

Immunological determinants of lung protection and repair
during viral infection

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A thesis submitted for the degree of

Doctor of Philosophy

Imperial College London

September 2020

Declaration

I Jack Major confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

During infection with respiratory viruses, destruction of the lung epithelium is due to cytopathic viral effects in host cells, and damage generated by the ensuing inflammatory immune response. Host response pathways that protect or exacerbate lung tissue damage are not fully understood, particularly with regard to the impact on lung regeneration during recovery from disease. Type I (IFN- α and IFN- β) and III (IFN- λ) interferons are host-produced cytokines exerting pleiotropic antiviral, antiproliferative, and immunomodulatory effects in target cells. Here, we examine the effects of type I and III IFN on lung repair during recovery from influenza. We show that IFN signalling specifically induced in respiratory epithelial cells disrupts epithelial proliferation and differentiation during lung repair. IFN- λ drives these effects most potently, activating p53-dependent cell cycle arrest. Disruption of lung repair processes results in exacerbated lung tissue damage, compromised protective barrier function, and increased susceptibility to bacterial superinfections. Owing to the pathogenic potential of aberrant host immune responses, including IFNs, these pathways require tight regulation by host factors to limit tissue damage. This includes the environmental sensor aryl hydrocarbon receptor (AHR), which has been shown to enhance barrier immunity, promote disease tolerance, and suppress IFN production during viral infection. Using genetic models to increase the availability of natural AHR ligands, we show that the AHR induces signals in lung barrier tissues to reduce pulmonary damage and dampen inflammation. Tissue-protective pathways induced by the AHR appear to occur independently from AHR-dependent regulation of IFN production. Taken together, our data highlights the importance of timing and regulation in generating protective antiviral immunity and lung barrier protection, in order to prevent the development of pathogenic, tissue-damaging responses.

Acknowledgements

I thought I would use this as an opportunity to tell everyone how much they really mean to me, although I do worry about embarrassment once the delirium wears off.

First up, Andreas. I couldn't have asked for a better supervisor. Aside from the boring stuff (top projects, scientific insight, guidance, support, trust), I feel privileged to be such good mates with my boss. Thanks for having faith, you'll smash the upcoming review. Stefania, where to begin! A testament to the extent in which I look up to you is the sheer number of times I've said to my mates: 'Well, Stefania thinks/does 'x''. I think you're brilliant, both in and out of science. Thanks for everything.

A huge shout-out goes to all lab members, past and present! Justina, Katja, Ashleigh, Julie, Milena, Konrad, Teresa and Claire. Special mention goes to Katja, for being there since day 1, and all the extra work you do behind the scenes. Justina, you genuinely brighten my day at work, so thanks for that. Helena, I'll not lie, I've missed you a lot since you left. In science, you helped me a ridiculous amount, and your passion is inspirational. It's rare to meet someone so like-minded, and a treat if you get to work with them. I'm content in knowing that we will always be close.

The STPs make the Crick what it is, so a huge thanks goes to those that work there. In particular, the BRF, FACS team, bioinformatics, advanced sequencing, and histology, who have all helped me out numerous times over the last four years. Thanks to Miriam and Probir in bioinformatics, and Simon and Alejandro in histology, for turning my data into something meaningful! Big thanks to all the BRF staff in Ex1—Tinashe, Jake, Craig, Lewis and Alex in particular, for your hard work. I also always loved having a friendly face downstairs, but mainly skiving off lab work and chatting about the mighty toon/the dross that is north London clubs. I'd also like to thank David Bacon, whose courses I always thought were brilliant, and for taking the time to help whenever I needed it. Thanks to the thesis committee for all the guidance along the way. I'd especially like to thank Gitta, for all the advice, reagents, and time spent to help me throughout the project. Our Italian collaborators across the pond, Achille and Ivan. I've loved working with you both, and love that we share our stories together in the same journal. Me crew (BBC), Liam, Eva and Marc. Love you guys, you've made the last 4 years immeasurably more enjoyable. Shout out to Hugo as well, my oldest friend at the Crick!

Alreet marras, your turn. Tin, without a doubt, the finest mind the Crick has ever had

walk through those pods (even you, Paul). You're the most important mention here. You are the perfect distraction for all things gloomy, and I have no idea what I'd do without you. Sean, there isn't a friend I miss more when I don't see you for a while. Yet I see you all the time, so that's embarrassing. You're an utter joy to be around wilsywils. Ali, I think I'm most content when I'm with you. You have an uncanny ability to make everything seem fine. Hanging out with you three is hands down my favourite thing to do. Sana, I couldn't ask for a better friend. You're the most caring and brilliant person I know. Thanks for everything. Leah, I've loved every second living with you. You always know how to pick me up when things are down without even trying to do so. You Zards are my family. Josie, I absolutely love when I get to hang out with you, and it makes me so happy seeing the way you and Ali are together. Hels, I love our friendship, I realised this most when you said you were staying in London. Dec, I don't think I've ever liked someone as much straight from the off. Everyone seems to feel a similar way, which is a testament to your character. Aidan, you're another one who just makes life more enjoyable when you're around them. Can't wait until you're home pal. Claire, you're an absolute treasure. You're the funniest person I know, and I look forward to every time I get to see you. Sofie, I'm so happy to have you as one of my closest friends, and I can't wait to see some gigs with you again. Cat, I was delighted when I heard you were finally moving down to London. I think you're great, and one of the kindest people I've ever met. Matt, I can't tell you how happy it makes me that we've become such good mates. You've become my closest confidant, and I feel like I can talk to you about anything, which really means a lot to me.

Mum, I'm so proud of you. You've not had it easy, yet you've been a rock the whole time. Dad, my role model in science! You've instilled in me a passion for the pursuit of knowledge. Thank you both for all the support, and everything you've done for me over the years. I love you both dearly.

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Abbreviations

3-MC	3-methylcholanthrene
ACE2	Angiotensin-converting enzyme 2
AEP	Alveolar epithelial progenitors
AHR	Aryl hydrocarbon receptor
AHRR	Aryl hydrocarbon receptor repressor
AIP	Aryl hydrocarbon receptor-interacting protein
ALI	Air–liquid interface
ARDS	Acute respiratory distress syndrome
ARNT	Aryl hydrocarbon receptor nuclear transporter
AT1	Type I alveolar
AT2	Type II alveolar
AEC	Airway epithelial cell
B6	C57BL/6
BALF	Bronchoalveolar lavage fluid
BASCs	Bronchioalveolar stem cells
bHLH	Basic helix-loop-helix
BM	Bone marrow
CARD	Caspase associated recruitment domain
CCSP	Club cell secretory protein
cDCs	Conventional dendritic cells
CDK	Cyclin-dependent kinase
CFU	Colony-forming unit
ChIP	Chromatin immunoprecipitation
CKI	Cyclin-dependent kinase inhibitor
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CTL	Cytotoxic T lymphocytes
DAMPs	Danger-associated molecular patterns
DAPI	4,6-diamidino-2-phenylindole
DASCs	Distal airway stem cells
DDR	DNA damage response
DCs	Dendritic cells

DR	Death receptor
EAE	Experimental autoimmune encephalitis
ECM	Extracellular matrix
EdU	5-ethynyl-2 deoxyuridine
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
FADD	Fas-associated death domain-containing protein
FasL	Fas-ligand
Fgf10	Fibroblast growth factor 10
FICZ	6-formylindolo[3,2-b]carbazole
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GO	Gene ontology
GSEA	Gene set enrichment analysis
HA	Haemagglutinin
HAH	Halogenated aromatic hydrocarbons
H&E	Hematoxylin and eosin
HIF1 α	Hypoxia-inducible factor-1 α
HIF1 β	Hypoxia-inducible factor-1 β
HSP90	Heat shock protein 90
I3C	Indole-3-carbinol
I3S	Indoxyl-3-sulfate
IAV	Influenza A virus
IBV	Influenza B virus
ICZ	Indole-3-carbazole
IDO	Indoleamine-2,3-dioxygenase
IECs	Intestinal epithelial cells
IFITM	Interferon-inducible transmembrane proteins
IFN	Interferon
IFNAR	IFN- α/β receptor
IFNLR	IFN- λ receptor
iNOS	Inducible nitric oxide synthase
IL	Interleukin
IL-22R	IL-22 receptor
ILCs	Innate lymphoid cell

IPA	Ingenuity Pathway Analysis
IRF	Interferon-regulatory factors
ISGF3	Interferon-stimulated gene factor 3
ISRE	Interferon-stimulated response elements
ITE	2-(1'H-indole-3-carbonyl)-thiazole-4-carboxylic acid methyl ester
JAK1	Janus kinase 1
ISG	Interferon-stimulated gene
KRT5	Keratin 5
Kyn	Kynurenine
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
M	Microfold
MAVS	Mitochondrial antiviral signalling protein
MERS-CoV	Middle East respiratory syndrome coronavirus
MEFs	Mouse embryonic fibroblasts
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
MYD88	Myeloid differentiation factor 88
NA	Neuraminidase
NETs	Neutrophil extracellular traps
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer
NLRP3	NOD-leucine rich repeat (LRR) and pyrin domain-containing 3
NOD	Nucleotide-binding oligomerisation domain
NS1	Non-structural protein-1
PAHs	Polycyclic aromatic hydrocarbons
PAMPs	Pattern-associated molecular pattern
PAS	Pern-Arnt-Sim
pDCs	Plasmacytoid dendritic cells
PBMCs	Peripheral blood mononuclear cells
PR8	H1N1 influenza A/Puerto Rico/8/34
PRRs	Pattern recognition receptors
qPCR	Quantitative polymerase chain reaction
RB	Retinoblastoma

RBC	Red blood cell
RIG-I	Retinoic acid-inducible gene-I
RIPLET	RING finger protein
RNAi	RNA interference
ROS	Reactive oxygen species
RSV	Respiratory syncytial virus
SARS-CoV	Severe acute respiratory syndrome coronavirus
SOCS	Suppressor of cytokine signalling
SP-C	Prosurfactant apoprotein-C
STAT1	Signal transducer and activator of transcription 1
ss	Single-stranded
TBK1	Tank binding kinase 1
TCDD	2,3,7,8-tetracholrodibenzo-p-dioxin
TDO	Tryptophan-2,3-dioxygenase
Th	T helper
TIPARP	TCDD-inducible poly(ADP-ribose)polymerase
T _{FH}	T follicular helper
TLR	Toll-like receptor
TNF α	Tumor necrosis factor- α
TPM	Transcripts per million
Tr1	Type 1 regulatory cells
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
Tregs	Regulatory T cell
TRIF	TIR-domain-containing adaptor protein inducing IFN β
TSLP	Thymic stromal lymphopoietin
TYK2	Tyrosine kinase 2
vRNP	Viral ribonucleoprotein
WHO	World Health Organisation
WT	Wild type
XREs	Xenobiotic response elements

Chapter 1. Introduction

1.1 Respiratory viruses: an overview

Respiratory infections are the leading cause of mortality by communicable diseases globally, accounting for approximately 4 million deaths per year (World Health Organization (WHO), 2018). Respiratory viruses account for approximately half of these infections (Legend *et al.*, 2013). Viruses that play an aetiological role in human respiratory disease include influenza virus, coronaviruses, respiratory syncytial virus (RSV), and rhinoviruses. Typical symptoms include a persistent cough, muscle aches, fever, rhinorrhoea and pharyngitis. However, exacerbation of respiratory viral disease can result in severe clinical manifestations, including pneumonia, acute respiratory distress syndrome (ARDS), bronchiolitis in young children, otitis media, myocarditis, encephalitis, gastrointestinal symptoms, asthma and chronic obstructive pulmonary disease (COPD) exacerbations, chronic lung damage, and multi-organ failure.

Respiratory viral diseases often occur in seasonal cycles. Evidence indicates that both environmental factors, such as changes in temperature and humidity, as well as changes in host behavioural patterns, are determinants of respiratory viral epidemics (Moriyama, Hugentobler & Iwasaki, 2020). The economic burden of respiratory viral infections is significant. Direct costs arise through expenditure on health care, treatments, and vaccination programmes, whilst indirect costs occur due to reduced productivity owing to a loss of working days (World Health Organization (WHO), 2016). In the United States alone, estimated costs are over \$87 billion per year for seasonal influenza (Molinari *et al.*, 2007).

In addition to reoccurring seasonal epidemics, novel zoonotic respiratory viruses harbour pandemic potential. In the decade following the turn of the millennium, emerging respiratory viruses resulted in several global outbreaks: the severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) outbreak in 2003; the Middle East respiratory syndrome coronavirus (MERS-CoV) outbreak in 2014; and the 2009 swine flu pandemic. Lack of prior exposure to novel respiratory viruses, and therefore a lack of pre-existing immunity upon exposure, renders the majority of the global population susceptible following zoonotic transmission. Additionally, the speed in which respiratory viruses spread in an era of globalisation, increased population densities, and frequent air travel, dramatically increases the risk of global transmission. In 2018, on the 100th anniversary of the 1918 influenza pandemic, Victor Dzau, president of the National Academy of Medicine, spoke of the threat emerging

viruses pose to society, stating, “The world is ill-prepared for another influenza pandemic” (Ogawa, Nicholson & Shah, 2019).

These warnings were realised following the emergence of a novel coronavirus in the Hubei province of China, late 2019 (Zhu *et al.*, 2020; Wang *et al.*, 2020a; Zhou *et al.*, 2020a). The virus, named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is genetically similar to the SARS-CoV, with the two coronavirus strains sharing approximately 75 to 80% of their genomes (Zhou *et al.*, 2020a). SARS-CoV-2 infection causes coronavirus disease 2019 (COVID-19). The majority of infected individuals are either asymptomatic or experience mild disease. Patients exhibiting moderate or severe disease typically suffer from pneumonia or hypoxic respiratory failure following progression to ARDS (Guan *et al.*, 2020; Wu & McGoogan, 2020). The COVID-19 crisis was declared a pandemic by the World Health Organisation (WHO) on March 11, 2020. Governments and local authorities implemented global health measures in an effort to curb the spread of SARS-CoV-2, resulting in an unprecedented situation whereby billions of people simultaneously remained indoors. Direct and indirect consequences of the COVID-19 crisis are yet to be fully realised, with over 32 million cases and more than 1 million deaths worldwide at the time of writing, in addition to a severe impact on the global economy that greatly surpasses that of the 2008–09 financial crisis.

The continued threat of future influenza or coronavirus pandemics underlines the importance of furthering our understanding of respiratory viral infection biology. Unanswered questions remain concerning the variation in disease severity between susceptible individuals, between certain demographics but also within particular demographics. Mounting evidence implicates both host-intrinsic genetic factors and environmental influences in determining the balance between protective and pathologic immune responses. Lack of an available vaccine or specific antivirals during the emergence of novel zoonotic viruses highlights the importance of generating and implementing effective host-directed therapeutic strategies. This requires an understanding of the processes underlying protective antiviral immunity, disease tolerance, and harmful immunopathological responses, during respiratory viral infection.

1.2 Influenza

1.2.1 Influenza A viruses

Influenza is an acute respiratory viral disease caused by influenza virus infection. Influenza viruses belong to the Orthomyxoviridae family that includes influenza A, B and C viruses. Influenza A virus (IAV) infection is the predominant cause of influenza disease in

humans, and uniquely circulate in humans, domestic animals (including pigs and poultry), and wild migratory birds (Webster *et al.*, 1992). Wild birds, in particular water birds, are the natural reservoir of IAV strains, with avian influenza viruses preferentially replicating in intestinal epithelial cells, mostly resulting in asymptomatic infection. IBV also circulates in humans, and generates seasonal epidemics (Paules & Subbarao, 2017).

Smith, Andrewes, and Laidlaw first isolated IAV in ferrets in 1933 (Smith, Andrewes & Laidlaw, 1933). Influenza viruses are enveloped negative-sense single-strand (ss) RNA viruses. The IAV genome is segmented, containing eight RNA segments encoding up to eleven viral proteins: viral nucleoprotein (NP), matrix protein (M1), membrane protein (M2), non-structural proteins (NS1 and PB1-F2), nuclear export protein (NEP), three RNA polymerase subunits (PB1, PB2, and PA), and viral glycoproteins (haemagglutinin (HA), and neuraminidase (NA)). The viral RNA segments, together with nucleoproteins and the polymerase subunits, make up the viral ribonucleoprotein (vRNP) complex. IAV subtypes are determined by expression of the antigenically variant HA and NA proteins on the viral surface. To date, there are 18 identified HA subtypes and 11 NA subtypes, and the specific combination of these surface proteins denominates each IAV strain (e.g. H1N1, H3N2).

The evolution of influenza virus strains typifies the host-pathogen arms race. The influenza RNA polymerase enzyme is error prone owing to a lack of proofreading activity, resulting in a high rate of mutation of approximately one error per replicated genome (Drake, 1993). Therefore, influenza viruses gradually accumulate mutations in HA and NA proteins in a process known as antigenic drift, allowing evasion of antibody- or T cell-mediated immunity induced through prior infection or vaccination (Webster *et al.*, 1992). The process of antigenic drift necessitates the continuous monitoring of circulating viruses for antigenic changes in human and animals through influenza virus surveillance programmes, to help predict IAV subtypes for vaccine development before seasonal epidemics.

Pandemic IAV strains are antigenically distinct to seasonal epidemic strains, and are closely associated with a process known as antigenic shift. Antigenic shift occurs when two IAV strains reassort their segmented genome within a host, producing an antigenically distinct strain. Swine express both avian and human influenza receptors in their respiratory tracts (Ito *et al.*, 1998), and can therefore act as a “mixing vessel” for multiple IAV strains from different origin, as was the case for the 2009 swine flu pandemic (Pappaioanou & Gramer, 2010). Pandemic influenza occurs once every 10 to 50 years. Since the beginning of the 20th century, four human influenza pandemics have occurred from swine or avian origin: H1N1 Spanish flu pandemic of 1918, H2N2 Asian flu pandemic of 1957, H3N2 Hong Kong pandemic of 1968,

and the H1N1 swine flu pandemic of 2009. In the years following a pandemic, emerging pandemic IAV strains often drift antigenically and replace or co-circulate with existing strains. Currently, pandemic 2009 H1N1 IAV co-circulates with H3N2 IAV in humans.

Direct zoonosis of antigenically diverse IAV strains from avian or swine origin is rare, and largely restricted to specific geographical regions where such strains are more prevalent and there is increased human contact with infected swine or poultry (de Wit & Fouchier, 2008; Krammer *et al.*, 2018). Avian H5N1 and H7N9 IAV strains can cause severe disease in humans (Uyeki & Cox, 2013), however reported cases of human-to-human transmission have been limited (Van Kerkhove *et al.*, 2011). The first detection of H5N1 IAV in humans occurred in 1997 in Hong Kong, and there have since been a confirmed 861 cases of human infection reported in 17 countries (World Health Organization (WHO), 2020). The emergence of a novel H7N9 IAV in China has since resulted in 1,568 confirmed cases of human infection (World Health Organization (WHO), 2020). Avian IAV strains are associated with highly pathogenic influenza and mortality rates as high as 60% for H5N1, and 20% for H7N9, causing respiratory and multi-organ failure in infected individuals (Abdel-Ghafar *et al.*, 2008; Wang *et al.*, 2017; Poovorawan *et al.*, 2013). The increased prevalence in human hosts furthers the possibility of avian IAV reassortment with seasonal strains, or adaption to allow efficient transmission between humans. As few as five amino acid substitutions are required for H5N1 IAV to become transmissible in ferrets: a host often used as a model to study human influenza virus transmission (Herfst *et al.*, 2012). Therefore, highly pathogenic avian influenza poses a significant pandemic threat.

1.2.2 Influenza virus life cycle

The significant threat respiratory viruses pose to society and global health is in large part due to their efficiency in person-to-person transmission. Human influenza viruses preferentially bind to sialyloligosaccharides terminated by a N-acetylsialic acid linked to a galactose by an α -2,6-linkage expressed mainly by epithelial cells of the upper respiratory tract (Rogers & Paulson, 1983). Influenza transmission therefore occurs via the respiratory route, thus increasing the likelihood for person-to-person transmission. Virus particles released by an infected individual are more easily spread through coughing, sneezing, or talking, in contrast to viruses transmitted only by direct contact with bodily fluids, for instance Ebola virus. Transmission occurs via three routes: large ($>10\ \mu\text{m}$) droplet particles depositing on the

mucosal surface of the upper airway epithelium; small ($<5\ \mu\text{m}$) aerosols by inhalation; and by direct or indirect contact transmission, via the transfer of virus particles in a mucus membrane (Killingley & Nguyen-Van-Tam, 2013).

Influenza virus tropism is dictated by tissue expression of different linkages sialyloligosaccharide receptors. α -2,6-links are expressed in humans by both upper airway and alveolar epithelial cells enabling human influenza virus entry, whilst α -2,3-linkages, expressed by epithelial cells lining the avian gastrointestinal tract, are also found on human alveolar epithelial cells (Shinya *et al.*, 2006). Absence of α -2,3-links in the human upper respiratory tract may explain the limited human-to-human transmission of avian influenza viruses, yet expression in the lower alveolar epithelium may contribute to the increased severity of disease caused by avian influenza virus strains. Swine express both α -2,6-linked and α -2,3-linked sialyloligosaccharide receptors in their respiratory tract, and are therefore susceptible to both avian and human influenza virus infection, and can act as an intermediary host for reassortment of multiple strains of different origin (Ito *et al.*, 1998).

Influenza virus entry is mediated by the viral envelope protein HA binding to sialic acid residues on respiratory epithelial cells. HA-mediated binding triggers virus internalisation via endosomal uptake. The low endosomal pH triggers a conformational change in HA, allowing fusion of the viral envelope with the endosome. The low pH also opens the M2 ion channel resulting in acidification the inner contents of the viral particle, triggering the release of the viral genetic material (eight vRNPs) into the cytoplasm, which are then subsequently imported to the host cell nucleus for viral transcription and replication. Viral RNA polymerase initiates transcription of viral RNAs generating a positive sense intermediate, which serves as a template to transcribe negative-strand mRNAs. Viral mRNAs are capped and polyadenylated before nuclear export into the cytoplasm, mediated by viral M1 and NEP proteins, and migrate to the plasma membrane by endosomal recycling. Host cell machinery-dependent translation synthesizes new viral proteins, which are assembled at the plasma membrane together with new vRNPs. Finally, viral budding from the plasma cell membrane results in the formation of a newly formed virion, with NA facilitating viral release and spread by catalysing the hydrolysis of sialic acid residues on the cell surface to prevent HA binding. Influenza virus replication and budding triggers cell death, contributing to respiratory epithelial damage and lung pathology.

1.2.3 Influenza disease in humans

Each year, there are an estimated 1 billion cases of influenza globally, 3–5 million of which are severe (World Health Organisation (WHO), 2019). Recent modelling estimates the global annual influenza-associated respiratory mortality rate is approximately 290,000–645,000 deaths per year from 1999–2015 (Iuliano *et al.*, 2018). Onset of symptoms usually occurs after an initial incubation period of 24–48 hours. Clinical manifestation of seasonal influenza ranges from asymptomatic infection or upper respiratory tract illness, to severe disease with infected individuals exhibiting viral pneumonia, ARDS, and secondary infection by bacterial pathogens.

Epidemic seasonal influenza is associated with increased severity and high death rates in the very young (<2 years old) and in the elderly (>65 years old), owing to an under-developed or aging immune system, respectively. Younger children are also important in enhancing community influenza transmission, in part due to limited pre-existing immunity and high virus titres (Antonova *et al.*, 2012). Indeed, vaccination of children reduces the incidence of severity in elderly (Cohen, Chui & Naumova, 2011). Additional risk factors include pregnancy, obesity, medical comorbidities, and immunosuppression or immunodeficiency (Peteranderl, Herold & Schmoltdt, 2016).

Pandemic influenza can be associated with increased virulence, owed at least in part to a degree of immunological naivety amongst the global human population. The Spanish flu of 1918–19, caused by an H1N1 IAV, was the most deadly influenza pandemic in modern history, infecting approximately one-third of the global population and resulting in at least 50 million deaths (Taubenberger & Morens, 2006). Incidentally, the term “Spanish” flu is a misnomer, as the virus did not originate in Spain. Rather, the neutrality of Spain during World War I meant increased Spanish media coverage of the deadly pandemic, as opposed to censorship of the outbreak in warring nations. Simultaneous outbreaks across North America, Asia, and Europe makes the exact origin difficult to place (Taubenberger, 2006). The 1918 flu was deadly, with mortality rates of those infected exceeding 2.5%, as opposed to 0.1% in other influenza pandemics (Taubenberger, 2006). Individuals experiencing severe disease following 1918 H1N1 infection often displayed viral pneumonia, with autopsies at the time revealing massive pulmonary damage (Rello & Pop-Vicas, 2009). A subset of patients died within a week of symptom onset, highlighting the degree of virulence and lethality of this pandemic influenza virus. A review of autopsies published during the pandemic suggested the leading cause of

mortality was secondary bacterial superinfection (Chien, Klugman & Morens, 2009), exacerbated by the fact that antibiotics were not available at the time.

The subsequent three influenza pandemics were significantly less deadly in nature. The 1957 H2N2 and 1968 H3N2 influenza pandemics each resulted in over 1 million deaths, and the 2009 H1N1 pandemic causing approximately 280,000 influenza-associated deaths (Honigsbaum, 2020; Peteranderl, Herold & Schmoldt, 2016). An important trend with pandemic influenza is the altered distribution of cases and mortalities by age. Whilst seasonal epidemic influenza particularly affects the very young and the elderly, pandemic influenza is associated with increased severity in young adults. Peak mortality during the 1918 pandemic was at the age of 28 in the United States of America and Canada 1918 (Gagnon *et al.*, 2013), whilst approximately 50% and 80% of lethal cases were in people below the age of 65 during the 1968 and 2009 pandemics, respectively (Honigsbaum, 2020; Karageorgopoulos *et al.*, 2011; Dawood *et al.*, 2012). Additionally, avian IAV H7N9 and H5N1 infections and severity in humans also differ according to age distribution (Qin *et al.*, 2015; Cowling *et al.*, 2013).

Reasons as to why there is a “pandemic age shift” are not fully understood (Simonsen *et al.*, 1998; Taubenberger & Morens, 2006). One hypothesis suggests an age-associated immunological protection depending to prior virus exposure. A recent study using statistical modelling revealed that immunological imprinting occurs in age groups following exposure to a virus, conferring protection against later emerging viruses of the same or related subtype (Gostic *et al.*, 2016). During the H1N1 1918 pandemic, it is predicted that the susceptible age group (20–40 year olds) had suboptimal immunity owing to mismatched immunological imprinting, having been exposed to an H3 virus in the 1890s, as opposed to older generations who were thought to have prior exposure to an H1 variant (Worobey, Han & Rambaut, 2014). Additionally, during the 2009 H1N1 pandemic very few younger individuals had cross-reactive antibodies, compared to those born before 1980 (Hancock *et al.*, 2009). Alternative hypotheses consider aberrant immune responses in driving pathology and enhancing disease severity. For instance, younger individuals displaying dysregulated pathogenic cellular immunity owing to cross-reactivity following previous exposure (Shanks & Brundage, 2012), or excessive immune cell signalling and production of proinflammatory cytokines (i.e. “cytokine storm”) in young adults with a robust and fully competent immune system (Kobasa *et al.*, 2007; Gagnon *et al.*, 2013).

1.2.4 Animal models of influenza

In order to study the complex physiological processes underpinning respiratory viral infection, including interplay between immune, respiratory, and circulatory systems, a mammalian host is required. Whilst still with its limitations, animal models provide an invaluable tool in which to study aspects of influenza biology *in vivo*, including transmission, viral kinetics, host immunity, vaccinology, therapeutic effectiveness (both antiviral and host-directed immunotherapy), tissue repair, and bacterial superinfection. Animal models used to study influenza biology include mice, rats, hamsters, guinea pigs, ferrets, domestic swine, mini-pigs, and non-human primates.

The success of an animal model for influenza research depends on its capacity for viral entry and replication. Ferrets have long been used as a model for human influenza virus infection owing to similarities in their lung physiology, including similar distribution of sialic acid glycans in the respiratory tract. Human IAV strains attach to ferret respiratory epithelial cells via the α -2, 6 glycosidic linkage to sialic acids (Herlocher *et al.*, 2001). Ferrets display similar clinical signs and progression of influenza disease to that in humans, with IAV manifesting in the upper airway respiratory tract before developing a febrile illness (Maher & DeStefano, 2004). They support replication of human influenza viruses, and can therefore be used to study transmission via aerosol droplets (Belser, Katz & Tumpey, 2011). Their size also allows for the effective measurement of parameters including pulse, temperature, and respiratory rate. Additionally, ferrets are a useful tool to study host immunity to influenza virus infection, displaying remarkable similarity and overlap to humans in their cellular immune response (Maher & DeStefano, 2004). The main drawback with ferrets as a model for human influenza, apart from limited analytical tools available, is their size, cost and husbandry requirements, therefore increasing the difficulty to perform experiments in large numbers. Similar to ferrets, guinea pigs are susceptible to human influenza virus strains, supporting viral replication and transmission (Bouvier & Lowen, 2010). Their small size enables easier handling, housing, and performance of experimental work. However, unlike ferrets, guinea pigs do not display typical clinical signs of human influenza disease. Nonhuman primates, including rhesus macaques, are also used to study host immunity following IAV infection owing to high-degree of genetic and physiological similarities with humans. However, performing biomedical research on rhesus macaques renders significant ethical concerns, in addition to their substantial cost of husbandry and maintenance, which resultantly reduces the number in experimental groups.

Laboratory mice are the most widely used mammalian model for biomedical research, with their usage dating back to the early twentieth century. Advantages include their low cost, small size, and ease of handling and housing, which has enabled the generation of numerous commercially available inbred strains and independent breeding on-site at biomedical research institutes across the globe, with C57BL/6 (B6) or BALB/C strains the most commonly used. The ease of maintenance coupled with our increasing ability to manipulate mice genetically has resultantly led to the generation of a wide-range of knockout, knock-in, and transgenic mice. In the context of influenza virus infection, this is an invaluable tool to study the host immunity in a mammalian host, through the genetic depletion or modification of specific cell populations and proteins. This can also be achieved through an expansive range of commercially available mouse-specific immunological reagents, including antibodies and recombinant proteins.

The main drawback with mouse influenza models is the requirement for mouse-adapted influenza strains in order to establish infection and viral replication (Bouvier & Lowen, 2010). Commonly used influenza virus strains in mice include the H1N1 influenza A/Puerto Rico/8/34 (PR8) isolate from the 1930s, and the X31 strain composed of HA and NA genes of the IAV/Hong Kong/1/1968 H3N2 on a PR8 background (Bouvier & Lowen, 2010). Mice display dissimilar clinical signs compared to humans, presenting with anorexia, hunching, piloerection, and hypothermia, as opposed to febrile illness (Bouvier & Lowen, 2010). Additionally, influenza virus infection in mice usually manifests as a lower respiratory tract infection and viral pneumonia, as opposed to the initial establishment of upper airway infections in humans.

A significant caveat to consider when using inbred laboratory mouse strains is their lack of a functional Mx1 protein; a crucial interferon (IFN)-inducible influenza virus restriction factor in both mice and humans (Staeheli *et al.*, 1986; Haller *et al.*, 2015). Defects of the *Mx1* gene in inbred laboratory mice are due to nonsense mutations and large deletions, resulting in their increased susceptibility to influenza viruses of the same strain and dose, in contrast with wild mice (Haller *et al.*, 1979; Staeheli *et al.*, 1988). Indeed, the lethal dose of a murine 1918 H1N1 virus increased over 100-fold in BALB/c mice expressing a functional *Mx1* allele targeted into the Mx1 locus (Tumpey *et al.*, 2007). Even the introduction of the intact mouse Mx1 gene leaves the infection system imperfect, as there was no co-evolution between pathogen and host, as is the case in natural pairings.

1.3 The immune response to influenza virus infection

1.3.1 Barrier immunity & viral recognition

The innate immune system functions primarily to prevent infection, or reduce the impact and limit the spread of invading pathogens, therefore buying time for the host to coordinate highly specific cellular responses of the adaptive immune compartment. The innate arm also is also crucial for the initiation of adaptive responses, providing signals necessary to activate cellular and humoral immunity. The skin provides front-line protection against invading pathogen, operating as both a physical barrier and a hub of skin-associated immune cell interactions (Matejuk, 2018). Mucosal surfaces are thin permeable barriers continuously exposed to the external environment to allow physiological activity, for instance gas exchange in the respiratory tract, and food absorption in the gastrointestinal tract. Mucosal sites therefore lack skin-associated protection, and instead depend on compartmentalised mucosal immunity and specialised barrier epithelial cells to defend internal tissues against any potentially harmful substances or microorganisms.

Respiratory virus diseases, like influenza, spread via virus inhalation or droplet transmission, and therefore, infection establishes in the upper airway respiratory tract. The respiratory epithelium forms a tight physical barrier, maintained by tight and adherens junction proteins forming cell-to-cell junctions, thus providing a barrier to virus entry and preventing dissemination in the airway submucosa (Whitsett & Alenghat, 2015). Barrier protection is enhanced by the production of cell-associated and secreted mucins, large glycoproteins produced by goblet and club cells lining the airways that compose mucus layers. The mucus gel layer prevents pathogen adhesion to cell surfaces, including respiratory viruses like influenza, enhancing their clearance via the mucociliary ‘escalator’ (Voynow & Mengr, 2009). Influenza virus infection induces mucin production (Vareille *et al.*, 2011), and mice over expressing one of the major secretory mucins, MUC5AC, have increased protection against influenza virus infection (Ehre *et al.*, 2012).

In vertebrates, the initiation of an innate immune response to pathogen is enabled by self/nonself discrimination. The ability to recognise ‘microbial nonself’—molecular patterns unique to pathogens—allows the host to discern between “infectious nonself” and “non-infectious self” (Medzhitov & Janeway, 2002). Innate responses are also initiated by detection of danger-associated molecular patterns (DAMPs): endogenous danger molecules released from infected, damaged, or dying cells. This includes misplaced self, such as ssRNA in the

cytosol. The detection of ‘danger’ signals forms the basis for ‘The danger theory’, pioneered by Polly Matzinger, which claims that self-constituents can trigger an immune response if they are deemed to be ‘dangerous’ (Matzinger, 2002). Viral detection occurs through the recognition of pathogen-associated molecular patterns (PAMPs) by germline-encoded pattern recognition receptors (PRRs). Viruses are intracellular pathogens, and therefore intracellular host-encoded PRRs detect influenza viruses upon virus entry into the cell. Retinoic acid-inducible gene-I (RIG-I) is a cytosolic helicase that detects ss viral RNA in influenza virus infected cells. Discrimination from self ssRNA by RIG-I is achieved by the recognition of a 5’-triphosphate group in uncapped viral RNA (Hornung *et al.*, 2006; Pichlmair *et al.*, 2006). One study suggests RIG-I detection of ssRNA occurs in antiviral stress granules, providing one possible explanation for how a cytosolic sensor detects influenza virus which replicates in the nucleus (Onomoto *et al.*, 2012). Following viral recognition, the RIG-I helicase domain binds to ATP, inducing a conformational change resulting in the exposure of its caspase associated recruitment domain (CARD) (Luo *et al.*, 2011). RIG-I CARD is then modified, including TRIM25- and RING finger protein (RIplet)-dependent ubiquitination (Gack *et al.*, 2007; Gao *et al.*, 2009), which enables its interaction with the signalling adaptor mitochondrial antiviral signalling protein (MAVS) (Jiang *et al.*, 2011). RIG-I recruitment to MAVS triggers the downstream activation of transcription factors, including interferon regulatory 3 (IRF3), IRF7, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), and subsequently production of antiviral cytokines, including type I and III interferons (IFN) (Kato *et al.*, 2006). RIG-I is crucial for influenza virus detection and IFN production in infected epithelial cells, fibroblasts, alveolar macrophages, and conventional dendritic cells (cDCs) (Kato *et al.*, 2005; Rehwinkel *et al.*, 2010; Crotta *et al.*, 2013).

The importance of RIG-I-dependent viral sensing and subsequent antiviral programme induction is highlighted by the fact that viruses have evolved mechanisms in order to evade these pathways (García-Sastre, 2017). This includes IAV NS1 protein, which functions to inhibit host innate immunity through several mechanisms. IAV NS1 protein inhibits RIG-I signalling by binding TRIM25 and preventing its E3 ligase activity (Gack *et al.*, 2009). Influenza virus NS1 also prevents host gene expression by posttranscriptional inhibition of RNA processing and trafficking (Qiu & Krug, 1994). For instance, IAV NS1 directly interacts with the cellular 30 kDa subunit of CPSF, an essential component of the 3’ end processing machinery of cellular mRNAs, resulting in the prevention of mRNA termination and polyadenylation (Nemeroff *et al.*, 1998). IAV NS1 is therefore an important influenza

virulence factor, allowing viral replication in IFN-competent systems (García-Sastre *et al.*, 1998). The divergence in NS1 amino acid sequences between influenza virus subtypes may affect protein function and ultimately the degree of viral strain pathogenicity. Recombinant IAV strains expressing heterotypic NS1 proteins from IBV, ICV and IDV strains have impaired viral fitness, suggesting IAV NS1 contributes to its increased pathogenicity in vivo (Nogales *et al.*, 2019).

The nucleotide-binding oligomerisation domain (NOD)-like receptor family member NOD-leucine rich repeat (LRR) and pyrin domain-containing 3 (NLRP3) inflammasome complex also detects viral PAMPs in the cytosol (Allen *et al.*, 2009). Inflammasomes are multiprotein complexes composed of an NLRP innate recognition receptor, the adaptor ASC, and pro-caspase 1 (Martinon, Burns & Tschopp, 2002). Inflammasome activation triggers pro-caspase 1 processing into its active form, which then cleaves pro-interleukin (IL)-1 β and pro-IL-18 for production and secretion of the proinflammatory cytokines IL-1 β and IL-18, respectively (Agostini *et al.*, 2004). NLRP3 also mediates antiviral protection by inducing caspase 1-dependent cell death (also known as pyroptosis) in virally infected cells: a pathogen inducible inflammatory mode of cell death to limit viral dissemination (Bergsbaken, Fink & Cookson, 2009). NLRP3 is expressed by myeloid cells including DCs, neutrophils, macrophages and monocytes (Thomas *et al.*, 2009), and additionally in human bronchial epithelial cells (Pothlichet *et al.*, 2013). NLRP3 is initially activated by PRRs like RIG-I, priming it for expression of the genes encoding IL-1 β and IL-18. Following priming, NLRP3 detects DAMPs, including endosomal injury, aberrant ionic fluxes driven by IAV M2 activity, and mitochondrial injury, triggering IL-1 β and IL-18 processing and production (Zhao & Zhao, 2020). Additionally, it has recently been shown that the IFN-inducible protein ZBP1 detects influenza virus proteins NP and polymerase subunit PB1, triggering NLRP3 inflammasome activation (Kuriakose *et al.*, 2016). ZBP1 also recognises viral Z-RNAs produced following IAV replication, resulting in nuclear envelope rupture and necroptosis (Zhang *et al.*, 2020). Similar to IAV-mediated suppression of RIG-I, the importance of NLRP3 inflammasome activity is made evident by the ability of IAV protein NS1 to suppress NLRP3 function (Moriyama *et al.*, 2016), in that evolutionary pressure promotes viral adaption for evasion of certain host antiviral responses.

Toll-like receptors (TLRs) are a family of transmembrane PRR. The host recognises viral PAMPs and DAMPs via endosomal TLR3, TLR7, and TLR8. TLR3 recognises double-stranded (ds)RNA in endosomes. However, the replication cycle of influenza viruses does not generate dsRNA (Pichlmair *et al.*, 2006), and so therefore, it is thought that TLR3 recognises

RNA structures in infected cells taken up by phagocytosis (Schulz *et al.*, 2005). TLR3-dependent recognition of viral RNA triggers downstream signalling via TIR-domain-containing adaptor protein inducing IFN β (TRIF), leading to IRF3- and NF- κ B-dependent production of IFNs and proinflammatory cytokines, respectively. TLR3 is expressed in DCs, macrophages, and airway epithelial cells (Le Goffic *et al.*, 2007). TLR7 is expression is predominantly expressed in human and murine plasmacytoid dendritic cells (pDCs) and B cells, and human monocytes (Chattergoon *et al.*, 2014; Hornung *et al.*, 2002), recognising ssRNA taken up in endosomes (Lund *et al.*, 2004; Diebold *et al.*, 2004; Heil *et al.*, 2004). TLR7 mediates downstream signalling via the adaptor protein myeloid differentiation factor 88 (MYD88) (Lande *et al.*, 2007; Honda *et al.*, 2005), which activates the transcription factors IRF7 and NF- κ B for the production of IFNs and other proinflammatory cytokines, respectively (Lund *et al.*, 2004). TLR8 is expressed in the endosomes of human monocytes and macrophages, whereby recognition of viral ssRNA induces production of IL-12 (Ablasser *et al.*, 2009).

The complexity of the innate immune recognition network has begun to unravel over the last two decades, yet further work is required to decipher the relative contribution of individual PRRs in influenza-related antiviral immunity. One study has shown mice deficient for MYD88, and therefore TLR7-dependent signalling, have impaired antiviral protection following influenza virus infection (Seo *et al.*, 2010), however another found no difference in mortality compared to WT controls (Le Goffic *et al.*, 2006). The contribution of TLR3 to protective antiviral protection is unclear, as TLR3 deficient mice have improved survival relative to WT controls, displaying reduced inflammation and lung pathology, despite improved viral control (Le Goffic *et al.*, 2006). In primary murine airway epithelial cells (AEC), the primary site for influenza virus infection, RIG-I/MAVS-dependent signalling alone was shown to be important for IFN production (Crotta *et al.*, 2013). Additionally, mice deficient for NLRP3, caspase 1, or IL-1R, have increased mortality following influenza challenge (Thomas *et al.*, 2009). These data suggest the importance of cytosolic influenza virus sensing, which may reflect early viral recognition in infected upper respiratory epithelial cells in mounting a robust immune response. However, potential redundancy between individual PRR pathways may overshadow findings in mice deficient for individual signalling components. In addition, the lack of a functional Mx1 protein in inbred mouse models may dilute protective antiviral immunity in PRR knockout mouse studies. Indeed, *Tlr7*^{-/-}*Mavs*^{-/-} double knockout mice carrying functional *Mx1* alleles are highly susceptible to influenza infection, relative to

individual knockout mice (Pillai *et al.*, 2017). Therefore, further research in both animal and human influenza models is required to dissect the contribution of innate recognition pathways in generating protective antiviral immunity.

1.3.2 The influenza cytokine response

Following viral detection, the ensuing proinflammatory cytokine and cellular response functions to limit viral dissemination. Activation of IRF and NF- κ B transcription factors and the NLRP3 inflammasome triggers production of antiviral type I (IFN- α and IFN- β , also known as IFN- α/β or IFN-I) and III IFNs (IFN- λ or IFN-III) (discussed in detail in the next section), proinflammatory cytokines, and chemokines. Cytokines are a diverse family of secreted extracellular signalling molecules with pleiotropic effects, enabling both innate and adaptive arms of the immune response. Chemokines orchestrate the recruitment of immune cells to the site of infection or secondary lymphoid tissues (lymph nodes, for instance) via chemotaxis. Influenza cytokine and chemokine responses operate quickly, promoting inflammation and the coordination of multiple modules of the immune response simultaneously. The non-specific manner in which innate immunity operates aids the host's ability to respond quickly following viral recognition, however this can have detrimental consequences for the host. The link between aberrant innate responses and the severity of influenza disease has been shown in macaques infected with a highly pathogenic reconstructed 1918 H1N1 virus, triggering extensive immune cell signalling and hypercytokinemia, along with significant lung tissue damage (Kobasa *et al.*, 2007).

In recent years, studies have begun to characterise the cytokine profiles of human influenza cases, in an attempt to identify pathogenic factors and biomarkers indicative of disease severity (Fiore-Gartland *et al.*, 2017; Dunning *et al.*, 2018; Gao *et al.*, 2013; De Jong *et al.*, 2006). This includes IL-6, a pleiotropic cytokine with both pro- and anti-inflammatory properties (Hunter & Jones, 2015). Elevated levels of IL-6 have been detected in the plasma of fatal influenza patients (De Jong *et al.*, 2006; Paquette *et al.*, 2012), yet its role in influenza pathology is unclear. Studies in mice have identified a role for IL-6 signalling in exacerbating influenza-induced lung damage (Dienz *et al.*, 2012; Lauder *et al.*, 2013), whilst others found no effect (Paquette *et al.*, 2012). Tumor necrosis factor- α (TNF- α) is another proinflammatory mediator implicated in influenza disease pathology; a cytokine with a range of proinflammatory effector functions, mediated by induction of NF- κ B activity. Despite the

antiviral functions of TNF- α (Seo & Webster, 2002), its activity is associated with pathology in murine influenza (Peper & Van Campen, 1995; Belisle *et al.*, 2010).

Proinflammatory cytokines, like TNF- α , IL-1 family members, IL-6, and type I IFNs, signal via an amplification loop, triggering further transcription factor activation and cytokine production (Biondo *et al.*, 2019). This feedforward inflammatory circuit can lead to unchecked cytokine signalling and immune cell recruitment (Brandes *et al.*, 2013), inducing a phenomenon known as “cytokine storm” and the perpetuation of inflammatory immune responses, ultimately inducing immunopathology and lung damage (Guo & Thomas, 2017; Tisoncik *et al.*, 2012). Conversely, cytokine storm-associated immunopathology can occur as a result of impaired innate responses and improper viral control, resulting in prolonged immune activation (To *et al.*, 2010). Therefore, host protection depends on a balanced innate response in order to control viral replication, whilst limiting the harmful effects of excessive inflammation.

1.3.3 Roles of the endothelium and epithelium in influenza immunity

As the primary site for viral infection and replication, respiratory epithelial cells play a crucial role in initiating early influenza responses. In addition to the aforementioned barrier and viral sensing functions, respiratory epithelial cells coordinate early immune cell recruitment through the production of cytokines and chemokines. In situ hybridization identified epithelial cells as a key producer of type I IFNs in vivo following influenza virus infection (Jewell *et al.*, 2007). These findings were corroborated by an independent study using an IFN- β -luciferase reporter mouse, which identified epithelial cells and macrophages as the main producers of early type I IFN (Kallfass *et al.*, 2013). In addition, epithelial cells are a major source of type III IFNs, highlighting the importance of barrier cells in promoting type I and III-dependent antiviral immunity (IFN-mediated antiviral immunity will be discussed in detail in the following section) (Jewell *et al.*, 2010; Crotta *et al.*, 2013).

In an effort to limit pathogen spread, respiratory epithelial cells orchestrate immune cell recruitment and transmigration to infected tissue sites. NLRP3-dependent IL-1 β production by epithelial cells promotes protective antiviral immunity through neutrophil recruitment (Niu *et al.*, 2019; Pothlichet *et al.*, 2013). Similarly, CCL2 production by alveolar epithelial cells directs monocyte recruitment and transepithelial migration following influenza virus infection (Herold *et al.*, 2006). To protect against potentially harmful proinflammatory

myeloid responses, alveolar epithelial cells express CD200, a ligand for the inhibitory receptor CD200R expressed by alveolar macrophages (Snelgrove *et al.*, 2008). Mice lacking CD200 exhibited pathogenic proinflammatory immunity, and increased influenza susceptibility (Snelgrove *et al.*, 2008). Recent evidence also suggests that respiratory epithelial cells indirectly coordinate adaptive immune responses to influenza virus infection by modulating DC function. Granulocyte-macrophage colony-stimulating factor (GM-CSF) produced by infected alveolar epithelial cells promotes DC expansion and activation, crucial for T cell-mediated antiviral protection (Unkel *et al.*, 2012). Additionally, IFN- λ signalling in respiratory M cells induces thymic stromal lymphopoietin (TSLP) synthesis, which in turn primes migratory CD103⁺ DCs for germinal centre reactions and IgG1 and IgA production (Ye *et al.*, 2019).

The endothelium, in close proximity to the respiratory epithelium, maintains vascular integrity and barrier function at steady state. Treatment of mice with a spingosine-1-phosphate (S1P₁) receptor agonist triggered the reduction of cytokine and chemokine production by lung endothelial cells during influenza, suggesting a role for the endothelium in coordinating early inflammatory responses (Teijaro *et al.*, 2011). In addition, activation of both the endothelial and epithelial barriers by cytokines like TNF α and IL-1 β induces the upregulation of adhesion molecules, including ICAM-1 and E-selectin, important for chemokine-dependent leukocyte transmigration (Oslund & Baumgarth, 2011; Herold *et al.*, 2006; Guo & Ward, 2002).

1.3.4 Innate immune influenza response

Alveolar macrophages are lung-resident phagocytes that line the inner epithelial alveolar surface. At a ratio of roughly one alveolar macrophage for every three alveoli (Westphalen *et al.*, 2014), they are the most abundant immune cell in the respiratory tract. Their proximity in relation to the lung epithelium and air spaces allow their sentinel function against inhaled pathogens (Laskin *et al.*, 2011). In naïve mice, alveolar macrophages are derived from foetal progenitors seeded during embryonic development (Guilliams *et al.*, 2013), with additional contribution by bone marrow (BM)-derived monocytes during adult life (Gibbings *et al.*, 2015; van de Laar *et al.*, 2016). Alveolar macrophages promote viral clearance through phagocytosis of IAV-infected epithelial cells (Tumpey *et al.*, 2005; Schneider *et al.*, 2014; Hashimoto *et al.*, 2007). Uptake of infected dead cells leads to viral PAMP recognition, triggering alveolar macrophage activation and the production of proinflammatory cytokines,

including type I and III IFNs, TNF α , IL-6, and CC chemokines, a process important for the coordination of early influenza responses in mice and humans (Wang *et al.*, 2012; Tate *et al.*, 2010; Chen *et al.*, 2018; Cheung *et al.*, 2002; Högner *et al.*, 2013; Kallfass *et al.*, 2013). Genetic mouse models which lack alveolar macrophages, or depletion by clodronate liposome, increases the susceptibility of mice to influenza virus infection (Tate *et al.*, 2010; Cardani *et al.*, 2017; Schneider *et al.*, 2014). Additionally, specific alveolar macrophage depletion in pigs increases influenza disease severity (Kim *et al.*, 2008), indicating their importance in controlling viral infection.

Influenza-infected epithelial cells produce CCL2 and TNF α , promoting the recruitment and transepithelial migration of proinflammatory CCR2⁺ BM-derived monocytes (Herold *et al.*, 2006; Serbina & Pamer, 2006). BM-derived monocytes contribute to alveolar macrophage replenishment in situ (Gibbings *et al.*, 2015; van de Laar *et al.*, 2016; Aegerter *et al.*, 2020), however, can also be associated with proinflammatory immunopathologic responses. Primary human monocyte-derived macrophages infected with H5N1 influenza virus produce high levels of proinflammatory cytokines, such as IFN- β and TNF α (Cheung *et al.*, 2002). In addition, monocyte-derived macrophages may exacerbate lung damage via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-dependent induction of apoptosis in alveolar epithelial cells (Herold *et al.*, 2008). Indeed, the reduced recruitment of proinflammatory monocytes in CCR2 deficient mice correlates with reduced lung damage during influenza virus infection (Lin *et al.*, 2008; Herold *et al.*, 2008), and exacerbates bacterial superinfections (Ellis *et al.*, 2015).

cDCs serve as immune sentinels, situated proximal to the respiratory epithelium where they continuously sample the airway lumen via protruding dendrites through epithelial cell tight junctions (Nelson *et al.*, 1994; Jahnsen *et al.*, 2006). Similar to monocyte-derived macrophages, monocyte-derived cDCs are recruited to the lung in a CCR2 dependent manner during influenza virus infection (Lin *et al.*, 2008; Serbina & Pamer, 2006). cDCs are defined as professional antigen-presenting cells that migrate upon antigen detection to draining lymph nodes in a CCL19/21-CCR7 dependent manner, to initiate T cell activation for specific adaptive immunity (Heer *et al.*, 2008; GeurtsvanKessel *et al.*, 2008). cDC activation promotes antiviral immunity through the production of both type I and III IFNs (Osterlund *et al.*, 2005). Additionally, cDCs are crucial for the maintenance of influenza-associated tertiary lymphoid structures in the lung following influenza virus infection through chemokine production (Geurtsvankessel *et al.*, 2009).

pDCs are important in antiviral immunity owing to their capacity to rapidly produce large amounts of type I (Cella *et al.*, 1999; Siegal *et al.*, 1999) and type III (Yin *et al.*, 2012; Coccia *et al.*, 2004) IFN. pDCs are produced in the BM, originating from a common DC progenitor (Naik *et al.*, 2007), maturing upon release into the periphery where they continuously recirculate through lymphoid organs (Reizis, 2019). pDCs have high expression of PRR sensors, and recognise influenza virus PAMPs via endosomal TLR7 (Lund *et al.*, 2004; Diebold *et al.*, 2004; Jewell *et al.*, 2007). However, murine models of pDC deficiency indicate that they are dispensable for influenza virus control (Wolf *et al.*, 2009), possibly because macrophages and epithelial cells are primary IFN producers following infection (Kallfass *et al.*, 2013). In fact, pDCs may promote lung pathology through excess production of type I IFNs, as depletion of PDCA-1⁺ cells, including all pDCs, decreased IFN-associated inflammation in 129 mice (Davidson *et al.*, 2014).

Neutrophils, the most abundant myeloid cell, continuously patrol the host in search for microbes to phagocytose and kill, representing a key component of innate immune defence. Influenza virus infection results in the production of neutrophil chemoattractants: KC, MIP-1 α , MIP-2, IL-8, and IP-10 (also known as CXCL1, CCL3, CXCL2, CXCL8, and CXCL10 respectively), triggering the rapid recruitment of neutrophils into the lungs, within the first 3 days following infection (Wareing *et al.*, 2007; Tate *et al.*, 2011). Neutrophils clean up infected epithelia and cellular debris by phagocytosis, however owing to their non-specific and proinflammatory functions, neutrophil activity and recruitment is associated with increased pathology and lung damage during influenza disease. Neutrophils produce reactive oxygen species (ROS) for phagocytic killing, including O₂⁻ and H₂O₂, through NADPH oxidase activity. However, ROS production has been linked with increased pulmonary inflammation and lung damage following influenza virus infection (Vlahos *et al.*, 2011). Evidence indicates that pulmonary neutrophilia promotes barrier disruption, with excessive and continuous neutrophil egress from the vasculature into infected alveoli disrupting tight junction integrity and thus contributing to ARDs progression (Zemans, Colgan & Downey, 2009). Additionally, neutrophil extracellular traps (NETs) have been identified in areas of lung tissue injury, contributing to influenza-induced viral pneumonia (Narasaraju *et al.*, 2011). Early neutrophil activity may be beneficial for viral control, as early or prophylactic antibody-mediated neutrophil depletion resulting in increased mortality of infected mice (Tumpey *et al.*, 2005; Tate *et al.*, 2011). However, other studies have found no effect following neutrophil depletion (Davidson *et al.*, 2014). In addition, excessive neutrophil activity, particularly in response to highly virulent influenza virus strains and late during the course of infection, promotes lung

pathology and inflammation in mice (Kulkarni *et al.*, 2019; Narasaraaju *et al.*, 2011; Perrone *et al.*, 2008; Brandes *et al.*, 2013). In humans, network analysis and expression profiling of influenza patient samples correlates excessive neutrophil activity with severe disease (Brandes *et al.*, 2013; Tang *et al.*, 2019).

Natural killer (NK) cells are innate lymphocytes with targeted antiviral function, owing to their ability to recognise and kill virally infected cells (Colonna, Jonjic & Watzl, 2011). Populations of tissue-resident NK cells exist in the lungs of healthy humans, in addition to lymphoid tissues, the liver, gut, uterus and in the peripheral blood (Carrega & Ferlazzo, 2012). Contrary to cytotoxic T cells, NK cells do not require antigen exposure to mediate their cytolytic effects (Kiessling, Klein & Wigzell, 1975). Instead, NK cells integrate signals from a system of activation and inhibitory receptors to discern self from non-self. Activation receptors detect viral-ligands, or aberrant expression of self-ligands on virally infected host cells, also known as “*induced-self*” (Raulet, 2003; Lanier, 2008). NK cell activation is further regulated to achieve self/nonself discrimination by its inhibitory receptors, which interact with self-major histocompatibility complex (MHC)-I (Valiante NM *et al.*, 1997). Infected cells downregulate MHC-I in an attempt to avoid recognition by CD8⁺ T cells, and NK cell inhibitory receptors detect this “*missing-self*”, which is thought to provide subsequent “*licensing*” for activation (Kim *et al.*, 2005). NK cell activation induces targeted antiviral cytotoxicity and the production of proinflammatory cytokines (Rajasekaran *et al.*, 2016). The role NK cells play during influenza virus infection is unclear, with mixed findings arising from studies in mice. For instance, *Ncr1*^{-/-} mice lacking NK cells have an increased susceptibility to influenza virus infection in both the 129 and B6 backgrounds (Gazit *et al.*, 2006), indicating a protective role. However, recent studies have shown influenza virus infected mice are better-protected following NK cell depletion (Abdul-Careem *et al.*, 2012; Zhou, Juang & Kane, 2013).

A recurring theme innate influenza immunity research is conflicting reports regarding the relative contribution of individual cell types in host protection. The dynamic and multifaceted nature of innate immune pathways during acute respiratory viral disease adds complexity to our understanding of the mechanisms that regulate disease progression. Collectively, studies indicate the importance of innate immune cell activity and recruitment in early viral control, whilst prolonged or aberrant signalling is associated with immunopathology, lung damage, and increased disease severity. Factors underlying these discrepancies are linked to both extrinsic factors, such as the IAV strain pathogenicity, and intrinsic host-factors, including genetic susceptibility and demographics.

1.3.5 Adaptive immune responses in influenza

A principal function of the innate immune system is to provide cytokine and antigen signals for initiation of pathogen-specific responses of the adaptive immune compartment. Innate protection also limits viral spread, thus, buying time for the initiation of adaptive immune pathways that require more time to manifest. Influenza virus infection is lethal in recombina-activating gene (RAG) deficient mice that lack mature T and B cells (Wu *et al.*, 2010; Davidson *et al.*, 2014), highlighting the importance of the adaptive immune compartment in viral clearance and host protection. Following uptake of viral antigen, lung-resident cDCs migrate to draining lymph nodes in a CCR7-CCL19/21 dependent manner in search for antigen-specific naïve T cells (Heer *et al.*, 2008). Exogenously sourced antigen taken up in endosomes via phagocytosis are presented by cDCs on MHC-II to naïve CD4⁺ T cells, whereas viral antigen detected in the cytosol following viral infection of cDCs are presented to naïve CD8⁺ T cells on MHC-I (Neyt & Lambrecht, 2013; Hargadon *et al.*, 2011; Manicassamy *et al.*, 2010). Additionally, CD103⁺ cDCs (also known as cDC1s, XCR1⁺ in humans) are efficient at a process known as cross-presentation, allowing the presentation of exogenous viral antigen on MHC-I complexes (Helft *et al.*, 2012), which is essential to induce naïve CD8⁺ T cell activation, and generate effector cytotoxic T lymphocytes (CTLs) (Joffre *et al.*, 2012; Jung *et al.*, 2002). Activated CD4⁺ T cells are skewed towards a type 1 T helper (Th1) profile owing to a virally-induced cytokine milieu, mainly antigen presenting cell production of IL-12, which in turn produces IL-2, IFN- γ and TNF α (Szabo *et al.*, 2000). IFN- γ and IL-2 promotes CTL differentiation and expansion, respectively (Whitmire, Tan & Whitton, 2005; D'Souza & Lefrançois, 2003). CTLs are potent antiviral effector cells, producing antiviral cytokines like IFN- γ , and target virally infected cells for killing by perforin-granzyme, Fas-ligand (FasL), or TRAIL dependent mechanisms (Brincks *et al.*, 2008; Topham, Tripp & Doherty, 1997).

B cells contribute antiviral protection as early as the point of infection, through innate-like B cell production of “natural” IgM antibodies during steady-state (Baumgarth *et al.*, 2000), which promote viral clearance via activation of classical complement pathway (Jayasekera, Moseman & Carroll, 2007). Antigen-stimulation of B cells occurs mainly in respiratory tract draining lymph nodes, inducing naïve B cell differentiation into short-lived plasma cells as part of the extra-follicular B cell response, producing class-switched IgM, IgG, and IgA antibodies (Sealy *et al.*, 2003). The generation of memory B cells and high-affinity antibody producing long-lived plasma cells occurs following a germinal centre reaction, dependent on signals from antigen-primed T follicular helper cells (T_{FH}). The kinetics of the extra-follicular B cell

response correlates with the peak of primary influenza virus infections and the onset of recovery (Kavaler *et al.*, 1991), suggesting that this fast-responding humoral response is important in viral clearance. This is in contrast with the germinal centre reaction which occurs closer to the resolution of infection, and plays an important role in immunological memory, forming the basis for vaccine-induced protection, along with memory CD8⁺ T cells (Lam & Baumgarth, 2019; Pizzolla & Wakim, 2019).

A swathe of studies in the 80s and 90s attempted to dissect the relative contribution of humoral (B cell) and cellular (T cell) responses to protective immunity in murine influenza models. CTL deficient mice are able to clear influenza virus infection, but clearance is delayed, and linked with increased mortality (Bender *et al.*, 1992; Eichelberger *et al.*, 1991). Similarly, depletion of CD4⁺ T cells does not affect viral clearance, however mice lacking both CD4⁺ and CD8⁺ T cells are incapable of influenza virus clearance (Wells *et al.*, 1981). Studies in μ MT mice deficient for B cells have produced contradicting results, with some reporting that T cell-mediated immunity provides antiviral protection in these mice (Topham & Doherty, 1998; Epstein *et al.*, 1998), whilst others claiming a humoral response is required for viral clearance (Graham & Braciale, 1997; Mozdzanowska, Maiese & Gerhard, 2000; Lee *et al.*, 2005). Collectively, these studies highlight an overlap between the humoral and cellular immune responses in providing antiviral immunity, with functional redundancy likely depending on the IAV strain and the severity of infection.

1.4 Type I and III interferons

Interferons provide front-line protection against viral pathogens. Prior to their discovery, it was known that inactivated viruses inhibited the growth of live infectious viruses, a phenomenon termed “viral interference”, however, the mechanism for this was unclear. Alick Isaacs and Jean Lindenmann discovered the antiviral activity of interferons over 60 years ago, when they incubated heat-inactivated IAV with fragments of chick chorio-allantoic membrane, and observed the release of an “interfering agent” with the capacity to inhibit the growth of live influenza virus (Isaacs & Lindenmann, 1957). They suitably named this agent interferon (IFN), setting the foundations for an entire field of antiviral research. The effectiveness of IFNs in providing antiviral host-protection is supported by the presence of an IFN system throughout evolutionary history, and it is thought that the earliest jawed vertebrates harboured IFN genes (Secombes & Zou, 2017).

1.4.1 IFN receptors & signalling

The IFN family is comprised of three classes in mammals: type I IFNs (including IFN- α and IFN- β , also referred to as IFN- α/β), type II IFNs (IFN- γ), and type III IFNs (the IFN- λ subtypes). Type I and III IFNs are best known for their innate antiviral function, whereas the direct antiviral activity of type II IFN is limited, with IFN- γ playing a more prominent role in coordinating innate and adaptive cellular responses. In humans, type I IFNs are composed of 13 IFN- α subtypes, a single IFN- β , and the less well characterised IFN- ϵ , IFN- τ , IFN- κ , IFN- ω , IFN- δ , and IFN- ζ (Ivashkiv & Donlin, 2014). Type III IFNs are comprised of four subtypes: IFN- λ 1 (IL-29), IFN- λ 2 (IL-28A), IFN- λ 3 (IL-28B), and IFN- λ 4 (Prokunina-Olsson *et al.*, 2013). Mice harbour two functional IFN- λ encoding genes (*Ifnl2* and *Ifnl3*) and two *Ifnl1* pseudogenes (*Ifnl1*-P1 and *Ifnl1*-P2) (Lasfar *et al.*, 2006; Wack, Terczyńska-Dyla & Hartmann, 2015).

Despite the existence of multiple Type I and III IFN subtypes, each class uses its own single cognate IFN receptor. The type I IFN receptor (IFN- α/β receptor, IFNAR) is composed of IFNAR1 and IFNAR2 subunits, and the receptor complex for IFN- λ (IFNLR or IL28R) consists of an IFNLR1 subunit, and the shared IL-10 receptor chain 2 (IL-10RB β). IFNAR is expressed on all nucleated cell types, and therefore type I IFNs have widespread functions in homeostasis and disease, including significant immunomodulatory and proinflammatory effects (McNab *et al.*, 2015; Ivashkiv & Donlin, 2014). Whereas IL-10RB β appears to be ubiquitously expressed (Gurney, 2004), the IFNLR1 subunit is largely restricted to epithelial cells, such as the respiratory and gastrointestinal barrier sites (Kotenko *et al.*, 2003; Sommereyns *et al.*, 2008), in addition to human and mouse hepatocytes (Dickensheets *et al.*, 2013; Diegelmann *et al.*, 2010), and neutrophils in mice (Blazek *et al.*, 2015; Broggi *et al.*, 2017; Espinosa *et al.*, 2017). The effects of IFN- λ -induced signalling are therefore more targeted, and in the context of respiratory viral disease, localised largely at the site of infection in epithelial cells.

IFN-receptor binding activates a downstream signalling cascade ultimately inducing the transcription of interferon-stimulated genes (ISGs): a battery of over 2000 genes that exert IFN-effector functions, most of which are uncharacterised (Hertzog, Forster & Samarajiwa, 2011). Despite using independent receptors, type I and III IFNs share considerable overlap in downstream signalling pathways and transcriptional responses (Zhou *et al.*, 2007; Crotta *et al.*,

2013; Marcello *et al.*, 2006; Doyle *et al.*, 2006). IFN receptor engagement triggers the activation of the receptor-associated Janus kinase family members: Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2). JAK1 and TYK2 then phosphorylate tyrosine residues in the intracellular receptor domain, creating docking sites for the recruitment and subsequent phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT2 signalling molecules (Murray, 2007). Phosphorylated STAT1 and STAT2 heterodimers then bind IRF9, forming a trimeric complex known as ISG factor 3 (ISGF3), which migrates to the nucleus binding to IFN-stimulated response elements (ISREs) in the promoter regions of ISGs. Additionally, IFN- β can uniquely bind, signal, and induce ISG expression through IFNAR1, in an IFNAR2/JAK-STAT-independent manner (De Weerd *et al.*, 2013).

The recent identification of mutations in genes regulating type I and III IFN signalling in cases of fatal human viral disease makes evident the importance of this pathway in antiviral protection. Whole-exome sequencing in a 7-year old girl who developed fatal influenza following primary 2009 H1N1 infection identified heterozygous null mutations in *IRF7*, resulting in a significantly dampened type I and III IFN response (Ciancanelli *et al.*, 2015). A similar case of life-threatening influenza identified a patient homozygous for a loss-of-function *IRF9* allele (Hernandez *et al.*, 2018). Another study identified two individuals with loss-of-function mutations in *IFNAR1* who developed life-threatening disease following measles, mumps, and rubella (MMR) vaccination (Hernandez *et al.*, 2019).

1.4.2 Negative regulation

Like all cytokines, IFN responses require regulatory feedback mechanisms to curtail potentially harmful effects associated with excessive or prolonged signalling. The ability for IFNs to induce gene transcription is dependent on receptor availability. The ubiquitous expression of the type I receptor, IFNAR, increases the likelihood of pathogenic inflammation and tissue damage (Trinchieri, 2010). Type I IFN responses are therefore subject to tight regulatory pathways, likely owing to evolutionary pressures promoting mechanisms that dampen type I IFN-associated immunopathology (Ivashkiv & Donlin, 2014; Arimoto *et al.*, 2018). On the other hand, mechanisms regulating type III IFN signalling are less stringent, as restricted expression of the IFNLR1 subunit is in itself a form of regulation, allowing targeted ISG induction by IFN- λ mainly at barrier epithelial sites. Interestingly, signals induced by IFN- λ have a slightly delayed yet prolonged kinetic compared to type I IFN (Bolen *et al.*, 2014; Lin

et al., 2016; Klinkhammer *et al.*, 2018). This extended signal duration can be explained, at least in part, by the reduced negative feedback mechanisms following IFNLR ligation (François-Newton *et al.*, 2011). It is important to note however, that whilst mechanisms of type I IFN control are well defined following decades of research, type III IFN research is still in its infancy, and is additionally hampered by the lack of a good quality commercially available IFNLR1 murine antibody.

The primary layer of IFN- α/β signal regulation is the rapid internalisation of the ternary IFNAR complex following ligand binding (Claudinon, Monier & Lamaze, 2007). So far, it has not been established how type III receptors are regulated following IFN- λ signal transduction. USP18 is one of the best characterised negative regulators of type I IFN signalling, and is induced by both type I and III IFN (Malakhova *et al.*, 2006). USP18 is an enzyme that removes the ubiquitin-like modifier ISG15 from conjugated proteins (Malakhov *et al.*, 2002). Regulation of IFN signalling is achieved by USP18 competing with JAK1 for IFNAR2 binding, causing destabilisation of the IFNAR complex and decreased ligand binding (Malakhova *et al.*, 2006; François-Newton *et al.*, 2011). USP18 deficiency in humans results in IFN-induced inflammation and type 1 interferonopathy underlying pseudo-TORCH syndrome (Meuwissen *et al.*, 2016). Additionally, ISG15-deficiency in patients prevents USP18 accumulation, resulting in IFN- α/β over-amplification and pathogenic inflammation (Zhang *et al.*, 2015), highlighting the importance of USP18-mediated type I IFN regulation. USP18 has been shown to inhibit type III IFN responses in a mouse mammary tumor model or if over expressed in vitro (Blumer *et al.*, 2017; Burkart *et al.*, 2013). However, IFNAR crosstalk was not excluded in these studies, and in normal physiological settings, USP18 has no effect on IFN- λ signalling (Blumer *et al.*, 2017). Other well-characterised negative feedback mechanisms include suppressor of cytokine signalling (SOCS) proteins. SOCS1 interferes with IFN signalling through interaction with TYK2, leading to its degradation (Fenner *et al.*, 2006; Piganis *et al.*, 2011). SOCS1 has a weak negative effect on type III IFN signalling, (Blumer *et al.*, 2017), which may be explained by the ability of IFN- λ to signal in the absence of TYK2 (Fuchs *et al.*, 2016). SOCS3 is also able to interfere with type I IFN signalling, but not type III IFN (Song & Shuai, 1998; Blumer *et al.*, 2017).

1.4.3 Interferon-stimulated genes

An important component of type I and III IFN activity is the ability to induce ISG transcription and an antiviral state not only in infected cells, but also in uninfected bystander cells. The resultant effect is the reduced availability of ‘hospitable’ cells for viral replication, thus reducing viral dissemination. This is opposed to alternative mammalian cell intrinsic antiviral mechanisms, such as RNA interference (RNAi), which utilises dicer/argonaute machinery to mediate viral RNA silencing within an infected cell (Maillard *et al.*, 2013), operating only within an infected cell. IFN-inducible ISG-mediated protection is initiated during steady-state conditions, priming the antiviral response prior to a viral infection. Commensal microbiota-derived signals trigger low level IFN- β production, which induces tonic IFNAR signals and the basal expression of IFN signalling molecules and PRRs (Gough *et al.*, 2012). Basal priming calibrates IFN responses for the rapid induction of antiviral programmes following infection (Abt *et al.*, 2012; Ganai *et al.*, 2012; Kawashima *et al.*, 2013). Recent studies have shown microbial-derived signals prime tonic ISG induction in the respiratory epithelium, promoting resistance to influenza virus infection (Steed *et al.*, 2017; Bradley *et al.*, 2019).

ISG activities encompass a wide range of biological functions to mediate antipathogen effects. ISG-encoded proteins can be classed into three main groups based on their mechanism of action: 1) antiviral effectors, interfering with specific stages of the viral life cycle or degradation of viral nucleic acids; 2) immunomodulatory factors, including cytokines, chemokines, signal transducing proteins, and transcription factors; 3) proteins modifying host cell processes, such as metabolism, cell cycle and apoptosis, to prevent or restrict viral spread from infected cells (Schneider, Chevillotte & Rice, 2014; MacMicking, 2012).

Two examples of important influenza virus restriction factors are Mx and IFN-inducible transmembrane (IFITM) family proteins. Mx proteins belong to a small family of dynamin like GTPases, which comprise MxA and MxB in humans and Mx1 and Mx2 in mice (Haller *et al.*, 2015). Human Mx proteins and mouse Mx2 are cytoplasmic, whereas rodent Mx1 localises to the nucleus (Pavlovic *et al.*, 1990). This is thought to restrict the antiviral effects of mouse Mx1 to viruses that replicate in the nucleus. In contrast, human MxA has broad antiviral activity irrespective of the subcellular site of virus replication (Haller *et al.*, 2015). MxA restricts influenza virus infection by inhibiting viral NP and PB2 interaction (Verhelst *et al.*, 2012). The importance of human MxA antiviral function has been demonstrated in mice expressing the human *MX1* locus as a transgene, which displayed

increased protection against highly pathogenic avian influenza, however, only a moderate increase in protection against influenza viruses of human origin (Deeg *et al.*, 2017). IFITM family proteins function by blocking virus entry into cells, and are potent inhibitors of influenza virus replication (Brass *et al.*, 2009). IFITM3 deficient mice are highly susceptible to otherwise low-pathogenicity influenza virus, and the same study found a significant number of hospitalised influenza patients had an enrichment for loss-of-function polymorphisms in the human *IFITM3* gene (Everitt *et al.*, 2012), suggesting its importance in human antiviral immunity.

