

Complex proteinopathies and neurodegeneration: insights from the study of transmissible spongiform encephalopathies

Proteinopatías complejas y neurodegeneración: conocimientos obtenidos del estudio de las encefalopatías espongiformes transmisibles.

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ABSTRACT

Protein misfolding diseases are usually associated with deposits of single “key” proteins that somehow drive the pathology; β -amyloid and hyperphosphorylated tau accumulate in Alzheimer’s disease, α -synuclein in Parkinson’s disease, or abnormal prion protein (PrP^{TSE}) in transmissible spongiform encephalopathies (TSEs or prion diseases). However, in some diseases more than two proteins accumulate in the same brain. These diseases might be considered “complex” proteinopathies. We have studied models of TSEs (to explore deposits of PrP^{TSE} and of “secondary proteins”) infecting different strains and doses of TSE agent, factors that control incubation period, duration of illness and histopathology. Model TSEs allowed us to investigate whether different features of histopathology are independent of PrP^{TSE} or appear as a secondary result of PrP^{TSE}. Better understanding the complex proteinopathies may help to explain the wide spectrum of degenerative diseases and why some overlap clinically and histopathologically. These studies might also improve diagnosis and eventually even suggest new treatments for human neurodegenerative diseases.

Keywords: Dementia; amyloidogenic proteins; prion diseases.

RESUMEN

La acumulación de proteínas con conformación anormal es observada en numerosas enfermedades degenerativas del sistema nervioso. Tales enfermedades están generalmente asociadas con el depósito de una proteína que es importante para la patogenia de la enfermedad; amiloide- β e hiperfosforilación de tau en la Enfermedad de Alzheimer, α -sinucleína en la Enfermedad de Parkinson, y acúmulo de proteína prion anormal (PrP^{TSE}) en las encefalopatías espongiformes transmisibles (EET). Sin embargo, en algunas enfermedades más de dos proteínas se acumulan en el sistema nervioso central. Estas enfermedades pueden considerarse “proteinopatías complejas”. Hemos estudiado varios modelos de EET para analizar los depósitos de PrP^{TSE} y la posible acumulación de otras proteínas (que podríamos llamar “proteínas secundarias”). La relación entre proteínas mal plegadas y neurodegeneración no es claro. La mayor parte de las enfermedades neurodegenerativas evolucionan por décadas; por lo tanto los acúmulos proteicos podrían generar diferentes efectos patogénicos en los diferentes estadios de la enfermedad. Alternativamente los acúmulos proteicos podrían ser el resultado de alteraciones del sistema nervioso y no su causa. Dado que la etiología de las EET es relativamente bien conocido y es atribuido a infección por agentes autoreplicantes que generan malformación de la proteína prion normal (la isoforma patológica, PrP^{TSE}, propuesta como el agente infeccioso) hemos estudiado varios modelos animales, cepas de agente infectante y dosis del agente causal de EET. Estos factores controlan el período de incubación, duración de la enfermedad e histopatología. Los modelos animales estudiados nos han permitido investigar si las diferentes características histopatológicas son independientes de PrP^{TSE} o podrían ser secundarias a la acumulación de la misma. Un mejor conocimiento de las proteinopatías complejas podría ayudar a analizar el espectro de enfermedades degenerativas y a su vez, investigar el motivo de la superposición clínico-patológico en algunas de ellas. Estos estudios podrían ayudar en el diagnóstico y eventualmente sugerir nuevas posibles terapéuticas para las enfermedades neurodegenerativas humanas.

Palabras-clave: Demencia; proteínas amiloidogénicas; enfermedades por prion.

