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Infection of neuronal cells by *Chlamydia pneumoniae* and Herpes simplex virus type 1 alters expression of genes associated with Alzheimer's disease

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Abstract

Several studies have suggested an infectious etiology for Alzheimer's disease (AD). We have been investigating a potential role for both *Chlamydia pneumoniae* and Herpes simplex virus type 1 (HSV1) in the initiation of sporadic late-onset AD. Our current study focuses on investigation of gene expression using Alzheimer-specific Real-Time PCR microarrays on RNA derived from SKNMC human neuronal cells infected with *C. pneumoniae* and/or HSV1. There are distinct differences in the patterns of gene regulation by the two pathogens. For example, *C. pneumoniae* induces expression of genes involved in amyloid production and processing, such as β-amyloid precursor protein (APP), β-site APP-cleaving enzyme 1 (BACE1), a γ-secretase complex protein (nicastrin [NCSTN]), NEDD8 activating enzyme E1 (NAE1), as well as a mitochondria-associated protein (hydroxysteroid (17-β) dehydrogenase 10 [HSD17B10]), α-2-macroglobulin (A2M) and the metallopeptidase ADAM9. Conversely, HSV1 tends to down-regulate expression of many genes, including those encoding a component of the γ-secretase complex (anterior pharynx defective 1 homolog A [APH1A]), low density lipoprotein related proteins (LRP1, LRP6, and LRP8), β-synuclein (SNCB) and ubiquinols (UQCRC1, UQCRC2). Co-infection with *C. pneumoniae* and HSV-1 produced a greater down-regulation of gene expression than that seen with HSV1 alone for several genes, including APP-like proteins (APLP1, APLP2) and kinases (cell division cycle 2 protein [CDC2], cyclin-dependent kinase [CDK5] and CDC2-related kinase [CDKL1]). Our data indicate that both *C. pneumoniae* and HSV1 can modulate expression of genes associated with AD, and thus could contribute to AD pathology, however these two pathogens likely act via different pathways. Furthermore, for several genes, co-infection with both *C. pneumoniae* and HSV1 appears to exacerbate the changes in gene expression seen with HSV1 alone.

Introduction

Alzheimer's disease (AD) is the leading cause of dementia, and while a genetic predisposition can account for a small percentage of the cases, the majority of cases are the result of a multifactorial etiology that may include infectious agents (Honjo et al., 2009). Infection within the central nervous system (CNS) by Herpes simplex virus type 1 (HSV1) and *Chlamydia pneumoniae* has been suggested to play a role in sporadic AD (Itzhaki et al., 2004). In this study we examine the possibility that *C. pneumoniae* and/or HSV1 will alter the expression of genes associated with AD. This would provide additional evidence that these pathogens might contribute to onset or progression of the pathology associated with AD, and thus play a role in the disease process.

Previous studies have indicated that *C. pneumoniae* is detected in the brains of patients with AD more frequently than in normal aged brains (Balin et al., 1998; Gerard et al., 2006). In the AD brains, *C. pneumoniae* antigens were present in macrophages, microglia, and astroglial cells of the temporal cortex, hippocampus, parietal, and pre-frontal cortices (Itzhaki et al., 2004). We postulate that *C. pneumoniae* might enter the central nervous system (CNS) through either an intravascular or olfactory route. In support of this hypothesis, *C. pneumoniae*-infected glial, macrophages, and monocytes were all identified around blood vessels and in olfactory bulbs (Balin et al., 2004).

Studies have also implicated HSV1 as a potential pathogen contributing to AD pathology (Itzhaki et al., 2004). HSV1 is ubiquitous and produces latent infection in neurons, with the potential for reactivation in the CNS. The chronic nature of this infection, with periodic reactivation of productive infection, may contribute to the gradual accumulation of pathology associated with AD. It has been shown that HSV1 can produce abnormal processing of amyloid precursor protein, which results in the deposition of amyloid plaques, an important pathological feature of AD (Dobson et al., 2002; Wozniak et al., 2007). Furthermore, herpesvirus DNA has been detected in plaques in AD brains (Wozniak et al. 2009), additional evidence that HSV1 may contribute to AD disease progression.

The role that *C. pneumoniae* and/or HSV1 plays in "triggering" events resulting in AD pathology has been further analyzed following in vitro infection of the human neuronal cell line, SKNMC. Experiments were designed to determine whether infection with *C. pneumoniae* and HSV1, either singly or in combination, influences the expression of cellular genes associated with AD. We have analyzed AD-associated gene expression in neuronal cells in the presence or absence of the two pathogens using microarray technology. These data may provide further insight into how these two pathogens might contribute to the onset or progression of Alzheimer's disease. The inclusion of co-infections by both *C. pneumoniae* and HSV1 in this study will also examine possible synergistic affects of multiple pathogens on the disease process.

