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Type-1 interferon signaling mediates neuro-inflammatory events in models of Alzheimer's disease

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ABSTRACT

A neuro-inflammatory response has been implicated in human patients and animal models of Alzheimer's disease (AD). Type-1 interferons are pleiotropic cytokines involved in the initiation and regulation of the pro-inflammatory response; however, their role in AD is unknown. This study investigated the contribution of type-1 IFN signaling in the neuro-inflammatory response to amyloid-beta (Aβ) in vitro and in the APP/PS1 transgenic mouse model of AD. Enzyme-linked immunosorbent assay confirmed a 2-fold increase in IFNα in APP/PS1 brains compared with control brains. Quantitative polymerase chain reaction also identified increased IFNα and IFNβ expression in human pre-frontal cortex from AD patients. In vitro studies in primary neurons demonstrated Aβ-induced type-1 IFN expression preceded that of other classical pro-inflammatory cytokines, IL1-β, and IL-6. Significantly, ablation of type-1 interferon-α receptor 1 expression in BE(2)M17 neuroblastoma cells and primary neurons afforded protection against Aβ-induced toxicity. This study supports a role for type-1 interferons in the pro-inflammatory response and neuronal cell death in AD and suggests that blocking type-1 interferon-α receptor 1 maybe a therapeutic target to limit the disease progression.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder worldwide accounting for >60% of all cases of dementia. Pathologically AD is characterized by the loss of pyramidal neurons and the intracellular accumulation of senile plaques containing amyloid-β (Aβ) deposits (Selkoe, 2001) and neuro-fibrillary tangles containing hyper-phosphorylated tau (Grundke-Iqbal et al., 1986). Features of neuro-inflammation including activated microglia (Tahara et al., 2006), reactive astrocytes (Apelt and Schliebs, 2001) and enhanced cytokine load (Salminen et al., 2008) have also been widely described in brain tissue from AD patients and transgenic mouse models. It is known that pro-inflammatory cytokines can not only induce neuropathic mechanisms and thereby contribute to neuronal death, but can also influence classical neurodegenerative pathways such as amyloid precursor protein processing. Tumor necrosis factor-alpha (TNF-α) can regulate amyloid precursor protein processing and the production of Aβ in vitro (Blasko et al., 1999). In addition, pro-inflammatory cytokines are induced by Aβ in cultured microglia contributing to a pro-inflammatory environment (Floden and Combs, 2006; Hanisch, 2002). Critically, Aβ triggers activation of the NALP3 inflammasome in vivo, responsible for caspase-1 mediated IL-1β production (Heneka et al., 2013). The subsequent microglial activation, astrogliosis, and further secretion of pro-inflammatory molecules and Aβ contribute to this damaging cascade (Sastre et al., 2003).

Interferons (IFNs) are a super-family of cytokine proteins that play an important role in host immune response to infections, pathogens, and various diseases (reviewed in [de Weerd and Nguyen, 2012]). Type-1 IFNs display pro-inflammatory properties via activation of multiple signaling cascades, including the classical JAK-Stat pathway. Specifically, type-1 IFNs (IFN-α and IFN-β) can induce pro-inflammatory gene transcription leading to the secretion of cytokines (including TNF-α, IL-6, and IL-1β), cellular infiltration and inflammatory progression. Importantly, type-1 IFNs are involved in the initiation and/or regulation of pro-inflammatory cytokines (de Weerd et al., 2007; Kawai and Akira, 2006) and without the presence of type-1 IFNs a robust innate immune response fails to manifest. These IFN-dependent

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functions differ based on cell and tissue type (reviewed in [de Weerd and Nguyen, 2012]). Indeed, much remains unclear about type-1 IFN signaling in various cell types, including those in the brain.

Recently, the Tyk2-STAT pathway has been implicated in soluble Aβ1–42 mediated neurotoxicity (Wan et al., 2010). This study confirmed STAT3 phosphorylation is elevated in both APP/PS1 mouse brains and postmortem brains of AD patients. Considering the potential of type-1 IFNs to drive these phosphorylation events, we hypothesized that they play a prominent role in mediating the neuro-inflammation in AD. In this study we used both in vivo and in vitro models of AD alongside postmortem human samples to characterize a role for type-1 IFNs in AD. We demonstrate elevated type-1 IFN levels and signaling in our models of AD and confirm their contribution to a neuro-inflammatory environment. Furthermore, we show decreased cellular apoptosis and Aβ1–42 neurotoxicity on removal of the type-1 IFN signaling cascade. Importantly, this study confirms a role for type-1 IFN production and signaling in the neuro-inflammation associated with the progression of AD.

