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## Biology of PrP<sup>sc</sup> accumulation in two natural scrapie-infected sheep flocks

Patrick Caplazi, Katherine O'Rourke, Cynthia Wolf, Daniel Shaw, Timothy V. Baszler<sup>1</sup>

**Abstract.** Sheep scrapie is a prion disease that requires interaction of exogenous prions with host prion protein (PrP) supporting prion formation. Disease is associated with deposition of a host-generated conformational variant of PrP, PrP<sup>sc</sup>, in a variety of tissues, including brain, resulting in fatal spongiform encephalopathy. Efficiency of PrP<sup>sc</sup> formation is determined by polymorphisms in the PrP-coding sequence. This article adds to previous data of natural sheep scrapie, concentrating on the effect of host genotype and age on PrP<sup>sc</sup> accumulation patterns during preclinical and clinical disease. Two entire scrapie-infected, predominantly Suffolk-cross, sheep flocks euthanized for regulatory purposes were genotyped and analyzed for PrP<sup>sc</sup> deposition in various tissues using single- and dual-label immunohistochemistry. Scrapie, as defined by PrP<sup>sc</sup> deposition, occurred in 13/80 sheep. Preclinical disease was evident in nearly 70% of infected sheep, ranging in age from 14 months to 7 years. PrP<sup>sc</sup> accumulated systemically in the nervous tissue, various lymphoid tissues, both alimentary tract related and non-alimentary tract related, and the placenta. Clinical neurological illness was always associated with spongiform encephalopathy and PrP<sup>sc</sup> deposition in the brain. Only 6 of 9 sheep with preclinical scrapie had PrP<sup>sc</sup> deposition in the brain but widespread PrP<sup>sc</sup> deposition in peripheral lymphoid tissue, supporting previous data showing peripheral PrP<sup>sc</sup> accumulation preceding deposition in the brain. PrP<sup>sc</sup> colocalized with a marker for follicular dendritic cells throughout the lymphoid system. PrP<sup>sc</sup> also accumulated in the peripheral nervous system, particularly the nervous supply of the gastrointestinal tract. Abundant PrP<sup>sc</sup> was evident in trophoblast cells of placentomes but not in the endometrium, myometrium, or associated nervous plexus. PrP<sup>sc</sup> deposits were not observed in the mammary parenchyma or bone marrow. Scrapie susceptibility was defined genetically by PrP codon 171: PrP<sup>sc</sup> deposition was restricted to PrP genotype AA<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub> in 12/13 cases or AV<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub> in 1/13 cases. The earliest accumulation was observed in the single VRQ/ARQ heterozygous animal, consistent with the reported high scrapie susceptibility and brief incubation period observed in breeds with predominance of the V<sub>136</sub>R<sub>154</sub>Q<sub>171</sub> allele. Disease occurred within, as well as independent of, mother-daughter lines, suggesting both maternal and nonmaternal transmission in the flocks.

### Introduction

Scrapie is the prototype of those transmissible spongiform encephalopathies (TSE) with a combined infectious and genetic pathogenesis and occurs naturally in sheep and goats.<sup>10–12</sup> The importance of oral uptake of infectious material for transmission<sup>12,29,30</sup> and the contribution of lymphoid tissue related to the alimentary tract for local amplification of PrP<sup>sc</sup>, a conformational variant of prion protein (PrP), have been recognized<sup>12,18</sup> and is supported by experimental models.<sup>3,4</sup> Data indicate that scrapie generally follows oral uptake of prions and includes a preclinical phase characterized by local amplification of PrP<sup>sc</sup> in the lym-

phoid tissue of the alimentary tract and transport through the peripheral nervous system into the central nervous system (CNS).<sup>8,13,14</sup> PrP<sup>sc</sup> accumulation patterns vary greatly among prion diseases, and susceptibility to scrapie is greatly modified by polymorphisms in the PrP gene, *PRNP*. This article, in support of previous reports, describes the biology of natural scrapie in 2 sheep flocks with specific reference to the tissue and cellular topography of PrP<sup>sc</sup> during preclinical and clinical disease, the role of host genotype in disease incubation, and potential modes of agent transmission.

