Investigating the role of intestinal IFN-γ expression on barrier immunity during enteric helminth infection

Olivia Jessica Ridgewell

BSc Immunology (Hons)

Submitted in fulfilment of the requirement for the degree of
Doctor of Philosophy (PhD)

School of Infection and Immunity
College of Medical, Veterinary and Life Sciences
University of Glasgow

June 2025

Abstract

Enteric helminth infections are a significant global health concern, with over a quarter of the world's population infected. The infecting parasites often migrate through host tissues and, as large multicellular organisms, they can cause substantial damage. The nematode *Heligmosomoides polygyrus bakeri* (*H. polygyrus*) causes a chronic roundworm infection in mice and during early stages of its lifecycle, it enters the intestinal tissue. The main aim of this thesis is to understand the factors that influence the immune responses at this early breach timepoint of infection, with particular interest in the role of IFN-γ production local to the helminth.

Our data show that the early phase of infection is accompanied by barrier disruption and an acute IFN-γ response which precedes the classical Th2 response to helminth infections. Through in vivo neutralisation of IFN-γ, we were able to show that the early production of IFN-γ contributes to increased expression of cell adhesion molecules found in *H. polygyrus* infection, suggesting a reparative role; and also coordinates and enhances local antimicrobial immunity characterised by increased antimicrobial protein expression and influx of innate myeloid cells to the site of infection.

Enteric helminth infections occur in the context of intestinal microbiota, and in environments where co-infection is frequent. We hypothesised that the antimicrobial responses and epithelial repair responses orchestrated by local IFN-γ could be in response to bacteria potentially invading the 'breach' points created by *H. polygyrus*. Using broad-spectrum antibiotics to eliminate microbiota during *H. polygyrus* infection, we found that the early IFN-γ response is dependent on bacterial presence. Our data suggest that bacterial translocation may not occur, perhaps reflecting the effective antimicrobial and tissue repair responses during the helminth infection. Together our data contribute to our overall understanding of the intestinal environment and immune responses in early *H. polygyrus* infection. By illustrating a microbiota-driven production of IFN-γ and characterising a role for this cytokine in coordinating local responses, we reveal a potential role for IFN-γ in protecting the host from secondary infection.

Table of Contents

List of Tables

List of Figures

Acknowledgements

Foremost, I want to thank my supervisor Prof Georgia Perona-Wright, for all of her support and supervision. You are one of the kindest people I have and will ever meet. I cannot thank you enough for your support throughout this PhD and with all the things you have helped me to achieve, thank you for the opportunity you gave me in joining your lab. Simon thank you for taking me under your wing and allowing me to join the Milling group when our GPW group moved to Edinburgh. Thanks for your support with my project in helping its final directions and for teaching me that you don't need to read papers on your weekends to be successful in academia. To you and Donal, thank you for your support in being my secondary supervisors.

Thanks to all the wonderful people I have collaborated with or shared an office with throughout my PhD. To all of the GPW lab members; thank you for everything and being such lovely people! Patrick, the pens out my hand! From ELISA stop songs in the lab and heardles in the office to wild swims after work, thanks so much for creating so many fun PhD memories with me. Jack, it's been lovely working with you over the years, thank you for being my MK (you'll have to work that one out). I learnt from working with you that everyone works differently to achieve their goals and not everyone likes to do lists and calendars. Jen, Jack M, Tasha, it was lovely to work with you all, thanks for all that you taught me and for making the lab and office a pleasure to work in at the start of my PhD. Holly, even though our paths didn't cross for too long, you taught me lots both in the lab and through your project ideas, thank you for letting me continue the *H. polygyrus* story! Thank you to all the members of the Milling lab, you all welcomed me into the group, thanks for all the fun memories in my final year and for all the lunchtime gossip while I borrowed the microwave! Thanks to everyone in our extension Milling office- Tom thanks for all the interesting chats both science-based and completely random, and for keeping office morale up with pics of Finlay. Anna A, you have been a role model, a supervisor and an incredible friend throughout my PhD, thank you for all that you have taught me in science and life- I never thought I would say it but I miss our early cull days together! Thanks to the Maloy group for all the science collabs and fun times. Anna H, Anna Ah and Annika, you are all such amazing scientists and bring a spark of enthusiasm to all that you do and it's a pleasure

to have worked with you all. Anna Ah I am so glad you joined the lab, what an addition to the MMP group, it's been lovely becoming your friend. Anna H, some of my favourite PhD memories will always be our Sardinia conference, I am so so happy that you came too- what a fun trip, thanks for always bringing laughter to the lab. To all members of the Maizel's lab, thank you for all the help with teaching me techniques and for including me in the wormy lab meetings. To the level 3 crew, thank you for all the help, guidance and memories. Becky, you brought such energy to work, you made sure the office always had an element of fun and were a joy to sit next to.

None of the work in this thesis would have been possible without the amazing staff working to support the School of Infection and Immunity. To all of the staff in the WRF facility, I am immensely thankful for all of the support you all contributed to ensuring the smooth operation of experiments, I literally couldn't have done it without you! Thanks to Diane, Alana and Daniel for running such an excellent Flow core. Thank you to Glasgow imaging facility, you all do a fantastic job, thanks for training and teaching me the wonders of microscope technology. To histology staff past and present; Fiona and Tyler you both taught me lots and processed countless samples for me, thank you! Stevie and the rest of the delivery team, you've been a big part of my PhD, always bringing a smile and energy to the lab bench and delivering antibodies right at the second I need them! Thanks to the John's in reception, and every cleaner and service member in the building contributing to maintaining a great work environment.

Thanks to Kevin Maloy, Allan Mowat, Simon Milling and Claire Bourke, your intense grilling during lab meetings (from some more than others) and advice has shaped both this project and me as a scientist. Thanks also to Allan for your Mowat master classes, it's crazy how much you forget in less than 4 years, thank you for your patience in reteaching us immunology throughout out PhDs. To both of my assessors during my PhD, Maggie and Rick, thank you for our science discussions and ideas for my project, thanks also Maggie for always checking in on us GPW members still in Glasgow.

Finally, a huge thank you to all my family and friends for supporting me in more ways that I can say. I am so lucky to have such an incredible group of friends in my life, every single one of you are amazing. To my main girls - I love you all,

thanks for bringing so much joy and happiness to my life. Emma, you checked in with me every few days whilst writing and although sometimes it stressed me out- it really got me through from start to finish, thank you!

To my wonderful sis- Ellie, we've been in our academic whirlwinds together recently, thanks for all of our facetime study sessions and celebrating all the small milestones along the way. Mum and Dad, you have provided such a loving home growing up, with creativity, learning and fun right in centre. You always encouraged me to never stop asking why and have always believed in me. Thank you for that and especially for supporting me during the writing stages of this thesis, we all made it through- a celebration to all of us. Oliver, you have provided me with love and support so much throughout my PhD and Uni beforehand, and I am so grateful for that. Thank you for always being my listening ear, even when I'm sure you didn't want to- I'd like to think that after listening to me ramble on that you have a reasonable understanding of immunology now! Thanks for always believing in me.

Publications

Andrusaite, A., **Ridgewell, O**., Ahlback, A., Webster, H., Yamaguchi, H., Peel, M., Frede, A., Al-Khalidi, S., Farthing, A., Heawood, A., Smith, A., Roberts, E., Mowat, A., Maizels, R., Perona-Wright, G., Milling, S., 2024. Intestinal helminth skews DC2 development towards regulatory phenotype to counter the anti-helminth immune response. *bioRxiv*, 2024.09.11.612410

Webster, H.C., Gamino, V., Andrusaite, A.T., **Ridgewell, O.J**., McCowan, J., Shergold, A.L., Heieis, G.A., Milling, S.W.F., Maizels, R.M., Perona-Wright, G., 2022. Tissue-based IL-10 signalling in helminth infection limits IFNγ expression and promotes the intestinal Th2 response. Mucosal Immunol 15, 1257–1269.

Conference Presentations

Canadian Society for Immunolofy Conference 2024,
Banff, Canada
Poster Presentation: "Investigating the impact of immunity to *Heligmosomoides* polygyrus on gut barrier integrity"

British Society for Immunology Congress 2023,
Belfast, UK
Poster Presentation: "Investigating the impact of immunity to *Heligmosomoides* polygyrus on gut barrier integrity"

Wellcome Centre for Integrative Parasitology: ISAB Meeting 2023,
Seamill, UK
Oral Presentation: "Investigating how *Heligmosomoides polygyrus* Infection affects gut barrier integrity"

ENII EFIS EJI Immunology Summer School Sardinia 2023,
Sardinia, Italy
Poster Presentation: "Investigating how *Heligmosomoides polygyrus* infection alters the immune response and susceptibility to concurrent infections"

Author's Declaration

I declare that, except where reference is made to the contribution of others, this thesis is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Name: Olivia Jessica Ridgewell

Signature:

Abbreviations

AAM	Alternatively activated macrophage
ABX	Antibiotics
AJD	Apical junctional complex
AMP	Antimicrobial protein
ANOVA	Analysis of variance
APC	Antigen presenting cell
ARG1	Arginase-1
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
CBA	Cytometric bead array
CCL	C-C chemokine ligand
CDH2	Cadherin 2
CLDN7	Claudin 7
CXCL	C-X-C motif chemokine ligand
DAMP	Damage associated molecular patterns
DC	Dendritic cell
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EdU	Ethynyl-2'-deoxyuridine
EGC	Enteric glial cell
ELISA	Enzyme-linked immunosorbent assay
ES	Excretory secretory
FB	FACS buffer
FISH	Fluorescence in situ hybridisation
FOXP3	Forkhead box P3
G	Granuloma
GALT	Gut associated lymphoid tissue
GAS	IFN-γ activation site
GATA3	Gata binding protein 3
GC	Germinal centre
H&E	Haematoxylin and eosin
HBSS	Hanks' balanced salt solution
HES	H. polygyrus excretory-secretory
HIV	Human immunodeficiency virus
IBD	Inflammatory bowel disease
ICOS	Inducible T cell costimulatory
IEC	Intestinal epithelial cell
IFN-γ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cell
iNOS	Inducible nitric oxide synthase
IP	Intraperitoneal
ISG	Interferon-stimulated genes
JAK	Janus kinase ____
JAM	Junctional adhesion molecules
JAM	Junctional adhesion molecules
KO	Knockout
L3	Third-stage larvae
LCN2	Lipocalin-2

LGR5	Leucine-rich repeat-containing G protein-coupled receptor 5
M	Microfold
mAb	Monoclonal antibody
MAdCAM-1	Mucosal addressin cell adhesion molecule-1
MBL	Mannose binding lectin
MCP1	Monocyte chemoattractant protein-1
MHC	Major histocompatibility complex
MIF	Migration inhibitory factors
mLN	Mesenteric lymph node
NBF	Neutral buffer formalin
NET	Neutrophil extracellular trap
NG	Non-granuloma
NK	Natural killer
NS	Non-significant
OCLN	Occludin
PAMP	Pathogen associated molecular patterns
PCR	Polymerase chain reaction
PI	Post-infection
PRR	Pattern recognition receptors
RELM-α/ß	Resistin-like-molecule-alpha/beta
RFU	Relative fluorescent unit
RORγT	Retinoic acid receptor-related orphan nuclear receptor gamma
ROS	Reactive oxygen species
RPS29	Ribosomal protein S29
RT	Room temperature
SCFA	Short chain fatty acid
SCID	Severe combined immunodeficiency
SD	Standard deviation
SEM	Scanning electron microscopy
SFB	Segmented filamentous bacteria
SILP	Small intestinal lamina propria
SIV	Simian immunodeficiency virus
SPF	Specific-pathogen-free
STAT	Signal transducer and activator of transcription
STH	Soil-transmitted helminth
T-BET	T-box transcription factor TBX21
TAC	Transit amplifying cell
TAMP	TJ-associated marvel proteins
TC	Tissue culture
TCR	T cell receptor
Tfh	T follicular helper
TGF-ß	Transforming growth factor beta
Th	T helper
TJ	Tight junction
TJP1	Tight junction protein 1
TLR	Toll-like receptor
TNF	Tumour necrosis factor
Treg	Regulatory T cell
TSLP	Thymic stromal lymphopoietin
Tvm	Virtual memory T cells
WNV	West Nile virus
WT	Wild type
ZO-1	Zonula occluden

Chapter 1 Introduction

1.1 Helminths

1.1.1 Helminth Infection

Parasites are one of the main pathogen types. The word originates from Greek in the 16th century: 'para' meaning alongside, 'sitos' meaning food and the phrase 'parasitos' together meaning to "eat at another's table". Parasites are defined as living inside or on another organism, stealing host nutrients for their own survival. Helminths are parasitic worms which can be classified into three groups: flukes, tapeworms and roundworms. Each of these groups have egg, larval and adult stages and contain species that infect a variety of tissues in a range of biological hosts (Castro, 1996; Joardar and Sinha Babu, 2020). Helminth infections are a major problem worldwide for a number of reasons, from disruption and losses to livestock farming due to animal infection, to the chronic and severe symptoms humans can face with infection. Globally, nearly a third of the human population is infected with helminths and, of that statistic, over 1.5 billion people are infected with soil-transmitted helminths (STH) specifically (McSorley and Maizels, 2012; W.H.O, 2023). STH species which infect humans include: most commonly *Ascaris lumbricoides*, a roundworm with highest prevalence of all STHs*; Trichuris trichiura*, a whipworm; and *Necator americanus* and *Ancylostoma duodenale,* both hookworms (Chen et al., 2024; Savioli and Albonico, 2004). Due to the common transmission route for these pathogens being faecal-oral, it is often poorer countries with reduced hygiene and sanitation who suffer most from infection (W.H.O, 2023). The global distribution and national prevalence of STH infection is shown in Figure 1-1 (Leta et al., 2020; Pullan et al., 2014).

Treatment programmes for controlling helminth infections often involve preventative measures such as improving sanitation and health education whilst also including anthelmintic drug treatment such as albendazole and mebendazole (Hedley and Wani, 2015; W.H.O, 2023). These drugs effectively clear worms by inhibiting the functions of microtubules within the parasites, consequently preventing glucose uptake by the worms and leading to their death (Chai et al., 2021).

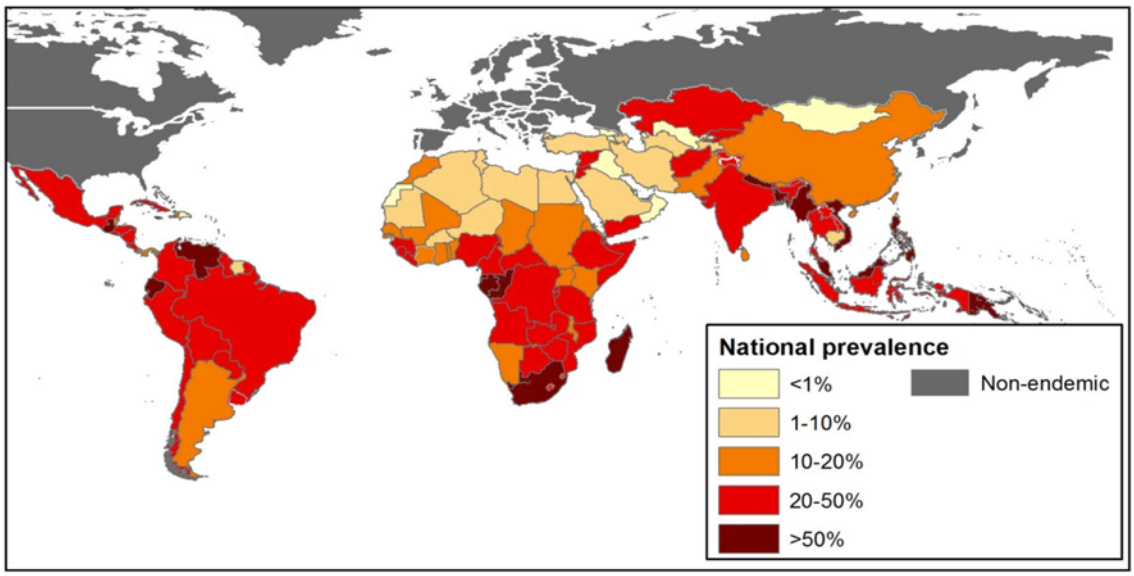

Figure 1-1 Global distribution of soil-transmitted helminth infection.
Distribution and national prevalence of soil-transmitted helminth infection based on empirical information available and geostatistical models for sub-Saharan Africa. Image taken from publication (Pullan et al., 2014).

Due to the nature of parasite infection, intestinal helminths most commonly cause symptoms related to lack of nutrients. Often an infected host would present with malnourishment, weakness, abdominal pain, diarrhoea, anaemia and in severe cases, intestinal obstruction (W.H.O, 2023). Children are most frequently infected and often with a higher abundance of helminths, which can lead to long-term complications such as stunted growth and impaired physical fitness (Hedley and Wani, 2015). These symptoms demonstrate that helminths as sole infections are harmful and can cause long-term consequences, however, when paired with co-infections they can be fatal. The host is often at a higher risk and susceptibility to co-infection by bacterial pathogens during helminth infection, although the understanding behind this is less well defined and thus an area of research importance (Hotez et al., 2008; Shea-Donohue et al., 2017).

Studying helminths has provided valuable insights which have not only improved treatment options but has also demonstrated the systemic influence of parasitic worms on their host's immune system. Similar to our bacterial microbiome, humans have co-evolved with parasitic worms throughout our existence. Therefore, the interactions between helminth and host provoke a complex immune response with both protective and harmful systemic effects.

Researchers in the field have proposed the 'hygiene hypothesis' that in our modern state of living, the cleaner environments we live in provide less exposure to microbes which can lead to increased risk of inflammatory/ autoimmune diseases. Specifically, the 'old friends hypothesis' builds on this, suggesting that the lack of infection with parasites which humans co-evolved with increases risk of autoimmune diseases (Rook, 2023). Current research is continuing to seek new evidence for ways in which helminths can be beneficial in preventing autoimmune conditions (Helmby, 2015). For example, using helminths or their secreted products, studies have shown that allergic airway inflammation and inflammatory bowel disease (IBD) can be inhibited (Elliott et al., 2004; Hang et al., 2010; McSorley et al., 2012). Therefore, continued research using helminth models is vital for further understanding the immune-pathogen interactions and how they can be harnessed when beneficial and prevented when harmful.

1.1.2 *Heligmosomoides polygyrus* model

As mentioned above, of all STH infections, roundworm nematodes have the highest prevalence. Mice are often used to model helminth infections and throughout the years have enabled researchers to understand and characterise local and systemic immune response. Several different mouse models can be used to study helminth infection, each with slightly different infection routes, lifecycles, and tissue migration patterns. *Heligmosomoides polygyrus bakeri* (*H. polygyrus*), previously known as *Nematospiroides dubius,* is a naturally occurring chronic roundworm infection in the small intestine of mice (Reynolds et al., 2012). This is a common infection in wild mice and is used in the laboratory as a model of human intestinal helminth infection. Additionally, the lifecycle of *H. polygyrus* includes interesting stages including two points whereby the helminth crosses the intestinal epithelium, 'breaching' the barrier (Figure 1-2). L3 *H. polygyrus* larvae infect the host via the oral route, travelling to the small intestine and burrowing into the duodenal wall at around day 2 of infection. The worm matures in the intestinal tissue until day 7 post-infection when it emerges from the duodenal wall and back into the gut lumen (Johnston et al., 2015; Pollo et al., 2023; Reynolds et al., 2012). Specific to this species, they possess a spiral shape (shown in Figure 1-3) which allows them to wrap around the villi in the gut lumen where they mate to produce eggs (Bąska and Norbury, 2022). Eggs pass

with faeces and hatch in soil upon warm and damp conditions at which larvae develop and await ingestion where the lifecycle is ready to begin again (summarised in Figure 1-2).

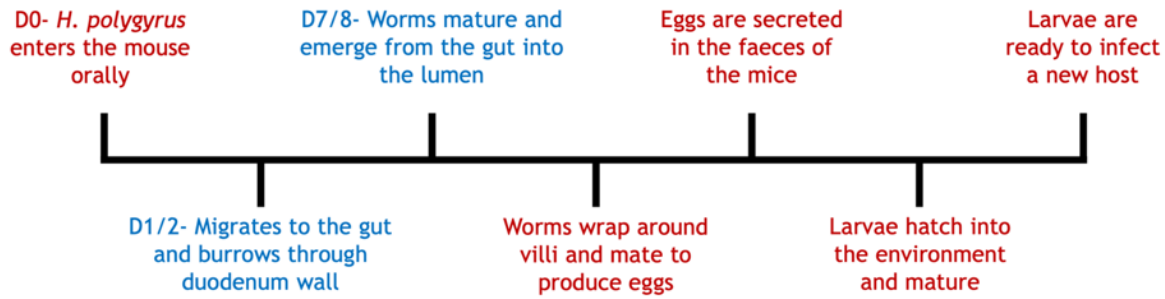

Figure 1-2 *H. polygyrus* life cycle
Timeline of each stage of *H. polygyrus* lifecycle. Text highlighted in blue to indicate 'breach' points of lifecycle.

Little is known about the process by which the worm burrows into the intestinal wall and later emerges, the extent of damage that this migration causes, how this damage is repaired, or the consequent effect on concurrent infections. Thus, further research is needed to explore how susceptibility to additional pathogens may be increased in *H. polygyrus* and how the immune responses initiated by the worm are able to both endanger and protect the host.

Figure 1-3 Image of *H. polygyrus* helminth.
Representative image of *H. polygyrus* helminth, demonstrating spiral-like shape used to attach to villi in intestinal lumen. Image produced by Dr Constance Finney.

In certain strains of mice, *H. polygyrus* infection can persist for months, making the model effective for studying not only innate and adaptive immunity but also

long-term consequences of infection. This helminth model alone has been beneficial in investigating the use of helminth products to treat other diseases, and has been more recently used to study distal immune effects and has been paired in co-infection experiments (Elliott et al., 2004; Hang et al., 2010; McSorley et al., 2012). Overall, helminth models such as this one have greatly contributed to our understanding of anthelminthic and overall intestinal immune responses. The first reports of mucus and wound healing as vital responses in immunity, the importance of innate lymphoid cell (ILCs), particularly ILC2s and tuft cells in promoting and amplifying anti-helminth responses and the ability of macrophages to proliferate in situ are examples of just a few key findings that helminth models have helped researchers to define (Perona-Wright and McSorley, 2022).

1.1.3 Anti-helminth immune responses

1.1.3.1 Intestinal inflammation

The early stages of *H. polygyrus* infection, when the parasite is moving into and out of the intestinal wall, are characterised by intestinal inflammation. Inflammation is a fundamental response of the immune system initiated upon injury, pathogen recognition or toxic compounds. This response aims to deplete harmful stimuli and repair damaged cells/ tissue. The response can be either acute or chronic, depending on the trigger and location of the induced inflammation, and, whilst the response should be beneficial, long-term inflammation and inflammation in certain tissues can be harmful to the host (Chen et al., 2017). Inflammation is an orchestrated response which requires coordination of various cell types, surface receptors, cytokine secretions and signalling pathways. Initially, inflammatory triggers are recognised by the appropriate cell receptors; pattern recognition receptors (PRRs) which can recognise conserved pathogenic structures known as pathogen-associated molecular patterns (PAMPs) or alarmins released by damaged cells known as damage-associated molecular patterns (DAMPs) (Chen et al., 2017; Li and Wu, 2021; Matta et al., 2017). Signalling through these PRRs triggers and promotes activation of immune cells and the release of pro-inflammatory cytokines and chemokines to recruit and activate other immune cells.

Depending on the tissue and immune cell, different chemokines are required to recruit cells to the specific location of injury or infection. The main chemokines important in myeloid cell influx to the small intestine are monocyte chemoattractant protein-1 (MCP-1, also known as CCL2), CXCL1&2, CXCL9, CXCL10, CCL5 and leukotriene B4 receptor 2 (ltb4r2). MCP-1 is an inflammatory-associated chemokine which promotes cell recruitment via activation of G-protein-coupled receptors (Deshmane et al., 2009; Singh et al., 2021). In times of inflammation it is often produced by macrophages and cells of the epithelium to recruit predominantly monocytes but also NK cells and memory T cells (Ruiz Silva et al., 2016). Epithelial cells are known to produce a variety of chemokines and can recruit myeloid cells upon damage or infection. Upon recruitment, many of the infiltrating immune cells are then co-ordinated and activated by the pro-inflammatory cytokines. Some of the main cytokines involved in the inflammatory response include IL-12, tumour necrosis factor (TNF), IL-6, IL-1β and interferon gamma (IFN-γ).

IL-12 (also known as IL-12p70) is a heterodimeric cytokine, mainly produced by antigen presenting cells (APCs) including macrophages and DCs. It enhances inflammatory immune responses by activating innate cells such as NK cells, which are then instructed to produce IFN-γ and contribute to host defence (Eley and Beatty, 2009). It also plays a crucial role in activating T cells and polarising Th1 differentiation to amplify inflammation (Athie-Morales et al., 2004). TNF (also known as TNF-α) has been shown to play a role in IL-12 inhibition although is a key cytokine involved in promoting and enhancing the inflammatory response. Although a beneficial and effective cytokine in many situations, TNF is one of the leading cytokines in the pathogenesis of IBD (Souza et al., 2023).It can be produced by a variety of immune cells and promotes the production of other inflammatory mediators including production of cytokines IL-6 and IL-1β and also chemokines to activate and recruit other cells (Harris et al., 2008). IL-6 and IL-1β are pro-inflammatory cytokines able to activate immune cells, contribute to tissue damage and regeneration, and enhance the overall inflammatory response. IL-6 specifically has been shown to modulate lectin production in Schistosoma infection (Antony et al., 2015). Lectins including mannose binding lectin (MBL) are a group of PRRs, key in recognising carbohydrates on pathogens and signalling inflammatory pathways. They are also

key molecules in activating the complement pathway to enhance local innate immunity (Fujita et al., 2004). IL-6 can also work in coordination with IFN-γ, influencing each other's production and immunity. IFN-γ is a known driver of the inflammatory immune response and a key cytokine involved in the host defence against bacterial pathogens. It is secreted by a number of cell types to promote a Th1 response and enhance pathogen clearance through antimicrobial effects and enhancing antigen presentation (discussed more in section 1.4.1) (Baldeón et al., 1997; Naoshi Hikawa et al., 1996). Each of these examples show effective ways in which cytokines are able to activate and enhance inflammatory responses, from increasing signalling signals, to activating and recruiting circulating cells to the affected area.

Tissue repair and regeneration are also important aspects of inflammation. Inflammatory cells such as macrophages and neutrophils can phagocytose apoptotic cells and debris. Additionally, both neutrophils and macrophages have been shown to play a role in wound healing by promoting rebuilding of the extracellular matrix (Choi et al., 2023; Cooke, 2019; Vicanolo et al., 2025). Macrophages can become polarised towards a phenotype known as alternatively activated macrophages (AAMs) which can secrete growth factors and interact with epithelial and stromal cells to promote their regeneration (Wynn and Vannella, 2016).

Most of the time, inflammation is beneficial, helping to clear pathogens by activating other immune cells and repair wounds by clearing debris and interacting with stromal and epithelial cells to promote tissue remodelling; all to protect the host. However, regulation of inflammation is also important, limiting its duration or intensity and preventing continuous amplification. When inflammation is chronic or occurring in response to stimuli such as self-antigens, inflammation can be dangerous. There are several regulatory mechanisms in place to suppress inflammation and promote resolution (Hanada and Yoshimura, 2002). These include production of anti-inflammatory cytokines such as IL-10 and (transforming growth factor beta) TGF-ß which can suppress immune cell activation, inhibit the production of more pro-inflammatory cytokines, limit antigen presentation and induce regulatory T cells (Tregs) (Moore et al., 2001). Tregs can interact with other T cells to suppress their function and inhibit

proliferation and can also produce additional anti-inflammatory cytokines (Goldmann et al., 2024).

1.1.3.2 Innate responses

Helminth infection is known to provoke a type 2 immune response in the host, important in clearance of the worms and coordinating wound repair (Allen and Wynn, 2011). Researchers have proposed that the Th2 response originated to facilitate wound repair caused by large parasites (Allen and Wynn, 2011). Additionally, it can contribute both systemically and distally to other infections. The anti-helminth response (summarised in Figure 1-4) is a well-coordinated response, involving crosstalk between a range of cells including epithelial cells, innate immune cells, T helper 2 (Th2) cells and the worm itself. As demonstrated through the lifecycle of *H. polygyrus* in section 1.1.2, enteric helminths are able to migrate across the intestinal epithelium and into the tissue where they reside during their maturation stage. Either helminths with lifecycle stages using similar migration processes or feeding methods in other helminth infections such as attachment to the intestinal wall using their 'cutting apparatus' can cause damage to epithelial cells (Cliffe and Grencis, 2004; Hotez et al., 2004; Loukas and Prociv, 2001; Monroy and Enriquez, 1992). The damaged cells release their stored cytokine alarmins, initiating innate immunity. The main alarmins involved in helminth infection are cytokines IL-25, IL-33 and thymic stromal lymphopoietin (TSLP). Each of these are involved in gut helminth immunity although IL-33 is more dominant in lung helminth responses, TSLP in the skin and IL-25 in enteric helminth responses (Stanbery et al., 2022). They can each act on local immune cells, inducing type 2 immunity (Inclan-Rico and Siracusa, 2018; Oyesola et al., 2020). Tuft cells are an important source of IL-25 in the gut. Tuft cells are located between epithelial cells in the intestinal wall and cell numbers expand upon helminth infection (Gerbe et al., 2016; von Moltke et al., 2016). These cells have been proven to be key in the induction of the type 2 immune response in helminth infections, (Inclan-Rico et al., 2022). Recently, a study has shown that receptors on tuft cells are able to recognise helminth derived products and binding results in calcium influx, subsequently causing their cellular secretion of IL-25 (Inclan-Rico et al., 2022; Luo et al., 2019). IL-25, IL-33 and TSLP are crucial for effective type 2 immunity against helminth infection. Each of them act on a variety of innate immune cells, mainly

ILC2s but also basophils, eosinophils, mast cells and DCs (Oyesola et al., 2020). Subsequently, these innate cells then produce IL-4, IL-5, IL-9 and IL-13 to develop a type 2 immune response and more specifically a T helper 2 (Th2) response. These cytokines are essential for this process and also provoke IgE class switching by B cells (Oyesola et al., 2020; Vacca and Le Gros, 2022).

In the past, research has reported that eosinophils can kill helminthic larvae *in vitro* using *T. spiralis* and *S. mansoni* species (Buys et al., 1981; Capron et al., 1979). In helminth infection, eosinophils have been shown to increase and contribute to developing the Th2 response by secreting cytokines including IL-4 (Huang and Appleton, 2016). Similarly, basophils mainly contribute to enhancing Th2 immunity through secretion of cytokines. Although, previous research argues a role for basophils acting as APCs to drive Th2 cell differentiation (Sokol et al., 2009). However, a requirement of either eosinophils or basophils for *in vivo* helminth clearance has not yet been demonstrated (Huang and Appleton, 2016; Voehringer, 2009).

ILC2s are also responsible for the production of IL-9 which works through autocrine signalling, enhancing both ILC2 and mast cell proliferation and survival (Bick et al., 2025). In experimental IL-9 depletion, intestinal helminth infection persists and tissue resolution is impaired in the lung stages of *N. brasiliensis* infection (Turner et al., 2013). Since IL-9 contributes to mast cell proliferation, the impaired immunity to helminths is likely due to a reduction in these cells. Mast cells are able to degranulate during early helminth infection which enhances alarmin production, amplifying the overall host response against the parasite (Hepworth et al., 2012). One study also suggested that effective Th2 responses were dependent on mast cell presence during helminth infection (Hepworth et al., 2012).

ILC2 produced IL-13 has been shown to directly interact with the intestinal epithelial cells to stimulate the ‘weep and sweep’ response, causing goblet cell hyperplasia to enhance mucous production (Campbell et al., 2019; Michla and Wilhelm, 2022). Researchers have also identified a role for IL-13 in increasing epithelial cell turnover in the intestine, thought to contribute to worm clearance by dislodging worms during *Trichuris muris* (*T. muris*) infection (Artis, 2006; Cliffe et al., 2005). Additionally, goblet cells produce RELMβ, a molecule that

can reduce helminth viability during infection by directly bind to the worms (Horsnell and Dewals, 2016). IL-13 is also able to promote an AAM phenotype in macrophages, contributing to tissue resolution and repair (Michla and Wilhelm, 2022).

During infection, intestinal helminths release many excretory-secretory (ES) molecules, some of which have immunomodulatory properties known to influence the host immune response (Maizels et al., 2018). In *H. polygyrus* infection, the ES secreted by the parasites (HES) contains a family of molecules that mimic TGF-β and induce Tregs to suppress the immune response and prevent their clearance (Grainger et al., 2010; Maizels and Newfeld, 2023; White et al., 2021). HES can also manipulate other aspects of the host response, for example, a study by Drurey et al. focussed on the influence they have on surrounding epithelial cells. The researchers found that HES inhibited the effects of type 2 cytokines, blocking the proliferation of tuft and goblet cells to prevent worm clearance from the host (Drurey et al., 2021).

As well as the induction of type 2 immunity, several other key cells and responses are involved in early helminth infection. In skin helminth infections, neutrophils are recruited to parasite entry sites and have been shown to play a key role in immobilisation and killing of *S. ratti* and *S. stercoralis* larvae (Turner et al., 2013). Similarly, neutrophil recruitment is important in lung and gut helminth infections and depletion of these cells was shown to increase worm burden and impair expulsion (Bouchery et al., 2020; Chen et al., 2014). During the tissue-dwelling stage of *H. polygyrus* infection, neutrophils accumulate in the tissue (Patel et al., 2009), although how exactly they contribute to immunity against this parasite remains unclear. There has been evidence recently to suggest that neutrophils, similarly to macrophages, can have an alternative, more type 2 phenotype and function during helminth infection, referred to in some literature as ‘N2s’ (Chen et al., 2014; Maizels and Gause, 2023). N2s specifically have been shown to contribute to shaping other cellular responses such as macrophage polarisation (Chen et al., 2022). Otherwise, neutrophils are known for their fast response to damage and pathogen clearance which is described further in section 1.3.2.2. In more recent years, roles have been identified for neutrophils in tissue repair. They can release mediators such as resolvins and protectins to promote tissue resolution and use mechanisms to

impair further neutrophil recruitment including trapping and taking up chemokines and proinflammatory cytokines to prevent amplification of the inflammatory response (Peiseler and Kubes, 2019). Evidence has also been demonstrated for them to produce proteins which help reconstruct the extracellular matrix upon injury (Vicanolo et al., 2025). NK cells are also effective at killing pathogens, particularly viruses. However, research has shown that in *H. polygyrus* infection when the helminth is undergoing maturation in the intestinal tissue, NK cells are recruited to the infection site to limit tissue damage. In *H. polygyrus* infected mice, depletion of these cells led to intestinal bleeding although the worm counts remained the same (Gentile et al., 2020). When assessing gene expression in these SILP NK cells, the authors found increased expression of genes previously identified to contribute to vascular integrity and remodelling (Gentile et al., 2020).

Granulomas encase larvae which have crossed the barrier and reside in the intestinal tissue. These consist of neutrophils, eosinophils, monocytes and AAMs (Anthony et al., 2006; Hewitson et al., 2015). A recent preprint in BioRxiv used spatial transcriptomics to analyse *H. polygyrus* granulomas, and showed a decrease in genes associated with intestinal homeostasis and an increase in tissue remodelling and inflammatory associated genes (Poveda et al., 2024). The aim of the granuloma formation appears to be tissue repair, immobilisation of the helminth and killing of larvae. One study suggested that AAMs use their CD11b complement receptor to directly bind parasite larvae using complement C3, immobilising the larvae (Esser-von Bieren et al., 2013). As an effective branch of innate immunity, complement is usually an effective mechanism to clear invading pathogens and damaged cells. However, helminths are often able to evade detection and clearance via complement by manipulating host immunity and inhibiting complement activation pathways (Shao et al., 2019). However, eosinophils have been shown to kill *S. mansoni* parasites through binding the same C3 receptor on larvae (Ariyaratne and Finney, 2019; Ramalho-Pinto et al., 1978). Whether eosinophils migrate to the granuloma or expand through proliferation in the granuloma remains unclear (Ariyaratne and Finney, 2019).

