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Genetic Depletion of Complement Receptors CD21/35 Prevents Terminal Prion Disease in a Mouse Model of Chronic Wasting Disease

Brady Michel,* Adam Ferguson,* Theodore Johnson,* Heather Bender,* Crystal Meyerett-Reid,* Bruce Pulford,* Adriana von Teichman,† Davis Seelig,* John H. Weis,‡ Glenn C. Telling,* Adriano Aguzzi,† and Mark D. Zabel*

The complement system has been shown to facilitate peripheral prion pathogenesis. Mice lacking complement receptors CD21/35 partially resist terminal prion disease when infected i.p. with mouse-adapted scrapie prions. Chronic wasting disease (CWD) is an emerging prion disease of captive and free-ranging cervids that, similar to scrapie, has been shown to involve the immune system, which probably contributes to their relatively facile horizontal and environmental transmission. In this study, we show that mice overexpressing the cervid prion protein and susceptible to CWD (Tg(cerPrP)5037 mice) but lack CD21/35 expression completely resist clinical CWD upon peripheral infection. CD21/35-deficient Tg5037 mice exhibit greatly impaired splenic prion accumulation and replication throughout disease, similar to CD21/35-deficient murine prion protein mice infected with mouse scrapie. TgA5037;CD21/35−/− mice exhibited little or no neuropathology and deposition of misfolded, protease-resistant prion protein associated with CWD. CD21/35 translocate to lipid rafts and mediates a strong germinal center response to prion infection that we propose provides the optimal environment for prion accumulation and replication. We further propose a potential role for CD21/35 in selecting prion quasi-species present in prion strains that may exhibit differential zoonotic potential compared with the parental strains. The Journal of Immunology, 2012, 189: 4520–4527.

C

Chronic wasting disease (CWD) is the only recognized naturally occurring transmissible spongiform encephalopathy (TSE), affecting captive and free-ranging cervids (1) in North America and captive cervids in South Korea. Similar to other TSEs, CWD is caused by prions, unusual infectious agents devoid of functional nucleic acid (2) and characterized by the accumulation of misfolded prion protein (PrPRES), a proteinase K (PK) resistant form of the normal cellular prion protein, PrPC. CWD and the sheep TSE scrapie can be transmitted relatively efficiently compared with other TSEs, probably contributing to their higher prevalences (3, 4). Prions have been detected in nervous and nonnervous tissues from infected animals, but PrPC expression has been shown to be essential for optimal capture and replication for prolonged periods, maximizing presentation to B cells, which can be positively selected, activated, undergo Ig affinity maturation, and become plasma cells. FDCs may retain Ag on their cell surfaces for prolonged periods, maximizing presentation to B cells and consequently affecting the humoral immune response.

FDC depletion significantly impairs prion replication, and FDC-specific PrPC expression has been shown to be essential for optimal peripheral prion infection (14, 17, 18, 20). B cells, although replicating little prion, also play an essential role in peripheral prion pathogenesis (21, 22). This requirement presumably relates to the ability of B cells to supply FDCs with critical cytokines important in FDC maturation and maintenance, but they may also be involved in lymphotropic and/or intranodal prion trafficking.

Substantial evidence supports a significant role for the complement system in expediting peripheral prion disease by mediating prion interaction with FDCs and B cells. Complement activation leads to asymmetrical cleavage of both C3 and C4 bound to pathogens. Complement receptors CD21/35 expressed on B cells and FDCs trap opsonized pathogens by binding cleaved C3 and C4 opsonins. Mice express CD21 and CD35 only on B cells and FDCs from alternatively spliced transcripts generated from a single gene, whereas humans express them on more cell types from separate genes (23, 24). Although complement-mediated Ag trapping enhances both innate and adaptive immune responses to microbial pathogens, it actually exacerbates prion pathogenesis. Elimination of complement receptors CD21/35 reduces prion trapping, replication, and disease (17). Interestingly, depletion of CD21/35 has a greater impact on disease progression than deleting an initial site of extracerebral prion accumulation and replication. Lymphoid follicles or inflammatory foci accumulate and replicate prions primarily on follicular dendritic cells (FDCs) that express relatively large amounts of PrPC (14–18). FDCs originate from perivascular precursor cells (19) and trap immune complexes on their elaborate projections and present them to B cells, which can be positively selected, activated, undergo Ig affinity maturation, and become plasma cells. FDCs may retain Ag on their cell surfaces for prolonged periods, maximizing presentation to B cells and consequently affecting the humoral immune response.