In addition to inducing an antiviral state in infected and noninfected cells, IFNs orchestrate innate and adaptive immune processes. This includes propagating the IFN response itself, as ISGs include several IFN pathway signalling proteins (Ivashkiv & Donlin, 2014). IRF9 and STAT1 are themselves IFN-inducible, and therefore induction enhances ISRE binding and IFNAR signalling, respectively (Levy *et al.*, 1990; Hu *et al.*, 2002). IFN-induced expression of various PRRs and IRF7 augments IFN signalling in a feedforward loop driving further downstream IFN expression (Levy *et al.*, 2002; Schneider, Chevillotte & Rice, 2014). Type I and III IFNs rapidly induce IRF1 (Novatt *et al.*, 2016), which helps maintain ISG expression early during antiviral responses (Panda *et al.*, 2019). Inflammation is further augmented through IFN-mediated cytokine and chemokine production. For instance, the production of CCL2 promotes monocyte and dendritic cell recruitment into infected tissues (Lehmann *et al.*, 2016; Shim *et al.*, 2017).

1.4.4 Antiproliferative & proapoptotic effects of IFNs

The transcriptional programme induced by IFNs includes genes that manipulate the host cell cycle machinery and induce apoptosis in an effort to limit viral replication and dissemination in infected host cells (Platanias, 2005; Peteranderl & Herold, 2017; Chawla-Sarkar *et al.*, 2002). The antiproliferative effects of IFN were first observed in 1962, where it was shown that a 24-hour IFN treatment reduced the growth of L cells in culture (Paucker, Cantell & Henle, 1962). Research efforts since have sought to delineate mechanisms by which IFN-induced signalling inhibits host cell growth. As a result, type I IFNs have a long history as antitumour therapeutics (Zitvogel *et al.*, 2015; Parker, Rautela & Hertzog, 2016). Following the observed IFN- λ -mediated antitumour capacity against B16 melanoma (Lasfar *et al.*, 2006),

our increasing understanding in IFN- λ biology has led to their inclusion in research and discussions regarding IFN cancer immunotherapy (Lasfar *et al.*, 2016).

The antitumour potential of IFNs resulted in an expansion of research looking to characterise the molecular mechanisms regulating growth inhibitory effects. Beginning roughly 40 years ago, research groups largely studied the effects of IFN treatment in immortalised cell lines, and have led to several proposed mechanisms by which IFNs disrupt cellular growth (Parker, Rautela & Hertzog, 2016). Cell cycle progression is coordinated by cyclin-dependent kinase activity (CDKs) interaction with their associated cyclins. These activities are subjected to a further layer of regulation by cyclin-dependent kinase inhibitors (CKIs) (Sherr & Roberts, 1995). CKI induction by IFN has been described as a mechanism to inhibit cell cycle progression, often resulting in G₁ arrest (Roos, Leanderson & Lundgren, 1984; Olle Sangfelt, 2000). IFN- α treatment in a human Burkitt lymphoma cell line induced expression of the CKI and tumour suppressor protein, p21, causing cell cycle arrest and accumulation of cells in G₁ phase (Subramaniam & Johnson, 1997). This has been corroborated by independent studies, in addition to p15 and p27 induction, following IFN treatment (Sangfelt *et al.*, 1999, 1997b). Additional described mechanisms of IFN-induced cell cycle arrest include induction and activation of the tumor suppressor retinoblastoma (RB) protein (Burke, Bybee & Thomas, 1992; Kumar & Atlas, 1992), and IFN-targeted c-myc suppression (Einat, Resnitzky & Kimchi, 1985).

The tumor suppressor protein, p53, also known as “the guardian of the genome”, has also been implicated in growth inhibitory IFN effects in response to cellular stress or oncogene activity. p53 is activated in response to cellular stress, including DNA damage and oncogene expression (Vousden & Lane, 2007). Mechanisms by which p53 activity mediates antiproliferative effects include direct induction of universal CDK/cyclin inhibitors, including p21 (Brown, Wei & Sedivy, 1997), in addition to specific CDK inhibitors, such as Gadd45, which targets the Cd2c/Cyclin B1 complex at the G2/M checkpoint (Zhan *et al.*, 1999). Prolonged type I IFN treatment induces p53 mRNA and protein in WT mouse embryonic fibroblasts (MEFs), in an IRF9 dependent manner (Takaoka *et al.*, 2003). Chromatin immunoprecipitation (ChIP) using an anti-STAT2 antibody revealed that p53 induction is regulated by ISGF3 complex binding to p53 ISRE sequences, thus, regulating antitumour IFN responses (Takaoka *et al.*, 2003). Interestingly, the IRF1 transcription factor has tumor suppressor function (Tanaka *et al.*, 1994), and p53/p21-dependent cell cycle arrest is IRF1-dependent following DNA damage (Tanaka *et al.*, 1996). Therefore, IRF1 activity may have implications in IFN-p53 interplay. Additionally, prolonged IFN- β treatment induces a p53-

dependent senescent cell cycle arrest in normal fibroblasts (Moiseeva *et al.*, 2006). IFN-induced p53-dependent cellular senescence or apoptosis is associated with activation of the ATM/CHK2 DNA damage signalling pathway (Moiseeva *et al.*, 2006) and the p53-p16/p21 axis (Yu *et al.*, 2015). However, these findings are largely correlative, and the precise downstream mechanism by which IFN-induces p53-dependent inhibition of cellular growth remains to be determined.

IFN-induced apoptosis has been shown to occur both dependent and independent to IFN-induced cell cycle arrest (Subramaniam *et al.*, 1998; Sangfelt *et al.*, 1997a). In the former, combined propidium iodide and TUNEL staining, measuring DNA content and apoptosis-related DNA fragmentation respectively, indicated G₁ arrest preceded apoptosis following IFN treatment, indicating cells underwent programmed cell death following cell cycle progression failure (Subramaniam *et al.*, 1998). Similar to the activation of antiproliferative effects, there are multiple mechanisms described for the induction of apoptosis by IFN. IFNs are capable of inducing both cell intrinsic and extrinsic apoptosis. Cell intrinsic apoptosis involves mitochondrial disruption and subsequent cytochrome *c* release. In malignant cells, IFN- α treatment induced caspase-8 dependent mitochondrial cytochrome *c* release (Thyrell *et al.*, 2002). Similar effects were observed following prolonged IFN treatment in WISH cells, independent from death receptor (DR) activation (Apelbaum *et al.*, 2013). Additionally, recent studies identified ISG54 as a key regulator for cell intrinsic apoptosis (Reich, 2013; Stawowczyk *et al.*, 2011). Cell extrinsic apoptosis is mediated by ligation of cell surface death receptors by TNF family members, such as TNF, TRAIL, or FasL. DR ligation induces Fas-associated death domain-containing protein (FADD) recruitment and activation of caspase-8, which results in further caspase activation and ultimately hallmark apoptosis, including DNA fragmentation. Type I IFN treatment triggers upregulation of proapoptotic proteins, including Fas, FasL and TRAIL (Chawla-Sarkar, Leaman & Borden, 2001; Kayagaki *et al.*, 1999; Choi *et al.*, 2003). The antiviral benefits of IFN-induced apoptosis have been shown in influenza virus infected murine fibroblasts, where IFN treatment sensitised infected cells to FADD-dependent apoptosis, thus limiting viral replication (Balachandran *et al.*, 2000).

The complexity underlying activation and regulation of growth inhibitory IFN signals as opposed to antiviral responses contributes to the lack of a well-defined mechanism(s), particularly in normal physiological conditions. Contributing factors regulating the IFN-dependent activation of antiproliferative pathways will be discussed in further detail later, in the short introduction to chapter 4. This complexity is exacerbated by the dynamic and multifaceted nature of ISG induction, as revealed by global analyses comparing IFN signatures

in human and mice across multiple cell types (Mostafavi *et al.*, 2016). A recent study looking at ISG–protein interactions highlighted another layer of intricacies, as the team characterised 104 ISG interactions using a global mass-spectrometry-based survey, and identified 1,401 cellular binding partners engaging in 2,734 interactions (Hubel *et al.*, 2019).

1.4.5 Pathogenic potential of IFN during respiratory viral disease I: influenza

Early in vivo studies in murine influenza models suggest a functional redundancy between the type I and III IFN systems. Single IFN receptor deficient mice (*Ifnar1*^{−/−} or *Ifnlr1*^{−/−}) have been shown to be resistant to influenza virus infection, with viral control comparable to WT mice (Price, Gaszewska-Mastarlarz & Moskophidis, 2000; Mordstein *et al.*, 2008). Type I and III IFN redundancy was confirmed in mice deficient for both type I and III receptors (*Ifnar1*^{−/−}*Ifnlr1*^{−/−} double-knockout) (Mordstein *et al.*, 2008), and in BM chimeric mice lacking IFN signalling specifically in epithelial cells (Crotta *et al.*, 2013). In both contexts, IFNR deficiency resulted in inefficient viral control and dramatically increased influenza susceptibility. As discussed earlier, the importance of both type I and III IFN protection in human influenza is made evident by the susceptibility of patients carrying mutations in crucial IFN signalling components (Ciancanelli *et al.*, 2015; Hernandez *et al.*, 2018).

In the past decade or so, emerging evidence has begun to challenge the dogma that IFN signalling is strictly beneficial during viral infection. A direct link between IFN signalling and pathogenesis during viral infection was first shown in chronic lymphocytic choriomeningitis virus (LCMV) infection (Teijaro *et al.*, 2013; Wilson *et al.*, 2013). IFNAR blockade using an α IFNAR antibody resulted in enhanced CD4⁺ T cell-dependent viral clearance (Teijaro *et al.*, 2013; Wilson *et al.*, 2013). The link between IFN and lung damage during acute influenza was established when it was observed that type I IFN signalling induces TRAIL expression on inflammatory monocytes (Högner *et al.*, 2013), which promotes alveolar epithelial damage through engagement of DRs during influenza virus infection (Herold *et al.*, 2008). A definitive link between type I IFN signalling and influenza pathology was confirmed in a comparison between highly susceptible 129 mice and their B6 counterparts (Davidson *et al.*, 2014). 129 mice were shown to produce significantly higher levels of both type I and type III IFN compared to B6 mice, and for a longer duration. This was shown to be detrimental, as IFNAR deficient 129s had improved survival and reduced lung damage compared to WT controls, independent from viral burden (Davidson *et al.*, 2014). This is significant, as it provides in vivo

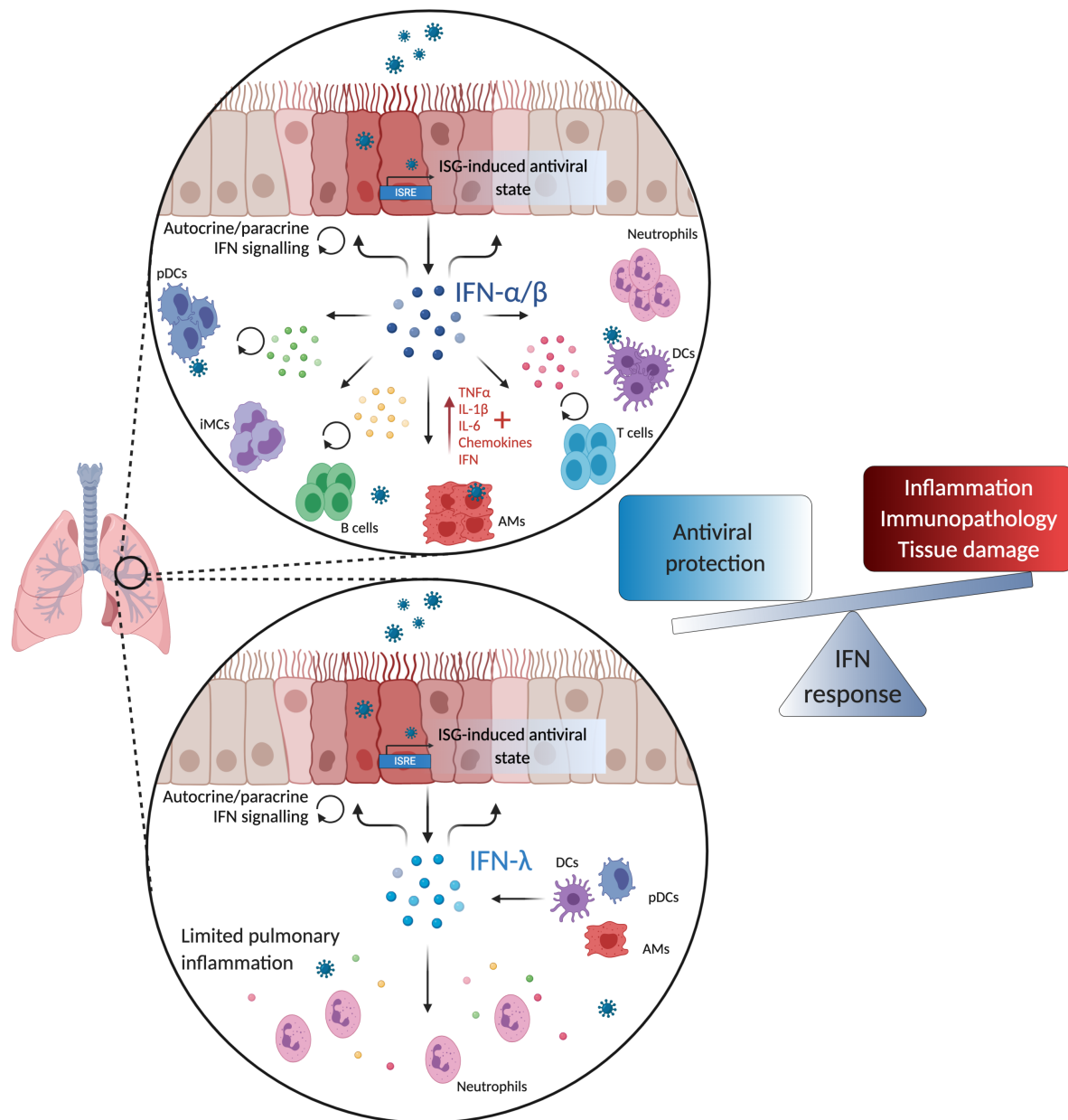
evidence of IFN-I-dependent immunopathology, in a context of excessive IFN signalling. Mechanistically, IFN-I produced by pDCs in 129 mice drove proinflammatory cytokine production and the recruitment of TRAIL⁺ inflammatory monocytes contributing to epithelial disruption (Davidson *et al.*, 2014). Type I IFN has additionally been linked with increased susceptibility to *S. pneumoniae* bacterial superinfection during influenza. IFN- α/β suppresses antibacterial responses through inhibition of CXCL1/2-dependent neutrophil recruitment and blocking IL-17 production by $\gamma\delta$ T cells (Li, Moltedo & Moran, 2012; Shahangian *et al.*, 2009).

Immunopathology associated with excessive IFN-I signalling led to an interest in the targeted antiviral function of type III IFN (Introduction Figure 1). Aside from activity in neutrophils (Blazek *et al.*, 2015; Broggi *et al.*, 2017; Espinosa *et al.*, 2017), IFN- λ signalling is thought to be restricted to epithelial cells in mice, and therefore provides targeted ISG induction in the epithelium during respiratory viral infection. Thus, IFN- λ lacks the pathogenic immunomodulatory effects described for type I IFN (Davidson *et al.*, 2014). Both IFN- α and IFN- λ treatment administered early during the course of influenza virus infection were effective in controlling viral replication in B6 mice expressing functional Mx1, however only IFN- λ provided protection (Davidson *et al.*, 2016). Instead, IFN- α treatment promoted pathogenic inflammation similar to that observed in 129 mice, and increased lung tissue apoptosis (Davidson *et al.*, 2016). It was recently suggested that IFN- λ plays a non-redundant role in mild influenza virus infection through the inhibition of early viral replication (Galani *et al.*, 2017). Galani *et al.* found IFN- λ induces an antiviral programme in neutrophils and epithelial cells, as opposed to proinflammatory activation induced by type I IFN. IFN- λ has also been shown to be important in controlling viral replication in the upper airways of mice, which was shown to block influenza transmission (Klinkhammer *et al.*, 2018). Collectively, evidence in murine influenza models suggests IFN- λ is the more protective antiviral cytokine, lacking the immunomodulatory and proinflammatory effects associated with excessive or prolonged IFN- α/β -induced signalling.

Two important studies sought to characterise the immune response to highly pathogenic 1918 H1N1 infection using a reconstructed recombinant virus (Kash *et al.*, 2006; Kobasa *et al.*, 2007). Kash *et al.* found the 1918 strain induced significant type I IFN induction in mice compared to less virulent strains (Kash *et al.*, 2006). In rhesus macaques, Kobasa *et al.* found that the reconstructed 1918 influenza virus infection triggered fatal ARDS, accompanied by significant lung tissue disruption and haemorrhaging, inflammatory immune cell infiltrate and prolonged cytokine production. Expression data analysis of bronchial tissues revealed a

dysregulated type I IFN response, characterised by low early IFN gene expression, yet sustained ISG signals late in the course of infection, implicating persistent IFN signalling in disease progression (Kobasa *et al.*, 2007). H5N1 avian influenza strains are associated with even higher mortality rates in humans. Primary human monocyte-derived macrophages infected with H5N1 IAV exhibited significant proinflammatory cytokine production, predominantly IFN- β and TNF α (Cheung *et al.*, 2002). In both ferrets (Cameron *et al.*, 2008) and humans (Beigel *et al.*, 2005), H5N1 induces a stronger IFN response than less pathogenic influenza strains.

Whilst the use of animal influenza models provides important mechanistic insight, it misses intrinsic and extrinsic components of immune function specific to human influenza disease. Patient blood and tissue samples have allowed us to begin characterising the immune profiles of individuals in response to influenza virus infection. Cytokine analysis of serially collected nasal lavage fluid collection revealed influenza symptom onset correlates directly with IFN- α production (Hayden *et al.*, 1998). Similarly, peripheral blood gene expression also detected IFN signalling early during acute influenza virus infection (Zhai *et al.*, 2015). This likely reflects viral PAMP recognition and early IFN production. Segregating patients into groups based on symptom characteristics and disease severity is a useful tool in understanding specific correlations between immune pathways and disease progression. Measurement of cytokine levels in nasal lavage fluid (Oshansky *et al.*, 2014) and blood expression profiling (Parnell *et al.*, 2012; Hoang *et al.*, 2014) found a significant IFN signature in the severe influenza patients. Conversely, a recent blood transcriptomic study found patients with severe disease had a diminished IFN-related signature (Dunning *et al.*, 2018).



Introduction figure 1. The type I and III IFN response during respiratory viral infection.

Type I and III IFNs are produced following viral recognition by respiratory epithelial cells and lung immune cells. Autocrine and paracrine IFN signalling generates an antiviral state in the respiratory epithelium, through induction of ISGs in infected and bystander epithelial cells. The IFN- α/β receptor IFNAR is ubiquitously expressed, and therefore type I IFN responses are associated with enhanced proinflammatory cytokine production and inflammation. The effects of IFN- λ are more targeted, owing to restricted expression of the IFNLR in epithelial cells and neutrophils, and are therefore thought to be less inflammatory. IFN responses require tight regulation in order to provide antiviral protection, without the induction of pathogenic responses that exacerbate lung tissue damage. Created using BioRender.com

1.4.6 Pathogenic potential of IFN during respiratory viral disease II: COVID-19

Late in 2019, a cluster of pneumonia disease cases emerged with unknown aetiology in Wuhan, in the Hubei province of central China. Samples from patients experiencing severe pneumonia were taken to the Wuhan Institute of Virology, where the novel SARS-CoV-2 was identified as the causative agent of the respiratory disease COVID-19 (Zhou *et al.*, 2020a). Since, the global scientific community has mobilised to help generate an understanding in aspects of COVID-19 biology. Given the time taken to generate vaccines or specific antiviral treatments against a novel respiratory viral disease, pre-developed host-targeted immunotherapies offer a more immediate therapeutic strategy to reduce morbidity and mortality, and help ease the burden on global healthcare. SARS-CoV-2 infects cells via the angiotensin-converting enzyme 2 (ACE2) receptor (Wan *et al.*, 2020), expressed mainly in the respiratory epithelium by type II alveolar (AT2) epithelial cells and ciliated cells (Jia *et al.*, 2005; Qi *et al.*, 2020). Clinical and lung radiological analyses of COVID-19 patients revealed massive alveolar damage in patients experiencing moderate or severe disease, with individuals exhibiting progressive hypoxic respiratory failure from ARDS (Qiu *et al.*, 2020; Xu *et al.*, 2020); the leading cause of mortality in COVID-19 patients (Wu & McGoogan, 2020). Additionally, histopathological findings in post-mortem lung biopsies revealed neutrophilic infiltration consistent with superimposed bacterial bronchopneumonia (Tian *et al.*, 2020). A screen for bacterial species in intensive-care COVID-19 patients found more than 50% of severe patients recorded bacterial superinfection (Dhesi *et al.*, 2020), indicating a failure of lung barrier function.

Given the extent to which loss of lung function contributes to COVID-19 morbidity and mortality, efforts currently underway seek to identify mechanisms propagating lung damage. Epithelial destruction occurs as a result of both cytopathic viral effects in host cells, and damage generated by the ensuing inflammatory immune response. The importance of host resistance to viral replication is still a matter of debate. Some studies have described a positive correlation between disease severity and viral burden, however others have found no difference (Liu *et al.*, 2020; He *et al.*, 2020; To *et al.*, 2020; Zheng *et al.*, 2020), suggesting host-intrinsic factors influence disease outcome. Viral loads were detected at highest levels in throat swabs at the time of symptom onset before gradually decreasing, resembling influenza kinetics (He *et al.*, 2020). Interestingly, a recent study found no difference in the viral burden of asymptomatic compared to symptomatic COVID-19 positive patients (Lavezzo *et al.*, 2020),

suggesting that aberrant immune responses, rather than cytopathic viral effects, may determine lung pathology.

The contribution of IFN responses to COVID-19 disease outcome is still unclear. Work by Stanley Perlman's group gave early indications into the pathogenic potential of type I IFN during coronavirus infection using mouse models of SARS-CoV-1 infection. Similar to our lab's findings in influenza (Davidson *et al.*, 2014), they show that *Ifnar1*^{-/-} BALB/c mice have reduced proinflammatory cytokine production and lung damage, and improved survival following SARS-CoV-1 infection (Channappanavar *et al.*, 2016). In vitro studies conducted early during the outbreak show SARS-CoV-2 is IFN-sensitive, with treatment reducing viral replication in Vero cells (Lokugamage *et al.*, 2020). SARS-CoV-2 infection has been shown to induce delayed type I and III IFN induction in primary AEC cultures (Pizzorno *et al.*, 2020), but not in human lung tissues ex vivo (Chu *et al.*, 2020). In one of the first studies characterising the innate antiviral response to SARS-CoV-2 infection, the tenOever group characterised the expression profile of serum and post-mortem lung tissue of COVID-19 patients, and found induction of a proinflammatory cytokine and chemokine profile, yet failed to detect IFNs (Blanco-Melo *et al.*, 2020). Similar findings were observed following whole-blood transcriptomic and cytokine profiling of fifty COVID-19 patients, which showed low IFN production and elevated NF-κB regulated cytokines, such as IL-6 (Hadjadj *et al.*, 2020). Coupled with the identification of virally encoded IFN-antagonists (Gordon *et al.*, 2020; Konno *et al.*, 2020), the argument of improper IFN-mediated antiviral protection in COVID-19 patients forms the basis for the use of IFNs therapeutically in ongoing COVID-19 clinical trials.

As the COVID-19 crisis progresses, emerging evidence from patient cohort sample studies suggests that the role of IFNs in COVID-19 disease is not so clear-cut. In contrast to the aforementioned studies, a significant IFN signature was identified in the bronchoalveolar lavage fluid (BALF) of severe COVID-19 patients (Zhou *et al.*, 2020b). High levels of IFN-λ expression have also been detected in the lower airways, but not in naso-oropharyngeal swabs (Broggi *et al.*, 2020). Additionally, single-cell RNA sequencing of peripheral blood mononuclear cells (PBMCs) from COVID-19 patients found an IFN signature in CD14⁺ monocytes (Wilk *et al.*, 2020). In a similar study, comparison of PBMC transcriptomic profiles between healthy donors, mild or severe COVID-19 patients, and severe influenza patients, found that monocytes produced type I IFNs in parallel with TNF and IL-1β in severe influenza and COVID-19 patients, but not in patients experiencing milder COVID-19 disease (Lee *et al.*, 2020). These findings were corroborated by a study from the Iwasaki lab, who employed

longitudinal analyses to samples from 113 COVID-19 patients (Lucas *et al.*, 2020). They also segregate patient samples by disease severity: healthy, moderate, or severe. Interestingly, they found severe COVID-19 patients maintained elevated expression of both type I and III IFNs, in addition to proinflammatory cytokines, whereas these cytokines steadily declined in patients with moderate disease (Lucas *et al.*, 2020).

Discrepancies in IFN detection between individual patient sample studies may be explained, at least in part, by the timing and location (serum, BALF, swabs) of sample collection. For instance, the tenOever study failed to detect IFNs in COVID-19 patient sera, however did detect ISG induction in post-mortem lung tissue (Blanco-Melo *et al.*, 2020), indicating IFN-dependent signalling. Additionally, differences in the individual susceptibility to infection may dilute IFN responses in global datasets. Segregation by disease severity provides useful insight into immunopathological mechanisms or biomarkers of respiratory viral disease pathogenesis. Further analyses and direct comparison of blood and lung tissue samples collected at multiple time intervals from patients experiencing various forms of disease severity would be highly beneficial to further our understanding of COVID-19 disease progression and pathology, and indeed other respiratory viral diseases.

1.5 Regeneration of the respiratory epithelium

The respiratory system is organised into two major compartments to allow gas exchange and the vital supply of oxygen to organs and tissues around the body. The upper respiratory tract functions as a conduit for air intake, composed of the nose, nasal cavity, mouth and throat (pharynx). The lower respiratory tract consists of the trachea, bronchi (primary, secondary and tertiary), bronchioles (terminal and respiratory), and the alveoli, where gas exchange occurs. Respiration requires an interface between the human body and the external environment, and at this interface, the respiratory system is continuously exposed to inhaled pollutants, allergen, microbes, and pathogen, including respiratory viruses and opportunistic bacterial species (Hogan *et al.*, 2014; Vareille *et al.*, 2011; McCullers, 2014). Frontline protection is provided by a continuous layer of epithelial cells that line the mammalian respiratory system, whilst simultaneously providing a site for oxygen-carbon dioxide gas exchange in alveolar sacs. Co-development of the respiratory system with the cardiovascular

system allows the intimate proximity between alveolar cells and pulmonary capillary endothelial cells, crucial for blood oxygenation and carbon dioxide removal.

Respiratory viral infection is typically limited to the upper respiratory tract in asymptomatic individuals or in cases of mild disease. However, spread to the lower respiratory tract can cause severe disease, resulting in viral pneumonia and ARDS, the latter being the leading cause of mortality in influenza and COVID-19 patients (Ramos & Fernandez-Sesma, 2015; Wu & McGoogan, 2020). Respiratory dysfunction occurs as a result of damage to the epithelial-endothelial barrier in the alveolus, induced by either cytopathic viral effects in host epithelial cells (Short *et al.*, 2016), or damage generated by the ensuing inflammatory immune response. Epithelial loss and alveolar collapse result in the filling of airspaces with fluid, red blood cells (RBCs), and leukocytes recruited during an inflammatory immune response. Indeed, escalation of immune cell signalling and resultant cytokine storm contributes to lung damage and respiratory dysfunction (Tisoncik *et al.*, 2012), as discussed earlier. Quantification and characterisation of cellular infiltrate and protein content in the air spaces by BALF extraction is therefore used as an indication of damage to epithelial and endothelial barriers (Henderson, 1984). Severe cases of respiratory viral disease are often exacerbated by secondary infection with bacterial species, including *Streptococcus pneumoniae* and *Staphylococcus aureus*. In homeostasis, bacteria colonise the upper respiratory tract, and are maintained in a state of balance owing to barrier protection and control by tissue-resident immune cells (Vernatter & Pirofski, 2013). Following infection, barrier dysfunction and virally-induced immunomodulatory effects enhance the extension of bacteria down into the lower respiratory tract, with the two pathogens showing remarkable synergy (McCullers, 2014).

At steady state, the mammalian lung has a remarkably low turnover rate and remains largely quiescent (Herriges & Morrissey, 2014). However, following severe lung injuries, such as those experienced in severe influenza, the immediate threat to host survival posed by ARDS-induced hypoxic respiratory failure, sepsis, and potential multi-organ failure, necessitates the rapid induction of tissue repair. This is enabled by a variety of lung repair programmes that coordinate the proliferation and differentiation of regional progenitor cells, in order to maintain lung function.

1.5.1 Repair of the airway epithelium

Recent single-cell profiling of the human and mouse airways revealed a significant degree of heterogeneity within airway epithelial cell populations (Montoro *et al.*, 2018; Plasschaert *et al.*, 2018). The trachea and proximal airways are lined by a pseudostratified epithelium, composed of multiple cell types of different lineages: progenitor basal cells, secretory cells, goblet cells (which are rare in mice, but abundant in human airways), multiciliated cells, and rare or highly specialised cell types such as pulmonary neuroendocrine cells, brush cells, and the newly discovered ionocytes (Montoro *et al.*, 2018; Plasschaert *et al.*, 2018; Zepp & Morrissey, 2019). Collective function of these cells constitutes elements of barrier protection and mucociliary clearance, and is dependent on a fine ratio and distribution of epithelial cell subtypes (Herriges & Morrissey, 2014). Mucus produced by goblet and secretory cells forms a layer for trapping inhaled particles, pollutants, and microorganisms. Trapped particles and microorganisms are shuttled away from the lungs and out of the airways by the coordinated beating of cilia protruding from the apical surface of multiciliated cells, in a process known as mucociliary clearance.

Turnover in the adult trachea is very low in homeostasis, however injury induces the rapid proliferation of basal cell populations to promote tissue repair (Kauffman, 1980). Quiescence and the response to injury are maintained by signals from local mesenchymal cell types and extracellular matrix (ECM) that constitute the local niche. Interactions between the respiratory epithelium and the local niche are important for the induction of tissue repair programmes. This includes the production of growth factors, such as fibroblast growth factor 10 (Fgf10) by airway smooth muscle cells, which promotes repair following binding to its receptor Fgfr2 on epithelial cells (Volckaert *et al.*, 2011; Lee *et al.*, 2017).

Progenitor basal cells are marked by expression of Trp63 and keratin 5 (Krt5) (Rock *et al.*, 2009), however recent findings reveal a heterogeneity within this population and their early origins in lung development (Yang *et al.*, 2018). Murine intrapulmonary airways lack basal cells, unlike in humans, where the pseudostratified epithelium containing basal cells continues down the respiratory tree into intermediate and respiratory airways, and therefore are more likely to have a more prominent role in airway epithelial recovery. Krt5⁺ basal cells have a high proliferative and self-renewal capacity, and lineage-tracing experiments confirmed this population can give rise to all airway cell types (Montoro *et al.*, 2018; Rock *et al.*, 2011; Garca *et al.*, 2019). Basal cell commitment to ciliated or secretory cell fate is thought to be determined by Notch signalling, as inhibition favours differentiation towards multiciliated cells (Mori *et*

al., 2015; Pardo-Saganta *et al.*, 2015). Multiciliated cells are a terminally differentiated, non-dividing cell type (Rawlins & Hogan, 2008). Commitment to the multiciliated cell lineage requires exit from the cell cycle to allow centriole amplification to produce basal bodies that provide the foundation for motile cilia (Quarmby & Parker, 2005; Al Jord *et al.*, 2017). Scgb1a1⁺ secretory cells on the other hand are capable of self-renewal (Rawlins *et al.*, 2009; Van Keymeulen & Blanpain, 2012), and can also dedifferentiate to contribute to the basal stem cell pool (Tata *et al.*, 2013). Additionally, antibody-mediated notch inhibition induces trans differentiation of secretory cells into multiciliated cell, indicating that these cells also play a role in maintaining independent cell lineages during repair (Lafkas *et al.*, 2015).

1.5.2 Repair of the distal lung

Lung branching morphogenesis is an embryonic developmental process that produces branched ductal tree-like structures from conducting airways, creating an extensive tubular network and formation of the respiratory tree (Zepp & Morrisey, 2019). Termination of branching morphogenesis coincides with post-natal alveologenesis, giving rise to tiny air sacs, known as alveoli. Alveoli consist of an alveolar epithelium, a connective tissue layer, and a continuous capillary endothelium. This intricate structural design is optimised for its primordial function in gas exchange, as it allows close proximity between air and the vasculature over a large surface area, which in the human lung comprises approximately 70 m² (Basil *et al.*, 2020). This is exemplified by measurements of the lung volume constituents following a deep inspiration: over 80% air, 10% blood, and only 10% tissue (Knudsen & Ochs, 2018). The alveolar epithelium consists of type I alveolar (AT1) and AT2 epithelial cells. Juxtaposed next to the capillary plexus, AT1 cells have a flattened and squamous morphology, covering 95% of the gas exchange surface of the lung. AT2 cells produce the lipid-dense pulmonary surfactant functioning to reduce surface tension and prevent alveolar collapse during respiration (Bachofen & Schürch, 2001).

Similar to the airway epithelium, the alveolar niche responds rapidly to injury, and can regenerate to its entirety within a few weeks (Zepp & Morrisey, 2019). AT2 cells have stem cell-like function, capable of both self-renewal and AT1 cell differentiation in homeostasis, and following injury (Barkauskas *et al.*, 2013). Lineage-tracing revealed an Axin2⁺ AT2 cell sublineage, termed alveolar epithelial progenitors (AEPs), that play a dominant role in alveolar repair following acute injury induced by influenza virus infection (Zacharias *et al.*, 2018).

Axin2 is a Wnt signalling transcriptional target, and plays an important role in AT2 activity during lung alveologenesis (Frank *et al.*, 2016). AEPs are primed for cell cycle entry and proliferation, responding to Wnt and Fgf7 signals induced by the alveolar niche during repair (Zacharias *et al.*, 2018). In contrast, AT1s play a limited role in alveolar epithelial regeneration (Jain *et al.*, 2015). Lung injury additionally induces a rapid proliferation of resident microvascular endothelial progenitor cells (Alvarez *et al.*, 2008). Alveolar regeneration is supported by endothelial cells through production of growth factors, including Vegfr2 and Fgfr1, which stimulate epithelial proliferation through induction of matrix metalloprotease MMP14 of epidermal growth factor (EGF)-like ligands (Ding *et al.*, 2011).

A series of recent publications have revealed a contribution of airway progenitor cells in regeneration of the distal lung. The lower airways of mice lack basal cells, and therefore, it was previously thought that repair in this region was mediated largely by secretory cells. However, two recent studies using genetic-lineage tracing systems identified a population of bronchioalveolar stem cells (BASCs) important for repair of the distal lung (Liu *et al.*, 2019; Salwig *et al.*, 2019). BASCs are located at the bronchioalveolar-duct junctions, and express club cell secretory protein (CCSP, or Scgb1a1) and the AT2 cell marker prosurfactant apoprotein-C (SP-C) (Bender Kim *et al.*, 2005). Following naphthalene-induced injury—a toxic aromatic hydrocarbon that ablates club cells once these cells have metabolised it—CCSP⁺SP-C⁺ BASCs become activated, and can repopulate club cell, ciliated cell, AT1 cell, and AT2 cell lineages (Liu *et al.*, 2019). This was also observed, albeit to a lesser extent, following influenza virus induced damage (Salwig *et al.*, 2019). BASC depletion resulted in delayed lung regeneration, although full regeneration was eventually observed (Salwig *et al.*, 2019), indicating that BASCs are not crucial for epithelial recovery.

The first identification of true airway stem cells contributing to alveolar repair followed the observation of Krt5⁺ cells forming pod-like structures in influenza infected lungs (Kumar *et al.*, 2011). This was followed by a series of high-impact publications detailing their origin, function, and importance following lung injury. Lineage-negative p63⁺Krt5⁺ stem cells are largely absent in a normal lung, and are thought to migrate from the proximal airways and proliferate in the lung parenchyma following influenza virus infection, populating heavily damaged tissue regions (Kumar *et al.*, 2011; Vaughan *et al.*, 2015). These progenitors, also known as distal airway stem cells (DASCs), have been further characterised as integrin β 4⁺CD200⁺Sox2⁺ (Vaughan *et al.*, 2015; Ray *et al.*, 2016), and are preferentially infected by influenza virus, resulting in suppressed Fgfr2b signalling and impairment of their capacity for self-renewal (Quantius *et al.*, 2016). p63⁺Krt5⁺ DASC ablation in mice compromises lung

regeneration following acute influenza virus infection, causing deficient oxygen exchange (Zuo *et al.*, 2014). Therefore, it is thought these airway progenitor cells provide crucial support for lung repair, in order to maintain gas exchange.

A recent study sought to define the molecular signals that govern airway stem cell expansion in distal lung repair. It is thought that local lung hypoxia, which often occurs following extensive epithelial necrosis, induces hypoxia-inducible factor (HIF1 α)-dependent Notch signalling, triggering DASC proliferation (Xi *et al.*, 2017). Notch signalling activates the Trp63 and Krt5 programme, producing an aberrant keratinised epithelial response and dysplastic alveolar epithelial repair in regions of severe alveolar damage (Vaughan *et al.*, 2015), with notch blockade promoting normal AT2 cell differentiation (Xi *et al.*, 2017). Therefore, it is thought these airway progenitor cells do not function primarily to promote normal repair, instead providing fast-responding repair support, in order to prevent alveolar collapse and significant tissue loss (Xi *et al.*, 2017). Krt5⁺ pods have been identified in humans that have experienced lung disease or injury, suggesting a mechanism of long-term lung scarring following respiratory viral infection (Taylor *et al.*, 2018a, 2018b).

A heterogeneity was revealed in DASCs by lineage-tracing experiments, which identified an additional rare p63⁺Krt5⁻ subset traced back to embryonic p63⁺ cells in the main bronchi, which constitutively express p63, yet switch on Krt5 following damage-induced migration (Yang *et al.*, 2018). In addition, a very recent single-cell transcriptomic analysis of EpCam⁺ β 4⁺CD200⁺ airway cells also identified a population of lineage-negative SP-C⁻ (therefore excluding BASCs) cells with high expression of MHC class I marker H2-K1 (Kathiriya *et al.*, 2020). This β 4⁺CD200⁺ subset expands and differentiates into AT1 and AT2 cells following bleomycin-induced lung injury in vivo, contributing significantly to alveolar epithelial regeneration (Kathiriya *et al.*, 2020).

The identification of airway progenitor/stem cell subsets and their roles in lung restoration highlights the complexity in nature of tissue repair programmes during influenza infection, particularly when you consider the additional inflammatory milieu in the lung microenvironment. The contribution of different stem cell populations to types of repair still require further work, particularly in the context of human lung repair. For instance, how overlapping are functions of the multiple DASC populations? How do these functions compare with those of AEP and AT2 cells, in both acute and chronic lung infections and disease? The importance in maintaining lung function during an infection gives reason to the multifaceted nature of responses that regulate tissue restoration, as an over reliance on one system would likely be detrimental in certain contexts.

1.5.3 Immune mediators in epithelial recovery

Inflammation also affects lung regeneration, including the promotion of repair pathways by resident and circulatory immune cells. Following chemical injury or *Nippostrongylus brasiliensis* hookworm infection, alveolar macrophages stimulate epithelial proliferation through the provision of Wnt signals (Hung *et al.*, 2019). Innate lymphoid cells (ILCs), a group of innate immune cells that lack T or B cell receptors, provide additional support through direct and indirect cues. CCL2 recruited monocytes following pneumonectomy-induced lung damage respond to IL-13 produced by ILC2s, skewing monocyte differentiation into an alternative macrophage phenotype, and promote AEC2 proliferation through ECM remodelling (Lechner *et al.*, 2017). ILC2s have also been shown to directly promote tissue recovery through production of the EGF family member, amphiregulin (Monticelli *et al.*, 2011). Epithelial-derived IL-33 induces ILC2 amphiregulin production resulting in tissue modelling, with ILC depletion leading to compromised epithelial integrity and diminished lung function (Monticelli *et al.*, 2011). Regulatory T cells are also an important source of amphiregulin during influenza recovery, as selective regulatory T cell deficiency in amphiregulin exacerbates lung damage and decreases blood oxygen levels (Arpaia *et al.*, 2015).

IL-22 is a cytokine produced by various immune cells, with important roles in mucosal barrier protection (Rutz, Eidenschenk & Ouyang, 2013). Belonging to the IL-10 family of cytokines, IL-22 promotes barrier integrity and tissue repair in the both the intestine (Zenewicz *et al.*, 2008; Pickert *et al.*, 2009) and the lung (Sonnenberg *et al.*, 2010; Barthelemy *et al.*, 2018) in a STAT3-dependent manner. Similar to the receptor for IFN- λ , the IL-22 receptor (IL-22R) is comprised of the ubiquitously expressed IL-10R β and a unique IL-22R α 1 chain largely restricted to epithelial sites (Gurney, 2004). In fact, IL-22 produced by ILC3s in the intestine has been shown to promote IFN- λ responses to rotavirus infection, through simultaneous IL-22R and IFNLR signalling (Hernández *et al.*, 2015). Barrier supporting functions include promoting epithelial cell proliferation, regulating tight-junction proteins, and the production of antimicrobial peptides, chemotactic factors, and mucins (Dudakov, Hanash & van den Brink, 2015). In the lung, IL-22 is thought to be produced by NK cells (Guo & Topham, 2010; Kumar *et al.*, 2013; Xu *et al.*, 2014), NKT cells (Paget *et al.*, 2012), and both $\gamma\delta$ and conventional $\alpha\beta$ T cells (Ivanov *et al.*, 2013; Potocnik *et al.*, 2014). Mice lacking the IL-22R were shown to have increased pulmonary damage and inflammation following influenza virus infection, correlating with impaired alveolar (Pociask *et al.*, 2013) and tracheal (Kumar *et al.*, 2013) epithelial recovery. Treatment with recombinant IL-22 promotes pulmonary barrier function,

protecting mice against bacterial pneumonia during influenza coinfection, independent from any antibacterial effects (Barthelemy *et al.*, 2018). Prolonged IL-22 responses can be associated with pathology (Zheng *et al.*, 2007). In the lung following bleomycin-induced injury this is driven by IL-17⁺ producing Th17 cells, as IL-22 was protective in this model in *Il17a*^{-/-} mice (Sonnenberg *et al.*, 2010).

1.5.4 Primary airway epithelial cell cultures – an in vitro model for lung regeneration

To study respiratory epithelial cell functions and regeneration, primary murine and human AECs can be used as an ex vivo culture system (You *et al.*, 2002; Fulcher *et al.*, 2005). As discussed previously, progenitor airway cells contribute to repair of both the airway and alveolar epithelium in vivo. To establish murine AEC cultures, progenitor basal cells are digested from mouse tracheas and plated on a collagen-coated transwell. Following plating, basal cells rapidly proliferate, reaching confluence in approximately 7 days. Subsequent exposure to air (termed air-liquid interface, or ALI) for a further 7 days induces a programme of further proliferation, enabled by simultaneous cellular morphogenesis, with basal cells shrinking in size, in addition to basal cell differentiation into secretory and multiciliated cell subtypes (Hackett *et al.*, 2011; Wu, Zhao & Chang, 1997; Kaartinen *et al.*, 1993). Thus, in primary AEC cultures, processes of progenitor cell proliferation and subsequent differentiation into specialised airway epithelial cell subsets encapsulates core components of respiratory epithelial regeneration in vivo, and therefore provides a physiological model to study this in culture.

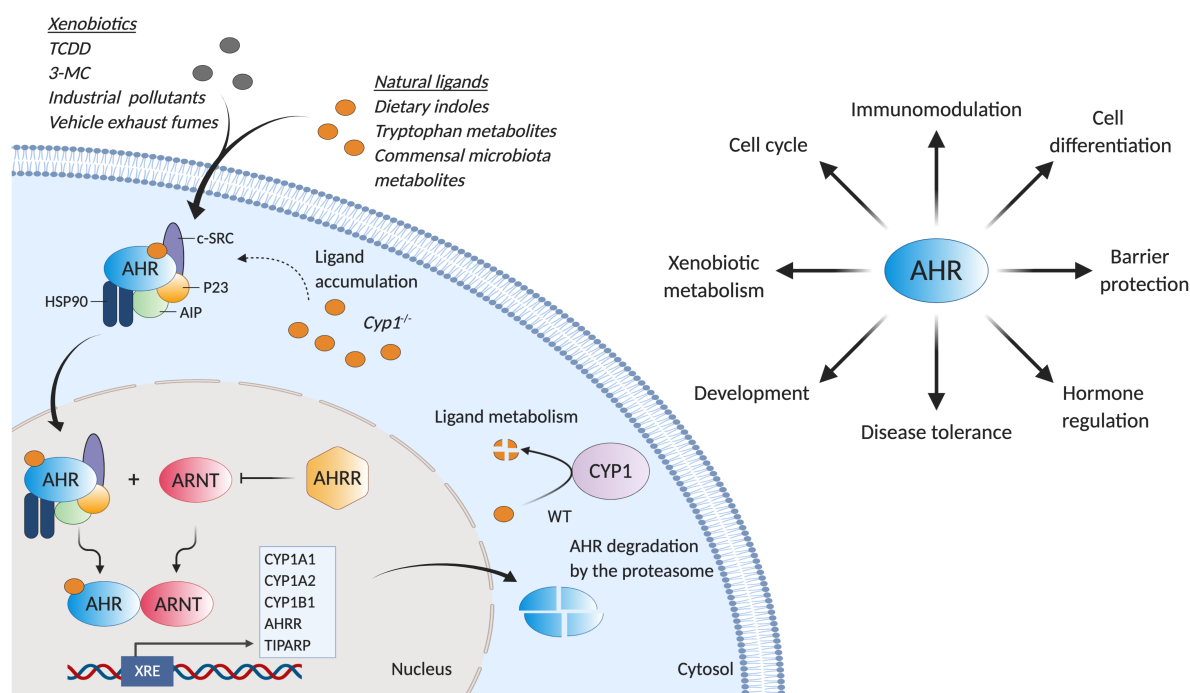
1.6 The aryl hydrocarbon receptor

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that coordinates responses to environmental and physiological stimuli. Originally identified as a dioxin receptor (Poland, Glover & Kende, 1976), AHR was studied for decades in the toxicology field for its ability to mediate the toxic effects of xenobiotics. However, evidence indicated a more prominent role in mammalian physiology, not least in relation to its evolutionary conservation from invertebrates onwards, with *Ahr* orthologues present in mammals, amphibians, reptiles and birds (Hahn *et al.*, 1997), and its widespread expression in different tissues (Frericks, Meissner & Esser, 2007). Early studies in AHR deficient mice

supported this notion, with knockout mice displaying abnormalities in the liver, eye, and vascular system (Fernandez-Salguero *et al.*, 1995; Schmidt *et al.*, 1996; Lahvis *et al.*, 2000), including consistent development of a patent ductus venosus (Lahvis *et al.*, 2000, 2005). The first indications of endogenous AHR activity regulating immune function came following the observation that *Ahr*^{-/-} mice had substantial decreased lymphocyte numbers in secondary lymphoid tissues (Fernandez-Salguero *et al.*, 1995). Since, research has unravelled numerous cell type- and context-specific roles for AHR in health and disease. Originally identified as an environmental xenobiotic sensor, defined AHR roles have expanded to include the coordination of responses to microbial, dietary, and metabolic stimuli. This includes important roles in regulating immune responses to infection, at barrier epithelial sites, and in promoting disease tolerance.

1.6.1 AHR activation

AHR belongs to the Pern-Arnt-Sim (PAS) superfamily of transcription factors, that also includes HIF proteins which detect and coordinate the response to hypoxia (Kewley, Whitelaw & Chapman-Smith, 2004). The AHR PAS and basic helix-loop-helix (bHLH) domains are involved in ligand and DNA binding, respectively (Fukunaga *et al.*, 1995). AHR is maintained in the cytosol in an inactive state by a protein complex that consists of the 90 kDa heat shock protein (HSP90), AHR-interacting protein (AIP), the co-chaperone p23, and the c-SRC protein kinase. AIP and p23 stabilise and maintain cellular AHR levels at steady-state, preventing its ubiquitination and degradation (Petrulis & Perdew, 2002; Lees, Peet & Whitelaw, 2003), and HSP90 maintains it in a high-affinity state for ligand binding (Pongratz, Mason & Poellinger, 1992). In addition, AIP additionally enhances its cytoplasmic localisation, and is released upon ligand binding, therefore exposing its nuclear localisation signal and triggering AHR translocation to the nucleus (Ikuta, Kobayashi & Kawajiri, 2004). Within the nucleus, the AHR-ligand complex partners with AHR nuclear transporter (ARNT), also known as HIF1 β , which enables its binding to AHR-responsive DNA sequences called xenobiotic response elements (XREs) (Yao & Denison, 1992), thus allowing control of target gene expression (Introduction Figure 2). AHR is also capable of influencing gene expression independent to



Introduction figure 2. Classic AHR signalling pathway and physiological activities.

The aryl hydrocarbon receptor (AHR) sits in the cytosol complexed to chaperone proteins in an inactive state. Following ligand binding, AHR translocates into the nucleus where it partners with ARNT, enabling xenobiotic response element (XRE) binding and the transcription of target genes which regulate a variety of physiological functions. Regulation of AHR signalling is achieved through induction of the aryl hydrocarbon repressor protein (AHRR), proteasomal degradation of AHR, and through induction of CYP1 enzymes which metabolise AHR ligands. Genetic depletion of CYP1 enzymes results in ligand accumulation and the enhancement of AHR signalling pathways, highlighting the importance of ligand availability in AHR regulation. Created using BioRender.com

canonical ARNT partnership and XRE binding. For instance, AHR can control NF- κ B activity by interaction with its various signalling components (Vogel *et al.*, 2007, 2014). In addition, AHR has E3 ubiquitin ligase function, and is capable of inducing proteasome-dependent degradation of proteins such as the oestrogen receptor (Ohtake *et al.*, 2007).

The best characterised response following agonist activation is AHR-mediated ligand metabolism, which is achieved by the direct induction of cytochrome p450 (CYP1) enzymes with monooxygenase activity, including CYP1A1, CYP1A2, and CYP1B1 (Schrenk, 1998). In addition to detoxifying effects, ligand metabolism promotes autoregulatory feedback. CYP-dependent ligand depletion, including both xenobiotics and endogenous ligands, effectively inactivates the activation signal for AHR, and therefore the level of active nuclear AHR-ARNT-ligand complexes. An additional layer of regulation is in the direct induction of AHR repressor (AHRR), which competes with the AHR-ligand complex for ARNT binding,

therefore operating as a negative regulatory feedback mechanism (Mimura *et al.*, 1999; Sakurai, Shimizu & Ohto, 2017). Finally, AHR is subject to proteosomal degradation following its activation (Davarinos & Pollenz, 1999).

Genome-wide RNA- and ChIP-sequencing studies have revealed that AHR responses vary in a species-, cell-, tissue-, and ligand-specific manner (Tijet *et al.*, 2006; Goodale *et al.*, 2013; Nault *et al.*, 2013; Dere *et al.*, 2011; Stockinger *et al.*, 2014; Denison & Faber, 2017). Combined with functional studies in vitro and in vivo, AHR has been shown to regulate a variety of cellular activities in addition to xenobiotic metabolism (Introduction Figure 2), including modulation of hormone receptor signalling (Denison *et al.*, 2011), proliferation and cell cycle (Yin *et al.*, 2016), inflammation, infection and immunity (Stockinger *et al.*, 2014; Gutiérrez-Vázquez & Quintana, 2018).

1.6.2 AHR ligands

First discovered as a receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), numerous other xenobiotic and also physiological AHR ligands have since been identified. Classic xenobiotic AHR agonists include a wide variety of ubiquitous hydrophobic environmental contaminants, largely categorised into halogenated aromatic hydrocarbons (HAHs), such as polychlorinated dibenzo-*p*-dioxins and biphenyls, and polycyclic aromatic hydrocarbons (PAHs) (Nguyen & Bradfield, 2008). Halogenated dioxins usually enter the environment through industrial activity, such as waste incineration. They are highly stable, toxic chemicals, and are often extremely potent AHR agonists with high binding affinity. For instance, in B6 mice, TCDD has a potency of about 1000-fold greater than that of PAHs like 3-methylcholanthrene (3-MC) (Niwa *et al.*, 1975). Therefore, TCDD and other stable xenobiotics are not easily or rapidly degraded by AHR activity, and often persist in the host (Bunger *et al.*, 2003). TCDD exposure in humans is associated with a range of toxic effects, including immunotoxicity, cardiotoxicity, hepatotoxicity, carcinogenesis (Denison *et al.*, 2011), and it was found to be the chemical agent responsible for chloracne outbreaks in chemical workers (Stockinger *et al.*, 2014). TCDD was also identified as the toxic contaminant of Agent Orange used by the U.S. army during the Vietnam war as part of its herbicidal warfare strategy, and is linked to a range of health problems in U.S. veterans, and Vietnamese citizens, including sarcomas, lymphomas, and leukaemia (Steele *et al.*, 1990). The extent to which metabolically stable agonists (i.e. TCDD), but not metabolically labile agonists (PAHs or endogenous

ligands), exert major toxic effects suggests that this toxicity is a product of a ligand's capacity to persistently activate or suppress AHR-target genes (Denison *et al.*, 2011). This is evident in mice deficient of AHR signalling, which are largely resistant to TCDD-induced toxicity (Bunger *et al.*, 2003). PAHs represent a large class of classic AHR agonists, however with a lower AHR-binding affinity compared to TCDD (Nguyen & Bradfield, 2008). Produced following combustion, PAHs are therefore often released as environmental pollutants following industrial activity in chimney soot and also diesel exhaust fumes, and are the main organic constituents of particulate matter. Researchers have therefore begun exploring possible links between environmental pollutant-induced AHR activation and adverse health effects (Hidaka *et al.*, 2016). In addition to the direct toxic effects of xenobiotics, AHR-dependent toxic ligand degradation can produce harmful by-products. For instance, the PAH, benzo(a)pyrene, is metabolised into a highly DNA-reactive procarcinogenic substance (Shimada & Fujii-Kuriyama, 2004).

In addition to the aforementioned physiological defects in AHR null mice (Fernandez-Salguero *et al.*, 1995; Schmidt *et al.*, 1996; Lahvis *et al.*, 2000), mounting evidence highlights a prominent role of AHR activation by endogenous ligands in health and disease (Rothhammer & Quintana, 2019). A variety of natural AHR ligands have been identified, largely sourced through dietary indole intake and metabolites of the tryptophan pathways (Gutiérrez-Vázquez & Quintana, 2018; Stockinger *et al.*, 2014). Cruciferous vegetables of the *Brassica* genus (including broccoli, cauliflower, Brussel sprouts and cabbages) are rich in glucosinolate glucobrassicin, which is enzymatically degraded to AHR agonist precursors, including indole-3-carbinol (I3C), and subsequently further metabolised to produce high-affinity AHR ligands indole-3-carbazole (ICZ) and 3,3'-diindolylmethane (Bjeldanes *et al.*, 1991; Degner *et al.*, 2009). Also sourced through dietary intake, the essential amino acid tryptophan serves as a precursor for potent AHR ligands. 90% of dietary tryptophan feeds into the kynurenine (Kyn) pathway (Seok *et al.*, 2018). Indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO) enzymes catalyse the conversion of tryptophan to its metabolite Kyn, which is itself an AHR agonist, although only at high concentrations (Mezrich *et al.*, 2010). Tryptophan is also upstream of the AHR agonist 2-(1'H-indole-30-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE); an endogenous ligand purified from porcine lung (Song *et al.*, 2002). In addition, exposure of tryptophan to ultraviolet light triggers its photo-oxidation to the potent AHR agonist, 6-formylindolo[3,2-b]carbazole (FICZ) (Rannug *et al.*, 1987). Microbial tryptophan metabolism provides additional AHR ligands independent from host-metabolic pathways. Bacteria expressing tryptophanase convert dietary tryptophan into indole,

which is then taken up by the host and metabolised in the liver into indoxyl-3-sulfate (I3S) (Wikoff *et al.*, 2009). Commensal *Lactobacillus* species provide further AHR ligands via tryptophan catabolism (Zelante *et al.*, 2013). Recent studies indicate the importance for commensal bacteria in sourcing tryptophan-derived AHR agonists to maintain intestinal homeostasis, with defects linked to the development of pathology in inflammatory bowel disease (IBD) (Lamas *et al.*, 2016).

The involvement of AHR in such a wide variety of cellular and host responses has led to speculation on factors that dictate these differential activities, including the importance of ligand specificity. Collective evidence suggests the differences observed between AHR agonists in ligand metabolism, receptor binding, and gene expression, influences a biological response outcome (Beischlag *et al.*, 2008; Denison *et al.*, 2011). This can be thought of with regard to TCDD-induced toxicity, which is not well defined mechanistically (Sorg, 2014). Classically, AHR-dependent toxic effects are thought to be linked with the metabolic persistence of AHR agonists and prolonged activation. However, structurally diverse ligands can stimulate AHR-dependent gene expression and biological responses similar to that of TCDD, without the associated toxicity (Denison & Faber, 2017). This tells us that there are likely further factors that potentiate distinct effects following AHR activation by different ligands. This might include non-canonical XRE-independent AHR activities (Vogel *et al.*, 2007, 2014), or differences in AHR-ligand complex structures. Site-directed mutagenesis and functional analysis of the AHR ligand-binding domain revealed binding of toxic TCDD-like compounds was different from that of structurally diverse non-toxic AHR ligands (Soshilov and Denison, 2014). With regard to AHR agonist-induced toxicity, AHR independent effects may also play a role. Despite the likely dependence of AHR in TCDD-induced toxicity (Bunger *et al.*, 2003), AHR-independent effects of TCDD have also been described (Park *et al.*, 2005; Tijet *et al.*, 2006; Patel *et al.*, 2009), which may be equally important in mediating TCDD-induced toxic effects. This is relevant with regard to physiological AHR functions driven by endogenous ligands, for instance during an infection. In particular, AHR activities following prolonged xenobiotic-induced activation may not reflect the physiological outcome following constitutive activation by endogenous agonists. This is supported by a growing body of literature generated over the past 15 years, which have identified important novel AHR functions *in vivo*, independent to exogenous stimuli activation.

1.6.3 AHR functions in the immune system

Two breakthrough studies in 2008 recognised the importance of AHR in directly regulating immune responses. AHR activation was shown to enhance the development and function of T_h17 cells, increasing their production of IL-17A, IL-17F, and IL-22 (Veldhoen *et al.*, 2008; Quintana *et al.*, 2008). T_h17 cells are defined by IL-17A production and expression of the transcription factor ROR γ t (Ivanov *et al.*, 2006), and are important in defence against extracellular pathogens, and in the progression of autoimmune inflammatory diseases (Korn *et al.*, 2009). In addition to identifying a direct link between AHR and the generation of mature T cells, the relevance of AHR in the context of autoimmunity was also realised. Using a murine model of multiple sclerosis (experimental autoimmune encephalitis; EAE), it was shown that AHR deficiency enhanced disease morbidity (Veldhoen *et al.*, 2008). Mechanistically, high AHR expression in T_h17 cells compared to naïve, T_h1 and T_h2 cells, promotes their differentiation and cytokine production (Veldhoen *et al.*, 2008), which may be induced by AHR interaction with ROR γ t, therefore promoting binding at the *Il22* promoter (Qiu *et al.*, 2012). Further T_h17 cell support is provided through AHR-dependent suppression of STAT1 and STAT5 signalling, which impair T_h17 cell differentiation (Kimura *et al.*, 2008). Conversely, AHR also promotes the differentiation of regulatory T cells (Tregs) and type 1 regulatory (Tr1) cells in a context-dependent manner (Gagliani *et al.*, 2015; Apetoh *et al.*, 2010; Gandhi *et al.*, 2010). Regulation of T cell function and polarisation is also mediated indirectly by AHR, through the modulation of DC function. AHR promotes a feedforward signalling loop in DCs, through the induction of IDO1 following its activation, increasing ligand and ligand precursor availability through Kyn production (Vogel *et al.*, 2008; Nguyen *et al.*, 2010). This pathway is thought to skew the polarisation of T cells to anti-inflammatory IL-10 producing Tregs dependent on DC production of TGF β (Mezrich *et al.*, 2010). Therefore, it is thought AHR is important in balancing inflammatory antipathogen responses mediated by T_h17 cells with regulatory activity, which is potentially significant in the development of inflammatory and autoimmune diseases in humans (Veldhoen, 2010).

AHR promotes barrier function through regulation of specialised tissue-resident T cell populations. All $\gamma\delta$ T cell subsets express AHR, however the V γ 3 and V γ 5 intraepithelial lymphocyte subsets in the skin and intestine, respectively, are dramatically reduced in AHR deficient mice (Li *et al.*, 2011; Kadow *et al.*, 2011). These can be increased in WT mice by supplementation with the dietary AHR ligand I3C (Li *et al.*, 2011). Functionally, AHR expression in $\gamma\delta$ T cells is essential for the production of IL-22, but not IL-17 (Martin *et al.*,

2009), supporting a role for AHR in the maintenance of tissue-resident T cells, and in promoting protective barrier functions. Other rare lymphoid cells regulated by AHR activity include ROR γ t⁺ ILC3s. AHR knockout mice have reduced numbers of intestinal ILC3s, resulting in increased susceptibility to infection by the extracellular mucosal bacterial pathogen, *Citrobacter rodentium* (Qiu *et al.*, 2012; Kiss *et al.*, 2011). Increased mortality in AHR deficient mice was attributed to a reduction in the number of IL-22 producing ILC3s (Qiu *et al.*, 2012; Kiss *et al.*, 2011). Mechanistically, subsets of IL-22 producing ILC3s are dependent on AHR-induction of Notch signalling (Lee *et al.*, 2012). A unique subset of CD4⁺IL-22⁺ IL-17⁻ T cells also depends on AHR for IL-22-dependent protection following *Citrobacter rodentium* infection (Basu *et al.*, 2012), and dextran sodium sulphate-induced colitis (Yeste *et al.*, 2014). The importance of AHR in converting environmental cues to barrier protective functions is highlighted by the maintenance of intestinal IL-22-producing ILC3s by dietary AHR ligands (Qiu *et al.*, 2012; Kiss *et al.*, 2011; Schiering *et al.*, 2017).

1.6.4 AHR regulating disease tolerance

Following an infection, host morbidity is determined by tissue damage inflicted by both the pathogen and the ensuing immune response. Resistance to infection is mediated by reducing pathogen burden, whilst tolerance reduces the negative impact of an infection on host fitness, without directly affecting pathogen burden (Medzhitov, Schneider & Soares, 2012). As discussed in the previous section, the AHR modulates various components of the immune system, both at a homeostatic level and following infection. The outcome is context-dependent, and, at least in part, influenced by the manner in which the AHR is activated. Apparent from studies in AHR deficient mice, it is becoming increasingly clear that dietary- and microbial-derived signals promote disease tolerance, particularly through the induction of CD4⁺ T cell and ILC3 production of IL-22, protecting the host against intestinal infection and inflammation (Qiu *et al.*, 2012; Kiss *et al.*, 2011; Basu *et al.*, 2012; Rothhammer & Quintana, 2019). This is also true with regard to balancing potentially pathogenic Th17 responses linked to autoimmunity (Veldhoen *et al.*, 2008; Quintana *et al.*, 2008) with anti-inflammatory responses as a result of Treg differentiation (Veldhoen, 2010). AHR also influences this balance indirectly, as AHR-dependent IL-22 production by ILC3s prevents the expansion of commensal segmented filamentous bacteria, which is known to promote the generation of Th17 cells (Qiu *et al.*, 2013). In AHR deficient mice this resulted in spontaneous Th17 driven colitis,

and therefore, the AHR is important in mediating intestinal homeostasis through interactions with the commensal flora.

Aberrant cytokine production is a hallmark of pathogenic inflammation during infection. Evidence from studies in AHR deficient mice highlight its role in balancing these effects, in order to preserve tissues from harmful host response pathways. For instance, AHR knockout mice challenged with the allergen ovalbumin have significantly increased pulmonary inflammation, owing to increased DC cytokine production (Thatcher *et al.*, 2016). *Ahr*^{-/-} mice have increased susceptibility to lipopolysaccharide (LPS) challenge, exhibiting a heightened inflammatory response characterised by excessive IL-6, TNF- α , and IL-12p40 production (Kimura *et al.*, 2009). Mechanistically, AHR forms a complex together with STAT1 and NF- κ B, leading to suppression at the *Il6* promoter (Kimura *et al.*, 2009). An eloquent study describes an AHR-IDO1 pathway that engages in long-term regulation of systemic inflammation following a secondary LPS challenge in B6 mice, through the induction of a state of LPS-refractoriness (Bessede *et al.*, 2014). Finally, the AHR is thought to regulate proinflammatory responses in the central nervous system by suppressing the production of proinflammatory cytokines by microglia in EAE (Rothhammer *et al.*, 2018).

By balancing potentially harmful proinflammatory cytokine and cellular responses, the AHR has important functions in limiting the severity of tissue damage. Therefore, the physiological regulation of supply of natural ligands has clinical significance. Microbial and dietary tryptophan catabolites are of known importance in both intestinal and systemic homeostasis in health and disease, including through AHR-dependent activities (Roager & Licht, 2018). However, dysfunction is increasingly linked with human inflammatory pathologies, such as IBD (Lamas *et al.*, 2016; Wikoff *et al.*, 2009; Natividad *et al.*, 2018; Mellor, Lemos & Huang, 2017).

1.6.5 AHR function at barrier epithelial sites

As an environmental sensor, the high expression levels of AHR at barrier epithelial sites, such as the lung, gut and skin, is not surprising (Esser & Rannug, 2015). As discussed previously, following activation by agonists derived from the commensal flora or the diet, the AHR indirectly promotes barrier immunity by promoting IL-22 production in various immune cells. Recent studies by Gitta Stockinger's lab have identified epithelial-specific AHR pathways that affect barrier function. Specific AHR deletion in intestinal epithelial cells (IECs)

increased the susceptibility to *Citrobacter rodentium* infection (Metidji *et al.*, 2018). Mechanistically, AHR was shown to directly induce expression of the E3 ubiquitin ligases *Rnf43* and *Znrf3*, which negatively regulate WNT signalling. Dysregulated AHR signalling resulted in the unrestricted proliferation of intestinal epithelial cells and disrupting barrier function (Metidji *et al.*, 2018). In an independent study, it was shown that induction of CYP1 enzymes following AHR activation in IECs *in vivo* regulates ligand passage to intestinal ILC3s and Th17 cells (Schiering *et al.*, 2017). *Cyp1a1* overexpression specifically in IECs resulted in the depletion of natural AHR ligands *in vivo*, thus blocking the supply of ligands from the intestinal lumen to IL-22 producing immune cells, consequently disrupting barrier protection following infection. This study importantly shows that AHR activity in epithelial cells regulates the supply of AHR agonists at barrier sites, where microbial and dietary derived ligands are more prevalent. In both studies, dietary I3C supplementation reversed the phenotype in *Cyp1a1* overexpression models (Metidji *et al.*, 2018; Schiering *et al.*, 2017).

The plethora of AHR ligands available in the intestine provided by microbial and dietary sources has likely led to the identification of a number of AHR pathways regulating intestinal immunity. However, recent evidence suggests AHR also regulates cellular responses in the lung. AHR was shown to be a crucial factor in AEC differentiation into multiciliated cells *in vitro*, through direct induction of cyclin O (Villa *et al.*, 2016). *In vivo*, AHR deficiency in mouse embryos led to aberrant cilia formation, indicating its importance in airway development. Interestingly, this study found that classic CYP1-inducing agonists (FICZ or 3-MC) impaired cilia formation, whereas exposure to an ALI in AEC cultures, independent from classic AHR activation, provided the necessary signals for AHR-dependent multiciliogenesis (Villa *et al.*, 2016). This suggests a possibility for ligand-independent AHR activity. Invertebrate species provide additional evidence for ligand-independent cellular regulation, as *Drosophila* AHR orthologues belonging to the bHLH family have physiological function, yet do not bind xenobiotics (Emmons *et al.*, 1999).

1.6.6 Respiratory viral disease

AHR response pathways during respiratory viral disease have nearly exclusively been studied in the context of xenobiotic-induced activation after influenza virus infection in mice. Continuous exposure of the lung to xenobiotics found in air pollution, vehicle exhaust fumes, and cigarette smoke, may dramatically impact lung physiology through AHR recognition (Sun,

Zeng & Ni, 2013), impacting host immunity during respiratory viral infection. Paige Lawrence's group have performed extensive work characterising the effects following TCDD treatment in influenza virus-infected mice. TCDD treatment was shown to induce a range of immunomodulatory effects, including pulmonary neutrophilia (Teske *et al.*, 2005), and immunosuppressive effects impairing DC (Jin *et al.*, 2010) and CD8⁺ T cell responses (Lawrence *et al.*, 2006). TCDD treatment increases morbidity and mortality in influenza virus infected mice in an AHR-dependent manner, independent from changes in viral burden (Vorderstrasse, Bohn & Lawrence, 2003; Luebke *et al.*, 2002). This suggests pathogenic inflammation or pathology driven by TCDD treatment contributes to the increase in susceptibility, possibly due to enhanced inducible nitric oxide synthase (iNOS) expression in the respiratory epithelium (Wheeler, Martin & Lawrence, 2013) promoting pathogenic neutrophilia (Teske *et al.*, 2005).

Whilst these studies are relevant in understanding how toxic agonists signalling through the AHR may exacerbate respiratory viral disease, whether physiological AHR cues affect lung immunity is currently unclear. Comparison of responses to various ligand-stimulations following influenza virus infection indicated that only TCDD increased mortality (Boule *et al.*, 2018). The effects of exogenous FICZ application were minimal, however this may be as this particular agonist is rapidly metabolised, and has little efficacy following in vivo administration (Boule *et al.*, 2018; Wheeler *et al.*, 2014). The toxic and AHR-independent effects following TCDD treatment makes dissecting relevant pathways difficult following respiratory viral infection in vivo.

An important study showed the endogenous role of AHR in suppressing type I IFN production during viral infections (Yamada *et al.*, 2016). In vivo, AHR deficient mice displayed enhanced IFN- β production, with a small decrease in viral titres. Mechanistically, AHR-mediated TCDD-inducible poly(ADP-ribose)polymerase (TIPARP) induction resulted in TIPARP-dependent ADP-ribosylation of tank binding kinase 1 (TBK1), a downstream signalling kinase in the RIG-I pathway. The resultant TBK1 post-translational modifications are thought to impair RIG-I-mediated viral sensing and IFN production (Yamada *et al.*, 2016). This study is important in showing for the first time that endogenous ligands signalling through the AHR influence antiviral immunity following respiratory viral infection.

A potential interplay between Kyn metabolism and antiviral immunity has been suggested following the observation that influenza induces IDO1 expression through an IFN-dependent mechanism (Fox *et al.*, 2015; Huang *et al.*, 2013). IDO1 deficient mice display enhanced CD8⁺ T cell viral suppression, resulting in improved outcome following influenza

virus infection (Huang *et al.*, 2013). Additionally, enhanced Kyn biosynthesis has been observed following influenza virus infection (Boergeling & Ludwig, 2017). Whether immunosuppressive effects of IDO1 are linked to AHR activity, remains unclear. However, a significant body of literature highlights the importance of AHR in converting physiological signals into barrier protective effects and the promotion of disease tolerance. In the context of pathogenic inflammation in severe cases of respiratory viral disease, for instance induced by prolonged IFN signalling (Davidson *et al.*, 2014; Channappanavar *et al.*, 2016), AHR may play a role in balancing the inflammatory pulmonary environment to limit tissue damage.

Host-specific factors influencing the progression and extent of lung damage are of great relevance in defining pathological mechanisms during respiratory viral infection. However, it is not yet known whether this includes immunological processes that directly impact lung tissue regeneration. The broad aims of this thesis are to better characterise these processes, by exploring a potential interplay between IFNs, AHR, and the respiratory epithelium, and understanding whether this interplay affects lung epithelial maintenance during influenza virus infection.

Chapter 2. Materials & Methods

2.1 Mice

All animal experiments were approved by the Home Office, UK, under project license P9C468066, and carried out in accordance with the Animals (Scientific Procedures) Act 1986. All experiments used male and female mice at 6–12 weeks of age, bred at the Francis Crick Institute (2016–2020) under specific pathogen-free conditions. For in vivo experiments, mice were age- and sex-matched where possible, between genotypes and treatment groups. All genetically modified mice were bred on a C57BL/6J background and maintained as homozygous lines. 129S7 WT mice were used where indicated. Genotypes used for in vivo experiments were C57BL/6J, *Ifnar1*^{-/-}, *Ifnlr1*^{-/-} (provided by Anne O’Garra at the Francis Crick Institute), B6.A2G-Mx1 congenic mice (Staeheli *et al.*, 1985) (a kind gift from Dr Peter Staeheli at Freiburg University), *Cyp1a1*^{-/-}*Cyp1a2*^{-/-}*Cyp1b1*^{-/-} (*Cyp1*^{-/-}) triple knockouts (Dragin *et al.*, 2008), *Cyp1a1*^{-/-}*Cyp1b1*^{-/-}*Cyp1a2*^{+/-} double knockouts, *Ahr*^{-/-}, WT^{*Cyp1a1-cre*}, and *Cyp1*^{-/-} *Cyp1a1-cre* mice (Schiering *et al.*, 2017). All AHR-related strains were provided by Gitta Stockinger at the Francis Crick Institute. *Cyp1a1* reporter mice were generated (Schiering *et al.*, 2017) through the breeding of *Cyp1a1-cre* mice (Henderson *et al.*, 2015) with *R2rR-eYFP* mice expressing eYFP constitutively under the ubiquitous *Rosa26* promoter (Srinivas *et al.*, 2001). This strain (WT^{*Cyp1a1-cre*}) was crossed with *Cyp1*^{-/-} mice to obtain *Cyp1*^{-/-} *Cyp1a1-cre* reporter mice. Tissues for in vitro studies were taken from C57BL/6J, *Tp53*^{-/-}, *Ahr*^{-/-}, and *Cyp1*^{-/-} mice.

