



HAL
open science

Inhibition of PrP^{Sc} formation by lentiviral gene transfer of PrP containing dominant negative mutations.

Carole Crozet, Yea-Lih Lin, Clément Mettling, Chantal Mourton-Gilles, Pierre Corbeau, Sylvain Lehmann, Véronique Perrier

► To cite this version:

Carole Crozet, Yea-Lih Lin, Clément Mettling, Chantal Mourton-Gilles, Pierre Corbeau, et al.. Inhibition of PrP^{Sc} formation by lentiviral gene transfer of PrP containing dominant negative mutations.. Journal of Cell Science, Company of Biologists, 2004, 117 (Pt 23), pp.5591-7. 10.1242/jcs.01484 . inserm-00143203

HAL Id: inserm-00143203

<https://www.hal.inserm.fr/inserm-00143203>

Submitted on 26 Apr 2007

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Inhibition of PrP^{Sc} formation by lentiviral gene transfer of PrP containing dominant negative mutations

Carole Crozet ¹, Yea-Lih Lin ², Clément Mettling ², Chantal Mourton-Gilles ⁴, Pierre Corbeau ², Sylvain Lehmann ^{1,3}, and Véronique Perrier ^{1,π}

¹Laboratoire de Biologie des Encéphalopathies Spongiformes - Institut de Génétique Humaine, CNRS UPR 1142, 141 rue de la Cardonille, 34396 Montpellier Cedex 5, France

²Laboratoire Lentivirus et Transfert de Gènes - Institut de Génétique Humaine, CNRS UPR 1142, 141 rue de la Cardonille, 34396 Montpellier Cedex 5, France

³Laboratoire de Biochimie. Hopital St Eloi, 80 avenue A. Fliche 34295 Montpellier Cedex 5, France

⁴BIO-RAD, Centre de Pharmacologie et Biotechnologies pour la Santé, CNRS UMR 5094, 15 avenue C. Flahault, 34060 Montpellier Cedex 2, France

^πCorresponding author : Véronique Perrier, Phone : 33 4 99 61 99 30, Fax : 33 4 99 61 99 01, e-mail : vperrier@igh.cnrs.fr

Running title: Prion inhibition by lentiviral gene transfer

Keywords: PrP, prion, dominant negative, lentivirus, therapy

Summary

Currently there is no treatment to cure Transmissible Spongiform Encephalopathies. By taking advantage of the prion “resistant” polymorphisms Q171R and E219K that naturally exist in sheep and humans, respectively, we have evaluated a lentiviral gene transfer therapeutic approach. Here we show that VSV-G (Vesicular Stomatitis Virus) pseudotyped FIV (Feline Immunodeficiency Virus) derived vectors carrying the mouse *Prnp* gene in which these mutations have been inserted, are able to inhibit prion replication in chronically prion infected cells. Since lentiviral tools are able to transduce post-mitotic cells such as neurons or cells of the lymphoreticular system, this result presents an insight into the development of gene or cell therapy approaches to prion disease.

Introduction

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative disorders, which include Creutzfeldt-Jakob disease in humans, scrapie in sheep and goats, and bovine spongiform encephalopathy in cattle. These diseases are characterized by the accumulation in the brain of an abnormal isoform of the prion protein called PrP^{Sc} (Prusiner et al., 1998).

According to the prion hypothesis, the infectious isoform PrP^{Sc} can trigger the autocatalytic conversion of PrP^C into PrP^{Sc} (Prusiner et al., 1990). However, certain cofactors, such as protein X or Laminin Receptor Precursor may also play a role in the conversion process (Kaneko et al., 1997; Leucht et al., 2003). Currently no treatment is available to cure TSEs. A number of chemical molecules, targeting PrP^{Sc} itself or PrP^{Sc} replication, were discovered either serendipitously, such as branched polyamines (Supattapone et al., 1999), phthalocyanines and porphyrin derivatives (Caughey et al., 1998), or by using a more rational approach with the help of structure-based drug design, such as the identification of compound 60 (Perrier et al., 2000). These molecules were able to cure scrapie infected cells of their infectivity, however, they were poorly efficient on mice once the clinical symptoms had developed. Since PrP^C is a major cellular requirement for the propagation of infectivity (Bueler et al., 1993), it

represents an attractive therapeutic target. Recent studies on conditional *Prnp* knock-out mice, showing that the inhibition of the PrP^C production in neurons was able to prevent or stop the development of the disease, emphasized the importance of therapeutic strategies targeting PrP^C (Mallucci et al., 2003).