### Materials and methods

**Animals and selection of tissue samples.** Sheep ( $n = 80$ ) from 2 privately owned flocks were euthanized and necropsied for disease regulatory purposes after observation of index clinical scrapie cases. Flock A, containing 50 sheep, originated from North Central United States, and flock B, containing 30 sheep, originated from the Pacific Northwest United States. Both flocks consisted predominately of Suffolk ( $n = 51$ ) or Suffolk-cross sheep ( $n = 22$ ), with the remainder being Black desert ( $n = 3$ ) or White desert ( $n = 4$ ). Sheep ranged in age from 12 mo to >6 yr, the majority

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**Table 1.** Age distribution of PrP<sup>sc</sup> and disease status in 2 scrapie-infected flocks.

Age	PrP <sup>sc</sup> negative*			PrP <sup>sc</sup> positive†					
	n	% total‡	Similar age (%)§	Clinical			Preclinical		
				n	% total	Similar age (%)	n	% total	Similar age (%)
≤14 mo	45	56.25	98	0	0	0	1	1.25	2
2 yr	4	5.00	50	0	0	0	4	5.0	50
3 yr	7	8.75	63.6	1	1.25	9	3	3.75	36.3
4 yr	6	7.50	75	2	2.5	25	0	0	0
5 yr	3	3.75	75	1	1.25	25	0	0	0
≥6 yr	2	2.50	66.6	0	0	0	1	1.25	33.3
Total	67	83.75	NA	4	5.0	NA	9	11.25	NA

\* No PrP<sup>sc</sup> immunoreactivity in any tissue.

† PrP<sup>sc</sup> immunoreactivity in at least 1 tissue.

‡ Percentage of total number of sheep in 2 flocks.

§ Percentage of number of sheep of similar age.

|| NA = not applicable.

being ≤2 yr old ( $n = 54$ ). Information on clinical signs and familial lineage was collected for individual sheep.

Various tissues were collected postmortem and fixed in 10% formalin for analysis of microscopic lesions by histopathology and PrP<sup>sc</sup> deposition by immunohistochemistry. Anatomic sites of tissues for PrP<sup>sc</sup> immunohistochemistry were 1) CNS (brain), 2) alimentary tract (jejunum, ileum, colon, rectum) and related lymph nodes, 3) lymphoid system not related to the alimentary tract (third eyelid, spleen, and various lymph nodes [superficial cervical, subiliac, medial iliac]), 4) mammary gland and related lymph node, 5) hematopoietic tissues (bone marrow), and 6) reproductive/fetal tissues (uterus, placenta, fetal spleen, fetal ileum).

**Determination of PrP genotype.** Whole blood was collected for isolation of genomic DNA from the leukocyte fraction. Amino acid polymorphisms at PrP codons 136, 154, and 171 were determined by DNA sequencing using commercially available services.<sup>a,b</sup> DNA sequencing followed PCR amplification using primers specific for the entire PrP-coding sequence, as described previously.<sup>38</sup>

**Immunohistochemical detection of PrP<sup>sc</sup>.** Single-label immunohistochemistry for PrP<sup>sc</sup> was performed on formalin-fixed, paraffin-embedded tissues using heat-induced antigen retrieval and 90% formic acid pretreatment as described previously.<sup>25</sup> F89.160.1.5 (F89), a monoclonal antibody (mAb) to ruminant PrP targeting a conserved epitope at residues 142 through 145 of ovine PrP<sup>26</sup> was used at a concentration of 1 µg/ml in a labeled streptavidin-biotin-based system.<sup>c</sup> Amino-ethyl-carbazole (AEC) was used as a chromogen.<sup>d</sup> Positive control tissue, consisting of brain or lymph node from a known scrapie-positive sheep, was included in each run to confirm immunoreactivity of the appropriate pattern and intensity in lymphoid follicle germinal centers and in the neuropil of the brain as described previously.<sup>26,27</sup> Negative controls consisted of a negative antibody control and a negative tissue control. Negative antibody was an irrelevant isotype-matched primary antibody (anti-*Neospora caninum* mAb Nc-5B6-25, 1 µg/ml) reacted with each test slide to ensure the lack of nonspecific binding by linker or signal amplification reagents to tissue sections. Negative tissue

control consisted of brain and lymph node of a known scrapie-negative sheep reacted with anti-PrP mAb F89 to ensure absence of PrP<sup>sc</sup> immunoreactivity.

