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Genetic background modulates outcome of therapeutic amyloid peptides in treatment of neuroinflammation

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ABSTRACT

Amyloid hexapeptide molecules are effective in the treatment of the murine model of neuroinflammation, known as experimental autoimmune encephalomyelitis (EAE). Efficacy however differs between two inbred mouse strains, C57BL/6J (B6) and C57BL/10SnJ (B10). Amyloid hexapeptide treatments improved the clinical outcomes of B6, but not B10 mice, indicating that genetic background influences therapeutic efficacy. Moreover, although previous studies indicated that prion protein deficiency results in more severe EAE in B6 mice, we observed no such effect in B10 mice. In addition, we found that amyloid hexapeptide treatments of B10 and B6 mice elicited differential IL4 responses. Thus, the modulatory potential of prion protein and related treatments with other amyloid hexapeptides in EAE depends on mouse strain.

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1. Introduction

Amyloid formation is evident in the pathology of numerous diseases, including neurodegenerative diseases such as Alzheimer's and prion disease. Molecules with amyloid structure are, however, a part of normal physiology. Functional amyloids occur naturally for storage of peptide hormones in the pituitary, for example (Maji et al., 2009). Emerging evidence suggests that endogenous expression of amyloid-forming proteins and exogenous administration of amyloid fibrils can reduce neuroinflammatory responses in autoimmune disease. Multiple lines of evidence demonstrate that amyloid fibrils can modulate autoimmune processes and decrease the severity of paralysis in the murine model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE) (Grant et al., 2012; Kurnellas et al., 2013; Kurnellas et al., 2014). Furthermore, targeted deletion of genes encoding proteins with the propensity to form amyloid results in more severe EAE disease in mice, implying a neuroprotective role for amyloid. For example, the absence of prion protein (PrP) results in exacerbated EAE disease (Tsutsui et al., 2008; Gourdain et al., 2012; Hu et al., 2010) indicating a role for PrP in the modulation of cellular immune responses. Genetic deletion of regions encoding other amyloid-forming proteins including tau (Weinger et al., 2012) and serum amyloid P component (Ji et al., 2012) also results in more severe EAE disease. Furthermore, recent work reveals that exogenous administration of peptide amyloids reduces EAE clinical severity. For example, amyloids formed from Alzheimer's β 1-40 and 1-42 peptides reduce EAE clinical scores and histopathological signs of inflammation (Grant et al., 2012). Additionally, amyloid fibril-forming hexapeptides reduce paralysis, inflammatory foci and serum IL-6 levels (Kurnellas et al., 2013). Collectively these data imply that amyloids can reduce inflammatory autoimmune processes.

Multiple studies have suggested that PrP has an anti-inflammatory role in EAE pathogenesis. However, there are differing reports as to the mechanism by which PrP modulates the disease. It has been reported that in the absence of PrP, there is increased T cell infiltration in the CNS and that PrP −/− T cells have an increased myelin oligodendrocyte glycoprotein (MOG) specific proliferative capacity ex vivo (Tsutsui et al., 2008; Ingram et al., 2009; Hu et al., 2010). Furthermore, PrP has been implicated in T cell activation, differentiation, and survival in EAE (Hu et al., 2010), suggesting a role in mediating T cell effects in EAE. Conversely, another study reported that MOG-challenged PrP −/− T cells from EAE mice had proliferation capacity and cytokine production that was similar to that of wild type T cells and attributed the exacerbation of EAE in PrP −/− animals to the absence of PrP in the CNS (Gourdain et al., 2012). Nevertheless, in all previous reports, the absence of PrP worsened EAE clinical disease, suggesting a protective role for the protein in regulating pathogenesis.

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Amyloid and its accumulation are typically associated with pathological outcomes. However, the beneficial effects of amyloid in autoimmune disease suggest previously unrecognized anti-inflammatory functions for amyloids and amyloid-forming proteins. To investigate the mechanism of amyloid fibrils in neuroinflammation and the reported neuroprotective role of PrP in autoimmunity, we have used both PrP knockout mice and exogenous administration of peptide amyloids to investigate the effects of PrP and amyloid treatments in EAE. We have found that these effects are strongly influenced by genetic background in two closely related lines of C57BL mice. These results suggest that these mouse models may be valuable for comparative studies to help unravel the mechanistic basis for the anti-inflammatory activities of amyloid forming structures.

2. Materials and methods

2.1. Mice

Mice were housed at Rocky Mountain Laboratories (RML) in an AAALAC-accredited facility. All experimentation was conducted using the NIH RML Animal Care and Use Committee approved protocols (NIH/RML Protocols #2012-014, #2012-067-E). C57BL/10SnJ mice were originally purchased from Jackson Laboratories and maintained at RML for several years as an inbred colony. C57BL/10SnJ-Prnp −/− mice were generated by serially backcrossing 129/Ola-Prnp −/− mice to C57BL/10SnJ mice for 11 generations as previously described (Striebel et al., 2013b). C57BL/6 mice were purchased from Jackson Laboratories.

