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Follicular Dendritic Cell-Specific Prion Protein (PrPc) Expression Alone Is Sufficient to Sustain Prion Infection in the Spleen

Laura McCulloch1, Karen L. Brown1, Barry M. Bradford1, John Hopkins1, Mick Bailey2, Klaus Rajewsky3, Jean C. Manson1, Neil A. Mabbott1*

1The Roslin Institute & Royal (Dick) School of Veterinary Sciences, University of Edinburgh, Midlothian, United Kingdom, 2Division of Veterinary Pathology, Infection and Immunity, School of Clinical Veterinary Science, University of Bristol, Avon, United Kingdom, 3Program in Cellular and Molecular Medicine, Children's Hospital, and Immune Disease Institute, Harvard Medical School, Boston, Massachusetts, United States of America

Abstract

Prion diseases are characterised by the accumulation of PrPSc, an abnormally folded isoform of the cellular prion protein (PrPc), in affected tissues. Following peripheral exposure high levels of prion-specific PrPSc accumulate first upon follicular dendritic cells (FDC) in lymphoid tissues before spreading to the CNS. Expression of PrPc is mandatory for cells to sustain prion infection and FDC appear to express high levels. However, whether FDC actively replicate prions or simply acquire them from other infected cells is uncertain. In the attempts to-date to establish the role of FDC in prion pathogenesis it was not possible to dissociate the Prnp expression of FDC from that of the nervous system and all other non-haematopoietic lineages. This is important as FDC may simply acquire prions after synthesis by other infected cells. To establish the role of FDC in prion pathogenesis transgenic mice were created in which PrPc expression was specifically “switched on” or “off” only on FDC. We show that PrPc-expression only on FDC is sufficient to sustain prion replication in the spleen. Furthermore, prion replication is blocked in the spleen when PrPc-expression is specifically ablated only on FDC. These data definitively demonstrate that FDC are the essential sites of prion replication in lymphoid tissues. The demonstration that Prnp-ablation only on FDC blocked splenic prion accumulation without apparent consequences for FDC status represents a novel opportunity to prevent neuroinvasion by modulation of PrPc expression on FDC.

Introduction

Prion diseases (Transmissible spongiform encephalopathies; TSE) are sub-acute neurodegenerative diseases that affect both humans and animals. Many prion diseases, including natural sheep scrapie, bovine spongiform encephalopathy, chronic wasting disease in mule deer and elk, and kuru and variant Creutzfeldt-Jakob disease in humans, are acquired by peripheral exposure (e.g. orally or via lesions to skin or mucous membranes). After peripheral exposure prions accumulate first upon follicular dendritic cells (FDC) as they make their journey from the site of infection to the CNS (a process termed, neuroinvasion) [1–7]. FDC are a unique subset of stromal cells resident within the primary B cell follicles and germinal centres of lymphoid tissues [8]. Prion accumulation upon FDC is critical for efficient disease pathogenesis as in their absence neuroinvasion are impaired [1–4]. From the lymphoid tissues prions invade the CNS via the peripheral nervous system [9].

During prion disease aggregations of PrPSc, an abnormally folded isoform of the cellular prion protein (PrPc) accumulate in affected tissues. Prion infectivity co-purifies with PrPSc [10] and is considered to constitute the major, if not sole, component of infectious agent [11]. Host cells must express cellular PrPc to sustain prion infection [12] and FDC appear to express high levels of PrPc on the cell membrane in uninfected mice [13,14]. Although prion neuroinvasion from peripheral sites of exposure is dependent upon the presence of FDC in lymphoid tissues, it is not known whether FDC actually replicate prions themselves. FDC characteristically trap and retain native antigen on their surfaces for long periods in the form of immune complexes, consisting of antigen-antibody and/or complement components. FDC are also known whether FDC actually replicate prions themselves. FDC are also considered to be acquired by FDC as complement-opsinized immune complexes [15–18]. Thus, during prion infection FDC might simply trap and retain PrPSc-containing immune complexes on their surfaces following synthesis by other infected cells such as neurones.

Many cell types including classical DC, lymphocytes, mast cells, platelets, reticulocytes and epithelial cells secrete membrane vesicles termed exosomes that are enriched in cell-specific protein [19,20]. Although the functions of exosomes are uncertain FDC can bind them on their surfaces. These microvesicles permit FDC to passively acquire and display proteins on their surfaces that they do not express at the mRNA level [21]. Studies have shown that prions only accumulated in the spleens of mice in which the FDC-
Author Summary

Prion diseases are infectious neurological disorders and are considered to be caused by an abnormally folded infectious protein termed PrPSc. Soon after infection prions accumulate first upon follicular dendritic cells (FDC) in lymphoid tissues before spreading to the brain where they cause damage to nerve cells. Cells must express the normal cellular prion protein PrPc to become infected with prions. However, whether FDC are infected with prions or simply acquire them from other infected cells is unknown. To establish the role of FDC in prion disease PrPc expression was specifically “switched on” or “off” only on FDC. We show that PrPc-expressing FDC alone are sufficient to sustain prion replication in the spleen. Furthermore, prion replication is blocked in the spleen when PrPc-expression is switched off only on FDC. These data definitively demonstrate that FDC are the essential sites of prion replication in lymphoid tissues.

Expression of Cre recombinase by the Cr2 promoter is not toxic to FDC

Cre toxicity can occur in some Cre transgenic mouse lines whereby Cre recombinase causes mis-recombination, DNA damage and death of Cre-expressing cells [30]. However, immunohistochemical (IHC) analysis of spleens from CD21-Cre ROSA26lox/lox mice and WT→CD21-Cre ROSA26lox/lox mice showed no significant effect of Cre-expression on the status of FDC networks and B cell follicles when compared to spleens from WT control mice and ROSA26lox/lox mice that lacked Cre expression (Figure 1C). Furthermore, the expression of Cre recombinase under the control of the Cr2 locus had no observable effect on CD21/35 expression (Figure 1C).

FDC express Pmp and do not acquire PrPc from neighbouring cells

Next, mice were created in which Pmp expression (which encodes PrPc) was restricted only to FDC. To do so, CD21-Cre mice were first bred onto a Prnp+/− (PrPc+/−) background. The resulting CD21-Cre Prnp−/− mice were then crossed with Prnp+/+ mice in which a floxed β-galo stop cassette was inserted into intron 2 of the Pnp gene upstream of exon 3 [31]. In the progeny CD21-Cre Pnp+/− mice, PrPc is only expressed in cells expressing Cre recombinase (CD21-expressing FDC and mature B cells). To restrict the Prnp-expression to FDC, CD21-Cre Pnp+/+ mice were lethally γ-irradiated and grafted with bone marrow from Cre-deficient Prnp+/− mice (Prnp+/−→CD21-Cre Prnp+/−/− mice). We also performed bone marrow transfers from CD21-Cre Prnp+/−/− donors into CD21-Cre Prnp+/−/− recipients (CD21-Cre Prnp+/−/−→CD21-Cre Prnp+/−/− mice), CD21-Cre Prnp+/−/− donors into Cre-deficient Prnp+/−/− mice (CD21-Cre Prnp+/−/−→CD21-Cre Prnp+/−/− mice) and Prnp+/− donors into Prnp−/− recipients (Prnp−/−→Prnp+/− mice) as controls (Figure 2A).