Interestingly, whilst many helminth host responses provoke a typical Th2 immune response, previous work in the laboratory and in recent research show

IFN-γ, a cytokine more typically associated with Th1 immune responses, is produced in some early helminth infections including *H. polygyrus* and *T. muris* (Bancroft et al., 1994; Gentile et al., 2020; Webster et al., 2022). What is known thus far about the relationship between IFN-γ, the host response and the helminth is described in section 1.4.3. Overall, the orchestration of the innate immune response includes alarmin secretion, granulocyte degranulation, chemokine production, immune cell recruitment and activation, and goblet and tuft cell hyperplasia. This co-ordinated response controls worm burden, and supports further host immunity to the helminth infection through the induction of adaptive, Th2 immunity.

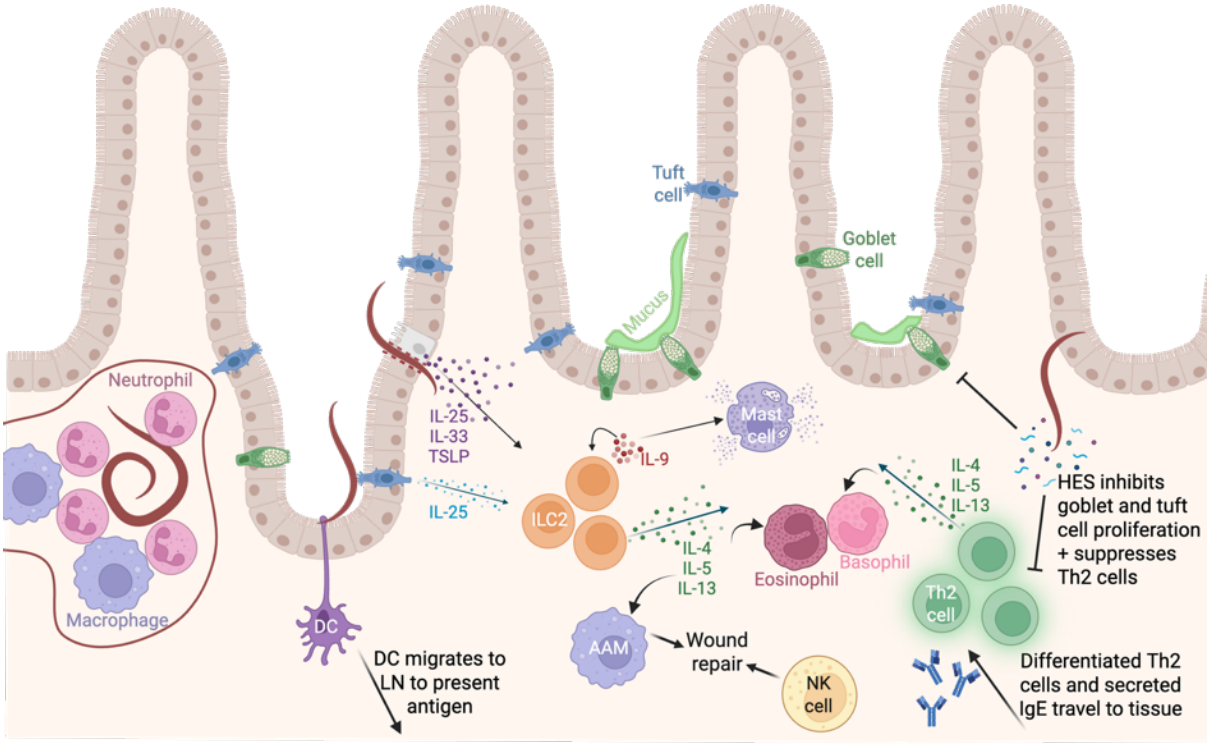

Figure 1-4 Anti-helminth immune responses in small intestine
Diagram showing a summary of the local anti-helminth immune responses in the small intestine during *H. polygyrus* infection. Created using BioRender.com.

1.1.3.3 Adaptive responses

DCs provide a link between the innate and adaptive immune responses, being shaped by other innate cells and essential for activating adaptive immune cells. ES molecules produced by helminths can be recognised by DCs through their

PRRs, and due to the stimulus and subsequent signalling, promote a Th2 polarisation in subsequent T cell responses (Motran et al., 2018). ILC2s can produce the neurotransmitter acetylcholine during helminth infection which contributes to eosinophil recruitment and can also induce OX40L expression on DCs (Vacca and Le Gros, 2022). This ligand is able to promote Th2 priming of T cells upon antigen presentation (Croft et al., 2013). For T cell priming, DCs from the small intestine sample for antigens and then travel to the mesenteric lymph nodes (mLNs) where they present antigen to T cells.

For CD4+ T cell differentiation and activation, three signals are required, shown in Figure 1-5. Initially, DCs will present antigen bound on their MHC class II receptor which binds to the T cell receptor (TCR). Upon binding, costimulatory molecules are the second required signal for differentiation. One of the co-stimulatory molecules involved in priming Th2 activation is CD28 and its ligand CD80/86, binding enhances IL-4 responsiveness, and increases inducible T cell costimulatory (ICOS) expression another important co-stimulatory molecule, thus promoting Th2 cell differentiation (Schorer et al., 2019). In helminth infection, blockade of ICOS shows hindered generation of effective Th2 cell responses (Kopf et al., 2000). The OX40L expression on DCs is also key for initiating IL-4 production by naïve T cells and when binding to OX40 on naïve T cells, it enhances IL-4 production, working through autocrine signalling to promote differentiation (Ohshima et al., 1998; Schorer et al., 2019; So et al., 2006). Researchers have previously suggested that the Th2 response is the default response upon T cell activation when IL-12 is not secreted (Everts et al., 2010). TSLP can inhibit the secretion of IL-12, in favour of suppressing Th1 and promoting Th2 responses (Massacand et al., 2009). However, IL-4 is key for Th2 differentiation. IL-4 and IL-2 cytokines are the final signal required for Th2 cell differentiation (Cote-Sierra et al., 2004). Stimulation with these enables differentiation and promotes cell survival, although the source of the IL-4 is a debated topic. Some papers have shown that ILC2s can produce IL-4 required for Th2 cell differentiation following *H. polygyrus* infection (Pelly et al., 2016) whilst other research has shown that eosinophils, basophils and Th2 cells were recruited to the lung as IL-4 producing cells for Th2 differentiation during *Nippostrongylus brasiliensis* infection (Voehringer et al., 2004). GATA-binding protein 3 (GATA-3) is the master transcription factor for Th2 cells and its

expression is promoted upon IL-4 binding (Paul and Zhu, 2010). Signal transducer and activator of transcription (STAT) proteins are activated and bind specific DNA loci to initiate upregulation of specific genes. IL-4 and IL-13 can activate STAT6 to upregulate GATA3 and TSLP and IL-2 activate STAT5 to initiate production of IL-4 and upregulate other Th2 cytokines.

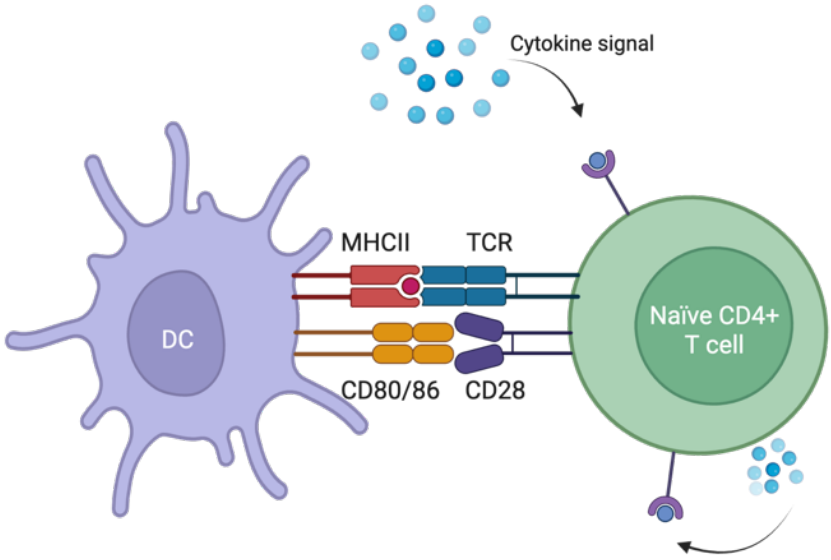

Figure 1-5 DC and Naïve CD4+ T cell interaction
Diagram showing three signals required for Th subset differentiation: MHCII-TCR, CD80/86-CD28 and cytokine stimulation to direct subset. Created with BioRender.com.

As effector differentiation occurs, Th2 cells migrate to the site of infection, the small intestine. Studies show that IL-25 and TSLP produced by epithelial cells can stimulate the migration process of DCs and also enhances T cell activation and differentiation by promoting upregulation of the OX40L on DCs (Fernandez et al., 2011; Ito et al., 2005). Additionally, TSLP can induce production of TARC (CCL17) and MDC (CCL22), chemokines known to attract Th2 cells (Soumelis et al., 2002). Once primed, many Th2 cells travel to the small intestine using increased expression of α4ß7 integrin on their cell surface to home to gut tissue through binding of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on endothelial cells (Hepworth et al., 2012).

When in the small intestine, the Th2 cells amplify ongoing immune responses to try and expel the helminths. Th2 cells secrete the key type 2 associated cytokines IL-4, IL-5, IL-13, IL-25 and also IL-2 for self-amplification. Each of

these can work locally in the small intestine to both promote tissue resolution and enhance immune responses, further activating AAMs, ILC2s and other type 2 components to promote worm expulsion. IL-5, IL-13 and IL-25 are predominantly tissue based. IL-5 can recruit granulocytes whilst IL-13 plays a wider role in wound repair and resolution, also driving AAM polarisation. IL-4 is most important in the lymph node tissue, driving new Th2 cell differentiation and guiding B cell class switching. Th2 cells interact with B cells in the mLN via the CD40: CD40L binding and by producing cytokines IL-4 and IL-13 they initiate immunoglobulin E (IgE) class switching (Janeway et al., 2001). B cells then produce parasite-specific IgE which is able to act upon helminths by targeting mast cell and basophil functions towards the parasites. Higher IgE levels have been shown to correlate with improved infection outcome and increased resistance to helminth infections in humans (Fitzsimmons et al., 2014). One particular study found that IgE is essential and works with basophils to trap *N. brasiliensis* larvae in the skin, preventing migration of this parasite to the host lungs (Obata-Ninomiya et al., 2013). Gurish et al also found that during the larval stages of *T. spiralis* infection, IgE-mediated mast cell activation promoted expulsion of helminths (Gurish et al., 2004). However, helminths are able to promote the production of polyclonal IgE which can dilute the parasite-specific IgE, preventing the helminth from being targeted (Cooper et al., 2008). Consequently, anti-IgE treatment has been shown to reduce worm burden in some infections, perhaps allowing for more targeted immune responses against the helminths (Cooper et al., 2008).

Whilst damaging the helminths through each of the described responses is important, the damaged worms still need to be cleared from the host intestines. As previously mentioned in section 1.2.2.1, goblet cells undergo hyperplasia and produce mucins, predominantly the glycoprotein Muc2, to contribute to the ‘weep and sweep’ environment to aid in parasite clearance. The ‘weep and sweep’ process also involves increased smooth muscle contractions which accelerates peristalsis. This develops in early infection to try and expel larvae and persists to later timepoints to try and detach and remove adult helminths (Bąska and Norbury, 2022).

Due to host co-evolution with parasites, there are numerous other ways in which helminths can modulate host immunity in their favour. For example, ES-62, a

molecule secreted by *Acanthocheilonema viteae* parasites is able to suppress CD4+ T cell and B cell proliferation and promote an anti-inflammatory environment (Doonan et al., 2019a; Harnett and Harnett, 2009). In other infections, research has shown that helminths can produce protease inhibitors to inhibit antigen processing, neutrophil functions and also to promote IL-10 production (Nutman, 2015). Another branch of the helminth immunomodulation is immune mimicry and involves the helminths producing molecules which resemble host proteins and act as agonists to promote certain responses (Hurford and Day, 2013). In *H. polygyrus* infection, the helminths produce TGF-ß mimicry molecules which can promote regulatory effects to dampen the local immune responses and promote parasite survival (White et al., 2021) (further described in section 1.1.3.2). Other helminths have also been shown to produce anti-inflammatory mimicry molecules such as SOCS-1, macrophage migration inhibitory factors (MIF) and even chemokine receptor-like proteins to affect cell recruitment (Nutman, 2015).

Although most research on helminth infection is carried out in controlled infection environments, often using murine models, human infections are often not as clearcut and involve repeated exposure and secondary infection from the same helminths after clearance. Th2 cells are essential for successful and rapid clearance of helminths upon secondary challenge (Harris and Gause, 2011). Research has shown that T follicular helper (Tfh) cells are also crucial for effective type 2 immunity and form germinal centres (GC) in the mLN to interact with B cells and promote antibody production and development of memory B cells that protect the host upon reinfection (Zaini et al., 2023). When B cells were depleted in mice, protective immunity against *H. polygyrus* reinfection was deficient (McCoy et al., 2008). Overall, the combination of innate and adaptive immunity together forms a carefully orchestrated response to combat helminth infection and provide protection from future infection.

1.2 The intestinal barrier

1.2.1 Small intestine environment

The digestive tract functions to digest and absorb nutrients from ingested food. Often referred to collectively as the ‘bowels’ or ‘guts’, the intestinal system is

composed of the small and large intestines. Following on from the stomach, the small intestine is primarily responsible for absorbing nutrients from food, whilst the large intestine (colon) absorbs remaining water and is the final stage of the digestive system (DeSesso et al., 2012). One of the main anatomical differences between mice and human large intestine is the caecum. In rodents, this is a separate compartmentalised structure where fermentation of food takes place, whilst in humans this plays a much less prominent role and is part of the continuous colonic structure instead of a separate compartment (DeSesso et al., 2012). To help with nutrient absorption, the small intestine has hair-like projections known as 'villi' which increase the surface area of the small intestine, hence increasing the amount of nutrients that can be absorbed from food. The small intestine can be classified into three sections, the duodenum (proximal upper section), jejunum (middle section) and the ileum (distal lower section) (Collins et al., 2025). Each section has slight changes in environment and structure to cater to different functions in digestion and nutrient and water absorption. Digested food from the stomach enters the duodenum and is met with enzymes which further break the food down. Glands within the duodenum release bicarbonate to neutralise the luminal contents and bile to digest and absorb lipids. Further nutrient absorption takes place in the jejunum and the ileum alongside uptake of bile acids which are recycled for future digestion (Collins et al., 2025). Surrounding the small intestine, the mesentery is the organ which attaches the digestive tract to the abdomen. It contains lymphatics, blood vessels and nerves which carry out their independent functions and allow crosstalk between each system to shape the environment of the small intestine. Overall, immunity in the small intestine has been highly conserved in evolution and remains very similar between humans and mice, even in the developmental process (Stanford et al., 2020). Both species develop tolerance to food antigen and commensal bacteria, maintain a strong barrier through tight junction proteins and respond with similar cells and antimicrobial properties to barrier breach (Gibbons and Spencer, 2011). Therefore, mouse models are an effective way of studying intestinal infections and diseases.

Dividing the luminal and tissue interface, the intestinal epithelium is a simple columnar epithelium, with only a single layer of cells (Kong et al., 2018). There are several differentiated types of intestinal epithelial cells (IECs) each with a

specific role to co-ordinate nutrient absorption, barrier protection and communication with other host systems. The types of IECs are enterocytes, goblet cells, Paneth cells, enteroendocrine cells, tuft cells, microfold (M) cells, neuroendocrine cells, transit-amplifying cells (TACs) and stem cells which are each further discussed in section 1.2.2.1 and shown in Figure 1-6 (Collins et al., 2025). Many of these specialised IECs also contribute to immune responses as has been described with examples in anti-helminth immunity in section 1.1.3. Also, there are a variety of immune cells residing in the small intestine to defend against infection and maintain homeostasis. These include myeloid cells such as macrophages, neutrophils and eosinophils, ILCs and adaptive cells such as effector T cells, Tregs and B cells. Additionally, gut associated lymphoid tissues (GALT) can be found throughout the small intestine in the form of large secondary lymphoid tissues called Peyer's patches or as isolated lymphoid follicles (Fenton et al., 2020; Lorenz and Newberry, 2004). Peyer's patches contain organised follicles which are predominantly made up of B cells with smaller amounts of other both innate and adaptive cells. These structures have M cells at the luminal interface and are important for immune surveillance in the gut and maintaining healthy gut tissue, defending against infections (Panneerselvam and Vaqar, 2025). Similarly, ILFs are also important in infection control, however their formation can be induced in response to infection challenges in the gut. They contain B and T cells and can produce antigen-specific immunoglobulin (Lorenz and Newberry, 2004). Lymph fluid from the small intestine is drained to and filtered by the mLN chain. This is the most crucial site for initiation of adaptive immune responses to combat infections in the small intestine (Macpherson and Smith, 2006). From the proximal to distal end of the small intestine, there is an increase in Peyer's patches, likely due to the increase in bacterial diversity and numbers further along the digestive tract (Martinez-Guryn et al., 2019). Whilst there are many cells and structures contributing to the small intestinal environment, bacteria are fundamental to overall host health, helping to digest food and facilitating vitamin production (further discussed in section 1.2.4).

On top of the single layer of epithelial cells providing a barrier is a thick layer of mucus to provide further protection. Mucus is made from glycoproteins that are produced by goblet cells. This layer enables nutrients to travel from the lumen

to the epithelium, whilst containing antimicrobial substances and providing a physical barrier to prevent direct contact with bacteria and harmful digestive enzymes (Macierzanka et al., 2019). Certain less virulent bacterial species can inhabit the mucus layer and the host facilitates this by providing digestible glycans as a food source and preventing influx of competing bacterial species (Johansson and Hansson, 2016). Studies have shown the importance of mucus through KO studies using mice with specific deletion of muc2, the main glycoprotein component of mucus. One study found that this KO led to the spontaneous onset of colitis and another found that the mucus-dependent niche for some commensal bacteria prevents colonisation by bacterial pathogens (Birchenough et al., 2023; Van der Sluis et al., 2006). In addition to bacterial species, parasites such as helminths can find suitable niches for growth along the intestinal tract due to the easy access of nutrients. Similarly to bacteria, certain parasites localise to different intestinal regions; in the case of *H. polygyrus* infection, larvae and adult worms reside in the duodenum of the small intestine.

1.2.2 Epithelium

1.2.2.1 Cells of the epithelium

Since the small intestine functions to absorb nutrients from digested food, most cells in the single layered epithelium are enterocytes which have microvilli structures on their luminal facing surface and focus their functions on nutrient uptake (Collins et al., 2025; Kong et al., 2018). Mixed in between these enterocytes are less abundant goblet cells and tuft cells. As previously mentioned, goblet cells are responsible for the production of mucins including the main component of mucus, Muc2. Their proliferation is enhanced by type 2 cytokine, IL-13 to increase mucus production in infections, an example being in helminth infection as described in section 1.1.3 (Zhang and Wu, 2020). Tuft cell differentiation and proliferation is also stimulated this way and these cells are able to promote a Th2 response upon helminth infection, as described, through their secretion of IL-25 (Howitt et al., 2016). Tuft cells express ‘taste’ receptors which enable them to directly sample their surroundings and the contents of the intestinal lumen and respond to infection (Howitt et al., 2016). Another cell known to sample its surroundings is the M cell. These are located on the luminal surface of Peyer’s patches and sample antigens in the lumen before transporting

them into the tissue to initiate immune responses in the Peyer's patch (Collins et al., 2025). A study by Kanaya et al has shown that deletion of M cells impairs the uptake of antigens into the Peyer's patches (Kanaya et al., 2018).

Enteroendocrine cells (also referred to as neuroendocrine cells) are also found in the epithelium of the small intestine (Gunawardene et al., 2011). Enteroendocrine cells have sensory receptors on their luminal surface to detect homeostatic changes such as pH, nutrient concentrations and physical changes in intestinal structure. Upon changes, these cells produce hormones and signalling molecules and use neural signals to communicate with local and systemic immune cells and connect the gut-brain axis (Atanga et al., 2023; Collins et al., 2025). Due to the ability of these cells to influence systemic responses, they have recently been studied in the context of targets for disease therapy (Atanga et al., 2023). For example, clinical trials have been ongoing to harness anti-inflammatory secreted products from enteroendocrine cells and use these to treat IBD patients (Atanga et al., 2023; Blonski et al., 2013).

Located in the crypts (the dipped areas between the villi) are Paneth cells, stem cells and TACs. Paneth cells have toll-like receptors (TLRs) on their cell surface, a subset of PRRs enabling them to detect bacteria. They have granules loaded with antimicrobial peptides such as defensins which can target bacteria. Paneth cells are also able to produce growth factors for other cell types and cytokines to promote immune responses (Bevins and Salzman, 2011; Collins et al., 2025; Kong et al., 2018). Several different enteric helminth infections result in Paneth cell hyperplasia, although whether this is a response to the helminth itself or the damage it causes is unclear (Kamal et al., 2002).

At the very base of the crypts are multipotent stem cells. They are identified by the expression of leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) (Bevins and Salzman, 2011). These cells continuously undergo proliferation and have the ability to differentiate into all of the IEC types described above (Collins et al., 2025). The cells in the intermediate stage between a stem cell and differentiated cell are the TACs (Sanman et al., 2021). Certain infections or autoimmune diseases can affect the rate of proliferation and differentiation by the stem cells, manipulating the overall functions of the epithelium. For example, in *H. polygyrus* infection, helminths can induce a

reprogramming of the stem cells to lessen the type 2 response and reduce their clearance (Karo-Atar et al., 2022). Several other studies have also shown reprogramming of the intestinal epithelium during *H. polygyrus* infection. Such reshaping of the epithelium by *H. polygyrus* was initially suggested by the researchers Nusse et al. Through analysis of whole crypts they found that local to the helminths, cells showed 'foetal-like programming' a process often during tissue regeneration in which epithelial cells can reactivate markers from developmental stages (Viragova et al., 2024). The authors suggested that this was a response to tissue injury following the burrowing of the helminth (Nusse et al., 2018a). Drurey et al also show that the ES products from *H. polygyrus* helminths are able to block type 2 cytokine function, further inhibiting expansion of goblet and tuft cells. The researchers found that these products were altering the intestinal epithelium composition (Drurey et al., 2021a). Likewise, another study found that the loss of Lgr5 expression on stem cells in *H. polygyrus* infection and the reduced numbers of Paneth cells were both effects that were mediated by IFN-γ signalling (Eriguchi et al., 2018). This set of papers together show that epithelial differentiation changes during infection, influenced both by immune signals and parasite instructions.

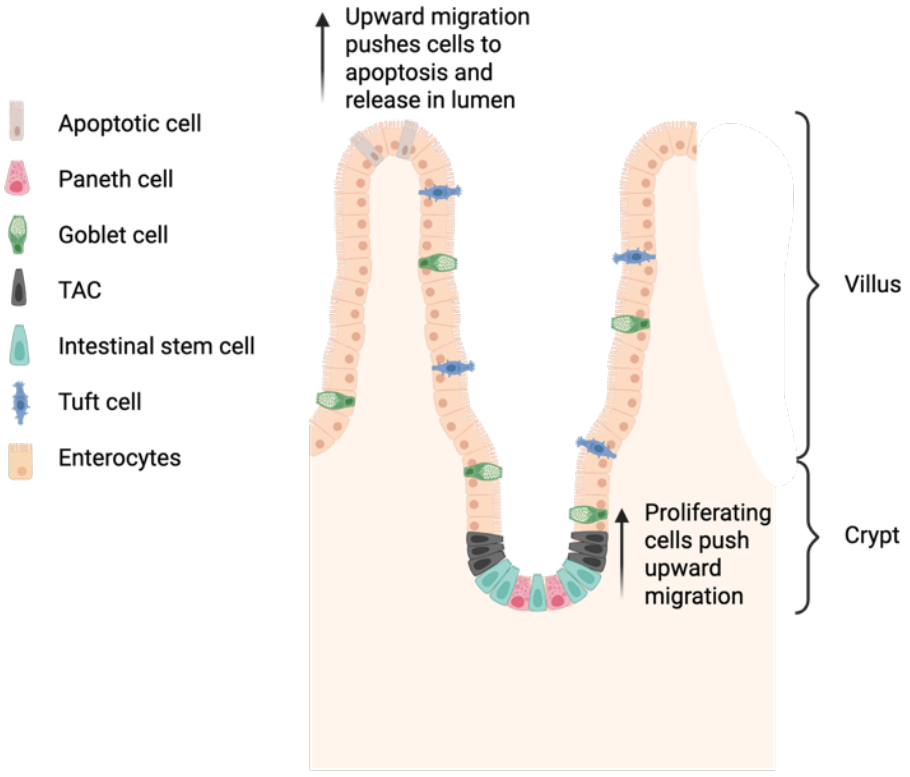

Figure 1-6 Small intestinal epithelial cells and their proliferation

Diagram showing different cell types of the intestinal epithelium and proliferative direction of cells. Adapted from (Bao et al., 2020) and created in BioRender.com.

1.2.2.2 Epithelial turnover

Epithelial cells in the small intestine have a very high turnover rate, with most cells being replaced every 3 to 5 days (Collins et al., 2025; Kong et al., 2018). The stem cells in the crypts divide and differentiate into each of the IEC types and, as they divide, they migrate upwards towards the top of the villi. Cells at the tips of the villi undergo apoptosis (shown in Figure 1-6) (Bao et al., 2020). This rapid renewal process ensures a strong and healthy epithelial barrier, important to prevent pathogen invasion. The IECs which have undergone apoptosis at the top of tip of the villi are shed into the gut lumen (Williams et al., 2015). The process of this cell extrusion has been studied visually using different pathological techniques. It is proposed that when an epithelial cell undergoes apoptosis, it detaches from a basement membrane and the cells either side redistribute their tight junction proteins as the cell protrudes upwards. As these redistribute, the neighbouring cells join their tight junction proteins to keep the barrier sealed and maintain barrier integrity (Guan et al., 2011; Madara, 1990; Williams et al., 2015). Intestinal stem cell proliferation is controlled by and dependent on Wnt signalling (Mah et al., 2016; Rao and Wang, 2010). In a study where a Wnt signalling inhibitor was produced by mice, not only was proliferation reduced but crypt structure was lost along with many differentiated IEC types (Pinto et al., 2003).

The rates of proliferation in intestinal stem cells can vary depending on a variety of factors. The microbiota and the host diet alter the proliferation rate through production of molecules such as short chain fatty acids (SCFA) or metabolites. As well as influencing Wnt signalling, microbiota can directly modulate proliferation signalling pathways (Brandi et al., 2024; Shiratori et al., 2024). Also, infections can significantly impact proliferation rates. A recent study found that in zebrafish, infection with the bacteria *V. cholerae* activates growth inhibitor proteins to inhibit epithelial cell proliferation in the intestine (Xu and Foley, 2024). On the contrary, another study found that damage to the intestine caused by *Salmonella pullorum* infection in chickens over-activated signalling pathways to induce an escalation in intestinal stem cell proliferation (Xie et al., 2020). In

Drosophila, infection with Drosophila A virus also promoted proliferation of intestinal stem cells, disrupting homeostasis (Nigg et al., 2024). In helminth infections, there is increasing evidence for accelerated intestinal epithelial cell turnover. As previously described, infections with helminths such as *H. polygyrus* can cause goblet and tuft cell hyperplasia. This is often beneficial to the host as it increases mucus production and the movement of the worms through the intestine. One particular study showed that, in severe combined immunodeficient (SCID) mice given *T. muris* infection, the response involved IFN-γ driven amplification epithelial cell turnover (Cliffe et al., 2005a). Additionally, *T. spiralis* infection has been shown to increase epithelial cell proliferation in the small intestine as well as the differentiation of stem cells into other cell types (Walsh et al., 2009).

1.2.2.3 Barrier integrity

The intestine is one of the parts of the body most exposed to infection. Although only a single layer, the intestinal epithelial barrier is tightly regulated and sealed to enable sufficient nutrient absorption and to prevent infections. The intestinal epithelial cells are adhered to a layer of extracellular matrix known as the basement membrane by adhesion molecules: integrins and hemidesmosomes. This attachment provides further structural support for the epithelium (Gilcrease, 2007). Between each of the IECs is a region called the apical junctional complex (AJC) which is a structure composed of molecules to adhere the epithelial cells together (Barbara et al., 2021). The main components of this structure are tight junction (TJ) proteins, which form a barrier between cells and regulate movement of molecules across it.

TJ proteins are split into four families: claudins, TJ-associated marvel proteins (TAMP), angulins and junctional adhesion molecules (JAM) (Liebing et al., 2025). There are over 24 members of the claudin family and each is a transmembrane protein, interacting at the cell walls between adjacent cells as either sealing proteins or pore forming proteins (Barbara et al., 2021; Suzuki, 2020). The pore forming protein, claudin-2, is responsible for enabling passage of cations and other molecules between the cells in the intestinal epithelium. A recent study by Oami et al found that claudin-2 is upregulated in patients with sepsis who also show increases in intestinal permeability. In the same paper, the

researchers use a claudin-2 KO mouse model to show that claudin-2 is responsible for the increased permeability in septic mouse models (Oami et al., 2024). Additionally, another role for claudin-7 in epithelial turnover has been reported by researchers who suggest that it is required for the Wnt signalling pathway, thus controlling proliferation and differentiation of IECs (Xing et al., 2020). In the TAMP family, members include occludin, tricellulin and marvelD3, all of which are important in TJ stability and barrier function (Liebing et al., 2025). One publication found that a double KO of both occludin and tricellulin proteins reduced the tight junction adherence points and led to an increased intestinal permeability (Saito et al., 2021). The same study also created cell lines which overexpressed these proteins and found that overexpression of occludin strengthened the intestinal barrier (Saito et al., 2021). Occludin is the primary TAMP and its phosphorylation state has been reported to influence its function and localisation (Wong, 1997). Additionally, occludin contains a binding domain for zonula occludens (ZO-1), a scaffold protein that can bind multiple proteins to the cytoskeleton (Barbara et al., 2021; Kuo et al., 2022). Deletion of this protein in cell lines can impair barrier function by preventing recruitment and attachment of TJ proteins (Kuo et al., 2022). The angulins TJ protein family function to aid tricellulin and cater for paracellular movement of macromolecules and water (Liebing et al., 2025). Finally, the JAM family play a role in barrier formation and are essential for effective barrier integrity as has been shown through impaired function in deletion mouse models (Rouaud et al., 2020). Additionally, calcium dependent adhesion molecules (cadherins) are transmembrane glycoproteins, also an important component of barrier integrity. Impairment of cadherins has been shown to negatively impact barrier function and intestinal homeostasis (Schneider et al., 2010; Schnoor, 2015).

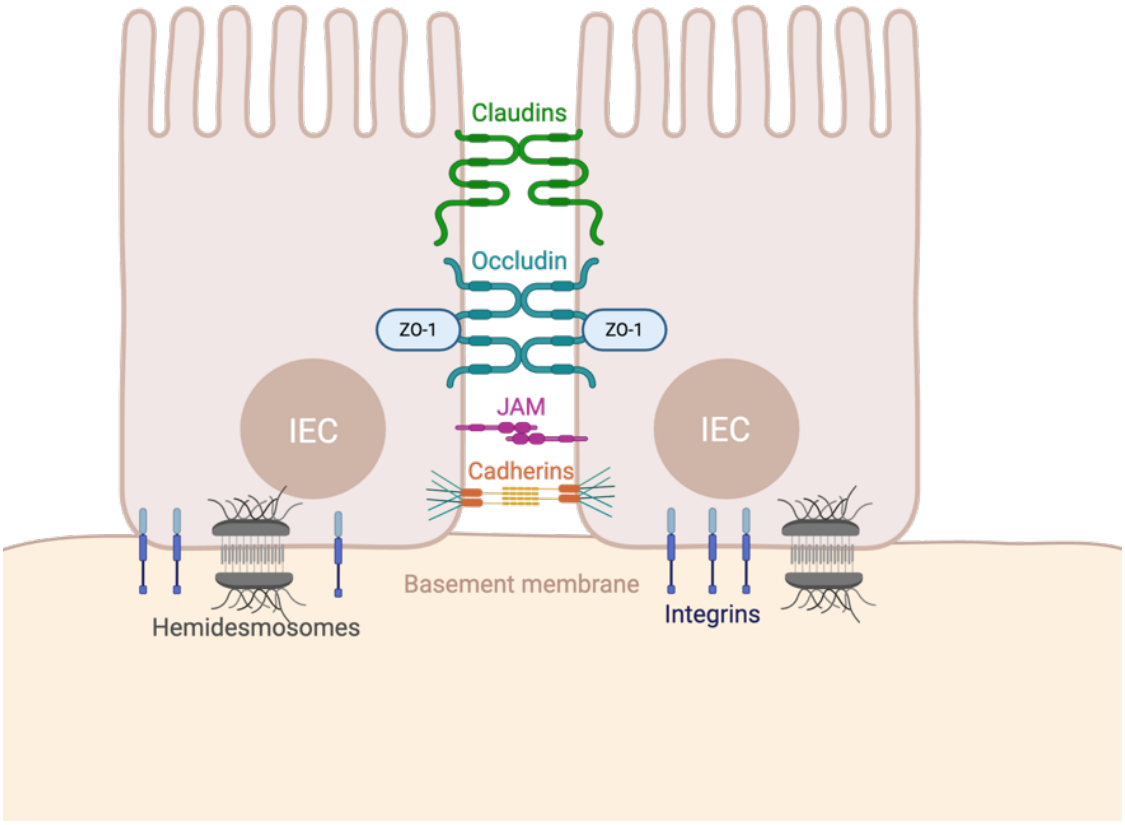

Figure 1-7 Apical junctional complex
Diagram showing the apical junctional complex, with examples of each of the structures important in maintaining barrier integrity. Created using BioRenderl.com.

Whilst all these molecules combine to create a strong intestinal barrier shown in Figure 1-7, permeability can be affected by numerous factors including microbiota, diet, stress, pathogens and autoimmune diseases. Commensal bacteria are able to strengthen the barrier through production of metabolites, tightening TJs and maintaining their structural integrity (Paradis et al., 2021). Pathogens have evolved ways to target the AJC and specific TJ proteins to support their ability to infect the host. Inflammatory cell signalling upon bacterial recognition, changes caused to commensal bacterial populations and causing stress and inflammatory responses by releasing harmful molecules are all ways by which bacteria can modulate cell adhesion. Each scenario could affect the expression of TJ genes, their phosphorylation and localisation or could alter cytoskeletal structures to disrupt the barrier (Paradis et al., 2021). For example, Astrovirus is able to increase intestinal permeability by delocalising occludin (Moser et al., 2007) and a toxin released by *Bacteriodes fragilis* can cleave

cadherin proteins (Wu et al., 1998). The proteins are also harnessed by pathogens in other ways; the intracellular parasite *T. gondii* is able to bind to extracellular loops of the occludin protein, supporting their cellular invasion (Weight et al., 2015) whilst SARS-CoV-2 can also harness occludin for cellular transmission (Zhang et al., 2023). In acute *H. polygyrus* infection, a recent publication found that TJ protein expression is significantly altered and intestinal permeability is increased (Mules et al., 2024a). Another study proposes that acetate, one of the ES molecules produced by *H. polygyrus,* is able to disrupt the claudin-7 protein to facilitate barrier breach by the helminth (Schälter et al., 2022a).