To generate BM chimeras, WT, *Ifnar1*^{-/-}, *Ifnlr1*^{-/-} and *Cyp1*^{-/-} mice recipient mice were lethally irradiated (2×6.5 Gy, 16-hour interval). 3 hours following the second irradiation, mice were reconstituted with 1×10⁶ donor BM cells from WT or *Ifnar1*^{-/-} donor mice (as indicated in the figures). Mice were maintained on antibiotics (Baytril) for 4 weeks following irradiation. To allow proper reconstitution of the hematopoietic compartment, irradiated chimeric mice were left for an additional 4 weeks (8 weeks total following irradiation) prior to experimental use.

2.2 Influenza viruses

X31 (a gift from John Skehel, MRC-NIMR) and hvPR8-ΔNS1(1-126) (another kind

gift from Dr Peter Staeheli at Freiburg University) (Kochs *et al.*, 2009) were grown in the allantoic cavity of 10-day-embryonated hen's eggs and were free of bacterial, mycoplasma, and endotoxin contamination. All viruses were stored at -80°C and were used only once following thawing. All viruses were titrated on Madin–Darby canine kidney cells. To quantify virus in naïve and infected mouse lungs, quantitative polymerase chain reaction (qPCR) was performed for the influenza *Matrix* gene on cDNA from whole lungs, normalized to the housekeeping gene *Hprt1*.

2.3 Infections

All infections were performed under light anesthesia (3% isoflurane) intranasally. C57BL/6J (B6), *Ifnar1*^{-/-}, and *Ifnlr1*^{-/-}, mice were infected with 10,000 TCID₅₀ X31 in 30 µl PBS, in experiments for data presented in chapters 1 and 2. B6, *Cyp1*^{-/-} and *Ahr*^{-/-} mice were infected with 35,000 TCID₅₀ X31 in 30 µl PBS in experiments for data presented in chapter 3. B6.A2G-Mx1 mice were infected with 2500 TCID₅₀ hvPR8-ΔNS1(1-126) in 30 µl PBS (Kochs *et al.*, 2009). BM chimeric mice (WT→WT or WT→*Ifnlr1*^{-/-}) for coinfections were infected with *Streptococcus pneumoniae* (TIGR4) (2×10^5 c.f.u. in 30 µl PBS) on day 8 post influenza virus infection for survival experiments, or on day 12 post influenza virus infection for colony-forming unit (CFU) quantification. With the exception of BM chimeras, mice were infected at 6–14 weeks of age. Chimeric mice were infected at 16 to 24 weeks of age. Prior to every infection, body weights were recorded. Following infection, mice were weighed daily in the morning and monitored for clinical symptoms. Mice reached a clinical endpoint following the loss of 20–25% of their initial starting weight (dependent on moderate or severe programme of work protocol), >10% weight loss in 24 hours, or a clinical score >5. For clinical scores, 1 point was given for presentation of the following symptoms: partial eye closure, hypothermia, piloerection, hunching, laboured breathing, decreased movement, and movement only on provocation. 2 points were given for an absence of movement on provocation, and 5 points for middle-ear infection and disrupted balance.

2.4 Mouse treatments

For repair experiments measuring lung epithelial proliferation, in vivo IFN treatments in WT, *Ifnar1*^{-/-} → WT BM chimeric, and B6.A2G-Mx1 mice were performed every 24 hours

from days 7 to 10 post infection (4 treatments in total). IFN used for in vivo experiments were used at equal biological potency based on in vitro titrations in AEC cultures (see below). Universal IFN- α was a kind gift from Dr Peter Staeheli at Freiburg University, IFN- β was purchased from PBL, and recombinant mouse IFN- λ 2 was a kind gift from Rune Hartmann and Hans Henrik Gad at Aarhus University (Dellgren *et al.*, 2009). On days 7, 8 and 9 post infection, IFN- α (8 μ g in 200 μ l PBS), IFN- β (10^6 U in 200 μ l PBS), and IFN- λ 2 (2.5 μ g in 200 μ l PBS) was administered intraperitoneally. On day 10 post infection, IFN treatment was administered intranasally to ensure activation of respiratory epithelial cells, at the same dose as intraperitoneal treatments in 50 μ l PBS, under light anesthesia (3% isoflurane). To study the effects of IFN in WT and *Cyp1^{-/-}* mice, universal IFN- α (3 μ g in 200 μ l PBS) was administered intraperitoneally on day 4 and 5 post infection. For neutrophil depletion, WT mice were treated with an α -Ly6G monoclonal antibody (clone 1A8) or isotype control (IgG2b) (150 μ g in 200 μ l PBS for both) intraperitoneally from days 5 to 10 post infection. B6 WT mice were treated intraperitoneally with 400 μ g (in 200 μ l PBS) anti-IFNAR (MAR1-5A3, Bio X Cell) on the day of infection, or from day 3 post infection, every 48 hours. For lung proliferation and weight loss experiments in 129S7 mice, anti-IFNAR was administered on days 6, 8, and 10 post infection at the same dose.

2.5 Diet studies

To measure the effects of AHR ligand-supplemented diet in mice, WT, WT^{*Cyp1a1-cre*}, and *Cyp1^{-/-} Cyp1a1-cre* mice were fed purified diet AIN-93M (TestDiet-IPS) or AIN-93M supplemented with 200mg/kg (200 parts per million). Mice were maintained on diets for four weeks, including throughout the duration of experiments.

2.6 Primary mouse and human AEC cultures

Isolation and culture of primary murine AECs were performed as previously described (You *et al.*, 2002). All murine AEC cultures were derived from B6 WT progenitor cells (or indicated genetically modified mice with a B6 background). In brief, isolation of murine tracheal progenitor cells was achieved following overnight enzymatic digestion (pronase 3 mg/ml, Roche) at 4°C. Tracheal basal cells were expanded in a T-75 flask to 100% confluence

with Rho kinase inhibitor Y27632 10 μ M (Horani *et al.*, 2013). Following expansion (5 to 7 days), cells were trypsinized and seeded on transwells (10^4 cells/transwell, unless indicated otherwise) on 0.4 μ m-pore size clear polyester membranes (Greiner) coated with a collagen solution. Cells were grown in submersion until confluent, and then exposed to air to establish an ALI and induce airway cell differentiation. Primary human bronchial epithelial cells were purchased from Lonza and cultured according to manufacturer's instructions. In brief, cells were expanded in a T-75 flask to 60% confluence and then harvested for seeding onto transwells at 5×10^4 cells per transwell. At confluence, liquid was removed from the apical side to establish ALI. Human AEC samples were sourced ethically and their research use was in accord with the terms of the informed consents.

2.7 In vitro IFN treatments and titrations

To compare IFN subtypes at equal biological potencies, titrations were performed. Fully differentiated murine and human AEC cultures were treated with IFN- α , IFN- β , and IFN- λ subtypes for 4 hours at indicated concentrations, and analysed for the induction of indicated ISGs by qPCR. Dose-response curves were generated to obtain an EC50 for each subtype, relative to ISG expression at the highest IFN concentration, in order to determine a conversion factor for equipotency between subtypes. Based on titrations for equal biological potency, concentration of mouse IFNs used for all other in vitro assays were as follows: 600 U/ml IFN- α_4 (PBL), 1000 U/ml IFN- β (PBL), and 5 ng/ml for both IFN- λ_2 and - λ_3 (R&D). Concentration of human IFNs at equal biological potencies: 1 ng/ml universal IFN- α , (Dr P. Staeheli), 2.3 ng/ml hIFN- β (PBL), 3.3 ng/ml hIFN- λ_3 (R&D) (Dellgren *et al.*, 2009).

For mouse and human AEC in vitro growth, proliferation, apoptosis assays and RNA sequencing, IFN treatments were initiated 24 hours post plating on both basolateral and apical sides, and changed in the media every 48 hours. Unless indicated otherwise, mouse and human AECs plated at 10^4 cells per transwell and 5×10^4 cells per transwell, respectively. In these conditions, AECs were exposed to an ALI 72 hours post plating (thus, 48 hours post IFN treatment), even if they had reached confluence prior to this timepoint, for 5 days total (ALI day -2 to +3). To maintain apical IFN stimulation during ALI exposure, cultures were pulsed with IFN for 4 hours on the apical side, before liquid was removed. For differentiation assays, IFN treatment was initiated 48 hours post ALI exposure, for 6 consecutive days.

2.8 AHR activation in influenza virus infected AEC cultures

For AHR activation in vitro, murine AEC cultures were treated with FICZ (Enzo, 250 nm) and 3-MC (Sigma, 1 μ m) 24 hours prior to influenza virus infection on the basolateral side, and maintained until experiment endpoint. 3-MC was changed with the media every 48 hours, and FICZ was added every 24 hours owing to its increased rate of metabolism. The apical surface of murine AECs was washed 3 $\times$ to remove accumulated mucins before influenza inoculation (PR8 at a MOI of 10^{-4}). Following a 1-hour incubation at 37°C, the virus inoculum was removed, and infected AECs were returned to culture for a further 48 hours, at which point RNA was collected (as described below).

2.9 RNA isolation and quantification of transcripts

AEC cultures were lysed in 350 μ l of RLT + β -mercaptoethanol. RNA was isolated using the Qiagen RNeasy mini kit, according to manufacturer's instructions. Lungs were harvested and stored in RNALater in -80°C . Lungs were homogenised with a Kinematica Polytron PT 10-35 homogenizer in 3 ml of RLT buffer (QIAGEN) + β -mercaptoethanol.

To isolate lung immune cells for RNA extraction, whole lungs were collected in gentleMACS C-tubes in 5 ml AB IMDM, and homogenised using a gentleMACS (Miltenyi Biotech) according to manufacturer's instructions. Lungs were digested with DNase I (50 μ g/ml) (Sigma) and Liberase TL (20 μ g/ml) Roche at 37°C for 30 minutes, and then further homogenised by gentleMACS. Lung homogenates were passed through a 70 μ m filter, and centrifuged at $300 \times g$ for 5 minutes at 4°C prior to red blood cell (RBC) lysis in 5 ml with 0.83% ammonium chloride. RBC lysis reaction was halted by addition of 20 ml AB-IMDM. To study ISG expression induced specifically by IFN- α/β (confirming effective α IFNAR blockade), lung immune cells were separated from single cell suspensions, with additional depletion of neutrophils (which express IFNLR in mice) by magnetic-activated cell sorting (MACS) column separation. Single-cell suspensions were preincubated with anti-Fc γ RIII/II (Fc block) in MACS buffer, before incubation with biotinylated anti-Ly6G. Cells were subsequently incubated with streptavidin microbeads (Miltenyi Biotech), and separated using an LS column (Miltenyi Biotech) on the magnetic field of a MACS Separator (Miltenyi Biotech) to deplete Ly6G⁺ neutrophils. The negative fraction was then incubated with CD45 microbeads (Miltenyi Biotech) to isolate lung immune cells, and separated again using a

MACS separator. CD45⁺ Ly6G-depleted lung immune cells were pelleted by centrifugation (300 × g), and lysed in 350 µl of RLT + β-mercaptoethanol.

RNA was isolated using the Qiagen RNeasy mini kit, according to manufacturer's instructions. 500 nanograms of total RNA was reverse-transcribed using the qPCRBIO cDNA synthesis kit according to manufacturer's instructions. RT-qPCR was performed on an Applied Biosystems Quantstudio 3 RT-qPCR machine with 1X qPCRBIO Probe Mix Lo-ROX, and 1X Taqman primers. Results were normalized to the housekeeping gene *Hprt1*.

Primers for influenza virus *Matrix* gene (Ward *et al.*, 2004):

- Forward: 5'-AAGACCAATCCTGTC ACCTCTGA-3';
- Reverse: 5'-CAAAGCGTCTACGCTGCAGTCC-3';
- Probe: 5'-TTTGTGTTACGCTCACCGT-3'.

2.10 Flow cytometry

For epithelial cell isolation from lung tissues, mice were euthanised (600 mg/kg pentobarbital + 17 mg/kg mepivacaine) and perfused with 10 ml of ice-cold PBS through the right ventricle of the heart. 1.5ml dispase II (5 mg/ml in AB-IMDM) (Sigma) was then injected into the lungs through catheter insertion intratracheally, followed by 0.4 ml 1% low-gelling agarose solution (in PBS) (Sigma). With the syringe and catheter held in situ to prevent agarose/dispase leakage, mice were placed on ice for 2 minutes to allow the agarose–dispase-filled lungs to set. Inflated lungs were dissected, and placed in 2 ml of Dispase II solution (5 mg/ml in AB-IMDM) for 30 minutes to dissociate epithelial cells. Lungs were passed through a 100 µm filter, before a 10-minute DNase I digestion (50 µg/ml) (Sigma). Following digestion, lung homogenates were passed through a 70 µm filter, and centrifuged at 300 × g. for 5 minutes at 4°C, before RBC lysis. Single-cell suspensions were preincubated with Fc block, before a 30-minutes incubation with fluorochrome-labelled antibodies. Cells were permeabilized with the Foxp3/Transcription factor staining kit (eBioscience), according to manufacturer's instructions, prior to intracellular staining for Ki67 or p53.

For analysis of whole lung immune cell populations, single cell suspensions were generated using gentleMACS as described above. Following surface marker staining, lung cells were fixed in 2% paraformaldehyde for 20 minutes at 4°C. Fixed lung cells were washed 1× in PBS prior to analysis.

To analyse AECs by flow cytometry, cells were washed 1× in PBS (apically and basolaterally) prior to a 15-minute trypsinisation. For EdU (5-ethynyl-2 deoxyuridine) assays, AECs were cultured with 2.5 µM EdU (Invitrogen) for 3 hours prior to trypsinisation. Trypsinised EdU⁺ AECs were labelled using the Click-iT Plus EdU Alexa Fluor 488 Flow Cytometry Assay Kit (Invitrogen), according to manufacturer's instructions. For CFSE assays, cells in culture were labelled with CFSE (1 µM) (Molecular Probes) for 8 minutes, followed by 3× washes with media + sera to wash out unincorporated CFSE. CFSE labelled AECs were trypsinised 24 hours post-labelling. Lung and AEC cells were analysed using a Fortessa X20 (Becton Dickinson) and data was analysed using FlowJo software (Treestar).

2.11 RNA-sequencing

Epithelial cells from the lungs from naïve and influenza virus infected WT and *Ifnlr1*^{-/-} mice were enriched through depletion of CD45⁺ immune cells by MACS separation. Further enrichment was achieved via cell sorting for live single EpCam⁺CD31⁻ cells using a FACS Aria III (BD Biosciences). RNA from sorted lung epithelial cells was isolated using the Qiagen RNeasy mini kit, according to manufacturer's instructions.

RNA-sequencing was performed on the HiSeq 4000 system (Illumina) with Single End 75 bp reads. Read quality trimming and adaptor removal was carried out using Trimmomatic (version 0.36). Reads were aligned to the mouse genome (Ensembl GRCm38 release 89) using STAR (version 2.5.2a) and gene level counts were obtained using the RSEM package (version 1.2.31). For RSEM, all parameters were run as default except "-forward-prob" that was set to 0 for AECs (RN200717) and 0.5 for the lung samples (RN19197). Differential expression analysis was carried out with DESeq2 package (version 1.20.0) within R version 3.5.1. Genes were considered to be differential expressed with $\text{padj} \leq 0.05$.

Ingenuity pathway analysis (IPA) was performed using differentially expressed genes with a fold change >1.5 with $P < 0.05$. Gene Set Enrichment analysis (GSEA, version 2.2.3) was performed for each pairwise comparison using gene lists ranked using the Wald statistic. Gene set pre-ranked analysis was carried out using C2 canonical pathways v5.2 and C5 biological processes v5.2. All parameters were kept as default except for enrichment statistic that was changed to classic and the max size which was changed to 500,000. Gene signatures were considered significant if FDR q-value % 0.05.

2.12 Quantification of bacterial loads

Lungs and spleens were collected in 2 ml PBS on ice. Tissues were processed and rinsed through a 70 μ M strainer to a total of 5 ml. Homogenates were stored at -80°C . Prior to plating, serial dilutions were performed on defrosted lung homogenates. Samples were spread on blood agar plates, divided into two halves for duplicates. Plates were incubated for 48 hours at 37° in 5% CO_2 . Following incubation, grey, alpha-hemolytic colonies were counted.

2.13 Quantification of cytokines, cells and damage markers in BALF

Mice were euthanised (600 mg/kg pentobarbital + 16 mg/kg mepivacaine) and BALF was collected through catheter insertion intratracheally, injecting and retracting 400–500 μ l of PBS. Following centrifugation ($300 \times g$ for 5 minutes at 4°C), supernatants were stored at -80°C . Pelleted cells in the BALF were resuspended in 200 μ l PBS for counting. If BALF cells were to be analysed by flow cytometry to determine the frequency of Ter-119⁺ RBCs and immune cells, cells were recentrifuged and resuspended in cell surface marker stain prior to Fortessa analysis.

Lung damage was assessed in BALF through quantification of lactate dehydrogenase (LDH) activity and serum album concentrations. LDH activity was assessed using the enzymatic detection step of the CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega), according to manufacturer's instructions. Serum albumin was quantified using an albumin enzyme-linked immunosorbent assay (ELISA) kit (eBioscience), according to manufacturer's instructions.

Lung cytokine concentrations were measured in BALF supernatants. Concentrations of IFN- α/β were measured using 2-Plex ProcartaPlex (Invitrogen). IFN- λ (R&D) was measured by ELISA, according to manufacturer's instructions. IL-6 and IL-22 were measured using mouse ELISA Ready-SET-Go! kits (eBioscience) according to manufacturer's instructions.

2.14 Evan's blue dye assay

To measure pulmonary vascular and epithelial barrier leakage, Evan's blue dye assay was used. Mice were injected intravenously with a 0.1% sterile solution of Evan's blue dye (in PBS) 30 minutes prior to euthanasia (600 mg/kg pentobarbital + 16 mg/kg mepivacaine). Mice

were perfused with 10 ml of ice-cold PBS through the right ventricle of the heart, and lungs were harvested. Extravascular Evan's blue was then extracted from lung tissues by overnight incubation in formamide at 56°C and quantified by spectrophotometric analysis at 620 nm absorbance.

2.15 Histology and immunofluorescence

Mice were euthanised (600 mg/kg pentobarbital + 16 mg/kg mepivacaine) and perfused with 10 ml of ice-cold PBS through the right ventricle of the heart. Whole lungs were inflated with intratracheal injection of 4% PFA, and added to a bijoux tube containing 2 ml 4% PFA for overnight fixation at 4°C. Lung samples were stained with hematoxylin and eosin (H&E) and prior to gross and microscopic pathologic analysis.

For immunofluorescent staining of paraformaldehyde-fixed AECs and dewaxed lung specimens, samples were permeabilised with 0.1% Triton X-100 for 15 minutes at room temperature and then blocked in 0.5% bovine serum albumin for 1 hour. Primary antibodies specific for acetylated α -tubulin (T7451, Sigma) were added in 0.5% bovine serum albumin and incubated for 1 hour at room temperature. Sections and transwells were washed 1× in PBS, and fluorochrome-conjugated secondary antibodies (Alexa Fluor, Life Technologies) were added for 1 hour at room temperature. Transwell filters were washed 1× in PBS, and mounted on slides using Vectashield mounting medium with 4,6-diamidino-2-phenylindole (DAPI) (Vector labs). Images were acquired on an Olympus VS120 slide scanner with a VC50 camera, a UPLSAPO lens, at magnification of 20× and a numerical aperture of 0.75. Acetylated α -tubulin staining was quantified using the whole transwell. Binary images were generated by global thresholding and measured using the ImageJ 1.4j application.

2.16 Histopathological analysis

Histopathological examination of lungs was performed by a board-certified veterinary pathologist (SLP). Sections from all lung lobes were examined, with histopathological scores based on regions with the most severe changes. A semi-quantitative scoring system was used (0; normal, 1; minimal, 2; mild, 3; moderate, and 4; marked changes) for each parameter. Bronchiole regeneration, represented by hyperplasia of the epithelium. Inflammation was assessed in both the perivascular and peribronchiolar compartments, and in each a score was

assigned for neutrophils and lymphocytes. The total histopathology score for inflammation was generated as the mean of the four separate inflammatory scores.

2.17 Statistical analysis

Data shown as the means $\pm$ SEM. Sample sizes were designed to give statistical power, while minimising animal use. All statistical comparisons were performed using Prism 8 (GraphPad). Figure legends denote the specific statistical tests used for each experiment. Statistical significance was determined as $P < 0.05$.

Table 1. Antibodies and other reagents used for flow cytometry and immunofluorescence.

Antibody	Clone	Label	Vendor	Dilution
EpCam	G8.8	APC	Invitrogen	2.5 µg/ml
CD45	30-F11	BV786	BioLegend	2.5 µg/ml
CD49f	eBioGoH3	PE-Cy7	eBioscience	20 µg/ml
CD31	RM5228	BV421	Invitrogen	2.5 µg/ml
CD24	M1/69	BV510	BioLegend	10 µg/ml
MHC-II	M5/114.15.2	BV711	BioLegend	2 µg/ml
Ki67	SolA15	PE	eBioscience	10 µg/ml
Ly6C	HK1.4	BV786	BioLegend	5 µg/ml
Ly6G	1A8	FITC	BioLegend	10 µg/ml
CD3	17A2	APC	TONBObiosciences	20 µg/ml
CD19	6D5	APC	BioLegend	20 µg/ml
NK1.1	PK136	APC	BioLegend	20 µg/ml
CD64	X54-5/7.1	PE-Cy7	BioLegend	20 µg/ml
Siglec F	E50-2440	APC-Cy7	BD Pharmingen	3.3 µg/ml
CD11b	M1/70	Pacific blue	BioLegend	10 µg/ml
CD11c	N418	BV605	BioLegend	5 µg/ml
CD4	RM4-5	BV605	BioLegend	5 µg/ml
CD8	53-6.7	BV510	BioLegend	5 µg/ml
Sca-1	D7	BV711	BioLegend	5 µg/ml
Siglec-H	551	PE	BioLegend	10 µg/ml
P53	GP59-12	PE	BD Pharmingen	10 µl/10 ⁶ cells
Ly6G	RB6-8C5	Biotin	eBioscience	0.5 µg/ml
Annexin V	N/A	FITC	BioLegend	10 µg/ml
TO-PRO-3	N/A	APC	Thermofisher	1 µM
Fixable blue dead stain	BUV395	UV	Thermo Fisher	1 µl/10 ⁶ cells
Acetylated α -tubulin	6-11B-1	N/A	Sigma	1 µg/ml

Table 2. Primers used for qPCR.

Gene	Catallgue number
<i>Hprt1</i>	Mm00446968_m1
<i>Irf7</i>	Mm00516791_g1
<i>Ccno</i>	Mm01297259_m1
<i>Mcidas</i>	Mm01308202_m1
<i>Muc5ac</i>	Mm01276718_m1
<i>Muc5b</i>	Mm00466391_m1
<i>Scb1a1</i>	Mm00442046_m1
<i>Dusp5</i>	Mm01266106_m1
<i>Gadd45g</i>	Mm0135255 0_g1
<i>Ifi203</i>	Mm00492601_m1
<i>Oasl2</i>	Mm00496187_m1
<i>Rsad2</i>	Mm00491265_m1
<i>RSAD2</i> (human)	Hs00369813_m1
<i>Mx1</i>	Mm00487796_m1
<i>MX1</i> (human)	Hs00895608_m1
<i>OAS1</i> (human)	Hs00973637_m1
<i>Cyp1a1</i>	Mm00487217_m1
<i>Ahrr</i>	Mm00477443_m1
<i>Tiparp</i>	Mm00724822_m1
<i>Ifna4</i>	Mm00833969_s1
<i>Ifnb1</i>	Mm00439552_s1
<i>Ifnl2/3</i>	Mm04204158_gh
<i>Il6</i>	Mm00446190_m1

Chapter 3. Results 1:

A novel IFN-mediated mechanism of immunopathology during recovery from respiratory viral infection

For the most part, results in this chapter are published, and largely include the expanded in vivo findings from the following publication:

Major, J., Crotta, S., Llorian, M., McCabe, T.M., Gad, H.H., Priestnall, S.L., Hartmann, R., and Wack, A. (2020). Type I and III interferons disrupt lung epithelial repair during recovery from viral infection. *Science* 369, 712–717.

3.1 Introduction

Our current understanding of the immune processes that exacerbate respiratory viral disease is largely based on immunomodulatory effects in immune cells, or pathogenic inflammation as a result of prolonged cytokine and chemokine signalling, often manifesting as a cytokine storm. This includes type I IFN, which, following recent studies in IFNAR deficient mice, has been directly linked to immunopathological consequences following respiratory viral infection. In influenza (Davidson *et al.*, 2014) and SARS-CoV-1 mouse models (Channappanavar *et al.*, 2016), IFN- α/β -induced signals drove a pathogenic proinflammatory response characterised by enhanced monocyte recruitment and cytokine production, including IL-6, CCL2, and TNF α . Davidson *et al.* additionally observed a strain-dependent effect in terms of pathological type I IFN. It is thought that the enhanced resistance in B6 mice compared to 129s is due to an improved capacity for disease tolerance, as viral burden was similar in both mouse strains. This is important, as it indicates that host-specific factors influencing disease outcome, and that immunopathologic events occur following prolonged and/or excessive IFN-induced signalling. This is likely not the case in B6 mice, which display greater regulation of IFN production, at least relative to 129s (Davidson *et al.*, 2014). Consistent with this notion, is that IFNAR deficient B6 mice show no improvement compared to WT controls, with similar viral levels of viral control (Price, Gaszewska-Mastarlarz & Moskophidis, 2000). This suggests that the B6 IFN response in influenza is less immunopathologic in comparison to the 129 IFN responses.

IFN- λ is widely regarded as the protective antiviral cytokine during respiratory viral infections, owing to its targeted activity mainly in epithelial cells. The lack of known proinflammatory and immunomodulatory IFN- λ effects supports this dogma, and is therefore regarded as the preferred host-directed antiviral therapeutic option (Prokunina-Olsson *et al.*, 2020). In mild influenza, IFN- λ is thought to provide non-redundant antiviral function, attributed to its increased rate of production early during infection (Galani *et al.*, 2017). This increased early IFN- λ production is in agreement with previous observations (Jewell *et al.*, 2010), and may be important in restricting viral replication in the upper airway epithelium, thus preventing spread to the lower lung (Klinkhammer *et al.*, 2018). However, changes in viral load or lung damage were small and transient during mild influenza (Galani *et al.*, 2017), and others have reported no difference in IFNLR deficient mice compared to WT (Mordstein *et al.*, 2008), again, indicative of overlapping antiviral function between endogenous type I and III IFNs.

An additional facet to consider regarding prolonged IFN responses is the induction of antiproliferative and proapoptotic pathways. Antiviral ISGs are induced rapidly and transiently following receptor binding, yet antiproliferative ISG induction requires continuous receptor stimulation over a prolonged period of time (Jaitin *et al.*, 2006). Reasons for this requirement are not known, yet it is likely that these responses are switched on following continuous IFN production as a result of viral persistence, or due to escalating inflammation during a cytokine storm. In a concerted effort to limit viral spread in infected host cells, antiproliferative and proapoptotic IFN pathways are also more likely to induce host tissue damage, and therefore require tighter regulation (Schreiber, 2017).

In this chapter, we explore the impact of IFN signalling on lung epithelial repair during influenza virus infection. Crucial for recovery from viral respiratory disease is the maintenance of lung function, which is dependent on an efficient repair process to restore lost epithelia (Gorski, Hufford & Braciale, 2012). Once a threshold of lung damage is surpassed, respiratory and barrier function may be compromised, resulting in hypoxic respiratory failure from ARDS or bacterial superinfection, respectively. As we looked at the effects of IFN specifically in the respiratory epithelium, which expresses both IFNAR and IFNLR, we compared the capacity IFN- α , IFN- β , and IFN- λ subtypes to disrupt lung regeneration during influenza recovery.

3.2 Temporal overlap of antiviral IFN responses and lung epithelial repair following influenza virus infection

We initially sought to characterise the kinetics of lung epithelial repair and IFN production in our murine influenza model. To induce acute lung damage, B6 WT mice were infected with a sublethal dose of the H3N2 influenza virus strain X31. Influenza virus matrix gene expression peaked on day 2 post infection following viral replication, before viral clearance was reached on day 12 post infection (Fig. 1A). Infection caused weight loss (Fig. 1B), accompanied by significant immune cell infiltration and lung damage as determined by histology (Fig. 1C and D) (histopathology scorings were determined by Simon Priestnall and Alejandro Suarez-Bonnet, Francis Crick Institute). Recovery from infection (approximately day 8 post infection; Fig. 1B) coincided with the onset of epithelial regeneration (Fig. 1C, right).

To further investigate lung repair dynamics during influenza recovery, proliferating lung epithelial cells were analysed by flow cytometry, using the marker of recent cell proliferation Ki67 (Fig. 2A). Differentiated AECs (EpCam⁺CD49^{high}CD24^{high}), including ciliated, goblet, and club cells (Quantius *et al.*, 2016), largely maintain a low rate of proliferation following influenza virus-induced lung damage (Fig. 2B). A population of highly proliferative progenitor AECs (EpCam⁺CD49^{high}CD24^{low}) (Quantius *et al.*, 2016; McQualter *et al.*, 2010) emerged in the lungs on approximately day 5 post infection (Fig. 2B and C). This is likely a heterogeneous DASC population, including p63⁺ and p63⁻ progenitors (Vaughan *et al.*, 2015; Xi *et al.*, 2017; Zuo *et al.*, 2014; Kumar *et al.*, 2011; Ray *et al.*, 2016), H2-K1^{hi} cells (Kathiriya *et al.*, 2020), and BASCs (Liu *et al.*, 2019; Salwig *et al.*, 2019). In steady-state, AT2 cells (EpCam⁺MHCII⁺CD49^{low}) (Hasegawa *et al.*, 2017) have a low rate of turnover and remain largely quiescent (Fig. 2B), consistent with previous findings (Nabhan *et al.*, 2018; Zacharias *et al.*, 2018). However, following influenza virus-induced lung damage, AT2 cells undergo rapid proliferation starting at days 5–7 post infection, correlating with mouse recovery and weight gain (Fig. 2B and Fig. 1B).

To compare the dynamics of epithelial repair with IFN production in our infection model, we analysed IFN subtypes (IFN- α , IFN- β , and IFN- λ) in the BALF at different timepoints throughout influenza virus infection (Fig. 3). IFNs were produced rapidly, peaking 2 days post infection (Fig. 3). The magnitude of IFN- λ production was greater than that of IFN- α/β , both in terms of total duration, and duration of maximal production. Whilst IFN- α/β production declined following the day 2 peak, high levels of IFN- λ production were maintained.

Importantly, only IFN- λ was detected 7–8 days post infection, coinciding with the onset of epithelial recovery (Fig. 3). Thus, following influenza virus infection, signalling triggered by IFNs, in particular by IFN- λ , overlaps with the onset of epithelial proliferation during lung repair.

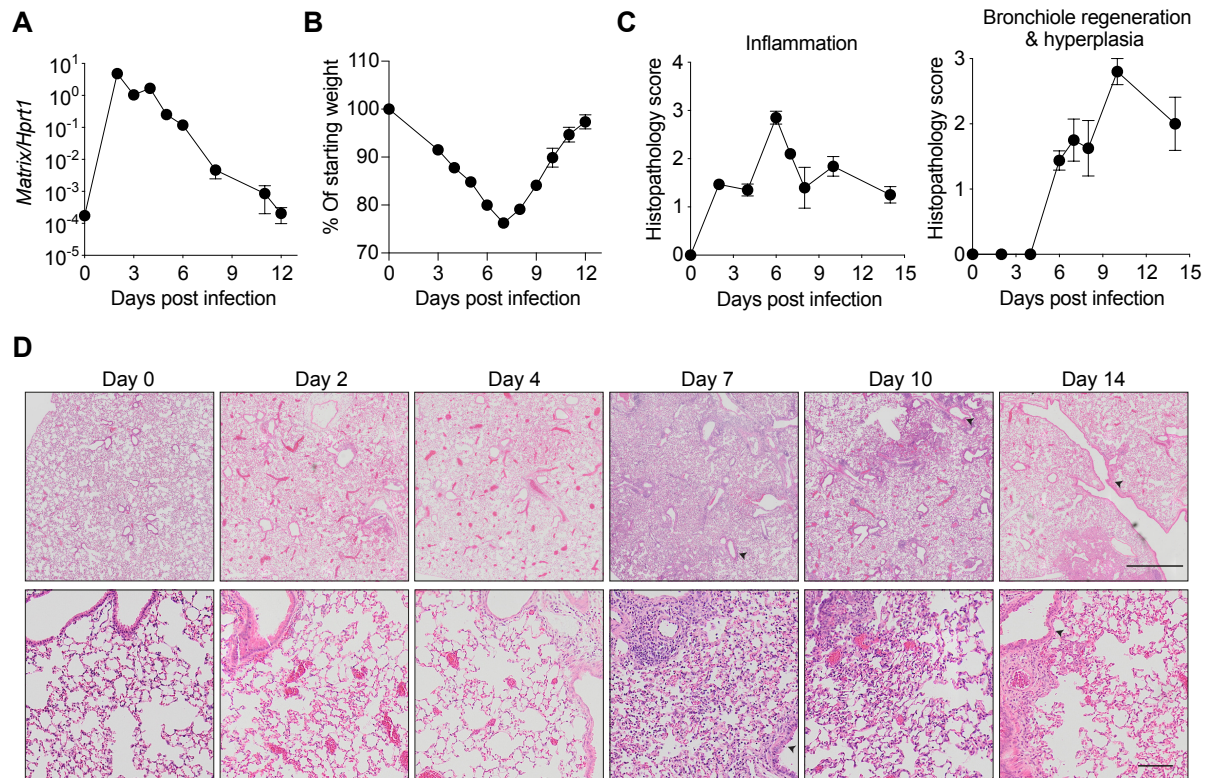


Figure 1. Influenza virus-induced lung damage and repair.

B6 WT mice were infected with influenza virus (10^4 TCID₅₀ X31 in 30 μ l PBS) and assessed for (A) influenza virus *MI* mRNA expression in total lung RNA, (B) weight loss, (C and D) and lung pathology at indicated days post infection. Black arrowheads indicate areas of bronchiole regeneration and epithelial hyperplasia. Scale bar represents 1 mm (top panel) and 100 μ m (bottom panel). Data are pooled from 3 independent experiments ($n = 5-6$). Data are shown as mean $\pm$ s.e.m.

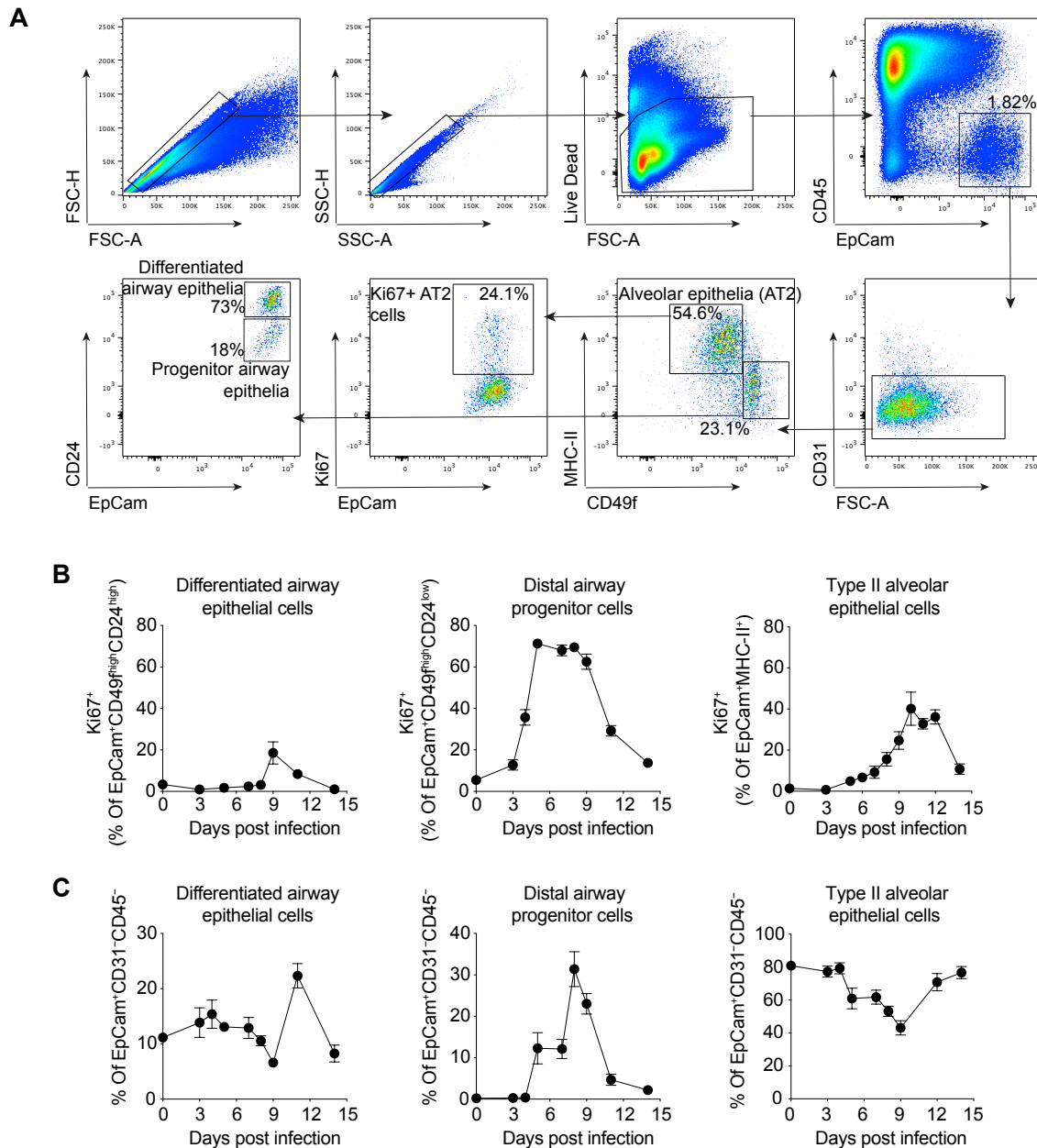


Figure 2. Kinetics of lung epithelial repair during recovery from influenza.

(A) Gating strategy for lung epithelial cell populations shown on lung cells from an influenza virus infected B6 mouse (day 9 post infection), analysed by flow cytometry. Mice were infected with influenza virus, and assessed for (B) Ki67⁺ and (C) frequency of (% of bulk epithelial cells) differentiated airway cells (EpCam⁺CD49f^{high}CD24^{high}), distal airway progenitor cells (EpCam⁺CD49f^{high}CD24^{low}), and AT2 cells (EpCam⁺MHCII⁺CD49f^{low}), measured by flow cytometry. Data are pooled from 5 independent experiments (n = 3–6). Data are shown as mean ± s.e.m.

3.3 IFN signalling during recovery from influenza impairs lung epithelial cell proliferation

To understand the effect of IFNs on lung epithelial repair, mice were treated with exogenous IFN during recovery from influenza virus infection (days 7 to 10 post infection) (Fig. 4A). To compare the effects of equipotent amounts of IFN- α , IFN- β , and IFN- λ , we performed titrations on AEC cultures and measured ISG induction (*Oasl2*, *ifi203*, *Rsad2*) by qPCR following a 4-hour treatment (Fig. 5A). Dose-response curves were then generated to determine a conversion factor for equipotency between the three IFN subtypes (Fig. 5B). Owing to the ubiquitous expression of IFNAR by immune cells, we generated irradiation BM chimeras to compare the effects of IFN- α/β . WT recipients were given *Ifnar1*^{-/-} BM cells, thus restricting IFNAR expression to the non-hematopoietic compartment. In chimeric mice, both IFN- α and IFN- β treatments significantly reduced the proliferation of AT2 cells on day 11 post influenza virus infection, compared to PBS-treated control mice (Fig. 4B). This reduction was independent from changes in viral burden, as influenza virus matrix gene expression remained unchanged following IFN treatment (Fig. 4C). We also observed a reduction in the proliferation of AT2 cells in WT mice following IFN- λ treatment (Fig. 4D), again independently from changes in viral load (Fig. 4E). To exclude the possibility of IFN- λ signalling in neutrophils (Blazek *et al.*, 2015; Broggi *et al.*, 2017; Espinosa *et al.*, 2017), we treated WT mice with a monoclonal antibody against Ly6G (clone 1A8). Even after depletion of neutrophils (Fig. 4F), we observed a significant reduction in AT2 cell proliferation following IFN- λ treatment (Fig. 4G). A caveat when using inbred mouse strains in the context of influenza virus infection is the lack of a functional Mx1 protein, a crucial IFN-inducible influenza virus restriction factor in both mice and humans (Haller *et al.*, 2015). We therefore infected mice that express functional Mx1 alleles (B6-Mx1) with the influenza virus strain hvPR8- Δ NS1, for a more clinically relevant model of IFN system function. IFN- λ treatment also significantly reduced lung epithelial proliferation in the presence of functional Mx1 (Fig. 4H).

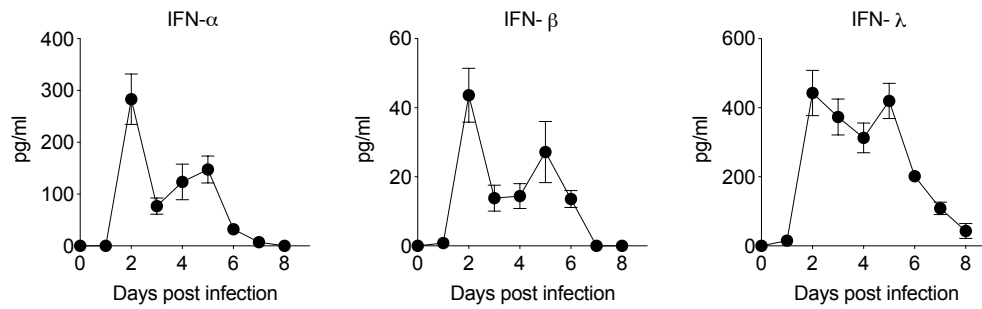


Figure 3. Kinetics of type I and III IFN production during influenza virus infection.

IFN-α, IFN-β, and IFN-λ protein concentrations were quantified in the BALF of influenza virus infected mice at indicated days post infection. All data are representative of two independent experiments (n = 3–6). Data are shown as mean±SEM.

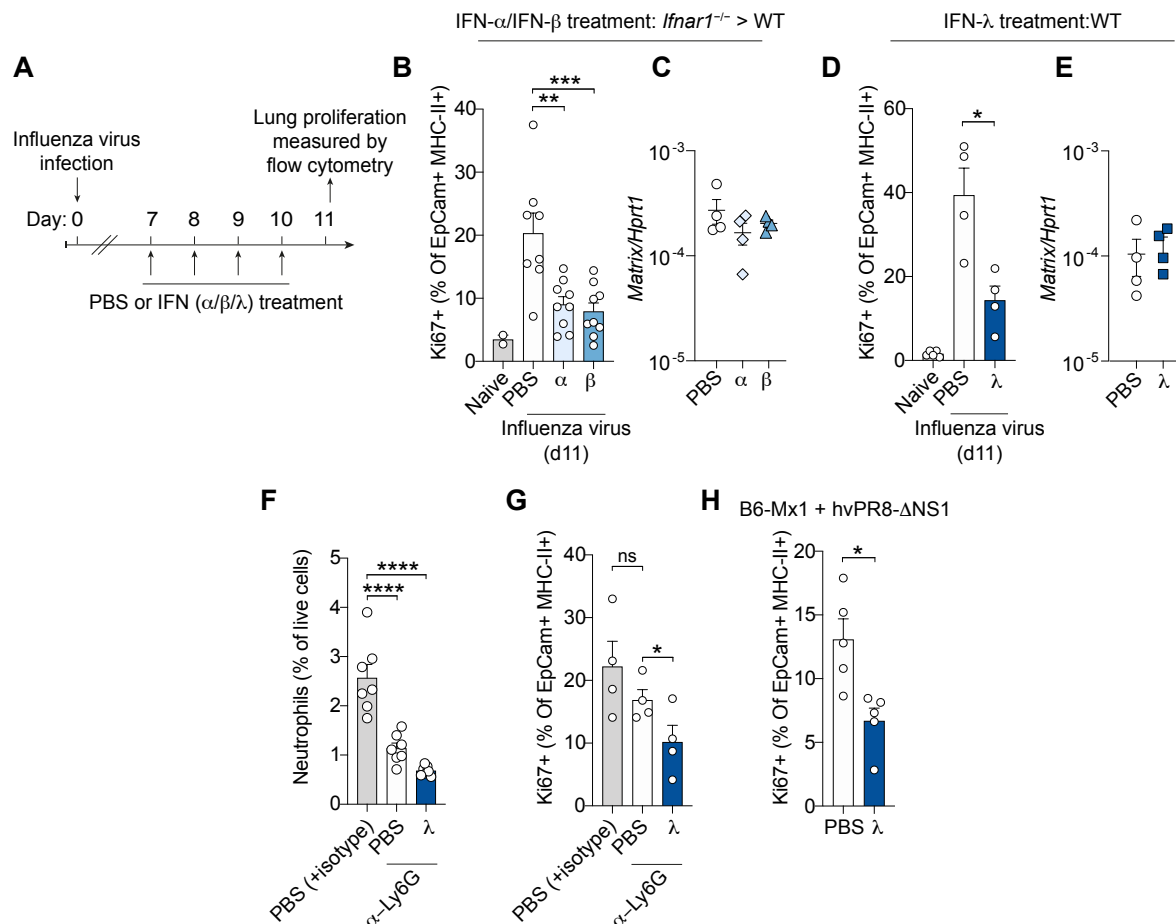


Figure 4. Exogenous type I and III IFN treatment during lung repair impairs epithelial cell proliferation.

(A) Schematic outlining IFN treatment strategy in influenza infected mice. (B to H) Mice were infected with influenza virus, and treated with IFN every 24 hours, on days 7 to 10 post infection. (B, D, G and H) Ki67⁺ AT2 cells were detected by flow cytometry on day 11 post

infection, and (C and E) influenza virus *M1* mRNA expression in total lung RNA on day 11 post infection. (B and C) Lethally irradiated WT mice were injected with *Ifnar1*^{-/-} BM cells. Following reconstitution, influenza virus-infected chimeric mice were treated with PBS (control), IFN- α , or IFN- β . Naïve controls are uninfected, untreated chimeric mice. (D and E) Influenza virus infected WT mice were treated with PBS or IFN- λ . (F and G) Influenza virus infected B6 mice were treated with anti-Ly6G monoclonal antibody or isotype control every 24 hours from days 5 to 10 post infection for neutrophil depletion. (F) Ly6C^{hi}CD11b⁺ lineage (CD3/CD19/NK1.1/CD64/Siglec F)⁻ neutrophil frequencies in lungs following isotype or anti-Ly6G treatment. (G) Anti-Ly6G treated mice were also treated with IFN- λ or PBS. (H) B6-Mx1 mice were treated with IFN- λ or PBS. All data are representative of three independent experiments, except for (H) which was two (n = 4–9). Data are shown as mean $\pm$ SEM and statistical significance was assessed by one-way ANOVA with Dunnett's post-test (B, D, F and G), or unpaired two-tailed Student's *t* test (H). ns, not significant ($P>0.05$); * $P\leq 0.05$, ** $P\leq 0.01$, *** $P\leq 0.001$, **** $P\leq 0.0001$.

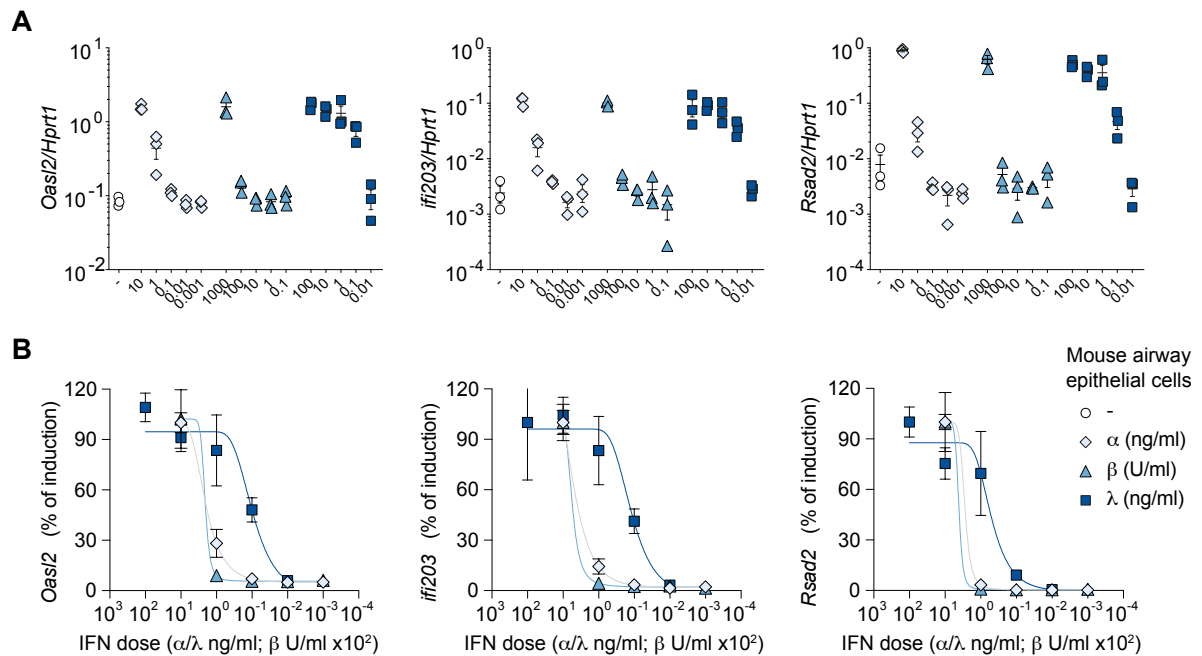


Figure 5. Titration of IFNs used in vivo for equal subtype dosage.

(A) Primary murine AEC cultures were treated for 4 hours at indicates concentrations with IFN- α , IFN- β , and IFN- λ subtypes (used for in vivo treatment experiments) at the indicated concentrations. In IFN and mock treated AECs, mRNA expression of indicated ISGs was assessed by qPCR. (B) Sigmoidal, 4PL, X is log(concentration) dose-response curves were generated for each IFN subtype, relative to ISG expression at the highest IFN concentration. (n = 3). Data are shown as mean $\pm$ SEM.

We next sought to determine the role of endogenous IFN during lung repair, using *Ifnar1^{-/-}* and *Ifnlr1^{-/-}* mice. AT2 cells were analysed on day 8 post influenza virus infection, the time when IFN signalling and epithelial cell proliferation overlapped (Fig. 2B and 3). Both *Ifnar1^{-/-}* and *Ifnlr1^{-/-}* mice had improved AT2 cell proliferation compared to WT controls (Fig. 6A and B). This was dependent on IFN signalling specifically through the epithelium, as receptor deficiency in the non-hematopoietic compartment alone was sufficient to increase lung epithelial cell proliferation (Fig. 6C, representative chimerism efficiency data in Appendix I). In contrast, we saw no effect of IFN- λ signalling on the proliferation of distal lung progenitor epithelial cells in the lung, across multiple timepoints (Fig. 6D). Improved proliferation was independent of major changes in viral burden, with only a slight increase in influenza virus matrix gene expression observed in *Ifnar1^{-/-}* mice on day 2 post infection, and no change in *Ifnlr1^{-/-}* mice, compared to WT controls (Fig. 7A). Viral control in individual IFN receptor-knockout mice was likely unaffected due to redundancy between type I and III IFN antiviral responses in epithelial cells (Mordstein *et al.*, 2008; Crotta *et al.*, 2013).

Despite type I and III IFN redundancy in viral control (Fig. 7A), the lack of redundancy in antiproliferative IFN responses, with both *Ifnar1^{-/-}* and *Ifnlr1^{-/-}* mice displaying enhanced epithelial proliferation (Fig. 6), led us to further interrogate the phenotype. IFNAR signalling has previously been shown to be important for the production of IFN- λ during influenza virus infection (Osterlund *et al.*, 2005; Jewell *et al.*, 2010). Consistent with previous findings, we observed a significant reduction in IFN- λ (and indeed IFN- α/β) production in *Ifnar1^{-/-}* mice compared to WT, yet we saw little change in IFN- α/β levels in *Ifnlr1^{-/-}* mice (Fig. 7B). Thus, the improved epithelial proliferation observed in *Ifnar1^{-/-}* mice could be attributed to a reduction in IFN- λ . Reduced IFN production in *Ifnar1^{-/-}* mice is linked with steady state priming defects in these mice owing to the absence of tonic IFNAR activation in immune cells (Gough *et al.*, 2012). Basal IFN- β expression is maintained in part by commensal microbes at barrier surfaces, resulting in the maintained expression of key IFN signal transducers, including STAT1 and IRF9 (Gough *et al.*, 2012; Abt *et al.*, 2012). To circumvent priming defects in *Ifnar1^{-/-}* mice, we used an anti-IFNAR monoclonal antibody (MAR1-5A3) administered only from the time of influenza virus infection (day 0). Anti-IFNAR treatment maintained steady-state priming required for IFN- λ production (Fig. 8A). To confirm that anti-IFNAR treatment effectively blocked IFN- α/β signalling, we measured ISG expression (*Irf7* and *Mx1*) in MACS-separated CD45⁺ cells following Ly6G depletion, to exclude IFN- λ -mediated signalling in neutrophils. Anti-IFNAR treatment blocked IFN- α/β signalling through IFNAR (Fig. 8B).

Importantly, anti-IFNAR treatment from day 0 or day 3 post infection had no effect on lung epithelial cell proliferation (Fig. 8C). Thus, in our model of influenza virus infection in B6 mice, IFN- λ responses are most effective in disrupting lung epithelial regeneration during influenza recovery. Furthermore, these effects are mediated directly in epithelial cells.

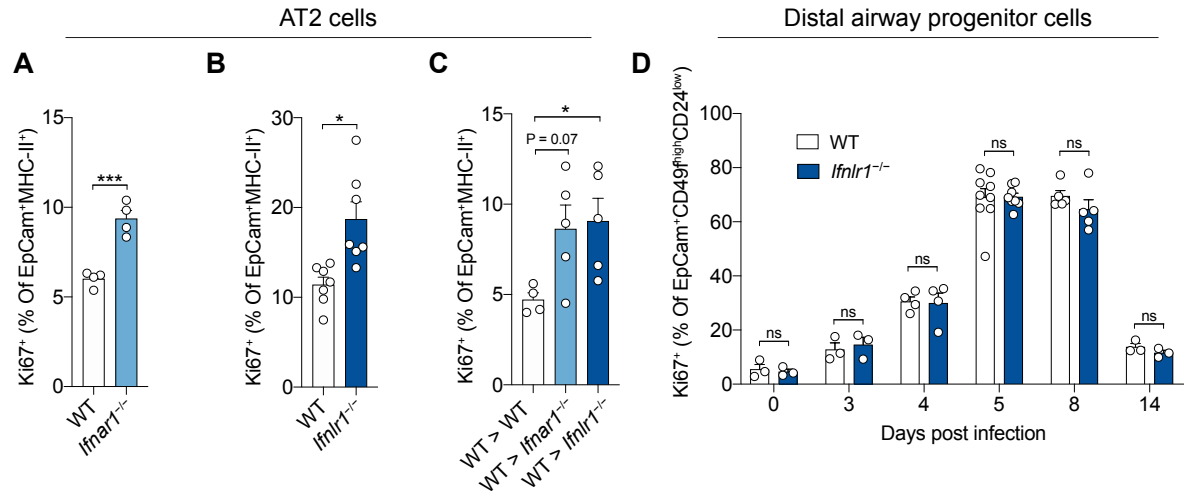


Figure 6. Host-produced IFN disrupts AT2 cell proliferation during lung repair, but not airway progenitor cells.

(A to C) Ki67⁺ AT2 cells (EpCam⁺MHCII⁺CD49^{low}) were measured in the lungs of influenza virus infected WT mice and (A) *Ifnar1*^{-/-}, (B) *Ifnlr1*^{-/-}, and (C) BM chimeric mice by flow cytometry on day 8 post infection. (D) Ki67⁺ distal airway progenitor cells (EpCam⁺CD49^{high}CD24^{low}) were measured in the lungs of influenza virus infected WT and *Ifnlr1*^{-/-} mice on indicated days post infection. All data are representative of at least three independent experiments (n = 3–9). Data are shown as mean±SEM and statistical significance was assessed by unpaired two-tailed Student's *t* test (A and B) and two-way ANOVA with Dunnett's post-test (C) or Sidak's post-test (D). ns, not significant (P>0.05); *P≤0.05, ***P≤0.001.

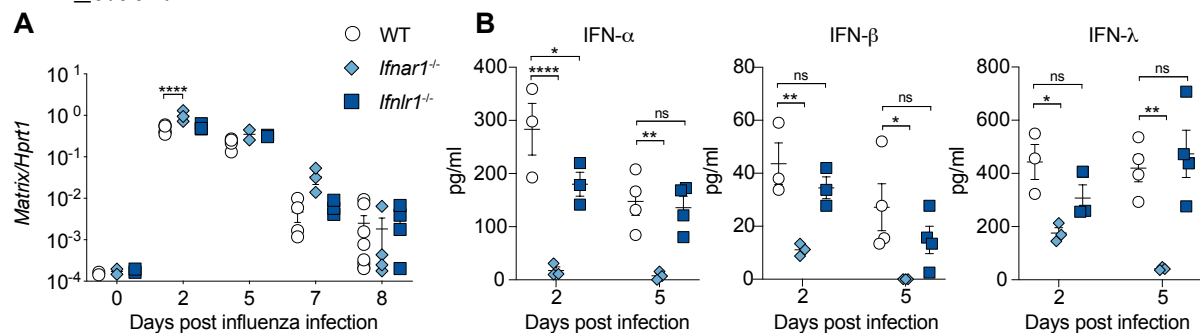


Figure 7. IFNAR deficiency impairs IFN production, but not antiviral immunity.

WT, *Ifnar1*^{-/-}, and *Ifnlr1*^{-/-} mice were infected with influenza virus. (A) Influenza *M1* mRNA expression was measured in whole lung RNA and (B) IFN- α , IFN- β and IFN- λ protein concentrations were measured in BALF. (A) Data are pooled three independent experiments or (B) representative of at least three independent experiments (n = 3–6). Data are shown as mean±SEM and statistical significance was assessed by two-way ANOVA with Dunnett's post-test (A) or Tukey's post-test (B). ns, not significant (P>0.05); *P≤0.05, **P≤0.01, ****P≤0.0001.

Owing to the observed reduction in lung epithelial proliferation following exogenous IFN- α or IFN- β treatment (Fig. 4B), we next sought to understand why endogenous type I IFNs had no effect (Fig. 8C). This may be due to the reduced magnitude of IFN- α and IFN- β production in B6 mice, relative to IFN- λ (Fig. 3). Specifically, on days 6 to 8 post infection, the time in which alveolar epithelial cell proliferative responses manifest (Fig. 2B, right), IFN- λ was detected in the BALF of mice, yet IFN- α and IFN- β levels are low, and undetectable past day 7 (Fig. 3). We have previously shown in the lab that 129 mice have increased levels of both IFN- α and IFN- β in BALF following influenza virus infection, compared to their B6 counterparts (Davidson *et al.*, 2014). This increase includes a second wave of IFN production late during infection, when epithelial repair likely takes place (Davidson *et al.*, 2014). Therefore, we utilised 129 mice as a model of severe influenza, to assess whether type I IFNs disrupt lung repair in a context of heightened immunopathology and excessive IFN production. 129 mice received either anti-IFNAR treatment or PBS control late during infection (day 6/8/10), to avoid blocking early antiviral IFN signalling, instead targeting potentially pathogenic signalling during the recovery phase of infection (Fig. 9A). Anti-IFNAR treatment triggered increased AT2 cell proliferation (Fig. 9B), which correlated with significantly improved recovery and weight gain (Fig. 9C). Therefore, in situations of excessive pulmonary inflammation and epithelial damage, type I IFNs have the potential to disrupt lung repair. However, as we used full IFNAR knockout mice as opposed to BM chimeras, the increase in proliferation and improved mouse recovery may occur as a result of reduced IFN-driven immune cell-mediated immunopathology (Davidson *et al.*, 2014), therefore indirectly allowing more efficient epithelial repair.

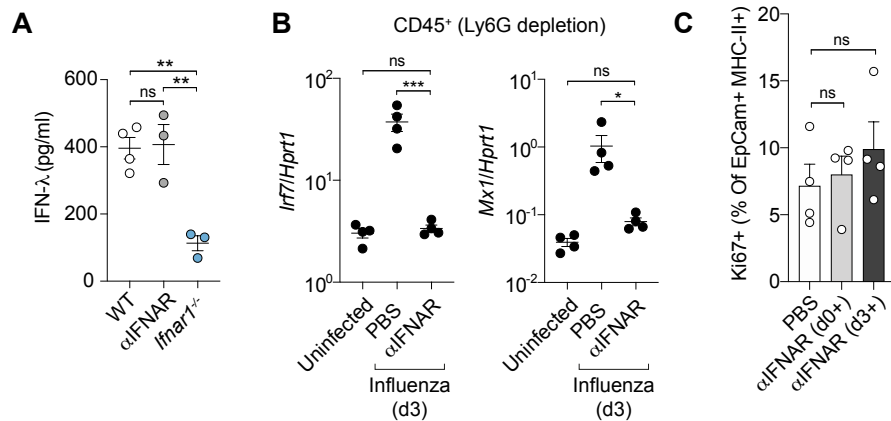


Figure 8. IFNAR signalling does not affect lung repair in B6 mice.

(A) IFN-λ protein concentrations were measured in the BALF of influenza virus infected WT *Ifnar1*^{-/-}, and αIFNAR treated mice on day 3 post infection. αIFNAR treatment was administered on day 0 and 2 post infection. (B) ISG expression was measured in Ly6G⁺ neutrophil-depleted CD45⁺ lung immune cells in WT *Ifnar1*^{-/-}, and αIFNAR treated mice on day 3 post influenza virus infection. αIFNAR treatment was administered on day 0 and 2 post infection. (C) Ki67⁺ AT2 cells were measured in the lungs of influenza virus infected WT and αIFNAR treated mice on day 8 post infection by flow cytometry. αIFNAR treatment was administered every 48 hours from day 0 or day 3 post infection. All data are representative of at least two independent experiments (n = 3–4). Data are shown as mean±SEM and statistical significance was assessed by one-way ANOVA with Tukey's post-test (A) or Dunnett's post-test (B and C). ns, not significant (P>0.05); *P≤0.05, **P≤0.01, ***P≤0.001.

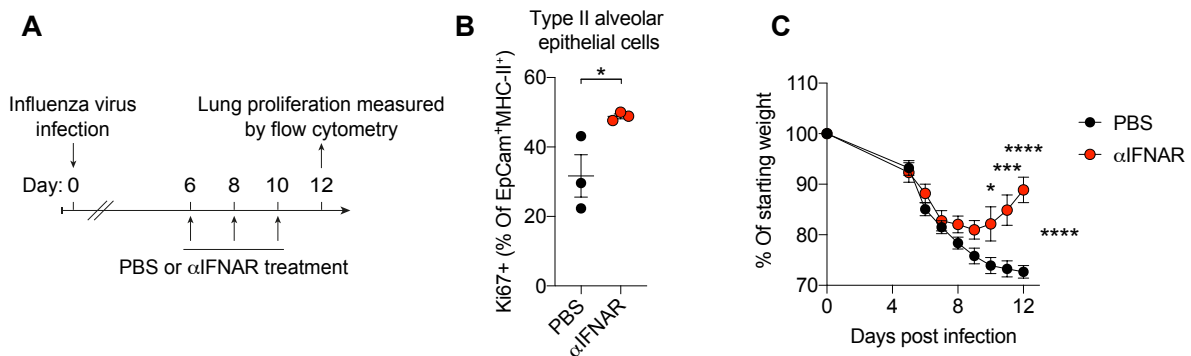


Figure 9. IFN-α/β-induced signalling disrupts lung repair in 129S7 mice.

(A) Schematic for αIFNAR treatment in 129 mice. (B and C) Influenza virus infected 129S7 mice were treated with αIFNAR treatment or PBS control on days 6, 8 and 10 post infection and (A) AT2 cell proliferation (EpCam⁺MHCII⁺CD49f^{low}) was measured on day 12 post infection by flow cytometry, or (B) weight loss. All data are representative of two independent experiments (n = 3–5). Data are shown as mean±SEM and statistical significance was assessed by unpaired two-tailed Student's *t* test (B) or two-way ANOVA with Sidak's post-test (C). *P≤0.05, ***P≤0.001, ****P≤0.0001.

3.4 IFN- λ impairs AEC differentiation during influenza recovery

We next sought to determine how IFN-induced signalling alters the state of lung epithelia during influenza virus infection by performing RNA sequencing analysis. We used *Ifnlr1*^{-/-} mice, owing to the increased potential of endogenous IFN- λ compared to IFN- α/β in blocking lung epithelial cell proliferation. RNA sequencing was performed on lung epithelial cells (EpCam⁺CD31⁻CD45⁻) sorted from naïve and influenza virus infected (day 8 post infection) WT or *Ifnlr1*^{-/-} mice (bioinformatics analyses were performed by Miriam Llorian Sopena, Francis Crick Institute). Samples clustered based on infection status, but, surprisingly, not by genotype (Fig. 10A). Accordingly, minimal differential gene expression was observed between WT and *Ifnlr1*^{-/-} lung epithelial cells during influenza (Fig. 10B). The similarity in expression profiles is potentially owing to the presence of type I IFN signals in both genotypes, the late timepoint used when IFN signals are decreasing/absent in WT mice (Fig. 3), or both. Likely differences in the expression profiles of WT and *Ifnlr1*^{-/-} AT2 cells earlier during the course of infection may account for the observed phenotypical changes later. Indeed, despite a lack in differential gene expression, gene set enrichment analysis (GSEA) showed *Ifnlr1*^{-/-} AT2 cells had an increase in pathways pertaining to cell cycle and proliferation (Fig. 10C, left plot). Interestingly, we also observed an upregulation of multiple epithelial cell differentiation and multiciliogenesis pathways in repairing lung epithelial cells lacking IFN- λ signalling (Fig. 10C, right plot). Following acute lung damage, populations of basal cells and Scgblal⁺ secretory cells give rise to secretory and multiciliated cell subtypes in the airways (Hogan *et al.*, 2014). To understand whether IFN- λ signalling affects airway epithelial cell differentiation during influenza-induced lung repair, we infected WT and *Ifnlr1*^{-/-} mice with influenza virus and stained lung sections with an antibody for acetylated α -tubulin⁺, to measure cilia on day 10 post infection. *Ifnlr1*^{-/-} mice displayed an increase in multiciliated cells in repairing conducting airways compared to WT controls (Fig. 11A). We quantified these findings by flow cytometry, and observed an increase in the frequency of differentiated AECs (EpCam^{hi}CD49f^{high}CD24^{high}) composed of ciliated, goblet, and club cells (Fig. 11B) (Quantius *et al.*, 2016). Therefore, IFN- λ signalling induces antiproliferative pathways, and additionally blocks multiciliogenesis in repairing lung epithelial cells in vivo. This reduces the capacity for basal cell differentiation in conducting airways during recovery from influenza virus infection.

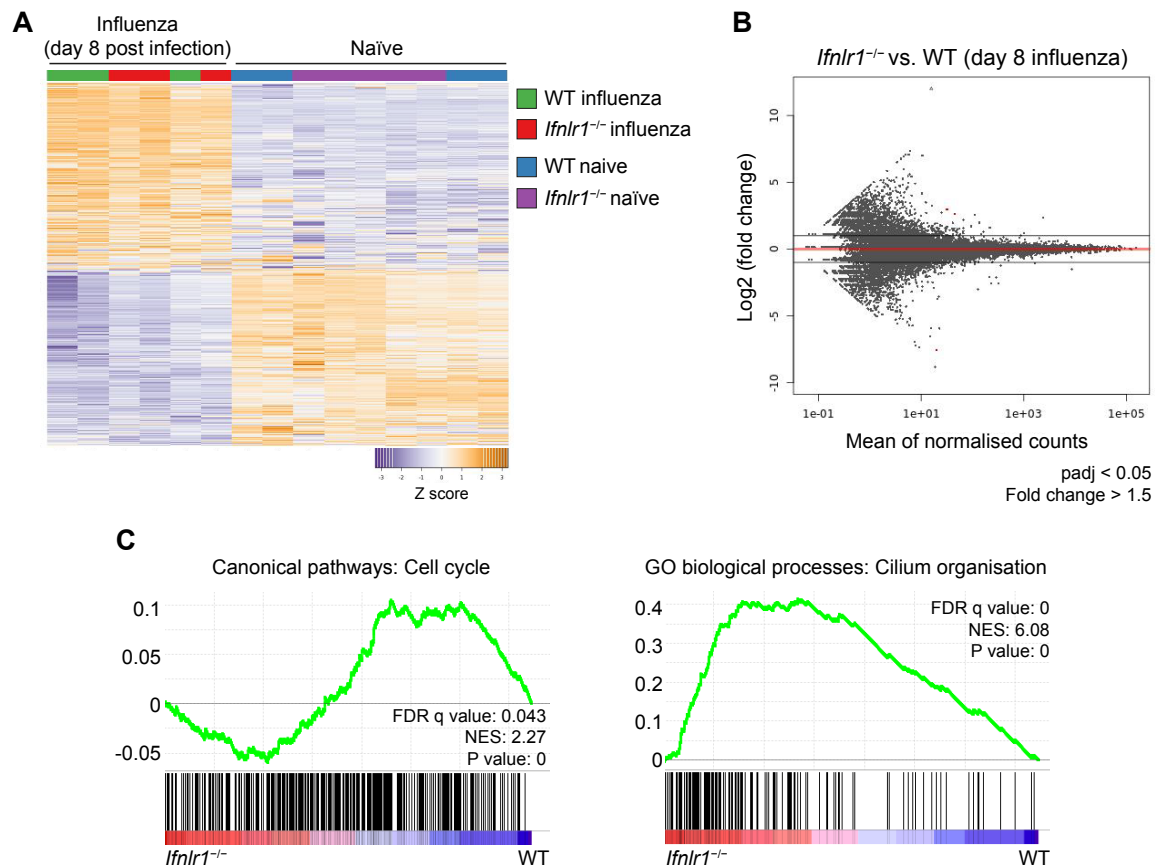


Figure 10. *Ifnlr1*^{-/-} mice have greater expression of proliferation and differentiation pathways in repairing lung epithelia.

EpCam⁺CD45⁻CD31⁻ bulk lung epithelial cells were isolated from naïve or influenza virus infected (day 8 post infection) WT and *Ifnlr1*^{-/-} mice, and RNA was isolated for analysis by RNA sequencing. (A) Heatmap of RNA sequencing datasets (one-way ANOVA with Benjamini–Hochberg correction, $P < 0.05$). (B) Volcano plots indicating fold changes between WT and *Ifnlr1*^{-/-} lung epithelial cells from influenza virus infected mice. Differentially expressed genes (p -adjusted < 0.05) with a $\log_2(\text{fold change})$ of more than 2 are indicated in red. (C) GSEA plots of RNA sequencing datasets from WT or *Ifnlr1*^{-/-} lung epithelial cells ($n = 3$).

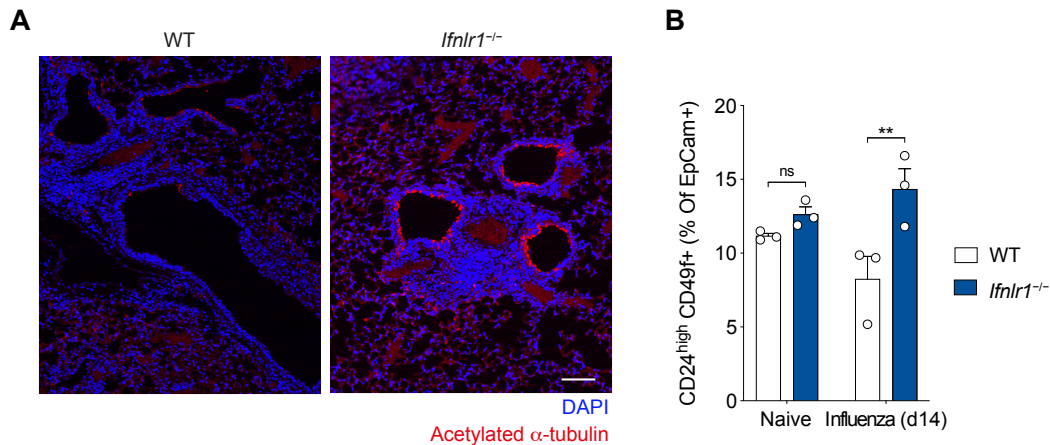


Figure 11. IFN- λ signalling disrupts airway epithelial cell differentiation during recovery from influenza.