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their ligand sources, C3 and C4, alluding to a role for CD21/35 in peripheral prion pathogenesis independent of their endogenous ligands. Genetic depletion of C1q also delays prion disease at high doses and prevents disease at low doses after i.p. infection (25, 26), and C1q has been shown to bind prions in vitro (27, 28).

In this study, we show that complete elimination of the complement receptors CD21/35 in transgenic mice susceptible to CWD significantly delays splenic prion accumulation and blocks progression to terminal disease upon inoculation with CWD prions. To assess the kinetics of prion accumulation in the spleen we developed a semiquantitative prion amplification scoring system based on protein misfolding cyclic amplification (PMCA), which allowed us to evaluate prion replication and/or accumulation at 15, 30, 70, and 140 dpi. Mice deficient in CD21/35 show a significant impairment in prion retention and replication compared with CD21/35-sufficient mice. We also observed significant germinal center (GC) formation during scrapie prion infection that was dependent on CD21/35 and PrP expression on FDCs. Lipid raft flotation experiments show movement of CD21/35 into lipid rafts on B cells upon prion infection. Overall, these data demonstrate that CD21/35-mediated prion trapping on FDCs and possibly B cells marks an important event in lymphoid prion pathogenesis that promotes terminal prion disease in these mouse models.

Materials and Methods

Mice

Prnp<sup>−/−</sup>CD21/35<sup>−/−</sup>, C3/C4<sup>−/−</sup>, Tg5037, and TgA20 mice were made as previously described (17, 29, 30). Prnp<sup>−/−</sup>CD21/35<sup>−/−</sup> mice were crossed with TgA20 or Tg5037 mice to produce TgA20;Prnp<sup>−/−</sup>CD21/35<sup>−/−</sup> (TgA20;CD21/35<sup>−/−</sup>) and Tg5037;Prnp<sup>−/−</sup>CD21/35<sup>−/−</sup> (Tg5037;CD21/35<sup>−/−</sup>) mice. All mice were bred and maintained at Laboratory Animal Resources, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, in accordance with protocols approved by the Institutional Animal Care and Use Committee at Colorado State University. Bone marrow chimeric mice were produced as previously described (17).

Preparation of inoculum

Brain homogenates (10%) were prepared in PMCA buffer (4 mM EDTA, 150 mM NaCl in PBS) from E2 homogenates derived from a terminally diseased elk brain. RML5 prions were prepared as previously described (17). Homogenates (10%) were diluted 1:10 (E2 and RML5) or 1:1000 (RML5) in 520 mM sucrose supplemented with 100 U/mI penicillin and 100 μg/ml streptomycin (Life Technologies) in PBS immediately prior to inoculation.

Inoculations, clinical scoring, and dissections

Mice were inoculated i.p. with 100 μl inoculum using a 28-gauge insulin syringe. Mice were monitored for clinical symptoms of prion disease, including tail rigidity, impaired extensor reflex, akinesia, tremors, ataxia, and weight loss. Mice with any four of these symptoms or paralysis were scored terminally sick and euthanized.

Mice were inoculated with inocula described above and euthanized at indicated time points by CO<sub>2</sub> inhalation. Brains and spleens were collected and divided sagittally. One brain hemisphere and half a spleen were fixed in 4% paraformaldehyde in PBS for histology and one was homogenized and used for sodium phosphotungstic acid (NaPTA) precipitation, PMCA, or PK digestion.