By taking advantage of prion “resistant” polymorphisms that naturally exist in sheep and humans, an alternative approach can be envisaged. Genotype analyses in sheep have shown that the polymorphism present at codon 171 of the ovine *Prnp* gene is correlated with the disease incidence and the modulation of its incubation time. The sheep carrying the polymorphisms Q/R or R/R at codon 171 of the PrP protein, were resistant to different isolates of scrapie, whereas sheep harbouring the Q/Q alleles developed prion disease (Belt et al., 1995; Bossers et al., 1996; Goldmann et al., 1994). Human genetic studies revealed that the prion protein gene has a large repertoire of polymorphisms and mutations. Among the Japanese population, 12% carried the polymorphism E/K at codon 219 of the PrP protein, whereas the remainder carried the E/E alleles. Since the heterozygosity E/K at codon 219 was not reported on the 85 autopsied sporadic CJD cases, this finding therefore suggests that the polymorphism E/K would protect humans from prion diseases (Shibuya et al., 1998). Kaneko et al., introduced the Q171R sheep mutation and E219K human mutation in the mouse *Prnp*

gene (MoPrPQ167R and MoPrPQ218K respectively). These mutated PrPs could not be converted into the abnormal PrP^{Sc} isoform, and significantly they also inhibited the formation of 3F4 tagged MoPrP^{Sc} molecules during co-transfection experiments (Kaneko et al., 1997). The substitutions Q167R and Q218K in MoPrP were therefore described as “dominant negative” mutants since they block the replication of their wild-type counterparts (Kaneko et al., 1997). Recently, Perrier et al., analyzed the “dominant negative” inhibition effect in transgenic mice (Perrier et al., 2002). Transgenic mice carrying PrP with Q167R or Q218K substitutions on a *Prnp*^{-/-} background, were unable to replicate and propagate prion diseases. In transgenic mice, in which the wild-type (wt) *Prnp* gene was re-inserted, the conversion of wt PrP^C into PrP^{Sc} was dramatically slowed down, and correlated with an increase in the incubation time in these animals. This dominant negative effect seemed to depend on a ratio between the mutated PrP molecules produced by the transgene and the endogenous wt PrP molecules.

These transgenic experiments demonstrate the relevance of the dominant negative mutants but since they are not directly applicable in gene therapy, it appeared necessary to develop vectors for their *in vivo* delivery. The lentiviral gene transfer system is one of the most appropriate systems that could be employed. We used the VSV-G (Vesicular Stomatitis Virus – G

Glycoprotein) pseudotyped FIV (Feline Immunodeficiency Virus) lentiviral vectors to evaluate a gene transfer therapeutic strategy. Here we show that FIV vectors carrying the MoPrPQ167R or MoPrPQ218K dominant negative mutants are able to abolish endogenous wt PrP^{Sc} replication in chronically prion infected N2a58/22L cells. In addition, injections of virion preparations into mice, using the intracerebral or intraveinuous routes, demonstrate that our lentiviral vectors can transduce cells from brain and spleen tissues. Therefore, these results present an advance in the development of gene or cell therapy approaches to combat prion disease.

Materials and Methods

Nomenclature

Residue 171 in ovine PrP corresponds to codon 167 of the mouse PrP (MoPrP). Residue 219 in human PrP (HuPrP) corresponds to codon 218 in MoPrP.

Cell lines description

The *Prnp*^{-/-} cerebellar cells are derived from mice knockout for the *Prnp* gene. They have been kindly provided by T. Onodera (Kuwahara et al., 1999). *Prnp*^{-/-} cells were cultured in DMEM/F12 containing 10% fetal calf serum and 1% penicillin/streptomycin, at 37°C in an atmosphere of 5% CO₂.

The mouse neuroblastoma cell line (N2a) was purchased from the American Type Culture Collection (ATCC CCL 131). N2a Cells were stably transfected with wild-type mouse *Prnp* cDNA. N2a58 subclone, overexpressing MoPrP proteins, was chronically infected with the mouse adapted scrapie strain 22L as described by Nishida et al. (Nishida et al., 2000). Cells were cultivated in MEM supplemented with 10% fetal calf serum, 300 µg/ml Geneticin and 1% penicillin/streptomycin, at 37°C with 5% CO₂.

Construction of lentiviral derived vector carrying the mutants PrP Q167R and Q218K

Mutagenesis was performed on the MoPrP open reading frame using the Quikchange Site Directed Mutagenesis kit (Stratagene) with primers containing the mutations Q167R (5'-GTACTACAGGCCAGTGGATCGGTACAGCAACCAGAACAACT-3' / 5'-GTTGTTCTGTTGCTGTACCGATCCACTGGCCTGTAGTAC-3') and Q218K (5'-GATGTGCGTCACCCAGTACAAAAAGGAGTCCCAGGCCTATTA-3' / 5'-TAATAGGCCTGGGACTCCTTTTGTACTGGGTGACGCACATC-3'). The resulting cDNAs (MoPrPQ167R and MoPrPQ218K) were subcloned in the gene transfer vector used for the virion production. This transfer vector was derived from the prototypic FIV-14-Petaluma genome (AIDS Research and Reference Reagent Program), by replacing the U3 region of the 5'LTR by a CMV promoter promoting the expression of the vector in the cells (Lin et al., 2004). Moreover, the *gag* gene was truncated at position 1243, and a frameshift introduced at position 926. The *pol*, *vif*, *orfA*, *env*, and *rev* genes were replaced by a cassette containing the mutated PrP sequences driven by the phosphoglycerate kinase promoter. The PrP sequence was followed by an Internal Ribosome Entry Sequence (IRES) and the enhanced Green Fluorescent Protein marker gene.