1.2.3 Barrier breach

A barrier breach is when the intestine becomes more permeable due to disruption of the barrier integrity, enabling substances and pathogens to cross the epithelium. Even though the intestinal epithelial barrier is highly effective at steady state, pathogens can disrupt this integrity by interfering with TJ proteins as discussed in section 1.2.2.3. Barrier breach can lead to translocation of bacteria or other pathogens from the lumen to the lamina propria (Di Tommaso et al., 2021). This then leaves a host susceptible to local and systemic infection and in severe cases, sepsis (Deitch, 2012).

In addition to the examples of pathogens that damage cell adhesion on the intestinal epithelium described previously, infections with pathogens such as parasites can also cause physical damage in other ways and allow bacterial translocation. One example of this is in host infection with *Toxoplasma gondii*. This parasite can cause intestinal damage by provoking an intense inflammatory response and researchers found that bacteria from the gut could then translocate and were identified in mLNs, the liver and the spleen (Hand et al., 2012a). Intestinal damage is frequently associated with helminth infections. When *T. muris* embeds in the intestinal epithelium, it causes intense local inflammation and damage. One study identified bacterial translocation and suggest that this is due to not only barrier damage but is directly facilitated by the movement of the helminths (de Oliveira et al., 2025a). In the *H. polygyrus* infection model, there is literature to suggest barrier breach may occur and

bacterial translocation has been proposed, although no evidence has been found for this yet (Gause and Maizels, 2016).

As well as disruption to the barrier, intestinal bacterial translocation can occur across an intact epithelium, when there is a large colonisation or due to weakened immune responses (Berg, 1999). The most common bacteria to affect the small intestine with high colonisation sometimes leading to translocation are *Escherichia coli, Aeromonas, and Klebsiella* species (Sorathia et al., 2025). In IBD, the persistent inflammatory profile causes disruption to the epithelial barrier, increasing the ability of bacteria to take advantage of a weakened barrier and colonise (Jin et al., 2022). Also, when a host has a weakened immune system due to primary infection with immunodeficiency viruses such as HIV or SIV, bacterial translocation is increased (Estes et al., 2010; Klatt et al., 2013). When the intestinal barrier is not disrupted, some pathogens are able to infect host cells intracellularly as a route into tissues (Berg, 1999); this is further discussed in section 1.3.1.

1.2.4 Microbiome

The gut microbiome is the collection of microorganisms residing in a host intestine and is composed of predominantly bacteria, but also fungi, viruses and other microbes (Sorboni et al., 2022). Although the microbiome comes into a contact with a range of virulent pathogens at any one time, most commensal microbes are fundamental to host survival. From fermenting and digesting food to protecting us from other pathogens, we rely on commensal bacteria in everyday life (Hou et al., 2022). As previously described, bacteria in the gut can contribute to strengthening intestinal barrier integrity and prevent colonisation by other virulent bacteria. They can also produce metabolites from food such as SCFA to contribute to immune regulation (Maciel-Fiuza et al., 2023).

Dysbiosis refers to the disruption of bacteria within the gut, either loss of bacterial diversity, lack of beneficial bacteria or overgrowth of pathogenic species. Several autoimmune diseases have been linked to dysbiosis, further demonstrating the importance of the commensal microbiota (DeGruttola et al., 2016). Additionally, pathogenic microorganisms are also able to alter the diversity of microbiota to their advantage. For example, certain bacterial

species including *Salmonella enterica* serovar Typhimurium and *Citrobacter rodentium* can promote inflammation in the intestine during infection which has been found to negatively impact the levels of beneficial commensal bacteria. This enables more space for the invading pathogen to colonise and hinders the protective role of the microbiota, thus increasing the chance of infection (Khosravi and Mazmanian, 2013). Viral infections are also capable of causing intestinal dysbiosis to favour their survival. During hepatitis infection, the virus is able to escape the immune defence mechanisms by promoting dysbiosis, although exact mechanisms of the virus to cause this dysbiosis remain unclear (Inoue et al., 2018).

On the other hand, pathogens may also increase the diversity of commensal bacteria. In the case of enteric helminth infection, this often occurs (Garcia-Bonete et al., 2023; Lee et al., 2014). Various studies have shown that *H. polygyrus* infection can alter the microbiota of its host during infection (Reynolds et al., 2014a; Su et al., 2018a; Walk et al., 2010a). Interestingly, while in C57BL/6 mice a significantly increased abundance in the bacterium *Lactobacillus* is shown during *H. polygyrus* infection (Reynolds et al., 2014a; Walk et al., 2010a), the most recent study by Su et al shows that Balb/C mice exhibit the opposite effect and show reduced levels of *Lactobacillus* (Su et al., 2018). Su et al further explore the mechanisms by which helminths alter microbiota in Balb/C mice and demonstrate that this process is Th2-dependent and involves suppression of protective responses to bacterial pathogens in the intestine by Tregs (Su et al., 2018a). Interestingly, with the promotion of *Lactobacillus* by *H. polygyrus* in C57BL/6 mice, the researchers found that the relationship is mutually beneficial as these bacteria increase susceptibility to *H. polygyrus* infection too (Reynolds et al., 2014a). Mechanisms for how the helminths promote the bacterial abundance in this paper are unclear, although they show that the Lactobacilli bacterium enhances Treg cell numbers and increase worm burden by limiting Th2 immunity (Reynolds et al., 2014a).

1.3 Bacteria

1.3.1 Bacterial infection

As discussed in the previous section, many bacteria in a host intestine are beneficial, however, it is important that the immune system is alert and ready to defend against pathogenic strains of bacteria. Therefore, there is a careful balance in the intestine so that defence and attacks against virulent species can be made whilst inhibiting responses against commensal bacteria. In general, the aim for bacterial pathogens is to multiply and colonise to increase their chances of survival. In the intestine, bacteria often translocate and invade the intestinal tissue either by paracellular (between epithelial cells) or transcellular (intracellular through epithelial cells) routes. Examples of bacterium which often use the paracellular route include *Vibrio cholerae* and *Pseudomonas aeruginosa* and in each case they can produce toxins to breakdown and disrupt the tight junction protein complexes, enabling their entry into intestinal tissue (Barreau and Hugot, 2014). The transcellular route involves intracellular invasion and is carried out by bacteria such as *Shigella* or *Salmonella*. There is evidence for *Salmonella* using specific receptors on the surface of M cells to enter these and cross the intestinal barrier (Hase et al., 2009). Although, there have also been studies proposing that they may also translocate through paracellular mechanisms disrupting tight junctions too (Broz et al., 2012; Jepson et al., 1995).

Different bacterial species can use a range of mechanisms for invasion and colonisation, known as virulence factors. Some species have outer capsules to protect themselves from host defences such as phagocytosis, other species use adherence molecules to attach to other cell surfaces as methods of invasion, and often bacterial species can produce toxins to weaken host cells, enabling their colonisation (Peterson, 1996). Even with each of these infective mechanisms, in the small intestine, the host has several layers of immune defence mechanisms in place to prevent and limit the colonisation of bacterial pathogens.

1.3.2 Antimicrobial responses

1.3.2.1 Physical / physiologic barrier

To prevent bacterial pathogens from causing infections, there are several physical barriers in place preceding the immune responses. As discussed in section 1.2, the epithelial barrier is composed of a range of specialised cells and involves tightly regulated junctions to carefully police what crosses from the lumen into the intestinal tissue. Also, the mucus produced by goblet cells creates an additional layer to prevent most bacteria from getting close contact with the barrier (Santaolalla and Abreu, 2012; Takiishi et al., 2017). As well as these mechanisms, the muscles in the small intestine work to stimulate continuous peristalsis, rhythmic contractions to push luminal contents along the intestinal tract. This not only aids in digestion but also continuously pushes bacteria in the lumen along and out of the host's system, preventing their ability to colonise and cause infection (Takiishi et al., 2017). Together, these functions make up the first line of defence in the small intestine. Pathogens successful in invading intestinal tissue are next met with innate and adaptive immune responses.

1.3.2.2 Innate

Upon recognition of invading pathogens by PRRs on the cell surface, or cytokine stimulation following another cell's response, antimicrobial defences are initiated. As described above, some epithelial cells including Paneth cells store antimicrobial proteins (AMPs) which can be released upon recognition and function to kill bacteria (Kinnebrew and Pamer, 2012). There are several types of AMP, each of which have different mechanisms of killing bacteria. They can interfere with bacterial cell wall structures or induce stress, and are frequently responsible for killing particular bacterial subsets; either gram-negative or gram-positive due to their mechanisms of killing (Hassan et al., 2022).

Neutrophils are often first responders to bacterial infection. They are an abundant cell type, small and quick moving, and they can rapidly release antimicrobial products including reactive oxygen species (ROS) which kill bacteria. Another kill mechanism they use is neutrophil extracellular traps (NETs), a projection of their DNA into the environment. This NET is able to trap

and kill bacteria (Mumy and McCormick, 2009). Neutrophils are also capable of phagocytosing pathogens although to a lesser extent than macrophages. Phagocytosis is the process by which immune cells can engulf other cell types; either invading bacteria or infected/ dying cells. Macrophages are the most phagocytic of immune cells and are very effective at eliminating bacterial cells via this mechanism. Macrophages also heavily contribute to the inflammatory response, secreting pro-inflammatory cytokines such as IL-12, IL-6 and TNF-α (previously discussed in section 1.1.3) (Yip et al., 2021). Despite this role in inflammation, macrophages are also critical for wound repair which aids in preventing further bacterial invasion across the intestinal barrier. Macrophages can either be tissue-resident or can be recruited to tissues upon infection. Monocytes circulate the blood and differentiate into macrophages once they migrate to the infection site. Complement is another important component of the innate defence against bacteria. In the gut components of this system are produced locally and work to combat pathogenic bacteria but not commensals (Wu et al., 2024). ILCs and NK cells are also quick to respond to pathogens. ILCs can produce cytokines to amplify the surrounding immune responses and mediate production of AMPs (Vivier et al., 2018). NK cells are effective at killing bacteria through the release of cytotoxic granules. They also secrete IFN-γ which is responsible for activating the antimicrobial responses fundamental in host defence against many bacterial species (Lieberman and Hunter, 2002). Additionally, the IFN-γ produced by NK cells is required to drive the adaptive Th1 response; enabling longer lasting and more effective immunity that just the innate response can provide. Likewise, DCs are also required to drive adaptive immunity, presenting antigen to T cells to initiate their differentiation and activation (Figure 1-5).

1.3.2.3 Adaptive

As shown earlier, when DCs present antigen to T cells, the outcome of the T cell differentiation depends on the cytokine signals given and the nature of the antigen presented. Th1 cells require IL-12 and also IFN-γ stimulation whilst Th17 cells are initiated by a combination of cytokines including TGF-ß, IL-6 and IL-23. Th17 cells are another subset of T helper cells which function to promote immune responses against extracellular pathogens, secreting IL-17, IL-22 and TNF-α. On the other hand, Th1 cells produce cytokines IFN-γ and TNF-α and are

induced to protect mainly against intracellular bacterial pathogens such as *Salmonella*. There is also evidence for combined Th1/ Th17 responses where these differentiated cell subsets can work inhibit or promote one another to together drive distinct immune responses (Damsker et al., 2010). Additionally, a subset of T cells positive for both IFN-γ and IL-17 production has been shown to provide protective immunity against both bacterial and fungal infections (Lin et al., 2009).

Th1 cells are a key subset involved in eliminating bacteria (Wu and Wu, 2012). They support the priming of CD8+ T cells by interacting with DCs to enhance their costimulatory molecule expression and cytokine production to help CD8+ T cell activation. Th1 cells can also produce cytokines such as IL-2 and IL-21 to promote and enhance activation of these cells directly (Sun et al., 2023). CD8+ T cells which have been primed and helped by the Th1 cells have enhanced efficacy with increased cytotoxic activity by their Fas ligands and granzyme release, and increased cytokine production amplifying overall innate responses against the bacteria (Ekkens et al., 2007; Sun et al., 2023). With the production of IFN-γ, Th1 cells also activate or super-activate macrophages, increasing their phagocytosis and killing of bacteria. Th1 cells are also essential for developing memory T cell responses (Sun and Bevan, 2003). Together, these studies highlight the importance of Th1 cells in contributing to antibacterial defence in the host.

Several studies provide evidence for Th17 responses, particularly IL-17 signalling being important in neutrophil recruitment and general protection against extracellular bacteria pathogens (Khader et al., 2009; Ouyang et al., 2008; Ye et al., 2001). Additionally, Th17 cells have been shown to induce AMP production by the epithelium (Peck and Mellins, 2010). In the gut, Th17 cells are required to protect against the mouse infecting bacterium, *C. rodentium* (Ishigame et al., 2009). Also, disruption of Th17 responses increase susceptibility to the bacterium, *Klebsiella pneumoniae* (Happel et al., 2005; Peck and Mellins, 2010). Some studies found that Th17 differentiation could be controlled by segmented filamentous bacterial (SFB) within the commensal bacteria population and are induced to maintain gut homeostasis (Atarashi et al., 2015; Ivanov et al., 2009; Schnupf et al., 2017). A particular paper demonstrated that C57BL/6 mice bought from different suppliers contained different levels of the SFB commensal

bacteria which in turn influenced Th17 cell proportions through inducing production of a specific protein, serum amyloid A (Ivanov et al., 2009). Overall these papers highlight that Th17 cells respond to and activate protective responses to extracellular bacteria.

1.3.3 Co-infection

Co-infection is when a host is simultaneously infected with two or more pathogen species (Dong and Xing, 2024). Due to the precise orchestration of host immune responses, each one tailored to the initiating pathogen, dealing with two types of pathogens at the same time can create a complicated environment. Depending on infection locations, the host may have to prioritise combating one infection first, or compromise host immune responses to fight both pathogen types. There is also a dynamic between the infecting pathogens whereby they try to manipulate host mechanisms to outlast other competing microbes. As previously described, *H. polygyrus* infection can induce hyperplasia of goblet and tuft cells in the host, as a mechanism of eliminating the worms. Single cell sequencing of the epithelium in a study by Haber et al confirmed this expansion of goblet and tuft cells, and highlighted the contrasting results of similar analysis of intestinal epithelium during *Salmonella* infection. In the bacterial infection, antimicrobial reprogramming, increased Paneth cells and increase in IECs were identified (Haber et al., 2017). This study reflects the diversity in responses dependent on pathogen type.

In areas where helminth infection prevalence is high, bacterial co-infections are frequent. There are a number of potential reasons for the high frequency of co-infections. Helminth infections are often spread through poor access to clean water, and other infections can spread this way too. Helminths are larger pathogens and cause disruption to barrier sites within a host, different pathogens require different responses to control them, and each microbe also secretes its own immunomodulatory molecules which could alter responses to other pathogens too. Another suggestion is that susceptibility to co-infection may be increased during helminth infection due to changes in nutrient availability. Many cases of helminth infection result in malnutrition and it has been shown that lack of nutrients can alter and impair immune system function in these cases (Shea-Donohue et al., 2017). In experimental models, this has also

been reflected. Mice fed reduced protein content in their diet or reduced overall calories showed inability to effectively drive Th2 responses, mice had lower antibody levels and were slower to clear parasites, resulting in worse infection outcomes (Ing et al., 2000; Shea-Donohue et al., 2017). In contrast, another paper found that vitamin deficiency resulted in impaired immunity to bacterial infections and enhanced ILC2 mediated anti-helminth immunity. The authors suggest that this is reflective of a selective activation of particular host responses due to nutrient deficiencies being commonly associated with helminth infection (Spencer et al., 2014).

Since helminths are much larger pathogens and their lifecycle can often involve a breach in barrier, this damage and the host response to the ongoing helminth infection could alter the outcome of infections with other pathogens. One study suggested that upon infection with both helminths and bacteria, these co-infections can mutually benefit each other by increasing both bacterial load and helminth burden and egg production (Lass et al., 2013). Research has focussed on modelling co-infections; in particular the *H. polygyrus* model has been used to study roundworm infection in various conditions. In a co-infection model of *H. polygyrus* and *C. rodentium*, one study found that the DCs primed by the helminth worsen bacterial infections, which the authors propose is due to their ability to inhibit development of Th1 immune responses or induce Th2 responses (Chen et al., 2006). Also, research using co-infection with *H. polygyrus* and S. Typhimurium resulted in worse disease outcomes with increased pathology and increased *Salmonella* colonisation due to limited neutrophil recruitment and chemokine recruitment compared with sole S. Typhimurium infection (Su et al., 2014a). The findings of this study were further supported by Brosschot et al who found that helminths increased *Salmonella* colonisation in the duodenum of mice and this colonisation persisted even after helminth clearance (Brosschot et al., 2021a). Another study demonstrated that *H. polygyrus* altered the metabolic environment in the small intestine which directly altered virulence gene expression in the bacteria and enhanced their ability to colonise (Reynolds et al., 2017a). In the case of viral infection, *H. polygyrus* can also worsen infection outcome. In a co-infection model using *H. polygyrus* and West Nile Virus (WNV), dual-infected mice had increased mortality when compared with sole WNV infection. This was due to the *H. polygyrus* associated changes in gut morphology

and the ability of commensal bacteria to translocate in the intestine, which increased apoptosis of WNV-specific CD8+ T cells, which would otherwise target and kill the virus (Desai et al., 2021). This impaired immunity to virus infection is supported by another study where researchers found that *T. Spiralis* infected mice had increased virus genome copies in the intestinal tissue and could not provide effective antiviral immunity against intestinal murine norovirus infection (Osborne et al., 2014).

In contrast, there is also evidence for systemic beneficial immune effects by helminths, influencing infection outcome of distal pathogens. Recent research has suggested a new subset of T cells, virtual memory T (Tvm) cells, which have a memory cell phenotype having undergone partial differentiation, but do not have experience with antigen. In contrast to the other studies mentioned, this study suggested that these Tvm cell populations are expanded by helminth infection, undergoing antigen-independent expansion upon direct IL-4 signalling (Lin et al., 2019). They also show that this expansion of cells are able to provide protection against additional infection with the bacterium, *Listeria monocytogenes* as shown by reduced bacterial load (Lin et al., 2019). In the lung, research shows that helminths can cause structural and immune changes. Goblet cells in the lung undergo hyperplasia and Th2 cytokines are increased as well as the overall number of Th cells and upon co-infection in the lung with bacteria, neutrophil recruitment is enhanced, resulting in increased survival rates in mice (Long et al., 2019). Additionally, another study shows that *H. polygyrus* infection causes an accumulation of *H. polygyrus* specific CD4+ Th2 cells in the skin of mice which accumulate and persist after infection, impairing recall responses to mycobacterium (Classon et al., 2022). This study implies that the host immune response to helminths tries to protect other host barrier sites from the invading parasite. Overall, these each show that helminths can influence not only local immunity but also systemic responses to other infections.

1.4 IFN-γ

1.4.1 IFN-γ production, function and signalling

One aspect that is quite common in these co-infection models is cytokine crosstalk between the immune responses against each pathogen, and IFN-γ (also known as type II IFN) is often a key mediator of this crosstalk. As one of the main pro-inflammatory cytokines and specifically a key driver of the Th1 response, IFN-γ can be produced by several cells and plays a key role in activating and instructing many different cell types to orchestrate inflammatory responses. It is mainly produced by activated T cells, natural killer (NK cells) and type 1 ILC1s, and under certain conditions has been shown to be produced at low levels by other innate cells including DCs (Darwich et al., 2009; Ivashkiv, 2018; Tau and Rothman, 1999; Vremec et al., 2007). IFN-γ plays a role in activating and super-activating macrophages, which enhances IL-12 and IL-18 secretion, activating NK cells to secrete IFN-γ, thus providing a positive feedback loop for production (Tau and Rothman, 1999). IFN-γ Is a homodimer, comprised of two polypeptide chains. The IFN-γ receptor is composed of two subunits, IFNGR1 and IFNGR2 which together signal through the JAK/STAT pathway (de Weerd and Nguyen, 2012; Walter et al., 1995). IFN-γ binds to the high affinity alpha subunit, IFNGR1, which then recruits IFNGR2, inducing a confirmational change in both receptor components. This brings together the protein-tyrosine kinases, Janus kinase 1 (JAK1) and JAK2, causing their activation. The active JAK1 and JAK2 then phosphorylate the tyrosine residue (Tyr701) of STAT1, enabling this molecule to translocate to the nucleus and bind to the IFN-γ activation site (GAS) in the promoter regions of target genes (Figure 1-8) (De Benedetti et al., 2021; Marsters et al., 1995; Platanias, 2005). Promoter binding activates the transcription of these target genes, the interferon-stimulated genes (ISGs), which encode chemokines, phagocytic receptors, antigen presentation molecules and several antibacterial and antiviral molecules (Ivashkiv, 2018).

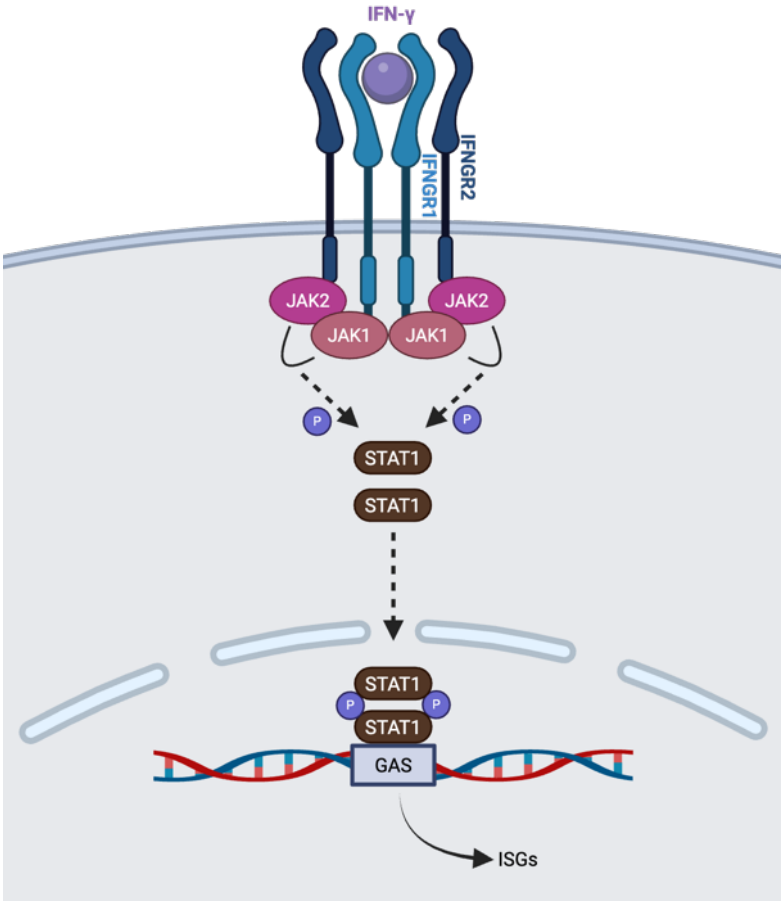

Figure 1-8 IFN-γ signalling pathway.
The JAK/STAT signalling pathway downstream of IFN-γ and IFNGR1 binding. Created with BioRender.com.

IFN-γ is a multi-functional molecule, able to promote activation of innate immune cells such as macrophages, neutrophils and NK cells, enhance antigen presentation, promote Th1 cell differentiation and, depending on the setting, can enhance inflammation or promote tissue repair. Macrophages are crucial defenders of many infections, undergoing phagocytosis, acting as antigen presenting cells and producing molecules such as inducible nitric oxide synthase (iNOS) to kill invading pathogens. The IFN-γ cytokine is the main macrophage activating factor and therefore is key in all of these functions (Tau and Rothman, 1999). IFN-γ is able to enhance not only macrophage antigen presentation through activation, but also widespread antigen presentation through upregulation of major histocompatibility complex I (MHC I) and other molecules involved in processing antigen (Zhou, 2009)(Baldeon et al., n.d.; Naoshi Hikawa et al., 1996; Tau and Rothman, 1999). Depending on other environmental

stimuli, IFN-γ can also promote recruitment and activation of neutrophils. Studies have shown that IFN-γ can enhance chemokine expression, in turn recruiting neutrophils to infection sites (Sun et al., 2007). Additionally, IFN-γ signalling in neutrophils can enhance phagocytic function, increase release of enzymes and ROS and promote further cytokine production to amplify the inflammatory response (Marchi et al., 2014). NK cell produced IFN-γ can activate several antimicrobial responses which control bacterial infections; including release of reactive oxygen species (ROS), production of AMPs and pro-inflammatory cytokines and enhanced function of phagocytic and antimicrobial immune cells including neutrophils and macrophages (Jessop et al., 2020; Lieberman and Hunter, 2002). Also, the cytokine is able to self-amplify its production through macrophage activation and subsequent increased IL-12 secretion, further activating other NK cells to produce more IFN-γ (Hodge-Dufour et al., 1998; Lin et al., 2021). In section 1.1.3.3, Figure 1-5 shows the DC interactions and signalling process for T cell differentiation. IFN-γ is one of the main cytokines involved in promoting Th1 differentiation, further amplifying inflammation and bacterial/ viral pathogen targeted killing.

In addition to promoting inflammation and pathogen clearance, several studies provide evidence for IFN-γ involvement in tissue repair. One study by Kanno et al, found that IFN-γ KO mice had defective wound healing both in the time taken and in the strength of the wound repair when compared with wild type (WT) mice (Kanno et al., 2019). On the contrary, an earlier study had described that IFN-γ KO led to faster wound healing due to antagonistic effects that IFN-γ can have on the TGF-β regulatory cytokine pathway in the reparative process (Ishida et al., 2004). In another setting, a study found that IFN-γ signalling was upregulated upon cartilage injury and was required for regeneration of the cartilage tissue (Kim et al., 2024). These data suggest that the surrounding environment and the tissue site can determine the outcome of IFN-γ signalling. The specific role of IFN-γ in the small intestine is further discussed in section 1.4.2. Each of the IFN-γ functions discussed so far demonstrates the importance of this cytokine as a fundamental part of the immune system, from initiation of inflammation to tissue resolution.

1.4.2 IFN-γ in the intestine

The previous section demonstrates the diverse range of functions of IFN-γ. In the context of the small intestine, IFN-γ contributes to immune responses to control pathogen invasion and can also promote intestinal homeostasis. The literature discussing the influence of IFN-γ on the intestinal epithelium remains controversial. Research has found that proinflammatory changes driven by IFN-γ can dysregulate homeostasis in the gut (Omrani et al., 2023); some studies show a IFN-γ driven hyperproliferation of epithelial cells and other studies suggest that IFN-γ can inhibit proliferation and cause apoptosis of the epithelial cells each discussed further below.

As part of the proinflammatory role that IFN-γ plays in pathogen clearance in the small intestine, IFN-γ can upregulate AMP expression through its STAT signalling pathway (Yue et al., 2021a). It can stimulate the release of chemokines to recruit immune cells to the intestinal tissue upon infection and it can then directly activate them to carry out their defensive antimicrobial functions (Evaristo and Alegre, 2013; Gomez et al., 2015). In the small intestine, microbiota can also impact IFN-γ production to enhance pathogen clearance. One study illustrates a microbiota driven production of IFN-γ during *Salmonella* Typhimurium infection in mice which is able to provide host protection, prolonging survival and reducing colonisation of the *Salmonella* bacterium (Thiemann et al., 2017). Molecules involved in the digestive process have also been shown to influence IFN-γ levels. For example, bile acids, SCFA butyrate and the amino acid, tryptophan, can each decrease IFN-γ production. On the other hand, IFN-γ can also influence microbiota colonisation. For example, the bacterium *Akkermansia muciniphila* has known benefits in host metabolism and has been shown to be regulated by IFN-γ in both mice and humans (Greer et al., 2016).

As described in section 1.2.2.2, Wnt signalling is required for intestinal proliferation. Nava et al found that IFN-γ is able to regulate the Wnt signalling pathway and prevent excessive proliferation of IECs with long term exposure (Nava et al., 2010). Research has also found that IFN-γ produced by T cells can directly signal to intestinal stem cells to upregulate proapoptotic gene expression and their cell death (Takashima et al., 2019). This apoptotic

reprogramming by IFN-γ has also been shown in a recent study where they suggest NKT cell production of IFN-γ induces these changes (Lebrusant-Fernandez et al., 2024). One paper shows evidence that IFN-γ induced apoptosis is a result of the inhibited Wnt signalling pathway (Laukoetter et al., 2008). Another study supports this regulatory role, showing that IFN-γ can reduce Paneth cell numbers and hinder IEC regeneration (Eriguchi et al., 2018). Interestingly, this paper also proposes a role for IFN-γ in modulating the markers expressed by the intestinal stem cells with loss of Lgr5 and Olfm4 expression upon IFN-γ treatment (Eriguchi et al., 2018). In contrast to the findings above, the same study by Nava et al found that, in short term exposure of an intestinal epithelial cell line to IFN-γ, the Wnt signalling pathway is induced and cells are hyperproliferative (Andrews et al., 2018a; Nava et al., 2010). In each case, IFN-γ often works synergistically with TNF-α to have effects on the intestinal epithelium as was shown in both this study and others (Nava et al., 2010; Woznicki et al., 2021). Additionally, several studies have shown that IFN-γ can increase intestinal permeability by disrupting the TJ protein structures (Beaurepaire et al., 2009; Rahman et al., 2018). However, Yue et al found that IFN-γ instead improved barrier function in the gut by strengthening the distribution of ZO-1 (Yue et al., 2021a). Again, each of these factors may be dependent on duration of exposure to this cytokine and on the additional environmental stimuli within the gut.

1.4.3 IFN-γ in helminth infection

IFN-γ is a known driver of the inflammatory response and has been identified in helminth infections, alongside type 2 immune responses, long before this project (Urban et al., 1996). On the theme of epithelial effects of IFN-γ signalling, in helminth infection, IFN-γ has been shown to induce proliferative changes to the intestinal epithelium. Artis et al demonstrated that IFN-γ production induced by the *T. muris* parasite promoted epithelial cell hyperproliferation (Artis et al., 1999b). Cliffe et al add to this finding by showing that the CXCL10 chemokine induced by IFN-γ during infection controls epithelial proliferation (Cliffe et al., 2005). Additionally, in *H. polygyrus* infection, IFN-γ has been identified to facilitate the upregulation of proliferative cells indicated by Sca1 expression and facilitate the reversion of cells to a foetal-like programming (Nusse et al., 2018a) (previously discussed in more detail in section 1.2.2.1).

Interestingly, through experimental IFN-γ depletion, a study revealed that IFN-γ is required for successful chronic infection with the parasite *T. muris* (Else et al., 1994a). This is because IFN-γ is able to promote a Th1 response and limit the expansion of the Th2 response to prevent parasite clearance (Dea-Ayuela et al., 2008). This finely balanced Th1/ Th2 response to determine the outcome of infection is also mediated by B cells in the mLNs (Sahputra et al., 2019). In *H. polygyrus* infection, similar results were found in several studies although IFN-γ was not required for chronic infection. IFN-γ and the subsequent Th1 cells were shown to hinder the Th2 responses, and blocking IFN-γ reduced worm counts, egg counts and parasite fecundity in later infection (Kapse et al., 2022a). A previous study in our laboratory demonstrated that IL-10 regulates and maintains IFN-γ levels during the later stages of *H. polygyrus* infection (Webster et al., 2022). This research was mirrored in the past with *Schistosoma mansoni* infection where researchers also found that IL-10 was responsible for regulating IFN-γ and Th1 levels (Montenegro et al., 1999).

Whilst evidence for IFN-γ in early *H. polygyrus* infection has been published by both our laboratory and others, the exact role of this cytokine remains unknown. One study found that increased IFN-γ expression in *H. polygyrus* also coincides with proliferation of enteric glial cells (EGCs). These cells function to control gut motility and play a role in the immune responses and wound repair. CXCL10 expression is increased in the EGCs and together the IFN-γ and EGC-CXCL10 signalling is able to promote tissue repair in early helminth infection (Progatzky et al., 2021). In line with this outcome, another study suggested a role for IFN-γ signalling in recruiting NK cells to the helminth infection sites and found that the NK cells were fundamental for effective tissue repair in *H. polygyrus* infection (Gentile et al., 2020). Helminth infection has been proposed to increase susceptibility to further infections from other pathogens both bacterial and viral (Donohue et al., 2019; Salgame et al., 2013). Additionally, there is evidence of increased susceptibility to further infection in *H. polygyrus* mouse models discussed in section 1.3.3. Although IFN-γ is commonly associated with antimicrobial responses and clearance of both bacteria and viruses, this cytokine has not been explored in this context during *H. polygyrus* infection.

1.5 Hypothesis and aims

The main focus of this thesis is to unravel the early shaping of the immune responses provoked by IFN-γ during helminth infection.

Whilst *H. polygyrus* itself is a well-defined helminth infection model, the threats posed whilst the worm burrows into the intestinal wall have not been thoroughly explored. With the helminth crossing the barrier, bacterial access to host tissue and easier colonising opportunities may arise. Certain immune changes have been characterised during *H. polygyrus* infection, although most research has focussed on later timepoints. More recent studies have confirmed IFN-γ production at day 2 of infection, with some of the roles for this cytokine suggested to be in epithelial and stromal cell changes. However, the involvement of IFN-γ in local protection from secondary infection has had little focus in the literature. Taken together we hypothesised that **IFN-γ is produced in early *H. polygyrus* infection to promote and provide local protection against bacteria which might otherwise invade the host via helminth barrier breach points**. To test our hypothesis, we addressed three main aims:

1. To examine barrier breaches and intestinal changes during early *H. polygyrus* infection. The lifecycle of this helminth is known to have a stage in which it lives in the intestinal tissue, and hence the parasite migrates across the intestinal barrier twice, although little is understood regarding its mechanisms. We aim therefore to investigate evidence of barrier disruption during early infection and monitor antimicrobial immune changes which may be provoked upon reduced barrier integrity.

2. To determine the role of IFN-γ and its potential involvement in antimicrobial defence at day 2 of *H. polygyrus* infection. Recent research shows evidence for IFN-γ production at day 2 of *H. polygyrus* infection, although the role it plays in the helminth response is not well understood. To investigate this, an anti-IFN-γ antibody will be used to neutralise IFN-γ signalling in *H. polygyrus* infected mice and immune and epithelial changes will be monitored.

3. To investigate whether bacterial presence is required for production of

IFN-γ and antimicrobial defence responses in early *H. polygyrus* infection. We hypothesise that helminth migration across the intestinal wall can create an entry point for bacteria, enhancing susceptibility to co-infection. To investigate whether commensal bacteria stimulate immune responses, broad-spectrum antibiotics will be given to *H. polygyrus* -infected mice to deplete microbiota and assess the changes in intestinal immunity.

Overall, focusing on these aims should enhance our understanding of the intestinal environment in early *H. polygyrus* infection, specifically characterising a role for IFN-γ and broadening our understanding of how this cytokine shapes the local immune response during helminth infection.

Chapter 2 Materials and Methods

2.1 Mice

Female C57BL/6 mice aged between 7-14 weeks (purchased from Envigo, UK) were used throughout this project. They were caged relative to their sex and kept in individually ventilated cages under the standard animal housing conditions in accordance with the University of Glasgow. All procedures used were performed in line with UK Home Office regulations which have been approved by the University of Glasgow Ethics committee and under UK Home Office Licenses (Project number PP4096415, Rick Maizels or Project Number PP2894536, Simon Milling). Mice were euthanised by cervical dislocation or inhalation of carbon dioxide and in both cases the femoral artery was cut for confirmation of death.

2.2 Infections and treatments

2.2.1 *Heligmosomoides polygyrus* infection

Claire Ciancia and subsequently Anne-Marie Donachie (Maizels laboratory, University of Glasgow) were responsible for maintaining, preparing and counting the *Heligmosomoides polygyrus* lifecycle. This was carried out using previously published methods from the same laboratory (Johnston et al., 2015). Upon arrival to the animal unit, mice were given a minimum of 5 days to acclimatise before infections were induced. In water, L3 larvae were prepared at a concentration of 1 larva/μL. Experimental mice were infected with 200μL of 200 L3 larvae by oral gavage (round ball stainless steel tip; Size:24G). In experiments lasting for a week or more, mice were weighed before infection and on days 7 and 8 post infection to monitor their health.