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Relationships between misfolded proteins and brain diseases remain unclear. Protein aggregates may accumulate in the central nervous system for years, probably decades, before the onset of overt illness and during the slow progression of various common neurodegenerative diseases. The protein aggregates may produce different pathologic effects at different stages of disease^{1,2}. Typical intracellular inclusions or extracellular deposits of proteins are commonly used to define specific neurodegenerative diseases. For example: β -amyloid (A β) plaques and neurofibrillary tangles composed of hyperphosphorylated tau (p-tau) both accumulate in Alzheimer's disease (AD), abnormal prion protein (PrP^{TSE}) in transmissible spongiform encephalopathies (TSEs, prion diseases), and intracellular deposits of α -synuclein form Lewy bodies in Parkinson's disease (PD)^{3,4}. However, more than one protein can interact, modifying the pathogenesis of disease by targeting different anatomical areas or altering signaling pathways in the brain. For example, the merging of A β , normal cellular PrP (PrP^C) and the nonreceptor tyrosine kinase Fyn on lipid rafts may result in synaptotoxicity⁵. Fyn associates with tau, sensitizing synapses to glutamate excitotoxicity⁶. These studies led to the hypothesis that the association between PrP^C and Fyn may couple A β and tau pathologies⁶. Tau and α -synuclein each promote aggregation of the other protein⁷, while A β seems to promote hyperphosphorylation of tau by downregulating the insulin signaling pathway⁸. Recent studies expanded this concept by showing that pre-aggregated A β can serve as a template that aggregates filamentous tau by cross-seeding⁹. While the interactions of normal endogenous proteins of the central nervous system are complex and poorly understood, certain common pathways appear to be activated in different neurodegenerative diseases¹⁰. The neuropathologic analysis of dementing diseases has become increasingly important: (i) clinical signs sometimes reflect the neuroanatomical distribution of lesions but do not necessarily predict specific histopathologic and biochemical alterations; (ii) development of new diagnostic tests and effective therapies require postmortem validation; (iii) increased prevalence of dementia in an aging population makes it a priority for clinical medicine, health-care and social support systems as well as for research; and (iv) diagnosis of dementing diseases often comes late in the course of the disease when brain damage is already severe and irreversible so that therapy is likely to offer little or no benefit. Taken together, these considerations affirm the need to study accessible markers of neurodegeneration in young populations and in healthy neurological controls. Clinico-pathologic and imaging studies have shown that some people without dementia have substantial AD pathology in the central nervous system¹¹. Recently, Perez-Nievas and colleagues found that A β plaques and neurofibrillary tangles did not always correlate with dementia; they identified glial activation as a more likely mediator of neurotoxicity¹¹. In conclusion, the study of autopsy cases remains necessary to

confirm antemortem clinical and neuroimaging diagnosis, to define new disease phenotypes, to assess the specificity and sensitivity of antemortem diagnostic tests and to evaluate the effects of potential therapeutic interventions. However, in an era when autopsies are no longer common—even in academic settings—ambitious programs to investigate post-mortem findings in familial and sporadic neurodegenerative diseases face obvious challenges not discussed further in this review. Fortunately, recent technological advances using transgenic animals, proteomics and experimental immunohistopathology have allowed development of sophisticated experimental models to study neurodegenerative diseases associated with protein misfolding¹².

ANIMAL MODELS

Animal models that recapitulate the pathogenesis of the human diseases AD, PD, and TSEs are especially needed to improve the understanding of chronic neurodegeneration. Only one group of human neurodegenerative diseases has been convincingly modeled in experimental animals: the TSEs or prion diseases¹³. Human prion diseases are unique because they occur in idiopathic (sporadic), familial and acquired forms. In humans, the most common TSE is sporadic Creutzfeldt-Jakob disease (CJD). Several other prion diseases have been described in farmed and wild animals: scrapie in sheep and goats, chronic wasting disease in deer and other cervids, bovine spongiform encephalopathy (BSE) and its feline derivative, transmissible mink encephalopathy and, most recently, a TSE of camels¹⁴. Animal models of prion diseases have been used to study neurodegeneration because they so closely resemble the human TSEs, are highly reproducible, and express several informative phenotypes. No transgenic-mouse model of AD, PD or the tauopathies so closely resembles the sporadic human neurodegenerative diseases¹³.

Animals with TSEs develop many neuropathologic hallmarks of other neurodegenerative diseases, e.g., protein accumulation, synaptic degeneration and neuronal loss, severe microgliosis and astroglia. Animal models can be precisely monitored from the time the agent is inoculated through advanced illness, facilitating study of both latent and overt phases of disease. We have shown that amyloid plaques containing PrP can form in mouse brains in the absence of infectivity detectable by inoculation of highly susceptible animals, suggesting that not all misfolded PrP is infectious in these experimental paradigms^{14,15,16,17}. Thus, proteinopathies sharing some similarities to AD and PD occur in brains of mice when PrP misfolds. First, we review recent data from experimental TSEs of nonhuman primates and mice; next we summarize data from a recently-reported natural complex proteinopathy of bovines (not caused by BSE or another TSE), the first tauopathy described in ruminants¹⁸.

