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The Effects of Host Age on Follicular Dendritic Cell Status Dramatically Impair Scrapie Agent Neuroinvasion in Aged Mice

Karen L. Brown,²* Gwennaelle J. Wathne,* Jill Sales,† Moira E. Bruce,* and Neil A. Mabbott²*

Following peripheral exposure, many transmissible spongiform encephalopathy (TSE) agents accumulate first in lymphoid tissues before spreading to the CNS (termed neuroinvasion) where they cause neurodegeneration. Early TSE agent accumulation upon follicular dendritic cells (FDCs) in lymphoid follicles appears critical for efficient neuroinvasion. Most clinical cases of variant Creutzfeldt-Jakob disease have occurred in young adults, although the reasons behind this apparent age-related susceptibility are uncertain. Host age has a significant influence on immune function. As FDC status and immune complex trapping is reduced in aged mice (600 days old), we hypothesized that this aging-related decline in FDC function might impair TSE pathogenesis. We show that coincident with the effects of host age on FDC status, the early TSE agent accumulation in the spleens of aged mice was significantly impaired. Furthermore, following peripheral exposure, none of the aged mice developed clinical TSE disease during their lifespan, although most mice displayed histopathological signs of TSE disease in their brains. Our data imply that the reduced status of FDCs in aged mice significantly impairs the early TSE agent accumulation in lymphoid tissues and subsequent neuroinvasion. Furthermore, the inefficient neuroinvasion in aged individuals may lead to significant levels of subclinical TSE disease in the population. The Journal of Immunology, 2009, 183: 5199–5207.

Transmissible spongiform encephalopathies (TSEs),³ 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 characteristically includes neuronal loss, spongiform pathology, glial activation, and amyloidogal aggregations of an abnormally folded host protein. The host prion protein (PrP⁰) 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 co-purifies 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 PrP⁰ to PrPSc.

Some TSE agents, including natural sheep scrapie, bovine spongiform encephalopathy (BSE), chronic wasting disease in mule deer and elk, and kuru and variant Creutzfeldt-Jakob (vCJD) disease in humans, are acquired by peripheral exposure (e.g., orally or via lesions to skin or mucous membranes). Experimental studies in mice (2–4) and analysis of tissues from sheep with natural scrapie (5), mule deer fawns with chronic wasting disease (6), and a patient with vCJD (7) 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 conventional hematopoietic dendritic cells, as they are considered to derive from stromal precursor cells, are nonphagocytic, and are nonmigratory (8). In mouse TSE models, agent accumulation upon PrP⁰-expressing FDCs within the lymphoid tissues is critical for disease pathogenesis (9, 10), as in their absence, neuroinvasion is significantly impaired (2, 11, 12). From the lymphoid tissues, translocation to the CNS occurs via the peripheral nervous system (13, 14).

Strong evidence indicates that the BSE epidemic in the United Kingdom most probably occurred and was sustained via the feeding of BSE-contaminated ruminant meat and bone meal to cattle (15). Furthermore, the consumption of BSE-contaminated meat products is considered the most likely source of the human TSE disease vCJD (16). During the United Kingdom BSE epidemic it was estimated that almost 500,000 infected cattle were slaughtered for human consumption (17). Despite this probable widespread exposure of the United Kingdom human population to the BSE agent, clinical cases of vCJD have predominantly occurred in young people (17). The possibility that young adults were exposed to greater levels of BSE by dietary preference has not been substantiated (18), suggesting that other age-related factors may influence susceptibility to peripherally acquired TSE agents.

FDCs characteristically trap and retain Ags, storing them on their surfaces for long periods (8). Ags are retained on FDCs in the...
form of immune complexes comprising Ag, Abs, and/or complement components. These complexes bind to FDCs via complement receptors (CRs) in naive mice and also Ab Fc receptors in immunized mice (19). Complement components C1q and C3, as well as cellular CRs, are likewise considered to play an important role in the localization of TSE agents to FDCs (20–22). In aged (600 days old) mice, FDC status is significantly impaired. Aged FDCs appear atrophic and have a reduced capacity to trap and retain immune complexes, leading to deficits in Ab responsiveness and germinal center formation (23–26). Therefore, using an experimental mouse TSE pathogenesis model (mouse-passaged scrapie agent) experiments were designed to determine whether the decline in FDC status in aged mice would influence the early accumulation of the TSE agent within lymphoid tissues and subsequent neuroinvasion.

