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Prions and Lymphocompetency

Pierre Sibille

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A large, light teal watermark of the letters 'INRAE' is visible in the background, partially overlapping the main text.

➤ Prions and lymphocompetency

Determinants for extra-neural prion replication

Pierre Sibille, Concepcion January 2023

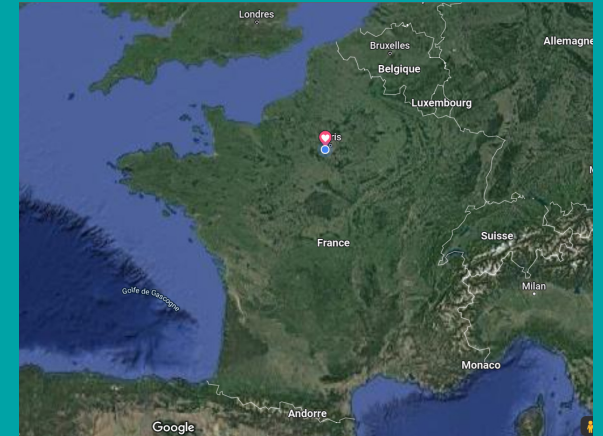
INRAE

➤ French institute for agricultural and alimentary research

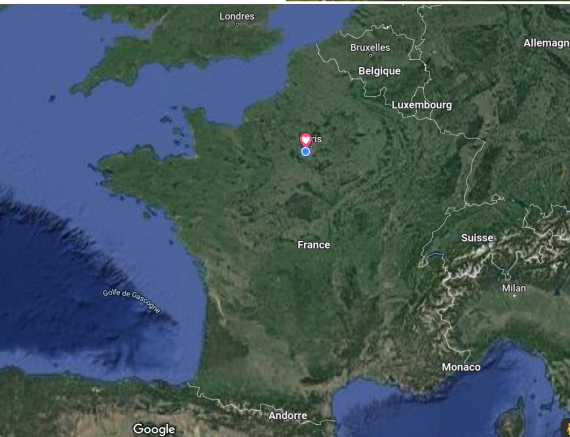
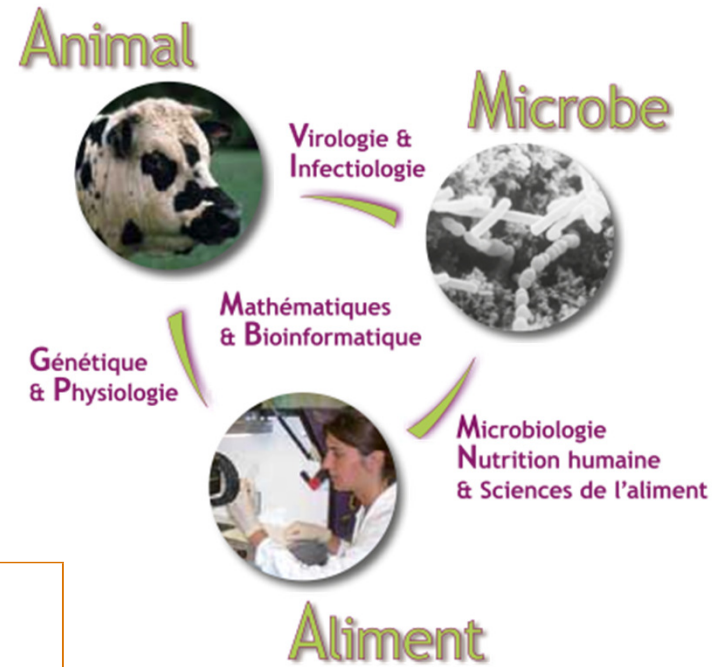
Jouy en Josas, France

DU: Sabine RIFFAULT

DUA: Pierre BOUDINOT



➤ Centre de Recherche Inrae de Jouy en Josas - Antony

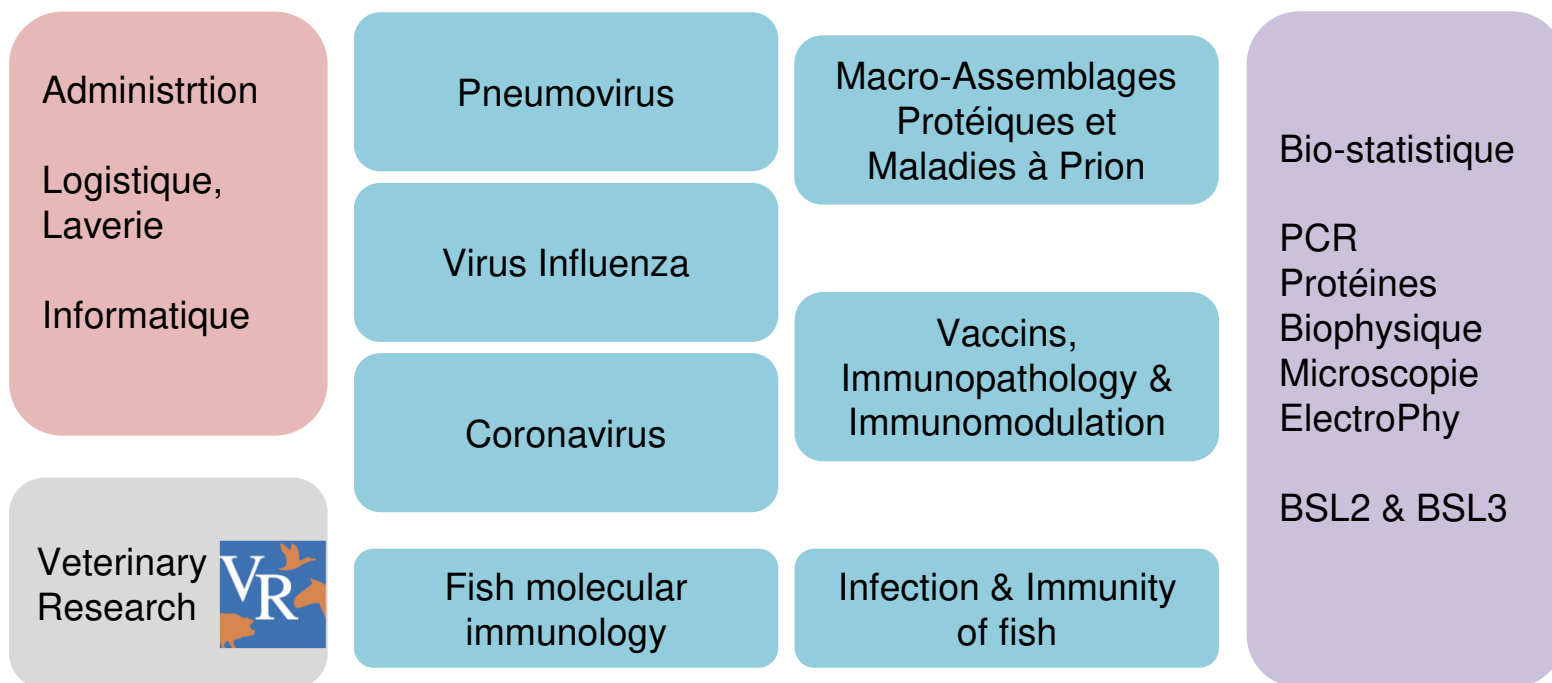


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➤ Organisation de la VIM

7 teams on immunology and infectious diseases of animal / Human



29 scientifiques (22 DR/CR INRAE, 1 CR INSERM, 6 PU-PH/MCU-PH/MC)

27 ITA (INRAE)

2 chercheurs PEPS, 8 chercheurs Post-doc INRAE, 15 doctorants, 2 CDD ITA



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➤ Open questions regarding peripheral prion replication

- What are prion diseases ?
- What is peripheral prion replication ?
 - PrP expression patterns
 - PrP in the immune system
 - Importance of follicular dendritic cells (FDCs)
- Why is peripheral prion replication relevant ?
 - Horizontal transmission : example of CWD in USA
 - adaptation to new host
- What are the determinants for prion lymphoid replication ?
 - Histological study on granuloma: a lymphocompetent model without FDCs ?
 - Strain selectivity and peripheral replication dependent on PrP expression levels



➤ Prion diseases : transmissible spongiform encephalopathies



Neurodegenerative disease

fatal issue

No treatment



Symptoms:

- Behavior disturbance, coordination dysfunction
- **Neuronal loss**, spongiosis, astrocytosis



Etiology:

- Sporadic
- Genetic
- Acquired



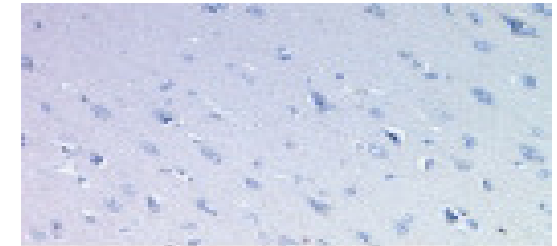
Non-conventional transmissible agent

= prion

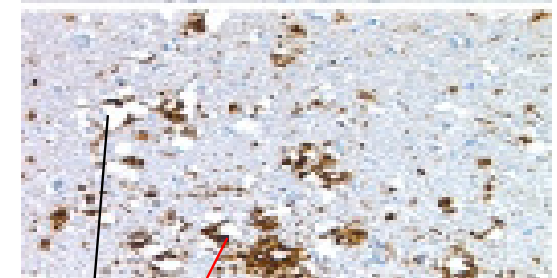


Parenchymal lesion

control



CJD



vacuole

accumulation of
amyloid plaques
composed by the
PrP protein

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➤ Triple determinism of human prion infections

85 % of unknown origin = sporadic 1 -2 cases/million inhabitant (with variations)

15 % genetic (over 30 mutations identified)

familial CJD

Gertsmann Straüssler Scheinker (GSS)

familial fatal insomnia (FFI)

ε % infectious, through iatrogenic, alimentary exposure

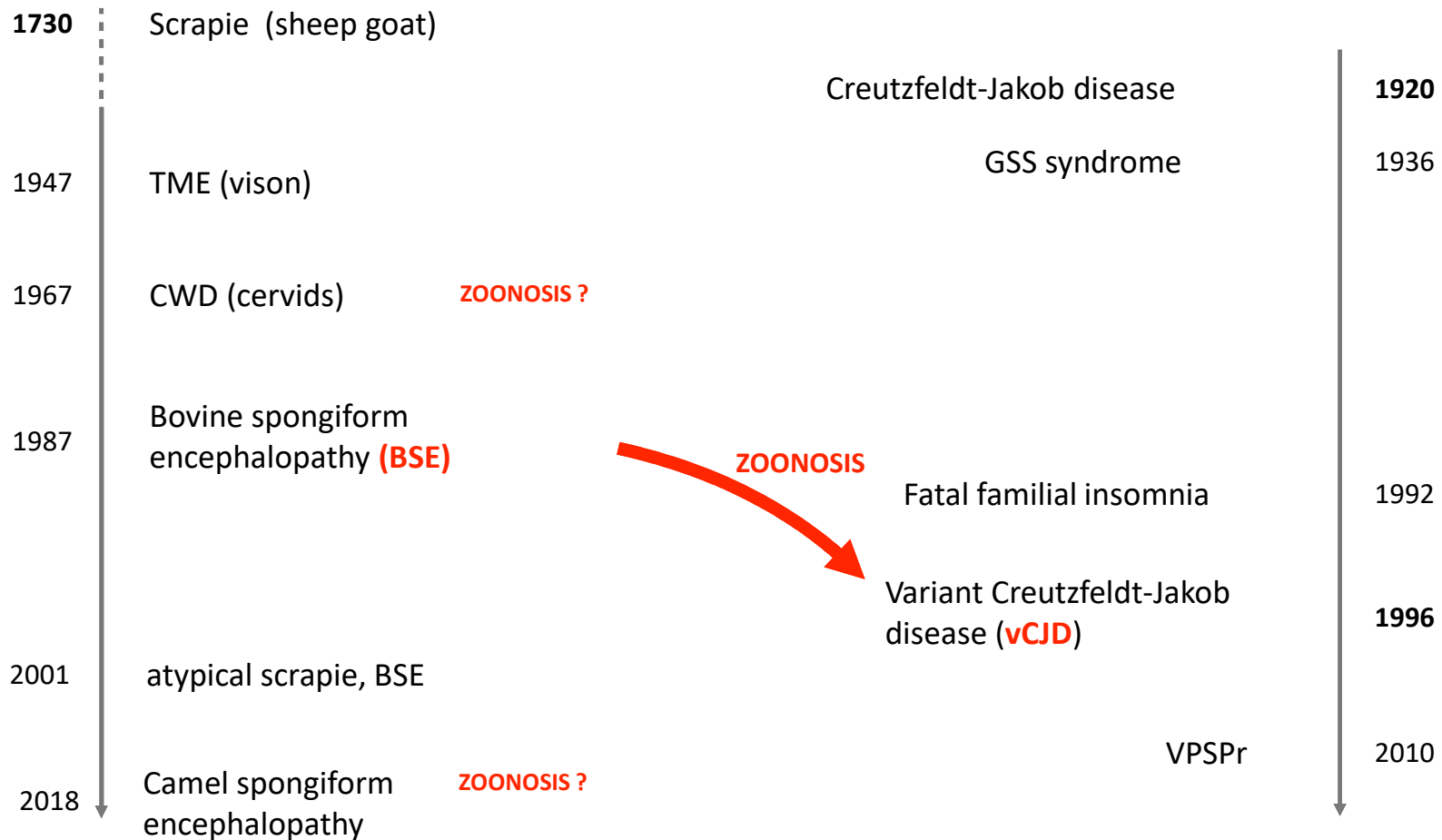
Kuru (1950s. ended after cannibalism rituals have been forbidden)

