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In Vivo Depletion of CD11c+ Cells Impairs Scapie Agent Neuroinvasion from the Intestine

Claudine R. Raymond,* Pierre Aucouturier, † and Neil A. Mabbott2*

Following oral exposure, some transmissible spongiform encephalopathy (TSE) agents accumulate first upon follicular dendritic cells (DCs) in the GALT. Studies in mice have shown that TSE agent accumulation in the GALT, in particular the Peyer’s patches, is obligatory for the efficient transmission of disease to the brain. However, the mechanism through which TSE agents are initially conveyed from the gut lumen to the GALT is not known. Studies have implicated migratory hemopoietic DCs in this process, but direct demonstration of their involvement in vivo is lacking. In this study, we have investigated the contribution of CD11c+ DCs in scrapie agent neuroinvasion through use of CD11c-dipheria toxin receptor-transgenic mice in which CD11c+ DCs can be specifically and transiently depleted. Using two distinct scrapie agent strains (ME7 and 139A scrapie agents), we show that when CD11c+ DCs were transiently depleted in the GALT and spleen before oral exposure, early agent accumulation in these tissues was blocked. In addition, CD11c+ cell depletion reduced susceptibility to oral scrapie challenge indicating that TSE agent neuroinvasion from the GALT was impaired. In conclusion, these data demonstrate that migratory CD11c+ DCs play a key role in the translocation of the scrapie agent from the gut lumen to the GALT from which neuroinvasion subsequently occurs.

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transmissible spongiform encephalopathies (TSEs), or “prion diseases,” are subacute neurodegenerative diseases that affect both humans and animals. In the TSE-affected host, pathology appears to be restricted to the CNS and characteristics includes neuronal loss, spongiform pathology, glial activation, and amyloidoid aggregations of an abnormally folded host protein. The host prion protein (PrP*) is widely expressed in both humans and animals, and its expression is crucial for TSE disease susceptibility. During TSE disease, changes occur to the secondary and tertiary conformation of PrP dramatically affecting the physicochemical and biological properties. This disease-specific isoform of the prion protein, termed PrPSc, accumulates in TSE-affected tissues in abnormal, detergent insoluble, relatively protease-resistant aggregates. The nature of the TSE agent is uncertain, but infectivity copurifies with PrPSc in diseased tissues and is a useful biochemical marker for the TSE agent (1). The “prion hypothesis” argues that PrPSc constitutes a major, or the sole, component of infectious agent and facilitates conversion of PrP* to PrPSc.

Some TSE agents, 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). Experimental studies in mice (2–5) and analysis of tissues from sheep with natural scrapie (6), mule deer fawns with chronic wasting disease (7), and a patient with variant Creutzfeldt-Jakob disease (8) suggest that after oral exposure TSE agents accumulate first upon follicular dendritic cells (FDCs) in B cell follicles within the GALT as they make their journey from the site of infection to the CNS (a process termed, neuroinvasion). FDCs are a distinct lineage from migratory hemopoietic DCs because they are considered to derive from stromal precursor cells, are nonphagocytic, and are nonmigratory (9–11). FDCs characteristically trap and retain native Ags on their cell surfaces in the form of immune complexes, through Fc and complement receptor binding (10). In mouse TSE models, agent accumulation upon PrPSc-expressing FDCs within the GALT is critical for disease pathogenesis after oral exposure, as in their absence, neuroinvasion is significantly impaired (2–5). From the lymphoid tissues, translocation to the CNS occurs via the peripheral nervous system (12).

How TSE agents are initially conveyed from the gastrointestinal tract to the GALT where they first accumulate before neuroinvasion is uncertain. The demonstration of the cells or mechanisms involved in TSE transport may identify an important process which influences disease susceptibility or to which therapeutic intervention can be directed. Following ingestion, the TSE agent must first cross the intestinal epithelium, but the luminal surface is made up of a single layer of epithelial cells bound by tight junctions which limits the access of pathogenic microorganisms to the underlying host tissues. Located within the follicle-associated epithelia (FAE) of Peyer’s patches (PPs), and isolated lymphoid follicles are microfold cells (M cells) which are specialized for the transepithelial transport of Ags (13, 14). Although M cells enable the host’s APCs to sample the contents of the intestinal lumen and initiate appropriate immune responses, some pathogenic microorganisms exploit them to gain entry into mucosal tissues (13). An in vitro study suggested that M cells are plausible sites for the transepithelial transport of TSE agents across the intestinal epithelium (15), however, intestinal epithelial cells themselves may also have the potential to transcytose TSE agents (16). Also, DCs can

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3 Abbreviations used in this paper: TSE, transmissible spongiform encephalopathy; PrP, prion protein; FAE, follicle-associated epithelium; DC, dendritic cell; FDC, follicular DC; PP, Peyer’s patch; MLN, mesenteric lymph node; DTR, dipheria toxin receptor; Ig, transgenic; DTx, dipheria toxin; WT, wild type; i.c., intracerebral; GFAP, glial fibrillary acid protein; pAb, polyclonal Ab.
insert dendrites through the tight junctions between intestinal epithelial cells to directly sample the luminal contents implying another potential route into the mucosa (17).