NaPTA precipitation of PrP<sup>RES</sup>

NaPTA precipitation was performed exactly as described previously (17). Briefly, gross cellular debris was removed by centrifugation at 80 × g and 500 μl supernatant was mixed 1:1 with 4% sarkosyl in PBS. Samples were incubated for 15 min at 37°C with constant agitation, then incubated with 50 U/ml benzonase and 12.75 mM MgCl<sub>2</sub> for 30 min at 37°C with constant agitation. Prewarmed NaPTA stock solution (pH 7.4) was added to a final concentration of 0.3% and the sample was incubated at 37°C for 30 min with constant agitation and centrifuged at 37°C for 30 min at maximum speed in an Eppendorf microcentrifuge. The pellet was resuspended in 30 μl 0.1% sarkosyl in PBS and digested with 20 μg/ml PK for 30 min at 37°C.

PMCA, PK digestion and Western blotting

PMCA was performed as previously described (13) with slight modifications. Samples were sonicated at 70–85% maximum power

FIGURE 1. Mice deficient in CD21/35 show resistance to CWD prion infection. (A) Tg5037 (blue line, n = 8) and Tg5037;CD21/35<sup>−/−</sup> (red line, n = 11) mice were inoculated with 100 μg brain homogenate from an elk terminally infected with CWD and monitored for terminal disease. Tg5037 mice show a median survival time of 301 dpi, compared with Tg5037;CD21/35<sup>−/−</sup> mice, all of which remained nonclinical to the end of the study (>500 dpi). Immunohistochemistry of brain sections from nonclinical Tg5037;CD21/35<sup>−/−</sup> mice 285 dpi (B, D, F) exhibited minimal vacuolization, prion deposition, and astrogliosis compared with dpi-matched Tg5037 mice (C, E, G). Original magnification ×200. (H) Western blot analysis of PrP<sup>RES</sup> content from Tg5037 and Tg5037;CD21/35<sup>−/−</sup> mice. All samples contain 100 μg protease-digested brain homogenate except lanes 1, 3, and 8, which contain 20 μg undigested brain homogenate (Tg5037 mice [lanes 3 and 8] express 5-fold more PrP than do cervids [E2, lane 1]). (I) Densitometric analyses of protease-resistant bands in the Western blot confirm that the brains from terminal Tg5037 mice contain significantly higher PrP<sup>RES</sup> content compared with Tg5037;CD21/35<sup>−/−</sup> mice. GFAP, Glial fibrillary acidic protein.
for 40 s in a microplate horn sonicator (Osonica, Framingham, MA), followed by a 30-min incubation at 37°C repeated for 24 h per round for up to five rounds total. PK digestion and Western blotting were performed as previously described (31), except that spleen homogenates were digested with 10 µg/ml PK. PrP was detected using HRP-conjugated Bar244 Ab (Bertin Pharma, Paris, France).

**Histology and immunohistochemistry**

Slides were prepared and stained as previously described (31). Briefly, 10-µm sections were cut from paraffin-embedded spleen tissue and mounted onto glass slides. Splenic follicles were stained with rat anti-mouse IgM (02031D; BD Pharmingen) followed by goat anti-rat IgG (H+L) followed by alkaline phosphatase-conjugated donkey anti-goat IgG (705-055-147; Jackson ImmunoResearch Laboratories) and visualized with fast blue (Polysciences, Warrington, PA). GCs were stained with biotin-conjugated peanut agglutinin (PNA) ABComplex/HRP (Dako) and visualized with 3-amino-9-ethylcarbazole (A5754; Sigma-Aldrich).

