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Peripherally Applied A β -Containing Inoculates Induce Cerebral β -Amyloidosis

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Abstract

The intracerebral injection of β -amyloid-containing brain extracts can induce cerebral β -amyloidosis and associated pathologies in susceptible hosts. We found that intraperitoneal inoculation with β -amyloid-rich extracts induces β -amyloidosis in the brains of β -amyloid precursor protein transgenic mice after prolonged incubation times.

Intracerebral (i.c.) inoculation with minute amounts of brain extract containing misfolded β -amyloid (A β) from patients with Alzheimer's Disease or from amyloid-bearing β -amyloid precursor protein (APP) transgenic (tg) mice induces cerebral β -amyloidosis and related pathologies in APP tg mice in a time- and concentration-dependent manner (1). However, oral, intravenous, intraocular, or intranasal inoculations have failed to induce cerebral β -amyloidosis in APP tg hosts (2). These findings suggest that A β -containing brain material in direct contact with the brain can induce cerebral β -amyloidosis, but that, unlike prions, either the inducing agent is not readily conveyed from peripheral sites to the brain or a higher concentration or longer incubation period is required for peripherally delivered A β seeds.

Intraperitoneal administration of prion-rich material is more efficient at transmitting prion disease than is oral administration (3,4). To test whether intraperitoneal inoculation of A β -rich material might similarly trigger A β misfolding and deposition in the brain, we administered two intraperitoneal injections (100 μ l each, one week apart) of A β -laden (10–20 ng/ μ l) brain extract from aged APP23 tg mice (Tg extract) to a cohort of young (2-month-old) female APP23 tg mice (5). After a 7-month incubation period, cerebral β -amyloidosis was robustly induced in all intraperitoneally inoculated mice compared with untreated littermate controls (Fig. 1). To confirm this finding, we inoculated a second cohort of 2-month-old female APP23 mice with a different batch of Tg brain extract in another laboratory (cohort 2: Tübingen, vs. cohort 1: Basel). After 6 to 7 months, mice injected intraperitoneally with the Tg extract exhibited robust cerebral β -amyloidosis, whereas

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intraperitoneal inoculation with phosphate-buffered saline (PBS) or brain extract from age-matched, non-tg wild-type mice (Wt extract) was ineffective (Fig. 1).

Induced β -amyloidosis was strongest in the anterior and entorhinal cortices, with additional deposition in the hippocampus, resembling the regional development of endogenous β -amyloidosis in aged APP23 mice (6). However, whereas normal aged APP23 mice manifest mostly parenchymal deposits, the induced β -amyloid in intraperitoneally seeded mice was predominantly associated with blood vessels [cerebral β -amyloid angiopathy (CAA)], often with massive spreading into the neighboring brain parenchyma (Fig. 1). The presence of A β was confirmed by immunoblotting, and amyloid fibrils were evident ultrastructurally; in addition, the induced β -amyloidosis was linked to gliosis, hyperphosphorylated tau, and other associated pathologies (Fig. 2), reminiscent of the cerebral β -amyloid deposition in aged APP23 mice (6,7).

To compare the efficiency and time course of intraperitoneal versus intracerebral inoculation, 2-month-old female APP23 mice were inoculated either intraperitoneally (2 x 100 μ l) or intracerebrally (2.5 μ l into the hippocampus) with Tg extract, and then analyzed 4 months later. No cerebral β -amyloid induction was found in any of the four intraperitoneally inoculated mice, whereas all six intracerebrally inoculated mice revealed β -amyloid induction identical to that previously reported (1,2). From this observation, together with previous time course and 1:20 dilution experiments for intracerebral inoculations (1), we estimate that intraperitoneal inoculations with 1000 times as much A β take 2 to 5 months longer to induce cerebral β -amyloidosis than do intracerebral inoculations.

The replication of peripherally applied prions and their translocation into the central nervous system depend on hematopoietic and stromal immune cells, in combination with sympathetic innervation of abdominal lymphoid organs (8). Both activation of the immune system and chronic inflammation promote prion replication (9,10). To assess the immune response to A β -rich brain extracts, additional APP23 mice were given single intraperitoneal injections of 200 μ l Tg or Wt extract and sacrificed 1 hour, 1 week, or 1 month after injection (5). An acute immune activation to the injected brain material was indicated by transient increases in plasma chemokines and cytokines [interleukin-6 (IL6), IL10, tumor necrosis factor- α , monocyte chemoattractant protein-1, and macrophage inflammatory protein-1 β] in both Tg and Wt extract-inoculated mice after 1 hour, with IL-6 still mildly elevated in Tg extract-injected mice 1 week after inoculation (fig. S1). However, no signs of chronic inflammation in various peripheral organs (e.g., liver, pancreas, kidney, and lung) or plasma titers of antibodies to A β were found in any mice investigated at 1 or 7 months after seeding (5). Moreover, no β -amyloid deposition was found in any of the peripheral tissues at any time point studied.

Thus, like prion disease, cerebral β -amyloidosis can be seeded in the brain by homologous protein aggregates delivered into the peritoneal cavity, although the intraperitoneal route required more time and was less efficient than was direct injection into the brain (1, 2). The amyloid-inducing factor in the Tg extract is probably a species of misfolded A β that is generated in its most effective form or composition in vivo (1). Because the expression of tg (human) APP is restricted to the nervous system in APP23 mice (7), in this model it is likely that the seed carried to the brain was the injected material itself, rather than A β aggregates that were first amplified in peripheral tissues.

