SCID mouse spleen does not support scrapie agent replication

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BALB/c and severe combined immunodeficiency (SCID) mice were inoculated intracerebrally or intraperitoneally with scrapie agent strain ME7 to examine the role of functional lymphocytes and follicular dendritic cells in splenic infectivity and PrP\(_{Sc}\) accumulation. Intracerebrally inoculated BALB/c and SCID mice developed the clinical signs and microscopic lesions characteristic of scrapie. Spleens from terminally affected BALB/c mice contained PrP\(_c\) which was detectable by immunoblot analysis; SCID mouse spleens did not contain detectable PrP\(_c\). SCID mouse spleens collected during the first 90 days after intraperitoneal infection contained neither infectivity nor PrP\(_c\).

Sheep scrapie is the archetype of a heterogeneous group of rare transmissible spongiform encephalopathies (TSEs) occurring naturally in humans, cattle, mink, cats and captive deer (Hartsough & Burger, 1965; Williams & Young, 1980; Wells et al., 1987; Leggett et al., 1990; Brown & Gajdusek, 1991). The histopathological lesions of TSEs are restricted to the nervous system (Chandler, 1969). However, immunohistochemical and biochemical analyses detect a host cellular membrane glycoprotein, the prion protein (PrP\(_c\)) (Prusiner, 1982; Oesch et al., 1985; Basler et al., 1986), that accumulates in lymphoid and nervous tissues as a relatively protease-resistant isoform [PrP\(_{Sc}\) in scrapie-infected animals and PrP\(_{Sc}^{CJD}\) in Creutzfeldt-Jakob disease (CJD)-infected animals] (Merz et al., 1987; Doi et al., 1988; Farquhar et al., 1989; Kitamoto et al., 1989; Race et al., 1992). PrP\(_c\) is tightly associated with infectivity and may either contain or be the infectious agent itself (Diringer et al., 1983; McKinley et al., 1983; Merz et al., 1984, 1987). Infectivity and PrP\(_{Sc}\) accumulation in the spleen are detectable within 2 weeks of experimental infection in rodents and persist throughout the incubation period (Eklund et al., 1967; Race & Ernst, 1992). Scrapie infectivity in the spleen is associated with post-mitotic non-lymphoid (stromal) cells and is unaffected by thymectomy (McFarlin et al., 1971; Worthington & Clark, 1971; Clarke & Kimberlin, 1984; Robinson & Gorham, 1990). PrP\(_c\) in normal mouse spleens and PrP\(_{Sc}\)/PrP\(_{Sc}^{CJD}\) accumulation in infected mouse spleens is restricted to cells with the morphological characteristics and antigen-trapping capacity of follicular dendritic cells (FDCs) (Kitamoto et al., 1991; McBride et al., 1992). In spite of these similarities, the splenic replication phase is not obligatory in all rodent TSE models. Prion mRNA is detectable in mouse spleens by Northern blot analysis (Caughey et al., 1988) and PrP\(_c\) is detectable by immunoblot and immunofluorescence analysis of hamster leukocytes and spleens (Bendheim et al., 1992).

Severe combined immunodeficiency (SCID) mice, which lack functional FDCs secondary to the absence of lymphocytes (Kapasi et al., 1993), do not develop clinical signs of CJD or accumulation of splenic PrP\(_{Sc}^{CJD}\) following intraperitoneal (i.p.) inoculation (Kitamoto et al., 1991), although CJD is readily transmitted to SCID mice by the intracerebral (i.c.) route. To extend this observation, we have examined splenic infectivity and PrP\(_{Sc}\) accumulation in SCID mice inoculated with scrapie agent strain ME7.

