

Stem Cell Therapy Extends Incubation and Survival Time in Prion-Infected Mice in a Time Window–Dependant Manner

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(See the editorial commentary by Carnaud, on pages 978–9.)

Prion diseases, which are mostly represented in humans by Creutzfeldt–Jakob disease, are transmissible neurodegenerative disorders characterized by vacuolization and neuronal loss, as well as by the accumulation of an abnormal form of the prion protein. These disorders have yet no effective treatment, and drugs that block prion replication *in vitro* do not significantly slow down the progression of the disease when used *in vivo* at late stages. Cell therapy that has been already tested in other neurodegenerative disorders therefore represents an interesting alternative approach. In this study, we showed for the first time in prion diseases that intracerebral transplantation of fetal neural stem cells significantly extended both incubation and survival time. This result was dependant on the time window chosen for the engraftment and was obtained with both genetically modified and wild-type stem cells, therefore forging a path toward efficient stem cell therapy for human prion diseases.

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative disorders that affect humans and animals [1]. The central feature of these disorders is the post-translational conversion of the host-encoded cellular prion protein (PrP^C) into an abnormal conformational isoform named PrP^{Sc} (scrapie-type PrP) [2]. The neuropathological characteristics of TSEs are vacuolization (spongiosis), neuronal death, astrogliosis, and PrP^{Sc} deposits [3]. Prion diseases progress toward a fatal issue, and to date there is no effective treatment in humans [4]. Importantly, their diagnosis is often late because symptoms progress

rapidly and leave a short therapeutic window. Since the 1990s, there have been significant efforts to develop treatments for prion diseases. The inhibition of the PrP^{Sc} accumulation is one of the most studied approaches, and mostly compounds, such as poly-sulphated polyanionic, polyamine, tetrapyrroles, polyene antibiotics, tetracyclic, tricyclic, peptides, and RNA aptamers, which have an antiprion activity, are able to cure scrapie-infected neuroblastoma (ScN2a) cells of their infectivity [5]. However, these compounds have no or limited therapeutic effect on the development of the disease *in vivo* [6]. Indeed, they have been shown to delay the disease progression only when they are administered prophylactically around the time of prion inoculation and only when infection is restricted to the lymphoreticular system [5]. Human therapeutic trials were performed with some molecules, but the results have so far been disappointing [4]. Some promising new therapeutic approaches of TSEs target directly PrP^C through RNA interference, passive or active immunization, or dominant negative inhibition of PrP^{Sc} formation [7–10]. *In vivo* studies performed by

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Mallucci et al [7], even showed that a single administration of lentivirus, expressing a short hairpin RNA targeting PrP, into the hippocampus of mice with established prion disease was able to promote an increase of the incubation time of 19%–24%. Another recent study has suggested that soluble dimeric PrP, also called PrP-Fc₂, may represent a new class of antiprion compounds [11]. The administration of recombinant purified soluble dimeric PrP-Fc₂ for postexposure prophylaxis treatment using a gene therapy system was evaluated in prion-infected mice [11]. Although the lentiviral injection into 1 telencephalic hemisphere was shown to be limited to the site of injection, the authors observed a decrease in PrP^{Sc} accumulation in most parts of the mouse brain. The delay of the disease progression was extended by 72 days when the lentiviruses were administered before prion inoculation and by 25 days when they were administered 30 days after prion inoculation. This was also accompanied by a reduction of prion infectivity in the PrP-Fc₂-treated brains [11]. Gene therapy targeting PrP^C/PrP^{Sc} interaction using dominant negative PrP was also shown to constitute a basis for novel gene therapy. The feline immunodeficiency virus (FIV) vectors carrying the MoPrPQ167R or MoPrPQ218K dominant negative mutants were able to abolish endogenous wild-type PrP^{Sc} (wtPrP^{Sc}) replication in chronically prion-infected N2a58/22L cells [10]. In addition, intracerebral injections of these lentiviral vectors into prion-infected mice demonstrated that the treatment was able to delay the survival time by 20%. This delay was correlated with (1) a strong reduction of spongiosis in the treated area and (2) a remarkable decrease in astrocytic gliosis in the whole brain [9].

The new and alternative therapeutic strategy that we explore here consists of the use of fetal neural stem cells (NSCs) to replace damaged neurons and eventually prolong lifespan [12]. Cell replacement-based therapy in prion disorders remains a poorly explored option, and there is only one study reporting that fetal neural cells freshly dissected from E14.5 PrP knockout (koPrP) embryos had an impact on neuronal survival but not on the disease itself [13]. Of note, NSCs such as those used in our study can be expanded in cell culture and have the ability to incorporate effectively into host brains and differentiate into oligodendrocytes, astrocytes, and neurons after grafting [12]. This raises hopes for the development of successful cell replacement therapies for the treatment of neurodegenerative diseases, and encouraging results were observed in Parkinson disease, Huntington disease, and amyotrophic lateral sclerosis [12].

