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B Cell-Specific S1PR1 Deficiency Blocks Prion Dissemination between Secondary Lymphoid Organs

Simon W. F. Mok,* Richard L. Proia, † Volker Brinkmann, ‡ and Neil A. Mabbott*

Many prion diseases are peripherally acquired (e.g., orally or via lesions to skin or mucous membranes). After peripheral exposure, prions replicate first upon follicular dendritic cells (FDC) in the draining lymphoid tissue before infecting the brain. However, after replication upon FDC within the draining lymphoid tissue, prions are subsequently propagated to most nondraining secondary lymphoid organs (SLO), including the spleen, by a previously underdetermined mechanism. The germinal centers in which FDC are situated produce a population of B cells that can recirculate between SLO. Therefore, we reasoned that B cells were ideal candidates by which prion dissemination between SLO may occur. Sphingosine 1-phosphate receptor (S1PR)1 stimulation controls the egress of T and B cells from SLO. S1PR1 signaling blockade sequesters lymphocytes within SLO, resulting in lymphopenia in the blood and lymph. We show that, in mice treated with the S1PR modulator FTY720 or with S1PR1 deficiency restricted to B cells, the dissemination of prions from the draining lymph node to nondraining SLO is blocked. These data suggest that B cells interacting with and acquiring surface proteins from FDC and recirculating between SLO via the blood and lymph mediate the initial propagation of prions from the draining lymphoid tissue to peripheral tissues. The Journal of Immunology, 2012, 188: 5032–5040.

Prion diseases (transmissible spongiform encephalopathies) are subacute neurodegenerative diseases that affect both humans and animals. Many prion diseases, including natural sheep scrapie, bovine spongiform encephalopathy, chronic wasting disease in cervids, and variant Creutzfeldt-Jakob disease in humans (vCJD), are acquired peripherally, such as by oral exposure. After exposure, prions first replicate upon follicular dendritic cells (FDC) as they make their journey from the site of infection to the CNS (a process termed neuroinvasion) (1–5). FDC are a unique subset of stromal cells resident within primary B cell follicles and germinal centers of lymphoid tissues (6). Prion accumulation and replication upon FDC are critical for efficient neuroinvasion (1–3, 7). During prion disease, aggregations of PrPSc, an abnormally folded isoform of cellular PrP (PrPc), accumulate in affected tissues. Prion infectivity copurifies with PrPSc and is considered to constitute the major, if not sole, component of the infectious agent (8, 9). Although low levels of prion infectivity are present in the bloodstream of infected animals (10), prions invade the CNS by spreading from lymphoid tissue via the peripheral nervous system as the depletion of sympathetic nerves impedes neuroinvasion (11).

Dietary exposure to bovine spongiform encephalopathy-contaminated meat products is considered the most likely source of vCJD in humans (12). However, in the United Kingdom, four cases of vCJD have been reported in recipients of blood or blood products derived from vCJD-infected donors (13–16). Initial concern that vCJD might have the potential to contaminate the bloodstream came from data from animal studies that demonstrated that many prion strains accumulate in lymphoid tissues prior to neuroinvasion (reviewed in Ref. 17). Subsequent findings that PrPSc could likewise be detected in the lymphoid tissues of vCJD patients further raised this concern (18, 19). Data from studies of experimental mice show that, following peripheral exposure (e.g., orally or via skin lesions), prions first replicate on FDC within the draining lymphoid tissue (e.g., Peyer’s patch or regional draining lymph node) and subsequently spread to most other nondraining secondary lymphoid organs (SLO), including the spleen. A similar situation appears to occur in patients with vCJD, because PrPSc accumulation in lymphoid tissues is restricted during the preclinical phase (14) but is widespread at the clinical stage of disease (18, 19). It is unknown how the propagation of prions occurs between SLO.

