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Review

Tracking prions: the neurografting approach

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Abstract. The physical nature of the agent that causes transmissible spongiform encephalopathies (the 'prion'), is the subject of passionate controversy. Investigation of it has benefited tremendously from the use of transgenic and knockout technologies. However, prion diseases present several other enigmas, including the mechanism of brain damage and how the affinity of the agent for the central nervous system is controlled. Here we show that such questions can be effectively addressed in transgenic and knockout systems, and that pathogenesis may be clarified even before we can be certain about the nature of the infectious agent. Availability of mice overexpressing the *Prnp* gene (which encodes the normal prion protein) and *Prnp* knockout mice allows for selective reconstitution experiments aimed at expressing PrP in specific portions of the brain or in selected populations of hemato- and lymphopoietic origin. We summarize how such studies can offer insights into how prions administered to peripheral sites can gain access to central nervous tissue, and into the molecular requirements for spongiform brain damage.
Key words. Prion diseases; neurografts; BSE; scrapie; transgenic mice; knockout mice; spongiform encephalopathies.

Introduction

The appearance of a new variant of Creutzfeldt-Jakob disease in humans [1, 2], which is thought to result from ingestion of bovine spongiform encephalopathy (BSE)-contaminated products, has dramatically highlighted the need for a better understanding of transmissible spongiform encephalopathies [3, 4]. Two unresolved questions continue to occupy the attention of researchers in the field. The first relates to the actual structure of the infectious agent, which has been named prion and which replicates in the central nervous system (CNS) and in some other tissues of infected animals and humans [5, 6]. The second question, however, is no less intriguing: By which mechanisms can prions bring about the damage to the central nervous system which is characteristic of transmissible spongiform encephalopathies? This raises many additional related questions. Is the damage related to the actual replication of the prion? Or is spongiform encephalopathy the result of accumulation of toxic metabolites within or around neurons?

One prime candidate for the latter hypothesis of toxicity could be PrP^{Sc}, the pathologically changed isoform of the normal prion protein, PrP^{C}. If so, would PrP^{Sc} be toxic only if generated within cells, or can it damage nervous cells when acting from without? And anyway, since prions appear to replicate, or at least to accumulate, in the organs of the lymphoreticular system, such as spleen, lymph nodes, and Peyer's patches of the intestine, why do we not observe immune deficiencies or structural pathologies of these organs after infection with prions? In other words, is susceptibility to prion toxicity a unique property of neural tissue, or is it rather the result of the 100-fold higher levels of PrP^{Sc} accumulation seen in brains of mice terminally ill with scrapie as compared to lymphoreticular organs?

The complexity of these questions, along with our limited understanding of the nature of the infectious agent, suggests that it may be very difficult to devise suitable systems to address them experimentally. However, the recent generation of genetic in vivo model systems, such as transgenic and knockout mice [7], has opened new, promising avenues of investigation. In particular, the experiments described below were made possible by the availability of mice expressing, at various levels, normal and mutated forms of the prion protein, as well as knockout mice which bear hetero- or homozygous ablations of the *Prnp* gene which encodes the prion protein. We have taken advantage of various strains of transgenic mice (produced in Weissmanm's laboratories) and have asked whether neurografting technology could be used to address the question of prion neurotoxicity. In this overview, we present a characterization of biological properties such as tissue growth, proliferation and differentiation in neuroepithelial grafts. Special emphasis is laid on the development of the blood-brain barrier (BBB) after grafting. We then describe how embryonic telencephalic grafting was applied to the study of scrapie pathogenesis.

Biological characteristics of mouse neuroectodermal grafts

Neural grafting has often been used to address questions related to developmental neurobiology [8–11].

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Several studies have investigated the establishment of neuronal organization within grafts and interactions with the host CNS [9, 12–14]. More recently, grafting studies have been aimed at questions related to neural plasticity. For example, it was asked whether and to what extent undifferentiated progenitor cells could integrate with and participate in the formation of the host CNS [15–17]. Other studies have addressed questions related to the tumorigenic potential of various oncogenes by grafting retrovirally transduced cells into the rodent CNS [18–20].

In the field of neurodegenerative disorders, grafting studies have been aimed mainly at reconstituting certain pathways or particular functions after surgical or toxic lesions to selected functional systems [8, 21–24]. In these models, an artificial lesion leads to degeneration of specific neuronal systems. Grafting of neural tissue or genetically engineered cells aims at functional repair of induced lesions [21]. Many such experiments were carried out in the rat system, which is well suited for developmental studies and allows stereotaxic surgical interventions with appropriate accuracy. However, with the advent of transgenic techniques, it has become possible to study in more detail the role played by single molecules during development and in pathological processes in mice [7, 25]. The production of knockout mice by targeted deletion of genes of interest [26] has further broadened our insight into the molecular mechanisms of neural development and pathogenesis of CNS diseases. A number of transgenic and knockout mice have provided valuable models for neurodegenerative diseases [7, 27–29]. Others, however, show early postnatal [30–32] or even embryonic [33–35] lethal phenotypes which can be difficult to interpret. Although these models provide evidence for a crucial role of the respective gene products during development and hint that these factors play an important role in the determination of cell fates during differentiation [33–36], they do not allow us to study the role these factors play in secondary pathologic processes such as neurodegeneration. To overcome this problem we have employed transplantation approaches for neural tissue derived from mouse embryos. Using grafting techniques, it has been possible to study neural tissue of mice with premature lethal genotypes at time points exceeding by far the life span of the mutant mice [37–39].