Primates inoculated with classical BSE agent (SQ-BSE) develop a vCJD-like disease

All primate tissues examined and described in this review were produced in previously-described transmission experiments¹⁹. All experiments were reviewed and approved by the Institutional Animal and Care Committee of the Center for Biologics, Evaluation and Research, of the US FDA. We infected squirrel monkeys (*Saimiri sciureus*) with the agent of classical bovine spongiform encephalopathy (SQ-BSE). Most animals (6/7) inoculated with a low dilution of BSE-infected bovine brain (generously provided by Torsten Seuberlich, University of Bern, Switzerland) became ill; two to four years later those animals developed cognitive and behavioral alterations with other neurological signs (tremors, bradykinesia, myoclonus, ataxia) typical of prion diseases. At necropsy, the brains from all sick monkeys showed pathological changes similar to those described in humans with variant Creutzfeldt-Jakob disease (vCJD)—a zoonosis transmitted to humans who ate meat or meat products from animals with BSE^{**} (**A few cases of vCJD were transmitted by transfusion of red blood cells and injections of a human plasma product prepared from donations by otherwise healthy blood donors presumed to have been silently incubating vCJD)¹⁹. All sick monkeys showed histopathological features typical of spongiform encephalopathy: widespread, severe astrogliosis, and accumulations of PrP^{TSE} in the cerebrum and cerebellum. There were no “florid” plaques (amyloid PrP-containing plaques surrounded by halos of vacuoles); such plaques have been a consistent, although not specific, feature in brains of patients with vCJD. Interestingly, florid plaques have never been observed in brains of cows with BSE, showing that the host determines some neuropathologic features of a TSE. Western blot analyses of brain homogenates from the six monkeys with disease confirmed that they all contained proteinase-K resistant PrP (PrP^{TSE})^{19,20}.

Brains of monkeys with SQ-BSE show severe tauopathy and synaptic pathology without A β deposits

Brain tissue sections were probed with a panel of anti-tau antibodies (AT8 [Ser202/Thr205], AT100 [Ser212/Thr205], AT180 [Thr231], AT270 [Thr181]) that bind to hyperphosphorylated tau (p-tau) and with an antibody to four-repeat tau isoforms [RD4]). The brains of all six monkeys with neurological signs and histopathology of TSE (Figure A, B, D, E, J, K) showed identical tau-positive immunostaining patterns with abundant round and rod-shaped aggregates of positive-staining material (Figure G, H). These lesions did not resemble the typical p-tau deposits seen in AD or tauopathies (i.e., neurofibrillary tangles, neuropil threads or diffuse intracytoplasmic staining (Table; Figure G, H). Positive tau staining was seen in the cerebral cortex, thalamus, hypothalamus, hippocampus, cerebellum and brain stem. The extensive accumulations of p-tau observed in the molecular, Purkinje and granule cell layers of the cerebellum were unexpected. This finding shows that the cerebellum, (an anatomical region of

the central nervous system previously considered refractory to tau pathology in humans) was targeted in an experimental encephalopathy accompanying PrP accumulation. The last squirrel monkey to become ill after an incubation period of approximately 8 years, SQ-736, showed more severe spongiform degeneration, PrP^{TSE}, p-tau and astrogliosis than SQ-735 (after an incubation period of 3.2 years) inoculated with the same dose of BSE agent (Figure). Similar patterns and distribution of PrP^{TSE} and p-tau-containing lesions were seen with all anti-PrP and anti-tau antibodies used²¹. The areas most affected were the frontal cortex and thalamus.