Spleens, tails and blood from six mice from each group were examined 100 days after bone marrow transfusion. PCR analysis of DNA isolated from the tails, blood and spleens of mice in each group was used to confirm the presence of Cre (Figure 2B, upper panel) and Cre-mediated DNA recombination (Figure 2B, lower panel) within the stromal, haematopoietic or both compartments (respectively). The detection of Cre in the tail and spleen but not...
Figure 1. Cre-mediated gene recombination in FDC in the spleens, lymph nodes and Peyer’s patches of CD21-Cre ROSA26<sup>flox/Flox</sup> mice. A) Analysis of the cellular sites of LacZ expression (blue) in the spleens, inguinal lymph nodes, Peyer’s patches and mesenteric lymph nodes of CD21-Cre ROSA26<sup>flox/Flox</sup> mice shows Cre-mediated recombination in a focus of cells within the B cell follicles. Sections were counterstained with nuclear fast red (red). B) IHC analysis of FDC (CD35<sup>+</sup> cells, upper row, red) and B cells (CD45R<sup>+</sup> cells, lower row, red) confirmed that Cre-mediated LacZ expression (blue) was associated with FDC in the spleens of WT→CD21-Cre ROSA26<sup>flox/Flox</sup> mice. No LacZ expression was associated with FDC in spleens from ROSA26<sup>flox/flox</sup> mice that lacked Cre. C) IHC analysis of the status of FDC (CD35<sup>+</sup> and C4-binding cells; red) and B cells expressing CD45R, CD19, and CD1d (red) in spleens from WT, CD21-Cre ROSA26<sup>flox/Flox</sup>, WT→CD21-Cre ROSA26<sup>flox/Flox</sup> and ROSA26<sup>flox/Flox</sup> mice. Scale bars 100 μm. n = 6 mice/group. doi:10.1371/journal.ppat.1002402.g001
FIGURE 2. FDC-restricted PrP<sup>C</sup> expression in the spleens of Prnp<sup>stop</sup>→CD21-Cre Prnp<sup>stop</sup> mice. A) The anticipated distribution of PrP<sup>C</sup> expression on FDC and B cells in tissues from each mouse group. B) The detection of Cre in the tail and spleen but not blood of the Prnp<sup>stop</sup>→CD21-Cre Prnp<sup>stop</sup> mice confirmed the restriction of the Cre-expression to the stromal but not haematopoietic compartments of these mice (upper panel). Efficient Cre-mediated recombination of Prnp<sup>stop</sup> when compared to control mice. Cre-mediated recombination by CD21-expressing lymphocytes was efficiently prevented in these mice by the irradiation and transfer of Prnp<sup>stop</sup> bone marrow as demonstrated by the lack of a Prnp<sup>stop</sup> band in DNA extracted from these mice (lower panel).
blood of the Prnp<sup>−/−</sup>/CD21-Cre Prnp<sup>stop</sup>/mice confirmed the restriction of the Cε-expression to the stromal but not hematopoietic compartments of these mice. In addition, PCR analysis also confirmed that in these mice efficient Cre-mediated recombination of the Prnp<sup>stop</sup>/alleles was restricted to the FDC-containing stromal compartment of the spleen (Figure 2B). In Prnp<sup>stop</sup>/CD21-Cre Prnp<sup>stop</sup>/mice the recombinant Prnp<sup>stop</sup>/allele (Prnp<sup>stop</sup>/B) was detected in the spleen, but not blood and tail. Thus these data indicate that in the spleens of Prnp<sup>stop</sup>/CD21-Cre Prnp<sup>stop</sup>/mice Cre-mediated recombination is restricted to FDC and not B cells.

As anticipated, in the spleens of Prnp<sup>−/−</sup>/Prnp<sup>−/−</sup> control mice high levels of PrP<sup>Sc</sup> expression were observed upon FDC and tyrosine hydroxylase (TH)-positive sympathetic nerves (Figure 2C). In contrast, in the spleens of Prnp<sup>stop</sup>/CD21-Cre Prnp<sup>stop</sup>/mice PrP<sup>Sc</sup> was only expressed on FDC (Figure 2C). In the absence of Cre-recombinase expression by FDC and peripheral nerves in CD21-Cre Prnp<sup>stop</sup>/Prnp<sup>stop</sup>/mice, PrP<sup>Sc</sup> expression was not expressed by either cell population (Figure 2C).

Morphometric analysis confirmed that the amount of the PrP<sup>Sc</sup> expression co-localized upon the surfaces of FDC in the spleens of Prnp<sup>stop</sup>/CD21-Cre Prnp<sup>stop</sup>/mice was not significantly different from that observed upon FDC in spleens from Prnp<sup>−/−</sup>/Prnp<sup>−/−</sup> control mice (P<0.69, n = 48 FDC networks/group; Figure 2D). In contrast, in the absence of Cre-recombinase expression by FDC in CD21-Cre Prnp<sup>stop</sup>/Prnp<sup>stop</sup>/mice, PrP<sup>Sc</sup> expression was substantially lower than that observed upon FDC in spleens from Prnp<sup>−/−</sup>/Prnp<sup>−/−</sup> control mice (P<1×10<sup>−25</sup>, n = 48 FDC networks/group). E) Morphometric analysis confirmed that PrP<sup>Sc</sup> expression upon the surfaces of sympathetic nerves in the spleens of Prnp<sup>stop</sup>/CD21-Cre Prnp<sup>stop</sup>/mice was significantly ablated when compared to that observed upon sympathetic nerves in spleens from Prnp<sup>−/−</sup>/Prnp<sup>−/−</sup> control mice (P<1×10<sup>−25</sup>, n = 48 sympathetic nerves/group). For all panels n = 6 mice/group.

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FDC-restricted PrP<sup>Sc</sup>-expression is sufficient to sustain prion replication in the spleen

Next, we determined the effect of FDC-restricted Prnp<sup>−/−</sup> expression on prion replication in the spleen. In this study, the normal cellular form of the prion protein is referred to as PrP<sup>C</sup>, and two distinct terms (PrP<sup>B</sup> or PrP<sup>C</sup>) are used to describe the disease-specific, abnormal accumulations of PrP that are characteristically found only in prion-affected tissues and considered a reliable biochemical marker for the presence of infectious prions [10]. Disease-specific PrP (PrP<sup>D</sup>) accumulations are relatively resistant to proteinase K (PK) digestion, whereas cellular PrP<sup>C</sup> is destroyed. Where we were able to confirm this resistance by treatment of samples with PK and subsequent paraformaldehyde-based tissue (PET) immunoblot analysis [34], PrP<sup>D</sup> is used as a biochemical marker for the presence of prions. Unfortunately, treatment of tissue sections with PK destroys the microarchitecture. Therefore, for IHC analysis tissue sections were fixed and pre-treated to enhance the detection of the disease-specific abnormal accumulations of PrP (PrP<sup>D</sup>), whereas cellular PrP<sup>C</sup> is denatured by these treatments [4]. We have repeatedly shown in a series of studies that these PrP<sup>D</sup>-accumulations occur only in prion-infected tissues, and correlate closely with the presence of ME7 scrapie prions [1,13,35–37].