Using a model of TSE infection, we have investigated the effects of transplanting fetal NSCs prepared from wtPrP, koPrP, and porcine PrP (poPrP) transgenic mice in prion-infected animals 20 days before the appearance of the clinical signs (100 days postinoculation–[dpi] grafted mice) or just after their appearance (120-dpi grafted mice). We hypothesized that the use of fetal koPrP NSCs would prevent transplanted cells from

being infected by prions. The wtPrP NSCs were employed not only as controls but also because it was important to check whether non-genetically modified cells could also be of interest for therapeutic purposes. Because there is no evidence of the transmission of bovine spongiform encephalopathy or other prion strains to pigs by natural means and because of the high species barrier in poPrP-Tg001 transgenic mice [14, 15], poPrP NSCs were also chosen in our strategy. Here we show, for the first time in prion diseases, a significant effect of this cell therapy marked by an increase in both incubation (20.1%) and survival times (13.6%) in mice grafted before the appearance of the clinical signs. In addition, these time delays were correlated with a reduction of the number of astrocytes in areas close to the NSC injection sites, suggesting not only cell graft benefits but also an impact on prion glial reaction events. These results may thus pave the way for a potential stem cell therapy strategy for prion diseases.

MATERIALS AND METHODS

NSC Isolation and Characterization

NSCs were isolated from E14.5 mice as fully described elsewhere [16]. They were derived from transgenic PrP^{0/0} mice with the 129/Ola background [17], transgenic porcine PrP (poPrP-Tg001) mice generated from the latter PrP^{0/0} mice [15], and 129/Ola wild-type mice [18]. NSCs were cultured as a neurosphere in an N2-based culture medium (Gibco) supplemented with (epidermal growth factor [EGF]; 20 ng/mL) and (basic fibroblast growth factor [bFGF]; 20 ng/mL). For ex vivo prion infection and differentiation, NSCs were plated on poly-L-ornithine/laminin-coated plates. Immunofluorescence analysis of the NSC marker (nestin 1/300, Chemicon), the neuronal marker (β -III-tubulin 1/500, Covance; microtubule-associated protein 2 [MAP2] 1/500, Sigma), and the glial marker (glial fibrillary acidic protein [GFAP] 1/500, Dako) was performed as follows: Cells were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 minutes, washed 3 times with PBS, and permeabilized in 0.1% Triton X-100-PBS for 3 minutes, then further incubated overnight at 4°C with primary antibodies diluted in 0.2% bovine serum albumin (BSA) in PBS. After 3 washes in PBS, cells were incubated with Alexa555 or Alexa888 Fluor secondary antimouse or antirabbit antibodies (Invitrogen) for 1 hour at 37°C. Nuclei were stained with Hoechst 33286. Cells were washed and mounted in FluorSave reagent (Calbiochem). Images were collected and processed using a Leica microscope.

Ex Vivo Infection of Fetal NSCs

The infection of each cellular type was performed with the Rocky Mountain Laboratory (RML) mouse adapted prion strain (0.1%), and PrP^{Sc} presence was checked by Western blot analysis using the specific primary antibody Anti-PrP Sha31 as described elsewhere [16].

