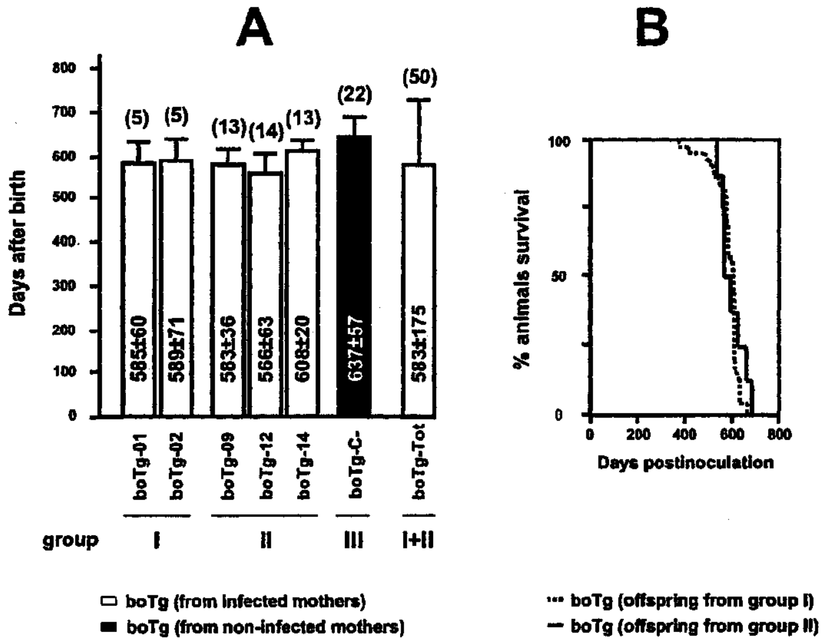

FIG. 2. Mean survival times of mice born from infected mothers. (A) Histograms showing survival times of the offspring of each female and of all offspring (boTg-Tot). BoTg-C-, offspring from uninoculated group III mothers. The values within the bars represent the days after inoculation ± standard deviations. The numbers of mice of each type inoculated are in parentheses. (B) Kaplan-Meier curves correspond to the overall groupings of the offspring (groups I and II)..

ing disease (CWD) (26). Recently it has been shown how vCJD and Gerstmann-Sträussler-Scheinker syndrome (strain Fukuoka-1) prions retaining full infectivity can be detected in the blood of mice after intracerebral inoculation (6). The role of blood in BSE prion dissemination became more evident after the demonstration of BSE transmission to sheep via blood transfusion even during the preclinical phase of an experimental oral BSE inoculation in sheep (18). Our results indicated that BSE prions could be transmitted to the offspring after intracerebral inoculation in a process that seems to be more efficient when detectable amounts of PrP^{res} are present in the brain. The way by which prion infectivity is transmitted through a next generation could be then, based on previous work, be identified as blood dissemination. Other investigated tissues (placenta, lymphoid tissues, and gastrointestinal tract) were negative for PrP^{res} either by Western blotting or by analysis with immunohistochemistry (data not shown). However, these negative results do not allow one to conclude that there is a lack of infectivity in these tissues. In our experimental model, other fluids cannot be disregarded as vehicles for prion spread. To asses whether the route of infection through milk feeding was involved, we carried out experimental inoculations of milk extracted from mothers. For this purpose, 0.5 ml of pooled milk extracted from both infected and uninfected mothers was delipidated and intracerebrally injected into boTg110 mice after a concentration step (centrifugation at 25,000 × *g* for 30 min). We estimate that the amount of milk used for the inoculations represents 25% of the milk intake during lactancy. Analysis of the survival times of mice inoculated or mock inoculated did not show any significant difference (Fig. 3). Brains from these mice were then analyzed with both histopathology and immunohistochemistry for the presence of PrP^{res}. Similarly, no PrP^{res} was detected (data not shown). This negative result does not exclude the potential of milk to transmit prions but suggests that the relevance of this fluid in infectivity might be very low if it exists at all. Thus, the centrifugal dispersion of prions together with the ability of blood to retain prion infectivity might account for the transmission of BSE prions to the offspring without excluding other possible ways.