2.2.2 IFN-γ monoclonal antibody blockade

To block IFN-γ signalling *in vivo*, we used a purified InVivoMAb rat anti-mouse IFNγ (BioXcell #BE0055) and as an isotype control, InVivoMAb rat IgG1, anti-horseradish peroxidase (BioXcell #BE0088). Stocks of both the IFN-γ mAb and the isotype control were prepared at a concentration of 2.5mg/mL in sterile Phosphate buffered saline (PBS). Each mouse received a total volume of 200μL

(equivalent to 500µg) of the appropriate antibody via intraperitoneal injection (26.5G needle). The treatments were administered on days -1 and 2 of *H. polygyrus* infection, and the mice were culled at various timepoints up to day 15 of infection. Throughout the study, *H. polygyrus* infected mice were housed together and separate to uninfected mice. Within cages, a mixture of anti-IFN-γ and isotype treated mice were housed together to limit variation between experimental groups as much as possible.

2.2.3 Antibiotics

Mixed in with their drinking water, mice were given a combination of ampicillin (1g/litre)(Sigma), metronidazole (1g/litre)(Sigma), neomycin (1g/litre)(Sigma), gentamicin (1g/litre)(Sigma), and vancomycin (0.5g/litre)(Wockhardt), a range of broad spectrum antibiotics (ABX) sweetened with 10 sweeteners per litre (Sweetex). This combination was previously optimised and established to be successful and effective in the laboratory and was used in published research (Scott et al., 2018).The ABX were given for up to 14 days and replaced with a fresh mixture every 3-4 days whilst control mice were provided with drinking water plus the 10 sweeteners. If any mouse weight dropped below 90% of their start weight, all mice from each sample group were supplemented with soft food diet and baby food to ensure consistent treatment. The reason for weight loss during antibiotics supplementation experiments is often due to uptake of less fluids, therefore the drinking water of their respective cage was mixed with their usual food, and a consistent amount of baby food (Ella's Kitchen) was added and mixed to encourage uptake of fluid.

2.3 Histology

2.3.1 Processing, Embedding, Sectioning

Small intestinal tissue was collected and processed as Swiss rolls or 1cm sections of the duodenum was collected. To form Swiss rolls, the top two thirds of the small intestine, the duodenum and jejunum were collected. Fat tissue was removed using tweezers and the intestinal tissue was gently washed through with 5mL of PBS. The intestine was inverted onto a kebab stick and placed in 10% neutral buffer formalin (NBF) for 5 hours and then a surgical blade was used to cut open the small intestinal tissue along the length of the kebab stick. Using

a toothpick, the intestinal tissue was then rolled into a Swiss roll shape so that the proximal intestine was located at the outer-most part. A needle was used to secure the intestinal tissue in place which was then placed in a cassette and back into the NBF. In total any collected intestinal tissue was left in 10% NBF for 24 hours and transferred to 70% ethanol until processed in a Leica ASP 300 (Leica Biosystems) and embedded in paraffin wax. The samples were sectioned at a thickness of 5 microns using a Shandon Finesse 325 rotary microtome (Thermo Fisher Scientific) and collected on frosted microscope slides, then ready for staining.

2.3.2 Staining

Firstly, samples were placed in a 60°C oven for 35 minutes to melt wax surrounding the tissue. In each case, slides went through a deparaffinisation and rehydration process before further staining. For Haematoxylin and eosin (H&E) staining, slides were taken through a series of reagents with Haematoxylin nuclei stain and Eosin a cytoplasm and extracellular matrix stain. (The preparation for staining slides from the Leica ASP 300 tissue processor stage onwards and occasionally the H&E staining was often carried out by staff in the histology core.) For saffron gram-staining, gram staining was used to identify bacteria and saffron was used as a counter-stain to improve tissue visualisation. The protocol was adapted from one shared by Dr Constance Finney and Lori Goodbrand, University of Calgary. The reagents used for immersion of slides were Crystal violet, Lugol's iodine, decolouriser, 2.5mg/mL Safranin-O and alcoholic saffron. In each staining procedure, slides were then dehydrated and mounted with glass cover slips using DPX mounting medium (CellPath). Slides were imaged on the NanoZoomer-SQ Digital slide scanner (Hamamatsu) and analysed using QuPath (Version 0.5.1) (Bankhead et al., 2017). In each case, these slides were analysed with a minimum of 3 slides per treatment group.

2.3.3 EdU staining

For this experiment, the Click-iT EdU Cell Proliferation Kit (Invitrogen) was used and contained all reagents required for *in vivo* EdU delivery and staining assays after sample processing. 40 hours prior to the cull, mice were administered with 200µL of 0.75µg/µL EdU diluted in PBS by intraperitoneal (IP) injection. After the

cull, the small intestine was rolled into a Swiss roll, processed, paraffin embedded and sliced as described (section 2.3.1).Using the Click-iT EdU Cell Proliferation Kit (Invitrogen), staining was carried out following manufacturer guidelines. Slides were imaged on the EVOS FL Auto 2 microscope (Thermo Scientific™) and analysis was carried out on QuPath (Version 0.5.1). For analysis, 20 villi were randomly selected from each microscope slide (one per mouse) and the length was measured. Additionally, the length from the base of the crypt to the top of the cells positive for EdU stain in the villus were measured as well as the length to the bottom section of the positively stained cells. This enabled calculation of the percentage of each villus positive for EdU staining and the distance at which stained cells were located from the base could be used to reflect proliferation since EdU labelling.

2.3.4 Scanning electron microscopy

Intestinal samples were collected from mice at days 8 and 21 post-infection with *H. polygyrus*. They were prepared and imaged by Dr Leandro Lemgruber Soares and my supervisor, Georgia Perona-Wright using scanning electron microscopy (SEM).

2.4 RT-PCR

2.4.1 Sample collection

Samples taken for ribonucleic acid (RNA) extraction were either 1cm cuts from the top of the duodenum with gut contents and fat removed, or isolated granuloma tissue. For the isolated granuloma tissue, worms were identified as red dots on the small intestine at day 7 (Figure 2-1). A small area surrounding the identified worm (maximum 0.3cm diameter) was cut out of the small intestine (Granuloma, G). Similar size tissue was taken from the same area where no worms were visualised (Non-granuloma, NG) and the same amount of tissue of comparable sizes were cut and taken from the naïve mice.

Figure 2-1 Representative image of small intestine at day 7 post *Hpb* infection.
Orientation duodenum (left) to jejunum (right), red arrows indicate worm (red dots) which are cut and isolated from tissue. (Photo also used later in Figure 3-1).

2.4.2 RNA extraction

Tissue samples were collected and stored in 700µL of RNAlater (Thermo Fisher Scientific) at 4˚C for up to 1 month. The fresh tissue was transferred to 700µL of Qiazol (Qiagen), with a 5mm steel ball and homogenised by the TissueLyser (Qiagen) for 2 minutes at 25Hz. Subsequently, the RNA extraction was carried out using the RNeasy Mini Kit and DNase protocol (Qiagen) as per the instructions of the manufacturer. 50µL of nuclease-free water was used to elute the RNA and quality, purity and concentration were analysed using the Spectrophotometer, NanoDrop-1000 (Labtech International).

2.4.3 Reverse transcription

Conversion to cDNA was then performed with 1000µg of RNA using the High-Capacity cDNA Reverse Transcription Kit as per the instructions (Applied Biosystems). The prepared samples were run on the Veriti Thermal Cycler (Applied Biosystems) using the conditions listed in the Applied Biosystems protocol, shown in Table 2-1. Once complete, the samples were diluted 1:20 with nuclease-free water and stored at -20˚C.

Settings	Step 1	Step 2	Step 3	Step 4
Temp.	25°C	37°C	85°C	4°C
Time	10 minutes	120 minutes	5 minutes	Hold

Table 2-1 Thermocycler parameters for reverse transcription protocol.
Table from Applied Biosystems 'High Capacity cDNA Reverse Transcription Kit'.

2.4.4 Quantitative RT-PCR

384-well PCR plates from Applied Biosystems were prepared by loading samples in triplicate. Master mixes were prepared, and each well was loaded with 4µL of the prepared cDNA samples, 5µL of PowerUP SYBR Green Master Mix (Thermo Fisher Scientific) and 1µL of the 10µM forward and reverse primer pair solution. Table 2-2 shows the primer sequences used for amplification of each gene of interest with ribosomal protein-S29 (RPS29) being the housekeeping gene used for normalisation of the values during analysis. The plate was run on the QuantStudio 7 Flex Real-time PCR System (Thermo Fisher Scientific) in the following conditions: 2 minutes at 50°C and 2 minutes at 95°C for the initial holding stage, a subsequent 15 seconds at 95°C and 1 minute at 60°C for the 40 cycles of the PCR stage repeated for 40 cycles. Finally, for validation, a melt curve was produced with 15 seconds at 95°C, a minute at 60°C and a further 15 seconds at 95°C. Ct values were normalised to RPS29 and averages were taken from the triplicate repeats with the $2^{-\Delta\Delta CT}$ method used for analysis (Livak and Schmittgen, 2001).

Gene	FW Primer Sequence	REV Primer Sequence
Ang4	CTCTGGCTCAGAATGTAAGGTACGA	GAAATCTTTAAAGGCTCGGTACCC
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
Cldn7	CTGCCTTGGTAGCATGTTCCTG	CCAGCCGATAAAGATGGCAGGT
Ifn-γ	TGAGTATTGCCAAGTTTGAG	CTTATTGGGACAATCTCTTCC
Il4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
Lcn2	AAGGCAGCTTTACGATGTACAGC	CTTGCACATTGTAGCTGTGTACC
Cdh2	AGGTAGCTGTAAACCTGAGC	CTTGGCAAGTTGTCTAGGGA
Nos2	CCCCGCTACTACTCCATCAG	CCACTGACACTTCGCACA
Ocln	CTCCCATCCGAGTTTCAGGT	GCTGTCGCCTAAGGAAAGAG
Relma	TATGAACAGATGGGCCTCCT	GGCAGTTGCAAGTATCTCCAC
Rps29	ACGGTCTGATCCGCAAATAC	CATGATCGGTTCCACTTGGT
Sprr2a	CCTTGTCCTCCCCAAGTG	AGGGCATGTTGACTGCCAT
Zo1	ACTCCCACTTCCCCAAAAAC	CCACAGCTGAAGGACTCACA

Table 2-2 List of the primer sequences used for RT-PCR
Ordered from Thermo Fisher Scientific or IDT and shown 5'-3'.

2.4.5 Bacterial RT-PCR

Spleen, liver, mLNs (avoiding adjacent LNs that drain the large intestine) and 1cm of the distal small intestinal tissue was collected in 700uL RNAlater. In a sterile cell culture hood, samples were dipped in absolute ethanol to sterilise their outer surface and subsequently, tissue was cut into smaller pieces and according to the manufacturer's instructions DNA was extracted from samples using the DNeasy Blood & Tissue kit (QIAGEN). The ND-1000 (Labtech International) spectrophotometer was used to measure the DNA concentration of the samples, and they were diluted to a concentration of 5ng/µL for RT-PCR. The plates were set up and run in the same conditions as described above (section 2.4.4). Since DNA concentrations were kept consistent between samples as loaded into the plate, the total bacteria was measured using pan-bacterial primers (forward: CGGTGAATACGTTCCCGG, reverse: TACGGCTACCTTGTTACGACTT)(Doonan et al., 2019b).

2.5 Protein Measurements

2.5.1 Sample collection

To measure proteins in the gut, sections of approximately 5cm was cut from the ileum of the small intestine of the mice and tweezers were used to squeeze gut contents out. The contents or in some cases fresh faecal samples were collected into an Eppendorf containing 700µL of PBS. Samples were processed through immediate homogenisation with a 5mm steel ball (Qiagen) using a TissueLyser (Qiagen) for 2 minutes at 25Hz. Samples were then centrifuged at 12,000xg for 15 minutes, and the supernatant collected and stored at -80°C.

To measure proteins secreted by gut tissue, granuloma and non-granuloma tissue were collected as described (1.4.1) and incubated for 24hours at 37°C in 1mL R10 media, an RPMI 1640 1X with addition of FBS, L-glutamine, Pen-Strep and beta-mercaptoethanol. The supernatant was collected and stored at -20°C.

To measure proteins in blood serum, blood was collected via cardiac puncture and samples were left at room temperature (RT) for 1-2 hours to clot. They were then centrifuged at 1000xg for 20 minutes to separate serum and clot. The sera samples (upper, clear liquid layer) were collected and stored at -20°C.

2.5.2 Calprotectin ELISA

An enzyme-linked immunosorbent assay (ELISA) was carried out using the Mouse S100A8/S100A9 Heterodimer DuoSet ELISA kit (R&D Systems). The protocol was carried out as per the manufacturer guidelines (R&D Systems). Supernatants collected from gut contents as described above and were diluted to appropriate concentrations by testing titrations until samples were comfortably within the range of the standard curves. Plates were read at 450nm using a VersaMax plate reader. Analysis was performed in GraphPad Prism (version 9 or 10). Blanks were subtracted from each of the sample readings to remove background. A standard curve was plotted, and values of the calprotectin concentrations extrapolated. Total protein concentration in each sample was also measured via a BCA assay (see below), and to normalise, calprotectin results were divided by the total protein concentrations.

2.5.3 BCA

To measure total protein concentration in samples, a bicinchoninic acid (BCA) assay was used. The Pierce™ BCA Protein Assay Kit (Thermo Scientific) was used, according to the manufacturer's guidelines. Plates were read at 605nm (Accuris™ SmartReader™ 96) and again, analysis was carried out using GraphPad Prism. Blanks were subtracted from each of the sample and standard readings to remove background. The standard curves were plotted and samples were extrapolated to provide protein concentration in µg/mL.

2.5.4 ELISA detection of commensal bacterial antigens

As an alternative measure of intestinal permeability, total Ig specific for commensal antigen was determined by ELISA as this should indicate whether commensal bacteria were able to access blood and systemic sites. The protocol used was adapted from a published study (Hepworth et al., 2013) and from an optimised format shared in communications with Andrew MacDonald's laboratory, University of Edinburgh. Caecal contents were taken from 3 uninfected, adult mice unrelated to our experiments but housed in the same conditions. 1mL of PBS and a 5mm steel ball were added to the caecal contents in an Eppendorf, and the tissue was vortexed and then lysed for 2 minutes at 25Hz using the TissueLyser (Qiagen). The tube was briefly centrifuged at 1000xg

for 10 seconds to remove larger caecal contents. 700µL of the supernatant was removed from above the pellet and added to a fresh tube with 600µL of fresh PBS and the tube was spun at 5000xg for 1 minute. The pellet was resuspended in 2mL PBS and sonicated for 2 minutes using Ultrawave steriliser. After sonication, the sample was centrifuged at 10,000xg for 10 minutes and supernatant was extracted and stored at -20°C. This sonicated supernatant is the antigen extract from the caecal contents, a BCA assay was carried out as described above to determine the antigen protein concentration.

To test whether antibodies reactive to caecal content antigens were present in the serum of experimental mice, I performed an ELISA on an antigen coated plate. A 96 well ELISA plate (R&D Systems) was coated with 0.1µg of caecal content antigen (50µL at 2µg/mL) and incubated at 4°C overnight. The plate was washed with wash buffer (0.1% Tween/PBS) and blocked with 100µL blocking buffer per well (1% BSA in PBS) (incubated for 3hrs at 4°C). Serum from experimental mice was collected and diluted 1:50 in blocking buffer as was recommended in the shared protocol, 100µL was added to each well and the plate was incubated at 4°C overnight. The plate was then thoroughly washed with wash buffer. An anti-mouse Ig conjugated with alkaline phosphatase (2BScientiifc) was diluted 1:2500 in blocking buffer and added at 100µL per well, and the plate incubated at RT for 1 hour. This Ig antibody was used to assess the total antibody reactivity to antigens found in caecal contents. The plate was washed, 75µL of PNPP Substrate solution (Thermo Scientific) added per well, and the plate incubated at RT in the dark for 25 minutes for colour to develop. The plate was then read at 405nm on the plate reader (Accuris™ SmartReader™ 96). For analysis, a BCA was carried out to determine total protein in the serum samples, and the OD readings from the caecal content binding assay were normalised to the total protein concentration.

2.5.5 Assessment of cytokine concentration by CBA

Cytokines were measured in serum, gut content or tissue supernatant samples using a cytometric bead array (CBA) assay. The BD® CBA Mouse Inflammation Kit (BD Biosciences) was used which contains beads pre-coated with antibodies against the cytokines; IL-6, IL-10, MCP-1, IFN-γ, TNF and IL-12p70. The beads are then distinguished by different fluorescence in the APC channel and a PE

detector is used to indicate how much cytokine has bound. The kit was used based on the manufacturer's guidelines, with extension of the standard curve to give a top concentration of 5000pg/mL and a bottom concentration of 1.2pg/mL, and using the same ratios of reagents but half of the volumes. Samples were diluted at 1:2 in appropriate assay diluent (sera) or run neat (supernatant). The CBA assay samples were acquired on a BD FACS Canto II (BD Biosciences) and analysis was performed in FlowJo (version 10) and GraphPad Prism (version 9 or 10).

2.6 FITC-dextran gut permeability assay

To investigate intestinal permeability, a FITC-dextran assay was carried out. 4 hours before blood samples were collected, food was removed from cages to briefly fast the mice to enable the dextran to travel along the intestine without obstruction. 30 minutes prior to the cull, 200uL of 15.2mg 4kDa FITC-dextran (Sigma-Aldrich) diluted in PBS (76mg/mL) was administered to mice by oral gavage. This size is commonly used to assess intestinal permeability, the sugar molecule is large enough that it has the potential to pass between cells in the intestinal barrier but under normal conditions should not freely cross it. Blood was collected and serum separated as described in section whilst taking particular care to keep samples in dark conditions to protect the FITC fluorescence. Samples were diluted 1:4 and 50µL was added to each well of a standard flat-bottom 96-well plate. The plate was read at 485nm using the PHERAstar FS (BMG Labtech) to enable detection of fluorescent emission and analysis was carried out using GraphPad Prism. A standard curve was plotted based on diluted concentrations of the FITC-dextran in PBS (Figure 2-2). This was then used to extrapolate values for each sample and determine the level of FITC-dextran present in the serum.

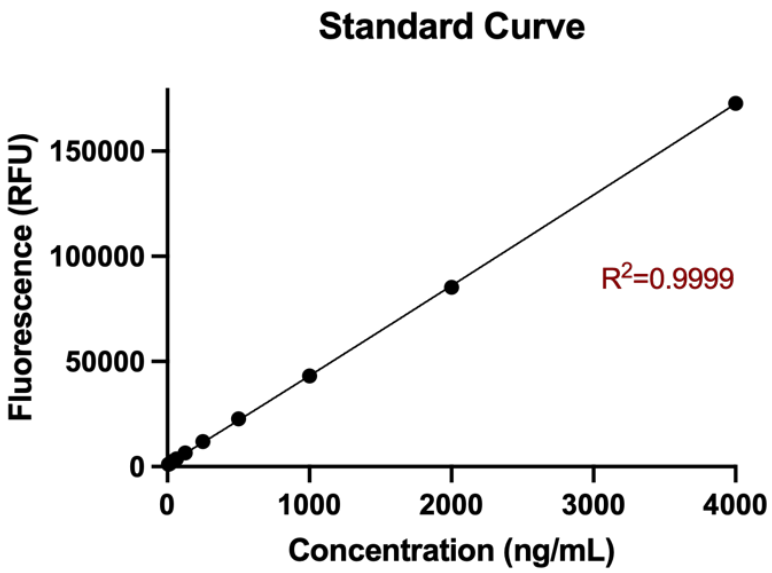

Figure 2-2 FITC-Dextran standard curve.
Standard curve of relative fluorescent units (RFU) plotted against known FITC-dextran concentrations. R squared value = 0.9999.

2.7 Flow Cytometry

2.7.1 Isolation of cells

To assess immune cell infiltration into intestinal tissues, the small intestine was collected assessed by flow cytometry. The tissue was digested and processed to provide a single cell suspension as previously described (Webster et al., 2020): the small intestine was cleaned of fat, and Peyer's patches were removed. Intestines were cut below the stomach and above the caecum and cut open, wiped clean of mucus and cut into small sections no bigger that 0.5cm long. The tissue was collected in R10 media. Samples were washed through nitex filter paper with warm 2mM EDTA HBSS then placed into the shaking incubator (37 degrees, 250 rpm) for 15 minutes. This process was repeated 3 times before a digestion mix of 15mL R10 and 0.5mg/mL of Collagenase VIII (enzyme activity; ≥125 CDU/mg solid) per sample was added and they were shaken for a further 20 minutes. After digestion, samples were mashed through a 70µm Easystrainer filter with additional R10 and washed twice, ready for counting.

2.7.2 Surface and intracellular staining

Live cells were counted using trypan blue and a range of 5-10 million cells were then resuspended in dilutions of viability dye to stain for live cells and Fc block

to prevent non-specific binding. Both antibodies were diluted in FACS buffer (FB) at concentrations shown in Table 2-3 and 200µL was used to resuspend the cells which were then kept in the dark at 4°C for the 20 minutes staining. The samples were washed with FB and then surface marker antibodies diluted using the FB at dilutions specified in Table 2-3 were added to samples at 200µL per sample and left in the dark for 30 minutes at 4 °C. Samples were then washed and either fixed using 400µL of Fixation Buffer (Biolegend) in the dark at RT for 45 minutes or permeabilised and fixed for intracellular staining.

To stain transcription factors in the nucleus, the eBioscience™ Foxp3/Transcription Factor Staining Kit (Thermo Fisher) was used. A concentration and diluent mix were provided and prepared in line with manufacturer instructions. 400µL of the buffer was added to cells and they were kept in the dark at RT for 1 hour. Cells were then washed with permeabilisation buffer, and the same buffer was used to dilute intracellular marker antibodies at concentrations specified in Table 2-3 and 200µL was added to the samples for 1 hour at RT.

To stain intracellular cytokines, a Cell Stimulation Cocktail containing protein transport inhibitors (eBioscience (00-4975-93)) was diluted 1/500 in R10 media and added at 200µL per sample and incubated at 37 °C for 4 hours. Washing and staining procedures then followed as previously described and 200µL of BD Cytofix/ Cytoperm Fixation/ permeabilisation solution was added and incubated in the dark at RT for 20 minutes. Permeabilisation buffer was then used to wash the cells and the cytokine and intracellular stains were diluted in the permeabilisation buffer and 200µL was added to samples for 1 hour at RT. Where IFN-γ was the cytokine measured, Rat IgG1 isotype control was used in a control sample to ensure true staining.

Target	Manufacturer	Clone	Dilution
T-bet (intracellular)	eBioscience	eBio4B10	1/100
CD16/32 (Fc block)	eBioscience	93	1/100
eFlour 780 (viability dye)	eBioscience	65-0865-14	1/1000
Ly6C	Biolegend	HK1.4	1/200
Ly6G	Biolegend	IA8	1/200
MHCII	Biolegend	M5/114.15.2	1/200

NK-1.1	Biolegend	PK136	1/200
Siglec-F	Biolegend	S17007L	1/200
CD19	Biolegend	6D5	1/200
CD3	Biolegend	17A2	1/200
CD45	Biolegend	30-F11	1/200
CD64	Biolegend	X54-5/7.1	1/200
CD11b	Biolegend	M1/70	1/200
CD11c	eBioscience	N418	1/200
B220	Biolegend	RA36B2	1/200
CD4	Biolegend	RM4-5	1/200
CD5	Biolegend	53-7.3	1/200
CD3e	eBioscience	eBio500A2	1/200
GATA3 (intracellular)	eBioscience	TWAJ	1/100
RORγT (intracellular)	eBioscience	AFKJS-9	1/100
IL-17Ra (intracellular)	Biolegend	A7R34	1/100
IFN-γ (intracellular cytokine)	eBioscience	XMG1.2	1/200

Table 2-3 List of antibodies used for analysis by flow cytometry.
Ordered from Biolegend or eBioscience.

All antibodies used for staining cells acquired by flow cytometry are shown in Table 2-3.

2.7.3 Acquisition and analysis

After staining, all samples were washed and resuspended in 400µL of FB. Then, they were filtered through nitex and acquired on the LSRFortessa (BD Biosciences) using the FACS-Diva software (BD Biosciences). Analysis was performed using FlowJo (version 10). Frequency (%) used as a label throughout the flow cytometry data in the results chapters is indicative of the proportion of cells gated from the parent population.

2.8 Analysis of intestinal organoids

Experimental assays were setup and carried out in Menno Oudoff's laboratory at the University of Science and Technology, Trondheim (his laboratory group have since relocated to Carleton University, Canada). In an independent experiment in Trondheim, crypts from the small intestine were isolated to set up small intestinal organoid models. These spheroid organoids were seeded for 24 hours and then treated apically with or without cytokine combinations including IFN-γ,

IL-13 or IL-22 at 10ng/mL and then samples were sent for bulk RNA-sequencing (Lindholm et al., 2022). The differential gene expression datasets were kindly shared with us, enabling us to do our own analysis, exploring our own research questions. Searchlight2 was the pipeline used to initially explore the overall differential expression of genes between the treatment groups (Cole et al., 2021). Subsequently, a previous postdoc in the Perona-Wright laboratory, Patrick Shearer, further analysed the data in R Studio using Metascape and selected pathway analysis and compared specific gene expression between treatment groups.

2.9 *H. polygyrus* and *Salmonella* co-culture

During a placement in the Reynolds laboratory at University of Victoria, *H. polygyrus* L3 larvae (maintained within the Reynolds laboratory using the same established protocols as Maizels laboratory) were cultured for 5 minutes in a petri dish with a fluorescently labelled strain of *Salmonella enterica* (strain SL1344), and visualised and imaged on the microscope.

2.10 Statistical Analysis

Throughout this thesis the trends discussed are based on small p-values deemed significant at less than 0.05 or on qualitative consistency of patterns within the data.

Sample size for each experimental design was determined most often as 5 mice per group to ensure sufficient power to detect differences in the key read-outs; whilst covering for the fact that certain treatments or assays could be subject to loss of a sample within a treatment group (e.g. antibiotics resulting in excessive weight loss and resulting in early culling). Within each experiment, each mouse was classified as an ‘independent’ datapoint even although mice were co-housed. This was still assumed as each individual mouse has its own social behaviour, genetic predispositions, environmental exposures, immune responses, unique gut bacteria colonisation and varied physical and behavioural traits.

All statistical analyses were performed using GraphPad Prism (version 9 or 10). Graphed data is typically presented as mean ± standard deviation, with details in

each figure legend. Normality of all data sets was assessed using the Shapiro–Wilk test. For normally distributed data, comparisons between two groups were made using Student's t-test, and comparisons among three or more groups were made using one-way or two-way ANOVA with Tukey's multiple comparison correction. For non-normally distributed data, comparisons between two groups were conducted using the Mann–Whitney *U* test, and comparisons among three or more groups were performed using the Kruskal–Wallis test with Dunn's multiple comparison correction. Significance levels are denoted as follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, and ns = not significant.

2.11 Illustrations

All illustrations throughout this thesis were created using Biorender.com with an academic subscription and are cited as used.

Chapter 3

3.1 Introduction

Maintaining a strong and intact epithelial barrier is essential in controlling infections. The apical junctional complex (AJC) is the network of molecules which ensure tight adhesion between neighbouring epithelial cells and is therefore a crucial component of the epithelium. Tight junction (TJ) proteins are a key part of this adhesion and are found in epithelial cells as either transmembrane or cytosolic proteins which together form strong and complex bonds with each other to seal and adhere cells together to tightly regulate the passage of molecules across the epithelium (Chelakkot et al., 2018; Lee, 2015). Several of the proteins involved in this include occludin, scaffold proteins, claudins and cadherins. Disruption of these proteins by pathogens has been shown in numerous studies, further discussed in section 1.2.2.3 as a mechanism to breach the intestinal barrier. In helminth infection specifically, a previous study proposed that the metabolite acetate is one of the secreted products of *H. polygyrus* and is able to facilitate the breakdown of TJ proteins to open the epithelial barrier and allow entry of the helminth (Schälter et al., 2022).

Since TJs are important for integrity, and helminths are able to break this, we hypothesise that breaking of this barrier by the helminth can allow bacteria to enter. Other intestinal infections including *Toxoplasma gondii* infection can cause high levels of intestinal damage, allowing commensal bacteria from the gut to 'leak' into the intestinal tissue (Hand et al., 2012b). Similarly, we hypothesise that as the *H. polygyrus* helminth crosses the intestinal wall, this causes a breach in the gut barrier, leading to a loss of integrity. The increased permeability could allow bacteria from the lumen of the gut to translocate into the intestinal wall with the helminth, using the disrupted epithelium as an entry point to colonise, infect host cells and evade the ordinary antimicrobial response.

Antimicrobial proteins (AMPs) are a large family of proteins, key in protecting the host from bacteria at barrier sites. They are predominantly secreted by epithelial cells, expressed continuously at low levels to keep bacterial presence controlled and prevent infection (Gallo and Hooper, 2012). Through a number of

mechanisms including membrane disruption, attacking cell walls, inhibiting iron uptake or activating other antimicrobial defences, they are able to kill and inactivate a range of bacterial pathogens. Certain AMPs including calprotectin and lipocalin-2 can also be produced by myeloid cells including neutrophils as these cells are effective first responders to bacterial invasion. By investigating the expression levels of these proteins and the myeloid cell infiltrate during early *H. polygyrus* infection, we thought these may serve of an indicator as to whether bacteria have invaded host tissue.

Helminths which have burrowed through intestinal tissue are encased in granulomas, an inflamed area surrounding the worm at the site of tissue entry. Within this space local to the worm we thought that presence of immune cells, and ongoing responses may also be as a reaction to bacterial translocation. In the granulomas of *H. polygyrus,* an infiltration of immune cells are present, composing of neutrophils, dendritic cells, eosinophils and macrophages. The macrophages present in *H. polygyrus* helminth infection have high plasticity to enable them to adapt to the changing environments between type 1 and type 2 immunity states (Reynolds et al., 2012b). Both arginase 1 (Arg1) and resistin-like molecule-alpha (Relm-α) are markers indicative of alternatively activated macrophage (AAM) phenotypes, activated by type 2 associated cytokines and involved in tissue repair and remodelling. Their presence in helminth infection is likely due to the damage caused by the worm when migrating through the intestinal barrier. In contrast, the more classically activated macrophage could develop as a result of IFN-γ stimulation or other pro-inflammatory cytokines. Assessing the cytokine profile in early infection and the changes in cell composition within the intestinal tissue throughout infection may indicate whether cells are responding to bacterial as well as helminth invasion.

In early *H. polygyrus* infection, the inflammatory cytokine prolife has not been widely researched. Since IFN-γ plays an important role in myeloid cell function and activation, assessing the presence of this cytokine in *H. polygyrus* infection and whether it is stimulated by and enhances responses to bacteria could develop our understanding of early helminth infection. When starting this project, previous findings in our laboratory and a recent publication had identified that IFN-γ is present in early *H. polygyrus* infection (Gentile et al., 2020). Other parasitic infections have described an initial type 1 phenotypic

immune response with an IFN-γ signature (Else et al., 1994b; Urban et al., 1996), yet little is known about its role in early helminth infection, especially *H. polygyrus*, and how local or systemic the response is. Additionally, what the IFN-γ is produced in response to, whether it is the worm itself, damage as a consequence of helminth breach, or in response to bacteria remains unclear. We hypothesised that locally produced IFN-γ in the intestinal wall could be an intrinsic defence to prevent bacterial invasion, and/ or a response induced by bacteria that successfully enter the tissue alongside *H. polygyrus*. To summarise, intestinal helminths are known to disrupt the epithelial barrier, but the consequences for barrier integrity, bacterial invasion and host immunity are not yet clear. In this chapter we explored whether early stages of *H. polygyrus* infection are associated with evidence of barrier breach, bacterial translocation and pro-inflammatory and anti-bacterial immune defences.

3.2 Aims;

Our overall objective in this chapter was to explore the intestinal changes during early *H. polygyrus* infection, answering these specific aims:

- To characterise the local area surrounding the *H. polygyrus* in the duodenum.

- To assess gut barrier integrity during early *H. polygyrus* infection.

- To test whether bacteria can enter the intestinal tissue more easily in *H. polygyrus* infection than in uninfected mice.

- To investigate the local intestinal immune responses at the timepoints of the *H. polygyrus* 'breaches', assessing whether there are indications of bacteria being present as well as immune responses typical of helminths.

3.3 Results

3.3.1 Exploring gut barrier integrity during *H. polygyrus* infection

We first aimed to examine the intestinal tissue surrounding the *H. polygyrus* worm as it enters the duodenum and assess the general changes in intestinal immune responses in early infection. The small intestine of mice was opened and rolled into a Swiss roll shape (Figure 3-1). This was H&E stained so that the worm could be identified, and pathology could be assessed.

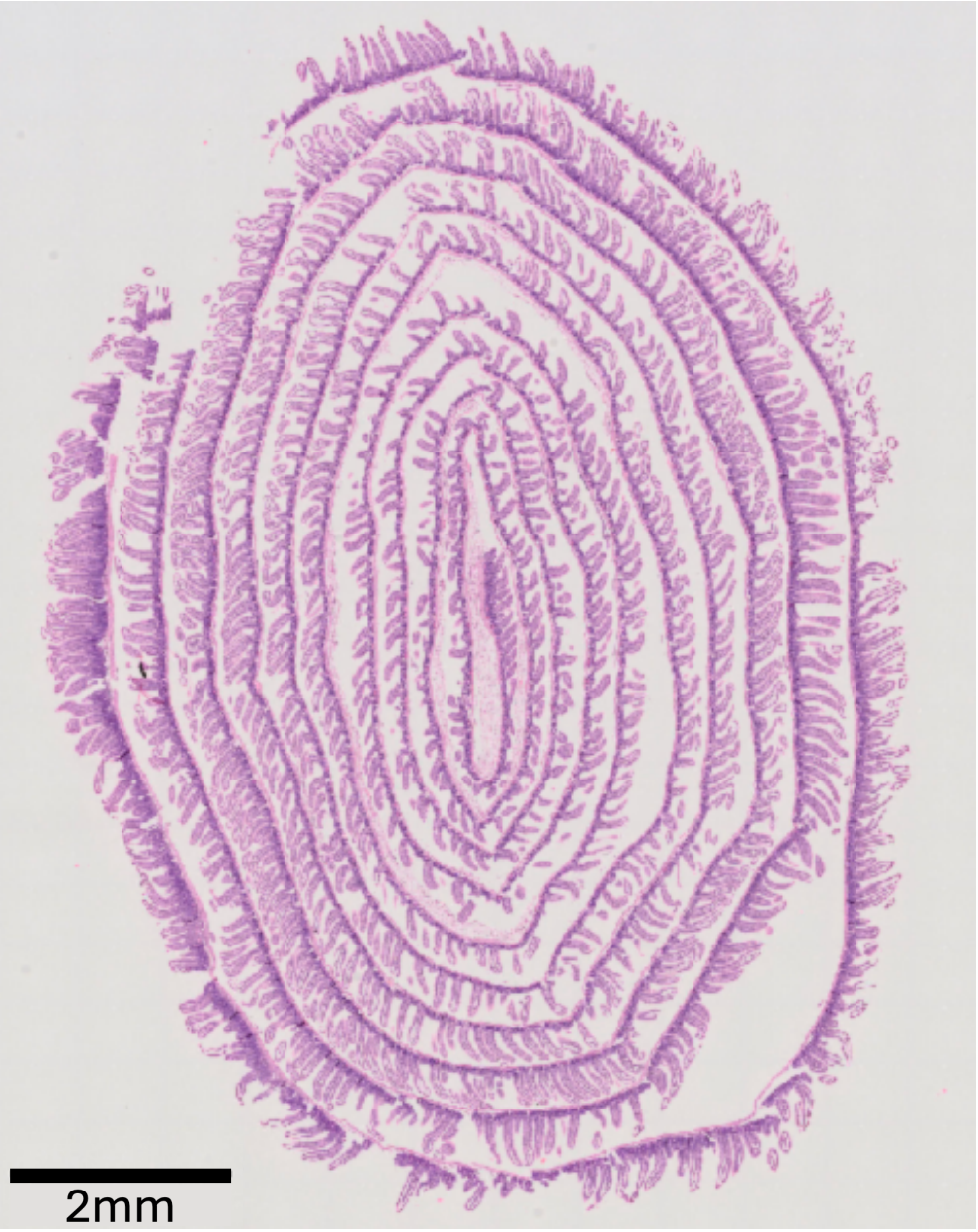

Figure 3-1 H&E staining of Swiss rolled small intestinal tissue from C57BL/6 mice. Representative image of Swiss roll shape and staining. Proximal section of small intestine (duodenum) on outer edge of roll, moving distally towards the centre of the image.