The grafting procedure is relatively simple [37, 38]. Embryos are harvested from timed pregnant dams at defined stages mid-gestation. Graft tissue can be radiolabelled for later identification by autoradiography [12, 13, 37] and injected into the caudoputamen or lateral ventricles of recipient mice using a stereotaxic frame [18, 37]. If histocompatible strains of mice are used, signs of graft rejection, such as lymphocytic infiltration and tissue necrosis, remain an exceptional finding and are detected in less than 5% of neural grafts [37].

To determine the optimal time point for embryonic tissue preparation and transplantation, we compared the final size of grafts resulting from tissue harvested at various embryonic stages. We found murine telencephalic tissue from embryonic day (E) 12.5 differentiated reliably into large neural grafts, which are suitable for detailed graft analysis. Tissue harvested at earlier embryonic stages often resulted in grafts containing non-neural tissue portions, because it was difficult to separate clearly mesenchymal tissue from the neural anlage at E9.5–E11.5. Such tissue portions induce permanent BBB leakage after grafting and were thus considered unsuitable. In contrast, neural tissue harvested at later embryonic stages (E13.5–E16.5) was easily separated from the meninges. However, proliferation and growth potential were markedly reduced, resulting in smaller transplants that were only partially accessible to thorough examination. Moreover, when tissue was harvested and transplanted at E12.5, the total number of neural grafts was higher than with tissue harvested at other embryonic stages [37].

Graft cell proliferation, as determined by immunocytochemical detection of incorporated 5-bromo-2′-deoxyuridine [40], showed that proliferation indices of graft cells decreased sharply from initially 35% of grafted cells to around 5% during the second week after transplantation and to less than 1% after more than 7 weeks [37]. At the same time, differentiation of grafted cells proceeds to the terminal postmitotic state. Thus, mature neuroepithelial grafts contain neurons with myelinated processes and a dense synaptic network, glia (astrocytes, oligodendrocytes and microglia), and blood vessels 4 weeks after grafting (projected age of grafted tissue: approx. P20) [37, 38]. Taken together, these findings indicate that embryonic neuroepithelial tissue grafted into an adult host brain follows a program of maturation and differentiation similar to the time course in vivo [37].

Blood-brain barrier and brain grafts

The BBB maintains the homeostatic environment in the brain by preventing blood-borne compounds from free entry into the CNS parenchyma. The barrier is formed by tight junctions in the vascular endothelia which are probably induced by astrocytes [41, 42]. A number of pathological CNS processes, such as inflammation, demyelination, tumour growth or degeneration can induce breakdown of the BBB. In turn, BBB leakage might induce CNS dysfunction caused by blood-borne neurotoxic compounds normally excluded from the brain parenchyma [43, 44].

Various investigators have reported controversial findings on the post-transplantation status of the BBB in rodents. An early but most valuable study has suggested that the type of donor tissue determines the characteris-

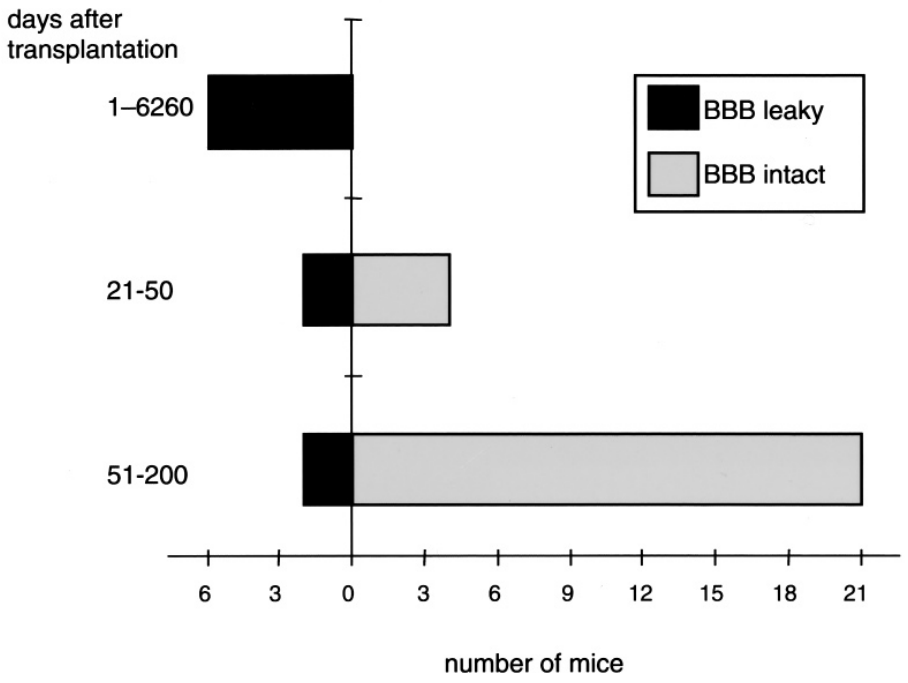

Figure 1. Reconstitution of the blood-brain barrier as a function of time after the grafting procedure. Nine weeks after transplantation, most animals exhibit tight, reconstituted barrier properties in the graft and its neighbourhood.

