

Analysis of *Chlamydia pneumoniae*-infected monocytes following incubation with a novel peptide, acALY18: A potential treatment for infection in Alzheimer's disease

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Abstract

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 observed in LOAD as a function of initiating neuroinflammation following entry of the organism into the brain. We have hypothesized that one entry mechanism may be by blood-borne infected monocytes trafficking the infection into the brain. Our current studies focus on infection of monocytes in vitro followed by analysis of infection using immunofluorescence labeling and RTPCR-microarray techniques. In addition, we are studying a novel approach utilizing a unique peptide, acALY-18, derived from the endogenously expressed endoplasmic reticulum protein TRPC1, to eradicate the organism at 24-48 hr post-infection, thereby limiting its capacity to develop into a chronic/persistent infection. The peptide appears to stimulate the innate immune system through activation of the inflammasome. **Results:** *C. pneumoniae* prominently and stably infected THP1 monocytes at 24-48hr. Numerous large inclusions were labeled using specific anti-chlamydial monoclonal antibodies. Monocyte gene expression, both for markers of innate and adaptive immunity as well as for Alzheimer disease, was significantly altered. For example, 6 genes were up-regulated at least 10 fold as compared to 4 genes down-regulated to the same extent the immunity array at 48hr post-infection. In the Alzheimer's array, 16 genes were up-regulated at least 4 fold as compared to 2 that were down-regulated to the same extent. Intriguingly, following incubation of *C. pneumoniae*-infected cells with acALY18 peptide (25-50nM) at 24hr post-infection, there was a dramatic clearance of the organism from the monocytes (80% infected and untreated to 13-15% infected after treatment). Furthermore, gene regulation was altered following peptide treatment, as there were 13 genes that were up-regulated at least 10 fold and no genes were down-regulated to that extent in the immunity array. **Conclusions:** Our data suggest that *C. pneumoniae*-infected monocytes are altered significantly to promote a chronic/persistent infection that may account for the presence of *C. pneumoniae* in LOAD. Furthermore, stimulating the innate immune response using the novel peptide, acALY18, promotes clearance of *C. pneumoniae* from infected monocytes; this peptide may be a viable candidate for treating *C. pneumoniae* infections in Alzheimer disease.

Introduction

Infection with the respiratory obligate intracellular bacterium *Chlamydia pneumoniae* has been shown to be involved in numerous human respiratory and non-respiratory diseases including: community-acquired pneumonia and bronchitis (1), atherosclerosis and heart disease (2; 3), and late-onset Alzheimer's disease (4;5). *C. pneumoniae* gains access to the circulation upon infection of monocytes, and potentially of lymphocytes (6;7). Chronic persistence of *C. pneumoniae* in circulating monocytes is likely, and may facilitate systemic infection (8) through transfer of *C. pneumoniae* between infected monocytes and peripheral endothelial cells (9). Our laboratory has evidence that the monocyte is the key cell type that may carry *C. pneumoniae* into the CNS (10; 11), and may implicate *C. pneumoniae* as a major risk factor in the development of Alzheimer disease (for review see 12). Impacting this infection with a therapeutic regimen may diminish the potential for infection in the CNS as well as elsewhere in the body. Antibiotics may be used in combating this type of infection; however, an antibiotic regimen would not necessarily be prudent given issues of antibiotic penetration to different anatomical sites, efficacy against chronic/persistent organisms and driving organisms into a persistent state, antibiotic resistance, and the potential for the development of super-infections. Therefore, we are studying an alternative method of combating infection by inducing the innate immune system to better respond and eradicate this type of infection. Specifically, we are using a recently discovered lipopeptide (acALY-18) derived from the endogenous endoplasmic reticulum TRPC1 protein that has been found to activate the innate immune response in fibroblasts and keratinocytes (13). Our preliminary data suggest that this lipopeptide activates *C. pneumoniae*-infected monocytes resulting in better clearance of the organism when compared to untreated infected cells. Therefore the lipopeptide (acALY-18) may be a viable candidate for treating *C. pneumoniae* infections in Alzheimer's disease.