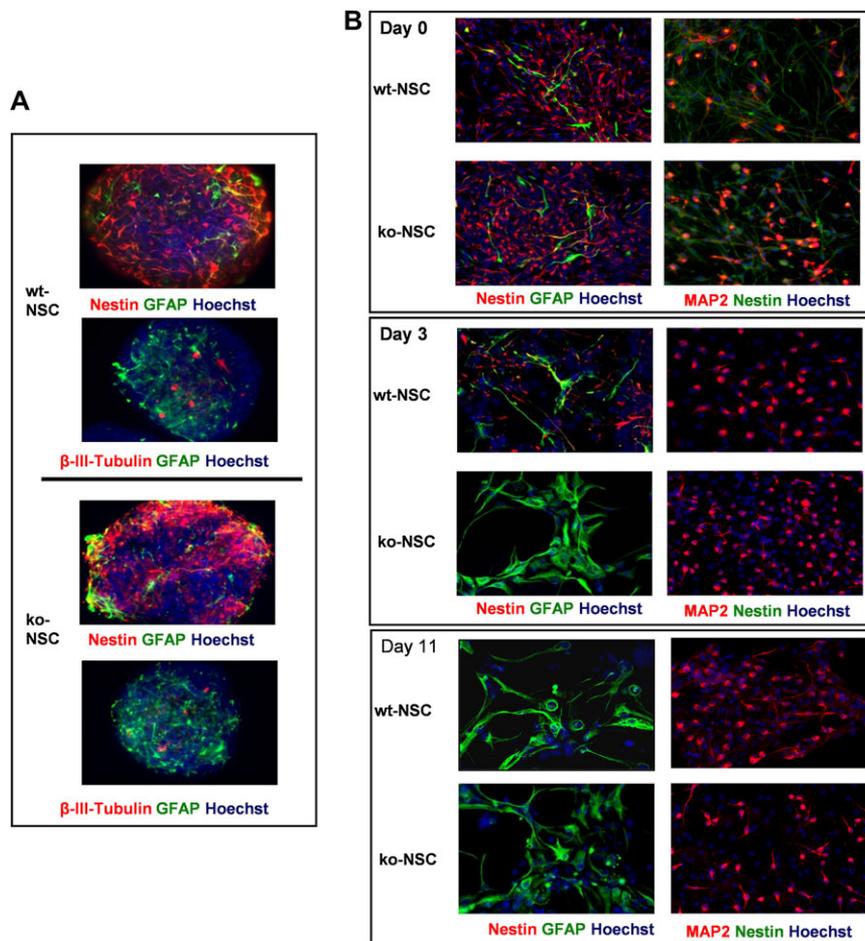


Figure 1. Immunofluorescence analysis of wild-type (wt) and knockout (ko) prion protein (PrP) neural stem cells (NSCs). The NSC neurosphere maintained in proliferation expressed mainly nestin, but some of the NSCs were also positive for β III-tubulin and glial fibrillary acidic protein (GFAP) markers (A). This suggested a small level of spontaneous differentiation in our culture. When plated on poly-L-ornithine/laminin and maintained in proliferative conditions, the initial (day 0) immunostaining showed a strong expression of nestin as well as some neuronal microtubule-associated protein 2 (MAP2)-positive and glial GFAP-positive cells (B). To induce neuronal differentiation, growth factors were removed from the medium. Immunofluorescence analysis for the different markers was also performed at different time points during the differentiation process. After 3 days (day 3), nestin expression started to decrease and was already completely absent in some cultures. In parallel, the proportion of glial cells (GFAP positive), as well as that of neuronal cells (MAP2 positive), increased during the course of differentiation until day 11. The nucleus appeared in blue after Hoechst DNA staining.

Animal Model and NSC Grafts

Female C57BL/6J mice were anesthetized intraperitoneally with 100 μ g/g of ketamine (Imalgène) and 5 μ g/g of xylazine (Rompun). They were then intracranially inoculated with the RML prion strain (1%; 20 μ L). Mice were housed in an A3 facility according to the European Community Directive 86/609/EEC and the Spanish RD 223/88. Infected mice were observed once a week for the appearance of TSE-related clinical signs (waddling gait, flattened back, rough coat, sticky eye discharge, weight loss, very jumpy behavior, hunched posture, and incontinence). At this point, mice were observed every day, and they were finally euthanized for ethical reasons when the progression of the disease was life-threatening. Prion-infected mice were grafted at 100 dpi with wtPrP NSCs, koPrP NSCs, or poPrP NSCs or injected with PBS alone as a control (mock-grafted

mice). An amount of 10^5 cells collected after 10 passages in flotation was grafted into the hippocampus and the lateral ventricle (stereotaxic coordinates of hippocampus: AP, -1.5 ; ML, $+0.2$; dV, $+2.2$; stereotaxic coordinates of lateral ventricle: AP, $+0.02$; ML, $+0.9$; dV, $+2.2$).

Preparation of Brains for Histological Analysis

Mice were anesthetized as described above and then perfused with 4% paraformaldehyde. The brains of the mice were collected and placed in 4% paraformaldehyde for 24 hours at 4°C. They were then embedded in paraffin (Tissue-Tek TEC III Embedding Center System) and cut in sections 5 μ m thick with the use of a Leica microtome. The sections were collected on microscope slides without treatment (Starfrost), dried, and left at 4°C until analysis.

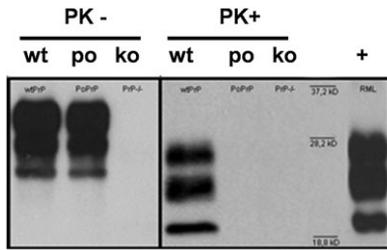


Figure 2. Western blot analysis of scrapie-type prion protein (PrP^{Sc}) in cell lysates after 12 days of neural stem cell (NSC) differentiation. Three types of NSCs were tested ex vivo for their susceptibility to Rocky Mountain Laboratory prion as described elsewhere [16]. Wild-type (wt) NSC prion infection was successful because these cells accumulated PrP^{Sc} , whereas, as expected, knockout PrP (koPrP) and porcine PrP (poPrP) NSCs were not susceptible to prion infection. Data are representative of ≥ 3 independent experiments). PK, proteinase K.