tics and BBB properties of graft-supplying vessels [45]. According to this hypothesis, neural grafts induce BBB properties in the supplying blood vessels. In fact, several authors described complete BBB reconstitution after neural grafting to the CNS. Some even find no residual BBB leakage as early as 1 week after grafting [46–49]. Other studies, however, have claimed that the BBB remains permanently disrupted after neural grafting to the CNS [50, 51]. Our group carried out studies in the model described using four independent marker molecules to detect damage to the BBB. The results obtained with various techniques were surprisingly consistent [39] (fig. 1). Magnetic resonance imaging (MRI) using a contrast agent in vivo indicates, in agreement with the histological data, that in our paradigm (grafting of tissue fragments as opposed to single-cell suspensions) the BBB is reconstituted in 67% of all grafts after 3 weeks, and in 90% of the grafts 7 weeks after grafting [39]. These findings are particularly important with respect to the use of neurografting techniques in neurodegenerative diseases. They indicate that the grafting procedure usually does not induce permanent BBB leakage that might expose the grafted tissue to a nonphysiological environment, and suggest that the genotype of the grafted tissue determines the BBB properties of the graft. Thus, a pathological condition affecting only the graft can result in secondary BBB disruption.

Neurografts in prion research

Having established that a neurograft from a donor which would have died early on may be kept alive in a healthy surrounding, we decided to apply this technique to the study of mouse scrapie. *Prnp*$^{0/0}$ mice, which are devoid of PrP^{C}, are resistant to scrapie and do not propagate prions [27, 52]. Because these mice show normal development and behaviour [53, 54], it has been argued that scrapie pathology may come about because PrP^{Sc} deposition is neurotoxic [55], rather than by depletion of cellular PrP^{C}. In the latter case, lack of PrP^{C} should result in embryonic or perinatal lethality, especially since PrP^{C} is encoded by a unique gene for which no related family members have been found. On the other hand, acute depletion of PrP^{C} may be much more deleterious than its lack throughout development since the organism may then not have the time to enable compensation mechanisms.

To address the question of neurotoxicity, we undertook to expose brain tissue of *Prnp*$^{0/0}$ mice to a continuous source of PrP^{Sc}. For the reasons given above, we thought a convenient approach would be to graft embryonic telencephalic tissue from transgenic mice overexpressing PrP into various structures (usually the caudoputamen, the lateral ventricles or the forebrain) of *Prnp*$^{0/0}$ mice and to inoculate it with scrapie prions (fig. 2). The donors, *tga20* mice [56], contain two arrays of

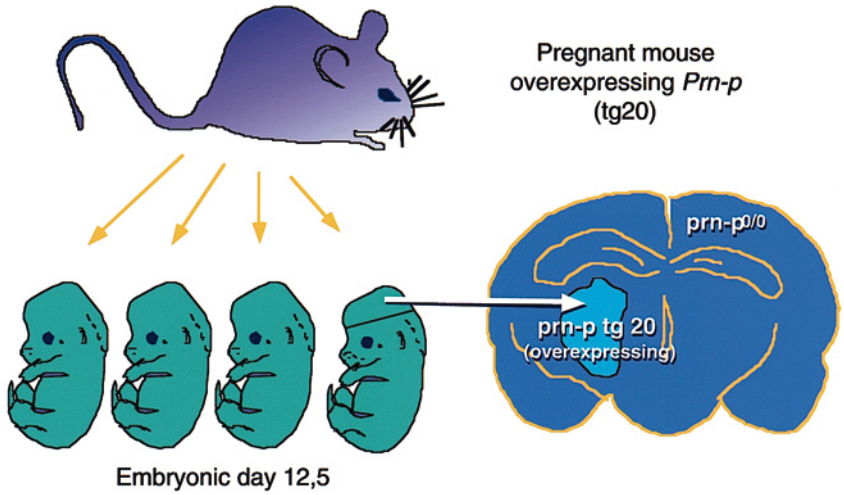

Figure 2. Use of the neurografting technique for the study of mouse scrapie. The neuroectodermal anlage from a PrP^C-overexpressing mouse is implanted into the brain of a *Prnp* deficient knockout mouse.

30–50 gene copies encoding PrP, overexpress PrP^C five- to eightfold and show incubation times of around 60 days as compared to 160 days for CD-1 wild-type mice. In the terminal stage of scrapie, both mouse strains exhibit similar prion titres.