Lung epithelial cell differentiation was assessed in influenza virus infected WT and *Ifnlr1*^{-/-} mice by (A) immunofluorescence (DAPI/acetylated α -tubulin) on day 10 post infection and (B) flow cytometry (EpCam⁺CD49f^{high}CD24^{high}) on day 14 post infection. Scale bar represents 100 μ m. All data are representative of three independent experiments (n = 3). Data are shown as mean $\pm$ SEM and statistical significance was assessed by two-way ANOVA with Dunnett's post-test. ns, not significant (P > 0.05); **P $\leq$ 0.01.

3.5 *Ifnlr1*^{-/-} mice have reduced lung damage and improved barrier function following influenza virus infection

Our data supports a significant role of IFN signalling, in particular IFN- λ , in disrupting epithelial cell proliferation and differentiation during lung repair. We therefore determined whether the improved capacity for repair in *Ifnlr1*^{-/-} mice affects lung damage or barrier function of the respiratory epithelium. Histopathological analysis of H&E stained lung sections on day 9 after infection indicated *Ifnlr1*^{-/-} mice had a significant reduction in inflammatory immune cells infiltrating lung tissue (Fig. 12A). This was consistent with reduced lung damage, with a reduction in both the total number of cells and the number of red blood cells (RBCs) in the BALF of *Ifnlr1*^{-/-} mice day 8 post infection (Fig. 12B and C). We did not observe differences in the frequencies of various infiltrating immune cells in the BALF between WT and *Ifnlr1*^{-/-} mice (Fig. 12D and gating strategy in appendix II), suggesting the differing total cell number (Fig. 12B) is likely owing to reduced RBCs and a general reduction in inflammation, as opposed to specific effects of IFN- λ on immune cell populations.

Influenza virus infections in humans are often exacerbated by opportunistic bacterial pathogen, including *S. pneumoniae* (McCullers, 2014). A contributing factor driving this

synergy, is a compromised epithelial barrier as a result of influenza virus-induced damage. To measure the effects of IFN- λ signalling on lung barrier function, we challenged mice with *Streptococcus pneumoniae* 8 days after influenza virus infection. All WT mice succumbed to the bacterial superinfection 2 days post-challenge (10 days post influenza virus infection), however *Ifnlr1*^{-/-} mice had prolonged and improved survival (Fig. 13A). To study specific effects of IFN- λ in epithelial cells, we used BM chimeric mice. Mice lacking IFNLR in the non-hematopoietic compartment (WT > *Ifnlr1*^{-/-}) also had improved survival following bacterial superinfection (Fig. 13B), indicating the pathology driven by IFN- λ during bacterial superinfection occurs independent to effects in immune cells. Improved survival in mice deficient for IFN- λ signalling in the non-hematopoietic compartment correlates with reduced bacteria dissemination in tissues, with mice displaying a lower bacterial load in both the lung and spleen (Fig. 13C). Thus, IFN- λ signalling reduces the capacity for epithelial repair following influenza virus infection, resulting in prolonged lung damage and compromised barrier function. This in turn increases susceptibility to bacterial superinfection.

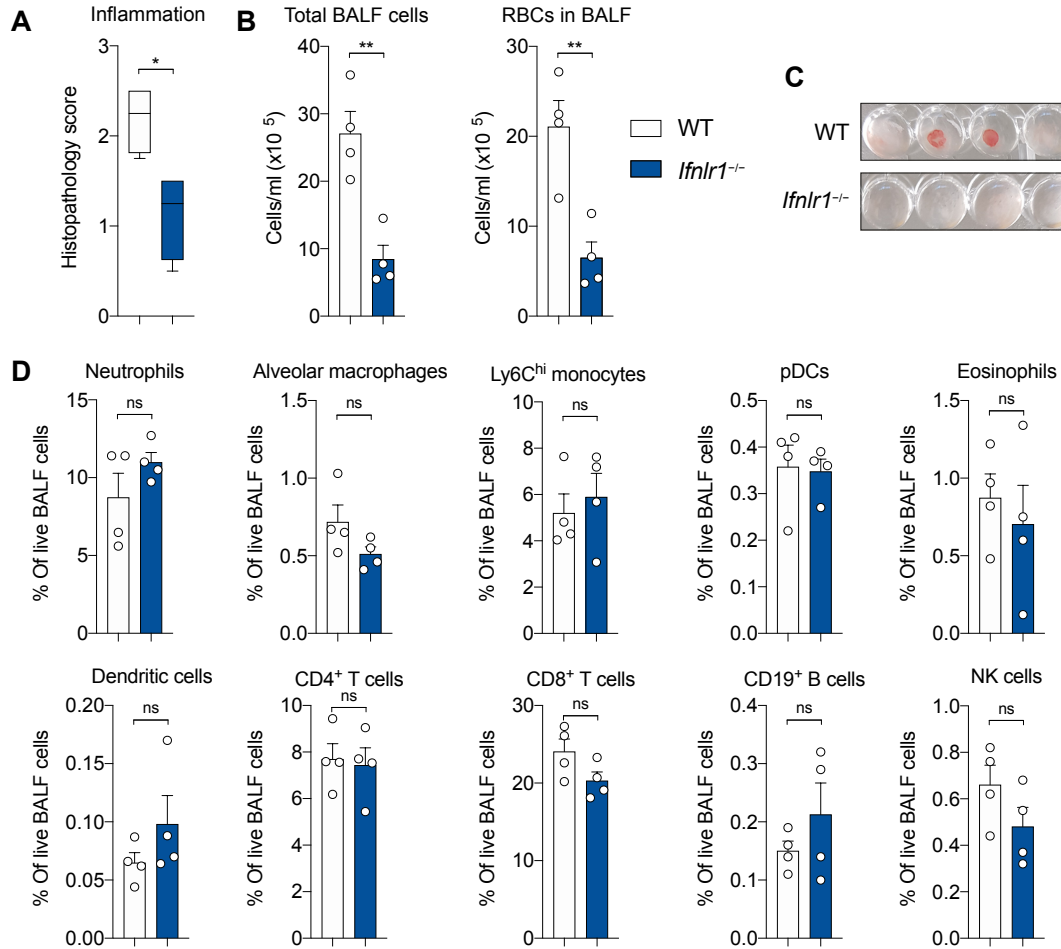


Figure 12. *Ifnlr1*^{-/-} mice have reduced lung damage.

WT and *Ifnlr1*^{-/-} mice were infected with influenza virus and assessed for lung damage and inflammation by (A) histopathological analysis of H&E lung sections on day 9 post infection, and (B to D) analysis of the cellular infiltrate in BALF on day 8 post infection. (B and C) BALF cell numbers were counted and the number of Ter119⁺ RBCs were determined by flow cytometry. (D) Frequencies of neutrophils (Ly6G⁺CD11b⁺), alveolar macrophages (Siglec-F⁺CD11c⁺), Ly6C^{hi} monocytes (Ly6C^{hi}CD11b⁺), pDCs (Siglec-H⁺), eosinophils (Siglec-F⁺CD11b⁺CD11c⁻), DCs (CD11c⁺MHCII⁺), CD4⁺ T cells (CD3⁺CD4⁺), CD8⁺ T cells (CD3⁺CD8⁺), CD19⁺ B cells, and NK cells (NK1.1⁺CD3⁻) in BALF were determined by flow cytometry. All data are representative of three independent experiments (n = 4). Data are shown as mean±SEM and statistical significance was assessed by Mann–Whitney *U* test (A) or unpaired two-tailed Student's *t* test (B and D). ns, not significant (*P*>0.05); **P*≤0.05, ***P*≤0.01.

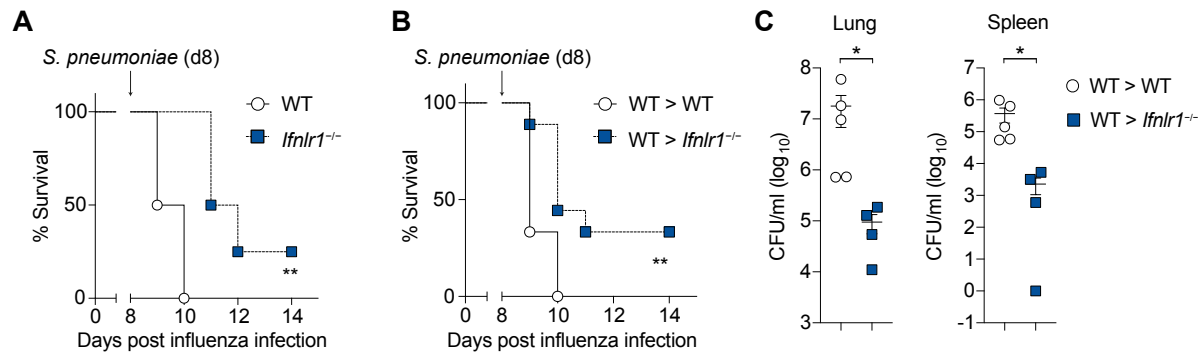


Figure 13. IFN- λ signalling specifically in epithelial cells increases susceptibility to bacterial superinfection.

(A and B) Influenza virus infected WT and *Ifnlr1*^{-/-} mice (A) or BM chimeric mice (B) were challenged with 2×10^5 CFU TIGR4 on day 8 post influenza infection and clinical scores were measured to determine survival. (C) Influenza virus infected BM chimeric mice were challenged with 2×10^5 CFU TIGR4 on day 12 post influenza infection, and CFUs were measured in spleen and lung tissues taken on day 14 post influenza infection. All data are representative of at least two independent experiments ($n = 4$). Data are shown as mean $\pm$ SEM and statistical significance was assessed by log-rank (Mantel–Cox) test (A and B, survival) or Mann–Whitney U test (C). * $P \leq 0.05$, ** $P \leq 0.01$.

3.6 Discussion

Here we describe a novel mechanism by which type I and III IFN signalling aggravates lung pathology during respiratory viral infection. By focusing on the importance of epithelial repair during the recovery phase of infection, we highlight the potential for both IFN- α/β and IFN- λ in exacerbating influenza disease.

Exogenous *in vivo* IFN application during respiratory viral disease provides a reductionist approach to study IFN and ISG kinetics in the context of protective antiviral immunity versus nonprotective or pathological effects. Here, we show the detrimental effects of IFN on lung epithelial proliferation by simply extending the time window of IFN signalling by exogenous treatment into that of mouse infection recovery. Prophylactic type I or III IFN treatment induces antiviral ISGs in epithelia prior to viral entry (Crotta *et al.*, 2013), thus severely inhibiting the capacity for viral replication and disease manifestation (Davidson *et al.*, 2016; Bradley *et al.*, 2019). However, this is not clinically relevant for the treatment of respiratory viral disease, only for prophylactic prevention. Regarding treatment administered post-symptom onset, numerous studies have described the benefits of *early* IFN- λ administration during murine influenza (Davidson *et al.*, 2016; Galani *et al.*, 2017), and IFN- α/β treatment in coronavirus (SARS-CoV-1 and MERS-CoV) infection models (Channappanavar *et al.*, 2016, 2019). The basis for protection is that ISG induction early in the infection, when viral replication is most prominent, curbs disease progression. Early IFN- α treatment during influenza was not protective in mice, instead promoting pathogenic pulmonary inflammation (Davidson *et al.*, 2016), highlighting the targeted antiviral benefits of IFN- λ . In addition, type I IFN administered *late* during the course of infection exacerbated MERS-CoV infection in mice, promoting similar pathology to that seen in severe influenza, with enhanced proinflammatory cytokine production and innate cell recruitment in the lung (Channappanavar *et al.*, 2019). Here, we show that late type I or III IFN treatment inhibited AT2 cell proliferation, independent to changes in viral burden (Fig. 4), thus, providing evidence that timing is a critical parameter to consider for exogenous IFN application, which has relevance for the clinical treatment of respiratory viral disease. Early ISG induction increases the likelihood of inhibiting viral replication, whilst late treatment bypasses the important window for inhibiting viral replication, and may exacerbate lung damage further through disrupting the efficiency of lung repair.

We additionally examine the effects of endogenously produced type I and III IFN on lung repair, and how this affects the extent of influenza-induced lung pathology. Through kinetics comparison between lung epithelial proliferation and IFN production, we were able to identify a crossover window between lung repair and IFN-induced signalling (Fig. 1 and 2). By comparing the capacity for lung repair in WT and individual IFN receptor deficient mice within this window, we found that AT2 cell proliferation was improved in B6 mice that lacked IFN- λ signalling (Fig. 6). Whilst *Ifnar1*^{-/-} mice also displayed improved AT2 cell proliferation, we attributed this to dysfunctional IFN- λ production in these mice. α IFNAR treatment effectively blocked type I IFN-induced signalling, whilst also maintaining steady state priming required for IFN production. In this situation, IFNAR blockade had no effect on lung epithelial proliferation (Fig. 8). However, we do not exclude a role for endogenously produced type I IFN in generating lung pathology via disruption of critical lung repair pathways. The observation that both IFN- α and IFN- β treatment reduced AT2 cell proliferation by signalling specifically through the epithelium (Fig. 4B) suggests that endogenous type I IFN lacks the in vivo antiproliferative capacity of type III IFN owing to its the comparably lower amounts produced (Fig. 3). This is supported by our studies in 129 mice, which provide a murine model for excessive type I IFN production, and IFN- α/β -driven lung pathology (Davidson *et al.*, 2014). Late (day 6 to 12 post infection) α IFNAR treatment in 129 mice improved AT2 cell proliferation (Fig. 9). However, this may occur indirectly as a result of reduced type I IFN-induced lung damage mediated by signalling in immune cells, as described previously by Davidson *et al.* Further work studying the effects of IFNAR depletion specifically in the epithelium of 129 mice would deliver insight into the capability of aberrant endogenous type I IFN responses in directly disrupting lung repair.

RNA sequencing analysis of the repairing lung epithelium yielded a surprisingly similar expression profile between WT and *Ifnlr1*^{-/-} mice (Fig. 10). As mentioned earlier, this may be a result of timing as we performed transcriptomic analysis on the same day as the observed improvement in proliferation in IFNLR knockout mice (day 8 post infection). We hypothesise that significant genotypic differences precede phenotypic differences, and this is supported by the upregulation of proliferation and differentiation pathways in *Ifnlr1*^{-/-} lung epithelial cells (Fig. 10C), despite such few significant differentially expressed genes. Findings by our collaborators in Ivan Zanoni's, lab published back-to-back with ours, supports this argument (Major *et al.*, 2020; Broggi *et al.*, 2020). Broggi *et al.* show a potent downregulation of an IFN signature in *Ifnlr1*^{-/-} lung epithelial cells and an upregulation of cell proliferation pathways, in

their model of poly (I:C) induced inflammation and lung damage. Importantly, their expression profiling was performed at the peak of IFN production, following 6 days of poly (I:C) treatment, indicating that at our timepoint, influenza virus infection has bypassed the window for significant gene expression differential.

Interestingly, we also observed a negative impact of IFN- λ signalling on AEC differentiation during lung repair. Influenza virus infection has previously shown to reduce mucociliary function and bacterial clearance (Pittet *et al.*, 2010). Therefore, direct inhibition of AEC differentiation by IFN- λ -induced signals may provide an additional mechanistic link underlying the enhanced synergy of bacterial pathogen and respiratory viruses (Brundage, 2006). Indeed, IFNLR deficiency in the non-hematopoietic compartment increased survival in mice following *Streptococcus pneumoniae* coinfection (Fig. 13B). This correlates with reduced bacterial dissemination in local (lung) and distal (spleen) tissue sites (Fig. 13C), indicating the immunopathological consequences of IFN- λ signalling on lung barrier function. We also found IFN- λ enhanced aberrant immune cell and RBC BALF infiltration, indicative of exacerbated lung damage (Fig. 12). Reduced pulmonary damage in IFNLR deficient mice likely occurs as a result of an earlier restoration of the epithelial lining, preventing cellular leakage into the air spaces. Therefore, endogenous IFN- λ both prolongs and exacerbates existing damage by delaying critical lung repair.

In summary, the data presented here highlights a mechanism by which immune signals can promote disease severity through the inhibition of lung tissue regeneration. In the next chapter, we utilise reductionist in vitro models to dissect these processes mechanistically.

Chapter 4. Results 2:

IFN- β and IFN- λ most potently disrupt airway epithelial cell growth via a p53-dependent mechanism

For the most part, results in this chapter are published, and largely include the expanded in vitro findings from the following publication:

Major, J., Crotta, S., Llorian, M., McCabe, T.M., Gad, H.H., Priestnall, S.L., Hartmann, R., and Wack, A. (2020). Type I and III interferons disrupt lung epithelial repair during recovery from viral infection. *Science* 369, 712–717.

4.1 Introduction

As discussed earlier, the growth inhibitory effects of IFNs have largely been studied using immortalised cell lines following in vitro treatment, in a concerted effort to define their antitumour potential. This has led to a variety of mechanisms attributed to the antiproliferative effects induced by IFN signalling. Collective evidence indicates a role for cell cycle arrest mediated by induction of CKIs (Roos, Leanderson & Lundgren, 1984; Olle Sangfelt, 2000; Subramaniam & Johnson, 1997; Sangfelt *et al.*, 1999, 1997b), activation of the tumor suppressor protein RB (Burke, Bybee & Thomas, 1992; Kumar & Atlas, 1992), and suppression of the cell division promoting transcription factor, *c-myc* (Einat, Resnitzky & Kimchi, 1985; Subramaniam & Johnson, 1997). However, many of these studies are correlative owing to a lack of effective genetic tools to study the individual contribution of host-derived cell cycle inhibitory proteins. This may in part explain the consistency in overlap between identified mechanisms. Global cell cycle shut down mediated by upstream regulators can generate an indirect and aberrant expression of mitotic and antimitotic proteins. In addition, the expansive ISG and protein-interaction networks induced by IFN adds further complexity to any attempt at delineating an IFN response (Hubel *et al.*, 2019; Mostafavi *et al.*, 2016; Schneider, Chevillotte & Rice, 2014).

Few physiological roles have been identified for the growth inhibitory effect of IFNs. Recent studies by the lab of Serge Fuchs utilised genetic mouse models to identify a role for IFNAR signalling in coordinating the DNA damage response (DDR) to aberrant cellular triggers, including telomere shortening (Katlinskaya *et al.*, 2015) and constitutive β -catenin

signalling (Katlinskaya *et al.*, 2016). In both situations, IFNAR signalling induced a p53/p21 axis and cellular senescence, preventing hyperproliferation of intestinal crypts. As discussed earlier, type I IFN-induced p53 has been described in regulating responses to stress signals, including oncogene-induced cell transformation and viral infection (Takaoka *et al.*, 2003). With regard to antiproliferative function during an infection, it has recently been shown that type I IFN negatively regulates the proliferation of inflammatory monocytes following influenza virus infection, again correlating with IFN-induced p53 (Pernet *et al.*, 2019).

Upstream factors important for the induction of growth inhibitory IFN pathways are better defined than specific molecular mechanisms. Gideon Schreiber's group studies the physico-chemical properties of IFN-receptor complexes that govern the stricter requirements for induction of antiproliferative and proapoptotic pathways, as opposed to antiviral responses. This is furthered by findings from Sandra Pellegrini's group, which dissects the negative regulatory components that control pathway induction. This important work has revealed regulatory factors required for the induction of growth inhibitory pathway by IFN: prolonged signalling, requiring days, as opposed to hours for antiviral pathways (Jaitin *et al.*, 2006); receptor binding affinity and stability of the ternary IFN-receptor complex (Kalie *et al.*, 2007, 2008; Thomas *et al.*, 2011); receptor density (Moraga *et al.*, 2009); and negative feedback control (François-Newton *et al.*, 2011, 2012). In addition, these kinetic, dynamic, and structural studies, have revealed an IFN subtype differential coordinating different responses following receptor binding. IFN- β has a binding affinity approximately 1000-fold greater than that of IFN- α subtypes (Lavoie *et al.*, 2011), thus, enhancing its ability to induce antiproliferative responses (Jaitin *et al.*, 2006). This enhanced binding affinity additionally renders IFN- β less susceptible to USP18-mediated destabilisation of the ternary IFNAR complex (François-Newton *et al.*, 2012). This also applies to IFN- λ , which is unaffected by USP18-mediated regulation (François-Newton *et al.*, 2011; Blumer *et al.*, 2017).

In the previous chapter, we described a negative impact of prolonged IFN signalling on epithelial regeneration during recovery from respiratory viral infection. In this chapter, we sought to delineate a mechanism by which IFNs mediate growth inhibition in respiratory epithelial cells. In addition, we aimed to compare and contrast the efficacy of IFN- α , IFN- β , and IFN- λ subtypes in impeding AEC growth, to add further clarification to the discrepancies observed between type I and III IFN *in vivo*. Here, we use primary AEC cultures derived from murine tracheal epithelial stem cells. Despite the lack of IFN effects observed on proliferating airway progenitor cells *in vivo*, airway stem cells contribute to regeneration of the airways,

bronchioles, and alveolar epithelium following lung damage (Rock *et al.*, 2009; Kumar *et al.*, 2011; Vaughan *et al.*, 2015; Zuo *et al.*, 2014; Kathiriya *et al.*, 2020; Zepp & Morrissey, 2019). By utilising AEC cultures, we sought to combine a reductionist model allowing the study growth inhibitory responses independent to viral infection, with a physiologically relevant model for epithelial regeneration, owing to the proliferation and differentiation of primary AECs.

4.2 IFN- β and IFN- λ most potently disrupt primary AEC growth in vitro

Upon plating onto a porous transwell membrane, progenitor basal cells derived from a mouse trachea proliferate until reaching confluence. Formation of a confluent monolayer allows us to establish an ALI, which induces basal cell differentiation into multiciliated, club, and secretory cells (You *et al.*, 2002; Zepp & Morrissey, 2019). Processes of pluripotent stem cell proliferation and differentiation in AEC cultures encapsulates repair dynamics observed in the lung and airway epithelium following damage in vivo, and thus provide a useful tool to study the effects of IFNs on regenerating respiratory epithelial cells.

Similar to IFNs used in vivo, IFNs used for in vitro assays were titrated on AEC cultures to compare IFN subtypes at equivalent biological potencies (Fig. 14A and B). AEC cultures seeded at a low density in the presence of IFN- β or - λ were unable to reach confluence, whereas IFN- α treatment significantly reduced AEC number and the time taken for cultures to reach confluence (Fig. 15A). To study growth inhibitory effects of IFNs without blocking the establishment of AEC cultures, we seeded cells at a higher density before treating with IFN for 5 days. All three IFN subtypes delayed the time taken for cultures to reach confluency, and impaired cell growth, with IFN- β and IFN- λ having the most potent effects (Fig. 15B). Growth inhibitory effects were dependent on the presence of the respective IFN receptor (15C).

To study IFN effects on human epithelial cells, we set up primary human AEC cultures. Similar to murine IFNs used, human IFN- α , IFN- β and IFN- λ were titrated on human AEC cultures and ISG expression was measured, to determine a conversion factor for equivalent biological potencies (Fig. 16A and B). Following 5 days of IFN treatment, all three IFN subtypes reduced human AEC growth, with IFN- β and IFN- λ treatments resulting in the most significant reduction (Fig. 16C). Thus, following prolonged IFN treatment at equal biological dosage, IFN- β and IFN- λ most significantly disrupt the growth of primary murine and human AEC cultures.

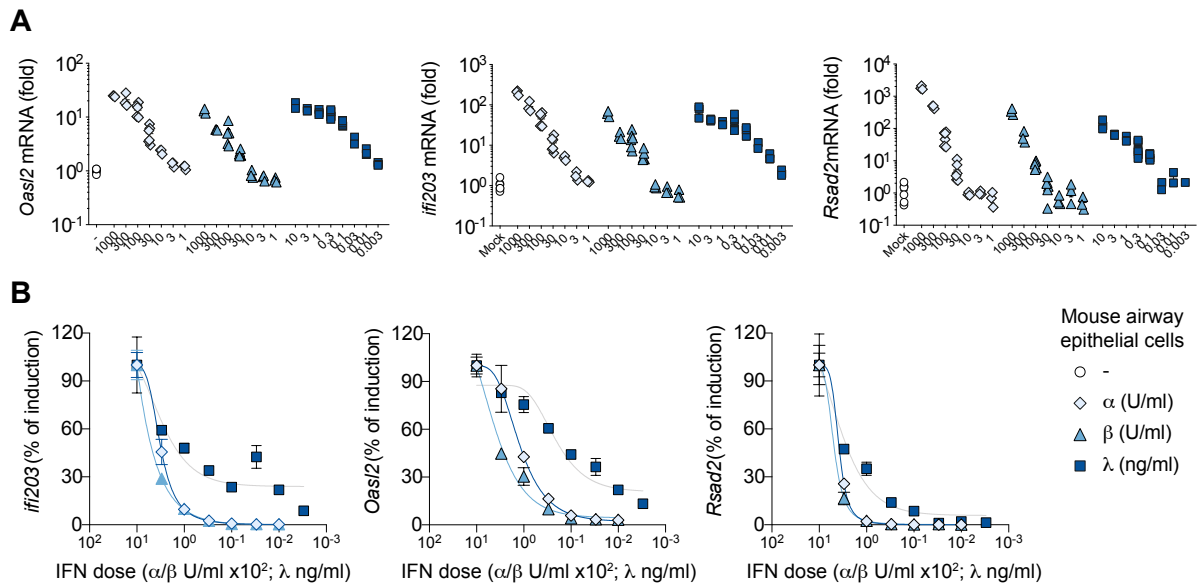


Figure 14. Titration of IFNs used during mouse AEC experiments in vitro for equal subtype dosage.

(A) Primary murine AEC cultures were treated for 4 hours at indicated concentrations with IFN- α , IFN- β , and IFN- λ subtypes (used for murine in vitro treatment experiments) at the indicated concentrations. In IFN and mock treated AECs, mRNA expression of indicated ISGs was assessed by qPCR. (B) Sigmoidal, 4PL, X is log(concentration) dose-response curves were generated for each IFN subtype, relative to ISG expression at the highest IFN concentration. ($n = 3$). Data are shown as mean $\pm$ SEM.

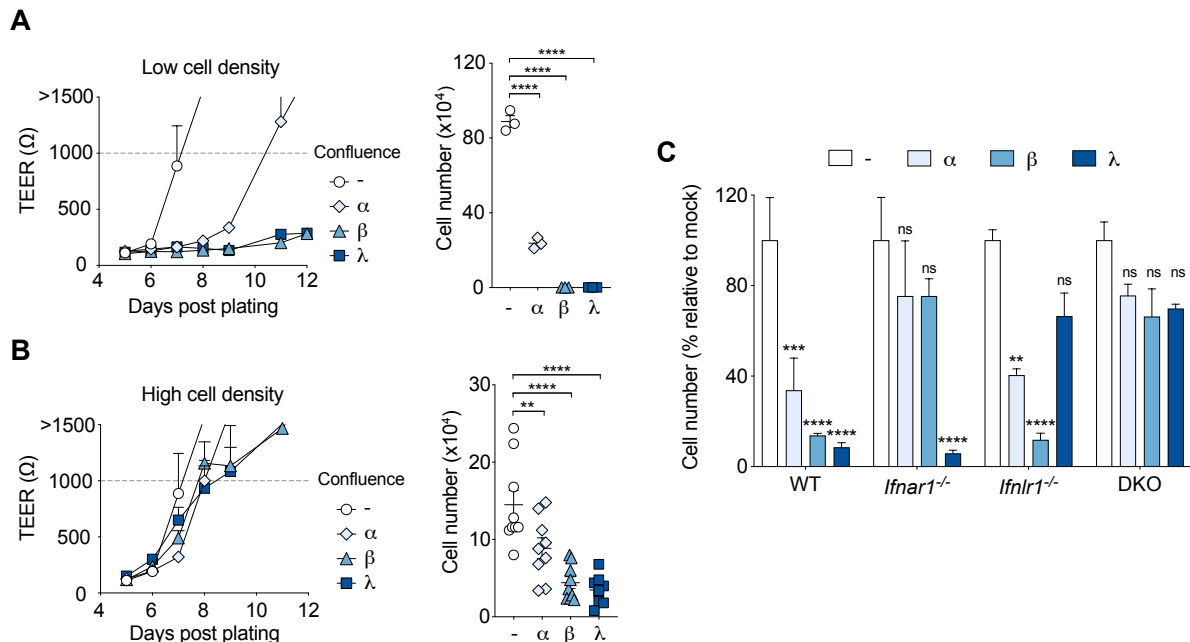


Figure 15. Type I and III IFNs disrupt AEC growth.

(A and B) Murine AECs were seeded at a low density (500 cells per transwell) (A) or a high density (10^4 cells per transwell) (B), and 24 hours post plating were treated for 5 days with equivalent IFN dosages (600 U/ml IFN- α , 1000 U/ml IFN- β , and 5 ng/ml IFN- λ). Confluence was determined by measuring transepithelial electrical resistance (TEER; $>1000\Omega$ = confluent cultures). (C) WT, *Ifnar1*^{-/-}, *Ifnlr1*^{-/-}, and *Ifnar1*^{-/-}*Ifnlr1*^{-/-} (double-receptor knockout) AECs were seeded at 10^4 cells per transwell and cell numbers were counted following 5 days of IFN

treatment. All data are representative of at least four independent experiments ($n = 3-9$). Data are shown as mean $\pm$ SEM and statistical significance was assessed by one-way (A and B) or two-way (C) ANOVA with Dunnett's post-test. ns, not significant ($P > 0.05$); $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $****P \leq 0.0001$.

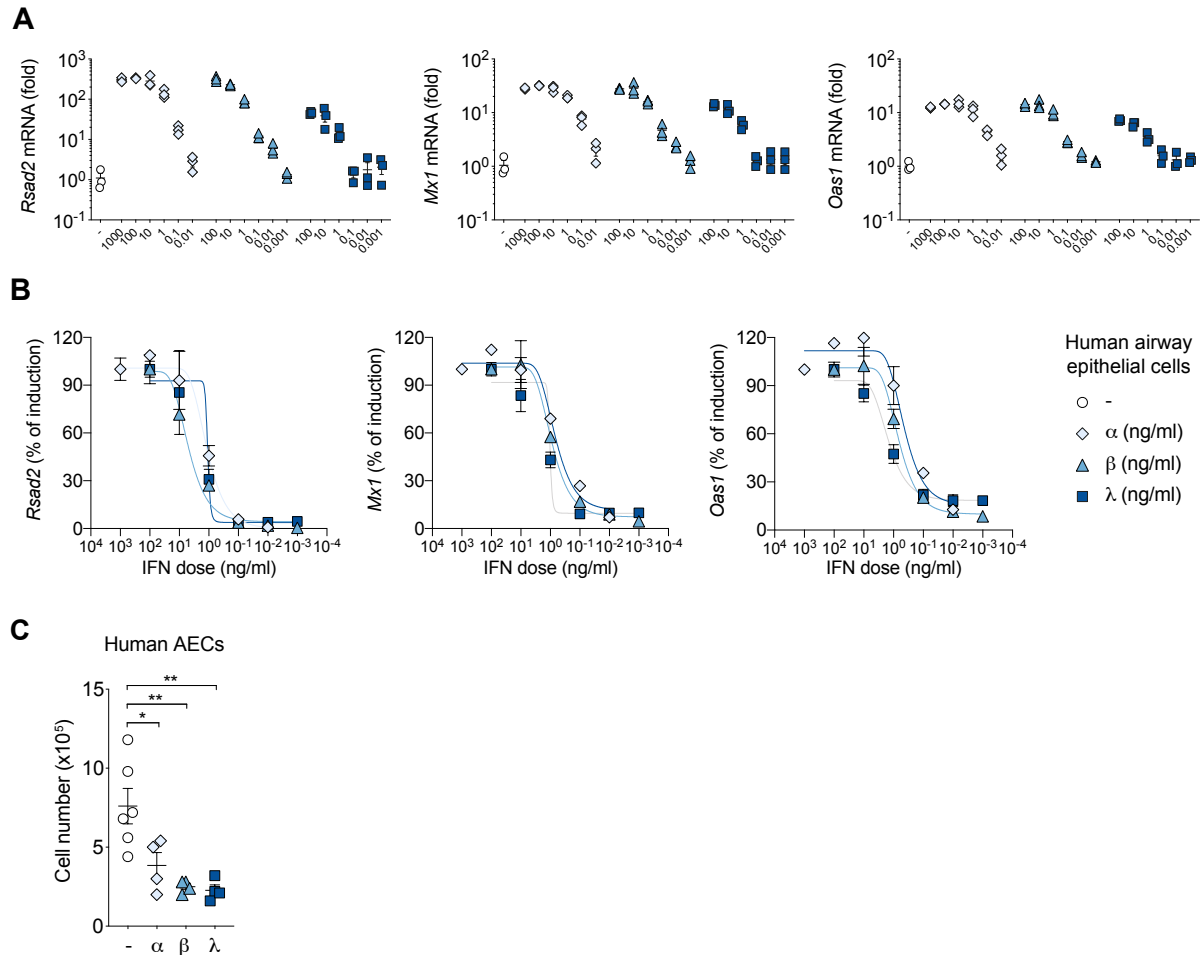


Figure 16. Type I and III IFNs disrupt the growth of primary human AEC cultures.

(A) Primary human AEC cultures (normal human bronchial epithelial cells) were treated for 4 hours at indicates concentrations with h-IFN- α , h-IFN- β , and h-IFN- λ subtypes (used for human. AEC in vitro experiments) at the indicated concentrations. In IFN and mock treated AECs, mRNA expression of indicated ISGs was assessed by qPCR. (B) Sigmoidal, 4PL, X is log(concentration) dose-response curves were generated for each IFN subtype, relative to ISG expression at the highest IFN concentration. ($n = 3$). (C) Primary human AECs were with treated with IFN for 5 days and cells were counted. All data are representative of at least two independent experiments. Data are shown as mean $\pm$ SEM and statistical significance was assessed by one-way ANOVA with Dunnett's post-test. $*P \leq 0.05$, $**P \leq 0.01$.

4.3 IFNs mediate disruption of AEC growth via blocking of cell cycle progression

IFNs are known inducers of proapoptotic and antiproliferative mediators which disrupt cellular growth (Parker, Rautela & Hertzog, 2016). To measure apoptosis and effects on cell cycle, we analysed murine AEC cultures treated with IFNs for 5 days by flow cytometry. Consistent with the observed reduction in cell number, IFN- β or - λ treatment resulted in a significant increase in the frequency of apoptotic or necrotic cells (Annexin V⁺/TO-PRO-3⁺) (Fig. 17A and B). We also observed a greater than 50% reduction in the number of actively proliferating cells, as measured by incorporation of the thymidine analogue EdU. By contrast, IFN- α treatment had only a modest effect (Fig. 18A and B). These findings were confirmed by measuring CFSE dilution (Fig. 18C and D, left panels). Interestingly, IFN-mediated antiproliferative effects were only observed in highly proliferative AEC cultures, as treating established cultures (days 7 to 12 post ALI exposure) that have a substantially reduced rate of turnover (approximately 20% in untreated controls) had no effect (Fig. 18C and D, right panels). Consistently, IFN treatment on established AEC cultures had no effect on cell number (Fig. 18E). Thus, IFNs appear to actively disrupt cell cycle progression without killing quiescent cells. Therefore, the increase in apoptosis observed may occur as a result of failed progression through the cell cycle following IFN treatment, as seen previously (Subramaniam *et al.*, 1998).

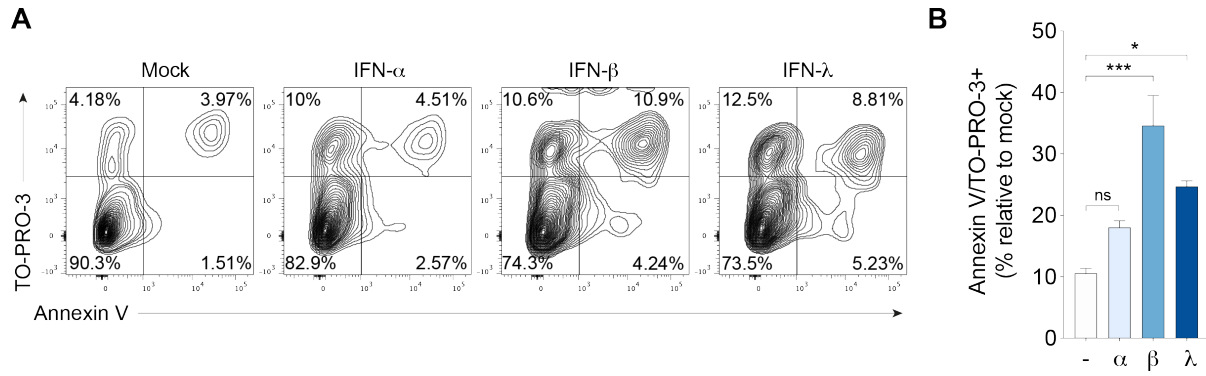


Figure 17. IFNs induce apoptosis in regenerating murine AECs.

AECs were treated for 5 days with IFN subtypes or media control and the frequency of apoptotic or necrotic AECs (Annexin-V⁺TO-PRO-3⁺) were assessed by flow cytometry. All data are representative of at least three independent experiments ($n = 3$). Data are shown as mean $\pm$ SEM and statistical significance was assessed by one-way ANOVA with Dunnett's post-test. ns, not significant ($P > 0.05$); * $P \leq 0.05$, *** $P \leq 0.001$.

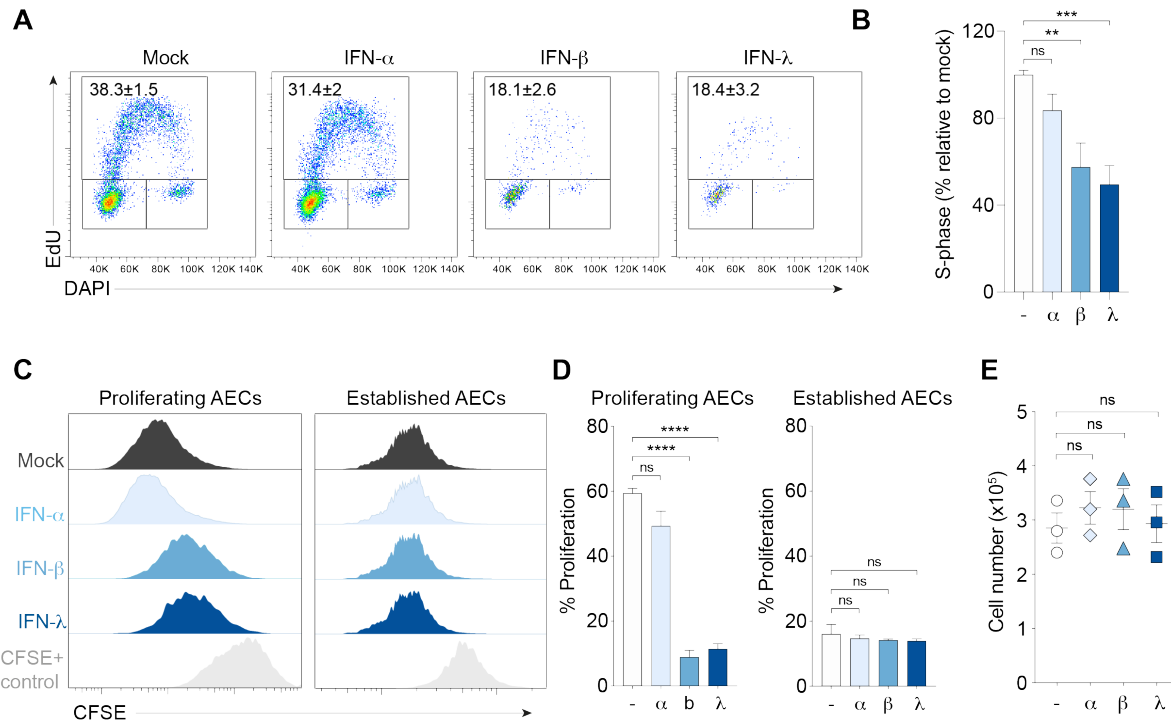


Figure 18. IFN-β and IFN-λ disrupt cell proliferation in regenerating AECs.

(A and B) Murine AECs were treated for 5 days with IFN subtypes or media control, and proliferation was quantified by measuring incorporation of the thymidine analogue EdU in diving cells. (C to E) Proliferating AECs were treated with IFNs from day -2 to +3 ALI exposure, and established AECs were treated with IFNs from day 7 to 12 ALI exposure. AECs were labelled with CFSE for the final 24 hours of culture, and proliferation was measured by quantifying CFSE dilution by flow cytometry. All data are representative of at least four independent experiments ($n = 3-6$). Data are shown as mean $\pm$ SEM and statistical significance was assessed by one-way ANOVA with Dunnett's post-test. ns, not significant ($P > 0.05$); ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

4.4 IFN- λ disrupts AEC differentiation and multiciliogenesis

In vivo, we observed that IFN- λ signalling interferes with multiciliogenesis pathways and reduces the capacity for basal cell differentiation in conducting airways during epithelial repair after influenza virus infection (Fig. 10 and 11). To compare IFN- α , IFN- β , and IFN- λ subtypes in their ability to affect AEC differentiation specifically, we initiated IFN treatment later during the course of AEC growth throughout exposure to an ALI—the time in which AEC differentiation is induced (Fig. 19A)—and measured expression of genes related to epithelial cell differentiation. Expression of the basal cell marker *Krt5* remained unchanged, or increased by IFN- λ treatment, suggesting maintenance of stemness (Fig. 19B). IFN- β treatment, and to a greater extent IFN- λ treatment, significantly reduced the expression of genes pertaining to multiciliated (*Mcidas*, *Ccno*) and secretory cell (*Muc5AC*, *Scb1a1*) differentiation (Fig. 19C). We also observed a significant reduction in the number of ciliated cells in AEC cultures (acetylated α -tubulin⁺) following IFN- λ treatment, but not with IFN- α or IFN- β (Fig. 19D and E). Thus, only IFN- λ reduces induction of genes important for AEC multiciliogenesis to an extent that effectively blocks the differentiation of multiciliated cells. Similar to observed IFN-inducible antiproliferative effects, these results collectively show that IFN- λ most potently disrupts AEC differentiation.

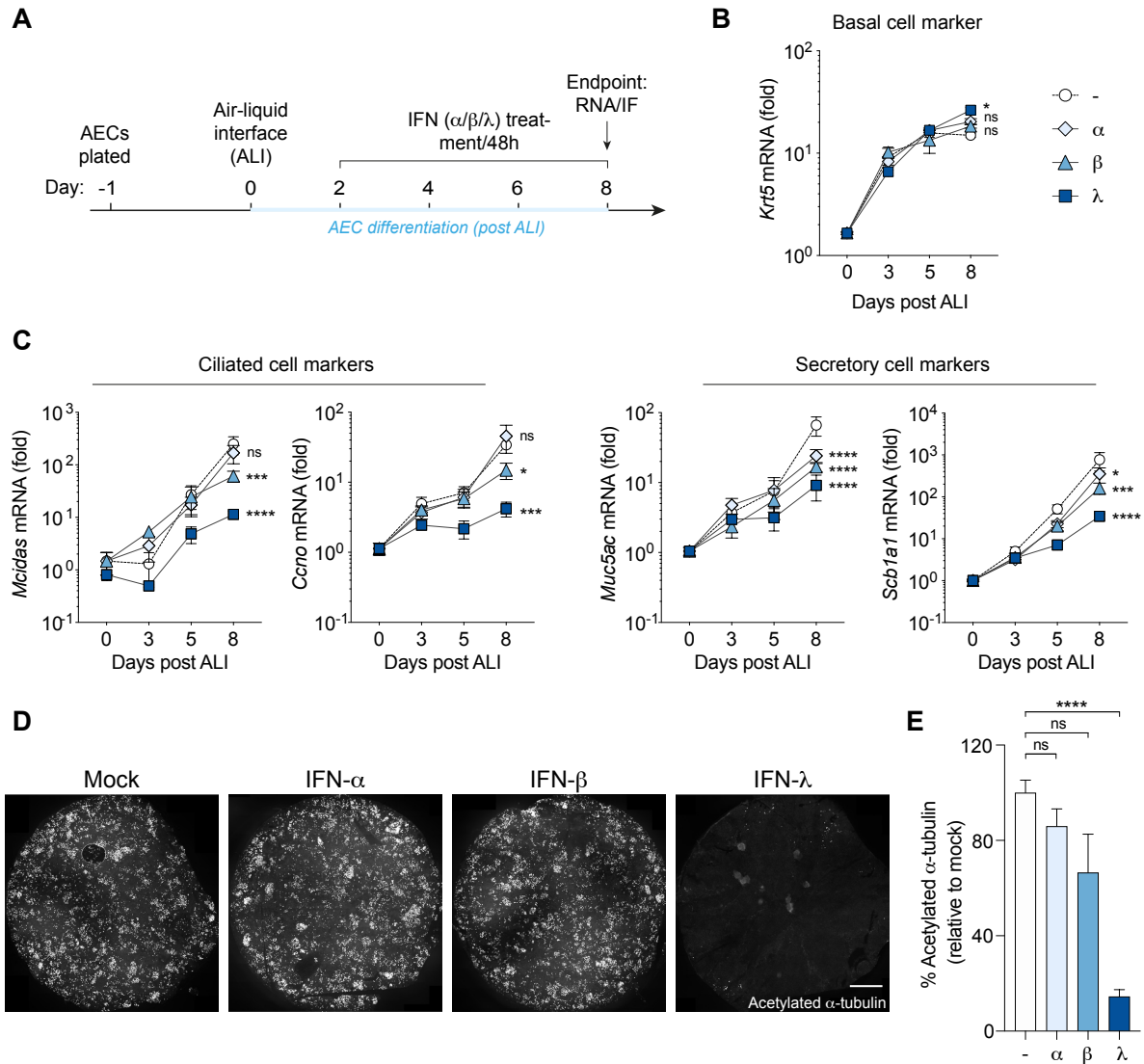


Figure 19. IFN- λ impairs AEC differentiation and the development of multiciliated cells.

(A) Schematic diagram for IFN treatment during murine AEC differentiation in ALI exposure. (B to E) AECs were treated for 6 days during ALI exposure (B and C) mRNA expression of indicated epithelial cell markers were determined by qPCR, and (D and E) multiciliated cells were measured by immunofluorescence staining of acetylated α -tubulin. Whole transwell membrane scans are shown. Scale bar represents 1 mm. All data are representative of at least three independent experiments ($n = 3$). Data are shown as mean $\pm$ SEM and statistical significance was assessed by two-way ANOVA with Dunnett's post-test (B and C) or one-way ANOVA with Dunnett's post-test. ns, not significant ($P > 0.05$); * $P \leq 0.05$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

4.5 Prolonged IFN signalling induces antiproliferative and proapoptotic pathways in regenerating AECs

To understand how IFN signalling disrupts AEC growth, we performed RNA sequencing on IFN-treated AEC cultures (bioinformatics analyses were performed by Miriam Llorian Sopena, Francis Crick Institute). AEC cultures were treated with IFN subtypes for: 4 hours; 2 days; 3 days; or 5 days (Fig. 20A). 4-hours of IFN treatment resulted in similar differential gene expression, confirming equal subtype dosage based on previous titrations (Fig. 14A and B). Principal component analysis (PCA) clustered 4-hour IFN treated samples together regardless of subtype away from their respective mock on principal component 1 (PC1) (Fig. 20C). This is consistent with previous findings, which show that ISGs are induced rapidly following IFN–receptor binding (Sadler & Williams, 2008; Wack, Terczyńska-Dyla & Hartmann, 2015). Using GeneSpring, we analysed differentially expressed genes contributing to the variance on PC1 and PC2 by gene ontology (GO) analysis. We confirmed that genes contributing to the variance on PC1 (46% of total variance) are involved in IFN signalling pathways (Fig. 20D, upper table). Notably, untreated AEC control samples clustered depending on the length of culture growth and ALI exposure along PC2 (35% of total variance) (Fig. 20C). GO analysis confirmed genes underlying this variance are involved in pathways of epithelial cell development and differentiation (Fig. 20D). It is also important to note, that 4-hour and 5-day IFN treated cultures shared the same experiment endpoint, and therefore share the same untreated controls (Fig. 20A and C).

AECs were also treated for 2, 3, or 5 days to understand the effects of prolonged IFN treatment in growing AEC cultures (Fig. 20A). 5 days of IFN- β or IFN- λ treatment clustered samples away from corresponding untreated controls on both PC1 and PC2, indicating maintained ISG induction (PC1) and a block of AEC proliferation and development (PC2) (Fig. 20C). Conversely, the response induced by IFN- α was significantly reduced after just 2 days of treatment, consistent with previously reported findings (Klinkhammer *et al.*, 2018). Factors that determine the antiproliferative differential of IFN subtypes include: ligand-receptor binding affinity, length of stimulation, receptor density, and negative feedback control, resulting in stronger and more sustained gene induction following treatment with IFN- β and IFN- λ subtypes (Jaitin *et al.*, 2006; Moraga *et al.*, 2009; Schreiber, 2017; François-Newton *et al.*, 2011, 2012; Schreiber & Piehler, 2015).

Ingenuity pathway analysis (IPA) of differentially expressed genes following prolonged IFN treatment (2, 3, and 5 days) revealed induction of pathways regulating cell cycle and cell death following prolonged IFN treatment, most significantly induced by IFN- λ across all timepoints (Fig. 21A). Predicted upstream transcriptional regulators identified typical regulators of IFN function, including interferon regulatory factors (IRFs) and signal transducer and activator of transcription (STAT) proteins, in addition to regulators of cell cycle (Fig. 21B). We identified the tumour suppressor protein p53 as a top candidate regulating IFN-inducible antiproliferative effects, owing to its significant predicted activity following prolonged IFN- β and - λ treatment in AECs (Fig. 3D). p53 has previously been shown to directly regulate IFN- α/β antitumour responses (Takaoka *et al.*, 2003). GSEA identified IFN-mediated induction of the p53 pathway (Fig. 21C), and we identified induction of p53-regulated downstream targets in expression data (Fig. 21D).

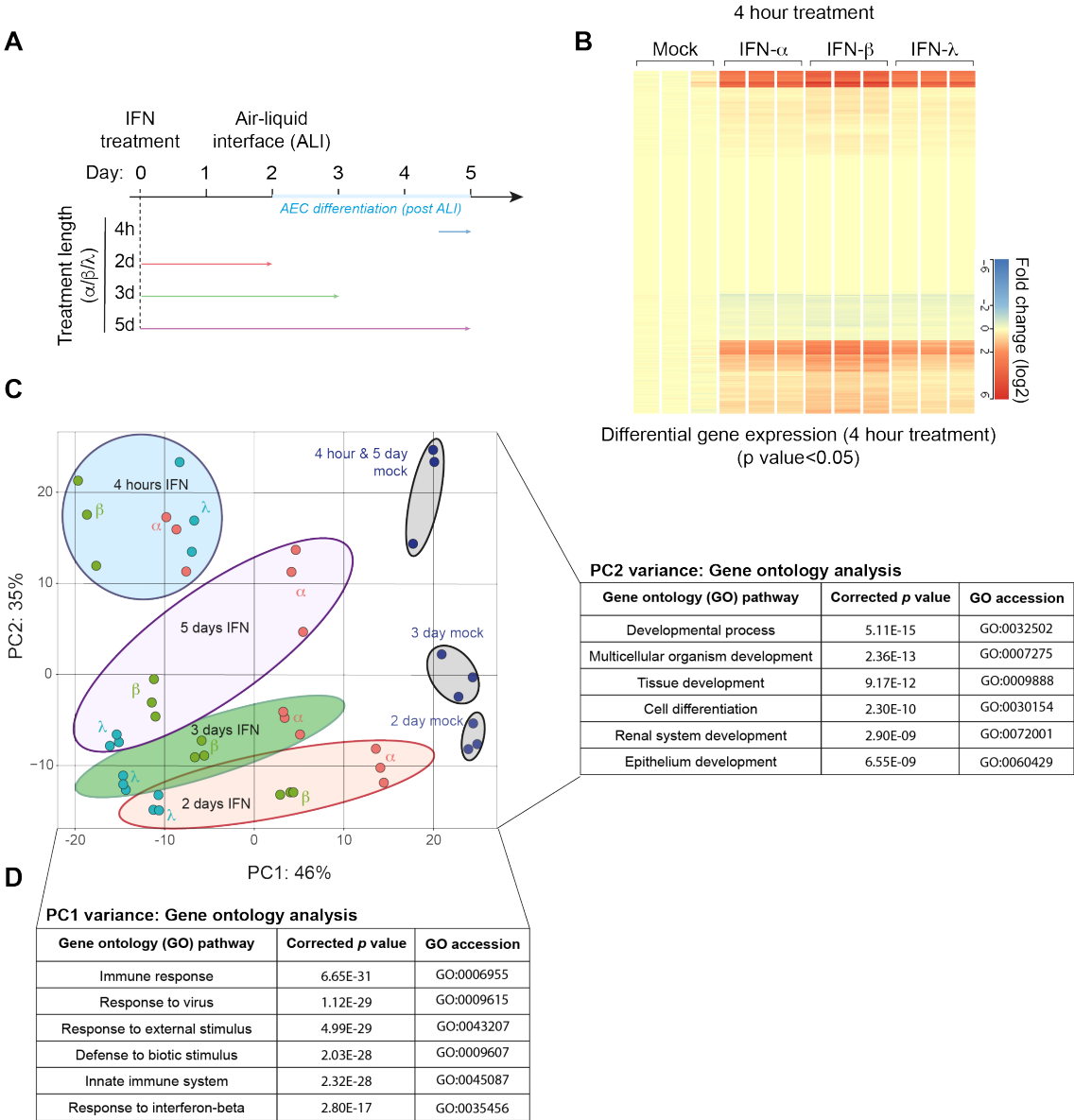


Figure 20. Type I and III IFNs activate antiproliferative and cell death pathways in regenerating AECs.

(A) Schematic diagram for IFN treatment of murine AECs (4 hours, 2 days, 3 days, and 5 day) for RNA sequencing analysis. (B) Heatmap for differentially expressed genes in AECs (one-way ANOVA with Benjamini–Hochberg correction, $P < 0.05$) following 4 hours of IFN treatment, relative to untreated controls. (C) PCA plot of IFN and control treated AECs. (D) Gene ontology analysis (GeneSpring) of the genes contributing to variance in PC1 and PC2.

4.6 IFNs block AEC proliferation and differentiation via induction of p53-mediated downstream pathways

To confirm the role of p53 in IFN-inducible antiproliferative effects, we utilized *Tp53*^{-/-} AEC cultures. IFN treatment had no effect on *Tp53*^{-/-} AEC number or proliferation following IFN treatment (Fig. 22A and B), and IFN- λ had a much-reduced negative effect on multiciliogenesis in differentiating *Tp53*^{-/-} cultures, compared to its effect on WT and *Tp53*^{+/-} cultures (Fig. 22C). In addition, IFN-mediated induction of antiproliferative downstream p53 target genes *Gadd45g* and *Dusp5* (Smith *et al.*, 1994; Ueda, Arakawa & Nakamura, 2003) was p53-dependent, as IFNs had no such effect in *Tp53*^{-/-} AEC cultures (Fig. 22D).

We next examined whether IFNs regulate p53 activity in epithelial cells during lung repair in vivo. To study specific effects of type I and III IFN specifically in the lung epithelium, we once again generated *Ifnar1*^{-/-} $\rightarrow$ WT BM chimeric mice treatment, or depleted neutrophils in WT mice with anti-Ly6G, respectively. In agreement with in vitro observations, IFN- β and IFN- λ , but not IFN- α , significantly upregulated p53 expression in repairing lung epithelial cells (Fig. 22E and F). Thus, IFN- β and - λ mediate antiproliferative effects in AECs via induction of p53. Additionally, our RNA-seq analyses confirm the potent antiproliferative activity of IFN- λ , compared to type I IFN subtypes.

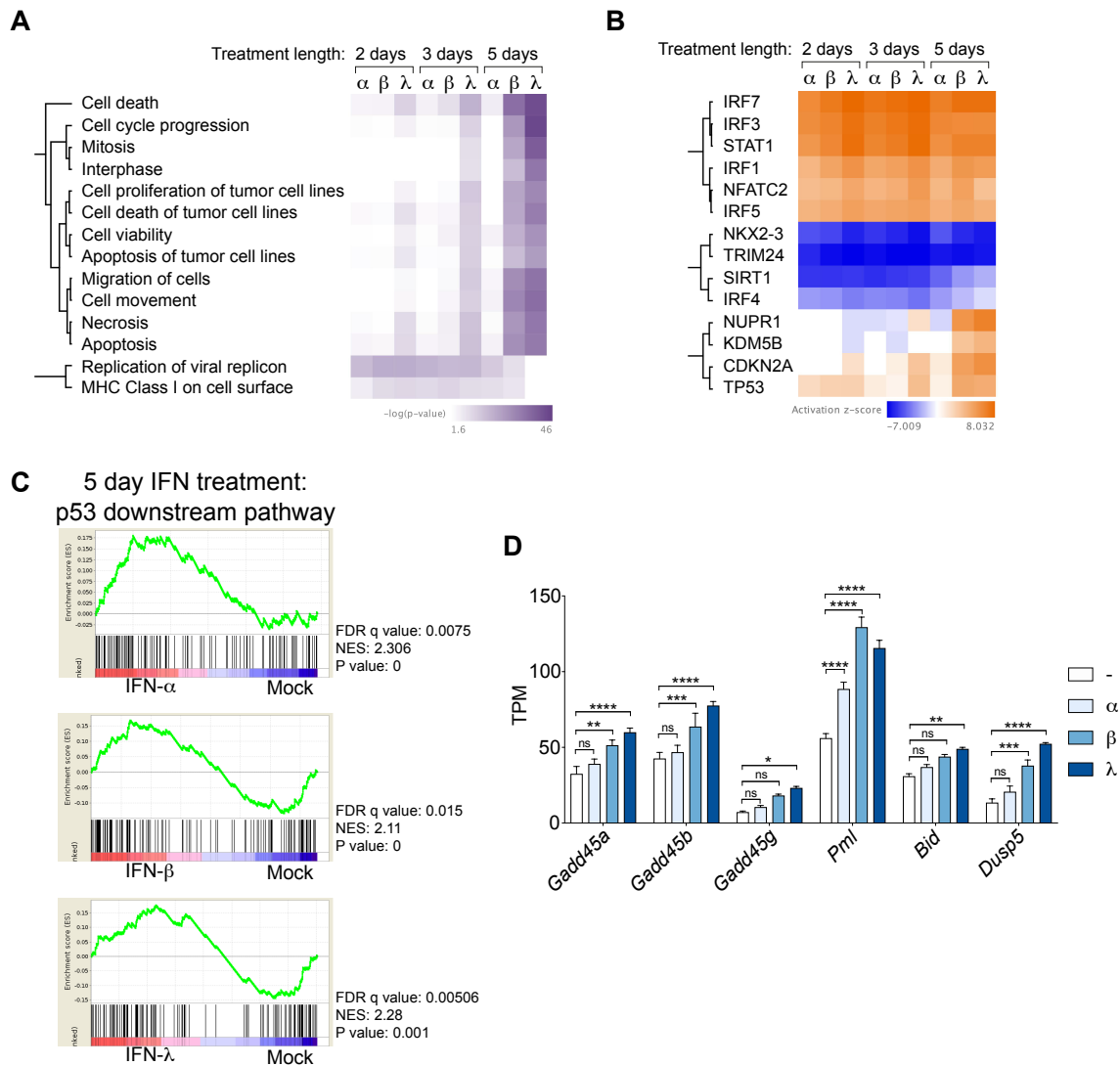


Figure 21. IFN- β and IFN- λ activate p53-mediated signalling pathways.

(A) Differentially expressed genes following prolonged IFN treatment (2, 3, and 5 days) were analysed using Ingenuity Pathway Analysis (IPA) for Canonical Pathway induction. Heatmap of comparisons between IFN treatment and the respective mock at each time point (fold change>1.5, one-way ANOVA with Benjamini–Hochberg correction, $P<0.05$). **(B)** IPA generated predicted upstream transcriptional regulators in prolonged IFN treated cultures (relative to the respective mock). **(C)** GSEA plots of 5-day IFN treated cultures relative to mock. **(D)** Transcripts per million (TPM) values for indicated p53-related genes following 5 days of IFN treatment. Data are shown as mean $\pm$ SEM and statistical significance was assessed by two-way ANOVA with Dunnett’s post-test ($n = 3$). ns, not significant ($P>0.05$); * $P\leq 0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

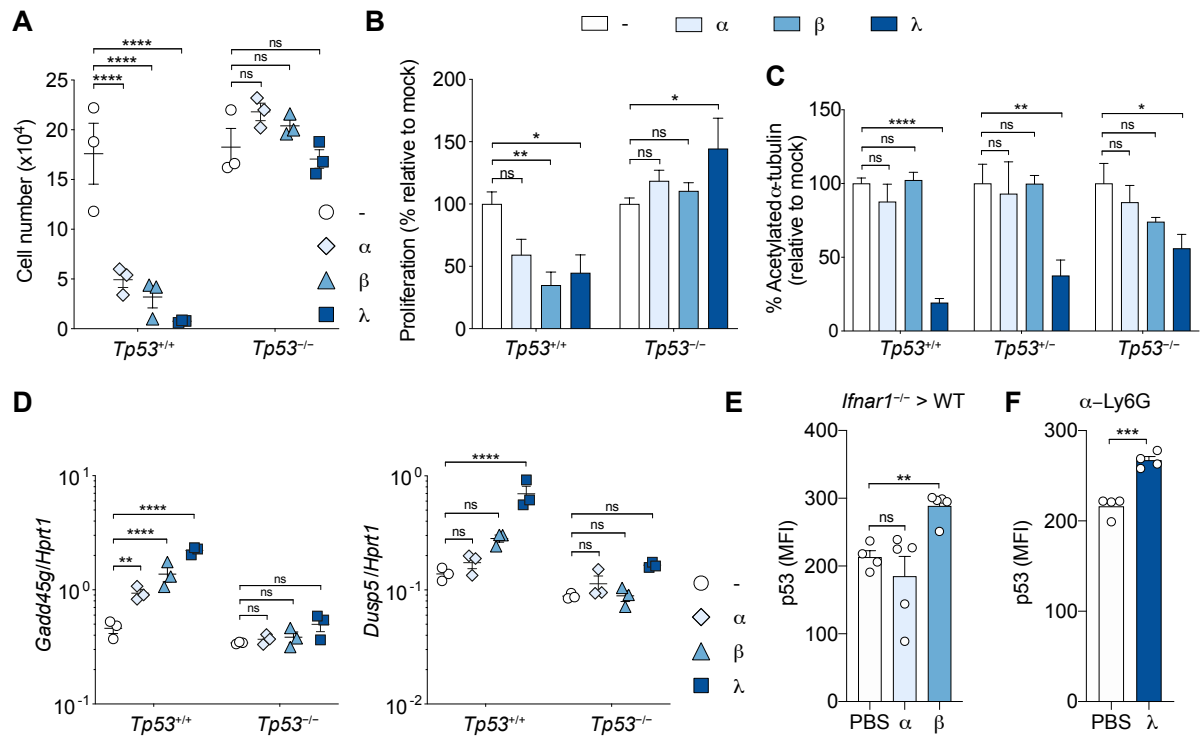


Figure 22. AEC growth inhibition mediated by IFNs is p53-dependent.

(A to D) $Tp53^{+/+}$ and $Tp53^{-/-}$ murine AEC cultures were treated for 5 days with IFN and cell numbers (A), proliferation determined by CFSE dilution (B), acetylated α -tubulin+ multiciliated cells (also $Tp53^{+/-}$) (C), and p53-related gene expression (D) were measured. (E and F) Influenza virus infected $Ifnar1^{-/-} \rightarrow WT$ BM chimeric mice (E) and α Ly6G treated mice (for neutrophil depletion) (F) were treated with IFNs every 24 hours from days 6 to 10 post infection. AT2 cells (EpCam⁺MHCII⁺CD49f^{low}) were analysed for p53 mean fluorescence intensity (MFI) on day 11 post infection by flow cytometry. All data are representative of at least two independent experiments (n = 3–5). Data are shown as mean $\pm$ SEM and statistical significance was assessed by two-way (A to D) or one-way (E) ANOVA with Dunnett's post-test, or unpaired two-tailed Student's *t* test (F). ns, not significant ($P > 0.05$); * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

4.7 Discussion

By studying the responses of IFN- α , IFN- β , and IFN- λ subtypes at equal biological potencies, we identified the enhanced potential of IFN- β and IFN- λ subtypes, compared to IFN- α , in impairing the growth of regenerating primary AECs. Comprehensive transcriptomic analysis following short and prolonged IFN treatments in AEC cultures revealed a markedly enhanced prolongation of signal induction primarily following IFN- λ treatment, but also IFN- β (Fig. 20 and 21). We believe this is best defined by the PCA, which clearly shows a marked differential in the profiles of prolonged IFN- α treated AECs, despite the similar effect of all three IFN subtypes after a 4-hour treatment (Fig. 20C). GO analysis confirmed the sustained activation of IFN pathways only following prolonged IFN- β or IFN- λ treatment, which coincided with a failure of these cells in continuing with proper epithelial development/regeneration (Fig. 20D). This was further made evident by pathway analysis of expression data, which highlighted the exceptional antiproliferative capacity of IFN- λ in particular at inducing antiproliferative and cell death pathways, even after following only 2 days of IFN treatment (Fig. 21C). It is important to note, that this data was collected using primary AEC cultures derived from trachea progenitor cells. To provide additional cross-comparisons with *in vivo* findings, it would be interesting to repeat kinetic and mechanistic studies performed here on primary alveolar organoid cultures.

Mechanistically, we identified the tumour suppressor protein p53 as a downstream regulator of IFN-induced antiproliferative effects. In primary AECs, we observed no difference between WT and *Tp53*^{-/-} cultures in expression of downstream effector genes (including *Gadd45g* and *Dusp5*) (Fig. 22D), indicating that these p53 pathways are inactive in AECs under normal conditions. In the first publication reporting p53 induction by type I IFN, it was additionally shown that p53 *activation* and induction of growth inhibitory pathways following IFN treatment (but not direct-induction of p53 mRNA and protein) only occurred in virally infected MEFs, not following IFN- β treatment alone in normal growing MEFs (Takaoka *et al.*, 2003). These findings are in contradiction to our observations, as we show that IFN-dependent p53-dependent growth inhibitory effects occur following treatment of normal growing primary AECs (Fig. 15, 16, and 22), independently from viral infection. This discrepancy is likely due to the differing IFN treatment lengths, with our observations recorded following a prolonged 5-day treatment, as opposed to 4 hours in MEFs (Takaoka *et al.*, 2003). It is also important to note that we did not measure p53 activation or protein induction *in vitro*, only pathway

activation and expression of downstream regulators (Fig. 21), so we cannot be sure of the dynamics of IFN-regulated p53 activity in our system.

Nonetheless, despite Takaoka *et al.* not measuring the effects in normal cells following prolonged IFN treatment, their findings and discussion raise an important point regarding IFN-mediated induction of growth inhibitory pathways: IFN-dependent p53 growth inhibitory responses (and possibly p53-independent growth inhibitory responses) are enhanced by stress signals, such as those induced by viral infection or tumorigenesis. This suggests that signal interplay provides an additional regulatory layer for potent induction of these pathways. A requirement may exist for a prolonged IFN stimulus (Jaitin *et al.*, 2006) in addition to signals induced by cellular stress, for instance following viral infection, tissue damage, or tumorigenesis. Selective pressures would arguably promote this, to only allow proper activation of these potentially harmful pathways in certain contexts. It would be interesting to measure whether the antiproliferative effects of IFNs in our in vitro AEC system would be augmented following influenza virus infection. Conversely, our in vivo findings may be dampened in a sterile model of lung damage. We also found that IFNs lack growth inhibitory function in less-proliferative, fully-established AEC cultures, even after a 5-day treatment (Fig. 18C to E). Therefore, we speculate that these pathways are most/only effective during active cell division, and also that in our system, induction of apoptosis occurs following a failure of cell cycle progression.

Consistent with our findings, type I IFN treatment induces rapid yet short-lived ISG induction, whereas IFN- λ induces a delayed but sustained response (Bolen *et al.*, 2014; Dellgren *et al.*, 2009; Dickensheets *et al.*, 2013; Egli *et al.*, 2014; Forero *et al.*, 2019). Differing kinetics are explained, at least in part, by the degree of negative regulation at the receptor level. IFN-induced USP18 desensitises the response to further IFN- α treatment, but not IFN- β or IFN- λ (François-Newton *et al.*, 2011). It is thought that USP18 destabilises weak IFN- α -IFNAR binding, whilst IFN- β and IFN- λ are unaffected due to tight receptor binding and lack of a negative regulatory effect, respectively (Blumer *et al.*, 2017; François-Newton *et al.*, 2012). In agreement, IFN- λ induces prolonged STAT1 and STAT2 phosphorylation relative to IFN- α , enabling maintained ISG expression (Maher 2008). This likely is responsible for the reduced induction of growth inhibitory pathways by IFN- α in vitro, including p53-dependent pathways, as well as p53 induction in repairing AT2 cells in vivo (Fig. 21 and 22). Activation of antiproliferative pathways by IFN is thought to be done in a transcription-dependent manner, requiring ISGF3 components IRF9 (Tsuno *et al.*, 2009) and transcriptionally active STAT1

(Chin *et al.*, 1996; Bromberg *et al.*, 1996). In addition, IRF9 is essential for IFN-dependent p53 induction, and an anti-STAT2 ChIP confirmed ISGF3 binding to *Tp53* ISRE sequences following IFN- β treatment in MEFs (Takaoka *et al.*, 2003).

In agreement with our in vitro observations, we found that IFN- β and IFN- λ , but not IFN- α , signalling specifically in the epithelium induced p53 expression in proliferating AT2 cells in vivo during influenza recovery (Fig. 22E and F). Interestingly, p53 has been shown to activate IFN-mediated antiviral immunity following Sendai virus infection in vivo, through transcriptional upregulation of IRF9 (Muñoz-Fontela *et al.*, 2008). In a follow-up study by the same group, it was shown that p53 deficient mice had increased viral titres and susceptibility to influenza virus infection compared to WT controls, attributed to impaired IFN and T cell driven antiviral protection (Munoz-Fontela *et al.*, 2011). This suggests a feed-forward loop driven by IFN and p53 interplay to promote antiviral immunity early during protection. However, our findings suggest elongation of this interplay into the window of lung repair may exacerbate lung pathology. To directly link IFN-induced p53 to inhibition of lung repair in vivo in our model, it would be interesting to inhibit p53 in WT and *Ifnlr1*^{-/-} mice during the recovery phase of infection, to study effects on lung regeneration independent to p53-dependent antiviral functions.

In contradiction with our findings here, an emerging body of literature outlines a role for IFN- λ in promoting barrier integrity. These are independent to the well-known indirect barrier integrity promoting effects as a result of antiviral protection (Broggi, Granucci & Zanoni, 2020). Regarding direct effects in intestinal epithelial cells, IFN- λ treatment in vitro was shown to enhance barrier integrity, measured by TEER (Odendall, Voak & Kagan, 2017), and promote intestinal organoid proliferation (Chiriac *et al.*, 2017). In addition, type I (Daniels *et al.*, 2014; Kraus *et al.*, 2008) and III (Lazear *et al.*, 2015) IFN enhances the barrier resistance in primary brain microvasculature endothelial cell cultures, which is thought to provide protection against neuroinvasive West Nile virus in vivo (Lazear *et al.*, 2015). Mechanistically, it is thought that a short-term IFN stimulus promotes the expression of tight junction proteins (Daniels *et al.*, 2014). These inconsistencies with our data are again likely explained by the differing lengths of IFN treatment used, with all studies indicated studying the effects of IFN following a maximal 24-hour treatment, with most observing effects within the first 4 hours. In addition, low *Ifnlr1* expression on endothelial cells (Lazear *et al.*, 2015) might reduce the capacity for the induction of growth inhibitory pathways, which are dependent on high receptor

density and a strong stimulus (Moraga *et al.*, 2009; Kalie *et al.*, 2007, 2008; Thomas *et al.*, 2011).

An additional type I and III IFN subtype differential has been observed with regard to the induction of proinflammatory chemokines, such as CXCL10. RNA sequencing analysis revealed comparable antiviral ISG induction in IFN- α and IFN- λ treated neutrophils, yet IFN- α uniquely drove the expression of various proinflammatory chemokines (Galani *et al.*, 2017). This was corroborated by an independent group, which suggested that the enhanced ability of type I IFNs to promote proinflammatory gene expression was due to increased expression of IRF1 (Forero *et al.*, 2019). These findings suggest a molecular mechanism for the promotion of proinflammatory IFN- α/β responses (Davidson *et al.*, 2014, 2016; Galani *et al.*, 2017), in addition to immunomodulation via ubiquitous IFNAR signalling. However, whilst the differential was clear in transformed human hepatocytes, attributed to a reduced expression of *Ifnlr1* (Forero *et al.*, 2019), findings in intestinal epithelial cells (Forero *et al.*, 2019) and neutrophils (Galani *et al.*, 2017) were observed using uncontrolled IFN concentrations, and only following a 24-hour treatment. It would be interesting to observe whether the enhanced proinflammatory chemokine induction following type I IFN treatment persisted after a longer stimulus, as we observe for IFN- λ -mediated activation of antiproliferative pathways. IFN- λ has also recently been shown to suppress intestinal inflammation by non-translational regulation, through Jak2-dependent ROS inhibition (Broggi *et al.*, 2017). However, this pathway operates independent to the activity of STAT proteins, and therefore is unlikely to regulate IFN-antiproliferative function, which is STAT1-dependent (Chin *et al.*, 1996; Bromberg *et al.*, 1996).