Conditions of antigen retrieval and pretreatment procedures were adapted for dual immunohistochemistry using antibodies toward cell-specific markers in conjunction with F89 for PrP<sup>sc</sup>. Antibodies toward cell markers included mAb CNA.42 for follicular dendritic cells (FDCs),<sup>e</sup> anti-CD3 rabbit antiserum for T lymphocytes,<sup>d</sup> anti-CD79a mAb for B lymphocytes,<sup>e</sup> anti-neuron-specific enolase rabbit antiserum for ganglion cells,<sup>d</sup> and mAb NCL-LN5 for macrophage/monocytes.<sup>f</sup> Dual labeling was carried out sequentially using cell-specific antibodies with peroxidase-based color reaction (red) in the first sequence and F89 with alkaline phosphatase-based color reaction (black) in the second sequence. After development of the red signal (AEC) under microscopic control, slides were incubated with 0.3 M glycine HCl at pH 1.8 at room temperature overnight to denature potential binding sites on linker reagents from the first sequence; then, residual biotin binding was blocked with an avidin-biotin-blocking kit.<sup>g</sup> As a control for the efficacy of blocking steps between sequences (i.e., nonspecific staining of the second immunostaining sequence), slides were treated with the full first sequence except for the color reaction and then subjected to the blocking steps and the full second sequence omitting primary antibody incubation. Absence of black signal (second signal) in these slides indicated effectiveness of disruptive glycine HCl treatment and avidin-biotin block between the sequences and, thus, absence of cross-reactivity between the 2 sequences (i.e., black signal represented PrP<sup>sc</sup> without cross-reactivity with cell markers).

## Results

**Age distribution of PrP<sup>sc</sup> accumulation.** Age of the sheep ranged from 12 months to 7 years of age; 67% of sheep were 24 months or younger (Table 1). Scrapie, as defined by neural or extraneural deposition of PrP<sup>sc</sup>, was diagnosed in 13 of 80 sheep (16.25%), whereas 67 of 80 sheep (83.75%) had no detectable

**Table 2.** Tissue distribution of PrP<sup>sc</sup> immunoreactivity in PrP<sup>sc</sup>-positive sheep.

Tissue*	Total†	% total	Clinical‡	Preclinical§
Central nervous system				
Brain	10/13	77	4/4	6/9
Alimentary tract and related lymphoid tissues				
Jejunum	7/13	54	3/4	4/9
Ileum	12/13	92	4/4	8/9
Colon	12/13	92	4/4	8/9
Rectum	9/12	75	3/4	6/8
Medial iliac LN	13/13	100	4/4	9/9
Anorectal LN	9/11	82	2/2	7/9
Palatine tonsil	12/12	100	4/4	8/8
Retropharyngeal LN	13/13	100	4/4	9/9
Lymphoid tissues not related to alimentary tract				
Third eyelid	12/13	92	4/4	8/9
Spleen	9/9	100	3/3	6/6
Subiliac LN	10/11	91	4/4	6/7
Mammary gland and related LN				
Mammary gland	1/13	8	1/4	0/9
Mammary LN	12/12	100	4/4	8/8
Hematopoietic				
Bone marrow	0/13	0	0/4	0/9
Reproductive/fetal				
Placenta	8/9	89	2/3	6/6
Uterus	0/13	0	0/4	0/9
Fetal spleen	0/2	0		
Fetal ileum	0/2	0		

\* LN = lymph node.

† Sheep with PrP<sup>sc</sup>-positive tissue/total sheep examined.

‡ Clinical scrapie sheep with PrP<sup>sc</sup>-positive tissue/total clinical scrapie sheep examined.

§ Preclinical scrapie sheep with PrP<sup>sc</sup>-positive tissue/total preclinical scrapie sheep examined.

|| PrP<sup>sc</sup> deposits restricted to the germinal center of an interstitial lymphoid follicle.

PrP<sup>sc</sup> in any tissue. Sheep with PrP<sup>sc</sup> deposits ranged from 14 months to more than 6 years. Disease occurred in 12 of 34 sheep aged  $\geq 2$  years or 12 of 18 genetically susceptible sheep aged  $\geq 2$  years, indicating high disease prevalence in this age category of 43 or 67%, respectively.

