

# Analysis by Real-Time PCR of Autophagy and Apoptosis in Neuronal Cells Infected with *Chlamydia Pneumoniae*

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## Abstract

**BACKGROUND:** Dysfunctions in cellular mechanisms such as apoptosis and autophagy have been implicated in the neurodegeneration associated with Alzheimer's disease (AD). Autophagy in AD pathogenesis has been linked to the endosomal-lysosomal system, which has been shown to play a role in amyloid processing. Studies have suggested that apoptosis may contribute to the neuronal cell loss observed in AD; however, there is no evidence of the apoptotic process leading to terminal completion. Aβ1-42 has been shown to induce apoptosis in neurons and may be an initiating factor in AD. Our previous studies demonstrated that neurons infected with *C. pneumoniae* are resistant to apoptosis, and that Aβ1-42 was increased by the infection. Additionally, studies have demonstrated the interactions of several pathogens on the autophagic pathway. The focus of the current studies was to determine if there is a relationship between the molecular mechanisms interconnecting autophagy and apoptosis following *C. pneumoniae* infection in neuronal cells that could lead to the pathologies observed in AD.

**METHODS:** Neuronal cells (SKNMC, obtained from ATCC) were infected with AR39 strain of *C. pneumoniae* at an MOI=1 for 24 to 72hrs and analyzed using Real-time PCR microarrays (SABiosciences) specific for autophagy and apoptosis markers. The cells were cytosol and immunolabeled with 61C75 directly conjugated antibody to FITC (Fitzgerald, Inc.) for verification of an infection with *Chlamydia pneumoniae*.

**RESULTS:** The major genes involved in autophagy and apoptosis regulation were suppressed in neuronal cells infected from 24 to 72 hrs with *C. pneumoniae*. The expression of prominent autophagy genes was further down-regulated at 72hrs than 24hrs. For example, the autophagy genes, ATG4D and ATG7, were down-regulated from -1.0 fold at 24hrs to -2.0 fold at 72hrs. Conversely, the apoptosis genes were further down-regulated at 24hrs than 72hr. The major apoptosis genes, caspase 3 (CASP3) and AKT (AKT1), were down-regulated to -3.0 to -3.5 fold at 24hrs post-infection, while these genes were down-regulated to -1.5 fold at 72hrs post-infection.

**CONCLUSIONS:** Our data suggest that *C. pneumoniae* exerts a control over changes in gene regulation affecting the apoptotic and the autophagic process in neuronal cells. This control over gene regulation creates a more stable environment for the pathogen. Both autophagic and apoptosis dysfunction have been observed in AD. The impairment of these normal cellular processes by a pathogen such as *C. pneumoniae* may contribute to the neuropathology seen in AD.

## Introduction

We are proposing that a pathogen, such as *Chlamydia pneumoniae*, could be a key factor in triggering the processes such as neuroinflammation and amyloid deposition, that leads to neurodegeneration seen in Sporadic AD. In separate studies, polymerase chain reaction detected *C. pneumoniae* DNA in 80 to 90% of postmortem brain samples examined from sporadic AD [1,2], but in only 5-11% of postmortem brain samples from age-matched, non-AD, control individuals. Furthermore, a murine model has been developed in which non-transgenic mice infected with *C. pneumoniae* demonstrate deposits of amyloid in areas of the brain typically affected in AD [3]. Recently, we have demonstrated that *C. pneumoniae* is capable of inhibiting apoptosis in neuronal cells thereby prolonging the viability of the infected neuronal cell [4].

Previous work demonstrated that some *chlamydiae*-infected host cells are resistant to proapoptotic stimuli such as TNFα, Fas antibody, staurosporine, and UV-light [5,6]. Inhibition of apoptotic activity may be important in the earlier stages of infection. This anti-apoptotic activity may block cytochrome c release from the mitochondrial membrane and subsequent inactivation of caspases [7]. Cytochrome c and caspases work in conjunction with each other to promote apoptosis. *C. pneumoniae* infection has been shown to modulate the pro-apoptotic cytoplasmic proteins, such as caspase-3 and cytochrome c, as well as the anti-apoptotic mitochondrial protein Bcl-2 and the anti-apoptotic nuclear protein NF-κB [8]. NF-κB is a transcriptional factor that is critical for the expression of multiple genes involved in inflammatory responses and anti-apoptotic mechanisms [9].