Virion production

VSV-G pseudotyped FIV lentiviral vectors were produced by transient transfections of HEK293T cells with the 3 following plasmids: the gene transfer vector, the packaging vector and the pMD.G vector (Lin et al., 2004). The packaging vector, was also derived from the FIV-14-Petaluma genome in which the 5' LTR is replaced by a CMV promoter, and the 3'LTR by a poly-A signal. The third vector pMD.G, used for pseudotyping, contains the VSV-G envelope gene, which encodes the envelope G protein of the Vesicular Stomatitis Virus.

By using the calcium phosphate method (Sambrook, et al., 1989), HEK293T cells, plated at a density of $3 \cdot 10^6$ in 100 mm plates, were transfected with 9 μg of the gene transfer plasmid harbouring the PrP mutant, 15 μg of the FIV-derived packaging plasmid which codes for virion structural protein and 6 μg of the VSV-G envelope plasmid for pseudotyping. After 18 hours, the medium was replaced with 10 ml of DMEM containing 125 mM Hepes/plate.

Virions were collected at day 2 and day 3. The virion suspension was cleared by filtration through a 0.45 μm filter and concentrated by successive ultra-centrifugations at 17 000 rpm for 90 min (SW28, Beckman). Virions were resuspended in PBS. The viral titer was determined by FACS analysis of the GFP in 293T cells transduced with virion preparations according to the protocol described by Curran et al. (Curran et al., 2000), and by measuring

the p24 levels in the virions with the Petchek Idexx kit (IDEXX Laboratories, Westbrook Maine, USA). As an indication, 20 μ l of virion suspension with a titer of 10^8 – 10^9 Transducing Unit /ml (TU/ml) can be obtained from a 100 mm plate of HEK293T.

In vitro lentivirus transduction

Cells were transduced with a multiplicity of infection (MOI) of 1 and 10 (1 to 10 Transducing Units per cell) in a medium supplemented with polybrene (8 μ g/ml), in a 12-well plate containing 10^5 cells/well. The plates were then centrifuged (2500 rpm) for 1h 30 min at 30°C. The following day the medium was replaced by a fresh one.

Immunoblotting of PrP^C and PrP^{Sc}

Six days after transduction, *Prnp*^{-/-} cells were lysed in a lysis buffer (10 mM Tris HCl pH 8.0, 100 mM NaCl, 0.5% NP-40 and 0.5% sodium deoxycholate) and samples were analyzed by immunoblotting (Perrier et al., 2004). PrP^C was revealed with SAF32 antibody directed against the 59-89 epitope of the human PrP.

For the detection of PrP^{Sc} in N2a58/22L cells, cellular lysates were collected at different times after transduction and PK treated. Immunoblotting was performed with SAF mix as described (Perrier et al., 2004).

GFP, PrP^C and PrP^{Sc} in situ detection

The fluorescence of the GFP protein was observed by indirect microscopy (Leica) after formaline (3.7%) fixation. PrP^C and PrP^{Sc} immunocytofluorescence were performed as previously described (Mange et al., 2004). The SAF61 antibody was directed against the 144-156 epitope of the human PrP.

Animal models

The C57Bl/6 mice were from Charles Rivers. They were housed in an independent, filtered and pressurised cabinet according to French Ethical Committee guidelines (Decree 87-848) and the European Community Directive 86/609/EEC.

Injection of the lentivirus in mice

Mice were deeply anaesthetized by a mixture of 100 µg/g of ketamine and 15 µg/g of Xylazine. For the intracerebral injection of the lentivirus, we used a 10 µl Hamilton syringe with a 26G gauge needle fixed on a stereotaxic frame and connected to a microinjection pump (David Kopf Instruments, Tujunga, CA). Five microliters of the lentiviral preparation expressing the betagalactosidase gene (10^8 TU/ml) were injected at a rate of 0.5 µl/min, either into the ventricle (anteroposterior 0, lateral -1, dorsoventral -2,5, see Figure 4C-D) or into the thalamus (-1.58, 0.2, -3.2, see Figure 4A-B) and (0.34, 0.5, -2.2, see Figure 4E). One to three weeks following the injections, the brains were removed and fixed in 2% PFA. For intravenous route, an hundred microliters of the lentiviral preparation expressing the betagalactosidase gene (2×10^8 TU/ml) were injected into the mice using a 26G gauge needle. Five weeks following the injections, the spleens were removed and fixed in 2% PFA.

Detection of betagalactosidase expression in C57Bl/6 mice

After a short 30 min fixation period in 2% PFA, betagalactosidase staining was carried out by incubating the entire brain at 37° C overnight in a X-gal-solution.