Immunohistochemistry

Tissues were dewaxed using Clearify solution (American Master Tech Scientific) and then rehydrated with decreasing degrees of ethanol washes. For histological studies, samples were stained with hematoxylin (Gill formula H-3401; Vector laboratories) for 3 minutes at room temperature, washed with H_2O , and treated with acid ethanol. Samples were then washed with H_2O and incubated with 2% eosin for 3 minutes at room temperature and finally washed again.

GFAP Immunohistochemistry. Sections were incubated with proteinase K (Roche; 20 $\mu\text{g}/\text{ml}$) for 10 minutes at 37°C and washed with H_2O . They were immersed in 0.5% H_2O_2 for 20 minutes at room temperature, then washed with H_2O and PBS and saturated with PBS, 0.1% BSA, and 0.1% Triton X-100 for 1 hour. Sections were incubated overnight at 4°C with the anti-GFAP (Dako) primary antibody (1:100 in PBS, 0.1% BSA, and 0.1% Triton X-100). The secondary antibody used was a biotinylated goat anti-rabbit antibody (Amersham; 1:1000 in PBS and 0.1% Triton X-100). The avidin-peroxidase complex (Vectastain Elite kit; Vector laboratories) was then added and revealed with 3,3'-diaminobenzidine (Vector laboratories), according to the manufacturer's instructions.

GFAP counting was realized in the specific area used to perform the reference Fraser prion lesion profile. For each area of interest, we analyzed 5 fields in 3–5 brain sections that had been taken 3 sections apart. Data were compared using a non-parametric statistical test (Kruskal–Wallis).

PrP^{Sc} Immunohistochemistry. Sections were treated with 98% formic acid for 10 minutes at room temperature and washed with H_2O . PrP^{Sc} was analyzed by immunohistochemistry using the Saf84 anti-PrP antibody as described elsewhere [19].

RESULTS

Derivation and In Vitro Differentiation Properties of Fetal NSCs

Fetal NSCs were prepared from E14.5 embryos of wtPrP, koPrP, [17] and poPrP [20] transgenic mice and maintained for

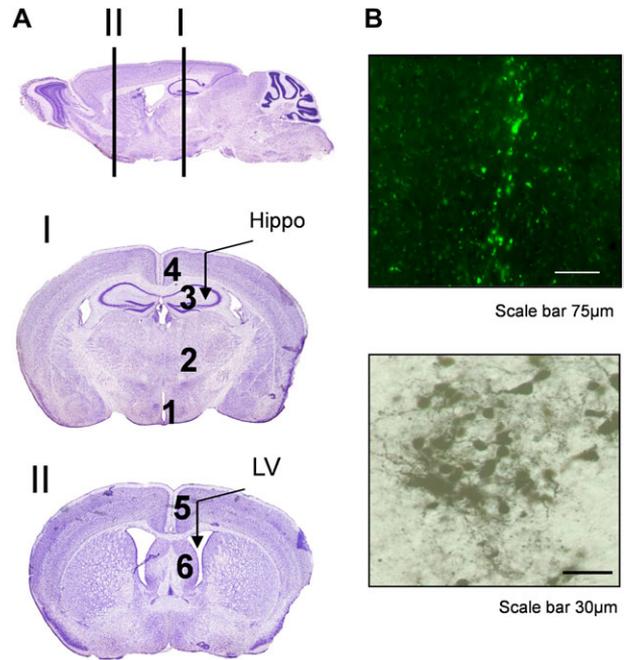


Figure 3. Stereotaxic grafts. *A*, Description of the brain regions where the grafts were performed (LV, lateral ventricle; Hippo, hippocampus). Areas 1–6 represent regions used as references to quantified astrogliosis: (1) hypothalamus, (2) thalamus (3) hippocampus (4) cortex close to or on the same histological section containing the first hippocampus injection site (5) cortex close to or on the same histological section containing the second lateral ventricle injection site, and (6) paraterminal body. *B*, Visualization of GFP fluorescence in brains grafted with green fluorescent protein (GFP) neural stem cells in the brain parenchyma (*top*) and GFP immunostaining using an anti-GFP antibody (Torrey Pines, Cliniscience) (*bottom*).

proliferation and amplification in N2 medium (Figure 1). NSCs expressed mainly nestin, but some of them were also positive for the other markers while they were maintained in proliferative conditions as floating or plated cells (day 0) (Figure 1). This suggested a small level of spontaneous differentiation in our cultures. To induce neuronal differentiation of the cells, neurospheres were plated on poly-L-ornithine/laminin. Immunofluorescence analyses for the different markers were also performed at different time points during the differentiation process. After 3 days, the level of nestin expression started to decrease in wtPrP (Figure 1) and poPrP (not shown) cells, whereas it was already completely absent in koPrP cells. In parallel, the proportion of glial cells (GFAP positive) and that of neuronal cells (MAP2 positive) increased during the course of differentiation until day 11 (Figure 1).