Within weeks after i.p. exposure of WT mice to ME7 scrapie prions, strong accumulations of prion-specific PrP<sup>D</sup> occur upon FDCs within the spleen and are sustained until the terminal stages of disease [1,13,35]. Here, mice were injected i.p. with ME7 scrapie prions and spleens from 4 mice from each group collected 35, 70 and 105 days after exposure. In spleens from control mice (Prnp<sup>−/−</sup>/Prnp<sup>−/−</sup>/mice) heavy PrP<sup>D</sup> accumulations, consistent with localisation upon FDC, were detected at 70 days after i.p. injection with the scrapie agent and had increased in intensity by 105 days after infection (Figure 4A & B). PET immunoblot confirmed the presence of PrP<sup>D</sup> upon the surfaces of the FDC in spleens from control mice (Figure 4C). Furthermore, in the spleens of Prnp<sup>stop</sup>/CD21-Cre Prnp<sup>stop</sup>/mice in which cellular PrP<sup>C</sup> was expressed only on FDC, heavy PrP<sup>D</sup> accumulations were likewise maintained upon FDC (Figure 4A & B). In contrast, in the absence of PrP<sup>C</sup> expression by FDC in the spleens of CD21-Cre Prnp<sup>stop</sup>/Prnp<sup>stop</sup>/mice, no PrP<sup>D</sup> accumulations were observed upon FDC. In the spleens of mice with PrP<sup>C</sup>-deficient FDC, if PrP<sup>D</sup> was detected at all, it was only occasionally observed within tangible body macrophages (Figure 4A and B, arrowheads; Figure S1). We also analysed prion infectivity levels in spleens collected 70 days after infection from control mice (Prnp<sup>−/−</sup>/Prnp<sup>−/−</sup>/mice) and Prnp<sup>stop</sup>/CD21-Cre Prnp<sup>stop</sup>/mice in which cellular PrP<sup>C</sup> was expressed only on FDC (Figure S2; n = 3/group). As anticipated high levels of prion infectivity were observed in each control.
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A

$Pmp^{cre}\rightarrow CD21$-cre $Pmp^{cre}$

CD45R / CD3

CD21/35

C4

B

Average area of FDC

$P = 0.755$

C

No. FDC networks/900 $\mu$m$^2$ field

$P = 0.249$

D

$Pmp^{cre}$

CD21-cre $Pmp^{cre}$

CD21-cre $Pmp^{cre}$

E

Mean distance (um) between FDC and nerves

$P = 0.932$
spleen. Furthermore, consistent with data above our analysis showed that PrP^C expression only of FDC was sufficient to sustain high levels of prion infectivity within the spleen (Figure S2). These data demonstrate that PrP^C expression only on FDC is sufficient to sustain prion replication in the spleen. In the absence of PrP^C expression on FDC the prions appeared to be scavenged by tingible body macrophages resident within the B cell follicles.

FDC-specific Prnp-ablation

Next, mice were created in which Prnp expression was specifically ablated in FDC. To do so, CD21-Cre Prnp^floxed/- mice were crossed with mice carrying a “floxed” Prnp gene (Prnp^floxed/- mice; [31]). In the progeny CD21-Cre Prnp^floxed/- mice, Prnp expression is conditionally ablated in cells expressing Cre recombinase (CD21-expressing FDC and mature B cells). To restrict the Prnp-ablation to FDC, CD21-Cre Prnp^floxed/- mice were lethally γ-irradiated and grafted with bone marrow from Cre-deficient Prnp^floxed/+ mice (Prnp^floxed/+→CD21-Cre Prnp^floxed/- mice). We also performed bone marrow transfers from CD21-Cre Prnp^floxed-/+ donors into CD21-Cre Prnp^floxed/- recipients (CD21-Cre Prnp^floxed-/+→CD21-Cre Prnp^floxed/- mice), CD21-Cre Prnp^floxed-/+ donors into Cre-deficient Prnp^floxed/+ mice (CD21-Cre Prnp^floxed-/+→Prnp^floxed/+ mice), and Prnp^floxed/+ donors into Prnp^floxed/- recipients (Prnp^floxed/+→Prnp^floxed/- mice) as controls (Figure 5A). Spleens, tails and blood from 6 mice from each group were examined 100 days after bone marrow transfusion. PCR analysis of DNA isolated from the spleens, blood and tails of Prnp^floxed/-→CD21-Cre Prnp^floxed/- mice confirmed that efficient Cre-mediated DNA recombination and Prnp-ablation was restricted to the FDC-containing stromal compartment of the spleen (Figure 5B). In Prnp^floxed/-→CD21-Cre Prnp^floxed/- mice the recombinated Prnp^floxed/- allele (Prnp^floxed/-) was detected in the spleen, but not blood and tail. Thus these data indicate that in the spleens of Prnp^floxed/-→CD21-Cre Prnp^floxed/- mice Cre-mediated recombination and Prnp-ablation is restricted to FDC and not B cells.

IHC analysis showed that in the spleens of Prnp^floxed/-→CD21-Cre Prnp^floxed/- and CD21-Cre Prnp^floxed/- mice FDC did not express PrP^F although high levels were associated with TH-positive sympathetic nerves (Figure 5C). In the absence of Cre-recombinase expression by FDC in CD21-Cre Prnp^floxed/-→Prnp^floxed/- mice, high levels of PrP^F were expressed by FDC and sympathetic nerves (Figure 5C).