Once TSE agents have crossed the epithelial cell barrier into the underlying lamina propria, they accumulate upon FDCs within the B cell follicles of the GALT (5). Ags that have been transcytosed by M cells exit into the intraepithelial pocket on the basolateral membrane where they are processed by the lymphocytes, macrophages, and DCs situated within it or immediately below in the subepithelial dome (13). Migratory hematopoietic DCs continually circulate throughout the host's tissues and tissue fluids sampling Ags and transporting them to lymphoid tissues. Indeed, DCs are centrally involved in the transport of proteins both within PPs and on into the mesenteric lymph nodes (MLNs) (18, 19). These characteristics suggest DCs are plausible candidates to transport TSE agents to the GALT. After intraintestinal exposure, PrPSc was observed in a population of DCs migrating in the mesenteric lymphatics (20). However, direct data on the role of DCs in TSE agent neuroinvasion are currently inconclusive and ultimate confirmation of their involvement in neuroinvasion will come from studies where these cells are depleted, or where their function or migration is compromised.

In this study, we have used CD11c-diphtheria toxin receptor (DTR)-transgenic (tg) mice (21) to determine whether CD11c+ DCs have a role in scrapie agent neuroinvasion from the intestine. In these mice, treatment with diphtheria toxin (DTX) transiently depletes CD11c+ cells in vivo (21) whereas FDCs are unaffected as they do not express CD11c (9). Using this model, we show that in the transient absence of CD11c+ cells at the time of oral exposure, early scrapie agent accumulation in the GALT is blocked and disease susceptibility reduced. Our data suggest that CD11c+ DCs play a critical role in the initial translocation of the TSE agent from the gut lumen to the GALT.

FIGURE 1. DTX treatment transiently depletes CD11c+ cells in the PPs, villus cores, MLNs, and spleens of CD11c-DTR-tg mice. A, CD11c+ cells (green) were undetectable in PPs (upper row) and villus cores (lower row) of CD11c-DTR-tg mice 1 day after treatment with DTX. Within 6 days of treatment, the distribution of CD11c+ cells in DTX-treated CD11c-DTR-tg mice appeared similar to that observed in control mice. The expression of PrPSc by FDCs within the follicles (upper row; red arrows) appeared unaffected by DTX treatment. SED denotes the location of the subepithelial dome. B, Status of FDCs (FDC-M2-binding cells; red), CD11b+ cells (green), F4/80+ cells (green), B cells (CD45R+ cells; red), macrophages/monocytes (MOMA-2-binding cells; green), and neutrophils (red) in the MLNs of DTX-treated WT mice (upper row) and CD11c-DTR-tg mice (lower row). Arrow denotes the presence of MOMA-2-binding tingible body macrophages in PPs of DTX-treated CD11c-DTR-tg mice, whereas MOMA-2-binding cells in the subepithelial dome (arrowhead) were absent. C, Effect of DTX treatment on CD11c+ cells (green; upper row), PrPSc expression within the follicles (red; upper row; arrows), neutrophils (red; lower row), and MOMA-1-binding metallophilic macrophages (green; lower row) in the MLNs of WT and CD11c-DTR-tg mice. No significant increase in the number of neutrophils was observed in the spleen following DTX treatment (red). Each PP image is orientated such that the intestinal lumen is uppermost. Original magnification: A, ×400; B, ×100; C and E, ×200.
Materials and Methods

Mice

CD11c-DTR-tg mice (21) (a gift from S. Jung, Weizmann Institute of Science, Rehovot, Israel) and tga20 mice (22) were maintained on a C57BL/6 background. Age- and sex-matched C57BL/6 mice were used as immunocompetent wild-type (WT) controls. Mice were maintained under strict specific-pathogen-free conditions. All protocols using experimental mice were approved by the Neurodegeneration Unit’s Protocols and Ethics Committee and conducted according to the strict regulations of the U.K. Home Office Animals (Scientific Procedures) Act 1986.

