Astrocytes Infected with *Chlamydia pneumonia* 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 neuritic plaques composed of toxic, insoluble fragments of amyloid protein (AB), including AB1-40 and AB1-42. Previously, our laboratory identified *Chlamydia pneumoniae* (Cpn) in autopsied 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 consistent with that observed in the human AD brain. These studies have led to the pathogen hypothesis of AD that implicates Cpn as a trigger for the cleavage of APP into AB1-40 and AB1-42.

Objective: Several studies have demonstrated the presence of astrocytes surrounding neuritic plaques within the AD brain; therefore, we speculate that astrocytes may be specifically involved in the pathological processes leading to AB deposition. This investigation addresses if an *in vitro* Cpn infection of human astrocytes affects processing of the B amyloid precursor protein (BAPP) and the enzyme B-APP cleaving enzyme-1 (BACE1), a type 1 transmembrane aspartyl protease implicated in numerous neurodegenerative diseases.

Methods: Human astrocytes (CCF-STTG1) were infected *in vitro* with the respiratory strain AR39 Cpn (MOI=1). Analysis of protein levels for AB and the enzyme BACE1 post-infection was detected by immunocytochemistry and captured with the Olympus Confocal FV1000 microscope.

Results: Amyloid processing in infected astrocytes was altered relative to that of uninfected astrocytes. BACE1 immunolabeling appeared more diffuse in the uninfected astrocytes as compared to membrane-localized BACE1 in the infected astrocytes.

Conclusions: Neurons have been presumed to be the primary source of beta-amyloid peptides in AD brains; however, when astrocytes are activated, as occurs during infection with Cpn, astrocytic beta-amyloid generation may contribute to amyloid plaque formation. These data imply that infection of human astrocytes with Cpn affects the processing of BAPP through altering the levels of the BACE1 protease. These data suggest an activation of BACE1 in the processing of amyloid by astrocytes as a major contributor to the neurotoxic amyloid deposition linked to pathology observed in AD.

Processing of Amyloid Precursor Protein

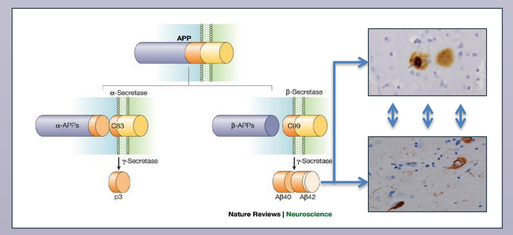

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Image: Image adapted from: Citron, M. (2010). Alzheimer's disease: strategies for disease modification. Nat. Rev. Drug Discov. 9, 387-398.