The temporal cortex was spared, showing no spongiform degeneration, severe gliosis or PrP deposits; no obvious accumulation of p-tau was seen. To explore another marker of neurodegeneration—the synaptic pathology observed in early stages of TSEs and other neurodegenerative diseases²²—we probed tissue sections with antibody to synaptophysin (Abcam, Cambridge, MA), a component of the membrane glycoprotein of synaptic vesicles that serves as a marker of presynaptic terminals. Synaptophysin, in the form of fine granular and evenly distributed deposits (as seen in preserved brain sections), was significantly reduced in all brain areas containing protein deposits and astrogliosis but remained unaffected in the temporal cortex, confirming that neurodegenerative histopathology was not evenly distributed. The p-tau deposits did not have the morphologic features of neurofibrillary tangles, neuropil threads, or diffuse intracytoplasmic tau described in some human patients with dementia²³. Brain sections from a control animal without prion disease showed no evidence of TSE, and p-tau deposits were completely absent (Figure C, F, I, L). Sections probed with 4G8 antibody, directed to amino acid residues 17-24 of β -amyloid (commonly used to detect A β in humans with AD, in animal models of AD and in nondemented persons with A β deposits^{24,25}), showed no immunostaining in the brain of any animal with prion disease. Adjacent sections treated with Congo red dye showed no congophilia and no birefringence under polarized light (confirming the absence of amyloid deposits). In short, all monkeys with BSE developed severe spongiform encephalopathy with parenchymal accumulations of PrP^{TSE} and p-tau in the cerebrum, cerebellum and brain stem. The areas most severely affected were the frontal cortex and thalamus, but no amyloid protein of any kind was observed in any of these animals.

Accumulation of α -synuclein and ubiquitin in SQ-BSE

To complete the neuropathologic characterization of SQ-BSE, we probed brain sections with antibodies to apolipoprotein-E, a risk factor for late-onset AD (also influencing A β and tau formation), to α -synuclein (present in Lewy bodies in PD), ubiquitin (a major component of the proteolytic quality control system), and synaptophysin (see above). In addition, we examined formalin-fixed samples of spleen, liver and kidney²¹. Analysis of adjacent brain tissue sections showed that