The recipient mice were clinically monitored for the development of scrapie symptoms. We also analyzed the grafts and their surroundings by conventional histology, immunohistochemistry, in situ hybridization, and determined the content of PrP^C and PrP^{SC} by Western blotting and histoblotting. Because PrP^{SC} levels cannot be equated with in-fectivity, we determined the amount of infectivity in the graft and in regions of the brain at various distances from the graft by bioassay titration using *tga20* recipient mice [58, 59]. A summary of these procedures is presented in figure 3.

We observed that all mice remained free of scrapie symptoms for at least 70 weeks; this exceeds at least sevenfold the survival time of scrapie-infected *tga20* mice. Therefore, the presence of a continuous source of PrP^{SC} and of scrapie prions does not exert any clinically detectable adverse effects on the physiological functions of a mouse devoid of PrP^C.

On the other hand, histological analysis revealed that *tga20* and wild-type grafts developed characteristic histopathological features of scrapie 70 and 160 days after inoculation (p.i.), respectively, reflecting the incubation time of scrapie in the respective donor animals [27, 56]. Uninfected or mock-infected *tga20* grafts occasionally showed mild gliosis but never spongiosis. Therefore, high expression of PrP by itself did not induce neurodegeneration in grafts.

Early stages of the disease in the graft (70–140 days p.i.) were characterized by spongiosis and gliosis (fig. 4). In addition, we observed reduced synaptophysin immunoreactivity, which we take as a sign of damage to the neuronal trees and subsequent decrease in the density of synaptic junctions. These changes are similar to

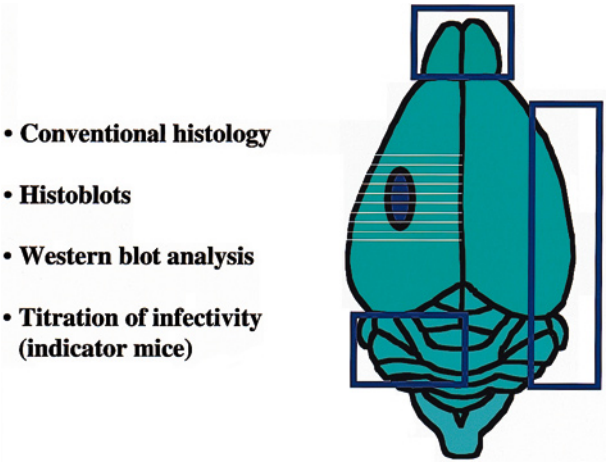

Figure 3. Analysis of grafted brains was performed using various methods on the grafts and on the shaded regions of the host brain (designated as 'frontal', 'parietal contralateral', and 'cerebellum').

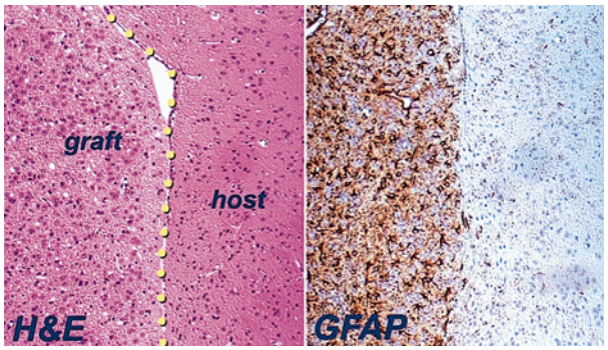

Figure 4. Typical histological appearance of a PrP^C-expressing graft (left side of the figure) at the interface to the knockout host brain (right side). Note the spongiform microcystic changes (upper panel) and the brisk astrocytic reaction evidenced by the immunocytochemical stain for glial fibrillary acidic protein (GFAP, lower panel).

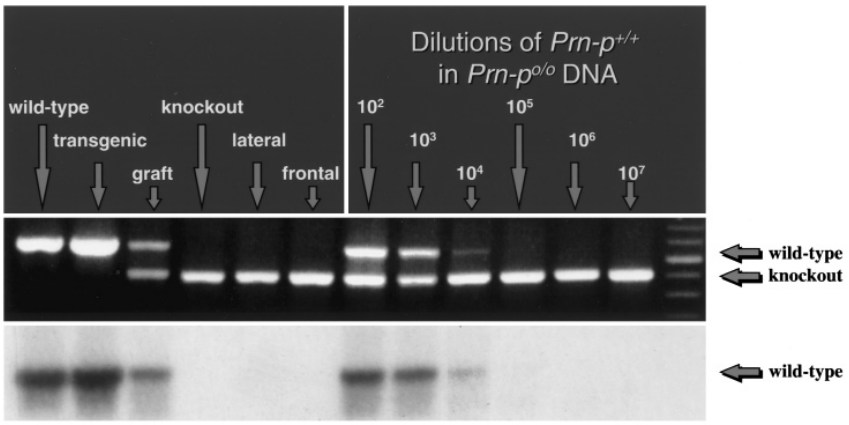

Figure 5. PCR analysis of grafts and surrounding host brain. The transgenic amplification product is identical to the product of the wildtype gene, and displays a slower electrophoretic mobility than the product of the knockout allele. No transgenic DNA was detected in the regions of the host brain in which PrP deposits were detected.