The germinal centers in which FDC are situated produce a population of recirculating Ag-specific memory B cells (20). These germinal center B cells preferentially migrate toward the B cell follicle-specific chemokine CXCL13, allowing B cells from one germinal center to seed other germinal centers via the bloodstream (20). B cells were shown to recirculate between lymphoid tissues for several weeks (21), and they can transfer Ag reactivity from the draining lymph node to nondraining lymph nodes within a few days of immunization (22). Several pathogens appear to exploit these characteristics to aid transmission. For example, migrating B cells play a key role in carrying retrovirus infection from lymph nodes to peripheral tissues (23). Naïve B cells were shown to often acquire FDC surface proteins during cognate Ag capture (24). Coupled with their capacity to migrate between lymphoid tissues (25), these data suggest that B cells are ideal candidates by which prion dissemination between SLO may occur. Thus, it is plausible that, soon after exposure, B cells become contaminated with prions within the draining lymphoid...
tissue and disseminate the agent via the bloodstream and lymph between SLO as they circulate around the host. Indeed, in the peripheral blood of scrapie-infected sheep, prion infectivity is associated with the lymphocyte-containing Buffy coat fraction (10). Studies also show that B cells within the peripheral blood of deer infected with chronic wasting disease are likewise associated with prion infectivity (26).

In the current study, the hypothesis was tested that recirculating B cells disseminate prions between SLO. To do so, early prion pathogenesis was studied in model mice in which lymphocyte egress from SLO was blocked. The splenomegaly 1-phosphate receptor (SIPR1) helps to control the egress of newly formed T cells from the thymus and the exit of mature T and B cells from SLO (27–29). SIPR1 is a G protein-coupled receptor that binds the lysophospholipid sphingosine 1-phosphate (SIP). Although ubiquitously synthesized, the concentration of SIP in the blood and lymph is higher compared with SLO. This concentration gradient is considered to promote lymphocyte egress from SLO into the blood and lymph via stimulation of lymphocyte SIPR1. The production of SIP by lymphatic endothelial cells likewise appears to provide an important source of SIP for the egress of lymphocytes from lymph nodes and Peyer’s patches (30). In the absence of SIPR1 stimulation, lymphocytes are sequestered in SLO, causing lymphopenia in the bloodstream and lymph (27). We show in this study that, in mice treated with the SIPR modulator FTY720 or with SIPR1 deficiency restricted to B cells, the dissemination of prions from the draining lymph node to non-draining lymph nodes and the spleen is blocked. These data suggest that B cells recirculating between lymphoid tissues via the blood and lymph play an important role in the initial transfer of prions between the draining lymph node and non-draining SLO.

Materials and Methods

Mice

CD19cre SIPR1loxPloxP mice (31) and tga20 mice (32) overexpressing PrPC were generated as described previously. All mice were bred on a C57BL/6 background and were maintained under specific pathogen-free conditions. All experiments using animal mice and regulatory licenses 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 U.K. Home Office Project License 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 U.K. Home Office Schedule One method. Prior to their use in experiments, the genotype of each CD19cre SIPR1loxPloxP mouse was confirmed by PCR analysis of tail DNA for the presence of Cre and Sippr1, as described (33).

Treatment with FTY720

Chronic SIPR1 blockade was achieved through treatment of mice with FTY720 (Novartis) via drinking water (2 mg/l). A parallel group of mice was provided with regular drinking water as a control.

Prion exposure and disease monitoring

Mice were exposed to ME7 scrapie prions by skin scarification of the medial surface of the left thigh, as previously described (34–36). Briefly, −1 cm² area of hair covering the site to be scarified was trimmed using curved scissors and then removed completely with an electric razor. Twenty-four hours later, a 23-gauge needle was used to create a 5-mm-long abrasion in the epidermal layers of the skin at the scarification site. Then, using a 26-gauge needle, one droplet (~6 μl) 1.0% (w/v) brain homogenate prepared from mice terminally affected with ME7 scrapie prions was applied to the abrasion and worked into the site using sweeping strokes. Every effort was made to ensure that the scarification did not cause bleeding. The scarification site was then sealed with OpSite (Smith & Nephew Medical, Hull, U.K.) and allowed to dry before the animals were returned to their final holding cages. Following exposure, mice were coded and assessed blindly for the signs of clinical prion disease and culled at a standard clinical endpoint (37). Scrapie diagnosis was confirmed blindly on coded sections by histopathological assessment of vacuolation in the brain. For the construction of lesion profiles, vacuolar changes were scored in nine gray matter areas of the brain, as described (38). Where indicated, some mice were culled at the times indicated postinjection with prions, and tissues were taken for further analysis.