acALY-18 has been determined to have identical sequence homology to the internal sequence of amino acids at positions 558-574 in the transient receptor potential channel-related protein 1 (TRPC1) (13). No significant homology with other known proteins or peptides has been noted. The TRPC family of proteins belongs to the TRP superfamily of non-voltage-gated cation channel proteins of which at least seven TRPC family members have been described in mammals (14). Members of the TRPC family, including TRPC1, appear to be conserved within the animal kingdom and the trp gene family also appears to be expressed in a wide variety of tissues and cell types including immune cells (for review see 15). The topology of TRPC1 has been deduced (16) and the amino acid sequence comprising the acALY-18 peptide appears to reside in the extracellular region between the sixth and seventh membrane spanning units. Sequence homology of this region of TRPC1 with the other members of the TRPC family is relatively low (~30%) as compared to ~80% for the N-terminal sequence (14).

The function of acALY-18 appears to involve the intracellular induction of specific cytokines/chemokines and this does not involve activation through toll-like receptors. The induction appears to activate the innate immune response by activating the inflammasome (17). Inflammasomes are cytosolic multiprotein complexes containing cytoplasmic receptors of the NALP (NACHT, LRR and pyrin domain-containing protein) family as central components (17). These inflammasomes can be activated by bacterial toxins, and by endogenous danger signals from intracellular pathogens (18; 19). Furthermore, lysosomal damage has been shown to induce NALP3 activation (20).

Using a peptide such as acALY-18, which was originally derived and identified from the naturally occurring TRPC1 protein (13), to induce the innate immune response, provides a unique opportunity to improve processing and clearance of intracellular pathogens, such as *Chlamydia pneumoniae*. Harnessing this more natural approach in combating infection may have implications for Alzheimer's disease therapy that incorporates a variety of environmental factors, including infection, as major risks and/or causative agents in disease pathogenesis.

Topology of TRPC1

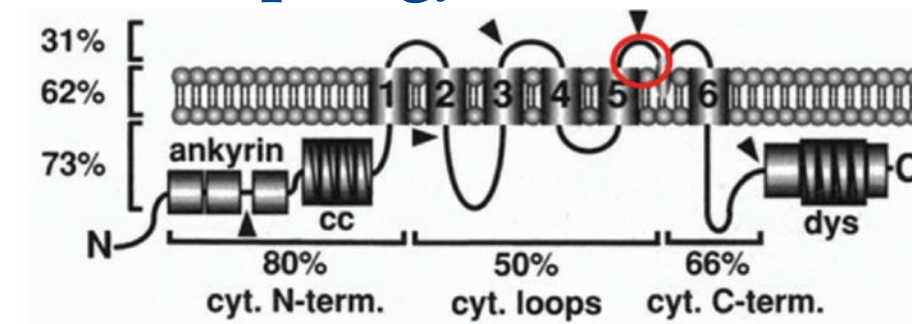


Figure 1: The PDAG peptide (acALY-18) sequence is homologous with an extracellular loop of TRPC1, a transmembrane Ca²⁺ channel protein. Percent homology between TRPC1 and other members of the TRP family is indicated. A putative pore loop is indicated between transmembrane segments S5 and S6. The region indicated by the red circle is the pDAG peptide sequence. Arrowheads indicate small gaps present in homology of TRPC1 relative to other TRP family members.

From Paul D. Wes, et al. Proc. Natl. Acad. Sci. USA Vol. 92, pp. 9652-9656, October 1995 Cell Biology

To test our hypothesis we sought to identify the effect of synthetic PDAG peptide (acALY-18) on *Chlamydia pneumoniae* infected human monocytes (MOI=1) at 48hrs post infection.