**Splenic isolation and flotation assays**

Individual whole spleens from at least five mice per group were ground through a nylon mesh to release lymphocytes into single-cell suspensions, which were then centrifuged 5 min at 200 × g and washed twice with ice-cold PBS. The remaining splenic tissue was digested for 20 min at 37°C in 1 mg/ml collagenase, 0.5 mg/ml dispase, and 40 µg/ml DNase I (Roche, Mannheim, Germany) with agitation. Supernatants were collected and the remaining tissue was digested for another 20 min. The samples were pooled and centrifuged 5 min at 200 × g. Cell pellets were washed twice with ice-cold PBS, combined with corresponding lymphocyte pellets, and incubated 30 min on ice in 400 µl 1% Triton X-100 in TNE buffer (25 mM Tris-HCl [pH 7.4], 150 mM NaCl, 5 mM EDTA, 5 mM DTT) containing protease inhibitors (Complete Mini tablet; Roche). Samples were centrifuged 10 min at 1000 × g at 4°C to pellet debris, and supernatants were transferred to new tubes. Lysates (133 µl) were mixed with 267 µl 60% (w/v) OptiPrep solution (Axis-Shield, Oslo, Norway) and pipetted to the bottom of 13 × 51-mm UltraClear centrifuge tubes (Beckman Coulter, Palo Alto, CA). Two hundred-microliter aliquots of 35, 30, 25, and 20% OptiPrep were gently layered on top of the lysates. Tubes were centrifuged 10 min at 12,000 × g at 4°C in an SS-55S Sorvall M150 SE rotor at 120,000 × g. Two hundred-microliter fractions were collected from the top of the tube. SDS-PAGE loading buffer was added to aliquots of each fraction with or without PK digestion (20 µg/ml for 30 min) and subjected to 4–12% gradient PAGE.

**GC counting and statistical and phylogenetic analyses**

Follicles were counted in three nonconsecutive sections from five distinct areas from at least five spleens as IgM+ B cells forming characteristic follicular foci. GCs were counted as PNA+ B cells (brown stain) within follicles (IgM+ blue stain). We derived percentages of follicles containing GCs by dividing the number of GCs by the number of follicles and multiplying by 100. Statistical analyses were performed using Excel (Microsoft) and Prism software (GraphPad Software). CD21 sequence alignment and phylogenetic analysis was performed using Geneious (Biomatters, Auckland, New Zealand).

**Results**

**Absence of CD21/35 protects mice from CWD**

To create a mouse deficient in complement receptors CD21/35 and susceptible to CWD prions, mice deficient in both CD21/35 (CD21/35−/−) and mouse PrP (Prnp(−/−)) were crossed to Tg(cerPrP)5037 mice that express high levels of elk, but no mouse, PrPC. Offspring were then screened and the resulting Tg5037;CD21/35−/− and Tg5037 littermates were inoculated with 100 µg brain homogenate from an elk terminally infected with CWD prions (E2). Tg5037;CD21/35−/− mice showed complete resistance to CWD prions, with no mice showing any clinical signs at the end of the study, whereas infected Tg5037 mice died of CWD at a median time of 301 dpi (Fig. 1A).

We next examined terminally sick Tg5037 and dpi-matched Tg5037;CD21/35−/− mice for characteristic signs of CWD neuropathology (Fig. 1B–G). Minimal astrogliosis and vacuolization...
with no PrPRES deposition were observed in nonclinical Tg5037; CD21/35−/− mice at 285 dpi (Fig. 1B, 1D, 1F). However, significant vacuolization, astrogliosis, and PrPRES deposition were observed in terminally sick Tg5037 mice at 285 dpi (Fig. 1C, 1E, 1G). Western blot and densitometric analyses revealed PrPRES in only 3 of 11 brains from Tg5037;CD21/35−/− mice, significantly less than in brains from terminally sick Tg5037 mice, all of which contained PrPRES and, when compiled together, contained 3.5-fold more PrPRES (Fig. 1H, 1I).

