PRP Expression in Schwann Cells is not Required for Transmissible Spongiform Encephalopathy Neuroinvasion

PrP Expression in Schwann Cells is Not Required for Transmissible Spongiform Encephalopathy Neuroinvasion

Barry Bradford[1], Peter Brophy[2], Jean Manson[1] and Nadia L. Tuzi[1]

[1]Neuropathogenesis Unit, Roslin Institute, Edinburgh, UK. [2]Centre for Neuroscience Research, University of Edinburgh, Edinburgh, UK.

Neuropathogenesis unit: Roslin Institute

1. Introduction

Transmissible spongiform encephalopathies (TSE) are transmissible neurodegenerative diseases characterised by long incubation periods and characteristic pathology which includes vacuolar spongiform changes, apoptotic neuronal loss and astrocytic proliferation in the central nervous system (CNS). A diagnostic feature of these invariably fatal diseases is the presence of a protease resistant isoform (PrP^{Sc}) of the host-encoded membrane glycoprotein PrP, of which the protease sensitive isoform (PrP^{c}) is necessary for disease susceptibility. Schwann cells are known to express PrP when associated with co-expressing peripheral nervous system axons and have also been shown to replicate and propagate TSE agent in culture.

2. Objectives

To test the hypothesis that Schwann cells are involved in TSE neuroinvasion, a line of transgenic mice were produced using Cre / LoxP technology in which PrP expression is removed from Schwann cells. We have infected these mice with TSE agents via various routes to determine the difference in incubation period and CNS pathology when compared to wild type mice.

3. Methods

By breeding mice (*P_0-Cre*) expressing Cre under the control of the peripheral myelin Protein Zero (MPZ or P_0) promoter (gift from M. Laura Feltri, Milan) and mice possessing *LoxP* sites flanking the PrP gene ($PrP^{fl/fl}$), we have generated mice expressing PrP normally, except where P_0 is expressed, i.e. myelinating Schwann cells. Mice were challenged with TSE agents 139A and ME7 intracerebrally (i.c.), orally and intraperitoneally (i.p.). Mice were clinically scored for signs of disease and analysed for terminal pathology. Disease incubation period, vacuolation in specific brain areas and presence of Proteinase K resistant PrP were analyzed as indicators of TSE disease.

4. Results

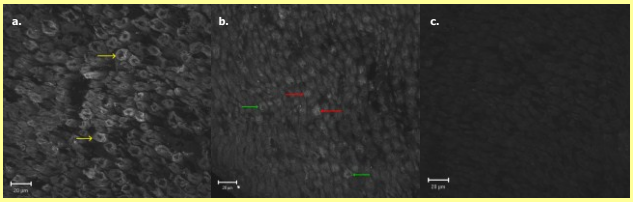

Figure 1. PrP^{c} (white) detected by fluorescence immunocytochemistry using monoclonal antibodies 7A12 and 8H4 (gift from Man Sun Sy, Cleveland). Sciatic nerve cross-section samples: **a**, wild type; **b**, *P_0-Cre* $PrP^{fl/fl}$ and **c**, $PrP^{0/0}$. Yellow arrows highlight strong PrP staining in myelin ring structures in wild type mice, red and green arrows show residual axonal and periaxonal staining respectively following conditional knockout of PrP expression from myelinating Schwann cells in *P_0-Cre* $PrP^{fl/fl}$ compound transgenic mice. Fluorescence detection level was gated using PrP knockout mice.

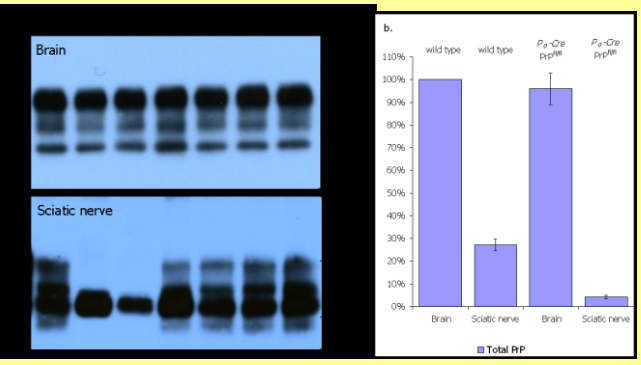

Figure 2. **a**, Western analysis of PrP^{c} expression in brain and sciatic nerve of *P_0-Cre* $PrP^{fl/fl}$ (lanes marked *) , *P_0-Cre*, wild-type and $PrP^{fl/fl}$ mice as detected by monoclonal antibody 7A12. Approximate protein size markers in kilodaltons (**kDa**). **b**, Densitometric analysis of PrP^{c} expression via Kodak 1D™ software showing reduction in PrP^{c} expression between sciatic nerve from wild type and *P_0-Cre* $PrP^{fl/fl}$ compound transgenic mice.