Depletion of CD11c+ cells and scrapie agent inoculation

To deplete CD11c+ cells, CD11c-DTR-tg mice were given a single i.p. injection of 100 ng of DTX (Sigma-Aldrich). Groups of WT mice were injected i.p. with DTX and groups of CD11c-DTR-tg mice were injected i.p. with PBS as controls. For oral scrapie agent inoculation, mice were fed individual food pellets doused with either 1) 50 µl of a 1.0% (w/v) scrapie brain homogenate prepared from mice terminally affected with ME7 scrapie, or 2) 100 µl of a 10.0% (w/v) scrapie brain homogenate prepared from mice terminally affected with 139A scrapie. Where indicated, separate groups of mice were inoculated by intracerebral (i.e.) injection with 20 µl of a 1.0% (w/v) scrapie mouse brain homogenate (containing −1 × 10^6 ID50 U).

Disease monitoring

Following scrapie agent inoculation, animals were coded and assessed weekly for signs of clinical disease and killed at a standard clinical endpoint (23). Scrapie diagnosis was confirmed by histopathological assessment of vacuolation in the brain. At the times indicated, some mice were culled and tissues taken for further analysis. For bioassay of scrapie agent infectivity, half spleens, MLNs, and spinal cords were homogenized in physiological saline. Groups of four tga20 indicator mice were injected i.c. with 20 µl of each homogenate. The scrapie titer in each sample was determined from the mean incubation period in the indicator mice, by reference to dose/incubation period response curves for ME7 scrapie-infected spleen and brain tissue serially titrated in tga20 mice. As the expression level of cellular PrP controls the TSE disease incubation period, tig mice expressing PrPα such as tga20 mice are extremely useful as indicator mice in scrapie agent infectivity bioassays as they succumb to disease with much shorter incubation times than conventional mouse strains (22).

Immunohistochemical and immunofluorescent analyses

Spleens and MLNs were removed and snap-frozen at the temperature of liquid nitrogen. Small intestines from each mouse were divided into three roughly equal parts, gently squeezed to remove gut contents, coiled, embedded in Tissue-Tek OCT compound (Bayer) and snap-frozen at the temperature of liquid nitrogen. Serial frozen sections (10 µm in thickness) were cut on a cryostat. Sections were stained with the following Abs: anti-CD11c (clone mAb HL3; BD Biosciences/BD Pharmingen); anti-CD11b (clone M1/70.15; Serotec); anti-F4/80 (clone CL:A3-1; Serotec). Metallophilic macrophages were identified using mAb MOMA-1 (Serotec). Macrophages/monocytes were detected using mAb MOMA-2 (Serotec). Neutrophils were detected using mAb 7/4 (Serotec). FDCs were visualized by staining with mAb 7G6 to detect CR2/CR1 (CD21/CD35; BD Biosciences) or mAb FDC-M2 to detect complement C4 (AMS Biotechnology). Cellular PrP was detected using PrP-specific polyclonal Ab (pAb) 1B3 (24). B cells were detected using mAb B220 that binds CD45R (Caltag Laboratories). For the detection of disease-specific PrP (PrPd) in brain tissue and small intestines, tissues were fixed in periodate-lysine-paraformaldehyde and embedded in paraffin wax. Sections (thickness, 6 µm) were deparaffinized, and pretreated to enhance the detection of PrPd by hydrated autoclaving (15 min, 121°C, hydration) and subsequent immersion in formic acid (98%) for 5 min (25). Sections of small intestine were then stained with the PrP-specific pAb 1B3 (24) and brain were stained with the PrP-specific mAb 6H4 (Prionics). The cell-surface fibrillar core protein (GFAP) was detected on adjacent brain sections using rabbit GFAP-specific antisera (DakoCytomation). To detect FDCs and B cells in paraffin-embedded small intestines, sections were deparaffinized and pretreated with Target Retrieval Solution (DakoCytomation) and immunostained with mAb 7G6 and mAb B220, respectively, as described above. Nerve fibers and ganglia in the intestine were also detected using protein-gene product 9.5 (PrP 9.5-specific pAb) (DakoCytomation).