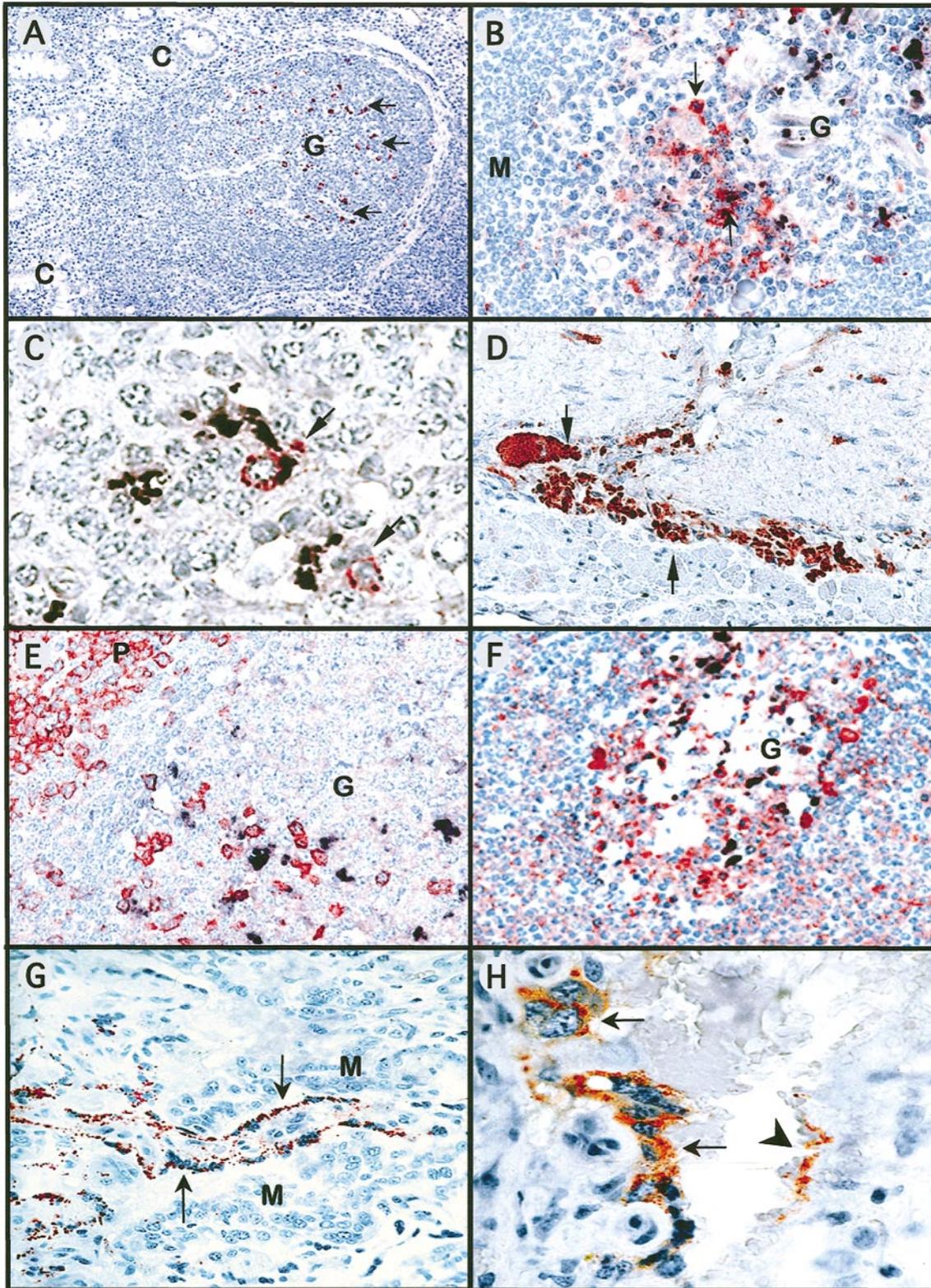
Clinical neurological disease typical of scrapie was observed in 4 of the 13 sheep with PrP<sup>sc</sup> deposits. PrP<sup>sc</sup> deposits and spongiform lesions were widespread within the 4 coronal planes examined (brain stem at the obex, middle cerebellar peduncle, rostral mesencephalic colliculi, and thalamus). The remaining 9 sheep with PrP<sup>sc</sup> deposits were preclinical and ranged in age from 14 months to more than 6 years.