Without magnification, the helminths become visible in the intestine at day 7 post-infection (PI) (Figure 3-2A, red arrows) and are more commonly found in the duodenum, decreasing in number into the jejunum. The worm resides in the lamina propria tissue which when imaged at a higher magnification in our H&E-stained Swiss rolls, are more clearly shown to be surrounded by a modest inflammatory response, forming a granuloma of infiltrating immune cells (Figure 3-2B, red circles). In line with the described lifecycle (section 1.1.2), *H. polygyrus* can be identified in the lamina propria as early as day 2 of infection where it then matures as is shown in the increase in size at both day 5 and day 7 until day 9 when most helminths have emerged back into the lumen of the gut. As indicated by the day 9 H&E-stained image, the granuloma remains present after the helminth has left and is still present even at day 14. Notably, the worms were much larger than the epithelial cells at both early and later time points, prompting us to investigate potential barrier breaches.

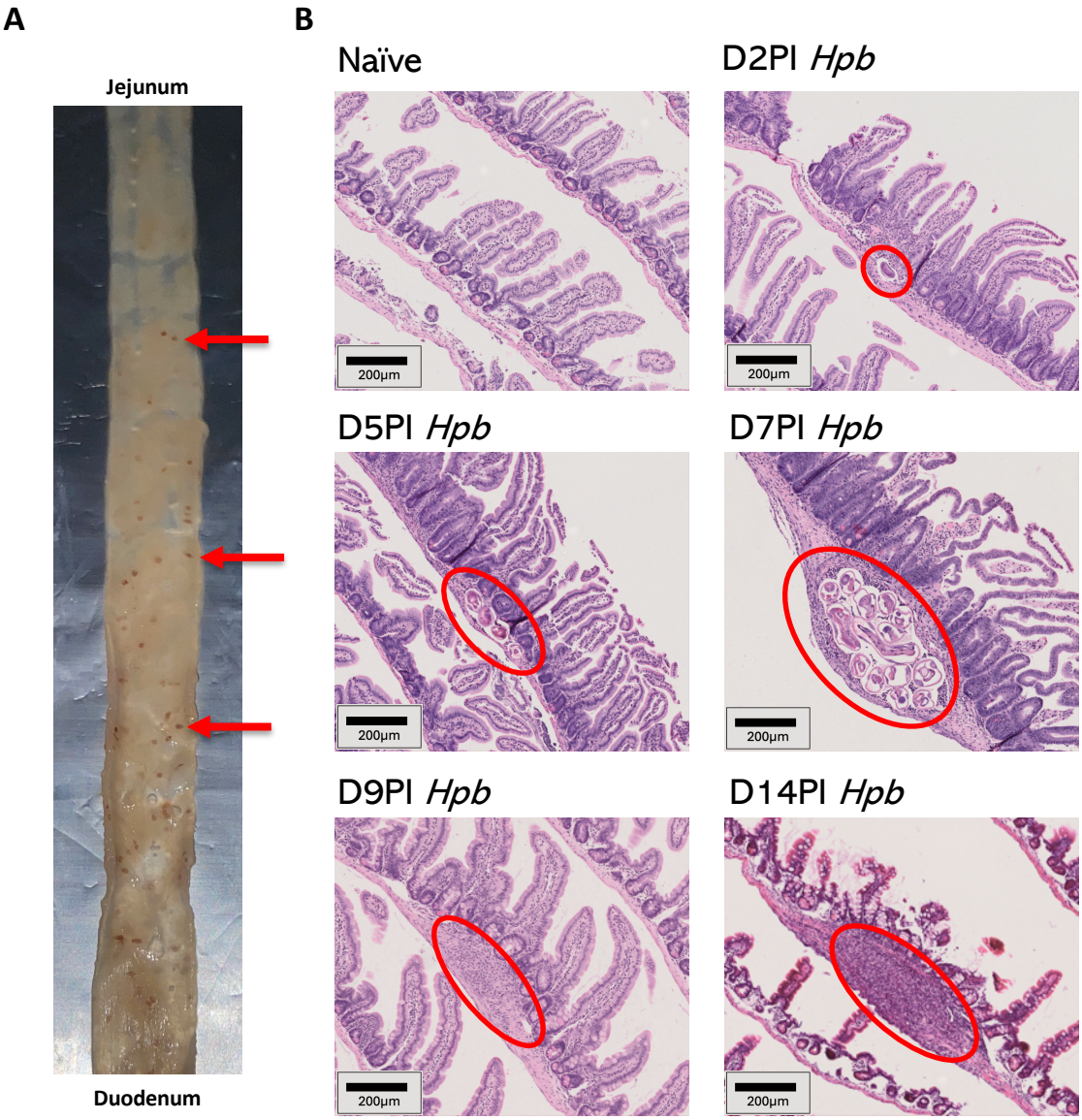

Figure 3-2 *Hpb* granuloma identified in the intestinal tissue.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and samples were collected at days 2, 5, 7 or 9 post infection. **A)** Image of small intestine at day 7 post-*Hpb* infection. Orientation jejunum (top) to duodenum (bottom), red arrows indicate worm (red dots) residing in lamina propria. **B)** H&E staining of small intestinal tissue at days 2, 5, 7, 9 and 14 post-infection (PI) with naïve control. Red ellipse, circling worm and formed granuloma within intestinal lamina propria. D14 is an image from early in my PhD and staining and tissue processing is not ideal but the main result is clear.

To explore evidence of intestinal barrier breach, we firstly collected gut contents of mice at days 2, 5, 7 and 9 of *H. polygyrus* infection and measured calprotectin (heterodimer of S100A8 and S100A9) protein concentration in these samples. Calprotectin is a known common faecal marker of intestinal inflammation and has been associated with intestinal permeability (Tyszka et

al., 2022). Our data show that calprotectin increased significantly in *H. polygyrus* infection at timepoints the worm is known to be residing in the intestinal tissue (Figure 3-3A). The calprotectin suggests that inflammation begins early and is already significant at day 2 of infection. We wanted to then assess whether the inflammation reflected barrier disruption so looked at TJ protein expression. TJ proteins are expressed by epithelial cells and facilitate cell adhesion, thereby maintaining barrier function (Chelakkot et al., 2018). Several of these including cadherin 2 (Cdh2), claudin 7 (Cldn7) and Occludin (Ocln) showed higher gene expression in the duodenum at day 2 of *H. polygyrus* infection (Figure 3-3B-D). This may suggest that the barrier has been disrupted, and higher gene expression is required as part of the repair and remodelling process in the epithelium. Consistent with this, lipocalin-2 (Lcn2) has a number of roles in immunity and has been speculated as a biomarker of intestinal permeability (Zhang et al., 2020). Measuring the gene expression of this protein at day 2 of *H. polygyrus* infection also reflected a significant increase. Altogether, Figure 3-3 suggests that *H. polygyrus* infection disrupts the intestinal barrier integrity.

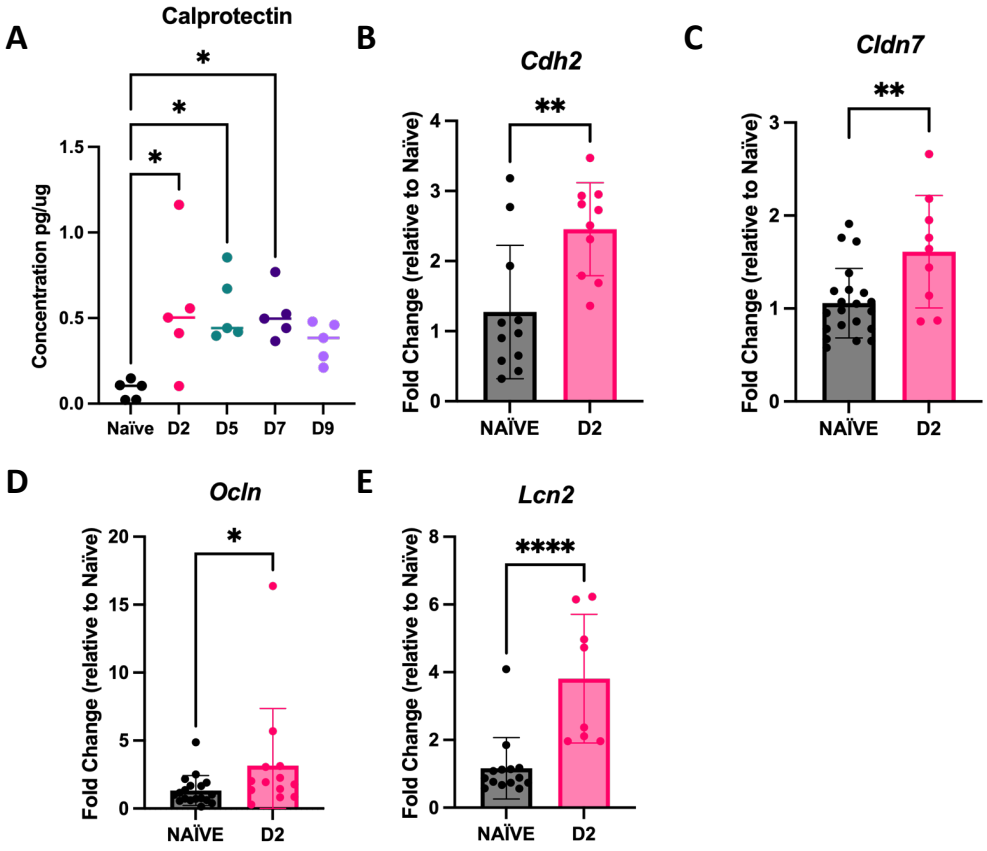

Figure 3-3 *Hpb* disrupts intestinal barrier integrity.

C57BL/6 mice were infected with 200 L3 *Hpb* larvae and samples were collected at days 2, 5, 7 or 9 post infection. **A)** Calprotectin concentration pg per ug of total protein in gut contents of mice. **B-E)** Fold change of **B)** Cdh2, **C)** Cldn7, **D)** Ocln and **E)** Lcn2 in the duodenum compared to housekeeping gene (Rps29) and normalised to the mean of the naïve samples. Data shown with 5 mice per experiment and statistical significance calculated by a One-Way ANOVA followed by a Tukey's multiple comparisons test (A). Or data shown with mean ±SD, 2-3 pooled experiments with 4-5 mice per experiment. Statistical significance was calculated by unpaired student t-test or Mann-Whitney U test depending on whether data was normally distributed. (Significance $*p < 0.05$, $**p < 0.01$, $****p < 0.0001$).

3.3.2 Antimicrobial responses in *H. polygyrus* infection

Given these changes in barrier integrity, we examined alterations in antimicrobial defence mechanisms indicative of increased bacterial threat to the host. Both calprotectin and lipocalin-2 have additional roles as antimicrobial proteins (AMPs) and are often secreted by neutrophils. Similarly, other AMPs have also been reported to be active in the duodenum, including Sprr2a (Hu et al., 2021). We measured gene expression in the duodenum at day 2 of infection, and saw higher expression of Sprr2a, Nos2 and Ang4 compared to uninfected mice, suggesting enhanced host defence mechanisms (Figure 3-4A-C).

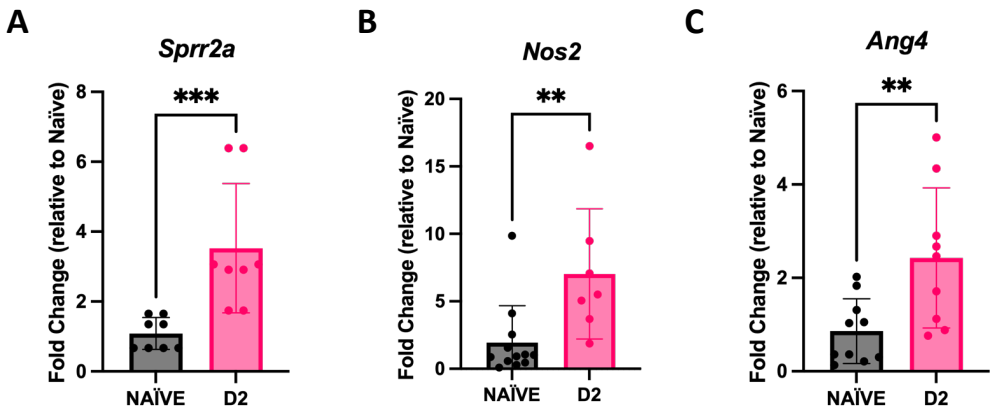

Figure 3-4 Increased AMP expression in early *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and samples were collected at day 2 post infection. Fold change of **A)** Sprr2a, **B)** Nos2 and **C)** Ang4 in the duodenum compared to housekeeping gene (Rps29) and normalised to the mean of the naïve samples. Data shown with mean ±SD, 2 pooled experiments with 4-5 mice per experiment. Statistical significance was calculated by unpaired student t-test or Mann-Whitney U test depending on whether data was normally distributed. (Significance $**p < 0.01$, $****p < 0.0001$, NS, Non-significant).

We also investigated myeloid populations throughout an infection time course including day 2, day 7 and day 14 of *H. polygyrus* infection so that we could

assess the immune cell infiltrate at the time of the early AMP increases, and in the days following as the granuloma matures. The small intestine was collected and digested for flow cytometry analysis using previously optimised methods (Webster et al., 2020). The gating strategy used to identify the different myeloid cell populations in the small intestinal lamina propria (SILP) is shown in Figure 3-5. Live, single, CD45+lineage- (lineage markers; CD3, CD19 and NK1.1) cells were gated in each case (Figure 3-5A) and CD64+ cells were further gated to show monocyte and macrophage populations in a 'waterfall' formation using the markers Ly6C and MHCII (Figure 3-5B). CD64- cells were further gated into eosinophils using SiglecF expression (Figure 3-5C), neutrophils based on CD11b and Ly6G expression (Figure 3-5D) and dendritic cells (DCs) based on MHCII and CD11c expression (Figure3-5E).

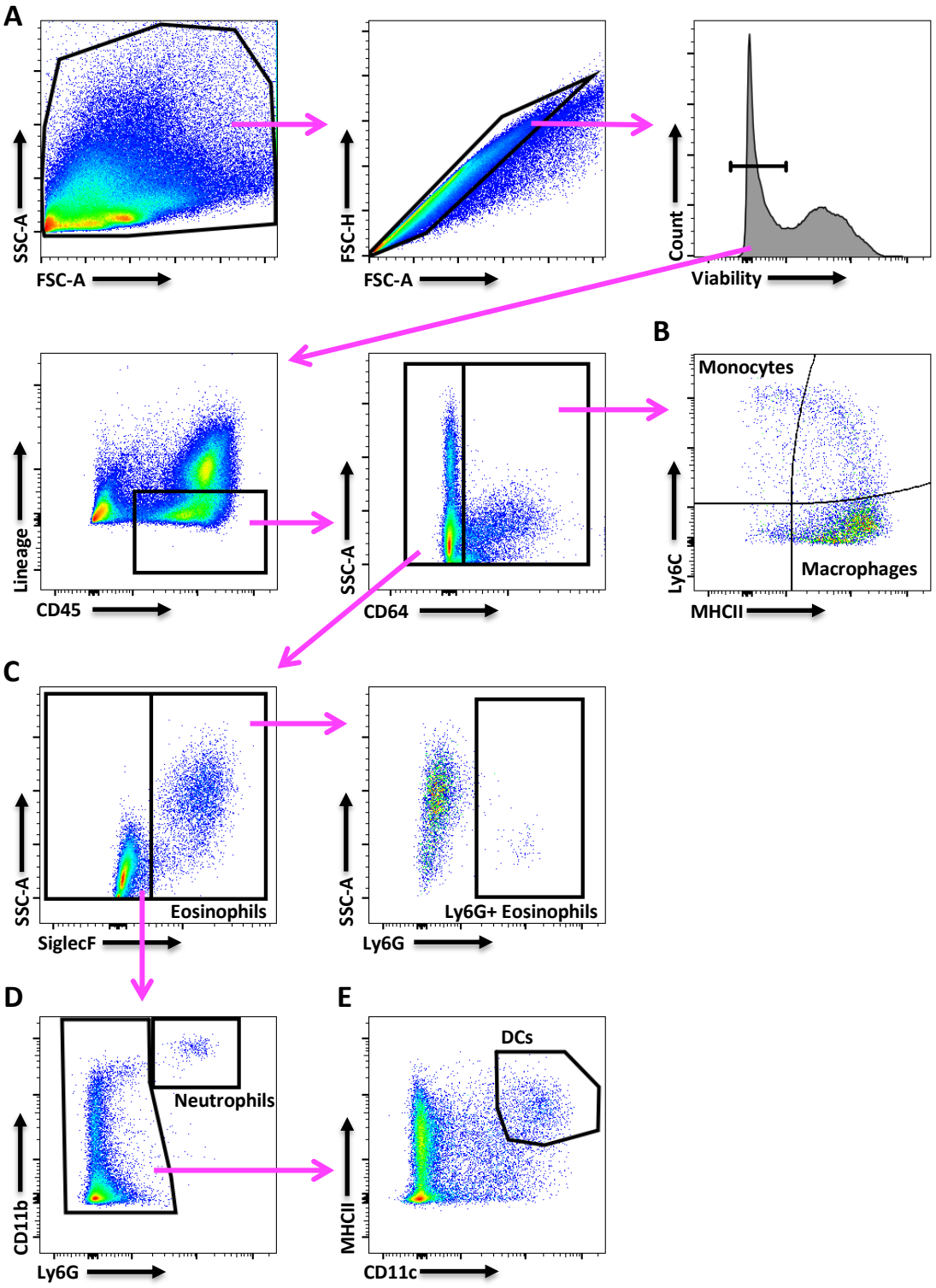

Figure 3-5 Gating strategy for identification of myeloid cells from the SILP tissue during *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and samples were collected. Small intestinal lamina propria digested and analysed by flow cytometry. **A)** Gating strategy shown with final gates for **B)** monocytes (live, single, CD45+, lineage-, CD64+, Ly6C+, MHCII- cells), intermediates (live, single, CD45+, lineage-, CD64+, Ly6C+, MHCII+ cells), macrophages (live, single, CD45+, lineage-, CD64+, Ly6C-, MHCII+ cells), **C)** eosinophils (live, single, CD45+, lineage-, CD64-, SiglecF+ cells), **D)** neutrophils (live, single, CD45+, lineage-, CD64-, SiglecF-, CD11b+, Ly6G+ cells), and **E)** DCs (live, single, CD45+, lineage-, CD64-, SiglecF-, Ly6G-, MHCII+, CD11c+ cells). Lineage channel for myeloid gating- CD3, CD19, NK1.1

DCs show no significant changes in cell numbers throughout infection, although there is a slight trend in increased numbers as infection progresses. The proportion of DCs within the recovered leukocytes showed a similar trend, although the increase at day 14 of infection reached significance compared to cells from uninfected mice, possibly in line with the role of DCs in adaptive immunity (Figure 3-6A). Moreover, eosinophils show no significant changes in frequencies or total cell counts in the small intestine throughout *H. polygyrus* infection (Figure 3-6B). We also decided to gate on Ly6G+ eosinophils specifically which are largely unreported in literature but have been recently described as the dominant eosinophil population in wild mice and have been found at low levels in lab mice as a result of acute infection with the *T. muris* parasite (Mair et al., 2021). Work in other laboratories has also recently identified these cells in laboratory mice infected with *H. polygyrus* and therefore we decided to include this gating in our time course (Dr Constance Finney, University of Calgary, personal communication). Our data found that Ly6G+ eosinophils show an increase during *H. polygyrus* infection, reaching significance at day 7 when compared with uninfected controls (Figure 3-6C).

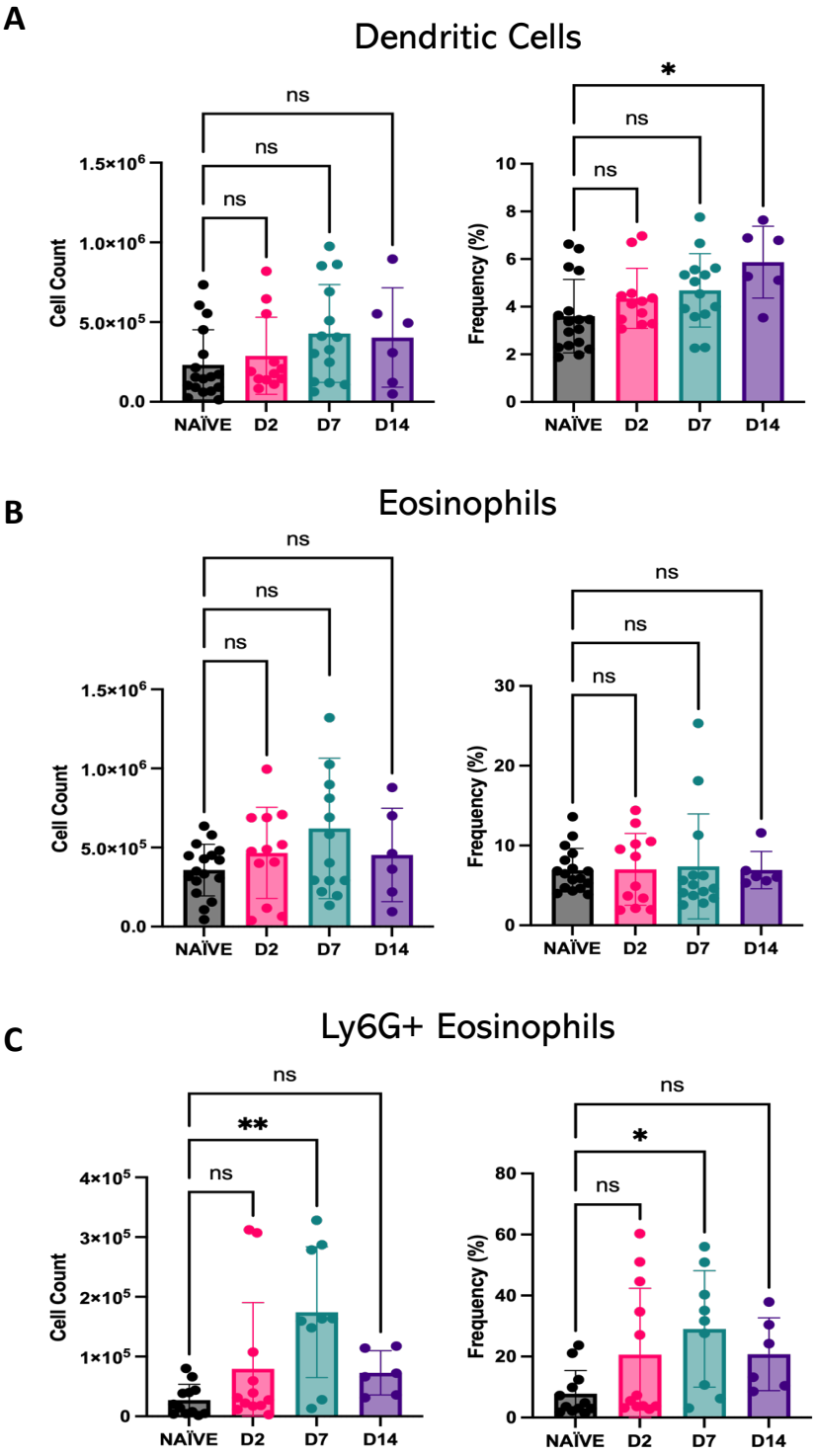

Figure 3-6 Myeloid cell population changes in the SILP during *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and samples were collected at days 2, 7 and 14 post infection. Small intestinal lamina propria digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 3-5). Frequency and absolute cell count of **A)** DCs, **B)** eosinophils and **C)** Ly6G+ eosinophils plotted. Data shown with mean ±SD, 2-3 pooled experiments with 3-6 mice per experiment. Statistical significance was calculated by a Kruskal-Wallis test followed by Dunn's multiple comparisons test. (Significance *$p < 0.05$, **$p < 0.01$, NS, Non-significant).

The monocyte/ macrophage 'waterfall' changes drastically in infection when compared with the naïve mice (Figure 3-7A), with a substantial increase in the total CD64+ cells at days 2 and 7 predominantly reflected in the monocyte (Ly6C+, MHCII-) and intermediate monocyte (Ly6C+, MHCII+) proportions (see top left and top right quadrants, Figure 3-7A). Interestingly, macrophages increased in total cell count by day 7 of infection but were significantly decreased in proportion/ frequency at days 2 and 7 PI in comparison to the naïve mice. Each of these fluctuations in cell population during infection returned to baseline levels by day 14 of *H. polygyrus* infection (Figure 3-7).

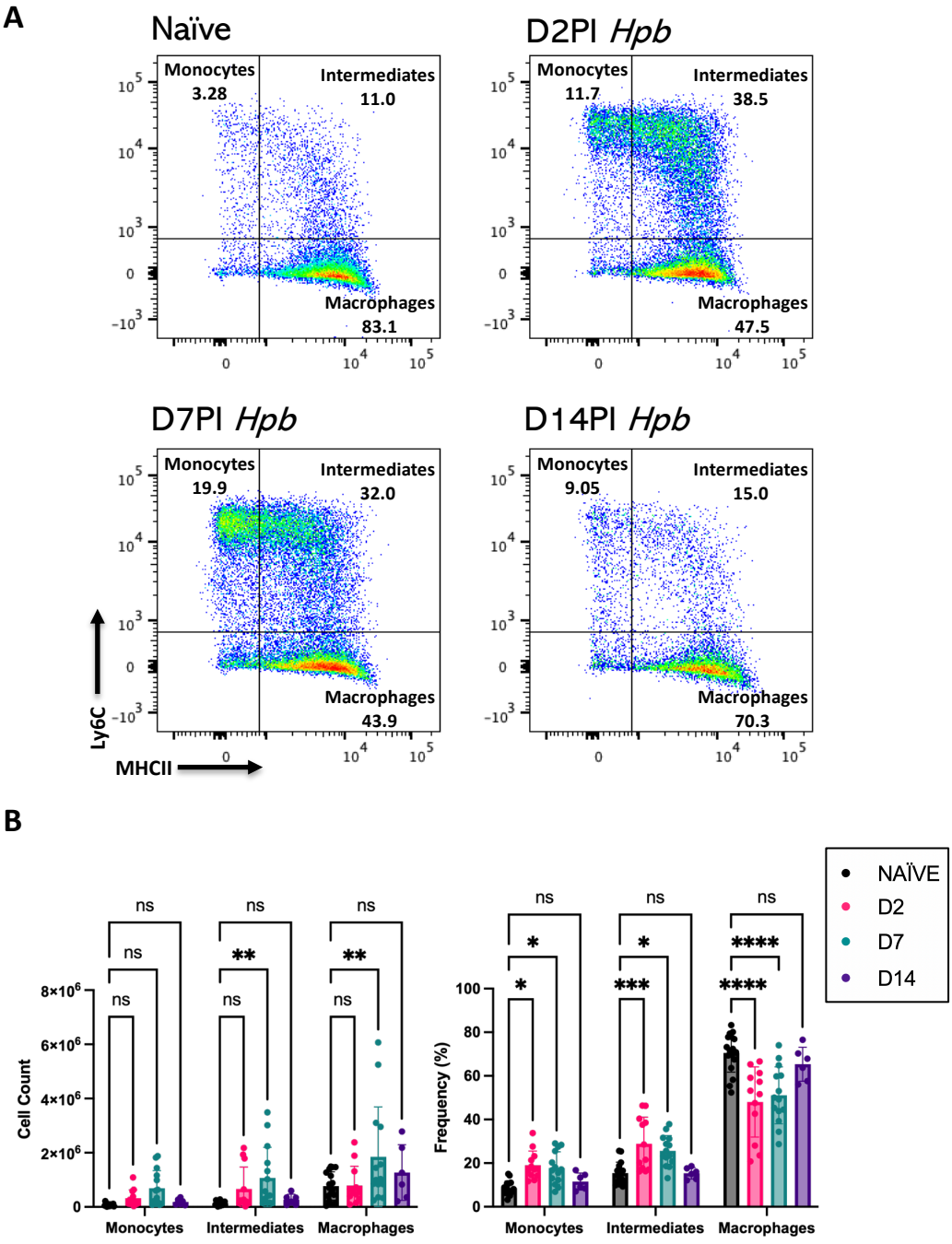

Figure 3-7 Monocyte and macrophage population changes in the SILP during *Hpb* infection. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and samples were collected at days 2, 7 and 14 post infection. Small intestinal lamina propria digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 3-5). **A)** Gating of the macrophage 'waterfall' with different populations identified and labelled with frequency of 'parent' population. **B)** Frequency and absolute cell count of monocytes (MHCII-Ly6C+), intermediates (MHCII+Ly6C+) and macrophages (MHCII+Ly6C-) plotted. Data shown with mean ±SD, 2-3 pooled experiments with 3-6 mice per experiment. Statistical significance was calculated by a Two-Way ANOVA followed by a Tukey's multiple comparisons test. (Significance *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, NS, Non-significant).

Next, we investigated neutrophil populations, as these are often first responders to bacterial infections and their presence could indicate that inflammatory

stimuli such as bacteria have invaded the intestinal tissue. Comparisons of their gated plots alone show a considerable increase in the neutrophil populations at days 2 and 7 of infection with the helminth (Figure 3-8A). These increases were shown to be significant in both frequency and cell count (Figure 3-8B) with a similar pattern of the changes, returning to comparable levels to that of the naïve mice by day 14 (Figure 3-8B). Overall, the myeloid population changes shown thus far confirm that early *H. polygyrus* infection is comprised of a highly active and defensive immune response in the small intestine, especially at the 'breach' timepoints of days 2 and 7 PI.

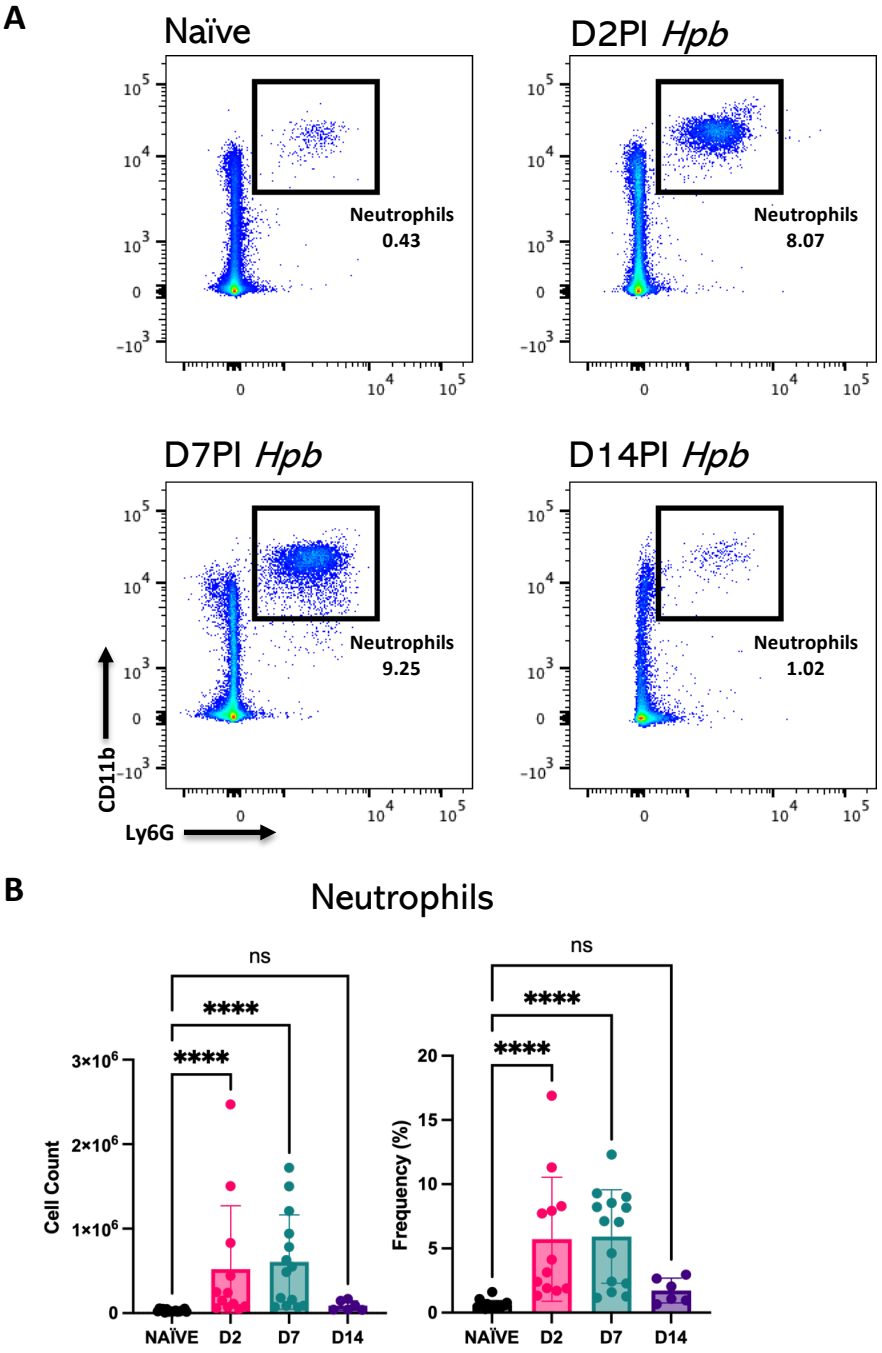

Figure 3-8 Neutrophils increased in the SILP during early *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and samples were collected at days 2, 7 and 14 post infection. Small intestinal lamina propria digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 3-5). **A)** Gating of neutrophils identified and labelled with frequency of 'parent' population. **B)** Frequency and absolute cell count of neutrophils plotted. Data shown with mean ±SD, 2-3 pooled experiments with 3-6 mice per experiment. Statistical significance was calculated by a Kruskal-Wallis test followed by Dunn's multiple comparisons test. (Significance ****$p < 0.0001$, NS, Non-significant).

With changes in the immune cell composition identified in the SILP during infection, we aimed to determine how local they are to the worm and whether they are each localised to the granulomas. Since we are unable to identify granulomas visually at day 2, we used only day 7 of *H. polygyrus* infection to investigate the localisation of responses. Granulomas were identified along the small intestine (Figure 3-2A) and small areas with a maximum of 0.3cm diameter were cut around them and they were collected (Figure 3-9: Granuloma, G). Separately, 0.3cm diameter sections with no visible granulomas on the same small intestines were also collected from similar areas, mainly along the duodenum (Non-granuloma, NG). The naïve tissue used were again cut isolates from the small intestine to ensure that similar amounts of tissue were used; the same digestion protocol and flow cytometry analysis as previously described was then carried out. Of the interesting changes described in bulk small intestinal tissue above, Ly6G+ eosinophils, monocytes, macrophages and neutrophils each show significant differences between the granuloma tissue and the naïve samples. Although similar trends are reflected when comparing granuloma to non-granuloma tissue, some of these comparisons did not reach statistical significance. This experiment was only carried out once in the interest of time and thus further repeats would help to strengthen this data. Although preliminary, these data in Figure 3-9 might suggest that the myeloid population changes in *H. polygyrus* infection are localised to the helminth granuloma.