Prion Susceptibility of wtPrP, koPrP, and poPrP Cells

The rationale for using koPrP and poPrP genetically modified cells was their expected resistance to prion infection, making them suitable for cell replacement strategies in TSEs. We first demonstrated that these NSCs could not propagate ex vivo the

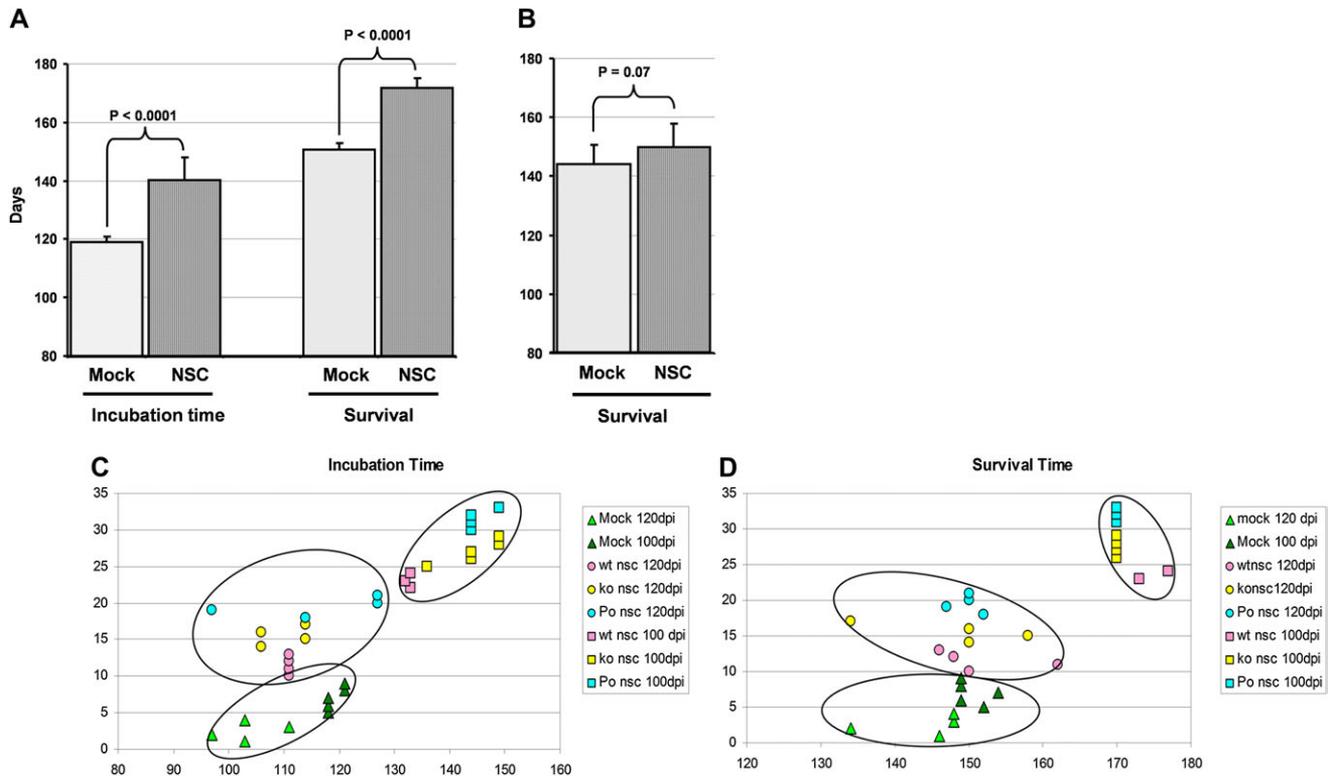


Figure 4. Incubation and survival time. *A*, Bar graph of incubation and survival time in mock-grafted and neural stem cell (NSC)-grafted mice at 100 days postinoculation (dpi). Significant differences between control and grafted mice were observed (Kruskal–Wallis test). *B*, Bar graph of survival time in mock-grafted mice and mice grafted with NSCs after the appearance of clinical signs. The difference between the 2 groups is not statistically significant (Kruskal–Wallis test). *C*, *D*, Incubation time and survival time, respectively, in days (*x*-axis) in mock-grafted mice and in mice grafted with wild-type (wt), knockout (ko), and porcine (po) prion protein NSCs before (100-dpi grafted mice) and after (120-dpi grafted mice) the appearance of clinical signs. The *y*-axis is only a numbering of individual mice based on their belonging to the different groups.