Materials and Methods

Mice

C57BL/Dk mice were aged to ~600 days under normal specific pathogen-free conditions before exposure to the scrapie agent. Six- to 8-wk-old C57BL/Dk mice were used as young adults. All experimental procedures were approved by the Institute’s ethical review committee and were conducted according to the strict regulations of the U.K. Home Office Animals (Scientific Procedures) Act 1986.

Scrapie agent exposure and disease monitoring

Where indicated, mice were injected i.p. or intracerebrally (i.c.) with 20 μl of a 1% (w/v) scrapie brain homogenate prepared from mice terminally affected with the ME7 scrapie agent strain (containing ~1 x 10^5 i.e. ID_{50}) (U). For oral exposure, mice were fed individual food pellets doused with 50 μl of a 10% (w/v) dilution of terminally affected scrapie brain homogenate. Following scrapie agent exposure, mice were coded and assessed weekly for signs of clinical disease and culled at a standard clinical endpoint (27). Survival times were recorded for mice that did not develop clinical signs of disease and were culled when they showed signs of intercurrent disease. Scrapie diagnosis was confirmed by histopathological assessment of vacuolation in the brain. For the construction of lesion profiles, vascular changes were scored in nine gray matter areas of brain as described (28). At the times indicated, some mice were culled and tissues taken for further analysis. For bioassay of scrapie agent infectivity, individual spleens were prepared as 10% homogenates in physiological saline. Groups of 10–12 C57BL/Dk indicator mice were injected i.c. with 20 μl of each homogenate. A dose/incubation period response curve was prepared for ME7 scrapie agent-infected spleen tissue titered serially in C57BL/Dk mice (supplemental Table I and supplemental Fig. 1). The scrapie titer in each young and aged spleen assayed was then estimated from the mean incubation period in each group of indicator mice, by reference to the dose/incubation period response curve using the relationship y = 13.879 – 0.0506x (where y indicates the log ID_{50}/20 μl of homogenate; x, incubation period time; supplemental Table II).

Immunohistochemistry

For the detection of disease-specific PrP (PrP{sup}) in brain and spleen, tissues were fixed in paraformaldehyde/formic acid/fixative and embedded in paraffin wax. Sections (thickness, 6 μm) were deparaffinized and dehydrated to enhance the detection of PrP{sup} by hydrated autoclaving (15 min, 121°C, hydration) and subsequent immersion in formic acid (98%) for 5 min (29). For oral exposure, mice were fed individual food pellets doused with 50 μl of a 10% dilution of terminally affected scrapie brain homogenate. Following scrapie agent exposure, mice were coded and assessed weekly for signs of clinical disease and culled at a standard clinical endpoint (27). Survival times were recorded for mice that did not develop clinical signs of disease and were culled when they showed signs of intercurrent disease. Scrapie diagnosis was confirmed by histopathological assessment of vacuolation in the brain. For the construction of lesion profiles, vascular changes were scored in nine gray matter areas of brain as described (28). At the times indicated, some mice were culled and tissues taken for further analysis. For bioassay of scrapie agent infectivity, individual spleens were prepared as 10% homogenates in physiological saline. Groups of 10–12 C57BL/Dk indicator mice were injected i.c. with 20 μl of each homogenate. A dose/incubation period response curve was prepared for ME7 scrapie agent-infected spleen tissue titered serially in C57BL/Dk mice (supplemental Table I and supplemental Fig. 1). The scrapie titer in each young and aged spleen assayed was then estimated from the mean incubation period in each group of indicator mice, by reference to the dose/incubation period response curve using the relationship y = 13.879 – 0.0506x (where y indicates the log ID_{50}/20 μl of homogenate; x, incubation period time; supplemental Table II).