Variant of Creutzfeldt-Jakob (~ 200 cases)

iatrogenic (growth Hormone, blood transfusion, contaminated electrodes)



➤ Prion diseases in mammals



Animals

Human



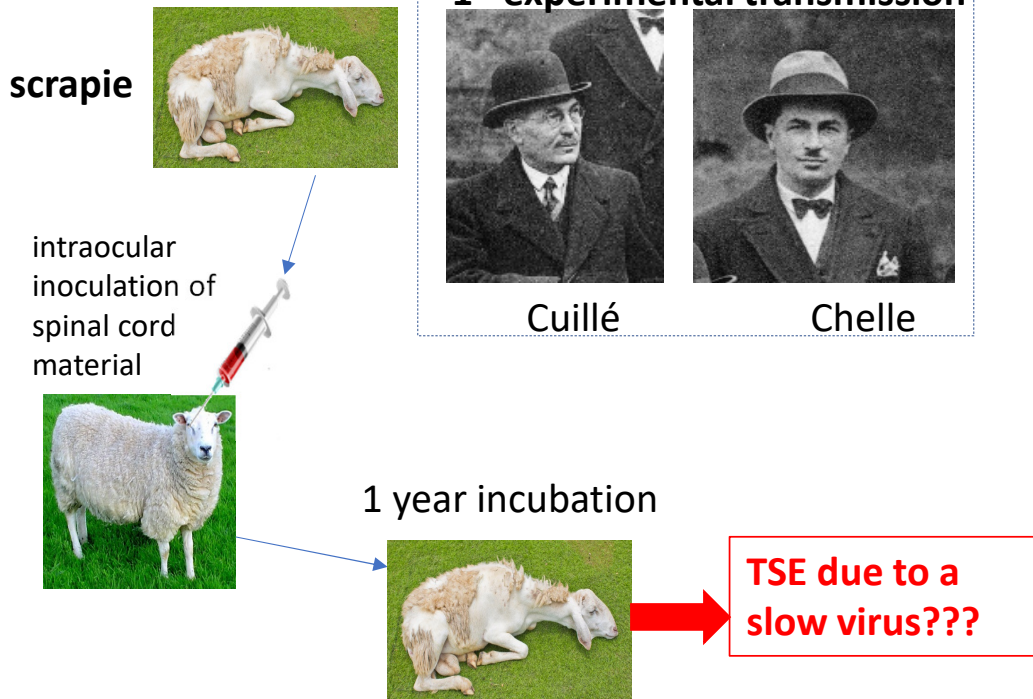
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➤ Prions are infectious

Two separate evidences for the infectious transmission of prions

- In sheep (1936)



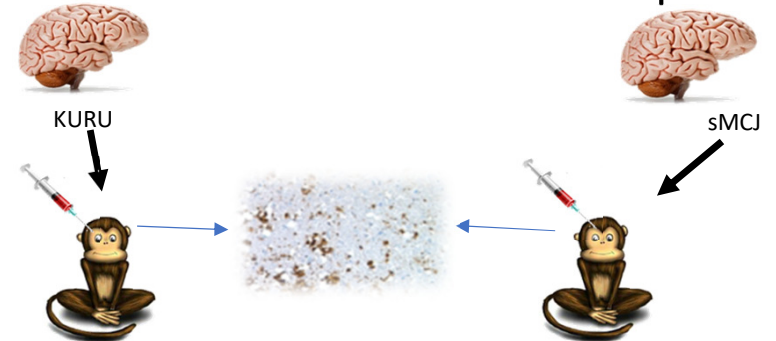
- In primates (1959, 1966)

- Kuru syndrome : ritual cannibalism



Daniel Carlton Gajdusek

- Experimental transmission to chimpanzee



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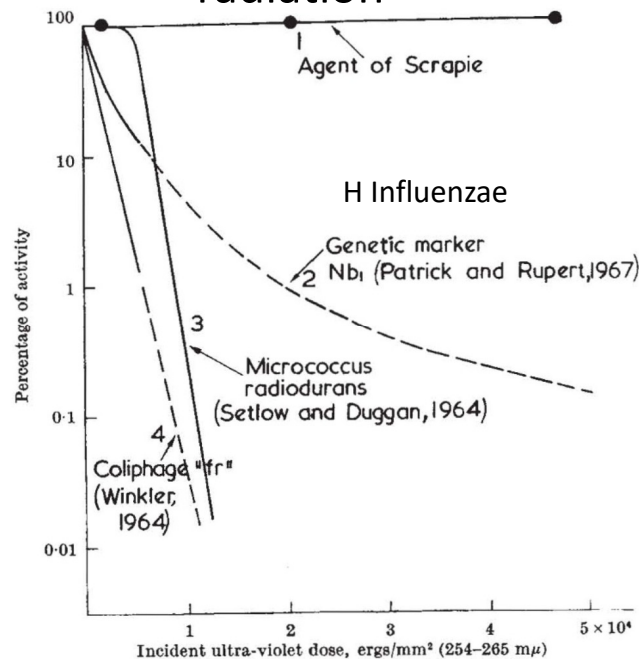
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➤ Prions are... proteins, only

Alper (1967), then Prusiner's seminal results (1982)

• Alper's work

- Scrapie agent is resistant to radiation

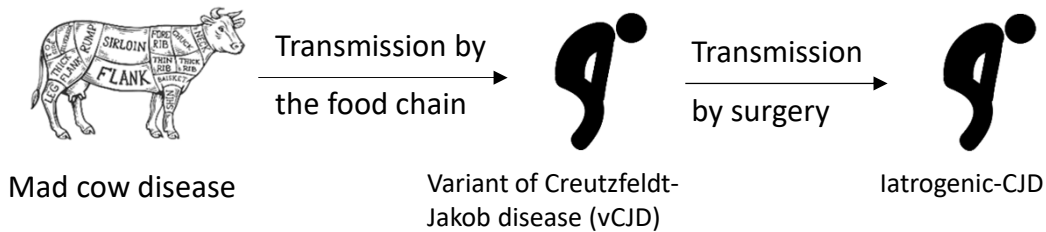


• Prusiner's

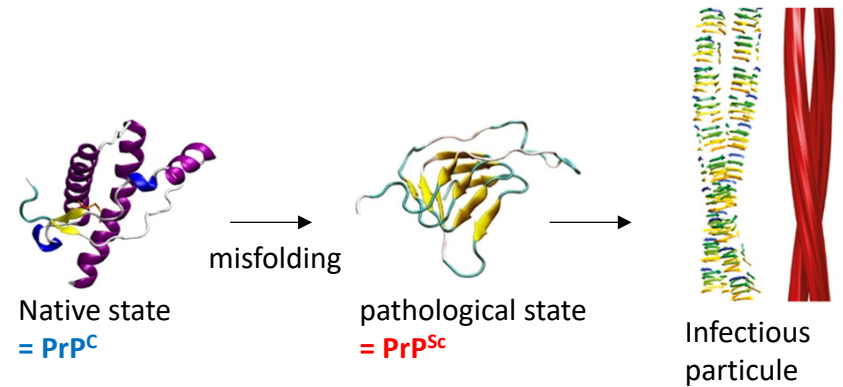
- Scrapie is sensitive to protein denaturing agents (SDS, Phenol, Urea, Guanidine, proteinase K...)
 - Stable in alcohol
 - Resistant to nuclease and ultraviolet
 - Too small to include both coding nucleic acid and protective protein
- ### • ... and definitive confirmation
- Infection with purified recombinant protein

➤ Prions are...

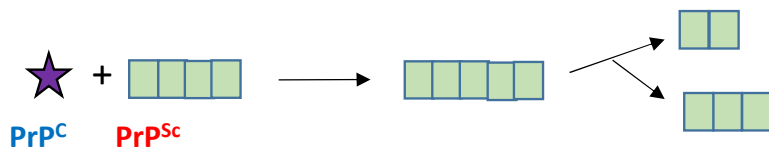
- Prions are **infectious**



- Prions are entirely composed by a “**misfolded form**” of a **naturally produced protein**.



- This misfolding is **auto-replicative**

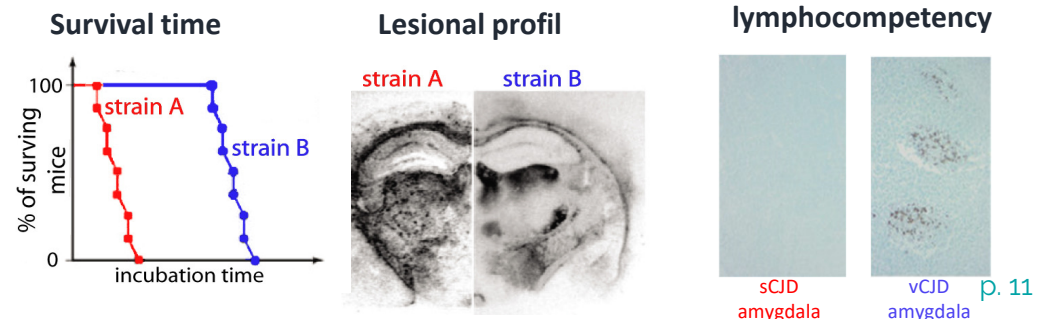


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- Structurally & phenotypically diverse = **strain phenomenon**



➤ Prions in the periphery ?



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➤ PrP peripheral expression

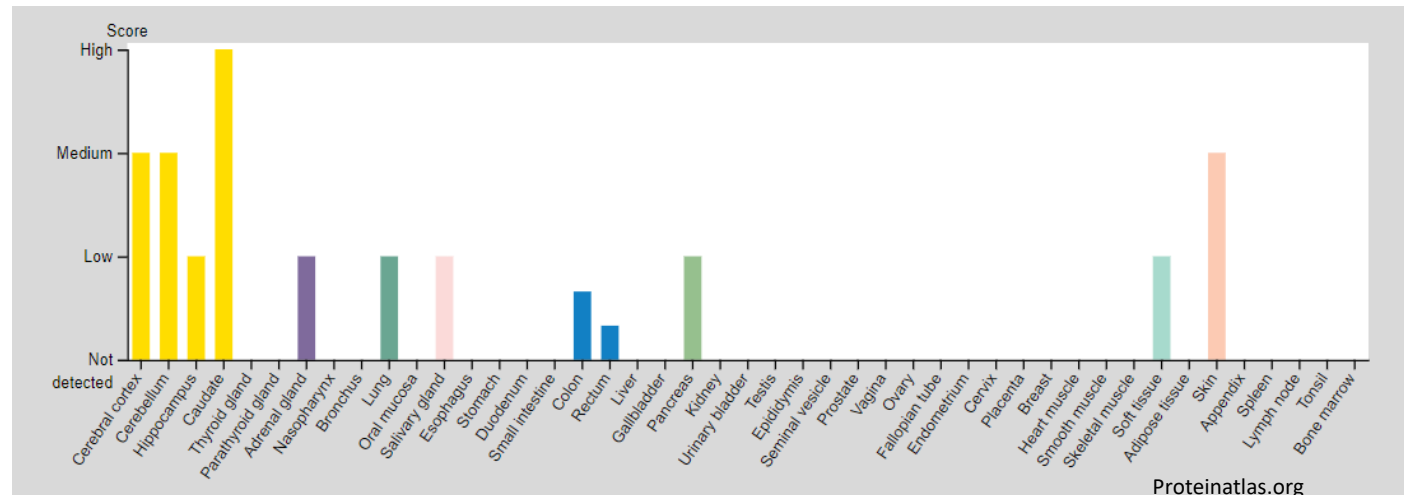
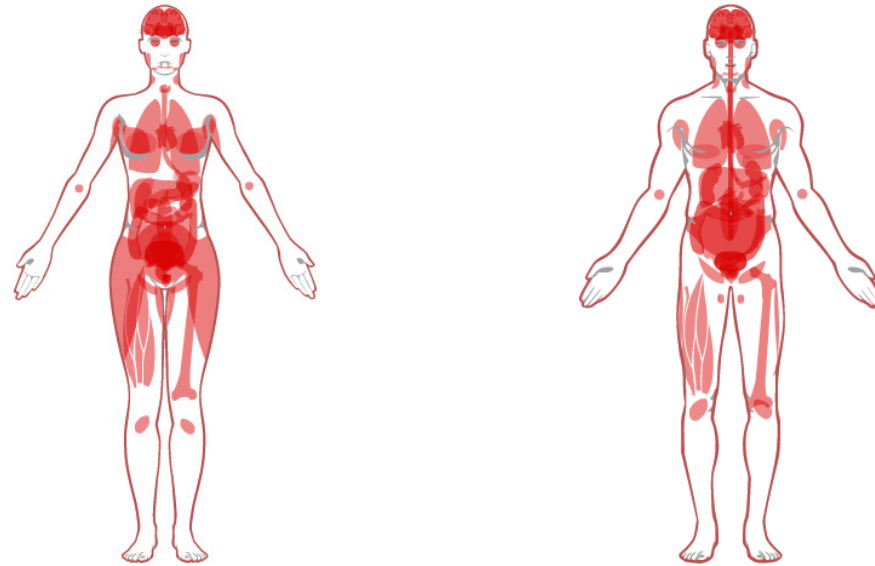
Prp expression is ubiquitous, but

MAINLY in

- Brain
- Spinal cord
- skin

Much lower amounts in :

- Lymph nodes
- Spleen
- Muscle (heart, uterus)
- ...