Synthetic PDAG peptide (acALY-18) decreases infection in monocytes

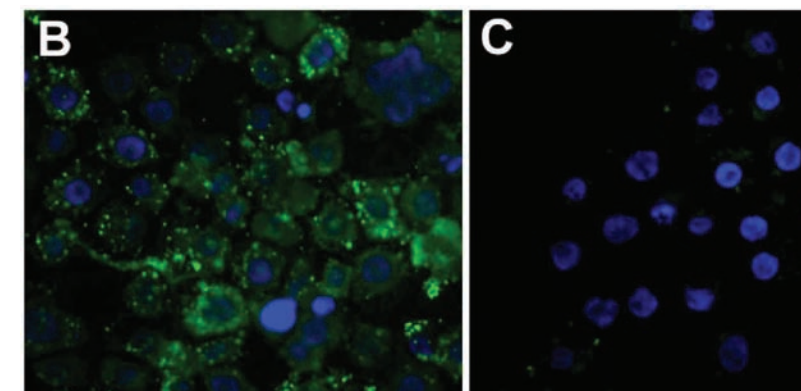
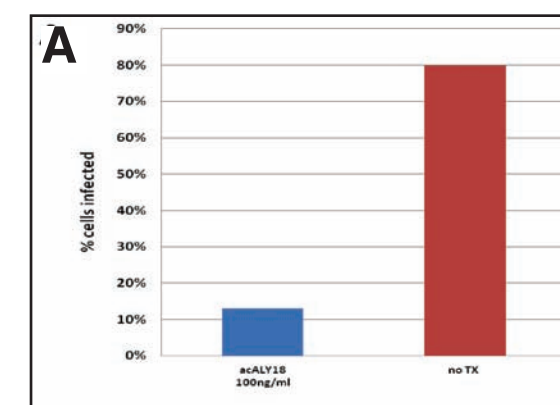


Figure 2: Percent cells infected with *Chlamydia pneumoniae* (A) as determined by counting infected cells (green labeling) in THP1 monolayers untreated (B) or treated (C) with acALY-18 peptide at 24hrs post-infection and processed at 48hrs post-infection.

PDAG peptide (acALY-18) induces gene expression in infected monocytes

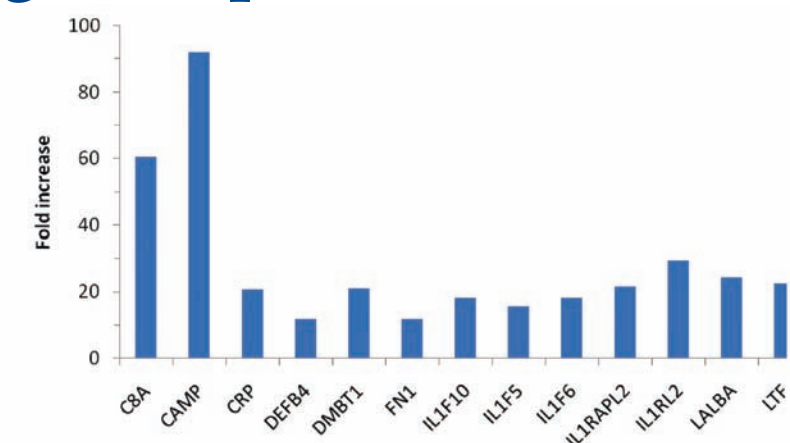


Figure 3: Fold increase of expression in a subset of genes in *Chlamydia pneumoniae* infected cells treated with acALY-18 relative to untreated infected cells. Genes were detected using an innate and adaptive immunity RT2PCR array from SABiosciences (Qiagen).