For immunohistochemical analysis, fixation of spleen tissue or brains was performed in phosphate buffered saline (0.1 M PBS, pH 7.4) with 2% PFA, overnight at 4° C. The brains

were subsequently dehydrated through graded alcohol concentrations and embedded in paraffin. Sections of 5 μm were collected onto pre-treated glass slides (Polylysine or StarFrost, Fisher Scientific). The slides were then dewaxed and treated with Proteinase K (20 $\mu\text{g}/\text{ml}$, Roche-Boehringer) for 10 min at 37° C. All these steps were carried out at room temperature (22°C-24°C). Endogenous peroxidase activity was inhibited with 2% H_2O_2 (Merck) in 0.1 M PBS for 5 min. Non-specific antigenic sites were blocked by a 30 min incubation in 4% normal goat serum. The anti-beta-galactosidase monoclonal antibody (Chemicon) was then applied overnight. The brain sections were rinsed before detection of the primary antibody using the ABC system (Vector). These steps were followed by rinsing in 0.1 M PBS and the peroxidase was finally revealed by incubating the sections in 0.1 M PBS containing aminoethylcarbazole (AEC, Dako) to give red deposits. The slides were weakly counterstained with aqueous hematoxylin before mounting (GelMount). Images were collected in section under Zeiss Axiophot microscope, using Nikon DXM1200 camera.

Results and discussion

Lentivirus mediated gene transfer provides a potentially useful tool for therapeutic strategies since it can transduce both dividing and non dividing cells such as neurons in a stable and long term fashion (Blomer et al., 1997; Blomer et al., 1996; Marr et al., 2003; Naldini et al., 1996). This system also allows the transfer of genes without any selection process, preventing an eventual undesirable clonal effect. We therefore developed FIV derived vectors to evaluate a gene transfer therapeutic strategy by using the inhibitory properties of PrP “dominant negative” mutants against prion replication. We prepared VSV-G and pseudotyped FIV vectors containing the mouse *Prnp* gene in which the Q167R or Q218K mutations were generated by PCR mutagenesis. In these vectors, the gene of interest is followed by an IRES sequence and a reporter gene coding for the Green Fluorescent Protein (GFP) in order to identify the transduced cells and measure the efficiency of transduction. Virion particles were produced by the transfection of 293T cells with a three-plasmid expression system (Figure 1) : the gene transfer vector carrying the mutated *Prnp* genes, the FIV-derived packaging plasmid which codes for structural proteins of virions, and the VSV-G envelope plasmid that determines the cellular pseudotyping. Virions produced in the culture medium were filtrated and concentrated by successive ultra-centrifugations (Curran et al., 2000). The viral titers

were determined by counting the number of GFP positive fluorescent cells using FACS analysis after transduction of 293T cells with different dilutions of the virions. These titers were about 3.5×10^8 to 8.8×10^8 TU /ml.

To evaluate whether viral particles were able to transduce our cellular models and efficiently express the mutated genes, *Prnp*^{-/-} cerebellar cells derived from *Prnp* knock-out mice (Kuwahara et al., 1999) were transduced with virion preparations with a multiplicity of infection (MOI) of 10 (10 TU/cell). The success of the gene transfer was assessed by the fluorescence properties of the GFP (Figure 2A). The transduced *Prnp*^{-/-} cells expressed the MoPrPQ167R and MoPrPQ218K proteins as demonstrated by immunoblotting analysis (Figure 2B) using the SAF32 antibody. The similar levels of PrP^C detected concord with the identical viral titers used for the transduction. Furthermore, immunofluorescence on these cells also showed that the mutated proteins were correctly exported at the cell surface suggesting that dominant negative mutations do not affect the cellular trafficking of the PrP protein (Figure 2C).

To investigate the inhibitory potential of lentivirus vectors carrying the dominant negative PrP mutants, the prion infected neuroblastoma cells (N2a58/22L) were transduced. This N2a58/22L cell line provides a model for prion replication since it constitutively produces

high levels of PrP^{Sc} proteins (Nishida et al., 2000). These cells were transduced with the different preparations of virions using a MOI of 1. By counting the number of GFP positive cells we were then able to determine an efficacy of transduction of 80% (Figure 3A), without any toxicity to the cells.

Transduced N2a58/22L cells were serially split in duplicates and cultivated for several weeks. Every four days, the cellular lysates were collected and proteinase K treated to analyse the inhibition kinetic of the endogenous MoPrP^{Sc} levels by immunoblotting.

After 8 and 12 days, the N2a58/22L cells expressing the "dominant negative" MoPrPQ167R and MoPrPQ218K mutants presented a strong reduction in endogenous wt MoPrP^{Sc} levels (Figure 3B). After 15 days of culture, the majority of PrP^{Sc} was cleared (Figure 3C). In order to confirm the elimination of PrP^{Sc}, we performed a SAF61 immunocytofluorescence of the PrP^{Sc} after 20 days. We used a standard guanidinium thiocyanate protocol allowing PrP^{Sc} staining by promoting antigen retrieval of the PrP^{Sc}, and the elimination of PrP^C staining (Mange et al., 2004). This method also showed a strong diminution of PrP^{Sc} in the cells containing the "dominant negative" construction (Figure 3D) and the inhibitory effect was reproduced in three independent experiments (data not shown). Globally, these results show that the virions harbouring the PrP dominant negative mutants are functional and efficient for

the inhibition of endogenous wt PrP^{Sc} conversion. These results are in accordance with those obtained in previous studies (Kaneko et al., 1997; Perrier et al., 2002) who showed that an inhibition of wt MoPrP^{Sc} formation can occur with the MoPrPQ167R and MoPrPQ218K mutants. In addition, the lentiviral gene transfer method allowed us to amplify the inhibitory effect of these dominant negative mutants in scrapie infected cells. The transient transfections of prion infected cells performed by Kaneko et al. (Kaneko et al., 1997), resulted only in a minority of cells expressing the PrP mutants, which only allowed for an inhibitory effect to be obtained on the conversion of 3F4 tagged MoPrP^C but not on the conversion of wild-type endogenous PrP^C into PrP^{Sc}. In our case, and because the majority of the cells express the PrP mutants, we were able to obtain a strong inhibition of the conversion of the endogenous wt PrP^C into PrP^{Sc}. Our results showed that the delivery of dominant negative PrP mutants through lentiviral vectors, can not only provide a strong expression of the transgene, but also transdominantly inhibit the accumulation of the endogenous wt MoPrP^{Sc} in chronically scrapie infected N2a58/22L cells.

