Studies of Respiratory *Chlamydia* Infections in Mouse Models of *Chlamydia* Infection And Alzheimer's Disease

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Statement of Originality

"I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo."

________________________, 01/05/2020

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"If you wish to make an apple pie from scratch, you must first invent the universe."

— Carl Sagan, *Cosmos*

Abstract

Alzheimer's disease (AD) is a neurodegenerative proteopathy associated with progressive cognitive impairment and characterised neuropathologically by neurofibrillary tangles and Aβ-amyloid plaques. Brain presence of the common respiratory bacteria *Chlamydia pneumoniae* (*Cpn*) is proposed to induce AD β-amyloidosis but this is controversial, with human studies giving inconsistent results. Mouse studies have also been inconclusive, having previously used only wild-type mice, which do not usually form Aβ-amyloid*,* together with methods that do not distinguish non-amyloid Aβ deposits from Aβ-amyloid deposits. In addition, *Cpn* is not a natural mouse pathogen, requiring large inocula that are not physiologically relevant.

If *Chlamydia* has roles in AD pathogenesis, treating AD patients for *Cpn* infection may be a viable strategy. This study tests the broad hypothesis that respiratory *Chlamydia* infection can gain entry to the brain, increase brain Aβ or Aβ-amyloid deposition, trigger AD-relevant gene expression changes and cause neuroinflammation. To address limitations of past research, both wild-type mice and the APP/PS1 transgenic mouse model of β-amyloidosis were infected with *Chlamydia muridarum* (*Cmu*), a related natural mouse pathogen more appropriately modelling respiratory *Chlamydia* infection. Also, since a defining feature of amyloid is affinity for the dye Congo red, with concomitant yellow-green birefringence under cross-polarised light, 'gold standard' Congo red polarisation microscopy was used to assess Aβ-amyloid.

Wild-type and APP/PS1 mice were intranasally infected with *Cmu*, or sham infected with vehicle alone, and brains examined at 6 months of age. There were three endpoint matched infection scenarios: i) adult mice (6 months) followed-up short-term 10 days post-infection to assess *Cmu* brain entry and acute responses during peak respiratory infection, ii) adult mice (3 months) followed-up long-term at 3 months post-infection to assess *Cmu* brain persistence and longer-term responses and iii) neonatal mice (24 hours) followed-up long-term at 6 months to assess if early-life exposure may cause long-term changes.

Adult mice infected with 100 inclusion forming units (IFU) and followed-up short-term (10 days) had high *Cmu* 16S rRNA signal in lungs by real-time reverse transcription polymerase chain reaction (RT-PCR) and considerable weight loss, consistent with severe infection. Adult mice followed-up long-term (3 months) were therefore given a slightly lower dose (75 IFU) and showed modest weight loss, consistent with moderate infection. Neonatal mice, which require higher *Cmu* doses to produce the same disease severity, probably due to differences in immune responses, received 400 IFU within 24 hours after birth and were followed-up long-term (6 months). There was no clear weight loss but alveolar damage (estimated with mean linear intercept method in lung sections) was consistent with successful infection.

Only adult mice followed-up short-term had evidence of *Cmu* brain entry by 16S rRNA RT-PCR. Signal was small but increased brain levels of innate immune response gene transcripts were detected by RT-PCR, suggesting *Cmu* may have entered the brain in small quantities in early infection but was subsequently cleared, with no evidence of *Cmu* in the brain at long-term follow-up. Peripheral immune responses signalling across brain barriers could feasibly alter brain Aβ deposition whether or not brain entry occurred. However, there was no evidence for changes in Aβ or Aβ-amyloid deposition in any infection scenario by antibody 4G8 immunolabelling, thioflavin S or Congo red staining by fluorescence or polarised light microscopy, the 'gold standard' for amyloid detection.

Notably, 75% of Aβ-immunoreactive structures and 20% of fluorescent structures were non-birefringent.

No changes clearly relevant to AD were observed in expression of the AD-related genes *App*, *Psen1*, *and Mapt* with RT-PCR. Microarray and bioinformatics analyses of neonatal mice followed-up long-term also showed minor long-term changes but none appeared relevant to AD. There were no differences in microglial proliferation or morphology in any scenario as assessed by morphometric analysis of antibody IBA1-immunolabelled microglia.

