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Role of the Goat K_{222}-PrP^C Polymorphic Variant in Prion Infection Resistance

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ABSTRACT

The prion protein-encoding gene (*prnp*) strongly influences the susceptibility of small ruminants to transmissible spongiform encephalopathies (TSEs). Hence, selective breeding programs have been implemented to increase sheep resistance to scrapie. For goats, epidemiological and experimental studies have provided some association between certain polymorphisms of the cellular prion protein (PrP^C) and resistance to TSEs. Among them, the Q/K polymorphism at PrP^C codon 222 (Q/K_{222}) yielded the most promising results. In this work, we investigated the individual effects of the K_{222}-PrP^C variant on the resistance/susceptibility of goats to TSEs. For that purpose, we generated two transgenic mouse lines, expressing either the Q_{222} (wild type) or K_{222} variant of goat PrP^C. Both mouse lines were challenged intracerebrally with a panel of TSE isolates. Transgenic mice expressing the wild-type (Q_{222}) allele were fully susceptible to infection with all tested isolates, whereas transgenic mice expressing similar levels of the K_{222} allele were resistant to all goat scrapie and cattle BSE isolates but not to goat BSE isolates. Finally, heterozygous K/Q_{222} mice displayed a reduced susceptibility to the tested panel of scrapie isolates. These results demonstrate a highly protective effect of the K_{222} variant against a broad panel of different prion isolates and further reinforce the argument supporting the use of this variant in breeding programs to control TSEs in goat herds.

IMPORTANCE

The objective of this study was to determine the role of the K_{222} variant of the prion protein (PrP) in the susceptibility/resistance of goats to transmissible spongiform encephalopathies (TSEs). Results showed that transgenic mice expressing the goat K_{222}-PrP polymorphic variant are resistant to scrapie and bovine spongiform encephalopathy (BSE) agents. This protective effect was also observed in heterozygous Q/K_{222} animals. Therefore, the single amino acid exchange from Q to K at codon 222 of the cellular prion protein provides resistance against TSEs. All the results presented here support the view that the K_{222} polymorphic variant is a good candidate for selective breeding programs to control and eradicate scrapie in goat herds.

Scrapie is an infectious neurodegenerative disease naturally affecting sheep and goats. It belongs to the group of transmissible spongiform encephalopathies (TSEs), or prion diseases, such as Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease in cervids. TSEs are characterized by the accumulation in the central nervous system (CNS) of a pathological aberrant form (PrP^{Sc}) of the host cellular prion protein (PrP^C) (1).

Scrapie occurrence is determined mainly by the host prion protein-encoding gene (*prnp*) (2, 3) and the prion strain (4), thus resulting in distinct disease phenotypes (showing differences in the PrP^{Sc} deposition pattern, incubation period, pathogenesis, or clinical signs). Sheep and goats share the same PrP^C amino acid sequence, but with a variety of polymorphisms. Sheep *prnp* genotypes $V_{136}R_{154}Q_{171}$ and $A_{136}R_{154}Q_{171}$ are associated with high-level susceptibility to classical scrapie, while the $A_{136}R_{154}R_{171}$ genotype is linked to resistance (5–8). This information was used in some European Union member states to implement selective breeding programs in order to promote the $A_{136}R_{154}R_{171}$ haplotype within ovine herds (9).

In goats, half a hundred polymorphisms in the open reading frame (ORF) of the *prnp* gene have been described (10–12), including some silent mutations, a three-octapeptide-repeat variant (13), and a nonsense mutation at codon 32 (14). Although several polymorphisms are shared by sheep and goats, to date, only some correlations between some variants of goat PrP^C (goPrP^C) and susceptibility to TSE have been published. The I/M_{142} amino acid substitution was associated with a slightly decreased risk of developing scrapie (15–17) and with prolonged incubation times after challenge with scrapie and BSE prions (18). The same effect on the incubation period was attributed to the three-repeat/glycine 102 genotype (13, 18). In natural outbreaks, goats carrying the H/R_{143} polymorphism showed a partial decrease in their susceptibility to scrapie prions (15, 19). Likewise, a low susceptibility to scrapie was also linked to the R/H_{154} and R/Q_{211} variants of goat PrP^C (15, 20), although the R/H_{154} polymorphism also arose as a risk factor for goat atypical scrapie (21). In addition, other PrP^C variants, such as the N/D_{146} and N/S_{146} polymorphisms, were linked to resistance to scrapie (22). However, the most encouraging results