*Tissue distribution of PrP<sup>sc</sup> deposition.* PrP<sup>sc</sup> deposits were widespread in all scrapie-infected sheep (Table 2). Detection of PrP<sup>sc</sup> in non-central nervous tissues was biased by the density of lymphoid follicles in the examined sample. For example, deposits were more frequently observed in ileum and colon (12 of 13 samples) than in rectum and jejunum (7 of 13 and 9 of 12 samples, respectively).

PrP<sup>sc</sup> deposits in the brain were detected in all (4 of 4) clinical sheep, but only in 6 of 9 preclinical sheep. Central nervous deposits were thus less frequent (10 of 13; 77% of affected sheep) than alimentary tract-related deposits (13 of 13; 100%). Deposits in extra-neural tissue always accompanied PrP<sup>sc</sup> deposits in the brain.

PrP<sup>sc</sup> deposits were generalized in alimentary tract-associated and non-alimentary tract-associated lymphoid tissue. PrP<sup>sc</sup> deposits occurred in the placentas of 8 of 9 scrapie-infected sheep that were pregnant. Of the 8 placenta-positive ewes, 6 were preclinical, 2 of which were without deposits in the brain. PrP<sup>sc</sup> was not detected in the bone marrow, secretory parenchyma of the mammary gland, uterus, or fetus of any scrapie-infected sheep.

*Cellular distribution of PrP<sup>sc</sup> deposition.* In lymphoid tissues, PrP<sup>sc</sup> deposits were localized to the germinal centers of lymphoid follicles in a random, multifocal pattern (Fig. 1A). Cells that accumulated PrP<sup>sc</sup> in lymphoid tissues were similar regardless of the anatomical location of the lymphoid tissue. The intensity



**Figure 1.** Microanatomic distribution of PrP<sup>sc</sup> accumulation in sheep with clinical and preclinical scrapie detected by single- and dual-label immunohistochemistry. **A**, ileum (preclinical). Multifocal, coarsely granular deposits of PrP<sup>sc</sup> (arrows) in the germinal center of a gut-associated lymphoid follicle (G) subjacent to intestinal crypts (C). PrP<sup>sc</sup> deposits were not detected in intestinal epithelium. Single-label immunohistochemistry using F89 mAb-red. 100 $\times$ . **B**, retropharyngeal lymph node (clinical). Multifocal PrP<sup>sc</sup> accumulation (black) in the germinal center (G) of a lymphoid follicle; no immunoreactivity in the mantle zone (M). PrP<sup>sc</sup> (black) mostly colocalized with dendrites and cell bodies of FDCs (red). Dual-label immunohistochemistry using mAbs CNA.42-red (FDC) and F89-black (PrP<sup>sc</sup>). 400 $\times$ . **C**, mes-

**Table 3.** PrP genotype frequency of PrP<sup>sc</sup> and disease status in 2 scrapie-infected flocks.

Genotype	PrP <sup>sc</sup> negative	PrP <sup>sc</sup> positive	Clinical	Preclinical
AA <sub>136</sub> RR <sub>154</sub> QQ <sub>171</sub>	34	12	4	8*
AA <sub>136</sub> RR <sub>154</sub> QR <sub>171</sub>	27	0	0	0
AA <sub>136</sub> RR <sub>154</sub> RR <sub>171</sub>	2	0	0	0
AA <sub>136</sub> RH <sub>154</sub> QQ <sub>171</sub>	1	0	0	0
AV <sub>136</sub> RR <sub>154</sub> QQ <sub>171</sub>	1	1	0	1†
AV <sub>136</sub> RR <sub>154</sub> QR <sub>171</sub>	2	0	0	0

\* PrP<sup>sc</sup> positive at ≥24 mo of age.

† PrP<sup>sc</sup> positive at 14 mo of age.

of immunoreactivity varied among individual sheep but did not correlate with age or clinical status. Most immunoreactivity was detected in multipolar cells characteristic of FDCs (Fig. 1B). Using dual-label immunohistochemistry, detecting specific cellular markers and PrP<sup>sc</sup>, PrP<sup>sc</sup> colocalized with CNA.42, a marker specific for FDC (Fig. 1B, 1C).<sup>19,22,33</sup> PrP<sup>sc</sup> did not colocalize with CD3, a marker for T lymphocytes (Fig. 1E), or CD79a, a B-lymphocyte marker (Fig. 1F). Rarely, PrP<sup>sc</sup> colocalized with a macrophage marker (NCL-LN5) in macrophages in the medulla of lymph nodes but not within germinal center macrophages (not shown).