Lentiviral vectors possess the ability to transduce post-mitotic cells such as differentiated neurons as well as the cells of the lymphoreticular system in a prolonged and stable manner (Aguzzi et al., 2003; Blomer et al., 1997; Blomer et al., 1996; Glatzel et al., 2004; Mabbott

and Bruce, 2003; Mangeot et al., 2002; Naldini et al., 1996). In order to analyse if our lentiviral vectors had the potential to deliver the gene in the brain or in peripheral organs, we performed intracerebral and intravenous injections of lentivirus expressing the betagalactosidase gene in mice. For intracerebral delivery we used a stereotaxic frame to inject the lentiviral preparation in the right lateral ventricle or in the thalamus of adult C57BL/6 mice. Betagalactosidase expression was assessed by direct X-gal staining of the entire brain. Following intrathalamic injection, we observed a betagalactosidase activity mainly in the injection site (Figure 4A, B and E), demonstrating the capacity of our lentivirus to transduce neural cells. Following intraventricular injection, Xgal-blue coloration was limited all along the syringe trajectory and around the lateral ventricle (Figure 4C-D). This was probably due to the diffusion of the lentiviral particle in the whole ventricle. However, close examination of the brain by immunohistochemical analysis allowed us to detect betagalactosidase staining in the striatum area, the cortex area and in some more distant sites as well as the cerebellum area (figure 4 I, J and K). After systemic lentiviral injections, betagalactosidase was essentially observed around the blood vessels in the spleen of the mice and in some dispersed liver cells, probably due to the intravenous injection (Figure 4 L-Q). The spleen localisation in the vicinity of the vessels is somehow very pertinent since these

structures are innervated by the peripheral autonomous nervous system and the noradrenergic system implicated in the neuroinvasion process (Aguzzi et al., 2003; Bencsik et al., 2001; Haik et al., 2004; Prinz et al., 2003). These data showed that our lentiviral vectors can be used to transduce cells in the brain and in the spleen. Since neurons are the main cells targeted in prion disease, the use of lentiviral vectors carrying dominant negative PrP mutants could slow or even prevent prion replication at least in the brain area transduced with the virions particles. In addition, the first stages of prion diseases are characterized by an increase of the PrP^{Sc} levels in the lymphoreticular system and in the spleen (Lasmezas et al., 1996; Maignien et al., 1999). Thus lentiviral vectors could be useful effectors for the development of a gene therapy through repeated peripheral injections with the aim of slowing down or preventing prion replication during the very early stages of disease, at least prior to neuroinvasion. Moreover, a gene therapy strategy was recently evaluated by Marr et al., as an alternative therapeutic approach for the treatment of Alzheimer's disease (Marr et al., 2003). Lentiviral vectors expressing the neprilysin, a major A β degrading enzyme identified in the human brain and implicated in the clearance of A β amyloids, was tested in transgenic mouse models of amyloidosis. A single lentiviral injection decreased the number and size of amyloid plaques by about 45%. Altogether, these data have encouraged us to further evaluate therapeutic

strategies based on injections of the lentivirus carrying dominant negative PrP mutants by both intracerebral and peripheral routes. Moreover, the recent and promising results of the neuroprotective cell therapy treatment have prompted us to consider an approach combining both cell therapy and gene therapy using these dominant negative PrP mutants (Bachoud-Levi et al., 2000; Brown et al., 2001; Brustle and McKay, 1996; Lindvall, 2003; Lindvall and McKay, 2003). Indeed, neural precursors obtained after *in vitro* differentiation from embryonic stem cells could thus be transduced with the dominant negative PrP virion preparations before injection in the prion infected brain. The recent description of soluble dimeric PrP (Meier et al., 2003) or RNAi interferences (Daude et al., 2003) that antagonize prion replication may also be engineered in lentiviral vectors to extend the inhibitory effect.

Acknowledgments

We are grateful to Claire Marianni; to T. Onodera for the gift of *Prnp*^{-/-} cells and J. Grassi for the anti-PrP antibodies, and to the AIDS Research and Reference Reagent Program for the FIV-F14-Petaluma strain. Dr Perrier's project and Carole Crozet were financially supported by government grants from the French Ministry of Research (GIS -"Infections à Prions" – 2001 project N° A62) and by a grant from the International Carrefour Foundation (Paris, France).