RML mouse adapted prion strain used in our study. As illustrated in Figure 2, infection of wild-type cells was successful because these cells accumulated PrP^{Sc} whereas, as expected, koPrP cells did not replicate PrP^{Sc}. The poPrP NSCs were also resistant to prion infection despite the poPrP^C expression in these cells. This result, which has been repeated 3 times, confirmed our hypothesis and justified the use of these cells for cell therapy.

Cells Grafts Increased the Incubation and Survival Time of Prion-Infected Mice

Stereotaxic grafting of NSCs in the hippocampus or around the lateral ventricle (the coordinates of the grafts are detailed in the Materials and Methods) (Figure 3) were previously optimized in the laboratory using green fluorescent protein NSCs as illustrated in Figure 3. These particular areas were known to contain a neural stem cell niche that could allow a good integration of the cells into the brain [21]. For the therapeutic trial, C57BL/6J female mice were intracranially inoculated with the RML prion strain [22]. Infected mice were observed once a week, using as a reference the appearance and progression of 8 clinical signs previously described for prion-infected mice [23, 24]. A mouse was considered to be clinically affected when 3 of the 8 clinical

signs were observed. At this point, mice were observed every day, and they were finally euthanized for ethical reasons when the progression of the disease was life-threatening.

NSCs were grafted at 120 days postinoculation (120-dpi grafted mice) after the appearance of the clinical signs and 20 days before the appearance of clinical signs (100-dpi grafted mice). Mock mice, used as controls, were injected with saline buffer ($n = 5$). They developed characteristic TSE clinical signs at 119.2 ± 1.6 dpi (median \pm SD) and were euthanized (survival time) at 150.6 ± 2.3 dpi (Figure 4; Table 1). In prion-inoculated mice that have been treated 20 days before the appearance of the clinical signs ($n = 12$), the clinical signs appeared later than in the control mice by 132.7 ± 0.6 dpi for wtPrP NSC-grafted mice ($P = .03$), 144.4 ± 2.9 dpi for koPrP-grafted mice ($P < .01$), and 145.3 ± 2.9 dpi for poPrP-grafted mice ($P = .03$), which resulted in increases from 11.3% (wtPrP) to 21% (koPrP and poPrP) of the incubation period (Figure 4). Three NSC 100-dpi grafted mice were also euthanized at 150 dpi to perform a histological comparison with controls. The remaining grafted mice ($n = 9$) were euthanized later than the control grafted mice, with survival times of 175 ± 2.8 dpi for wtPrP and 170 dpi for koPrP and poPrP (Figure 4; Table 1),

Table 1. Time of Appearance of Typical Transmissible Spongiform Encephalopathy Clinical Signs and Survival Time

Dpi, group	Time, days		
	Appearance of clinical signs	Euthanasia ^a	Survival
100			
Mock-grafted mice	118	...	152
	118	...	149
	118	...	154
	121	...	149
	121	...	149
Mice grafted with wt-PrP NSCs	133	150	...
	132	...	173
	133	...	177
Mice grafted with ko-PrP NSCs	136	150	...
	144	...	170
	144	...	170
	149	...	170
	149	...	170
Mice grafted with po-PrP NSCs	144	150	...
	144	...	170
	144	...	170
	149	...	170
120			
Mock-grafted mice	103	...	146
	97	...	134
	111	...	148
	103	...	148
Mice grafted with wt-PrP NSCs	111	...	150
	111	...	162
	111	...	148
	111	...	146
Mice grafted with ko-PrP NSCs	106	...	150
	114	...	158
	106	...	150
	114	...	134
Mice grafted with po-PrP NSCs	114	...	152
	97	...	147
	127	...	150
	127	...	150

NOTE. Dpi, days postinoculation; koPrP, knockout prion protein; NSC, neural stem cell; poPrP, porcine prion protein, wt-PrP, wild-type prion protein.

^a Mice were euthanized when the control mice died.

which resulted in increases from 13.3% (koPrP and poPrP) to 16.6% (wtPrP; $P < .01$; Kruskal–Wallis test) of the incubation period. Taken together, these differences in incubation and survival times were significant between the group of 100-dpi grafted mice and control mice ($P < .001$; Kruskal–Wallis test). The group of mice grafted after the appearance of the clinical signs (120-dpi grafted mice) exhibited a slight increase in survival (Figure 4) that was not statistically significant, as illustrated in Figure 4.

These results indicated that the grafting of NSCs into the prion-injured brain, shortly before the appearance of the clinical signs, resulted in an increase of both incubation and survival times (Figure 4). Importantly, this was observed independently of the stem cell type origin as mice grafted with wtPrP NSCs had even a slightly longer survival period than that of mice grafted with prion-nonsusceptible koPrP or poPrP NSCs.