Morphometric analysis confirmed that the magnitude of the PrP^F expression co-localized upon the surfaces of FDC in the spleens of Prnp^floxed/-→CD21-Cre Prnp^floxed/- mice and CD21-Cre Prnp^floxed/-→CD21-Cre Prnp^floxed/- mice was substantially lower than that observed upon FDC in spleens from Prnp^floxed/-→Prnp^floxed/- control mice (P<0.001; Figure 5D). In contrast, in the absence of Cre-recombinase expression by FDC in CD21-Cre Prnp^floxed/-→Prnp^floxed/- mice, PrP^F expression was not significantly different from the level observed upon FDC in spleens from Prnp^floxed/-→Prnp^floxed/- control mice (P=0.016; Figure 5D). In contrast, morphometric analysis showed that the magnitude of the PrP^F expression co-localized upon the surfaces sympathetic nerves in the spleens of Prnp^floxed/-→CD21-Cre Prnp^floxed/-→CD21-Cre Prnp^floxed/-→Prnp^floxed/-→Prnp^floxed/- mice. In the spleens of Cre-deficient Prnp^floxed/-→CD21-Cre Prnp^floxed/-→Prnp^floxed/-→Prnp^floxed/-→Prnp^floxed/- mice, no PrP accumulations were observed upon FDC (Figure 8A−C). Consistent
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with data above (Figure 4), in spleens of mice with PrP\textsuperscript{C}-deficient FDC PrP\textsubscript{Sc} accumulations were only occasionally observed within tingible body macrophages (Figure 4A and B, arrowheads; Figure S1). We also analysed prion infectivity levels in spleens from Prnp\textsuperscript{flox/\textminus}\textsuperscript{-}\textsuperscript{-}CD21-Cre Prnp\textsuperscript{flox/\textminus}\textsuperscript{-}\textsuperscript{-} mice in which cellular PrP\textsuperscript{C} expression was ablated only on FDC (Figure S2; n = 3). Consistent with data above this analysis showed that in the absence of PrP\textsuperscript{C} expression only on FDC the accumulation of high levels of prion infectivity in the spleen was blocked (Figure S2). Taken together, these data show that in the specific absence of PrP\textsuperscript{C} expression FDC are unable to sustain prion replication upon their surfaces and as a consequence the agent is scavenged by tingible body macrophages.

FDC-restricted PrP\textsuperscript{C}-ablation does not influence prion disease and susceptibility when infection is established directly within the CNS

When mice with PrP\textsuperscript{C}-ablated FDC (Prnp\textsuperscript{flox/\textminus}\textsuperscript{-}\textsuperscript{-}CD21-Cre Prnp\textsuperscript{flox/\textminus}\textsuperscript{-}\textsuperscript{-} mice) were injected intracerebrally (i.c.) with the ME7 scrapie agent strain directly into the CNS all mice succumbed to clinical signs of scrapie approximately 300 days after exposure with incubation periods indistinguishable from those of Prnp\textsuperscript{+}\textsuperscript{-}\textsuperscript{-}\textsuperscript{-} control mice [43] (Prnp\textsuperscript{+}\textsuperscript{-}\textsuperscript{-}CD21-Cre Prnp\textsuperscript{+}\textsuperscript{-}\textsuperscript{-} mice, 297 ± 4 days, n = 4; Prnp\textsuperscript{+}\textsuperscript{-}, 290 ± 4 days, n = 5; P = 0.386). Histopathological analysis showed that brains from all clinically-affected mice from each group displayed the characteristic spongiform pathology, astrogliosis, microgliosis and PrP\textsuperscript{C} accumulation typically associated with terminal infection with the ME7 scrapie agent (Figure S3, third and fourth columns). In contrast, none of the mice with PrP\textsuperscript{C}-ablated FDC (Prnp\textsuperscript{flox/\textminus}\textsuperscript{-}\textsuperscript{-}CD21-Cre Prnp\textsuperscript{flox/\textminus}\textsuperscript{-}\textsuperscript{-} mice, n = 0/6; CD21-Cre Prnp\textsuperscript{+}\textsuperscript{-}\textsuperscript{-} mice, n = 0/7) succumbed to clinical prion disease during their life-spans (Table S1). Although we cannot exclude the possibility that if the clinically-negative mice with PrP\textsuperscript{C}-ablated FDC mice had lived longer some may have succumbed to clinical prion disease after substantially extended incubation periods, no PrP\textsuperscript{D} or other characteristic histopathological hallmarks of prion disease were detected in their brains (Figure S3, first two columns). Together, these data suggest that in the specific absence of PrP\textsuperscript{D} expression on FDC neuroinvasion following peripheral exposure is impaired.

Discussion

These data definitively demonstrate that FDC are essential sites of prion replication in lymphoid tissues. In order to precisely establish the role of FDC in prion pathogenesis two unique compound transgenic mouse models were created in which PrP\textsuperscript{D} expression was specifically “switched on” or “off” only on FDC. Our data confirm that FDC express high levels of PrP\textsuperscript{D} and do not simply acquire it from other host cells. Furthermore, we show that following peripheral exposure PrP\textsuperscript{D}-expressing FDC alone are sufficient to sustain high levels of prion replication in the spleen. Accordingly, when PrP\textsuperscript{D}-expression was specifically ablated only on FDC prion replication in the spleen was blocked. These data likewise demonstrate that FDC do not simply acquire prions after their release from other infected host cells. Our analysis showed that the effects of Prnp-ablation on prion replication in the spleen were specific to FDC and had no effect on prion neuropathogenesis when the infection was established directly in the CNS.

Effect of FDC-restricted PrP\textsuperscript{D}-ablation on prion neuroinvasion after i.p. exposure

Studies in mice show that efficient prion neuroinvasion from peripheral sites of exposure is dependent upon the presence of FDC in lymphoid tissues [1,4,35,44–46]. Next, the effect of FDC-specific Prnp-ablation on prion neuroinvasion via the peritoneal route was determined. Unfortunately, due to the advanced ages of the mice in this experiment, some succumbed to age-related inter-current illness. As there was a 100 days interval between the time of lethal \( \gamma \)-irradiation/bone marrow reconstitution and prion infection, many mice were approximately 500–600 days old when cullled. However, most mice with PrP\textsuperscript{D}-expressing FDC in their spleens succumbed to clinical prion disease after i.p. injection (Prnp\textsuperscript{+}\textsuperscript{-}→ Prnp\textsuperscript{+}\textsuperscript{-} control mice, n = 5/7; CD21-Cre Prnp\textsuperscript{+}\textsuperscript{-}→ Prnp\textsuperscript{+}\textsuperscript{-} mice, n = 3/6; Table S1). Histopathological analysis showed that brains from all clinically-affected mice from these groups displayed the characteristic spongiform pathology, astrogliosis, microgliosis and PrP\textsuperscript{D} accumulation typically associated with terminal infection with the ME7 scrapie agent (Figure S3, third and fourth columns). In contrast, none of the mice with PrP\textsuperscript{D}-ablated FDC (Prnp\textsuperscript{flox/\textminus}\textsuperscript{-}\textsuperscript{-}CD21-Cre Prnp\textsuperscript{flox/\textminus}\textsuperscript{-}\textsuperscript{-} mice, n = 0/6; CD21-Cre Prnp\textsuperscript{+}\textsuperscript{-}\textsuperscript{-}→ CD21-Cre Prnp\textsuperscript{+}\textsuperscript{-}\textsuperscript{-} mice, n = 0/7) succumbed to clinical prion disease during their life-spans (Table S1). Although we cannot exclude the possibility that if the clinically-negative mice with PrP\textsuperscript{D}-ablated FDC mice had lived longer some may have succumbed to clinical prion disease after substantially extended incubation periods, no PrP\textsuperscript{D} or other characteristic histopathological hallmarks of prion disease were detected in their brains (Figure S3, first two columns). Together, these data suggest that in the specific absence of PrP\textsuperscript{D} expression on FDC neuroinvasion following peripheral exposure is impaired.