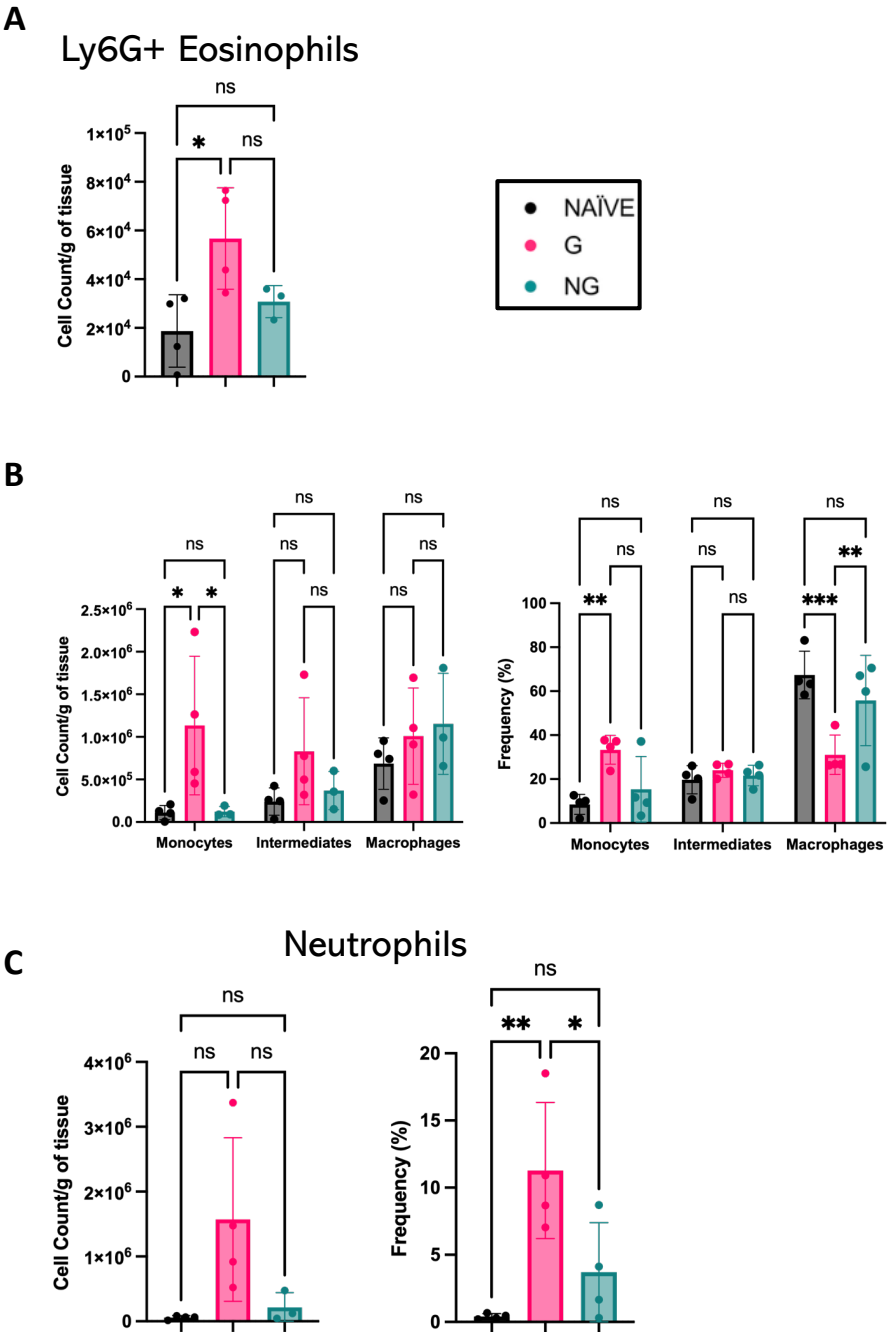

Figure 3-9 Myeloid population changes localised to *Hpb* granuloma during early infection. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and granulomas (shown in Figure 3-2A) and non-granuloma equivalents were isolated from mice at D7 post-infection then digested and analysed by flow cytometry. Populations gated and measured as previously identified (Figure 3-5). Absolute cell count per gram of tissue plotted for **A)** Ly6G+ eosinophils, and with frequency for **B)** monocytes (MHCII-Ly6C+), intermediates (MHCII+Ly6C+), macrophages (MHCII+Ly6C-) and **C)** neutrophils. Data shown with mean ±SD, one experiment with 3-4 mice per sample group. Statistical significance was calculated by a One-Way Anova or in cases where data was not normally distributed a Kruskal-Wallis test followed by Dunn's multiple comparisons test was used. (Significance *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS, Non-significant).

3.3.3 Bacterial Translocation

Altogether, my data thus far indicates that barrier integrity is disrupted, and antimicrobial defences are increased through AMP expression and infiltration of innate immune cells. Both of these findings may indicate bacterial entry into the intestinal tissue. Since the *H. polygyrus* helminth is large compared with epithelial cells and yet resides within the lamina propria for a portion of its lifecycle, we wondered whether the 'breach' points at days 2 and 7-8 of infection may act as an entry point for bacteria, increasing the susceptibility of the host to co-infection. Therefore, we decided to directly gram-stain our Swiss roll sections to see if bacteria could be detected in the intestinal tissue, surrounding the invading worm (Figure 3-10). Different from an ordinary gram-stain, the method used an alcoholic saffron counterstain which is able to highlight collagen fibres and is an effective contrasting dye to allow easier visualisation of bacteria (Becerra et al., 2016). The red circles indicate the helminth, and the red arrows indicate the bacteria (purple). The naïve sample shows a layer of bacteria in the luminal section of the tissue, some of which can also be identified in the lumen of the day 2 image. In each slide, bacteria could be identified in the lumen however, this did not always align with the frame used to capture the worm (the number of bacteria in the luminal sections are variable in these samples due to the wash methods used in forming the Swiss roll: most of the mucus and bacterial layers will be cleared). However, the granuloma and pocket hosting the worm itself remain clear of bacteria. The absence of detectable bacteria around the worms suggests that bacteria do not translocate at these entry point opportunities in *H. polygyrus* infection, or that perhaps they are rapidly cleared by a protective immune response.

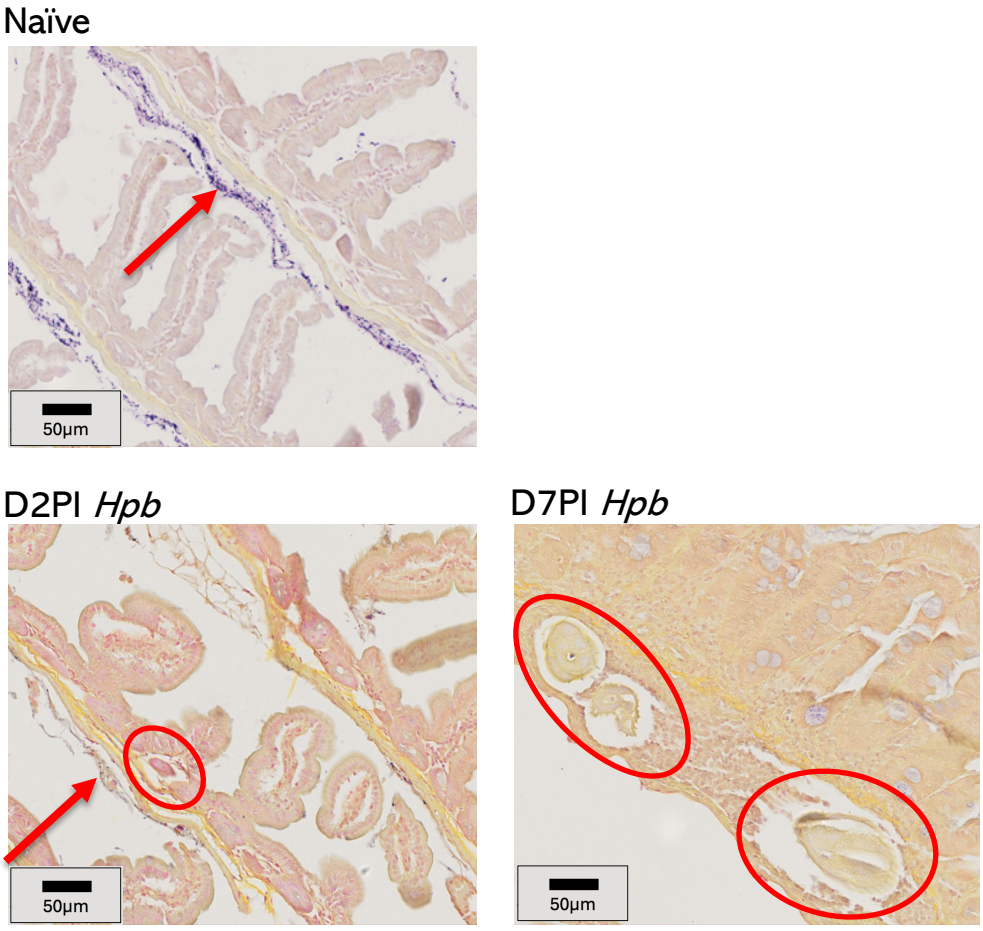

Figure 3-10 Bacteria not detected in intestinal wall during *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and small intestine was harvested and saffron gram-stained at days 2 and 7 of infection. Red arrows indicate bacteria (stained purple), red circles indicate helminth.

3.3.4 Immune changes in *H. polygyrus*

Since no bacteria were detected in the intestinal tissue, we decided to characterise the immune response in more detail. Some of our data so far had suggested an inflammatory influx and therefore we wanted to further explore this and determine whether there is evidence of anti-bacterial activity in the intestine during *H. polygyrus*, corresponding with the recruited cells. Usually helminths, *H. polygyrus* included, elicit a type 2 immune response (McGinty et al., 2020; Smith et al., 2012). We confirmed evidence of type 2 responses in the small intestine of infected mice compared with naïve mice by qPCR. We show increased gene expression of the type 2 cytokine IL-4 and type 2 inflammatory

markers Arg1 and Relm-α (Figure 3-11A-C), both associated with an AAM phenotype. Expression of these genes was increased only at day 7 of infection, indicating that the type 2 immune response is not yet elicited as early as day 2 of *H. polygyrus* infection.

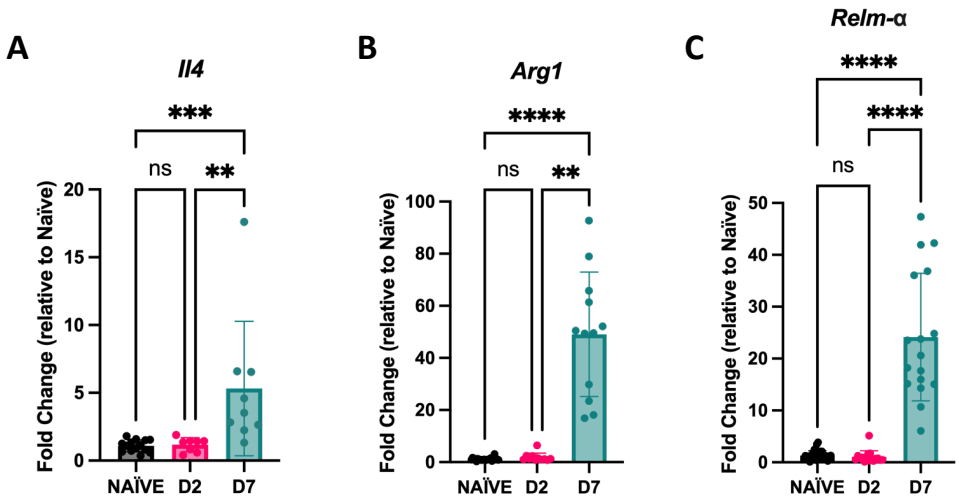

Figure 3-11 Increased expression of type 2 markers confirmed in *Hpb* infection. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and samples were collected at days 2 and 7 post infection. Fold change of **A)** Il4, **B)** Arg1, **C)** Relm-α in the duodenum compared to housekeeping gene (Rps29) and normalised to the mean of the naïve samples. Data shown with mean ±SD, 2-3 pooled experiments with 3-6 mice per experiment. Statistical significance was calculated by a Kruskal-Wallis test followed by Dunn's multiple comparisons test. (Significance $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, NS, Non-significant).

Following this confirmation, we aimed to investigate the inflammatory profile of the host during infection. Using the same timepoints of interest as before, serum was extracted from the blood of mice at days 2 and 7 PI and a CBA assay was carried out to measure the protein levels of six different inflammatory cytokines; IFN-γ, IL-6, IL-12p70, MCP-1, TNF and IL-10 (Figure 3-12A-F). IFN-γ was significantly increased in serum at day 2 of infection, compared to naïve; and IL-6 was significantly increased at days 2 and 7 PI (Figure 3-12A&B). However, the other proteins measured did not show any significant changes during infection compared with the serum of the naïve mice.

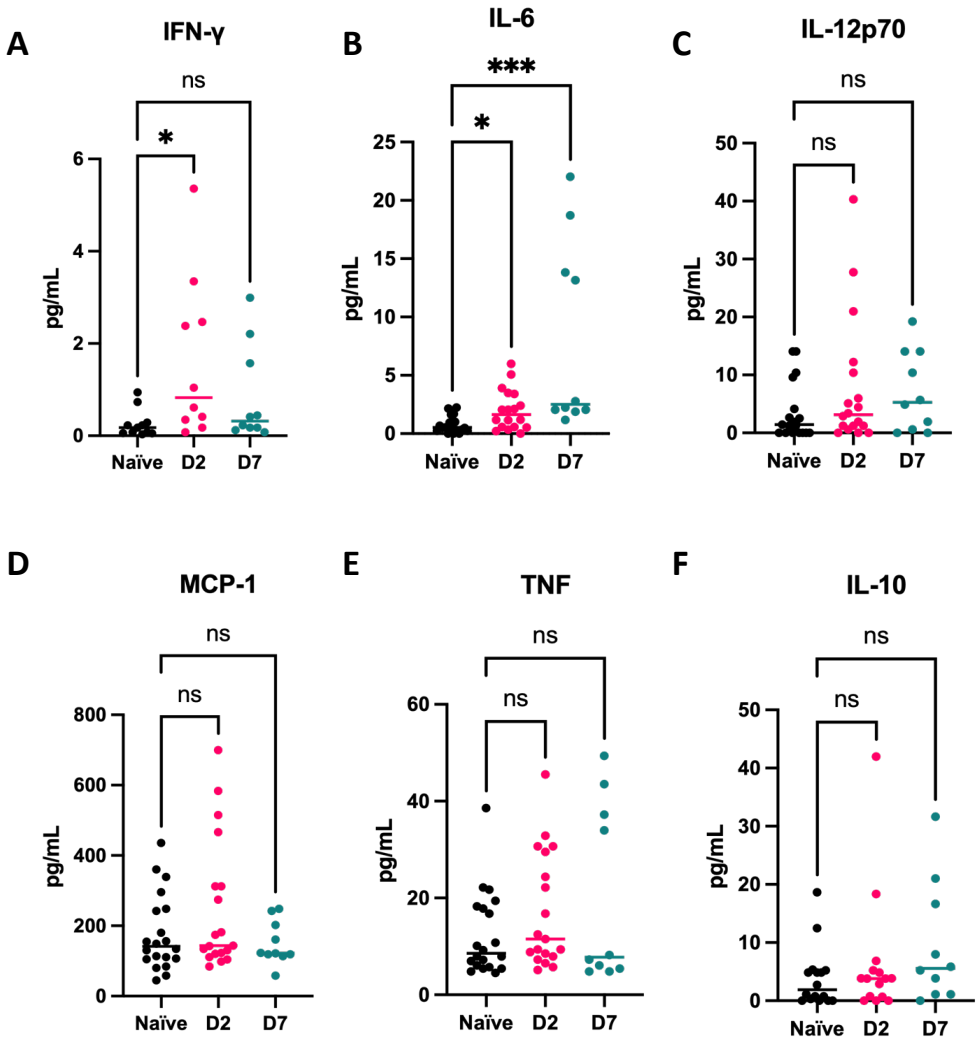

Figure 3-12 Inflammatory profile of proteins in serum during early *Hpb* infection. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and blood samples were collected at days 2 and 7 post infection. Protein levels of **A)** IFN-γ, **B)** IL-6, **C)** IL-12p70, **D)** MCP-1, **E)** TNF and **F)** IL-10 measured in serum of mice using CBA assay. Data shown with line indicating median data point. 2-4 pooled experiments with 3-6 mice per experiment. Statistical significance was calculated by a Kruskal-Wallis test followed by Dunn's multiple comparisons test. (Significance $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$, NS, Non-significant).

Since IFN-γ is a known driver of the anti-bacterial and inflammatory response and has been reported in early helminth infection before (Else et al., 1994; Gentile et al., 2020; Nusse et al., 2018; Urban et al., 1996), we decided to primarily focus on this cytokine, to confirm these data and investigate the proximity of the expression to the worm. We first aimed to confirm higher presence of IFN-γ in the small intestine in early *H. polygyrus* infection. A qPCR was used to measure the gene expression of IFN-γ in the duodenum during *H. polygyrus* infection and showed a significant increase at days 2 and 7 PI compared to naïve mice (Figure 3-13A). Additionally, 1cm sections of the

duodenum were collected at days 2 and 7 of infection, cultured overnight, and the supernatant assayed for secreted IFN-γ protein. Secreted IFN-γ showed a significant increase at day 2 of infection but not at day 7 PI compared with non-infected mice (Figure 3-13B). To investigate whether IFN-γ expression was local to the worm's location, granuloma and non-granuloma sections were collected at day 7 as described earlier and shown in Figure 3-2A. Gene expression of IFN-γ was significantly higher in the granuloma when compared with the naïve and non-granuloma tissues (Figure 3-13C). Secreted protein did not reach significance at D7 even in the granuloma-specific tissue, although a trend towards an increase can perhaps be seen (Figure 3-13D). Altogether this figure shows that IFN-γ is higher in the *H. polygyrus* granuloma at day 2 of infection, prior to the dominant expression of type 2 markers shown in Figure 3-11. With the known roles of IFN-γ in promoting anti-bacterial defence, we hypothesise that this increase may suggest a role for the cytokine in clearing invading bacteria or preventing their entry, thus preventing their visualisation by gram-staining earlier.

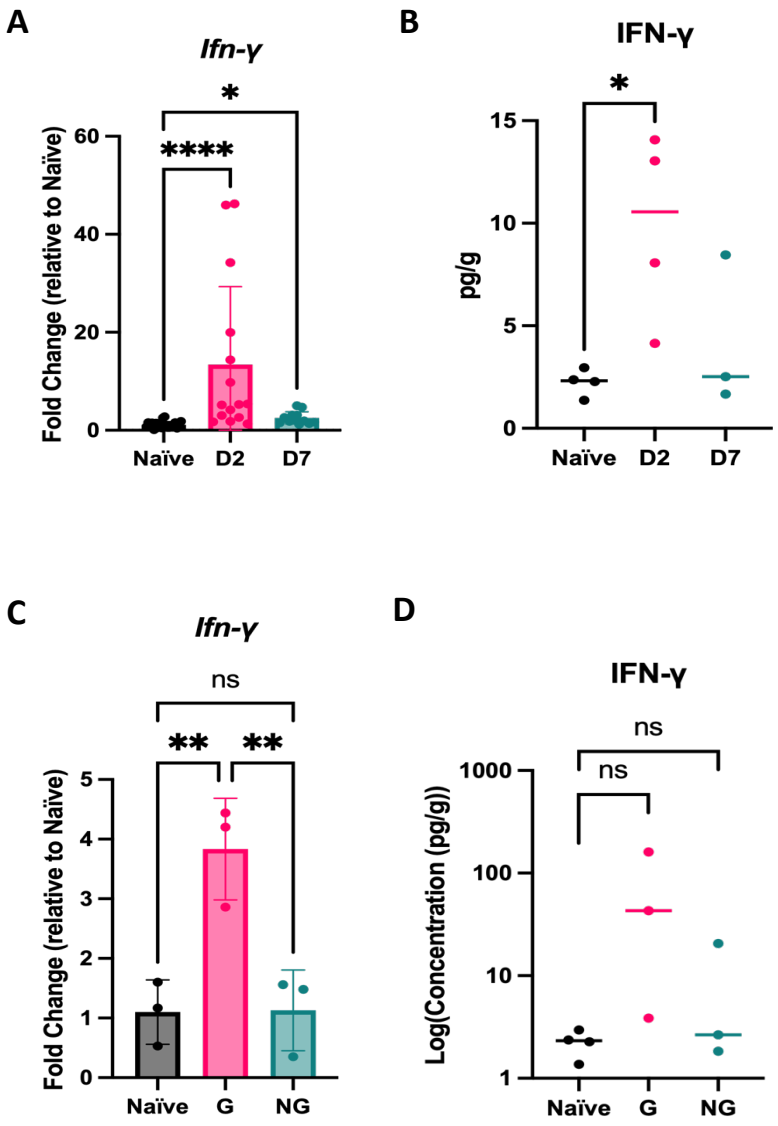

Figure 3-13 IFN-γ expression increases in *Hpb* granuloma during early infection. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and samples were collected at days 2 and 7 post infection. **A)** Fold change of Ifn-γ in the duodenum compared to housekeeping gene (Rps29) and normalised to the mean of the naïve samples. **B)** 1cm sections of duodenum isolated and cultured in R10 media overnight, supernatant was collected and IFN-γ protein levels measured using CBA assay. **C & D)** Granulomas (shown in Figure 3-2A) and non-granuloma equivalents were isolated from mice at D7 post-infection and used either for **C)** qPCR (analysed as in A) or **D)** cultured as described above for measurement of secreted IFN-γ protein levels. Data shown with mean ±SD, or with line indicating median data point. 1-3 pooled experiments with 3-6 mice per experiment. Statistical significance was calculated by a One-Way Anova or in cases where data was not normally distributed a Kruskal-Wallis test followed by Dunn's multiple comparisons test was used. (Significance *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, NS, Non-significant).

3.4 Discussion

In this first chapter, I aimed to investigate the local environment of *H. polygyrus*; specifically exploring indication of disruption to barrier integrity, suggestion of bacterial invasion and defining the local immune responses. Overall, we show that *H. polygyrus* disrupts gut barrier integrity and initiates local increases in antimicrobial responses including antimicrobial protein (AMP) expression and innate immune cell recruitment. Although the helminth disrupts the epithelial barrier, bacteria do not appear to translocate with the helminth during infection, perhaps because of these antimicrobial responses. *H. polygyrus* also induces an increased inflammatory profile with a specific increase in the production of IFN-γ both locally and systemically in early infection before type 2 cytokine responses begin to dominate.

3.4.1 Disruption of the barrier

As indicated by the images in Figure 4-2B, the worm is encased in a sizeable granuloma throughout its maturation in the lamina propria, especially days 5-7, and a considerable inflammatory response is underway with high immune cell infiltrate. The size of the *H. polygyrus* helminth is staggeringly large when compared with the intestinal epithelial cells and thus, we hypothesised that there must be disruption at the barrier interface. At the timepoints which the helminth migrates across the intestinal wall, our data collectively indicate that the gut barrier integrity is affected. TJ proteins are crucial for maintaining barrier integrity, acting as a “glue” between epithelial cells. Their increased expression is suggestive of a role of repair upon damage (Namrata and Bai, 2021). Other recent studies have reported changes in tight junction protein expression during *H. polygyrus* infection (Mules et al., 2024; Schälter et al., 2022). One study suggests that through the production of metabolites, the helminth can break down tight junction proteins to facilitate its transportation across the epithelial barrier (Schälter et al., 2022). However, other publications speculate that short-chain fatty acids produced by the worm can facilitate assembly and strengthening of tight junction proteins (Mules et al., 2024; Peng et al., 2009), perhaps to strengthen the barrier to protect the host from further infection or to protect the worm from clearance throughout the maturation stages of its lifecycle. Taken together, these studies suggest evidence for

disruption of the intestinal barrier in helminth infection and provide insight into mechanisms by which the helminth can manipulate TJ proteins. Perhaps the worm breaks down TJ proteins to enter the intestinal tissue and is able to facilitate their assembly and reinforce the barriers once through, to protect the host from additional infection and evade mass disruption which may lead to clearance by the immune system. We assessed the dynamics of TJ components only by measuring gene expression at particular timepoints. To get a more complete understanding of how local these TJ changes are to the helminth and whether the helminth appears to disrupt the structure between cells, a next step would be to carry out immunofluorescent staining of TJs in the small intestine at the 'breach' timepoints. Determining the localisation of TJ expression within the intestinal epithelium would help to clarify the involvement of these proteins in barrier breach during *H. polygyrus* infection.

Both calprotectin and lipocalin-2 are proteins usually produced in inflammatory environments and are known to also positively correlate with intestinal permeability (Berstad et al., 2000; Zhang et al., 2020). Since this project started, other laboratories have also explored intestinal permeability using a FITC-dextran assay and have shown significant increase in permeability at day 7 of *H. polygyrus* infection (Mules et al., 2024). This supports our data indicating that the worm is indeed causing disruption to the gut barrier integrity. We hypothesised that this disruption may allow for bacteria to translocate across the barrier.

3.4.2 Antimicrobial responses

Lipocalin-2 and calprotectin are also AMPs, produced by cells of the intestinal epithelium (Gallo and Hooper, 2012). Lipocalin-2 functions to inhibit the growth of bacteria by binding to siderophores on the bacteria directly to sequester their iron acquisition (Furci and Secchi, 2018). Similarly, calprotectin is able to starve bacteria of essential metal ions to inhibit expansion and further growth (Nakashige et al., 2015). Due to the indicated barrier disruption in helminth infection by our data and others, we decided to further investigate AMP expression in *H. polygyrus* as altered expression could indicate bacterial presence in the intestinal tissue (Duarte-Mata and Salinas-Carmona, 2023; Gallo and Hooper, 2012; Lai and Gallo, 2009). When measuring AMP expression in the

duodenum, our data show that other AMPs including SPRR2A, NOS2 and ANG4 were also increased in early *H. polygyrus* infection when compared with the naïve mice. SPRR2A is an AMP produced predominantly by Paneth and goblet cells and functions to kill gram-positive bacteria (Hu et al., 2021). NOS2 is an antimicrobial gene which produces large amounts of nitric oxide in a short amount of time, causing direct targeting of pathogens (Mühl et al., 2011; Okda et al., 2025). ANG4 is a ribonuclease that functions to kill bacteria by binding to and disrupting their membrane (Abo et al., 2023; Sultana et al., 2022). As is evident by their functions, AMPs are fundamental in protecting the host from bacterial infections and have even been shown to protect the host from further infection during *H. polygyrus* (Horsnell and Oudhoff, 2022; Hu et al., 2021; Muniz et al., 2012). One particular study showed that specifically SPRR2A was increased at day 14 PI with *H. polygyrus* and was required to prevent bacterial invasion during infection (Hu et al., 2021). Thus, we propose that the increased AMP expression is stimulated in response to bacterial invasion or as a protective measure upon the barrier disruption.

To test the hypothesis that bacteria are infiltrating the intestinal tissue by using 'breach' points created by the *H. polygyrus* worm, we decided to explore the myeloid cell infiltrate to the small intestinal tissue during infection as myeloid infiltration is often associated with bacterial invasion. A recent publication from our laboratory showing an optimised method for isolating leukocytes from the lamina propria in *H. polygyrus* infection has enabled the field to study cell populations in this context (Webster et al., 2020). The methods paper also reported that neutrophils are increased in the lamina propria at day 7 of *H. polygyrus* infection. Our data in this chapter show that neutrophil and monocyte/ macrophage populations have significant increases in cell number during early *H. polygyrus* infection, whilst DCs and eosinophils show small trends in increase but do not reach statistical significance until later in infection. Crucial, quick responders to infection, neutrophils are key in protecting the host from bacterial translocation (Fournier and Parkos, 2012). When neutrophils are depleted, bacterial translocation is greater, leaving the host more vulnerable to infection (Kühl et al., 2007). Interestingly, aside from their involvement in anti-bacterial action, recent work has proposed an important role for neutrophils in the repair process (Peiseler and Kubes, 2019; Vicanolo et al., 2025). One

particular study used a mouse model of injury in the skin and found that infiltrating neutrophils could produce proteins for building the extracellular matrix around a wound and also protected the area from bacterial invasion, suggesting that neutrophils may play a more diverse role in helminth infection than suspected (Vicanolo et al., 2025). Our data show an increase in monocytes and intermediate monocytes in early infection with no change to macrophage numbers. The proportions however reflect a significant decrease in macrophage frequency, likely due to the influx of monocytes. Monocytes are often recruited in inflammatory environments and perhaps this increase may be suggestive of a response to invading bacteria. Although the increased neutrophils and monocytes shown in our data do not prove that bacteria are able to enter host tissue through breach points created by the helminth, they are consistent with this hypothesis, suggesting a host response to increased bacterial contact.

The proportions and numbers of the monocytes and intermediate monocytes returns to baseline by D14 of infection, with higher macrophage proportions at this timepoint. AAMs are often associated with helminth infection and, spatially, have been found locally to helminths (Anthony et al., 2006; Poveda et al., 2024). These cells are involved in tissue homeostasis, controlling inflammation and can contribute to the overall elimination of helminths from the host (Anthony et al., 2006). They are induced predominantly by Th2 cells and would therefore be expected late in infection, aligning with our cell numbers and frequencies and also with our qPCR data showing increased Arg1 and Relm-α gene expression during later infection. DCs are fundamental to kickstarting the Th2 response and enhancement of type 2 immunity during helminth infection (Maizels et al., 2012; Phythian-Adams et al., 2010). Due to their role, it is perhaps unsurprising that they increase as infection progresses at the same time as the Th2 response.

Many studies demonstrate that the host experiences eosinophilia during helminth infection (Löscher and Saathoff, 2008). Specifically in *H. polygyrus* infection, a study has shown that eosinophils aid in the clearance of adult worms (Hewitson et al., 2015). Our experiments found a trend towards increased eosinophil number in *H. polygyrus* infection, although this did not reach statistical significance. The lack of significance here is likely due to the fact that eosinophils are recruited and activated by IL-5, a type 2 cytokine, and our data show that the type 2 cytokines were not significantly increased until later in

infection. Furthermore, Ly6G+ eosinophils specifically were increased at day 7 of infection. Commonly immune cells have subsets within subsets, but little is known about the difference in functional role that these Ly6G+ eosinophils may have compared with Ly6G- eosinophils (Berdnikovs, 2021). The Ly6G+ marker is thought to be induced by IL-5, a cytokine known to be present in the local intestinal response to *H. polygyrus* infection and known to be key in general eosinophil recruitment and activation (Lacy, 2020; Limkar et al., 2020). Whether this marker is in fact a role of a more mature/ immature or active eosinophil in helminth immunity remains unknown. Although little is known about these cells, they are reported to have a reduced responsiveness to eotaxin-1, the chemoattractant known to recruit eosinophils to inflammatory sites (Lacy, 2020). This may suggest that the increase in the Ly6G+ cells we show in *H. polygyrus* infection is instead an increased proliferation of eosinophils within the intestinal tissue. The Ly6G+ eosinophils are also mainly identified in wild mice compared with laboratory mice (Mair et al., 2021). Since *H. polygyrus* is a naturally, common occurring infection (Marchiondo et al., 2019), maybe these cells play a much larger role in helminth infection than we are able to identify and understand with researching laboratory mice.

The myeloid changes we characterise here in *H. polygyrus* infection we also confirm are local to the helminth in our granuloma specific flow cytometry, possibly suggestive of bacteria presence in the granuloma of the intestinal tissue, at the sites of *H. polygyrus* barrier disruption. Surprisingly little is known about the functionality and coordination of cell populations local to the helminth in *H. polygyrus* infection. Further assessment of their phenotype, cytokine production, activation state and phagocytic ability could be investigated by sorting these cells and culturing/ running functionality tests on them. This could help to further our understanding of the role they are playing in helminth infection and whether their functions appear to be targeted towards antimicrobial, reparative or anti-helminth responses. The interface between the host and nematode has been an expanding topic of research (Patel et al., 2009). Neutrophils and eosinophils were first characterised in *H. polygyrus* infection as early as 1974 (Liu et al., 1974). In more recent years these cells were visualised in the intestinal tissue surrounding the worm by H&E staining and pathological examination (Morimoto et al., 2004). However, neutrophils appeared less

prevalent in cases where the host had increased resistance (Reynolds et al., 2012). This indicates that they are perhaps not key in immunity against helminths, and thus we propose their recruitment is to instead target bacteria at the disrupted barrier site.

Although we hypothesise that bacterial access to the intestinal tissue is provoking these antimicrobial responses, it is important to note that helminths may also drive these responses alongside the Th2 response. As previously mentioned, helminths have been known to alter microbiota composition within the host and mechanism for this has been proposed to involve altering host AMP production, perhaps suggesting helminths are responsible for the higher expression of these proteins in our data (Brosschot and Reynolds, 2018). Additionally, whilst there is no literature examining the direct recruitment of neutrophils and other immune cells by the helminths, there are several studies which show myeloid cell influx during infection with parasitic worms (Ajendra, 2021). Therefore, it may be that helminths are directly able to trigger this influx.

3.4.3 Bacterial Translocation

Barrier breach could lead to bacterial entry, and both AMP expression and inflammatory cell influx could be indicators of bacterial presence in the tissue. Studies have shown that dysbiosis or infection in the gut can cause disruption in the intestinal epithelial barrier which can lead to bacterial translocation, identifiable in the intestinal tissue (Hand et al., 2012b; Mouries et al., 2019). We therefore decided to use a straightforward staining method to gram-stain for bacterial presence in the vicinity of the worm. Although we hypothesised that the breach in barrier would allow bacterial entry, we did not detect any bacteria in the intestinal lamina propria. As this method uses a simple gram stain and goes through several washes and tissue processing steps, perhaps a more sensitive assay such as 16S rRNA fluorescence in situ hybridisation (FISH) would be more effective. Commensal bacteria do not infect the host at steady state and perhaps would not translocate even where other more virulent strains of bacteria with more effective immune evasion strategies would. Using a pathogenic bacterial strain which infects the small intestine in a co-infection model alongside the *H. polygyrus* helminth could also more appropriately

address our hypothesis that pathogenic bacteria use the breach points in *H. polygyrus* as an opportunity to invade the intestinal tissue and colonise local to the helminth. One study shows that even in scenarios of intestinal permeability, neutrophils remain effective enough to prevent infection (Fournier and Parkos, 2012; Laukoetter et al., 2007). The presence of neutrophil influx we identified during *H. polygyrus* infection is consistent with an immune response that would rapidly clear incoming bacteria. Therefore, we hypothesised that commensal bacteria may be able to translocate with the helminth but are cleared effectively by the host's effective immune response.

3.4.4 Cytokine profile

To explore this hypothesis that the host can clear translocating bacteria, we also looked at other evidence of inflammatory deterrents to bacteria, aiming to further characterise the early immune profile in *H. polygyrus* infection. Although we only found statistical significance in our IFN-γ and IL-6 cytokines in serum, there is a definitive trend of increased expression of the other inflammatory cytokines measured (MCP-1, TNF, IL-12p70 and IL-10). It was interesting to find inflammatory cytokines and chemokines in early helminth infection due to the more commonly understood type 2 immune response involved in anti-helminth immunity.

Monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) can be produced in inflammatory environments by macrophages and epithelial cells to recruit innate immune cells such as monocytes and NK cells (Ruiz Silva et al., 2016). Although we have measured the protein levels in serum, this cytokine will likely be responsible for the local influx of monocytes to the helminth in our tissue-based flow cytometry data. The production of MCP-1 can be induced in several innate immune cells by TNF signalling. Interestingly, in a *Trichinella spiralis* enteric helminth infection, a study found that TNF was required for the expulsion of this parasite. Although TNF is often associated as a type 1 response cytokine, a couple of studies have found that it can regulate Th2 responses in helminth infections such as *T. muris* by working to regulate IL-13 (Artis et al., 1999). Additionally, in *H. polygyrus* infection, mice with TNF depleted B cells were unable to properly eliminate the helminth (Wojciechowski et al., 2009). TNF can also inhibit the production of IL-12p70 by macrophages which ordinarily

promotes polarisation towards a Th1 cell response (Gilmour et al., 2024; Hamza et al., 2010), thus contributing to the role of TNF in promoting Th2 responses. There is a slight increase in serum levels of IL-12p70 found in our data which is interesting in the context of helminth infection alongside IL-6 and IFN-γ increases. IL-6 is a key in enhancing the inflammatory response, activating immune cells and contributing to tissue damage and has been shown to control PRR expression in Schistosoma infection (Antony et al., 2015). Often the secretion of IL-6 can be enhanced by IFN-γ signalling (McLoughlin et al., 2003). Hence, increased systemic secretion of IL-6 and IFN-γ in combination may be a host protection mechanism to prevent additional infection. The cytokine IL-10 plays a role in anti-inflammation, regulating and limiting the inflammatory response (Moore et al., 2001). Previous research in our laboratory showed that at 2 weeks PI with *H. polygyrus*, IL-10 was able to regulate IFN-γ secretion (Webster et al., 2022). Both our lab and other publications have previously shown the increase in IFN-γ during early *H. polygyrus* infection (Gentile et al., 2020). Our data confirm an IFN-γ spike at day 2 of *H. polygyrus* infection and additionally we show that this response could be detected both systemically and locally to the helminth. While type 2 immune responses are typically associated with helminth infections, our data show that the timings of type 1 and 2 immune responses are exclusive with IFN-γ produced early in infection and type 2 associated cytokines produced later. Although it is important to note that the measurements we make use duodenal gene expression and protein secretion measurements in blood sera which are not direct matches for one other. The IL-4, Arg1 and Relm-α markers associated with type 2 immunity that we show indicate that the type 2 responses do not appear in early infection but rather develop by day 7, whilst IFN-γ is seen as early as day 2 by both gene expression and protein secretion. The role of this IFN-γ is interesting considering the characteristic type 2 response associated with helminth infection and although it has been a recent topic of research interest, its role in helminth infection is largely undefined. IFN-γ is well-known for orchestrating inflammatory responses and contributing to type 1 immunity and previous studies have shown its ability in manipulating the intestinal epithelium during helminth infection. In *H. polygyrus* infection, Nusse et al. found that epithelial proliferation appeared to be increased and the epithelium had a more foetal like profile, which they evidence to be driven by IFN-γ. Recently, several other laboratories have

identified more niche roles for IFN-γ during helminth infection in contributing to repair (*H. polygyrus*) and even encouraging helminth survival (*T. Muris*). Our own research priorities were to understand and characterise the role of this increased IFN-γ in protecting the host from further infection, specifically at day 2 PI. The next two chapters aimed to therefore understand the role of IFN-γ in this context and whether it is produced in response to the damage caused to the epithelium, bacterial invasion or to the worm itself. This chapter has provided evidence for disrupted barrier integrity and enhanced antimicrobial responses in the form of AMP expression and immune cell influx during early *H. polygyrus* infection. Although, translocation of bacteria was not identified, we characterised a strong inflammatory profile with anti-microbial responses suggestive of bacterial invasion. We aimed to explore the role IFN-γ plays in the coordination of these responses and to investigate whether bacteria influence any of the changes in *H. polygyrus* infection that we describe.

Chapter 4

4.1 Introduction

We have shown that during *H. polygyrus* helminth infection, gut barrier integrity is reduced, and antimicrobial defences are increased. As a fundamental cytokine, IFN-γ is known to coordinate many immune responses, but its specific role in the changes we see in *H. polygyrus* infection remain unclear. In this chapter we set out to explore whether the IFN-γ produced in early stages of *H. polygyrus* infection is responsible for changes in barrier integrity, anti-bacterial defences and preventing bacterial translocation.

Despite its role in suppressing type 2 immunity, studies have shown that IFN-γ is produced in helminth infections. In *H. polygyrus* infection, studies have shown that depletion of IFN-γ by either signalling blockade or genetic knockout (KO) models can increase resistance to *H. polygyrus* infection with reduced worm and egg counts (Kapse et al., 2022b; Reynolds and Maizels, 2012). Both studies highlight that absence of IFN-γ enables stronger Th2 responses which are effective at reducing parasite burden. Although, the reason for the production of IFN-γ during helminth infection remains unclear; whether it is a response to epithelial damage, or is induced by the helminth, allowing larval entry into the intestinal barrier and providing protection against Th2 immunity or is produced in response to bacterial invasion, playing an overlooked role in protecting the host from further infection upon barrier disruption.

The function of IFN-γ in intestinal repair in other contexts is controversial: a study using a human intestinal epithelial cell line has shown that short term IFN-γ exposure can induce proliferation, whilst long-term exposure inhibits and reduces proliferation. A study using another epithelial cell line showed that IFN-γ is able to directly damage the epithelium, promoting apoptosis of epithelial cells (Woznicki et al., 2021). Whereas, using a model of graft-versus-host disease, research by Mowat, illustrates that IFN-γ can promote epithelial cell proliferation. Other studies using *T. muris* infection support this research, and found that IFN-γ could promote epithelial proliferation during infection (Artis and Grencis, 2008; Cliffe et al., 2005c). Recent research has shown that IFN-γ is also able to manipulate the intestinal epithelium in helminth infection, driving a

foetal-like reversion in stem cells in the intestinal crypts (Nusse et al., 2018b) which might control tissue repair and remodelling as helminth-inflicted damage to the gut wall is resolved. Another paper established that natural killer (NK) cells localise to *H. polygyrus* granulomas in infection and contribute to wound healing in a IFN-γ signalling-dependent manner (Gentile et al., 2020). Together, these data indicate that IFN-γ can be a key factor in both epithelial damage and repair. We therefore aimed to further define the relationship between IFN-γ and the intestinal epithelium, and to determine whether the changes to barrier integrity identified at day 2 of *H. polygyrus* infection were controlled by this cytokine.

Intestinal epithelial cells are not only important in barrier integrity but also contribute to antimicrobial defence as the predominant secretors of antimicrobial proteins (Gallo and Hooper, 2012). Thus, we wondered whether the increased AMP changes that we observe in early infection are due to the effects of IFN-γ on the epithelium. Other cells which express AMPs and have other antimicrobial functions include neutrophils and macrophages and are known to be stimulated by IFN-γ. However, IFN-γ is not always directly associated with recruitment of neutrophils and monocytes to inflammatory sites and has been shown to attenuate their recruitment in some cases, maintaining tissue homeostasis (Dallagi et al., 2015; Hoeksema et al., 2015; Nandi and Behar, 2011). The recruitment of these cells to the intestine during helminth infection has not been studied in the context of IFN-γ. We hypothesise that IFN-γ has a beneficial role in coordinating local immune responses to limit and prevent bacterial infection during ongoing *H. polygyrus* infection.

As previously mentioned, IFN-γ can be produced by NK cells, T cells and ILCs (Mosmann and Coffman, 1989; Perussia, 1991; Sad et al., 1995). ILCs are most commonly found in the intestinal tissue and can initiate immune responses reflective of their T cell counterparts. As mentioned in section 1.1.3, ILC2s are key in helminth infection: when activated in response to alarmins upon epithelial damage, they initiate the ‘weep and sweep’ response that clears the worm. ILC1s are most similar to Th1 cells and form part of the type 1 immune response, key in fighting viral and bacterial pathogens and amplifying responses via IFN-γ production. The researchers who established roles for NK cells in

wound repair in *H. polygyrus* infection also suggest that these NK cells may be responsible for the increased expression of IFN-γ (Gentile et al., 2020).

NK cells can be activated and recruited via IFN-γ signalling and this is their most abundantly produced cytokine. Also, as IFN-γ activates macrophages, enhancing IL-12 production, there is a positive feedback loop occurring for IFN-γ secretion to maintain and amplify an active inflammatory response (Tau and Rothman, 1999). Both NK cells and ILC1 can also be directly activated upon bacterial contact, further increasing local IFN-γ production. Therefore, to examine the source and the regulation of IFN-γ during *H. polygyrus* infection, we aimed to characterise these populations and assess their activation and IFN-γ production in the presence and absence of IFN-γ.

Our overarching hypothesis in this chapter is that IFN-γ is produced in response to translocating bacteria during helminth infection and is contributing to both repair and antimicrobial defence to locally protect the host from secondary infection. To test this hypothesis, we analysed epithelial changes under IFN-γ influence to address direct changes. We also used an *in vivo* monoclonal antibody against IFN-γ to neutralise this cytokine in *H. polygyrus* infection and monitored some of our previously identified changes. We analysed TJ changes and intestinal permeability to assess the influence of IFN-γ on damage/ repair. We also assessed the expression of AMPs and characterised changes in the immune cell populations upon IFN-γ blockade. Additionally, we confirmed a source for the IFN-γ expression. Overall, the data in this chapter helped to characterise a protective role for IFN-γ in the context of early *H. polygyrus* infection.

4.2 Aims

Our overlying aim for this chapter was to understand and characterise the role of IFN-γ at day 2 of *H. polygyrus* infection and to explore its involvement in the immune changes characterised in Chapter 3. To do this, we answered these specific aims:

- To investigate whether IFN-γ instructs and alters the intestinal epithelium in early *H. polygyrus* infection.

- To explore whether IFN-γ is involved in the damage or repair to the gut barrier integrity that we observe in early *H. polygyrus* infection.

- To determine whether IFN-γ is co-ordinating and promoting the antimicrobial defences that we characterised in early *H. polygyrus* infection in Chapter 3.

- To identify cells responsible for IFN-γ production during early *H. polygyrus* infection and understand what may drive their IFN-γ secretion.

4.3 Results

4.3.1 Exploring IFN-γ effects on the intestinal epithelium

Due to the local expression of IFN-γ in the small intestine, we first investigated the effects of this cytokine in directly instructing the intestinal epithelium. This work was performed in collaboration with Menno Oudoff's laboratory (the experiments were conducted at the Norwegian University of Science and Technology, Trondheim; and the Oudhoff group have since relocated to Carleton University, Canada), who kindly shared a dataset generated in recent experiments culturing murine intestinal organoid systems with a variety of cytokines including IFN-γ individually and in combination with either IL-13 or IL-22. Crypts from the small intestine were used to set up small intestinal organoids. These organoids were seeded for 24 hours and then cultured with each cytokine combination for a further 24 hours, after which the samples were sent for bulk RNA sequencing (Lindholm et al., 2022). I ran Searchlight2 (Cole et al., 2021) on this sequenced dataset to look at the differential expression of genes between the treatment groups, and my colleague Patrick Shearer (a postdoc in the Perona-Wright group) further analysed the data using R Studio and generated the figures shown in Figure 4-1.

Our analysis shows first that IFN-γ stimulation of these intestinal epithelial organoids upregulates predicted pathways of IFN-γ signalling and MHC upregulation (Figure 4-1A). We then looked to see if tight junction repair and barrier integrity were affected and, interestingly, an IFN-γ stimulated cell adhesion pathway and pathways associated with antimicrobial defence were upregulated (Figure 4-1A). Figure 4-1B illustrates changes in gene expression, for genes within these pathways that were differentially expressed in untreated and IFN-γ treated organoids. All are upregulated by IFN-γ. We also wanted to know if IFN-γ was altering the epithelium to coordinate the neutrophil attraction we observed in Chapter 3. The volcano plot in Figure 4-1C shows the overall pattern of genes either up or downregulated in the IFN-γ treated organoid culture compared with the control. Some of the genes with the highest fold change and statistical significance are labelled and include a number of chemokines known to attract monocytes and neutrophils into the tissue such as CXCL9, CXCL10, CCL5 and leukotriene B4 receptor 2 (ltb4r2), and the expression level of these

four genes is further detailed in Figure 4-1D. Together these data show that IFN-γ can signal directly to intestinal epithelial cells, promoting barrier integrity and antibacterial immunity, including recruitment of innate immune cells into the tissue.

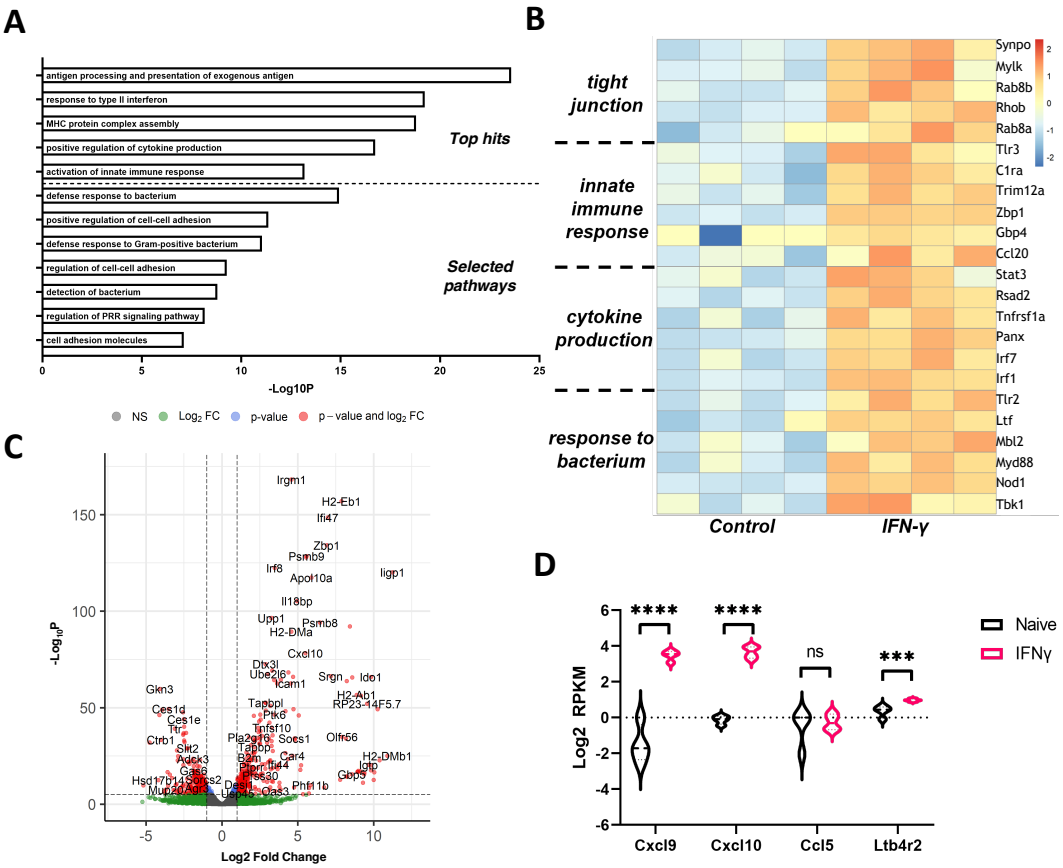

Figure 4-1 IFN-γ upregulates TJs, cell adhesion and the ability of the epithelium to recruit neutrophils in a gut organoid system.
Small intestine organoids were stimulated with recombinant IFN-γ and sequenced by bulk RNA-seq. (A) Significantly upregulated pathways calculated by Metascape pathway analysis reported as the reciprocal of p-value *($log_{10}P$).* (B) Differentially expressed genes from selected pathways in (A) from control and interferon treated organoids, z-score scaled Reads Per Kilobase per Million mapped reads (RPKM) values shown. (C) Most differentially expressed genes after interferon treatment highlights genes involved in neutrophil recruitment which are presented in (D). Log scaled RPKM of neutrophil recruitment genes, statistical testing carried out using a two-tailed unpaired t-test. *P < 0.05, **P < 0.01, ***P < 0.001 and ***P < 0.0001. FC, fold change.

To explore the role that IFN-γ plays in *H. polygyrus* infection, we decided to block this cytokine in the early phase of infection and to investigate whether this blockade affects the altered reduction of barrier integrity and increased antimicrobial defences which we identified previously. We infected mice with *H.*

polygyrus as before and additionally administered an *in vivo* monoclonal antibody specific for IFN-γ at day -1 and day 2 of infection (Figure 4-2A).

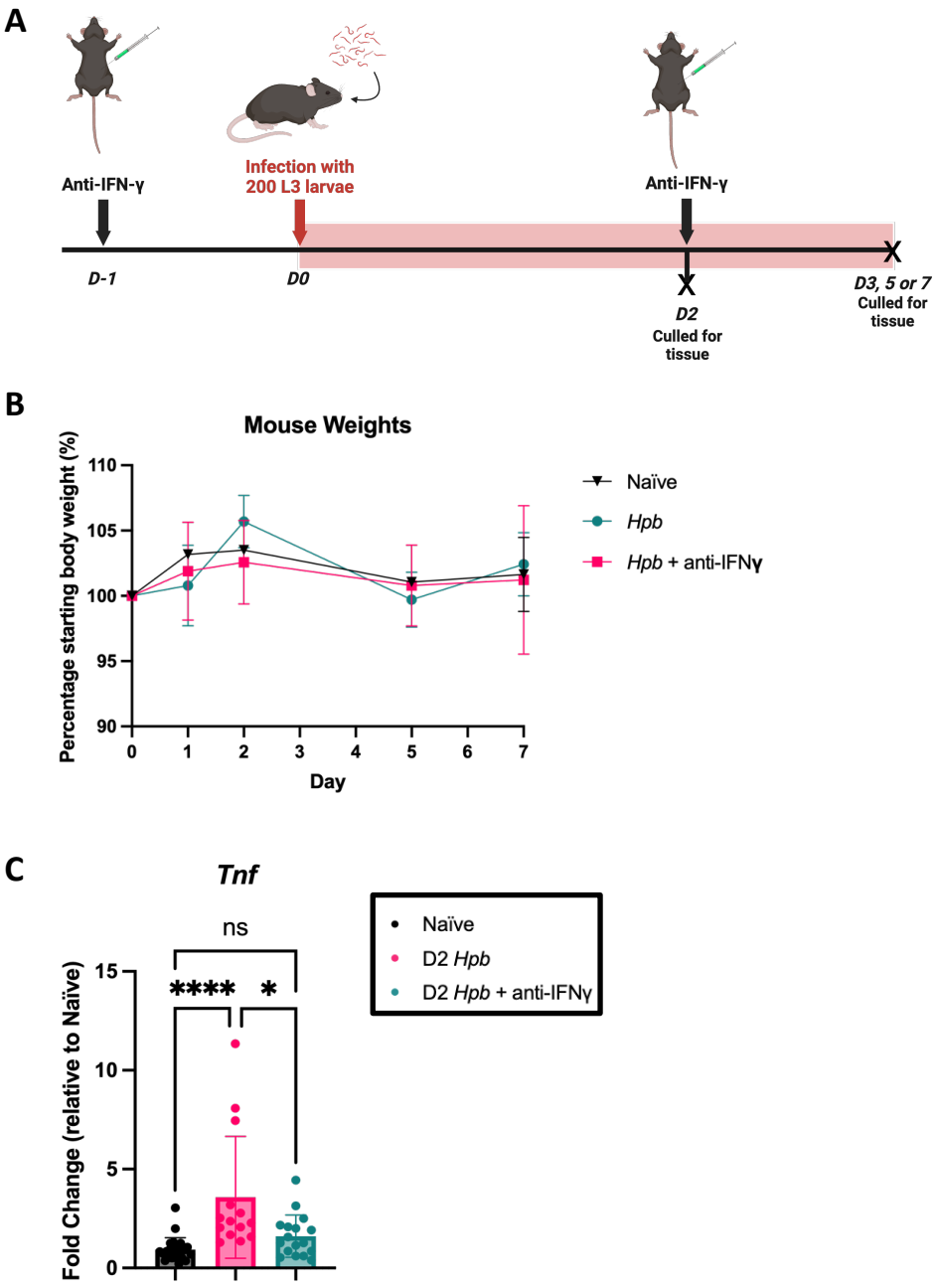

Figure 4-2 Successful *in vivo* Hpb infection with IFN-γ blockade.
A) C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at days -1 and 2 of infection, samples were collected at days 2, 3, 5 or 7 post infection. **B)** Mouse weights during infection and anti-IFN-γ treatment, plotted as percentage of starting body weight. **C)** Fold change of Tnf in the duodenum compared to housekeeping gene (Rps29) and normalised to the mean of the naïve samples. Data shown with mean ±SD, 3 pooled experiments with 3-7 mice per experiment. Statistical significance was calculated by a Kruskal-Wallis test followed by Dunn's multiple comparisons test. (Significance $*p < 0.05$, $****p < 0.0001$, NS, Non-significant).

Mice were weighed throughout initial experiments to monitor their health and showed no significant changes when anti-IFN-γ was administered alongside *H. polygyrus* infection (Figure 4-2B). The antibody used is well cited and, throughout experiments, changes were observed in gene expression that are indicative of successful neutralisation of the IFN-γ protein. As an example, the gene expression of TNF is usually increased alongside the IFN-γ at day 2 of *H. polygyrus* infection as these cytokines often work synergistically. TNF is often induced as a downstream result of IFN-γ signalling (Del et al., 1998). We saw that, when anti-IFN-γ was administered, the expected increase in TNF is suppressed (Figure 4-2C), reflecting that the resulting IFN-γ changes usually identified are limited, suggesting effective IFN-γ neutralisation. Other measurements which could have been carried out to validate this include measuring MHC upregulation or NK cells, both of which are more difficult to measure in vivo than the TNF gene expression. Examination of H&E-stained slides from *H. polygyrus* infected mice with and without IFN-γ blockade revealed that infection was successful in both cases, with no inflammatory differences between intestines from infected mice treated with anti-IFN-γ and their infected, untreated controls (Figure 4-3).

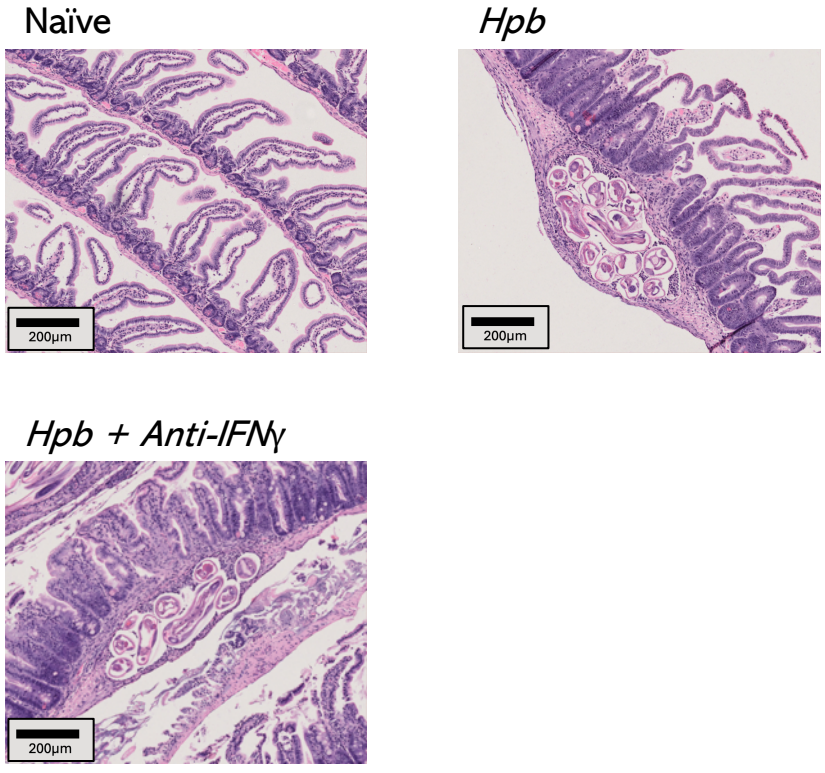

Figure 4-3 *Hpb* granuloma identified, representing successful and ordinary visual infection alongside IFN-γ blockade.

C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at days -1 and 2 of infection, samples were collected at day 7 post infection. H&E staining of small intestinal tissue at day 7 post-infection with and without anti-IFN-γ treatment and naïve control.

With the intestinal organoid data showing us that IFN-γ can influence gene expression of epithelial cells *in vitro*, we decided to look at the direct effects of IFN-γ on the proliferation of the epithelial cells during early *H. polygyrus* infection. Often in the intestinal epithelium, increased proliferation is an indication of repair as the body tries to replace damaged tissue and restore the intestinal barrier upon disruption (Iizuka and Konno, 2011). Thus, how intestinal epithelial proliferation is affected during *H. polygyrus* infection and assessing whether it is influenced by IFN-γ could help to define the role of this cytokine during infection. Recent research has shown that during *H. polygyrus* infection, the stem cells in the intestinal epithelium appear to revert to a more foetal-like state. The researchers found that these changes were indicated by Sca-1 and induced in a IFN-γ signalling dependent pathway (Nusse et al., 2018b). We decided to further investigate the influence of IFN-γ on the epithelial proliferation directly using our *in vivo* blockade model and an additional 5-ethynyl-2'-deoxyuridine (EdU) stain. EdU is administered to mice by IP injection and incorporates into newly synthesised DNA, allowing detection of proliferating cells by a fluorescently labelled azide group. In the small intestine, 24 hours after EdU is administered, the stain shows at the base of the villi and by 96 hours after administration, the tips of the villi show as stained (Salic and Mitchison, 2008). This is due to the direction of the epithelial cell proliferation: cells proliferate in the crypts of the villi, and this then pushes older cells upwards towards the tips of the villi. The rate of proliferation since EdU-injection can therefore be measured by the distance that the stained cells have travelled upwards along the villi. By measuring how large the stained section is as a percentage of the total villi length, this indicates the proliferation rates at the time of injection.

A pilot study was carried out to determine how long after giving EdU to mice provided the easiest visualisation of proliferating cells. Administering EdU treatment 40 hours prior to the cull was decided to be the most suitable timepoint due to the EdU stained cells being in an optimal location on the villi.

Additionally, administering EdU 40 hours prior to the cull on day 3 of infection allowed us to capture the proliferation during the day 2 timepoint when the helminth is breaching the intestinal barrier and the IFN-γ is at peak expression. Figure 4-4A shows the successful imaging of the EdU stained small intestine, with Hoeschst (a common DNA stain used to identify each cell nucleus) labelled in blue and the EdU stain (showing labelled proliferating cells) in green. With further magnification, the proliferating cells can be seen lining the lower half of the villi (Figure 4-4B). The analysis of the EdU stained slides was carried out by Jay Biggart, an undergraduate student working with me during her investigative project in the Milling laboratory. For analysis, 20 villi per mouse sample were picked at random, the villi length was measured and the lengths from the base of the crypt to the top of the EdU stained cells and from the base of the crypt to the bottom of the EdU stained cells were measured. These measurements allowed us to determine the overall percentage of each villus stained by EdU and, by measuring the height at which the stained cells had travelled along the villus, allows us to measure how much proliferation had occurred after the EdU labelling.

No significant differences were found between the control and IFN-γ blockade experimental group in the length of villi (Figure 4-5A). Additionally, quantification of EdU staining showed no significant differences in the percentage of villus containing proliferating cells (stained), or in the percentage of the villus the stained cells had travelled up (Figure 4-5B). However, there was a slight trend in the data suggesting that the EdU stained cells had not travelled as far along the villi in the *H. polygyrus* infected anti-IFN-γ treated mice when compared with *H. polygyrus* infected and isotype treated mice, perhaps indicating less proliferation. Together our data so far suggest that although IFN-γ is able to alter the gene expression of epithelial cells, it does not appear to change the proliferation of these cells during *H. polygyrus* infection.

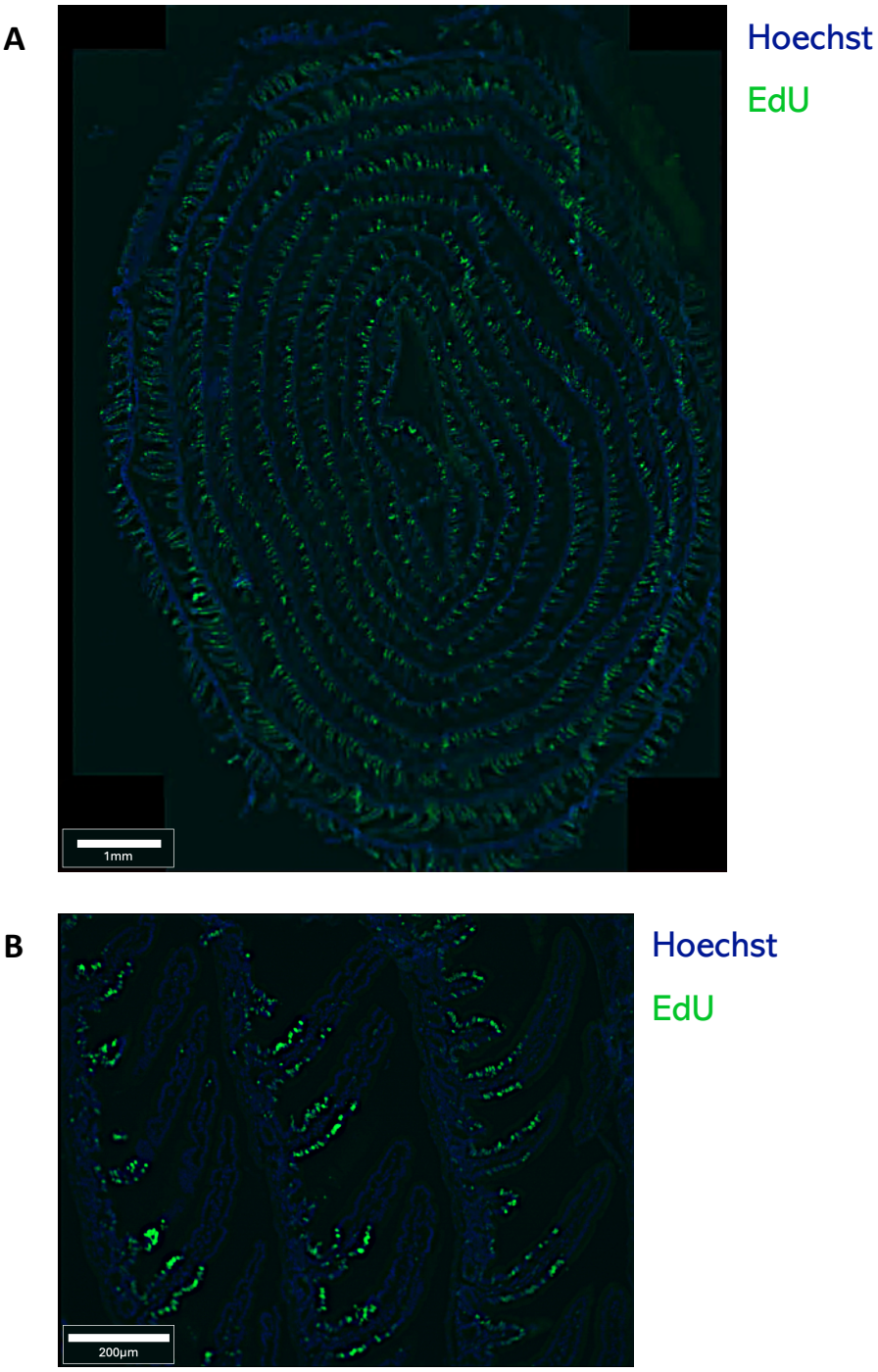

Figure 4-4 Representative images of EdU staining of the small intestinal tissue. C57BL/6 mice were infected with 200 L3 *Hpb* larvae. A monoclonal antibody of IFN-γ was administered at days -1 and 2 of infection and EdU was administered 40 hours prior to the cull. Samples were collected at day 3 post infection. Small intestine was stained with the Clickit reaction kit to capture EdU incorporated into DNA of proliferating cells (green). Hoechst was used to stain cell nuclei (blue). **A)** Shows overview of staining in Swiss roll. **B)** shows staining at higher magnification to show localisation of proliferating cells on the villi. Images shown are representative of experiment using 3-4 mice per experimental group (data in Figure 4-5).

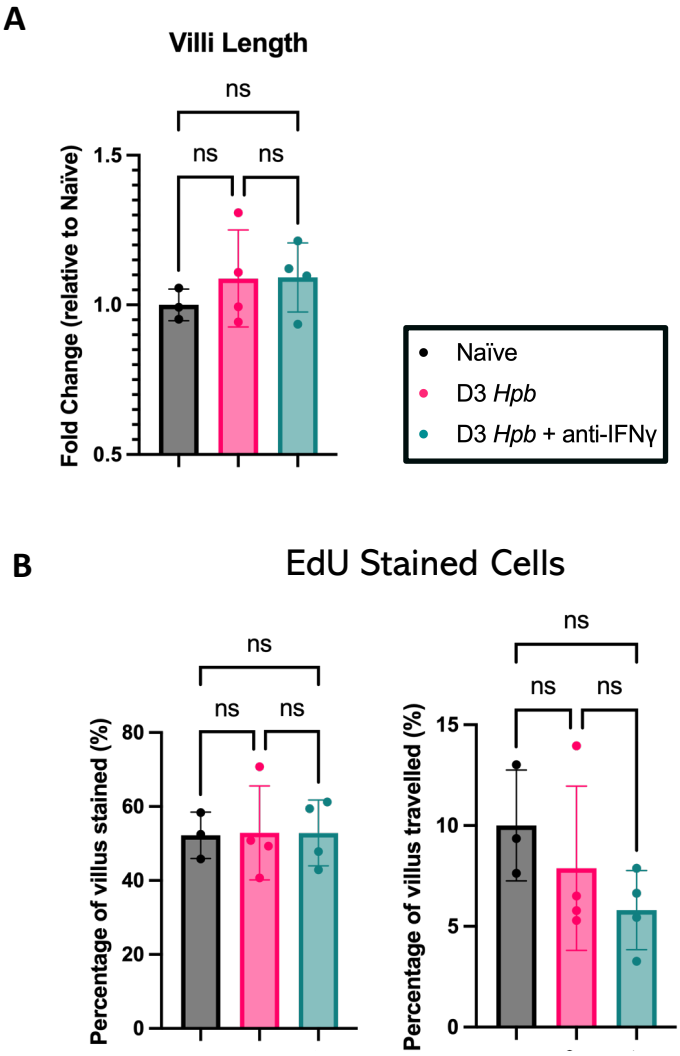

Figure 4-5 IFN-γ does not affect proliferation of intestinal epithelial cells in *Hpb* infection. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at days -1 and 2 of infection and EdU was administered 40 hours prior to the cull. Samples were collected at day 3 post infection. Small intestine was stained with the Clickit reaction kit to capture EdU incorporated into DNA of proliferating cells. **A)** Fold change of villi length normalised to naïve samples. **B)** Percentage of villi stained, and percentage of height stain begins from crypt of villi plotted. Data shown with mean ±SD, experiments used 3-4 mice. Statistical significance was calculated by a One-Way Anova followed by a Tukey's multiple comparisons test or in cases where data was not normally distributed a Kruskal-Wallis test followed by Dunn's multiple comparisons test was used. (NS, Non-significant).

4.3.2 IFN-γ effects on gut barrier integrity in *H. polygyrus* infection

Although epithelial cell proliferation remained unaffected by IFN-γ signalling, we next examined some of our previously identified changes in epithelial barrier structure during *H. polygyrus* infection, starting with tight junction (TJ) protein expression. We looked at the gene expression of cadherin 2 (Cdh2), Tight

junction protein 1 (Tjp1) (also known as zonula occludens-1 (ZO-1)) and occludin (Ocln). As we found before, the duodenum had higher gene expression of these TJ proteins at day 2 of *H. polygyrus* infection compared to uninfected mice. However, blockade of the IFN-γ alongside infection with *H. polygyrus* ablated the significant changes, particularly in the case of Ocln: for Ocln, gene expression was significantly lower when IFN-γ was neutralised compared to our isotype control infected mice (Figure 4-6A-C). We proposed in Chapter 3 that the increased expression suggests a repair process in the epithelium following barrier disruption caused by the helminth and our data in this figure suggest that this repair process, at least at the level of TJ gene expression, is normally directed by the locally produced IFN-γ.