Quantification of FDC networks

For enumeration of PrP{sup}-expressing FDC networks, coded spleen sections from aged (n = 19) and young (n = 9) mice were immunolabeled for cellular PrP{sup} as described above and examined using a Zeiss confocal microscope. The total number of PrP{sup}-expressing follicles within each entire spleen section was then counted. Data are presented as the mean number of PrP{sup}-expressing follicles per spleen section (±SEM). Statistical analysis was performed using Student’s t test.

For analysis of C4 and PrP{sup} colocalization in the spleens of aged and young aged mice, coded sections were double-immunostained to detect C4 and PrP{sup} as described above. For each section, five to eight randomly selected fields of view from each spleen were measured using ImageJ software (National Institutes of Health, http://rsb.info.nih.gov/ij/). Data are expressed as the mean percentage of the area occupied by PrP-positive cells (±SEM). Statistical analysis was performed using Student’s t test.

Coded spleen sections from young and aged mice were immunostained to detect PrP-positive nerves as described above, and the area occupied by these cells was quantitated. To do so, six fields of view from each spleen when viewed with the ×10 objective were captured, and the area covered by TH-positive cells in each field of view was measured using ImageJ software (National Institutes of Health, http://rsb.info.nih.gov/ij/). Data are expressed as the mean percentage of the area occupied by TH-positive cells (±SEM). Statistical analysis was performed using Student’s t test.

Quantification of TH-positive nerve fibers in the spleen

Differences in the proportions of mice having positive vascular pathology (young vs aged) were investigated using a Fisher’s exact two-tailed test. To compare the severity of the vacuolation between aged and young mice, the extent of the vacuolation was assessed in each of nine brain regions, with scoring done on a scale of 0–5. As a summary measure, the areas under the lesion profile curves (AUC) were estimated using the trapezoidal rule, for those mice that could be scored in at least eight of the regions (i.p. route, n = 12 young, n = 3 aged; oral route, n = 12 young, n = 5 aged; i.c. route, n = 6 young, n = 3 aged). For each route separately, the AUC was found for each mouse using the trapezoidal rule. Differences in median AUC between the aged and young animals were tested using a two-tailed Mann-Whitney U test. Analysis of the incubation period data obtained from the spleen bioassay studies was conducted on all mice that showed positive pathology (n = 130). A linear mixed model was fitted to the data with status (whether the spleen came from a young or aged mouse), spleen age (how long after infection the spleen was collected) and sex of recipient mouse (whether the spleen came from a young or aged mouse) as fixed effects, and the time (when viewed with the ×10 objective) as random factor. Parameters of the model were estimated using the residual maximum likelihood (REML) directive in GenStat 10th edition, and the fixed effects were tested using F tests with appropriately modified degrees of freedom.

Results

Effect of host age on FDC status

First we compared the status of FDCs within the spleens of young adult (6–8 wk old) and aged (~600 days old) C57BL/Dk mice by immunohistochemistry. High levels of complement component C4 in association with FDCs expressing CR2 and CR1 (CD21/CD35) and the cellular form of the prion protein, PrP{sup}, were detected in the spleens of young mice (Fig. 1, A and B). The association of C4 on FDCs is considered to provide a reliable reflection of the level of immune complex trapping on these cells (24, 25, 32). The detection of complement component C4 upon most FDCs from aged mice was substantially reduced (Fig. 1, A and C), indicating that the retention of immune complexes on the FDCs was impaired. Furthermore, our analysis indicated that these networks were disrupted (Fig. 1C) and lacked the typical follicular structure observed in young mice (Fig. 1B). Expression of cellular PrP{sup} by FDCs is critical for the accumulation of the TSE agent within the
spleen and efficient neuroinvasion (9, 10). In most FDC networks from aged mice, the reduced detection of complement component C4 coincided with a similar reduction in PrPc expression (Fig. 1C). However, some FDC networks from aged mice were intact and expressed levels of PrPc and FDC-associated C4 similar to those in the spleens of young mice (Fig. 1D).