Figure 4. Effect of FDC-restricted PrP\textsuperscript{D}-expression on PrP\textsuperscript{Sc} accumulation in the spleen. Mice were injected i.p. with the ME7 scrapie agent and tissues collected 35, 70 days and 105 days after exposure. A and B) High levels of PrP\textsuperscript{D} were detected in association with FDC (CD21/35 positive cells) in the B cell follicles (CD45R positive cells) of spleens of mice with PrP\textsuperscript{D}-expressing FDC. Prnp\textsuperscript{flox/\textminus}\textsuperscript{-}→ CD21-Cre Prnp\textsuperscript{flox/\textminus}\textsuperscript{-} mice, CD21-Cre Prnp\textsuperscript{+}\textsuperscript{-}→ CD21-Cre Prnp\textsuperscript{+}\textsuperscript{-} and Prnp\textsuperscript{+}\textsuperscript{-}→ Prnp\textsuperscript{+}\textsuperscript{-} control mice. B) High magnification images of the sites of PrP\textsuperscript{D} accumulation (red) at 70 days post-injection with scrapie. Arrowheads show PrP-accumulation within tingible body macrophages. C) Analysis of adjacent sections by PET-macrophages.
Figure 5. FDC-restricted PrP\(^{\text{c}}\)-ablation in the spleens of \textit{Prnp}\(^{\text{flox/flox}}\)-CD21-Cre \textit{Prnp}\(^{\text{flox/flox}}\) mice. A) The anticipated distribution of PrP\(^{\text{c}}\) expression on FDC and B cells in tissues from each mouse group. B) PCR analysis of DNA isolated from the spleens, blood and tails of \textit{Prnp}\(^{\text{flox/flox}}\)-CD21-Cre \textit{Prnp}\(^{\text{flox/flox}}\) mice confirmed that efficient Cre-mediated DNA recombination and \textit{Prnp}\(^{\text{c}}\)-ablation (\textit{Prnp}\(^{\text{deflox}}\)) was restricted to the FDC-containing stromal compartment of the spleen. Cre-mediated recombination of CD21-expressing lymphocytes was efficiently prevented in these mice by the irradiation and transfer of \textit{Prnp}\(^{\text{flox/flox}}\) bone marrow as demonstrated by the lack of a \textit{Prnp}\(^{\text{deflox}}\) band in DNA extracted from blood (lower panel). B, blood; S, spleen; T, tail; M, DNA size markers; a, b, c, d control DNA samples for each transgene combination tested which were (a) \textit{Prnp}\(^{\text{flox/flox}}\), (b) \textit{Prnp}\(^{\text{flox/flox}}\), (c) \textit{Prnp}\(^{-/-}\) and (d) \textit{Prnp}\(^{\text{flox/flox}}\) with complete recombination of the floxed exon 3. C) IHC analysis of PrP\(^{\text{c}}\) expression (blue) by FDC (CD35\(^{+}\) cells; Role of Follicular Dendritic Cells in Prion Pathogenesis

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and sympathetic nerves (TH + cells, green) confirmed the PrP 0-ablation was restricted to FDC in spleens of Prnp flox/-→CD21-Cre Prnp flox/- mice. Scale bar, 100 μm. D) Morphometric analysis confirmed that the magnitude of the PrP 0 expression co-localized upon the surfaces of FDC in the spleens of Prnp flox/-→CD21-Cre Prnp flox/- mice was significantly lower than that observed upon FDC from Prnp flox/-→Prnp cre/- control mice (p<0.106, n = 48 FDC networks/group). In contrast, in the absence of Cre-recombinase expression by FDC in CD21-Cre Prnp flox/-→Prnp cre/- mice, PrP 0 expression was similar to that observed upon FDC in spleens from Prnp flox/-→Prnp cre/- control mice (p=0.106, n = 48 FDC networks/group). E) Morphometric analysis confirmed that the magnitude of the PrP 0 expression co-localized upon the surfaces sympathetic nerves in the spleens of Prnp flox/-→CD21-Cre Prnp flox/- and CD21-Cre Prnp cre/-mice was not significantly different when compared to that observed upon sympathetic nerves in spleens from Prnp 0→Prnp 0 control mice (p=0.400, n = 48 sympathetic nerves/group). For all panels n = 6 mice/group. doi:10.1371/journal.ppat.1002402.g005

antibody and/or complement components. The longevity of FDC ensures that antigen is retained upon their surfaces for long periods [50,51]. Antigens trapped on the surface of FDC are considered to play an important role in the retention of prions by FDC [15,16,18]. Thus FDC may simply act as concentrating depots for prion-containing complement-opsonized immune complexes.

The practical hurdles that are encountered when attempting to isolate highly purified FDC from lymphoid tissues have made detailed analysis of their pathobiological functions extremely difficult. The main issues include: contamination with other cell types such as B cells and tingible body macrophages which express MFGE8 (FDC-M1), a common marker used to identify FDC [32,56], low yield, and their dependence on constitutive lymphotixin β receptor-stimulation to maintain their differentiated state [57]. FDC and mature B cells express high levels of C2 which encodes the complement receptors CR2/CR1 (CD21/35) [18,27]. A previous study used CD21-crc mice to study FDC-specific gene function [27]. In the current study, our data confirm that Cre/loxP-mediated DNA recombinase was specific to FDC and mature B cells in CD21-crc mice, and could be restricted to FDC by transfecting the mice with Cre-deficient bone marrow. In some Cre transgenic mouse lines Cre-toxicity is encountered whereby Cre recombinase causes mis-recombination, DNA damage and death of Cre-expressing cells [30]. However, our analysis suggested no significant effect of Cre-expression on the number, size and status of FDC networks and B cell follicles. CD21-crc mice are therefore a powerful in vivo tool in which to study FDC-specific gene expression and function.

Expression of PrP 0 is mandatory for host cells to sustain prion infection [43]. In the current study to establish whether FDC actively amplify prions a compound transgenic mouse model was created using the CD21-crc mouse line to specifically “switch on” PrP 0 expression only on FDC (Prnp flox/-→CD21-Cre Prnp 0stop/stop - mice). As a consequence, only FDC in these mice had the potential to be actively infected with and replicate prions. Our analysis showed that expression of PrP 0 only on FDC was sufficient to sustain high levels of PrP 0 accumulation upon FDC in the spleen after peripheral prion exposure. These data definitively demonstrate that the FDC are the critical sites of prion replication in lymphoid tissues. Ultrastructural analysis of the cellular compartments within which PrP 0 localizes upon/within FDC has failed to show any intracellular accumulation. Instead the PrP 0 appears to be restricted to the plasmalemma of their dendritic processes [58]. This implies that early de novo PrP 0 conversion occurs upon the surface of FDC.