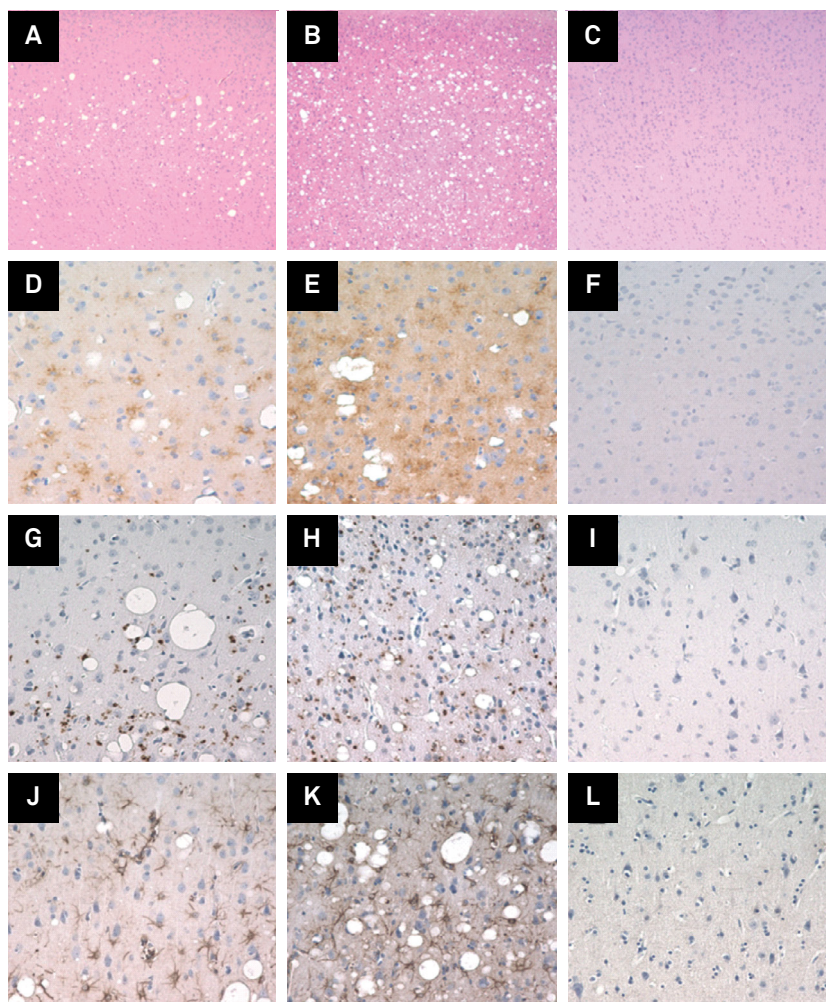


Figure. Squirrel monkeys (SQ) inoculated with classical BSE (SQ-BSE) develop TSE and a complex proteinopathy. (A, D, G, J) SQ-BSE 735 with incubation period of 3.2 years; (B, E, H, K) SQ-736 with incubation period 8.1 years; (C, F, I, L) SQ-718 (control) with no TSE confirmed neuropathologically. Moderate (A) and severe (B) spongiform degeneration, brain tissue sections stained with hematoxylin-eosin (HE). Moderate (D) and severe (E) PrP^{TSE} accumulation in the neuropil. Moderate (G) and severe (H) p-tau immunopositivity in adjacent sections of the frontal cortex. Moderate (J) and severe (K) astrogliosis in the same region of the neocortex. (C, F, I, L) No spongiform degeneration, PrP^{TSE}, p-tau or astrogliosis are seen in the frontal cortex of SQ without TSE. (A-C) HE; (D-F) PrP (antibody 6H4); (G-I) p-tau (antibody AT8); and anti-gial fibrillary acidic protein antibody (J-L). All panels 40x magnification.

PrP^{TSE} (mostly as coarse pericellular aggregates), p-tau (rod-shaped), α -synuclein (small punctate) and ubiquitin (small punctate) were deposited mainly in the neuropil of areas with severe spongiform degeneration; deposits were also prominent at the periphery of vacuoles; no PrP^{TSE} was observed in peripheral organs²¹. The brains of all six monkeys contained more PrP^{TSE} than p-tau or α -synuclein. Similar observations in human cases and in some other animal models have led others to suggest that the dose-dependent toxicity triggered by PrP^{TSE} might be the primary cause of neurodegeneration²⁶. Further studies will be needed to explore a possible role for other factors, such as inflammation, in TSE pathogenesis.

P-tau in rodents with PrP accumulation

To explore further the association between p-tau and PrP while eliminating the potential confounding effects of aging, unrelated diseases, drug treatment, agonal changes and

postmortem delay inevitable in human autopsy cases and to expand the study from nonhuman primates to small animals, we analyzed several murine models. All rodent tissues examined and described in this review were produced in previous transmission experiments^{27,28,29,30,31} performed under licence from the UK Home Office in accordance with the Animals (Scientific Procedures) Act 1986. The severe tauopathy seen in squirrel monkeys infected with classical SQ-BSE¹⁹ led us to study a line of transgenic mice from which the murine *PRNP* gene was deleted and then engineered to express a bovine PrP with a six-octapeptide repeat region (“bovinized knock-in” B6 mice)²⁸; we analyzed B6 mice intracerebrally inoculated with classical BSE agent from a bovine brain and also analyzed B6 mice inoculated with brain suspensions from cattle with two atypical forms of BSE: “heavy” or H-type BSE and bovine amyloidogenic spongiform encephalopathy (also called “light” or L-type BSE)²⁹. To study possible correlation between PrP

amyloid and p-tau, we used the following models: (i) wild-type (Wt) VM mice (an inbred Wt line of mice with known susceptibility to scrapie) inoculated intracerebrally with 87V mouse-adapted scrapie agent (87V-VM)^{30,32}; (ii) transgenic mice overexpressing mutant PrP-101L (equivalent to a mutation found in Gerstmann-Sträussler-Scheinker disease, GSS-22) that spontaneously develop a severe spongiform degeneration with abundant diffuse and amyloid PrP deposits in most areas of the central nervous system^{17,27}; and (iii) knock-in mice expressing a murine mutant PrP (101LL, corresponding to human *PRNP* P102L) inoculated with recombinant wild-type PrP (recWt-PrP) or recombinant mutant PrP (recPrP-101L) fibrils³³. To study p-tau accumulation in animals with large amounts of widespread diffuse nonamyloid PrP^{TSE} deposits, we inoculated Wt C57BL mice with ME7 mouse-adapted scrapie agent (ME7-C57BL)³⁰. Uninoculated mice of the same types described above served as normal controls.