PrP<sup>sc</sup> deposition in nonlymphoid tissues (outside the CNS) was restricted to neurons and chorionic epithelium. In the gut, PrP<sup>sc</sup> was regularly detected within the perikarya and neurites of the submucosal and myenteric plexus, colocalizing with neuron-specific enolase, a marker for neural cells (Fig. 1D). Axonal and somatic PrP<sup>sc</sup> deposits were also detected in the mesenteric ganglia (not shown). No PrP<sup>sc</sup> deposits were detected in gut epithelial cells, including M cells of Peyer's patches, smooth muscle cells, endothelial cells, or round cells in the lamina propria. Placental PrP<sup>sc</sup> deposition was present multifocally in placentomes within groups of chorionic epithelial cells of the cotyledon but not within adjacent endometrial epithelial cells of the interdigitating maternal caruncle (Fig. 1G). The deposits were present within the cytoplasm of

chorionic mononuclear and multinuclear trophoblast cells (as identified by morphology) and were apparently not cell associated in the space between the fetal cotyledon and the maternal caruncle (Fig. 1H). In the brain, PrP<sup>sc</sup> deposits were associated with glial cells and neurons within various nuclei of the diencephalon, mesencephalon, metencephalon, and myelencephalon. Detailed, scrapie strain-specific topography of central nervous PrP<sup>sc</sup> deposits has been reported<sup>9</sup> and was not the subject of this study.

*Effect of PrP genotype on scrapie status of the sheep.* All PrP<sup>sc</sup>-positive sheep were homozygous for glutamine at codon 171 and homozygous for arginine at codon 154 of the PrP gene (RR<sub>154</sub>QQ<sub>171</sub>) (Table 3). One PrP<sup>sc</sup>-positive sheep was heterozygous for alanine and valine at codon 136 (AV<sub>136</sub>), whereas the remaining 12 PrP<sup>sc</sup>-positive sheep were homozygous for alanine (AA<sub>136</sub>). Thus, 12/13 PrP<sup>sc</sup>-positive sheep were genotype AA<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub> and 1/13 was genotype AV<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub>. Consistent with published data on the effect of genotype on susceptibility and incubation period,<sup>16,28</sup> the single PrP<sup>sc</sup>-positive AV<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub> sheep was younger (14 mo) than the 4 youngest PrP<sup>sc</sup>-positive AA<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub> sheep (24 months). Tissue distribution, cell specificity, or intensity of PrP<sup>sc</sup> deposition did not differ between genotypes. Representation in the study was good for PrP genotypes AA<sub>136</sub> (76/80), QQ<sub>171</sub> (49/80), and QR<sub>171</sub> (29/80), whereas genotypes AV<sub>136</sub> (4/80), VV<sub>136</sub> (0/80), RH<sub>154</sub> (1/80),

←

enteric lymph node (preclinical). Higher magnification of PrP<sup>sc</sup> (black) colocalized (arrows) with dendrites and cell bodies of FDCs (red). Dual-label immunohistochemistry using CNA.42-red (FDC) and F89 mAb-black (PrP<sup>sc</sup>). 630×. **D**, ileum (clinical). Finely granular deposits of PrP<sup>sc</sup> (black) occur throughout the myenteric plexus (red). Colocalization (arrows) of PrP<sup>sc</sup> (black) with nerve fibers of the myenteric plexus (red). Dual-label immunohistochemistry using anti-neuron-specific enolase-red (ganglia neurons and fibers) and F89 mAb-black (PrP<sup>sc</sup>). 100×. **E**, medial iliac lymph node germinal center (clinical). PrP<sup>sc</sup> deposits (black) do not colocalize with T lymphocytes (red) that are sparsely scattered throughout the germinal center (G) or packed within the adjacent paracortex (P). Dual-label immunohistochemistry using anti-CD3-red (T-lymphocyte) and F89 mAb-black (PrP<sup>sc</sup>). 400×. **F**, subiliac lymph node germinal center (clinical). PrP<sup>sc</sup> (black) does not colocalize with B lymphocytes (red) in the germinal center (G). Dual-label immunohistochemistry using anti-CD79a-red (B-cell) and F89 mAb-black (PrP<sup>sc</sup>). 400×. **G**, placenta (placentome) (clinical). PrP<sup>sc</sup> (red) localized to chorionic epithelium of the fetal cotyledon (arrows). No immunoreactivity of maternal caruncle epithelium (M). Single-label immunohistochemistry using F89 mAb-red (PrP<sup>sc</sup>). 200×. **H**, placenta (placentome) (clinical). PrP<sup>sc</sup> (red) localized to the cytoplasm of mononuclear and multinuclear chorionic epithelial cells of the fetal cotyledon (arrows). Some immunoreactivity free in the extracellular space between fetal cotyledon and maternal caruncle (arrowhead). Single-label immunohistochemistry using F89 mAb-red (PrP<sup>sc</sup>). 630×.