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

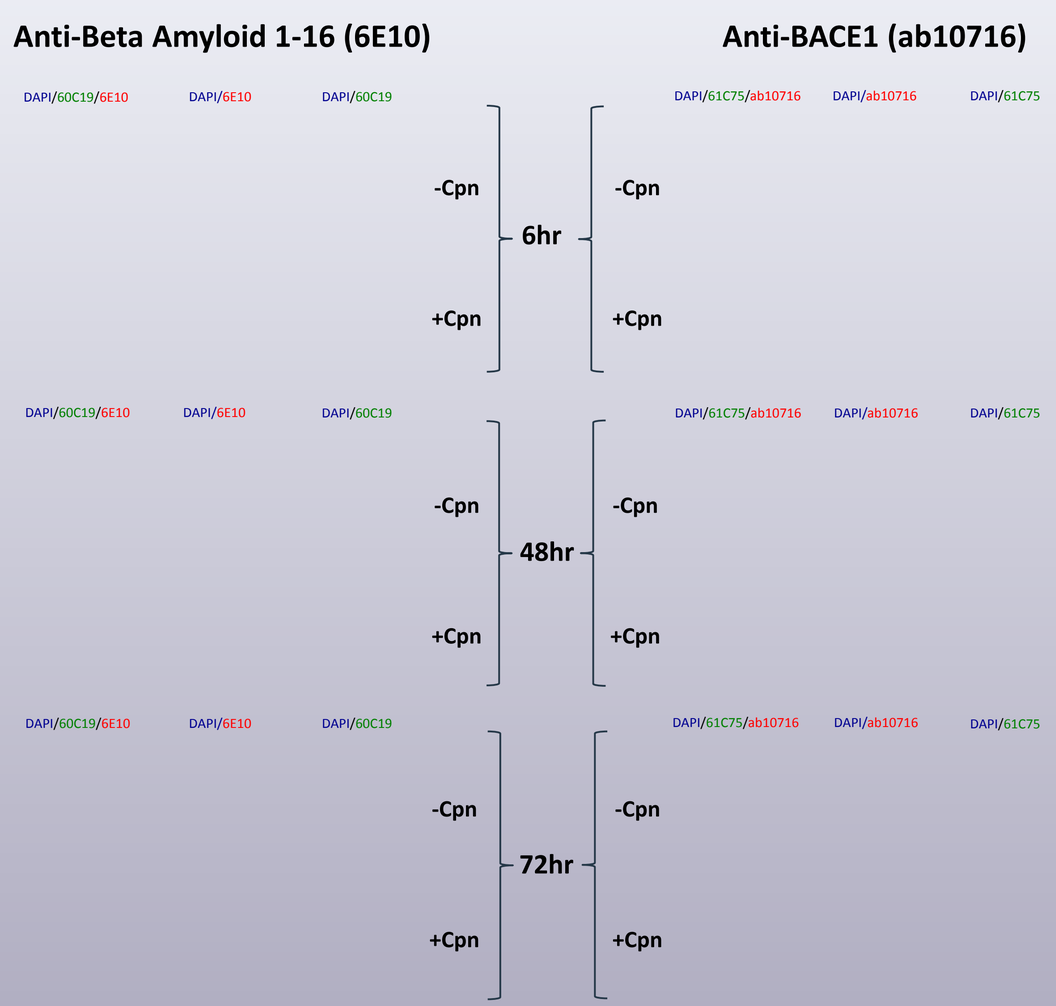

Figure 1: Human CCF-STTG1 astrocytes immunolabeled with anti-*Chlamydial,* anti-amyloid, and anti-BACE1 antibodies. CPn was labeled using FITC direct tag anti-*Chlamydia* antibodies 60C19 and 61C75A. Amino acids 1-16 of Aβ was labeled with 6E10 (mouse, monoclonal) while amino acids 485-501 of BACE1 was labeled with ab10716 (rabbit, polyclonal). Secondary antibodies were Alexa-Fluor 594-tagged.

Background

Alzheimer's Disease (AD) is a chronic, progressive neurodegenerative disease that afflicts nearly 5.2 million Americans and ranks among the top 10 causes of death in the US that can neither be prevented nor cured (8). The pathogenesis of AD centers on the abnormal proteolytic processing of amyloid precursor protein (APP) by proteases such as β-APP cleaving enzyme 1 (BACE1), resulting in the formation of toxic and insoluble fragments of amyloid protein (Aβ) of variable lengths, including Aβ1-40 and Aβ1-42 (6,7) that are primarily localized to the hippocampus and amygdala (4). BACE1, as the first protease to initiate Aβ production, has been shown to increase in activity and protein levels in the tissue and CSF of AD brains (3). Within astrocytes specifically, BACE1 is regulated post-translationaly in a feed-forward mechanism in response to inflammatory cytokines (i.e. TNF-α and IFN-γ) and Aβ oligomers (3, 9).

Previous studies using autopsied AD brain tissue have identified genetic material of viable *Chlamydia pneumoniae* (CPn), an obligate intracellular, gram-negative respiratory pathogen (2). Once intranasally acquired, CPn is thought to pass through the olfactory bulb and into the entorhinal and perirhinal cortices, hippocampus, and amygdala (5). This proposed route of CPn travel is also supported by the clinically apparent loss of smell (anosmia) notable in the earlier stages of the disease (1). *In vitro* studies describing the glial response to infection, however, are lacking. Therefore, this study will attempt to profile APP processing and the localization of BACE1 in human CCF-STTG1 astrocytes infected with CPn AR39.

Materials and Methods

Human astrocytoma cells, CCF-STTG1 (ATCC, CRL1718), were infected with *Chlamydia pneumonia* (*CPn*, ATCC, 53592) at an MOI of 1 for 6 to 72 hours. The cells were grown on 12mm glass coverslips (Neuvitro, GG-12) and stained using CPn direct tag antibodies: 60C19 FITC-direct tag (Fitzgerald, 5861188) or 61C75A FITC-direct tag (Fitzgerald, 5861504) at a dilution of 1:100. Coverslips were washed and labeled with primary amyloid antibodies: 6E10 detecting amino acids 1-16 of Aβ (BioLegend, sig-39320) or anti-BACE1 detecting amino acids 485-501 of BACE1 (Abcam, ab10716) at a dilution of 1:500. Secondary goat-derived antibodies tagged with Alexa-Fluor 594 (Life Technologies, A-11005) were applied after additional washing and blocking steps. The labeled cells were mounted on glass slides using FLUORO-GEL II with DAPI (EMS, 17985-50). Samples were observed using a Olympus Confocal FV1000 microscope.

Conclusions

- The present study investigates the downstream products of βAPP processing in human astrocytoma cells infected with a respiratory strain of *Chlamydia pneumoniae* (CPn). Infected astrocytes showed a minimal increase in amyloid labeling from 6 hours to 72 hours post infection as compared to that of uninfected astrocytes, indicating enhanced βAPP processing at these timepoints.
- BACE1 labeling was also more robust along the astrocytic membrane after 6 hours of infection and progressed through 72 hours post infection.
- The mechanism by which βAPP processing and BACE1 protein levels increase in response to cellular stress (e.g. infection) remains to be elucidated. Quantification of intracellular and secreted amyloid and enzymatically active BACE1 protease would validate these findings. Additionally, mRNA expression assays would provide further insight into how both APP and secretase genes are regulated in response to an infectious process.

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