With regard to BSE in cattle, previous fieldwork studies

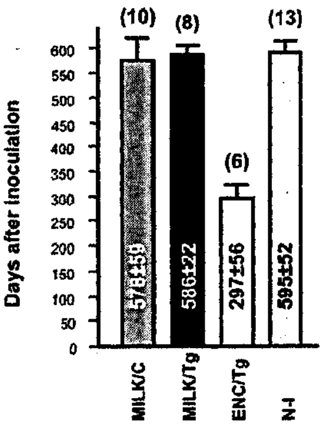

FIG. 3. Survival times of boTg110 mice inoculated with donor milk samples. Survival times for mice inoculated with milk from healthy female boTg110 mice (MILK/C) or milk (MILK/Tg) and brain (ENC/Tg) pools from BSE_I-infected female boTg110 mice are shown. The values within the bars indicate the days postinoculation ± standard deviations. The numbers of mice inoculated with each type of sample are indicated in parentheses.

suggested that the disease may be passed from cow to calf (29, 30). However, there has been controversy and uncertainties regarding whether or not maternal transmission has implications in the prevalence of this disease similar to those that it has for sheep scrapie (9, 10). Our results reveal an enhanced risk of disease in mice born from BSE-infected mothers at the end stage of the incubation time. The same type of risk may apply to the offspring from BSE-infected cattle, as has been suggested from the epidemiological data (9). However, it is necessary to point out here some differences between our transgenic mouse model and bovine species. Firstly, boTg110 mice express boPrP at a level eight times that of bovine PrP in cattle brain; therefore, there is more PrP^C substrate available for conversion to PrP^{Sc}. Secondly, there are some evident differences with respect to the architectural anatomies of mouse and cattle placentations. In cattle, the placenta is bridged to the uterus by a cotyledonary form of attachment, and the structure is of the syndesmochorial type, in which the embryo trophoblastic layer and the maternal uterine epithelium are not fused. In contrast, mouse embryonic and uterine epithelia are completely fused (hemochorial). This type of structure allows blood from the uterine endothelium to be in close contact with the fetal placenta, therefore facilitating the chances for prion dissemination and embryonic contamination.

The BSE agent can propagate efficiently in sheep (11), and the possibility of sheep flocks becoming infected with BSE was raised (21). However, in contrast to findings for sheep scrapie, no evidence of PrP^{Sc} has been found in the reproductive tissues of sheep infected with BSE (13), nor has BSE been reported in the offspring of experimentally infected ewes (12). Since transmission of BSE prions to the offspring occurs in the mouse model, it is reasonable to assume that host-specific restrictions may compromise the ability of BSE prions to be vertically transmitted.

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研究報告の概要	慢性消耗病(CWD)はシカ及びヘラジカの新興のプリオン病である。シカ及びヘラジカのCWDの地域分布は広がっており、ヒトとの接触頻度の増加により伝播が起こる可能性がある。これまでCWDと関連付けられたヒトプリオン病の発生は無かったが、ヒトに対するCWDの異種間伝播のリスクは明確にされていない。ヒトへのCWD伝播リスクを調べるためには、霊長類での感染実験が有用である。CWDのミュールジカの20%（W/V）脳ホモジネート200μlを2匹の成熟した雌のリスザルに大脳内へ接種した。接種されたリスザルは2匹とも進行性神経系疾患を発症し、疾患末期の31ヶ月及び34ヶ月目に安楽死させた。ウエスタンブロットによる分析の結果、脳における異常プリオン蛋白（PrP-res）量は、34ヶ月目に安楽死させたリスザルの方が31ヶ月目に安楽死させたより多かった。31ヶ月目に安楽死させたリスザルの脳、脳幹、脊髄の組織学的検査によって、CWD感染による神経変性に一致する広範囲の海綿状変化が明らかになった。リスザルのプリオン病感染物質を脳内接種後の疾患末期までの期間は、ミンクの伝染性脳症（9〜12ヶ月)、スクレイピー（16ヶ月）の病原体を接種されたものより長く、散発的CJD(11〜37ヶ月)およびクール病(10〜48ヶ月)を接種された範囲内であった。	使用上の注意記載状況・その他参考事項等 2. 重要な基本的注意 (1)略 1)略 2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
リスザルを用いたCWDの感染実験により、霊長類にCWDが感染する可能性を示唆した初めての報告である。 これまで血漿分画製剤によってCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一CWD感染者の血液が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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NOTES

Interspecies Transmission of Chronic Wasting Disease Prions to Squirrel Monkeys (*Saimiri sciureus*)

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Chronic wasting disease (CWD) is an emerging prion disease of deer and elk. The risk of CWD transmission to humans following exposure to CWD-infected tissues is unknown. To assess the susceptibility of nonhuman primates to CWD, two squirrel monkeys were inoculated with brain tissue from a CWD-infected mule deer. The CWD-inoculated squirrel monkeys developed a progressive neurodegenerative disease and were euthanized at 31 and 34 months postinfection. Brain tissue from the CWD-infected squirrel monkeys contained the abnormal isoform of the prion protein, PrP-res, and displayed spongiform degeneration. This is the first reported transmission of CWD to primates.