References

Aguzzi, A., Heppner, F. L., Heikenwalder, M., Prinz, M., Mertz, K., Seeger, H. and

Glatzel, M. (2003). Immune system and peripheral nerves in propagation of prions to CNS.

Br. Med. Bull. **66**, 141-159.

Bachoud-Levi, A. C., Remy, P., Nguyen, J. P., Brugieres, P., Lefaucheur, J. P., Bourdet,

C., Baudic, S., Gaura, V., Maison, P., Haddad, B. et al. (2000). Motor and cognitive

improvements in patients with Huntington's disease after neural transplantation. *Lancet* **356**,

1975-1979.

Belt, P. B., Muileman, I. H., Schreuder, B. E., Bos-de Ruijter, J., Gielkens, A. L. and

Smits, M. A. (1995). Identification of five allelic variants of the sheep PrP gene and their

association with natural scrapie. *J. Gen. Virol.* **76 (Pt 3)**, 509-517.

Bencsik, A., Lezmi, S. and Baron, T. (2001). Autonomic nervous system innervation of

lymphoid territories in spleen: a possible involvement of noradrenergic neurons for prion

neuroinvasion in natural scrapie. *J. Neurovirol.* **7**, 447-453.

Blomer, U., Naldini, L., Kafri, T., Trono, D., Verma, I. M. and Gage, F. H. (1997).

Highly efficient and sustained gene transfer in adult neurons with a lentivirus vector. *J. Virol.*

71, 6641-6649.

Blomer, U., Naldini, L., Verma, I. M., Trono, D. and Gage, F. H. (1996). Applications of gene therapy to the CNS. *Hum. Mol. Genet.* **5 Spec No**, 1397-1404.

Bossers, A., Schreuder, B. E., Muileman, I. H., Belt, P. B. and Smits, M. A. (1996). PrP genotype contributes to determining survival times of sheep with natural scrapie. *J. Gen. Virol.* **77**, 2669-2673.

Brown, K. L., Brown, J., Ritchie, D. L., Sales, J. and Fraser, J. R. (2001). Fetal cell grafts provide long-term protection against scrapie induced neuronal loss. *NeuroReport* **12**, 77-82.

Brustle, O. and McKay, R. D. (1996). Neuronal progenitors as tools for cell replacement in the nervous system. *Curr. Opin. Neurobiol.* **6**, 688-695.

Bueler, H., Aguzzi, A., Sailer, A., Greiner, R. A., Autenried, P., Aguet, M. and Weissmann, C. (1993). Mice devoid of PrP are resistant to scrapie. *Cell* **73**, 1339-1347.

Caughey, W. S., Raymond, L. D., Horiuchi, M. and Caughey, B. (1998). Inhibition of protease-resistant prion protein formation by porphyrins and phthalocyanines. *Proc. Natl. Acad. Sci. U S A* **95**, 12117-12122.

Curran, M. A., Kaiser, S. M., Achacoso, P. L. and Nolan, G. P. (2000). Efficient transduction of nondividing cells by optimized feline immunodeficiency virus vectors. *Mol. Ther.* **1**, 31-38.

Daude, N., Marella, M. and Chabry, J. (2003). Specific inhibition of pathological prion protein accumulation by small interfering RNAs. *J. Cell Sci.* **116**, 2775-2779.

Glatzel, M., Giger, O., Seeger, H. and Aguzzi, A. (2004). Variant Creutzfeldt-jakob disease: between lymphoid organs and brain. *Trends Microbiol.* **12**, 51-53.

Goldmann, W., Hunter, N., Smith, G., Foster, J. and Hope, J. (1994). PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *J. Gen. Virol.* **75**, 989-995.

Haik, S., Faucheux, B. A. and Hauw, J. J. (2004). Brain targeting through the autonomous nervous system: lessons from prion diseases. *Trends Mol. Med.* **10**, 107-112.

Kaneko, K., Zulianello, L., Scott, M., Cooper, C. M., Wallace, A. C., James, T. L.,

Cohen, F. E. and Prusiner, S. B. (1997). Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. *Proc. Natl. Acad. Sci.*

U S A **94**, 10069-10074.

Kuwahara, C., Takeuchi, A. M., Nishimura, T., Haraguchi, K., Kubosaki, A.,

Matsumoto, Y., Saeki, K., Matsumoto, Y., Yokoyama, T., Itohara, S. et al. (1999). Prions prevent neuronal cell-line death. *Nature* **400**, 225-226.

Lasmezas, C. I., Cesbron, J. Y., Deslys, J. P., Demaimay, R., Adjou, K. T., Rioux, R.,

Lemaire, C., Locht, C. and Dormont, D. (1996). Immune system-dependent and -independent replication of the scrapie agent. *J. Virol.* **70**, 1292-1295.

Leucht, C., Simoneau, S., Rey, C., Vana, K., Rieger, R., Lasmezas, C. I. and Weiss, S.

(2003). The 37 kDa/67 kDa laminin receptor is required for PrP(Sc) propagation in scrapie-infected neuronal cells. *EMBO Rep.* **4**, 290-295.

Lin, Y. L., Noël, D., Mettling, C., Réant, B., Clot, J., Jorgensen, C. and Corbeau, P.