2.2. Induction of EAE

Active EAE was induced as per Kurnellas et al. (2014). Briefly, EAE was induced in B6 (Jackson Laboratories) or B10 female mice 8–12 weeks of age by subcutaneous immunization mid-back with an emulsion of complete Freund's adjuvant containing 4 μg/mL *Mycobacterium tuberculosis* H37RA with an equal volume of 200 μg myelin oligodendrocyte glycoprotein$_{35–55}$ (Genemed) in PBS. Mice were given 400 ng pertussis toxin dissolved in PBS intraperitoneally at 0 and 48 h after immunization. Clinical disease was scored independently by two blinded investigators. Scoring was as per Stromnes and Goverman (2006): 0, no clinical disease; 0.5, partially limp tail; 1, paralyzed tail; 1.5, mild gait disturbance; 2, hindlimb weakness; 2.5, one hind limb paralyzed; 3, complete hindlimb paralysis; 3.5 hind limb paralysis, weakness in forelimbs; 4, hindlimb and forelimb paralysis. Due to animal protocol requirements, mice were euthanized at a clinical score of 4 and marked as such for the duration of the experiment.

2.3. Amyloid preparation

Lyophilized Aβ1-42 peptide (California peptide) was resuspended in DMSO to a final concentration of 30 mg/mL. The dissolved peptide was incubated at 37 °C overnight. Prior to injection, Aβ1-42 peptide was diluted in PBS to a final concentration of 1 mg/mL. The vehicle control was the equivalent dilution of DMSO in PBS. Tau and tau shuffle were prepared by diluting 1 mg of peptide in 15 μL (351 μM) of formic acid and then diluting in 5 mL of water. An equivalent molar quantity of NaOH was added and the pH adjusted to 7. 1 mL of 10× PBS was added and water added to reach 10 mL for a final peptide concentration of 100 μg/mL. Amylin was diluted to a final concentration of 100 μg/mL in PBS.

2.4. Tau characterization

ThT fluorescence was used to measure amyloid formation of tau or tau shuffle hexapeptides. 25 μg of peptide was diluted in PBS to 100 μL and 10 μM ThT added. ThT fluorescence was measured (450 +/− 10 nm excitation and 480 +/− 10 nm emission; bottom read) on a BMG Fluostar plate reader. The ability of tau amyloid to inhibit insulin aggregation was assessed as previously described (Kurnellas et al., 2013).

2.5. Histology

Sections were stained with Luxol Fast Blue as per (Kluver and Barrera, 1953; Sheehan and Barbara, 1980) with the following modifications. Tissue was sectioned at 10 μm and the slides dried overnight at 42 °C. Slides were deparaffinized and hydrated to 95% EtOH. Slides were placed in Luxol Fast Blue solution, heated and maintained at 65 °C in a BioPro Microwave (Pelco) at 750 W for 30 min and then allowed to cool for an additional 20 min at room temperature. Slides were rinsed in 95% EtOH to remove excess stain, followed by distilled water. Differentiation was carried out in a 0.05% lithium carbonate solution for 20 s followed by continued differentiation in 70% EtOH. Slides were rinsed in distilled water. If nuclear detail was present, we stained in instant hematoxylin (Thermo Scientific) for 15 s, quickly blued the slides in 70% ammonia alcohol, and dehydrated in several changes of 100% EtOH, cleared in xylene, and coverslipped in Cytoseal XYL (Thermo Scientific).

2.6. Complete blood count (CBC) analysis

Blood samples were collected into dipotassium EDTA microtainers (BD) and the CBC analyzed on a Hemavet 950 (Drew Scientific).

2.7. Splenocyte cultures

Mice were induced with EAE and following the onset of hindlimb weakness (average clinical score of approximately 1.5) mice were treated with 10 μg tau or tau shuffle for 3 days. Mice were euthanized using isofluorane inhalation and spleens collected into PBBS. Tissue was ground through 70 μm mesh filters and filters rinsed twice with 1 mL PBBS. Cells were pelleted at 1500 RPM for 5 min in an Eppendorf 5810 R centrifuge. 3 mL of ACK (Ammonium-Chloride-Potassium) buffer was added to the cell pellet and incubated for 5 min at room temperature. The cell pellet was resuspended in 10 mL PBBS, refiltered through a 70 μm mesh filter and repelleted at 1500 RPM for 5 min. The pellet was resuspended in 5 mL RPMI 1640 media and the cells counted in the presence of trypan blue with a hematocytometer. Cells were repelleted and sufficient volume of RPMI 1640 media containing 10 mM Hepes, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, penicillin, streptomycin, 0.5 μM 2-mercaptoethanol and 10% FBS was added to reconstitute at 10^7 cells/mL. For stimulation with CD3/CD28, 24 well plates were pre-coated with 2.5 μg/mL CD3e and 1.0 μg/mL CD28 diluted in PBS and incubated overnight at 4 °C. Plates were washed with ice-cold PBS to remove unbound antibody prior to plating cells. 5×10^6 cells were added per well. For MOG$_{35–55}$ restimulation, 20 μg/mL MOG$_{35–55}$ peptide was included in media. After 24 h in culture, an additional 1 mL of media (1 vol; including 20 μg/mL MOG$_{35–55}$ if appropriate) was added. For sample collection, trituration was used to remove cells from the wells and media collected. Cells were pelleted at 2000 g for 5 min. Supernatant was decanted and frozen at −80 °C for multiplex analysis. Cell pellet was resuspended in ZR buffer for RNA purification and vortexed briefly to resuspend. Cell solutions were frozen at −80 °C until RNA isolation.

2.8. RNA purification and real-time PCR

Total RNA was purified using RNA spin purification columns (Zymo Research). RNA clean-up and real-time PCR analysis was carried out as previously described (Du et al., 2010). Primers used in this study are as follows: IL4 5′ CCTCACAGCAACGAAGAACA 3′, 5′ GATGAATCCAGGCATCGAAA 3′.