Chronic wasting disease (CWD) is a prion disease of elk and deer in North America that was first identified at cervid research facilities in Colorado and Wyoming in the late 1960s (17, 18). CWD has been identified on cervid game farms from Montana to New York and has been diagnosed in wild deer and elk in Colorado, Wyoming, Nebraska, South Dakota, Wisconsin, New Mexico, Illinois, and Utah and in Saskatchewan, Canada (1, 14, 15). The geographic distribution of CWD in deer and elk has been expanding and will likely result in an increase in human exposure to the CWD agent. Although there have been no cases of human prion disease linked to CWD infection, the risk of interspecies transmission of CWD to humans following consumption of CWD-infected tissues is uncertain (5, 13).

One approach to assess the susceptibility of humans to animal prion diseases is by experimental transmission to nonhuman primates (9–11). To investigate the susceptibility of nonhuman primates to CWD, two adult female squirrel monkeys (*Saimiri sciureus*) were intracerebrally (i.c.) inoculated with 200 μl of a 20% (wt/vol) brain homogenate from a female mule deer in the clinical phase of CWD (inoculum was a gift from Elizabeth Williams, Department of Veterinary Sciences, University of Wyoming, Laramie, WY). Both CWD-inoculated squirrel monkeys developed a progressive neurological disease and were euthanized at the terminal stages of disease at 31 and 34 months postinfection, respectively (data on clinical symptoms and the time to onset of disease were not available).

To determine whether the abnormal form of the prion protein, PrP-res, was present in the CWD-infected squirrel monkeys, brain homogenates were analyzed by Western blotting as previously described using the anti-PrP monoclonal antibody 6H4 (Prionics AG, Switzerland) (2). For this analysis, a 5% (wt/vol) brain homogenate in Dulbecco's phosphate-buffered saline (Mediatech, Inc.) from CWD-infected squirrel monkeys, a CWD-infected elk, or an uninfected mouse was either digested with proteinase K (PK) (4 U/ml; United States Biochemical) for 1 h at 37°C with agitation or was not digested with PK. In the samples that were not digested with PK, PrP migrated between 21 and 35 kDa in the CWD-infected squirrel monkeys (Fig. 1, lanes 1 and 2) and between 30 and 35 kDa in the CWD-infected elk (Fig. 1, lane 3) and in the uninfected mouse sample (Fig. 1, lane 4). In the samples that were digested with PK, PrP-res were detected in the two CWD-infected squirrel monkeys (Fig. 1, lanes 5 and 6) and in the CWD-infected elk sample (Fig. 1, lane 7). In the PK-digested uninfected mouse brain, PrP was not detected (Fig. 1, lane 8), indicating that PK digestion completely removed the PK-sensitive isoform of PrP. In both CWD-infected squirrel monkeys, the migration of the three PrP-res polypeptides on sodium dodecyl sulfate-polyacrylamide gels was similar. The diglycosylated PrP-res polypeptide migrated at 30 kDa similar to what has been reported for squirrel monkeys infected with sporadic Creutzfeldt-Jakob disease (CJD), kuru, and scrapie (4). The relative abundance of PrP-res in the brain from the squirrel monkey that was sacrificed at 34 months postinfection (Fig. 1, lane 5) was greater than that in the squirrel monkey sacrificed at 31 months postinfection (Fig. 1, lane 6) and may represent differences in the state of disease progression when the animals were sacrificed.

Histological examination of the brain, brain stem, and spinal cord from the squirrel monkey that was euthanized at 31 months postinfection revealed widespread spongiform changes that are consistent with CWD-induced neurodegeneration.

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† Deceased.