Histological Analysis Revealed a Decrease of Astrocytic Gliosis in Grafted Infected Mice

Histological examination of the vacuolization and immunohistochemical analysis of PrP^{Sc} deposits and astrogliosis were performed to confirm the development of TSE in all animals (Figure 5). Astrocytes were counted after GFAP immunostaining (Figures 5 and 6) in 6 different brain areas (Figure 3): (1) hypothalamus, (2) thalamus, (3) hippocampus, (4) cortex close to or on the same histological section containing the first hippocampus injection site (Figures 5 and 6), (5) cortex, and (6) paraterminal body, also close to or on the same section of the second injection site (lateral ventricle) in mock- and NSC-grafted mice euthanized at 150 dpi (Figure 6). In mice grafted before the appearance of clinical signs (100-dpi grafted mice), we observed a statistically significant ($P < .001$) astrogliosis decrease in areas surrounding the treated lateral ventricles or hippocampus (Figures 5 and 6) compared with those in 120-dpi grafted mice (Figures 5 and 6) or mice treated with PBS (Figures 5 and 6). These results were not reminiscent of the results of Brown et al [13] and suggested a diminution of the inflammatory response.

DISCUSSION

To date, there is no effective treatment for TSEs despite the fact that a lot of effort has been made in this area. For a long time, research in this field focused on the discovery of chemical agents able to decrease PrP^{Sc} replication in cellular models. Unfortunately, these compounds were not successful in vivo when administered at the late stage of the disease. Other approaches have consisted of the selection of molecules that could interfere with in vivo prion propagation by targeting PrP^C, PrP^{Sc}, PrP^C/PrP^{Sc}, or cofactors of the replication. These antiprion molecules included, for example, small interfering RNA, antibodies against PrP or 37kDa/67kDa laminin receptor (LRP/LR), soluble dimeric PrP (PrP-Fc2), or PrP dominant negative mutants [8]. Recent strategies have proposed the development of gene therapy systems of antiprion molecule delivery. However, these approaches target only the prion conversion process, not the brain damage that has already occurred before the appearance of clinical symptoms. For that reason, we have addressed this particular point and have developed a therapeutic approach based on the replacement of damaged neurons by grafting neural stem cells.

In the present therapeutic strategy, different types of stem cells were grafted at 2 different times during the course of the disease

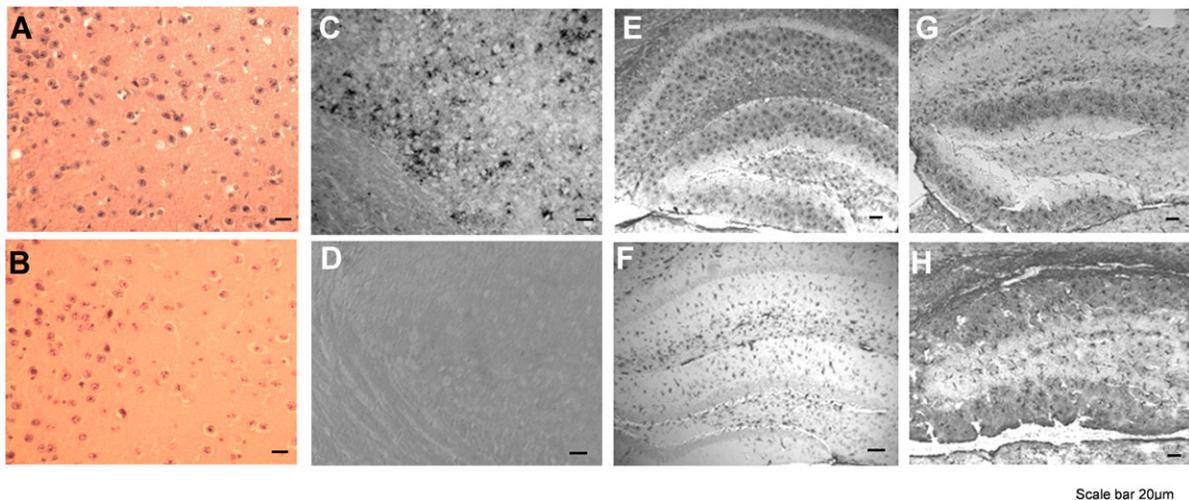


Figure 5. Histological features of prion diseases. Histological analysis for the presence of vacuolization (A, B), scrapie-type prion protein (PrP^{Sc}) deposition (C, D), and astrocytosis (E–H) revealed the hallmark of transmissible spongiform encephalopathy in prion-infected animals (A, C, E, G, H) in comparison with noninfected control mice (B, D, F). Astrocytes were immunostained in mice treated with phosphate-buffered saline (E), mice grafted at 100 days postinoculation (G), and mice grafted 120 days postinoculation (H).

