Analysis of autophagy and inflammasome regulation in neuronal cells and monocytes infected with Chlamydia pneumoniae: Implications for Alzheimer’s disease

B. Balin, C. Hammond, J. Zoga, A. Cader, A. Slutter, J. Anzmann, I. Kohler, S. Hingley, D. Appelt
Center for Chronic Disorders of Aging, Philadelphia College of Osteopathic Medicine, Philadelphia, PA, USA

Abstract

Objectsives: Our laboratory has been studying the role of infection with the obligate intracellular bacterium Chlamydia pneumoniae in sporadic late-onset Alzheimer disease (LOAD). This infection may be a trigger for the formation and progression in LOAD as a function of innate immune gene regulation following entry of the organism into the brain. As such, we are probing whether this infection can promote changes in autophagy and inflammasome gene regulation as both have been shown to be altered in LOAD.

Methods: Human SK-N-MC neuronal cells and THP-1 monocytes were infected in vitro for 24-72 hrs with a laboratory strain of Chlamydia pneumoniae followed by RNA extraction, cDNA synthesis and analysis using Real-Time PCR microarrays for autophagy and inflammasome genes.

Results: Gene expression for autophagy and inflammasome pathways was altered dramatically following infection. Genes encoding for co-regulation of autophagy, apoptosis, and the cell cycle that were significantly changed included BAX, FAS, PIK3CG, APP, and TP53. In addition, ATG3 and GABARAP, genes encoding for protein transport & ubiquitination and autophagic vacuole formation were significantly deregulated. Of the inflammasome genes, 4 NOD-like receptor genes were significantly up-regulated. IL-1β, IL18, CCL2, and CCL7 genes were all dramatically up-regulated in monocytes during the 72 hrs of infection.

Conclusions: Our data suggest that Chlamydia pneumoniae-infected human SKMNC neuronal cells and THP-1 monocytes exhibit specific changes in gene regulation for both autophagy and inflammasome pathways. These gene changes appear to correlate with pathologic changes previously reported in AD and further support the contention that infection with Chlamydia pneumoniae plays a role in LOAD pathogenesis.

Introduction

Neurodegeneration has been well documented in the CNS of Alzheimer individuals. Strong evidence suggests that abnormalities of autophagy and apoptosis pathways as well as activation of inflammasomes are contributing factors in Alzheimer’s disease (AD) pathogenesis. Our laboratory has focused on infection with Chlamydia pneumoniae (Cpn) as a risk factor/pathological feature in LOAD. Cpn is an obligate, intracellular, parasitic bacterium. Cpn is transmitted from person to person via respiration. Once inhaled, Cpn may enter the brain along 2 pathways, directly through olfaction and/or blood-borne in monocytes. In studies of AD brain tissues, we have identified Cpn in areas of neopatohology and demyelination. Cpn was detected in 80%-90% of post-mortem LOAD brain samples, but only in 5%-11% of brains from age-matched non-AD controls (Balin et al., 1998; Gerard et al., 2000; Hammond et al., 2010). Inflamed glia, perivascular macrophages, monocytes, and neurons have been observed in the AD brain. Infection may result in early neuroinflammation and neuronal damage, in specific vulnerable regions of the brain (Balin et al., 1998). In analyzing cellular responses following infection, we have demonstrated that Cpn can inhibit apoptosis in neuronal cells thereby protecting the viability of the infected cells (Appelt et al., 2008). Other laboratories have demonstrated that chlamydiae-infected host cells are resistant to proapoptotic stimuli such as TNFα, Fas antibodies, and UV-light (Fischer et al., 2006). Further, as Cpn is an intracellular bacterium, we have started investigating autophagy and inflammasome activation of the host cell as these mechanisms are commonly employed by eukaryotic cells to eradicate intracellular organisms.