When we assessed the number of intact PrPc-expressing FDC networks in the spleen, highly significantly reduced numbers were observed in those from all aged mice (Fig. 2). Spleens from aged (n = 19) and young (n = 9) mice were immunolabeled with antisera specific for cellular PrPc and complement component C4. Entire sections were coded, examined microscopically, and the total number of FDC networks were counted, their status was recorded (intact or disrupted), and the numbers of each type expressing PrPc and C4 were counted. A highly significant reduction in the total number of PrPc-expressing FDC networks in the spleens of aged mice was found when compared with those from young mice (p < 0.0003). B. Although a small number of intact FDC networks were observed in spleens from aged mice, a significantly greater proportion of the FDC networks were disrupted (Disrupt.) and showed reduced levels of complement component C4 on their surfaces (p < 0.027). C. All of the FDC networks from young mice expressed cellular PrPc. In spleens from aged mice none of the disrupted FDC networks expressed PrPc. Only the small number of intact FDC networks expressed cellular PrPc. Each bar represents mean ± SEM.

Taken together, these data demonstrate that the number of intact PrPc-expressing FDC networks in the spleens of aged mice is substantially reduced. Furthermore, a significant majority of the FDC networks in aged spleens lack PrPc expression, are disrupted, and demonstrate an impaired ability to retain complement-opsonized immune complexes.
The precise nature of the scrapie agent is uncertain, but PrPSc, a proteinase K (PK)-resistant form of the cellular prion protein, PrPc, copurifies with agent infectivity in diseased tissues (1). Within 10 wk after peripheral exposure of young adult mice to the ME7 scrapie agent, strong accumulations of PrPSc and agent infectivity accumulate upon FDCs within the spleen and are sustained until the terminal stages of disease (2, 10, 11). This early accumulation within lymphoid tissues is critical for efficient neuroinvasion (2, 12). As PrPc expression (9, 10) and opsonizing complement within lymphoid tissues is critical for efficient neuroinvasion (2, 12). During processing, tissue sections are treated with PK to destroy the scrapie agent (Fig. 3A), and were maintained until the terminal stages of disease. The distribution of PrPc, leaving only the TSE agent-specific PK-resistant PrPSc (Fig. 3B). In contrast, PrPc was not detected in the spleens of aged mice until 15 wk after i.p. exposure. By 15 wk after exposure our data indicate that the levels of agent infectivity in the spleens of aged mice were ~100-fold lower than those in the spleens from young adult mice (Fig. 4A). Spleens were also analyzed from two clinically scrapie-affected young mice and four aged mice that remained free of the signs of clinical disease at the end of the experiment (Fig. 4B). Our data show that TSE agent infectivity levels in spleens from aged mice were likewise reduced and never reached the levels observed in spleens from young mice. Furthermore, in the spleen of one aged animal only trace levels of agent infectivity were observed. These data show that the early accumulation of the scrapie agent upon FDCs is significantly reduced in the spleens of aged mice.

**Effect of host age on the early accumulation of the scrapie agent upon FDCs**

The nature of the scrapie agent is uncertain, but PrPSc, a proteinase K (PK)-resistant form of the cellular prion protein, PrPc, copurifies with agent infectivity in diseased tissues (1). Within 10 wk after peripheral exposure of young adult mice to the ME7 scrapie agent, strong accumulations of PrPSc and agent infectivity accumulate upon FDCs within the spleen and are sustained until the terminal stages of disease (2, 10, 11). This early accumulation within lymphoid tissues is critical for efficient neuroinvasion (2, 12). As PrPc expression (9, 10) and opsonizing complement components (20–22) are critical for the accumulation of the scrapie agent upon FDCs, their reduced association with FDCs in the spleens of aged mice suggested to us that scrapie agent accumulation in aged spleens would likewise be reduced. In this study, PrPc is used to describe the disease-specific PrP accumulations detected by immunohistochemistry when PK treatment was not used (4). Heavy PrPc accumulations were detected in the spleens of all young adult mice at 5 wk (not shown) and 10 wk after i.p. injection with the scrapie agent (Fig. 3A) and were maintained until the terminal stages of disease. The distribution of PrPc within these tissues was consistent with accumulation upon FDCs (2, 10, 11). Paraffin-embedded tissue immunoblot analysis of tissue sections can be used to directly demonstrate the presence of TSE agent-specific PK-resistant PrPSc on histological sections (31). During processing, tissue sections are treated with PK to destroy all the cellular PrPc, leaving only the TSE agent-specific PK-resistant PrPSc (if present). Sites of PrPSc are then readily detected immunohistochemically using PrP-specific antisera. Paraffin-embedded tissue immunoblot analysis of adjacent sections confirmed that these PrPc accumulations contained PK-resistant PrPSc (Fig. 3B). In contrast, in the spleens of aged mice the accumulation of PrPSc appeared to be delayed and was not detected until 15 wk after exposure (Fig. 3B).