2. Methods

2.1. Antibodies

Primary antibodies used for immunohistochemistry: rabbit anti-p-STAT-3 (1:50, Santa Cruz, sc-135649, Santa Cruz, CA, USA), mouse anti-FOX-3 (1:250, Abcam, ab104224, Cambridge, MA, USA), mouse anti-GFAP (1:1000, Cell Signaling, 3670S, Danvers, MA, USA). Secondary antibodies used for immunohistochemistry: Alexa fluor 488 goat anti-rabbit and Alexa fluor 594 goat anti-mouse (1:1000, Life Technologies, A11008 and A11012, Carlsbad, CA, USA). Primary antibodies used for western blot analysis: rabbit anti-p-STAT-3 (1:1000, Cell Signaling, 9145S), rabbit anti-STAT-3 (1:1000, Cell Signaling, 4904S), rabbit anti-IRF-7 (1:500, Cell Signaling, 4920S, Oakville, ON, Canada), mouse anti-β-Tubulin (1:20,000, Millipore, MAB3408), mouse anti-β-actin (1:5000, Sigma-Aldrich, A5441). Secondary antibodies used for western blot: horseradish peroxidize conjugated goat anti-rabbit and goat anti-mouse (1:1000, Dako, P0448 and P0447, Carpinteria, CA, USA).

2.2. Mice

APP/PS1 mice on a dual C57Bl/6 and C3H/Hej genetic background were sourced from JAX (stock #: 004462) (9–12 months of age; 2 males and 2 females for each genotype). IFNAR1-/- mice on a pure C57Bl/6 background were initially generated by (Hwang et al., 1995). Age-matched littermate wild type control mice were used in all experiments.

2.3. Mixed cortical and hippocampal neuron isolation

Mixed cortical and hippocampal neurons were isolated from embryonic day 14–16 embryos as previously described (Taylor et al., 2005). Cultures were treated at 7 days in vitro. Purity of cultures was confirmed to be >95% neurons by NeuN and GFAP staining to identify neurons and glia, respectively.

2.4. Mouse brain immunohistochemistry

Immunohistochemistry was performed on 10 μm sagittal cryostat sections. Brain sections were permeabilized in phosphate buffered saline (PBS) and/or Tween-20 for 15 minutes at 25 °C, before a blocking incubation using CAS-Block (Life Technologies) for 60 minutes at 25 °C. After a brief PBS wash, primary antibodies were diluted in 1% (wt/vol) bovine serum albumin (BSA) in PBS and incubated overnight at 4 °C. Sections were then washed in 3 changes of PBS before being incubated for 2 hours at 25 °C with fluorescent secondary antibodies diluted in 1% (wt/vol) BSA. Following a further 3 PBS washes, sections were mounted using VECTASHIELD Mounting Medium with 4′,6-diamidino-2-phenylindole (DAPI) (Vector laboratories, Burlingame, CA, USA). Images were taken under water immersion on a Leica (Wetzlar, Germany) DMI 6000 B fluorescence microscope. Aβ plaques were identified using the ProteoStat Amyloid plaque detection kit (Enzo Lifesciences, Farmingdale, NY, USA) according to manufacturers protocol. Plaques were imaged under water immersion on a Leica DMI 6000B fluorescence microscope (Texas Red filter).

2.5. M17 neuroblastoma cells

Human BE(2)M17 neuroblastoma cells (ATCC number: CRL-2267) were cultured in T75 flasks with culture medium (Opti-MEM Gibco, Carlsbad, CA, USA), 5% fetal bovine serum, 0.5% penicillin-streptomycin (Gibco) at 37 °C and/or 5% CO_2 until 90% confluent. Cells were plated at 2×10^5cells/mL in 6 cm dishes for 24 hours before the treatment.

2.6. Generation of M17 IFNAR1 knockdown cell line

IFNAR1 (IFNAR1-KD) or negative control (NC) knockdown M17 cells were generated using commercially available shRNA plasmid constructs (Origene, Madison, WI, USA). Briefly, M17 cells were transfected using FugeneHD (Promega) with HuSH shRNA plasmids containing an IFNAR1 specific shRNA cassette or noneffective 29-mer scrambled shRNA cassette with a green fluorescent protein (GFP) tag. Clonal cell lines were generated using the selectable marker puromycin (0.5 mg/mL, Gibco). Successful knockdown of IFNAR1 expression (>70%) was confirmed by quantitative-polymerase chain reaction (qPCR) where IFNAR1 levels in IFNAR1-KD M17cells were compared with NC M17cells (Supplementary Fig. 1).

2.7. Human samples

Pre-frontal cortex postmortem samples from human AD patients (age 70–90 years) were sourced from the Australian brain bank (n = 9; 5 male and 5 female). Control tissue (n = 9) was sourced from patients where a demented state was known not to contribute to the cause of death.

2.8. Amyloid-beta preparation and treatment

Amyloid peptide stocks were created according to protocol by (Barnham et al., 2003). Aβ1–42 (peptide corresponding to amino acids 1–42) and the reversed Aβ42–1 (American Peptide Co, Sunnyvale, CA, USA; 62-080 and 62-0-81) were initially reconstituted in 1,1,1,3,3,3-Hexafluoro-2-propanol at 0.5 mg/mL, lyophilized, and stored at −80 °C until required. The peptide was then dissolved in a 5 mM NaOH vehicle and protein concentration determined by absorbance spectrophotometry at 214 nm. Primary neuronal cultures were then treated with 0.5–10 μM Aβ1–42, Aβ42–1 or vehicle for up to 72 hours in treatment medium (neurobasal media containing anti-oxidant free B27 supplement). M17 NC shRNA and M17 IFNAR1-KD cultures were treated with 5 μM Aβ1–42 for up to 96 hours in fresh culture medium. The final NaOH concentration in the culture medium was <5nM and shown to be nontoxic.