Review article

Rodent models for prion diseases

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Abstract – Until today most prion strains can only be propagated and the infectivity content assayed by experimentally challenging conventional or transgenic animals. Robust cell culture systems are not available for any of the natural and only for a few of the experimental prion strains. Moreover, the pathogenesis of different transmissible spongiform encephalopathies (TSE) can be analysed systematically by using experimentally infected animals. While, in the beginning, animals belonging to the natural host species were used, more and more rodent model species have been established, mostly due to practical reasons. Nowadays, most of these experiments are performed using highly susceptible transgenic mouse lines expressing cellular prion proteins, PrP, from a variety of species like cattle, sheep, goat, cervidae, elk, hamster, mouse, mink, pig, and man. In addition, transgenic mice carrying specific mutations or polymorphisms have helped to understand the molecular pathomechanisms of prion diseases. Transgenic mouse models have been utilised to investigate the physiological role of PrP^C, molecular aspects of species barrier effects, the cell specificity of the prion propagation, the role of the PrP glycosylation, the mechanisms of the prion spread, the neuropathological roles of PrP^C and of its abnormal isoform PrP^D (D for disease) as well as the function of PrP *Doppel*. Transgenic mouse models have also been used for mapping of PrP regions involved in or required for the PrP conversion and prion replication as well as for modelling of familial forms of human prion diseases.

prion / scrapie / BSE / transgenic mouse

Table of contents

1. INTRODUCTION

Prion diseases in humans and animals can occur either spontaneously or following an infection. They are caused by prions, mainly if not entirely composed of PrP^D. PrP^D, commonly also designated PrPsc in reference to scrapie pions, is a misfolded form of a physiological protein [68] that is expressed in neuronal cells and many other cell types. PrP^D

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is used as a diagnostic marker for transmissible spongiform encephalopathies (TSE) and studied in basic research. It has been reported that the accumulation of PrP^D broadly correlates with the infectivity levels in a given tissue or sample [5], and it has even been shown for the hamster 263K scrapie model that 14-28 coaggregated PrP^D molecules are contained in one infectious unit [79]. However, it is well feasible that this correlation is valid only for particular combinations of prion strains and species, whereas correlations for other combinations are quite different. Indeed prion diseases have been described with no or only marginally detectable PrP^D depositions in the brain of infected mice [23, 52]. On the contrary, transmission of human TSE cases to transgenic mice can result in a massive PrP^D accumulation in these mice without causing clinical disease [67]. Moreover, in some cases of bovine spongiform encephalopathy (BSE) in cattle, the intensity of the PrP^D signal as determined by immunoblot does not mirror the amount of infectivity in the sample[1]. Therefore, there is no universal equation known to date for the number of PrP^D molecules to one infectious unit. One major prerequisite for finding such an equation would be a better understanding of the infectious nature of prions *eo ipso* and the molecular mechanisms involved in their replication. This would also allow a better understanding and definition of PrP^D, which is primarily only defined biochemically by a prion strain specific partial protease resistance. For most prion strains, the experimental transmission to recipient animals is therefore still the only practical way to quantitatively determine the infectivity load and to describe the transmissibility properties. Until now, no cell culture system has been established that can be easily infected with prions derived from the original host species (cattle, sheep, goats, humans, etc.). So far only experimentally adapted TSE strains can be efficiently propagated in vitro.

In order to study the infectious nature of prions as well as the TSE susceptibility and pathogenesis in humans and animals, the experimental use of in vivo models is a *sine qua non*. In vivo studies can either be carried out in the original animal host species or by transmission of the prions into heterologous species like laboratory rodents which are much easier to handle than ruminants for instance. Moreover, the recent progress in molecular biology techniques has allowed the design of transgenic mice, which are in particular aspects comparable to the natural host species. Other transgenic mouse models expressing prion protein mutants can be used to answer questions regarding the physiological function of the cellular prion protein and the pathomolecular mechanisms of prion diseases.

On the following pages an overview of the most recent advances for the different rodent models is given – with special emphasis on the use of genetically modified mice. Reviews on experimental ruminant and non-human primate models can be found in other papers in the special issue "Prion diseases in animals".