A second compound transgenic mouse model was created in which PrP 0 expression was specifically “switched off” only on FDC (Prnp flox/-→CD21-Cre Prnp 0stop/stop - mice). If, as shown above, FDC do actively amplify prions, then one would also expect the specific ablation of PrP 0 expression only on FDC to block prion replication in the spleen. Our data confirmed this to be the case. As PrP 0 expression in all other host cells (eg: neurones) in these mice was unaffected, these data clearly show that FDC do not simply acquire prions following release from other infected host cells, even in mice with clinical prion disease in the brain.
analysis implied that in the spleens of mice with PrP-deficient FDC the prions appeared to be scavenged by tangible body macrophages resident within the B cell follicles. The lack of detection of PrP\(^{\text{Sc}}\) within tangible body macrophages in the spleens of clinically-affected mice with PrP-deficient FDC (Figure 9) clearly demonstrates that these cells are not alternative sites of replication of ME7 scrapie prions. High levels of prions rapidly accumulate within the spleen and other lymphoid tissues within weeks of peripheral exposure. The magnitude of the prion accumulation within the spleen rapidly reaches a plateau level which is maintained for the duration of the disease [13,44]. The maintenance of this plateau may be the consequence of a competitive state whereby FDC act to amplify prions above the threshold required to achieve neuroinvasion, whereas phagocytic cells such as macrophages act to destroy them [59,60]. Indeed increased numbers of PrP\(^{\text{Sc}}\)-containing tangible body macrophages are found within the B cell follicles of TSE-affected animals [58]. This contrast with the specific absence of PrP\(^{\text{Sc}}\) expression by FDC: the initial inoculum is phagocytosed and gradually degraded by mononuclear phagocytes such as tangible body macrophages [59,60]. These data are congruent with data from our earlier study which likewise occasionally detected trace levels of prions from the initial inoculum within tangible body macrophages in the spleens of mice with a PrP\(^{\text{Sc}}\)-deficient FDC-containing stromal compartment [13].

The density of sympathetic nerves can significantly influence the amount of prion accumulation in the spleen [33]. In the current study the distribution of TH-positive sympathetic nerves in the spleens of the FDC-specific gene targeted mouse lines was not adversely affected. Furthermore, when prions were injected directly to the brain, FDC-specific Prnp ablation had no influence on the onset of clinical disease or the neuropathology. These data provide strong evidence that the effects of Cre-mediated Prnp ablation on prion replication in the spleen were specific to FDC and not due to unregulated ablation of PrP\(^{\text{Sc}}\) expression within the nervous system. In the current study PrP\(^{\text{Sc}}\) accumulation upon PrP\(^{\text{Sc}}\)-ablated FDC (Prnp\(^{\text{flox/flox}}\)→CD21-Cre Prnp\(^{\text{flox/flox}}\/) was blocked even in spleens from i.c. injected clinically-scrapie affected mice. These data contrast those reported by Crozet and colleagues [61] which used Tg(OvPrP4) mice that express the ovine Prnp gene under the control of the neuron-specific enolase promoter on neurones it is plausible that greater prion replication occurred within the peripheral nervous system, which may have been subsequently trapped on the surface of the FDC and scavenged by macrophages as the prion burden increased. Similarly, hyper-innervation of the spleen likewise leads to increase prion burden in this tissue [33].

In conclusion, our data demonstrate that PrP\(^{\text{Sc}}\)-expressing FDC are the essential sites of prion replication in lymphoid tissues. Indeed, PrP\(^{\text{Sc}}\)-expression on FDC alone was sufficient to sustain high levels of prion replication. In contrast, the specific ablation of PrP\(^{\text{Sc}}\) expression on FDC blocked prion replication. Although FDC have the capacity to bind exosomes and immune complexes which may contain PrP\(^{\text{Sc}}\), this finding clearly demonstrates that FDC do not simply passively acquire prions from other infected cell populations such as neurones. Previous data show treatments which impair the status or immune complex-trapping function of FDC reduce prion susceptibility after peripheral exposure [1,16,35,46,62]. The demonstration that Prnp-ABLATION only on FDC blocked splenic prion replication without apparent consequences for FDC status represents a novel opportunity to prevent neuroinvasion by modulation of PrP\(^{\text{Sc}}\) expression on FDC.

Materials and Methods

Ethics statement

All studies using experimental mice and regulatory licences were approved by both The Roslin Institute’s and University of Edinburgh’s Protocols and Ethics Committees. All animal experiments were carried out under the authority of a UK Home Office Project Licence within the terms and conditions of the strict regulations of the UK Home Office ‘Animals (scientific procedures) Act 1986’. Where necessary, anaesthesia appropriate for the procedure was administered, and all efforts were made to minimize harm and suffering. Mice were humanely culled using a UK Home Office Schedule One method.

Mice

The CD21-Cre [26], ROSA26\(^{\text{lox/lox}}\) reporter strain [28], Prnp\(^{-/-}\) [12] mice and tga20 mice over-expressing PrP\(^{\text{Sc}}\) [63] were generated as described previously. Prnp\(^{\text{flox/lox}}\) mice have loxP sites flanking exon 3 of the Prnp gene [31]. Prnp\(^{\text{flox/flox}}\) mice have a floxed β-gro cassette inserted into intron 2 of the Prnp gene upstream of exon 3 [31]. Mice were maintained under SPF conditions.

Genotype confirmation by PCR analysis

Prior to their use in experiments, the genotype of each mouse was confirmed by PCR analysis. DNA was prepared from tails, blood and spleens using the DNaseasy blood and tissue kit (Qiagen, Crawley, UK) according to the manufacturer’s instructions. Where indicated DNA samples were analysed for presence of Cre, lacZ, Prnp\(^{-/-}\), Prnp\(^{+/+}\), Prnp\(^{\text{flox/flox}}\), recombinant Prnp\(^{\text{flox/flox}}\) (Prnp\(^{\text{flox/flox}}\)), Prnp\(^{\text{flox/flox}}\) and recombinant Prnp\(^{\text{flox/flox}}\) (Prnp\(^{\text{flox/flox}}\)). Using the primers listed in Table 1. PCR products were resolved by electrophoresis through a 1.0% agarose gel containing 0.002% GelRed (Biotium, Cambridge Biosciences Ltd, Cambridge, UK).
Figure 7. Effect of FDC-restricted PrP<sup>C</sup>-ablation on immune complex trapping. A) Mice were passively immunized with preformed PAP immune complexes, and 24 h later, the presence of immune complexes (red) upon FDC (CD35<sup>+</sup> cells, green) assessed by IHC. Scale bar, 100 μm. B) Morphometric analysis confirmed that the magnitude of the immune complex-trapping co-localized upon the surfaces of FDC from Prnp<sup>−/−</sup> mice was not significantly different from that observed in spleens from control mice. This analysis also confirmed that the immune complexes were preferentially associated with FDC in these tissues and significantly greater than the null hypothesis that the pixels were randomly distributed. *, P<1×10<sup>−21</sup>; **, P<1×10<sup>−32</sup>; ***, P<9×10<sup>−28</sup>. n = 40 FDC networks/group. For all panels n = 6 mice/group. doi:10.1371/journal.ppat.1002402.g007
Bone-marrow from the femurs and tibias of donor mice was prepared as single-cell suspensions (3 × 10^7–4 × 10^7 viable cells/ml) in HBSS (Invitrogen, Paisley, UK). Recipient adult (6–8 weeks old) mice were γ-irradiated (950 rad) and 24 h later reconstituted with 100 μl bone-marrow by injection into the tail vein. Recipient mice were used in subsequent experiments as described 100 days after bone marrow reconstitution to allow sufficient time for removal of...
Figure 9. Effect of FDC-restricted PrP<sup>C</sup>-ablation on PrP<sup>Sc</sup> accumulation in the brains and spleens of scrapie-affected mice. Control mice (Prnp<sup>+</sup> / / mice) and Prnp<sup>+/</sup> →CD21-Cre Prnp<sup>+/</sup> mice that lacked PrP<sup>C</sup>-expressing FDC were injected i.c. with the scrapie agent directly into the CNS. Brains and spleens were collected from clinically scrapie-affected mice to compare the neuropathology and cellular sites of PrP<sup>Sc</sup> accumulation.