Similar tauopathy seen in PrP-bovinized mice infected with agents from typical and atypical forms of BSE

Similar patterns and distribution of p-tau were seen in brains of B6 mice inoculated with BSE agents of all three types: classical, H-type, and L-type³¹. The largest amounts of p-tau were observed in the brains of B6 mice inoculated with L-BSE agent. In the brains of mice inoculated with BSE agents all three types, the cerebral cortex was consistently affected. Confocal microscopic images of isolated p-tau deposits showed that PrP^{TSE} co-localized with p-tau; Imaris 3D reconstruction confirmed that observation and revealed p-tau deposited throughout PrP plaques in classical BSE and H-type BSE as well. No p-tau was detected in brain sections of uninoculated age-matched control B6 mice probed with anti-tau antibodies (AT8 or Thr231) or in BSE-infected B6 mice incubated without primary antibody. In short, p-tau accumulated widely in the brains of B6 mice infected with both typical and atypical BSE agents³¹ (Table) in the same places as PrP^{TSE} deposits.

P-tau in wild-type mice inoculated with mouse-adapted ME7 and 87V scrapie agents

The strains ME7 and 87V are mouse-passaged scrapie agents that elicit disease after very different incubation periods: 160 days for ME7 in C57BL mice and 320 days for 87V in VM mice. The two models also have distinct histopathologies³⁰. The brains of terminally ill C57BL mice infected with ME7 scrapie showed severe gliosis and accumulated large amounts of diffusely distributed PrP^{TSE} with only small numbers of amyloid plaques. In contrast, the brains of VM mice infected with 87V scrapie contained abundant amyloid plaques and both coarse and fine-punctate deposits of PrP^{TSE}. Brains of terminally ill C57BL mice infected with ME7 scrapie contained small amounts of p-tau in most areas with spongiform degeneration³¹. The brains of terminally ill VM Wt mice

Table. Neuropathologic characterization of rodents and bovines with tauopathy.

	IP (days)	p-tau	SD	PrP ^{TSE}	α -syn	Ub	A β
Bov6.H.BSE	590	+	+	+	NA	NA	NA
Bov6.C.BSE	540	+	+	+	NA	NA	NA
Bov6.BASE	635	+	+	+	NA	NA	NA
87V/VM	320	+	+	+	NA	NA	NA
ME7/C57	160	+	+	+	NA	NA	NA
GSS22	198	-(#1)	+	+	NA	NA	NA
101LLrec.PrP-101LL	NA	-	-	+	NA	NA	NA
101LL rec.PrP-Wt	NA	-	-	+	NA	NA	NA
IBNC	NA	+	+	+	+	+	-
SQ-BSE	870-2960	+	+	+	+	+	-

IP: incubation period; p-tau: hyperphosphorylated tau detection by immunohistochemistry (IHC) with antibody AT8 (epitope human tau protein Ser202/Thr205); SD: spongiform degeneration; α -syn: α -synuclein detection by IHC (immunogen recombinant human α -synuclein); Ub: ubiquitin detection by IHC with antibody Ubiquitin-1 (raised against purified ubiquitin conjugated with glutaraldehyde to keyhole limpet hemocyanin). (#1) Minimal p-tau immunopositive deposits were observed in one animal. H.BSE: heavy (H-type) bovine spongiform encephalopathy (BSE); C.BSE: classical BSE; BASE: bovine amyloidogenic spongiform encephalopathy (light, or L-type BSE); IBNC: idiopathic brainstem neuronal chromatolysis; SQ-BSE: squirrel monkey classical BSE

infected with 87V scrapie contained deposits of p-tau within and adjacent to the margins of PrP^{TSE} amyloid plaques; p-tau was also observed in the same regions where diffuse nonamyloid PrP^{TSE} deposits occurred³¹ (Table). Confocal and Imaris 3D reconstruction of double-labeled sections showed that p-tau colocalized with PrP^{TSE} aggregates throughout the plaques in both 87V/VM and ME7/C57 models. In short, two mouse-adapted scrapie strains elicited widespread formation of p-tau in multiple brain areas in two lines of inbred Wt mice; 87V scrapie infection of VM mice induced the largest amounts of p-tau in the cerebral cortex, deposited in the same areas containing PrP-amyloid. In a time course study, PrP^{TSE} deposits in the brain preceded the appearance of p-tau in both animal models. Others have reported similar results²⁶.

