Role of Mechanical Forces in Asthma Pathogenesis

Punnam Chander Veerati

B. Pharm., M. Pharm. (Pharmacology)

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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.

Punnam Chander Veerati

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Conference Presentations

1. **Punnam Chander Veerati,** Kristy S Nichol, Andrew T Reid, Nathan W Bartlett, Peter A B Wark, Darryl A Knight, Chris L Grainge. *Mechanical Forces Suppress Innate Anti-Viral Immunity in Primary Human Airway Epithelial Cells Obtained from Asthmatic Donors.* American Thoracic Society (ATS) conference 2018 (Rapid Poster) – awarded ATS abstract scholarship.
2. **Punnam Chander Veerati,** Niamh M Troy, Andrew T Reid, Kristy S Nichol, Peter A B Wark, Darryl A Knight, Anthony Bosco, Nathan W Bartlett, Chris L Grainge. *Physiological Relevant Rhinovirus Infection Model in Differentiated Human Primary Bronchial Epithelial Cells from Healthy, Asthmatic and COPD Donors.* American Thoracic Society (ATS) conference 2018 (Poster presentation).
3. **Punnam Chander Veerati**, Nathan Bartlett, Kristy Parsons, Fatemeh Moheimani, Peter Wark, Darryl Knight, Chris Grainge. *Mechanical Forces Attenuate Anti-viral Immunity in Asthmatics.* Thoracic Society of Australia and New Zealand (TSANZ) Annual Scientific Meeting 2017 (Oral presentation).
4. **Punnam Chander Veerati**, Nathan Bartlett, Darryl Knight, Chris Grainge. *Defining the roles of airway mechanical stress during viral exacerbations of asthma.* Australian Respiratory Epithelium Consortium Workshop 2016 (Post Graduate Short Course) (Oral presentation).

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Abstract

Asthma is characterised by bronchoconstriction which leads to clinical symptoms and generates mechanical forces within the airway. During virally induced asthma exacerbations, bronchoconstriction and viral infection occur simultaneously; these factors may interact in the airway leading to the apparent innate immune deficiencies demonstrated in some asthmatics. This lead to our overarching hypothesis that “airway compressive forces suppress innate immunity following viral infection in asthmatics”.

To investigate this in *in-vitro* using a full experimental time course, there was a requirement for high cell numbers from individual patients with respiratory diseases such as asthma. Initially, we performed cell line optimisation to try to establish air-liquid interface (ALI) cultures. Later we used a conditionally reprogrammed (CR) technique on primary bronchial epithelial cells (pBECs) to proliferate indefinitely for extended passages and then used those at ALI.

In order to investigate the effects of apical compression on cells during viral infection, we wished to use a model which as closely as possible mimicked human airway infection *in-vivo*. Therefore we developed a physiologically relevant RV1B infection model and demonstrated for the first time that ultra-low multiplicity of infection induces delayed innate immune responses from cells obtained from asthma and COPD donors in comparison to healthy controls. Here we used cells obtained from COPD donors along with asthma to investigate anti-

viral responses; as previously it was shown that the anti-viral responses from COPD cells were also altered.

In order to investigate the effect of bronchoconstriction on anti-viral responses, we exposed pBECs obtained from asthmatic donors, grown at ALI to apical compressive stress mimicking bronchoconstriction along with physiologically relevant RV infection. We examined the effect of mechanical forces occurring prior to infection (mimicking poorly controlled asthma), or during viral infection (mimicking virally induced asthma exacerbation).

We demonstrate that apical compression suppresses innate immune responses from asthmatic pBECs under these conditions. Our data may explain why the patients with less well-controlled asthmatics appear more vulnerable to viral infection and why some asthmatics appear to have deficient Interferon (IFN) responses to natural infections.