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are those related to the Q/K_{222} polymorphism. The absence or marked underrepresentation of scrapie-positive goats expressing the K_{222} variant in numerous herds from different countries (11, 12, 15, 20, 22–25) suggests that the K_{222}-PrP^C variant strongly influences goat susceptibility to TSE agents. Recently, heterozygous Q/K_{222} goats were reported to show a strong resistance to classical scrapie after intracerebral (i.c.) inoculation (26), as also reported in the accompanying article by Lacroux et al. (27). This evidence should be reinforced by experimental data, but the large number of genetic variants harbored in the goat *prnp* gene hinders *in vivo* determination of the individual effects of the K_{222}-PrP^C variant in this matter.

In this work, we analyzed the role of the K_{222} variant of goat PrP^C in the susceptibility to prion infection. For that purpose, two transgenic mouse lines, one expressing the wild-type (wt) goat PrP^C sequence and another expressing the same PrP^C sequence, but with a single amino acid exchange from Q to K at codon 222, were generated. Both mouse lines were challenged i.c. with a panel of TSE isolates, and their susceptibilities were assessed and then compared.

MATERIALS AND METHODS

Ethics statement. Animal experiments were carried out in strict accordance with the recommendations in the guidelines of European Community Council Directive 86/609/EEC, and all efforts were made to minimize suffering. Experiments were approved by the Committee on the Ethics of Animal Experiments of INIA (permit number CEEA2009/003).

Transgenic mice. The procedures used to generate transgene constructs have been described elsewhere (28). Briefly, the ORF of goat wt *prnp* was isolated by PCR amplification and sequenced, and the sequence was identical to the goat *prnp* sequence previously reported (GenBank accession number AF117316). The primers used created an XhoI restriction enzyme site adjacent to the translation start and stop sites of the goat PrP gene ORF (5′-CTCGAGATCATGGTGAAAAGCCACATAGGC-3′ and 3′-CTCGAGCTATCCTACTATGAGAAAAATGAG-5′, respectively). The PCR fragment was subcloned into the pGEM-T Easy vector system (Promega) following the manufacturer's instructions. The expression vector MoPrP.Xho (29) was used for the production of transgenic mice. This vector contains the murine PrP (muPrP) promoter, exon 1, intron 1, exon 2, and 3′-untranslated sequences. The PrP ORF fragment was excised from the pGEM-T vector by using the restriction enzyme XhoI and was inserted into the MoPrP.Xho expression vector (29), also digested with the XhoI enzyme, resulting in the plasmid pMo-GoPrP.Xho. This plasmid was mutated to generate a K_{222}-PrP plasmid (pMo-GoK_{222}-PrP.Xho) by using a QuikChange II XL kit (Stratagene, CA) with specific oligonucleotides (5′-GTGCATCACCCAGTACAAGAGAGAATCCCAGGC-3′ and 3′-GCCTGGGATTCTCTCTTGTACTGGGTGATGCAC-5′), following the procedures described by the manufacturer. The MoPrP.Xho expression vector contains the murine wt *prnp* gene, including the murine *prnp* promoter, exon 1, intron 1, exon 2, and 3′-untranslated sequences, but not intron 2 and the murine PrP ORF.

Transgenic mouse lines were generated as previously described (28). Briefly, transgenes were excised from their expression vectors (pMo-GoPrP.Xho and pMo-GoK_{222}-PrP.Xho) by use of the restriction endonuclease NotI, leading to DNA fragments of approximately 12 kb. DNAs were then purified using sodium chloride gradients as previously described (30) and resuspended in TE (10 mM Tris, pH 7.4, 0.1 mM EDTA) at a final concentration of 2 to 6 ng/ml. Finally, DNAs were microinjected into pronuclear-stage embryos collected from superovulated B6CBAF1 females mated with 129/Ola males carrying a null mutation in their endogenous PrP gene (31).