Modulation of the apoptotic process may be regulated by molecular mechanisms stimulated by autophagy. An increase in the number of autophagic vacuoles (AVs) was identified in neurons from AD brains implicating autophagy as a pathological process in AD [10]. Autophagy is associated with the endosomal-lysosomal system, in which an autophagosome will fuse with an endosome or lysosome. The lysosome will degrade the contents of the autophagosome [11]. Studies have found that the activity of the lysosomal system is enhanced in patients with AD by observing an increase in the production of hydrolases present in lysosomes [12]. The lysosomal system is also related to the endosomal pathway since early endosomes that are formed will fuse with late endosomes or lysosomes [11]. Neurons from AD brains have been found to exhibit enlarged early endosomes. This is significant in AD because early endosomes take in proteins such as apolipoprotein E and APP, and it has been determined that Aβ is formed in early endosomes [12].

Neuronal cell death in Alzheimer's disease may occur through several mechanisms. Neurodegeneration may begin following the initiation of early apoptotic events and alterations in autophagy. Typically, apoptosis and autophagy are common pathways by which infected cells, incapable of eliminating the infectious agent, undergo cell death. Intriguingly, these pathways appear to be altered in *C. pneumoniae* infected neuronal cells. This study will further dissect the mechanisms for cellular function that may be important in both the pathogenesis of AD and infection by *C. pneumoniae*. A modification by *C. pneumoniae* could render the cell resistant to apoptosis following modification of the autophagy pathway.

