severe scrapie encephalopathy, including typical histopathological features (Fig. 1 g and h) and accumulation of protease-resistant PrP<sup>Sc</sup> (Fig. 2 c and d). The grafted region contained at least 5.7 log ID<sub>50</sub> of infectivity (19). In contrast, none of 7 mice inoculated intraocularly showed spongiosis, gliosis, synaptic loss (Fig. 1 i and k), or PrP<sup>Sc</sup> (Fig. 2 a and b). Identical results were obtained with 5 mock-inoculated and 17 uninfectected mice (Table 1). In one instance, the graft of an intraocularly inoculated mouse was assayed and found to be devoid of infectivity (Table 1). We conclude that infectivity administered to the eye of PrP-deficient hosts cannot induce scrapie in a PrP-expressing brain graft.

Engraftment of Prnp<sup>o/o</sup> mice with PrP<sup>C</sup>-producing tissue might lead to an immune response to PrP (27) and possibly to neutralization of infectivity. Indeed, analysis of sera from grafted mice revealed significant anti-PrP antibody titers. Because 1 of 2 mock-inoculated and 3 of 3 uninoculated Prnp<sup>o/o</sup> mice showed an immune response to PrP 5–50 weeks after neurografting, whereas nongrafted, intracerebrally inoculated Prnp<sup>o/o</sup> mice did not develop detectable antibody titers (15), PrP<sup>C</sup> presented by the intracerebral graft rather than the inoculum or graft-born PrP<sup>Sc</sup> was the offending antigen. To test whether grafts would develop scrapie if infectivity was administered before establishment of an immune response, we inoculated mice 24 h after grafting. Again, no disease was detected in the graft of two mice inoculated intraocularly.

intracerebral infection with mouse prions. Spongiosis (g) and gliosis (h) are conspicuous within the graft and were not observed in uninoculated or mock-inoculated grafts (not shown). (i and k) Neural grafts (tg20) 217 days after transplantation in a Prnp<sup>o/o</sup> host brain and 305 days after intraocular inoculation show no neuropathological changes. The asterisks label the border between graft and host tissue.
PrPC-expressing grafts (Protease-resistant PrP was formed only in intracerebrally infected tissue) were mounted on nitrocellulose, and PrP was displayed immunohistochemically without (a, c, and e) or with (b, d, and f) previous protease digestion (25). Arrows pinpoint the position of grafts. Intracerebrally inoculated mice engrafted with PrP-overexpressing tg20 neuroectodermal tissue did not develop antibodies to PrP after intracerebral or intracocular inoculation (n = 9) even 31 weeks after grafting, presumably because of clonal deletion of PrP-immunoreactive lymphocytes (Fig. 3). As before, intracocular inoculation with prions did not provoke scrapie in the graft (n = 5; data not shown), supporting the conclusion that lack of PrPC, rather than immune response to PrP, prevented spread.

**DISCUSSION**

Scrapie pathology and replication of infectivity after intraocular injection of wild-type mice occur along the anatomical structures of the visual system (14) and spread to transsynaptic structures such as the contralateral superior colliculus, lateral geniculate nucleus, and visual cortex: this has been taken as evidence for axonal transport of the agent. However, although PrPC seems to travel with the fast axonal transport (28), the very slow kinetics of disease development caused by prions, as opposed to canonical neurotropic viruses (29), argues against the hypothesis that prions follow fast or perhaps even slow axonal transport. Because intraocular inoculation failed to infect grafts even in the absence of an immune response to PrP, PrPC appears to be necessary for the spread of prions along the retinal projections and within the intact CNS. The prion itself is therefore surprisingly sessile.