DNAs were extracted from founders' tail biopsy specimens by use of an Extract-N-Amp tissue PCR kit (Sigma-Aldrich) following the manufacturer's instructions. The presence of the goat transgene in these founders was identified by PCR amplification using specific primers for mouse PrP exon 2 and the goat PrP ORF. The primers used were 5′-CATTCTGCCTTCCTAGTGGTACC-3′ and 5′-GCTTGTTCCACTGACTGTGGC-3′. $muPrP^{+/-}$ $goPrP^{+/-}$ founders were backcrossed with homozygous PrP null animals ($muPrP^{-/-}$) to obtain mice homozygosis for the null mutation ($muPrP^{-/-}$ $goPrP^{+/-}$). The absence of the murine PrP ORF in the transgenic mice thus generated was confirmed by PCR amplification using the primers 5′-ATGGCGAACCTTGGCTACTGGC-3′ and 5′-GATTATGGGTACCCCCTCCTTGG-3′.

Analysis of PrP^C expression in transgenic mice. Whole brains from either mice or goats were homogenized in extraction buffer (0.5% NP-40, 1% sodium deoxycholate, 10 mM EDTA in phosphate-buffered saline [PBS], pH 7.4, with Complete protease inhibitor cocktail [Roche]). Samples were precleared by centrifugation at 2,000 × *g* for 5 min, after which an equal volume of 2× SDS reducing sample loading buffer was added to all samples, and each one was boiled for 5 min before being loaded onto an SDS-12% polyacrylamide gel. For immunoblotting experiments, the monoclonal antibodies (MAbs) FH11 (32) and 12B2 (33) were used at a concentration of 1 μg/ml. FH11 recognizes the goat PrP amino-terminal region (amino acids 23 to 85), and 12B2 recognizes the $_{93}$WGQGG$_{97}$ epitope of the goat PrP sequence. Immunocomplexes were detected using horseradish peroxidase-conjugated anti-mouse IgG. Immunoblots were developed with enhanced chemiluminescence.

Transmission studies. Transgenic mice were challenged with a panel of TSE agents, including cattle BSE, goat BSE, and different goat scrapie isolates (see Table 1 for isolate information). Inocula were prepared from infected brain tissues as 10% (wt/vol) homogenates in 5% glucose.

Groups of 6 to 9 individual identified animals (6 to 7 weeks old) were anesthetized and inoculated intracerebrally with 20 μl of 10% brain homogenate in the right parietal lobe, using a 25-gauge disposable hypodermic needle. As a control, 6 or 7 animals of each line were inoculated with healthy goat brain to discard the possibility of appearance of a spontaneous prion disease. Mice were observed daily and their neurological status assessed twice a week. When the progression of the disease was evident, or at the end of their life span (≈650 days), mice were euthanized for ethical reasons. During necropsy, the brain was harvested at −20°C for determination of the presence of proteinase K-resistant PrP (PrP^{res}) by Western blotting (WB). Survival time was expressed as the mean number of survival days postinoculation (dpi) for all the PrP^{res}-positive mice, with the standard error included. Attack rate was determined as the proportion of PrP^{res}-positive mice among all the mice inoculated.

Western blotting. A total of 175 mg of brain tissue was homogenized in 5% glucose in distilled water in grinding tubes (Bio-Rad) and adjusted to 10% (wt/vol) by using a TeSeETM Precess 48TM homogenizer (Bio-Rad) following the manufacturer's instructions. To determine the presence of PrP^{res} in transgenic mouse brains, 100 μl of 10% brain homogenate was analyzed by Western blotting as previously described (34). For immunoblotting, membranes were incubated with the Sha31 MAb (35), which recognizes the $_{148}$YEDRYYRE$_{155}$ epitope of the goat PrP sequence. Immunocomplexes were detected with horseradish peroxidase-conjugated anti-mouse IgG (Amersham Pharmacia Biotech) after incubating the membranes for 1 h, and immunoreactivity was visualized by chemiluminescence with ECL Plus (GE Healthcare Amersham Biosciences).