## Material & Methods

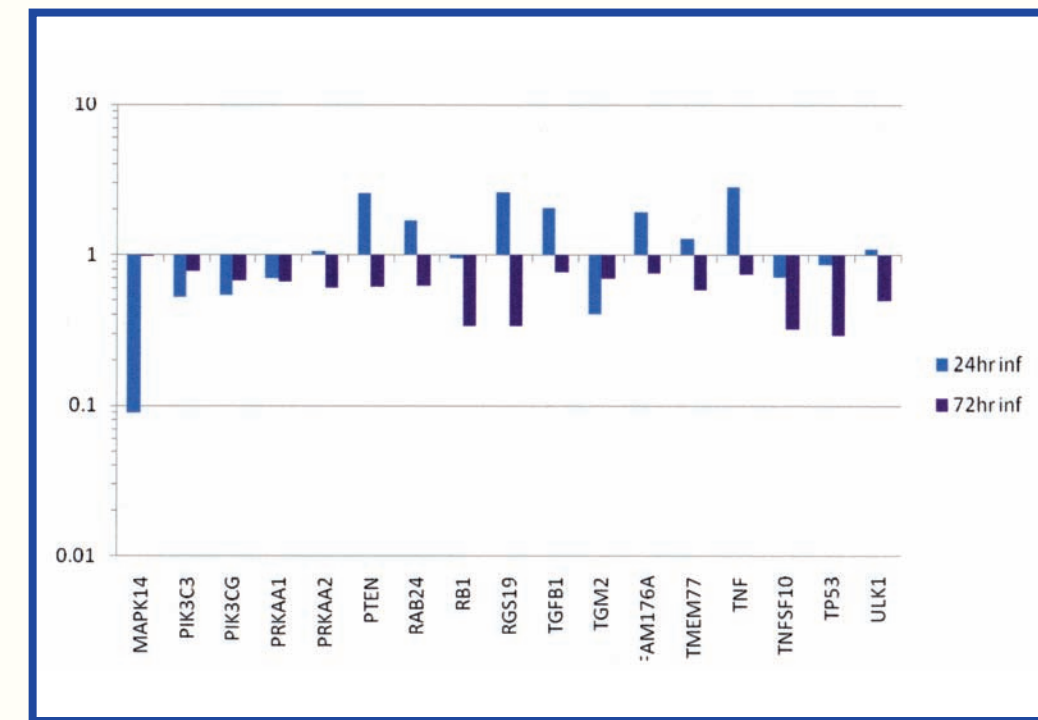
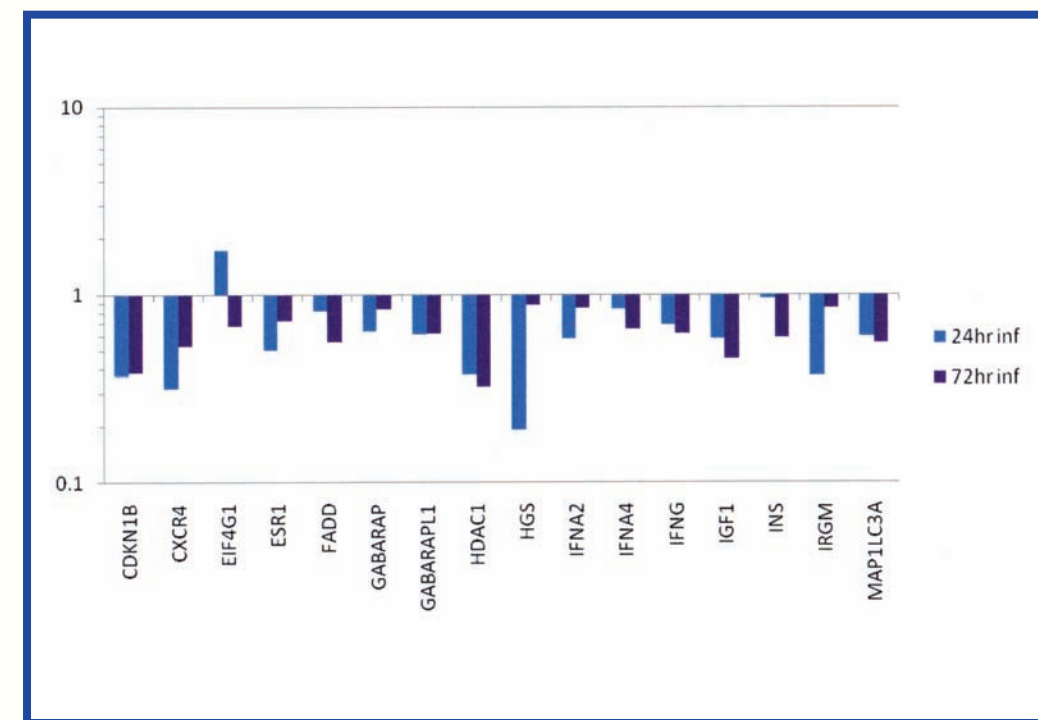
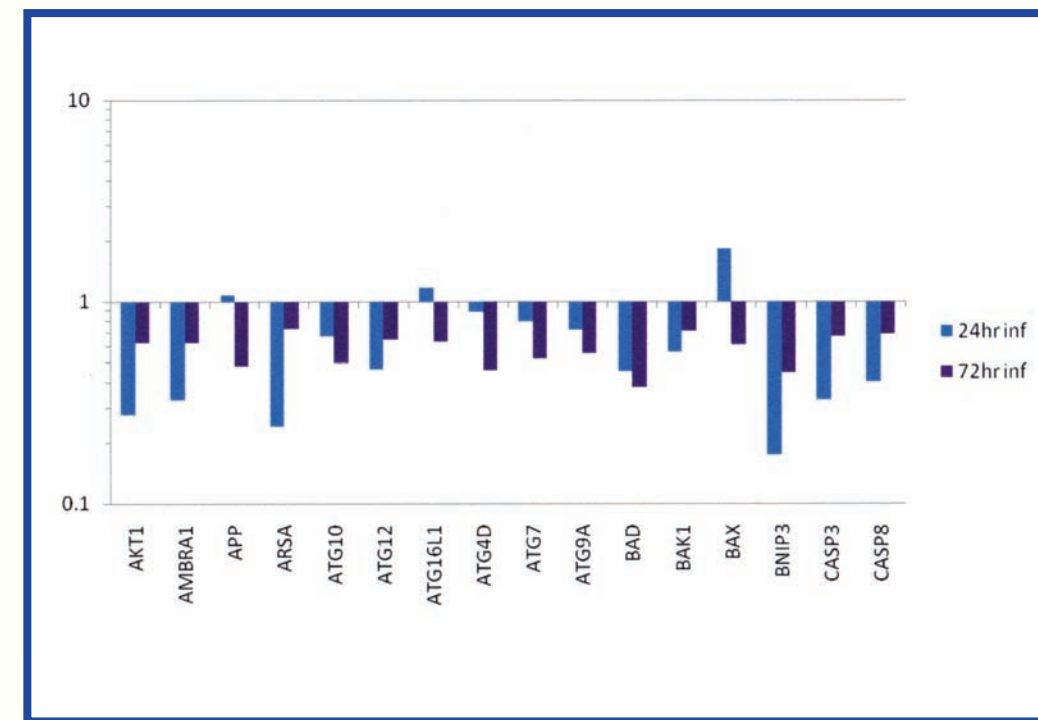
The SKNMC (ATCC) human neuroblastoma cell line was infected with ATCC's AR39 strain of *Chlamydia pneumoniae* at an MOI of 1 for 24 and 72 hrs. Cells were immunolabeled with the direct tag FITC chlamydia antibody 61C75 (Fitzgerald) for verification of infection. The Human Autophagy RT<sup>2</sup> Profiler<sup>TM</sup> PCR Array from SABiosciences was used to analyze the expression of a focused panel of genes involved in autophagy and apoptosis.