We next compared scrapie agent infectivity levels in spleens from young adult and aged mice taken 5, 7, 10, and 15 wk after i.p. exposure. In spleens of young adult mice, high levels of scrapie agent infectivity were detected from 5 wk after exposure (Fig. 4A). However, significantly lower scrapie agent infectivity levels were detected in aged spleens, and in many instances infectivity was undetectable or at trace levels ($p < 0.001$; general linear mixed model; Fig. 4A). By 15 wk after exposure our data indicate that the levels of agent infectivity in the spleens of aged mice were ~100-fold lower than those in the spleens from young adult mice (Fig. 4A). Spleens were also analyzed from two clinically scrapie-affected young mice and four aged mice that remained free of the signs of clinical disease at the end of the experiment (Fig. 4B). Our data show that TSE agent infectivity levels in spleens from aged mice were likewise reduced and never reached the levels observed in spleens from young mice. Furthermore, in the spleen of one aged animal only trace levels of agent infectivity were observed. These data show that the early accumulation of the scrapie agent upon FDCs is significantly reduced in the spleens of aged mice.

**Sympathetic innervation in spleens and intestines of young and aged mice**

Following accumulation upon FDCs, the scrapie agent subsequently spreads to the brain via the peripheral nervous system (13, 14). Immunohistochemical analysis suggested that host age did not appear to impair the status of TH-positive sympathetic nerves, as the location and density of these cells in the spleens of uninfected young and aged mice appeared similar (Fig. 6. A and B, respectively). Furthermore, quantitative assessment of the area occupied by TH-positive sympathetic nerves within the spleens of young and aged mice indicated that their density was similar in each group ($p = 0.9, n = 5$, Student’s $t$ test; Fig. 6C). Previous studies have shown that both the relative distance between FDCs and sympathetic nerves (33, 34), as well as their overall density (35), significantly influences the rate of neuroinvasion from the spleen. As each of these parameters was similar in the spleens of young and aged mice, our data imply that any potential effects of host age on...
the status of sympathetic nerves were unlikely to influence disease pathogenesis. Immunohistochemical analysis likewise suggested that host age did not appear to impair the status of PrPc-expressing (Fig. 6D) and TH-positive (Fig. 6E) sympathetic nerves in the small intestine, as their distribution and density appeared similar in tissues from young and aged mice. Taken together, these data suggest that the effects of host age on the ability of FDCs to acquire and accumulate the TSE agent were the major influence on neuroinvasion.

Effect of host age on the accumulation of the scrapie agent within the brain and spleen at the terminal stages of disease

During TSE disease, the deposition of PrP<sub>d</sub> in the brain typically precedes the vacuolar changes (36). We therefore determined whether PrP<sub>d</sub> was present in the brains of the peripherally exposed aged mice despite the absence of positive vacuolar pathology. Large accumulations of PrP<sub>d</sub> were detected in the brains and spleens of all clinically scrapie-affected young mice after i.p. injection (Fig. 7, A and D, respectively), oral exposure, and i.c. injection (data not shown). Within the brains of the clinically negative aged mice, PrP<sub>d</sub> was detected in most brains from the i.p.-injected mice (six of nine mice; Fig. 7, B and C) and all of the orally exposed mice (Table I).