For light microscopy, following the addition of primary Abs, biotin-conjugated species-specific secondary Abs (Stratech) were applied followed by alkaline phosphatase or HRP coupled to the avidin/biotin

Flow cytometry

Spleens were removed and injected with 0.5 ml of collagenase D solution (1 mg/ml in sterile PBS; Roche Diagnostics), placed in small petri-dishes with sufficient collagenase D solution to completely cover the tissue (~3–5 ml), and incubated for 30 min at 37°C. The released cells and remaining tissue fragments were then passed through a 100 µm cell strainer (BD Biosciences) and cells collected. Mononuclear cells were centrifuged on Histopaque-1077 (Sigma-Aldrich) and washed with PBS containing 0.5% BSA and 2.5 mM sodium EDTA. Cells were resuspended in FACS buffer (PBS (pH 7.4) containing 0.1% BSA, 0.1% sodium azide and 0.02% EDTA) to 1 × 10^6 cells per 100 µl of buffer. Nonspecific binding of IgGs to FcγRIII and FcγRI receptor was blocked with 0.1 µg of mouse Ab CT-17.2 specific for CD16/32 (FcγRIII/FcγRII; Caltag Laboratories). Cells were then incubated with biotin-conjugated anti-CD11c antiserum followed by Alexa Fluor 488-conjugated streptavidin (Invitrogen Life Technologies). An appropriate Alexa Fluor 488-conjugated Ab was used as a nonspecific Ig-isotype control (Serotec). Samples were fixed in 1% paraformaldehyde and flow cytometry was conducted on a FACS caliber (BD Biosciences) and data were analyzed using CellQuest software (BD Biosciences).

Immunohistoblot detection of PrPSc

Spleens, MLNs, and portions of intestine containing Peyer’s patches (PPs) were snap-frozen and maintained at the temperature of liquid nitrogen. Serial 10-µm sections were cut on a cryostat, applied directly to polyvinylidene difluoride membranes (Bio-Rad), and thoroughly air-dried. For the detection of PrPSc, membranes were rehydrated and treated with 50 µg/ml proteinase K for 3 h at 37°C, as previously described (26). Following processing, membranes were immunostained with the PrP-specific Ab 1B3 (24), counterstained with alkaline phosphatase-conjugated goat-anti-rabbit antisera, and bound alkaline phosphatase activity was detected with SigmaFast NBT/BCIP solution.

Statistical analyses

Data are presented as mean ± SE. Significant differences between samples in different groups were sought by one-way ANOVA. Values of p < 0.05 were accepted as significant.
To determine the in vivo contribution of DCs in the translocation of the TSE agent from the gut lumen to lymphoid tissues, CD11c-DTR-tg mice were used in which CD11c⁺/H11001 DCs can be transiently depleted (21). Previous studies show that within 24 h of i.p. injection with DTX, 85% of CD11chigh cells in the spleens of CD11c-DTR-tg mice are depleted (21). In the current study, CD11c⁺ cells were also depleted in PPs, villus cores (Fig. 1A), and MLNs (Fig. 1C) within 24 h of treatment of CD11c-DTR-tg mice with DTX. The effect of DTX treatment on CD11c⁺ cells was transiently depleted.
transient as their presence within PPs, villus cores (Fig. 1A), MLNs (data not shown), and the spleen (Fig. 1D; Ref. 21) was restored by 6 days after DTX treatment. Virtually all DCs appeared to be depleted in the FAE of PPs, villi, and MLNs within 24 h of treatment with DTX treatment as visualized by immunohistochemical analysis of CD11c<sup>+</sup> cells (Fig. 1, A and C), whereas FACS analysis suggested that their numbers in the spleen were depleted by ~60% (Fig. 1D). The reasons for this discrepancy are uncertain, but may represent the rapid replacement of CD11c<sup>+</sup> cells by hemopoietic precursors directly within the spleen or the greater of sensitivity FACS analysis when compared with immunohistochemistry. In some sections, villus epithelial cells appeared to show CD11c immunolabeling. However, as shown in Fig. 1A this was quite variable. Comparisons with adjacent control Ig-stained sections suggested this staining was not CD11c specific and most likely due to the binding of streptavidin to the high levels of endogenous biotin in the intestine. Expression of the cellular form of PrP, PrP<sub>c</sub>, upon FDCs within PPs is critical for efficient scrapie agent neuroinvasion from the intestine (2, 5). FDC status and PrP<sub>c</sub> expression in FDCs within PPs is critical for efficient scrapie agent neuroinvasion up to at least this time after inoculation. Italicized values represent incubation periods for individual clinically and pathologically scrapie-positive mice.