Because prion infectivity is consistently detectable earlier in the spleen than in the brain, even after intracerebral inoculation (26), it could be argued that prion replication in lymphoreticular organs may be involved in the neuroinvasiveness of intraocular administered prions. Enucleation as late as 7 days after intraocular inoculation resulted in scrapie but prevented targeting to the visual system (30), suggesting that systemic infection and secondary neuroinvasion can bypass the neural spread of prions if the visual pathway is interrupted before prions colonize the brain through the retinotectal projection. Therefore, the lack of graft infection described in

**Table 1.** Analysis of engrafted and control mice after intracerebral and intraocular inoculation

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Graft</th>
<th>Inoculum</th>
<th>Site</th>
<th>Time of analysis, days after inoculation</th>
<th>Scrapie pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prnp&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>tg20</td>
<td>RML</td>
<td>i.c.</td>
<td>222–467</td>
<td>17/17</td>
</tr>
<tr>
<td>Prnp&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>wt</td>
<td>RML</td>
<td>i.c.</td>
<td>147–245</td>
<td>3/3</td>
</tr>
<tr>
<td>Prnp&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>tg20</td>
<td>RML</td>
<td>i.o.</td>
<td>147–293</td>
<td>0/5</td>
</tr>
<tr>
<td>Prnp&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>wt</td>
<td>RML</td>
<td>i.o.</td>
<td>238</td>
<td>0/2</td>
</tr>
<tr>
<td>Prnp&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>tg20</td>
<td>Mock- or not inoculated</td>
<td>i.o.</td>
<td>250–473</td>
<td>0/20</td>
</tr>
<tr>
<td>Prnp&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>tg20</td>
<td>Mock- or not inoculated</td>
<td>i.o.</td>
<td>266, 295</td>
<td>0/2</td>
</tr>
<tr>
<td>tg20</td>
<td>No graft</td>
<td>RML</td>
<td>i.c.</td>
<td>62–65</td>
<td>4/4</td>
</tr>
<tr>
<td>tg20</td>
<td>No graft</td>
<td>RML</td>
<td>i.o.</td>
<td>74,112</td>
<td>2/2</td>
</tr>
<tr>
<td>wt</td>
<td>tg20</td>
<td>RML</td>
<td>i.c.</td>
<td>104–148</td>
<td>10/10</td>
</tr>
<tr>
<td>wt</td>
<td>tg20</td>
<td>RML</td>
<td>i.o.</td>
<td>187–224</td>
<td>3/3</td>
</tr>
<tr>
<td>tg33</td>
<td>tg20</td>
<td>RML</td>
<td>i.o.</td>
<td>130–218</td>
<td>5/5</td>
</tr>
<tr>
<td>tg33</td>
<td>tg20</td>
<td>RML</td>
<td>i.o.</td>
<td>130–218</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Mice were engrafted and inoculated as described in the text. No Prnp<sup>−/−</sup> animal inoculated intraocularly and no animal either inoculated with mock inoculum or left untreated developed spongiosis and gliosis (diagnosed by histological and immunohistochemical examination of paraffin sections) or showed accumulation of protease-resistant protein within the graft, as determined by histoblot analysis (19, 25). The infectious titer of the graft of one intraocularly inoculated animal was determined by injecting 20 µl of a 10% dilution prepared as described (19) intraocularly into 2 tg20 indicator mice; no clinical or histopathological signs of scrapie were found after 186 days (the incubation time at end-point dilution is about 110 days; ref. 20), i.e., Intracerebral; i.o., intracocular; wt, wild type.

*One animal analyzed 104 days after intraocular inoculation did not show graft pathology.*
the present study suggests that the absence of extracerebral PrPC impairs prion spread from extracerebral sites to the CNS, in addition to blocking neural spread.

The present results indicate that intracerebral spread of prions is based on a PrPC-paved chain of cells, perhaps because they are capable of supporting prion replication. When such a chain is interrupted by interposed cells that lack PrPC, as in the case described here, no propagation of prions to the target tissue can occur. Perhaps prions require PrPC for propagation across synapses; PrPC is present in the synaptic region (31) and certain synaptic properties are altered in Prnp mice (32, 33). It is also possible that transport of prions within (or on the surface of) neuronal processes is PrPC-dependent. Within the framework of the protein-only hypothesis (4, 6), these findings may be accommodated by a “domino-stone” model in which spreading of scrapie prions in the CNS occurs per continuitatem through conversion of PrPSc by adjacent PrPC.

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