2. CONVENTIONAL RODENT MODELS

TSE transmission experiments to mice were first introduced in the 1960s [29, 90] and to hamsters in the 1970s [30]. Mice and hamsters have since then been the most frequently used experimental animals in TSE research, due to their much easier maintenance and shorter incubation times, and because they allow to discriminate and characterise prion strains. Prion strains are characterised by their biological features (transmissibility, incubation times, brain spongiform lesion profiles, and the topology and quality of the PrP^D depositions) following transmission to a panel of conventional inbred (RIII, C57Bl, VM) mice [14, 17, 38, 42]. The same strain typing methodology has now also been applied to transgenic mice (e.g. tg*a*20, TgbovXV, Tg338, Tgshp, Tgshp) as well as to bank voles [26, 66]. Bank voles (*Clethrionomys glareolus*) have recently been described to be as susceptible to a Creutzfeldt-Jakob disease (CJD) infection as human PrP transgenic mice [66], although there is only a comparably low sequence homology between the PrP of humans and bank voles. Research with this species will therefore be intensified in the future. The

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[1] Buschmann A., Groschup M.H., Unpublished data.

availability of inbred mouse strains played a very important role in the standardisation of brain lesion profiling and was crucial for the definition of the TSE strains. However, it must be noted that such a congruency in their genetic backgrounds does not usually exist for transgenic mice that commonly represent crosses of different inbred lines. TSE isolates can also be characterised biochemically on the basis of the glycosylation pattern, and proteinase K digestion profile (cleavage site as well as general resistance) of PrP^D. Most prion strains are stable when propagated within a given host species.

3. TRANSGENIC MOUSE MODELS

3.1. PrP knockout models

After the announcement of the prion hypothesis in 1982, considerable efforts were made to prove the involvement of this cellular protein in the prion pathogenesis and replication. This can best be shown by PrP knockout mice in which the expression of the physiological protein is genetically ablated. At least four different PrP knockout mice have been generated to date which differ in their genetic design [86]. They all prove that the PrP^C expression is dispensable for the development and function of mammalian cells and organisms. However, the PrP^C expression is crucially important for the prion replication and development of the disease since experimental challenge experiments on PrP knockout mice were unsuccessful [19]. Moreover, the successive inbreeding of a single PrP^C allele into these knockout mice, which leads to an intermediate PrP^C expression and in later studies the transgenic overexpression of mouse PrP^C driven by the introduction of multiple prion gene copies into the mouse genome, showed that the PrP^C expression rate is an important modulator for TSE susceptibility [36].

However, since animals of two PrP knockout mouse models also suffered from a Purkinje cell loss and ataxia, lessons were learned about the genetic regulation of another protein, the so-called Doppel protein. Doppel is encoded by a gene in the close vicinity of the prion gene. The prion gene ablation in these particular knock out mouse models led to an up-regulation of the Doppel expression in adult animals which causes these harmful effects [8]. Doppel is, however, not involved in prion pathogenesis and propagation [69].

3.2. Mutant PrP expression models

Reverse genetics on PrP knockout was used to introduce modified mouse or hamster genes to address a variety of questions:

– mapping PrP regions involved in or required for PrP conversion and prion replication [36, 37, 44, 56, 57, 63, 78, 80];
– studies on the physiological role of PrP^C and the contributions of individual molecular regions to these functions [65];
– studies on molecular aspects of species barrier effects by mutation of single or limited numbers of positions within the PrP [20, 41, 51, 76, 77, 82];
– modelling of familial forms of human prion diseases [3, 4, 60];
– analysis of the cell specificity of prion propagation [48, 71, 72];
– analysis of the role of PrP glycosylation [22, 64];
– studies on the mechanisms of prion spread [11, 12];
– studies on the neuropathological roles of PrP^C and of PrP^D in prion disease [39];
– studies on the function of PrP *Doppel* [34].

All these complex studies have contributed enormously to a better understanding of the nature of prions.