For bioassay of prion infectivity, individual half spleens were prepared as 10% (w/v) homogenates in physiological saline. Groups of four tga20 indicator mice were injected intracerebrally with 20 μl each homogenate. The scrapie titer in each sample was determined from the mean incubation period in the indicator mice, by reference to a dose/incubation period response curve for ME7 scrapie prions-infected spleen tissue serially titrated in tga20 mice using the relationship: $y = 9.4533 - 0.0595x$ (where $y = \log ID_{50}$ U.K. homogenate; $x = \text{time (days)}$; $x = 100$). Minimum expression level of cellular PrPSc controls the prion disease incubation period, tga20 mouse overexpressing PrPSc are extremely useful as indicator mice in prion infectivity bioassays; they succumb to disease with much shorter incubation times than do conventional mouse strains (32).

Flow cytometric analysis

Peripheral blood samples were prepared at 4°C in FACSM buffer (PBS [pH 7.4] containing 5% FCS). RBCs were removed using RO lysing buffer (Sigma). After washing in FACSM buffer, nonspecific Ab binding to FcRs was reduced using Sippr1-specific rabbit FcγR IIb Ab (AbD Serotec). B cells were identified using FITC-conjugated rat anti-mouse CD19 mAb (clone 6D5; Invitrogen) and T cells were detected using R-phycocerythrin–conjugated rat anti-mouse CD4 mAb (clone YTS191.1; AbD Serotec) and analyzed on a FACSCalibur flow cytometer (BD Biosciences). Viable cells were gated by forward and side light scatter.

Immunohistochemistry and immunofluorescent analyses

Lymph nodes and 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 Abs: FDC were visualized by staining with anti-mouse FcγRIIIa mAb (BD Serotec); lymphocytes were identified using FITC-conjugated rat anti-mouse CD19 mAb (clone 6D5; Invitrogen); T cells were detected using R-phycocerythrin–conjugated rat anti-mouse CD4 mAb (clone YTS191.1; AbD Serotec) and analyzed on a FACSCalibur flow cytometer (BD Biosciences). Viable cells were gated by forward and side light scatter.

Paraffin-embedded tissue immunoblot detection of PrPSc

PrPSc was detected in paraffin-embedded-tissue (PET) sections of lymph nodes, as described previously (40). Briefly, tissues were fixed in periodate-lysine-paraformaldehyde fixative and embedded in paraffin wax. Sections (6 μm in thickness) were deparaffinized, pretreated to enhance the detection of PrP by hydrated autoclaving (15 min, 121°C, 1.2 kg/cm²), and subsequently immersed in formic acid (98%) for 5 min. Sections were then immunostained with 1B3 PrP-specific pAb. For the detection of astrocytes, brain sections were immunostained with anti-glial fibrillary acidic protein (Dako). For the detection of microglia, deparaffinized brain sections were pretreated with Target Retrieval Solution (DAKO) and subsequently immunostained with anti-ionized calcium-dependent adhesion molecule 1 (MADCAM1). For a control, sections were pretreated by autoclaving (120°C, 15 min) in Ag-retrieval solution (Dako, Ely, U.K.). To avoid the following addition of primary Ab, streptavidin–conjugated or species-specific secondary Abs (anti-mouse IgG2a; H+L conjugated to Alexa Fluor 488 (green) or Alexa Fluor 594 (red) dyes (Invitrogen)) were used. Sections were mounted in fluorescent mounting medium (DAKO) and examined using a Zeiss LSM5 confocal microscope (Zeiss, Welwyn Garden City, U.K.).

Brains were fixed in periodate-lysine-paraformaldehyde followed and embedded in paraffin wax. Sections (6 μm in thickness) were deparaffinized, pretreated to enhance the detection of PrP by hydrated autoclaving (15 min, 121°C, 1.2 kg/cm²), and subsequently immersed in formic acid (98%) for 5 min. Sections were then immunostained with 1B3 PrP-specific pAb. For the detection of astrocytes, brain sections were immunostained with anti-glial fibrillary acidic protein (Dako). For the detection of microglia, deparaffinized brain sections were pretreated with Target Retrieval Solution (DAKO) and subsequently immunostained with anti-ionized calcium-dependent adhesion molecule 1 (Wako Chemicals, Neuss, Germany). Immunohistochemistry was carried out using a 6 mAb 7.1+ 6.3 (NovoRED kit; Vector Laboratories, Peterborough, U.K.).