Histopathological analysis. All procedures concerning the histopathological analysis of infected mouse brains were performed as previously described (36). Brain samples were immediately fixed in neutral-buffered 10% formalin (4% 2-formaldehyde) during mouse necropsy and paraffin embedded later. After deparaffinization, 2-μm-thick tissue slices were stained with hematoxylin and eosin. Lesion profiles of the brains were established following the standard method of Fraser and Dickinson (37). Paraffin-embedded tissue (PET) blots and immunohistochemistry (IHC) were conducted as described by Andreoletti et al. (38, 39).

RESULTS

Goat PrP^C expression in transgenic mice. Several mouse lines (founders) were obtained for each transgene (Q_{222}-PrP^C or K_{222}-

TABLE 1 Description of the different isolates used in this work

Isolate	Origin (local code)	Goat PrP genotype[a]	Description (reference)	Supplier[b]
Goat-BSE1	France (CH0064)	$I_{136}Q_{154}S_{240}/I_{136}R_{154}S_{240}$	Isolate from a terminally ill goat intracerebrally inoculated with Ca-BSE isolate	INRA
Goat-BSE2	France (CH1075)	$I_{136}R_{154}S_{240}/I_{136}R_{154}P_{240}$	Isolate from a terminally ill goat intracerebrally inoculated with Ca-BSE isolate	INRA
Sheep-BSE	France (ARQ_0)	wt	Isolate from a pool of terminally ill ARQ/ARQ sheep inoculated with Ca-BSE isolate (45)	INRA
Ca-BSE	France (139)		BSE isolate from a naturally infected cow (45)	INRA
Goat-Sc F2	France (CP40)	wt; S/P_{240}	Classical scrapie isolate from an experimentally infected goat	INRA
Goat-Sc F10	France (2143)	wt; S/P_{240}	Classical scrapie isolate from a naturally infected goat	INRA
Goat-Sc I3	Italy (121429/1/1)	wt	Classical scrapie isolate from a naturally infected goat	IZSTO
Goat-Sc I9	Italy (85792/1/1)	wt; S/P_{240}	Classical scrapie isolate from a naturally infected goat	IZSTO
Goat-Sc S2	Spain (C-163)	wt; S/P_{240}	Classical scrapie isolate from a naturally infected goat	UNIZAR
Goat-Sc S3	Spain (C-645)	wt	Classical scrapie isolate from a naturally infected goat	UNIZAR
Healthy goat brain		wt	Brain from a noninfected goat	INRA

[a] The wild-type (wt) goat prion protein genotype is $A_{136}R_{154}P_{240}/A_{136}R_{154}P_{240}$.
[b] INRA, French National Institute for Agricultural Research, Nouzilly, France; IZSTO, Istituto Zooprofilattico Sperimentale del Piemont, Italy; UNIZAR, Universidad de Zaragoza, Spain.

PrP^C). Founder animals also expressing the endogenous murine *prnp* gene (mu$prnp^{+/-}$ go$prnp^{+/-}$) were crossed with *prnp* null mice (mu$prnp^{-/-}$) to obtain transgene-hemizygous lines in a murine *prnp* null background (mu$prnp^{-/-}$ go$prnp^{+/-}$). The absence of murine *prnp* was determined by PCR using specific primers (data not shown). The PrP^C expression levels in brain homogenates were then determined by serial dilution and Western blotting using the 12B2 MAb and were compared with the PrP^C levels found in goat brain homogenates. The Q_{222}-Tg501 and K_{222}-Tg516 lines were selected based on the fact that they had PrP^C expression levels in the brain similar to those in goats (Fig. 1). In addition, both Q_{222}-PrP^C and K_{222}-PrP^C expressed in brains of transgenic mice showed electrophoretic profiles similar to that observed for PrP^C from goat brain (Fig. 1). Finally, the hemizygous Q_{222}-Tg501 and K_{222}-Tg516 mouse lines were used for transmission studies.

