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

**Anti-Beta Amyloid 1-16 (6E10)**

<table>
<thead>
<tr>
<th>DAPP50CCTG</th>
<th>DAPP56TGG</th>
<th>DAPP60C19</th>
</tr>
</thead>
<tbody>
<tr>
<td>+Cpn</td>
<td>-Cpn</td>
<td>6hr</td>
</tr>
</tbody>
</table>

**Anti-BACE1 (ab10716)**

<table>
<thead>
<tr>
<th>DAPP50CCTG</th>
<th>DAPP56TGG</th>
<th>DAPP60C19</th>
</tr>
</thead>
<tbody>
<tr>
<td>+Cpn</td>
<td>-Cpn</td>
<td>48hr</td>
</tr>
<tr>
<td>+Cpn</td>
<td>+Cpn</td>
<td>72hr</td>
</tr>
</tbody>
</table>

**Background**

Alzheimer's Disease (AD) is a chronic, progressive neurodegenerative disease that affects 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 BACE1. Disease progression is characterized by the formation of toxic, insoluble fragments of amyloid protein (Aβ) of variable lengths, including Aβ40 and Aβ42 (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-translationally in a feed-forward manner in response to inflammatory cytokines (i.e. TNF-a and IFN-γ) and Aβ oligomers (1, 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 intracellularly captured, 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 traversal is also supported by the clinically apparent loss of smell (anosmia) notable in the earlier stages of the disease (1). In vivo studies describing the gial response to infection, however, are lacking. Therefore, this study will attempt to profile APP processing and the localization of BACE1 in human CCF-STT1G astrocytes infected with CPn-AK39.

**Materials and Methods**

Human astrocytoma cells, CCF-STT1G (ATCC, CRL-19280), were infected with *Chlamydia pneumoniae* (CPn. ATCC, B-744) on an MOI of 1 for 48 to 72 hours. The cells were grown on 24 well glass coverslips that were coated with 3.5% gelatin in PBS and incubated at 37°C, 5% CO2 in humidified atmosphere. Multiplicity of infection was determined using optical microscopy (1, 6, 8, 9). Detection of bacterial antigens and Alzheimer's disease was achieved using IFN-γ (BioLegend, 501101) at a dilution of 1:100. Secondary goat anti-mouse antibody conjugated with Alexa Fluor 647 (Life Technologies, A-21235) was added to the cells. Immunolabeling from 6 hours to 72 hours post infection as compared to that of uninfected astrocytes. BACE1 immunolabeling appeared more diffuse in the uninfected astrocytes as compared to membrane-labeled 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 AD.

**Processing of Amyloid Precursor Protein**

**References**

2. Vassar, R., Zheng, B. J. L., C.S.; Hammond, C.J.; Portier, D.M.; Delatour, D.; and Whittum, J. (2014). Detection of bacterial antigens and Alzheimer’s disease in the central nervous system of BALB/c mice following infection with *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 clearance of APP into Aβ1 and Aβ42.
4. Previously, our laboratory identified a 36"x60" presentation — save online to —
5. AR39. Cpn (MOI=1). Analysis of protein levels for Aβ and the enzyme BACE1 post-infection was detected by immunocytochemistry and captured with the Olympus Confocal FV1000 microscope.

**Conclusions**

The present study investigates the downstream mechanisms of APP 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 BACE1 processing at these timepoints. BACE1 labeling was also more robust along the astrotic membrane after 6 hours of infection and progressed through 72 hours post infection. The mechanism by which BACE1 processing and BACE1 protein level increases in response to cellular stress (i.e. infection) is still 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.

**Funding**

This work was funded by Dr. Gupta, the Center for Chronic Disorders of Aging (CCDA), and the Division of Research in the Philadelphia College of Osteopathic Medicine and the Adolph and Rose Leventhal Foundation for Alzheimer’s disease research.

**Acknowledgments**

We would like to thank Marcel Valer and Gannia Pareau for their assistance in the maintenance of our cell cultures.

---

**Image 1:** Human CCF-STT1G astrocytes immunolabeled with anti-Chlamydia, anti-amyloid, and anti-BACE1 antibodies. Cpn was labeled using FITC direct tag anti-Chlamydia antibodies B2C2 and B1C7A. 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-Flour 594-tagged.