Further analysis revealed that there was considerable variation in the detection of PrP<sub>d</sub> in the spleens of the clinically negative aged survivors (Fig. 7, E and F, and Table I). All of the peripherally exposed aged mice that displayed positive vacuolar pathology (except one orally exposed animal) had detectable PrP<sub>d</sub> in their brains and spleens (Fig. 7, B and E, respectively). In contrast, the aged mice that did not show signs of vacuolar pathology had detectable PrP<sub>d</sub> in the brain but not in the spleen (Fig. 7, C and F, respectively). The reasons for these differences are uncertain. In mice deficient in FDCs, neuroinvasion can occur in some mice by an FDC-independent process such as direct uptake by peripheral nerves (37–39), implying direct transfer of the TSE agent to the nervous system in some aged mice. Alternatively, it is plausible that the TSE agent initially accumulated upon FDCs, facilitating subsequent neuroinvasion, but that it was later cleared from the spleen as the status of the FDCs declined.

Discussion

Here we show that the reduced status of FDCs in aged mice significantly impairs TSE agent neuroinvasion following peripheral exposure. We show that coincident with the effects of host age on FDC status, the early TSE agent accumulation in the spleens of
Effects in immune complex trapping and PrPc expression dramatically reduce the ability of aged FDCs to acquire and replicate TSE agents. Therefore, our data suggest that the combined agents provide costimulatory signals to B cells and stimulate germinal center formation (23–26). In the present study the mAb FDC-M2, which detects complement C4 on FDC-associated immune complexes (32), was used to determine the ability of aged FDCs to trap immune complexes, small numbers of intact PrPc-expressing FDC networks were observed. Therefore, it is plausible that in some of these aged mice, these small populations of functional FDC networks were disrupted, lacked PrPc-expression, and diminished to the time of exposure were sufficient to allow a limited degree of neuroinvasion to occur for a transient period before their eventual decline. Although a significant majority of the FDC networks in the spleens of aged mice were disrupted, lacked PrPc-expression, and diminished at the time of exposure were sufficient to allow a limited degree of neuroinvasion to occur for a transient period before their eventual decline. Although a significant majority of the FDC networks in the spleens of aged mice were disrupted, lacked PrPc-expression, and diminished at the time of exposure were sufficient to allow a limited degree of neuroinvasion to occur for a transient period before their eventual decline. Therefore, our data suggest that the combined defects in immune complex trapping and PrPc expression dramatically reduce the ability of aged FDCs to acquire and replicate TSE agents.

Following peripheral exposure to the scrapie agent many factors will act on the inoculum to aid its elimination from the host. For example, after oral exposure much will be eliminated by factors including digestion (by enzymes secreted into the gastrointestinal tract) and excretion. As a consequence, very little of the original inoculum will be available to be translocated to FDCs within lymphoid tissues. In young adult mice it is likely that a competitive state exists whereby cells such as macrophages aid the clearance of the TSE agent (41, 42), whereas FDCs act to expand the levels of the TSE agent above the threshold required to achieve neuroinvasion. Thus, as the status of FDCs declines in aged mice, our data suggest that although host age may present a significant barrier against TSE transmission, the inefficient neuroinvasion in aged individuals may lead to significant levels of subclinical TSE disease in the population.

Previous studies suggest that the Ag trapping capacity of FDCs becomes defective with aging, markedly impairing their ability to provide costimulatory signals to B cells and stimulate germinal center formation (23–26). In the present study the mAb FDC-M2, which detects complement C4 on FDC-associated immune complexes (32), was used to determine the ability of aged FDCs to trap and retain immune complexes. We show that the decline in the Ag trapping capacity of FDCs coincided with the loss of cellular PrPc expression. Host cells must express cellular PrPc to sustain TSE infection (40). Therefore, our data suggest that the combined defects in immune complex trapping and PrPc expression dramatically reduce the ability of aged FDCs to acquire and replicate TSE agents.

