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CORRESPONDENCE

Infectious prions do not induce Aβ deposition in an in vivo seeding model

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An increasing number of studies have suggested that certain cases of iatrogenic Creutzfeldt–Jakob disease (iCJD) that harbor significant β-amyloid (Aβ) pathology are the result of aggregated Aβ transmission to patients during the same procedure that caused prion disease [2, 4, 7, 8, 11, 13, 17]. The source of iatrogenic contamination has been observed both for human growth hormone infusions and dura mater grafts, arguing against a treatment specific effect. Intriguingly, recent work has also observed suspected Aβ pathology transmission in post-mortem samples that received growth hormone treatments but did not develop CJD [17]. Yet another study suggested that neurosurgery with Aβ-contaminated tools can transmit Aβ pathology and lead to intracerebral hemorrhage [12]. These findings have been debated in the context of whether Aβ pathology is truly transmissible and whether Alzheimer's disease could subsequently develop.

It is well known that aggregated Aβ can nucleate the misfolding and aggregation of naïve Aβ monomers both in vitro and in vivo, in a process termed seeding [15, 20]. Thus, it appears plausible that exogenous Aβ seeds could induce pathology in human subjects. However, it has been reported that the cellular prion protein, PrP^{C}, can bind different Aβ species, especially oligomers [3, 14]. Furthermore, conflicting reports have suggested that infectious prions (PrP^{Sc}) can exacerbate Aβ deposition at a time point where plaques are already present, with both pathologies working synergistically, but also that misfolded Aβ can actually interfere with prion pathogenesis [16, 18, 19]. Given these findings, it is important to consider that PrP^{Sc} inoculations could influence Aβ pathology, but the role of PrP^{Sc} in initiating cerebral β-amyloidosis is still unclear. To this end, we inoculated APP transgenic mice on a PrP^{C} wild-type or heterozygous knockout background with infectious PrP^{Sc} prions to investigate whether PrP^{Sc} alters the onset of Aβ pathology.

APP23 mice expressing human amyloid precursor protein (APP) with a Swedish mutation (KM670/671NL) were intracerebrally inoculated (hippocampus, bregma: 2.5 mm posterior; ±2.0 mm lateral; 1.8 mm ventral) with infectious RML-PrP^{Sc} or wild-type (WT) brain extracts (1% w/v) prepared from RML-PrP^{Sc} infected or healthy CD1 mice, respectively, using the Precellys system (5500 rpm, 2×20 s, Bertin Instruments) (Fig. 1a, Supplemental Methods). All PrP^{Sc}-inoculated mice developed terminal prion disease and were sacrificed after 176 days post injection (median). Histological analysis with Hematoxylin & Eosin and SAF84 (PrP^{Sc}, 1:250) revealed that brains displayed typical vacuolation (spongiosis) and PrP-immunoreactive deposits in sick animals, which was not detectable in WT-injected animals of the same age (Fig. 1a). Using an in-house pan-Aβ antibody (CN6, 1:1000) (Supplemental Methods), no induced Aβ pathology was detected in the hippocampus of PrP^{Sc} prion-injected animals, while conversely, the injection of minimal Aβ seeds and a similar incubation period does yield Aβ deposition as demonstrated previously [20].

Given that the incubation period of the suggested transmission of human Aβ pathology is 10–40 years for both transmission via contaminated dura grafts and growth hormone [2, 4, 8,