3.3. Transgenic mice overexpressing PrP^C from heterologous species

Many prion diseases can only be transmitted rather inefficiently from diseased to recipient animals, even under experimental conditions where inocula are injected parenterally by a simultaneous intraperitoneal (i.p.) and intracerebral (i.c.) route. The transmission efficiency is particularly low, if the recipient animal belongs to another species. This is usually the case since mostly rodent recipients were used for practical or ethical reasons. In such an experimental setup prions have to cross a considerable species barrier which results in a

lower transmission efficiency (leading to extended incubation times). The primary amino acid sequence of a protein determines its folding structure and therefore defines the potential interaction sites on the molecular surface. In the case of prions, there is considerable evidence that the amino acid sequence homology of PrP^C from the donor versus that of the recipient species determines the transmission efficiency. However, this efficiency is sometimes also influenced by strain specific effects. In order to overcome this sequence effect, a number of transgenic mouse models have been generated during the last two decades, which express PrP^C of heterologous species. The seminal experiment was conducted by Scott et al. who have generated transgenic mice overexpressing hamster PrP^C that proved other than conventional mice susceptible to 263K hamster prions [76]. Meanwhile a battery of transgenic mouse lines are available which express human, mink, bovine, ovine, caprine, cervid, and mouse PrP^C. A register of published susceptible transgenic mouse lines is compiled in Table I.

In principle, there are two approaches to generating transgenic mice: (a) by homologous recombination after transfecting murine stem cells which introduces the gene of interest exactly at the defined target site in the mouse genome; or (b) by microinjection of a transgenic DNA sequence encoding the gene of interest into the pronucleus of a mouse oocyte which leads to a multiple (usually head to tail) insertion array of the trangene randomly into the genome. Both techniques have their advantages and disadvantages.

An interesting phenomenon was observed when humanised transgenic mice were generated and challenged with CJD prions [76]. These mice were only susceptible when the murine PrP^C expression was ablated or when the transgenically expressed human PrP^C carried the murine aminoacid sequences at the carboxy-terminus. Scott et al. [76] postulated that there is a so-called factor X necessary for the conversion of human PrP^C that is captured and blocked by the C-terminus of mouse PrP^C if present. In order to avoid any interference with wild-type mouse PrP^C, most transgenic lines used today are therefore on a mouse PrP^C ablated background.

4. TRANSGENIC MICE AS ULTRA-SENSITIVE BIOLOGICAL PRION DETECTION MODELS

According to the prion hypothesis, infectivity should be detectable using transgenic mice expressing the PrP^C of the donor species at least as good as if animals of the original species were used. The best prion transmission rates were shown in transgenic mice in which PrP^C is heavily overexpressed due to a multiple transgene copy insertion after pronucleus microinjection. However, it has been argued that the random insertion does not mimic the normal mouse PrP^C expression pattern so that the prion disease pathogenesis may be affected. In order to at least partly overcome such random integration effects, large transgene vectors like the half genomic hg-PrP vector (which is composed of the mouse prion gene (12 kb) in which the second intron is deleted) [10] or a cosmid encompassing the total hamster prion gene (45 kb) [75] are used for the generation of transgenic mice. Alternatively, PrP^C is expressed under the control of cytomegalovirus (CMV) [85] or neuron specific enolase (NSE) [33] gene derived promoters. However, these transgenic mice usually do not display an authentic mouse PrP^C expression pattern in body tissues like conventional mice.

The susceptibility of a transgenic mouse line can best be determined by a comparative parallel titration of a defined original inoculum in the transgenic mouse model and in the donor host species. Interestingly, a PrP^C overexpression can even result in a higher sensitivity as compared to the original species. Comparative bioassays have been performed for CJD in monkeys [73] and for BSE in cattle [21, 87], and in each case the transgenic mouse bioassays revealed a 1-2 log LD_{50} higher infectivity titre than the respective homologous species. Tgbov XV mice are currently the only transgenic mouse model for ruminant prions for which such a parallel titration is available. These mice were challenged by the i.c. route with a limited dilution series of a BSE brain

Table I. Register of all published transgenic mouse lines expressing wild-type PrP^C (frequently found non-mutated) which have been shown to be susceptible experimentally to a homologous species challenge.