those found in terminally ill *tga20* mice, and since they occurred at graft ages similar to the life expec-tancy of scrapie-exposed donor mice, we concluded that neurografted PrP^C-expressing tissue indeed constitutes a realistic model for the scrapie encephalopathy (data not shown). Intermediate-stage grafts (140–280 days p.i.) showed *status spongiosus* with dramatic ballooning and loss of neurons, gliosis and stripping of neuronal processes. At late stages (280–480 days p.i.), cellular density increased from 900 to over 4000 cells/mm^2. Astrocytes constituted the main cell population and synaptophysin im-munoreactivity was almost completely absent. Intriguingly, the grafts underwent progressive disruption of the BBB during the course of the disease (S. Brandner, S. Isenmann, G. Kühne, C. Weissmanm and A. Aguzzi, unpublished data).

Although grafts had extensive contact with the recipient $Prnp^{0/0}$ brain, histopathology never extended into host tissue, even at the latest stages. Wild-type mice engrafted with *tga20* tissue showed severe histopathology in the graft and milder changes in the recipient brain, in accordance with the general observation that the level of PrP^C determines the speed of onset. Surprisingly, histoblot analysis [57] of noninoculated, engrafted $Prnp^{0/0}$ brain revealed that PrP immunoreactivity extended to the white matter of the recipient brains. In infected grafts, PrP^{Sc} was detected in both grafts and recipient brain, where it formed fine granules along white matter tracts and even in the contralateral hemisphere. Further, immunohistochemistry revealed PrP deposits in the host hippocampus and occasionally in the parietal cortex of all animals harbouring PrP-expressing grafts [57]. Up to 35 clusters of PrP deposits per section appeared late in infection, each consisting of 25–120 globules of 2–4 μm diameter closely associated to astrocytic processes; no deposits were observed in inoculated or mock-inoculated nonen-grafted $Prnp^{0/0}$ brains or in $Prnp^{0/0}$ brains engrafted with $Prnp^{0/0}$ tissue. It is unlikely that such deposits were produced locally by *tga20* cells emigrating from the graft, since the graft borders were always sharply demarcated. PCR analysis of host brain regions containing PrP deposits failed to reveal PrP-encoding DNA (fig. 5, detection limit $< 1{:}10^4$), and graft-derived cells were never detected distant from the graft by in situ hybridization (fig. 6) or autoradiography of brain engrafted with ^{3}H-thymidine-labelled [38] tissue. We conclude that graft-derived PrP^C and PrP^{Sc} are transferred from the graft to distant areas of the host brain.

It could be argued that pathology does not spread to the surroundings because there may not be sufficient physiological connections between the graft and the surrounding tissue. To address this potential issue, we grafted *tga20* PrP^C overexpressing tissue in wild-type mice and determined the effects of intracerebral prion inoculations. This procedure resulted in histopathologically verifiable scrapie in both graft and host tissue and in clinical scrapie of the host mouse without any modulation of the time course of the disease. However, the

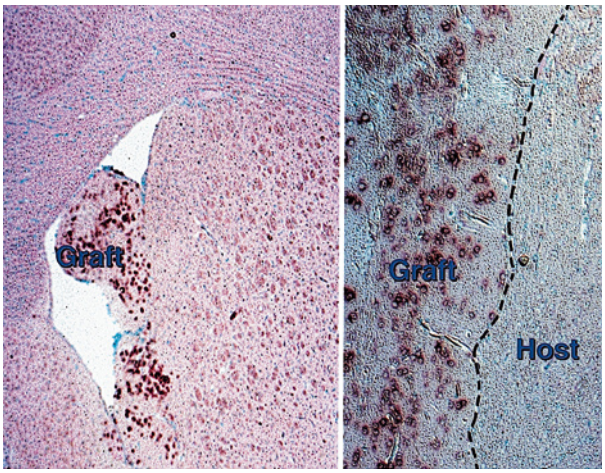

Figure 6. In situ hybridization analysis of grafts and surrounding brain. The grafted cells are 'well-behaved' and did not migrate from the graft into distant areas of the brain.

Table 1. Determination of scrapie prion infectivity in the grafts and in regions of the host brain adjacent to, or distant from, the graft. The presence of prion infectivity correlates with the presence of histologically detectable PrP deposits.

Source of infectivity	Days after inoculation	Transmission	Incubation time of recipients
A. Standard prion inoculum			
RML, 10-1	–	4/4	58, 59, 62, 67
RML, 10-3	–	2/2	65, 65
RML, 10-5	–	4/4	83, 84, 84, 95
RML, 10-7	–	1/3	109, >217, >217
RML, 10-9	–	0/4	>217, >217, >217, >217
RML, 10-11	–	0/3	>217, >217, >217
B. Mouse tissue			
graft region	245	2/2	75, 75
Contralateral frontal cortex	336	0/2	>170, >170
Contralateral frontal cortex	350	0/2	>170, >170
Contralateral frontal cortex	428	0/2	>170, >170
Contralateral frontal cortex	454	0/2	>170, >170
Contralateral parietal cortex	285	2/2	103/121
Cerebellum	285	0/3	>170, >170, >170
Spleen	285	0/1	>170

extent of pathology was more pronounced in the graft than in the host, which reinforces the general observation that the availability of PrP^{C}, rather than the deposition of PrP^{Sc}, is the rate-limiting step and the major pathogenetic deter-minant in the development of scrapie [57]. This accords with the fact that in several instances, and especially in fatal familial insomnia [59], spongiform pathology is detectable although very little PrP^{Sc} is present.