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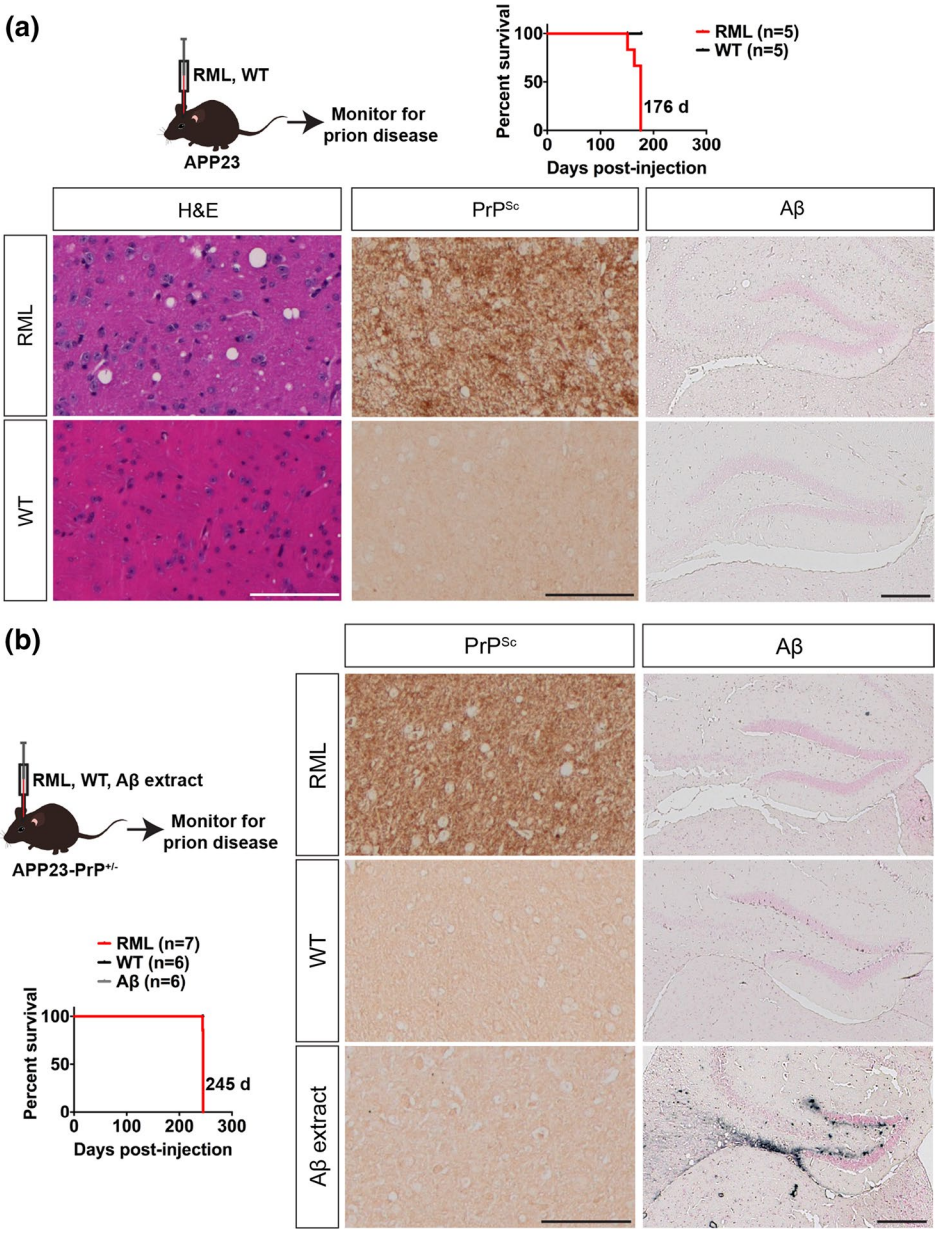