Paraffin-embedded tissue immunoblot detection of PrPSc

Paraffin-embedded tissue immunoblot detection of PrPSc was performed in paraffin-embedded-tissue (PET) sections of lymph nodes, as described previously (40). Briefly, tissues were fixed in periodate-lysine-paraformaldehyde and embedded in paraffin wax. Serial sections (6 μm thickness) were mounted on polyvinylidene difluoride membrane (Bio-Rad, Hemel Hempstead, U.K.) and fixed by incubation at 55°C overnight. Membranes were then deparaffinized and digested with proteinase K (PK; 20 μg/ml) at 65°C for 16 h at 55°C (to confirm the presence of PrPSc), washed in TBS/Tween (10 mM Tris-HCl [pH 7.8], 100 mM NaCl, 0.5% Tween), and denatured in 3 M guanidine isothiocyanate (10 mM Tris-HCl [pH 7.8]) for 10 min. Membranes were blocked in 2% casein, and
PrP was detected with PrP-specific pAb 1B3, followed by alkaline phosphatase-conjugated goat anti-rabbit antiserum (Jackson Immunoresearch Laboratories, West Grove, PA). Bound alkaline phosphatase activity was detected with SigmaFast NBT/BCIP solution (Sigma, Poole, U.K.). Immunostained membranes were assessed using an Olympus dissecting microscope.

**Immunoblot detection of PrPSc**

Spleen fragments (~20 mg) were prepared as 10% (w/v) tissue homogenates, and PrPSc was enriched by sodium phosphotungstic acid (NaPTA) precipitation (41) and treated in the presence of proteinase K (40 μg/ml, 60 min, 37˚C; VWR, Lutterworth, U.K.). Following enrichment, pellets were resuspended and diluted to an approximate protein concentration of 0.5 mg protein/ml, and 10 μl was electrophoresed through SDS/PAGE gels (12%) (Invitrogen). Proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad) by semidry blotting. PrP was detected with the rabbit PrP-specific mAb (clone EP1802Y; Epitomics, Burlingame, CA), followed by HRP-conjugated goat anti-mouse antiserum. Bound HRP activity was visualized by using SuperSignal West Dura Extended Duration Substrate (Pierce Biotechnology, Rockford, IL).

**Statistics**

Data are presented as mean ± SD, and significant differences between samples in different groups were assessed with the Student t test. The p values < 0.05 were accepted as significant.

**Results**

**Effect of FTY720 treatment on B and T cells**

To study the requirement for recirculating lymphocytes in the dissemination of prions from the draining lymph node to non-draining lymph nodes and the spleen, S1PR1 blockade was used to induce lymphopenia in the blood and lymph by impeding the egress of B and T cells from SLO. Chronic S1PR1 blockade was achieved through continual exposure of C57BL/6 mice to the S1PR modulator FTY720 via drinking water. FTY720 is ideally suited for use in the experiments described below because it is extremely stable in aqueous solution and has been used in long-term studies (up to 12 mo) without adverse affects (42–44). Parallel groups of mice were given normal drinking water as a control. As anticipated, the number of B and T cells (CD19+ and CD4+ cells, respectively) in the bloodstream of FTY720-treated mice was rapidly and significantly reduced compared with controls (Fig. 1A, p < 0.007, n = 4) (27). The lymphopenia was maintained for the duration of the exposure to FTY720. Immunohistochemical (IHC) analysis suggested that no observable effects of FTY720 treatment were observed in the density and overall distribution of B and T cells in lymph nodes and spleens (Fig. 1B). In control mice, most lymphocytes appeared to exhibit a low-intensity homogenous membrane expression of S1PR1 (Fig. 1C). In tissues from FTY720-treated mice, this immunostaining appeared to be more punctate, implying internalization of S1PR1 (Fig. 1C) (29). Together, these data confirmed that chronic FTY720 treatment caused a prolonged lymphopenia by blocking the S1PR1-mediated egress of lymphocytes from SLO.

