Astrocytes Infected with Chlamydia pneumoniae Alter Amyloid Processing Implicated in Alzheimer’s Disease

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Abstract

Background and Significance: Alzheimer’s Disease (AD) is a chronic, progressive neurodegenerative disease whose pathogenesis centers around the abnormal processing of amyloid precursor protein (APP) by proteases, resulting in the formation of toxic plaques composed of toxic, insoluble fragments of amyloid protein (Aβ), including Aβ1-40 and Aβ1-42. Previously, our laboratory identified Chlamydia pneumoniae (Cpn) in autopsyed sporadic AD brains. Additionally, an infection-based animal model was developed using BALB/c mice that were intranasally inoculated with Cpn, in which the deposition of amyloid was constant with that observed in the human AD brain. These studies have led to the hypothesis that AD is caused by Cpn producing an infectious stress on brain cells, leading to the formation of Aβ.

Objective: Several studies have demonstrated in vivo and in vitro the impact of Cpn infection on amyloid formation and processing. In the present study, the impact of Cpn infection on human astrocytes and in vitro processing of APP was evaluated.

Methods: Human astrocytes (CCF-STTG1) were infected with Cpn and processed for Aβ plaque formation, protein levels for Aβ, and the enzyme BACE1.

Results: The results demonstrate that Cpn infection results in an increase in Aβ plaque formation and a decrease in BACE1 levels.

Conclusions: The results demonstrate that Cpn infection results in an increase in Aβ plaque formation and a decrease in BACE1 levels, which may contribute to the progression of AD.

Keywords: Alzheimer’s Disease (AD), Chlamydia pneumoniae (Cpn), Amyloid, Astrocytes, BACE1

Human Astrocytes Infected with Chlamydia pneumoniae (Cpn) and Immunolabeled with Antibodies Specific for Isoforms of Aβ and BACE1

Anti-Beta Amyloid 1-16 (6E10)

Anti-BACE1 (ab10716)

Figure 1: Human CCF-STTG1 astrocytes immunolabeled with anti-Chlamydia, anti-amyloid, and anti-BACE1 antibodies. Aβ1-16 and BACE1 were labeled with 6E10 (mouse, monoclonal) while amino acid 485-501 of BACE1 was labeled with ab10716 (rabbit, polyclonal). Secondary antibodies were Alexa-Fluor 594-conjugated.

Materials and Methods

Human astrocyte cultures, CCF-STTG1 (ATCC, CRL-1330), were infected with Chlamydia pneumoniae (Cpn; ATCC, B-1528) on a MOI of 5 for 48 to 72 hours. The cells were grown on 12x75mm glass coverslips and cultured in DMEM/F12 (1:1) supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, and 100 units/ml penicillin/streptomycin. Cells were infected with Cpn at an MOI of 1 for 72 hours. Control cultures were mock-infected with DMEM/F12. Six hours post-infection, cells were fixed with 4% paraformaldehyde (PFA) for 20 minutes and permeabilized with 0.3% Triton X-100 for 5 minutes. The cells were labeled for Aβ1-16 using 6E10 (mouse, monoclonal) and BACE1 using ab10716 (rabbit, polyclonal). Secondary antibodies were Alexa Fluor 594-conjugated (Invitrogen). The cells were imaged using a Leica TCS-SP5 confocal microscope.

Conclusions

1. The present study investigates the downstream effects of APP processing in human astrocytes infected with Cpn. The results demonstrate that Cpn infection results in an increase in Aβ plaque formation and a decrease in BACE1 levels, which may contribute to the progression of AD.

2. The mechanism by which Cpn infection results in a reduction in BACE1 levels is being further investigated.

3. The results suggest that Cpn infection may contribute to the progression of AD.

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