To determine infectivity, various portions of brains containing grafts with severe histopathology were inoculated into *tga20* indicator mice. Samples of graft led to terminal scrapie after 74 days, indicating a titre of approximately 5.7 log LD_{50} units per ml 10% homogenate (table 1). While frontal brain and cerebellum, in which no deposits were detected, did not show infectivity, about 1.5 log LD_{50} units per ml 10% homogenate were detected in the contralateral hemisphere. Infectivity is not due to residual inoculum, because within 4 days after inoculation no infectivity can be detected in recipient brain [27]. Thus, infectious prions move from the grafts to some regions of the PrP-deficient host brain without causing pathological changes or clinical disease. The distribution of PrP^{Sc} in white matter tracts of the host brain suggests diffusion within the extra-cellular space [60] rather than axonal transport (S. Brandner and A. Aguzzi, un-published results).

Why was no scrapie pathology observed in PrP^{C}-deficient tissue even in regions ad-joining the graft, which contained high levels of PrP^{Sc} and was clearly leaking this material? Perhaps PrP^{Sc} is inherently nontoxic and PrP^{Sc} plaques found in spongiform encephalopathies are an epiphenomenon rather than a cause of neuronal damage. Indeed, the extent of PrP deposition in the brains of humans succumbing to prion diseases with similar clinical presentation is extremely variable [61, 62].

Alternatively, PrP^{Sc} may only be toxic when it is formed and accumulated within the cell, but not when it is presented from without. Finally, it may be that PrP^{Sc} is pathogenic when presented from without, but only to cells expressing PrP^{C}, either because it initiates conversion of PrP^{C} to PrP^{Sc} at the cell surface and/or because it is internalized by association with PrP^{C}, which is endocytosed efficiently [63]. Along with previous transgenetic studies [54, 64] which show delayed onset of clinical disease in $Prnp^{0/+}$ mice despite massive accumulation of PrP^{Sc}, reports of typical scrapie histopathology in FFI-inoculated mouse brains devoid of detectable levels of PrP^{Sc} [65] and the lack of PrP^{Sc} accumulation in BSE-inoculated wild-type mice [66], the data discussed above imply that it is not deposition of PrP^{Sc}, but rather availability of PrP^{C} for some intracel-lular process elicited by the infectious agent, which is directly linked to spongiosis, gliosis and neuronal death.

Spread of prions in the central nervous system

Intracerebral inoculation of tissue homogenate into suitable recipients is the most effective method for transmission of spongiform encephalopathies and may allow for efficient circumvention of the species barrier. However, spongiform encephalopathies have also been transmitted by feeding [67–69] as well as by intravenous, intraperitoneal [70] and intramuscular injection [71]. Prion diseases can also be initiated from the eye by conjunctival instillation [72], corneal grafts [73] and intraocular injection [74]. The latter method has proved particularly useful in studying neural spread of the agent, since the retina is a part of the CNS and intraocular injection