Table I. Effect of CD11c<sup>+</sup> cell depletion on susceptibility to the ME7 scrapie agent

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Oral Inoculation Incidence&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mean incubation period (days) ± SE</th>
<th>i.c. Inoculation Incidence</th>
<th>Mean incubation period (days) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>DTX</td>
<td>6/6</td>
<td>326 ± 2</td>
<td>6/6</td>
<td>183 ± 2</td>
</tr>
<tr>
<td>CD11c-DTR-tg</td>
<td>PBS</td>
<td>6/6</td>
<td>336 ± 11</td>
<td>6/6</td>
<td>174 ± 3</td>
</tr>
<tr>
<td>CD11c-DTR-tg</td>
<td>DTX</td>
<td>5/8</td>
<td>321, 344, 370, 379, 536</td>
<td>6/6</td>
<td>172 ± 2</td>
</tr>
</tbody>
</table>

<sup>a</sup> CD11c<sup>+</sup> cells were depleted in CD11c-DTR-tg mice by treatment with DTX and 24 h later inoculated orally with the ME7 scrapie agent.

<sup>b</sup> Incidence = number of animals affected/number of animals tested. The notation “NX > 538” means that mice were free of the clinical and pathological signs of scrapie up to at least this time after inoculation. Italicized values represent incubation periods for individual clinically and pathologically scrapie-positive mice.

Next, the effect of transient CD11c<sup>+</sup> cell depletion on the accumulation of the scrapie agent in the GALT and the spleen was examined. CD11c-DTR-tg mice were injected with DTX to deplete their CD11c<sup>+</sup> cells and 24 h later inoculated orally with either the ME7 or 139A scrapie agents. In addition, two control groups of mice were treated as follows 24 h before scrapie inoculation: CD11c-DTR-tg mice were injected with PBS; WT mice were injected with DTX.

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>Sc</sup> or PrP<sup>d</sup>) are used to describe the disease-specific, abnormal accumulations of PrP that are characteristically found only in TSE-affected tissues and considered to reflect the presence of the TSE agent (1). Disease-specific PrP accumulations are relatively resistant to proteinase K digestion, whereas cellular PrP<sup>c</sup> is destroyed by this treatment.

Table II. Effect of CD11c<sup>+</sup> cell depletion on susceptibility to the 139A scrapie agent

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Oral Inoculation Incidence&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mean incubation period (days) ± SE</th>
<th>i.c. Inoculation Incidence</th>
<th>Mean incubation period (days) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>DTX</td>
<td>6/6</td>
<td>244 ± 3</td>
<td>6/6</td>
<td>155 ± 4</td>
</tr>
<tr>
<td>CD11c-DTR-tg</td>
<td>PBS</td>
<td>6/6</td>
<td>260 ± 4</td>
<td>6/6</td>
<td>150 ± 6</td>
</tr>
<tr>
<td>CD11c-DTR-tg</td>
<td>DTX</td>
<td>4/9</td>
<td>258, 272, 279, 300, 5X &gt; 531</td>
<td>6/6</td>
<td>151 ± 4</td>
</tr>
</tbody>
</table>

<sup>a</sup> CD11c<sup>+</sup> cells were depleted in CD11c-DTR-tg mice by treatment with DTX and 24 h later inoculated orally with the 139A scrapie agent.

<sup>b</sup> Incidence = number of animals affected/number of animals tested. The notation “NX > 531” means that mice were free of the clinical and pathological signs of scrapie up to at least this time after inoculation. Italicized values represent incubation periods for individual clinically and pathologically scrapie-positive mice.
Where we were able to confirm this resistance by proteinase-K treatment of samples and analysis by immunohisto blot (26), PrPSc is used as a biochemical marker for the presence of the TSE agent. Unfortunately, treatment of tissue sections with proteinase-K destroys the microarchitecture. Therefore, for immunohistochemical analysis, tissue sections were fixed and pretreated to enhance the detection of the disease-specific abnormal accumulations of PrP (PrPSc), whereas cellular PrP is denatured by these treatments (25). We have shown in a series of studies that these PrPSc accumulations occur only in the tissues of TSE-affected animals, and correlate closely with the presence of the TSE agent (2, 5, 29, 30).