**FIGURE 1.** FTY720 treatment causes lymphopenia. (A) Frequency of B cells (CD19+) and T cells (CD4+) in the blood of mice administered FTY720 via drinking water. Data are presented as percentage of control group. Each bar represents mean ± SD (n = 4/group). (B) IHC analysis of the effect of FTY720 treatment on the distribution of B cells (B220+ cells, red) and T cells (CD4+ cells, green) in lymph nodes and spleen. Scale bar, 100 μm. (C) IHC analysis of the effect of FTY720 treatment on S1PR1 expression (red) by lymphocytes within lymph nodes. Arrowheads indicate apparent punctate immunostaining in cells from FTY720-treated mice indicative of the internalization of S1PR1. Scale bar, 20 μm. *p < 0.003, **p < 0.005, ***p < 0.007.

FTY720 treatment does not affect FDC status

Following peripheral exposure, prions first replicate upon the surfaces of FDC in the draining lymphoid tissue (3, 34, 36). Host cells must express cellular PrPSc to sustain prion infection, and FDC express high levels of PrPSc on their surfaces (7, 45, 46). FDC in mice also characteristically express high levels of CD35 (complement receptor 1), which was also shown to aid the retention of prions upon FDC (47–49). Therefore, we next determined the effect of FTY720 treatment on FDC status. IHC analysis suggested there was no observable difference in the status of CD35- and PrPSc-expressing FDC within the lymph nodes and spleens of FTY720-treated and control mice (Fig. 2).
without initial contamination of the bloodstream (34–36). This was important because it would not be possible to study any potential effects on prion dissemination between SLO if the inoculation route itself directly contaminated the bloodstream, causing hematogenous spread.

In this study, the normal cellular form of the prion protein is referred to as PrP\text{C}, and PrP\text{Sc} is used to describe the disease-specific, abnormal accumulations of PrP that are characteristically found only in prion-affected tissues (8). Prion disease-specific PrP\text{Sc} accumulations are relatively resistant to PK digestion, whereas cellular PrP\text{C} is destroyed. To confirm the presence of PrP\text{Sc} in lymph nodes, histological sections were applied to nitrocellulose membrane, treated with PK, and subsequently analyzed by PET immunoblot analysis (40).

As anticipated, dense PrP\text{Sc} accumulations, consistent with localization upon FDC (7, 34, 50), were detected at 80 d after prion exposure in the draining inguinal lymph nodes of control mice (Fig. 3A) and FTY720-treated mice (Fig. 3A). Furthermore, FTY720 treatment had no significant effect on the number of PrP\text{Sc}-positive follicles in the draining lymph node compared with controls (Fig. 3A, \( p = 0.705 \), Student \( t \) test; \( n = 4 \)). These data show that S1PR1 blockade had no observable effect on the initial accumulation of prions within the draining lymph node. In control mice, dense PrP\text{Sc} accumulations were also observed in the non-draining inguinal lymph node (Fig. 3A) and the spleen (Fig. 3B), consistent with the dissemination of prions to nondraining SLO by

![FIGURE 2. FTY720 treatment does not affect the status of FDC within SLO. IHC analysis of the expression of CD35 (red) and PrP\text{C} (green) by FDC in lymph nodes and spleens from FTY720-treated and control mice. Data are representative of tissues from four to six mice/group. Scale bar, 100 \( \mu \)m.](image)