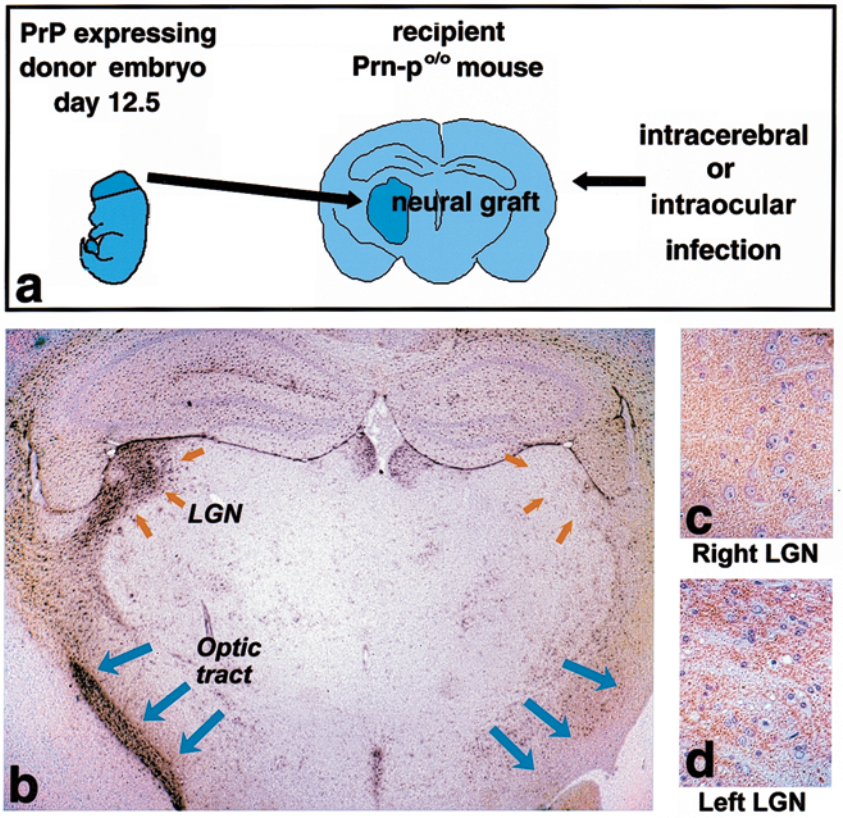

Figure 7. Tracing the spread of prions in the nervous system of *Prnp*-deficient hosts. (*a*) Schematic drawing of the transplantation procedure. (*b–d*) Coronal section of the thalamus of a PrP-overexpressing *tga20* mouse 78 days after inoculation into the right eye. At time of analysis, the animal showed clinical symptoms of scrapie. (*b*) Pronounced gliosis in the visual pathway (optic tract and lateral geniculate nucleus, LGN) is visualized by immunocytochemistry for GFAP. (*c, d*) Asymmetric neurodegeneration of the LGN is visualized by synaptophysin immunostain. Coarse granular deposits and patchy staining reflect significant synaptic loss in the affected left LGN while the right, unaffected LGN displays the fine granular synaptic stain typical of normal neural tissue. Because scrapie infection starts in the visual system and is followed by generalized disease in the CNS, the LGN and superior colliculus show a more prominent astrocytic reaction and severe loss of neuronal processes than other regions of the brain, as for example the hippocampus.

does not produce direct physical trauma to the brain, which may disrupt the BBB and impair other aspects of brain physiology. The assumption that the spread of prions occurs axonally rests mainly on the demonstration of diachronic spongiform changes along the retinal pathway following intraocular infection [74].

It has been shown repeatedly that expression of PrP^C is required for prion replication [27, 52, 54, 75] and also for neurodegenerative changes to occur [57]. We set out to investigate whether spread of prions within the CNS is also dependent on PrP^C. For the reasons mentioned above, the visual pathway lends itself ideally to the study of this question. We transplanted embryonic neuroectoderm derived from midgestation *tga20* embryos overexpressing PrP^C [57] into the caudoputamen of *Prnp*$^{0/0}$ mice which are not susceptible to scrapie [7, 50, 51]. As mentioned above, intracerebral (i.c.) inoculation of scrapie prions invariably produces transmissible spongiform encephalopathy in the graft after 70 days but not in the surrounding *Prnp*$^{0/0}$ tissue [57]; neural grafts are there-fore 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^C was then studied by inoculating prions into the eye of *Prnp*$^{0/0}$ mice carrying a *tga20* graft (fig. 7). In *tga20* mice, uni-lateral intraocular inoculation led to the progressive appearance of scrapie pathology along the optic nerve and optic tract to the contralateral superior colliculus and lateral geniculate nucleus, and was followed by generalized encephalopathy and death after 74–112 days. In agreement with earlier studies [74, 76], these results suggest that the infectious agent travels along fibre tracts of the CNS, such as the retinotectal projection. After inoculation, *Prnp*$^{0/0}$ mice grafted with *tga20* tissue were sacrificed after 222–467 days. By this time, all intracerebrally infected grafts ($n = 17$) developed severe scrapie encephalopathy, including typical histopathological features, and accumulation of protease-resistant PrP^{Sc}. The grafted region contained at least 5.7 log ID_{50} of infectivity [57]. In contrast, none of the seven mice inoculated intraocularly showed spongiosis, gliosis, synaptic loss or PrP^{Sc}. Identical results were obtained with 5 mock-inoculated and 17 uninfected mice. In one instance, the graft of an intraocularly inoculated mouse was assayed and found to be