Within 70 days of oral inoculation of WT mice with the ME7 scrapie agent, strong accumulations of PrPSc and agent infectivity are found within PPs and sustained until the terminal stages of disease (Refs. 2 and 5 and N. A. Mabbott, unpublished observation). In the current study, heavy PrPSc accumulations were likewise detected in the PPs of control mice (DTX-treated WT mice and PBS-treated CD11c-DTR-tg mice) 70 days after inoculation with the ME7 scrapie agent strain (Fig. 2). The distribution of the PrPSc within the B cell areas of PPs was consistent with accumulation upon FDCs (Fig. 2; Refs. 5, 29, 30). Immunohisto blot analysis confirmed that these PrPSc accumulations were proteinase-K-resistant PrPSc (Fig. 3A). Following temporary CD11c+ cell depletion, no PrPSc or PrPSc was detected in any of the PPs from DTX-treated CD11c-DTR-tg mice assayed 70 days (Figs. 2 and 3A, respectively) or 105 days after inoculation (Fig. 3B). Temporary CD11c+ cell depletion before oral inoculation with the 139A scrapie agent strain likewise blocked the early accumulation of PrPSc and PrPSc within the PPs of DTX-treated CD11c-DTR-tg mice (data not shown).

High levels of PrPSc and agent infectivity are also found within the MLNs of control mice within 70 days of exposure (Fig. 3, A and C, respectively) and are sustained until the terminal stage of disease (2, 5). However, CD11c+ cell depletion blocked the accumulation of PrPSc (Fig. 3A, B and D) and agent infectivity (Fig. 3C) in the MLNs of DTX-treated CD11c-DTR-tg mice.

In the spleen, low levels of PrPSc and agent infectivity were detected in tissues from control mice assayed 70 days after inoculation (Fig. 3, A and C, respectively), which increased by 105 days after inoculation (Fig. 3, B and D, respectively). However, PrPSc and agent infectivity were undetectable in the spleens of DTX-treated CD11c-DTR-tg mice (Fig. 3). Scarpie agent infectivity was undetectable in spinal cords from most mice at the times assayed, although low levels were detected in tissue from one DTX-treated WT mouse 105 days after inoculation (Fig. 3D).

Thus, the absence of CD11c+ cells at the time of oral exposure blocks the initial accumulation of the scrapie agent in the GALT and its subsequent distribution to the spleen.

**Effect of CD11c+ cell depletion on scrapie susceptibility**

We next determined the effects of temporary CD11c+ cell depletion on scrapie susceptibility. All control mice succumbed to clinical TSE disease with similar incubation periods —330 days after oral inoculation with the ME7 scrapie agent (Table I). In contrast, CD11c+ cell depletion dramatically affected disease susceptibility as three of eight DTX-treated CD11c-DTR-tg mice remained free of the signs of scrapie to at least 538 days after oral inoculation (Table I). Three of the five DTX-treated CD11c-DTR-tg mice that developed clinical scrapie had individual incubation periods substantially beyond the range observed in control mice (Table I). Likewise, all control mice inoculated orally with the 139A scrapie agent succumbed to clinical TSE disease with similar incubation periods —250 days after inoculation with the 139A scrapie agent (Table II). In contrast, five of nine DTX-treated CD11c-DTR-tg mice remained free of the signs of scrapie up to 531 days after exposure (Table II), at least twice the duration of the incubation period observed in control-treated mice. The individual incubation periods observed in three of the four DTX-treated CD11c-DTR-tg mice that developed clinical scrapie were beyond the range observed in control mice (Table II). Characteristic PrPSc accumulation, reactive astrocytes and spongiform pathology were detected in the brains of all clinically affected control mice (Fig. 4). The severity and distribution of the spongiform pathology and PrPSc
accumulation in the brains of CD11c-DTR-tg mice that developed clinical scrapie were not significantly different to those of clinically affected control mice. However, none of the pathological characteristics of TSE disease were detected within the brains of any of the surviving DTX-treated CD11c-DTR-tg mice in which CD11c<sup>+</sup> cells were depleted before oral inoculation with the ME7 scrapie agent or 139 scrapie agent (Fig. 4, A and B, respectively).

When inoculated with the scrapie agent directly into the CNS (by i.c. injection), all control mice and DTX-treated CD11c-DTR-tg mice developed clinical scrapie with similar incubation periods (Tables I and II, respectively). Thus, the dramatically reduced susceptibility following oral exposure of CD11c<sup>+</sup> cell-depleted mice could not be attributed to a role for these cells in the development of pathology directly within the CNS.