Species	Designation of transgenic line	HR / MI	Transgene[a]	Transgenic vector[b]	Expression rate[c]	Research group[d]	Reference
Mouse	Tga20	MI	MoPrP-A	phgPrP	10	Weissmann	[36]
	Tg 94	MI	MoPrP-B	cosSHa.Tet	4	Prusiner	[46]
	Tg 117	MI	MoPrP-B	cosSHa.Tet	4	Prusiner	[46]
	Tg4053/FVB	MI	MoPrP-A	cosSHa.Tet	8	Prusiner	[25]
	129/Ola PrP A	HR	MoPrP-A	n.a.	1	Manson	[62]
	129/Ola PrP gtB	HR	MoPrP-B	n.a.	1	Manson	[62]
	Tg46 MloxP	MI	MloxP *Prnp a*	cosSHa.Tet	1	Collinge	[58, 59]
	Tg37 MloxP	MI	MloxP *Prnp a*	cosSHa.Tet	3	Collinge	[58, 59]
Hamster	Tg7	MI	HaPrP	cosHaPrP	1	Prusiner	[46]
	Tg10	MI	HaPrP	cosSHa.Tet	Nd	Oldstone	[70]
	Tg81	MI	HaPrP	cosHaPrP	1	Prusiner	[74]
	Tg71	MI	HaPrP	cosHaPrP	1	Prusiner	[74]
	Tg52NSE	MI	HaPrP	NSE-promotor	Nd	Oldstone	[70]
Human	Tg152	MI	HuPrPV	cosSHaTet	4-8	Prusiner	[83]
	Tg110	MI	HuPrPV	cosSHaTet	4-8	Prusiner	[82]
	Tg440	MI	HuPrPM	cosSHaTet	2	Prusiner	[83]
	HuMTg	HR	HuPrPM	n.a.	1	Manson	[9]
	HuVTg	HR	HuPrPM	n.a.	1	Manson	[9]
	Tg35	MI	HuPrPM	cosSHaTet	4-8	Collinge	[3]
	Tg45	MI	HuPrPM	cosSHaTet	4-8	Collinge	[3]
	Tg1	MI	HuPrPM	phgPrP	2	Gambetti/Kong	[50]
	Tg40	MI	HuPrPM	phgPrP	1	Gambetti/Kong	[50]
Sheep (Goat)	TgshpXI	MI	OvPrP ARQ allele (= *CaprPrP IRQ allele*)	phgPrP	2-4	Groschup	[51]
	TgshpIX	MI	OvPrP ARQ allele (= *CaprPrP IRQ allele*)	phgPrP	4-8	Groschup	[51]
	Tg301	MI	OvPrP VRQ allele	sheep DNA BAC	16	Laude	[84]
	Tg338	MI	OvPrP VRQ allele	sheep DNA BAC	8-10	Laude	[55]
	Tg(ShePrP) 1855	MI	OvPrP ARQ allele (= *CaprPrP IRQ allele*)	cosSHa.Tet		Prusiner	[88]
	Tg(ShePrP) 83669	MI	OvPrP ARQ allele (= *CaprPrP IRQ allele*)	cosSHa.Tet		Prusiner	[88]
	TgovPrP4	MI	Ov PrP ARQ allele (= *CaprPrP IRQ allele*)	NSE-promotor	3	Baron	[33]
	TgovPrP59	MI	Ov PrP ARQ allele (= *CaprPrP IRQ allele*)	NSE-promotor	3	Baron	[32]

Table II. Continued.