**Absence of CD21/35 delays prion propagation in the spleen**

We next used a semiquantitative prion amplification assay to estimate prion loads in spleens of CWD-infected Tg5037 and Tg5037;CD21/35−/− mice. PMCA, a technique used to amplify prions in vitro, takes advantage of a prion’s ability to self-propagate, using seeded protein fibrillization (Fig. 2A). We employed PMCA to amplify and quantify minute amounts of PrPRES from spleen homogenates at various intervals postinfection. Tg5037 mice at 15 dpi show a significant difference in the amount of splenic PrPRES (75.49 ± 4.43 relative PMCA units [rpu]; Fig. 2B, 2C) compared with Tg5037;CD21/35−/− mice (25.56 ± 4.24 rpu). Using our standard curve for this assay generated previously, we estimate that splenic PrPRES load in Tg5037 mice is ∼20,000 pg/g spleen tissue (Fig. 2C). The PMCA score for Tg5037;CD21/35−/− mice falls just out of the dynamic range of our assay (29 rpu), so we can only estimate the load to be <100 pg/g. We detected much less PrPRES in spleens from Tg5037 mice at 30 dpi (17.17 ± 0.16 rpu) whereas Tg5037;CD21/35−/− spleens showed only a slight decrease in PrPRES load (21.88 ± 0.15 rpu). Both scores fall just below the range of the PMCA score standard curve. Accumulation of PrPRES differed drastically between the two groups at 30 dpi. PrPRES load increased significantly more in Tg5037 mice from 70 (37.50 ± 0.15 rpu, 250 pg/g) to 140 dpi (55.56 ± 0.11 rpu, 2000 pg/g) to terminal disease (71.00 ± 0.2 rpu, 10,000 pg/g). We detected significantly less PrPRES in spleens from nonclinical Tg5037;CD21/35−/− mice at 70 (20.00 ± 0.14 rpu, <100 pg/g) and 140 dpi (41 ± 0.16 rpu, 500 pg/g) and at dpi matched to sick Tg5037 mice (47.00 ± 0.50 rpu, 900 pg/g).

**CD21/35 mediate a strong GC response during prion infection**

Increased PrPRES retention could be mediated via formation of GCs. Ag trapping by Fcy receptors and CD21/35 on FDCs, as well as concomitant B cell signaling through the BCR and the CD21/CD19/CD81 coreceptor, stimulates lymphoid follicles to generate GCs (32–35) containing arborized FDCs. Prolonged Ag presentation by FDCs and additional signals (CD40/CD40L) activates B cells to become plasma or memory B cells (36, 37). In mice that were only nominally increased and less than WT spleens upon prion infection (D). Scale bar, 100 μm. (E) CD21/35 are greatly enriched in NaPTA-precipitated PrPRES preparations from spleens of terminally sick mice compared with PrPRES−/− and mock-infected WT mice. PrPRES was precipitated from 500 μg total spleen homogenate and equal volumes were loaded into each lane.

**Figure 3.** Prion infection stimulates GC formation and increases CD21/35 presence in prion-enriched spleen preparations. (A–E) Mice (n ≥ 10) were inoculated i.p. with 0.1% prion-infected brain homogenate, sacrificed 60 dpi, and frozen spleen sections with stained with IgM (blue stain) and PNA (brown) that reveal GCs in splenic follicles from uninfecte

or without CD21/35 expression, after infecting them with RML5 mouse-adapted scrapie prions. We discovered that i.p. inoculation of high and low doses of prions, but not uninfected (mock) brain homogenate, stimulated significant GC formation in spleens of WT, but not CD21/35−/−, mice (Fig. 3A–D, Table I; n ≥ 5). Interestingly, prion infection, but not 109 CFU heat-killed Escherichia coli or DNP-keyhole limpet hemocyanin (data not shown), induced GC formation in C3/4−− mice, suggesting that CD21/35 can mediate prion-induced GC reactions independent of its endogenous ligands.

### Table I. GC formation in peripheral prion disease

<table>
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<tr>
<th>Inoculum</th>
<th>dpi</th>
<th>WT</th>
<th>CD21/35−/−</th>
<th>C3/4−/−</th>
</tr>
</thead>
<tbody>
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<td>1% NBH</td>
<td>120</td>
<td>34 ± 5</td>
<td>14 ± 3*</td>
<td>15 ± 4*</td>
</tr>
<tr>
<td>0.1% IBH</td>
<td>30</td>
<td>33 ± 8</td>
<td>25 ± 3</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>60</td>
<td>44 ± 14</td>
<td>17 ± 5*</td>
<td>37 ± 7</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>55 ± 6</td>
<td>21 ± 4*</td>
<td>51 ± 5</td>
<td></td>
</tr>
<tr>
<td>1% IBH</td>
<td>Terma</td>
<td>67 ± 4</td>
<td>23 ± 3*</td>
<td>62 ± 6</td>
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<tr>
<td>109 E. coli</td>
<td>Terma</td>
<td>96 ± 16</td>
<td>27 ± 5*</td>
<td>72 ± 14</td>
</tr>
</tbody>
</table>

*aNumber of GCs/number of follicles.

bFor term, dpi ranged from 280 to 385.

cp < 0.01.