### Discussion

Mice in which CD11c<sup>+</sup> DCs can be specifically depleted, such as DTX-treated CD11c-DTR-tg mice (21), have provided an important model in which the precise contribution of DCs in immunity and disease pathogenesis can be studied. Indeed, because CD11c is expressed by all known subsets of mouse DCs, DTX treatment of CD11c-DTR-tg mice results in impairment of DC specific functions such as Ag cross-presentation and priming of naive T cells (21, 31). Here, we have used CD11c-DTR-tg mice to determine the contribution of CD11c<sup>+</sup> DCs in the transmission of the scrapie agent from the gastrointestinal tract to the CNS, at the very early stage of propagation. Using two distinct scrapie agent strains (ME7 and 139A scrapie), we show that when CD11c<sup>+</sup> cells were temporarily depleted in the GALT and spleen before oral exposure, early agent accumulation in these tissues was blocked. In addition, CD11c<sup>+</sup> cell depletion reduced susceptibility to oral scrapie challenge indicating that agent neuroinvasion from the GALT was impaired. Together, these data demonstrate that migratory CD11c<sup>+</sup> cells, most likely DCs, play a key role in the translocation of the scrapie agent from the gut lumen to the GALT from which neuroinvasion subsequently occurs.

TSE agent accumulation upon PrP<sup>+</sup>-expressing FDCs within the B cell follicles of PPs is crucial for neuroinvasion following oral exposure (2, 5). FDCs appear to expand the levels of the TSE agent above the threshold required to achieve neuroinvasion. Accordingly, when FDCs (2) or PPs (3–5) are absent at the time of inoculation, scrapie agent transmission from the intestine is blocked. However, as FDCs form immobile networks and are situated distally from the FAE within PPs, little is known of how TSE agents are initially conveyed to them from the gut lumen. Several mechanisms have been implicated including transcytosis by M cells (15) or intestinal epithelial cells (16), and capture and transport by migratory hemopoietic DCs (20).

The central involvement of DCs in the transport of Ags both within PPs and on into MLNs (18, 19) implied that these cells were credible candidates through which the TSE agent might be initially conveyed to the GALT. The DCs that patrol peripheral tissues are immature cells that continuously endocytose Ags from the local environment. Within the DC, Ags usually rapidly enter the lysosomal compartment where they are processed into short peptides for presentation on the cell surface in association with MHC class II molecules (18). Some DCs likewise acquire and degrade TSE agents following in vitro exposure implying such cells would be unlikely to efficiently deliver them to lymphoid follicles in infectious form (32, 33). However, some DCs can acquire and retain Ags (34–36) and TSE agents (20, 33) in their native, nondegraded state for several days. Indeed, ex vivo studies of splenic DCs isolated from scrapie-affected mice showed that they bear TSE infectivity and are capable of transmitting disease to recipient mice after i.v. injection (37). The prion protein fragment PrP<sub>106–126</sub> appears to act as a chemottractant for DCs (38) suggesting that DCs might migrate toward PrP<sup>+</sup> in host tissues, but few studies have investigated whether DCs actually transport TSE agents, and data from those that have are currently inconclusive. After intraintestinal administration, PrP<sup>+</sup> was observed in a population of DCs migrating in the afferent mesenteric lymphatics (20) suggesting a role in neuroinvasion, but it was not certain whether the level of TSE agent associated with these DCs was sufficient to mediate disease pathogenesis. In the current study, specific and transient depletion of CD11c<sup>+</sup> DCs before oral exposure with two distinct scrapie agent strains blocked the early agent accumulation upon FDCs in the GALT and reduced disease susceptibility. Although the period of CD11c<sup>+</sup> cell depletion was transient and restored within 6 days of DTX treatment, this resulted in a substantial impairment in TSE agent neuroinvasion, indicating that the critical period of DC involvement was complete within this time period. Although the early TSE agent accumulation upon FDCs within the GALT of DTX-treated CD11c-DTR-tg mice was blocked, FDC status and PrP<sup>+</sup> expression appeared to be unaffected. Together, these data suggest a crucial role for CD11c<sup>+</sup> DCs in the initial conveyance of the scrapie agent from the gut lumen to lymphoid follicles within the GALT.

Our recent data suggest that TSE agent neuroinvasion from the intestine occurs directly from PPs (5). In the mouse, specific DC subsets localize to distinct regions of PPs (39): CD11b<sup>+</sup>/CD8α<sup>+</sup>-DCs are present within the subepithelial dome but absent from the T cell-rich interfollicular region; CD11b<sup>+</sup>/CD8α<sup>+</sup> DCs are mainly present in the interfollicular region and rarely the subepithelial dome; whereas the “double-negative” DC subset (CD11b<sup>+</sup>/CD8α<sup>+</sup>/CD11c<sup>+</sup> cells) is located in both the subepithelial dome and the interfollicular region. Which of these DC subsets is involved in the early stages of TSE pathogenesis within PPs is uncertain. The location of the CD11b<sup>+</sup>/CD8α<sup>+</sup>-DCs from the subepithelial dome would suggest these cells are ideally situated to acquire TSE agents following their translocation across the intestinal epithelium. The exclusion of CD11b<sup>+</sup>/CD8α<sup>+</sup>- DCs from the subepithelial dome would imply that these cells are unlikely to play a role. Indeed, a recent study has shown that specific depletion of CD8α<sup>+</sup> DCs does not influence TSE agent neuroinvasion from the intestine (40). As the same study also showed that the depletion of CD8α<sup>+</sup> DCs significantly delayed TSE agent neuroinvasion from the peritoneal cavity, this implies that the contribution of different DC subsets may differ according to the route of exposure and/or that they might be involved in other steps of pathogenesis (40).