![FIGURE 3. FTY720 treatment impedes prion dissemination between SLO. (A) PET-immunoblot analysis of PrP\text{Sc} accumulation (black) within the draining and nondraining lymph nodes of control mice (i, ii) and FTY720-treated mice (iii, iv) collected 80 d after prion exposure by skin scarification (\( n = 4 \)/group). Arrowheads indicate sites of PrP\text{Sc} accumulation in association with FDC. Scale bar, 0.5 mm. Right panel, Number of PrP\text{Sc}-positive follicles/sections in the draining and nondraining lymph nodes from all mice from each group. (B) Western blot analysis of PrP\text{Sc} accumulation within the spleens of control and FTY720-treated mice collected 80 d after prion exposure by skin scarification (\( n = 4 \)/group). Samples were treated with PK prior to electrophoresis to destroy cellular PrP\text{C}. After PK treatment, a typical three-band pattern was observed between molecular mass values of 20 and 40 kDa, representing unglycosylated, monoglycosylated, and diglycosylated isomers of PrP (in order of increasing molecular mass). Each lane represents an individual spleen (\( n = 4 \)/group). (C) Prion infectivity levels were assayed in spleens from control and FTY720-treated mice (\( n = 4 \)/group) collected 44 and 80 d after exposure to ME7 scrapie prions via skin scarification. Prion infectivity titers were determined by transmission of tissue homogenates into groups of four indicator tga20 indicator mice. Each symbol 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.)
this time after exposure. We also analyzed prion infectivity levels in spleens from each group of mice. By 44 d after exposure, only trace levels of prion infectivity were detected in spleens from either group of mice, indicating that significant prion dissemination to nondraining SLO had not occurred at this time. However, high levels of prion infectivity were detected within control spleens by 80 d after exposure (Fig. 3C). In contrast, S1PR1 blockade prevented the dissemination of prions to nondraining SLO. In tissues from FTY720-treated mice, taken at 80 d after prion exposure, no PrPSc was detected in nondraining lymph nodes (Fig. 3Aiv), and no PrPSc or prion infectivity was detected in the spleen (Fig. 3B, 3C). Together, these data clearly show that the FTY720-mediated blockade of lymphocyte egress from SLO impeded the dissemination of prions from the draining lymph node to nondraining SLO.

**S1PR1 blockade does not influence prion neuroinvasion**

We next determined the effect of chronic S1PR1 blockade on the spread of prions to the CNS (neuroinvasion). All control mice succumbed to clinical prion disease, with a mean incubation period of 357 ± 10 d (n = 6). Chronic S1PR1 blockade had no significant influence on neuroinvasion, because all FTY720-treated mice succumbed to clinical prion disease with similar incubation periods (359 ± 9 d, p = 0.534, Student t test, compared with controls; n = 6).

Characteristic disease-specific PrP accumulation, astrocytosis, microgliosis, and spongiform pathology (vacuolation), typically associated with terminal infection with ME7 scrapie prions, were detected in the brains of all clinically affected control and FTY720-treated mice (Fig. 4A). The severity and distribution of the spongiform pathology within the brains of the clinically affected mice from each group were similar and typical of mice clinically affected with ME7 scrapie prions (Fig. 4B).

**Mice with B cell-restricted S1PR1 deficiency**

To determine whether the effects of FTY720 treatment on prion pathogenesis were specifically due to the impairment of B cell egress from SLO, a Cre/LoxP approach was used to create mice in which S1PR1 deficiency was restricted to B cells (31). To do so, CD19cre mice, in which the Cd19 locus directs Cre recombinase expression in B cells, were crossed with mice containing a floxed S1pr1 allele, which encodes S1PR1 (33). In the progeny CD19cre S1PR1flox/flox mice, S1pr1 expression is conditionally ablated only in Cre recombinase-expressing cells (B cells). Cre recombinase-deficient littersmates were used as controls.

As anticipated, B cells were dramatically reduced in the blood of CD19cre S1PR1flox/flox mice compared with Cre-deficient controls, whereas T cells were unchanged (Fig. 5A). No apparent differences were observed by IHC analysis in the overall density and distribution of B and T cells in the spleens (Fig. 5B) and lymph nodes (data not shown). Consistent with the above data (Fig. 2), IHC analysis suggested there was no observable difference in the status of CD35 and PrPC-expressing FDC in CD19cre S1PR1flox/flox and Cre-deficient control mice (Fig. 5C).