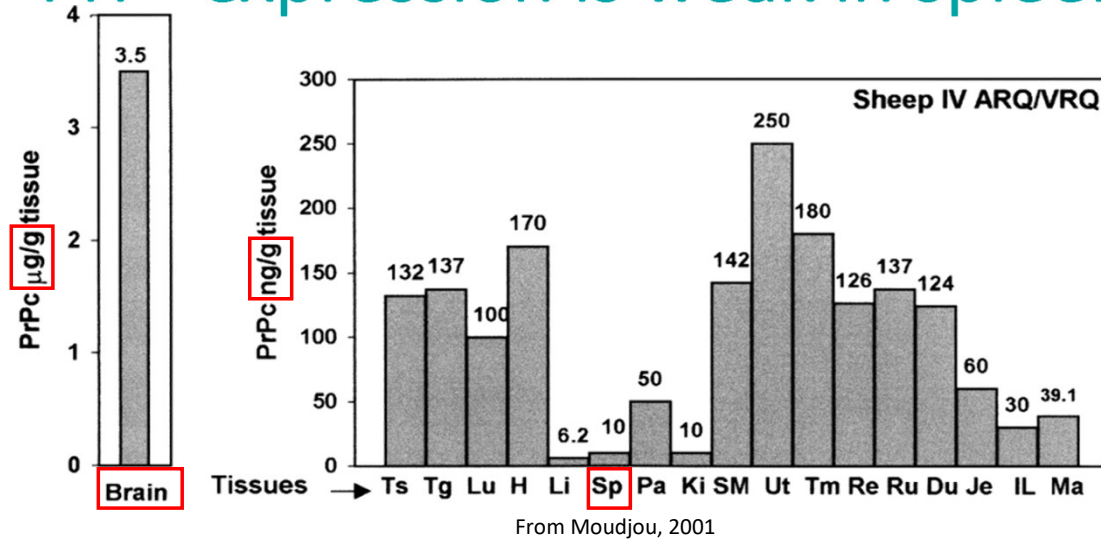
Proteinatlas.org



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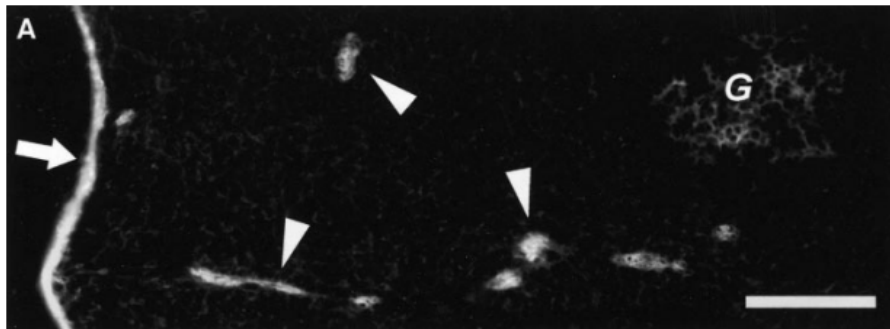
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➤ PrP^C expression is weak in spleen...

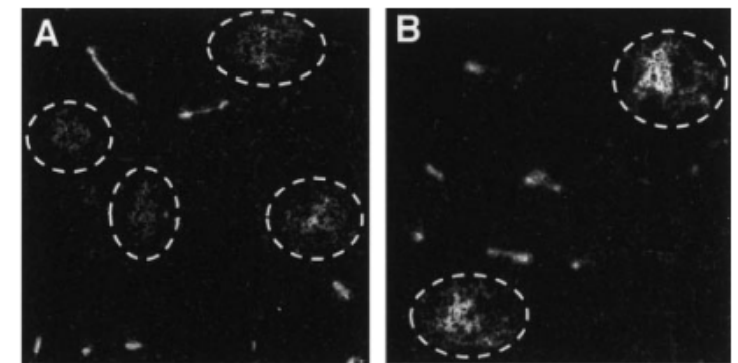


- ... mostly located in the capsule and trabeculae...

... and inducible



Spleen capsule (arrow) and trabecula (arrowhead), Lötcher 2003

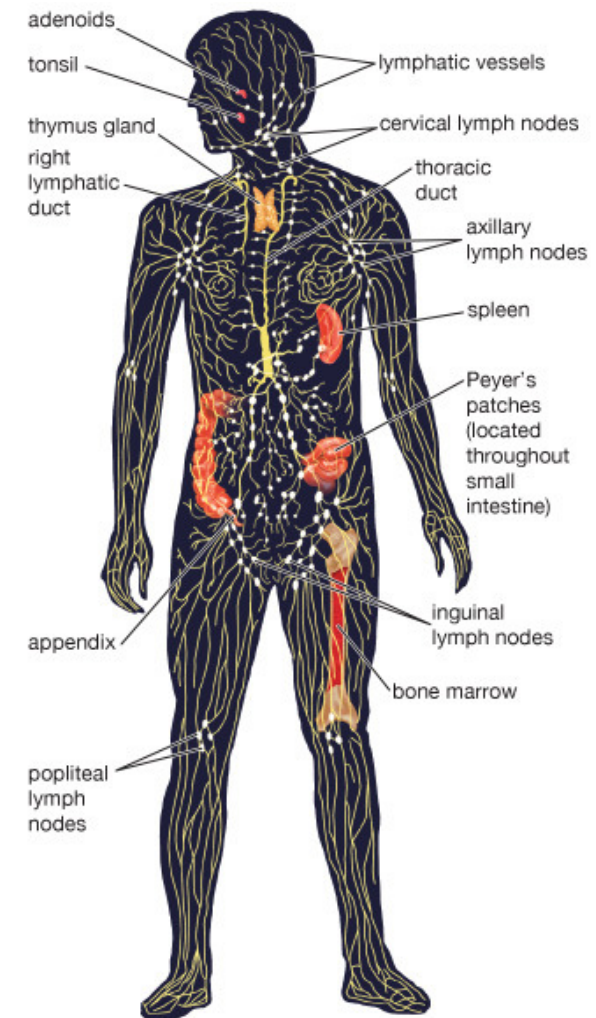


Naive

IC-treated

➤ Lymphoid system is...

- ... a tissue network in charge with immune functions
 - Production of immune cells : primary lymphoid organs
 - bone marrow
 - thymus
 - Education and amplification of specific immune response in secondary lymphoid organs
 - Lymph nodes
 - Peyer's patches
 - Spleen...
- ... an extraneural site for early and efficient prion amplification



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➤ Natural entry sites for prions

Apart from the experimental / iatrogenic intracerebral route, prions can find their way to CNS through

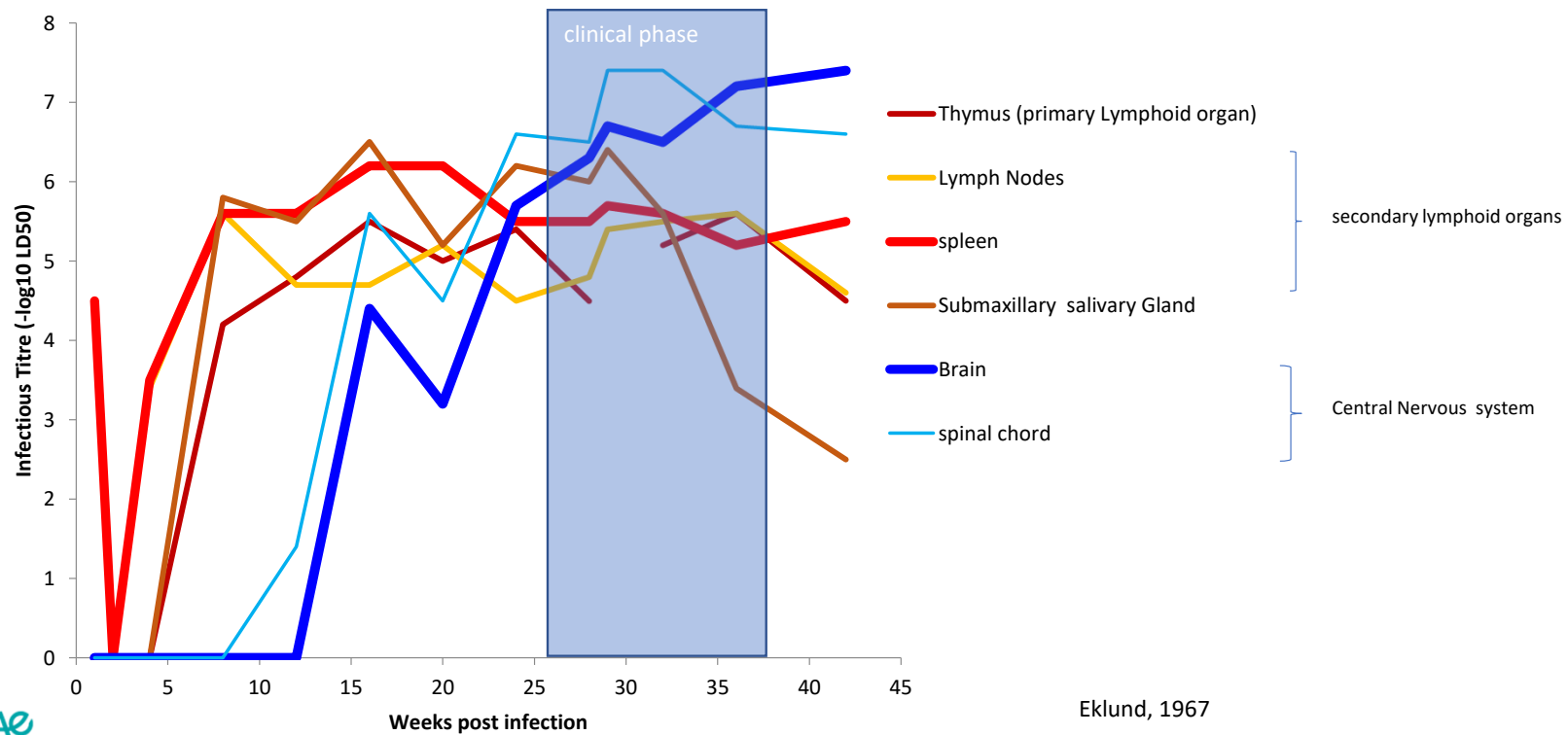
- Oral route
 - Kuru
 - Mad cow outbreak
 - Oral and olfactory mucosa
 - Incubation time equivalent to peritoneal route (>500 days vs 170 with ic)
 - Role of the reticular lymphoid system (with role of FDCs, Bessen 2009)
- CWD inoculation results in Tg(cerPrP) mice (Denkers 2010)

Route	Exposure dose	Attack rate*
Aerosol	24 µg†	6/7 (86%)
IN	100 µg‡	2/9 (22%)
Oral	100 µg§	0/10 (0%)

- Skin scarification and more generally skin or mucosal lesions
 - (Taylor 1996)
 - Dependency upon FDCs but not Langerhans cells (Mohan 2004, 2005)
- Eye (conjunctiva)
 - Scott 1993
 - Attack rate: 50%, Incubations times similar to intraperitoneal inoculation



➤ Precocious accumulation of PrP^{Sc} in the lymphoid tissue



Eklund, 1967



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➤ Splenic prion replication and barrier species transmission

When the spleens turn out to be more permissive to allotypic prion than the brain

- BSE transmitted to « homotypic animals

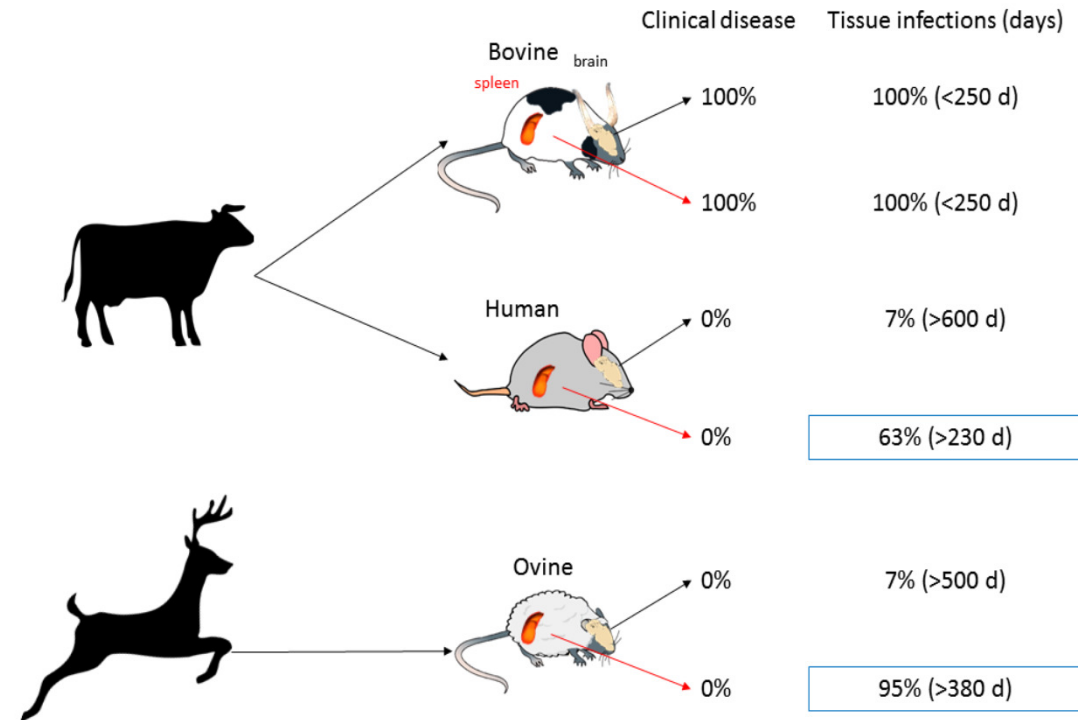
- 100% attack rate
- Spleen and brain positive

- BSE transmitted to humanized mouse

- 0% attack rate
- BUT 63% of positive spleens

- CWD transmitted to ovine mouse

- 0% attack rate
- BUT 95% of positive spleens



Béringue 2012



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➤ Prions in the periphery ?