## References

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## Results

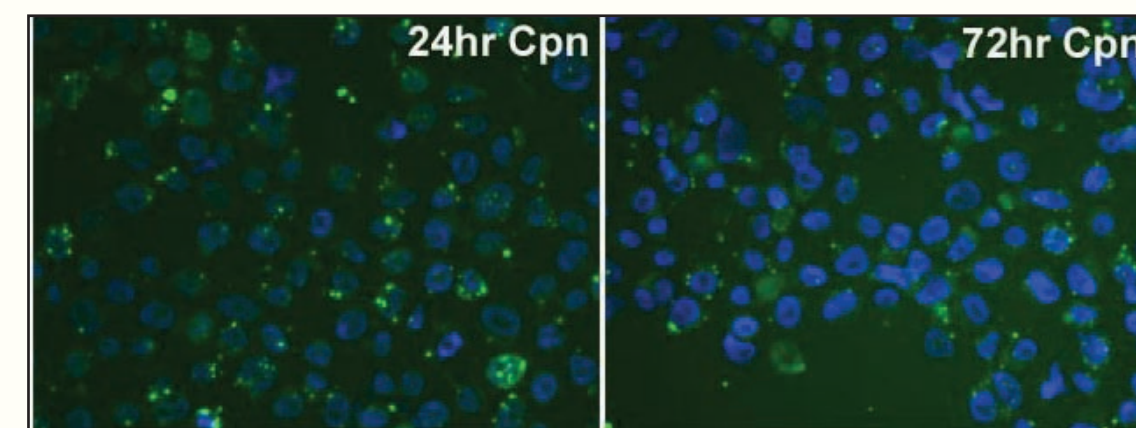
### Autophagy and Apoptosis Gene Expression Analysis by RT-PCR Microarrays of Neuronal Cells Infected with *Chlamydia Pneumoniae* for 24 and 72 hrs.