(2004). FIV vectors for efficient transduction of primary human synoviocytes: application to an original model of rheumatoid arthritis. *Hum. Gene Ther.* *in press*

Lindvall, O. (2003). Stem cells for cell therapy in Parkinson's disease. *Pharm. Res.* **47**, 279-

287.

Lindvall, O. and McKay, R. (2003). Brain repair by cell replacement and regeneration.

Proc. Natl. Acad. Sci. U S A **100**, 7430-7431.

Mabbott, N. A. and Bruce, M. E. (2003). Prion disease: bridging the spleen-nerve gap. *Nat.*

Med. **9**, 1463-1464.

Maignien, T., Lasmezas, C. I., Beringue, V., Dormont, D. and Deslys, J. P. (1999).

Pathogenesis of the oral route of infection of mice with scrapie and bovine spongiform encephalopathy agents. *J. Gen. Virol.* **80 (Pt 11)**, 3035-3042.

Mallucci, G., Dickinson, A., Linehan, J., Klohn, P. C., Brandner, S. and Collinge, J.

(2003). Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis. *Science* **302**, 871-874.

Mange, A., Crozet, C., Lehmann, S. and Beranger, F. (2004). Scrapie-like prion protein is

translocated to the nuclei of infected cells independently of proteasome inhibition and interacts with chromatin. *J. Cell Sci.* **117**, 2411-2416.

Mangeot, P. E., Duperrier, K., Negre, D., Boson, B., Rigal, D., Cosset, F. L. and Darlix,

J. L. (2002). High levels of transduction of human dendritic cells with optimized SIV vectors. *Mol. Ther.* **5**, 283-290.

Marr, R. A., Rockenstein, E., Mukherjee, A., Kindy, M. S., Hersh, L. B., Gage, F. H.,

Verma, I. M. and Masliah, E. (2003). Nepriylsin gene transfer reduces human amyloid pathology in transgenic mice. *J. Neurosci.* **23**, 1992-1996.

Meier, P., Genoud, N., Prinz, M., Maissen, M., Rulicke, T., Zurbriggen, A., Raeber, A.

J. and Aguzzi, A. (2003). Soluble dimeric prion protein binds PrP(Sc) in vivo and antagonizes prion disease. *Cell* **113**, 49-60.

Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F. H., Verma, I. M. and

Trono, D. (1996). In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* **272**, 263-267.

Nishida, N., Harris, D. A., Vilette, D., Laude, H., Frobert, Y., Grassi, J., Casanova, D.,

Milhavet, O. and Lehmann, S. (2000). Successful transmission of three mouse-adapted scrapie strains to murine neuroblastoma cell lines overexpressing wild-type mouse prion protein. *J. Virol.* **74**, 320-325.

Perrier, V., Kaneko, K., Safar, J., Vergara, J., Tremblay, P., DeArmond, S. J., Cohen,

F. E., Prusiner, S. B. and Wallace, A. C. (2002). Dominant-negative inhibition of prion replication in transgenic mice. *Proc. Natl. Acad. Sci. U S A* **99**, 13079-13084.

Perrier, V., Solassol, J., Crozet, C., Frobert, Y., Mourton-Gilles, C., Grassi, J. and

Lehmann, S. (2004). Anti-PrP antibodies block PrPSc replication in prion infected cell cultures by accelerating PrPC degradation. *J. Neurochem.* **89**, 454-463.

Perrier, V., Wallace, A. C., Kaneko, K., Safar, J., Prusiner, S. B. and Cohen, F. E.

(2000). Mimicking dominant negative inhibition of prion replication through structure-based drug design. *Proc. Natl. Acad. Sci. U S A* **97**, 6073-6078.

Prinz, M., Heikenwalder, M., Junt, T., Schwarz, P., Glatzel, M., Heppner, F. L., Fu, Y.

X., Lipp, M. and Aguzzi, A. (2003). Positioning of follicular dendritic cells within the spleen controls prion neuroinvasion. *Nature* **425**, 957-962.

Prusiner, S. B., Scott, M., Foster, D., Pan, K. M., Groth, D., Mirenda, C., Torchia, M.,

Yang, S. L., Serban, D., Carlson, G. A. et al. (1990). Transgenic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* **63**, 673-686.

Prusiner, S. B., Scott, M. R., DeArmond, S. J. and Cohen, F. E. (1998). Prion protein biology. *Cell* **93**, 337-348.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). Molecular Cloning. In *Molecular Cloning*, vol. 3 (ed. C. Nolan), pp. 16.33-16.37. Cold Spring Harbor Laboratory Press.

Shibuya, S., Higuchi, J., Shin, R. W., Tateishi, J. and Kitamoto, T. (1998). Codon 219

Lys allele of PRNP is not found in sporadic Creutzfeldt-Jakob disease. *Ann. Neurol.* **43**, 826-828.

Supattapone, S., Nguyen, H. O., Cohen, F. E., Prusiner, S. B. and Scott, M. R. (1999).

Elimination of prions by branched polyamines and implications for therapeutics. *Proc. Natl.*

Acad. Sci. U S A **96**, 14529-14534.