Transgenic mice expressing the K_{222}-PrP variant are resistant to a broad panel of goat scrapie isolates. The Q_{222}-Tg501 mouse line, expressing wild-type goat PrP^C, was susceptible to all inoculated scrapie isolates. Intracerebrally challenged animals displayed a 100% attack rate, and mean survival times ranged from 250 to 650 dpi (Table 2). No differences were observed between the electrophoretic profiles of PrP^{res} in the inoculated isolates and those of the PrP^{res} present in the brains of challenged Q_{222}-Tg501 mice (Fig. 2).

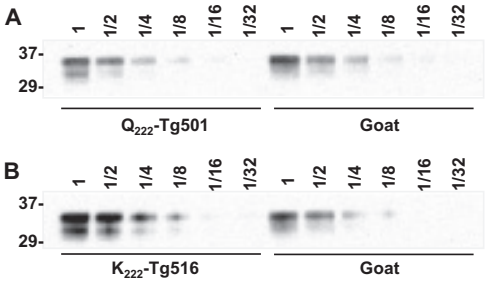

FIG 1 Brain PrP^C expression in hemizygous Q_{222}-Tg501 (A) and K_{222}-Tg516 (B) mouse lines in comparison with that in goat brain. Immunoblotting of PrP^C was performed with the 12B2 MAb. Direct samples (10% brain homogenates) and 1/2 serial dilutions were loaded onto 12% Bis-Tris gels. The figure illustrates a representative set of data for three independent experiments. Relative molecular masses (kDa) are indicated on the left.

In contrast, none of the K_{222}-Tg516 mice succumbed to inoculation with any of the scrapie isolates (Table 2). These animals were sacrificed at 650 dpi, without clinical signs, and were scored PrP^{res} negative in the brain by Western blotting. The absence of PrP^{res} in the brain was confirmed by either IHC or PET blotting. These animals exhibited some vacuolation, mainly at the level of the dorsal medulla and the mesencephalic tegmentum. However, this vacuolation was also observed in the same areas in old Q_{222}-Tg501 and K_{222}-Tg516 mice inoculated with healthy goat brain. In contrast, all the infected Q_{222}-Tg501 mice displayed PrP^{res} deposits in the brain which were accompanied by histopathological alterations characteristic of prion infection (data not shown).

It is important that the Q_{222}-Tg501 and K_{222}-Tg516 mouse lines, both expressing similar amounts of PrP^C in the brain, differ only in the Q/K_{222} substitution in the PrP^C sequence, thus demonstrating that this single amino acid exchange is the only muta-

TABLE 2 Transmission of a panel of prion isolates to Q_{222}-Tg501, K_{222}-Tg516, and heterozygous Q/K_{222} mice

Isolate	Mean survival time (days) ± SEM (n/n_0)[a]		
	Q_{222}-Tg501 mice ($Q_{222}/-$)	K_{222}-Tg516 mice ($K_{222}/-$)	Tg501 × Tg516 mice (Q/K_{222})
Goat-BSE1	497 ± 31 (5/5)	519 ± 42 (5/5)	533 ± 44 (5/5)
Goat-BSE2	484 ± 34 (7/7)	478 ± 31 (4/4)	470 ± 14 (5/5)
Sheep-BSE	485 ± 62 (7/7)	418 ± 11 (4/4)	ND
Ca-BSE	583 ± 57 (6/6)	>650 (0/6)	617 ± 47 (5/5)
Ca-BSE/Tg501[b]	326 ± 26 (6/6)	434 ± 30 (5/5)	ND
Goat-Sc F10	465 ± 19 (7/7)	>650 (0/5)	>650 (6/6)
Goat-Sc F2	250 ± 36 (4/4)	>650 (0/7)	630 ± 26 (5/6)
Goat-Sc I3	659 ± 10 (5/5)	>650 (0/5)	>650 (0/6)
Goat-Sc I9	600 ± 43 (5/5)	>650 (0/5)	ND
Goat-Sc S2	449 ± 62 (9/9)	>650 (0/6)	>650 (0/6)
Goat-Sc S3	298 ± 22 (6/6)	>650 (0/6)	ND
Healthy goat brain	>650 (0/6)	>650 (0/6)	>650 (0/6)

[a] n/n_0, number of diseased, PrP^{res}-positive animals/number of inoculated animals. ND, no data.
[b] Isolate from a pool of terminally ill wt Tg501 mice inoculated with the Ca-BSE isolate.

