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

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Abstract

Objectives: Our laboratory has been studying the role of inflammation with the obligate intracellular bacterium Chlamydia pneumoniae in sporadic late-onset Alzheimer disease (LOAD). This infection may be a trigger for the pathology observed in LOAD as a function of initiating changes in gene regulation following entry of the organism into the brain. As such, we are analyzing how 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: BCL2L1, 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, NOD2, 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 SKMC neuronal cells and THP-1 monocytes exhibit specific changes in gene regulation for both autophagy and inflammasomes 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's individuals. Strong evidence suggests that alterations of autophagy and apoptosis pathways as well as addition of inflammation are contributing factors in Alzheimer's disease (AD) pathogenesis. Our laboratory has focused on infection with Chlamydia pneumoniae (Cpn) as a risk factor causative agent in LOAD. Cpn is an intracellular, parasitic bacterium. Cpn is transmitted from person to person via respiration. Once infected, Cpn may enter the brain along 2 pathways, directly through olfaction or through blood-borne in monocytes. In studies of AD brain tissues, we have identified Cpn in areas of neurophagitis by PCR and immunochemistry. Cpn was detected in 80 to 90% of post-mortem LOAD brain samples, but only in 5-11% of brains from age-matched non-AD controls (Bain et al., 1998; Gerad et al., 2006; Harmund et al., 2010). Infected glia, peryvascular 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 (Bain et al., 1998). In analyzing cellular changes following infection, we have demonstrated that Cpn can inhibit apoptosis in neuronal cells thereby prolonging the viability of the infected cells (Appelt et al., 2008). Other laboratories have demonstrated that chlamydia-infected host cells are resistant to pro-apoptotic stimuli such as TNFα, Fas antibody, staurosporine, and UV-light (Fischer et al., 2004). 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 cell death and cell survival and remains the lysosomal system. The endosomal pathway is linked to the lysosomal system as early endosomes fuse 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 AD brains implicating autophagy as a pathological 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 that have been demonstrated to be formed in early endosomes (Wisen et al., 2011).

An additional prominent feature in AD is neuroinflammation (Akhyama et al., 2000). Recent evidence implicates the pre-inflammation process with production of IL-1β in mid cognitive impairment and early AD (Aposthi et al., 2010). A proinflammatory signal typically follows from an infection leading to NF-kB activation and synthesis of pro-IL-1β. A second signal activates caspase-1 which cleaves pro-IL-1β into its mature form, IL-1β. Caspase-1 is derived from pro-caspsa-1 following cleavage by a multiprotein complex called an inflammasome (He et al., 2010). The inflammasome contains three proteins, caspase-1, apoptosis associated speck-like protein containing a caspase recruitment domain (ASC), and a nucleotide-binding oligomerization domain-like receptor (NLR). When an inflammasome is recruited, the three proteins will aggregate in order to cleave pro-caspsa-1 and initiate the inflammatory response (He et al., 2010).

One specific inflammasome, NLRP3, when activated produces IL-1β, in response to various fungal, viral, and 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 NLRP3 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) - SK-N-MC and THP-1 cells were used to infect all cultures in 24, 48, and 72hrs. For the neuronal cells, Cpn was added to a subconfluent monolayer followed by centrifugation at 800 rpm for 5 min and incubated for the allotted time periods. Monocots were centrifuged, washed, and resuspended, 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 First Strand Synthesis - RNA was extracted using the RNeasy Plus Mini kit from Qiagen, followed by cDNA production from RNA (1µg) using the RT2 First Strand Kit from SABiosciences (Qiagen), all following manufacturer’s directions.

Real-Time – Polymerase Chain Reaction (RT-PCR) - RT-PCR for gene expression analysis of autophagy (PAHS-084A) and inflammasome (PAHS-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.com/pcrdataanalysis/pctools.html. Arrays were run on Cpn infected SK-N-MC neuronal cells, THP-1 monocytes, and their uninfected controls at 24, 48, and 72hs with each experiment performed in triplicate.

Data Analysis - For autophagy genes, in significance of p<0.05 and p<0.10. For inflammasome genes, only those greater than 4-fold change with significance of p<0.05 are represented on the charts.

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Data Analysis - For autophagy genes, in significance of p<0.05 and p<0.10. For inflammasome genes, only those greater than 4-fold change with significance of p<0.05 are represented on the charts.

• Genes encoding for inflammasomes are up-regulated in Cpn-infected neuronal and monocytes.
• Up-regulation of inflammasome genes may lead to pro-inflammatory cytokines and chemokine increase.
• These inflammasome genes (NFKB, MAPK, and NLRB 4) were significantly altered.

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