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2-ME	2-mercaptoethanol
AB	Alcian blue
Ad-SV40	Adenovirus – simian virus 40
ALI	Air-liquid interface
ASM	Airway smooth muscle
BAL	Bronchoalveolar lavage
BCi-NS1.1	Basal cell immortalised – non-smoker 1.1
BEBM	Bronchial epithelial cell base medium
BEGM	Bronchial epithelial cell growth medium
BPE	Bovine pituitary extract
BSA	Bovine serum albumin
$CaCl_2$	Calcium chloride
CBA	Cytometric bead array
CDK 4	Cyclin-dependent kinase 4
cDNA	Complementary deoxyribonucleic acids
CFM	Complete freezing media
CK19	Cytokeratin 19
CK5	Cytokeratin 5
cm H_2O	Centimetres of water
CO_2	Carbon dioxide
CPE	Cytopathic effect
CR	Conditional reprogramming
DAB	3,3′-diaminobenzidine
DEG	Differentially expressed genes
DMEM	Dulbecco's modified eagle medium
DMEM/F12	Dulbecco's modified eagle medium/nutrient mixture F-12
DMSO	Dimethyl sulfoxide
D-PBS	Dulbecco’s phosphate buffered saline
EGF	Epithelial growth factor
EGFR	Epidermal growth factor receptor
Egr-1	Early growth response protein 1

ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinase
ET-1	Endothelin-1
EVOM	Epithelial volt/ohm meter
FBS	Fetal bovine serum
FDR	False discovery rate
FEV1	Forced expiratory volume in one second
FITC	Fluorescein isothiocyanate
FVC	Forced vital capacity
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Good manufacturing practice
H&E	Hematoxylin and eosin
HB-EGF	Heparin binding-Epidermal growth factor
HBSS	Hank's balanced salt solution
HC	Hydrocortisone
HCO_3^-	bicarbonate
hEGF	Human epidermal growth factor
HLF	Human lung fibroblasts
hTERT	Human telomerase reverse transcriptase
ICAM-1	Intercellular adhesion molecule 1
ICS	Inhaled corticosteroids
IF	Immunofluorescence
IFN	Interferon
IFNAR	Interferon-alpha/beta receptor
IFN-β	Interferon beta
IFN-λ	Interferon lambda
IHC	Immunohistochemistry
IL-13	Interleukin-13
IL-6	Interleukin-6
IL-8	Interleukin-8
IP-10	Interferon gamma-induced protein 10
IRF-3	Interferon regulatory transcription factor -3
IRF-7	Interferon regulatory transcription factor -7

ISGs	Interferon stimulatory genes
ITS	Insulin-Transferrin-Selenium
JAK-1/TYK-2	Janus kinase 1/tyrosine kinase 2
KSFM	Keratinocyte serum-free media
LA	Linoleic acid
LDL	Low-density lipoprotein
LGA	Low gelling agarose
LIS	Lateral intercellular space
m^2	Square metre
MDA5	Melanoma differentiation associated protein 5
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
MMP-9/TIMP-1	Matrix metalloproteinase-9/tissue inhibitor of metalloproteinases-1
MOI	Multiplicity of infection
N	Newtons
NBF	Neutral buffered formalin solution
NF-κB	Nuclear factor kappa light chain enhancer of activated B cells
Pa	Pascals
PAI-1	Plasminogen activator inhibitor-1
PAS	Periodic acid schiff
pBECs	Primary bronchial epithelial cells
PBS	Phosphate-buffered saline
PCLS	Precision cut lung slice
PDE-4	Phosphodiesterase-4
PKR	Protein kinase R
PLC	Programmable logic controller
qPCR	Quantitative polymerase chain reaction
RIG-1	Retinoic acid inducible gene 1
RNA	Ribonucleic acid
ROCK	Rho-associated, coiled-coil containing protein kinase
RT-PCR	Reverse transcriptase - polymerase chain reaction
RV1B	Rhinovirus 1B

SI	International system
SOCS3	Suppressor of cytokine signalling 3
STAT-1	Signal transducer and activator of transcription 1
STAT-2	Signal transducer and activator of transcription 2
T_3	Triiodothyronine
TACE	Transforming growth factor alpha converting enzyme
TBS-T	Tris-buffered saline –tween 20
$TCID_{50}$	50% tissue culture infective dose
TEER	Trans-epithelial electrical resistance
TF	Tissue factor
TGF-α	Transforming growth factor alpha
TGF-β_1	Transforming growth factor beta 1
TGF-β_2	Transforming growth factor beta 2
TLR	Toll-like receptors
TMB	3,3’,5,5’-trimethylbenzoate
TNS	Trypsin neutralizing solution
tPA	Tissue plasminogen activator
uPA	Urokinase plasminogen activator
uPAR	Urokinase-type plasminogen activator receptor
VEGF	Vascular endothelial growth factor
ZO-1	Zonula occludens-1