Autophagy and apoptosis are common pathways by which infected cells attempt to rid themselves of an infectious agent and cells incapable of eliminating the infectious agent undergo cell death. Autophagy is associated with the endocytosomal-lysosomal system. The endosomal pathway is linked to the lysosomal system as early endosomes with late endosomes or lysosomes. Contents of an autophagosome are degraded as a result of fusing with lysosomes (Fischer et al., 2004, Funderburk et al., 2010). An increase in the number of autophagic vacuoles (AVs) has been identified in neurons from AD brains implicating autophagy as a physiological process in AD (Lee et al., 2011). Neurons from AD brains do have enlarged early endosomes which is significant because early endosomes take in proteins such as apolipoprotein E and APP, and Aβ has been demonstrated to be formed in early endosomes (Neen et al., 2011). An additional prominent feature in AD is neuroinflammation (Akaiyama et al., 2000). Recent evidence implicates the pro-inflammatory process with production of (IL-1β) in mild cognitive impairment and early AD (Agostinho et al., 2010). A proinflammatory signal typically follows from an infection leading to NF-κB activation and synthesis of (IL-1β). A second signal activates caspase-1, which cleaves pro-IL-1β into its mature form, IL-1β (He et al., 2010). The inflammasome contains three proteins, caspase-1, apoptosis-associated speck-like containing a caspase recruitment domain (ASC), and a nucelds base-binding oligomerization domain-like receptor (NLR). When an inflammatory response is needed, these three proteins will aggregate in order to cleave pro-caspase-1 and initiate the inflammatory response (He et al., 2010).

One specific inflammasome, NLRP-3, when activated produces IL-1β in response to various fungal, viral, and/or bacterial infections including those caused by Cpn (He et al., 2010). Chlamydia utilize a type III secretion system to secrete virulence factors into the host cell cytosol to control intracellular reactions. These factors cause K+ efflux and formation of reactive oxygen species. This rise in reactive oxygen species is sufficient to initiate assembly of the NLRP-3 inflammasome (Abdul-Sater et al., 2009).

How Cpn infection affects autophagy and inflammasome gene expression in eukaryotic cells is important for understanding the role that infection plays in initiating acute damage and eventual chronic inflammatory responses resulting in AD pathogenesis.

Materials and Methods

Cell Lines: Human SK-N-MC neuronal cells and THP-1 monocytes obtained from the ATCC were used in these studies.

Infection with Chlamydia pneumoniae (Cpn)
Cpn-infected neuronal cells and THP-1 monocytes were infected at a multiplicity of infection (MOI) of 1. Infected neuroblastoma cells (NBL-1) were used for all infections to 24, 48, and 72hrs. For the neuronal cells, Cpn was added to a substrate monolayer followed by centrifugation at 800 g for 5 min and incubated at the desired timepoints. Monocytes were centrifuged, washed, and repopulated, followed by the addition of Cpn and incubated for the same time periods. Parallel uninfected control cells were grown under the same conditions for the times indicated.

RNA Isolation and cDNA Synthesis
RNA was extracted using the RNeasy Plus Micro kit from Qiagen, followed by cDNA production from RNA (1ug) using the RTi First Strand Kit from SABiosciences (Gaithersburg, MD), following manufacturer’s directions.

Real Time- Polymerase Chain Reaction (RT-PCR)
RT-PCR for gene transcription used TaqMan (Hs-PNLC-96AC) and Inflammasome (PAHSS-097A) PCR Arrays from SABiosciences (Qiagen). Arrays were run on the ABI Prism 7000 Sequence Detection System from Applied Biosciences. The results were analyzed using software from SABiosciences (www.sabiosciences.com/primers/realtime/). Arrays were run on Cpn-infected SK-N-MC neuronal cells, THP-1 monocytes, and their uninfected controls at 24, 48, and 72hrs with each experiment performed in triplicate.

Data Analysis:
- For autophagy genes, a significance of (p<0.05) and fold changes of ≥2.0 were represented in the charts.
- For inflammasome genes, only those greater than 4-fold change will significance of (p<0.05) are represented in the charts.

References

Our study provides a mechanistic foundation for infection as an initiator and propagator in the pathogenesis of Alzheimer’s disease.