IBH, Infected brain homogenate; NBH, normal brain homogenate.

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Moreover, we detected significant amounts of CD21/35 in PrPRES preparations enriched by NaPTA precipitation from spleen homogenates of infected mice at 60 dpi, but very little CD21/35 from NaPTA precipitates from mock-infected spleens (Fig. 3E). We recovered far less PrPRES from CD21/35−/− spleens, consistent with our PMCA data from Tg5037;CD21/35−/− spleens.

CD21/35 translocate to lipid rafts on B cells upon prion infection

GC formation by the traditional primary immune response requires CD21/35 expression on B cells but not on FDCs (35). Closer examination of GC formation in bone marrow (BM) chimeric mice infected with prions divulged a pattern of GC formation predominantly dependent on both CD21/35 and PrP expression (Table II; n ≥ 5), suggesting that CD21/35 Ag presentation is more important than CD21/35 signaling to mediate this reaction. To resolve this discrepancy in GC formation we assessed whether prion infection alters localization of CD21/35 on the plasma membrane. In a typical primary immune response, C3d- or C4d-opsonized Ag binds to specific short consensus repeats of C4d-opsonized Ag binds to specific short consensus repeats of CD21/35, but not IgH, translocate to lipid rafts and colocalize with PrPRES and the raft marker flotillin on prion-infected WT splenocytes (Figs. 4A and 4B). In contrast, PrP was detected in detergent-insoluble fractions containing lipid rafts that float to the top of the density gradient and colocalize with the raft marker flotillin. Upon prion infection, a significant amount of CD21/35 moved into detergent-insoluble fractions (Fig. 4B). CD21/35 were present in the same fractions as flotillin and PK-resistant PrPRES, but not the IgH, indicating that CD21/35 translocation occurred independent of the BCR. Furthermore, whereas a T cell-dependent Ag failed to stimulate CD21/35 translocation to lipid rafts in C3/4−/− mice (Fig. 4C), prion infection induced a significant amount of CD21/35 translocation (Fig. 4D). These data strongly suggest that CD21/35 can interact with prions independent of its endogenous ligands, which could explain why CD21/35−/− mice exhibit a more significant delay in disease progression than do C3/4−/− mice.

![Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** Prion infection stimulates CD21/35 translocation to lipid rafts on B cells in CD21/35-expressing mice. Splenocytes (2 × 10^7) were lysed in ice-cold Triton X-100 and cleared lysates were centrifuged at the bottom of a density gradient (see Materials and Methods). (A) CD21/35 and the BCR IgH reside outside lipid rafts on mock-infected WT splenocytes. (B) CD21/35, but not IgH, translocate to lipid rafts and colocalize with PrPRES and the raft marker flotillin on prion-infected WT splenocytes. (C) CD21/35 reside outside lipid rafts on C3/4−/− splenocytes from mice inoculated i.p. with 10^8 CFU heat-killed *E. coli*. (D) Upon prion infection, CD21/35 moves into lipid rafts independent of its endogenous ligands in C3/4−/− mice. (E) Infected CD21/35−/− splenocytes retain less PrPRES than do infected WT splenocytes [compare with (B)]. (F) Irradiated WT mice reconstituted with CD21/35−/− bone marrow express CD21 only on FDCs. Upon prion infection, CD21 remains outside lipid rafts, indicating that prion-stimulated CD21/35 translocation occurs only on B cells. Data are representative of at least five independent experiments.

