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 infection with the obligate intracellular bacterium Chlamydia pneumoniae in sporadic late-onset Alzheimer disease (LOAD). This infection may be a trigger for the pathology seen in LOAD as a function of initiating 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 SKMNC neuronal cells and THP1 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 arrays for autophagy and inflammasome genes.

Results: Gene expression for autophagy and inflammasome pathways was altered dramatically following infection. Genes encoding for key regulation of autophagy, apoptosis, and the cell cycle were significantly changed including BCL2, MAP1LC3B, and MAP1LC3A. In addition, ATG3 and GABARAP genes encoding for protein transport & ubiquilinization and autophagic vacuole formation were significantly deregulated. Of the inflammasome genes, 4 NOD-like receptor genes were significantly up-regulated. IL-1beta, AIM2, 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 THP1 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) pathology. Our laboratory has focused on infection with Chlamydia pneumoniae (Cpn) as a risk factor causative 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 tissue, we have identified Cpn in areas of neuroinflammation in postmortem brain tissue. 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 (Balin et al., 1998; Gerhard et al., 2008; Hammond et al., 2010). Infected 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 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 chlamydiae-infected host cells are resistant to proapoptotic stimuli such as TNFα, Fas-associated death domain, 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 a nuclear-enlarged lysosomal system. The endosomal turnover pathway is linked to early autophagy, 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 autophagosomes and autolysosomes has been observed in response to various fungal, viral, and/or bacterial infections including those caused by Cpn (He et al., 2010). Inflammasomes bridge signaling between pathogen identification and the immune response.

Materials and Methods

Cell lines - Human SKMNC neuronal cells and THP1 monocytes obtained from the ATCC were used in these studies.

Infection with Chlamydia pneumoniae (Cpn)

Infection of neuronal cells was carried out by inoculation with Cpn for 24, 48, and 72h. 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. Monolayers were centrifuged, washed, and resuspended, followed by addition of Cpn and incubated for the same period time. Parallel uninfected control cells were grown under the same conditions for the lines indicated.

RNA Isolation and cDNA Synthesis

RNA was extracted using the RNeasy Plus Mini kit from Qiagen, followed by cDNA synthesis from RNA (1ug) using the iScript First Strand Kit from BioRad (California, CA) following manufacturer’s directions.

Real-Time - Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed using the Viho RT-PCR kit. PCR was performed using primers specific for each gene. Amplification conditions for all primers were 15 cycles of 95°C for 60 sec, 59°C for 1 min, and 72°C for 1 min. PCR products were separated on a 2% agarose gel and visualized under UV light.

Data Analysis - For autophagy genes, * p value < 0.05 and ** p value < 0.10

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

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Introduction

Figures

• Genes encoding for inflammasomes are up-regulated in Cpn-infected neuronal cells

• Genes encoding for protein transport & ubiquilinization and autophagic vacuole formation are significantly deregulated in Cpn-infected neuronal cells

• Up-regulation of inflammasome genes may lead to pro-inflammatory cytokines and chemokine increase

Inflammasomes

Materials and Methods

Biological Factors

Autophagy

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References