Results

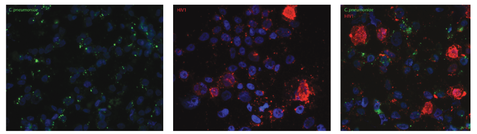


Figure 1 Immunofluorescence labeling of SKNMC cells infected by *C. pneumoniae* (green) for 48 hours (left panel), HSV1 (red) for 24 hours (middle panel) or co-infected (right panel) with both *C. pneumoniae* (48 hours) and HSV1 (24 hours). Nuclei are shown in blue.

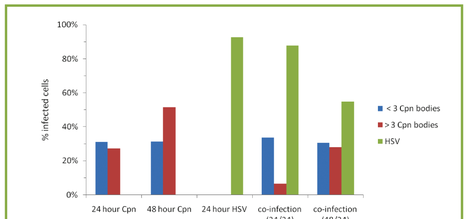


Figure 2 Representative infection of SKNMC cells by *C. pneumoniae* (Cpn) and HSV1. Cells were infected with *C. pneumoniae* for 24 or 48 hours or with HSV1 for 24 hours at an MOI of 0.5. For co-infections, cells were inoculated with *C. pneumoniae* and HSV together and incubated for 24 hours (co-infection [24/24]) before harvesting, or inoculated first with *C. pneumoniae* alone for 24 hours, then with HSV1 for an additional 24 hours (co-infection [48/24]) before harvesting. Cells were labeled for *C. pneumoniae* and/or HSV1 by immunofluorescence and the % of cells labeled for each pathogen was quantified. Cells showing low levels of *C. pneumoniae* labeling (< 3 Cpn bodies) were scored separately from cells demonstrating more extensive *C. pneumoniae* infection (> 3 Cpn bodies). Four fields, each typically containing between 75 and 100 cells/field, were counted for each infected monolayer.

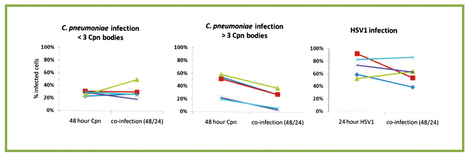
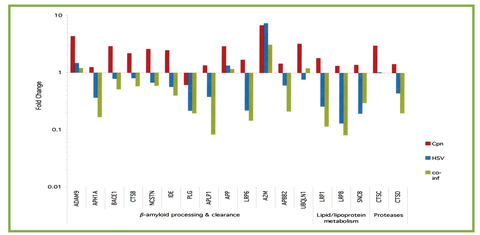


Figure 3 Comparison of % *C. pneumoniae* (Cpn)- or HSV1-infected cells in monolayers inoculated with a single pathogen or co-infected with both pathogens from 5 separate infections. Cells were infected with *C. pneumoniae* for 48 hours and HSV1 for 24 hours at an MOI of 0.5. For co-infections, cells were inoculated with *C. pneumoniae* first, inoculated with HSV1 after 24 hours, then harvested after an additional 24 hours (co-infection [48/24]). Cells were labeled for *C. pneumoniae* and/or HSV1 by immunofluorescence and the % of cells labeled for each pathogen was quantified. Cells showing low levels of *C. pneumoniae* labeling (< 3 Cpn bodies) were scored separately from cells demonstrating more extensive *C. pneumoniae* infection (> 3 Cpn bodies). Four fields, each typically containing between 75 and 100 cells/field, were counted for each infected monolayer.

- It is possible for a single cell to be infected with both *C. pneumoniae* and HSV1
- When both pathogens are added simultaneously, HSV1 infection of SKNMC cells appear to dominate over that of *C. pneumoniae*
- The % of SKNMC cells showing low levels of infection with *C. pneumoniae* (< 3 Cpn bodies) remained relatively constant over 24 and 48 hours, regardless of whether HSV1 was present or not
- The % of SKNMC cells showing more extensive infection with *C. pneumoniae* (> 3 Cpn bodies) increased over time in the absence of HSV1, but was consistently lower in 48 hour co-infections compared to 48 hour infections with *C. pneumoniae* alone
- The affect of *C. pneumoniae* or HSV1 infection on SKNMC cells was more variable, with 3 of 5 infections showing a decrease in % HSV1 positive cells, while 2 of 5 infections showing an increase.