Requirements & consequences for a natural route

- Prp peripheral expression is
 - low but significant
 - Associated with the immune system (skin, gut, mucosae...)
- Peripheral entry
 - Low to high attack rate
 - Slow pace (as compared to intracerebral route)
 - Not necessarily connected with CNS infection (a question of time ?)
 - Very efficient colonization of the lymphoreticular system
- PrP^{Sc} in the periphery is
 - Detected sooner or in absence of CNS contamination
 - Silent in a large portion (1/2000) of the BSE-exposed UK population
 - Associated with prion mutation / species barrier crossing
 - Associated to environmental contamination with Scrapie / CWD
- Consequences for global health
 - Human health: blood-derived pharmaceuticals, blood transfusion, graft surgery...
 - Food & environment safety



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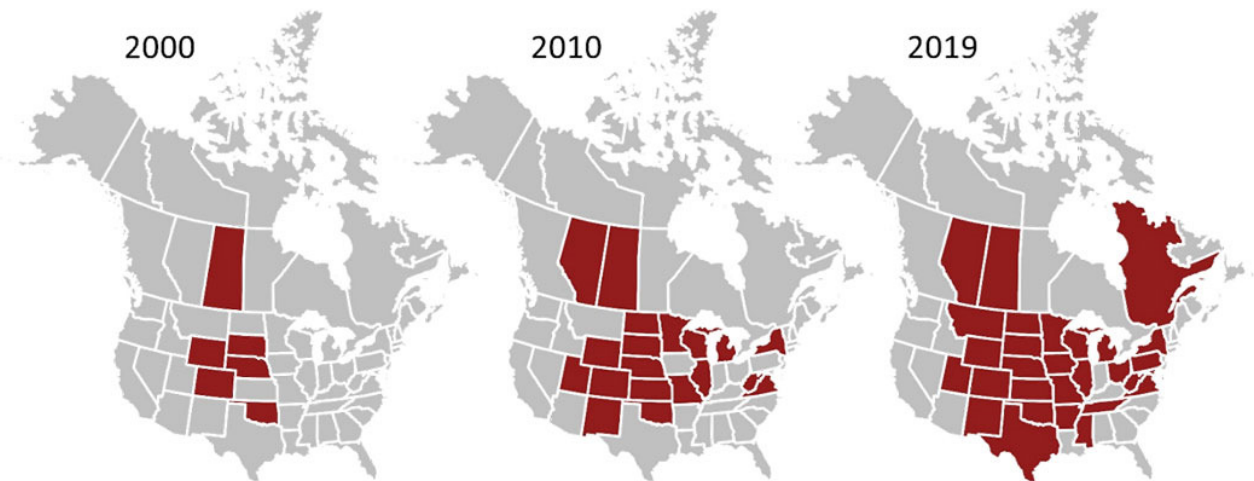
➤ Peripheral exposure in Chronic wasting disease

PrP^{Sc} shedding and horizontal contaminations : case of CWD in north America

- CWD: a prion disease affecting wild cervids
- Reported in Colorado, USA in 1967
- Spreading in USA within 30 years
- Detected now in Scandinavia (Benestad, 2016)



Williams, 1980



<https://cwhl.vet.cornell.edu/>

➤ PrP^{Sc} shedding in CWD-infected animals

A source of environmental prion exposure

- Accumulation of infectious prions in body fluids (review Moreno 2018)
 - Saliva,
 - blood,
 - urine
 - feces (Plummer 2017)
 - antlers consumption (Mysterud 2020)
- ... and durable contamination of environmental reservoirs
 - Water
 - Forage
 - Mineral licks (Plummer 2018), clay (revault 2005)
 - All (but brass) anthropic materials: wood, concrete, steel, plastic, glass...



➤ What is a lymphotropic strain ?

Existence lymphotropic- and exclusively neurotropic-prion strains

- Similar prion levels in
 - Nerves
 - Muscles
 - Enteric nervous system
- Marked contrast in
 - Spleen
 - Tonsils
 - Lymph nodes
 - Peyer's patches

Agent	Lymphoid system				Peripheral nervous system			
	Spleen	Tonsils	LN	Intestine (PP)	Nerves	Muscle	Adrenal gland	Intestine (ENS)
BSE	+++	+++	+++	++	++	+	++	+
vCJD	+++	+++	+++	++	++	+	+	+
sCJD	+	-	-	-	++	+	++	+
iCJD	ND	+	-	-	++	+	+	+

Herzog 2005

- Extrinsic or intrinsic features of the lymphotropic prion strains ?
 - Expression level is 100 times less in lymphoid organs as compared with the CNS
 - Presence of follicular dendritic cells in the lymphoid organs

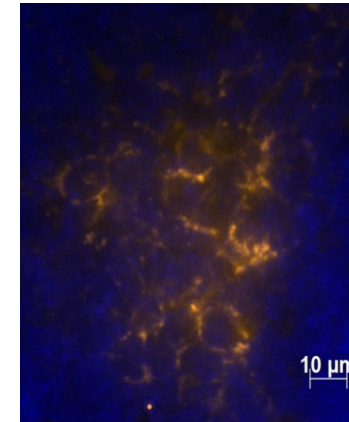


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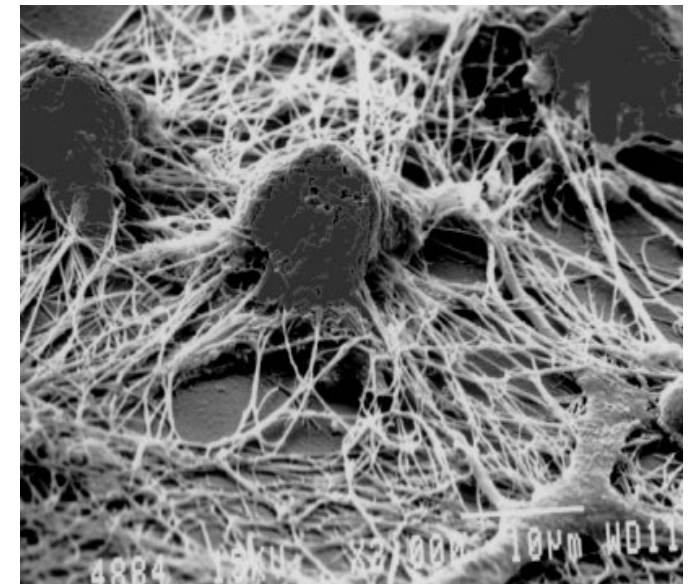
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➤ Focus on Follicular dendritic cells (FDCs)

- Stromal cells
- Keystone of the germinal center architecture



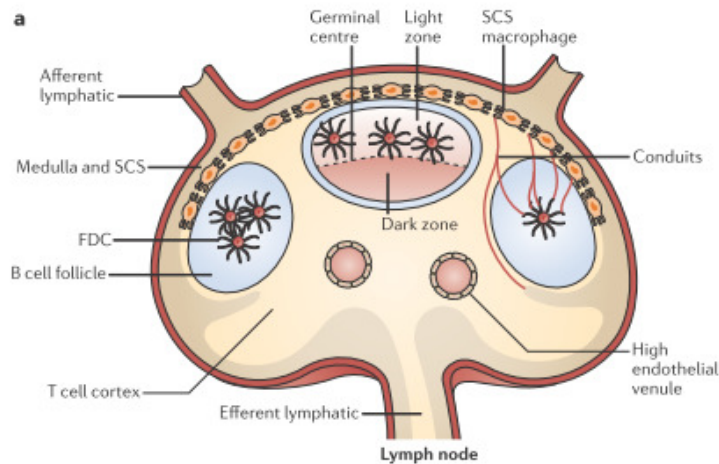
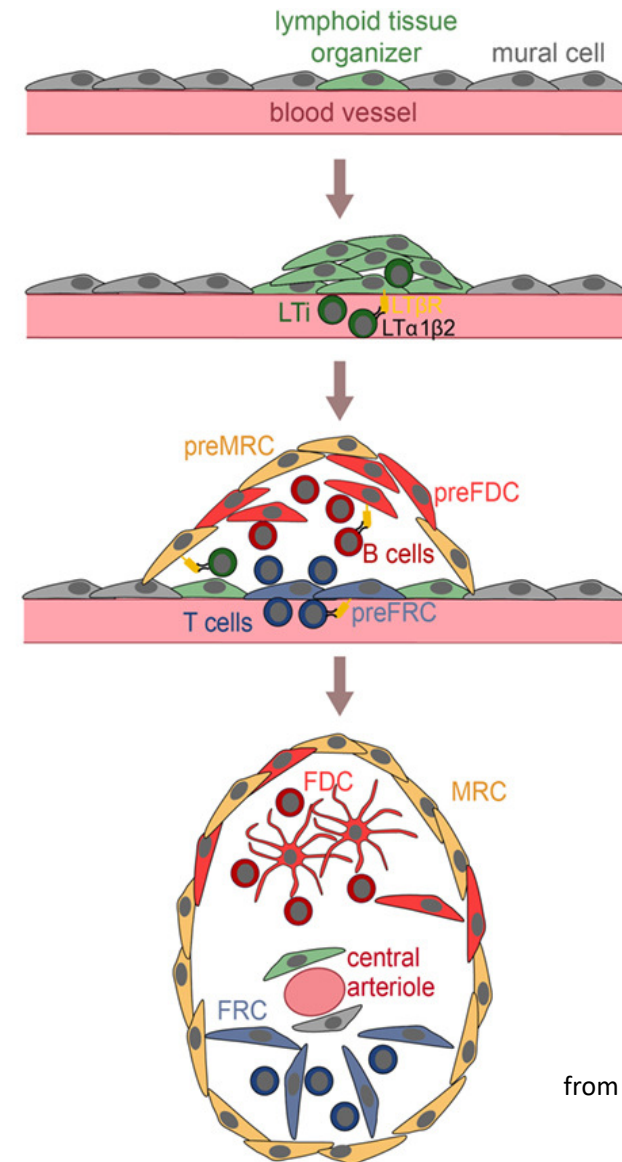
Spleen X40- mfg8 labeling



➤ Stromal origin of FDC

originate from vascular pericytes

- Pericytes are mural vascular cells
- Trophic and shaping role : blood flow maintenance and homeostasis
- Endothelium precursor for remodelling of the vasculature
- Contribute to the formation of skeletal muscle
- Can differentiate into neural cells (Karow 2021)
- DIFFERENTIATE into FOLLICULAR DENDRITIC CELLS (FDC)