Finally, we show in this chapter the effects of IFN signalling on AEC differentiation, using our in vitro primary AEC culture system. Consistent with the improvement in airway cell differentiation during lung repair in *Ifnlr1*^{-/-} mice (Fig. 11), IFN- λ treatment during exposure to ALI suppressed genes important in multiciliogenesis and secretory cell differentiation (Fig. 19C), which corresponds with a reduction in the proportion of multiciliated cells (Fig. 19D). Inhibition of multiciliogenesis by IFN- λ signalling was partially rescued in *Tp53*^{-/-} AEC cultures (Fig. 19C). Antiproliferative effects exerted by IFN- λ signalling may indirectly disrupt multiciliogenesis by reducing progenitor cell numbers, which give rise to multiciliated cells (Rock *et al.*, 2009). In contradiction to this, is the increased expression of basal cell marker *Krt5*, inverse to the decrease in ciliated cell markers (Fig. 22B and C). This suggests an IFN-induced maintenance of stemness in progenitors. In addition, IFN treatments were administered

late during the AEC culture lifespan for differentiation experiments (days 2 to 8 post ALI onset), when growth inhibitory effects are less potent (Fig. 22C to E). An alternative indirect mechanism is through p53-dependent modulation of cell cycle regulatory proteins independent to growth inhibition. Development of multiciliated cells requires a tight coordination of mitotic proteins to allow cell cycle exit and centriole amplification to produce cilia-supporting basal bodies (Quarmby & Parker, 2005; Al Jord *et al.*, 2017). p53 distortion of mitotic regulatory proteins, for instance CDKs and CKIs, may disrupt the strict cellular requirements for multiciliogenesis.

In chapters 3 and 4, we describe a mechanism by which IFNs exacerbate influenza lung pathology through the induction of signals specifically in epithelial cells, which consequently, are detrimental for lung tissue regeneration. In the following and final results chapter of this thesis, we explore the relationship between IFN and the aryl hydrocarbon receptor, specifically in the context of influenza disease tolerance.

Chapter 5. Results 3:

Constitutive aryl hydrocarbon receptor signalling promotes disease tolerance following influenza virus infection

5.1 Introduction

Disease outcome following respiratory virus infection is dependent on host antiviral resistance and disease tolerance. For the latter, tolerating the response to an infection serves as a host defence strategy to prevent tissue damage, independently from anti-pathogen mechanisms. Failure to establish disease tolerance can have detrimental consequences for the host, including immunopathology and resultant tissue damage. This is reflected by reported inconsistencies in the correlation (or lack thereof) between viral load and disease severity, including influenza and COVID-19 patients (Granados *et al.*, 2017; Gorini da Veiga, Kretzmann & Correa, 2012; Zheng *et al.*, 2020; To *et al.*, 2020; Lavezzo *et al.*, 2020). The fact that some individuals experience severe disease independently of excessive viral replication indicates alternate mechanisms that compromise host fitness, such as immunopathology or comorbidities.

The AHR coordinates a variety of host responses following environmental and endogenous ligand sensing. This includes prominent immunological functions (Stockinger *et al.*, 2014), with recent evidence highlighting a role for the AHR in balancing proinflammatory cytokine responses and promoting disease tolerance (Kimura *et al.*, 2009; Rothhammer *et al.*, 2018; Bessede *et al.*, 2014; Shinde *et al.*, 2018). AHR functions are well characterised in the intestine, owing to a rich source of natural AHR ligands derived from microbial and dietary compounds, including tryptophan metabolites (Rothhammer & Quintana, 2019). In particular, AHR responds to physiological ligands to induce production of IL-22 in immune cells, thus promoting barrier resistance and protection in response to intestinal pathogen (Qiu *et al.*, 2012; Kiss *et al.*, 2011; Basu *et al.*, 2012).

Owing to wide expression in the immune system and barrier epithelial surfaces, including the lung (Frericks, Meissner & Esser, 2007), the AHR is optimally positioned to balance immunological and proinflammatory pathways initiated following influenza virus infection. However, AHR-influenza studies have thus far have largely been focused on characterising responses to the particularly toxic xenobiotic, TCDD, also known as dioxin (Head & Lawrence, 2009), which essentially binds the AHR irreversibly (Bradfield, Kende &

Poland, 1988). As discussed earlier, TCDD significantly increases influenza susceptibility in mice, characterised by excessive immunopathology, tissue damage, and pulmonary neutrophilia (Vorderstrasse, Bohn & Lawrence, 2003; Luebke *et al.*, 2002). An important study recently identified a physiological role for the AHR in negatively regulating IFN responses following viral infection (Yamada *et al.*, 2016). However, the importance of endogenous AHR activity in influenza responses is not well understood. Microbial, dietary, and tryptophan-related metabolic signals, have all been shown to alter influenza disease outcome (Abt *et al.*, 2012; Steed *et al.*, 2017; Bradley *et al.*, 2019; Trompette *et al.*, 2018; Goldberg *et al.*, 2019; Gaelings *et al.*, 2016; Fox *et al.*, 2013). Therefore, it is possible that these natural AHR ligand-rich sources also influence influenza responses in an AHR-dependent manner.

Here, we sought to understand the role natural AHR ligands play in lung AHR function, specifically following influenza virus infection. Given the toxic effects induced by TCDD in an AHR-dependent, and importantly, -independent manner (Bunger *et al.*, 2003; Park *et al.*, 2005; Tijet *et al.*, 2006; Patel *et al.*, 2009), we wanted to examine the effects of constitutive AHR activation by endogenously sourced physiological ligands. AHR ligand-binding triggers the induction of CYP family enzymes and their ligand-metabolising effects. This effectively results in autoregulatory feedback via the depletion of natural AHR ligands (Stockinger *et al.*, 2014; Ito *et al.*, 2007). We therefore amplified endogenous AHR signals using mice deficient for the three AHR-regulated CYP family enzymes: CYP1A1, CYP1A2, and CYP1B1, termed *Cyp1^{-/-}* mice (Dragin *et al.*, 2008). Using *Cyp1^{-/-}* mice, we aimed to study the effects of prolonged AHR activation by natural ligands on host immunity following influenza virus infection. Owing to the barrier promoting and anti-inflammatory effects attributed to physiological AHR function, we hypothesised that constitutive activation will promote disease tolerance, thus reducing pulmonary inflammation and tissue damage. This includes examining AHR-dependent effects on both IFN-dependent and IFN-independent immunopathology, including pathogenic immunomodulation (Davidson *et al.*, 2014; Channappanavar *et al.*, 2016), and epithelial-specific consequences on lung epithelial repair (as outlined in chapters 3 and 4).

5.2 AHR activation regulates innate antiviral responses in AECs

Initially, we aimed to understand whether AHR activation by endogenous ligands regulates immune signalling processes in the respiratory epithelium. We therefore employed whole genome expression analysis on steady-state WT and *Ahr*^{-/-} AEC cultures (bioinformatics analyses were performed by Probir Chakravarty, Francis Crick Institute). GSEA revealed *Ahr*^{-/-} AECs had increased expression of immune system and interferon signalling pathways (Fig. 23), indicating basal AHR functions in regulating innate antiviral responses in respiratory epithelial cells. Therefore, we speculated that treating AEC cultures with AHR agonists would augment endogenous AHR functions involved in innate responses to viral infection. It has previously been reported that AHR activation in MEFs by the tryptophan metabolites, FICZ and Kyn, suppresses IFN- β production following viral infection (Yamada *et al.*, 2016). To study effects in AECs, we treated control or influenza virus infected cultures with either the cancerogenic aromatic hydrocarbon, 3-MC, or the endogenous ligand FICZ. Both 3-MC (Fig. 24A) and FICZ (Fig. 24B) treatment strongly upregulated expression of the AHR target gene *Cyp1a1* (Fig. 24A and B, left panel). AHR activation resulted in a reduction of type I IFN (IFN- α and IFN- β) and IL-6 mRNA expression, but not type III IFNs (IFN- λ) (Fig. 24A and B). Therefore, AHR activation in AECs by either xenobiotics or physiological ligands suppresses IFN and proinflammatory cytokine production following influenza virus infection.

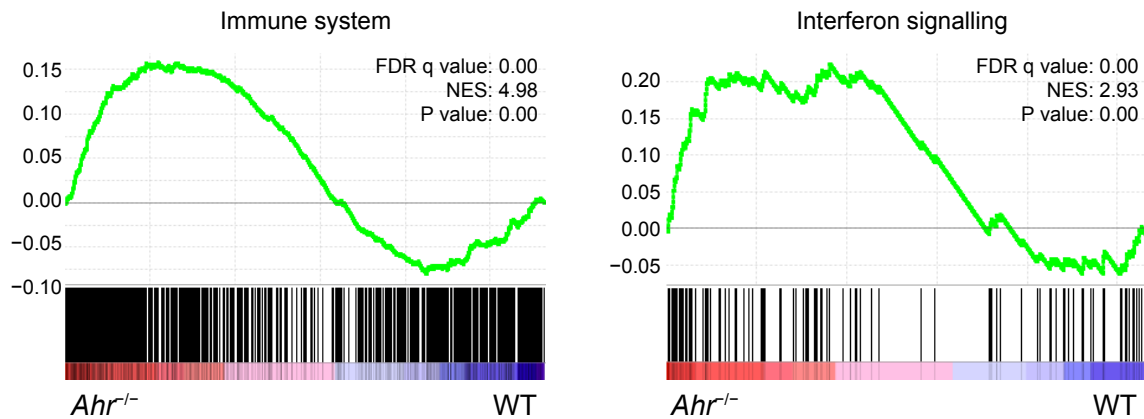


Figure 23. AHR regulates IFN and immune signalling pathways in steady state AEC cultures.

GSEA plots of RNA sequencing datasets from WT or *Ahr*^{-/-} murine fully differentiated (+day 8 ALI) AEC cultures (n = 3).

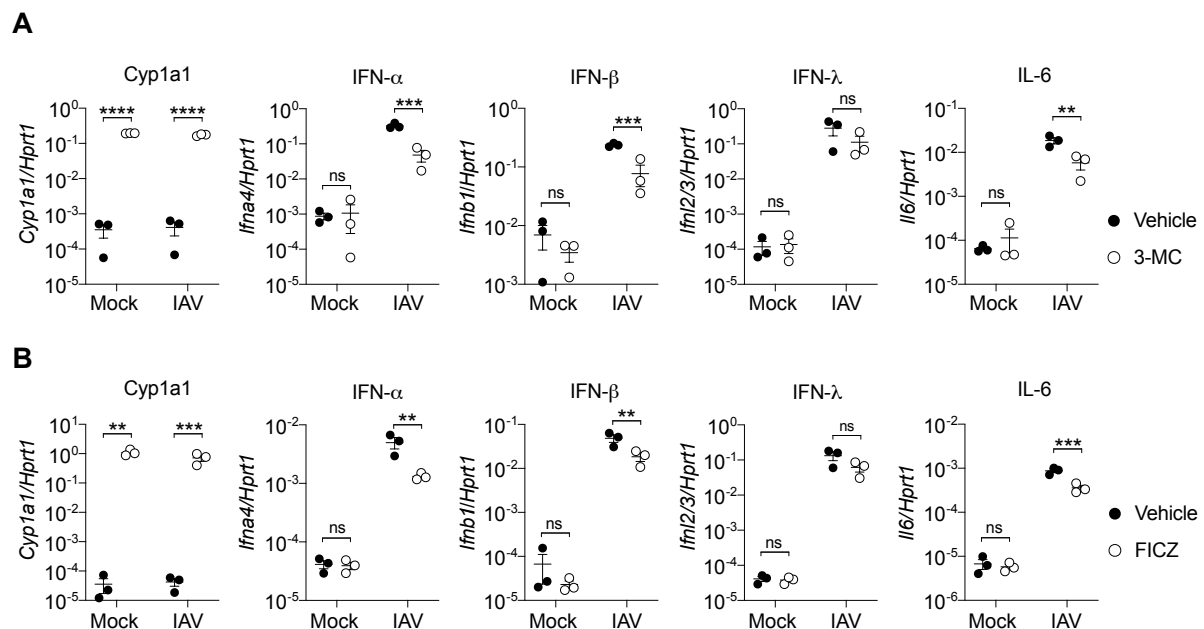


Figure 24. AHR agonist treatment suppresses IFN and IL-6 responses in influenza virus infected AECs.

(A and B) Fully differentiated (day 8 ALI) WT AECs were treated with AHR agonists 250 nM FICZ (A) or 1 μ M 3-MC (B) 24 hours prior to influenza virus infection (PR8 10^{-4} MOI). RNA was collected 48 hours post infection, and mRNA expression of the indicated genes were measured by qPCR. All data are representative of at least two independent experiments (n = 3). Data are shown as mean $\pm$ SEM and statistical significance was assessed by two-way ANOVA with Sidak's post-test. ns, not significant ($P > 0.05$); ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

5.3 Increased endogenous ligand availability as a model to study functions of AHR in the lung

AHR functions during respiratory viral infections have mainly been studied following TCDD treatment in influenza virus infected mice. We therefore utilised CYP1 deficient AECs to augment natural ligand availability, with the aim of enhancing physiological AHR functions. *Cyp1^{-/-}* AEC cultures had increased expression of the AHR target gene *Tiparp* both in mock and influenza virus infected cultures (Fig. 25A). Consistently, *Ahr^{-/-}* AECs had reduced *Tiparp* expression compared to WT cultures following influenza virus infection, suggesting that AHR functions at a basal level in WT AECs (Fig. 25A). Similar findings were observed in the naïve whole lung, with decreased expression of the AHR target gene *Ahr* in *Ahr^{-/-}* mice (although not significant), and a significant increase in *Cyp1^{-/-}* mice (Fig. 25B). Therefore, increasing the availability of natural AHR ligands provides us with a tool to study the effects of enhanced AHR activity in AECs, and in the lung in vivo.

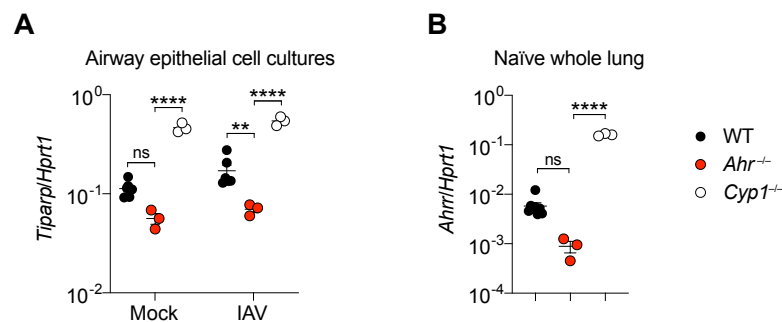


Figure 25. Endogenous ligands regulate AHR activity in AECs in vitro and in the lung in vivo.

Total RNA was isolated from WT, *Ahr^{-/-}*, and *Cyp1^{-/-}* (A) fully differentiated (day 8 ALI) murine AEC cultures and (B) naïve whole lung, and mRNA expression of the indicated genes were measured by qPCR. All data are representative of at least three independent experiments (n = 3–6). Data are shown as mean ± SEM and statistical significance was assessed by two-way ANOVA with Tukey's post-test. ns, not significant (P > 0.05); **P ≤ 0.01, ****P ≤ 0.0001.

5.4 Suppressed IFN production in *CypI*^{-/-} mice correlates with reduced lung damage and dampened inflammation during influenza virus infection

To understand if increased natural AHR ligand availability affects IFN production in vivo, we infected WT and *CypI*^{-/-} mice with the influenza virus strain X31, and measured cytokine concentrations in the BALF days 2 to 6 post infection. Consistent with previous findings (Yamada *et al.*, 2016) and our in vitro observations in AECs, *CypI*^{-/-} mice had decreased type I and III IFN levels in BALF on day 2 post infection, however this difference did not persist throughout the course of infection (Fig. 26A). Similar to IFN concentrations, IL-6 was also decreased in the BALF of *CypI*^{-/-} mice on day 2 post infection (Fig. 26A). Consistently, *Ahr*^{-/-} mice had increased type I and III IFN, and IL-6, on day 2 post influenza virus infection (Fig. 26B).

To understand whether increasing AHR ligand availability affects influenza disease severity, we measured the extent of lung damage in WT and *CypI*^{-/-} mice on day 6 post infection. *CypI*^{-/-} mice had a significant reduction in the total cell number, lactate dehydrogenase (LDH), and serum albumin in BALF (Fig. 27A). We also observed a reduction in Evan's blue extravasation in the lungs of *CypI*^{-/-} mice, indicating reduced vascular leakage and epithelial damage (Fig. 27B). Consistently, *CypI*^{-/-} mice had a marked reduction of RBCs in the BALF (Fig. 27C). Histopathological analysis of H&E stained lung sections revealed a significantly reduced pulmonary immune cell infiltrate dispersed in lung tissues (Fig. 27D) (histopathology scorings were determined by Simon Priestnall and Alejandro Suarez-Bonnet, Francis Crick Institute). To confirm these observations, we measured total lung cell number throughout the course of influenza virus infection as an indicator of pulmonary inflammation. Whilst the total lung cell number of WT mice increased by more than 100% over the course of the infection (approximately 1.1×10^7 cells), *CypI*^{-/-} mice had no increase (Fig. 27E). Reduced lung damage and inflammation correlates with improved protection, as *CypI*^{-/-} mice had reduced weight loss and mortality following influenza virus infection (Fig. 27F). Overall, these data show that amplifying AHR signalling through expanded natural ligand availability suppresses the production of IFNs and proinflammatory cytokines (IL-6). This correlates with reduced barrier tissue damage, and improved survival following influenza virus infection.

To understand whether the increased protection in *CypI*^{-/-} mice occurs as a result of improved antiviral function, we measured expression of the influenza virus matrix (*MI*) gene

expression. However, we observed no difference in viral burden between WT and *Cyp1^{-/-}* mice (Fig. 28). This suggests that the dampened antiviral IFN response in *Cyp1^{-/-}* mice (Fig. 26) is still a sufficient level to control viral replication (Fig. 28). Therefore, the reduced lung damage and mortality observed in *Cyp1^{-/-}* mice is potentially due to an increase in disease tolerance, possibly as a result of reduced IFN-dependent or -independent immunopathology, including disruption in epithelial repair.

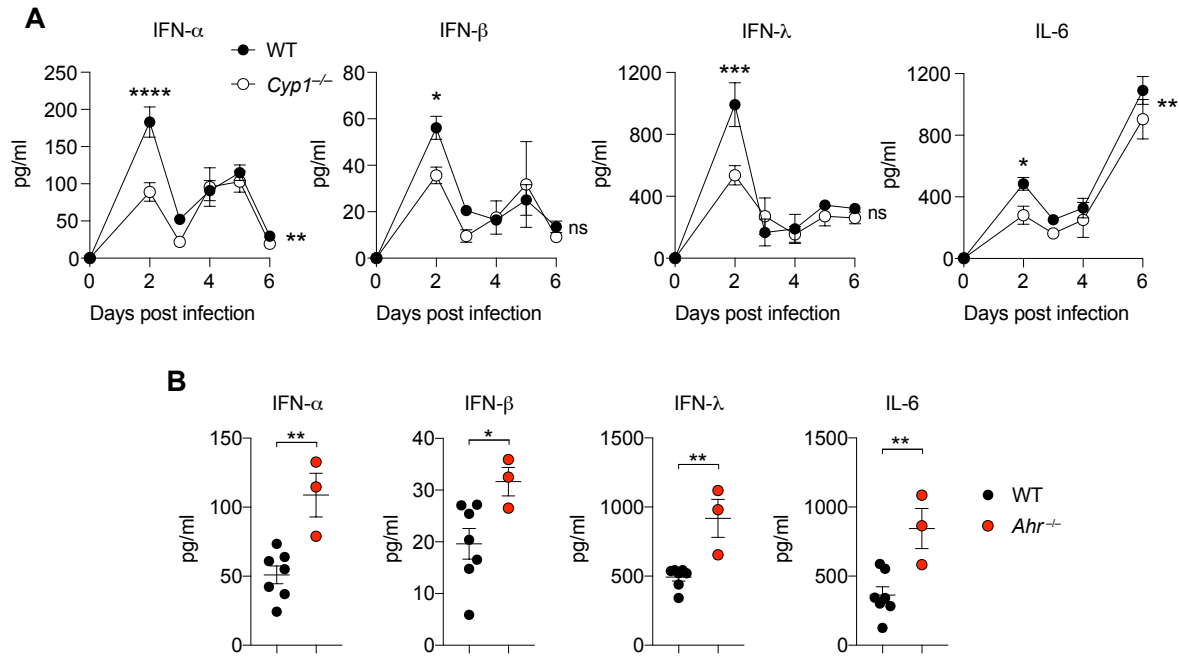


Figure 26. Endogenous AHR activity suppresses early IFN and IL-6 production during influenza infection in vivo.

IFN- α , IFN- β , IFN- λ and IL-6 protein concentrations were measured in the BALF of influenza virus (3.5×10^4 TCID₅₀ X31 in 30 μ l PBS) infected WT, *Cyp1^{-/-}* (at indicated days post infection) (A) and *Ahr^{-/-}* (day 2 post infection) (B) mice. All data are representative of at least three independent experiments ($n = 3-7$). Data are shown as mean $\pm$ SEM and statistical significance was assessed by two-way ANOVA with Sidak's post-test (A) or unpaired two-tailed Student's *t* test (B). ns, not significant ($P > 0.05$); * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

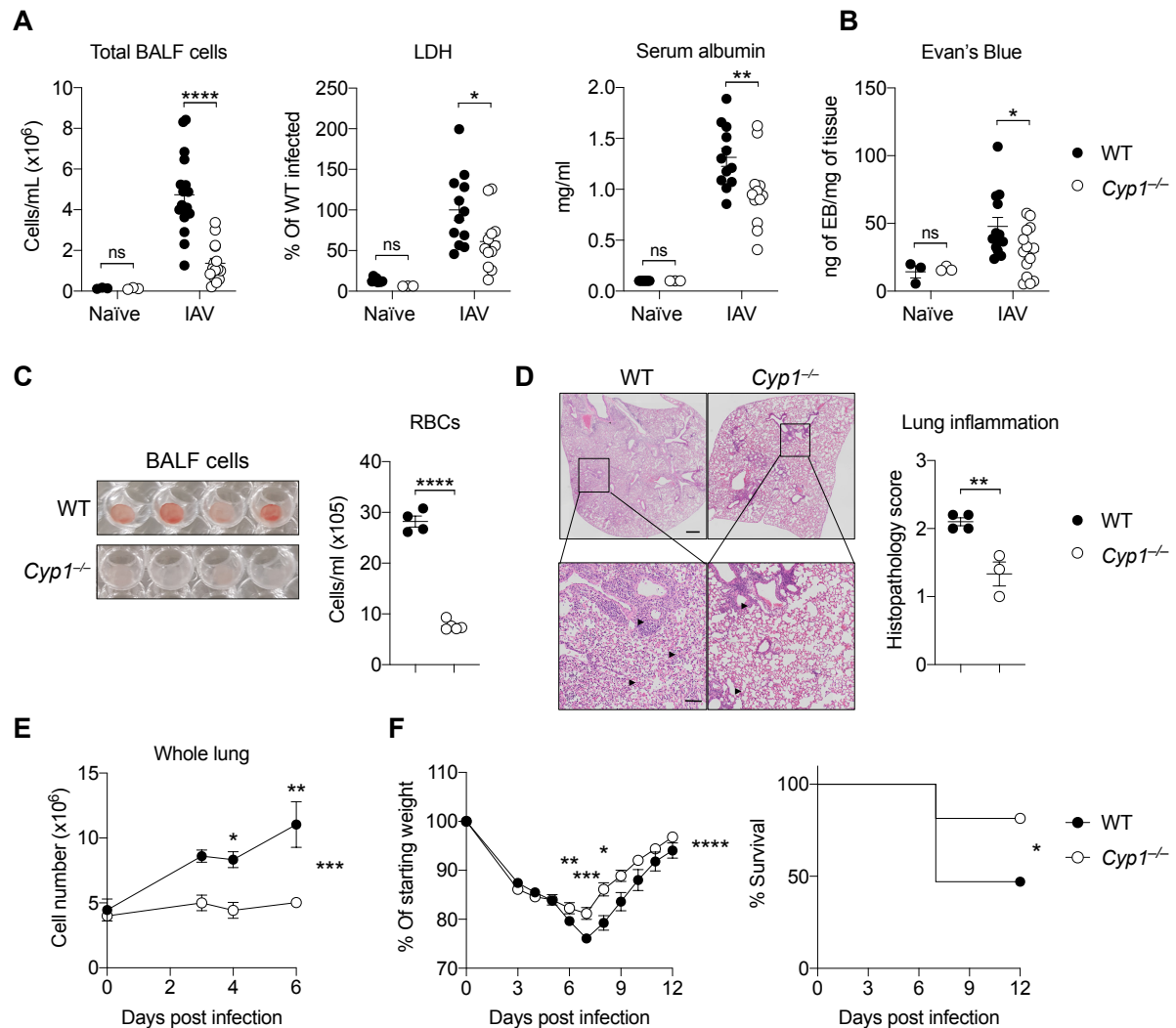


Figure 27. Genetic increase in the availability of natural AHR ligands reduces lung damage during influenza virus infection.

(A to D) Naïve and influenza virus infected WT and *Cyp1*^{-/-} mice were assessed for lung damage by measurement of (A) total cells, LDH release, and serum albumin in BALF, (B) Evan's blue dye extravasation in lung tissues, (C) RBCs in BALF, and (D) histopathological examination of H&E lung sections, on day 6 post infection. (E) Total lung cell numbers were measured in naïve and influenza virus infected WT and *Cyp1*^{-/-} mice on indicated days post infection. (F) Weight loss and survival were measured in influenza virus infected WT and *Cyp1*^{-/-} mice. Data are representative of or pooled from four independent experiments (n = 3–27). Data are shown as mean±SEM and statistical significance was assessed by two-way ANOVA with Sidak's post-test (A, B, E and F), unpaired two-tailed Student's *t* test (C), Mann–Whitney *U* test (D), or log-rank (Mantel–Cox) test (F, survival). ns, not significant ($P>0.05$); * $P\leq 0.05$, ** $P\leq 0.01$, *** $P\leq 0.001$, **** $P\leq 0.0001$.

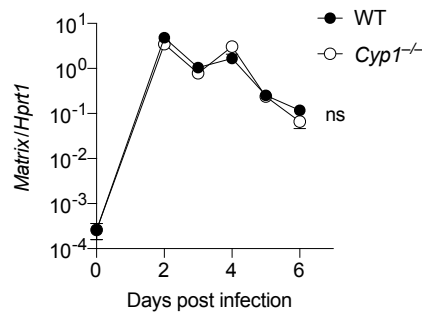


Figure 28. Increased protection in *Cyp1*^{-/-} mice is independent to viral burden.

Influenza virus *M1* mRNA expression in total lung RNA from naïve and infected WT and *Cyp1*^{-/-} mice was measured by qPCR at indicated days post infection. Data are representative of three independent experiments (n = 4–7). Data are shown as mean±SEM and statistical significance was assessed by two-way ANOVA with Sidak's post-test. ns, not significant (P>0.05).

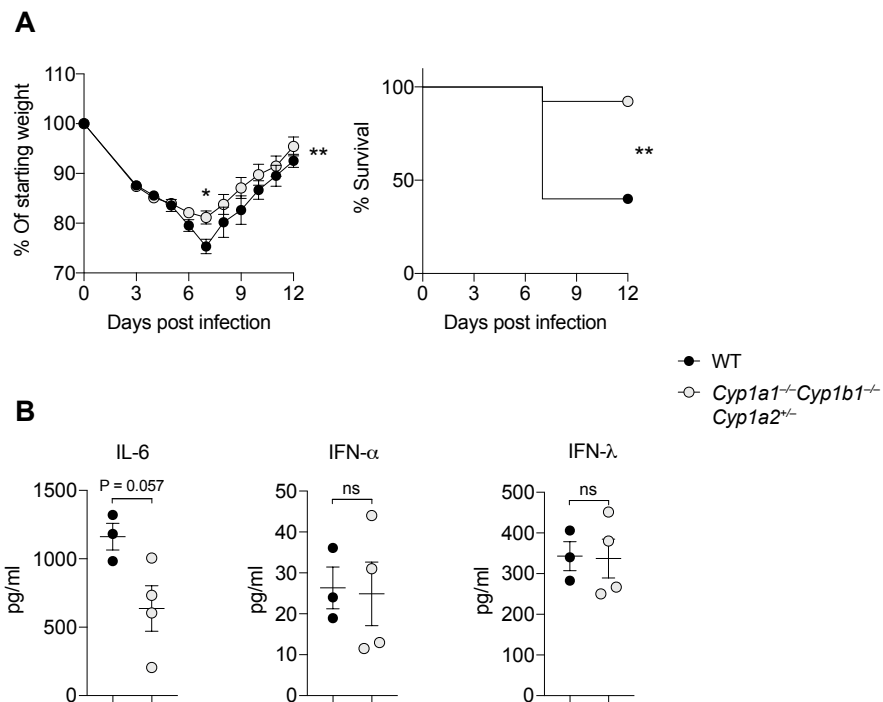


Figure 29. *Cyp1a1*^{-/-}*Cyp1b1*^{-/-}*Cyp1a2*^{+/-} double knockout mice have a comparable influenza phenotype to that of *Cyp1*^{-/-} mice.

Influenza virus infected WT and *Cyp1a1*^{-/-}*Cyp1b1*^{-/-}*Cyp1a2*^{+/-} mice were measured for (A) weight loss and survival or (B) IFN and cytokine protein concentration in BALF on day 6 post infection. All data are representative of two independent experiments (n = 3–5). Data are shown as mean±SEM and statistical significance was assessed by two-way ANOVA with Sidak's post-test (A), log-rank (Mantel–Cox) test (A, survival), or unpaired two-tailed Student's *t* test (B). ns, not significant (P>0.05), **P≤0.01.

CYP1 family enzymes (*Cyp1a1*, *Cyp1b1*, and *Cyp1a2*) play important physiological roles and are involved in the metabolism of endogenous hormones, in addition to xenobiotics and drugs (Go, Hwang & Choi, 2015). Therefore, a caveat to consider when using *Cyp1^{-/-}* mice is their altered physiological state. Mice deficient for CYP1 family enzymes have an altered urinary metabolite profile, in addition to perturbed expression of genes in the liver involved in lipid, steroid, and cholesterol biosynthesis and metabolism (Dragin *et al.*, 2008). During steady-state conditions, *Cyp1a2* expression is almost exclusively restricted to the liver (Jorge-Nebert *et al.*, 2010; Kapelyukh *et al.*, 2019). To examine effects of increased AHR ligand availability during influenza virus infection independent to ablation of physiological *Cyp1a2* activity in the liver, we infected WT mice and mice heterozygous for *Cyp1a2* (*Cyp1a1^{-/-}Cyp1b1^{-/-}Cyp1a2^{+/-}*). *Cyp1a1^{-/-}Cyp1b1^{-/-}Cyp1a2^{+/-}* mice had reduced weight loss and mortality following influenza virus infection (Fig. 29A), as well as a reduction in IL-6 on day 6 post infection (Fig. 29B). Type I and III IFNs were not significantly different compared to WT controls (Fig. 29B), consistent with *Cyp1^{-/-}* mice on day 6 post infection, which only showed a reduction when IFN production was at its peak, on day 2 (Fig. 26A). Although further experiments are required to assess effects on lung damage in mice with partial or full CYP1A2 function, these data suggest that improved protection and suppression of IL-6 during influenza in *Cyp1^{-/-}* mice occurs independently of CYP1A2 activity in the liver.

To determine factors that may contribute to the reduced pathology observed in *Cyp1^{-/-}* mice, we characterised the profile of inflammatory immune cells recruited to the lung throughout the course of influenza virus infection by flow cytometry (Gating strategies: appendix II and III). Owing to the reduction in the total lung cell number of *Cyp1^{-/-}* mice following infection, we quantified cell frequencies in the lung to examine specific effects on immune cell populations. Notably, *Cyp1^{-/-}* mice showed a significant increase in the frequency of neutrophils across all days following influenza virus infection (Fig. 30A). This observation is in line with previous findings, where it was shown AHR-dependent neutrophilia occurred in mice treated with TCDD (Teske *et al.*, 2005; Wheeler, Martin & Lawrence, 2013). We also recorded consistent reductions in pDCs, eosinophils, and CD4⁺ T cells (Fig. 30A), potentially due to cell-intrinsic AHR regulatory functions in these cell types (Jeong *et al.*, 2012; Liu, Ramachandran & Gabrilovich, 2014; Stockinger *et al.*, 2014). *Cyp1^{-/-}* mice additionally exhibited an increase in the frequency of NK cells during steady-state and early in the course of infection (Fig. 30A, lower-bottom right), however these differences dissipated as the disease progressed. *Ahr^{-/-}* mice have reduced liver-resident NK cells (Zhang *et al.*, 2016), suggesting

AHR may be important in the maintenance of tissue-resident NK cells. When we compare WT and *Cyp1^{-/-}* mice for the absolute number of different immune cell populations in the lung, we found a broad suppression of immune cell infiltration in pulmonary tissues in *Cyp1^{-/-}* mice (Fig. 30B), with the exceptions being alveolar neutrophils, alveolar macrophages, and NK cells (which had comparable numbers to WT). Taken together, these results demonstrate that augmented AHR signals through improved ligand availability promotes influenza disease tolerance. *Cyp1^{-/-}* mice displayed an early reduction in IFN and IL-6 production, which potentially dampens the proinflammatory cellular response in lung tissues. Reduced inflammation in *Cyp1^{-/-}* mice correlated with a substantial reduction in the level of pulmonary epithelial and vascular damage.

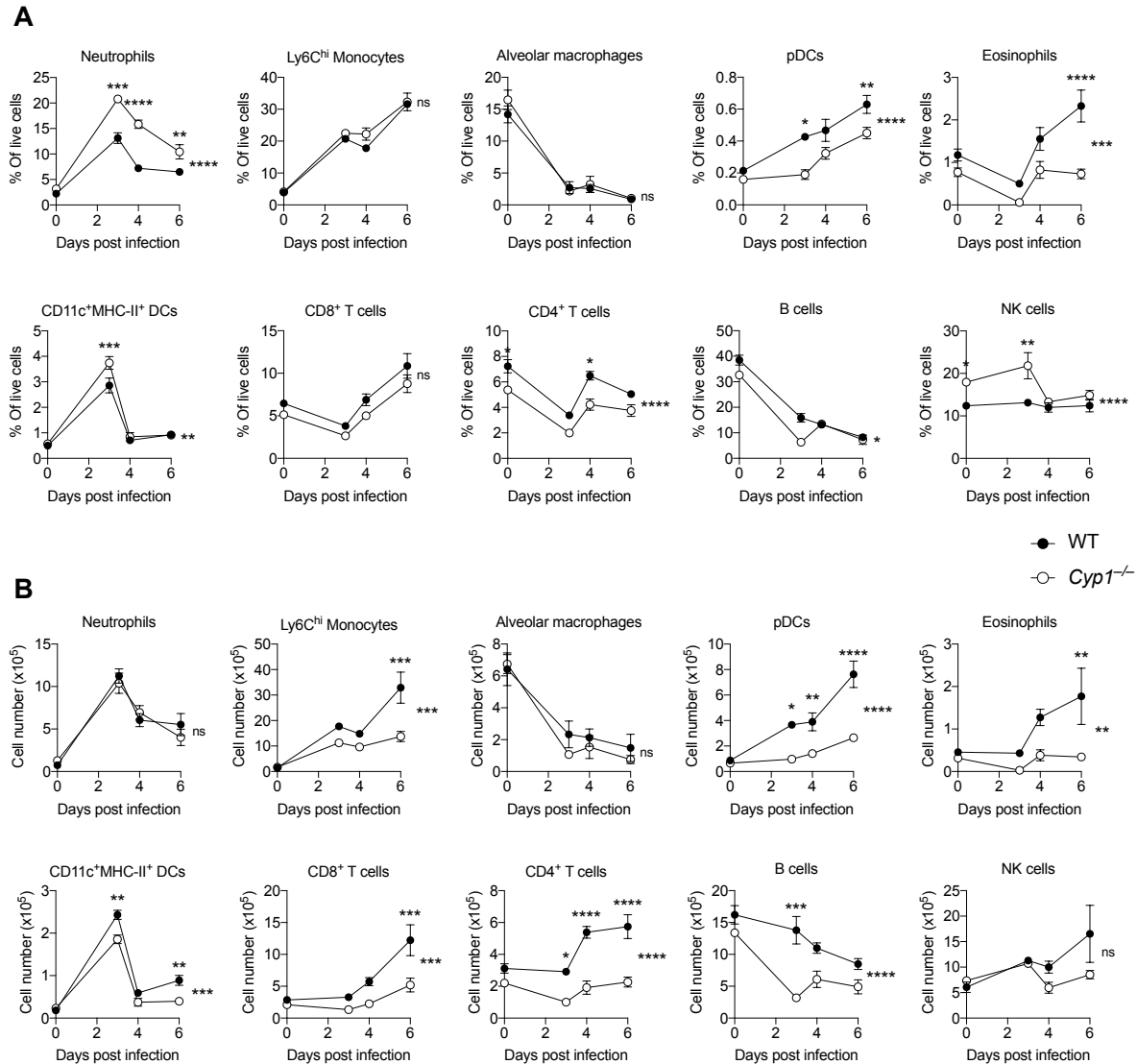


Figure 30. *Cyp1*^{-/-} mice have reduced influenza virus-induced pulmonary inflammation.

Frequencies (A) and cell numbers (B) of neutrophils (Ly6G⁺CD11b⁺), alveolar macrophages (Siglec-F⁺CD11c⁺), Ly6C^{hi} monocytes (Ly6C^{hi}CD11b⁺), pDCs (Siglec-H⁺), eosinophils (Siglec-F⁺CD11b⁺CD11c⁻), DCs (CD11c⁺MHCII⁺), CD4⁺ T cells (CD3⁺CD4⁺), CD8⁺ T cells (CD3⁺CD8⁺), CD19⁺ B cells, and NK cells (NK1.1⁺CD3⁻) in the lungs of naïve and influenza virus infected WT and *Cyp1*^{-/-} mice. All data are representative of three independent experiments (n = 3–7). Data are shown as mean±SEM and statistical significance was assessed by two-way ANOVA with Sidak's post-test. ns, not significant (P>0.05); *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001.

5.5 IFN treatment does not substantially exacerbate pulmonary inflammation or lung damage in *CypI*^{-/-} mice

Our results and conclusions from Chapters 1 and 2 outline a novel mechanism by which IFNs exacerbate influenza disease pathology via the disruption of critical lung epithelial repair (Major *et al.*, 2020). In *CypI*^{-/-} mice we have a context of both reduced IFN production and lung damage, and emerging evidence over the last decade highlight the pathogenic potential of type I and III IFNs during respiratory viral infections (Davidson *et al.*, 2014, 2016; Channappanavar *et al.*, 2016, 2019; Major *et al.*, 2020). To understand whether the reduced lung damage observed in *CypI*^{-/-} mice occurs as a result of AHR-mediated suppression of type I and III IFN production (Fig. 26), we treated influenza virus infected mice with IFN- α (days 4 and 5 post infection) and measured lung damage. IFN- α treatment successfully triggered additional IFNAR signalling, as CD19⁺ B cells increased surface expression of the ISG Sca-1 (Fig. 31A). However, we did not observe a significant change in the total BALF, RBC (not quantified) and lung cell number, with IFN- α treatment only causing a mild increase (Fig. 31B to D). Additionally, we did not see alterations in the number of proinflammatory IFN-associated immune cells (Fig. 31E). Thus, the increased protection in *CypI*^{-/-} mice with augmented AHR signalling is likely not due to dampened IFN production, as high-dose exogenous IFN- α treatment only mildly increases lung inflammation and damage.

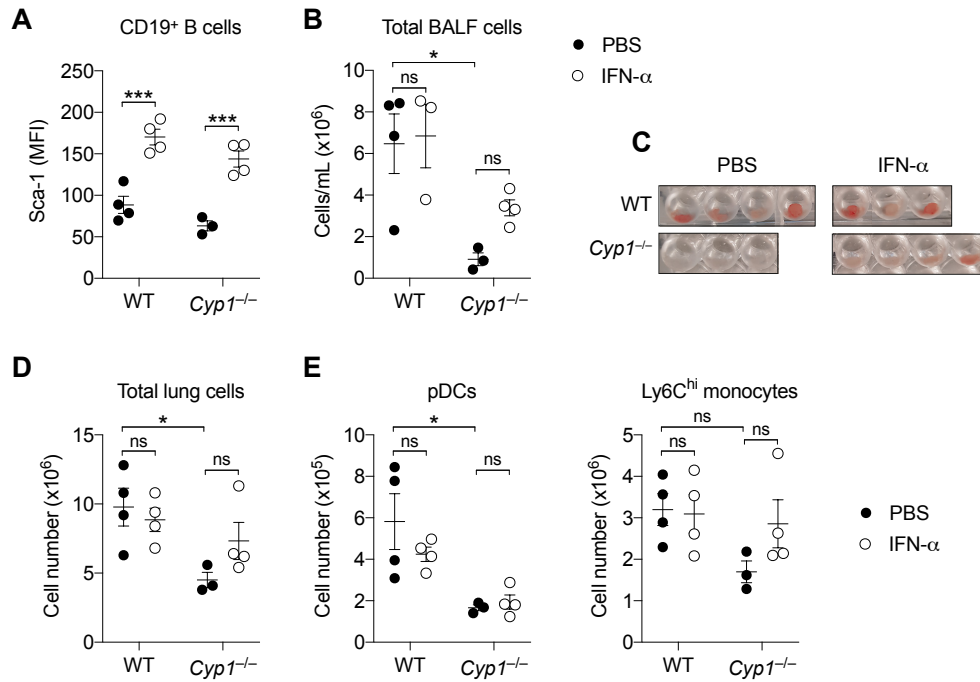


Figure 31. IFN treatment does not exacerbate lung damage in influenza infected WT or *Cyp1*^{-/-} mice.

Influenza virus infected WT and *Cyp1*^{-/-} mice were treated with IFN- α on days 4 and 5 post infection, and lung and BAL samples were analysed for damage on day 6 post infection. (**A** and **E**) Sca-1 MFI in CD19⁺ B cells, pDCs (Siglec-H⁺) and Ly6C^{hi} monocyte (Ly6C^{hi}CD11b⁺) cell numbers were measured by flow cytometry. (**B** to **D**) Total BALF (**B**), RBCs in BALF (**C**) and total lung cell numbers (**D**) were counted on day 6 post infection. All data are representative of three independent experiments (n = 3–4). Data are shown as mean $\pm$ SEM and statistical significance was assessed by two-way ANOVA with Tukey's post-test (A) or Sidak's post-test (B to E). ns, not significant ($P > 0.05$); * $P \leq 0.05$, *** $P \leq 0.001$.

5.6 Ablation of AHR signalling has no effect on lung pathology

So far, we have seen increased AHR signalling in *Cyp1^{-/-}* mice results in a dampened proinflammatory response and less lung damage, occurring independently to changes in antiviral immunity and viral clearance. Therefore, we next wanted to examine whether a lack of AHR signalling would increase susceptibility to influenza virus infection. WT and *Ahr^{-/-}* mice were infected with influenza virus, and we measured inflammation and damage on day 6 post infection. *Ahr^{-/-}* mice had a significant increase in total lung cell number (Fig. 32A), possibly owing to the increased production of IFNs and IL-6 (Fig. 26B). However, we did not see an increase in an inflammatory immune cell infiltrate, quantified by analysis of H&E stained lungs (Fig. 32B). We also saw no change in the degree of lung damage in *Ahr^{-/-}* mice, with no increase in the total or RBC number in BALF (Fig. 32C). Consistently, *Ahr^{-/-}* mice had comparable weight loss and survival to WT mice following influenza virus infection (Fig. 32D). Taken together, these data suggest that AHR signalling in WT mice does not significantly contribute to protection, and only following an augmentation of the natural AHR ligand pool (in *Cyp1^{-/-}* mice) do protective responses and disease tolerance promoting effects initiate. In addition, this provides further indication that AHR regulation of IFNs and IL-6 do not alter lung pathology, as increased cytokine production in AHR deficient mice (Fig. 26B) does not correlate with increased lung damage (Fig. 32B and C).

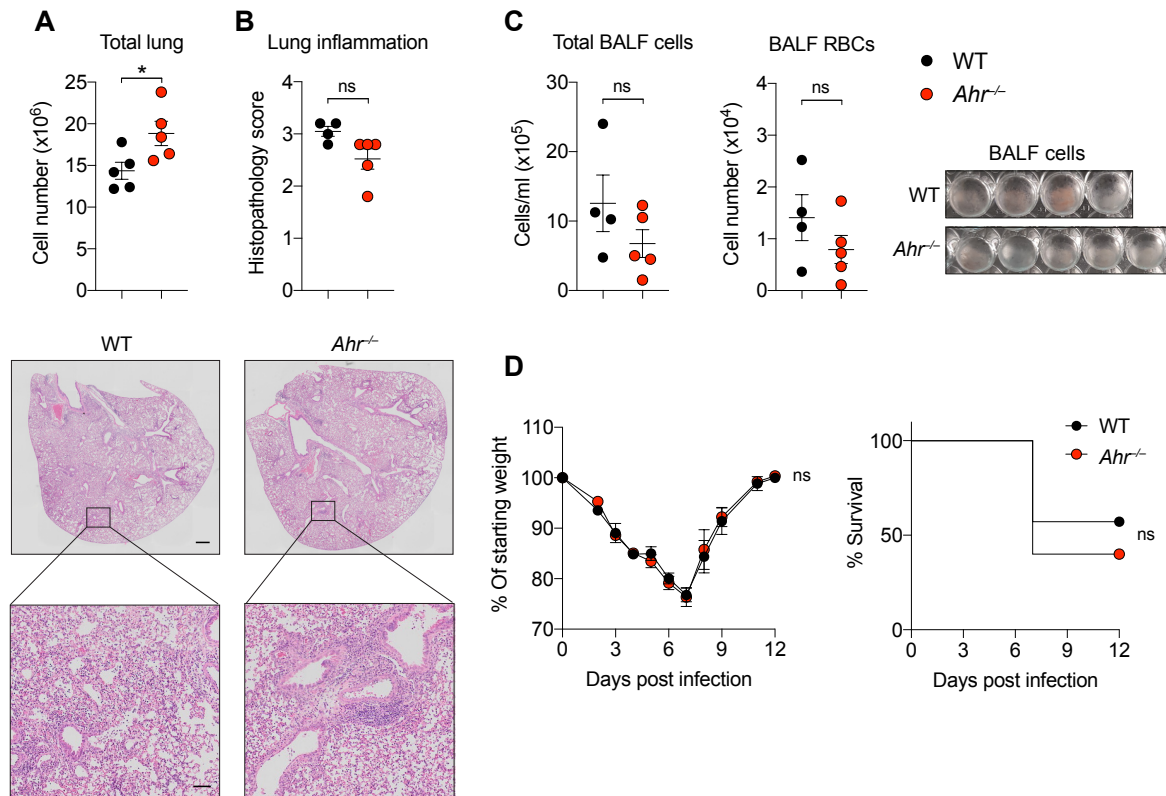


Figure 32. AHR deficiency does not affect lung damage or influenza susceptibility.

Influenza virus infected WT and *Ahr*^{-/-} mice assessed for lung damage by measurement of (A) total lung cell number, (B) histopathological examination of H&E lung sections (Scale bar represents 500 μ M (top) or 100 μ M bottom), (C) Total and RBC numbers in BALF, (D) weight loss and survival. (A to C) samples were taken on day 6 post infection. All data are representative of three independent experiments ($n = 4-5$). Data are shown as mean $\pm$ SEM and statistical significance was assessed by unpaired two-tailed Student's *t* test (A and C), Mann-Whitney *U* test (B), two-way ANOVA with Sidak's post-test (weight loss) or by log-rank (Mantel-Cox) test (survival) (D). ns, not significant ($P > 0.05$); $*P \leq 0.05$.

5.7 Increased AHR signalling may confer protection through IL-22 production, but independent to improved epithelial repair

Recent studies highlight the importance of AHR signalling pathways in intestinal epithelial barrier protection (Metidji *et al.*, 2018; Schiering *et al.*, 2017), including production of IL-22 by various immune cell (Basu *et al.*, 2012; Qiu *et al.*, 2012; Kiss *et al.*, 2011; Schiering *et al.*, 2017; Martin *et al.*, 2009). The importance of IL-22 during influenza virus infection is highlighted by studies using *Il22*^{-/-} mice, which exhibit increased lung damage and dampened epithelial repair responses (Pociask *et al.*, 2013). Comparison of WT, *Ahr*^{-/-}, and *Cyp1*^{-/-} mice revealed increased levels of IL-22 in the BALF on day 6 post infection in *Cyp1*^{-/-} mice (Fig. 33A). Interestingly, WT and *Ahr*^{-/-} mice had no difference in IL-22 levels, which correlates with the similar levels of lung pathology in these mice (Fig. 32). However, we recorded no difference in alveolar or bronchial epithelial repair between WT and *Cyp1*^{-/-} mice, as determined by histopathological analysis of H&E sections (Fig. 33B). To understand whether the improved protection in *Cyp1*^{-/-} mice was due to enhanced lung epithelial repair, we measured AT2 (EpCam⁺MHC-II⁺) and airway (EpCam⁺MHC-II⁻) epithelial cell proliferation on day 6 post infection. However, *Cyp1*^{-/-} mice had similar lung epithelial cell proliferation to WT controls (Fig. 33C). Lung regeneration only begins to initiate around day 6 post infection (Fig. 2B), however, reduced lung damage in *Cyp1*^{-/-} mice is apparent on day 6 (Fig. 27). Therefore, as *Cyp1*^{-/-} mice do not have improved or an earlier onset repair response, this data suggests that the increased influenza protection as a result of enhanced AHR signalling is independent of an improved capacity for lung regeneration. Taken together, these data indicate that increased IL-22 levels correlate with AHR activation and disease tolerance following influenza virus infection, however this occurs independently from enhanced epithelial proliferation. IL-22 signalling may promote other mechanisms of barrier function, yet its contribution in host protection remains to be determined.

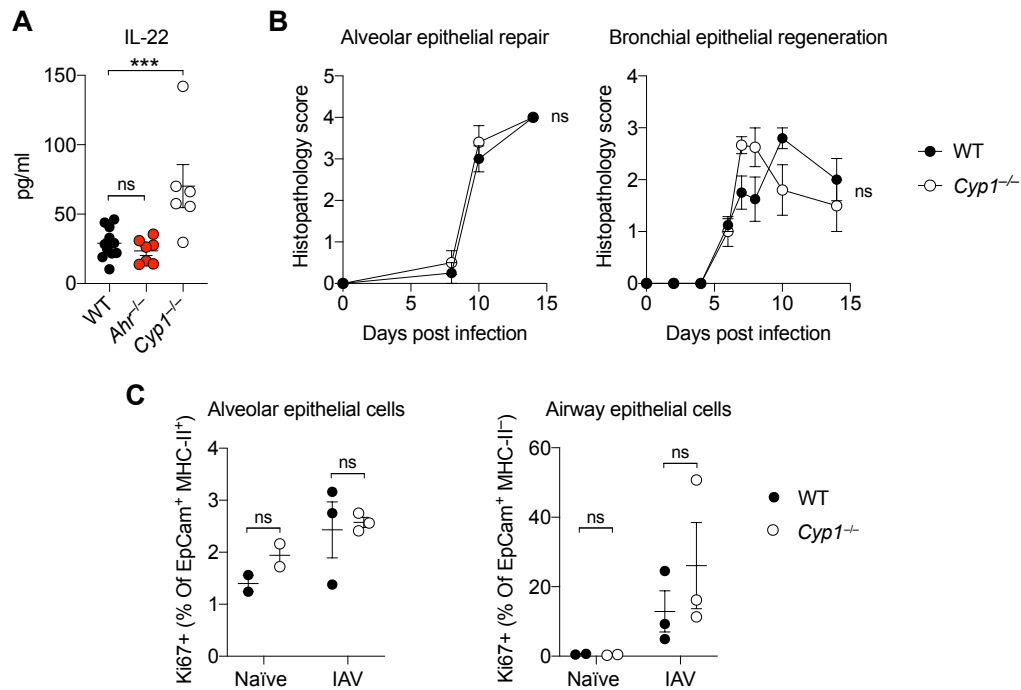


Figure 33. Enhanced endogenous AHR ligand availability increases IL-22 production, but does not affect lung repair during influenza.

(A) IL-22 protein concentrations in the BALF of influenza virus infected WT, *Ahr*^{-/-}, and *Cyp1*^{-/-} mice on day 6 post infection. (B) Histopathological scores of H&E lung sections in WT and *Cyp1*^{-/-} mice on. (C) Ki67⁺ AT2 (EpCam⁺MHC-II⁺) and airway epithelial cells (EpCam⁺MHC-II⁻) in naïve and influenza infected WT and *Cyp1*^{-/-} mice (day 6 post infection), measured by flow cytometry. All data are representative of at least two independent experiments (n = 2–12). Data are shown as mean±SEM and statistical significance was assessed by one-way ANOVA with Dunnett's post-test (A) or two-way ANOVA with Sidak's post-test (B and C). ns, not significant (P>0.05); ***P≤0.001.

5.8 Enhanced AHR signalling in the non-hematopoietic compartment confers protection during influenza virus infection

To define the mechanism underlying protection in *Cyp1^{-/-}* mice, we sought to define which cell populations experience enhanced AHR activity as a result of the increase in available natural ligands. We utilised a CYP1A1 fate-reporter mouse strain, allowing us to measure induction of an enhanced yellow fluorescent protein (eYFP) reporter activated through targeted insertion of a Cre recombinase in the mouse *Cyp1a1* locus (Henderson *et al.*, 2015). Whilst deficient for the CYP1A1 enzyme, *Cyp1^{-/-}* mice still reported AHR activation and induction of Cre-mediated eYFP expression (Fig. 34). In naïve WT and *Cyp1^{-/-}* mice, we detected no or very low levels of eYFP expression in alveolar epithelia, alveolar macrophages, or other CD45⁺ immune cells (Fig. 34). Strikingly, more than 90% of CD31⁺ endothelial cells were eYFP⁺ in both WT and *Cyp1^{-/-}* mice, indicating constitutive physiological AHR activity in the endothelium during development or in steady-state. Additionally, *Cyp1^{-/-}* mice had a population of eYFP⁺ AECs, unlike WT AECs which had nearly no eYFP expression (Fig. 34). Owing to the transient nature of AHR signalling, particularly in WT mice, Cre-mediated induction of eYFP expression may not fully reflect the extent of AHR activation in these reporter mice. In addition, eYFP expression includes prior CYP1A1 expression, and infection may also alter AHR activity. Therefore, further investigation is required to identify the cellular source(s) of AHR signals in *Cyp1^{-/-}* mice following influenza virus infection.

CYP1A1 fate-reporter mice indicate AHR is active in non-hematopoietic cells of the lung, namely airway epithelial and endothelial cell populations (Fig. 34). To test the importance of AHR signalling in the non-hematopoietic compartment during influenza virus infection, we generated BM chimeras, where irradiated WT or *Cyp1^{-/-}* hosts were administered WT BM cells intravenously (chimerism efficiency in Appendix I). Thus, the expanded pool of available natural AHR ligands is restricted to the non-hematopoietic of WT→*Cyp1^{-/-}* chimeric mice. Restricting AHR ligand availability to the non-hematopoietic compartment was sufficient to reduce lung inflammation and damage following influenza virus infection (Fig. 35A and B). Differences were less pronounced, yet still consistent with findings in normal WT and *Cyp1^{-/-}* mice (Fig. 27). We also observed a similar pattern in disease progression, with WT→CYP1 mice displaying less severe weight-loss and improved recovery (Fig. 35C). We did not see differences in the level of RBCs in BALF (Fig. 35D), however this may be a result of an increased resilience to infection in chimeric mice, as WT→WT controls did not have similar

damage to that of non-irradiated mice (Fig. 27C). In addition, CYP1 deficiency in the non-hematopoietic compartment did not affect IFN or IL-6 production, however we still saw an increase in IL-22 levels (Fig. 35E). Finally, to characterise the pulmonary immune cell landscape in chimeric mice, we analysed lung cells by flow cytometry. To identify specific effects in immune cell populations, as opposed to a general decrease owing to reduced lung inflammation (Fig. 35B), we looked at cell frequencies. Consistent with normal *Cyp1^{-/-}* mice, chimeras had reduced pDCs, DCs, and increased NK cell frequencies (Fig. 35F), indicating that enhanced AHR ligand availability in the non-hematopoietic compartment still has effects on immune cell balances. In chimeric mice, no longer observing differences in IFN and IL-6 levels, as well as the proportion of eosinophils (compared to non-chimeric *Cyp1^{-/-}* mice), indicates that these changes require AHR activation in immune cells. Overall, these data indicate that increasing natural AHR ligand availability in the non-hematopoietic compartment alone is sufficient to reduce lung pathology during influenza virus infection.

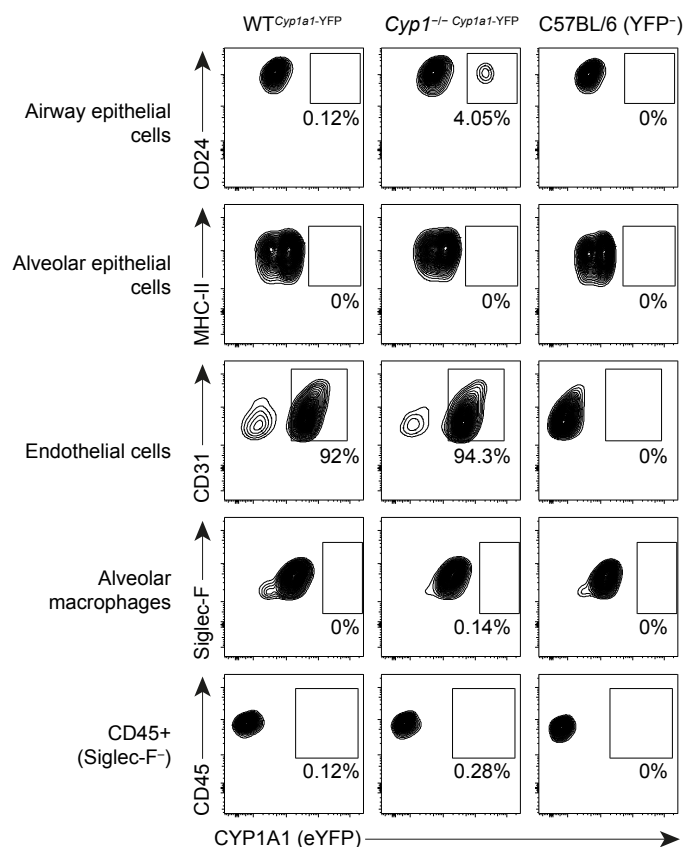


Figure 34. CYP1A1-reporter mice reveal AHR signalling in lung epithelial and endothelial compartments.

YFP⁺ cells were identified in steady state lung cells of WT^{Cyp1a1-YFP}, *Cyp1^{-/-}* ^{Cyp1a1-YFP}, and B6 WT (YFP negative controls) mice by flow cytometry. Lung cells analysed: differentiated airway epithelial cells (EpCam⁺CD49f^{high}CD24^{high}); AT2 cells (EpCam⁺MHCII⁺CD49f^{low}); endothelial cells (CD31⁺CD45⁻EpCam⁻); alveolar macrophages (Siglec-F⁺CD11c⁺); and Siglec-F⁻CD45⁺ cells (lung immune cells except for eosinophils and alveolar macrophages). All data are representative of two independent experiments (n = 2–3).

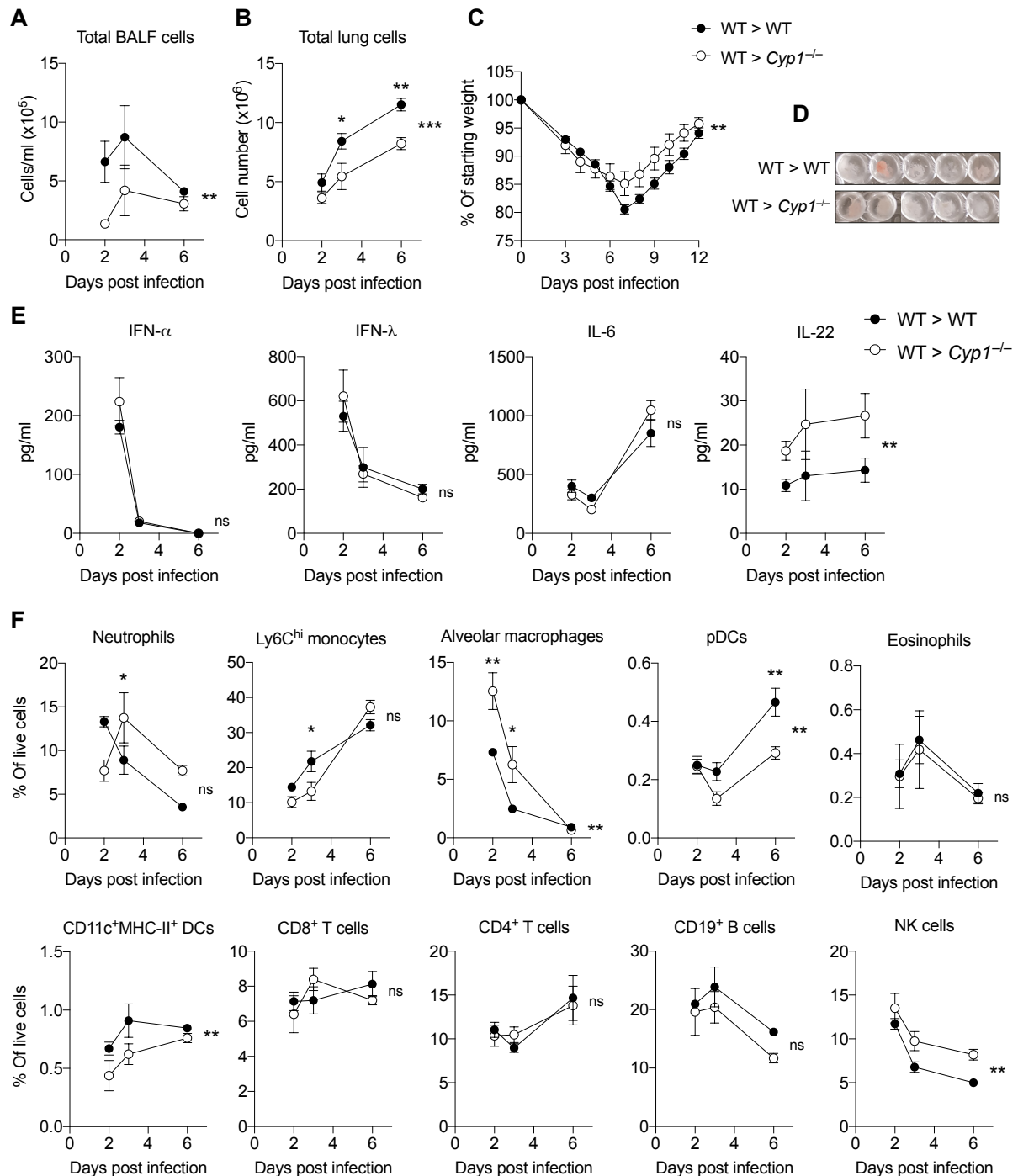


Figure 35. CYP1 deficiency in the non-hematopoietic compartment alone is sufficient to provide protection during influenza.

Lethally irradiated WT mice were injected with WT or *Cyp1*^{-/-} BM cells. Following reconstitution, chimeric mice were infected with influenza virus, and on indicated days post infection, measured for (A) total BALF cell numbers, (B) total lung cell numbers, (C) weight loss, (D), BALF RBCs (day 6 post infection), (E) IFN and IL-22 protein concentrations in BALF, and (F) lung immune cell frequencies by flow cytometry. Lung immune cells analysed: neutrophils (Ly6G⁺CD11b⁺); alveolar macrophages (Siglec-F⁺CD11c⁺); Ly6C^{hi} monocytes (Ly6C^{hi}CD11b⁺); pDCs (Siglec-H⁺); eosinophils (Siglec-F⁺CD11b⁺CD11c⁻); DCs (CD11c⁺MHCII⁺); CD4⁺ T cells (CD3⁺CD4⁺); CD8⁺ T cells (CD3⁺CD8⁺); CD19⁺ B cells; and NK cells (NK1.1⁺CD3⁻). All data are representative of at least three independent experiments

($n = 4-5$). Data are shown as mean $\pm$ SEM and statistical significance was assessed by two-way ANOVA with Sidak's post-test. ns, not significant ($P > 0.05$); * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

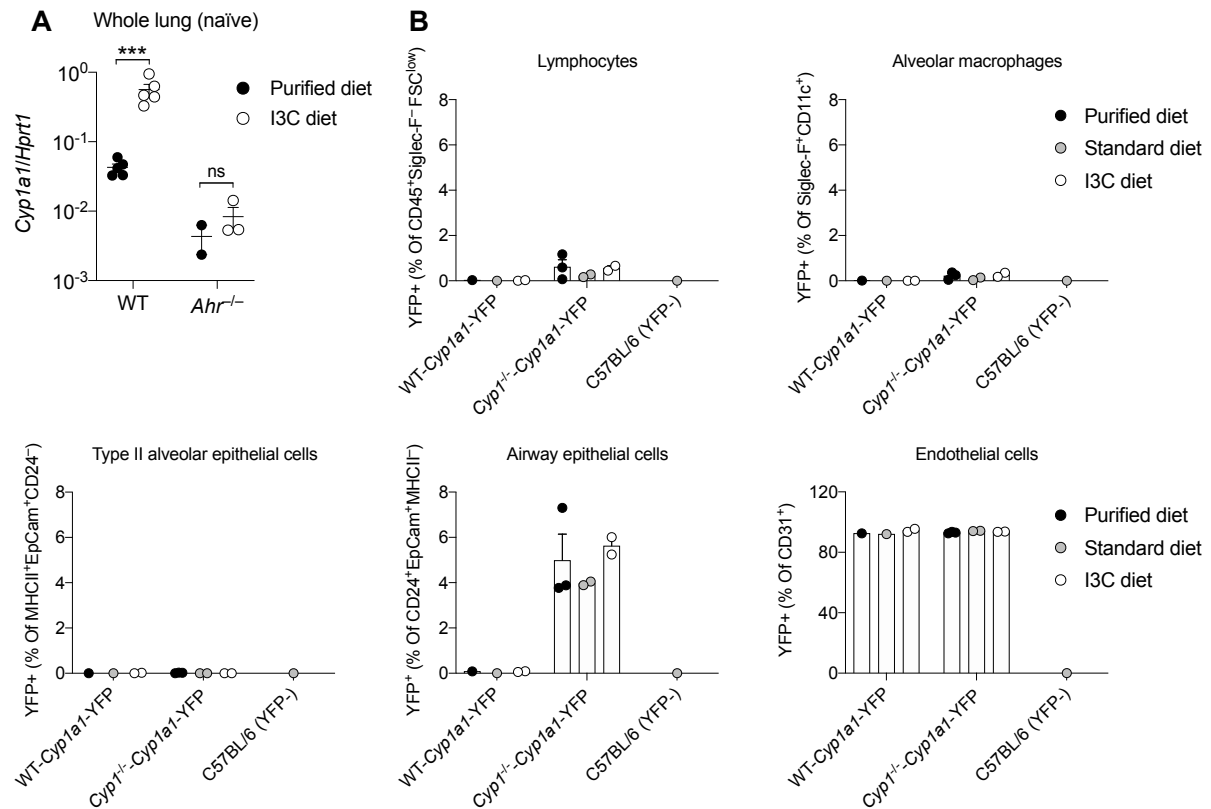


Figure 36. Dietary AHR ligands activate AHR signalling in the lung.

(A) Whole lung *Cyp1a1* mRNA expression in steady state WT and $Ahr^{-/-}$ mice fed purified or I3C-supplemented diet for four weeks. (B) YFP⁺ cells were identified in steady state lung cells of WT- $Cyp1a1$ -YFP, $Cyp1^{-/-}$ - $Cyp1a1$ -YFP, and B6 WT (YFP negative controls) mice fed purified or I3C-supplemented diet for four weeks by flow cytometry. Lung cells analysed: Siglec-F⁻CD45⁺ cells (lung immune cells except for eosinophils and alveolar macrophages); alveolar macrophages (Siglec-F⁺CD11c⁺); AT2 cells (EpCam⁺MHCII⁺CD24⁻); differentiated airway epithelial cells (EpCam⁺CD24⁺MHCII⁻); endothelial cells (CD31⁺CD45⁻EpCam⁻). Data in (A) are representative of two independent experiments ($n = 2-5$), and (B) of one experiment ($n = 1-3$). Data are shown as mean $\pm$ SEM and statistical significance was assessed by two-way ANOVA with Sidak's post-test (A). ns, not significant ($P > 0.05$); *** $P \leq 0.001$.

5.9 Dietary supplementation with natural AHR ligands activates AHR pathways in the lung

The similarity in disease severity between WT and *Ahr*^{-/-} mice suggests that AHR signalling in WT mice is not sufficient to promote disease tolerance or reduce pathology during influenza virus infection (Fig. 32). Sources of natural AHR ligands include microbial compounds, tryptophan metabolites, and dietary ligands (Stockinger *et al.*, 2014). To understand whether dietary ligands induce AHR activity in the lungs of WT mice, we supplemented the diet with I3C, a tryptophan-derived phytochemical that is converted to high-affinity AHR ligands by exposure to stomach acid (Bjeldanes *et al.*, 1991). I3C dietary supplementation enhanced *Cyp1a1* mRNA expression in the lung in an AHR-dependent manner, compared to mice fed a purified control diet (Fig. 36A). To define which cell populations experienced increased AHR activity following dietary supplementation, we administered I3C-supplemented diets to WT and *Cyp1*^{-/-} CYP1A1 fate-reporter mice and measured eYFP expression. Notably, we did not detect any difference in eYFP expression across cell types between control mice on a purified diet control mice and mice on I3C diet (Fig. 36B). As mentioned previously, this may reflect limitations of these reporter mice, particularly considering the increase in AHR-dependent *Cyp1a1* lung mRNA expression (Fig. 36A). These limitations include that the transient nature of AHR signalling processes may not be sufficient to drive Cre-mediated *Cyp1a1* expression, and eYFP expression in CYP1A1 fate-reporter mice may not reflect current AHR activation. It is also important to note that diet experiments in reporter mice were only performed once, and therefore repeats are required before definitive conclusions can be drawn.

To determine the effects of dietary AHR ligand supplementation following influenza virus infection, we gave purified or I3C-supplemented diet to mice for four weeks, continuing the diet throughout the duration of infection. I3C diet supplementation did not affect lung damage or inflammation (Fig. 37A and B), and of the measured cytokines, only IFN- λ had a significant reduction (Fig. 37C). When we characterised the lung immune cell profile, we again saw little differences in cellular recruitment, with only a slight decrease in the frequency of NK cells (Fig. 37D). Whilst these are preliminary findings ($n = 1$), our early experimental data suggests that despite a gut-lung axis in natural ligand supply and AHR activation (Fig. 36A), this may not increase signalling enough to confer protection during influenza virus infection.

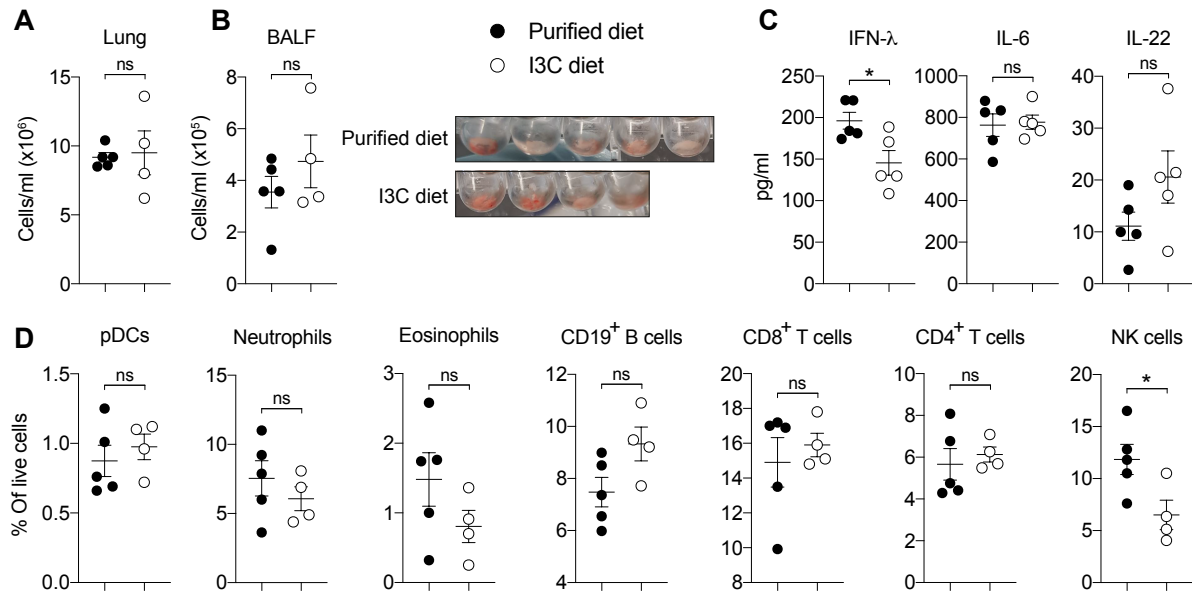


Figure 37. Dietary AHR ligands do not affect lung damage or inflammation during influenza virus infection.