HH<sub>154</sub> (0/80), and RR<sub>171</sub> (2/80) were underrepresented (Table 3).

**Potential modes of transmission.** Potential modes of scrapie transmission were investigated by analyzing genealogy within flock A and by examining fetuses and offspring of PrP<sup>sc</sup>-positive sheep when available. Association of clinical scrapie with PrP<sup>sc</sup>-positive status by genealogy was not possible in all PrP<sup>sc</sup>-positive sheep. Not all sheep culled or removed from the flocks because of wasting of suspect scrapie were necropsied to definitively determine scrapie status. However, in 7/13 PrP<sup>sc</sup>-positive sheep, in which accurate genealogy analysis was possible, positive scrapie status followed mother-to-daughter lineage in 57% (4/7) and was not associated with mother-to-daughter lineage in 43% (3/7). The single 7-year-old sheep with preclinical scrapie was part of the latter group. Two fetuses from PrP<sup>sc</sup>-positive ewes with clinical scrapie were available for examination. Both ewes had PrP<sup>sc</sup> deposition in the placenta, proving the scrapie-susceptible genotype of the fetus,<sup>36</sup> but neither fetus had PrP<sup>sc</sup> deposition in fetal spleen or intestine (Table 2).

### Discussion

This study demonstrates the spatial and cell-specific preclinical accumulation pattern of PrP<sup>sc</sup> in sheep that resemble in their PrP genotype the American Suffolk, a breed with limited PrP gene polymorphism and prolonged preclinical phase. As expected from experimental models,<sup>4,5,23</sup> a buildup of PrP<sup>sc</sup> throughout the lymphoid system can be observed during the preclinical period after a lag phase during which PrP<sup>sc</sup> is not detectable by immunohistochemistry. After the lag phase, PrP<sup>sc</sup> accumulates rapidly in lymphoid tissues throughout the body as well as the intestinal nervous supply, which offers the possibility of biopsy-based preclinical diagnosis.<sup>24,25,34,35</sup> On the other hand, preclinical dissemination of PrP<sup>sc</sup> deposits into the placenta indicates a risk of horizontal and vertical transmission by clinically healthy ewes. The finding that most cases within scrapie-affected flocks are preclinical underscores the significance of observing PrP<sup>sc</sup> accumulation in the placentas of a high percentage of infected sheep.

Based on this study, the lag phase needed for PrP<sup>sc</sup> deposits to become detectable by immunohistochemistry is about 12–14 months, assuming perinatal infection by oral exposure to PrP<sup>sc</sup>. This lag phase is longer in Suffolk-type sheep with predominance of the A<sub>136</sub>R<sub>154</sub>Q<sub>171</sub> allele than the 2–6 months reported in breeds with predominance of the V<sub>136</sub>R<sub>154</sub>Q<sub>171</sub> allele.<sup>1,21,35</sup> The data thus support the notion that the primary structure of PrP determines the rate of PrP<sup>sc</sup> accumulation. From this it follows that the residues at positions 136 and 171 determine disease susceptibility

by altering accumulation kinetics rather than accumulation sites or cellular specificity. Further research is needed to determine the nature of this kinetic barrier and whether parameters such as time and infectious dose modify its permissibility to accumulation and disease.