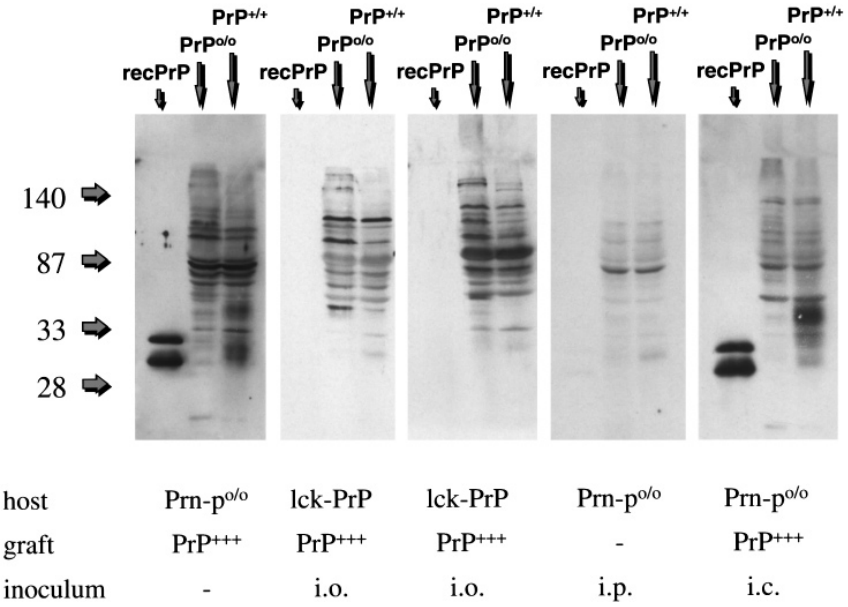

Figure 8. Detection of immune response in sera of mock and scrapie-inoculated grafted and nongrafted mice. Immunoblots containing purified recombinant PrP (recPrP), brain homogenate derived from *Prnp*$^{0/0}$, and from *tga20* mice (in the second and third lane of each strip), were incubated with sera from mock- and scrapie-inoculated grafted and control mice (dilution 1:100) and visualized by enhanced chemiluminescence. Presence of PrP-specific antibodies in the serum is indicated by 27–30 kD bands in lane 'recPrP' and by a cluster of bands present in the second lane but absent from the third lane. *tga20*-engrafted *Prnp*$^{0/0}$ mice develop a strong humoral immune response to PrP both prior to and following inoculation with scrapie prions, while engrafted *tg33* mice (*Prnp*$^{0/0}$, lck-PrP) do not develop antibodies to PrP even after intraocular inoculation (second and third strip).

devoid of infectivity. We conclude that infectivity admin-istered to the eye of PrP-deficient hosts cannot induce scrapie in a PrP-expressing brain graft. Engraftment of *Prnp*$^{0/0}$ mice with PrP^{C}-producing tissue might lead to an immune re-sponse to PrP [77] and possibly to neutralization of infectivity. Indeed, analysis of sera from grafted mice revealed significant anti-PrP antibody titres. Because one of two mock-inoculated and three of three uninoculated *Prnp*$^{0/0}$ mice showed an immune response to PrP 5–50 weeks after neurografting, while nongrafted, intracerebrally inoculated *Prnp*$^{0/0}$ mice did not develop detectable antibody titres [27], we conclude that PrP^{C} presented by the intracerebral graft (rather than the inoculum or graft-borne PrP^{Sc}) was the offending antigen (fig. 8). To test whether grafts would develop scrapie if infectivity were 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.

In order to rule out the possibility that prion transport was disabled by a neutralizing immune response, we repeated the experiments in mice tolerant to PrP. We used *Prnp*$^{0/0}$ mice transgenic for multiple copies of a hybrid gene consisting of a PrP coding sequence under the control of the *lck*-promoter. These mice (designated *tg33*) overexpress PrP on T lymphocytes, but are resistant to scrapie and do not contain scrapie infectivity in brain and spleen after inoculation with scrapie prions (A. Raeber, A. Sailer and C. Weissmann, unpublished results). *tg33* mice engrafted with PrP-overexpressing *tga20* neuroectoderm did not develop antibodies to PrP after intracerebral or intraocular inoculation ($n = 9$) even 31 weeks after grafting (fig. 8), presumably due to clonal deletion of PrP-immunoreactive lymphocytes. As before, intraocular inoculation with prions did not provoke scrapie in the graft, supporting the conclusion that lack of PrP^{C}, rather than immune response to PrP, prevented spread.

Scrapie pathology and replication of infectivity after intraocular injection of wild-type mice occur along the anatomical structures of the visual system [74] and spread to trans-synaptic 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 PrP^{C} seems to travel with the fast axonal transport [78], the very slow kinetics of disease development caused by prions, as opposed to canonical neurotropic viruses [79], argues against the hypothesis that prions follow fast or perhaps even slow axonal transport. Since intraocular inoculation failed to infect grafts even in the absence of an immune response to PrP, PrP^{C} 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.

Since prion infectivity is consistently detectable in the spleen earlier than in the brain, even after intracerebral inoculation [76], it could be argued that prion replication in lymphoreticular organs may be involved in the neuroinvasiveness of intraocularly administered prions.

Enucleation as late as 7 days following intraocular inoculation resulted in scrapie but prevented targeting to the visual system [80]. This suggests that systemic infection and secondary neuroinvasion can bypass the neural spread of prions if the visual pathway is interrupted before prions colonize the brain via the retinotectal projection. Therefore, the lack of graft infection described in the present study suggests that the absence of extracerebral PrP^C 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 PrP^C-paved chain of cells, perhaps because they are capable of supporting prion replication [81]. When such a chain is interrupted by interposed cells that lack PrP^C, as in the case described here, no propagation of prions to the target tissue can occur. Perhaps prions require PrP^C for propagation across synapses: PrP^C is present in the synaptic region [82], and certain synaptic properties are altered in *Prnp*0/0 mice [83, 84]. Perhaps transport of prions within (or on the surface of) neuronal processes is PrP^C-dependent. Within the framework of the protein-only hypothesis [85, 86], these findings may be accommodated by a ''domino'' model in which spreading of scrapie prions in the CNS occurs per continuitatem through conversion of PrP^C by adjacent PrP^{Sc} [87].

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