Mechanisms of Increased Susceptibility to Influenza Infection in Mouse Models of Chronic Lung Diseases

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Statement of originality

This thesis contains no material which has been accepted 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 in the text.

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The works presented in this thesis have been done in collaboration with other researchers. I have included the Statement of Collaboration which clearly outlines the extent of the collaborations.

Irwan Hanish bin Warsanah
Statement of collaboration

I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers. I have included below a statement clearly outlining the extent of collaboration, with whom and under what auspices.

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List of abbreviations

AAD: Allergic airway disease

AHR: Airway hyperresponsiveness

Akt-in: Akt inhibitor NH(2)-AVTDHPDLWAEKF-COOH

APC: Antigen presenting cell

BALF: Bronchoalveolar lavage fluid

CD: Cluster of Differentiation

cDNA: Complementary DNA

CARD: Caspase-recruitment domain

CLDSI: Chronic lung disease severity index

COPD: Chronic obstructive pulmonary disease

DC: Dendritic cell

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic acid

Dpi: Days post infection

ELISA: Enzyme linked immunosorbent assay

FcεRI: Fc epsilon receptor I

FEV₁: Forced expiratory volume in 1 second
170  FVC: Forced vital capacity
171  GOLD: Global Initiative for Chronic Obstructive Lung Disease
172  GPCR: G protein-coupled receptor
173  GTP: Guanosine triphosphate
174  H₂O₂: Hydrogen peroxide
175  H&E: Haematoxylin and eosin
176  HDM: House dust mite
177  HEK293: Human embryonic kidney 293
178  HO-1: Heme oxygenase 1
179  HPRT: Hypoxanthine-guanine phosphoribosyltransferase
180  Ig: Immunoglobulin
181  IFN: Interferon
182  pfu: Plaque forming unit
183  IKKi: IκB kinase-i
184  IL: Interleukin
185  IL-13Rα1: Interleukin-13 receptor alpha 1
186  ILC: Innate lymphoid cell
187  ILC2: Group 2 innate lymphoid cell
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<tr>
<td>188</td>
<td>i.n: Intranasal</td>
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<tr>
<td>189</td>
<td>i.p: Intraperitoneal</td>
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<tr>
<td>190</td>
<td>IP-10: IFN-γ-induced protein-10</td>
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<tr>
<td>191</td>
<td>IPS-1: IFN-β promoter stimulator 1</td>
</tr>
<tr>
<td>192</td>
<td>JAK: Janus kinase</td>
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<tr>
<td>193</td>
<td>KC: Keratinocyte-derived chemokine</td>
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<tr>
<td>194</td>
<td>MDA-5: Melanoma Differentiation-Associated protein-5</td>
</tr>
<tr>
<td>195</td>
<td>MDCK: Madin-Darby Canine Kidney</td>
</tr>
<tr>
<td>196</td>
<td>MHC: Major histocompatibility complex</td>
</tr>
<tr>
<td>197</td>
<td>MIP-1α: Macrophage inflammatory protein-1α</td>
</tr>
<tr>
<td>198</td>
<td>mRNA: Messenger ribonucleic acid</td>
</tr>
<tr>
<td>199</td>
<td>miRNA: MicroRNA</td>
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<tr>
<td>200</td>
<td>MSC: Mucus secreting cell</td>
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<tr>
<td>201</td>
<td>NF-κB: Nuclear factor kappa light chain enhancer of activated B cells</td>
</tr>
<tr>
<td>202</td>
<td>NK cell: Natural killer cell</td>
</tr>
<tr>
<td>203</td>
<td>NKT cell: Natural killer T cell</td>
</tr>
<tr>
<td>204</td>
<td>Nrf2: Nuclear factor (erythroid-derived 2)-like 2</td>
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<tr>
<td>205</td>
<td>NO: Nitric oxide</td>
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O$_2^-$: Superoxide

Ova: Ovalbumin

PAS: Periodic acid–Schiff

PAMP: Pathogen-associated molecular pattern

PBS: Phosphate-buffered saline

PC: Physical containment

PIP$_2$: Phosphatidylinositol 4,5–bisphosphate

PIP$_3$: Phosphatidylinositol 3,4,5-triphosphate

PIV-3: Parainfluenza virus type 3

PKB: Protein Kinase B

PRR: Pattern recognition receptor

PVDF: Polyvinylidene difluoride

qPCR: Quantitative real-time Polymerase Chain Reaction

RIG-I: Retinoic acid-inducible gene-I

ROS: Reactive oxidant species

rIL-13: Recombinant Interleukin-13

RIPA: radio-immunoprecipitation assay

RLR: RIG-I-like receptor
RNA: Ribonucleic acid

RSV: Respiratory syncytial virus

RV: Rhinovirus

SDS-PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis

SEM: Standard error of the mean

SPF: Specific pathogen free

SH: Src Homology

STAT6: Signal transducer and activator of transcription 6

TANK: TRAF family member-associated NF-κB activator

TBK1: TANK binding kinase-1

Th: T helper lymphocyte

TLC: Total lung capacity

TLR: Toll-like receptor

TNF: Tumour necrosis factor

TRAF: TNF receptor associated factor

VEGF: Vascular endothelial growth factor

VPg: Virion protein genome linked protein
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Influenza infections are of major importance as they have a significant impact on
the health of individuals and impart substantial socio-economic ramifications on
society. Prevention and treatment of influenza infections are complicated by frequent
genetic mutations of the influenza virus. Patients with underlying chronic lung diseases,
such as chronic obstructive pulmonary disease (COPD) and asthma are more susceptible
to influenza infection, and infection with influenza exacerbates these diseases.
Therefore, elucidation of the mechanisms underpinning increased susceptibility to
influenza in these patients is vital. Here, we established an experimental mouse model
of COPD and utilised an existing ovalbumin-induced allergic airways disease (AAD)
model to investigate the effects of influenza infection in COPD and asthma,
respectively. Influenza infection in experimental COPD resulted in increased viral titre,
exaggerated airway inflammation and further impaired lung function. These effects
were accompanied by decreased neutrophil influx into the airways, reduced antiviral
interferon responses, and the suppression of a range of cytokines and chemokines,
including interferon (IFN)-γ, tumour necrosis factor (TNF)-α, IFN-γ-induced protein
(IP)-10, macrophage inflammatory protein (MIP)-1α, keratinocyte-derived chemokine
(KC, or IL-8 in humans) and interleukin (IL)-10, as well as increased IL-6. This
increased susceptibility was mediated by an increase in phosphoinositide 3-kinase
(PI3K) protein expression. The inhibition of PI3K effectively reduced viral titre,
enhanced antiviral IFNs and improved lung function.

Influenza infection in recombinant IL-13-treated or ovalbumin (Ova)-induced
AAD models led to increased viral titre, impaired antiviral responses and increased
airway hyper-responsiveness (AHR). It also resulted in exaggerated airway
inflammation, more severe histopathology, increased mucus secreting cell numbers and
increased IL-13. Importantly, we also showed that inhibition of IL-13 by administration
of anti-IL-13 (αIL-13) monoclonal antibody during influenza infection reduced viral
titre, AHR, eosinophil infiltration and MSCs, which were associated with improved
antiviral IFN responses.

In summary, these data highlight the important roles of PI3K and IL-13 in the
increased susceptibility to influenza infection in experimental models of COPD and
asthma, respectively. Such findings offer evidence for new and promising avenues for
influenza disease management in these chronic lung diseases. In fact, both PI3K
inhibitors and anti-IL-13 antibodies have already entered clinical trials and may be
utilised as novel therapeutic approaches for influenza infections in the future.