PrP<sup>sc</sup> was identified in FDCs and peripheral neurons. PrP<sup>sc</sup> was only rarely detected in macrophages. Scant, macrophage-associated PrP<sup>sc</sup> was occasionally detected in the medulla of lymph nodes, rather than in the germinal centers of lymphoid follicles. This observation contrasts somewhat to a similar study that reports early accumulation of PrP<sup>sc</sup> in tingible body macrophages of the germinal center.<sup>17</sup> Failure to observe PrP<sup>sc</sup> in tingible body macrophages may be because of examination of tissues at a later stage of disease because PrP<sup>sc</sup> derives from scavenging exogenous PrP<sup>sc</sup> rather than synthesis within that cell type.<sup>15</sup>

The distribution of PrP<sup>sc</sup> observed in this study underscores the importance of the germinal center and FDC for the peripheral amplification of PrP<sup>sc</sup> and is consistent with neurogenic spread from sites of primary amplification to the CNS. Our observations support the role of FDC for scrapie pathogenesis<sup>6</sup> and are in line with experiments that report drastically reduced susceptibility of mice to scrapie on dedifferentiation of FDC.<sup>20</sup> Furthermore, the proximity of nerve endings and PrP<sup>sc</sup> in lymphoid follicles has been demonstrated.<sup>14,31</sup> Conversely, in this study the authors failed to observe a pattern of PrP<sup>sc</sup> accumulation in lymphoid tissues that would suggest travel of PrP<sup>sc</sup> through the lymphatic vessels or blood stream. For instance, PrP<sup>sc</sup> was not detected in the glandular tissue of the mammary gland, the tributary region of the mammary lymph nodes; yet, the mammary lymph node regularly accumulated PrP<sup>sc</sup>. Likewise, PrP<sup>sc</sup> was not detected in the bone marrow or splenic red pulp and was only rarely detected in macrophages within lymph nodes. The mode by which generalized depositional disease throughout the lymphoid tissue is initiated and synchronized has been elusive; however, episodic hematogenous dispersion of cells carrying PrP<sup>sc</sup> below the detection level of immunologic assays is a possibility.

Large, intrauterine PrP<sup>sc</sup> deposits occurred during preclinical and clinical disease and were restricted to the (fetal) chorionic epithelium of the placentome, whereas no PrP<sup>sc</sup> was detected in adjacent maternal tissues or tissues of the fetus proper. This observation is consistent with the well-recognized infectivity of fetal membranes.<sup>32</sup> Similar to Andreoletti et al.,<sup>2</sup> PrP<sup>sc</sup> was found to be restricted to fetal tissues. Because placentomes were not analyzed specifically for macrophages, the possibility of PrP<sup>sc</sup> deposits occurring in macrophages of the (maternal) caruncle or at the interface between fetal and maternal tissues remains.

The finding of Andreoletti et al.<sup>2</sup> is in contrast to those of Tuo et al.,<sup>37</sup> who observed PrP<sup>sc</sup> deposits also in the caruncular epithelium, albeit the latter study did not use double immunohistochemistry to ascertain cellular specificity

Natural transmission of sheep scrapie is thought to occur most commonly by perinatal maternal transmission through oral exposure of neonates to prions within the secundines and placental fluids,<sup>7,32</sup> a mechanism supported by the findings of this study. Infection of placental membranes is reportedly restricted to susceptible fetal genotypes.<sup>36</sup> In this study, transmission followed ewe-to-lamb lines in most scrapie-affected sheep, and there was abundant PrP<sup>sc</sup> deposition within and around placental trophoblast cells. PrP<sup>sc</sup> was not evident in 2 genetically susceptible fetuses, supporting other studies arguing against direct intrauterine transmission of scrapie or infectivity of the fetus proper.<sup>2,12</sup> However, horizontal transmission is reported to occur in heavily infected flocks.<sup>39</sup> Similarly, this study showed transmission outside the ewe-to-lamb lines in 43% of cases; for example, preclinical scrapie was diagnosed in a 7-year-old ewe. Understanding how PrP polymorphism affects PrP conversion and PrP<sup>sc</sup> deposition in specific tissues and cell types, especially those at potential sites of preclinical accumulation, is critical to the development of treatment or control measures for the scrapie-type TSE.

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- d. DakoCytomation Inc., Carpinteria, CA.
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