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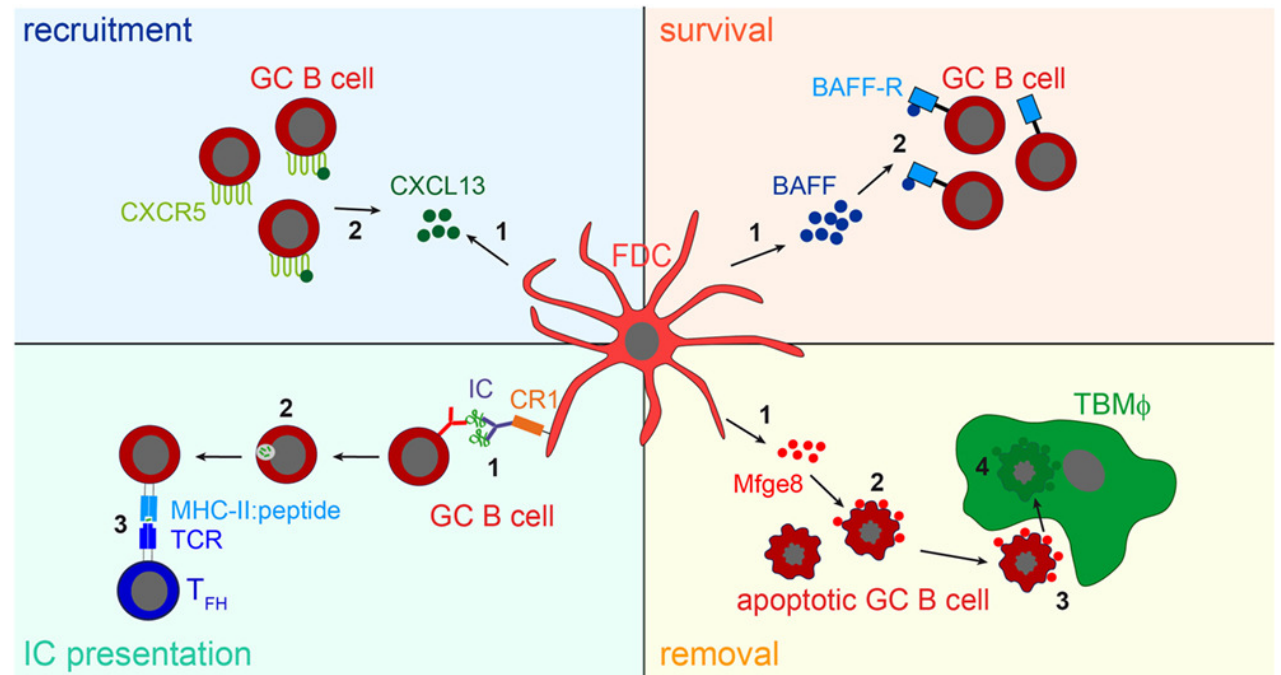
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from Kranich Front. Immunol.
2016

p. 24

➤ FDC: Keystone of the germinal center architecture

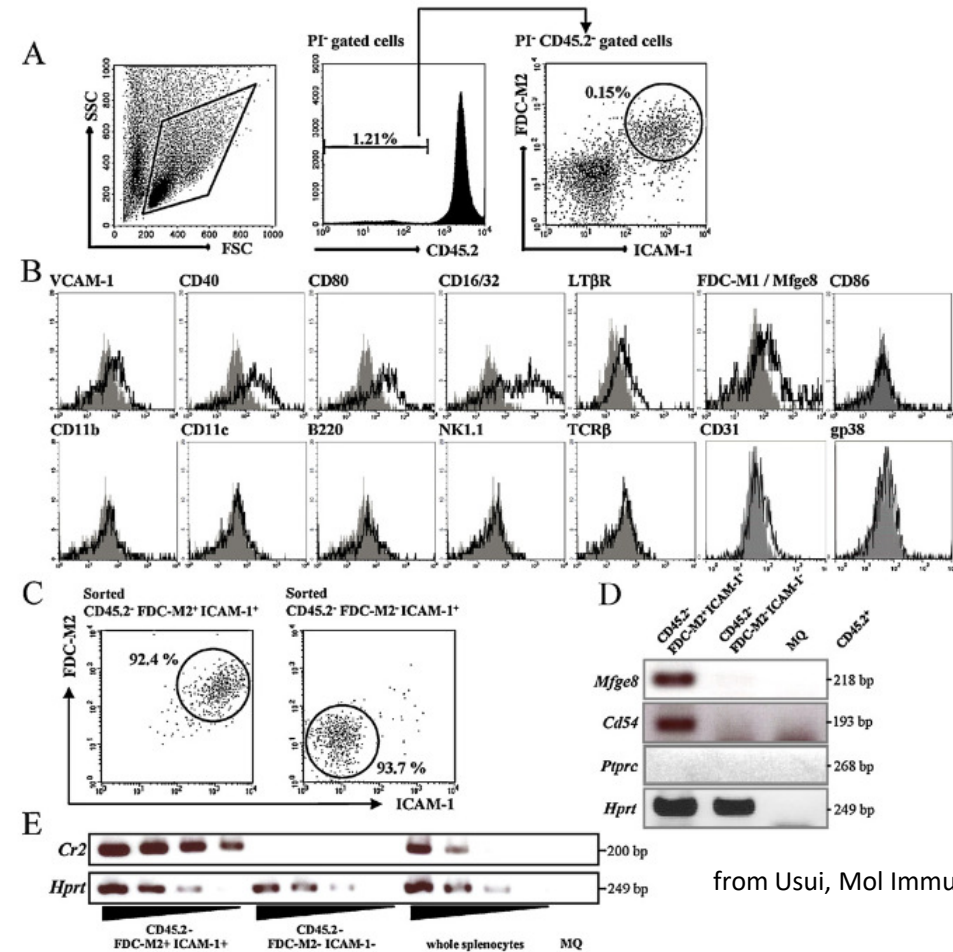
- Essential role in germinal center formation/maintenance (Prinz, 2002)
- Education/activation of reactive B Cells (Kosko-Vilbois, 2003, Kranich 2008)
 - Recruitment of pre-B Cells
 - affinity maturation and survival
 - clonal selection of B cells or
 - Apoptotic removal of unwanted B cells



from Kranich Front. Immunol. 2016

➤ Consequences for Follicular Dendritic Cells studies

- Abundance is less than 0.2%
- No monospecific cellular marker
 - FDC purification uneasy
- Trophic interdependence between B cells and FDCs
 - Depletion, KO tricky to perform



from Usui, Mol Immunol 2012

➤ Peripheral replication of prions in immune system is associated to FDCs

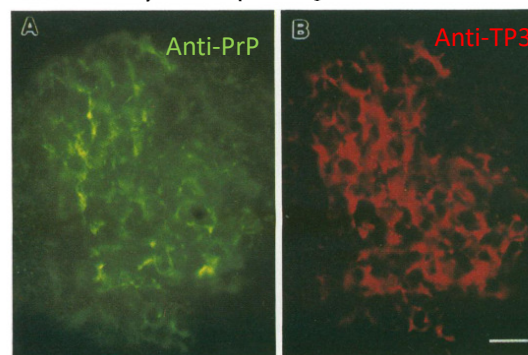
A long journey, fiercely debated...

Abnormal Isoform of Prion Protein Accumulates in Follicular Dendritic Cells in Mice with Creutzfeldt-Jakob Disease

TETSUYUKI KITAMOTO,^{1*} TAMAKI MURAMOTO,¹ SHIROU MOHRI,² KATSUMI DOH-URA,¹ AND JUN TATEISHI¹

Department of Neuropathology¹ and Laboratory Animal Center,² Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

Received 28 May 1991/Accepted 5 August 1991



Lymph nodal prion replication and neuroinvasion in mice devoid of follicular dendritic cells

Marco Prinz^{*,†}, Fabio Montrasio^{*,†}, Michael A. Klein^{*}, Petra Schwarz^{*}, Josef Priller^{*,†}, Bernhard Odermatt[‡], Klaus Pfeffer[§], and Adriano Aguzzi[¶]

^{*}Institute of Neuropathology, University Hospital of Zurich, Schmelzbergstrasse 12, CH-80 University (Charité) Berlin, Schumannstrasse 20/21, D-10098 Berlin, Germany; [†]Laboratory University Hospital of Zurich, Schmelzbergstrasse 12, CH-8091 Zurich, Switzerland; and [¶]in Technical University of Munich, Trogerstrasse 9, D-81675 Munich, Germany

Communicated by Charles Weissmann, Imperial College of Science, Technology, and Medi (received for review October 2, 2001)

Positioning of follicular dendritic cells within the spleen controls prion neuroinvasion

Marco Prinz^{1,*,†}, Mathias Heikenwalder^{1,*,†}, Tobias Junz², Petra Schwarz¹, Markus Glatzel¹, Frank L. Heppner¹, Yang-Xin Fu¹, Martin Lipp¹ & Adriano Aguzzi¹

¹Institute of Neuropathology, and ²Institute of Experimental Immunology, University Hospital of Zurich, Schmelzbergstrasse 12, CH-8091 Zurich, Switzerland

³Department of Pathology and Committee on Immunology, The University of Chicago, 5841 S. Maryland, Chicago, Illinois 60637, USA

⁴Department of Molecular Tumor Genetics and Immunogenetics, Max Delbrück Center for Molecular Medicine (MDC), Robert-Rössle-Strasse 10, 13092 Berlin, Germany

*These authors contributed equally to this work

†Present address: Institute of Neuropathology, Georg-August-University Göttingen, Göttingen, Germany

Peripheral infection is the natural route of transmission in most prion diseases¹. Peripheral prion infection is followed by rapid prion replication in lymphoid organs, neuroinvasion² and progressive neurological disease. Both immune cells and nerves are involved in pathogenesis^{3,4}, but the mechanisms of prion transfer from the immune to the nervous system are unknown. Here we show that ablation of the chemokine receptor CXCR5 juxtaposes follicular dendritic cells (FDCs) to major splenic nerves, and accelerates the transfer of intraperitoneally administered prions into the spinal cord. Neuroinvasion velocity correlated exclu-



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➤ FDC-specific PrP^C expression alone is sufficient to sustain prion infection in the spleen

An on/off PrP^C expression model on FDC (Cre-lox system with CD21 promoter gene)

- FDCs readily express PRP^C
 - PrP^C –expressing FDC alone are sufficient to sustain prion replication in the spleen
- FDCs readily amplify prions upon infection
 - Prion replication in the spleen is blocked when PrP^C expression is ablated from FDC

OPEN ACCESS Freely available online

PLoS PATHOGENS

Follicular Dendritic Cell-Specific Prion Protein (PrP^C) Expression Alone Is Sufficient to Sustain Prion Infection in the Spleen (2011)

Laura McCulloch¹, Karen L. Brown¹, Barry M. Bradford¹, John Hopkins¹, Mick Bailey², Klaus Rajewsky³, Jean C. Manson¹, Neil A. Mabbott^{1*}

¹ The Roslin Institute & Royal (Dick) School of Veterinary Sciences, University of Edinburgh, Midlothian, United Kingdom, ² Division of Veterinary Pathology, Infection and Immunity, School of Clinical Veterinary Science, University of Bristol, Avon, United Kingdom, ³ Program in Cellular and Molecular Medicine, Children's Hospital, and Immune Disease Institute, Harvard Medical School, Boston, Massachusetts, United States of America



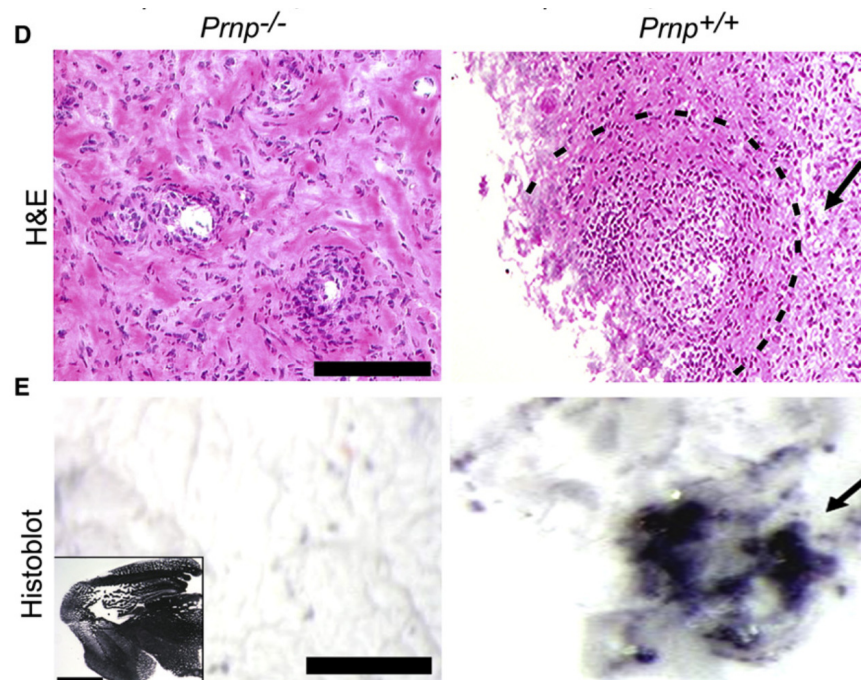
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➤ Do we have peripheral prion replication without FDCs ?