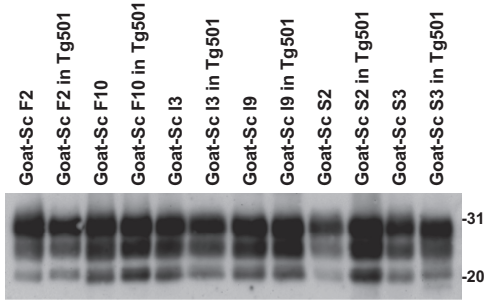

FIG 2 PrP^{res} of goat scrapie isolates both before and after transmission in Q_{222}-Tg501 mice. Immunoblots show brain PrP^{res} detected with the Sha31 MAb. Similar quantities of PrP^{res} were loaded for adequate comparison. Molecular masses (kDa) are shown on the right.

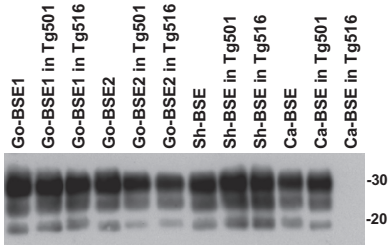

FIG 3 PrP^{res} of BSE isolates before and after transmission in either Q_{222}-Tg501 or K_{222}-Tg516 mice. Immunoblots show brain PrP^{res} detected with the Sha31 MAb. Similar quantities of PrP^{res} were loaded for adequate comparison. Molecular masses (kDa) are shown on the right.

tion responsible for the differential susceptibility/resistance to scrapie observed between these mouse lines.

None of the Q_{222}-Tg501 or K_{222}-Tg516 mice inoculated with healthy brain samples succumbed to disease (Table 2). They were euthanized at the end of their life span (650 dpi), without either clinical signs or PrP^{res} in the brain.

Transgenic mice expressing the K_{222}-PrP variant are resistant to cattle BSE but not to goat or sheep BSE. Both the Q_{222}-Tg501 and K_{222}-Tg516 mouse lines were fully susceptible (100% attack rate) to goat and sheep BSE isolates, displaying similar mean survival times ranging from 400 to 500 dpi (Table 2). However, inoculation of cattle BSE isolates into Q_{222}-Tg501 and K_{222}-Tg516 mice produced markedly different results. While Q_{222}-Tg501 mice showed a 100% attack rate and a survival time of 583 ± 57 dpi, K_{222}-Tg516 mice were sacrificed at the end of their life span (650 dpi), without either clinical signs or PrP^{res} in the brain (Table 2). Moreover, K_{222}-Tg516 mice were totally susceptible to the cattle BSE agent passaged in Q_{222}-Tg501 mice, with a mean survival time of 434 ± 30 dpi.

Western blot analysis of brain PrP^{res} in goat BSE isolates revealed a typical PrP^{res} banding pattern characterized by small fragments (19-kDa fragment for the aglycosylated band) and prominent diglycosylated species. This pattern was indistinguishable from those observed for both Q_{222}-Tg501 and K_{222}-Tg516 mice infected with goat or sheep BSE isolates. Moreover, an identical PrP^{res} banding pattern was observed for Q_{222}-Tg501 mice inoculated with cattle BSE isolates (Fig. 3).

Histopathological analysis confirmed the absence of both PrP^{res} deposits and spongiform changes in the brains of all K_{222}-Tg516 mice inoculated with cattle BSE isolates. However, when these mice were infected with goat BSE isolates, they exhibited PrP^{res} deposition patterns and lesion profiles similar to those observed in Q_{222}-Tg501 mice infected with either cattle or goat BSE isolates (data not shown).