### Table II. GC formation in BM chimeric mice infected with prions

| Donor BM | Host Genotype | CD21/35 Expression | PrP Expression | % GCc
|----------|---------------|--------------------|---------------|---
| WT       | WT            | Both               | Both          | 72 ± 12
| WT       | CD21/35−/−    | B cells            | Both          | 22 ± 4
| CD21/35−/− | WT            | FDCs               | Both          | 78 ± 13
| CD21/35−/− | CD21/35−/−    | Neither            | Both          | 23 ± 3
| WT       | PrP−/−        | Both               | B cells       | 15 ± 11
| CD21/35−/− | PrP−/−        | FDCs               | B cells       | 11 ± 7
| PrP−/−   | WT            | Both               | FDCs          | 73 ± 9
| PrP−/−   | CD21/35−/−    | B cells            | FDCs          | 21 ± 2
| PrP−/−   | CD21/35−/−    | Neither            | FDCs          | 19 ± 2
| WT       | PrP−/−CD21/35−/− | FDCs               | B cells       | 13 ± 6
| PrP−/−   | CD21/35−/−    | Neither            | FDCs          | 10 ± 4
| PrP−/−   | CD21/35−/−    | Neither            | FDCs          | 12 ± 2
| PrP−/−   | CD21/35−/−    | Neither            | FDCs          | 13 ± 4

*BM was isolated from mice of the indicated genotype.

**Hemopoietic systems of sublethally irradiated mice of the indicated genotypes were reconstituted with donor BM.**

**Materials and Methods**

**CD21/35 or PrP expression was restricted to the indicated cell types for each reconstitution group.**

**Mice inoculated with 1% infected brain homogenate analyzed 90 dpi.**

**p < 0.01.**
mice. Although the lack of CD21/35 expression does not completely prevent PrPRES accumulation in lipid rafts, it significantly decreases PrPRES load (Fig. 4E). Flotation assays on spleens from BM chimeric mice revealed that FDCs appear to express only the short form of the complement receptor CD21, and that prion infection fails to translocate CD21 to lipid rafts on FDCs (Fig. 4F). Thus, prion infection provokes CD21/35 translocation on B cells, which express all members of the CD21/CD19/CD81 coreceptor complex, but not on FDCs, which only express CD21.

**Discussion**

We investigated the role of the complement receptors CD21/35 in CWD prion accumulation, replication, and disease progression. We observed a complete rescue from terminal CWD of Tg5037 mice lacking CD21/35. Only 3 of 11 nonclinical Tg5037;CD21/35 

mice displayed detectable, yet reduced, prion neuropathology and PrPRES deposition in their brains. These results reveal a more dramatic outcome than earlier studies showing only a partial rescue of CD21/35-deficient mice from scrapie infection, despite those mice expressing only WT (i.e., 5-fold less) PrP levels. This could reflect differences between mouse and cervid CD21 expression, as are apparent between mouse and human CD21. However, little is known about cervid CD21. The gene has yet to be cloned, so comparative analyses with murine CD21/35 are impossible at present. We can, however, compare CD21 sequence homology and phylogeny among other species that are susceptible to TSEs. For example, sheep, which are susceptible to scrapie, a TSE that closely resembles CWD in transmission efficiency, and lymphotropism, express a CD21 molecule that shares 65% sequence identity with murine CD21/35, including their ligand binding domains (Fig. 5A). This may explain the similar lymphotropic characteristics of murine and ovine scrapie. Ovine CD21 also shares 65% identity with human CD21/35. Overall, CD21/35 from these three species share 52% identity and 64% similarity. In contrast, bovine CD21, which is 40% larger than the other three CD21/35 molecules (∼1400 compared with ∼1000 aa, respectively), shares <20% similarity to the other three CD21/35 molecules. Phylogenetic analysis reveals a clustering of murine, ovine, and human CD21/35 proteins, with bovine CD21 much more distantly related (Fig. 5B). Interestingly, bovine spongiform encephalopathy has been shown to have little or no lymphotropic characteristics (44–47), perhaps owing to the vastly different CD21 molecule that bovids express.