Experimental Inflammatory Granuloma

- Inflammatory granuloma replicate prions without FDCs

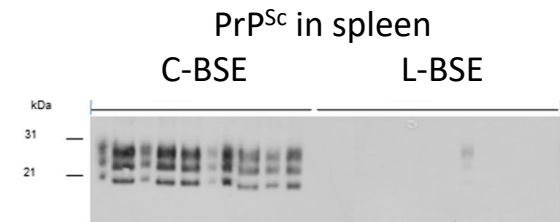
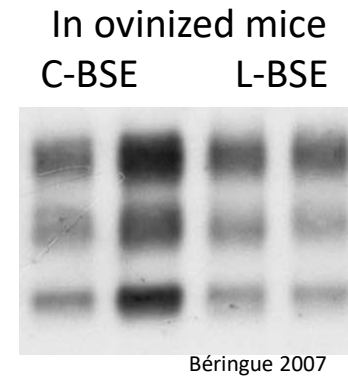


From Heikenwalder 2008

➤ Requirements for prion lymphotropism

Use of the granuloma model in the context of BSE-L or BSE-C prion infections

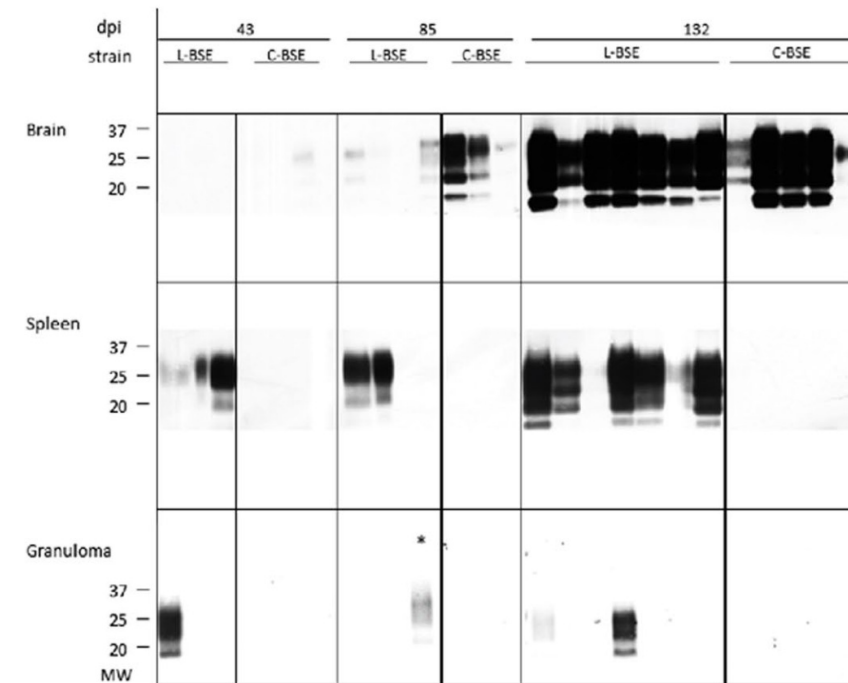
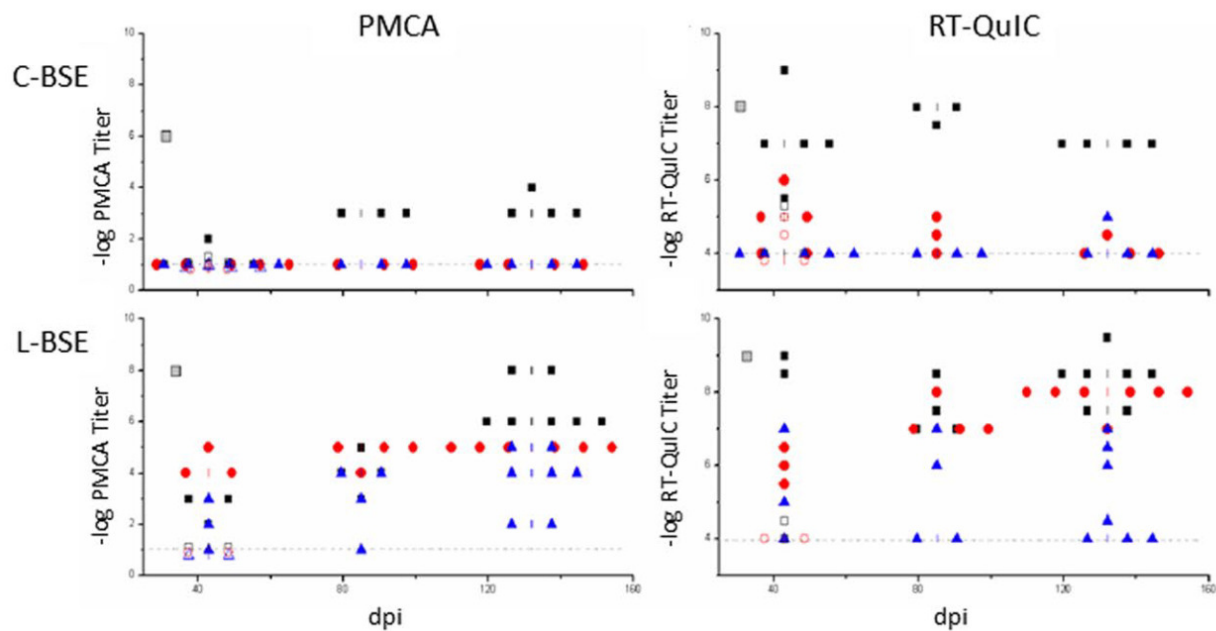
- Two strains of BSE adapted to ovinized mice
 - **Identical** in ovine EXCEPT
 - No Peripheral replication of C-BSE in ovinized mice
- Inoculated in ovinized mice with granuloma



➤ Detection of L-BSE in granuloma

C-BSE not detected

- As for the spleen, granuloma are competent for lymphotropic L-BSE replication, NOT for C-BSE



Al Dybiat 2019

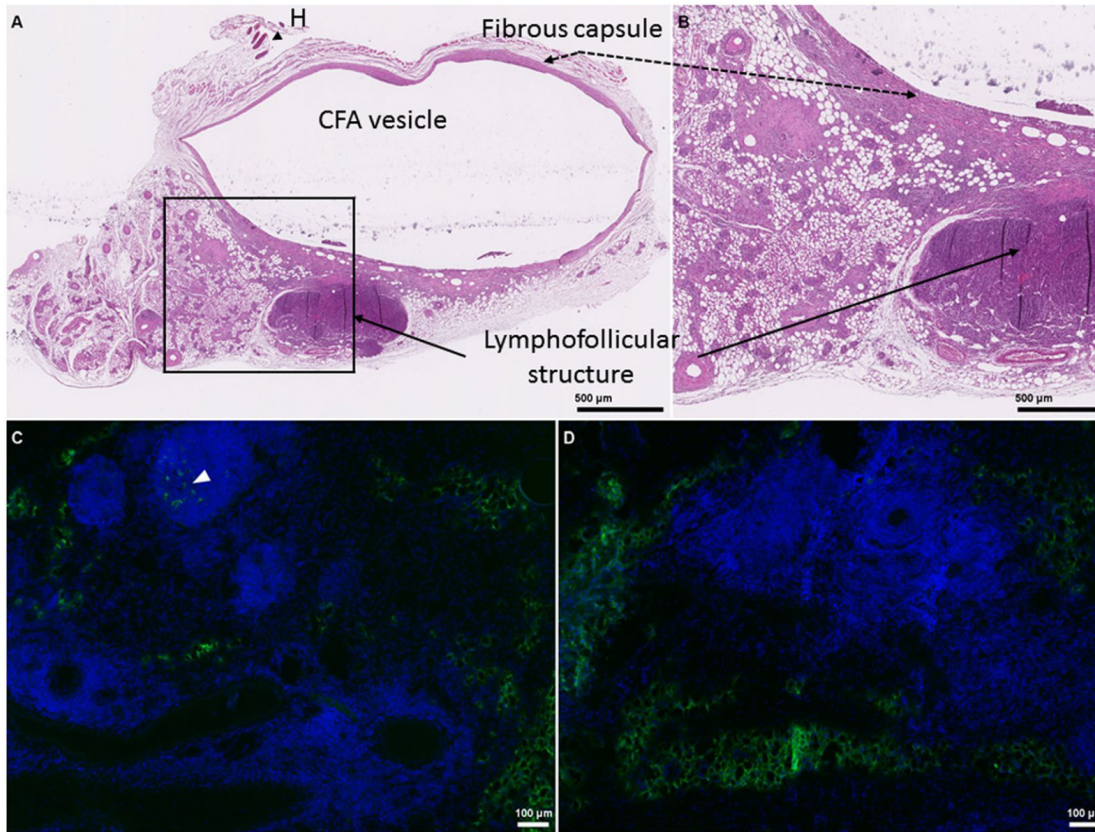


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■ brain
● spleen
▲ granuloma

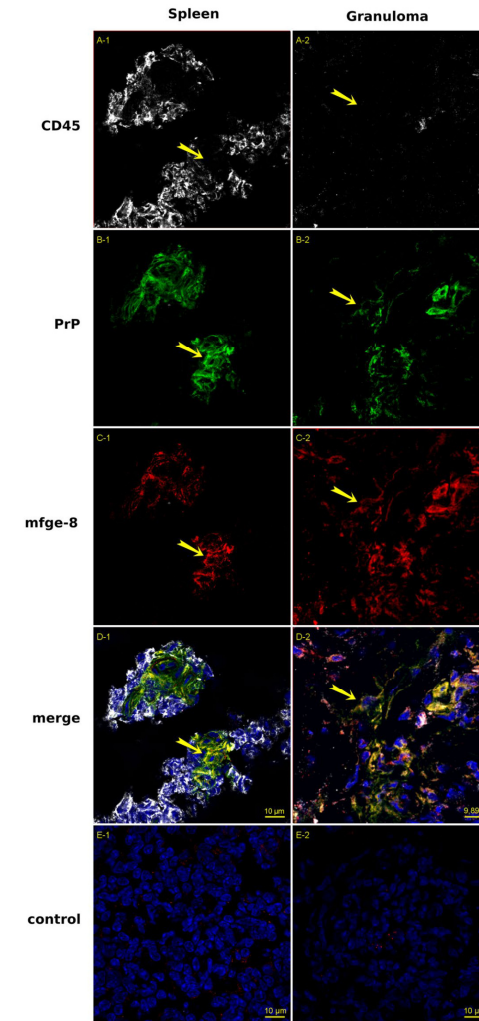
➤ PrP^{Sc} detection is linked to lymphoreticular structures near the granuloma



adjacent lymphoid structures

replicate prions

contain FDCs



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➤ Conclusions

Granuloma are not appropriate for the study of FDC's role in prion lymphoselectivity

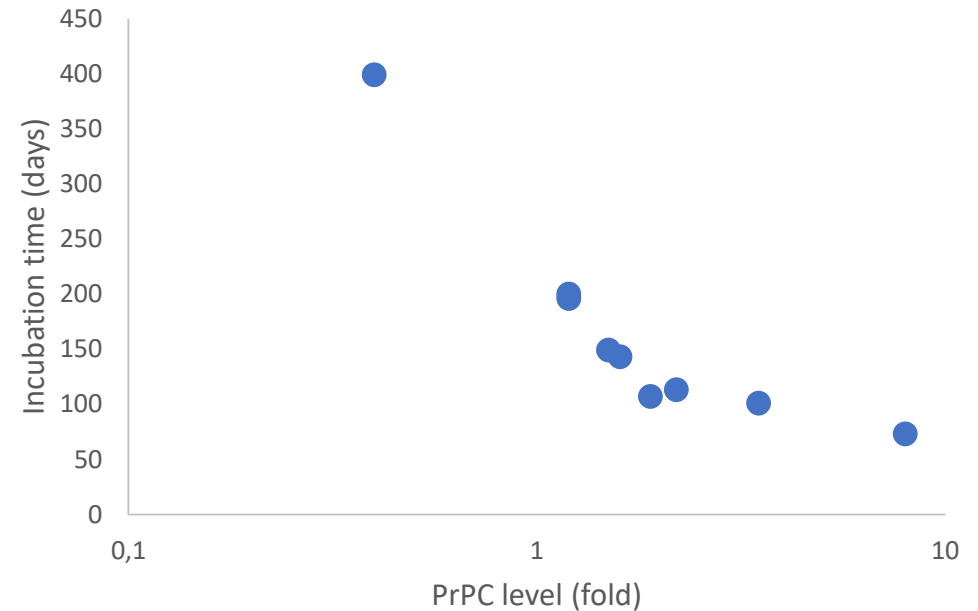
- Granuloma behave like spleens
 - Same selectivity
 - Amplification level is lower
 - Presence of FDCs
- Transient detection of C-BSE in the spleens and granuloma
 - Inoculum detection
 - FDCs probably can trap C-BSE prions but do not replicate



➤ Prion accumulation in relation with PrP expression levels

Marked importance of the PrP expression level in prion infections

- PrP expression level is related to incubation times
 - Essential contribution of mouse transgenesis
 - Several mouse lines with various PrP expression levels
 - The higher levels of PrP, the shortest incubation times