Species	Designation of transgenic line	HR / MI	Transgene[a]	Transgenic vector[b]	Expression rate[c]	Research group[d]	Reference
Pig	PoTg001	MI	Porcine PrP	phgPrP	4	Torres	[28]
	PoTg027	MI	Porcine PrP	phgPrP	16	Torres	[28]
Cow	TgbovXV	MI	bovine PrP (6 OR)	phgPrP	8	Groschup	[20]
	BoPrP4125	MI	bovine PrP (6 OR)	cosSHa.Tet	8-16	Prusiner	[77]
	BoPrP4092	MI	bovine PrP (6 OR)	cosSHa.Tet	8-16	Prusiner	[77]
	tg540	MI	bovine PrP (6 OR)	CMV promotor	8-10	Laude	[6]
	boTg110	MI	bovine PrP (6 OR)	phgPrP	8	Torres	[27]
	boTg035	MI	bovine PrP (6 OR)	phgPrP	6	Torres	[27]
	boTg078	MI	bovine PrP (6 OR)	phgPrP	2	Torres	[27]
	boTg022	MI	bovine PrP (6 OR)	phgPrP	0.5	Torres	[18]
	boTg004	MI	bovine PrP (5 OR)	phgPrP	0.5	Torres	[18]
	boTg008	MI	bovine PrP (5 OR)	phgPrP	1	Torres	[18]
	BovTg	HR	bovine PrP (6 OR)	mouse gene	1	Manson	[9]
Mink	Mk7	MI	mink PrP	CosSHaTet	2	Kretzschmar	[89]
	Mk45	MI	mink PrP	CosSHaTet	1	Kretzschmar	[89]
Cervidae	TgCerPrP1536	MI	Cervid PrP	CosSHaTet	5	Telling	[13]
	TgCerPrP1534	MI	Cervid PrP	CosSHaTet	3	Telling	[13]
	TgElk	MI	ElkPrP	CosSHaTet	2.5	Rubenstein	[53]
	TgElkPrP 12577	MI	ElkPrP	CosSHaTet	2	Prusiner	[81]
	TgElkPrP 12580	MI	ElkPrP	CosSHaTet	2	Prusiner	[81]
	TgElkPrP 3934	MI	ElkPrP	CosSHaTet	1.5	Prusiner	[81]
	TgElkPrP 12584	MI	ElkPrP	CosSHaTet	3	Prusiner	[81]
	TgDePrP 10945	MI	DeerPrP (E226K)	CosSHaTet	1	Prusiner	[81]
	TgDePrP 10969	MI	DeerPrP (E226K)	CosSHaTet	1	Prusiner	[81]
	Tg Line 33	MI	DeerPrP (G96)	phgPrP	1	Chesebro	[61]
	Tg Line 60	MI	DeerPrP (S96)	phgPrP	0.7	Chesebro	[61]
	Tg Line 15	MI	DeerPrP (G96)	phgPrP	0.5	Chesebro	[61]
	Tg12	MI	ElPrP-132M	phgPrP	2	Gambetti/Kong	[50]

HR : homologous recombination; MI : microinjection; n.a.: not applicable; n.d.: not done.

a Wild-type PrP allele which was inserted.

b Vector backbone used.

c Compared to PrP expression in the original host species (ususally on a heterozygous (+/−) background).

d Research group in which the transgenic line was generated and characterised.

stem pool homogenate and an infectivity titre of $10^{7.8}$ ID_{50}/g was revealed. The same inoculum when titrated intracerebrally in cattle gave an infectivity titre of 10^6 ID_{50}/g. Since a titration for each inoculum is not always feasible in the original host species, a titration in a defined transgenic mouse line can be used and the transgenic line of interest can then be compared to this reference model. However, even such data are not available for most transgenic mouse lines at present. It must therefore be emphasised that the standardisation by comparative titration of inocula in the original host species and in transgenic mouse models is one of the major tasks for the future in order to facilitate a better appreciation of the many experimental data published on infectivity levels in tissues and bodily fluids of prion diseased animals and humans.

The determination of the susceptibility of transgenic mice is even more important in the light of conflicting evidence speaking against the amino acid homology hypothesis that has been published lately. In a comparative titration of sheep-passaged BSE infectivity in Romney sheep and in RIII mice, the sheep-to-mouse species barrier was lower than anticipated and similar infectivity titres were obtained for both sheep $10^{5.4}$ (i.c.) ID_{50}/g and mice $10^{5.0}$ (i.c + i.p.) ID_{50}/g [40]. Moreover, it has been suggested that the increased susceptibility of transgenic mice results rather from the heterologous PrP^C overexpression than from a homology effect. However, challenging transgenic mice overexpressing murine PrP^C (tga20 mice) with BSE did not result in a remarkable reduction of the incubation time [20, 21]. Answering these questions will not only help define to the most sensitive detection models for human and animal prions but also will shed light on the mechanisms of the prion replication itself.

5. ASSESSMENT OF SPECIES BARRIER EFFECTS IN HETEROLOGOUS TRANSGENIC MOUSE INFECTIONS

Species barrier effects are frequently estimated on the basis of the incubation times in primary transmission studies and on the reduction in the second passage. However, these data must be interpreted with particular care, since incubation times are crucially dependent on the infectivity levels in the samples used. A low infectivity load in the inoculum causes a long incubation time, while a high challenge dose causes a short incubation time in the recipient transgenic mice. Likewise, an efficient initial prion replication in transgenic mice generates a highly infectious subpassage inoculum, which itself yields a shorter incubation time as compared to that of the original inoculum, independently from any species adaptation effect. *In stricto sensu* incubation time differences can therefore only be interpreted as species barrier effects, if TSE isolates of compatible infectivity titres are used in all transmission studies and if it can be verified that compatible PrP^C expression patterns are present in the transgenic mice.