The results support the hypothesis that respiratory *Chlamydia* infection can gain entry to the brain in early or later life but do not provide evidence for short- or long-term effects on brain Aβ deposition. Future studies could assess the validity of these findings by intracerebral injection of larger inocula of *Cmu*, or by infecting older mice with age-related immunosenescence. However, based on present evidence, there is no justification for screening and treating AD patients without concurrent respiratory illness for *Cpn* infection.

This project highlights the need for future AD studies to complement other methods with polarised light microscopy of Congo red staining, which is currently rarely performed in AD studies. Differentiating Aβ-amyloid and non-amyloid Aβ structures should improve understanding of the mechanisms underlying β-amyloidosis and of the actions of new drugs on Aβ deposition and β-amyloidosis in animal models before proceeding to human trials.

List of Abbreviations

4-HNE	4-hydroxynonenal
Aβ	amyloid-β
ABC	avidin-biotin complex
AD	Alzheimer’s disease
ADAM	a disintegrin and metalloprotease
AICD	amyloid intracellular domain
ANOVA	analysis of variance
APOE	apolipoprotein E
APP	amyloid precursor protein
AUD	Australian dollar
BACE	β-site APP cleaving enzyme (also known as β-secretase)
BACT	β-actin
BLAST	Basic Local Alignment Search Tool
C1QA	complement C1q subcomponent subunit A
CA	cornu Ammonis (of hippocampus)
CASP	caspase
CD	cluster of differentiation
CERAD	Consortium to Establish A Registry for Alzheimer’s Disease
CI	confidence interval
Cmu	*Chlamydia muridarum*
CNS	central nervous system
Cpn	*Chlamydia pneumoniae*
CR	Congo red
CS	cubic spline
CSF	cerebrospinal fluid
CXCL	C-X-C motif chemokine ligand
DAB	3,3'-diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole
DAVID	Database for Annotation, Visualisation, and Integrated Discovery
Db	fractal dimension
ddH_2O	deionised, distilled water
DG	dentate gyrus
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DPX	di-n-butylphthalate in xylene
EB	elementary body
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
EOAD	early onset Alzheimer’s disease
FAD	familial Alzheimer’s disease
FDR	false discovery rate
FGF	fibroblast growth factor
FITC	fluorescein isothiocyanate
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GFAP	glial fibrillary acidic protein
GSEA	gene set enrichment analysis
GSK-3β	glycogen synthase kinase-3β
GWAS	genome-wide association studies
HIV	human immunodeficiency virus
HPRT	hypoxanthine-guanine phosphoribosyl transferase
HRP	horseradish peroxidase
HSV	herpes simplex virus
IBA1	ionized calcium binding adaptor molecule 1

IHC	immunohistochemistry
IF	immunofluorescence
IFN	interferon
IFU	inclusion forming unit
IL	interleukin
iNOS/NOS2	inducible nitric oxide synthase
IVT	*in vitro* transcription
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LOAD	late onset Alzheimer's disease
LPS	lipopolysaccharide
MAPT	microtubule-associated protein tau
MCI	mild cognitive impairment
M.O.M.	mouse-on-mouse
MS	multiple sclerosis
NCBI	National Centre for Biotechnology Information
NDS	normal donkey serum
NES	normalised enrichment score
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NGS	normal goat serum
NFT	neurofibrillary tangle
NMDA	N-methyl-D-aspartate
NSAID	non-steroidal anti-inflammatory drug
NTP	nucleotide triphosphate
O.C.T.	optimal cutting temperature
PAMP	pathogen-associated molecular pattern
PANTHER	Protein Analysis through Evolutionary Relationships
PB	persistent body
PBS	phosphate buffered saline
PET	positron emission tomography
PP2A	protein phosphatase 2A
p-tau	phospho-tau
PS/PSEN	presenilin
r^2	coefficient of determination
RB	reticulate body
RNA	ribonucleic acid
rRNA	ribosomal RNA
ROS	reactive oxygen species
RT	room temperature
RT-PCR	real-time reverse transcription polymerase chain reaction
RTP	Research Training Program
Saa3	serum amyloid A 3
S.E.M.	standard error of the mean
SPG	sucrose-phosphate-glutamate
Stat	signal transducer and activator of transcription
tau	microtubule-associated protein tau
Th1	Type 1 T helper
TLR	toll like receptor
TM	transmembrane
TNF	tumour necrosis factor
TREM	triggering receptor expressed on myeloid cells
TS	thioflavin S
TSPO	translocator protein 18 kDa
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labelling
TX-100	Triton X-100
WT	wild-type