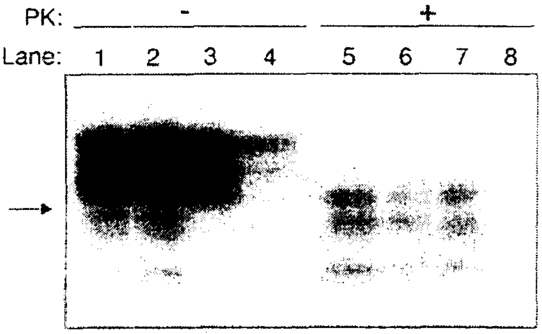

FIG. 1. Deposition of the abnormal isoform of the prion protein, PrP-res, in the brain of squirrel monkeys inoculated with chronic wasting disease. Western blot analysis of 250-μg tissue equivalents of brain homogenates digested with proteinase K or not digested with proteinase K was performed. The brain homogenates were from a CWD-infected squirrel monkey that was sacrificed at 34 months (lanes 1 and 5) or at 31 months postinfection (lanes 2 and 6), a CWD-infected elk (lanes 3 and 7), and an uninfected mouse (lanes 4 and 8). The arrow indicates the location of the 29-kDa molecular mass marker.

Spongiform lesions in the neuropil were predominantly located in subcortical gray matter structures of the forebrain. There was widespread spongiform change in the putamen, caudate nucleus, nucleus accumbens, lateral and medial hypothalamus, hippocampal formation (CA 1), amygdala, and dorsomedial thalamus (Fig. 2). Diffuse spongiosis was found in the interpeduncular nucleus and substantia nigra in the midbrain and in the reticular formation of the pons and medulla. Due to the limited number of histological sections, a detailed comparison of the neuropathology in CWD-infected squirrel monkeys and other prion transmission studies in squirrel monkeys was not possible.

The time to terminal disease following inoculation of squirrel monkeys with the CWD agent, 31 and 34 months, was longer than for squirrel monkeys that were i.c. inoculated with transmissible mink encephalopathy agent (9 to 12 months) and scrapie agent (16 months) but is within the reported range of the time to terminal disease following i.c. inoculation with sporadic CJD (11 to 37 months) and kuru (10 to 48 months) (6, 8). This variation in disease progression following experimental transmission of sporadic CJD, kuru, and CWD to squirrel monkeys could be due to differences in the inoculation dose, strain of the prion agent, or the ability to establish infection upon interspecies transmission. Regardless, this study illustrates that a nonhuman primate can develop a prion disease following i.c. inoculation with a brain homogenate from a CWD-infected mule deer.

Direct comparison of the ability of the CWD agent to cause disease in squirrel monkeys following experimental i.c. inoculation and the susceptibility of humans to CWD infection must be interpreted with caution. Although squirrel monkeys are susceptible to experimental infection with kuru and CJD, they are also susceptible to experimental infection with scrapie (8), and there is no epidemiological evidence to suggest that scrapie can be transmitted to humans (16). These data suggest, following direct cerebral inoculation, squirrel monkeys may not be a good experimental model for assessing human susceptibility to animal prion diseases. Oral exposure is the likely natural route of human exposure to CWD, and in experimental animals, this route is much less efficient at causing disease than i.c. inoculation (3, 7, 12). Therefore, the ability of scrapie and CWD to cause disease in primates by oral infection needs to be established to further resolve the issue of susceptibility of humans to CWD infection.