Gene Symbol	Gene Description	Fold Regulation	Gene Symbol	Gene Description	Fold Regulation
C8A	Complement component 8, alpha polypeptide	60.5	IL1F5	Interleukin 1 family, member 5 (delta)	15.8
CAMP	Cathelicidin antimicrobial peptide	92.1	IL1F6	Interleukin 1 family, member 6 (epsilon)	18.2
CRP	C-reactive protein, pentraxin-related	20.8	IL1TRAP2	Interleukin 1 receptor accessory protein-like 2	21.4
DEFB4	Defensin, beta 4	11.9	IL1RL2	Interleukin 1 receptor-like 2	29.4
DMBT1	Deleted in malignant brain tumors 1	21.0	LALBA	Lactalbumin, alpha-	24.4
FN1	Fibronectin 1	11.8	LTF	Lactotransferrin	22.5
IL1F10	Interleukin 1 family, member 10 (theta)	18.1			

Innate and adaptive immunity gene expression changes in *C. pneumoniae* infected vs uninfected monocytes

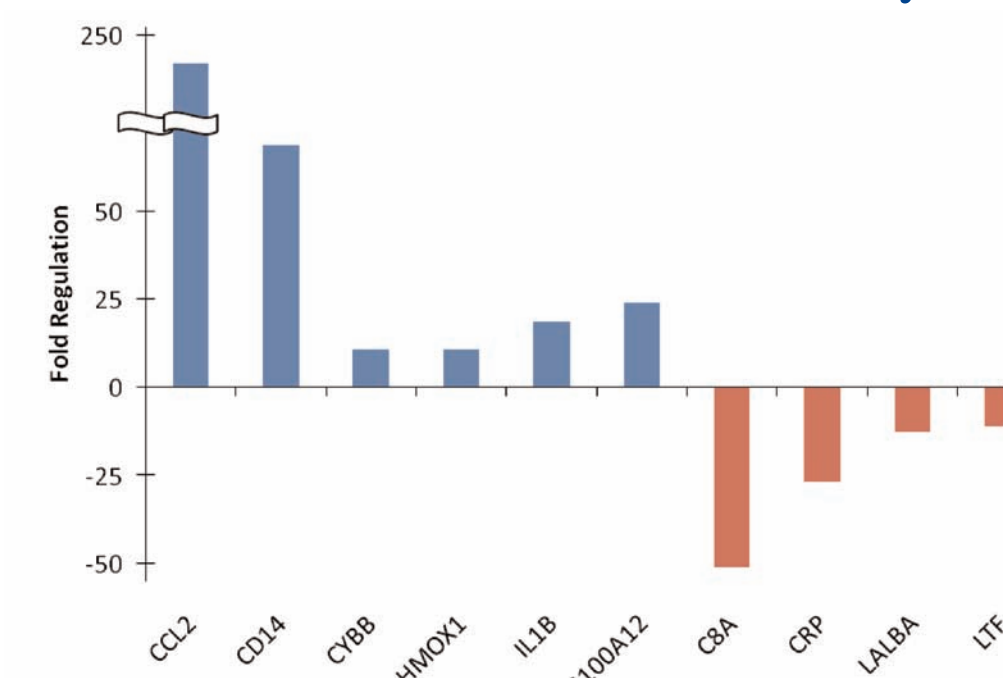


Figure 4: Fold regulation of expression in a subset of genes in *Chlamydia pneumoniae* infected cells relative to uninfected cells at 48hrs. Data were derived from 3 sets of experiments. Genes were detected using innate and adaptive immunity RT2PCR array from SABiosciences (Qiagen).