### Table I. Effect of host age on scrapie agent susceptibility and disease pathogenesis

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Exposure Route</th>
<th>Individual Disease Incubation Periods or Survival Times (days)</th>
<th>Mean Disease Incubation Period (days ± SEM) or Survival Range</th>
<th>Clinical Disease</th>
<th>Vacuolar Pathology in Brain</th>
<th>PrPc in Brain</th>
<th>PrPc in Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>9/12 (p &lt; 0.001)</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Young</td>
<td>i.p.</td>
<td>244, 262, 262, 267, 267, 267, 268, 282, 298, 302, 323</td>
<td>247–323 ± 14</td>
<td>No</td>
<td>0/9</td>
<td>3/9</td>
<td>6/9</td>
</tr>
<tr>
<td></td>
<td>i.c.</td>
<td>168, 177, 194, 254, 254, 301, 301, 323, 314, 321, 321</td>
<td>(179 ± 8)</td>
<td>No</td>
<td>12/12 (p = 0.002)</td>
<td>6/9</td>
<td>3/9</td>
</tr>
<tr>
<td>Young</td>
<td>i.c.</td>
<td>163, 168, 176, 176, 176, 176, 176, 176, 176, 176, 176, 176</td>
<td>174 ± 3</td>
<td>No</td>
<td>3/9</td>
<td>6/9</td>
<td>3/9</td>
</tr>
</tbody>
</table>

a Young C57BL/Dk mice were 6–8 wk old at the time of scrapie agent exposure. Aged C57BL/Dk mice were ~600 days old at the time of scrapie agent exposure.

b Only aged mice that were culled after the first positive TSE case in the corresponding young mouse group were included in the analysis.

c Incidence = no. animals affected/no. animals tested. Only aged mice that were culled after the first clinically positive TSE case in the young mice were included in the analysis.

d Statistical differences between the numbers of aged mice and young mice with positive vacuolar pathology were determined using a Fisher’s exact two-tailed test.
The first positive TSE cases in the young mice were observed 244 days after i.p. exposure and 282 days after oral exposure. Although the corresponding ages of the aged mice were 844 and 882 days old (for i.p. and oral TSE-exposed mice, respectively) at these times, 9 aged mice survived between 247 and 323 days after i.p. exposure (between 847 and 923 days old), and 12 aged mice survived between 282 and 366 days after oral exposure (between 882 and 966 days old). None of these peripherally TSE-exposed aged mice developed clinical signs of disease during its lifespan, although a proportion of the aged mice did display histopathological signs of TSE disease in their brains. This suggests that in some mice, neuroinvasion may have occurred independently of FDCs, albeit by a much less efficient route that did not allow the level of the TSE agent in the brain to reach the magnitude required to cause clinical disease during the host’s lifespan (37–39). As histopathological signs of TSE disease were detected in the brains of many of the aged mice, the possibility cannot be excluded that if the mice had survived longer some may have developed clinical signs of disease after substantially extended incubation periods. However, all of the aged mice were at least 2.3 years old when culled.

At present, no TSE-specific preclinical diagnostics are available, compounding the problems for disease treatment and eradication.

**FIGURE 5.** Effect of host age on TSE-specific neuropathology in the brain. Pathological assessment of the spongiform change (vacuolation) in the brains of young (closed circles) and aged (open circles) mice with positive vacuolar pathology. Vacuolation was scored on a scale of 0–5 in nine gray matter areas: 1, dorsal medulla; 2, cerebellar cortex; 3, superior colliculus; 4, hypothalamus; 5, thalamus; 6, hippocampus; 7, septum; 8, retrosplenial and adjacent motor cortex; 9, cingulate and adjacent motor cortex. Each point represents mean vacuolation score ± SEM for groups of 3–12 mice. To determine whether the severity of the vacuolation was significantly altered in aged mice, the area under the lesion profile curve (AUC) was found for each mouse using the trapezoidal rule. The differences in the median AUC between the aged and young mice were tested using a two-tailed Mann-Whitney U test. The severity of the vacuolated pathology was significantly lower in the aged mice following exposure to the scrapie agent by i.p. injection (A, \( p = 0.022, n = 12 \) young, \( n = 3 \) aged) and oral exposure (B, \( p = 0.001, n = 12 \) young, \( n = 5 \) aged). Although both aged and young mice injected i.c. with the scrapie agent developed clinical disease with similar incubation periods, the severity of vacuolar pathology was significantly lower in the brains of the aged mice (C, \( p = 0.024, n = 6 \) young, \( n = 3 \) aged).