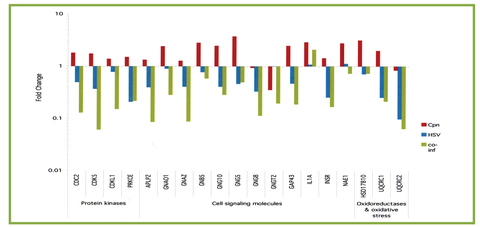


Figure 4 Changes in gene expression following infection of SKNMC cells with *C. pneumoniae* or HSV1 alone, or co-infected with both pathogens. Cells were infected with *C. pneumoniae* for 48 hours and HSV1 for 24 hours. For co-infections, cells were inoculated with *C. pneumoniae* first, inoculated with HSV1 after 24 hours, then harvested after an additional 24 hours (co-infection [48/24]). RNA was isolated and analyzed by RT^2-PCR using a microarray for Alzheimer-associated genes (SABiosciences). The results shown are pooled from 6 separate infections. Only select genes with the greatest changes, either up- or down-regulation, are shown.

Function	Gene symbol	Gene name
β-amyloid processing & clearance		
Secretases	ADAM9	ADAM metallopeptidase domain 9
	APH1A	Anterior pharynx defective 1 homolog A (C. elegans)
	BACE1	β-site APP-cleaving enzyme 1
	CTSB	Cathepsin B
	NCSTN	Nicastrin
Other peptidases	IDE	Insulin-degrading enzyme
	PLG	Plasminogen
β-amyloid clearance through endocytosis	APLP1	β-amyloid precursor-like protein 1
	APP	β-amyloid precursor protein
	LRP6	LDL receptor-related protein 6
Other genes involved in β-amyloid metabolism	A2M	α-2-macroglobulin
	APBB2	β-amyloid precursor protein-binding, family B, member 2
	UBQLN1	Ubiquilin 1
Lipid/lipoprotein metabolism		
	LRP1	LDL-related protein 1 (α -2-macroglobulin receptor)
	LRP8	LDL receptor-related protein 8 (apolipoprotein e receptor)
	SNCB	Synuclein, β
Proteases		
	CTSC	Cathepsin C
	CTSD	Cathepsin D
Protein kinases		
	CDC2	Cell division cycle 2, G1 to S & G2 to M
	CDK5	Cyclin-dependent kinase 5
	CDKL1	Cyclin-dependent kinase-like 1 (CDC2-related kinase)
	PRKCE	Protein kinase C,
Cell signaling molecules		
G-protein coupled receptor	APLP2	β-amyloid precursor-like protein 2
	GNAO1	G protein, α activating activity polypeptide O
	GNAZ	G protein, α z polypeptide
	GNB5	G protein, β 5
	GNG10	G protein, γ 10
	GNG5	G protein, γ 5
	GNG8	G protein, γ 8
	GNGT2	G protein, γ transducing activity polypeptide 2
Other signaling molecules	GAP43	Growth associated protein 43
	IL1A	Interleukin 1, α
	INSR	Insulin receptor
	NAE1	NEDD8 activating enzyme E1 subunit 1
Oxidoreductases & oxidative stress		
	HSD17B10	Hydroxysteroid (17-β) dehydrogenase 10
	UQCRC1	Ubiquinol-cytochrome c reductase core protein I
	UQCRC2	Ubiquinol-cytochrome c reductase core protein II

- Although the changes were not dramatic, infection of SKNMC cells with *C. pneumoniae* resulted in the up-regulation of numerous genes, especially genes involved in theprocessing of β-amyloid and select cell signaling genes
- Infection of SKNMC cells with HSV1 resulted in the down-regulation of most genes in the array, though genes involved in β-amyloid processing were generally not affected to the same extent as genes involved in lipoprotein metabolism.
- The expression of genes in co-infected monolayers was down-regulated as seen following infection with HSV1 alone, with numerous genes, such as several of the protein kinases and select cell signaling genes, showing greater down-regulation in co-infections than in HSV1 infection alone

Conclusions

- HSV1 appears to inhibit *C. pneumoniae* growth in SKNMC cells
  - The decrease in % of cells with multiple *C. pneumoniae* bodies may reflect an inhibition of chlamydial attachment to SKNMC cells
- The down-regulation of most of the genes in the Alzheimer disease microarray by infection with HSV1 may reflect a general ability of viruses to shut down host gene synthesis
- Gene regulation in co-infected monolayers is similar to that seen with HSV1 alone, as opposed to being intermediate between the up-regulation seen with *C. pneumoniae* and the down-regulation seen with HSV1
  - HSV1 infection dominates over that of *C. pneumoniae* in co-infected monolayers, consistent with quantification of infection in single vs co-infections
- *C. pneumoniae* could directly contribute to amyloid plaque formation, an important pathological feature of Alzheimer's disease, by enhancing expression of genes involved in the processing of β-amyloid
- The most likely scenario to account for a *C. pneumoniae*/HSV1 co-infection would be dissemination of *C. pneumoniae* to the CNS of an individual with latent HSV1 infection. Our data suggest:
  - HSV1 would be able to reactivate in the presence of active *C. pneumoniae* infection
  - Productive HSV1 infection, however, might limit the *C. pneumoniae* infection

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