There is now persuasive evidence that the aggregation of A β is a key pathogenic feature of Alzheimer's disease and A β -CAA (11–14), although the majority of these cases are initiated by unknown causes. The possibility that mechanisms exist allowing for the transport of A β aggregates (and possibly other seeds) from the periphery to the brain justifies further studies

to better understand the cellular and molecular origin of these diseases and to clarify the basis of infectious vs. noninfectious proteopathies (15,16).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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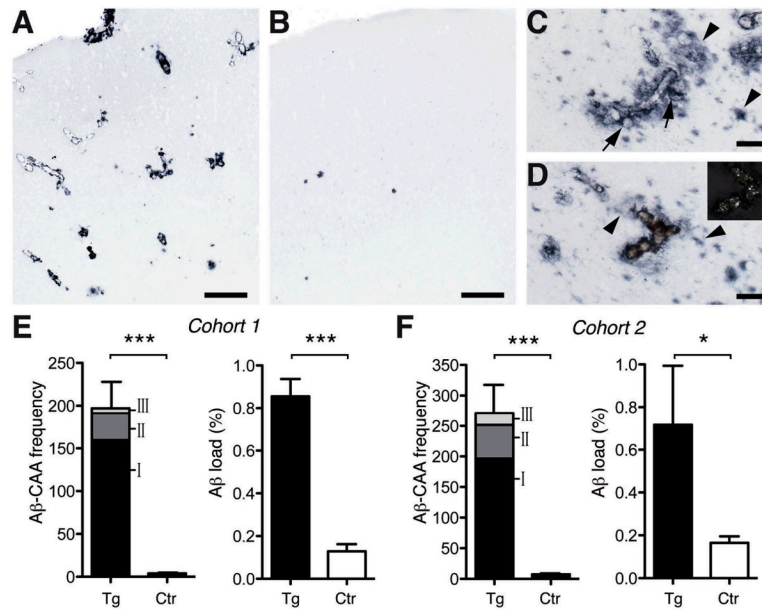


Fig. 1. Induced A β deposition

(A, B) A β -immunostained frontal cortex of APP23 mice inoculated intraperitoneally with Tg extract (A) or Wt extract- (B). (C, D) Most induced β -amyloid was vascular (A β -CAA), with A β -immunoreactivity extending into the brain parenchyma (arrows). Amyloid-laden vessels were congophilic [red in (D); birefringent under cross-polarized light in insert] and often were surrounded by diffuse, Congo red-negative A β deposits (arrowheads). (E, F) Analysis of the entire neocortex for A β -CAA frequency [indicated are all three (I-III) CAA severity grades (5)], and for total A β load in Tg extract-inoculated mice compared with control (Ctr) mice. *Cohort 1* consisted of six Tg extract-inoculated mice versus seven untreated control mice. A β -CAA: $t(11)=6.78$ (all severity grades combined), $***p<0.0001$; A β load: $t(11)=8.79$, $***p<0.0001$. *Cohort 2* consisted of five Tg extract-inoculated mice versus five Wt extract-inoculated mice and four PBS-injected mice. These latter two (control) groups did not differ significantly and were combined for analysis. A β -CAA: $t(12)=7.79$, $***p<0.0001$; A β load $t(12)=2.71$, $*p<0.05$. The occasional parenchymal A β -deposits in control mice are normal for 9-month-old APP23 mice. Error bars, means \pm SEM. Scale bars: [(A) and (B)] 200 μ m; [(C) and (D)] 50 μ m.

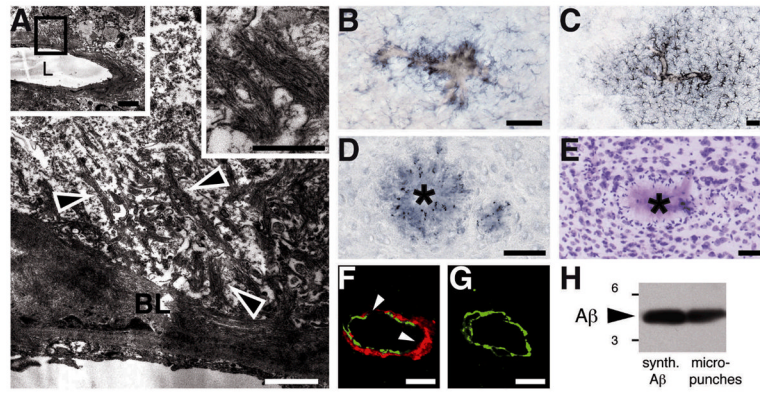


Fig. 2. Induced A β deposition was linked to multiple associated pathologies

(A) Ultrastructural analysis showed amyloid deposition within the vascular basal lamina (BL), with typical amyloid fibrils (arrowheads) extending into the brain parenchyma. Insets are low- and high-magnification views of the examined vessel (L, lumen) and the typical nonbranching amyloid fibrils. (B to E) Vascular amyloid [stained by Congo Red in (B) and (C)] and parenchymal plaques were surrounded by hypertrophic, Iba1-positive microglia (B), Glial fibrillary acidic protein (GFAP)-positive astrocytes (C), hyperphosphorylated tau-positive neurites [(D); asterisk indicates amyloid core], but a paucity of proximate neurons [cresyl-violet stain (E)]. (F and G) Vessels with CAA types II and III showed smooth muscle cell loss at the site of amyloid deposition (arrowheads; confocal image, maximum projection of 5 μ m z-stack: red, A β ; green, smooth muscle actin). A normal vessel (G) has a complete ring of smooth muscle cells. (H) Immunoblotting of micropunches of A β -immunoreactive material revealed the expected A β band. Synthetic A β 40/42 is shown as control. Markers, 3 and 6 kD. Scale bars: (A) 1 μ m (insets, 5 and 0.5 μ m); [(B) to (E)] 50 μ m; [(F) and (G)] 10 μ m.