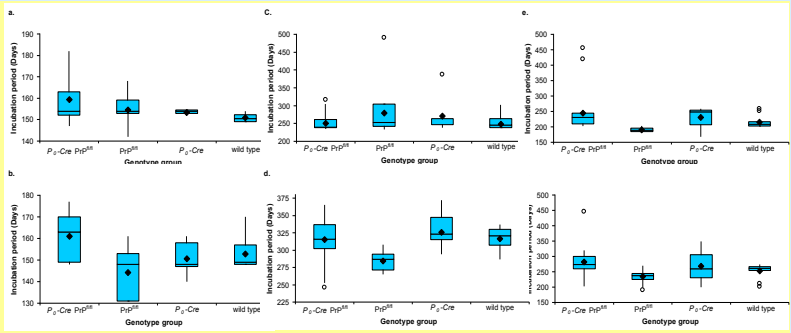

Figure 3. Box-and-whisker diagram showing incubation period distribution following transmission of mouse-adapted Scrapie strains 139A (**a**, **c**, **e**) and ME7 (**b**, **d**, **f**) via intracerebral (**a**, **b**), oral (**c**, **d**) and intraperitoneal (**e**, **f**) routes. Animals were grouped following post-mortem genotype confirmation, ANOVA revealed no significant differences between *P_0-Cre* $PrP^{fl/fl}$ and control groups: Q statistical outliers; ◆, group mean incubation period.

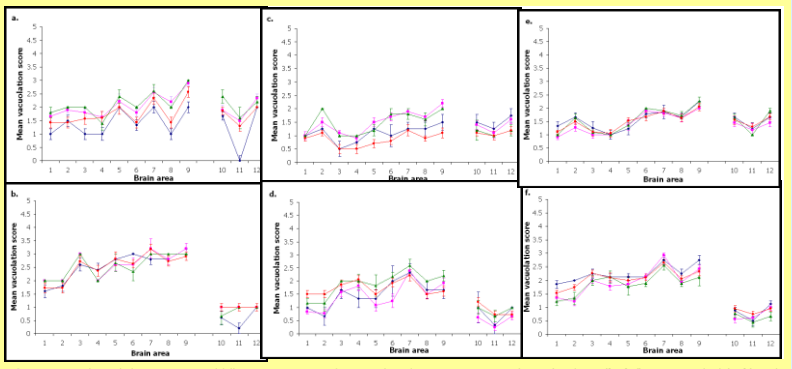

Figure 4. Vacuolar pathology was assessed following transmission of mouse-adapted Scrapie strains 139A (**a**, **c**, **e**) and ME7 (**b**, **d**, **f**) via intracerebral (**a**, **b**), oral (**c**, **d**) and intraperitoneal (**e**, **f**) routes. Brains were scored on a scale of 0—5 in nine grey matter areas and 0–3 in three white matter areas, animals were grouped following post-mortem confirmation of genotype and mean scores calculated to produce lesion profiles (error bars representing ±SEM). Brain scoring areas: **1**, dorsal medulla; **2**, cerebellar cortex; **3**, superior colliculus; **4**, hypothalamus; **5**. medial thalamus; **6**, hippocampus; **7**, septum; **8**, cerebral cortex; **9**, forebrain cerebral cortex; **10**, cerebellar white matter; **11**, midbrain white matter; **12**, cerebral peduncle. ◆, *P_0-Cre*; ■, $PrP^{fl/fl}$; ▲, wild type; ● *P_0-Cre* $PrP^{fl/fl}$.

5. Discussion

Characterisation of these mice show that PrP is effectively excised, resulting in a 90 % reduction in PrP^{c} expression in axonal lengths of peripheral nerves. This finding is particularly interesting as no differences have been observed in disease susceptibility or ability to traffic the infectious agent to the CNS in these mice. No alteration was seen in either incubation period or end pathology via agents 139A and ME7 after i.c., oral and i.p. challenges. These results suggest that TSE neuroinvasion may occur via peripheral neurons alone with no involvement of peripheral glia despite their contribution to overall PrP expression in peripheral neurons. Also peripheral PrP glycosylation is not required for neuroinvasion and has no impact on strain specific properties such as the final targeting of CNS vacuolation and deposition of PrP^{Sc}.

6. Conclusion

Removal of PrP expression in Schwann cells has no effect on TSE neuroinvasion of the two strains studied. We can therefore discount Schwann cells as a viable therapeutic target during the long incubation period between infection and clinical presentation. This study has revealed some very interesting biochemical and cell biological properties of PrP. The results from this study question what role PrP and its glycosylation status may play in disease. These results also offer insights into possible roles for PrP in glial / axonal interaction and cell-cell signalling.

Funding: MRC. **e-mail**: barry.bradford@bbsrc.ac.uk