These results indicate a significant role in prion pathogenesis for CD21/35, the importance of which may vary by prion strain. Complement components C1q and C3 have recently been shown to exhibit similar strain preferences in vitro and in vivo (48). We are currently investigating other prion strains to determine the contribution of CD21/35 to prion pathogenesis in those infection models. Interestingly, cross-species prion transmission was recently shown to result in a higher infection rate of the lymphoreticular system than the CNS in the xenohost (49). This cross-species infection resulted in distinct lymphotropic and neurotropic strains with differential host ranges. These strains may result from tissue-specific strain selection or mutation. The higher efficiency of prion infection in the spleen (which harbors CD21/35-expressing FDCs and B cells) compared with the brain (which lacks them) alludes to a critical role for CD21/35 in prion retention, replication, and possibly strain selection in trans-species prion infection. The lack of CD21/35 that delays peripheral prion accumulation might further limit the lymphoid replication of neurotropic prion strains, resulting in delayed or aborted disease progression. If so, this would have profound implications for prion xenotransmission and possible therapeutic approaches aimed at CD21/35. For example, targeting CD21/35 to slow the spread of neurotropic prions could be an attractive alternative to most prion disease therapeutics developed to date that target the CNS, which can complicate drug delivery. Interfering with CD21/35-mediated...
prion strain selection could also mitigate emergence of new prion strains with expanded host ranges and prevent a breach of the species barrier similar to the one that likely caused the bovine spongiform encephalopathy and subsequent new-variant Creutzfeldt-Jakob disease outbreak 15 y ago in the United Kingdom.

To study the kinetics of extraneural CWD prion accumulation, we amplified PrPRES from spleens of CWD prion-infected Tg5037 and Tg5037;CD21/35−/− mice at various time points throughout infection. At 15, 70, and 140 dpi and at terminal disease, prion accumulation was significantly lower in CD21/35-deficient mice. The extremely high prion load detected at 15 dpi most likely reflects increased retention of prion inocula early after infection. This delay in extraneural prion accumulation strongly correlates with abrogation of prion neuropathology and terminal disease. These results coincide with our previous data from scrapie mouse models (17), further strengthening evidence that CD21/35 play an integral part in prion accumulation in peripheral lymphoid organs that ultimately facilitates neuroinvasion.

Furthermore, we show that CD21/35 are present in prion preparations enriched from spleen homogenates by NaPTA precipitation. We also demonstrate GC formation in spleens during prion infection primarily dependent on CD21/35 and PrPC expression on FDCs. It may seem surprising that CD21/35 expression on FDCs, rather than B cells, correlates with prion-induced GC formation, because CD21/CD19/CD81 B cell coreceptor ligation helps activate B cells to form GCs. However, maximal B cell activation and GC formation require signaling from both the BCR and B cell coreceptor (32, 33). In this study, we show that although prion infection stimulates CD21/35 translocation to lipid rafts on B cells, signaling appears to be suboptimal for GC formation in the absence of concomitant BCR translocation. We observed a strong dependence on both PrP and CD21/35 expression on FDCs for a strong GC response. Paradoxically, CD21/35 translocation did not occur on FDCs, which are the major prion trappers and replicators but lack other B cell coreceptor components required for CD21/35 movement. One could therefore argue that GC formation represents an artifact, rather than being a driver of splenic prion replication. Elimination of GCs had no effect on peripheral prion replication and disease progression in mice infected i.p. with RML (50), supporting this interpretation. However, GC-deficient mice infected intracranially with 139A mouse-adapted scrapie prions exhibited a significant delay to terminal disease (51). Thus, distinct prion strains may differentially influence GC formation and subsequent prion pathogenesis. Additionally, this discrepancy further highlights potential preferences of distinct tissues for different prion strains. CD21/35-expressing cells within GCs may facilitate this selection process in the lymphoid system. Increased retention of prions on FDCs could induce a persistent state of prion presentation to adjacent B cells sufficient to cause a atypical GC response (40). FDCs may cox B cells to linger there, providing increased lymphoatom signaling to FDCs that may promote formation of hypertrophic dendrites that efficiently retain and replicate prions. Consistent with their role as long-lived, long-term APCs, FDCs may also dendrites that efficiently retain and replicate prions. Consistent with their role as long-lived, long-term APCs, FDCs may also.

Terminal disease (51). Thus, distinct prion strains may differen-

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Disclosures
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References