WT mice were fed purified or I3C-supplemented diet for four weeks (inclusive of experiment duration), and infected with influenza virus to assess lung damage and immune response profiles on day 6 post infection. **(A)** Total lung cell numbers, **(B)** BALF cell numbers and RBCs, **(C)** IFN- λ , IL-6, and IL-22 protein concentrations in BALF, **(D)** lung immune cell frequencies. Immune cells analysed by flow cytometry: pDCs (Siglec-H⁺); neutrophils (Ly6G⁺CD11b⁺); eosinophils (Siglec-F⁺CD11b⁺CD11c⁻); CD19⁺ B cells; CD8⁺ T cells (CD3⁺CD8⁺); CD4⁺ T cells (CD3⁺CD4⁺); and NK cells (NK1.1⁺CD3⁻). All data are representative of one experiment. Data are shown as mean $\pm$ SEM and statistical significance was assessed by unpaired two-tailed Student's *t* test. ns, not significant ($P > 0.05$); * $P \leq 0.05$.

5.10 Discussion

Here, we link the availability of natural AHR ligands to the degree of influenza-induced inflammation and lung damage. In contrast to TCDD-induced influenza susceptibility (Vorderstrasse, Bohn & Lawrence, 2003; Luebke *et al.*, 2002), augmenting the natural reservoir of physiological ligands through CYP1 depletion triggers sustained AHR activity, generating a significantly dampened inflammatory lung environment and reduced barrier damage.

Initially, we hypothesised that sustained AHR activation would taper immunopathology driven by IFN, either by reducing pathogenic IFN- α/β signalling in immune cells, or through enhanced lung repair. Consistent with previous findings (Yamada *et al.*, 2016), we show AHR-dependent suppression of influenza-induced type I and III IFN responses following in vitro ligand treatment (Fig. 24), and regulated by endogenous ligands in vivo (Fig. 26). Suppression of IFN responses in CYP1 deficient mice correlates with a significant reduction in both lung immune cell infiltration and lung barrier disruption (Fig. 27). However, lung inflammation and damage were not exacerbated in *Cyp1*^{-/-} mice following exogenous IFN- α treatment (Fig. 31), suggesting alternative mechanisms in the promotion of host fitness. This is consistent with the kinetics of AHR-dependent IFN regulation in these mice, considering pathogenic responses are associated with IFN signalling late in disease course (Channappanavar *et al.*, 2019), yet we observed differences in *Ahr*^{-/-} and *Cyp1*^{-/-} mice early, on day 2 post infection (Fig. 26). Finally, despite increased IFN production in AHR deficient mice (Fig. 26B), this did not correlate with changes in the degree of lung damage or influenza susceptibility (Fig. 32). Thus, we conclude that the improved influenza protection in CYP1 deficient mice occurs independently of AHR regulation of IFN responses.

Anti-inflammatory and IFN suppressive effects in CYP1 deficient mice do not change antiviral immunity (Fig. 28), suggesting AHR-dependent promotion of disease tolerance. Mechanistically, data from CYP1A1 fate-reporting and BM chimera experiments suggests AHR activation in the non-hematopoietic compartment is important for lung protection (Fig. 34 and 35). AHR is known to regulate cell cycle processes (Yin *et al.*, 2016), and has recently been shown to promote intestinal barrier function through modulation of Wnt signalling pathways (Metidji *et al.*, 2018). However, *Cyp1*^{-/-} mice displayed normal rates of lung repair during influenza recovery (Fig. 33). In addition, improved repair would not reflect the kinetics of influenza-induced lung regeneration in our model, as reduced lung damage in CYP1

deficient mice is observed on day 6 post infection (Fig. 27), at which point alveolar regeneration is only just beginning to establish (Fig. 2).

CYP1A1-reporter mice revealed a striking level of AHR activity in endothelial cells of both WT and *Cyp1^{-/-}* mice (Fig. 34). AHR deficient mice have vascular defects (Lahvis *et al.*, 2000), suggesting a role in vascular development, which may explain the high degree of Cre-mediated eYFP induction. In addition, AHR is highly expressed in endothelial cells, and mice with AHR deficiency specifically in the endothelium exhibit hypotension (Agbor, Elased & Walker, 2011). However, genetic dissection of transcriptional activity in zebrafish revealed a physiologic role for AHR in the endothelium, including the regulation of *cxc4a* chemokine receptor expression (Sugden *et al.*, 2017). Whether AHR regulates endothelial immune function following infection is not known. It would be interesting to characterise the expression profiles of CYP1 deficient mice in individual immune and non-immune cell populations during influenza virus infection, to define pathways influenced by increased AHR activation that may contribute to host protection.

Sustained AHR activation promoted IL-22 production, which may induce lung barrier protection independent to proliferative repair responses (Fig. 22A). Recombinant IL-22 treatment reduces lung damage through the induction of barrier resistance and protection pathways in influenza virus infected mice (Barthelemy *et al.*, 2018). We also observed greater IL-22 levels in chimeric mice that lacked CYP1 enzymes in the non-hematopoietic compartment alone (WT $\rightarrow$ *Cyp1^{-/-}*) (Fig. 35E). IL-22 is thought to be exclusively produced by immune cells (Dudakov, Hanash & van den Brink, 2015), therefore the increase in chimeric mice may occur following indirect effects mediated by AHR activity in the non-hematopoietic compartment. Alternatively, CYP1 deficiency in the non-hematopoietic compartment may allow enhanced AHR ligand trafficking across barrier tissues to IL-22-producing immune cells. This ligand-trafficking effect was observed in genetically modified mice with constitutive CYP1A1 expression in intestinal epithelial cells, which consequently depleted the supply of natural AHR ligands to intestinal immune cells, thus compromising IL-22-dependent barrier protection (Schiering *et al.*, 2017). Measurement of AHR-target gene expression in the individual cell populations (including immune cells, epithelia, and endothelia) of BM chimeric mice may reveal whether pulmonary AHR activation is influenced by ligand-trafficking. NK cells were one of the few immune cell types with increased frequencies in the lungs of *Cyp1^{-/-}* mice following infection, suggesting a potential source for enhanced production of IL-22 (Cella *et al.*, 2009; Kumar *et al.*, 2013).

Despite detecting increased AHR activity in the lungs of *Cyp1^{-/-}* mice (Fig. 25 and 34), we have not directly attributed AHR to influenza protection in these mice. This may be achieved by use of the AHR antagonist, CH223191. However, the mechanism by which CH223191 inhibits ligand binding is not fully understood, and it operates in a ligand-selective manner (Soshilov & Denison, 2014; Zhao *et al.*, 2010). Alternatively, crossing *Cyp1^{-/-}* and *Ahr^{-/-}* mice would deliver insight into the direct contribution of AHR in delivering protection. It would also be interesting to identify the source of augmented natural ligands in CYP1 deficient mice, and subsequently starve that source, whether it be dietary or microbial, in an attempt to reduce AHR-dependent effects in influenza.

Cyp1^{-/-} mice provide a valuable genetic model to enhance and examine AHR pathways through sustained receptor activation, without resorting to potent xenobiotics and their associated toxicity. Even so, their uses as a model are limited, owing to well-documented physiological problems, with mice exhibiting various birth-defects and metabolic imbalances, including liver dysfunction (Dragin *et al.*, 2008). The similar extent to which WT and *Ahr^{-/-}* mice exhibit lung damage during influenza virus infection suggests that in our system, physiological levels of AHR signalling are insufficient to provide tissue protection. Protective cell-specific effects may be diluted by the multifaceted nature of AHR biology, with identified functions in multiple different cell types. This is reflected in our BM chimeras, as when CYP1 deficiency was restricted to the non-hematopoietic compartment, we no longer observed differences in eosinophil and CD4⁺ T cell lung frequencies, or in IFN and IL-6 levels (Fig. 35E and F). AHR function in multiple cell types may consequently counterbalance one another, resulting in a dilution of phenotypic effects during infection. Lung epithelial- or endothelial-specific genetic AHR depletion might reveal important barrier functions independent of effects mediated in immune cells, as has been shown in the intestinal epithelium (Metidji *et al.*, 2018).

In order to provide physiological context to the protective effects observed in mice lacking CYP1 activity, we aimed to induce AHR activity through natural ligand supplementation in the diet. We observed significant AHR activation in the lungs of WT mice fed I3C-supplemented diet (Fig. 36A), suggesting a gut-lung axis in the provision of natural ligands. In CYP1A1 reporter mouse, we failed to detect differential AHR activity induced by the I3C diet, with the only signal in WT mice (both on purified and I3C diet) emanating from endothelial cells (Fig. 36B). In addition, I3C supplementation had no effect on the development of lung damage in influenza virus infected mice (Fig. 37), although these results are preliminary. To identify in which cells AHR is activated in the lung following dietary supplementation—

be it tissue resident immune cells, structural (endothelia, epithelia), or circulating lymphocytes—it would be beneficial to quantify AHR target gene mRNA levels in individual lung cell types. Following this, understanding the kinetics of lung damage progression following physiological dietary AHR activation, as opposed to constitutive action in *Cyp1^{-/-}* mice, may reveal an alternative course of disease progression. For instance, it could be postulated that the transient nature of AHR signalling induced by natural ligands that are efficiently metabolised (Bergander *et al.*, 2004) may require an extended period to influence lung inflammation and barrier integrity. Therefore, protective dietary-induced pathways may manifest later in the course of an infection, and it would be interesting to measure effects during the influenza recovery phase. In addition, AHR-induced ligand metabolism in the intestinal epithelium has gateway function for ligand supply to the rest of the body (Ito *et al.*, 2007; Schiering *et al.*, 2017). It would be interesting to examine whether genetic ablation of AHR-induced ligand metabolism specifically in the intestinal epithelium would enhance AHR activities in the lung, and whether this may be enhanced by dietary I3C supplementation.

In conclusion, our data highlights protective AHR function during respiratory viral disease, through the preservation of lung barrier tissues. Endogenous AHR ligands—ubiquitous in the diet and the commensal flora—are influential in regulating these effects, suggesting AHR incorporates both host and environmental cues to influence lung disease progression. Our data contrast with studies using xenobiotic ligands, despite both inducing sustained AHR activation, which suggests that responses are influenced by ligand-specificities. In addition, this provides a possible mechanistic link between the prevalence of air pollutants and compromised respiratory health (Gordon *et al.*, 2014), including respiratory infections and chronic lung disease.

Chapter 6. Discussion

During the early stages of my graduate research project, we were interested in host response pathways in influenza that impact the severity of lung damage. Specifically, we explored contextual aspects of IFN and AHR biology, and their contribution to lung barrier protection and epithelial regeneration. In this thesis, we describe a novel mechanism by which type I and III IFNs exacerbate lung pathology, signalling specifically in epithelial cells to disrupt vital lung regeneration. Of the three IFN subtypes analysed, we found type III IFNs (IFN- λ) harboured the most potent antiproliferative potential, in both in vitro and in vivo settings. We additionally show that the AHR suppresses influenza virus-induced IFN production, yet this appears to be independent from its protective effects exerted following the increase of available natural ligands. Enhanced AHR activity triggers signals in epithelial and endothelial cells, resulting in a dampened inflammatory environment and tissue protection.

In the discussion sections following individual results chapters, I focused on more technical aspects of the data shown, including discrepancies and similarities with previous publications, evidence gaps within our findings, and short-term experimental ideas for how these might be addressed. Here, in the final chapter of this thesis, I attempt to discuss findings that form the basis of this thesis in a wider context of infection, immune, and tissue repair biology.

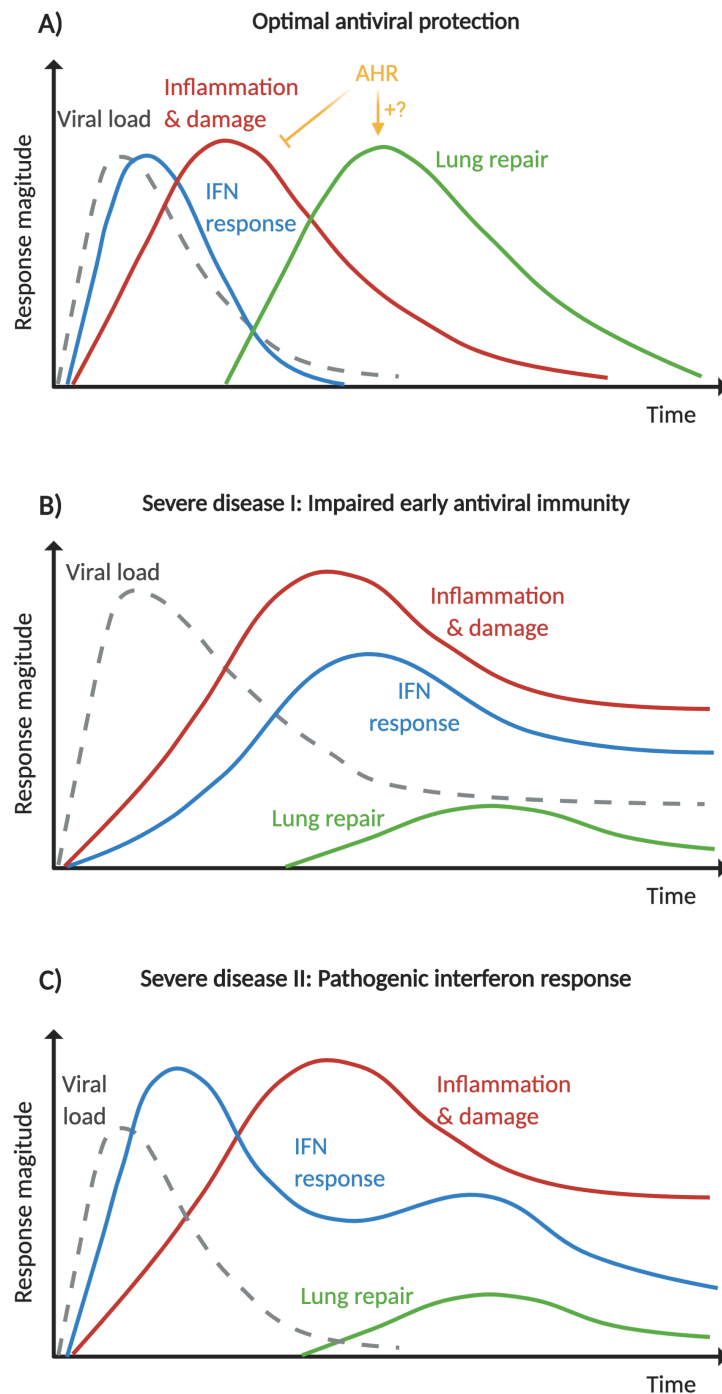
6.1 Host factors as determinants of disease severity

Given the drastic health and economic impact imposed by the ongoing COVID-19 pandemic and endemic respiratory viruses, in addition to the constitutive threat of future viral pandemics, an unprecedented global research effort is currently underway to identify factors that influence the severity of human disease caused by viral pathogens. Cases of severe disease are often characterised by extensive loss of respiratory epithelial and endothelial tissues, resulting in the disruption of critical gas exchange and lung barrier function. During an infection, the host endures two types of damage: that inflicted directly by cytopathic viral effects, and also as a consequence of immunopathology. Evidence put forward in this thesis focuses on the latter, as genetic alterations of IFN and AHR pathways in our models did not affect viral burden (Fig. 7 and 28).

For years, a persistent dogma maintained that the effects induced by IFNs are largely

protective during respiratory viral infection, owing to their frontline antiviral activity. Over the last decade or so, this has been called into question, due to the emergence of evidence identifying disease-promoting IFN mechanisms in animal models of viral infection (Teijaro *et al.*, 2013; Wilson *et al.*, 2013; Davidson *et al.*, 2014; Channappanavar *et al.*, 2016), and positive correlations of IFN signatures with severe disease in humans (Hoang *et al.*, 2014; Lucas *et al.*, 2020). A consistent pattern from murine influenza studies indicates a pathogenicity induced by *excessive* IFN responses. The comparable capacity for viral control in individual IFN receptor knockout mice compared to WT mice highlights IFN system redundancy, and importantly, that there is an endogenous threshold of antiviral ISG induction required for IFN-induced antiviral protection (Price, Gaszewska-Mastarlarz & Moskophidis, 2000; Mordstein *et al.*, 2008; Channappanavar *et al.*, 2016; Davidson *et al.*, 2014; Crotta *et al.*, 2013). Put simply, one IFN system is likely sufficient for viral control, and therefore, ISG induction beyond this is either redundant, or in certain contexts, pathogenic. This *excess* in IFN function is usually attributed to immunopathology induced by IFN- α/β signals in immune cells.

Our findings in chapters 3 and 4 add scope to this discussion, emphasising the importance of timing, and therefore regulation, in maintaining a balanced endogenous IFN response (Discussion figure 1). Kinetic comparison of IFN production and epithelial proliferation revealed a temporal overlap between the two, prior to the onset of lung repair and mouse recovery. We identified a window in which signals induced by IFN- λ inhibited and/or delayed AT2 cell proliferation, thus, exacerbating pulmonary inflammation and damage. In addition to independent observations, published at the same time (Broggi *et al.*, 2020); these findings, to our knowledge, provide the first mechanistic evidence linking the actions of type III IFN to pathological consequences during a viral infection. These findings indicate a degree of incompatibility between signals induced by IFN and lung regeneration. Therefore, IFN-induced ISG expression requires regulation to balance antiviral function and disease recovery processes, in order to maintain lung function.



Discussion figure 1. Optimal and pathogenic IFN responses during respiratory viral infection.

A) Early and rapid IFN production allows ISG induction to limit viral replication and spread in host tissues. Timely downregulation of IFN responses necessitates an efficient epithelial repair process. AHR-dependent signalling pathways reduce early inflammation and damage in an IFN-independent manner, and may promote repair pathways later during recovery from influenza. B) Impaired early antiviral immunity owing to host-intrinsic factors or virally encoded IFN antagonists results in improper viral control and a delayed IFN kinetics, which subsequently exacerbates inflammation and tissue damage. IFN signalling late during a viral infection impairs regenerative tissue repair programmes. C) Excessive and prolonged IFN production owing to host-intrinsic factors, as seen in 129 mice, generates pathogenic inflammation and lung damage, and disrupts epithelial repair. Created using BioRender.com

In infectious disease, a phenomenon exists in the enormous inter-individual variability in severity of human disease (Casanova & Abel, 2013). This includes the nature of an individual's IFN response to infection, be it protective, or pathogenic. Species-comparison in influenza identified 129 mice as potent IFN producers, relative to their B6 counterparts, following influenza virus infection at the same dose (Davidson *et al.*, 2014), implicating host genetic factors as a determinant of response magnitude. Variation between individuals in the

cell-intrinsic mechanisms that regulate IFN production may therefore be important in determining disease outcome in humans (Ciancanelli *et al.*, 2016; To *et al.*, 2015).

IFN responsiveness contributes to the activation of pathogenic IFN pathways, particularly growth inhibitory effects that can disrupt tissue regeneration, which require a sustained ISG signal in order to manifest. Whether this varies between hosts and contributes to influenza susceptibility, is not clear. However, studies examining the physico-chemical properties and binding kinetics at the IFN receptor level suggest so, as they show that the induction of these pathways is heavily influenced by the nature of an individual's type I and III IFN system. Type I and III IFNs regulate overlapping sets of ISGs (Bolen *et al.*, 2014), yet differential signalling (antiviral versus antiproliferative) is achieved by multiple layers of regulatory mechanisms that determine the magnitude of IFN signal transduction. This includes ternary complex stability, which differs for each subtype due to receptor binding affinity; receptor abundance and distribution; binding duration; and induction and effectiveness of negative regulatory mechanisms (Schreiber & Piehler, 2015; Schreiber, 2017; Mendoza *et al.*, 2017). Sustained IFN signals required for antiproliferative effects can also be affected by extrinsic cellular factors, which again, can vary between individuals. A hyperinflammatory pulmonary environment, as seen in severe influenza (Kobasa *et al.*, 2007), is often characterised by the recruitment of cytokine-producing immune cells, including IFN (Davidson *et al.*, 2014), contributing to the elongation of IFN-induced signals. The nature of a host's IFN response is therefore dependent on numerous intrinsic factors at the molecular and cellular levels, in addition to external variables like viral strain.

Here, our data makes evident the importance of intrinsic mechanisms in dictating the outcome of an IFN response. In particular, the response magnitude of individual IFN subtypes produced during a viral infection. We show that IFN- λ production was produced at higher concentrations and for a longer duration, relative to IFN- α/β in vivo (Fig. 3), and also had the most potent effects in vitro in activating ISGs and antiproliferative pathways (Fig. 20 and 21). Selection pressures likely resulted in the tighter regulation of IFN- α/β responses due to ubiquitous cellular IFNAR expression. Thus, a signalling response with a more 'unchecked' kinetic is induced by IFN- λ subtypes owing to reduced regulation at the receptor level, which is additionally enhanced by increased cytokine levels during infection in vivo. Through α IFNAR blockade, we show that in B6 mice IFN- λ mediates epithelial disruption. However, our findings in 129 mice show that IFN-mediated inhibition of lung repair is host- and context-dependent, as α IFNAR treatment late during infection in 129 mice was sufficient to improve

repair. This therefore highlights how intrinsic variances affecting the nature of an IFN response can subsequently impact lung repair.

Inter-individual variations in IFN-associated pathogenicity could be influenced by genetic factors that alter IFN signal transduction regulatory mechanisms. Indeed, overactive IFN pathways are heritable traits linked to the severity of autoimmune disease in humans, such as systemic lupus erythematosus and Sjögren's syndrome (Ghodke-Puranik & Niewold, 2013; Miceli-Richard *et al.*, 2007). With regard to potential genetic risk factors during viral infections, the genetic condition that causes Down syndrome, trisomy 21, also activates the IFN pathway (Sullivan *et al.*, 2016), and could be linked to the more severe consequences in people with Down syndrome during lung viral infections (Pérez-Padilla *et al.*, 2010; Espinosa, 2020). Interestingly, genome-wide association studies have identified expression of functional *IFNL4* (encoding IFN- λ 4) as a risk factor in human hepatitis C virus infection therapies (Ge *et al.*, 2009; Prokunina-Olsson *et al.*, 2013). Individuals expressing IFN- λ 4 exhibit delayed viral clearance, and reduced responsiveness to IFN therapies, yet expression of a non-functional IFN- λ 4 variant is associated with protection (Terczyńska-Dyla *et al.*, 2014). Whilst the mechanism underlying this association is unclear, the increased susceptibility is linked to enhanced ISG induction, and IFN- λ 4 also induces antiproliferative and cell death pathways (Onabajo *et al.*, 2015). Thus, this provides evidence for inter-individual variations in IFN subtype expression affecting disease severity, which may alter expression of pathogenic tissue-damaging programmes.

The strict, multi-layered regulatory mechanisms that control induction of growth inhibitory pathways are a testament to their pathogenic potential, which are activated with the intent to enhance viral clearance during pathogen persistence. However, the way in which these regulatory processes influence differential gene expression past the receptor level, remains unknown. It seems antiproliferative proteins can be directly induced as ISGs (Takaoka *et al.*, 2003), and are dependent on sustained ISG activation and transcriptional machinery (Tsuno *et al.*, 2009; Chin *et al.*, 1996; Bromberg *et al.*, 1996). One possibility, is that specific receptor complex kinetics enhance the recruitment and accessibility of downstream signalling components, such as JAK and TYK proteins, resulting in the induction of distinct effector programmes. An example of distinct downstream programmes is the differential activity mediated by IRF1, which is induced by IFN- α/β , but not IFN- λ (Forero *et al.*, 2019). However, we do not believe this mechanism applies for our findings, as IFN- λ failed to induce IRF1 in hepatocytes owing to their decreased expression of *Ifnlr1*, which is not the case in our system.

Our studies examining AHR signalling effects in influenza suggest a potential mechanistic link for IFN-dependent and IFN-independent pathways as determinants of respiratory viral disease severity. Despite our conclusions that AHR-mediated tissue protection occurs independently from IFN regulatory effects, we do not exclude the possibility that this may occur in other systems. In agreement with previous observations (Yamada *et al.*, 2016), we describe an endogenous role of AHR in suppressing type I and III IFN production during a viral infection (Fig. 26). Aside from epithelial-specific lung repair inhibition, IFN-related pathology appears to be limited in influenza virus infected B6 mice (Price, Gaszewska-Mastarlarz & Moskophidis, 2000; Davidson *et al.*, 2014). This may explain the lack of IFN suppression-dependent AHR protection in our model of enhanced AHR signalling. In a system or individual where pathological IFN activities are more prevalent during a viral infection, endogenous AHR signalling pathways may play a more prominent role in influencing disease severity. In addition, variations in the functional capacity of the AHR between individuals could be a host-intrinsic genetic factor that influences disease severity. For example, the AHR in B6 mice with high affinity ligand-binding potential is encoded by the *Ah^{b-l}* allele, whilst the AHR in 129 mice encoded by the low-responder *Ahr^d* allele has significantly diminished binding affinity for ligands (Poland, Palen & Glover, 1994). Therefore, it is possible that the pathogenic effects of IFNs in 129 mice may be exacerbated by weakened AHR ligand-binding, resulting in reduced negative regulation of IFN production. Whilst genetic AHR-related risk factors have not yet been identified for viral infections, genome-wide association studies have identified single nucleotide polymorphisms near AHR-regulated genes that may contribute to AHR-dependent disease mechanisms in inflammatory immune disorders (Neavin *et al.*, 2018). For instance, *AHR* has been identified as a susceptibility locus in inflammatory bowel disease (Liu *et al.*, 2015). Thus, variations in AHR activity between individuals could contribute to disease outcome during viral infection.

We also describe here a mechanism of AHR activation in promoting IFN-independent lung tissue protection. By genetically amplifying endogenous AHR responses *in vivo*, we show natural ligands can promote disease tolerance, thus protecting mice during influenza virus infection. The identification of AHR pathways in protective influenza responses provides a possible mechanistic link for the influence environmental signals have on host-response pathways during viral infection. Recent studies have identified a dietary gut-lung axis affecting pulmonary immunity and inflammation modulated by dietary compounds (Trompette *et al.*, 2014, 2018; Goldberg *et al.*, 2019). Our evidence here suggests natural AHR responses can be

augmented through AHR ligand supplementation in the diet (Fig. 36). In addition, tryptophan metabolic pathways are switched on during viral infection (Fox *et al.*, 2015; Huang *et al.*, 2013), suggesting a potential feedback mechanism in the AHR-dependent curtailing of pathogenic inflammatory responses.

The differential AHR activity in response to toxic xenobiotics compared to natural ligands suggests the type of ligand and the dynamics of AHR–ligand interactions dictate response outcome. This is made evident when comparing our findings with the effects of TCDD treatment in influenza infected mice. CYP1 deficient mice have sustained AHR signalling (Fig. 25), as is the case following TCDD–AHR binding (Niwa *et al.*, 1975). However, whilst we observed significant protection of lung barrier tissues through enhancing the availability of natural AHR ligands (Fig. 27), TCDD treatment significantly increases the susceptibility of mice to influenza (Vorderstrasse, Bohn & Lawrence, 2003; Luebke *et al.*, 2002). Site-directed mutagenesis in the AHR ligand binding domain has confirmed distinct binding patterns between structurally diverse ligands (Soshilov and Denison, 2014). Differential AHR activities are also affected by cell-specific activation. Augmenting the availability of endogenous ligands seemed to induce AHR signalling in endothelial and epithelial cells (Fig. 34). The increased protection in WT → Cyp1 chimeric mice supports this (Fig. 35), and indicates the importance of AHR signalling in barrier lung tissues during viral infection. Conversely, TCDD administered by oral gavage, as was the case in influenza studies (Vorderstrasse, Bohn & Lawrence, 2003), is likely to induce systemic effects (Ozeki *et al.*, 2011).

The diversity in AHR-dependent functions is also influenced by molecular cofactors that influence transcriptional activity. This includes HIF1 α , that competes with the AHR for ARNT binding (Vorrink & Domann, 2014). Local lung hypoxia occurs as a consequence of extensive epithelial necrosis in influenza (Xi *et al.*, 2017), and influenza virus triggers a hypoxic response through the stabilisation of HIF1 α protein (Ren *et al.*, 2019). Therefore, lung hypoxia may exacerbate pathology mediated by HIF1 α -dependent reduction of AHR activation through ARNT competition. This could promote a break in disease tolerance, with lung damage inhibiting tissue protective AHR functions.

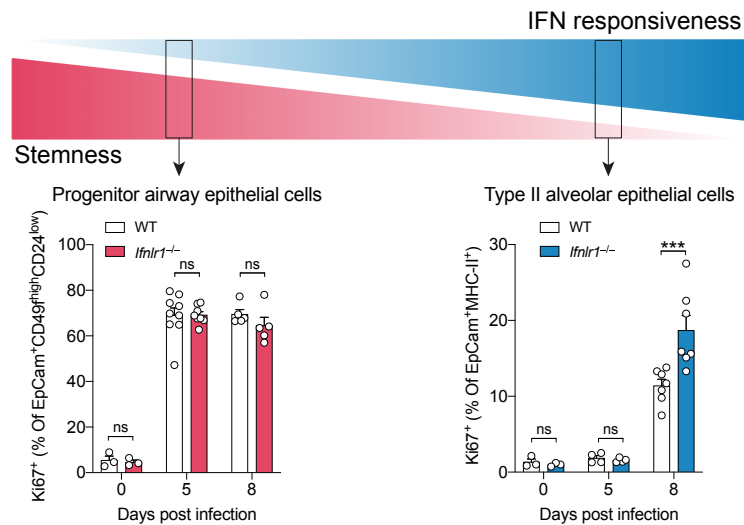
How differential AHR activities are regulated and the effects they induce during viral infection in humans remains to be determined. A very recent study has also shown that Zika virus infection induces Kyn production and AHR activation in human liver cells with correlating alterations in IFN response pathways (Giovannoni *et al.*, 2020). Interestingly,

metatranscriptome analysis of BALF from COVID-19 patients detected a significant upregulation of the AHR signalling pathways (Zhou *et al.*, 2020b). This suggests that the AHR response pathway regulates lung immunity during respiratory viral disease in humans, and this may be altered due to host-intrinsic and environmental factors, which could affect disease severity in individuals.

Overall, our findings along with others suggest a commonality and also interplay between AHR and IFN pathways during viral infections. Both are influenced by receptor-binding dynamics which regulate differential signalling mechanisms and therefore the cellular response. They both serve protective functions during an infection; however, aberrant responses can result in pathology and tissue damage. Deviations away from an optimum in these pathways can be influenced by host-intrinsic and extrinsic factors, which may ultimately affect the severity of disease in humans.

6.2 Tissue-resident IFN-nonresponsive stem cells? A potential mechanism for emergency lung repair

During our lung repair studies *in vivo*, we could not detect an effect of endogenous IFN signals on the proliferation of distal airway progenitor cells (Fig. 6D). This intriguing observation is in contrast to the increased AT2 cell proliferation observed in IFNR knockout mice (Fig. 6A to C). Assuming these results are correct, one possible reason for this discrepancy is the different kinetics between the two epithelial cell populations. Distal airway progenitor cells expand in the lung on day 4 post infection in a highly proliferative state (Fig. 2B and C), and therefore, may not have received an IFN signal of sufficient duration to induce growth inhibitory effects. An alternative hypothesis is that these distal airway progenitor cells are IFN-nonresponsive, which would allow them to avoid IFN-inducible antiproliferative effects, thus, promoting efficient lung repair (Discussion figure 2).



Discussion figure 2. IFN responsiveness in respiratory epithelial stem cells.

Surprisingly, we did not observe differences in the proliferation of airway stem cells in IFNLR deficient mice during influenza virus-induced lung repair. On the other hand, lack of IFN- λ signalling enhanced the proliferation of differentiated AT2 cells. Highly proliferative airway progenitors may be refractory to IFN signalling, which would enable the efficient and rapid restoration of destructed epithelia in order to maintain lung function. Created using BioRender.com

To discern whether or not these progenitor cells are in fact refractory to IFN, it is important to define this epithelial cell population. In our studies, we identify this population by EpCam⁺CD49^{high}CD24^{low} flow cytometry staining (Fig. 2A). This population has been further characterised, and is defined by high expression of the stem cell antigen Sca-1, positive β 4 integrin and CD200 staining, and upregulation of the Krt5/p63 programme following influenza virus infection (Quantius *et al.*, 2016). Our identified EpCam⁺CD49^{high}CD24^{low} cells therefore likely constitute the lineage-negative p63/Krt5⁺ stem cells that mobilise from distal airways to repopulate severely damaged alveoli in influenza virus infected mice (Kumar *et al.*, 2011; Vaughan *et al.*, 2015). This corresponds with the cellular kinetics of progenitor cells in our model, which are at very low levels in an uninfected lung (Fig. 2B). In addition, EpCam⁺CD49^{high}CD24^{low} cells may be a heterogenous population that harbours additional stem cell types. Single-cell transcriptomics analysis of EpCam⁺ β 4⁺CD200⁺ cells identified additional known and unknown lung stem cell (Kathiriya *et al.*, 2020). It may be that only a subset of distal airway progenitor cells is IFN refractory.

Distal airway progenitor cells (also referred to as DASCs) mobilise following severe lung injury, aiding restoration of the alveolar niche to maintain lung structure and prevent

respiratory failure (Kumar *et al.*, 2011; Vaughan *et al.*, 2015; Zuo *et al.*, 2014; Kathiriya *et al.*, 2020). This process has been likened to an emergency tissue repair response, owing to their emergence in the lung following severe injury and their highly proliferative nature. Genetic depletion of p63⁺Krt5⁺ lung stem cells during recovery from influenza significantly compromises gas exchange (Zuo *et al.*, 2014). In the context of emergency tissue repair, the inability of stem cells to respond to IFN and avoid IFN-inducible antiproliferative effects would, arguably, be highly beneficial. To confirm whether lung stem cells are IFN-nonresponsive, it would be interesting to measure the downstream signalling response and ISG expression in individual cells. This could be compared to the responses of mature lung epithelia, for instance AT2 cells, which do respond to antiproliferative effects induced by IFN in our model (Fig. 6A to C).

The notion that stem cells are refractory to IFN stimulation has been recognised for over 40 years (Burke, Graham & Lehman, 1978). It also known that some stem cells are resistant to viral infection (Swartzendruber & Lehman, 1975; Wu, Kwong & Rice, 2019). This begs the question: how do IFN non-responsive stem cells promote antiviral resistance? Several mechanisms have been suggested: 1) IFN-independent RNA interference (RNAi) (Maillard *et al.*, 2013); 2) IFN-independent epigenetic repression of endogenous retroviruses (Yang *et al.*, 2015); and 3) constitutive intrinsic expression of an ISG subset in pluripotent stem cells (Wu *et al.*, 2018). If our hypothesis is correct, distal airway progenitor cells in our system would rely on one of these alternative cell-intrinsic antiviral mechanisms. Two club-like distal airway progenitor cell populations in a steady-state lung have recently been identified as possessing high baseline expression of an ISG subset (Kathiriya *et al.*, 2020), and therefore, we suggest the third option may apply, with lung stem cells in an intrinsic antiviral state due to IFN-independent ISG subset expression.

Both embryonic stem cells and tissue resident stem cells have been identified as having high intrinsic expression of a subset of antiviral ISGs (Wu *et al.*, 2018). The idea behind alternative antiviral responses in stem cells is that proliferative self-renewal functions are not compatible with the full battery of IFN-inducible effects, including antiproliferative and proapoptotic functions. Selective expression of potent antiviral molecules (such as IFITM1) therefore promotes intrinsic antiviral resistance and avoids sensitivity to more harmful IFN-inducible pathways. Mechanistically, high basal ISG subset expression is thought to be JAK/STAT independent given their lack of IFN responsiveness. It is likely histone modifications are involved, as H3K4me3 and H3K27ac modifications which commonly mark

active promoters are enriched in the promoters of highly expressed ISGs in stem cells, relative to silent ISGs (Wu, Kwong & Rice, 2019).

An additional interesting observation in the study by Wu *et al.* is the decrease in high intrinsic ISG expression as stem cells progress to terminal differentiation. Consistently, a functional IFN system appears to impair the differentiation potential of pluripotent stem cells (Eggenberger *et al.*, 2019), and only following terminal differentiation is this function restored and intrinsic expression of ISG subsets wane (Wu *et al.*, 2018). This fits to the notion that a fully functional IFN system is incompatible with stem cell proliferative and cellular differentiation function. Our data here supports this concept, as we show the detrimental IFN-inducible effects on AT2 cell proliferation and airway cell differentiation during lung repair (Fig. 6 and 11). Therefore, tissue-resident IFN-nonresponsive airway stem cells would provide a lifeline for repair, compensating for epithelial regenerative gaps where IFN-sensitive AT2 cells cannot. It would be interesting to cross-examine ISG profiles in individual stem and differentiated cell subsets by single-cell RNA sequencing analysis, during recovery from influenza. If the hypothesis is correct, one might expect an ISG profile gradient inversely correlating with degree of stemness by an epithelial cell.

The increased susceptibility of lung progenitor cells to influenza virus infection compared to alveolar and differentiated airway cells (Quantius *et al.*, 2016) conflicts with the above hypothesis, contradicting the inherent antiviral resistance in stem cells. However, this may be explained by a heterogeneity in the EpCam⁺CD49f^{high}CD24^{low} population, as it may only be a small group of stem cells that harbour intrinsic antiviral immunity. In addition, stem cells may become susceptible to infection as they begin to differentiate to restore damaged alveoli (Wu *et al.*, 2018). It would be interesting to perform-lineage tracing experiments to delineate the specific contribution of stem cell precursors to lung repair in our model, and understand how these pathways are affected by IFN. It is also important to note that a role of tissue-resident IFN-nonresponsive stem cells has not been identified *in vivo*, where stem cell antiviral resistance may not apply to the same extent.

6.3 Host-targeted therapies for respiratory virus disease

Modulation of host immune responses through therapeutic intervention can significantly alleviate disease burden in individuals. Effective implementation of such therapeutic strategies requires a thorough understanding of disease progression, and how this

varies between susceptible individuals and demographics. During respiratory viral infection, successful host-targeted therapies manipulate host pathways to either aid viral clearance or reduce immunopathology. To devise a strategy for effective host-targeted therapies, it is important to identify mechanisms that drive pathology in different patient cohorts. For instance, a positive correlation between increased viral load and disease severity indicates that the use of antiviral therapeutics would be beneficial (Liu *et al.*, 2020). On the other hand, lack of such a correlation suggests that pathology is driven by other factors, namely immunopathology and comorbidities (Gorini da Veiga, Kretzmann & Correa, 2012; Lavezzo *et al.*, 2020). As previously discussed, disease heterogeneity is driven by both host-intrinsic and environmental factors, including the virulence of the infecting agent. This heterogeneity is particularly prevalent in COVID-19 patients (Vabret *et al.*, 2020), and therefore, blanket treatment strategies that are not tailored to the specific manifestation of disease are likely to be less effective, and can even induce further pathology if used improperly. The use of patient biomarkers is invaluable in such a context, allowing clinicians to understand the stage of disease, including immune status, to then implement treatment options accordingly.

The potent antiviral effects induced by type I and III IFNs make them prime candidates for the treatment of respiratory viral infections. IFNs are administered in humans through subcutaneous injection, or directly into the airways via an aerosol nebuliser (Sallard *et al.*, 2020). Due to the pathogenic effects associated with IFN, in particular IFN- α/β , the use of IFN therapies for influenza and COVID-19 patients is a matter of ongoing debate (Lee & Shin, 2020). As is the case with all antiviral therapies, earlier IFN administration is more likely to be efficacious through the disruption of initial viral replication. During respiratory viral infections, progression of disease severity is linked to spread of the virus from the airways into the lungs. In this context, IFN treatments may help to control viral replication to prevent alveolar damage, particularly when host antiviral immunity is impaired due to host genetic factors or virally encoded IFN antagonists (Blanco-Melo *et al.*, 2020). In late stages of disease, IFNs must be used with caution to avoid exacerbating inflammation and lung pathology. IFN- λ therapies therefore have the additional benefit of inducing epithelial-targeted antiviral effects (Prokunina-Olsson *et al.*, 2020).

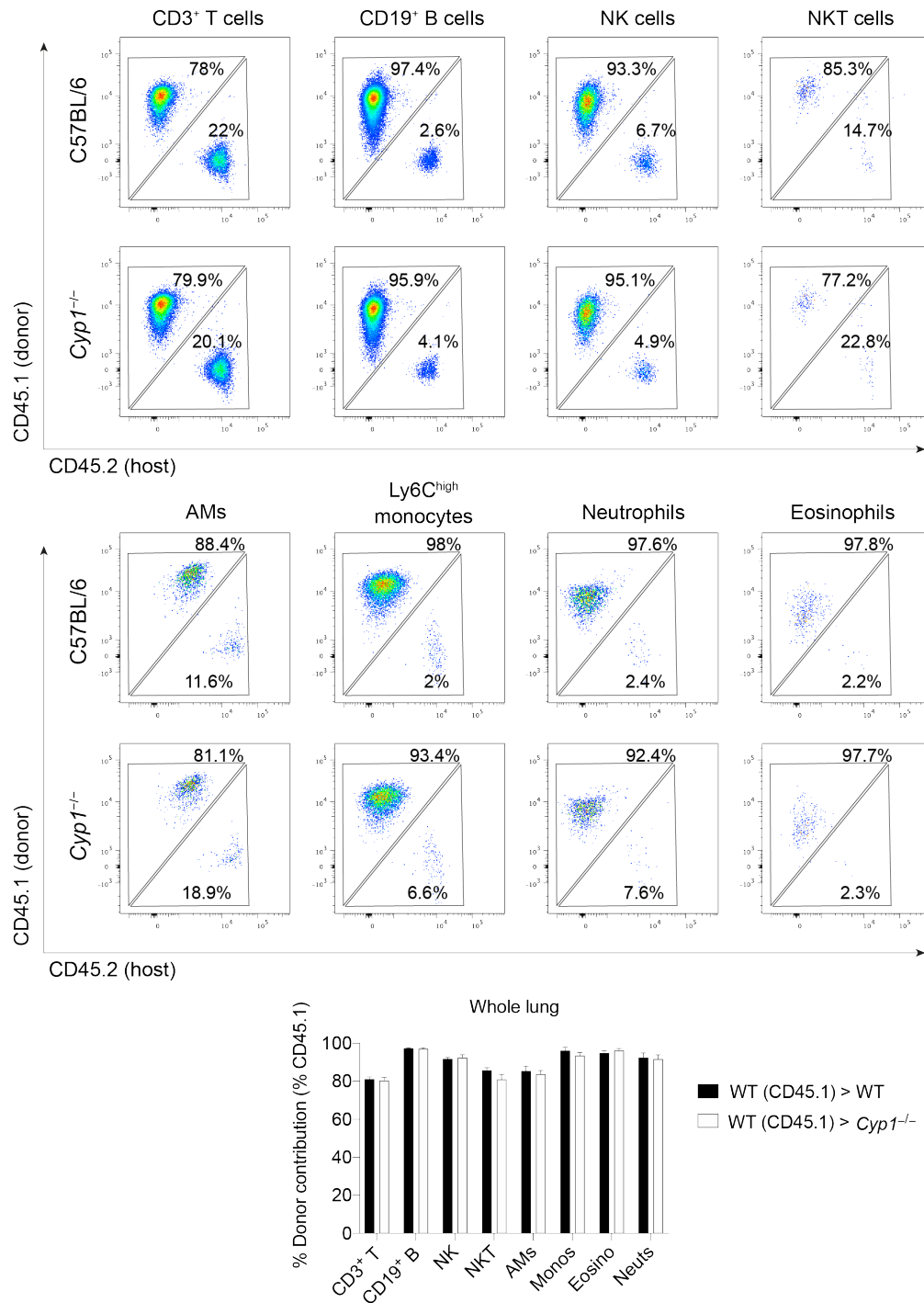
By identifying a detrimental consequence of sustained IFN signalling on lung repair, our findings highlight an additional caveat to consider in the application of host-directed IFN therapies. This includes IFN- λ , which has not previously been attributed to pathogenic effects during respiratory viral infection. By studying the effects of IFNs specifically in epithelial cells

during the recovery phase of disease, we were able to identify a mechanism by which IFNs exacerbate lung pathology independently from immunomodulation. In our mouse influenza model, further extension of the IFN response into the window of epithelial recovery, through exogenous treatment, significantly dampens alveolar cell proliferation (Fig. 4). Late treatment has no effect on viral clearance (Fig. 4C and E), suggesting that there is a break-even point in the progression of disease, after which IFN treatment is no longer beneficial, and potentially detrimental for recovery. This is supported by recent evidence from a retrospective COVID-19 patient cohort study (Wang *et al.*, 2020b). Early administration of IFN- α was associated with reduced in-hospital mortality, whereas late IFN- α therapy was associated with delayed recovery and increased mortality (Wang *et al.*, 2020b).

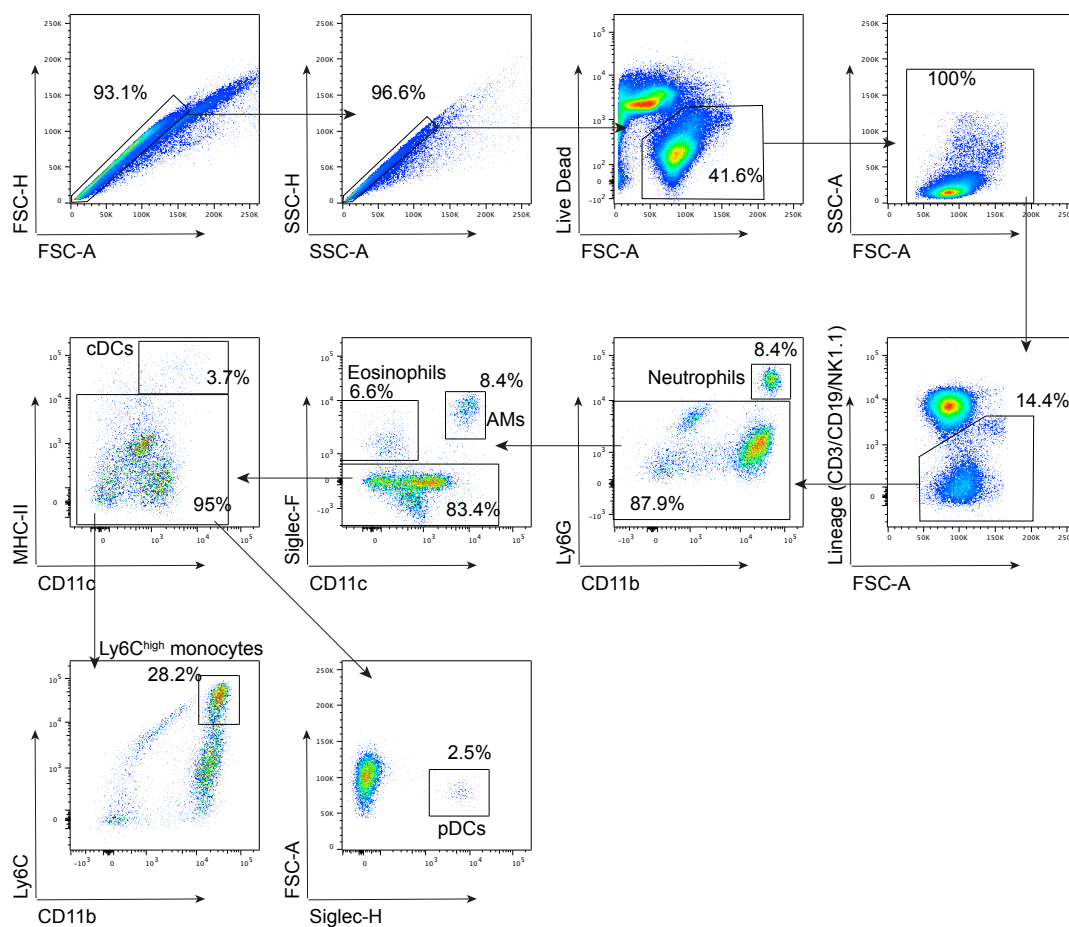
Given the particular signalling thresholds that dictate differential activities induced by IFN, it is possible that these pathways could be optimised for advantageous effects in the clinic. The vastly different signalling thresholds required for induction of antiviral and antiproliferative pathways can be regulated by engineered IFN molecules to fine-tune specific responses (Levin *et al.*, 2014), and even promote cell-marker specific targeting (Garcin *et al.*, 2014). Regulatory mechanisms controlling differential activity can be further exploited to enable optimal and efficient lung repair. For instance, the use of JAK/STAT inhibitors late in the course of disease could effectively block sustained ISG signals required for the disruption of lung repair. Optimal protection induced by host-targeted IFN-related therapies would be achieved by strong induction of ISGs early during infection to curb viral replication, followed by timely downregulation of IFN responses, enabling efficient lung epithelial repair.

Evidence put forward in this thesis furthers our understanding in the underlying mechanisms that govern immunopathology during viral infection. By focusing on the importance of epithelial repair during the recovery phase of infection, we identify a novel mechanism by which type I and III IFN-induced signals aggravate lung pathology. We also highlight a tissue-protective role of AHR mediated by endogenous ligands, which has implications for host-intrinsic and environmental factors that influenza disease severity.

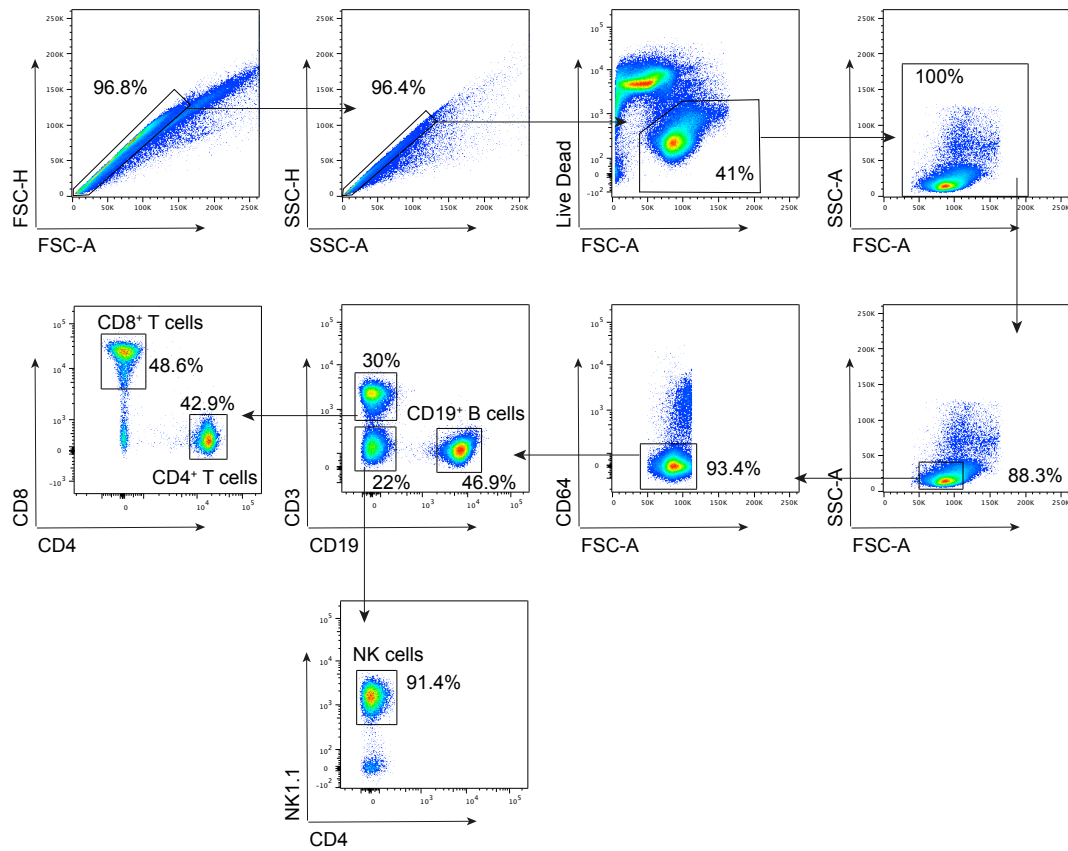
Chapter 7. Appendix



Appendix I. Data representative to the degree of chimerism in bone marrow chimera experiments. The frequency of CD45.1⁺ immune cells was assessed by flow cytometry in irradiated B6 WT or *Cyp1^{-/-}* CD45.2⁺ mice host mice.



Appendix II. Myeloid gating strategy (shown on a naïve B6 WT lung).



Appendix III. Lymphoid gating strategy (shown on a naïve B6 WT lung).

Reference List

- Abdel-Ghafar, A.N., Chotpitayasunondh, T., Gao, Z., Hayden, F.G., et al. (2008) Update on avian influenza A (H5N1) virus infection in humans. *New England Journal of Medicine*. 358 (3). Available from: doi:10.1056/NEJMra0707279.
- Abdul-Careem, M.F., Firoz Mian, M., Yue, G., Gillgrass, A., et al. (2012) Critical role of natural killer cells in lung immunopathology during influenza infection in mice. *Journal of Infectious Diseases*. 206 (2), 167–177. Available from: doi:10.1093/infdis/jis340.
- Ablasser, A., Poeck, H., Anz, D., Berger, M., et al. (2009) Selection of Molecular Structure and Delivery of RNA Oligonucleotides to Activate TLR7 versus TLR8 and to Induce High Amounts of IL-12p70 in Primary Human Monocytes. *The Journal of Immunology*. 182 (11), 6824–6833. Available from: doi:10.4049/jimmunol.0803001.
- Abt, M.C., Osborne, L.C., Monticelli, L.A., Doering, T.A., et al. (2012) Commensal Bacteria Calibrate the Activation Threshold of Innate Antiviral Immunity. *Immunity*. 37 (1), 158–170. Available from: doi:10.1016/j.immuni.2012.04.011.
- Aegerter, H., Kulikauskaite, J., Crotta, S., Patel, H., et al. (2020) Supp: Influenza-induced monocyte-derived alveolar macrophages confer prolonged antibacterial protection. *Nature Immunology*. 21 (2), 145–157. Available from: doi:10.1038/s41590-019-0568-x.
- Agbor, L.N., Elased, K.M. & Walker, M.K. (2011) Endothelial cell-specific aryl hydrocarbon receptor knockout mice exhibit hypotension mediated, in part, by an attenuated angiotensin II responsiveness. *Biochemical Pharmacology*. 82 (5), 514–523. Available from: doi:10.1016/j.bcp.2011.06.011.
- Agostini, L., Martinon, F., Burns, K., McDermott, M.F., et al. (2004) NALP3 Forms an IL-1-Processing Inflammasome with Increased Activity in Muckle-Wells Autoinflammatory Disorder containing protein called ASC binds and activates pro-caspase-1 (Martinon et al ASC contains a C-terminal CARD motif as well as an N-terminal CAR. *Immunity*. 20, 319–325.
- Allen, I.C., Scull, M.A., Moore, C.B., Holl, E.K., et al. (2009) The NLRP3 Inflammasome Mediates In Vivo Innate Immunity to Influenza A Virus through Recognition of Viral RNA. *Immunity*. 30 (4), 556–565. Available from: doi:10.1016/j.immuni.2009.02.005.
- Alvarez, D.F., Huang, L., King, J.A., Elzarrad, M.K., et al. (2008) Lung microvascular endothelium is enriched with progenitor cells that exhibit vasculogenic capacity. *American Journal of Physiology - Lung Cellular and Molecular Physiology*. 294 (3),

- 419–430. Available from: doi:10.1152/ajplung.00314.2007.
- Antonova, E.N., Rycroft, C.E., Ambrose, C.S., Heikkinen, T., et al. (2012) Burden of paediatric influenza in Western Europe: A systematic review. *BMC Public Health*. 12 (1), 1. Available from: doi:10.1186/1471-2458-12-968.
- Apelbaum, A., Yarden, G., Warszawski, S., Harari, D., et al. (2013) Type I interferons induce apoptosis by balancing cFLIP and caspase-8 independent of death ligands. *Molecular and cellular biology*. 33 (4), 800–814. Available from: doi:10.1128/MCB.01430-12.
- Apetoh, L., Quintana, F.J., Pot, C., Joller, N., et al. (2010) The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. *Nature Immunology*. 11 (9), 854–861. Available from: doi:10.1038/ni.1912.
- Arimoto, K.I., Miyauchi, S., Stoner, S.A., Fan, J.B., et al. (2018) Negative regulation of type I IFN signaling. *Journal of Leukocyte Biology*. 103 (6), 1099–1116. Available from: doi:10.1002/JLB.2MIR0817-342R.
- Arpaia, N., Green, J.A., Molledo, B., Arvey, A., et al. (2015) A Distinct Function of Regulatory T Cells in Tissue Protection. *Cell*. 162 (5), 1078–1089. Available from: doi:10.1016/j.cell.2015.08.021.
- Bachofen, H. & Schürch, S. (2001) Alveolar surface forces and lung architecture. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*. 129 (1), 183–193. Available from: doi:10.1016/S1095-6433(01)00315-4.
- Balachandran, S., Roberts, P.C., Kipperman, T., Bhalla, K.N., et al. (2000) Alpha/Beta Interferons Potentiate Virus-Induced Apoptosis through Activation of the FADD/Caspase-8 Death Signaling Pathway. *Journal of Virology*. 74 (3), 1513–1523.
- Barkauskas, C.E., Noble, P.W., Brigid, L.M., Barkauskas, C.E., et al. (2013) Type 2 alveolar cells are stem cells in adult lung. *The Journal of Clinical Investigation*. 123 (7), 3025–3036. Available from: doi:10.1172/JCI68782.ing.
- Barthelemy, A., Sencio, V., Soulard, D., Deruyter, L., et al. (2018) Interleukin-22 immunotherapy during severe influenza enhances lung tissue integrity and reduces secondary bacterial systemic invasion. *Infection and Immunity*. 86 (7), 1–17. Available from: doi:10.1128/IAI.00706-17.
- Basil, M.C., Katzen, J., Engler, A.E., Guo, M., et al. (2020) The Cellular and Physiological Basis for Lung Repair and Regeneration: Past, Present, and Future. *Cell Stem Cell*. 26 (4), 482–502. Available from: doi:10.1016/j.stem.2020.03.009.
- Basu, R., O’Quinn, D.B., Silberger, D.J., Schoeb, T.R., et al. (2012) Th22 Cells Are an

- Important Source of IL-22 for Host Protection against Enteropathogenic Bacteria. *Immunity*. 37 (6), 1061–1075. Available from: doi:10.1016/j.immuni.2012.08.024.
- Baumgarth, N., Herman, O.C., Jager, G.C., Brown, L.E., et al. (2000) B-1 and b-2 cell-derived immunoglobulin m antibodies are nonredundant components of the protective response to influenza virus infection. *Journal of Experimental Medicine*. 192 (2), 271–280. Available from: doi:10.1084/jem.192.2.271.
- Beigel, J.H., Farrar, J., Han, A.M., Hayden, F.G., et al. (2005) Avian influenza A (H5N1) infection in humans. *New England Journal of Medicine*. 353 (13), 1374–1385. Available from: doi:10.1056/NEJMra052211.
- Beischlag, T. V, Luis Morales, J., Hollingshead, B.D. & Perdew, G.H. (2008) The aryl hydrocarbon receptor complex and the control of gene expression. *Critical reviews in eukaryotic gene expression*. 18 (3), 207–250. Available from: doi:10.1615/critreveukargeneexpr.v18.i3.20.
- Belisle, S.E., Tisoncik, J.R., Korth, M.J., Carter, V.S., et al. (2010) Genomic Profiling of Tumor Necrosis Factor Alpha (TNF- α) Receptor and Interleukin-1 Receptor Knockout Mice Reveals a Link between TNF- α Signaling and Increased Severity of 1918 Pandemic Influenza Virus Infection. *Journal of Virology*. 84 (24), 12576–12588. Available from: doi:10.1128/jvi.01310-10.
- Belser, J.A., Katz, J.M. & Tumpey, T.M. (2011) The ferret as a model organism to study influenza A virus infection. *DMM Disease Models and Mechanisms*. 4 (5), 575–579. Available from: doi:10.1242/dmm.007823.
- Bender, B.S., Croghan, T., Zhang, L. & Small, P.A.J. (1992) Transgenic Mice Lacking Class I Major Histocompatibility Complex-restricted T Cells Have Delayed Viral Clearance and Increased Mortality after Influenza Virus Challenge. *Journal of Experimental Medicine*. 175 (4), 1143–1145.
- Bender Kim, C.F., Jackson, E.L., Woolfenden, A.E., Lawrence, S., et al. (2005) Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell*. 121 (6), 823–835. Available from: doi:10.1016/j.cell.2005.03.032.
- Bergander, L., Wincent, E., Rannug, A., Foroozesh, M., et al. (2004) Metabolic fate of the Ah receptor ligand 6-formylindolo[3,2-b]carbazole. *Chemico-biological interactions*. 149 (2–3), 151–164. Available from: doi:10.1016/j.cbi.2004.08.005.
- Bergsbaken, T., Fink, S.L. & Cookson, B.T. (2009) Pyroptosis: Host cell death and inflammation. *Nature Reviews Microbiology*. 7 (2), 99–109. Available from:

- doi:10.1038/nrmicro2070.
- Bessedé, A., Gargaro, M., Pallotta, M.T., Matino, D., et al. (2014) Aryl hydrocarbon receptor control of a disease tolerance defence pathway. *Nature*. 511 (7508), 184–190. Available from: doi:10.1038/nature13323.
- Biondo, C., Lentini, G., Beninati, C. & Teti, G. (2019) The dual role of innate immunity during influenza. *Biomedical Journal*. 42 (1), 8–18. Available from: doi:10.1016/j.bj.2018.12.009.
- Bjeldanes, L.F., Kim, J.Y., Grose, K.R., Bartholomew, J.C., et al. (1991) Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: Comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proceedings of the National Academy of Sciences of the United States of America*. 88 (21), 9543–9547. Available from: doi:10.1073/pnas.88.21.9543.
- Blanco-Melo, D., Nilsson-Payant, B.E., Liu, W.C., Uhl, S., et al. (2020) Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*. 181 (5), 1036–1045.e9. Available from: doi:10.1016/j.cell.2020.04.026.
- Blazek, K., Eames, H.L., Weiss, M., Byrne, A.J., et al. (2015) IFN- λ resolves inflammation via suppression of neutrophil infiltration and IL-1 β production. *The Journal of experimental medicine*. 212 (6), 845–853. Available from: doi:10.1084/jem.20140995.
- Blumer, T., Coto-Llerena, M., Duong, F.H.T. & Heim, M.H. (2017) SOCS1 is an inducible negative regulator of interferon λ (IFN- λ)-induced gene expression in vivo. *Journal of Biological Chemistry*. 292 (43), 17928–17938. Available from: doi:10.1074/jbc.M117.788877.
- Boergeling, Y. & Ludwig, S. (2017) Targeting a metabolic pathway to fight the flu. *FEBS Journal*. 284 (2), 218–221. Available from: doi:10.1111/febs.13997.
- Bolen, C.R., Ding, S., Robek, M.D. & Kleinstein, S.H. (2014) Dynamic expression profiling of type I and type III interferon-stimulated hepatocytes reveals a stable hierarchy of gene expression. *Hepatology*. 59 (4), 1262–1272. Available from: doi:10.1002/hep.26657.
- Boule, L.A., Burke, C.G., Jin, G.B. & Lawrence, B.P. (2018) Aryl hydrocarbon receptor signaling modulates antiviral immune responses: Ligand metabolism rather than chemical source is the stronger predictor of outcome. *Scientific Reports*. 8 (1826), 1–15. Available from: doi:10.1038/s41598-018-20197-4.
- Bouvier, N.M. & Lowen, A.C. (2010) Animal models for influenza virus pathogenesis and transmission. *Viruses*. 2 (8), 1530–1563. Available from: doi:10.3390/v20801530.

- Bradfield, C.A., Kende, A.S. & Poland, A. (1988) Kinetic and equilibrium studies of Ah receptor-ligand binding: use of [125I]2-iodo-7,8-dibromodibenzo-p-dioxin. *Molecular pharmacology*. 34 (2), 229–237.
- Bradley, K.C., Finsterbusch, K., Schnepf, D., Crotta, S., et al. (2019) Microbiota-Driven Tonic Interferon Signals in Lung Stromal Cells Protect from Influenza Virus Infection. *Cell Reports*. 28 (1), 245–256.e4. Available from: doi:10.1016/j.celrep.2019.05.105.
- Brandes, M., Klauschen, F., Kuchen, S. & Germain, R.N. (2013) A systems analysis identifies a feedforward inflammatory circuit leading to lethal influenza infection. *Cell*. 154 (1), 197. Available from: doi:10.1016/j.cell.2013.06.013.
- Brass, A.L., Huang, I.C., Benita, Y., John, S.P., et al. (2009) The IFITM Proteins Mediate Cellular Resistance to Influenza A H1N1 Virus, West Nile Virus, and Dengue Virus. *Cell*. 139 (7), 1243–1254. Available from: doi:10.1016/j.cell.2009.12.017.
- Brincks, E.L., Katewa, A., Kucaba, T.A., Griffith, T.S., et al. (2008) CD8 T Cells Utilize TRAIL to Control Influenza Virus Infection. *The Journal of Immunology*. 181 (7), 4918–4925. Available from: doi:10.4049/jimmunol.181.7.4918.
- Broggi, A., Ghosh, S., Sposito, B., Spreafico, R., et al. (2020) Type III interferons disrupt the lung epithelial barrier upon viral recognition. *Science*. 369 (6504), 706–712. Available from: doi:10.1126/science.abc3545.
- Broggi, A., Granucci, F. & Zanoni, I. (2020) Type III interferons: Balancing tissue tolerance and resistance to pathogen invasion. *The Journal of experimental medicine*. 217 (1), 1–12. Available from: doi:10.1084/jem.20190295.
- Broggi, A., Tan, Y., Granucci, F. & Zanoni, I. (2017) IFN- λ suppresses intestinal inflammation by non-translational regulation of neutrophil function. *Nature Immunology*. 18 (August), 1084–1093. Available from: doi:10.1038/ni.3821.
- Bromberg, J.F., Horvath, C.M., Wen, Z., Schreiber, R.D., et al. (1996) Transcriptionally active Stat1 is required for the antiproliferative effects of both interferon alpha and interferon gamma. *Proceedings of the National Academy of Sciences of the United States of America*. 93 (15), 7673–7678. Available from: doi:10.1073/pnas.93.15.7673.
- Brown, J.P., Wei, W. & Sedivy, J.M. (1997) Bypass of senescence after disruption of p21(CIP1)/(WAF1) gene in normal diploid human fibroblasts. *Science*. 277 (5327), 831–834. Available from: doi:10.1126/science.277.5327.831.
- Brundage, J.F. (2006) Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. *Lancet Infectious Diseases*. 6 (5), 303–312.

- Available from: doi:10.1016/S1473-3099(06)70466-2.
- Bunger, M.K., Moran, S.M., Glover, E., Thomae, T.L., et al. (2003) Resistance to 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity and abnormal liver development in mice carrying a mutation in the nuclear localization sequence of the aryl hydrocarbon receptor. *Journal of Biological Chemistry*. 278 (20), 17767–17774. Available from: doi:10.1074/jbc.M209594200.
- Burkart, C., Arimoto, K. ichiro, Tang, T., Cong, X., et al. (2013) Usp18 deficient mammary epithelial cells create an antitumour environment driven by hypersensitivity to IFN- λ and elevated secretion of Cxcl10. *EMBO Molecular Medicine*. 5 (7), 1035–1050. Available from: doi:10.1002/emmm.201201864.
- Burke, D.C., Graham, C.F. & Lehman, J.M. (1978) Appearance of interferon inducibility and sensitivity during differentiation of murine teratocarcinoma cells in vitro. *Cell*. 13 (2), 243–248. Available from: doi:10.1016/0092-8674(78)90193-9.
- Burke, L.C., Bybee, A. & Thomas, N.S. (1992) The retinoblastoma protein is partially phosphorylated during early G1 in cycling cells but not in G1 cells arrested with alpha-interferon. *Oncogene*. 7 (4), 783–788.
- Cameron, C.M., Cameron, M.J., Bermejo-Martin, J.F., Ran, L., et al. (2008) Gene Expression Analysis of Host Innate Immune Responses during Lethal H5N1 Infection in Ferrets. *Journal of Virology*. 82 (22), 11308–11317. Available from: doi:10.1128/jvi.00691-08.
- Cardani, A., Boulton, A., Kim, T.S. & Braciale, T.J. (2017) Alveolar Macrophages Prevent Lethal Influenza Pneumonia By Inhibiting Infection Of Type-1 Alveolar Epithelial Cells. *PLoS Pathogens*. 13 (1), 1–25. Available from: doi:10.1371/journal.ppat.1006140.
- Carrega, P. & Ferlazzo, G. (2012) Natural killer cell distribution and trafficking in human tissues. *Frontiers in Immunology*. 3 (NOV), 1–6. Available from: doi:10.3389/fimmu.2012.00347.
- Casanova, J.-L. & Abel, L. (2013) The genetic theory of infectious diseases: a brief history and selected illustrations. *Annual review of genomics and human genetics*. 14, 215–243. Available from: doi:10.1146/annurev-genom-091212-153448.
- Cella, M., Fuchs, A., Vermi, W., Facchetti, F., et al. (2009) A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature*. 457 (7230), 722–725. Available from: doi:10.1038/nature07537.
- Cella, M., Jarrossay, D., Facchetti, F., Alebardi, O., et al. (1999) Plasmacytoid monocytes

- migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nature Medicine*. 5 (8), 919–923. Available from: doi:10.1038/11360.
- Channappanavar, R., Fehr, A.R., Vijay, R., Mack, M., et al. (2016) Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. *Cell Host and Microbe*. 19 (2), 181–193. Available from: doi:10.1016/j.chom.2016.01.007.
- Channappanavar, R., Fehr, A.R., Zheng, J., Wohlford-Lenane, C., et al. (2019) IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. *Journal of Clinical Investigation*. 129 (9), 3625–3639. Available from: doi:10.1172/JCI126363.
- Chattergoon, M.A., Latanich, R., Quinn, J., Winter, M.E., et al. (2014) HIV and HCV Activate the Inflammasome in Monocytes and Macrophages via Endosomal Toll-Like Receptors without Induction of Type 1 Interferon. *PLoS Pathogens*. 10 (5), 1–12. Available from: doi:10.1371/journal.ppat.1004082.
- Chawla-Sarkar, M., Leaman, D.W. & Borden, E.C. (2001) Preferential induction of apoptosis by interferon (IFN)-beta compared with IFN-alpha2: correlation with TRAIL/Apo2L induction in melanoma cell lines. *Clinical cancer research*. 7 (6), 1821–1831. Available from: doi:10.1146/annurev.biochem.67.1.227.
- Chawla-Sarkar, M., Linder, D.J., Liu, Y.-F., Williams, B.R., et al. (2002) Apoptosis and interferons: Role of interferon-stimulated genes as mediators of apoptosis. *Apoptosis*. 8 (3), 237–249.
- Chen, X., Liu, S., Goraya, M.U., Maarouf, M., et al. (2018) Host immune response to influenza A virus infection. *Frontiers in Immunology*. 9 (MAR), 1–13. Available from: doi:10.3389/fimmu.2018.00320.
- Cheung, C.Y., Poon, L.L.M., Lau, A.S., Luk, W., et al. (2002) Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: A mechanism for the unusual severity of human disease? *Lancet*. 360 (9348), 1831–1837. Available from: doi:10.1016/S0140-6736(02)11772-7.
- Chien, Y.W., Klugman, K.P. & Morens, D.M. (2009) Bacterial pathogens and death during the 1918 influenza pandemic. *New England Journal of Medicine*. 361 (26), 2582–2583. Available from: doi:10.1056/NEJMc0908216.
- Chin, Y.E., Kitagawa, M., Su, W.-C.S., You, Z.-H., et al. (1996) Cell Growth Arrest and Induction of Cyclin-Dependent Kinase Inhibitor p21WAF1/CIP1 Mediated by STAT1.