Chemokines and chemokine receptors play important roles in attracting lymphocytes and DCs to lymphoid tissues and controlling their positioning within them. Chemokines CCL19 and CCL21 are constitutively expressed within T cell zones and mediate the homing of chemokine receptor CCR7-expressing naive T cells and mature DCs into these regions (41). In the GALT, the localization of DCs within the interfollicular T cell region of PPs, and their steady-state migration from the lamina propria and the subepithelial dome of PPs to the MLNs is likewise dependent upon CCR7-CCL19/CCL21 signaling (42, 43). Following oral exposure, it is plausible that DCs might acquire the TSE agent within the lamina propria or subepithelial dome following its initial translocation across the intestinal epithelium. Therefore, as the presence of DCs within the subepithelial dome is independent of CCR7 signaling (42, 44), and neuroinvasion appears to occur directly following accumulation within PPs (2, 5, 45), this suggests that CCR7-CCL19/CCL21-dependent DC migration is unlikely to significantly influence TSE agent neuroinvasion from PPs. Indeed, a
recent report shows that TSE agent accumulation in the GALT and subsequent neuroinvasion are not affected in *plh/plh* mice with impaired CCL19/CCL21-mediated DC migration (46).

Specific DC subsets have been identified that can migrate into B cell follicles (47–49), but further studies are necessary to determine whether these DCs also acquire the TSE agent. Intriguingly, some DCs can capture and retain protein Ags in native form for sufficient time to facilitate their migration to lymphoid tissues and interaction with B cells (34). The mechanisms controlling DC migration into B cell follicles are less well-defined. In the spleen and lymph nodes, chemokine receptor CXCR5-expressing DCs appear to localize to B cell follicles in response to the chemokine CXCL13 produced by follicular stromal cells and FDCs (48–50). Whether the same stimuli regulate the migration of DCs into B cell follicles in the GALT is uncertain, but the expression of CXCL13 by FDCs within the lymphoid follicles of human GALT has recently been described (51).

Further studies are also necessary to determine how DCs subsequently transmit the scrapie agent to FDCs. Such transfer might occur through direct DC-FDC contact, secretion, or passive diffusion. Many cells including DCs secrete small membrane vesicles termed exosomes, which originate from late endosomes, are 30–100 nm in diameter and are enriched in cell-specific proteins (52, 53). The functions of exosomes are uncertain, but may include immune regulation and Ag transfer between cells (52). For example, the presence of MHC class II molecules on the surface of FDCs was shown to be passively acquired through capture of exosomes (54). Some pathogens including HIV and TSE agents also appear to be released from infected cells in association with exosomes (53, 55). Therefore, intercellular TSE agent transfer might occur via exosomes drifting from infected DCs toward uninfected FDCs. As a subset of DCs can transfer native Ag directly to B cells in vivo (34, 36), it is also possible that the TSE agent might indirectly accumulate upon FDCs following transfer from DCs to B cells within the follicle.

In the current study, our animals ate the scrapie agent to model oral pathogenesis as closely as possible. Following ingestion, it is anticipated that factors including digestion (by enzymes secreted in the stomach or small intestine) and excretion will act on the scrapie agent within the inoculum to aid its elimination from the host. As a consequence, very little of the original inoculum will be available to be translocated across the gut epithelium. In WT mice, it is likely that once the remaining fraction of the inoculum enters the lamina propria a competitive state exists whereby cells such as macrophages sequester and degrade the TSE agent (56, 57). Some macrophages with prolonged incubation periods and a small number with sustained neuroinvasion are not affected in *CD11c-DTR-tg* mice; and Christine Farquhar (Neuropathogenesis Unit) for excellent technical support; Steffen Jung (Weizmann Institute of Science, Rehovot, Israel) for supply of CD11c-DTR-tg mice; and Christine Farquhar (Neuropathogenesis Unit) for provision of pAb 1B3.

**Disclosures**

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**References**