A) High levels of spongiform pathology (H&E, upper row), heavy accumulations of PrP<sup>d</sup> (brown, second row), reactive astrocytes expressing GFAP (brown, third row) and active microglia expressing Iba-1 (brown, bottom row) were detected in the hippocampi of the brains of all clinically scrapie-affected mice. Scale bars, 500 μm.

B) High levels of PrP<sup>d</sup> (red) were detected in association with FDC in spleens from clinically scrapie-affected control mice that contained PrP<sup>C</sup>-expressing FDC.

C) PET blot analysis of analysis of adjacent sections by PET-immunoblot analysis confirmed the presence of PK-resistant PrP<sup>Sc</sup> (blue/black). In contrast, no PrP<sup>d</sup> or PrP<sup>Sc</sup> was detected in spleens of Prnp<sup>+/</sup> →CD21-Cre Prnp<sup>+/</sup> that lacked PrP<sup>C</sup>-expressing FDC. Scale bars = 500 μm.

doi:10.1371/journal.ppat.1002402.g009
Table 1. PCR primers used to confirm the genotypes of mice used in this study.

<table>
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<tr>
<td>Cre Rev</td>
<td>GCTAATGTCCTCTCCACTACCTG</td>
<td>300</td>
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<tr>
<td>Prnp Wt Fwd</td>
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<td>Prnp Wt Rev</td>
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<td>Prnpde-flx Rev</td>
<td>GTGTTGTTATGTCGACATGGC</td>
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Fwd, forward primer; Rev, reverse primer; (R), Cre-mediated DNA recombined allele.

long-lived B lymphocyte populations and their replacement from the donor bone marrow.

Histological assessment of LacZ expression

Tissues were first immersed in LacZ fixative (PBS [pH 7.4] containing 2% paraformaldehyde, 0.2% glutaraldehyde, 0.02% Nonidet P40, 0.01% sodium deoxycholate, 5 mM MgCl2) and washed in LacZ wash buffer (PBS [pH 7.4] containing 0.02% Nonidet P40, 0.01% sodium deoxycholate, 2 mM MgCl2). Tissues were subsequently incubated in 15% (wt/vol) sucrose in PBS overnight followed by a further overnight incubation in 30% (wt/vol) sucrose in PBS and embedded in Tissue-Tek O.C.T. compound (Bayer PLC, Newbury, UK). Serial sections (thickness, 6 μm) were cut on cryostat and stained with the following antibodies, respectively, and sections were counterstained with nuclear fast red (Vector Laboratories, Peterborough, UK).

Prion exposure and disease monitoring

For i.c. or i.p. exposure, mice were injected with 20 μl of a 1% (v/v) scrapie brain homogenate prepared from mice terminally-affected with ME7 scrapie-infected spleen tissue serially titrated in tga20 mice using the relationship: γ = -0.4533–0.0.0595x (γ = log ID50 U/μl of homogenate; x = incubation period; R2 = 0.9562). As the expression level of cellular PrP controls the prion disease incubation period, tga20 mice overexpressing PrP are extremely useful as indicator mice in prion infectivity bioassays as they succumb to disease with much shorter incubation times than convention mouse strains [63].

IHC and immunofluorescent analyses

Spleens were removed and snap-frozen at the temperature of liquid nitrogen. Serial frozen sections (10 μm in thickness) were cut on a cryostat and immunostained with the following antibodies: FDCs were visualized by staining with mAb 7G6 to detect CR2/CR1 (CD21/CD35; BD Biosciences Pharmingen), mAb FDC-M2 to detect C4 (AMS Biotechnology, Oxon, UK) or mAb 8C12 to detect CR1 (CD35; BD Biosciences Pharmingen). Cellular PrP was detected using PrP-specific polyclonal antibody (pAb) I83 [66]. B cells were detected using mAb B220 to detect CD45R (Caltag, Towcester, UK), or anti-CD19 (BD biosciences Pharmingen). Marginal zone B cells were detected using mAb 1B1 to detect CD1d (BD Biosciences Pharmingen). Sympathetic nerves were detected using tyrosine hydroxylase (TH)-specific pAb (Chemicon Europe).

For the detection of disease-specific PrP (PrPΔ) in spleens and brains, tissues were fixed in periodate-lysine-paraformaldehyde fixative and embedded in paraffin wax. Sections (thickness, 6 μm) were deparaffinised, and pre-treated to enhance the detection of PrPΔ by hydrated autoclaving (15 min, 121°C, hydration) and subsequent immersion formic acid (98%) for 5 min [67]. Sections were then immunostained with 1B3 PrP-specific pAb. For the detection of EGF-like module-containing mucin-like hormone receptor-like 1 (EMR1)-expressing macrophages, paraffin-embedded spleen sections were micro-waved in citric acid buffer (pH 6.0) for 10 min. Endogenous peroxidase activity was blocked using 1% hydrogen peroxidase in methanol, and macrophages detected using rat mAb F4/80 to detect EMR1 (clone CI:A3-1, AbD Serotec). For the detection of astrocytes, brain sections were immunostained with anti-glial fibrillary acidic protein (GFAP; DAKO, Ely, UK). For the detection of microglia, deparaffinised brain sections were first pre-treated with Target Retrieval Solution (DAKO) and subsequently immunostained with anti-ionized calcium-binding adaptor molecule 1 (iba-1; Wako Chemicals GmbH, Neuss, Germany). Immunolabelling was revealed using HRP-conjugated to the avidin-biotin complex (Novared kit, Vector laboratories, Peterborough, UK).