Function	Gene Symbol	Gene Name
Autophagic Vacuole Formation	AMBRA1	Autophagy/beclin-1 regulator 1
	ATG12	ATG12 autophagy related 12 homolog (S. cerevisiae)
	ATG16L1	ATG16 autophagy related 16-like 1 (S. cerevisiae)
	ATG4D	ATG4 autophagy related 4 homolog D (S. cerevisiae)
	ATG9A	ATG9 autophagy related 9 homolog A (S. cerevisiae)
	GABARAP	GABA(A) receptor-associated protein
	GABARAPL1	GABA(A) receptor-associated protein like 1
	IRGM	Immunity-related GTPase family, M
	MAP1LC3A	Microtubule-associated protein 1 light chain 3 alpha
	RGS19	Regulator of G-protein signaling 19
ULK1	Unc-51-like kinase 1 (C. elegans)	
Protein Targeting to Membrane/Vacuole	ATG4D	ATG4 autophagy related 4 homolog D (S. cerevisiae)
	GABARAP	GABA(A) receptor-associated protein
Protease Activity	ATG4D	ATG4 autophagy related 4 homolog D (S. cerevisiae)
Protein Ubiquitination	ATG7	ATG7 autophagy related 7 homolog (S. cerevisiae)
Autophagosome to Lysosome	GABARAP	GABA(A) receptor-associated protein
Protein Transport	FAM176A	Family with sequence similarity 176, member A
	ATG10	ATG10 autophagy related 10 homolog (S. cerevisiae)
Co-Regulators of Autophagy and Apoptosis	ATG16L1	ATG16 autophagy related 16-like 1 (S. cerevisiae)
	ATG4D	ATG4 autophagy related 4 homolog D (S. cerevisiae)
	ATG7	ATG7 autophagy related 7 homolog (S. cerevisiae)
	ATG9A	ATG9 autophagy related 9 homolog A (S. cerevisiae)
	GABARAP	GABA(A) receptor-associated protein
	RAB24	RAB24, member RAS oncogene family
	AKT1	V-akt murine thymoma viral oncogene homolog 1
APP	Amyloid beta (A4) precursor protein	
ATG12	ATG12 autophagy related 12 homolog (S. cerevisiae)	
BAD	BCL2-associated agonist of cell death	
BAK1	BCL2-antagonist/killer 1	
BAX	BCL2-associated X protein	
BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3	
CASP3	Caspase 3, apoptosis-related cysteine peptidase	
CASP8	Caspase 8, apoptosis-related cysteine peptidase	
CASP9	Caspase 9, apoptosis-related cysteine peptidase	
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	
CXCR4	Chemokine (C-X-C motif) receptor 4	

Function	Gene Symbol	Gene Name
Co-Regulators of Autophagy and Apoptosis (cont)	FADD	Fas (TNFRSF6)-associated via death domain
	HDAC1	Histone deacetylase 1
	IFNA2	Interferon, alpha 2
	IFNG	Interferon, gamma
	IGF1	Insulin-like growth factor 1 (somatomedin C)
	INS	Insulin
	PIK3CG	Phosphoinositide-3-kinase, catalytic, gamma polypeptide
	PRKAA1	Protein kinase, AMP-activated, alpha 1 catalytic subunit
	PTEN	Phosphatase and tensin homolog
	TGFB1	Transforming growth factor, beta 1
TGM2	Transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	
TNF	Tumor necrosis factor (TNF superfamily, member 2)	
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	
TP53	Tumor protein p53	
Co-Regulators of Autophagy and the Cell Cycle	BAX	BCL2-associated X protein
	CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)
	IFNG	Interferon, gamma
	PTEN	Phosphatase and tensin homolog
	RB1	Retinoblastoma 1
TGFB1	Transforming growth factor, beta 1	
TP53	Tumor protein p53	
Autophagy Induction by Intracellular Pathogens	IFNA2	Interferon, alpha 2
	IFNA4	Interferon, alpha 4
	IFNG	Interferon, gamma
Autophagy in Response to Other Intracellular Signals	ARSA	Arylsulfatase A
	EIF4G1	Eukaryotic translation initiation factor 4 gamma, 1
	ESR1	Estrogen receptor 1
	HGS	Hepatocyte growth factor-regulated tyrosine kinase substrate
	MAPK14	Mitogen-activated protein kinase 14
	PIK3C3	Phosphoinositide-3-kinase, class 3
	PRKAA2	Protein kinase, AMP-activated, alpha 2 catalytic subunit
TMEM77	Transmembrane protein 77	

### *Chlamydia pneumoniae* infected Neuronal Cells used in RT-PCR Analysis



SKNMC neuronal cells have been infected with AR39 strain of *Chlamydia pneumoniae* (green) at an MOI=1 for 24 and 72 hrs. These cells represent the infection profile used for RT-PCR analysis. MAC40X

## Conclusions

A *Chlamydia pneumoniae* infection in neuronal cells alters autophagy and apoptosis gene regulation. For both apoptosis and autophagy genes, there were marked differences between 24 and 72hrs infection with *Chlamydia pneumoniae*. These changes in gene regulation are consistent with alterations in apoptosis and autophagy as observed in late onset Alzheimer' disease.

### Funding:

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