- Science*. 272 (5262), 719–722. Available from: doi:10.1126/science.272.5262.719.
- Chiriac, M.T., Buchen, B., Wandersee, A., Hundorfean, G., et al. (2017) Activation of Epithelial STAT1 by Interleukin 28 Controls Mucosal Healing in Mice with Colitis and is Increased in Mucosa of Patients With Inflammatory Bowel Disease. *Gastroenterology*. (May), 1–16. Available from: doi:10.1053/j.gastro.2017.03.015.
- Choi, E.A., Lei, H., Maron, D.J. & Wilson, J.M. (2003) Stat1-dependent induction of tumor necrosis factor-related apoptosis-inducing ligand and the cell-surface death signaling pathway by interferon beta in human cancer cells. *Cancer Res*. 63, 5299–5307.
- Chu, H., Chan, J.F.W., Wang, Y., Yuen, T.T.T., et al. (2020) Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. (Xx), 1–10. Available from: doi:10.1093/cid/ciaa410.
- Ciancanelli, M.J., Abel, L., Zhang, S.Y. & Casanova, J.L. (2016) Host genetics of severe influenza: From mouse Mx1 to human IRF7. *Current Opinion in Immunology*. 38 (January 2015), 109–120. Available from: doi:10.1016/j.coi.2015.12.002.
- Ciancanelli, M.J., Huang, S.X.L., Luthra, P., Garner, H., et al. (2015) Life-threatening influenza and impaired interferon amplification in human IRF7 deficiency. *Science*. 348 (6233), 448–453. Available from: doi:10.1126/science.aaa1578.
- Claudinon, J., Monier, M.N. & Lamaze, C. (2007) Interfering with interferon receptor sorting and trafficking: Impact on signaling. *Biochimie*. 89 (6–7), 735–743. Available from: doi:10.1016/j.biochi.2007.03.014.
- Coccia, E.M., Severa, M., Giacomini, E., Monneron, D., et al. (2004) Viral infection and toll-like receptor agonists induce a differential expression of type I and λ interferons in humans plasmacytoid and monocyte-derived dendritic cells. *European Journal of Immunology*. 34 (3), 796–805. Available from: doi:10.1002/eji.200324610.
- Cohen, S.A., Chui, K.K.H. & Naumova, E.N. (2011) Influenza vaccination in young children reduces influenza-associated hospitalizations in older adults, 2002–2006. *Journal of the American Geriatrics Society*. 59 (2), 327–332. Available from: doi:10.1111/j.1532-5415.2010.03271.x.
- Colonna, M., Jonjic, S. & Watzl, C. (2011) Natural killer cells: Fighting viruses and much more. *Nature Immunology*. 12 (2), 107–110. Available from: doi:10.1038/ni0211-107.
- Cowling, B.J., Jin, L., Lau, E.H.Y., Liao, Q., et al. (2013) Comparative epidemiology of

- human infections with avian influenza A H7N9 and H5N1 viruses in China: A population-based study of laboratory-confirmed cases. *The Lancet*. 382 (9887), 129–137. Available from: doi:10.1016/S0140-6736(13)61171-X.
- Crotta, S., Davidson, S., Mahlakoiv, T., Desmet, C.J., et al. (2013) Type I and type III interferons drive redundant amplification loops to induce a transcriptional signature in influenza-infected airway epithelia. *PLoS pathogens*. 9 (11), e1003773. Available from: doi:10.1371/journal.ppat.1003773.
- D'Souza, W.N. & Lefrançois, L. (2003) IL-2 Is Not Required for the Initiation of CD8 T Cell Cycling but Sustains Expansion. *The Journal of Immunology*. 171 (11), 5727–5735. Available from: doi:10.4049/jimmunol.171.11.5727.
- Daniels, B.P., Holman, D.W., Cruz-orengo, L., Jujjavarapu, H., et al. (2014) Viral Pathogen-Associated Molecular Patterns Regulate Blood-Brain Barrier Integrity via Competing Innate Cytokine Signals. *mBio*. 5 (5), e01476-14. Available from: doi:10.1128/mBio.01476-14.Editor.
- Davarinos, N.A. & Pollenz, R.S. (1999) Aryl hydrocarbon receptor imported into the nucleus following ligand binding is rapidly degraded via the cytoplasmic proteasome following nuclear export. *The Journal of biological chemistry*. 274 (40), 28708–28715. Available from: doi:10.1074/jbc.274.40.28708.
- Davidson, S., Crotta, S., McCabe, T.M. & Wack, A. (2014) Pathogenic potential of interferon $\alpha\beta$ in acute influenza infection. *Nature Communications*. 5 (3864). Available from: doi:10.1038/ncomms4864.
- Davidson, S., McCabe, T.M., Crotta, S., Gad, H.H., et al. (2016) IFN λ is a potent anti-influenza therapeutic without the inflammatory side effects of IFN α treatment. *EMBO Molecular Medicine*. 8 (9), 1–14. Available from: doi:10.15252/emmm.201606413.
- Dawood, F.S., Iuliano, A.D., Reed, C., Meltzer, M.I., et al. (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: A modelling study. *The Lancet Infectious Diseases*. 12 (9), 687–695. Available from: doi:10.1016/S1473-3099(12)70121-4.
- Deeg, C.M., Hassan, E., Mutz, P., Rheinemann, L., et al. (2017) In vivo evasion of MxA by avian influenza viruses requires human signature in the viral nucleoprotein. *Journal of Experimental Medicine*. 214 (5), 1239–1248. Available from: doi:10.1084/jem.20161033.
- Degner, S.C., Papoutsis, A.J., Selmin, O. & Romagnolo, D.F. (2009) Targeting of aryl

- hydrocarbon receptor-mediated activation of cyclooxygenase-2 expression by the indole-3-carbinol metabolite 3,3'-diindolylmethane in breast cancer cells. *The Journal of nutrition*. 139 (1), 26–32. Available from: doi:10.3945/jn.108.099259.
- Dellgren, C., Gad, H.H., Hamming, O.J., Melchjorsen, J., et al. (2009) Human interferon- λ 3 is a potent member of the type III interferon family. *Genes and Immunity*. 10 (2), 125–131. Available from: doi:10.1038/gene.2008.87.
- Denison, M.S. & Faber, S.C. (2017) And now for something completely different: Diversity in ligand-dependent activation of Ah receptor responses. *Current Opinion in Toxicology*. 2, 124–131. Available from: doi:10.1016/j.cotox.2017.01.006.
- Denison, M.S., Soshilov, A.A., He, G., Degroot, D.E., et al. (2011) Exactly the same but different: Promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor. *Toxicological Sciences*. 124 (1), 1–22. Available from: doi:10.1093/toxsci/kfr218.
- Dere, E., Lee, A.W., Burgoon, L.D. & Zacharewski, T.R. (2011) Differences in TCDD-elicited gene expression profiles in human HepG2, mouse Hepa1c1c7 and rat H4IIE hepatoma cells. *BMC Genomics*. 12. Available from: doi:10.1186/1471-2164-12-193.
- Dhesi, Z., Enne, V.I., Brealey, D., Livermore, D.M., et al. (2020) Organisms causing secondary pneumonias in COVID-19 patients at 5 UK ICUs as detected with the FilmArray test. *medRxiv*.
- Dickensheets, H., Sheikh, F., Park, O., Gao, B., et al. (2013) Interferon-lambda (IFN- λ) induces signal transduction and gene expression in human hepatocytes, but not in lymphocytes or monocytes. *Journal of Leukocyte Biology*. 93 (3), 377–385. Available from: doi:10.1189/jlb.0812395.
- Diebold, S.S., Kaisho, T., Hemmi, H., Akira, S., et al. (2004) Innate Antiviral Responses by Means of TLR7-Mediated Recognition of Single-Stranded RNA. *Science*. 303 (5663), 1529–1531. Available from: doi:10.1126/science.1093616.
- Diegelmann, J., Beigel, F., Zitzmann, K., Kaul, A., et al. (2010) Comparative analysis of the lambda-interferons IL-28A and IL-29 regarding their transcriptome and their antiviral properties against hepatitis C virus. *PLoS ONE*. 5 (12). Available from: doi:10.1371/journal.pone.0015200.
- Dienz, O., Rud, J.G., Eaton, S.M., Lanthier, P.A., et al. (2012) *Essential role of IL-6 in protection against H1N1 influenza virus by promoting neutrophil survival in the lung*. 5 (3), 258–266. Available from: doi:10.1038/mi.2012.2.

- Ding, B. Sen, Nolan, D.J., Guo, P., Babazadeh, A.O., et al. (2011) Endothelial-derived angiocrine signals induce and sustain regenerative lung alveolarization. *Cell*. 147 (3), 539–553. Available from: doi:10.1016/j.cell.2011.10.003.
- Doyle, S.E., Schreckhise, H., Khuu-Duong, K., Henderson, K., et al. (2006) Interleukin-29 uses a type 1 interferon-like program to promote antiviral responses in human hepatocytes. *Hepatology*. 44 (4), 896–906. Available from: doi:10.1002/hep.21312.
- Dragin, N., Shi, Z., Madan, R., Karp, C.L., et al. (2008) Phenotype of the Cyp1a1/1a2/1b1(-/-) triple-knockout mouse. *Molecular Pharmacology*. 73 (6), 1844–1856. Available from: doi:10.1124/mol.108.045658.
- Drake, J.W. (1993) Rates of spontaneous mutation among RNA viruses. *Proceedings of the National Academy of Sciences of the United States of America*. 90 (9), 4171–4175. Available from: doi:10.1073/pnas.90.9.4171.
- Dudakov, J.A., Hanash, A.M. & van den Brink, M.R.M. (2015) Interleukin-22: Immunobiology and Pathology. *Annual Review of Immunology*. 33 (1), 747–785. Available from: doi:10.1146/annurev-immunol-032414-112123.
- Dunning, J., Blankley, S., Hoang, L.T., Cox, M., et al. (2018) Progression of whole-blood transcriptional signatures from interferon-induced to neutrophil-associated patterns in severe influenza. *Nature Immunology*. 19 (6), 625–635. Available from: doi:10.1038/s41590-018-0111-5.
- Eggenberger, J., Blanco-Melo, D., Panis, M., Brennand, K.J., et al. (2019) Type I interferon response impairs differentiation potential of pluripotent stem cells. *Proceedings of the National Academy of Sciences of the United States of America*. 116 (4), 1384–1393. Available from: doi:10.1073/pnas.1812449116.
- Egli, A., Santer, D.M., O’Shea, D., Tyrrell, D.L., et al. (2014) The impact of the interferon-lambda family on the innate and adaptive immune response to viral infections. *Emerging Microbes & Infections*. 3 (May), e51. Available from: doi:10.1038/emi.2014.51.
- Ehre, C., Worthington, E.N., Liesman, R.M., Grubb, B.R., et al. (2012) Overexpressing mouse model demonstrates the protective role of Muc5ac in the lungs. *Proceedings of the National Academy of Sciences*. 109 (41), 5753. Available from: doi:10.1073/pnas.1404711111.
- Eichelberger, M., Allan, W., Zijlstra, M., Jaenisch, R., et al. (1991) Clearance of Influenza Virus Respiratory Infection in Mice Lacking Class I Major Histocompatibility Complex-restricted CD8 + T Cells. *Journal of Experimental Medicine*. 174 (4), 875–880.

- Einat, M., Resnitzky, D. & Kimchi, A. (1985) Close link between reduction of c-myc expression by interferon and G0/G1 arrest. *Nature*. 313 (6003), 597–600. Available from: doi:10.1038/313597a0.
- Ellis, G.T., Davidson, S., Crotta, S., Branzk, N., et al. (2015) TRAIL+ monocytes and monocyte-related cells cause lung damage and thereby increase susceptibility to influenza-Streptococcus pneumoniae coinfection. *EMBO reports*. 16 (9), 1203–1218. Available from: doi:10.15252/embr.201540473.
- Emmons, R.B., Duncan, D., Estes, P.A., Kiefel, P., et al. (1999) The spineless-aristopedia and tango bHLH-PAS proteins interact to control antennal and tarsal development in Drosophila. *Development (Cambridge, England)*. 126 (17), 3937–3945.
- Epstein, S.L., Lo, C., Misplon, J.A. & Bennink, J.R. (1998) Mechanism of Protective Immunity Against Influenza Virus Infection in Mice Without Antibodies. *The Journal of Immunology*. 160 (1), 322–327.
- Espinosa, J.M. (2020) Down Syndrome and COVID-19: A Perfect Storm? *Cell Reports Medicine*. 1 (2), 100019. Available from: doi:10.1016/j.xcrm.2020.100019.
- Espinosa, V., Dutta, O., Mcelrath, C., Du, P., et al. (2017) Type III interferon is a critical regulator of innate antifungal immunity. 5357 (October). Available from: doi:10.1126/sciimmunol.aan5357.
- Esser, C. & Rannug, A. (2015) The aryl hydrocarbon receptor in barrier organ physiology, immunology, and toxicology. *Pharmacological Reviews*. 67 (2), 259–279. Available from: doi:10.1124/pr.114.009001.
- Everitt, A.R., Clare, S., Pertel, T., John, S.P., et al. (2012) IFITM3 restricts the morbidity and mortality associated with influenza. *Nature*. 484 (7395), 519–523. Available from: doi:10.1038/nature10921.
- Fenner, J.E., Starr, R., Cornish, A.L., Zhang, J.G., et al. (2006) Suppressor of cytokine signaling 1 regulates the immune response to infection by a unique inhibition of type I interferon activity. *Nature Immunology*. 7 (1), 33–39. Available from: doi:10.1038/ni1287.
- Fernandez-Salguero, P., Pineau, T., Hilbert, D.M., McPhail, T., et al. (1995) Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science*. 268 (5211), 722–726. Available from: doi:10.1126/science.7732381.
- Fiore-Gartland, A., Panoskaltsis-Mortari, A., Agan, A.A., Mistry, A.J., et al. (2017) Cytokine Profiles of Severe Influenza Virus-Related Complications in Children. *Frontiers in*

- Immunology*. 8 (November), 1423. Available from: doi:10.3389/FIMMU.2017.01423.
- Forero, A., Ozarkar, S., Li, H., Lee, C.H., et al. (2019) Differential Activation of the Transcription Factor IRF1 Underlies the Distinct Immune Responses Elicited by Type I and III Interferons. *Immunity*. 1–14. Available from: doi:10.1016/j.immuni.2019.07.007.
- Fox, J.M., Crabtree, J.M., Sage, L.K., Tompkins, S.M., et al. (2015) *Interferon Lambda Upregulates IDO1 Expression in Respiratory Epithelial Cells After Influenza Virus Infection*. 35 (7), 554–562. Available from: doi:10.1089/jir.2014.0052.
- Fox, J.M., Sage, L.K., Huang, L., Barber, J., et al. (2013) Inhibition of indoleamine 2,3-dioxygenase enhances the T-cell response to influenza virus infection. *Journal of General Virology*. 94 (PART7), 1451–1461. Available from: doi:10.1099/vir.0.053124-0.
- François-Newton, V., de Freitas Almeida, G.M., Payelle-Brogard, B., Monneron, D., et al. (2011) USP18-based negative feedback control is induced by type I and type III interferons and specifically inactivates interferon α response. *PLoS ONE*. 6 (7). Available from: doi:10.1371/journal.pone.0022200.
- François-Newton, V., Livingstone, M., Payelle-Brogard, B., Uzé, G., et al. (2012) USP18 establishes the transcriptional and anti-proliferative interferon α/β differential. *Biochemical Journal*. 446 (3), 509–516. Available from: doi:10.1042/BJ20120541.
- Frank, D.B., Peng, T., Zepp, J.A., Snitow, M., et al. (2016) Emergence of a Wave of Wnt Signaling that Regulates Lung Alveologenesis by Controlling Epithelial Self-Renewal and Differentiation. *Cell Reports*. 17 (9), 2312–2325. Available from: doi:10.1016/j.celrep.2016.11.001.
- Frericks, M., Meissner, M. & Esser, C. (2007) Microarray analysis of the AHR system: Tissue-specific flexibility in signal and target genes. *Toxicology and Applied Pharmacology*. 220 (3), 320–332. Available from: doi:10.1016/j.taap.2007.01.014.
- Fuchs, S., Kaiser-Labusch, P., Bank, J., Ammann, S., et al. (2016) Tyrosine kinase 2 is not limiting human antiviral type III interferon responses. *European Journal of Immunology*. 46 (11), 2639–2649. Available from: doi:10.1002/eji.201646519.
- Fukunaga, B.N., Probst, M.R., Reisz-Porszasz, S. & Hankinson, O. (1995) Identification of functional domains of the aryl hydrocarbon receptor. *The Journal of biological chemistry*. 270 (49), 29270–29278. Available from: doi:10.1074/jbc.270.49.29270.
- Fulcher, M.L., Gabriel, S., Burns, K.A., Yankaskas, J.R., et al. (2005) Well-Differentiated Human Airway Epithelial Cell Cultures. In: Joanna Picot (ed.). *Human Cell Culture*

- Protocols*. Totowa, NJ, Humana Press. pp. 183–206. Available from: doi:10.1385/1-59259-861-7:183.
- Gack, M.U., Albrecht, R.A., Urano, T., Inn, K.S., et al. (2009) Influenza A Virus NS1 Targets the Ubiquitin Ligase TRIM25 to Evade Recognition by the Host Viral RNA Sensor RIG-I. *Cell Host and Microbe*. 5 (5), 439–449. Available from: doi:10.1016/j.chom.2009.04.006.
- Gack, M.U., Shin, Y.C., Joo, C.H., Urano, T., et al. (2007) TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature*. 446 (7138), 916–920. Available from: doi:10.1038/nature05732.
- Gaelings, L., Söderholm, S., Bugai, A., Fu, Y., et al. (2016) Regulation of Kynurenine Biosynthesis during Influenza Virus Infection. *The FEBS Journal*. 284, 222–236. Available from: doi:10.1111/febs.13966.
- Gagliani, N., Amezcu Vesely, M.C., Iseppon, A., Brockmann, L., et al. (2015) TH17 cells transdifferentiate into regulatory T cells uring resolution of inflammation. *Nature*. 523 (7559), 221–225. Available from: doi:10.1038/nature14452.
- Gagnon, A., Miller, M.S., Hallman, S.A., Bourbeau, R., et al. (2013) Age-Specific Mortality During the 1918 Influenza Pandemic: Unravelling the Mystery of High Young Adult Mortality. *PLoS ONE*. 8 (8). Available from: doi:10.1371/journal.pone.0069586.
- Galani, I.E., Triantafyllia, V., Eleminiadou, E.-E.E., Koltsida, O., et al. (2017) Interferon- λ mediates non-redundant front-line antiviral protection against influenza virus infection without compromising host fitness. *Immunity*. 46 (5), 875-890.e6. Available from: doi:10.1016/j.immuni.2017.04.025.
- Ganal, S.C., Sanos, S.L., Kallfass, C., Oberle, K., et al. (2012) Priming of Natural Killer Cells by Nonmucosal Mononuclear Phagocytes Requires Instructive Signals from Commensal Microbiota. *Immunity*. 37 (1), 171–186. Available from: doi:10.1016/j.immuni.2012.05.020.
- Gandhi, R., Kumar, D., Burns, E.J., Nadeau, M., et al. (2010) Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3+ regulatory T cells. *Nature Immunology*. 11 (9), 846–853. Available from: doi:10.1038/ni.1915.
- Gao, D., Yang, Y.K., Wang, R.P., Zhou, X., et al. (2009) REUL is a novel E3 ubiquitin ligase and stimulator of retinoic-acid-inducible gene-I. *PLoS ONE*. 4 (6). Available from: doi:10.1371/journal.pone.0005760.

- Gao, R., Bhatnagar, J., Blau, D.M., Greer, P., et al. (2013) Cytokine and chemokine profiles in lung tissues from fatal cases of 2009 pandemic influenza A (H1N1): Role of the host immune response in pathogenesis. *American Journal of Pathology*. 183 (4), 1258–1268. Available from: doi:10.1016/j.ajpath.2013.06.023.
- García-Sastre, A. (2017) Ten Strategies of Interferon Evasion by Viruses. *Cell Host and Microbe*. 22 (2), 176–184. Available from: doi:10.1016/j.chom.2017.07.012.
- García-Sastre, A., Egorov, A., Matassov, D., Brandt, S., et al. (1998) Influenza A virus lacking the NS1 gene replicates in interferon- deficient systems. *Virology*. 252 (2), 324–330. Available from: doi:10.1006/viro.1998.9508.
- Garcin, G., Paul, F., Staufenbiel, M., Bordat, Y., et al. (2014) High efficiency cell-specific targeting of cytokine activity. *Nature communications*. 5, 3016. Available from: doi:10.1038/ncomms4016.
- García, S.R., Deprez, M., Lebrigand, K., Cavard, A., et al. (2019) Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures. *Development*. 146 (20). Available from: doi:10.1242/dev.177428.
- Gazit, R., Gruda, R., Elboim, M., Arnon, T.I., et al. (2006) Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1. *Nature Immunology*. 7 (5), 517–523. Available from: doi:10.1038/ni1322.
- Ge, D., Fellay, J., Thompson, A.J., Simon, J.S., et al. (2009) Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*. 461 (7262), 399–401. Available from: doi:10.1038/nature08309.
- Geurtsvankessel, C.H., Willart, M.A.M., Bergen, I.M., Van Rijt, L.S., et al. (2009) Dendritic cells are crucial for maintenance of tertiary lymphoid structures in the lung of influenza virus-infected mice. *Journal of Experimental Medicine*. 206 (11), 2339–2349. Available from: doi:10.1084/jem.20090410.
- GeurtsvanKessel, C.H., Willart, M.A.M., Van Rijt, L.S., Muskens, F., et al. (2008) Clearance of influenza virus from the lung depends on migratory langerin+CD11b- but not plasmacytoid dendritic cells. *Journal of Experimental Medicine*. 205 (7), 1621–1634. Available from: doi:10.1084/jem.20071365.
- Ghodke-Puranik, Y. & Niewold, T.B. (2013) Genetics of the type I interferon pathway in systemic lupus erythematosus. *International Journal of Clinical Rheumatology*. 8 (6), 657–669. Available from: doi:10.2217/ijr.13.58.
- Gibbings, S.L., Goyal, R., Desch, A.N., Leach, S.M., et al. (2015) Transcriptome analysis

- highlights the conserved difference between embryonic and postnatal-derived alveolar macrophages. *Blood*. 126 (11), 1357–1366. Available from: doi:10.1182/blood-2015-01-624809.
- Giovannoni, F., Bosch, I., Polonio, C.M., Torti, M.F., et al. (2020) AHR is a Zika virus host factor and a candidate target for antiviral therapy. *Nature Neuroscience*. 23 (8), 939–951. Available from: doi:10.1038/s41593-020-0664-0.
- Go, R.E., Hwang, K.A. & Choi, K.C. (2015) Cytochrome P450 1 family and cancers. *Journal of Steroid Biochemistry and Molecular Biology*. 147, 24–30. Available from: doi:10.1016/j.jsbmb.2014.11.003.
- Le Goffic, R., Balloy, V., Lagranderie, M., Alexopoulou, L., et al. (2006) Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. *PLoS Pathogens*. 2 (6), 0526–0535. Available from: doi:10.1371/journal.ppat.0020053.
- Le Goffic, R., Pothlichet, J., Vitour, D., Fujita, T., et al. (2007) Cutting Edge: Influenza A Virus Activates TLR3-Dependent Inflammatory and RIG-I-Dependent Antiviral Responses in Human Lung Epithelial Cells. *The Journal of Immunology*. 178 (6), 3368–3372. Available from: doi:10.4049/jimmunol.178.6.3368.
- Goldberg, E.L., Molony, R.D., Kudo, E., Sidorov, S., et al. (2019) Ketogenic diet activates protective gd T cell responses against influenza virus infection. *Science immunology*. 4, eaav2026.
- Goodale, B.C., Tilton, S.C., Corvi, M.M., Wilson, G.R., et al. (2013) Structurally distinct polycyclic aromatic hydrocarbons induce differential transcriptional responses in developing zebrafish. *Toxicology and Applied Pharmacology*. 272 (3), 656–670. Available from: doi:10.1016/j.taap.2013.04.024.
- Gordon, D.E., Jang, G.M., Bouhaddou, M., Xu, J., et al. (2020) A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature*. 583 (7816), 459–468. Available from: doi:10.1038/s41586-020-2286-9.
- Gordon, S.B., Bruce, N.G., Grigg, J., Hibberd, P.L., et al. (2014) Respiratory risks from household air pollution in low and middle income countries. *The Lancet Respiratory Medicine*. 2 (10), 823–860. Available from: doi:10.1016/S2213-2600(14)70168-7.
- Gorini da Veiga, A.B., Kretzmann, N.A. & Correa, L.T. (2012) Viral load and epidemiological profile of patients infected by pandemic influenza A (H1N1) 2009 and seasonal influenza A virus in southern brazil. *Journal of Medical Virology*. 84

- (November 2005), 371–379. Available from: doi:10.1002/jmv.
- Gorski, S.A., Hufford, M.M. & Braciale, T.J. (2012) Recent insights into pulmonary repair following virus-induced inflammation of the respiratory tract. *Current Opinion in Virology*. 2 (3), 233–241. Available from: doi:10.1016/j.coviro.2012.04.006.
- Gostic, K.M., Ambrose, M., Worobey, M. & Lloyd-Smith, J.O. (2016) Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting. *Science*. 354 (6313), 722–726. Available from: doi:10.1126/science.aag1322.
- Gough, D.J., Messina, N.L., Clarke, C.J.P., Johnstone, R.W., et al. (2012) Constitutive type I interferon modulates homeostatic balance through tonic signaling. *Immunity*. 36 (2), 166–174. Available from: doi:10.1016/j.immuni.2012.01.011.
- Graham, M.B. & Braciale, T.J. (1997) Resistance to and recovery from lethal influenza virus infection in B lymphocyte-deficient mice. *Journal of Experimental Medicine*. 186 (12), 2063–2068. Available from: doi:10.1084/jem.186.12.2063.
- Granados, A., Peci, A., McGeer, A. & Gubbay, J.B. (2017) Influenza and rhinovirus viral load and disease severity in upper respiratory tract infections. *Journal of Clinical Virology*. 86, 14–19. Available from: doi:10.1016/j.jcv.2016.11.008.
- Guan, W.-J., Ni, Z.-Y., Hu, Y., Liang, W.-H., et al. (2020) Clinical Characteristics of Coronavirus Disease 2019 in China. *The New England journal of medicine*. 1–13. Available from: doi:10.1056/NEJMoa2002032.
- Guilliams, M., De Kleer, I., Henri, S., Post, S., et al. (2013) Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *Journal of Experimental Medicine*. 210 (10), 1977–1992. Available from: doi:10.1084/jem.20131199.
- Guo, H. & Topham, D.J. (2010) Interleukin-22 (IL-22) Production by Pulmonary Natural Killer Cells and the Potential Role of IL-22 during Primary Influenza Virus Infection. *Journal of virology*. 84 (15), 7750–7759. Available from: doi:10.1128/JVI.00187-10.
- Guo, R.-F. & Ward, P.A. (2002) Mediators and regulation of neutrophil accumulation in inflammatory responses in lung: Insights from the IgG immune complex model. *Free Radical Biology & Medicine*. 33 (3), 303–310.
- Guo, X.J. & Thomas, P.G. (2017) New fronts emerge in the influenza cytokine storm. *Seminars in Immunopathology*. Available from: doi:10.1007/s00281-017-0636-y.
- Gurney, A.L. (2004) IL-22, a Th1 cytokine that targets the pancreas and select other peripheral tissues. *International Immunopharmacology*. 4 (5), 669–677. Available from:

- doi:10.1016/j.intimp.2004.01.016.
- Gutié Rrez-Vá Zquez, C. & Quintana, F.J. (2018) Regulation of the Immune Response by the Aryl Hydrocarbon Receptor. *Immunity*. 48, 19–33. Available from: doi:10.1016/j.immuni.2017.12.012.
- Hackett, N.R., Shaykhiev, R., Walters, M.S., Wang, R., et al. (2011) The human airway epithelial basal cell transcriptome. *PLoS ONE*. 6 (5). Available from: doi:10.1371/journal.pone.0018378.
- Hadjadj, J., Yatim, N., Barnabei, L., Corneau, A., et al. (2020) Impaired type I interferon activity and exacerbated inflammatory responses in severe Covid-19 patients. *medRxiv*. <https://doi.org/10.1101/2020.05.07.20093184>.
- Hahn, M.E., Karchner, S.I., Shapiro, M.A. & Perera, S.A. (1997) Molecular evolution of two vertebrate aryl hydrocarbon (dioxin) receptors (AHR1 and AHR2) and the PAS family. *Proceedings of the National Academy of Sciences of the United States of America*. 94 (25), 13743–13748. Available from: doi:10.1073/pnas.94.25.13743.
- Haller, O., Arnheiter, H., Gresser, I. & Lindenman, J. (1979) Genetically determined, interferon-dependent resistance to influenza virus in mice. *Journal of Experimental Medicine*. 149 (February), 601–612.
- Haller, O., Staeheli, P., Schwemmle, M. & Kochs, G. (2015) Mx GTPases: Dynamin-like antiviral machines of innate immunity. *Trends in Microbiology*. 23 (3), 154–163. Available from: doi:10.1016/j.tim.2014.12.003.
- Hancock, K., Veguilla, V., Lu, X., Zhong, W., et al. (2009) Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. *New England Journal of Medicine*. 361 (20), 1945–1952. Available from: doi:10.1056/NEJMoa0906453.
- Hargadon, K.M., Zhou, H., Albrecht, R.A., Dodd, H.A., et al. (2011) Major Histocompatibility Complex Class II Expression and Hemagglutinin Subtype Influence the Infectivity of Type A Influenza Virus for Respiratory Dendritic Cells. *Journal of Virology*. 85 (22), 11955–11963. Available from: doi:10.1128/jvi.05830-11.
- Hasegawa, K., Sato, A., Tanimura, K., Uemasu, K., et al. (2017) Fraction of MHCII and EpCAM expression characterizes distal lung epithelial cells for alveolar type 2 cell isolation. *Respiratory Research*. 18 (1), 1–13. Available from: doi:10.1186/s12931-017-0635-5.
- Hashimoto, Y., Moki, T., Takizawa, T., Shiratsuchi, A., et al. (2007) Evidence for Phagocytosis of Influenza Virus-Infected, Apoptotic Cells by Neutrophils and

- Macrophages in Mice. *The Journal of Immunology*. 178 (4), 2448–2457. Available from: doi:10.4049/jimmunol.178.4.2448.
- Hayden, F.G., Fritz, R.S., Lobo, M.C., Alvord, W.G., et al. (1998) Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. *Journal of Clinical Investigation*. 101 (3), 643–649. Available from: doi:10.1172/JCI1355.
- He, X., Lau, E.H.Y., Wu, P., Deng, X., et al. (2020) Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nature Medicine*. 26 (5), 672–675. Available from: doi:10.1038/s41591-020-0869-5.
- Head, J.L. & Lawrence, B.P. (2009) The aryl hydrocarbon receptor is a modulator of anti-viral immunity. *Biochemical Pharmacology*. 77 (4), 642–653. Available from: doi:10.1016/j.bcp.2008.10.031.
- Heer, A.K., Harris, N.L., Kopf, M. & Marsland, B.J. (2008) CD4+ and CD8+ T Cells Exhibit Differential Requirements for CCR7-Mediated Antigen Transport during Influenza Infection. *The Journal of Immunology*. 181 (10), 6984–6994. Available from: doi:10.4049/jimmunol.181.10.6984.
- Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., et al. (2004) Species-Specific Recognition of Single-Stranded RNA via Toll-like Receptor 7 and 8. *Science*. 303 (5663), 1526–1529. Available from: doi:10.1126/science.1093620.
- Helft, J., Manicassamy, B., Guermonprez, P., Hashimoto, D., et al. (2012) Cross-presenting CD103+ dendritic cells are protected from influenza virus infection. *J Clin Invest*. 122 (11), 4037–4047. Available from: doi:10.1172/JCI60659.
- Henderson, C.J., McLaughlin, L.A., Osuna-Cabello, M., Taylor, M., et al. (2015) Application of a novel regulatable Cre recombinase system to define the role of liver and gut metabolism in drug oral bioavailability. *Biochemical Journal*. 465 (3), 479–488. Available from: doi:10.1042/BJ20140582.
- Henderson, R.F. (1984) Use of bronchoalveolar lavage to detect lung damage. *Environmental Health Perspectives*. 56, 115–129. Available from: doi:10.1289/ehp.8456115.
- Herfst, S., Schrauwen, E.J., Linster, M., Chutinimitkul, S., et al. (2012) Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets. *Science*. 336, 1534–1541. Available from: doi:10.1038/nature12476.
- Herlocher, M.L., Elias, S., Truscon, R., Harrison, S., et al. (2001) Ferrets as a transmission model for influenza: Sequence changes in HA1 of type A (H3N2) virus. *Journal of*

- Infectious Diseases*. 184 (5), 542–546. Available from: doi:10.1086/322801.
- Hernandez, N., Bucciol, G., Moens, L., Le Pen, J., et al. (2019) Inherited IFNAR1 deficiency in otherwise healthy patients with adverse reaction to measles and yellow fever live vaccines. *Journal of Experimental Medicine*. 216 (9), 2057–2070.
- Hernandez, N., Melki, I., Jing, H., Habib, T., et al. (2018) Life-threatening influenza pneumonitis in a child with inherited IRF9 deficiency. *The Journal of experimental medicine*. 215 (10), 2567–2585. Available from: doi:10.1084/jem.20180628.
- Hernández, P.P., Mahlakdöv, T., Yang, I., Schwierzeck, V., et al. (2015) Interferon- λ and interleukin 22 act synergistically for the induction of interferon-stimulated genes and control of rotavirus infection. *Nature Immunology*. 16 (7), 698–707. Available from: doi:10.1038/ni.3180.
- Herold, S., Steinmueller, M., Von Wulffen, W., Cakarova, L., et al. (2008) Lung epithelial apoptosis in influenza virus pneumonia: the role of macrophage-expressed TNF-related apoptosis-inducing ligand. *Journal of Experimental Medicine*. 205 (13), 3065–3077. Available from: doi:10.1084/jem.20080201.
- Herold, S., von Wulffen, W., Steinmueller, M., Pleschka, S., et al. (2006) Alveolar Epithelial Cells Direct Monocyte Transepithelial Migration upon Influenza Virus Infection: Impact of Chemokines and Adhesion Molecules. *The Journal of Immunology*. 177 (3), 1817–1824. Available from: doi:10.4049/jimmunol.177.3.1817.
- Herriges, M. & Morrissey, E.E. (2014) Lung development: orchestrating the generation and regeneration of a complex organ. *Development*. 141 (3), 502–513. Available from: doi:10.1242/dev.098186.
- Hertzog, P., Forster, S. & Samarajiwa, S. (2011) Systems biology of interferon responses. *Journal of Interferon and Cytokine Research*. 31 (1), 5–11. Available from: doi:10.1089/jir.2010.0126.
- Hidaka, T., Ogawa, E., Suzuki, T., Funayama, R., et al. (2016) The aryl hydrocarbon receptor AhR links atopic dermatitis and air pollution via induction of the neurotrophic factor artemin. *Nature Immunology*. 18 (1). Available from: doi:10.1038/ni.3614.
- Hoang, L.T., Tolfvenstam, T., Ooi, E.E., Khor, C.C., et al. (2014) Patient-based transcriptome-wide analysis identify interferon and ubiquitination pathways as potential predictors of influenza a disease severity. *PLoS ONE*. 9 (11), 1–15. Available from: doi:10.1371/journal.pone.0111640.
- Hogan, B.L.M., Barkauskas, C.E., Chapman, H.A., Epstein, J.A., et al. (2014) Repair and

- regeneration of the respiratory system: Complexity, plasticity, and mechanisms of lung stem cell function. *Cell Stem Cell*. 15 (2), 123–138. Available from: doi:10.1016/j.stem.2014.07.012.
- Högner, K., Wolff, T., Pleschka, S., Plog, S., et al. (2013) Macrophage-expressed IFN- β Contributes to Apoptotic Alveolar Epithelial Cell Injury in Severe Influenza Virus Pneumonia. *PLoS Pathogens*. 9 (2). Available from: doi:10.1371/journal.ppat.1003188.
- Honda, K., Ohba, Y., Yanai, H., Hegishi, H., et al. (2005) Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. *Nature*. 434 (7036), 1035–1040. Available from: doi:10.1038/nature03547.
- Honigsbaum, M. (2020) Revisiting the 1957 and 1968 influenza pandemics. *The Lancet*. 395 (10240), 1824–1826. Available from: doi:10.1016/S0140-6736(20)31201-0.
- Horani, A., Nath, A., Wasserman, M.G., Huang, T., et al. (2013) Rho-associated protein kinase inhibition enhances airway epithelial basal-cell proliferation and lentivirus transduction. *American Journal of Respiratory Cell and Molecular Biology*. 49 (3), 341–347. Available from: doi:10.1165/rcmb.2013-0046TE.
- Hornung, V., Ellegast, J., Kim, S., Brzozka, K., et al. (2006) 5'-Triphosphate RNA is the ligand for RIG-I. *Science*. 314 (5801), 994–997. Available from: doi:10.1126/science.1132505.
- Hornung, V., Rothenfusser, S., Britsch, S., Krug, A., et al. (2002) Quantitative Expression of Toll-Like Receptor 1–10 mRNA in Cellular Subsets of Human Peripheral Blood Mononuclear Cells and Sensitivity to CpG Oligodeoxynucleotides. *The Journal of Immunology*. 168 (9), 4531–4537. Available from: doi:10.4049/jimmunol.168.9.4531.
- Hu, X., Herrero, C., Li, W.P., Antoniv, T.T., et al. (2002) Sensitization of IFN- γ Jak-STAT signaling during macrophage activation. *Nature Immunology*. 3 (9), 859–866. Available from: doi:10.1038/ni828.
- Huang, L., Li, L., Klonowski, K.D., Tompkins, S.M., et al. (2013) Induction and Role of Indoleamine 2,3 Dioxygenase in Mouse Models of Influenza A Virus Infection. *PLoS ONE*. 8 (6). Available from: doi:10.1371/journal.pone.0066546.
- Hubel, P., Urban, C., Bergant, V., Schneider, W.M., et al. (2019) A protein-interaction network of interferon-stimulated genes extends the innate immune system landscape. *Nature Immunology*. Available from: doi:10.1038/s41590-019-0323-3.
- Hung, L., Sen, D., Oniskey, T.K., Katzen, J., et al. (2019) Macrophages promote epithelial proliferation following infectious and non-infectious lung injury through a Trefoil factor

- 2-dependent mechanism. *Mucosal Immunology*. (August 2017). Available from: doi:10.1038/s41385-018-0096-2.
- Hunter, C.A. & Jones, S.A. (2015) IL-6 as a keystone cytokine in health and disease. *Nature Immunology*. 16 (5), 448–457. Available from: doi:10.1038/ni.3153.
- Ikuta, T., Kobayashi, Y. & Kawajiri, K. (2004) Phosphorylation of nuclear localization signal inhibits the ligand-dependent nuclear import of aryl hydrocarbon receptor. *Biochemical and biophysical research communications*. 317 (2), 545–550. Available from: doi:10.1016/j.bbrc.2004.03.076.
- Isaacs, A. & Lindenmann, J. (1957) Virus interference. I. The interferon. *Proceedings of the Royal Society of London. Series B - Biological Sciences*. 147 (927), 258–267. Available from: doi:10.1098/rspb.1957.0048.
- Ito, S., Chen, C., Satoh, J., Yim, S., et al. (2007) Dietary phytochemicals regulate whole-body CYP1A1 expression through an arylhydrocarbon receptor nuclear translocator-dependent system in gut. *Journal of Clinical Investigation*. 117 (7), 1940–1950. Available from: doi:10.1172/JCI31647.
- Ito, T., Nelson, J., Couceiro, S.S., Kelm, S., et al. (1998) Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. *Journal of Virology*. 72 (9), 7367–7373.
- Iuliano, A.D., Roguski, K.M., Chang, H.H., Muscatello, D.J., et al. (2018) Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *The Lancet*. 391 (10127), 1285–1300. Available from: doi:10.1016/S0140-6736(17)33293-2.
- Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., et al. (2006) The Orphan Nuclear Receptor ROR γ t Directs the Differentiation Program of Proinflammatory IL-17+ T Helper Cells. *Cell*. 126 (6), 1121–1133. Available from: doi:10.1016/j.cell.2006.07.035.
- Ivanov, S., Renneson, J., Fontaine, J., Barthelemy, A., et al. (2013) Interleukin-22 Reduces Lung Inflammation during Influenza A Virus Infection and Protects against Secondary Bacterial Infection. *Journal of Virology*. 87 (12), 6911–6924. Available from: doi:10.1128/JVI.02943-12.
- Ivashkiv, L.B. & Donlin, L.T. (2014) Regulation of type I interferon responses. *Nature reviews. Immunology*. 14 (1), 36–49. Available from: doi:10.1038/nri3581.
- Jahnsen, F.L., Strickland, D.H., Thomas, J.A., Tobagus, I.T., et al. (2006) Accelerated Antigen Sampling and Transport by Airway Mucosal Dendritic Cells following Inhalation of a Bacterial Stimulus. *The Journal of Immunology*. 177 (9), 5861–5867.

- Available from: doi:10.4049/jimmunol.177.9.5861.
- Jain, R., Barkauskas, C.E., Takeda, N., Bowie, E.J., et al. (2015) Plasticity of Hopx⁺ type I alveolar cells to regenerate type II cells in the lung. *Nature Communications*. 6.
Available from: doi:10.1038/ncomms7727.
- Jaitin, D.A., Roisman, L.C., Jaks, E., Piehler, J., et al. (2006) Inquiring into the Differential Action of Interferons (IFNs): an IFN- α 2 Mutant with Enhanced Affinity to IFNAR1 Is Functionally Similar to IFN- β . *Molecular and Cellular Biology*. 26 (5), 1888–1897.
Available from: doi:10.1128/MCB.26.5.1888.
- Jayasekera, J.P., Moseman, E.A. & Carroll, M.C. (2007) Natural Antibody and Complement Mediate Neutralization of Influenza Virus in the Absence of Prior Immunity. *Journal of Virology*. 81 (7), 3487–3494. Available from: doi:10.1128/jvi.02128-06.
- Jeong, K.T., Hwang, S.J., Oh, G.S. & Park, J.H. (2012) FICZ, a Tryptophan photoproduct, suppresses pulmonary eosinophilia and Th2-type cytokine production in a mouse model of ovalbumin-induced allergic asthma. *International Immunopharmacology*. 13 (4), 377–385. Available from: doi:10.1016/j.intimp.2012.04.014.
- Jewell, N.A., Cline, T., Mertz, S.E., Smirnov, S. V., et al. (2010) Lambda interferon is the predominant interferon induced by influenza A virus infection in vivo. *Journal of Virology*. 84 (21), 11515–11522. Available from: doi:10.1128/jvi.01703-09.
- Jewell, N.A., Vaghefi, N., Mertz, S.E., Akter, P., et al. (2007) Differential Type I Interferon Induction by Respiratory Syncytial Virus and Influenza A Virus In Vivo. *Journal of Virology*. 81 (18), 9790–9800. Available from: doi:10.1128/jvi.00530-07.
- Jia, H.P., Look, D.C., Shi, L., Hickey, M., et al. (2005) ACE2 Receptor Expression and Severe Acute Respiratory Syndrome Coronavirus Infection Depend on Differentiation of Human Airway Epithelia. *Journal of Virology*. 79 (23), 14614–14621. Available from: doi:10.1128/jvi.79.23.14614-14621.2005.
- Jiang, F., Ramanathan, A., Miller, M.T., Tang, G.Q., et al. (2011) Structural basis of RNA recognition and activation by innate immune receptor RIG-I. *Nature*. 479 (7373), 423–427. Available from: doi:10.1038/nature10537.
- Jin, G.B., Moore, a J., Head, J.L., Neumiller, J.J., et al. (2010) Aryl hydrocarbon receptor activation reduces dendritic cell function during influenza virus infection. *Toxicol Sci*. 116 (2), 514–522. Available from: doi:10.1093/toxsci/kfq153.
- Joffre, O.P., Segura, E., Savina, A. & Amigorena, S. (2012) Cross-presentation by dendritic cells. *Nature Reviews Immunology*. 12 (8), 557–569. Available from:

- doi:10.1038/nri3254.
- De Jong, M.D., Simmons, C.P., Thanh, T.T., Hien, V.M., et al. (2006) Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nature Medicine*. 12 (10), 1203–1207. Available from: doi:10.1038/nm1477.
- Al Jord, A., Shihavuddin, A., D'Aout, R.S., Faucourt, M., et al. (2017) Calibrated mitotic oscillator drives motile ciliogenesis. *Science*. 358 (6364), 803–806. Available from: doi:10.1126/science.aan8311.
- Jorge-Nebert, L.F., Jiang, Z., Chakraborty, R., Watson, J., et al. (2010) Analysis of human CYP1A1 and CYP1A2 genes and their shared bidirectional promoter in eight world populations. *Human Mutation*. 31 (1), 27–40. Available from: doi:10.1002/humu.21132.
- Jung, S., Unutmaz, D., Wong, P., Sano, G.I., et al. (2002) In vivo depletion of CD11c⁺ dendritic cells abrogates priming of CD8⁺ T cells by exogenous cell-associated antigens. *Immunity*. 17 (2), 211–220. Available from: doi:10.1016/S1074-7613(02)00365-5.
- Kaartinen, L., Nettesheim, P., Adler, K.B. & Randell, S.H. (1993) Rat tracheal epithelial cell differentiation in vitro. *In vitro cellular & developmental biology. Animal*. 29A (6), 481–492.
- Kadow, S., Jux, B., Zahner, S.P., Wingerath, B., et al. (2011) Aryl Hydrocarbon Receptor Is Critical for Homeostasis of Invariant $\gamma\delta$ T Cells in the Murine Epidermis. *The Journal of Immunology*. 187 (6), 3104–3110. Available from: doi:10.4049/jimmunol.1100912.
- Kalie, E., Jaitin, D.A., Abramovich, R. & Schreiber, G. (2007) An interferon α 2 mutant optimized by phage display for IFNAR1 binding confers specifically enhanced antitumor activities. *Journal of Biological Chemistry*. 282 (15), 11602–11611. Available from: doi:10.1074/jbc.M610115200.
- Kalie, E., Jaitin, D.A., Podoplelova, Y., Piehler, J., et al. (2008) The stability of the ternary interferon-receptor complex rather than the affinity to the individual subunits dictates differential biological activities. *Journal of Biological Chemistry*. 283 (47), 32925–32936. Available from: doi:10.1074/jbc.M806019200.
- Kallfass, C., Lienenklaus, S., Weiss, S. & Staeheli, P. (2013) Visualizing the Beta Interferon Response in Mice during Infection with Influenza A Viruses Expressing or Lacking Nonstructural Protein 1. *Journal of Virology*. 87 (12), 6925–6930. Available from: doi:10.1128/jvi.00283-13.
- Kapelyukh, Y., Henderson, C.J., Scheer, N., Rode, A., et al. (2019) Defining the contribution

- of CYP1A1 and CYP1A2 to drug metabolism using humanized CYP1A1/1A2 and Cyp1a1/Cyp1a2 knockout mice. *Drug Metabolism and Disposition*. 47 (8), 907–918. Available from: doi:10.1124/dmd.119.087718.
- Karageorgopoulos, D.E., Vouloumanou, E.K., Korbila, I.P., Kapaskelis, A., et al. (2011) Age distribution of cases of 2009 (H1N1) pandemic influenza in comparison with seasonal influenza. *PLoS ONE*. 6 (7). Available from: doi:10.1371/journal.pone.0021690.
- Kash, J.C., Tumpey, T.M., Prohl, S.C., Carter, V., et al. (2006) Genomic analysis of increased host immune and cell death responses induced by 1918 influenza virus. *Nature*. 443 (7111), 578–581. Available from: doi:10.1038/nature05181.
- Kathiriya, J.J., Brumwell, A.N., Jackson, J.R., Tang, X., et al. (2020) Distinct Airway Epithelial Stem Cells Hide among Club Cells but Mobilize to Promote Alveolar Regeneration. *Cell Stem Cell*. 26 (3), 346–358.e4. Available from: doi:10.1016/j.stem.2019.12.014.
- Katlinskaya, Y. V., Carbone, C.J., Yu, Q. & Fuchs, S.Y. (2015) Type 1 interferons contribute to the clearance of senescent cell. *Cancer Biology and Therapy*. 16 (8), 1214–1219. Available from: doi:10.1080/15384047.2015.1056419.
- Katlinskaya, Y. V., Katlinski, K. V., Lasri, A., Li, N., et al. (2016) Type I Interferons Control Proliferation and Function of the Intestinal Epithelium. *Molecular and cellular biology*. 36 (7), 1124–1135. Available from: doi:10.1128/MCB.00988-15.
- Kato, H., Sato, S., Yoneyama, M., Yamamoto, M., et al. (2005) Cell type-specific involvement of RIG-I in antiviral response. *Immunity*. 23 (1), 19–28. Available from: doi:10.1016/j.immuni.2005.04.010.
- Kato, H., Takeuchi, O., Sato, S., Yoneyama, M., et al. (2006) Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature*. 441 (1), 101–105. Available from: doi:10.1038/nature04734.
- Kauffman, S.L. (1980) Cell proliferation in the mammalian lung. *International review of experimental pathology*. 22, 131–191.
- Kavaler, J., Caton, A.J., Staudt, L.M. & Gerhard, W. (1991) A B cell population that dominates the primary response to influenza virus hemagglutinin does not participate in the memory response. *European Journal of Immunology*. 21 (11), 2687–2695. Available from: doi:10.1002/eji.1830211107.
- Kawashima, T., Kosaka, A., Yan, H., Guo, Z., et al. (2013) Double-Stranded RNA of Intestinal Commensal but Not Pathogenic Bacteria Triggers Production of Protective

- Interferon- β . *Immunity*. 38 (6), 1187–1197. Available from: doi:10.1016/j.immuni.2013.02.024.
- Kayagaki, N., Yamaguchi, N., Nakayama, M., Hiroshi, E., et al. (1999) Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: A novel mechanism for the antitumor effects of type I IFNs. *Journal of Experimental Medicine*. 189 (9), 1451–1460. Available from: doi:10.1084/jem.189.9.1451.
- Van Kerkhove, M.D., Mumford, E., Mounts, A.W., Bresee, J., et al. (2011) Highly pathogenic avian influenza (H5N1): Pathways of exposure at the animal-human interface, a systematic review. *PLoS ONE*. 6 (1), 1–8. Available from: doi:10.1371/journal.pone.0014582.
- Kewley, R.J., Whitelaw, M.L. & Chapman-Smith, A. (2004) The mammalian basic helix-loop-helix/PAS family of transcriptional regulators. *International Journal of Biochemistry and Cell Biology*. 36 (2), 189–204. Available from: doi:10.1016/S1357-2725(03)00211-5.
- Van Keymeulen, A. & Blanpain, C. (2012) Tracing epithelial stem cells during development, homeostasis, and repair. *Journal of Cell Biology*. 197 (5), 575–584. Available from: doi:10.1083/jcb.201201041.
- Kiessling, R., Klein, E. & Wigzell, H. (1975) “Natural” killer cells in the mouse. 1. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype*. *European Journal of Immunology*. 5 (2), 112–117.
- Killingley, B. & Nguyen-Van-Tam, J. (2013) Routes of influenza transmission. *Influenza and other Respiratory Viruses*. 7 (SUPPL.2), 42–51. Available from: doi:10.1111/irv.12080.
- Kim, H.M., Lee, Y.-W., Lee, K.-J., Kim, H.S., et al. (2008) Alveolar Macrophages Are Indispensable for Controlling Influenza Viruses in Lungs of Pigs. *Journal of Virology*. 82 (9), 4265–4274. Available from: doi:10.1128/jvi.02602-07.
- Kim, S., Poursine-Laurent, J., Truscott, S.M., Lybarger, L., et al. (2005) Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature*. 436 (7051), 709–713. Available from: doi:10.1038/nature03847.
- Kimura, A., Naka, T., Nakahama, T., Chinen, I., et al. (2009) Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. *The Journal of Experimental Medicine*. 206 (9), 2027–2035. Available from: doi:10.1084/jem.20090560.

- Kimura, A., Naka, T., Nohara, K., Fujii-Kuriyama, Y., et al. (2008) Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. *Proceedings of the National Academy of Sciences of the United States of America*. 105 (28), 9721–9726. Available from: doi:10.1073/pnas.0804231105.
- Kiss, E.A., Vonarbourg, C., Kopfmann, S., Hobeika, E., et al. (2011) Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. *Science*. 334 (6062), 1561–1565. Available from: doi:10.1126/science.1214914.
- Klinkhammer, J., Schnepf, D., Ye, L., Schwaderlapp, M., et al. (2018) IFN- λ prevents influenza virus spread from the upper airways to the lungs and limits virus transmission. *eLife*. 7, e33354. Available from: doi:10.7554/eLife.33354.
- Knudsen, L. & Ochs, M. (2018) The micromechanics of lung alveoli: structure and function of surfactant and tissue components. *Histochemistry and Cell Biology*. 150 (6), 661–676. Available from: doi:10.1007/s00418-018-1747-9.
- Kobasa, D., Jones, S.M., Shinya, K., Kash, J.C., et al. (2007) Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. *Nature*. 445 (7125), 319–323. Available from: doi:10.1038/nature05495.
- Kochs, G., Martínez-Sobrido, L., Lienenklaus, S., Weiss, S., et al. (2009) Strong interferon-inducing capacity of a highly virulent variant of influenza A virus strain PR8 with deletions in the NS1 gene. *Journal of General Virology*. 90 (12), 2990–2994. Available from: doi:10.1099/vir.0.015727-0.
- Konno, Y., Kimura, I., Urui, K., Fukushi, M., et al. (2020) SARS-CoV-2 ORF3b is a potent interferon antagonist whose activity is further increased by a naturally occurring elongation variant. *bioRxiv*. 1–24. Available from: doi:https://doi.org/10.1101/2020.05.11.088179.
- Korn, T., Bettelli, E., Oukka, M. & Kuchroo, V.K. (2009) IL-17 and Th17 cells. *Annual Review of Immunology*. 27, 485–517. Available from: doi:10.1146/annurev.immunol.021908.132710.
- Kotenko, S. V., Gallagher, G., Baurin, V. V., Lewis-Antes, A., et al. (2003) IFN- λ s mediate antiviral protection through a distinct class II cytokine receptor complex. *Nature Immunology*. 4 (1), 69–77. Available from: doi:10.1038/ni875.
- Krammer, F., Smith, G.J.D., Fouchier, R.A.M., Peiris, M., et al. (2018) Influenza. *Nature Reviews Disease Primers*. 4 (1), 1–21. Available from: doi:10.1038/s41572-018-0002-y.
- Kraus, J., Voigt, K., Schuller, A.M., Scholz, M., et al. (2008) Interferon- β stabilizes barrier

- characteristics of the blood-brain barrier in four different species in vitro. *Multiple Sclerosis*. 14 (6), 843–852. Available from: doi:10.1177/1352458508088940.
- Kulkarni, U., Zemans, R.L., Smith, C.A., Wood, S.C., et al. (2019) Excessive neutrophil levels in the lung underlie the age-associated increase in influenza mortality. *Mucosal Immunology* 2019. (October 2018), 1. Available from: doi:10.1038/s41385-018-0115-3.
- Kumar, P., Thakar, M.S., Ouyang, W. & Malarkannan, S. (2013) IL-22 from conventional NK cells is epithelial regenerative and inflammation protective during influenza infection. *Mucosal Immunology*. 6 (1), 69–82. Available from: doi:10.1038/mi.2012.49.
- Kumar, P.A., Hu, Y., Yamamoto, Y., Hoe, N.B., et al. (2011) Distal airway stem cells yield alveoli in vitro and during lung regeneration following H1N1 influenza infection. *Cell*. 147 (3), 525–538. Available from: doi:10.1016/j.cell.2011.10.001.
- Kumar, R. & Atlas, I. (1992) Interferon α induces the expression of retinoblastoma gene product in human Burkitt lymphoma Daudi cells: Role in growth regulation. *Proceedings of the National Academy of Sciences of the United States of America*. 89 (14), 6599–6603. Available from: doi:10.1073/pnas.89.14.6599.
- Kuriakose, T., Man, S.M., Subbarao Malireddi, R.K., Karki, R., et al. (2016) ZBP1/DAI is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways. *Science Immunology*. 1 (2), 1–12. Available from: doi:10.1126/sciimmunol.aag2045.
- van de Laar, L., Saelens, W., De Prijck, S., Martens, L., et al. (2016) Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages. *Immunity*. 44 (4), 755–768. Available from: doi:10.1016/j.immuni.2016.02.017.
- Lafkas, D., Shelton, A., Chiu, C., de Leon Boenig, G., et al. (2015) Therapeutic antibodies reveal Notch control of transdifferentiation in the adult lung. *Nature*. 528 (7580), 127–131. Available from: doi:10.1038/nature15715.
- Lahvis, G.P., Lindell, S.L., Thomas, R.S., McCuskey, R.S., et al. (2000) Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*. 97 (19), 10442–10447. Available from: doi:10.1073/pnas.190256997.
- Lahvis, G.P., Pyzalski, R.W., Glover, E., Pitot, H.C., et al. (2005) The Aryl Hydrocarbon Receptor Is Required for Developmental Closure of the Ductus Venosus in the Neonatal Mouse. *Molecular Pharmacology*. 67 (3), 714–720. Available from:

- doi:10.1124/mol.104.008888.
- Lam, J.H. & Baumgarth, N. (2019) The Multifaceted B Cell Response to Influenza Virus. *The Journal of Immunology*. 202 (2), 351–359. Available from: doi:10.4049/jimmunol.1801208.
- Lamas, B., Richard, M.L., Leducq, V., Pham, H.-P.P., et al. (2016) CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nature Medicine*. 22 (6), 598–605. Available from: doi:10.1038/nm.4102.
- Lande, R., Gregorio, J., Facchinetti, V., Chatterjee, B., et al. (2007) Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature*. 449 (7162), 564–569. Available from: doi:10.1038/nature06116.
- Lanier, L.L. (2008) Up on the tightrope: Natural killer cell activation and inhibition. *Nature Immunology*. 9 (5), 495–502. Available from: doi:10.1038/ni1581.
- Lasfar, A., Gogas, H., Zloza, A., Kaufman, H.L., et al. (2016) IFN- λ cancer immunotherapy: New kid on the block. *Immunotherapy*. 8 (8), 877–888. Available from: doi:10.2217/imt-2015-0021.
- Lasfar, A., Lewis-Antes, A., Smirnov, S. V., Anantha, S., et al. (2006) Characterization of the mouse IFN- λ ligand-receptor system: IFN- λ s exhibit antitumor activity against B16 melanoma. *Cancer Research*. 66 (8), 4468–4477. Available from: doi:10.1158/0008-5472.CAN-05-3653.
- Laskin, D.L., Sunil, V.R., Gardner, C.R. & Laskin, J.D. (2011) Macrophages and tissue injury: Agents of defense or destruction? *Annual Review of Pharmacology and Toxicology*. 51, 267–288. Available from: doi:10.1146/annurev.pharmtox.010909.105812.
- Lauder, S.N., Jones, E., Smart, K., Bloom, A., et al. (2013) Interleukin-6 limits influenza-induced inflammation and protects against fatal lung pathology. *European Journal of Immunology*. 43 (10), 2613–2625. Available from: doi:10.1002/eji.201243018.
- Lavezzo, E., Franchin, E., Ciavarella, C., Cuomo-Dannenburg, G., et al. (2020) Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo'. *Nature*. 584 (August), 425–429. Available from: doi:10.1038/s41586-020-2488-1.
- Lavoie, T.B., Kalie, E., Crisafulli-Cabatu, S., Abramovich, R., et al. (2011) Binding and activity of all human alpha interferon subtypes. *Cytokine*. 56 (2), 282–289. Available from: doi:10.1016/j.cyto.2011.07.019.
- Lawrence, B.P., Roberts, A.D., Neumiller, J.J., Cundiff, J.A., et al. (2006) Aryl Hydrocarbon

- Receptor Activation Impairs the Priming but Not the Recall of Influenza Virus-Specific CD8 + T Cells in the Lung. *The Journal of Immunology*. 177 (9), 5819–5828. Available from: doi:10.4049/jimmunol.177.9.5819.
- Lazear, H.M., Daniels, B.P., Pinto, A.K., Huang, A.C., et al. (2015) Interferon- λ restricts West Nile virus neuroinvasion by tightening the blood-brain barrier. *Science Translational Medicine*. 7 (284), 1–14. Available from: <http://stm.sciencemag.org/content/7/284/284ra59.full.pdf>.
- Lechner, A.J., Driver, I.H., Lee, J., Conroy, C.M., et al. (2017) Recruited Monocytes and Type 2 Immunity Promote Lung Regeneration following Pneumectomy. *Cell Stem Cell*. 21 (1), 120-134.e7. Available from: doi:10.1016/j.stem.2017.03.024.
- Lee, B.O., Rangel-Moreno, J., Moyron-Quiroz, J.E., Hartson, L., et al. (2005) CD4 T Cell-Independent Antibody Response Promotes Resolution of Primary Influenza Infection and Helps to Prevent Reinfection. *The Journal of Immunology*. 175 (9), 5827–5838. Available from: doi:10.4049/jimmunol.175.9.5827.
- Lee, J.H., Tammela, T., Hofree, M., Choi, J., et al. (2017) Anatomically and Functionally Distinct Lung Mesenchymal Populations Marked by Lgr5 and Lgr6. *Cell*. 170 (6), 1149-1163.e12. Available from: doi:10.1016/j.cell.2017.07.028.
- Lee, J.S., Cella, M., McDonald, K.G., Garlanda, C., et al. (2012) AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. *Nat Immunol*. 13 (2), 144–151. Available from: doi:10.1038/ni.2187.
- Lee, J.S., Park, S., Jeong, H.W., Ahn, J.Y., et al. (2020) Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19. *Science immunology*. 5 (49), 1–17. Available from: doi:10.1126/sciimmunol.abd1554.
- Lee, J.S. & Shin, E.-C. (2020) The type I interferon response in COVID-19: implications for treatment. *Nature Reviews Immunology*. Available from: doi:10.1038/s41577-020-00429-3.
- Lees, M.J., Peet, D.J. & Whitelaw, M.L. (2003) Defining the role for XAP2 in stabilization of the dioxin receptor. *The Journal of biological chemistry*. 278 (38), 35878–35888. Available from: doi:10.1074/jbc.M302430200.
- Legend, A., Briand, S., Shindo, N., Brooks, W.A., et al. (2013) Addressing the public health burden of respiratory viruses: the Battle against Respiratory Viruses (BRaVe) Initiative.

- Future Virology*. 8 (10), 953–968. Available from: doi:10.2217/fvl.13.85.
- Lehmann, M.H., Torres-Domínguez, L.E., Price, P.J.R., Brandmüller, C., et al. (2016) CCL2 expression is mediated by type I IFN receptor and recruits NK and T cells to the lung during MVA infection. *Journal of Leukocyte Biology*. 99 (6), 1057–1064. Available from: doi:10.1189/jlb.4ma0815-376rr.
- Levin, D., Schneider, W.M., Hoffmann, H.H., Yarden, G., et al. (2014) Multifaceted activities of type I interferon are revealed by a receptor antagonist. *Science Signaling*. 7 (327). Available from: doi:10.1126/scisignal.2004998.
- Levy, D.E., Lew, D.J., Decker, T., Kessler, D.S., et al. (1990) Synergistic interaction between interferon- α and interferon- γ through induced synthesis of one subunit of the transcription factor ISGF3. *EMBO Journal*. 9 (4), 1105–1111. Available from: doi:10.1002/j.1460-2075.1990.tb08216.x.
- Levy, D.E., Marié, I., Smith, E. & Prakash, A. (2002) Enhancement and diversification of IFN induction by IRF-7-mediated positive feedback. *Journal of Interferon and Cytokine Research*. 22 (1), 87–93. Available from: doi:10.1089/107999002753452692.
- Li, W., Moltedo, B. & Moran, T.M. (2012) Type I Interferon Induction during Influenza Virus Infection Increases Susceptibility to Secondary Streptococcus pneumoniae Infection by Negative Regulation of T Cells. *Journal of Virology*. 86 (22), 12304–12312. Available from: doi:10.1128/JVI.01269-12.
- Li, Y., Innocentin, S., Withers, D.R., Roberts, N.A., et al. (2011) Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. *Cell*. 147 (3), 629–640. Available from: doi:10.1016/j.cell.2011.09.025.
- Lin, J. Da, Feng, N., Sen, A., Balan, M., et al. (2016) Distinct Roles of Type I and Type III Interferons in Intestinal Immunity to Homologous and Heterologous Rotavirus Infections. *PLoS Pathogens*. 12 (4), 1–29. Available from: doi:10.1371/journal.ppat.1005600.
- Lin, K.L., Suzuki, Y., Nakano, H., Ramsburg, E., et al. (2008) CCR2+ Monocyte-Derived Dendritic Cells and Exudate Macrophages Produce Influenza-Induced Pulmonary Immune Pathology and Mortality. *The Journal of Immunology*. 180 (4), 2562–2572. Available from: doi:10.4049/jimmunol.180.4.2562.
- Liu, H., Ramachandran, I. & Gabrilovich, D.I. (2014) Regulation of plasmacytoid dendritic cell development in mice by aryl hydrocarbon receptor. *Immunology and Cell Biology*. 92 (2), 200–203. Available from: doi:10.1038/icb.2013.65.

- Liu, J.Z., Van Sommeren, S., Huang, H., Ng, S.C., et al. (2015) Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nature Genetics*. 47 (9), 979–986. Available from: doi:10.1038/ng.3359.
- Liu, Q., Liu, K., Cui, G., Huang, X., et al. (2019) Lung regeneration by multipotent stem cells residing at the bronchioalveolar-duct junction. *Nature Genetics*. 51 (4), 728–738. Available from: doi:10.1038/s41588-019-0346-6.
- Liu, Y., Yan, L.-M., Wan, L., Xiang, T.-X., et al. (2020) Viral dynamics in mild and severe cases of COVID-19. *The Lancet Infectious Diseases*. 2019 (20), 2019–2020. Available from: doi:10.1016/S1473-3099(20)30232-2.
- Lokugamage, K., Hage, A., de Vries, M., Valero-Jimenez, A., et al. (2020) Type I interferon susceptibility distinguishes SARS-CoV-2 from SARS-CoV. *bioRxiv: the preprint server for biology*. Available from: doi:10.1101/2020.03.07.982264.
- Lucas, C., Wong, P., Klein, J., Castro, T.B.R., et al. (2020) Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature*. 584 (August). Available from: doi:10.1038/s41586-020-2588-y.
- Luebke, R.W., Copeland, C.B., Bishop, L.R., Daniels, M.J., et al. (2002) Mortality in dioxin-exposed mice infected with influenza: Mitochondrial toxicity (Reye's-Like Syndrome) versus enhanced inflammation as the mode of action. *Toxicological Sciences*. 69 (1), 109–116. Available from: doi:10.1093/toxsci/69.1.109.
- Lund, J.M., Alexopoulou, L., Sato, A., Karow, M., et al. (2004) Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proceedings of the National Academy of Sciences of the United States of America*. 101 (15), 5598–5603. Available from: doi:10.1073/pnas.0400937101.
- Luo, D., Ding, S.C., Vela, A., Kohlway, A., et al. (2011) Structural insights into RNA recognition by RIG-I. *Cell*. 147 (2), 409–422. Available from: doi:10.1016/j.cell.2011.09.023.
- MacMicking, J.D. (2012) Interferon-inducible effector mechanisms in cell-autonomous immunity. *Nature Reviews Immunology*. 12 (5), 367–382. Available from: doi:10.1038/nri3210.
- Maher, J.A. & DeStefano, J. (2004) The ferret: An animal model to study influenza virus. *Lab Animal*. 33 (9), 50–53. Available from: doi:10.1038/labani1004-50.
- Maillard, P.V., Ciaudo, C., Marchais, A., Li, Y., et al. (2013) Antiviral RNA Interference in

- Mammalian Cells. *Science*. 342 (6155), 235–238. Available from: doi:10.1126/science.1241930.
- Major, J., Crotta, S., Llorian, M., Teresa, M.M., et al. (2020) Type I and III interferons disrupt lung epithelial repair during recovery from viral infection. *Science*. 369 (6504), 712–717.
- Malakhov, M.P., Malakhova, O.A., Il Kim, K., Ritchie, K.J., et al. (2002) UBP43 (USP18) specifically removes ISG15 from conjugated proteins. *Journal of Biological Chemistry*. 277 (12), 9976–9981. Available from: doi:10.1074/jbc.M109078200.
- Malakhova, O.A., Kim, K. Il, Luo, J.K., Zou, W., et al. (2006) UBP43 is a novel regulator of interferon signaling independent of its ISG15 isopeptidase activity. *EMBO Journal*. 25 (11), 2358–2367. Available from: doi:10.1038/sj.emboj.7601149.
- Manicassamy, B., Manicassamy, S., Belicha-Villanueva, A., Pisanelli, G., et al. (2010) Analysis of in vivo dynamics of influenza virus infection in mice using a GFP reporter virus. *Proceedings of the National Academy of Sciences of the United States of America*. 107 (25), 11531–11536. Available from: doi:10.1073/pnas.0914994107.
- Marcello, T., Grakoui, A., Barba-Spaeth, G., Machlin, E.S., et al. (2006) Interferons α and λ Inhibit Hepatitis C Virus Replication With Distinct Signal Transduction and Gene Regulation Kinetics. *Gastroenterology*. 131 (6), 1887–1898. Available from: doi:10.1053/j.gastro.2006.09.052.
- Martin, B., Hirota, K., Cua, D.J., Stockinger, B., et al. (2009) Interleukin-17-Producing $\gamma\delta$ T Cells Selectively Expand in Response to Pathogen Products and Environmental Signals. *Immunity*. 31 (2), 321–330. Available from: doi:10.1016/j.immuni.2009.06.020.
- Martinon, F., Burns, K. & Tschopp, J. (2002) The Inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proIL- β . *Molecular Cell*. 10 (2), 417–426. Available from: doi:10.1016/S1097-2765(02)00599-3.
- Matejuk, A. (2018) Skin Immunity. *Archivum Immunologiae et Therapiae Experimentalis*. 66 (1), 45–54. Available from: doi:10.1007/s00005-017-0477-3.
- Matzinger, P. (2002) The danger model: A renewed sense of self. *Science*. 296 (5566), 301–305. Available from: doi:10.1126/science.1071059.
- McCullers, J.A. (2014) The co-pathogenesis of influenza viruses with bacteria in the lung. *Nature Reviews Microbiology*. 12 (4), 252–262. Available from: doi:10.1038/nrmicro3231.
- McNab, F., Mayer-Barber, K., Sher, A., Wack, A., et al. (2015) Type I interferons in

- infectious disease. *Nat Rev Immunol.* 15 (2), 87–103. Available from: doi:10.1038/nri3787.
- McQualter, J.L., Yuen, K., Williams, B. & Bertoncello, I. (2010) Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung. *Proceedings of the National Academy of Sciences of the United States of America.* 107 (4), 1414–1419. Available from: doi:10.1073/pnas.0909207107.
- Medzhitov, R. & Janeway, C.A. (2002) Decoding the patterns of self and nonself by the innate immune system. *Science.* 296 (5566), 298–300. Available from: doi:10.1126/science.1068883.
- Medzhitov, R., Schneider, D.S. & Soares, M.P. (2012) Disease Tolerance as a Defense Strategy. *Science.* 335 (6071), 936–942. Available from: doi:10.1126/science.1214935.
- Mellor, A.L., Lemos, H. & Huang, L. (2017) Indoleamine 2,3-Dioxygenase and tolerance: Where Are We Now? *Frontiers in Immunology.* 8 (OCT), 1–6. Available from: doi:10.3389/fimmu.2017.01360.
- Mendoza, J.L., Schneider, W.M., Hoffmann, H.-H., Vercauteren, K., et al. (2017) The IFN- λ -IFN- λ R1-IL-10R β Complex Reveals Structural Features Underlying Type III IFN Functional Plasticity. *Immunity.* 46 (3), 379–392. Available from: doi:10.1016/j.immuni.2017.02.017.
- Metidji, A., Omenetti, S., Crotta, S., Li, Y., et al. (2018) The Environmental Sensor AHR Protects from Inflammatory Damage by Maintaining Intestinal Stem Cell Homeostasis and Barrier Integrity. *Immunity.* 1–10. Available from: doi:10.1016/j.immuni.2018.07.010.
- Meuwissen, M.E.C., Schot, R., Buta, S., Oudesluijs, G., et al. (2016) Human USP18 deficiency underlies type 1 interferonopathy leading to severe pseudo-TORCH syndrome. *The Journal of Experimental Medicine.* 213 (7), 1163–1174. Available from: doi:10.1084/jem.20151529.
- Mezrich, J.D., Fechner, J.H., Zhang, X., Johnson, B.P., et al. (2010) An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *Journal of immunology (Baltimore, Md. : 1950).* 185 (6), 3190–3198. Available from: doi:10.4049/jimmunol.0903670.
- Miceli-Richard, C., Comets, E., Loiseau, P., Puechal, X., et al. (2007) Association of an IRF5 gene functional polymorphism with Sjögren's syndrome. *Arthritis and rheumatism.* 56

- (12), 3989–3994. Available from: doi:10.1002/art.23142.
- Mimura, J., Ema, M., Sogawa, K. & Fujii-Kuriyama, Y. (1999) Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. *Genes & development*. 13 (1), 20–25. Available from: doi:10.1101/gad.13.1.20.
- Moiseeva, O., Mallette, F.A., Mukhopadhyay, U., Moores, A., et al. (2006) DNA damage signaling and p53-dependent senescence after prolonged β -interferon stimulation. *Molecular biology of the cell*. 17 (4), 1583–1592. Available from: doi:10.1091/mbc.E05.
- Molinari, N.A.M., Ortega-Sanchez, I.R., Messonnier, M.L., Thompson, W.W., et al. (2007) The annual impact of seasonal influenza in the US: Measuring disease burden and costs. *Vaccine*. 25 (27), 5086–5096. Available from: doi:10.1016/j.vaccine.2007.03.046.
- Monticelli, L.A., Sonnenberg, G.F., Abt, M.C., Alenghat, T., et al. (2011) Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat Immunol*. 12 (11), 1045–1054. Available from: doi:10.1031/ni.2131.
- Montoro, D.T., Haber, A.L., Biton, M., Vinarsky, V., et al. (2018) A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature*. Available from: doi:10.1038/s41586-018-0393-7.
- Moraga, I., Harari, D., Schreiber, G., Uze, G., et al. (2009) Receptor Density Is Key to the Alpha2/Beta Interferon Differential Activities. *Molecular and Cellular Biology*. 29 (17), 4778–4787. Available from: doi:10.1128/MCB.01808-08.
- Mordstein, M., Kochs, G., Dumoutier, L., Renauld, J.C., et al. (2008) Interferon- λ contributes to innate immunity of mice against influenza A virus but not against hepatotropic viruses. *PLoS Pathogens*. 4 (9), 1–7. Available from: doi:10.1371/journal.ppat.1000151.
- Mori, M., Mahoney, J.E., Stupnikov, M.R., Paez-Cortez, J.R., et al. (2015) Notch3-Jagged signaling controls the pool of undifferentiated airway progenitors. *Development*. 142 (2), 258–267. Available from: doi:10.1242/dev.116855.
- Moriyama, M., Chen, I.-Y., Kawaguchi, A., Koshiba, T., et al. (2016) The RNA- and TRIM25-binding domains of influenza virus NS1 protein are essential for suppression of NLRP3 inflammasome-mediated interleukin-1 β secretion. *Journal of Virology*. 90 (8), 4105–4114. Available from: doi:10.1128/JVI.00120-16.Editor.
- Moriyama, M., Hugentobler, W.J. & Iwasaki, A. (2020) Seasonality of respiratory viral infections. *Annual Review of Virology*. 7, 1–19. Available from: doi:10.1146/annurev-virology-012420-022445.
- Mostafavi, S., Yoshida, H., Moodley, D., Leboité, H., et al. (2016) Parsing the Interferon

- Transcriptional Network and Its Disease Associations. *Cell*. 164 (3), 564–578. Available from: doi:10.1016/j.cell.2015.12.032.
- Mozdzanowska, K., Maiese, K. & Gerhard, W. (2000) Th Cell-Deficient Mice Control Influenza Virus Infection More Effectively Than Th- and B Cell-Deficient Mice: Evidence for a Th-Independent Contribution by B Cells to Virus Clearance. *The Journal of Immunology*. 164 (5), 2635–2643. Available from: doi:10.4049/jimmunol.164.5.2635.
- Muñoz-Fontela, C., Macip, S., Martínez-Sobrido, L., Brown, L., et al. (2008) Transcriptional role of p53 in interferon-mediated antiviral immunity. *The Journal of Experimental Medicine*. 205 (8), 1929–1938. Available from: doi:10.1084/jem.20080383.
- Munoz-Fontela, C., Pazos, M., Delgado, I., Murk, W., et al. (2011) p53 Serves as a Host Antiviral Factor That Enhances Innate and Adaptive Immune Responses to Influenza A Virus. *The Journal of Immunology*. 187 (12), 6428–6436. Available from: doi:10.4049/jimmunol.1101459.
- Murray, P.J. (2007) The JAK-STAT Signaling Pathway: Input and Output Integration. *The Journal of Immunology*. 178 (5), 2623–2629. Available from: doi:10.4049/jimmunol.178.5.2623.
- Nabhan, A., Brownfield, D.G., Harbury, P.B., Krasnow, M.A., et al. (2018) Single-cell Wnt signaling niches maintain stemness of alveolar type 2 cells. *Science*. 1123 (March), 1–12. Available from: doi:10.1126/science.aam6603.
- Naik, S.H., Sathe, P., Park, H.Y., Metcalf, D., et al. (2007) Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nature Immunology*. 8 (11), 1217–1226. Available from: doi:10.1038/ni1522.
- Narasaraju, T., Yang, E., Samy, R.P., Ng, H.H., et al. (2011) Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. *American Journal of Pathology*. 179 (1), 199–210. Available from: doi:10.1016/j.ajpath.2011.03.013.
- Natividad, J.M., Agus, A., Planchais, J., Lamas, B., et al. (2018) Impaired Aryl Hydrocarbon Receptor Ligand Production by the Gut Microbiota Is a Key Factor in Metabolic Syndrome. *Cell Metabolism*. 28 (5), 737–749.e4. Available from: doi:10.1016/j.cmet.2018.07.001.
- Nault, R., Forgacs, A.L., Dere, E. & Zacharewski, T.R. (2013) Comparisons of differential gene expression elicited by TCDD, PCB126, β NF, or ICZ in mouse hepatoma Hepa1c1c7 cells and C57BL/6 mouse liver. *Toxicology Letters*. 223 (1), 52–59.