Homozygous C.B.-17-SCID/SCID female mice, 3 weeks of age, were obtained from Taconic Laboratories and normal 3-week-old female BALB/c mice were obtained from B&K Universal. All mice were housed in groups of two to four animals in microisolator cages and given autoclaved food and water. Scrapie agent strain ME7 was obtained as whole frozen brains from terminally infected C57BL mice. A 10% (w/v) brain suspension was prepared using sterile Tris-buffered saline (TBS; 0.05 M-Tris-HCl pH 7.8, 0.1 M-NaCl) in a sterile glass Dounce homogenizer and frozen at -20 °C until required. Mice were inoculated by the i.c. route with 30 µl or by the i.p. route with 100 µl of freshly thawed 10% brain homogenate or with dilutions made in sterile TBS. Controls included untreated mice and mice inoculated with similar volumes of a 10% homogenate of uninfected C57BL brain or with TBS alone. Mice were inoculated intracerebrally or intraperitoneally with scrapie agent strain ME7 to examine the role of functional lymphocytes and follicular dendritic cells in splenic infectivity and PrP\(_{Sc}\) accumulation. Intracerebrally inoculated BALB/c and SCID mice developed the clinical signs and microscopic lesions characteristic of scrapie. Spleens from terminally affected BALB/c mice contained PrP\(_c\) which was detectable by immunoblot analysis; SCID mouse spleens did not contain detectable PrP\(_c\). SCID mouse spleens collected during the first 90 days after intraperitoneal infection contained neither infectivity nor PrP\(_c\).
Table 1. Titration of ME7 in BALB/c and SCID mice

<table>
<thead>
<tr>
<th>Dilution of whole ME7 brain (log_{10})</th>
<th>Animals affected/number inoculated [incubation range (days)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BALB/c</td>
</tr>
<tr>
<td>-1</td>
<td>3/3 (146–147)</td>
</tr>
<tr>
<td>-2</td>
<td>3/3 (161–180)</td>
</tr>
<tr>
<td>-3</td>
<td>4/4 (161–182)</td>
</tr>
<tr>
<td>-4</td>
<td>4/4 (171–196)</td>
</tr>
<tr>
<td>-5</td>
<td>4/4 (189–198)</td>
</tr>
<tr>
<td>-6</td>
<td>3/3 (243)</td>
</tr>
<tr>
<td>-7</td>
<td>4/4 (252)</td>
</tr>
<tr>
<td>-8</td>
<td>0/4†</td>
</tr>
</tbody>
</table>

* ND, Not done.
† Animals euthanized at 426 days p.i.

Table 2. Infectivity bioassay of spleens from i.p. inoculated BALB/c and SCID mice

<table>
<thead>
<tr>
<th>Time p.i. (days)</th>
<th>Animals affected/number inoculated [incubation range (days)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BALB/c donor</td>
</tr>
<tr>
<td>30</td>
<td>16/16 (167–196)</td>
</tr>
<tr>
<td>60</td>
<td>15*/15 (164–174)</td>
</tr>
<tr>
<td>90</td>
<td>16/16 (174–201)</td>
</tr>
</tbody>
</table>

* Excludes one mouse which died within 24 h of inoculation.

Infectivity was detectable in all spleens collected from ME7-inoculated BALB/c mice. Mean incubation times for recipients of spleens collected at 30, 60 and 90 days p.i. were 187, 168 and 184 days respectively (Table 2), consistent with the observation that splenic infectivity levels reach a plateau within 2 months following subcutaneous (Eklund et al., 1967) or i.c. inoculation (Race & Ernst, 1992). Recipients of inocula from the spleens of ME7-inoculated SCID mice were clinically normal throughout the 360 day observation period. Recipients of inocula from the spleens of uninoculated BALB/c and SCID mice also remained clinically normal. These observations demonstrate that scrapie infectivity, as defined by mouse subinoculation, was not detectable in the spleens of scrapie-inoculated SCID mice during the first 90 days p.i.