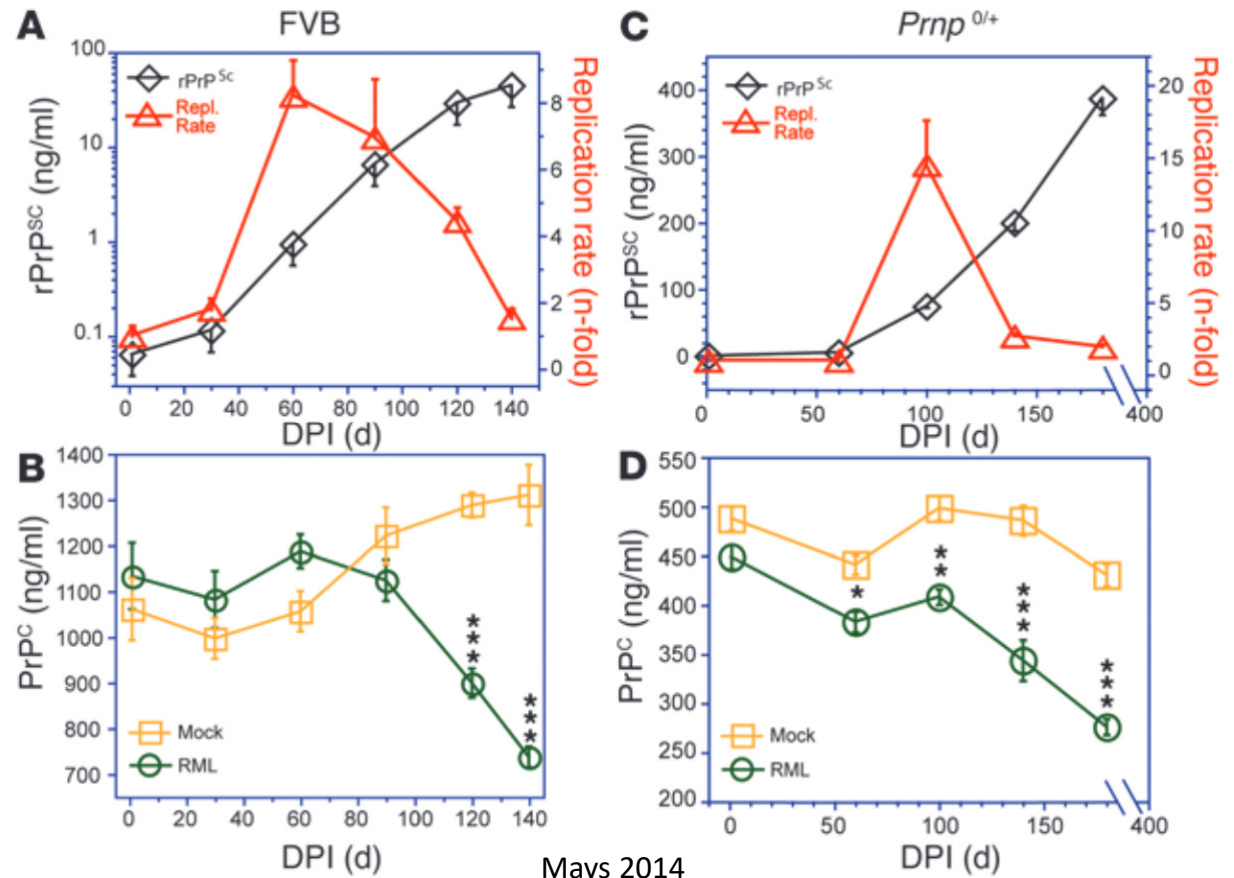


From Vilotte 2001

➤ Prion accumulation in relation with PrP expression levels

Modulation of the PrP expression level during prion infections

- PrP^C expression level and PrP^{Sc} accumulation are modulated by infection
 - Decrease in PrP^C expression
 - Transient increase in PrP^{Sc} production rate



Mays 2014

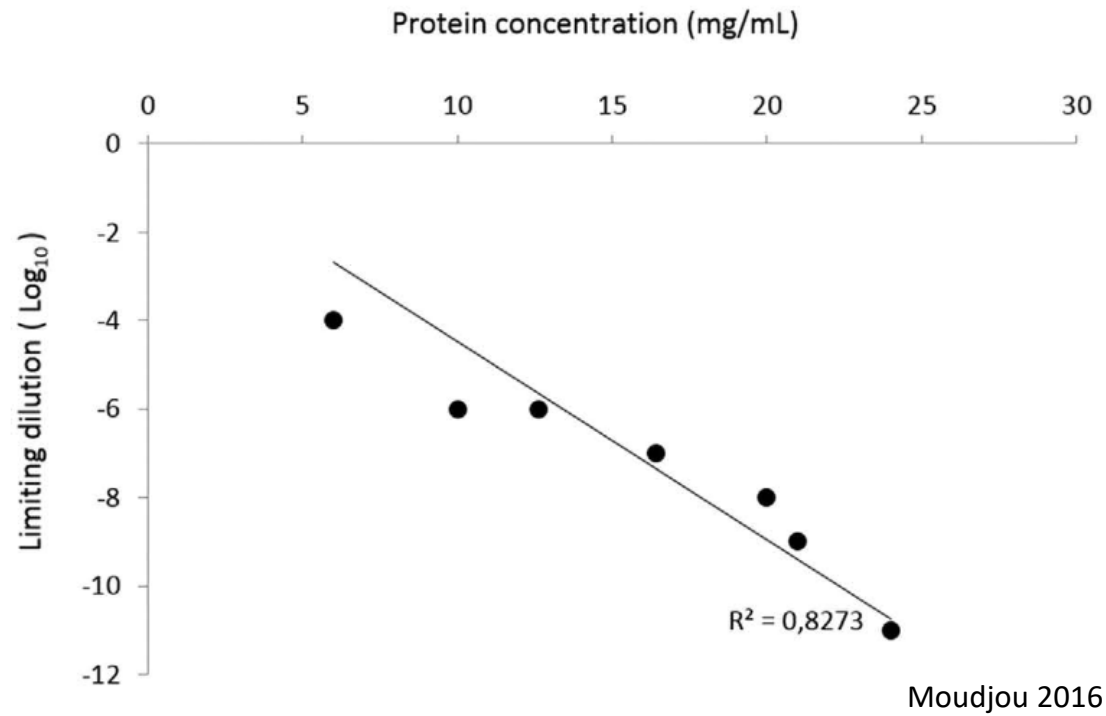


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➤ PMCA efficiency modulated by PrP^C concentration

In vitro amplification of prions is highly sensitive to substrate concentration



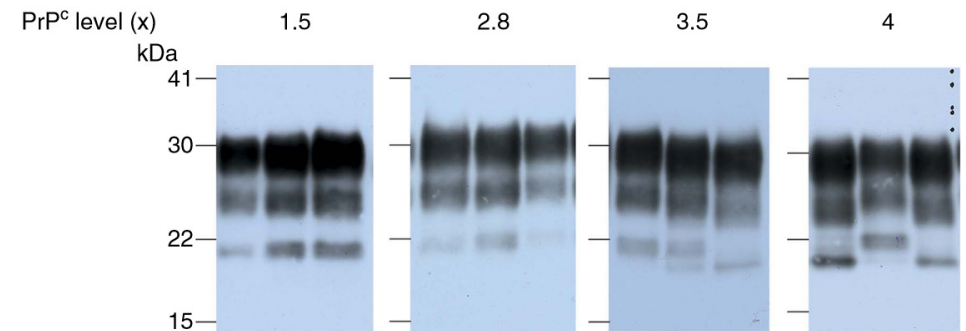
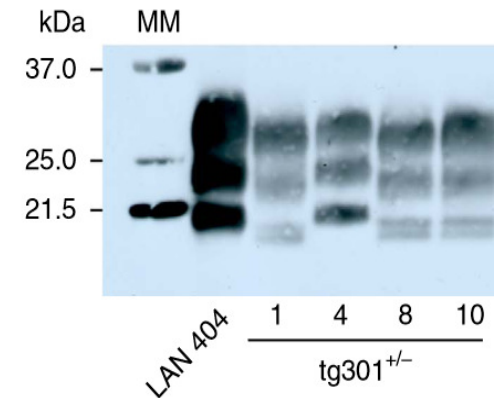
➤ Prion strain selection rely on expression levels

A case of homotypic transmission experiment (le Dur, 2017)

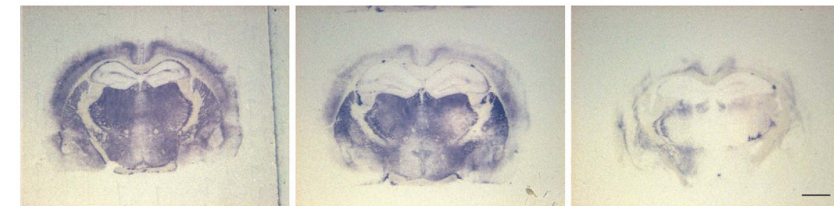
- Scrapie isolate (Lan 404, sheep) transmission to ovine mice $tg301^{+/-}$ (4x overexpression)
 - Change in the biochemical and histological features

- Change in the features depend on the PrP^C expression level

- PrP^C expression level is critical for the selection and emergence of particular prion strains



profile 21K 21K 21K / 19K 21K / 19K
PrP^C level (x) 1.5 2.8 8



Hippocampus histoblots



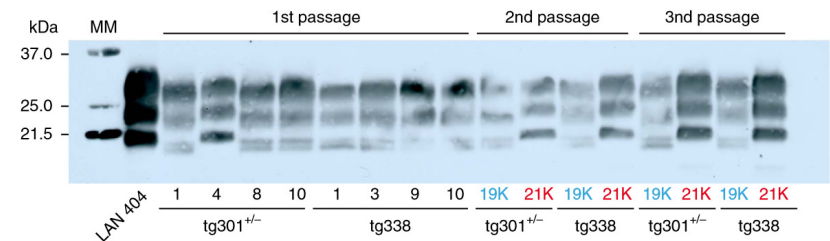
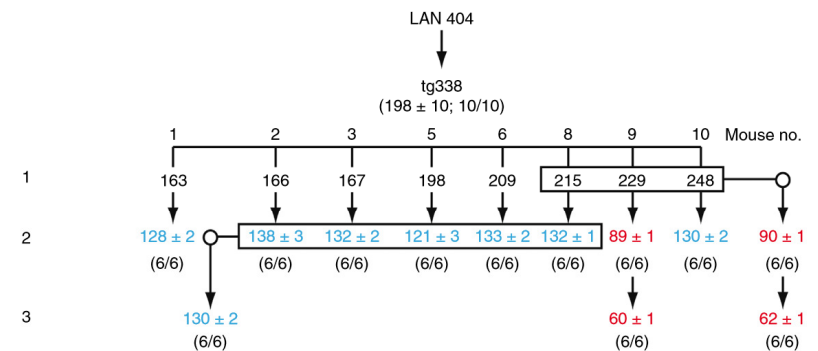
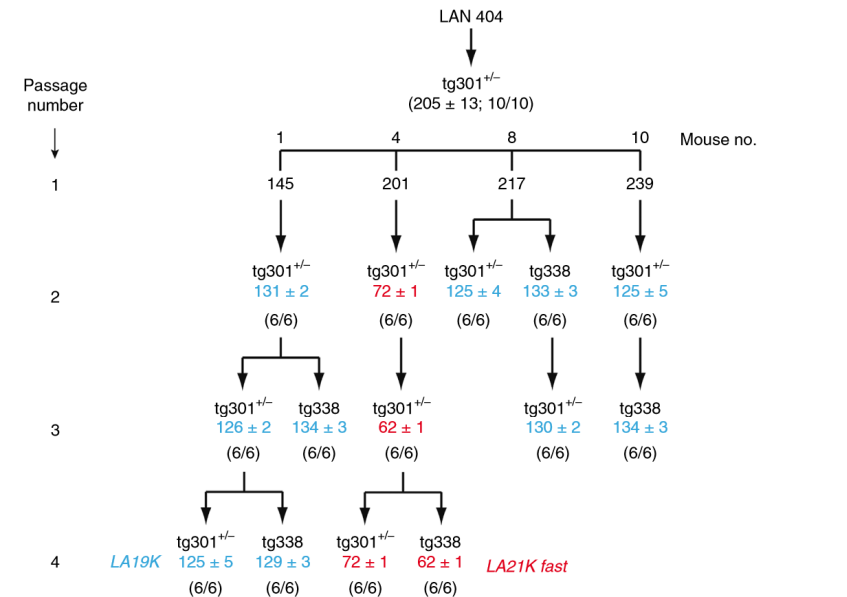
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➤ The intriguing case of Lan19K / Lan21K prions

How co-evolution of prion strains is influenced by PrP^C expression level (Le dur 2017)

- Lan 404 (scrapie) isolate inoculated to over expressor mice (Tg301^{+/-}, ovinized)
 - On 1st passage, biochemical profile is different from isolate : most mouse brains show a 19K profile
 - On 2nd passage (and subsequent), stabilization for most mice. Some show drastic reduction of incubation time associated with a change of biochemical profile (21K fast). Change is fixed