Moreover, the following aspects should be kept in mind when interpreting such transmission data:

– Depending on the experimental setup, there can be quite a range of incubation time variations in cross species barrier transmission experiments. This is particularly true if only low numbers of recipient mice are used, if the general incubation time in this model is quite long and if the differences in the individual mice of the challenge group are comparatively wide. From the authors experience, it is advised to use at least ten mice per group if incubation times are expected to lie within half of the mouse's natural life span, and 15 animals if longer incubation times are expected.

– The reduction of the incubation time after primary passage versus subpassage is dependent on prion strain specific effects. This has been described in detail for BSE and sheep scrapie in transmission studies to RIII, C57Bl, and VM mice [16]. While the primary transmission can be accompanied with quite long incubation times, the subpassages can, depending on the prion strain, result in short, intermediate, or long incubation times.

– An interspecies transmission to transgenic mice (e.g. scrapie transmission to

bovinised mice) does not necessarily lead to prolonged incubation times as compared to an intraspecies transmission (e.g. BSE transmission to bovinised mice). The underlying mechanism is still largely unknown, but from various experiments, it can be assumed that this effect is not only due to the infectivity titres of the applied inocula.

– The absolute length of the incubation period must not necessarily correlate with the susceptibility of the mouse line but is also prion strain dependent. Even in bovinised transgenic mice serially passaged BSE prions show longer incubation times than many scrapie strains. Transgenic mouse lines displaying a short incubation period with a given prion strain must therefore not necessarily have a high susceptibility to all other strains derived from the same species. For example in Tg338 mice overexpressing ovine PrP^C (VRQ allele), incubation times for primary ovine scrapie isolates can vary from 60 days (ARQ allele PG127) [84] to well over 600 days (for individual Italian scrapie isolates)[2]. However, a prion strain with a shorter incubation time has the potential to be detected more efficiently since the natural life span of a mouse is limited to approximately two years. Low infectivity doses of prion strains with extremely long incubation times may therefore simply be missed when the mice die naturally before the end of the incubation time.

6. PHENOTYPIC AND/OR BIOLOGICAL EVOLUTION OF PRION STRAINS AS A CONSEQUENCE OF TRANSMISSIONS TO NEW HOST SPECIES

Transmissions across species barriers can alter the characteristics of prion strains. It has been suggested that the transmission of scrapie between species may be associated with a permanent change in the strain phenotype, due to a selection or to a mutation of the prion strain [15, 31]. According to the selection concept, prion strains are composed of a mix of different variants and the propagation across

a transmission barrier favours one or another subset of variants with a higher or lower efficiency. Since changes in prion strains were observed even when cloned scrapie strains were used, this was attributed to a mutation in the infectious agent. However, the discrimination between selection and mutation processes seems somewhat arbitrary as long as it cannot be excluded that even cloned prion strains might be composed of a variety of subsets [49]. It can be assumed that for most if not all published transmission experiments, accidental transmissions of a different TSE strain by contamination of instruments or other equipment can be excluded, as usually stated in the methods section. This enigma will only be resolved when the nature of prion strain determining factors are completely understood and can be measured directly (and not only by bystander effects). Evolution of strain characteristics following interspecies transmissions have been reported for experimental hamster scrapie strains and for sheep scrapie isolates [49]. The transmission of cattle-derived BSE to C57Bl6 mice has produced the experimental BSE strain 301C while the transmission to VM mice generated 301V prions. However, BSE proved quite stable, i.e. represented only one defined strain when reisolated from a variety of species (including vCJD in humans) which were infected during the course of the BSE epidemic in the UK. Asante et al. [3] have reported that the transmission of BSE to Tg35 mice overexpressing human PrP^C produced primarily the well known vCJD PrP^D phenotype, but also in low numbers a second phenotype (distinguished by biochemical and anatomical analysis of the PrP^D deposits) which was reminiscent of sporadic CJD PrP^D. Another publication describes the transmission of a new bovine prion strain called bovine amyloidotic spongiform encephalopathy (BASE), which can be discriminated from BSE mainly on the basis of a lower molecular mass of the unglycosylated form of PrP^D, a different anatomical distribution of the accumulated PrP^D in the brain, and by the presence of PrP immunoreactive amyloid plaques, bovine PrP transgenic mice. Surprisingly, passage of this strain in conventional