Gene Symbol	Description	Fold Regulation
CCL2	Chemokine (C-C motif) ligand 2	241.8
CD14	CD14 molecule	68.7
CYBB	Cytochrome b-245, beta polypeptide	10.7
HMOX1	Heme oxygenase (decycling) 1	10.5
IL1B	Interleukin 1, beta	18.4
S100A12	S100 calcium binding protein A12	23.9
C8A	Complement component 8, alpha polypeptide	-51.2
CRP	C-reactive protein, pentraxin-related	-27.0
LALBA	Lactalbumin, alpha-	-12.7
LTF	Lactotransferrin	-11.0

PDAG peptide (acALY-18) promotes specific gene upregulation to combat *C. pneumoniae* infection

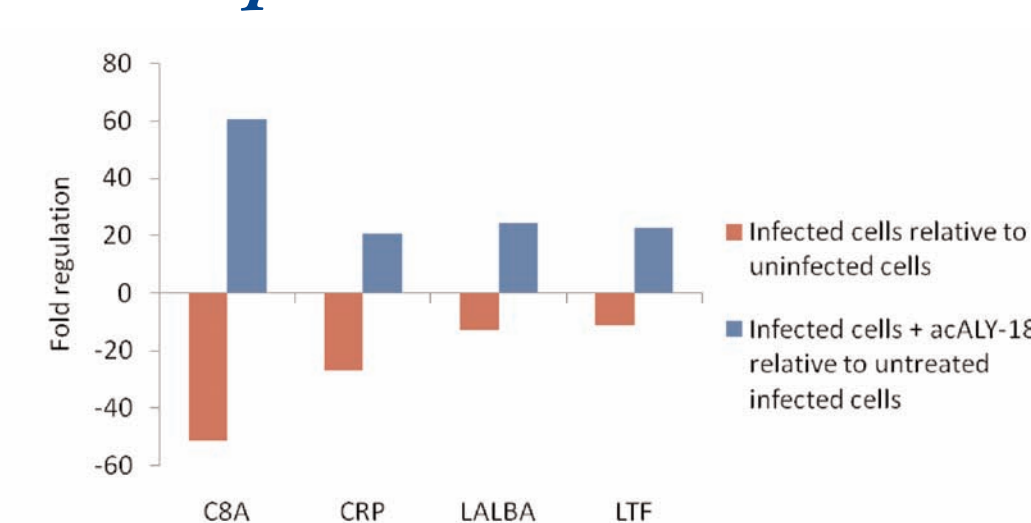


Figure 5: Fold regulation of expression in a subset of genes following infection of THP1 cells with *C. pneumoniae* (red bars) or following treatment of infected cells with acALY-18 (blue bars). Genes were detected using an innate and adaptive immunity RT2PCR array from SABiosciences (Qiagen).

Gene Symbol	Description	Fold Regulation	
		Infected cells relative to uninfected cells	Infected cells with acALY-18 relative to untreated infected cells
C8A	Complement component 8, alpha polypeptide	-51.2	60.5
CRP	C-reactive protein, pentraxin-related	-27.0	20.8
LALBA	Lactalbumin, alpha-	-12.7	24.4
LTF	Lactotransferrin	-11.0	22.5

Alzheimer gene expression in *c. pneumoniae* infected vs uninfected human monocytes

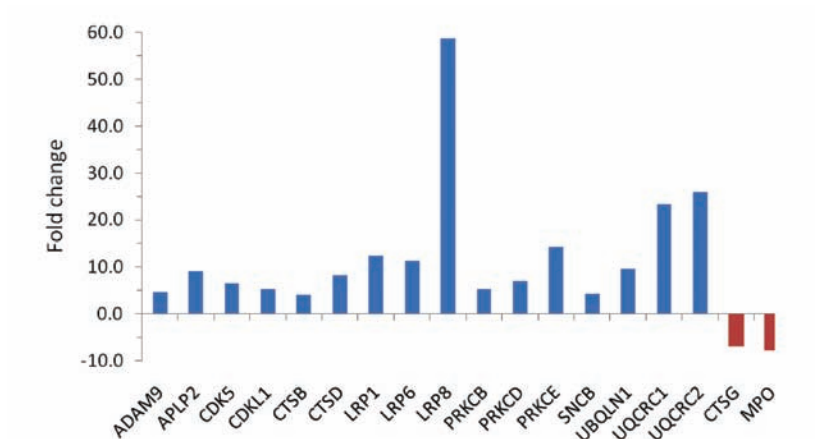
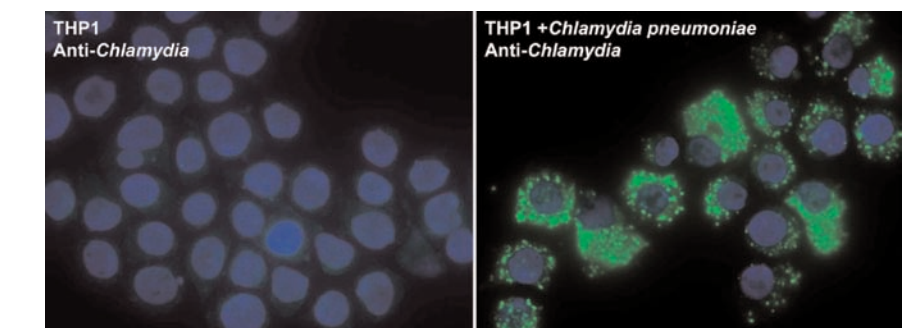