Over expression of mutant PrP and neurodegeneration in mouse brains was not accompanied by accumulation of p-tau

Mice overexpressing PrP-P101L (GSS-22 mice)²⁷ have spontaneously developed severe spongiform degeneration accompanied by large numbers of PrP amyloid plaques and widespread gliosis in the brain. Although GSS-22 mice expressed a neurological disease with all the hallmarks of a spongiform encephalopathy, suspensions of their brains have not transmitted prion disease to either Wt or 101LL mice and therefore appear to be devoid of infectivity. The GSS-22 mice provide a model to study possible correlations between PrP aggregates and p-tau in animals that express mutant PrP throughout their lifespan but are not exposed to the trauma of intracerebral inoculation and do not develop a transmissible disease. We observed that, despite the severe spongiform

degeneration and accumulation of large PrP plaques in their brains at terminal disease, these animals had almost no p-tau in any brain areas (Table). Confocal microscopy confirmed this unexpected finding. Similar results were obtained in sections probed with either of two antibodies to p-tau: AT8 and Thr231. In short, we observed almost no p-tau in any brain areas at terminal disease.

Brains of animals inoculated with recombinant PrP accumulated PrP-amyloid without detectable p-tau

To develop a model of seeded PrP proteinopathy, we inoculated synthetic PrP amyloid fibrils into *PRNP*-knock-in mice homozygous for a proline-to-leucine mutation at PrP codon 101 (101LL mice); the amyloid fibrils were composed of either wild-type recombinant PrP (recWt-PrP) or mutant recombinant PrP (rec101LL-PrP)³³. The inoculated 101LL mice developed no overt signs of prion disease, no histopathological spongiform degeneration of the brain, and had no replication of infectivity as evidenced by failure to transmit any illness (overt or histopathological) to 101LL mice; the inoculated 101LL mice did, however, accumulate large PrP amyloid plaques in and adjacent to the area of inoculation (in corpus callosum and hippocampus), but no p-tau was detectable by immunolabeling with anti-tau antibodies AT8 and Thr231³¹ (Table). This model is important because it eliminated possible artifacts resulting from (i) overexpression of PrP (since PrP expression levels were normal), (ii) pathology resulting from a replicating infectious agent (because there was no demonstrable prion-related infectivity), or (iii) damage from spongiform encephalopathy (which was absent). In addition, because we injected only synthetic recombinant PrP, there could be no effect—enhancing or inhibiting—on formation of p-tau by any of the other molecules that inevitably “contaminate” extracts of human or animal brains. In short, although we found that p-tau co-localized with PrP^{TSE} amyloid plaques in the brains of 87V-VM mice infected with a scrapie agent absence of p-tau in the brains of 101LL mice inoculated with recombinant PrP fibrils shows that PrP amyloidogenesis alone does not invariably lead to formation of p-tau in the brain.

Natural tauopathy in ruminants

Idiopathic brainstem neuronal chromatolysis (IBNC) is a neurological disease first recognized in 1986 in the UK among some adult cattle slaughtered because of suspected BSE¹⁸. The clinical presentation of IBNC includes behavioral and locomotor changes¹⁸. This disorder is characterized neuropathologically by neuronal chromatolysis and degeneration in the brainstem plus hippocampal sclerosis and spongiform changes in the gray matter of the cerebrum¹⁸. Investigations of possible causes of IBNC included testing for viruses and for deficiencies in vitamins, trace elements and minerals; all results were negative. Abnormal PrP was detected in the brains of several IBNC cases¹⁸; however, IBNC was not