in prion-infected mice. In this study, wtPrP, koPrP, and poPrP cells were selected. Functional recovery was supposed to be potentiated by the fact that koPrP-implanted cells could not be infected and poPrP NSCs were not susceptible to prion infection *in vitro*. We even hypothesized that the transplantation of poPrP NSCs could impair the progression of the disease, not only because of their intrinsic resistance to prion infection like koPrP NSCs but also in relation to a heterotypic prion inhibitor mechanism as observed in heterozygote poPrP/wtPrP mice. NSCs were transplanted at 2 different stages of the progression of the disease: 100 dpi, which corresponded to 20 days before the appearance of clinical signs; and 120 dpi, just after the appearance of clinical signs. A significant therapeutic effect was observed in mice grafted at 100 dpi, because their incubation period was increased by 11.3%–21.9% and their survival by 13.3%–16.6%. Importantly, no major differences were observed between the different types of stem cells used. In particular, wtPrP NSCs resulted in a strong therapeutic effect, suggesting that these cells could be beneficial although one could expect that they eventually will be infected by prions. This result may be related to a neuroprotective effect mediated by trophic factors (brain-derived neurotrophic factor [BDNF], ciliary neurotrophic factor [CNTF], and glial cell-derived neurotrophic factor [GDNF]) produced by the cells present in the graft [25, 26]. The transplanted cells could have modulated the microenvironment by protecting against excitotoxicity, secreting support proteins [25] and thus providing a permissive growth substrate for regeneration. They could also have been able to suppress the destructive inflammatory processes, as has been shown in some studies using marrow stromal cells [27] or stem cells [25]. Interestingly, astrocytosis was also strongly reduced in mice

grafted before the appearance of clinical signs, in the grafted areas and more particularly in the area surrounding the treated hippocampus, which was reminiscent of the results of Brown et al [13]. These decreases of GFAP labeling in the grafted brain suggest that the cell transplantation might play a key role by interfering with the mechanism that activates the inflammatory process. A recent study on prion-infected cells showed that only the PrP^{Sc} isoform interacted with neurons and thereby triggered the recruitment of microglia [28]. Activated microglia releases various factors such as cytokines and free radicals that induce neuronal apoptosis but maintain glial survival [29]. In our approach, the accumulation of PrP^{Sc} may be less important

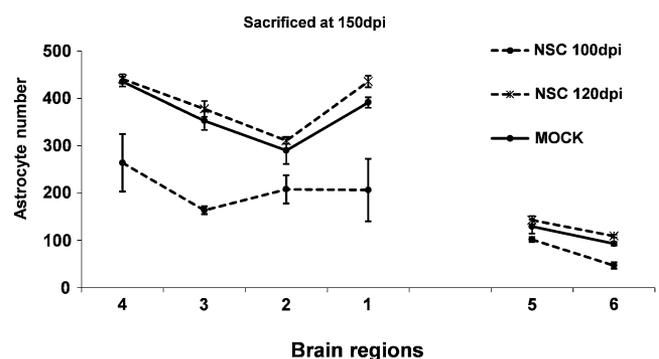


Figure 6. Number of astrocytes present in different sections of mock-grafted and neural stem cell (NSC)-grafted mice. Astrocytosis was quantified after glial fibrillary acidic protein immunostaining at 150 days postinoculation (dpi) in mock-grafted and NSC-grafted mice in the following brain areas: (1) hypothalamus (2) thalamus, (3) hippocampus, (4) cortex close to or on the same histological section containing the hippocampus injection site, (5) cortex, and (6) paraterminal body.

in the grafted area of mice grafted before the clinical signs appeared than in control mice, and as a consequence this may diminish the microglia recruitment that activates the inflammatory processes as well decrease the proliferation of astrocytes.

Because the protection effect is not total, it could have been limited by an immune response toward the grafted cells, although numerous studies have consistently shown engraftment of cells of 129/Ola origin in the brain of C57BL/6 mice with only a limited rejection of the grafted cells [30]. Immune cells infiltration was, however, difficult to analyze in the present study because T-cell or macrophage infiltration into the brain as well as microglial response are increased during the course of a prion disease [31, 32].

In conclusion, it is the first time in prion therapeutics research that a stem cell graft approach led to an increase of both incubation and survival time in a preclinical model of the disease. Importantly, this effect was also apparent with unmodified stem cells, which is interesting in the perspective of future clinical applications. Our results also suggested that there is a time window in which such a treatment could be more efficient. This emphasizes the need for an early TSE diagnosis. Efficacy could probably be improved in further studies by increasing the number and sites of stem cells grafted. Moreover, recent data using gene therapy also showed improvements in the delay of the disease [7, 9], suggesting that gene therapy could be performed in addition to cell therapy.

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