**B cell-specific S1PR1 deficiency blocks prion dissemination between SLO**

We next determined whether B cell-restricted S1PR1 deficiency impeded the dissemination of prions to nondraining SLO. Groups of CD19cre S1PR1flox/flox mice and Cre-deficient control mice were exposed to prions via skin scarification, and tissues were collected 105 d after exposure. As anticipated, dense PrPSc accumulations, consistent with localization upon FDC, were detected within the draining inguinal lymph nodes of control and CD19cre S1PR1flox/flox mice (Fig. 6Ai, 6Aiii). Thus, as observed for FTY720-mediated S1PR1 blockade (Fig. 3A), B cell-restricted S1PR1 deficiency did not influence the initial delivery of prions to, or their accumulation within, the draining lymph node. In control mice, the dissemination of prions to nondraining SLO had also occurred, because dense PrPSc accumulations were detected in the nondraining inguinal lymph node (Fig. 6Aii) and the spleen (Fig. 6B). In contrast, in CD19cre S1pr1flox/flox mice with B cell-
restricted S1PR1 deficiency, the dissemination of prions to the nondraining inguinal lymph node (Fig. 6Aiv) and the spleen (Fig. 6B) was blocked. These data clearly show that the inhibition of S1PR1-mediated B cell egress from SLO blocks the dissemination of prions from the draining lymph node to nondraining SLO. Taken together, these data suggest that the recirculation of B cells between SLO via the blood and lymph plays an important role in the initial dissemination of prions between SLO.

S1PR1-signaling blockade displaces marginal zone B cells from the splenic marginal zone

Within the spleen, S1PR1 signaling was shown to promote the positioning of B cells within the marginal zone (28). In mice, marginal zone B cells express high levels of the nonclassical MHC molecule CD1d (28, 51). The splenic marginal zone is delineated by a distinct channel of MADCAM1-expressing sinus-lining cells. In C57BL/6 control mice (Fig. 7A) and Cre-deficient control mice (Fig. 7B), CD1d-expressing marginal zone B cells were present within the marginal zone and B cell follicles. In contrast, FTY720-mediated S1PR1-signaling blockade (Fig. 7A) or B cell-restricted S1PR1 deficiency (Fig. 7B) caused the displacement of marginal zone B cells into the B cell follicles.

Discussion

Following peripheral exposure, prions accumulate first within the draining lymphoid tissue. However, after replication upon FDC within these tissues, prions are propagated to most nondraining SLO, including the spleen, by a previously undetermined mechanism. Lymphocyte S1PR1 stimulation controls the egress of T and B cells from SLO (27–29). When S1PR1 signaling is blocked, lymphocytes are sequestered within SLO, resulting in lymphopenia in the blood and lymph. In this article, we show that, in mice treated with the S1PR modulator FTY720 (52) or with S1PR1 deficiency restricted to B cells (31), the dissemination of prions from the draining lymph node to nondraining lymph nodes and the spleen is blocked. These data suggest that B cells interacting with and acquiring surface proteins from FDC (24), as well as recirculating between lymphoid tissues via the blood and lymph, play an important role in the initial propagation of prions to nondraining SLO.

Taken together, data from the current study and elsewhere suggest that the following factors influence the initial propagation of prions within the periphery. Following peripheral exposure, prions first accumulate and replicate upon the surfaces of FDC within the germinal centers of the draining lymphoid tissue (7, 53, 54). FDC are considered to amplify the prions above the threshold level required for neuroinvasion. Following their expansion upon FDC, prions infect neighboring nerve fibers of the peripheral nervous system from which they spread to the CNS, where they ultimately cause neurodegeneration (11, 55, 56). Within weeks of accumulating within the draining lymphoid tissue, prions are subsequently propagated to most other SLO, including the spleen, implying dissemination via the blood and lymph (3, 34). Within the germinal centers, B cells were shown to
often acquire FDC surface proteins during cognate Ag capture (24). Furthermore, B cells can recirculate between lymphoid tissues for several weeks (21) and can transfer Ag reactivity from the draining lymph node to nondraining lymph nodes within a few days of immunization (22). In the current study, when B cell egress from SLO was specifically blocked, the dissemination of prions to non-draining lymph nodes and the spleen was likewise impeded. These data clearly demonstrate that B cells recirculating between lymphoid tissues via the blood and lymph are crucial for the transfer of prions between the draining lymph node and non-draining SLO.

