Contribution of Infection with Cytomegalovirus (CMV) to the Production of Amyloid Precursor Protein (APP) and Alzheimer's Disease-associated Beta Amyloid

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ABSTRACT

The accumulation of harmful proteins in the brain, particularly amyloid-β peptides, is a hallmark of Alzheimer's disease, a neurodegenerative disorder for which the specific cause is unknown. Infection with cytomegalovirus (CMV) may be a factor contributing to the development of Alzheimer's disease because disease incidence is higher in persons who have been infected with CMV. In previous work, we determined that CMV infection increased phosphorylation of tau, the protein that forms neurofibrillary tangles in Alzheimer's disease. Here, we determine if CMV infection of neuron-like cells also contributes to the production of beta-amyloid. To address this, we will infect B35 neuroblastoma cells with murine CMV, and determine whether the production of amyloid precursor protein (APP) and beta-amyloid is altered using western blotting. If so, we will design experiments to determine how CMV infection alters APP processing and beta amyloid production, shedding light on the pathological mechanisms of Alzheimer's disease.

HYPOTHESIS

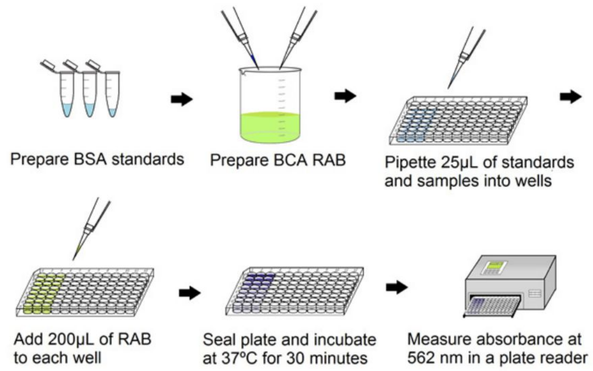
We hypothesize that CMV infection will differentially modify expression or processing of amyloid precursor protein in cells such as B35 neuroblastoma cells, salivary gland epithelial cells, macrophages, and fibroblasts, all of which represent cell types which may be infected with the virus. We will address this hypothesis by observing the virological features of cytomegalovirus (CMV) infection, particularly changes in amyloid precursor protein or amyloid peptides after CMV infection.

INTRODUCTION

Human cytomegalovirus (CMV) infection is prevalent in causing serious neurological complications. CMV is a major common virus that causes mental defects and hearing loss in neonates. Consequently, studies show that it, and other herpesviruses, may contribute to the development of Alzheimer's disease. Alzheimer disease is the world's leading cause of dementia, and it is important to understand how infection with CMV can increase incidence of Alzheimer's disease. Our goal is to analyze amyloid-beta precursor proteins (APP) and possible changes in cellular amyloidogenic pathways. It is important to study what is happening to amyloid-beta precursor proteins in cytomegalovirus infection because when alterations are detected associated with infection, we could move onto the viral interactions of proteins to determine other neural functional consequences. Henceforth, we will be using western blotting to compare cell lines levels of APP within 48 hours, with or without infection to see if there is evidence of changes in synthesis or degradation. We are also including analysis of samples including treatment with LiCl, which inhibits replication of the virus, to see how this inhibitor, which is already approved for use in people with bipolar disorder, affects any changes in APP or the amyloid pathways.

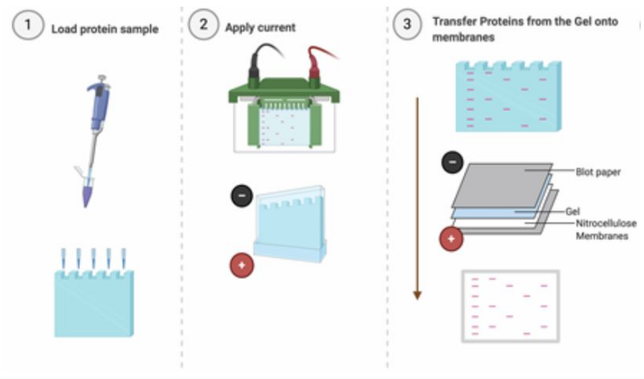
METHODS

Experiment 1, Part 1: Pierce BCA Protein Assay Kit (Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 20 - 2,000 µg/mL)



- We prepare the diluted bovine serum albumin (BSA) standards and used pre-prepared cell lysates.
- Pipetted 25 µL of each standard and unknown samples (B35, SCG-1, or NIH 3T3 cells either mock-infected or infected with mCMV. LiCl-treated B35 cells were also tested) in duplicate into a microplate well.
- Added working reagent, incubated 30 min at 37°C and read absorbance at 562 nm.

Experiment 1, Part 2: Western Blotting (reducing, denaturing)

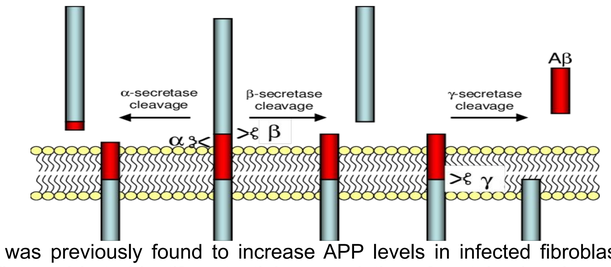


- Lysed samples were provided/prepared by Laura Hanson's lab. Equivalent amounts of protein were loaded on SDS/PAGE gels, electrophoresed and transferred to nitrocellulose.
- Blots were probed with rabbit anti-APP and IRDye700 goat anti-rabbit secondary antibodies and visualized on a LiCor Odyssey Clx infrared imager.

DISCUSSION AND CONCLUSION

To find the contribution of cytomegalovirus (CMV), we used antibodies specific for amyloid-beta precursor proteins (APP) to analyze levels in the different cell types, with or without infection or treatment with the inhibitor lithium chloride (LiCl). Amyloid-beta precursor proteins is a type-I transmembrane protein that is extensively processed by different proteases that result in fragments of proteins that have recently been shown to have antimicrobial activity. For Alzheimer disease there is a sequence of secretases that cut the precursor proteins into pieces to produce the beta amyloid **(as explained in Figure 1).** There are two pathways of processing, one, which involves beta secretase and gamma secretase results in the pathological amyloid beta peptides. There is also an alternate pathway, involving alpha secretase, which does not lead to the peptides associated with the amyloid plaques associated with Alzheimer's disease. Therefore, by conducting a western blotting protocol which would examine levels of APP of cell lines from salivary gland cells, B-35 neuroblastoma cells, fibroblasts, and macrophages will dictate the next step to study potential up regulation after the cell lines are infected.

Figure 1: Processing of the amyloid precursor protein and generation of its derivatives APP, sAPP and A-beta.



Cytomegalovirus (CMV) was previously found to increase APP levels in infected fibroblasts and is associated with Alzheimer's pathology. The goal is to identify potential up-regulation of proteins by observing the difference in band intensities from infected SGC-1 salivary gland cells, B-35 neuroblastoma cells, fibroblasts, and macrophages vs non infected cells of the same type. Mechanisms of beta-amyloid production that are regulated by CMV infection have not been addressed. However, our lab has revealed that following CMV infection, there is a considerable increase in tau phosphorylation, including at regions linked with tau tangles, in either fibroblast cells or neuronal cells in culture. We also discovered that microtubule stability is impaired in infected neuronal-type cells (unpublished results). The studies that I am conducting right now examine moderating the levels of APP to identify if there is increasing the synthesis or decreasing degradation. Should CMV infection alter APP levels, we will next investigated whether infection alters the activity of secretases involved in APP processing.

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