PrPSc accumulation was monitored by immunoblot analysis of tissues using the procedure described by Race & Ernst (1992) with slight modifications. Brains and spleens were collected from i.c. inoculated mice when they were terminally affected and from clinically normal, i.p. inoculated mice at 60 days p.i.Brains from individual mice or 200 to 250 mg aliquots of spleens from groups of three mice were prepared as 10% (w/v) homogenates in sterile 0.01 M-Tris–HCl pH 7.5, 0.005 M-MgCl2 using disposable homogenizers, digested with DNase (final concentration 1 U/ml) for 1 h at 37 °C, and kept at −20 °C until assayed. Samples were adjusted to 10% Sarkosyl, clarified at 10000 g for 30 min at 20 °C and pelleted at 215000 g for 2 h at 4 °C. Each pellet was resuspended in 1 ml of 0.01 M-Tris–HCl pH 7.5 and incubated with proteinase K (10 μg per g starting tissue) for 30 min at 37 °C. The suspension was adjusted to 5 mM-PMSF and centrifuged at 212000 g for 1 h at 4 °C. Pellets were boiled in SDS–PAGE sample buffer (final SDS concentration 5%) and stored at −20 °C until used for Western immunoblots. PrPSc preparations (representing 1/2 spleen or 1/4 brain per lane) were electrophoresed on 12% polyacrylamide gels (Pharmacia PhastSystem), transferred to PVDF membranes (Immobilon-P, Millipore) and probed with anti-PrP or control rabbit serum. Anti-PrP serum was prepared by inoculation of rabbits with the pentadecapeptide GQGGGTHNQWNKPSK (Shinagawa et al., 1986) synthesized as a multimeric macromolecule on a heptad lysine backbone (serum R2843) (Tam, 1988) or covalently coupled to keyhole limpet haemocyanin (serum R27) (Wiley et al., 1987; Race et al., 1992). Bound rabbit antibody was detected with goat anti-rabbit IgG conjugated to horseradish peroxidase followed by incubation with a chemiluminescent substrate (Amersham) diluted 1:2 in distilled water. Membranes were exposed to ECL.
Hyperfilm (Amersham) for intervals varying from 5 to 60 s. Both antisera gave comparable staining patterns, although R27 was 50- to 100-fold more sensitive.

PrP\textsuperscript{sc} was detected in the brain but not the spleen of terminally affected, i.e. inoculated SCID mice (Fig. 1) and in both the brain and spleen of i.e. inoculated BALB/c mice. PrP\textsuperscript{sc} was detected in BALB/c mouse spleen but not in SCID mouse spleen collected 60 days after i.p. inoculation. These findings are consistent with the observation that SCID mouse spleen does not accumulate PrP\textsuperscript{GJD} following either i.e. or i.p. inoculation (Kitamoto et al., 1991). In that study, PrP\textsuperscript{GJD} was undetectable in the spleen of SCID mouse collected 500 days after i.p. inoculation.

In this study, we have demonstrated that the scrapie agent does not replicate in the spleens of SCID mice. Furthermore, PrP\textsuperscript{sc} does not accumulate in SCID mouse spleen following i.p. or i.e. inoculation with the scrapie agent. Failure of SCID mouse spleen to accumulate PrP\textsuperscript{sc} or to harbour infectivity may be due to the lymphocyte deficiency, the failure of FDCs to mature, or other less well characterized defects in SCID mice. Immunohistochemical evidence for PrP\textsuperscript{C} and PrP\textsuperscript{sc}/PrP\textsuperscript{GJD} in FDCs is consistent with early experiments using cyclophosphamide-treated mice, which suggested that the tissue-associated non-mitotic cells (Worthington & Clark, 1971), rather than the circulating lymphocytes, are the site of agent replication.

The association of the scrapie agent with antigen-trapping cells such as FDCs is of interest because the agent may replicate in that cell type or it may accumulate there after replication in other tissues and transport via the peripheral circulation (Tenner-Racz et al., 1985; Mori et al., 1991). Pre-clinical diagnostic testing of sheep by identification of PrP\textsuperscript{sc} in lymphoid tissue has been proposed (Ikegami et al., 1991; Race et al., 1992; Onodera et al., 1993). The feasibility of such testing increases significantly if a circulating cell type containing infectivity or PrP\textsuperscript{sc} can be identified. Further discrimination between endogenous and exogenous sources of the FDC-associated scrapie agent will be critical to our understanding of the extraneural pathogenesis of the TSEs.

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References


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