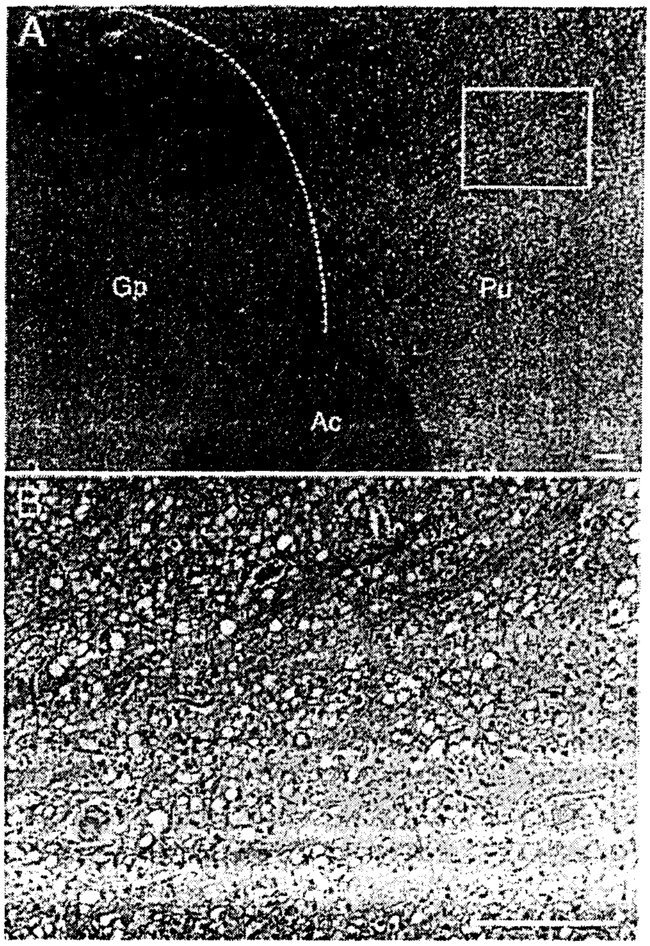

FIG. 2. Spongiform degeneration in brain tissue from a squirrel monkey inoculated with chronic wasting disease and euthanized at 31 months postinfection. (A) Low-power view of the lentiform nucleus, showing the distribution of spongiform changes in the putamen (Pu) and lack of spongiosis in the globus pallidus (Gp). Ac, anterior commissure. (B) High-power view of the area outlined in panel A that exhibits widespread spongiosis. Bars = 100 microns.

Richard Marsh, who performed the experimental transmission of CWD to squirrel monkeys, died in 1997 before these experiments were completed. Due to the emergence of CWD in deer and elk and the potential risk for CWD transmission to humans, we present his findings with additional tissue analysis.

We thank Al Jenny, USDA-APHIS-VS-NVSL for the gift of the CWD-infected elk tissue.

We dedicate the manuscript to Elizabeth Williams for her pioneering work on CWD.

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医薬品
医薬部外品　研究報告　調査報告書
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識別番号・報告回数			**報告日**	**第一報入手日** 2005年10月14日	**新医薬品等の区分** 該当なし
一般的名称	人ハプトグロビン	**研究報告の公表状況**	SCIENCE 310, 324-326, 2005	**公表国** スイス	**厚生労働省処理欄**
販売名（企業名）	ハプトグロビン注－ヨシトミ(ベネシス)				
研究報告の概要	プリオンの感染は、通常、宿主の中枢神経系とリンパ系にしか及ばない。しかし、慢性的な炎症反応がプリオンの分布を拡大しうる。今回われわれは、慢性炎症性腎疾患が感染能のあるプリオンを尿中へ排泄する引き金となるかどうかを検討した。まずリンパ性腎炎のスクレイピー感染マウス由来の尿蛋白を感染していない指標マウスに接種すると、スクレイピーに感染した。また、プリオンを含む尿は、症状発現前のスクレイピー感染マウス及び発症したスクレイピー感染マウスで認められたが、一方、プリオン尿も尿中プリオン PrP^{Sc} もプリオン感染野生型マウス、PrP^{c} 過剰発現マウスもしくは非感染脳を接種された腎炎マウスでは検出されなかった。したがって、尿はプリオンの水平感染のベクターとなり、排泄臓器の炎症はプリオンの拡大に影響を及ぼす可能性がある。				**使用上の注意記載状況・その他参考事項等** 2. 重要な基本的注意 (1)略 1)略 2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。
報告企業の意見				**今後の対応**	
スクレイピーに感染した腎炎罹患マウスの尿中たん白質を非感染マウスに脳内接種するとスクレイピーが伝播した。また、スクレイピー感染と腎炎が共存するとプリオンの尿排出をきたしたという報告である。 これまで血漿分画製剤によってvCJDが伝播したとの報告はない。しかしながら、万一vCJD感染者の血液が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。				本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	

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ate (50-kb) scales (*24*), comparisons over larger scales will require either a chimpanzee genetic map or coalescent analyses of much larger chimpanzee polymorphism surveys than are currently available. Another prediction relates to hotspots detected by sperm typing that are polymorphic among men. In a set of men who do not have a particular hotspot, the model would predict increased activity in other hotspots and a similar total amount of recombination over large regions containing the polymorphic hotspot.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5746/321/DC1
Materials and Methods
Tables S1 to S11
Figs. S1 to S4
References

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Coincident Scrapie Infection and Nephritis Lead to Urinary Prion Excretion

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Prion infectivity is typically restricted to the central nervous and lymphatic systems of infected hosts, but chronic inflammation can expand the distribution of prions. We tested whether chronic inflammatory kidney disorders would trigger excretion of prion infectivity into urine. Urinary proteins from scrapie-infected mice with lymphocytic nephritis induced scrapie upon inoculation into noninfected indicator mice. Prionuria was found in presymptomatic scrapie-infected and in sick mice, whereas neither prionuria nor urinary PrP^{Sc} was detectable in prion-infected wild-type or PrP^{C}-overexpressing mice, or in nephritic mice inoculated with noninfectious brain. Thus, urine may provide a vector for horizontal prion transmission, and inflammation of excretory organs may influence prion spread.

