J. Comp. Path. 2006, Vol. 134, 126–133



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# Short-term Study of the Uptake of $PrP^{Sc}$ by the Peyer's Patches in Hamsters after Oral Exposure to Scrapie

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## Summary

The disease-associated prion protein ($PrP^{Sc}$) has been detected in the ileal Peyer's patches of lambs as early as one week after oral exposure to scrapie. In hamsters, the earliest reported time of $PrP^{Sc}$ detection in the Peyer's patches after oral exposure to scrapie is 69 days post-infection. To evaluate the acute uptake of inoculum and to investigate whether the Peyer's patches constitute the primary site of entry for scrapie after oral exposure, hamsters were each exposed orally to 1 ml of a 10% brain homogenate from hamsters in the terminal stage of infection with the 263 K strain of the scrapie agent. $PrP^{Sc}$ was demonstrated in the Peyer's patches only a few days after exposure, i.e., much earlier than previously reported. This study supports the view that the Peyer's patches constitute at least one of the primary entry sites of $PrP^{Sc}$ after oral exposure to scrapie.



*Keywords:* hamster; Peyer's patches; prion disease; scrapie; sheep

## Introduction

Scrapie is a transmissible spongiform encephalopathy (TSE) of sheep and goats occurring endemically in large parts of Europe and the Northern American continent (Detwiler and Baylis, 2003). Transmission of scrapie is, together with chronic wasting disease of cervids (Miller and Williams, 2004), relatively efficient as compared with transmission of other TSEs and follows both horizontal (i.e., from one flock-member to another) and vertical (i.e., transplacental, germinal or lactogenic) infection routes (Detwiler and Baylis, 2003; Miller and Williams, 2004). Oral uptake of scrapie infectivity followed by gastrointestinal tract absorption is considered the main entry route in horizontal transmission (Detwiler and Baylis, 2003), and the gut-associated lymphoid tissue (GALT) has a central role in this process. Peyer's patches, which are aggregated GALT follicles localized within the intestines, play a major role in the absorption of macromolecules from the gut. Histologically, Peyer's patches consist of (1) a central lymphoid follicle containing B and T lymphocytes and follicular dendritic cells (FDCs), and (2) domes, facing the gut lumen and made up of follicle-associated epithelium (Kelsall and Strober, 1996; Owen, 1999). The epithelium overlying the dome houses the specialized antigen-sampling M cells (Bye *et al.*, 1984; Owen, 1999).

Scrapie infectivity is generally related to the presence of the disease-associated prion protein ($PrP^{Sc}$) (McKinley *et al.*, 1983; Race *et al.*, 1998), a misfolded variant of the normal prion protein ($PrP^{C}$) (Prusiner *et al.*, 1998; Prusiner, 2001). This has for instance been demonstrated for the 263 K strain of scrapie in hamsters (Beekes *et al.*, 1996), that was used in the present study. $PrP^{C}$ is a prerequisite for scrapie-infection (Brandner *et al.*, 1996) and is expressed in the gastrointestinal tract (Heggebo *et al.*, 2000;

0021-9975/$ - see front matter
doi:10.1016/j.jcpa.2005.08.002

Pammer *et al.*, 2000). Preclinical detection of $PrP^{Sc}$ in the ileal Peyer's patches has been reported in sheep one week after oral exposure to scrapie (Heggebo *et al.*, 2000, 2002, 2003). In hamsters, the earliest recorded uptake of $PrP^{Sc}$ after oral exposure to scrapie has so far been at 69 days post-infection (dpi), $PrP^{Sc}$ being clearly detected in the Peyer's patches and other GALT components as well as in ganglia of the enteric nervous system (Beekes and McBride, 2000). In cervids, $PrP^{Sc}$ was detected in the Peyer's patches 69 days after oral exposure to chronic wasting disease (Sigurdson *et al.*, 1999). In cattle and non-human primates orally exposed to bovine spongiform encephalopathy (BSE), $PrP^{Sc}$ was detected in the Peyer's patches after 6 and 5 months, respectively (Bons *et al.*, 1999; Terry *et al.*, 2003). From the Peyer's patches and other GALT, $PrP^{Sc}$ appears to be spread to the enteric nervous system and further to sympathetic and parasympathetic autonomic nerves (especially the efferent components of vagus and splanchic nerves). Retrograde transport then transfers $PrP^{Sc}$ to the brain stem and the rest of the central nervous system (CNS) (McBride *et al.*, 2001).