Fig. 1 RML-PrP^{Sc} inoculations into APP23 transgenic mice do not induce Aβ deposition. **a** APP23 mice were injected with either RML-PrP^{Sc} ($n=5$; 4 males, 1 female) or a WT control brain extract ($n=5$; 3 males, 2 females) and monitored until RML-injected animals became sick (median survival: 176 days), at which time point all animals were sacrificed. Representative histological stains for Hematoxylin–Eosin (H&E), PrP^{Sc} (SAF84) and Aβ (CN6) are presented. None of the RML-PrP^{Sc} (0/5) or WT-inoculated (0/5) APP23 mice revealed any detectable Aβ deposits in the hippocampus. H&E, PrP^{Sc} scale bars = 100 µm; Aβ scale bar = 200 µm. **b** APP23 mice heterozygous for PrP^{C} knockout (APP23-PrP$^{+/-}$) were injected with either RML-PrP^{Sc} ($n=7$; 4 males, 3 females), WT control ($n=6$; 4 males, 2 females) or Aβ seeding extract ($n=6$; 3 males, 3 females). Mice were monitored until RML-injected animals became sick, then all animals were sacrificed (median survival: 245 d). Representative histological stainings of PrP^{Sc} (SAF84) and Aβ (CN6) are presented. None of the RML-PrP^{Sc} (0/7) or WT-inoculated (0/6) APP23-PrP$^{+/-}$ mice revealed any detectable induced Aβ deposits, whereas all Aβ seed-inoculated (6/6) APP23-PrP$^{+/-}$ mice showed induced Aβ deposition. PrP^{Sc} scale bar = 100 µm; Aβ scale bar = 200 µm

10, 11], we hypothesized that the incubation period in our mouse model may not be long enough to detect induced Aβ pathology caused by PrP^{Sc} prions. Thus, APP23 mice were crossed to a *Prnp*-null line to produce PrP^{C} heterozygous knockout mice (APP23-PrP$^{+/-}$), which is known to increase the prion incubation period until terminal sickness [1, 6]. Indeed, APP23-PrP$^{+/-}$ mice injected with PrP^{Sc} prions survived an extra 69 days after intrahippocampal inoculation as described above (median survival: 245 days post injection) before being sacrificed due to prion disease with the expected PrP^{Sc}-positive staining using SAF84 (Fig. 1b). Nevertheless, none of the animals injected with PrP^{Sc} prions presented with detectable seeded Aβ deposition after staining sections for Aβ (CN6) (Fig. 1b). In parallel, APP23-PrP$^{+/-}$ mice were also inoculated with brain extracts prepared from aged APP23 Aβ-laden brains homogenized with the Precellys system as above (10% w/v) followed by a 3000 g centrifugation (5 min). All Aβ-injected animals showed obvious induced Aβ pathology as expected [15, 20] (Fig. 1b).

The potential of Aβ pathology transmission under specific circumstances in humans is interesting both from a basic biology and human health perspective [2, 4, 7, 8, 10–13, 17]. However, given that these human studies are observational, there are questions on the mechanism behind the increased Aβ deposition. We have found that intrahippocampal inoculations of infectious RML prions into APP23 transgenic mice caused prion disease but did not induce Aβ pathology in the hippocampus after long incubation periods that are sufficient to detect seeded Aβ pathology caused by nanomolar amounts of Aβ (one seeding unit) [20]. This argues against a direct cross-seeding effect of PrP^{Sc} prions on Aβ or an indirect effect of prion disease leading to

Aβ pathology. This conclusion is supported by the recent report that patients who received growth hormone treatments but did not have prion disease still contained significant Aβ pathology and other instances suggesting Aβ pathology in prion disease patients is an age-related phenomenon [9, 17].

It is worth noting that although PrP^{Sc} did not induce Aβ pathology before terminal prion disease, these inoculations were intracerebral and were meant to provide a model system for dura mater grafting or for contaminated surgical instruments. Instances of Aβ pathology after peripheral growth hormone treatment in humans with iCJD could be caused by Aβ seeds in the growth hormone extract traveling to the brain similar to studies in APP transgenic mice [2, 4, 5, 11, 17]. However, it cannot be excluded that a peripheral prion infection indirectly influences Aβ pathology [18], and thus mechanistically would contrast intracerebral exposure. It is also important to consider that a different PrP^{Sc} prion strain may harbor Aβ pathology inducing activity.

From our work, we can conclude that misfolded Aβ introduced during treatment may be responsible for induction of Aβ pathology in these human cases as opposed to being the by-product of iCJD prion infection. Future work will need to determine whether cases of Aβ pathology transmission could eventually develop into clinical Alzheimer's disease.

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