The prion, the infectious agent of transmissible spongiform encephalopathies (TSEs), is detectable at extraneural sites long before clinical symptoms appear (*1*). PrP^{Sc}, a protease-resistant isoform of the host protein PrP^{C}, accumulates mostly in central nervous system and lymphoid organs of infected organisms and may represent the infectious principle (*2*, *3*). In addition to PrP^{C} (*4*), splenic prion replication requires follicular dendritic cells (FDCs), the maintenance of which depends on B cells expressing lymphotoxins (LT) α and β (*5*). By activating local LTα/β signaling, which induces lymphoneogenesis, chronic inflammation enables ectopic prion replication (*6*). Inflammatory kidney conditions induced by bacteria, viruses, or autoimmunity are frequent in animals and humans, and urosepsis can occur in terminally demented patients (*7*). We therefore wondered whether renal inflammatory conditions might lead to urinary prion excretion.

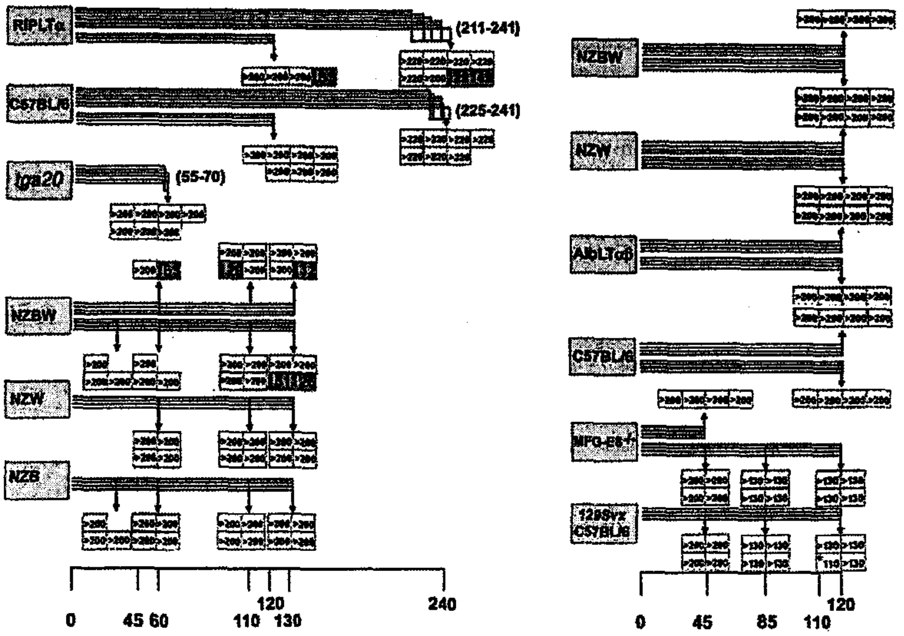

Fig. 1. Transmission of prions through urine. Urine samples were collected from individual donors (horizontal lines) at time points after inoculation, denoted by vertical lines, and pooled (intersections between lines, arrows). Squares represent individual *tga20* mice inoculated i.c. with urinary proteins. White squares: no scrapie symptoms; red squares: histopathologically confirmed scrapie; green squares: positive PrP^{Sc} immunoblot. Numbers within squares: days to terminal disease. Clinical disease: red line. Prion incubation time is expressed in days. Asterisk: intercurrent death without clinical scrapie signs.

To probe this possibility, we administered prions to RIPLTα and NZB × NZW F_1 mice (henceforth termed NZBW) suffering from lymphocytic nephritis (figs. S1 and S2 and table S1), as well as NZW mice and milk fat globule–epidermal growth factor 8 (MFG-E8)–deficient mice, which develop glomerulonephritis but lack lymphofollicular inflammation (fig. S1).