Figure Legends

Figure 1: Characteristics of the lentiviral vectors. The packaging vector, was derived from the prototypic FIV-14-Petaluma genome (AIDS Research and Reference Reagent Program), in which the 5' LTR is replaced by a CMV promoter, and the 3'LTR by a poly-A signal. The transfer vector was also derived from FIV-14-Petaluma by replacing the U3 region of the 5'LTR by a CMV promoter in order to enable the expression of the vector in cells. Moreover, the *gag* gene was truncated at position 1243, and a frameshift introduced at position 926. The *pol*, *vif*, *orfA*, *env*, and *rev* genes were replaced by a cassette containing the mutated PrP sequences driven by the phosphoglycerate kinase promoter. The PrP sequence is followed by an IRES sequence and the enhanced Green Fluorescent Protein marker gene. The third vector pMD.G, used for pseudotyping, contains the VSV-G envelope gene, which encodes the envelope G protein of the Vesicular Stomatitis Virus. (PGK, phosphoglycerate kinase promoter; CTE, constitutive transport element; cPPT CTS, central DNA flap sequence; SD, splice donor site.

Figure 2: Transduction of *Prnp*^{-/-} cells with lentivirus carrying the PrP dominant negative mutants MoPrPQ167R and MoPrPQ218K. (A) Transduction efficacy of *Prnp*^{-/-} cells with

lentiviral vectors assessed by GFP fluorescence. A1, A2, A3, A4 correspond to non-transduced, GFP, MoPrPQ167R and MoPrPQ218K transduced cells, respectively. (B) Detection of PrP^C by immunoblotting using SAF32 anti-PrP antibody (C) SAF32 immunocytofluorescence of the PrP^C in *Prnp*^{-/-} cells: no staining is visible in non-transduced (C5) and GFP (C6) lentiviral transduced cells, whereas cells transduced with the MoPrPQ167R (C7), or MoPrPQ218K (C8) lentivirus exhibit a strong rhodamine fluorescence. C1, C2, C3, C4 correspond to the Hoechst nuclear staining of the cells represented in figure C5, C6, C7, C8 respectively. Bar: 8µm.

Figure 3: Transduction of N2a58/22L cells with the MoPrPQ167R and MoPrPQ218K virions. (A) GFP analysis after transduction of N2a58/22L cells: non transduced cells (A1), cells transduced with GFP vector (A2), MoPrPQ167R (A3) or MoPrPQ218K (A4) PrP mutants. (B) Inhibition of endogenous MoPrP^{Sc} levels by dominant negative mutants in N2a58/22L cells. PK digested cell lysates were analyzed by immunoblotting and the wild-type MoPrP^{Sc} levels revealed with SAF mix. N2a58/22L cells expressing the MoPrPQ218K mutant showed a strong inhibition of the PrP^{Sc} level at 8 (D8) and 12 (D12) days after transduction, while cells expressing MoPrPQ167R mutants showed a slight diminution. (C)

Kinetic analysis of PrP^{Sc} inhibition in N2a58/22L cells by western blot analysis, at 8 (1), 12 (2) and 15 days (3) after transduction. Non-transduced cells (NT), cells transduced with virions carrying GFP (GFP) only, or virions carrying MoPrPQ167R (Q167R) or MoPrPQ218K (Q218K). These results are representative of several independent experiments.

(D) Immunofluorescence of the PrP^{Sc} accumulation using SAF61 anti-PrP antibody 20 days after transduction. (D5 non-transduced, D6: GFP, D7: MoPrPQ167R, D8: MoPrPQ218K).

D1, D2, D3, D4 correspond to the Hoechst nuclear staining of the cells presented in figure D5, D6, D7, D8 respectively. Bar: 8µm.

Figure 4: In vivo delivery of FIV lentiviral vectors.

(A-E) Brain slices of mice intracerebrally injected with FIV lentiviral vectors carrying the betagalactosidase gene after X-gal staining (blue coloration), 1 week after injection. (A-B) According to the site of injection (red arrow), the blue coloration was observed in the mediodorsal thalamic nucleus. (C-E) X-gal staining was also observed along the needle passage : (C) in the cortex as well as in the fibers of the corpus callosum, (D) in the wall of the ventricles and (E) in the anteroventral thalamic nucleus.

(F-K) After intraventricular injection, close immunohistochemical examination using antibetagalactosidase antibody, allowed the detection of the betagalactosidase expression (red staining) : (I) in the cortex, (J) the striatum, and (K) the cerebellum, 3 weeks after injection. No staining was observed in non injected mice used as control (F - cortex, G - striatum, H - Cerebellum).

(L-Q) Sections of spleen and liver from intraveinously injected mice were analysed by immunocytochemistry. Positive cells were detected in the vicinity of the blood vessels : (M-N) in the spleen, (Q) in the liver as well as (P) in some isolated cells in the liver. No staining was observed in non injected mice (L - spleen, O - Liver).

(Cx: Cortex; Hp: Hippocampus; MDTh: Mediodorsal thalamic nucleus; CC: Corpus Callosum; LV: Lateral Ventricule; vhc: ventral hippocampal commissure; AV Th: anteroventral thalamic nucleus; BV: Blood vessel. A-E: Bar 1mm; F-N: Bar 10 μ m.