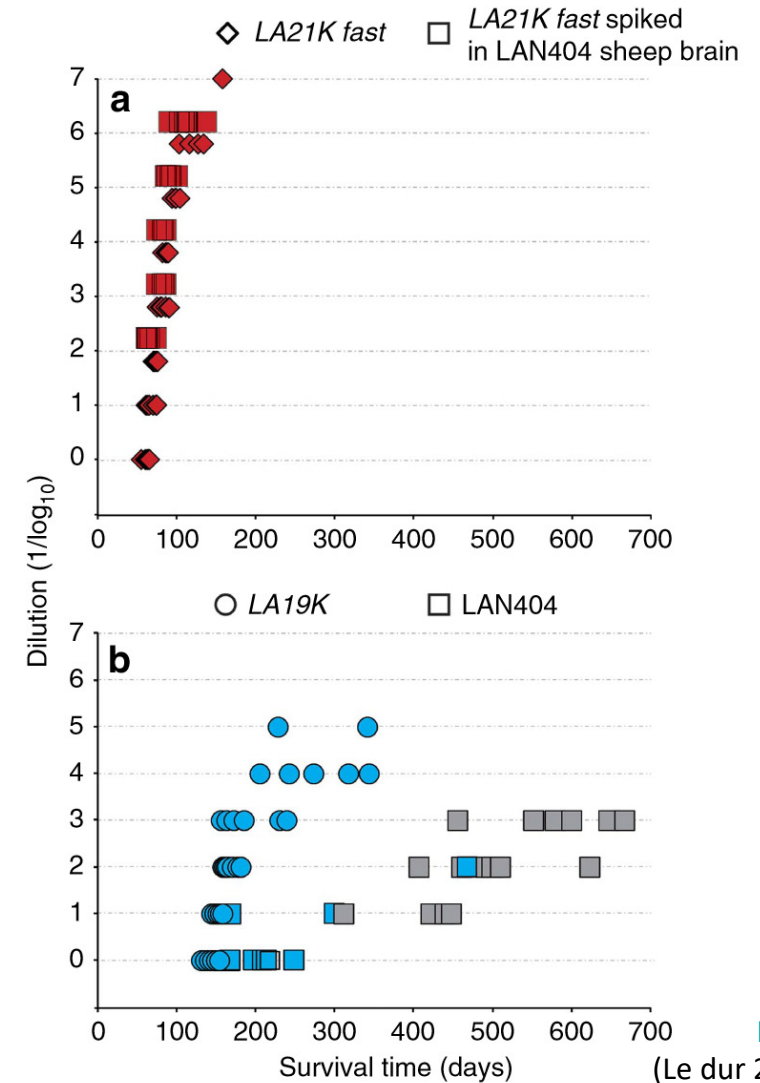
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➤ 21Kfast does not pre-exist within Lan 404 isolate

Spiking of **21K fast** in isolate results in 100% 21Kfast profiles

- **21K fast** prion is a mutant appearing stochastically
- Diluting Lan404 initial isolate material results in acquisition of Lan21K (not fast) profile
- Diluting Lan19K prions does not change the profile
- The agent initially present in Lan404 isolate should have a replication pace at least **3 times slower** than that of 19K



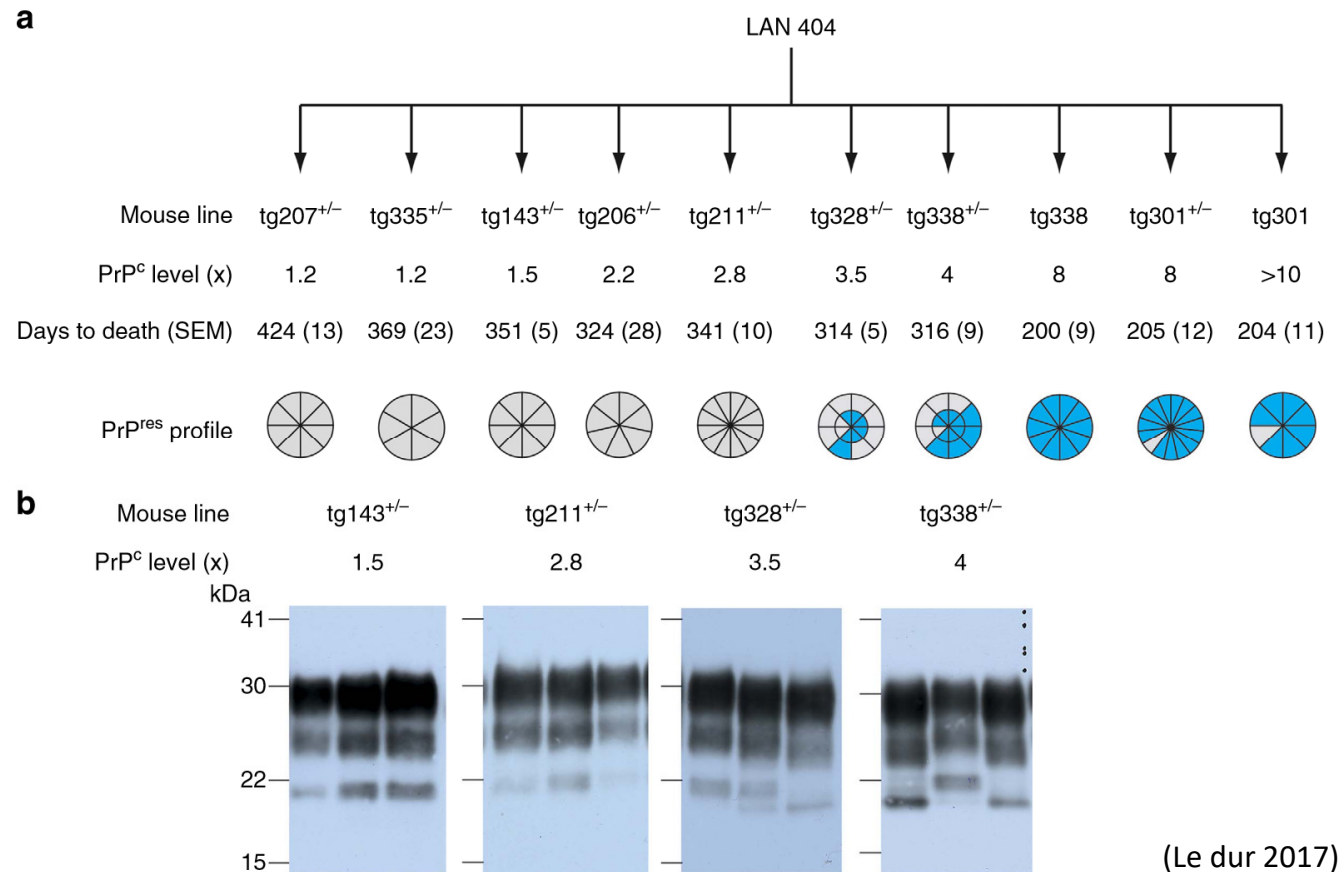
➤ Influence of the PrP expression level on the strain selection

Prion strains replicating in the brain depend on the PrP expression level

- Variation of the biochemical profiles is linked to the expression level

- Physiological levels of PrP are associated with the same profile as the starting isolate (21K)
- The more intense the expression the more often 19K profile is observed

- What happens in the spleen ??



(Le dur 2017)

p. 40

➤ Can differential expression level result in organ specific strain selection ?

Divergent biochemical profile of 19K-inoculated mice in brains and spleens

- Lan404 isolate or Lan 19K strain inoculated to high expressor mice (8x)

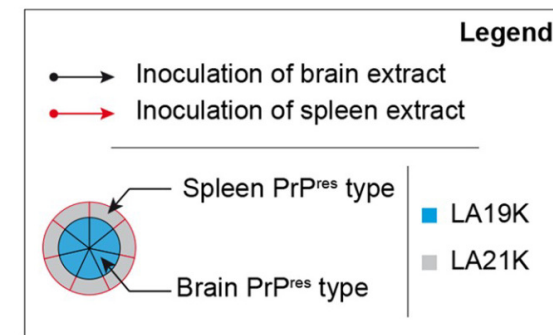
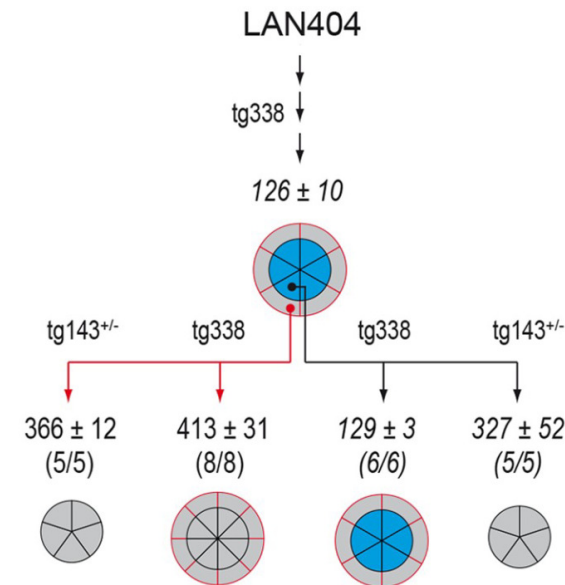
- 19K profile in the brain
- 21K profile in the spleen

- Brain 19K inoculum turns

- 21K on low expressors (1.2x)
- 19K in brain and 21K in spleen on high expressors

- Spleen 21K inoculum turns

- 21K on either low or high expressors (spleen and brain : cloning effect)

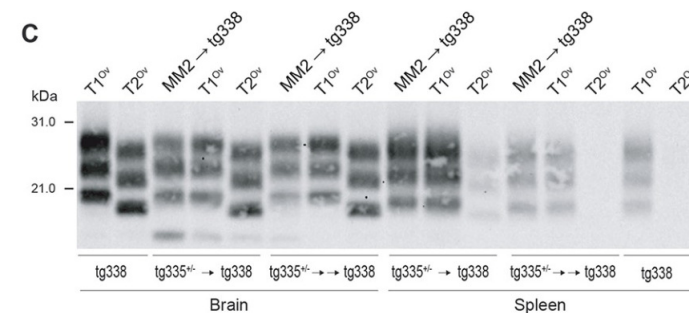
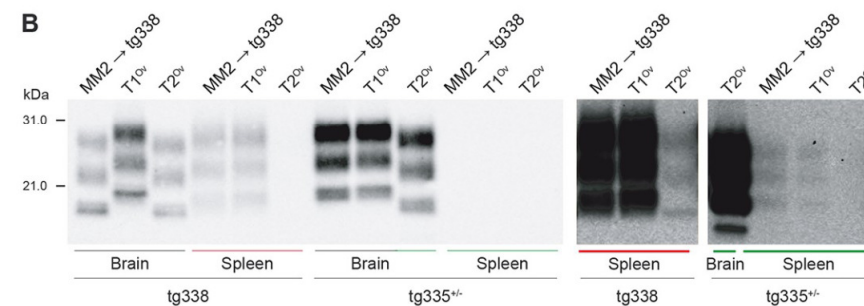
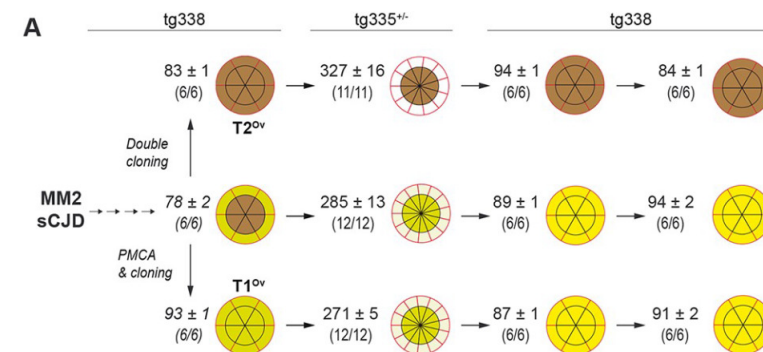


(Béringue 2020)

➤ Similar results upon adaptation of MM2 sCJD to ovine high expressor

Adaptation of MM2 sCJD to ovine tg338 high expressor mice results in two divergent profiles in the spleen and the brain of the mice

- MM2 sCJD, type 2 profile
- In tg 338 (8x expressor)
 - Keeps type 2 profile in the brain
 - Acquires type 1 profile in the spleen
- In tg335 (1,2x expressor)
 - Acquires type 1 profile in both brain and spleen
- Both profiles can be stabilized (cloned)



(Béringue 2020)

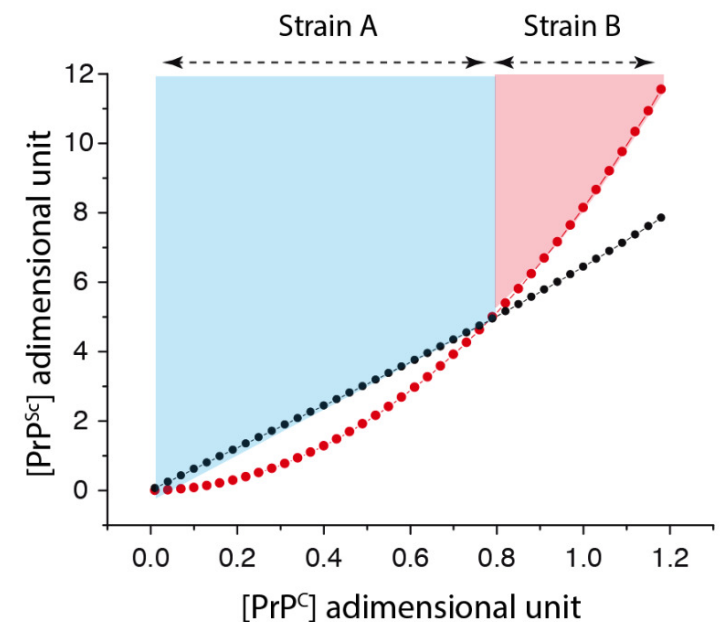


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➤ Conclusions

- Prion strain selection is greatly influenced by the PrP expression level
 - Biochemical profile
 - Histological profile
 - Incubation times
- Endogenous PrP expression level can favor or repress the emergence of particular prion strains
- The selected strain may or may not replicate in the spleen of the host, and therefore determine its lymphocompetency
- Portfolio is wide but not infinite



(Le dur 2017)



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➤ Take Home messages

- Prions actively replicate outside the CNS
- Extraneural replication facilitate:
 - Dissemination and horizontal contamination
 - Crossing of species barrier
- Lymphoid prion replication relies on follicular dendritic cells
- Prion strain selection relies on PrP^C expression level



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