For light microscopy, following the addition of primary antibodies, biotin-conjugated species-specific secondary antibodies (Stratech, Soham, UK) were applied followed by alkaline phosphatase or HRP coupled to the avidin/biotin complex (Vector Laboratories). Vector Red (Vector Laboratories) and diamonobenzidine (DAB; Sigma Aldrich, Dorset, UK) were used as substrates, respectively, and sections were counterstained with haematoxylin to distinguish cell nuclei. For fluorescent microscopy, following the addition of primary antibody, species-specific secondary antibodies coupled to Alexa Fluor 488 (green), Alexa Fluor 594 (red) or Alexa Fluor 647 (blue) dyes (Invitrogen, Paisley, UK) were used. Sections were mounted in fluorescent mounting medium.
mounting medium (DakoCytomation) and examined using a Zeiss LSM5 confocal microscope (Zeiss, Welwyn Garden City, UK).

Image analysis

Digital microscopy images were analyzed using ImageJ software (http://rsbweb.nih.gov/ij/) as described [68]. Intensity thresholds were first applied and then the number of pixels of each colour (black, red, green, yellow) were then automatically counted and presented as a proportion of the total number of pixels in each area under analysis. The preferential co-localisation of fluorochromes was determined by comparisons of the observed distribution of colours with those predicted by the null hypothesis that each element of positive staining was randomly and independently distributed. Values found to be significantly greater than the null hypothesis confirm significant co-localisation of fluorochromes. Spleens from 6 mice from each group were analyzed. From each spleen, 2 sections were studied and on each section data from 4 individual FDC networks collected. Thus, for each mouse group data from a total of 48 individual FDC were analysed. Similarly, data from 48 images from each group were analyzed to determine the preferential co-localisation of fluorochromes upon TH-positive sympathetic nerves within the spleen. A one-way ANOVA test was then used to compare the null hypothesis (that the pixels were randomly distributed) to the observed levels of co-localisation.

Passive immunization

To assess antigen trapping by FDC in vivo, mice were passively immunized by intravenous injection with 100 μL preformed PAP immune complexes (Sigma). Spleens were removed 24 h later and the presence of FDC-associated immune complexes identified by IHC.

Statistical analyses

Data are presented as mean ± SE. Unless indicated otherwise, significant differences between samples in different groups were sought by one-way ANOVA. Values of P<0.05 were accepted as significant.

Supporting Information

Figure S1 In the absence of PrP⁰ expression by follicular dendritic cells prions are scavenged by tangible body macrophages in the spleen. Mice were injected i.p. with ME7 scaprio prions. Splens from CD21-Cre Prnp<sup>flox/-</sup>→Prnp<sup>stop/-</sup> mice (in which cellular PrP<sup>⁰</sup> was expressed only on B cells, upper row), Prnp<sup>flox/-</sup>→CD21-Cre Prnp<sup>stop/-</sup> mice (with FDC-restricted PrP<sup>⁰</sup> ablation, middle row) and CD21-Cre Prnp<sup>flox/-</sup>→CD21-Cre Prnp<sup>stop/-</sup> mice (in which PrP<sup>⁰</sup> expression was ablated on FDC and B cells, lower row) were collected 70 days after i.p. infection. Due to the absence of PrP⁰-expressing FDC prion replication in these tissues was blocked. However, in the spleens of some of these mice, low levels of PrP⁰ (left-hand column, red) were occasionally localised within cells with characteristics typical of tangible body macrophages. These cells contained the remnants of many phagocytosed apoptotic lymphocytes (tangible bodies, arrowheads) and expressed the tissue macrophage marker EGF-like module-containing mucin-like hormone receptor-like 1 (EMR1) detected by mAb F4/80 (right-hand column, brown).

Data are representative of spleens from at least 4 mice from each group. Sections are counterstained with hematoxylin (blue). Scale bar, 20 μm.

PDF

Figure S2 Follicular dendritic cell-specific PrP⁰ expression alone is sufficient to sustain high levels of prion infectivity in the spleen. Prion infectivity levels were assayed spleens from control mice (Prnp<sup>flox/-</sup>→Prnp<sup>stop/-</sup> mice, Prnp<sup>flox/-</sup>→CD21-Cre Prnp<sup>stop/-</sup> mice in which cellular PrP<sup>⁰</sup> was expressed only on FDC and Prnp<sup>flox/-</sup>→CD21-Cre Prnp<sup>stop/-</sup> mice with FDC-restricted PrP<sup>⁰</sup> ablation (n = 3/group) collected 70 days after i.p. with ME7 scrapie prions. Prion infectivity titres were determined by transmission of tissue homogenates into groups of 4 indicator tg20a mice. Each point represents data derived from an individual spleen. Data below the horizontal line indicate disease incidence in the recipient mice <100% and considered to contain trace levels of prion infectivity. High levels of prion infectivity were detected in spleens of control mice and those in which cellular PrP<sup>⁰</sup> was expressed only on FDC (left-hand and middle panels, respectively). However, this accumulation was blocked in spleens with FDC-restricted PrP<sup>⁰</sup> ablation as only trace levels of infectivity were detected (right-hand panel).

PDF

Figure S3 Effect of FDC-restricted PrP<sup>⁰</sup>-ablation on disease pathogenesis within the brain after i.p. prion exposure. Mice were injected i.p. with ME7 scrapie prions. Brains were collected from mice with PrP<sup>⁰</sup>-ablated and control mice and which were free of the clinical signs of prion disease at the time of cull and the neuropathology within each brain compared. High levels of spongiform pathology (H&E, upper row), heavy accumulations of PrP<sup>⁰</sup> (brown, second row), reactive astrocytes expressing GFAP (brown, third row) and active microglia expressing Iba-1 (brown, bottom row) were detected in the hippocampi of the brains of all clinically scrapie-affected mice (right-hand column, n = 5) and mice in which PrP<sup>⁰</sup> expression was ablated in B cells only (CD21-Cre Prnp<sup>flox/-</sup>→Prnp<sup>stop/-</sup> mice, third column, n = 3). In contrast, none of the mice with PrP<sup>⁰</sup>-ablated FDC (FDC-restricted, Prnp<sup>flox/-</sup>→CD21-Cre Prnp<sup>stop/-</sup> mice, first column, n = 6; FDC and B cells, CD21-Cre Prnp<sup>flox/-</sup>→CD21-Cre Prnp<sup>stop/-</sup> mice, second column, n = 7) developed clinical signs of prion disease during their life-spans or histopathological signs of prion disease in their brains. Scale bar, = 500 μm. Clin., presence of clinical signs of scrapie at the time of cull; Path., histopathological detection of spongiform pathology in the brain; dpi, days post i.p. prion infection.

PDF

Table S1 Effect of FDC-restricted PrP<sup>⁰</sup> ablation on prion disease pathogenesis after i.p. exposure.

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Author Contributions

Conceived and designed the experiments: NAM LM BMB KLB JCM KR JH MB. Performed the experiments: LM KLB NAM. Analyzed the data: LM NAM MB JH. Contributed reagents/materials/analysis tools: MB JCM KR. Wrote the paper: NAM LM BMB KLB JCM KR MB JH.
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