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[2] Laude H., personal communication.

mice produced PrP^D of the BSE phenotype, while bovine PrP transgenic mice revealed the PrP^D signature of the BASE type [24]. Moreover, in a similar study, BASE prions acquired strain features closely similar to those of BSE-type agents when propagated in mice expressing ovine PrP (Tg338), although they retained the BASE-associated phenotypic traits in other lines, including bovine PrP mice [7]. Finally, Espinosa et al. [35] studied the effect of an interim passage of BSE prions in sheep carrying a PrP allele encoding alanine at codon 136, arginine at codon 154 and glutamine at codon 171 (PrP^{ARQ}) in homozygosity prior to the inoculation into bovinised transgenic mice, and observed that this interim passage altered the transmission phenotype of the BSE prions. Cattle derived BSE produced an incubation time of 303 days in the bovinised mice, while sheep derived BSE was shorter with only 234 days. Infectivity titre effects are improbable, since this incubation time difference was similar after subpassage (290 days and 234 days) in the same transgenic mouse line. Biochemical signature, lesion profile, and PrP^D deposition pattern of both cattle and sheep BSE were similar. The observation that ovine BSE but not ovine scrapie isolates lack a species barrier to bovinised transgenic mice illustrates how little the concept of species barrier and strain specific effects is understood to date.

7. TRANSGENIC MICE AS ANIMAL MODELS TO STUDY THE PRION PATHOGENESIS

The question of TSE pathogenesis has been addressed by using transgenic mice produced by microinjection or by homologous recombination. The latter approach is certainly leading to a more authentic PrP^C expression in the transgenic mouse as compared to conventional mice. However, most factors which modulate the pathogenesis of prion infections in vivo are still an enigma. It is well feasible that prions enter the organism most frequently via the oral route. The infectious agents thereby have to overcome the gut wall barrier, replicate locally in the lymphoreticular system (LRS) of the gastrointestinal tract and then travel by one or more routes to the central nervous

system. In the gut associated lymphoid tissues, early agent-specific PrP^D accumulation occurs within the germinal centres of B-cell follicles on follicular dendritic cells (FDC) and also within macrophages. Since neuroinvasion is delayed and disease susceptibility is reduced in the absence of FDC, the initial prion replication in FDC seems to be crucial. Likewise are B-cells that provide important cytokine stimuli such as membrane lymphotoxin (LT) α1β2 and tumour-necrosis factor (TNF) α, which maintain the FDC in their differentiated state. It is clear today that FDC in the LRS play the decisive role at least at the site of entry, if not in the whole organism for the success and effectiveness of the prion infection. Various transgenic mouse models carrying mutations that affect their immune system have played an important role in elaborating today's understanding of these events (for review see [1]). However, most of these studies have been carried out by using a limited number of experimental murine prion strains, whereas little is known about the pathogenesis and infectivity distribution of other prion strains. Moreover, there is a number of major or minor differences (mostly still to be discovered) in the prion pathogenesis in other mammalian species given their evolutionary distance (from humans, bovines, sheep, goats to cervids). Grossly speaking, prions seem to replicate preferentially, if not entirely, in the nervous system in cattle, while there can be a strong lymphatic component in sheep, goats, and cervids. However, this is not a universal rule, because within the same species differences can be enormous, e.g. depending on the prion haplotype that an animal carries. For example, prions replicate in the LRS of sheep carrying two PrP^{VRQ} or PrP^{ARQ} haplotypes while the replication seems to be more restricted to the nervous system in sheep carrying at least one PrP^{ARR} haplotype [2, 43, 45, 47, 54].

8. CONCLUSIONS

The development of transgenic mouse models for prion diseases has been a major step forward for the detection of infectivity in naturally affected species for which intraspecies

transmission studies are unavailable or impratical. While transgenic mouse (independent of whether they were generated by homologous recombination or by microinjection), bank vole, or hamster models are not *bona fide* models for the prion pathogenesis in humans and animals, they can allow to make better exposure and transmission risk assessments for animals and humans. Studying the scrapie pathogenesis in these rodent models can shed light on the principal mechanisms of prion replication and disease. This knowledge may be useful to explain the pathogenetic mechanisms in the original host species and strain combinations.

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