After intraperitoneal (i.p.) prion inoculation [3 and 5 logLD_{50} (50% lethal dose) units of the Rocky Mountain Laboratory (RML) scrapie strain (passage 5, henceforth called RML5) (*8*)], brains and spleens of RIPLTα, NZBW, MFG-E8$^{-/-}$, and control mice displayed similar prion and PrP^{Sc} loads (fig. S3, A to C). Whereas RIPLTα and NZBW kidneys progressively accumulated PrP^{Sc} and prion infectivity at 60 to 90 days postinoculation (dpi), presymptomatic (66 dpi) and terminally sick MFG-E8$^{-/-}$ mice lacked renal PrP^{Sc} (fig. S3D). Histoblot and immunohistochemical analysis identified PrP^{Sc} in renal lymphofollicular infiltrates of RIPLTα and NZBW mice (*6*).

RIPLTα, AlbLTαβ, C57BL/6 (4 to 6 months old), NZW, NZB, NZBW, MFG-E8$^{-/-}$, *tga20*, and 129Sv × C57BL/6 (8 to 16 weeks old) were inoculated i.p. with 3 or 5 logLD_{50} scrapie prions. We dialyzed and purified urinary proteins from pools of three to six mice of each genotype at 30, 45, 60, 85, 110, 120, and 130 dpi (all presymptomatic) and from terminally scrapie-sick mice (Fig. 1). Each urine donor was confirmed to contain brain or spleen PrP^{Sc} and/or infectivity upon necropsy (fig. S3, A to C).

Next, we quantified the recovery of spiked PrP^{Sc} and infectivity from urinary proteins (fig. S4). Scrapie cell endpoint assay (*9*) revealed a higher prion titer in dialyzed samples (fig. S4, C and D), possibly because dialysis removed biocontaminants inhibiting infection of PK1 cells.

Urinary proteins were purified by ultrafiltration followed by dialysis (~600 μg pooled from groups of three to six mice), or by dialysis followed by ultracentrifugation, and inoculated intracerebrally (i.c.) into groups of three to eight *tga20* mice that overexpress PrP^C (*10*). We found prion infectivity within pools of presymptomatic (120 dpi, $n = 3$) and scrapie-sick RIPLTα ($n = 6$) and NZBW mice ($n = 16$). However, we did not find infectivity in C57BL/6 ($n = 18$), MFG-E8$^{-/-}$ ($n = 8$), 129Sv × C57BL/6 ($n = 4$), NZW ($n = 12$), or NZB ($n = 4$) urine at any time point after prion inoculation (Fig. 1). Urine from terminally scrapie-sick NZBW, NZW, and NZB mice could not be collected because the incubation