**FIGURE 6.** Comparison of sympathetic innervation in the spleens (A–C) and intestines (D and E) of young and aged mice. A and B, Immunohistochemical detection of TH-positive sympathetic nerves (green) and FDCs (CR1/CR2-positive cells; red) in the spleens of uninfected young (A) and aged (B) C57BL/Dk mice. The location of sympathetic nerves (white arrow) within the spleens of both young and aged mice appeared similar and was predominantly located within the T cell areas, around the central arteriole. FO indicates location of FDC-containing follicles; original magnification \( \times 200 \); scale bar = 100 \( \mu \)m. C, Quantitative analysis of the area occupied by TH-positive sympathetic nerves in the spleens of uninfected aged mice and young mice (\( n = 5 \)). The area occupied by TH-positive nerves was measured in six random fields of view from each spleen when viewed with the \( \times 10 \) objective. No significant difference between the area occupied by TH positive nerves in spleens from young and aged mice was observed (\( p = 0.9 \); Student’s t test). D and E, Immunohistochemical analysis of the status of PrP\(^c\)-expressing (D) and TH-positive (E) sympathetic nerves in the small intestines of uninfected young and aged C57BL/Dk mice. No differences were observed in the distribution and density of sympathetic nerves (white arrow) within the intestines of both young and aged mice. Scale bar = 100 \( \mu \)m.
The detections of some TSE agents in blood (43) or lymphoid tissue biopsy specimens such as tonsils, appendix (7, 44), and reticul-associated lymphoid follicles (45–48) have each been proposed as useful methods to detect some preclinical TSE diseases. Our data presented herein revealed that there was considerable variation in the detection of the TSE agent in the spleens of the clinically negative aged survivors. Although most TSE-exposed aged mice had detectable levels of PrP\textsuperscript{\textsc{d}} in their brains, PrP\textsuperscript{\textsc{d}} was absent in the spleens of many mice. These data have important implications for the development of reliable preclinical diagnostics, as tests based on the detection of the TSE agent in blood or lymphoid tissues may be less sensitive when used on elderly patients and livestock species.

Our data show that when the TSE agent was delivered directly to the brain, host age had no significant influence on the onset of clinical disease. Furthermore, our data show that host age did not influence the distribution of TH-positive sympathetic nerves in the spleen and small intestine. These data provide strong evidence that the effects of age observed on susceptibility to peripherally acquired TSE infection were not due to effects on the central and peripheral nervous systems. Our recent data suggest that conventional dendritic cells may play an important role in the initial translocation of TSE agents from the gut lumen to the GALT (49). Moretto and colleagues have recently shown that mucosal dendritic cells have a reduced capacity to prime T cells and secrete IL-15 (50). Whether aging likewise affects the distribution of DCs at the sites of TSE agent exposure and their ability to acquire them and deliver them to draining lymphoid tissues is not known. Therefore, although the effects of age on other factors cannot be entirely excluded, our data imply that following peripheral exposure, the effects of host age on TSE pathogenesis are most likely due to effects on FDC status dramatically impairing TSE agent accumulation in lymphoid tissues and subsequent neuroinvasion. Data from the present study agree with data from a comparative study of Peyer’s patches from sheep, cattle, and humans that suggested an age-related association between Peyer’s patch development and susceptibility to natural TSE infection (51).

In conclusion, although the reasons associated with the predominance of vCJD cases in young people may be complex, our data suggest that the pathogenesis of peripherally acquired TSE agents such as natural sheep scrapie, chronic wasting disease in cervids, and vCJD in humans are much less efficient in the aged than in young individuals. Although host age may represent an important barrier to the efficient transmission of peripherally acquired TSE agents, our data suggest there may be significant levels of subclinical disease in the elderly population. Furthermore, our data suggest that the tissue distribution of some TSE agents may differ significantly between the young and elderly. These data raise important issues for the development of reliable preclinical TSE-specific tests, the assessment of risk tissues, and the control of iatrogenic spread.

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Disclosures
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References