Altogether, these results suggest a low transmission barrier to cattle BSE in transgenic mice expressing wild-type (Q_{222}) goat PrP^C. Conversely, the K_{222} amino acid substitution seemed to give rise to a transmission barrier that limited the propagation of cattle BSE prions. Nevertheless, this transmission barrier was abolished if the isolate was previously passaged in a wt goat PrP^C-expressing host (sheep, goat, or Q_{222}-Tg501 mouse) (Table 2).

Heterozygous Q/K_{222} mice show lower attack rates and/or longer survival times depending on the inoculated isolate. To accurately determine the role of the K_{222}-PrP variant in goat resistance/susceptibility to TSEs, K_{222}-Tg516 mice were crossbred with Q_{222}-Tg501 mice to generate heterozygous (Q/K_{222}) mice. These animals were inoculated intracerebrally with the same panel of TSE isolates used previously (Table 2). Heterozygous Q/K_{222} animals were resistant to the Goat-Sc I3 and Goat-Sc S2 isolates. These animals were sacrificed at 650 dpi, without having shown clinical signs, and no detectable PrP^{res} was found in their brains by WB, IHC, or PET blotting. However, Q/K_{222} mice succumbed to infection with the Goat-Sc F2 isolate, displaying a reduced attack rate (83.3%) and longer survival times than those observed in Q_{222}-Tg501 mice. Moreover, Q/K_{222} mice challenged with the Goat-Sc F10 isolate were also sacrificed at 650 dpi, without clinical signs, but PrP^{res} was detected in their brains (Table 2). Taken together, these results suggest that in heterozygous Q/K_{222} mice, the K_{222}-PrP^C variant interferes with the replication of all tested scrapie agents.

When inoculated with the cattle BSE agent, heterozygous Q/K_{222} animals showed prolonged survival times compared to Q_{222}-Tg501 mice (Table 2). However, goat BSE isolates displayed similar transmission features in all transgenic mouse models challenged (Q_{222}-Tg501, K_{222}-Tg516, and Q/K_{222} mice), independent of the expressed PrP^C variant, thus indicating low or nonexistent interference of the K_{222}-PrP^C variant with BSE agent replication.

Histopathological studies confirmed the presence of PrP^{res} and spongiform changes in the brains of heterozygous Q/K_{222} mice infected with Goat-Sc F2, Goat-Sc F10, and goat and cattle BSE isolates. PrP^{res} deposits and vacuolation profiles in these mice were similar but not identical to those determined for Q_{222}-Tg501 mice infected with the same isolates (data not shown). However, we cannot exclude the possibility that these slight differences were linked to the old age of Q/K_{222} mice, since they displayed longer survival times than those of Q_{222}-Tg501 mice. Further passages are necessary to make a conclusion on this point.

DISCUSSION

In this study, we use a transgenic mouse model to assess the role of the K_{222}-PrP^C variant in the resistance/susceptibility of goats to TSEs. For that purpose, we generated two transgenic mouse lines, expressing either the wt (Q_{222}-Tg501) or the K_{222} variant (K_{222}-Tg516) of goat PrP^C. Both mouse lines expressed similar amounts of PrP^C and were challenged intracerebrally with a panel of prion isolates.

When TSE agent transmission efficiency was assessed in Q_{222}-Tg501 mice, these animals showed high-level susceptibility (100%

attack rate) to all the tested scrapie and BSE isolates. PrP^{res} profiles in Western blots of all scrapie and BSE isolates were not altered after passage in these Q_{222}-Tg501 mice (Fig. 2 and 3). Goat BSE isolates displayed a weak decrease in survival times compared with cattle BSE isolates, confirming the low transmission barrier of goats to cattle BSE previously reported for experimentally challenged goats (40). According to these data, we propose Q_{222}-Tg501 transgenic mice expressing wild-type goat PrP^C as a valuable model for both studying the susceptibility/resistance of small ruminants to TSEs and characterizing TSE strains affecting these species.

