Background and Significance: The pathogenic Chlamydia pneumoniae (Cp) and herpes simplex virus type 1 (HSV-1) have been studied as pathogens contributing to neurodegenerative disorders of the CNS, such as Alzheimer’s disease. Although many individuals are presumed to be exposed to both pathogens during their lifetime, it is currently unknown whether co-infections occur in vivo or in vivo, and if so, whether this combination affects the intracellular environment that induces persistence in astrocytes.

Introduction:
The ongoing studies investigate the potential role of herpes simplex virus type 1 (HSV-1) and Chlamydia pneumoniae (Cp) in the context of neurodegenerative disorders, such as Alzheimer’s disease. Since both HSV-1 and Cp are able to establish and have the ability to coexist in the CNS with the potential for reactivation to a productive infection, it is necessary to understand how infection by either or both pathogens might contribute to the gradual accumulation of pathology associated with neurodegeneration. This current study examines whether an astrocytic cell line can be simultaneously infected with these two pathogens and if co-infection alters pathogens replication or/and facilitates the pathogenesis associated with infection by either pathogen individually.

Results:
1) HSV-1 and Cp can utilize heparin sulfate as receptors, thus the presence of one pathogen might interfere with attachment and internalization of the second pathogens. Furthermore, Cp enters a state of persistence in response to changes in the CNS environment and we speculate that production of the Cp infection-regulated as an intracellular environment that induces persistence in CNS. It is possible that both Cp and HSV-1 might cycle through the same cycle of changes in the intracellular environment and thereby contributing to progressive neurodegeneration.

2) To assess whether the presence of HSV-1 alters Cp gene expression, the relative levels of 6 different Cp genes involved in different stages of the replication cycle were examined in RNA from cells infected with Cp alone or dually infected with Cp and HSV-1. The genes examined are listed in Table 1. LH1, 7TIF, and ABCB were designated as potential chlamydial housekeeping genes whose expressions remain relatively constant throughout the replication cycle (13). In this study, we use a 6-plex as an efficient method to determine the real-time gene expression levels simultaneously within an experiment. Immunofluorescence and determination of viral titers provide additional methodologies to further evaluate HSV-1 infection of astrocytes.

Conclusions:
• The presence of Cp may inhibit viral attachment or entry.
• Cp does not prevent HSV-1-induced cytopathology.
• HSV-1 can alter gene expression in astrocytes.
• The infection of one pathogen does not prevent intracellular replication of the other one.
• Differential Cp gene expression is not observed in single vs dual infections with the same pathogen.