- Available from: doi:10.1016/j.toxlet.2013.08.013.
- Neavin, D.R., Liu, D., Ray, B. & Weinshilboum, R.M. (2018) The role of the aryl hydrocarbon receptor (AHR) in immune and inflammatory diseases. *International Journal of Molecular Sciences*. 19 (12). Available from: doi:10.3390/ijms19123851.
- Nelson, B.D.J., Mcmenamin, C., Mcwilliam, A.S., Brenan, M., et al. (1994) Development of the airway intraepithelial dendritic cell network in the rat from class II major histocompatibility (Ia)-negative precursors: differential regulation of Ia expression at different levels of the respiratory tract. *Journal of Experimental Medicine*. 179 (1), 203–212.
- Nemeroff, M.E., Barabino, S.M.L., Li, Y., Keller, W., et al. (1998) Influenza virus NS1 protein interacts with the cellular 30 kDa subunit of CPSF and inhibits 3' end formation of cellular pre-mRNAs. *Molecular Cell*. 1 (7), 991–1000. Available from: doi:10.1016/S1097-2765(00)80099-4.
- Neyt, K. & Lambrecht, B.N. (2013) The role of lung dendritic cell subsets in immunity to respiratory viruses. *Immunological Reviews*. 255 (1), 57–67. Available from: doi:10.1111/imr.12100.
- Nguyen, L.P. & Bradfield, C.A. (2008) The search for endogenous activators of the aryl hydrocarbon receptor. *Chemical Research in Toxicology*. 21 (1), 102–116. Available from: doi:10.1021/tx7001965.
- Nguyen, N.T., Kimura, A., Nakahama, T., Chinen, I., et al. (2010) Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proceedings of the National Academy of Sciences of the United States of America*. 107 (46), 19961–19966. Available from: doi:10.1073/pnas.1014465107.
- Niu, J., Wu, S., Chen, M., Xu, K., et al. (2019) Hyperactivation of the NLRP3 inflammasome protects mice against influenza A virus infection via IL-1 β mediated neutrophil recruitment. *Cytokine*. 120 (January), 115–124. Available from: doi:10.1016/j.cyto.2019.04.019.
- Niwa, A., Kumaki, K., Nebert, D.W. & Poland, A.P. (1975) Genetic expression of aryl hydrocarbon hydroxylase activity in the mouse. Distinction between the 'responsive' homozygote and heterozygote at the Ah locus. *Archives of biochemistry and biophysics*. 166 (2), 559–564. Available from: doi:10.1016/0003-9861(75)90420-8.
- Nogales, A., Aydillo, T., Ávila-Pérez, G., Escalera, A., et al. (2019) Functional Characterization and Direct Comparison of Influenza A, B, C, and D NS1 Proteins in

- vitro and in vivo. *Frontiers in Microbiology*. 10 (December), 1–16. Available from: doi:10.3389/fmicb.2019.02862.
- Novatt, H., Theisen, T.C., Massie, T., Massie, T., et al. (2016) Distinct Patterns of Expression of Transcription Factors in Response to Interferon β and Interferon λ 1. *Journal of Interferon and Cytokine Research*. 36 (10), 589–598. Available from: doi:10.1089/jir.2016.0031.
- Odendall, C., Voak, A.A. & Kagan, J.C. (2017) Type III IFNs Are Commonly Induced by Bacteria-Sensing TLRs and Reinforce Epithelial Barriers during Infection. *The Journal of Immunology*. 199 (9), 3270–3279. Available from: doi:10.4049/jimmunol.1700250.
- Ogawa, V.A., Nicholson, A. & Shah, C.M. (2019) *Exploring Lessons Learned from a Century of Outbreaks: Readiness for 2030*. Available from: https://www.ncbi.nlm.nih.gov/books/NBK544054/pdf/Bookshelf_NBK544054.pdf.
- Ohtake, F., Baba, A., Takada, I., Okada, M., et al. (2007) Dioxin receptor is a ligand-dependent E3 ubiquitin ligase. *Nature*. 446 (7135), 562–566. Available from: doi:10.1038/nature05683.
- Olle Sangfelt, S.E. and D.G. (2000) Mechanisms of interferon-induced cell cycle arrest. *Frontiers in Bioscience*. (2), 479–487.
- Onabajo, O.O., Porter-Gill, P., Paquin, A., Rao, N., et al. (2015) Expression of Interferon Lambda 4 Is Associated with Reduced Proliferation and Increased Cell Death in Human Hepatic Cells. *Journal of Interferon & Cytokine Research*. 35 (11), 888–900. Available from: doi:10.1089/jir.2014.0161.
- Onomoto, K., Jogi, M., Yoo, J.S., Narita, R., et al. (2012) Critical role of an antiviral stress granule containing RIG-I and PKR in viral detection and innate immunity. *PLoS ONE*. 7 (8). Available from: doi:10.1371/journal.pone.0043031.
- Oshansky, C.M., Gartland, A.J., Wong, S.S., Jeevan, T., et al. (2014) Mucosal immune responses predict clinical outcomes during influenza infection independently of age and viral load. *American Journal of Respiratory and Critical Care Medicine*. 189 (4), 449–462. Available from: doi:10.1164/rccm.201309-1616OC.
- Oslund, K.L. & Baumgarth, N. (2011) Influenza-induced innate immunity: Regulators of viral replication, respiratory tract pathology & adaptive immunity. *Future Virology*. 6 (8), 951–962. Available from: doi:10.2217/fvl.11.63.
- Osterlund, P., Veckman, V., Siren, J., Klucher, K.M., et al. (2005) Gene expression and antiviral activity of alpha/beta Interferons and interleukin-29 in virus-infected human

- myeloid dendritic cells. *Journal of Virology*. 79 (15), 9608–9617. Available from: doi:10.1128/jvi.79.15.9608-9617.2005.
- Ozeki, J., Uno, S., Ogura, M., Choi, M., et al. (2011) Aryl hydrocarbon receptor ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin enhances liver damage in bile duct-ligated mice. *Toxicology*. 280 (1–2), 10–17. Available from: doi:10.1016/j.tox.2010.11.003.
- Paget, C., Ivanov, S., Fontaine, J., Renneson, J., et al. (2012) Interleukin-22 is produced by invariant natural killer T lymphocytes during influenza A virus infection: Potential role in protection against lung epithelial damages. *Journal of Biological Chemistry*. 287 (12), 8816–8829. Available from: doi:10.1074/jbc.M111.304758.
- Panda, D., Gjinaj, E., Bachu, M., Squire, E., et al. (2019) IRF1 maintains optimal constitutive expression of antiviral genes and regulates the early antiviral response. *Frontiers in Immunology*. 10 (MAY), 1–16. Available from: doi:10.3389/fimmu.2019.01019.
- Pappaioanou, M. & Gramer, M. (2010) Lessons from pandemic H1N1 2009 to improve prevention, detection, and response to influenza pandemics from a one health perspective. *ILAR Journal*. 51 (3), 268–280. Available from: doi:10.1093/ilar.51.3.268.
- Paquette, S.G., Banner, D., Zhao, Z., Fang, Y., et al. (2012) Interleukin-6 is a potential biomarker for severe pandemic H1N1 influenza a infection. *PLoS ONE*. 7 (6). Available from: doi:10.1371/journal.pone.0038214.
- Pardo-Saganta, A., Law, B.M., Tata, P.R., Villoria, J., et al. (2015) Injury induces direct lineage segregation of functionally distinct airway basal stem/progenitor cell subpopulations. *Cell Stem Cell*. 16 (2), 184–197. Available from: doi:10.1016/j.stem.2015.01.002.
- Park, S.-J., Yoon, W.-K., Kim, H.-J., Son, H.-Y., et al. (2005) 2,3,7,8-Tetrachlorodibenzo-p-dioxin activates ERK and p38 mitogen-activated protein kinases in RAW 264.7 cells. *Anticancer research*. 25 (4), 2831–2836.
- Parker, B.S., Rautela, J. & Hertzog, P.J. (2016) Antitumour actions of interferons: implications for cancer therapy. *Nature reviews. Cancer*. 16 (3), 131–144. Available from: doi:10.1038/nrc.2016.14.
- Parnell, G.P., McLean, A.S., Booth, D.R., Armstrong, N.J., et al. (2012) A distinct influenza infection signature in the blood transcriptome of patients with severe community-acquired pneumonia. *Critical Care*. 16 (4), R157. Available from: doi:http://dx.doi.org/10.1186/cc11477.
- Patel, R.D., Murray, I.A., Flaveny, C.A., Kusnadi, A., et al. (2009) Ah receptor represses

- acute-phase response gene expression without binding to its cognate response element. *Laboratory Investigation*. 89 (6), 695–707. Available from: doi:10.1038/labinvest.2009.24.
- Paucker, K., Cantell, K. & Henle, W. (1962) Quantitative studies on viral interference in suspended L cells. III. Effect of interfering viruses and interferon on the growth rate of cells. *Virology*. 17 (2), 324–334. Available from: doi:10.1016/0042-6822(62)90123-X.
- Paules, C. & Subbarao, K. (2017) Influenza. *The Lancet*. 6736 (17), 1–12. Available from: doi:10.1016/S0140-6736(17)30129-0.
- Pavlovic, J., Zürcher, T., Haller, O. & Staeheli, P. (1990) Resistance to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. *Journal of Virology*. 64 (7), 3370–3375. Available from: doi:10.1128/jvi.64.7.3370-3375.1990.
- Peper, R.L. & Van Campen, H. (1995) Tumor necrosis factor as a mediator of inflammation in influenza a viral pneumonia. *Microbial Pathogenesis*. 19 (3), 175–183. Available from: doi:10.1006/mpat.1995.0056.
- Pérez-Padilla, R., Fernández, R., García-Sancho, C., Franco-Marina, F., et al. (2010) Pandemic (H1N1) 2009 virus and Down syndrome patients. *Emerging infectious diseases*. 16 (8), 1312–1314. Available from: doi:10.3201/eid1608.091931.
- Pernet, E., Downey, J., Vinh, D.C., Powell, W.S., et al. (2019) Leukotriene B4–type I interferon axis regulates macrophage-mediated disease tolerance to influenza infection. *Nature Microbiology*. Available from: doi:10.1038/s41564-019-0444-3.
- Perrone, L.A., Plowden, J.K., García-Sastre, A., Katz, J.M., et al. (2008) H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. *PLoS Pathogens*. 4 (8). Available from: doi:10.1371/journal.ppat.1000115.
- Peteranderl, C. & Herold, S. (2017) The Impact of the Interferon/TNF-Related Apoptosis-Inducing Ligand Signaling Axis on Disease Progression in Respiratory Viral Infection and Beyond. *Frontiers in Immunology*. 8 (March), 1–18. Available from: doi:10.3389/fimmu.2017.00313.
- Peteranderl, C., Herold, S. & Schmoldt, C. (2016) Human Influenza Virus Infections. *Seminars in respiratory critical care medicine*. 37 (4), 487–500. Available from: doi:http://dx.doi.org/ 10.1055/s-0036-1584801. ISSN 1069-3424.
- Petrulis, J.R. & Perdew, G.H. (2002) The role of chaperone proteins in the aryl hydrocarbon receptor core complex. *Chemico-Biological Interactions*. 141 (1–2), 25–40. Available

- from: doi:10.1016/S0009-2797(02)00064-9.
- Pichlmair, A., Schulz, O., Ping Tan, C., Naslund, T.I., et al. (2006) RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates. *Science*. 314 (5801), 997–1001. Available from: doi:10.1017/CBO9781107415324.004.
- Pickert, G., Neufert, C., Leppkes, M., Zheng, Y., et al. (2009) STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. *Journal of Experimental Medicine*. 206 (7), 1465–1472. Available from: doi:10.1084/jem.20082683.
- Piganis, R.A.R., De Weerd, N.A., Gould, J.A., Schindler, C.W., et al. (2011) Suppressor of Cytokine Signaling (SOCS) 1 inhibits type I interferon (IFN) signaling via the interferon α receptor (IFNAR1)-associated tyrosine kinase tyk2. *Journal of Biological Chemistry*. 286 (39), 33811–33818. Available from: doi:10.1074/jbc.M111.270207.
- Pillai, P.S., Molony, R.D., Martinod, K., Dong, H., et al. (2017) Mx1 reveals innate pathways to antiviral resistance and lethal influenza disease. *Science*. 352 (6284), 463–466. Available from: doi:10.1126/science.aaf3926.Mx1.
- Pittet, L.A., Hall-Stoodley, L., Rutkowski, M.R. & Harmsen, A.G. (2010) Influenza virus infection decreases tracheal mucociliary velocity and clearance of *Streptococcus pneumoniae*. *American Journal of Respiratory Cell and Molecular Biology*. 42 (4), 450–460. Available from: doi:10.1165/rcmb.2007-0417OC.
- Pizzolla, A. & Wakim, L.M. (2019) Memory T Cell Dynamics in the Lung during Influenza Virus Infection. *The Journal of Immunology*. 202 (2), 374–381. Available from: doi:10.4049/jimmunol.1800979.
- Pizzorno, A., Padey, B., Julien, T., Trouillet-Assant, S., et al. (2020) Characterization and Treatment of SARS-CoV-2 in Nasal and Bronchial Human Airway Epithelia. *Cell Reports Medicine*. 1 (4), 100059. Available from: doi:10.1016/j.xcrm.2020.100059.
- Plasschaert, L.W., Žilionis, R., Choo-wing, R., Savova, V., et al. (2018) A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature*. 560 (7718), 377–381. Available from: <https://doi.org/10.1038/s41586-018-0394-6>.
- Platanias, L.C. (2005) Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nature reviews. Immunology*. 5 (5), 375–386. Available from: doi:10.1038/nri1604.
- Pociask, D.A., Scheller, E. V., Mandalapu, S., McHugh, K.J., et al. (2013) IL-22 is essential for lung epithelial repair following influenza infection. *American Journal of Pathology*. 182 (4), 1286–1296. Available from: doi:10.1016/j.ajpath.2012.12.007.
- Poland, A., Glover, E. & Kende, A.S. (1976) Stereospecific, High Affinity Binding of

- 2,3,7,8-Tetrachlorobidibenzo-p-dioxin by Hepatic Cytosol. *The Journal of biological chemistry*. 251 (27), 4936–4946.
- Poland, A., Palen, D. & Glover, E. (1994) Analysis of the four alleles of the murine aryl hydrocarbon receptor. *Molecular pharmacology*. 46 (5), 915–921. Available from: doi:0028-895X/94/050915-07.
- Pongratz, I., Mason, G.G. & Poellinger, L. (1992) Dual roles of the 90-kDa heat shock protein hsp90 in modulating functional activities of the dioxin receptor. Evidence that the dioxin receptor functionally belongs to a subclass of nuclear receptors which require hsp90 both for ligand binding activity a. *The Journal of biological chemistry*. 267 (19), 13728–13734.
- Poovorawan, Y., Pyungporn, S., Prachayangprecha, S. & Makkoch, J. (2013) Global alert to avian influenza virus infection: From H5N1 to H7N9. *Pathogens and Global Health*. 107 (5), 217–223. Available from: doi:10.1179/2047773213Y.00000000103.
- Pothlichet, J., Meunier, I., Davis, B.K., Ting, J.P.Y., et al. (2013) Type I IFN Triggers RIG-I/TLR3/NLRP3-dependent Inflammasome Activation in Influenza A Virus Infected Cells. *PLoS Pathogens*. 9 (4). Available from: doi:10.1371/journal.ppat.1003256.
- Potocnik, A.J., Di Meglio, P., Ahlfors, H., Stockinger, B., et al. (2014) IL-22 Fate Reporter Reveals Origin and Control of IL-22 Production in Homeostasis and Infection. *The Journal of Immunology*. 193 (9), 4602–4613. Available from: doi:10.4049/jimmunol.1401244.
- Price, G.E., Gaszewska-Mastarlarz, a & Moskophidis, D. (2000) The role of alpha/beta and gamma interferons in development of immunity to influenza A virus in mice. *Journal of virology*. 74 (9), 3996–4003. Available from: doi:10.1128/JVI.74.9.3996-4003.2000.
- Prokunina-Olsson, L., Muchmore, B., Tang, W., Pfeiffer, R.M., et al. (2013) A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nature Genetics*. 45 (2), 164–171. Available from: doi:10.1038/ng.2521.
- Prokunina-Olsson, L., Noemie, A., Dickeonson, R.E., Durbin, J.E., et al. (2020) COVID-19 and emerging viral infections : The case for interferon lambda. *Journal of Experimental Medicine*. 217 (5), 5–8.
- Qi, F., Qian, S., Zhang, S. & Zhang, Z. (2020) Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. *Biochemical and Biophysical Research Communications*. 526 (1), 135–140. Available from:

- doi:10.1016/j.bbrc.2020.03.044.
- Qin, Y., Horby, P.W., Tsang, T.K., Chen, E., et al. (2015) Differences in the Epidemiology of Human Cases of Avian Influenza A(H7N9) and A(H5N1) Viruses Infection. *Clinical Infectious Diseases*. 61 (4), 563–571. Available from: doi:10.1093/cid/civ345.
- Qiu, H., Wu, J., Hong, L., Luo, Y., et al. (2020) Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: An observational cohort study. *Lancet Infect Dis*. (20), 1–8. Available from: doi:https://doi.org/10.1016/S1473-3099(20)30198-5. [Published Online First: 2020/03/25].
- Qiu, J., Guo, X., Chen, Z. ming E., He, L., et al. (2013) Group 3 innate lymphoid cells inhibit T-cell-mediated intestinal inflammation through aryl hydrocarbon receptor signaling and regulation of microflora. *Immunity*. 39 (2), 386–399. Available from: doi:10.1016/j.immuni.2013.08.002.
- Qiu, J., Heller, J.J., Guo, X., Chen, Z.M.E., et al. (2012) The Aryl Hydrocarbon Receptor Regulates Gut Immunity through Modulation of Innate Lymphoid Cells. *Immunity*. 36 (1), 92–104. Available from: doi:10.1016/j.immuni.2011.11.011.
- Qiu, Y. & Krug, R.M. (1994) The influenza virus NS1 protein is a poly(A)-binding protein that inhibits nuclear export of mRNAs containing poly(A). *Journal of Virology*. 68 (4), 2425–2432. Available from: doi:10.1128/jvi.68.4.2425-2432.1994.
- Quantius, J., Schmoldt, C., Vazquez-Armendariz, A.I., Becker, C., et al. (2016) Influenza virus infects epithelial stem/progenitor cells of the distal lung: impact on Fgfr2b-driven epithelial repair. *PLoS Pathogens*. 12 (6). Available from: doi:10.1371/journal.ppat.1005544.
- Quarmby, L.M. & Parker, J.D.K. (2005) Cilia and the cell cycle? *Journal of Cell Biology*. 169 (5), 707–710. Available from: doi:10.1083/jcb.200503053.
- Quintana, F.J., Basso, A.S., Iglesias, A.H., Korn, T., et al. (2008) Control of Treg and TH17 cell differentiation by the aryl hydrocarbon receptor. *Nature*. 453 (7191), 65–71. Available from: doi:10.1038/nature06880.
- Rajasekaran, K., Riese, M.J., Rao, S., Wang, L., et al. (2016) Signaling in effector lymphocytes: Insights toward safer immunotherapy. *Frontiers in Immunology*. 7 (MAY), 1–11. Available from: doi:10.3389/fimmu.2016.00176.
- Ramos, I. & Fernandez-Sesma, A. (2015) Modulating the innate immune response to influenza A virus: Potential therapeutic use of anti-inflammatory drugs. *Frontiers in*

- Immunology*. 6 (JUL), 1–19. Available from: doi:10.3389/fimmu.2015.00361.
- Rannug, A., Rannug, U., Rosenkranz, H.S., Winqvist, L., et al. (1987) Certain photooxidized derivatives of tryptophan bind with very high affinity to the Ah receptor and are likely to be endogenous signal substances. *The Journal of biological chemistry*. 262 (32), 15422–15427.
- Raulet, D.H. (2003) Roles of the NKG2D immunoreceptor and its ligands. *Nature Reviews Immunology*. 3 (10), 781–790. Available from: doi:10.1038/nri1199.
- Rawlins, E.L. & Hogan, B.L.M. (2008) Ciliated epithelial cell lifespan in the mouse trachea and lung. *American journal of physiology. Lung cellular and molecular physiology*. 295 (May 2008), L231–L234. Available from: doi:10.1152/ajplung.90209.2008.
- Rawlins, E.L., Okubo, T., Xue, Y., Brass, D.M., et al. (2009) The Role of Scgb1a1+ Clara Cells in the Long-Term Maintenance and Repair of Lung Airway, but Not Alveolar, Epithelium. *Cell Stem Cell*. 4 (6), 525–534. Available from: doi:10.1016/j.stem.2009.04.002.
- Ray, S., Chiba, N., Yao, C., Guan, X., et al. (2016) Rare SOX2+ Airway Progenitor Cells Generate KRT5+ Cells that Repopulate Damaged Alveolar Parenchyma following Influenza Virus Infection. *Stem Cell Reports*. 7 (5), 817–825. Available from: doi:10.1016/j.stemcr.2016.09.010.
- Rehwinkel, J., Tan, C.P., Goubau, D., Schulz, O., et al. (2010) RIG-I Detects Viral Genomic RNA during Negative-Strand RNA Virus Infection. *Cell*. 140 (3), 397–408. Available from: doi:10.1016/j.cell.2010.01.020.
- Reich, N.C. (2013) A Death-Promoting Role for ISG54/IFIT2. *Journal of Interferon & Cytokine Research*. 33 (4), 199–205. Available from: doi:10.1089/jir.2012.0159.
- Reizis, B. (2019) Plasmacytoid Dendritic Cells: Development, Regulation, and Function. *Immunity*. 50 (1), 37–50. Available from: doi:10.1016/j.immuni.2018.12.027.
- Rello, J. & Pop-Vicas, A. (2009) Clinical review: Primary influenza viral pneumonia. *Critical Care*. 13 (6). Available from: doi:10.1186/cc8183.
- Ren, L., Zhang, W., Han, P., Zhang, J., et al. (2019) Influenza A virus (H1N1) triggers a hypoxic response by stabilizing hypoxia-inducible factor-1 α via inhibition of proteasome. *Virology*. 530 (February), 51–58. Available from: doi:10.1016/j.virol.2019.02.010.
- Roager, H.M. & Licht, T.R. (2018) Microbial tryptophan catabolites in health and disease. *Nature Communications*. 9 (1), 1–10. Available from: doi:10.1038/s41467-018-05470-4.

- Rock, J.R., Gao, X., Xue, Y., Randell, S.H., et al. (2011) Notch-dependent differentiation of adult airway basal stem cells. *Cell Stem Cell*. 8 (6), 639–648. Available from: doi:10.1016/j.stem.2011.04.003.
- Rock, J.R., Onaitis, M.W., Rawlins, E.L., Lu, Y., et al. (2009) Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proceedings of the National Academy of Sciences of the United States of America*. 106 (31), 12771–12775. Available from: doi:10.1073/pnas.0906850106.
- Rogers, G.N. & Paulson, J.C. (1983) Receptor determinants of human and animal influenza virus isolates: Differences in receptor specificity of the H3 hemagglutinin based on species of origin. *Virology*. 127 (2), 361–373. Available from: doi:10.1016/0042-6822(83)90150-2.
- Roos, G., Leanderson, T. & Lundgren, E. (1984) Interferon-induced Cell Cycle Changes in Human Hematopoietic Cell Lines and Fresh Leukemic Cells. *Cancer Research*. 44 (6), 2358–2362.
- Rothhammer, V., Borucki, D.M., Tjon, E.C., Takenaka, M.C., et al. (2018) Microglial control of astrocytes in response to microbial metabolites. *Nature*. 557 (7707), 724–728. Available from: doi:10.1038/s41586-018-0119-x.
- Rothhammer, V. & Quintana, F.J. (2019) The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. *Nature Reviews Immunology*. 19, 184–197. Available from: doi:10.1038/s41577-019-0125-8.
- Rutz, S., Eidenschenk, C. & Ouyang, W. (2013) IL-22, not simply a Th17 cytokine. *Immunological Reviews*. 252 (1), 116–132. Available from: doi:10.1111/imr.12027.
- Sadler, A.J. & Williams, B.R.G. (2008) Interferon-inducible antiviral effectors. *Nature Reviews Immunology*. 8 (7), 559–568. Available from: doi:10.1038/nri2314.
- Sakurai, S., Shimizu, T. & Ohto, U. (2017) The crystal structure of the AhRR-ARNT heterodimer reveals the structural basis of the repression of AhR-mediated transcription. *The Journal of biological chemistry*. 292 (43), 17609–17616. Available from: doi:10.1074/jbc.M117.812974.
- Sallard, E., Lescure, F., Yazdanpanah, Y., Mentre, F., et al. (2020) Type 1 interferons as a potential treatment against COVID-19 Erwan. *Antiviral Research*. 178 (104791).
- Salwig, I., Spitznagel, B., Vazquez-Armendariz, A.I., Khalooghi, K., et al. (2019) Bronchioalveolar stem cells are a main source for regeneration of distal lung epithelia in vivo. *The EMBO Journal*. 38 (12), 1–16. Available from:

- doi:10.15252/emj.2019102099.
- Sangfelt, O., Erickson, S., Castro, J., Heiden, T., et al. (1997a) Induction of apoptosis and inhibition of cell growth are independent responses to interferon- α in hematopoietic cell lines. *Cell Growth and Differentiation*. 8 (3), 343–352.
- Sangfelt, O., Erickson, S., Castro, J., Heiden, T., et al. (1999) Molecular mechanisms underlying interferon-alpha-induced G0/G1 arrest: CKI-mediated regulation of G1 Cdk-complexes and activation of pocket proteins. *Oncogene*. 18 (June 1998), 2798–2810. Available from: doi:10.1038/sj.onc.1202609.
- Sangfelt, O., Erickson, S., Einhorn, S. & Grandér, D. (1997b) Induction of Cip/Kip and Ink4 cyclin dependent kinase inhibitors by interferon- α in hematopoietic cell lines. *Oncogene*. 14 (4), 415–423. Available from: doi:10.1038/sj.onc.1200832.
- Schiering, C., Wincent, E., Metidji, A., Iseppon, A., et al. (2017) Feedback control of AHR signalling regulates intestinal immunity. *Nature*. 542 (7640), 242–245. Available from: doi:10.1038/nature21080.
- Schmidt, J. V, Su, G.H., Reddy, J.K., Simon, M.C., et al. (1996) Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. *Proceedings of the National Academy of Sciences of the United States of America*. 93 (June), 6731–6736. Available from: doi:10.1073/pnas.93.13.6731.
- Schneider, C., Nobs, S.P., Heer, A.K., Kurrer, M., et al. (2014) Alveolar Macrophages Are Essential for Protection from Respiratory Failure and Associated Morbidity following Influenza Virus Infection. *PLoS Pathogens*. 10 (4). Available from: doi:10.1371/journal.ppat.1004053.
- Schneider, W.M., Chevillotte, M.D. & Rice, C.M. (2014) Interferon-Stimulated Genes: A Complex Web of Host Defenses. *Annual Review of Immunology*. 32 (1), 513–545. Available from: doi:10.1146/annurev-immunol-032713-120231.
- Schreiber, G. (2017) The molecular basis for differential type i interferon signaling. *Journal of Biological Chemistry*. 292 (18), 7285–7294. Available from: doi:10.1074/jbc.R116.774562.
- Schreiber, G. & Piehler, J. (2015) The molecular basis for functional plasticity in type I interferon signaling. *Trends in Immunology*. 36 (3), 139–149. Available from: doi:10.1016/j.it.2015.01.002.
- Schrenk, D. (1998) Impact of dioxin-type induction of drug-metabolizing enzymes on the metabolism of endo- and xenobiotics. *Biochemical pharmacology*. 55 (8), 1155–1162.

- Available from: doi:10.1016/s0006-2952(97)00591-1.
- Schulz, O., Diebold, S.S., Chen, M., Naslund, T.I., et al. (2005) Toll-like receptor 3 promotes cross- priming to virus-infected cells. *Nature*. 433 (7028), 887–892. Available from: doi:10.1038/nature03292.1.
- Sealy, R., Surman, S., Hurwitz, J.L. & Coleclough, C. (2003) Antibody response to influenza infection of mice: Different patterns for glycoprotein and nucleocapsid antigens. *Immunology*. 108 (4), 431–439. Available from: doi:10.1046/j.1365-2567.2003.01615.x.
- Secombes, C.J. & Zou, J. (2017) Evolution of interferons and interferon receptors. *Frontiers in Immunology*. 8 (209), 2–11. Available from: doi:10.3389/fimmu.2017.00209.
- Seo, S.-U., Kwon, H.-J., Song, J.-H., Byun, Y.-H., et al. (2010) MyD88 Signaling Is Indispensable for Primary Influenza A Virus Infection but Dispensable for Secondary Infection. *Journal of Virology*. 84 (24), 12713–12722. Available from: doi:10.1128/jvi.01675-10.
- Seo, S.H. & Webster, R.G. (2002) Tumor Necrosis Factor Alpha Exerts Powerful Anti-Influenza Virus Effects in Lung Epithelial Cells. *Journal of Virology*. 76 (3), 1071–1076. Available from: doi:10.1128/jvi.76.3.1071-1076.2002.
- Seok, S.-H., Ma, Z.-X., Feltenberger, J.B., Chen, H., et al. (2018) Trace derivatives of kynurenine potentially activate the aryl hydrocarbon receptor (AHR). *The Journal of biological chemistry*. 293 (6), 1994–2005. Available from: doi:10.1074/jbc.RA117.000631.
- Serbina, N. V. & Pamer, E.G. (2006) Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nature Immunology*. 7 (3), 311–317. Available from: doi:10.1038/ni1309.
- Shahangian, A., Chow, E.K., Tian, X., Kang, J.R., et al. (2009) Type I IFNs mediate development of postinfluenza bacterial pneumonia in mice. *The Journal of Clinical Investigation*. 119 (7), 1910–1920. Available from: doi:10.1172/JCI35412.1910.
- Shanks, G. & Brundage, J. (2012) Pathogenic Responses among Young Adults during the 1918 Influenza Pandemic. *Emerging Infectious Diseases*. 18 (2), 201–207. Available from: http://wwwnc.cdc.gov/eid/article/18/2/10-2042_article.
- Sherr, C. & Roberts, J.M. (1995) Inhibitors of mammalian cyclin-dependent kinases. *Genes & development*. 9 (10), 1149–1163.
- Shim, J., Kim, J., Tenson, T., Min, J.-Y., et al. (2017) Influenza Virus Infection, Interferon Response, Viral Counter-Response, and Apoptosis. *Viruses*. 9 (8), 223. Available from:

doi:10.3390/v9080223.

- Shimada, T. & Fujii-Kuriyama, Y. (2004) Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. *Cancer science*. 95 (1), 1–6. Available from: doi:10.1111/j.1349-7006.2004.tb03162.x.
- Shinde, R., Hezaveh, K., Halaby, M.J., Kloetgen, A., et al. (2018) Apoptotic cell-induced AhR activity is required for immunological tolerance and suppression of systemic lupus erythematosus in mice and humans. *Nature Immunology*. Available from: doi:10.1038/s41590-018-0107-1.
- Shinya, K., Ebina, M., Yamada, S., Ono, M., et al. (2006) Influenza virus receptors in the human airway. *Nature*. 440, 435–436.
- Short, K.R., Kasper, J., van der Aa, S., Andeweg, A.C., et al. (2016) Influenza virus damages the alveolar barrier by disrupting epithelial cell tight junctions. *European Respiratory Journal*. 47 (3), 954–966. Available from: doi:10.1183/13993003.01282-2015.
- Siegal, F.P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P.A., et al. (1999) The nature of the principal Type 1 interferon-producing cells in human blood. *Science*. 284 (5421), 1835–1837. Available from: doi:10.1126/science.284.5421.1835.
- Simonsen, L., Clarke, M.J., Schonberger, L.B., Arden, N.H., et al. (1998) Pandemic versus epidemic influenza mortality: A pattern of changing age distribution. *Journal of Infectious Diseases*. 178 (1), 53–60. Available from: doi:10.1086/515616.
- Smith, M.L., Chen, I.T., Zhan, Q., Bae, I., et al. (1994) Interaction of the p53-regulated protein GADD45 with proliferating cell nuclear antigen. *Science*. 266 (5189), 1376–1380. Available from: doi:10.1126/science.7973727.
- Smith, W., Andrewes, C.H. & Laidlaw, P.P. (1933) A virus obtained from influenza patients. *The Lancet*. 222, 66–68.
- Snelgrove, R.J., Goulding, J., Didierlaurent, A.M., Lyonga, D., et al. (2008) A critical function for CD200 in lung immune homeostasis and the severity of influenza infection. *Nature Immunology*. 9 (9), 1074–1083. Available from: doi:10.1038/ni.1637.
- Sommereyns, C., Paul, S., Staeheli, P. & Michiels, T. (2008) IFN- λ is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. *PLoS Pathogens*. 4 (3), 1–12. Available from: doi:10.1371/journal.ppat.1000017.
- Song, J., Clagett-Dame, M., Peterson, R.E., Hahn, M.E., et al. (2002) A ligand for the aryl hydrocarbon receptor isolated from lung. *Proceedings of the National Academy of Sciences of the United States of America*. 99 (23), 14694–14699. Available from:

- doi:10.1073/pnas.232562899.
- Song, M.M. & Shuai, K. (1998) The suppressor of cytokine signaling (SOCS) 1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities. *Journal of Biological Chemistry*. 273 (52), 35056–35062. Available from: doi:10.1074/jbc.273.52.35056.
- Sonnenberg, G.F., Nair, M.G., Kirn, T.J., Zaph, C., et al. (2010) Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A. *Journal of Experimental Medicine*. 207 (6), 1293–1305. Available from: doi:10.1084/jem.20092054.
- Sorg, O. (2014) AhR signalling and dioxin toxicity. *Toxicology Letters*. 230 (2), 225–233. Available from: doi:10.1016/j.toxlet.2013.10.039.
- Soshilov, A.A. & Denison, M.S. (2014) Ligand promiscuity of aryl hydrocarbon receptor agonists and antagonists revealed by site-directed mutagenesis. *Molecular and cellular biology*. 34 (9), 1707–1719. Available from: doi:10.1128/MCB.01183-13.
- Srinivas, S., Watanabe, T., Lin, C.S., William, C.M., et al. (2001) Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC developmental biology*. 1, 4. Available from: doi:10.1186/1471-213x-1-4.
- Staeheli, P., Dreiding, P., Haller, O. & Lindenmann, J. (1985) Polyclonal and monoclonal antibodies to the interferon-inducible protein Mx of influenza virus-resistant mice. *Journal of Biological Chemistry*. 260 (3), 1821–1825.
- Staeheli, P., Grob, R., Meier, E., Sutcliffe, J.G., et al. (1988) Influenza virus-susceptible mice carry Mx genes with a large deletion or a nonsense mutation. *Molecular and Cellular Biology*. 8 (10), 4518–4523. Available from: doi:10.1128/mcb.8.10.4518.
- Staeheli, P., Haller, O., Boll, W., Lindenmann, J., et al. (1986) Mx protein: Constitutive expression in 3T3 cells transformed with cloned Mx cDNA confers selective resistance to influenza virus. *Cell*. 44 (1), 147–158. Available from: doi:10.1016/0092-8674(86)90493-9.
- Stawowczyk, M., Van Scoy, S., Kumar, K.P. & Reich, N.C. (2011) The interferon stimulated gene 54 promotes apoptosis. *Journal of Biological Chemistry*. 286 (9), 7257–7266. Available from: doi:10.1074/jbc.M110.207068.
- Steed, A.L., Christophi, G.P., Kaiko, G.E., Sun, L., et al. (2017) The microbial metabolite desaminotyrosine protects from influenza through type I interferon. *Science*. 357 (6350), 498–502. Available from: doi:10.1126/science.aam5336.

- Steele, E.J., Bellett, A.J., McCullagh, P.J. & Selinger, B. (1990) Reappraisal of the findings on Agent Orange by the Australian Royal Commission. *Toxicology letters*. 51 (3), 261–268. Available from: doi:10.1016/0378-4274(90)90068-w.
- Stockinger, B., Di Meglio, P., Gialitakis, M. & Duarte, J.H. (2014) The aryl hydrocarbon receptor: multitasking in the immune system. *Annu Rev Immunol*. 32, 403–432. Available from: doi:10.1146/annurev-immunol-032713-120245.
- Subramaniam, P.S., Cruz, P.E., Hobeika, A.C. & Johnson, H.M. (1998) Type I interferon induction of the Cdk-inhibitor p21 is accompanied by ordered G1 arrest, differentiation and apoptosis of the Daudi B-cell line. *Oncogene*. 16 (14), 1885–1890.
- Subramaniam, P.S. & Johnson, H.M. (1997) A role for the cyclin-dependent kinase inhibitor p21 in the G1 cell cycle arrest mediated by the type I interferons. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research*. 17 (1), 11–15. Available from: doi:10.1089/jir.1997.17.11.
- Sugden, W.W., Leonardo-Mendonça, R.C., Acuña-Castroviejo, D. & Siekmann, A.F. (2017) Genetic dissection of endothelial transcriptional activity of zebrafish aryl hydrocarbon receptors (AHRs). *PLoS ONE*. 12 (8), 1–22. Available from: doi:10.1371/journal.pone.0183433.
- Sullivan, K.D., Lewis, H.C., Hill, A.A., Pandey, A., et al. (2016) Trisomy 21 consistently activates the interferon response. *eLife*. 5 (JULY). Available from: doi:10.7554/eLife.16220.
- Sun, J.-L., Zeng, H. & Ni, H.-G. (2013) Halogenated polycyclic aromatic hydrocarbons in the environment. *Chemosphere*. 90 (6), 1751–1759. Available from: doi:10.1016/j.chemosphere.2012.10.094.
- Swartzendruber, D.E. & Lehman, J.M. (1975) Neoplastic differentiation: Interaction of simian virus 40 and polyoma virus with murine teratocarcinoma cells in vitro. *Journal of Cellular Physiology*. 85 (2), 179–187. Available from: doi:10.1002/jcp.1040850204.
- Szabo, S.J., Kim, S.T., Costa, G.L., Zhang, X., et al. (2000) A novel transcription factor, Tbet, directs Th1 lineage commitment. *Cell*. 100 (6), 655–669. Available from: doi:10.1016/S0092-8674(00)80702-3.
- Takaoka, A., Hayakawa, S., Yanai, H., Stoiber, D., et al. (2003) Integration of interferon- α/β signalling to p53 responses in tumour suppression and antiviral defence. *Nature*. 424 (6948), 516–523. Available from: doi:10.1038/nature01850.
- Tanaka, N., Ishihara, M., Kitagawa, M., Harada, H., et al. (1994) Cellular commitment to

- oncogene-induced transformation or apoptosis is dependent on the transcription factor IRF-1. *Cell*. 77 (6), 829–839. Available from: doi:10.1016/0092-8674(94)90132-5.
- Tanaka, N., Ishihara, M., Lamphier, M.S., Nozawa, H., et al. (1996) Cooperation of the tumour suppressors IRF-1 and p53 in response to DNA damage. *Nature*. 382 (6594), 816–818. Available from: doi:10.1038/382816a0.
- Tang, B.M., Shojaei, M., Teoh, S., Meyers, A., et al. (2019) Neutrophils-related host factors associated with severe disease and fatality in patients with influenza infection. *Nature Communications*. 10 (1), 1–13. Available from: doi:10.1038/s41467-019-11249-y.
- Tata, P.R., Mou, H., Pardo-Saganta, A., Zhao, R., et al. (2013) Dedifferentiation of committed epithelial cells into stem cells in vivo. *Nature*. 503 (7475), 218–223. Available from: doi:10.1038/nature12777.
- Tate, M.D., Ioannidis, L.J., Croker, B., Brown, L.E., et al. (2011) The role of neutrophils during mild and severe influenza virus infections of mice. *PLoS ONE*. 6 (3), 2–11. Available from: doi:10.1371/journal.pone.0017618.
- Tate, M.D., Pickett, D.L., van Rooijen, N., Brooks, A.G., et al. (2010) Critical Role of Airway Macrophages in Modulating Disease Severity during Influenza Virus Infection of Mice. *Journal of Virology*. 84 (15), 7569–7580. Available from: doi:10.1128/jvi.00291-10.
- Taubenberger, J.K. (2006) The origin and virulence of the 1918 ‘Spanish’ influenza virus. *Proceedings of the American Philosophical Society*. 150 (1), 86–112.
- Taubenberger, J.K. & Morens, D.M. (2006) 1918 Influenza: the Mother of All Pandemics. *Emerging Infectious Diseases*. 12 (1), 15–22. Available from: doi:10.3201/eid1209.050979.
- Taylor, M.S., Chivukula, R.R., Myers, L.C., Jeck, W.R., et al. (2018a) A Conserved Distal Lung Regenerative Pathway in Acute Lung Injury. *American Journal of Pathology*. 188 (5), 1149–1160. Available from: doi:10.1016/j.ajpath.2018.01.021.
- Taylor, M.S., Chivukula, R.R., Myers, L.C., Jeck, W.R., et al. (2018b) Delayed alveolar epithelialization: A distinct pathology in diffuse acute lung injury. *American Journal of Respiratory and Critical Care Medicine*. 197 (4), 522–524. Available from: doi:10.1164/rccm.201706-1094LE.
- Teijaro, J.R., Ng, C., Lee, A.M., Sullivan, B.M., et al. (2013) Persistent LCMV Infection Is Controlled by Blockade of Type I Interferon Signaling. *Science*. 340 (6129), 207–211. Available from: doi:10.1126/science.1235214.

- Teijaro, J.R., Walsh, K.B., Cahalan, S., Fremgen, D.M., et al. (2011) Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. *Cell*. 146 (6), 980–991. Available from: doi:10.1016/j.cell.2011.08.015.
- Terczyńska-Dyla, E., Bibert, S., Duong, F.H.T., Krol, I., et al. (2014) Reduced IFN λ 4 activity is associated with improved HCV clearance and reduced expression of interferon-stimulated genes. *Nature Communications*. 5. Available from: doi:10.1038/ncomms6699.
- Teske, S., Bohn, A. a, Regal, J.F., Neumiller, J.J., et al. (2005) Activation of the aryl hydrocarbon receptor increases pulmonary neutrophilia and diminishes host resistance to influenza A virus. *American journal of physiology. Lung cellular and molecular physiology*. 289 (1), L111–L124. Available from: doi:10.1152/ajplung.00318.2004.
- Thatcher, T.H., Williams, M.A., Pollock, S.J., McCarthy, C.E., et al. (2016) Endogenous ligands of the aryl hydrocarbon receptor regulate lung dendritic cell function. *Immunology*. 147 (1), 41–54. Available from: doi:10.1111/imm.12540.
- Thomas, C., Moraga, I., Levin, D., Krutzik, P.O., et al. (2011) Structural linkage between ligand discrimination and receptor activation by Type I interferons. *Cell*. 146 (4), 621–632. Available from: doi:10.1016/j.cell.2011.06.048.
- Thomas, P.G., Dash, P., Aldridge, J.R., Ellebedy, A.H., et al. (2009) The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. *Immunity*. 30 (4), 566–575. Available from: doi:10.1016/j.immuni.2009.02.006 [Accessed: 1 October 2014].
- Thyrell, L., Erickson, S., Zhivotovsky, B., Pokrovskaja, K., et al. (2002) Mechanisms of interferon-alpha induced apoptosis in malignant cells. *Oncogene*. 21 (8), 1251–1262. Available from: doi:10.1038/sj/onc/1205179.
- Tian, S., Xiong, Y., Liu, H., Niu, L., et al. (2020) Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. *Modern Pathology*. 10–12. Available from: doi:10.20944/preprints202003.0311.v1.
- Tijet, N., Boutros, P.C., Moffat, I.D., Okey, A.B., et al. (2006) Aryl hydrocarbon receptor regulates distinct dioxin-dependent and dioxin-independent gene batteries. *Molecular Pharmacology*. 69 (1), 140–153. Available from: doi:10.1124/mol.105.018705.
- Tisoncik, J.R., Korth, M.J., Simmons, C.P., Farrar, J., et al. (2012) Into the Eye of the Cytokine Storm. *Microbiology and Molecular Biology Reviews*. 76 (1), 16–32. Available from: doi:10.1128/mmbr.05015-11.

- To, K.K.W., Hung, I.F.N., Li, I.W.S., Lee, K., et al. (2010) Delayed Clearance of Viral Load and Marked Cytokine Activation in Severe Cases of Pandemic H1N1 2009 Influenza Virus Infection. *Clinical Infectious Diseases*. 50 (6), 850–859. Available from: doi:10.1086/650581.
- To, K.K.W., Tsang, O.T.Y., Leung, W.S., Tam, A.R., et al. (2020) Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *The Lancet Infectious Diseases*. 20 (5), 565–574. Available from: doi:10.1016/S1473-3099(20)30196-1.
- To, K.K.W., Zhou, J., Chan, J.F.W. & Yuen, K.Y. (2015) Host genes and influenza pathogenesis in humans: An emerging paradigm. *Current Opinion in Virology*. 14, 7–15. Available from: doi:10.1016/j.coviro.2015.04.010.
- Topham, D.J. & Doherty, P.C. (1998) Clearance of an Influenza A Virus by CD4+ T Cells Is Inefficient in the Absence of B Cells. *Journal of Virology*. 72 (1), 882–885. Available from: doi:10.1128/jvi.72.1.882-885.1998.
- Topham, D.J., Tripp, R.A. & Doherty, P.C. (1997) CD8+ T cells clear influenza virus by perforin or Fas-dependent processes. *Journal of immunology (Baltimore, Md. : 1950)*. 159 (11), 5197–5200. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9548456>.
- Trinchieri, G. (2010) Type I interferon: friend or foe? *Journal of Experimental Medicine*. 207 (10), 2053–2063. Available from: doi:10.1084/jem.20101664.
- Trompette, A., Gollwitzer, E.S., Pattaroni, C., Lopez-Mejia, I.C., et al. (2018) Dietary Fiber Confers Protection against Flu by Shaping Ly6c–Patrolling Monocyte Hematopoiesis and CD8+T Cell Metabolism. *Immunity*. 48 (5), 992-1005.e8. Available from: doi:10.1016/j.immuni.2018.04.022.
- Trompette, A., Gollwitzer, E.S., Yadava, K., Sichelstiel, A.K., et al. (2014) Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature Medicine*. 20 (2), 159–166. Available from: doi:10.1038/nm.3444.
- Tsuno, T., Mejido, J., Zhao, T., Schmeisser, H., et al. (2009) IRF9 is a key factor for eliciting the antiproliferative activity of IFN- α . *Journal of Immunotherapy*. 32 (8), 803–816. Available from: doi:10.1097/CJI.0b013e3181ad4092.
- Tumpey, T.M., García-Sastre, A., Taubenberger, J.K., Palese, P., et al. (2005) Pathogenicity of Influenza Viruses with Genes from the 1918 Pandemic Virus: Functional Roles of Alveolar Macrophages and Neutrophils in Limiting Virus Replication and Mortality in Mice. *Journal of Virology*. 79 (23), 14933–14944. Available from:

- doi:10.1128/jvi.79.23.14933-14944.2005.
- Tumpey, T.M., Szretter, K.J., Van Hoeven, N., Katz, J.M., et al. (2007) The Mx1 Gene Protects Mice against the Pandemic 1918 and Highly Lethal Human H5N1 Influenza Viruses. *Journal of Virology*. 81 (19), 10818–10821. Available from: doi:10.1128/jvi.01116-07.
- Ueda, K., Arakawa, H. & Nakamura, Y. (2003) Dual-specificity phosphatase 5 (DUSP5) as a direct transcriptional target of tumor suppressor p53. *Oncogene*. 22 (36), 5586–5591. Available from: doi:10.1038/sj.onc.1206845.
- Unkel, B., Hoegner, K., Clausen, B.E., Lewe-Schlosser, P., et al. (2012) Alveolar epithelial cells orchestrate DC function in murine viral pneumonia. *Journal of Clinical Investigation*. 122 (10), 3652–3664. Available from: doi:10.1172/JCI62139.
- Uyeki, T.M. & Cox, N.J. (2013) Global Concerns Regarding Novel Influenza A (H7N9) Virus Infections. *New England Journal of Medicine*. 368 (18), 1862–1864.
- Vabret, N., Britton, G.J., Gruber, C., Hegde, S., et al. (2020) Immunology of COVID-19: current state of the science. *Immunity*. 52 (1), 1–16. Available from: doi:10.1016/j.immuni.2020.05.002.
- Valiante NM, Uhrberg M, Shilling HG, Lienert-Weidenbach K, et al. (1997) Functionally and structurally distinct NK cell receptor repertoires in the peripheral blood of two human donors. *Immunity*. 7 (6), 739–751. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9430220.
- Vareille, M., Kieninger, E., Edwards, M.R. & Regamey, N. (2011) The airway epithelium: Soldier in the fight against respiratory viruses. *Clinical Microbiology Reviews*. 24 (1), 210–229. Available from: doi:10.1128/CMR.00014-10.
- Vaughan, A.E., Brumwell, A.N., Xi, Y., Gotts, J.E., et al. (2015) Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature*. 521 (7536), 621–625. Available from: doi:10.1038/nature14112.
- Veldhoen, M. (2010) A toxin-sensitive receptor able to reduce immunopathology. *Nature Immunology*. 11 (9), 779–781. Available from: doi:10.1038/ni0910-779.
- Veldhoen, M., Hirota, K., Westendorf, A.M., Buer, J., et al. (2008) The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature*. 453 (7191), 106–109. Available from: doi:10.1038/nature06881.
- Verhelst, J., Parthoens, E., Schepens, B., Fiers, W., et al. (2012) Interferon-Inducible Protein

- Mx1 Inhibits Influenza Virus by Interfering with Functional Viral Ribonucleoprotein Complex Assembly. *Journal of Virology*. 86 (24), 13445–13455. Available from: doi:10.1128/jvi.01682-12.
- Vernatter, J. & Pirofski, L.A. (2013) Current concepts in host-microbe interaction leading to pneumococcal pneumonia. *Current Opinion in Infectious Diseases*. 26 (3), 277–283. Available from: doi:10.1097/QCO.0b013e3283608419.
- Villa, M., Crotta, S., Dingwell, K.S., Hirst, E.M.A., et al. (2016) The aryl hydrocarbon receptor controls cyclin O to promote epithelial multiciliogenesis. *Nature Communications*. 7, 1–11. Available from: doi:10.1038/ncomms12652.
- Vlahos, R., Stambas, J., Bozinovski, S., Broughton, B.R.S., et al. (2011) Inhibition of Nox2 oxidase activity ameliorates influenza a virus-induced lung inflammation. *PLoS Pathogens*. 7 (2). Available from: doi:10.1371/journal.ppat.1001271.
- Vogel, C.F.A., Goth, S.R., Dong, B., Pessah, I.N., et al. (2008) Aryl hydrocarbon receptor signaling mediates expression of indoleamine 2,3-dioxygenase. *Biochemical and biophysical research communications*. 375 (3), 331–335. Available from: doi:10.1016/j.bbrc.2008.07.156.
- Vogel, C.F.A., Khan, E.M., Leung, P.S.C., Gershwin, M.E., et al. (2014) Cross-talk between aryl hydrocarbon receptor and the inflammatory response: a role for nuclear factor- κ B. *The Journal of biological chemistry*. 289 (3), 1866–1875. Available from: doi:10.1074/jbc.M113.505578.
- Vogel, C.F.A., Sciallo, E., Li, W., Wong, P., et al. (2007) RelB, a new partner of aryl hydrocarbon receptor-mediated transcription. *Molecular endocrinology (Baltimore, Md.)*. 21 (12), 2941–2955. Available from: doi:10.1210/me.2007-0211.
- Volckaert, T., Dill, E., Campbell, A., Tiozzo, C., et al. (2011) Parabronchial smooth muscle constitutes an airway epithelial stem cell niche in the mouse lung after injury. *Journal of Clinical Investigation*. 121 (11), 4409–4419. Available from: doi:10.1172/JCI58097.
- Vorderstrasse, B.A., Bohn, A.A. & Lawrence, B.P. (2003) Examining the relationship between impaired host resistance and altered immune function in mice treated with TCDD. *Toxicology*. 188 (1), 15–28. Available from: doi:10.1016/S0300-483X(02)00749-7.
- Vorriink, S.U. & Domann, F.E. (2014) Regulatory crosstalk and interference between the and hypoxia sensing pathways at the AhR-ARNT-HIF1 α signaling node. *Chemico-Biological Interactions*. 218, 82–88. Available from: doi:10.1016/j.cbi.2014.05.001.

- Vousden, K.H. & Lane, D.P. (2007) P53 in health and disease. *Nature Reviews Molecular Cell Biology*. 8 (4), 275–283. Available from: doi:10.1038/nrm2147.
- Voynow, J.A. & Mengr, B.K.R. (2009) Mucins, mucus, and sputum. *Chest*. 135 (2), 505–512. Available from: doi:10.1378/chest.08-0412.
- Wack, A., Terczyńska-Dyla, E. & Hartmann, R. (2015) Guarding the frontiers: the biology of type III interferons. *Nature Immunology*. 16 (8), 802–809. Available from: doi:10.1038/ni.3212.
- Wan, Y., Shang, J., Graham, R., Baric, R.S., et al. (2020) Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *Journal of Virology*. 94 (7), 1–9. Available from: doi:10.1128/jvi.00127-20.
- Wang, C., Horby, P.W., Hayden, F.G. & Gao, G.F. (2020a) A novel coronavirus outbreak of global health concern. *The Lancet*. 395 (10223), 470–473. Available from: doi:10.1016/S0140-6736(20)30185-9.
- Wang, J., Nikrad, M.P., Travanty, E.A., Zhou, B., et al. (2012) Innate immune response of human alveolar macrophages during influenza a infection. *PLoS ONE*. 7 (3). Available from: doi:10.1371/journal.pone.0029879.
- Wang, N., Zhan, Y., Zhu, L., Hou, Z., et al. (2020b) Retrospective Multicenter Cohort Study Shows Early Interferon Therapy Is Associated with Favorable Clinical Responses in COVID-19 Patients Clinical and Translational Report Retrospective Multicenter Cohort Study Shows Ear. *Cell Host and Microbe*. 28, 1–10. Available from: doi:10.1016/j.chom.2020.07.005.
- Wang, X., Jiang, H., Wu, P., Uyeki, T.M., et al. (2017) Epidemiology of avian influenza A H7N9 virus in human beings across five epidemics in mainland China, 2013–17: an epidemiological study of laboratory-confirmed case series. *The Lancet Infectious Diseases*. 17 (8), 822–832. Available from: doi:10.1016/S1473-3099(17)30323-7.
- Ward, C.L., Dempsey, M.H., Ring, C.J.A., Kempson, R.E., et al. (2004) Design and performance testing of quantitative real time PCR assays for influenza A and B viral load measurement. *Journal of Clinical Virology*. 29 (3), 179–188. Available from: doi:10.1016/S1386-6532(03)00122-7.
- Wareing, M.D., Shea, A.L., Inglis, C.A., Dias, P.B., et al. (2007) CXCR2 is required for neutrophil recruitment to the lung during influenza virus infection, but is not essential for viral clearance. *Viral Immunology*. 20 (3), 369–377. Available from:

- doi:10.1089/vim.2006.0101.
- Webster, R.G., Bean, W.J., Gorman, O.T., Chambers, T.M., et al. (1992) Evolution and ecology of influenza A viruses. *Microbiological reviews*. 56 (1), 152–179. Available from: doi:10.1007/82_2014_396.
- De Weerd, N.A., Vivian, J.P., Nguyen, T.K., Mangan, N.E., et al. (2013) Structural basis of a unique interferon- β signaling axis mediated via the receptor IFNAR1. *Nature Immunology*. 14 (9), 901–907. Available from: doi:10.1038/ni.2667.
- Wells, M.A., Albrecht, P., Ennis, F.A., Wells, M.A., et al. (1981) Recovery from a viral respiratory infection I. Influenza pneumonia in normal and T-deficient mice. *The Journal of Immunology*. 126 (3).
- Westphalen, K., Gusarova, G.A., Islam, M.N., Subramanian, M., et al. (2014) Sessile alveolar macrophages communicate with alveolar epithelium to modulate immunity. *Nature*. 506 (7489), 503–506. Available from: doi:10.1038/nature12902.
- Wheeler, J.L., Martin, K.C. & Lawrence, B.P. (2013) Novel cellular targets of AhR underlie alterations in neutrophilic inflammation and inducible nitric oxide synthase expression during influenza virus infection. *J Immunol*. 190, 659–668. Available from: doi:10.4049/jimmunol.1201341.
- Wheeler, J.L.H., Martin, K.C., Resseguie, E. & Paige Lawrence, B. (2014) Differential consequences of two distinct AhR ligands on innate and adaptive immune responses to influenza A virus. *Toxicological Sciences*. 137 (2), 324–334. Available from: doi:10.1093/toxsci/kft255.
- Whitmire, J.K., Tan, J.T. & Whitton, J.L. (2005) Interferon- γ acts directly on CD8⁺ T cells to increase their abundance during virus infection. *Journal of Experimental Medicine*. 201 (7), 1053–1059. Available from: doi:10.1084/jem.20041463.
- Whitsett, J.A. & Alenghat, T. (2015) Respiratory epithelial cells orchestrate pulmonary innate immunity. *Nature immunology*. 16 (1), 27–35. Available from: doi:10.1038/ni.3045.
- Wikoff, W.R., Anfora, A.T., Liu, J., Schultz, P.G., et al. (2009) Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proceedings of the National Academy of Sciences of the United States of America*. 106 (10), 3698–3703. Available from: doi:10.1073/pnas.0812874106.
- Wilk, A.J., Rustagi, A., Zhao, N.Q., Roque, J., et al. (2020) A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nature Medicine*. 26

- (7), 1070–1076. Available from: doi:10.1038/s41591-020-0944-y.
- Wilson, E.B., Yamada, D.H., Elsaesser, H., Herskovitz, J., et al. (2013) Blockade of chronic type I interferon signaling to control persistent LCMV infection. *Science*. 340 (6129), 202–207. Available from: doi:10.1126/science.1235208.
- de Wit, E. & Fouchier, R.A.M. (2008) Emerging influenza. *Journal of Clinical Virology*. 41 (1), 1–6. Available from: doi:10.1016/j.jcv.2007.10.017.
- Wolf, A.I., Buehler, D., Hensley, S.E., Cavanagh, L.L., et al. (2009) Plasmacytoid Dendritic Cells Are Dispensable during Primary Influenza Virus Infection. *The Journal of Immunology*. 182 (2), 871–879. Available from: doi:10.4049/jimmunol.182.2.871.
- World Health Organization (WHO) (2020) *Avian Influenza Weekly Update - Human infection with avian influenza A(H5) viruses*. Available from: <https://iris.wpro.who.int/bitstream/handle/10665.1/14460/AI-20200103.pdf>.
- World Health Organization (WHO) (2016) *WHO Manual for estimating the economic burden of seasonal influenza*. Available from: doi:WHO/IVB/16.04.
- World Health Organization (WHO) (2018) *World health statistics 2018*. Available from: doi:10.1051/mateconf/201712107005.
- Worobey, M., Han, G.Z. & Rambaut, A. (2014) Genesis and pathogenesis of the 1918 pandemic H1N1 influenza A virus. *Proceedings of the National Academy of Sciences of the United States of America*. 111 (22), 8107–8112. Available from: doi:10.1073/pnas.1324197111.
- Wu, H., Haist, V., Baumgärtner, W. & Schughart, K. (2010) Sustained viral load and late death in Rag2^{-/-} mice after influenza A virus infection. *Virology Journal*. 7, 6–11. Available from: doi:10.1186/1743-422X-7-172.
- Wu, R., Zhao, Y.H. & Chang, M.M. (1997) Growth and differentiation of conducting airway epithelial cells in culture. *The European respiratory journal*. 10 (10), 2398–2403. Available from: doi:10.1183/09031936.97.10102398.
- Wu, X., Dao Thi, V.L., Huang, Y., Billerbeck, E., et al. (2018) Intrinsic Immunity Shapes Viral Resistance of Stem Cells. *Cell*. 172 (3), 423–438.e25. Available from: doi:10.1016/j.cell.2017.11.018.
- Wu, X., Kwong, A.C. & Rice, C.M. (2019) Antiviral resistance of stem cells. *Current Opinion in Immunology*. 56, 50–59. Available from: doi:10.1016/j.coi.2018.10.004.
- Wu, Z. & McGoogan, J.M. (2020) Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of

- 72314 Cases from the Chinese Center for Disease Control and Prevention. *JAMA - Journal of the American Medical Association*. 323 (13), 1239–1242. Available from: doi:10.1001/jama.2020.2648.
- Xi, Y., Kim, T., Brumwell, A.N., Driver, I.H., et al. (2017) Local lung hypoxia determines epithelial fate decisions during alveolar regeneration. *Nature Cell Biology*. (June). Available from: doi:10.1038/ncb3580.
- Xu, X., Weiss, I.D., H. Zhang, H., Singh, S.P., et al. (2014) Conventional NK Cells Can Produce IL-22 and Promote Host Defense in *Klebsiella pneumoniae* Pneumonia. *The Journal of Immunology*. 192 (4), 1778–1786. Available from: doi:10.4049/jimmunol.1300039.
- Xu, Z., Shi, L., Wang, Y., Zhang, J., et al. (2020) Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet Respiratory Medicine*. 2600 (20), 19–21. Available from: doi:10.1016/S2213-2600(20)30076-X.
- Yamada, T., Horimoto, H., Kameyama, T., Hayakawa, S., et al. (2016) Constitutive aryl hydrocarbon receptor signaling constrains type I interferon–mediated antiviral innate defense. *Nature Immunology*. 17 (6), 687–694. Available from: doi:10.1038/ni.3422.
- Yang, B.X., El Farran, C.A., Guo, H.C., Yu, T., et al. (2015) Systematic Identification of Factors for Provirus Silencing in Embryonic Stem Cells. *Cell*. 163 (1), 230–245. Available from: doi:10.1016/j.cell.2015.08.037.
- Yang, Y., Riccio, P., Schotsaert, M., Mori, M., et al. (2018) Spatial-Temporal Lineage Restrictions of Embryonic p63+Progenitors Establish Distinct Stem Cell Pools in Adult Airways. *Developmental Cell*. 752–761. Available from: doi:10.1016/j.devcel.2018.03.001.
- Yao, E.F. & Denison, M.S. (1992) DNA sequence determinants for binding of transformed Ah receptor to a dioxin-responsive enhancer. *Biochemistry*. 31 (21), 5060–5067. Available from: doi:10.1021/bi00136a019.
- Ye, L., Schnepf, D., Becker, J., Ebert, K., et al. (2019) Interferon- λ enhances adaptive mucosal immunity by boosting release of thymic stromal lymphopoietin. *Nature Immunology*. 20 (5), 593–601. Available from: doi:10.1038/s41590-019-0345-x.
- Yeste, A., Mascanfroni, I.D., Nadeau, M., Burns, E.J., et al. (2014) IL-21 induces IL-22 production in CD4+ T cells. *Nature Communications*. 5 (May). Available from: doi:10.1038/ncomms4753.
- Yin, J., Sheng, B., Qiu, Y., Yang, K., et al. (2016) Role of AhR in positive regulation of cell

- proliferation and survival. *Cell Proliferation*. 49 (5), 554–560. Available from: doi:10.1111/cpr.12282.
- Yin, Z., Dai, J., Deng, J., Sheikh, F., et al. (2012) Type III IFNs Are Produced by and Stimulate Human Plasmacytoid Dendritic Cells. *The Journal of Immunology*. 189 (6), 2735–2745. Available from: doi:10.4049/jimmunol.1102038.
- You, Y., Richer, E.J., Huang, T. & Brody, S.L. (2002) Growth and differentiation of mouse tracheal epithelial cells: selection of a proliferative population. *American journal of physiology Lung cellular and molecular physiology*. 283 (4), L1315–L1321. Available from: doi:10.1080/08858198909528020.
- Yu, Q., Katlinskaya, Y. V., Carbone, C.J., Zhao, B., et al. (2015) DNA-Damage-Induced Type I Interferon Promotes Senescence and Inhibits Stem Cell Function. *Cell Reports*. 11 (5), 785–797. Available from: doi:10.1016/j.celrep.2015.03.069.
- Zacharias, W.J., Frank, D.B., Zepp, J.A., Morley, M.P., et al. (2018) Regeneration of the lung alveolus by an evolutionarily conserved epithelial progenitor. *Nature*. 555 (7695), 251–255. Available from: doi:10.1038/nature25786.
- Zelante, T., Iannitti, R.G., Cunha, C., DeLuca, A., et al. (2013) Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity*. 39 (2), 372–385. Available from: doi:10.1016/j.immuni.2013.08.003.
- Zemans, R.L., Colgan, S.P. & Downey, G.P. (2009) Transepithelial migration of neutrophils: Mechanisms and implications for acute lung injury. *American Journal of Respiratory Cell and Molecular Biology*. 40 (5), 519–535. Available from: doi:10.1165/rcmb.2008-0348TR.
- Zenewicz, L.A., Yancopoulos, G.D., Valenzuela, D.M., Murphy, A.J., et al. (2008) Innate and Adaptive Interleukin-22 Protects Mice from Inflammatory Bowel Disease. *Immunity*. 29 (6), 947–957. Available from: doi:10.1016/j.immuni.2008.11.003.
- Zepp, J.A. & Morrissey, E.E. (2019) Cellular crosstalk in the development and regeneration of the respiratory system. *Nature Reviews Molecular Cell Biology*. Available from: doi:10.1038/s41580-019-0141-3.
- Zhai, Y., Franco, L.M., Atmar, R.L., Quarles, J.M., et al. (2015) Host transcriptional response to influenza and other acute respiratory viral infections – a prospective cohort study. *PLoS Pathogens*. 11 (6), 1–29. Available from: doi:10.1371/journal.ppat.1004869.
- Zhan, Q., Antinore, M.J., Wang, X.W., Carrier, F., et al. (1999) Association with Cdc2 and

- inhibition of Cdc2/Cyclin B1 kinase activity by the p53-regulated protein Gadd45. *Oncogene*. 18 (18), 2892–2900. Available from: doi:10.1038/sj.onc.1202667.
- Zhang, L.H., Shin, J.H., Haggadone, M.D. & Sunwoo, J.B. (2016) The aryl hydrocarbon receptor is required for the maintenance of liver-resident natural killer cells. *The Journal of Experimental Medicine*. 213 (11), 2249–2257. Available from: doi:10.1084/jem.20151998.
- Zhang, T., Yin, C., Boyd, D.F., Quarato, G., et al. (2020) Influenza Virus Z-RNAs Induce ZBP1-Mediated Necroptosis. *Cell*. 180 (6), 1115–1129.e13. Available from: doi:10.1016/j.cell.2020.02.050.
- Zhang, X., Bogunovic, D., Payelle-Brogard, B., Francois-Newton, V., et al. (2015) Human intracellular ISG15 prevents interferon- α/β over-amplification and auto-inflammation. *Nature*. 517 (7532), 89–93. Available from: doi:10.1038/nature13801.
- Zhao, B., Degroot, D.E., Hayashi, A., He, G., et al. (2010) CH223191 is a ligand-selective antagonist of the Ah (Dioxin) receptor. *Toxicological sciences : an official journal of the Society of Toxicology*. 117 (2), 393–403. Available from: doi:10.1093/toxsci/kfq217.
- Zhao, C. & Zhao, W. (2020) NLRP3 Inflammasome—A Key Player in Antiviral Responses. *Frontiers in Immunology*. 11 (February), 1–8. Available from: doi:10.3389/fimmu.2020.00211.
- Zheng, S., Fan, J., Yu, F., Feng, B., et al. (2020) Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–March 2020: Retrospective cohort study. *The BMJ*. 369 (March), 1–8. Available from: doi:10.1136/bmj.m1443.
- Zheng, Y., Danilenko, D.M., Valdez, P., Kasman, I., et al. (2007) Interleukin-22, a TH17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature*. 445 (7128), 648–651. Available from: doi:10.1038/nature05505.
- Zhou, G., Juang, S.W.W. & Kane, K.P. (2013) NK cells exacerbate the pathology of influenza virus infection in mice. *European Journal of Immunology*. 43 (4), 929–938. Available from: doi:10.1002/eji.201242620.
- Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., et al. (2020a) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. Available from: doi:10.1038/s41586-020-2012-7.
- Zhou, Z., Hamming, O.J., Ank, N., Paludan, S.R., et al. (2007) Type III Interferon (IFN) Induces a Type I IFN-Like Response in a Restricted Subset of Cells through Signaling

- Pathways Involving both the Jak-STAT Pathway and the Mitogen-Activated Protein Kinases. *Journal of Virology*. 81 (14), 7749–7758. Available from: doi:10.1128/jvi.02438-06.
- Zhou, Z., Ren, L., Zhang, L., Zhong, J., et al. (2020b) Heightened innate immune responses in the respiratory tract of COVID-19 patients. *Cell Host & Microbe*. 27, 1–8. Available from: doi:10.1016/j.chom.2020.04.017.
- Zhu, N., Zhang, D., Wang, W., Li, X., et al. (2020) A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine*. 382 (8), 727–733. Available from: doi:10.1056/NEJMoa2001017.
- Zitvogel, L., Galluzzi, L., Kepp, O., Smyth, M.J., et al. (2015) Type I interferons in anticancer immunity. *Nat Rev Immunol*. 15 (7), 405–414. Available from: doi:10.1038/nri3845.
- Zuo, W., Zhang, T., Wu, D.Z., Guan, S.P., et al. (2014) p63(+)Krt5(+) distal airway stem cells are essential for lung regeneration. *Nature*. 517 (7536), 616–620. Available from: doi:10.1038/nature13903.