Investigating the Role of Interferon-Inducible Transmembrane 3 (IFITM3) in Infection.

By

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Abstract

My thesis focuses on the interaction between interferon-inducible transmembrane 3 (IFITM3) and influenza viruses. IFITM3 confers cells *in vitro* with resistance to multiple pathogenic viruses, including influenza, dengue and West Nile virus amongst others (Brass *et al.* 2009; Huang *et al.* 2011). Although the current mechanism of restriction is unknown, it is thought that aggregation of IFITM3 within the late endosomes prevents the membrane fusion necessary for the release of viral nucleic acids and proteins into the cells' cytoplasm (Feeley *et al.* 2011; John *et al.* 2013).

My thesis aims to further understanding of IFITM3 through the use of a knockout mouse model with an ablation of the *Ifitm3* allele (*Ifitm3*$^{-/-}$). Challenge of the mouse with sub-lethal doses of influenza A virus showed that the loss of Ifitm3 resulted in heightened susceptibility to the virus, which resulted in accelerated weight loss, fulminant viral pneumonia, a persistent viral burden and ultimately death. These phenotypic effects are more commonly associated with infections using highly pathogenic 1918 'Spanish' influenza and avian H5N1 influenza viruses.

These findings were taken further by analysing the prevalence of single nucleotide polymorphisms (SNPs) in the *IFITM3* locus of humans hospitalised during the 2009 H1N1 pandemic. Through international collaboration, SNP rs12252-C, which is thought to be associated with sub-optimal IFITM functioning, was identified as being over-represented in these patients. Typically, 0.3% of the European Caucasian population are homozygous for the rs12252-C allele; however, the study showed that in patients hospitalised with influenza virus this proportion increased to 5.7%: a significant enrichment.

Furthering this observation, the thesis also investigates the effects and interactions of IFITM3 on medically-relevant treatments. Primarily, studies were employed to test the safety and efficacy of live attenuated influenza virus vaccines in *Ifitm3*$^{-/-}$ mice to assess the potential for vaccine-associated morbidity in individuals possessing sub-optimally functioning IFITM3, and if protection is elicited against subsequent infection. This showed the vaccine was safe in these mice, and induced a normal, robust immune response that protected mice from a lethal challenge with pandemic H1N1 influenza virus. Furthermore, the mouse model was employed to assess the

effects of AmBisome, a commonly used antifungal agent, on Ifitm3 function, as it had been shown to cause a bypass of IFITM3-based restriction *in vitro*. The wild type mice treated with AmBisome prior to, and during, influenza virus infection show weight loss and morbidity similar to *Ifitm3*$^{-/-}$ mice; suggesting that AmBisome may heighten viral susceptibility in patients treated with this drug.

The thesis concludes with a meta-analysis investigating the *in vivo* effects of Ifitm3 in restricting a range of bacterial, viral and protozoan pathogens. This demonstrates the specificity of Ifitm3 for restricting only specific viral pathogens, despite the fact that a variety of pathogens utilise the endosomal pathway for entry into cells.

In conclusion, the thesis furthers our knowledge of IFITM3 by showing for the first time its *in vivo* effects on viral restriction and the criticality of IFITM3 in preventing the morbidity and mortality associated with influenza viruses.

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Here's to the next step and whatever that may bring...

Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

Dr. S. Clare from the Wellcome Trust Sanger Institute assisted the author in all live animal work and conducted all inoculations, immunisations and animal procedures during the course of experiments. Dr. A.L. Brass gave guidance and practical help with *in vitro* MEF transductions and infections, immunofluorescence imaging of *in vitro* influenza infections, and in RNA immunohistochemistry of tissue sections whilst I was part of his lab at the Ragon Institute, USA. Dr. D. Goulding performed the GMA-embedded protein immunohistochemistry.

All other protocols relating to influenza challenge, including *in vitro* and *ex vivo* work, were conducted by the author.

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Table of contents

List of tables

List of figures

Abbreviations

aDC	Alveolar dendritic cell
APC	Antigen presenting cell
BAL	Bronchoalveolar lavage
CFU	Colony forming unit
CTL	Cytotoxic T-lymphocyte
DC	Dendritic cell
ELISA	Enzyme-linked immunosorbant assay
g	Force of gravity
GenISIS	Genetics of *Influenza* Susceptibility in Scotland
GMA	Glycol methacrylate
HA	Hemagglutinin
HCV	Hepatitis C virus
HP	High pathogenicity
HTS	High throughput screening
IFN	Interferon
Ig	Immunoglobulin
i.v.	Intravenous
IL	Interleukin
i.m.	Intramuscular
i.n.	Intranasal
i.p.	Intraperitoneal
ISG	Interferon-stimulated gene
i.v.	Intravenous
kDa	Kilo Dalton
LAIV	Live attenuated influenza vaccine
LCL	Lymphoblastoid cell line
LD	Lethal dose
LP	Low pathogenicity
MEF	Murine embryonic fibroblast

MHC	Major histocompatability complex
MOSAIC	Mechanisms of Severe Acute Influenza Consortium
NA	Neuraminidase
NK	Natural killer
PCR	Polymerase chain reaction
PFU	Plaque forming unit
PRR	Pathogen recognition receptor
QIV	Quadrivalent influenza vaccine
QTL	Quantitative trait loci
RNAi	RNA interference
RSV	Respiratory syncytial virus
SA	Sialic acid
siRNA	Small interfering RNA
SNP	Single nucleotide polymorphism
TCID	Tissue culture infective dose
TIV	Trivalent influenza vaccine
TLR	Toll-like receptor
pDC	Plasmacytoid dendritic cell
VRDF	Viral replication dependence factor
VRF	Viral restriction factor
WHO	World Health Organisation
WTSI	Wellcome Trust Sanger Institute