successfully transmitted to transgenic mice expressing a bovine PrP gene¹⁸. Recently, we performed an immunohistochemical analysis of brains from 15 bovines with IBNC, looking for accumulations of several important proteins³⁴. Brain sections from all animals, probed with six different anti-tau antibodies directed against hyperphosphorylated serine or threonine phospho-epitopes, showed positive staining of astroglia in gray and white matter, dendrites and neuronal cell bodies (Table). The most intense p-tau staining was seen in cases with severe spongiform degeneration; however, p-tau was not observed in chromatolytic neurons. P-tau was detected in the cerebrum, gray matter nuclei of the neuroaxis, and cerebellum. P-tau-positive structures also contained ubiquitin and α -synuclein but not A β peptide. Alpha-synuclein labeling was conspicuous in the dentate gyrus and surrounding dendrites of the substantia nigra, globus pallidus and entorhinal cortex. It has been proposed that the pattern of α -synuclein accumulation in IBNC resembles that occurring secondary to synaptic loss in human brains, rather than resulting from a primary accumulation of abnormal α -synuclein³⁴. Punctate staining of ubiquitin was also seen, mostly in the cerebral cortex, hippocampus, thalamus, white matter and brain stem, largely in the same areas with hyperphosphorylated tau deposits. The brains of age-matched control cattle were negative when stained with antibodies against all the proteins studied. In conclusion, the neuropathology of IBNC did not correspond to any complex proteinopathy described in humans. While the etiology of IBNC remains unknown, results of epidemiological and experimental studies so far suggest that it is unlikely to result from an infection. Environmental factors, including exposure of cattle to agricultural chemicals, should be investigated.

Conclusion

In the experimental models described above (SQ-BSE, B6-BSE, ME7/C57, 87V/VM) complex protein aggregates containing both PrP^{TSE} and p-tau were evident when disease was transmitted to susceptible recipient animals and serially passaged (i.e., was confirmed to have an infectious mechanism). In contrast, in the other animal models, p-tau was never observed in animal brains containing misfolded PrP when no infectious agent was detected (rec101LL, rec101Wt). Therefore, it is evident that p-tau accumulated only in brains containing both misfolded PrP and infectivity and not in brains with misfolded PrP alone. One possible explanation is that replication of infectivity plus host factors together elicit the formation, deposition and aggregation of heterogeneous protein, including p-tau, in the brain.

In aged cattle, IBNC is characterized by widespread deposits of p-tau in most areas of the brain, the rare example of a naturally-occurring tauopathy in a nonprimate species. Idiopathic brainstem neuronal chromatolysis is the first tauopathy of ruminants in which α -synuclein and ubiquitin also accumulate. While the etiology of IBNC is unknown,

its occurrence in several breeds of cattle argues against a genetic etiology. This disease has not been transmitted experimentally (M. Stack, personal communication)³⁴, leading to a preliminary conclusion that environmental exposures are more likely than infection to cause IBNC.

Several age-related neurodegenerative diseases are associated with the accumulation of specific proteins in the nervous system. Protein aggregates clearly induce the misfolding of similar proteins to form characteristic lesions. The apparent simplicity of this molecular process and consistency in histopathology contrast dramatically with the remarkable clinical-pathological heterogeneity seen in patients with Alzheimer's, Parkinson's, and prion diseases. New data suggest that this variability may be mediated by the formation of diverse protein architectures referred to as "proteopathic strains"³⁵. In turn, such proteopathic strains could be influenced by genetic, cellular/post-translational and environmental factors. We and

others suspect that the genesis of complex proteinopathies may also involve participation of other molecules (e.g., heparan sulfate proteoglycan^{31,36}). Transcellular propagation of protein aggregates has been proposed to mediate neurodegeneration. Glycosaminoglycans are required for cellular uptake of tau, α -synuclein and β -amyloid proteins. Stopschinski et al. recently reported considerable specificity for interaction of aggregated tau protein with glycosaminoglycans, while the interaction of α -synuclein and β -amyloid is less stringent³⁶. These findings might explain some of the phenotypic heterogeneity so characteristic of the histopathology in the brains of humans with age-related neurodegenerative diseases. A better understanding of these factors and their interactions should improve our knowledge of disease processes, lead to development of new diagnostic methods, and even suggest new targets for therapy. Continued study of animal models will support those efforts.

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