It is unknown how B cells may acquire prions. Prion disease does not invoke a specific humoral response (57), and disease pathogenesis is unaffected in mice deficient in Ab FcRs or circulating Igs (48). This suggests that cognate (Ag [prion]-specific) capture by B cells via their BCRs is highly unlikely. Prions are considered to be initially acquired by FDC as complement-bound complexes (47–49, 58). B cells were also shown to acquire complement-opsonized Ags in a noncognate (Ag-independent) manner via their complement receptors and deliver them intact to FDC (59, 60), implying a potential mechanism by which B cells may also acquire prions as they travel through the germinal center. Indeed, prion pathogenesis is impaired in the specific absence of complement receptors on B cells (47).

Data from the current study and elsewhere show that B cells influence the prion disease pathogenesis in the periphery in distinct ways. B cells themselves are not sites of prion replication in SLO (7, 46, 61). Data also suggest that prion-contaminated B cells do not play a key role in the transfer of prions directly to the nervous system (62). Instead, B cells indirectly influence prion replication in SLO by their provision of important maturation stimuli, in the form of lymphokines and TNF-α, which maintain FDC in their differentiated state (63). Without these stimuli, FDC rapidly dedifferentiate, and prion accumulation in SLO is blocked (1, 53, 54). Data in the current study add to this by showing how the migratory nature of B cells mediates the initial transfer of prions between SLO.

Data suggest that the intrasplenic positioning of immature hematopoietic classical dendritic cells (DC; a distinct cell lineage from FDC (6)) in the spleen is also regulated by S1P signaling (64). Furthermore, treatment with FTY720 impaired the migration of classical DC from the skin to the draining lymph node (65). Although other effects of S1P1 blockade on prion pathogenesis cannot be entirely excluded, previous data suggest that potential effects on the positioning or migration of classical DC are unlikely to be the major influence. Prion disease pathogenesis was not affected when Langerhans cell migration out of the skin was blocked (35), and, in the current study, FTY720 treatment was not initiated until 14 days post-infection, by which time the prions had been delivered to the draining lymph node and had begun to replicate upon the FDC with them (34). Furthermore, in the current study, the propagation of prions between SLO was also blocked in mice with S1P1 deficiency restricted to B cells.

The positioning of marginal zone B cells in the marginal zone of the spleen is essential for their ability to capture blood-borne Ags. Marginal zone B cells shuttle back and forth rapidly between the marginal zone and follicles, providing an efficient mechanism for systemic Ag capture and delivery to FDC (66). The expression of S1P receptors on marginal zone B cells is important for their positioning within the splenic marginal zone (Fig. 7) (28, 66, 67). Accordingly, in mice treated with FTY720 or with S1PR1-deficient B cells, the capture of blood-borne Ag by marginal zone B cells and their subsequent deposition on FDC are diminished. These data suggest that a possible role for S1PR1 signaling in the capture of prions by marginal zone B cells in the spleen and their delivery to FDC cannot be excluded. However, data in the current study suggest that this is unlikely to be the major influence on disease pathogenesis, because the dissemination of prions to the non-draining lymph nodes was also blocked in mice treated with FTY720 or with S1PR1-deficient B cells.

Chronic S1PR1-signaling blockade had no significant effect on prion neuroinvasion because control and FTY720-treated mice all developed clinical prion disease at similar times after exposure. These data are consistent with the conclusion that B cells do not directly mediate the transfer of prions to the nervous system (62). SLO are highly innervated with sympathetic nerves (68), and their depletion dramatically impairs prion neuroinvasion (11). Data in the current study are also consistent with the peripheral nervous system being the major route of prion transfer to the CNS (11), as well as the demonstration that, following peripheral exposure (orally or via skin lesions), neuroinvasion appears to initially occur directly from the draining lymphoid tissue after replication upon FDC (3, 34, 36, 69).

In conclusion, data in the current study suggest a novel B cell-dependent mechanism by which prions are initially propagated between SLO via the blood and lymph after peripheral exposure. Host inflammation was shown to significantly influence prion disease pathogenesis, either through enhancing prion uptake or expanding their tissue distribution (3, 70–72). This implies that active germinal center responses (e.g., after immunization or in response to concomitant infection with a pathogen) may likewise influence prion pathogenesis by enhancing the propagation of prions within the host by stimulating their dissemination by circulating B cells.

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