The immune system plays a central role in the uptake, spread and replication of $PrP^{Sc}$; especially in early replication in lymphoid organs (Aguzzi and Heikenwalder, 2005). The localization of $PrP^{Sc}$ in the Peyer's patches generally seems to be associated with FDCs, and mice devoid of FDCs show a highly reduced efficiency of prion replication after peripheral exposure to scrapie (Prinz *et al.*, 2002). Lymphoid organs are innervated by sympathetic nerves (Felten *et al.*, 1988) and the proximity of these nerves to the FDCs is essential for susceptibility to peripheral prion infection (Forster *et al.*, 1996; Prinz *et al.*, 2003). Other cells of the immune system, such as macrophages and dendritic cells, also play a role in TSE pathogenesis, especially in the uptake and early replication of $PrP^{Sc}$ (Kelsall and Strober, 1996; Beringue *et al.*, 2002; Huang *et al.*, 2002).

To investigate whether the Peyer's patches represent a primary entry site of scrapie in the gut, the present study was designed to detect $PrP^{Sc}$ in the Peyer's patches of hamsters only a few days after oral exposure to the scrapie strain 263 K. The techniques used consisted of immunahistochemistry (IHC) and paraffin wax-embedded tissue blot (PET-blot), the latter having been described as the more sensitive method (Schulz-Schaeffer *et al.*, 2000).

## Materials and Methods

### *Animals, Infection and Tissue Preparation*

Ten outbred male Syrian hamsters aged 6 weeks (Charles River Deutschland, Sulzfeld, Germany) were housed in Class II facilities at the Danish Institute for Food and Veterinary Research. Eight overnight-starved hamsters were each fed 1 ml of a 10% (w/v) brain homogenate in phosphate-buffered saline (PBS). The homogenate, prepared from hamsters in the terminal stage of infection with strain 263 K (Beekes *et al.*, 1996), was administered on food pellets to individually caged animals. At 1, 2, 4 and 8 days post-infection, hamsters were anaesthetized in pairs and killed by decapitation. Two non-exposed hamsters served as controls. The gut was removed and sections of intestine containing Peyer's patches were dissected and placed in periodate lysine paraformaldehyde fixative (McBride *et al.*, 1998) for 24 h, then in 70% ethanol for 48 h, after which they were dehydrated and embedded in paraffin wax. Sections (4 µm) were cut and collected on glass slides for IHC or on nitrocellulose (NC) membranes (Advantec MFS, Inc., CA, USA) for PET blot. NC membrane-bound brain sections (8 µm) from 263 K-affected hamsters were used as positive controls, the sections having been prepared from animals inoculated intracerebrally (ic) 90 days earlier with 50 µl of a 1% 263 K brain homogenate. Sections from non-exposed hamsters were used as negative controls.

### *IHC*

After dewaxing and rehydration, endogenous peroxidase activity was blocked with $H_2O_2$ 3% in methanol for 15 min, followed by washing for 5 min in distilled water. To enhance the antigenicity of $PrP^{Sc}$, the sections were treated for 20 min with 96% formic acid, rinsed and then autoclaved in distilled water for 10 min at 121° C. After cooling, washing was performed twice in washing buffer (PBS containing bovine serum albumin 0.2%). Blocking was performed with normal horse serum (Vectastain kit PK-6102; Vector Laboratories, Burlingame, CA, USA) for 30 min. Excess blocking buffer was removed before adding primary antibody, 1 µg/ml; the latter consisted of either 3F4 (P1115; Sigma-Aldrich, Brøndby, Denmark, Brøndby, Denmark), 1.5D7 (see below), or an IgG2b-isotype control antibody (X0944; Dako, Glostrup, Denmark). Incubation was performed overnight at 4 °C. After washing, incubation with secondary antibody (horse anti-mouse; Vectastain kit PK-6102) was performed for 1 h at room