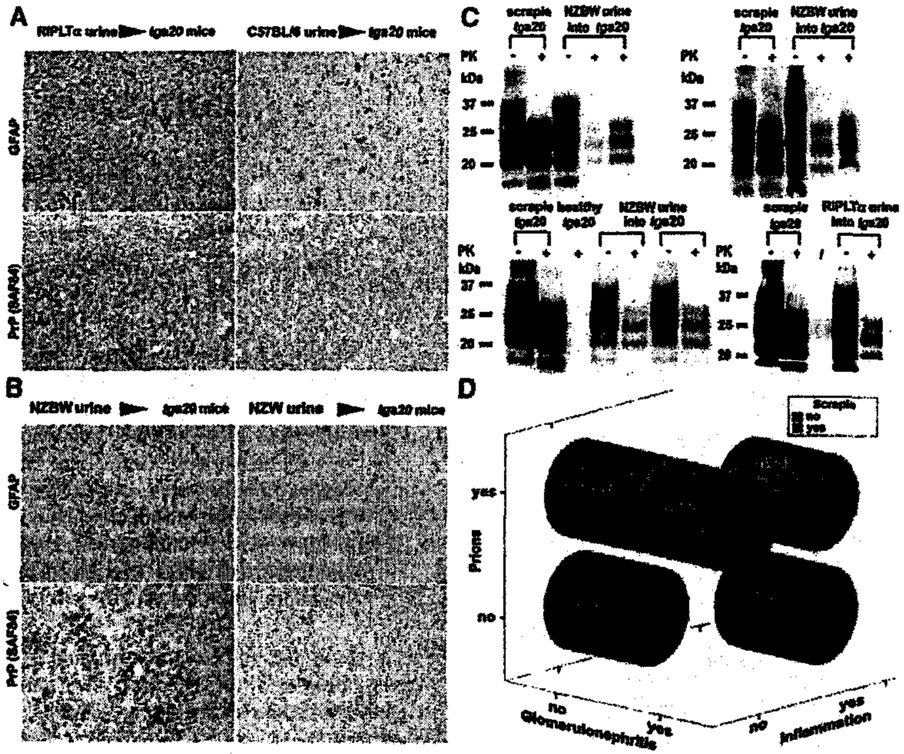

Fig. 2. Scrapie pathology in mice exposed to urine of nephritic mice. (**A** and **B**) Brain sections of *tga20* mice that succumbed to scrapie after i.c. inoculation with urinary proteins from RIPLTα (terminal) (A) or NZBW mice (130 dpi) (B), showing gliosis (GFAP, glial fibrillary acidic protein) and PrP deposition (SAF84). *Tga20* brains inoculated with urine from terminally sick C57BL/6 or presymptomatic NZW mice showed little or no astrogliosis and no PrP deposition. (**C**) (Upper panels) PrP^{Sc} in brains of *tga20* mice inoculated i.c. with NZBW urinary proteins (130 dpi). Ten micrograms (left) or 20 μg (right) of *tga20* brain were digested with proteinase K and immunoblotted. (Lower left panel) PrP^{Sc} in brains of *tga20* mice inoculated i.c. with NZBW or RIPLTα urinary proteins. Lanes 4 to 7: Inoculation with NZBW urinary proteins at 60 dpi (lanes 4 and 5) and 110 dpi (lanes 6 and 7). Positive controls: scrapie-sick *tga20* brain homogenate (left two lanes of each blot). Negative control: brain homogenate of a healthy *tga20* mouse. (Lower right panel) Inoculation with RIPLTα urinary proteins at 120 dpi. (**D**) Prions were detected in *tga20* mice exposed to urine from mice with lymphocytic nephritis (18.2%), but not in mice without kidney pathology or with isolated glomerulonephritis.

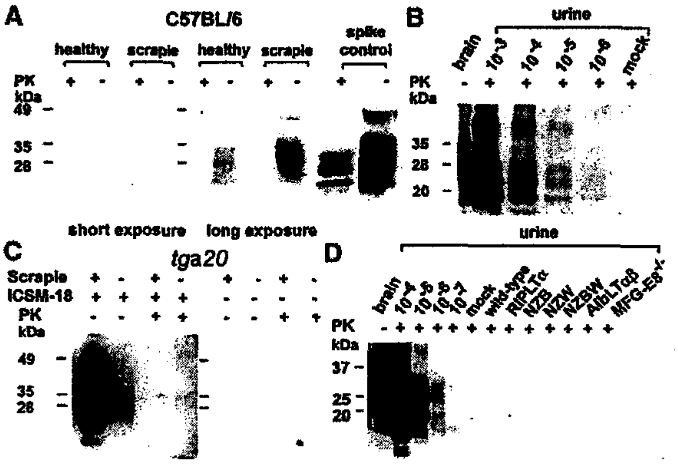

Fig. 3. Failure to detect urinary PrP^{Sc}. (**A**) Immunoblot analysis of urinary proteins from terminally scrapie-sick C57BL/6 mice. No PrP^{Sc} was found after ultracentrifugation. For control, *Prnp*$^{o/o}$ urine was spiked with scrapie brain homogenate. (**B**) Threshold of PrP^{Sc} detection in urinary proteins purified by dialysis and ultracentrifugation. C57BL/6 urine was spiked with serial dilutions of brain homogenate. Assay sensitivity: ≥100 ng of terminal brain homogenate per milliliter of urine (≅10^3 ID_{50} units/ml). (**C**) Immunoblot analysis of urinary proteins after ultracentrifugation. Scrapie-sick *tga20* mice lacked $UPrP^{Sc}$. PK, proteinase K digestion; ICSM-18, primary antibody to PrP. Omission of primary antibody (right) abolished all signals. (**D**) Immunoblot analysis of urinary proteins from presymptomatic [NZB, NZW, and NZBW (100 dpi)] and terminally scrapie-sick mice. No PrP^{Sc} was detected after ultracentrifugation (long exposure). Controls: scrapie brain homogenate used for spiking (lane 1); urine spiked with brain homogenate from scrapie-sick (lanes 2 to 5) or healthy mice (lane 6).

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