In contrast to the high-level susceptibility of Q_{222}-Tg501 mice, K_{222}-Tg516 mice were fairly resistant to all the classical scrapie isolates inoculated, independent of their origin or PrP^{res} signature. Since Q_{222}-Tg501 mice and K_{222}-Tg516 mice express exactly the same PrP^C sequence, except for the Q/K_{222} amino acid substitution, and have similar PrP^C expression levels, we can assume that the lack of transmission efficiency of classical scrapie is linked to the K_{222} amino acid. This conclusion is consistent with the results reported in the accompanying article by Lacroux et al. (27) and previous epidemiological studies carried out in different European goat scrapie outbreaks where the K_{222}-PrP^C variant was detected only in healthy goats (12, 15, 23, 24). Taken together, these results suggest that the K_{222} variant of goat PrP^C is strongly resistant to a wide range of classical scrapie strains.

On the other hand, heterozygous Q/K_{222} mice were clearly more resistant to the different scrapie isolates than Q_{222}-Tg501 mice. These results support the contention that the K_{222}-PrP^C variant provides a dominant negative effect over the wild-type PrP^C sequence. This phenomenon is consistent with the stone fence model (41), which predicts that for a given TSE agent, the incorporation of a conversion-incompetent PrP^C variant will interfere with wild-type PrP^{Sc} replication, resulting in a lower efficacy of prion propagation. This decreased propagation efficacy would result in reduced attack rates and/or prolonged survival times, as shown in our study, and could explain the limited epidemiological evidence linking scrapie with the K_{222} allele (11, 15, 22).

BSE transmission experiments showed that mice expressing the K_{222}-PrP^C variant (K_{222}-Tg516 mice) were resistant to cattle BSE but not to goat BSE or sheep BSE isolates (Table 2). The failure of the cattle BSE isolates to be transmitted in K_{222}-PrP^C mice can be explained by the dual influence of both the PrP^C sequence differences in prion cross-species transmission (2, 3) and the effect of the Q/K_{222} amino acid substitution. In this sense, the effect of the PrP^C primary sequence of the donor (bovine) is not strong enough to avoid cattle BSE replication in goat wt Q_{222}-Tg501 mice. However, after the Q/K_{222} substitution in K_{222}-Tg516 mice, the cattle BSE agent is unable to replicate. This view is fully consistent with the fact that K_{222}-Tg516 mice were susceptible to BSE only after passage in goats or Q_{222}-Tg501 mice (Table 2). Therefore, the transmission barrier toward the BSE agent is complex, being modulated not only by the K_{222} allele but also by other determinants, such as the PrP amino acid differences between host and donor (goats versus cattle). Interestingly, this transmission barrier was abolished when the K_{222}-PrP^C variant was present in heterozygosis, indicating that for BSE, the K_{222} amino acid substitution does not interfere with the conversion of wild-type Q_{222}-PrP^C into PrP^{Sc}.

An inhibitory effect on prion replication of equivalent K_{222} variants has also been described for other species. The human K_{219}-PrP^C allele (homologous to the codon 222 allele in goat PrP^C) was reported to protect humans against Creutzfeldt-Jakob disease (42), while the equivalent K_{218} variant of mouse PrP^C significantly reduced the fibril aggregation kinetics and generated non-proteinase K-resistant PrP (43). A plausible explanation for this inhibitory effect could be the insertion of an additional positive charge at codon 222, provided by the lysine amino acid (K), thus interfering with the PrP^C-PrP^{Sc} interaction and resulting in abolished or low conversion rates of PrP^{Sc} (44). All these considerations suggest that codon 222 must play an important role in PrP^C conformation and point it out as a target for future TSE studies.

The use of transgenic mice in our transmission studies allowed us to pinpoint the Q/K_{222} amino acid substitution as the unique mutation responsible for the observed resistance to TSEs, excluding other genetic factors. In addition, the intracerebral inoculation route provided the best scenario for prion replication, as the inoculum was placed directly in the target tissue. Therefore, although our procedure mimics neither the natural route of scrapie infection nor the complex pathogenesis involving prion replication in peripheral tissues, our results offer solid arguments supporting the hypothesis that the K_{222} variant of goat PrP^C provides strong resistance against a wide range of classical scrapie isolates and reinforce the view that the K_{222} allele is a good candidate for the development of breeding programs for resistance against scrapie in commercial goat populations.

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