Figure 6: Fold regulation of expression in a subset of genes in *Chlamydia pneumoniae* infected cells relative to infected cells. Data were derived from 3 sets of experiments. Genes were detected using an Alzheimer's disease RT2PCR array from SABiosciences (Qiagen).

Gene Symbol	Gene Description	Fold change	Gene Symbol	Gene Description	Fold change
ADAM9	ADAM metalloproteinase domain 9 (meltrin gamma)	4.7	PRKCB	Protein kinase C, beta	5.4
APLP2	Amyloid beta (A4) precursor-like protein 2	9.1	PRKCD	Protein kinase C, delta	7.0
CDK5	Cyclin-dependent kinase 5	6.6	PRKCE	Protein kinase C, epsilon	14.3
CDKL1	Cyclin-dependent kinase-like 1 (CDC2-related kinase)	5.4	SNCB	Synuclein, beta	4.3
CTSB	Cathepsin B	4.1	UBQLN1	Ubiquilin 1	9.7
CTSD	Cathepsin D	8.3	UQCRC1	Ubiquinol-cytochrome c reductase core protein I	23.3
LRP1	Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)	12.5	UQCRC2	Ubiquinol-cytochrome c reductase core protein II	26.0
LRP6	Low density lipoprotein receptor-related protein 6	11.4	CTSG	Cathepsin G	-6.9
LRP8	Low density lipoprotein receptor-related protein 8, apolipoprotein e receptor	58.7	MPO	Myeloperoxidase	-7.8

Conclusions

- C. pneumoniae* infection of human monocytes alters gene expression for markers of the innate and adaptive immune response
 - Altered gene expression prevents adequate clearance of the organism from the professional killing cells thereby promoting persistent infection.
 - Ineffective clearance of infection maintains chronic turn-on of an inflammatory response by infected monocytes as reflected by specific gene expression (see figure 4)
- C. pneumoniae* infection of human monocytes alters gene expression for markers of Alzheimer's disease
 - Increased expression of specific genes (see figure 6) demonstrates the effect of intracellular infection on genes associated with all aspects of cellular function
 - These data support our contention that *C. pneumoniae* infection is a "trigger" for altered gene expression in the pathogenesis of Alzheimer's disease
- The novel PDAG peptide acALY-18 induces expression of genes associated with innate and adaptive immunity to effectively reduce *C. pneumoniae* infection of monocytes
 - acALY-18 may be a viable candidate for treating this infection in Alzheimer's disease.

Funding

This work was funded by the Center for Chronic Disorders of Aging at the Philadelphia College of Osteopathic Medicine, and the Adolph and Rose Levis Foundation for Alzheimer's Disease Research.

Acknowledgments

We would like to thank Kathryn Hingley, Chris Cappellini, Charlie Lim, and Catherine Fusco for their assistance with the RTPCR experiments.