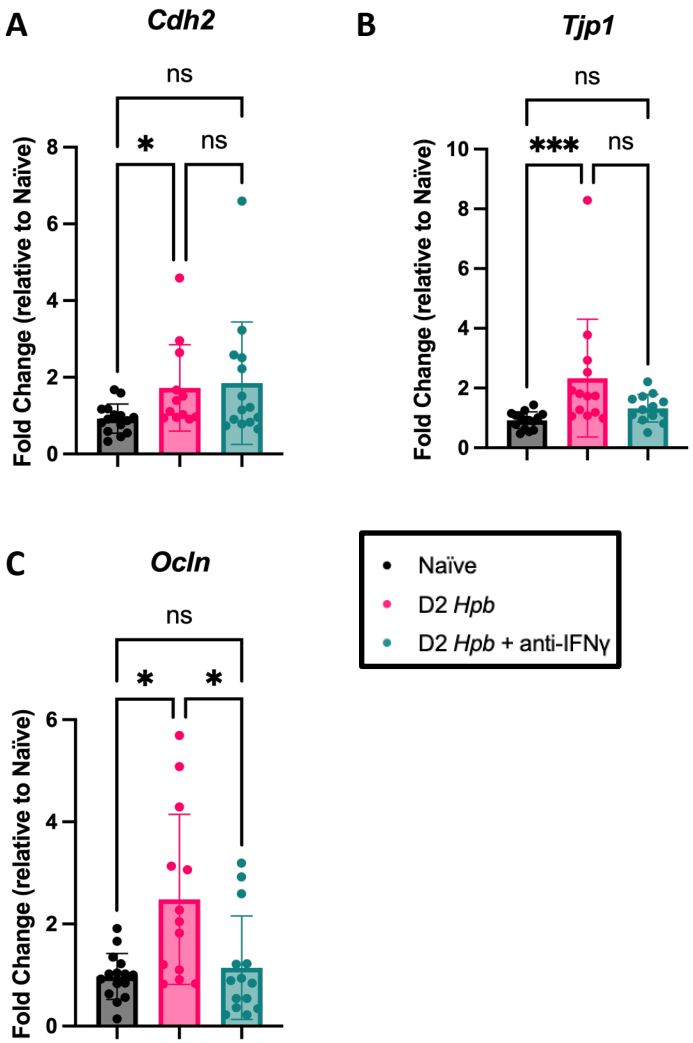

Figure 4-6 Disrupter barrier integrity in *Hpb* infection is altered by IFN-γ.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at day -1 of infection, samples were collected at day 2 post infection. Fold change of **A)** Cdh2, **B)** Tjp1, and **C)** Ocln in the duodenum compared to housekeeping gene (Rps29) and normalised to the mean of the naïve samples. Data shown with mean ±SD, 3 pooled experiments with 3-5 mice per experiment. Statistical significance was calculated by a Kruskal-Wallis test

followed by Dunn's multiple comparisons test. (Significance *$p < 0.05$, ***$p < 0.001$, NS, Non-significant).

To further examine whether IFN-γ is playing a role in repair and influencing gut barrier integrity, we carried out a FITC-dextran experiment in order to assess intestinal permeability in both the *H. polygyrus* infection alone and in the context of IFN-γ blockade. The FITC-dextran used is a 4kDa sugar molecule, commonly used to assess intestinal permeability. Its size means that it has the potential to pass between cells but should not freely cross the barrier under normal conditions. Mice were orally administered FITC-dextran 30 minutes prior to the cull at day 2 of *H. polygyrus* infection. The image in Figure 4-7A shows a small intestine from a FITC-dextran treated mouse at the time of the cull. The fluorescence can be seen along the length of the small intestine, confirming that the permeability of our area of interest, where the helminths are located, is being assessed with the assay. In Figure 4-7B, the data points plotted have been quantified measurements of FITC-dextran from the blood serum of mice, so these data are a direct measurement of permeability. The assay did not show any significant differences in the permeability between the naïve and day 2 of *H. polygyrus* infection sample groups. However, the anti-IFN-γ treated infected mice had significantly lower FITC-dextran measured in their blood serum compared with *H. polygyrus* infected, isotype treated mice (Figure 4-7B). This data suggests that neutralisation of IFN-γ in *H. polygyrus* infected mice strengthens the barrier, reducing any permeability and possibly implying that IFN-γ may be causing slight disruption to the barrier in both steady state and *H. polygyrus* infection. To further explore permeability, we ran an assay which was adapted from both Andrew MacDonald's laboratory at the University of Edinburgh and from published work (Hepworth et al., 2013). The principle of the assay was that antibodies would only exist to commensal bacteria or in much higher concentrations, if gut permeability enabled commensal bacteria to access blood and systemic sites. Serum was taken from mice and Ig specific to commensal bacteria was measured by an ELISA. Here, no differences were seen between sample groups (Figure 4-7C). Taken together, these data indicate that at day 2 of *H. polygyrus* infection there is not enough barrier disruption to detect by either FITC-dextran or serum antibody measures of intestinal permeability. Intestinal permeability may instead be more prominent later in

infection when the worm is larger, as shown by other recent studies (Mules et al., 2024).

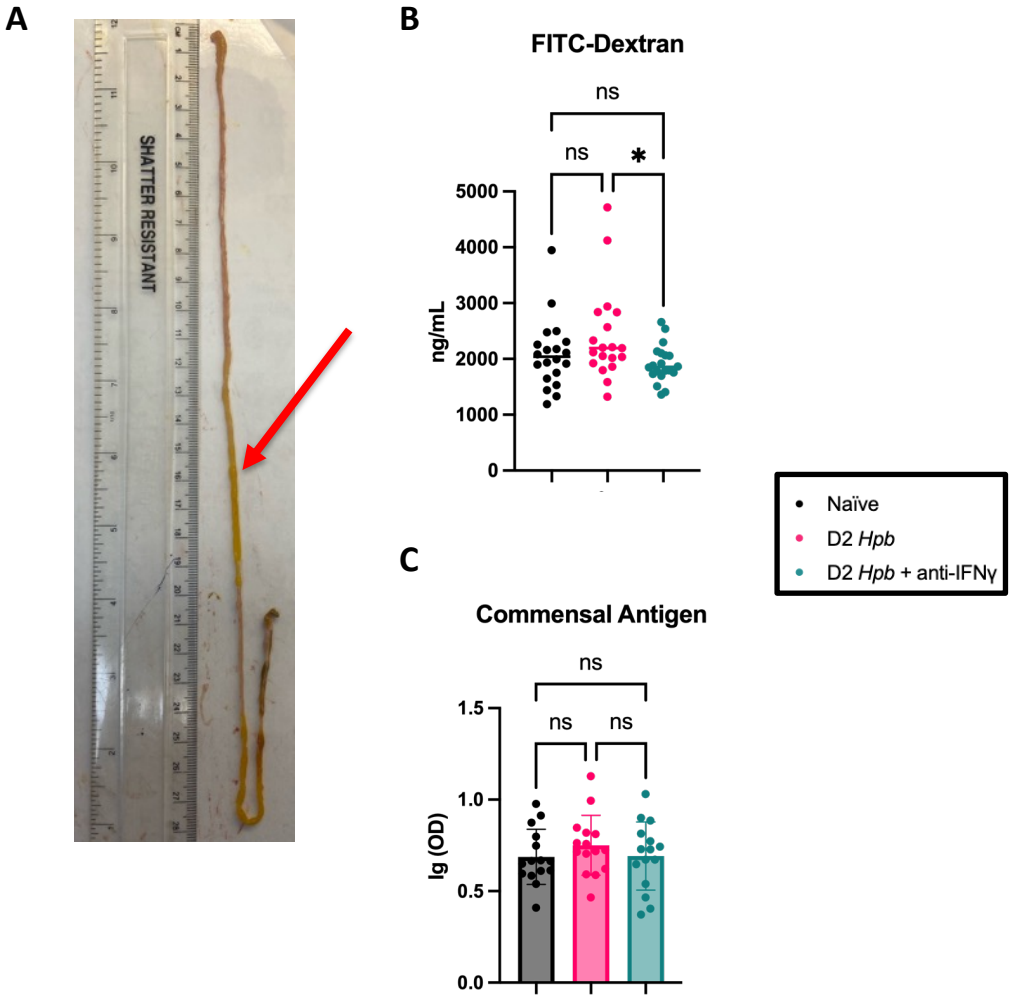

Figure 4-7 Intestinal permeability remains intact during *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at day -1 of infection, samples were collected at day 2 post infection. **A&B)** 4kDa FITC-dextran was given to mice 30 minutes prior to the cull. **A)** Image of small intestine at time of cull, arrow indicates fluorescent colour to confirm administered FITC-dextran has travelled beyond the area of interest for examining intestinal permeability. **B)** Amount of FITC-dextran detected in serum at D2 of infection. **C)** serum Ig specific to commensal bacteria plotted with OD readings compared between sample groups. Statistical significance was calculated by a One-Way Anova followed by a Tukey's multiple comparisons test or in cases where data was not normally distributed a Kruskal-Wallis test followed by Dunn's multiple comparisons test was used. (*$p < 0.05$, NS, Non-significant).

4.3.3 IFN-γ effects on antimicrobial responses in *H. polygyrus* infection

Next, we aimed to explore how IFN-γ affects the local antimicrobial responses in the context of early *H. polygyrus* infection. We first assessed Lcn2, which is both

a marker of intestinal inflammation and an AMP. We showed in Figure 3-3 that Lcn2 expression is increased in the duodenum during infection. When we compared *H. polygyrus* infected mice with and without IFN-γ blockade, Lcn2 expression had a decreased trend in expression in the absence of IFN-γ signalling, but the data was not significant (Figure 4-8A). Additionally, we identified an increase in the gene expression of AMPs Nos2 and Ang4 again in the duodenum at day 2 of *H. polygyrus* infection when compared with naïve mice (Figure 4-8B&C). In the case of Ang4, no significant changes in gene expression were measured between the isotype treated infected and anti-IFN-γ treated infected mice, but there was a possible trend towards lower levels when IFN-γ is neutralised. However, with Nos2, IFN-γ blockade reverted the infection-induced increase in gene expression back to levels similar to those in uninfected mice (Figure 4-8B). Overall, these changes in gene expression show that IFN-γ is essential for the induction of Nos2 expression upon *H. polygyrus* infection, and may contribute to the induction of other AMPs too, suggesting that IFN-γ is contributing to host defence in *H. polygyrus* infection.

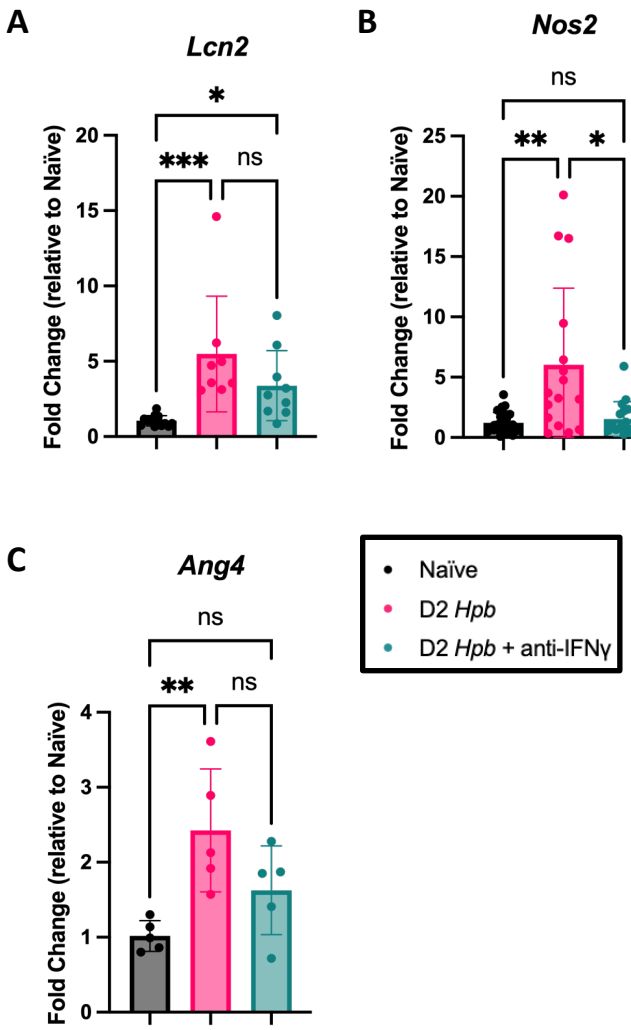

Figure 4-8 Increased AMP expression in early *Hpb* enhanced by IFN-γ.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at day -1 of infection, samples were collected at day 2 post infection. Fold change of **A)** Lcn2, **B)** Nos2, and **C)** Ang4 in the duodenum compared to housekeeping gene (Rps29) and normalised to the mean of the naïve samples. Data shown with mean ±SD, 1-3 pooled experiments with 3-8 mice per experiment. Statistical significance was calculated by a Kruskal-Wallis test followed by Dunn's multiple comparisons test. (Significance *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS, Non-significant).

Our data in Chapter 3 found an influx in immune cells local to the infecting helminths, specifically with high numbers of cells commonly involved in bacterial defence. We therefore aimed to determine how IFN-γ might be involved in regulating the inflammatory environment during *H. polygyrus* infection. We focussed particularly on day 2 of infection, due to this being the peak of IFN-γ production. Previously we found that monocytes, intermediate monocytes and neutrophils all had significant increases at day 2 post infection with *H. polygyrus* when compared to naïve mice, whilst each of the other cell populations measured had trending increase in numbers and proportion (excluding

macrophages) but did not reach statistical significance. We again used flow cytometry with the gating strategy defined in Figure 3-5 to classify myeloid cell populations in the small intestine. Although we didn't identify any visible changes in pathology (Figure 4-3), IFN-γ significantly impacted the myeloid influx that occurs during infection. Firstly, the number of DCs, eosinophils and Ly6G+ eosinophils in the small intestine were all significantly higher at day 2 of *H. polygyrus* infection in the isotype treated mice, compared to small intestines in uninfected mice; contrasting our results in Chapter 3 (Figure 3-6) where statistical significance is not reached until later in infection. The increases of each of these cell populations was ablated when infection was accompanied with IFN-γ blockade (Figure 4-9A-C). The impact of IFN-γ was not seen when only the frequency of each cell population was considered: neither infection nor IFN-γ blockade altered the frequency of DCs, eosinophils or Ly6G+ eosinophils in the small intestine (Figure 4-9A-C).

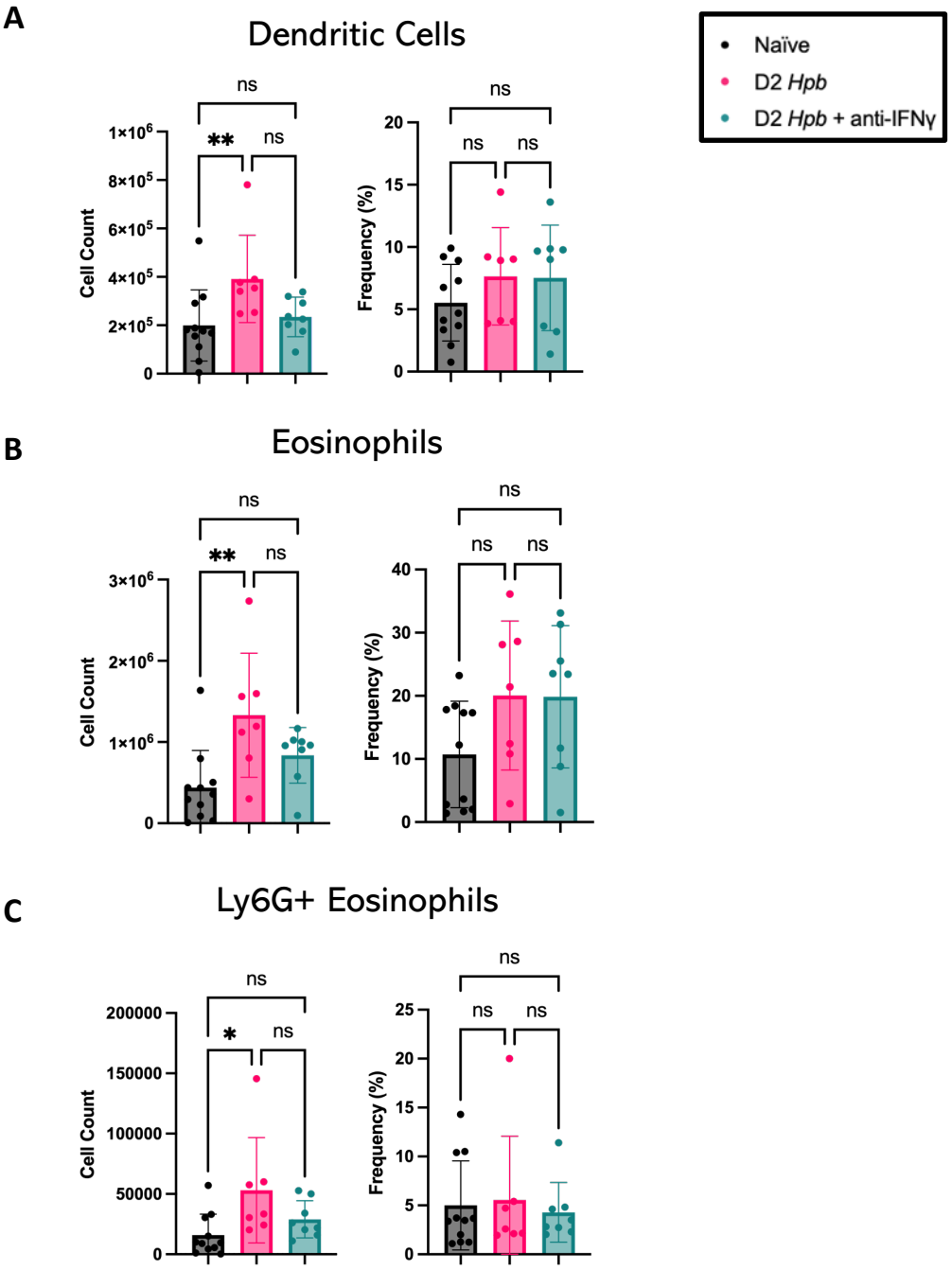

Figure 4-9 IFN-γ enhances DC and eosinophil cell numbers during early *H. polygyrus* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at day -1 of infection, samples were collected at day 2 post infection. Small intestinal lamina propria digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 3-5). Frequency and absolute cell count of **A)** DCs, **B)** eosinophils and **C)** Ly6G+ eosinophils plotted. Data shown with mean ±SD, 2 pooled experiments with 3-6 mice per experiment. Statistical significance was calculated by a Kruskal-Wallis test followed by Dunn's multiple comparisons test. (Significance *$p < 0.05$, **$p < 0.01$, NS, Non-significant).

Further identification of monocyte/ macrophage populations found that the significant increase in macrophage cell number we see in *H. polygyrus* infection

disappears in the IFN-γ blocked mice (Figure 4-10A). As we found in *H. polygyrus* infection alone in Figure 3-7B, frequencies of macrophages are significantly lower at day 2 of infection and interestingly although IFN-γ appeared to influence cell number, it does not seem to affect the frequency of these cells. Although the monocyte and intermediate monocyte populations do not show any significant changes in cell number between any of the sample groups, the graph suggests an increase in both cell types during *H. polygyrus* infection which disappears in anti-IFN-γ treated infected mice. Perhaps explaining why macrophage frequencies are reduced. Similarly to the macrophage data, neutrophils again reflected significant increase at day 2 of *H. polygyrus* infection both by cell count and proportion, however, neutralising IFN-γ impaired the neutrophil cell counts, whilst frequency remained unaffected by IFN-γ (Figure 4-10B). Taken together these results confirm that IFN-γ is playing a role in the coordination of local protection for the host during early *H. polygyrus* infection.

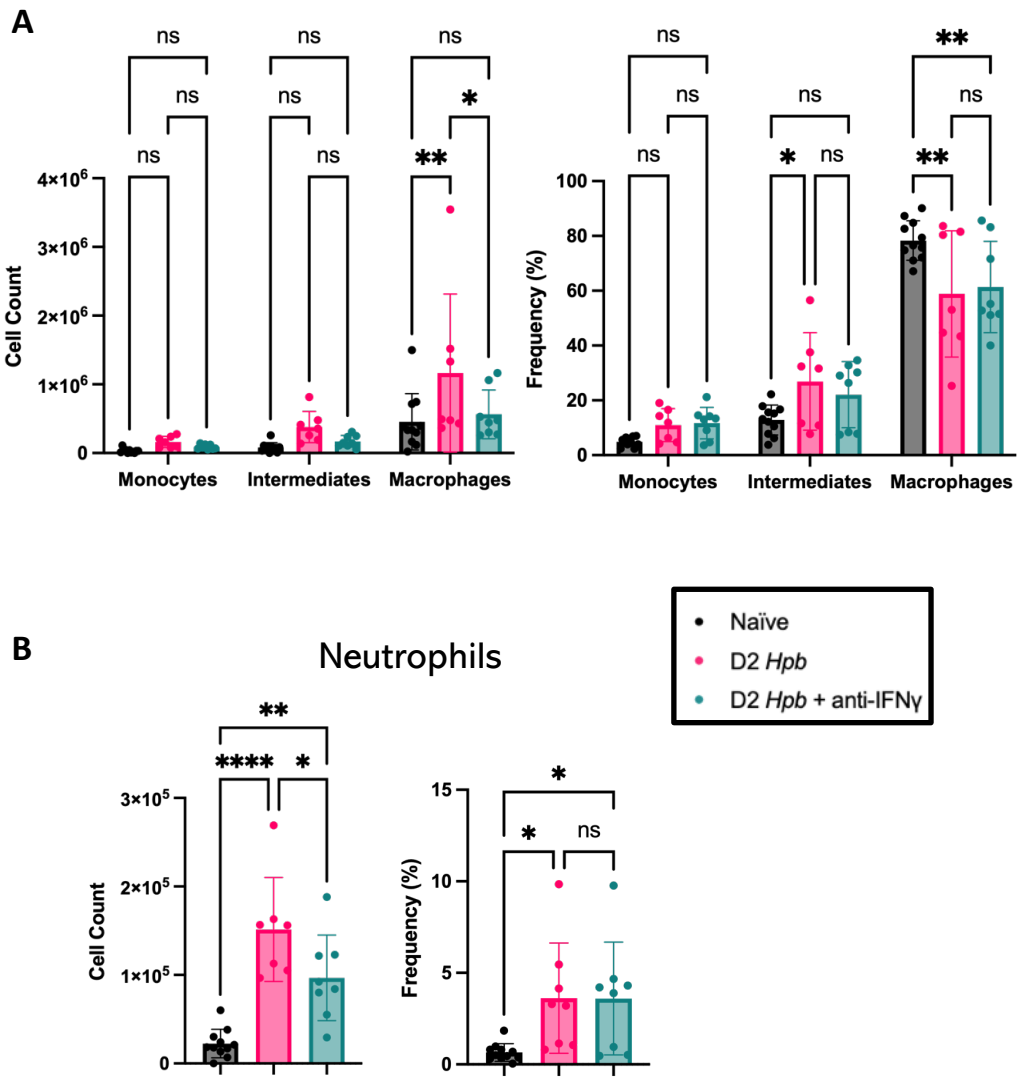

Figure 4-10 IFN-γ enhances neutrophil recruitment in early *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at day -1 of infection, samples were collected at day 2 post infection. Small intestinal lamina propria digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 3-5). Frequency and absolute cell count of **A)** monocytes (MHCII-Ly6C+), intermediates (MHCII+Ly6C+), macrophages (MHCII+Ly6C-) and **B)** neutrophils plotted. Data shown with mean ±SD, 2 pooled experiments with 3-6 mice per experiment. Statistical significance was calculated by a Two-Way ANOVA **(A)** or a One-Way ANOVA **(B)** followed by a Tukey's multiple comparisons test. (Significance *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, NS, Non-significant).

Together our data suggest that IFN-γ is contributing to the coordination of antimicrobial responses; stimulating epithelial cell changes, increasing TJ gene expression, increasing AMP expression and increasing immune cell infiltration. Considering these data, we asked whether IFN-γ is providing a local protective response for the host which is able to prevent further infection by invading bacteria. To test this, we used the saffron gram-staining approach as shown in

Chapter 3 to stain for bacterial translocation surrounding the worm in our IFN-γ blockade model. If IFN-γ is able to enhance antimicrobial defence and contribute to repair, perhaps neutralising this cytokine in early *H. polygyrus* infection will hinder the host's ability to clear and prevent commensal bacterial invasion. We again show that bacteria can be identified in the lumen of the small intestine as indicated by arrows in the naïve image. We looked at both day 2 of infection and day 7 to enable time for potential translocated bacteria to colonise and be visualised. Both timepoints show that in the intestinal tissue surrounding the worm (indicated by red circles), no bacteria are detected, suggesting no translocation in both isotype treated infected mice and the anti-IFN-γ treated infected mice (Figure 4-11A). In case the washing and fixation steps of this method were too harsh for bacteria to survive, we decided to carry out bacterial qPCRs to further assess bacterial translocation. Multiple tissues including mLNs, spleen, liver and small intestine as a positive control were collected at days 2, 5 or 7 PI and a DNA extraction was performed on the tissues. Using a pan-bacterial primer pair which targets specific regions of the 16S ribosomal RNA gene, this enabled determination of total bacterial burden. This assay is very sensitive and therefore tissue processing was carried out in a tissue culture (TC) hood for conditions to be as sterile as possible. For each of the tissues, no changes were found in the bacterial DNA measurements between each of the sample groups. Data is shown for liver only (Figure 4-11B). These two data sets do not suggest any enhanced bacterial translocation when IFN-γ is blocked.

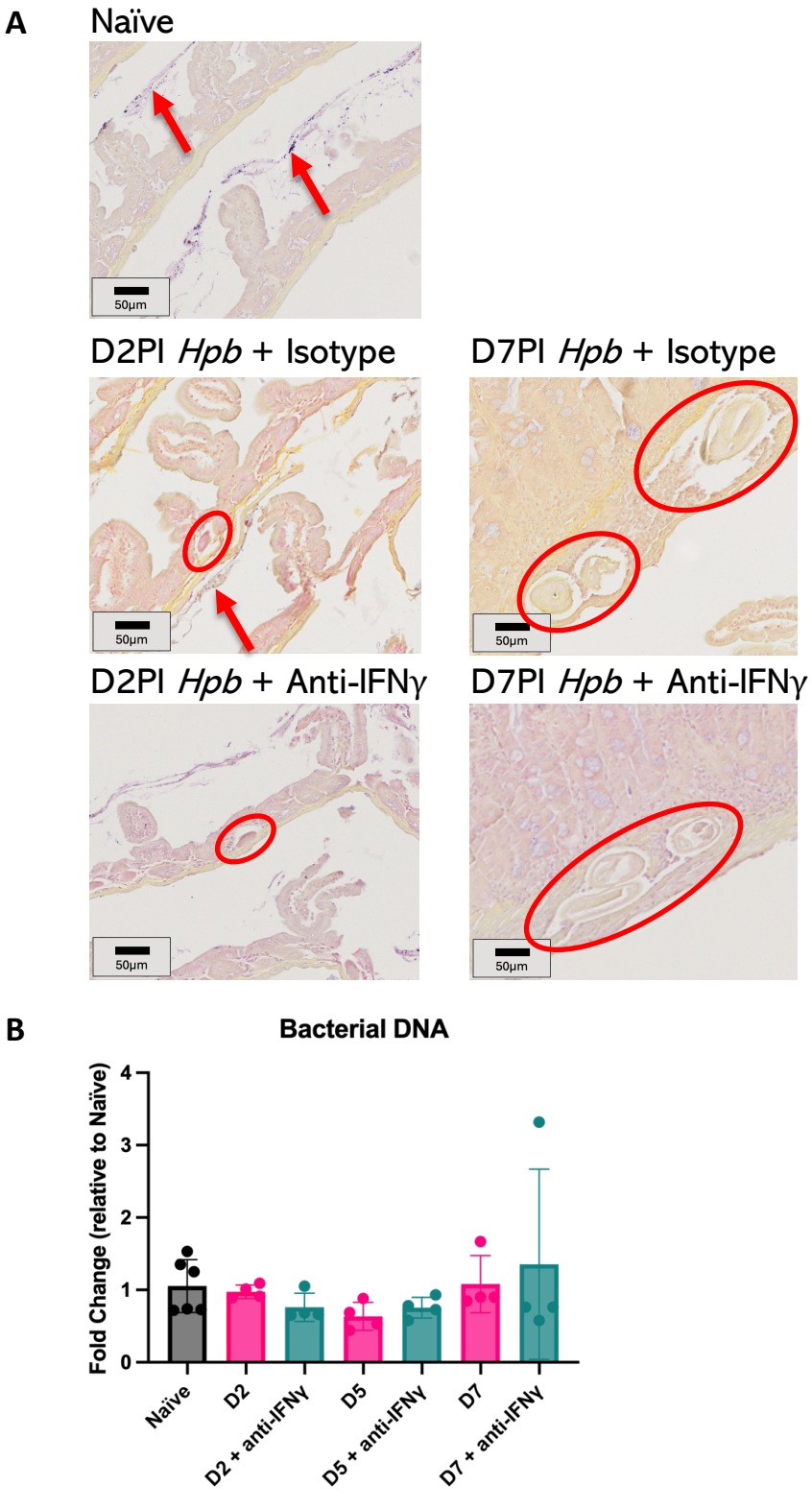

Figure 4-11 Bacterial translocation not detected in *Hpb* infection, irrespective of IFN-γ. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at days -1 and 2 of infection, samples were collected at days 2 and 7 post infection. **A)** Small intestine was saffron gram-stained at days 2 and 7 of infection. Red arrows indicate bacteria (stained purple), red circles indicate helminth. Images show naïve control group and days 2 and 7 post *H. polygyrus* infection with either isotype control or anti-IFN-γ treatment. **B)** Fold change of total bacterial burden in the liver normalised and compared to the mean of the naïve samples. Data shown with mean ±SD, 1 experiment with 4-6 mice per sample group. Statistical significance was calculated by a Kruskal-Wallis test followed by Dunn's multiple comparisons test.

4.3.4 Production of IFN-γ in *H. polygyrus* infection

Having shown the effects of IFN-γ in early *H. polygyrus* infection, we were next interested in where the IFN-γ comes from. Although other studies have also identified the higher expression of IFN-γ during *H. polygyrus* infection, no definitive cell has been recognised as responsible for this cytokine's production. One study suggested NK cells as the dominant producer of IFN-γ at day 2 of infection, whilst other recent research suggests that memory T cells are responsible (Gentile et al., 2020; Westfall et al., 2025). Innate lymphoid cells (ILCs) are cells commonly found in the small intestine and known to interact with the epithelial cells to maintain homeostasis, barrier function and control bacterial pathogens. ILC1s are also known producers of IFN-γ, alongside NK cells which can also be activated and recruited by IFN-γ. Therefore, to investigate the source of IFN-γ and to see if it stimulates a positive feedback loop to enhance its own production, we decided to investigate the populations of ILCs and NK cells in the SILP during early *H. polygyrus* infection and determine the consequent effects that IFN-γ blockade has on them. Figure 4-12 shows the gating strategy used to identify the different populations during flow cytometry analysis. ILCs were identified and gated as live, single, CD45+, Lineage-, IL-7Rα+, CD3ϵ/TCRß - cells (Figure 4-12B) which were further subset into ILC1s, ILC2s and ILC3s based on the expression of their respective transcription factors, T-bet (ILC1s), GATA3 (ILC2s) and RORγT (ILC3s) (Figure 4-12C&D). NK cells were instead gated as live, single, CD45+, CD3ϵ/TCRß-, NK1.1+ cells (Figure 4-12E).

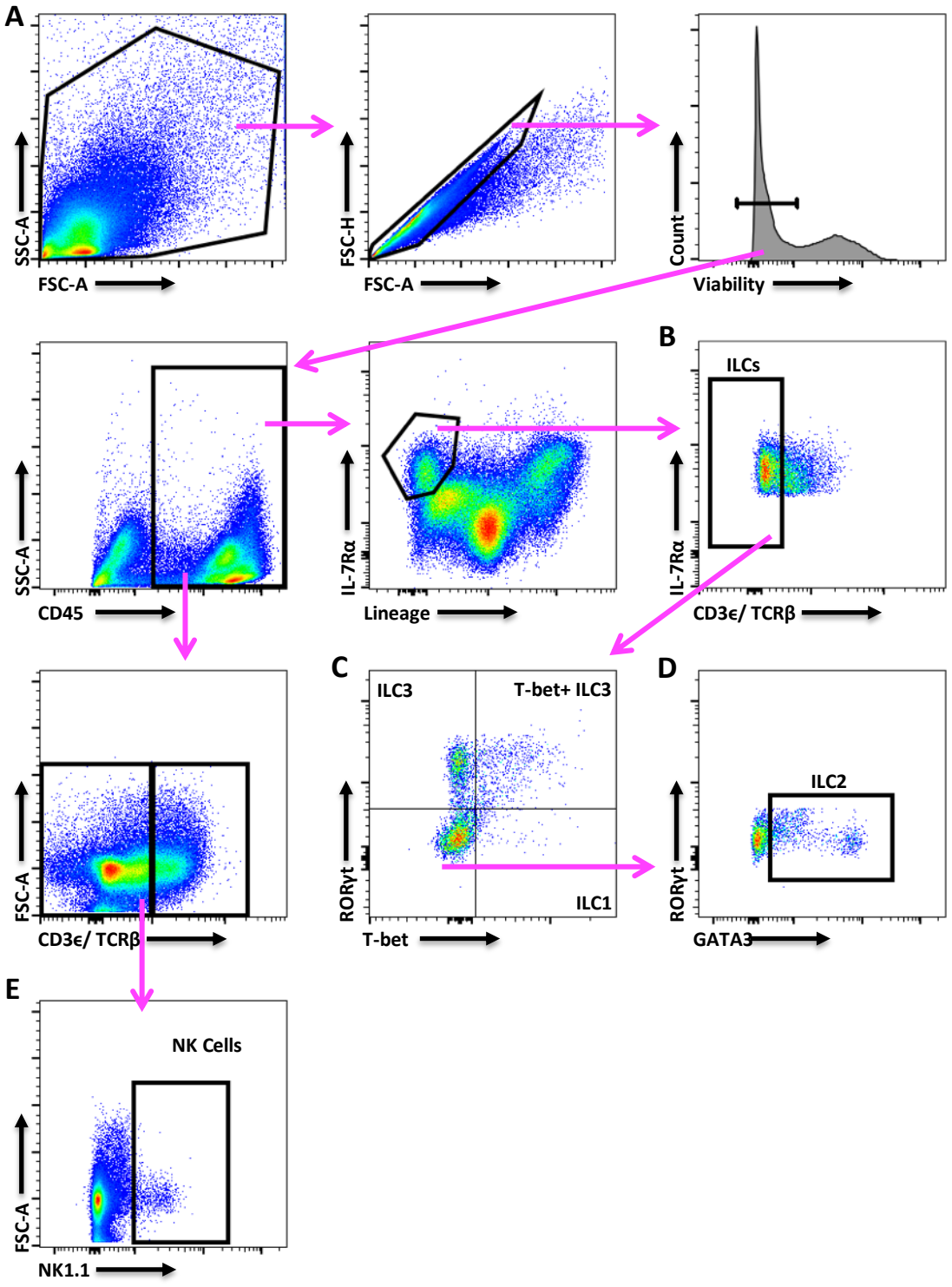

Figure 4-12 Gating strategy for identification of ILCs and NK cells from the SILP tissue during *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at day -1 of infection and samples were collected. Small intestinal lamina propria digested and analysed by flow cytometry. **A)** Gating strategy shown with final gates for **B)** total ILCs (live, single, CD45+, IL-7Rα+, lineage-, CD3ε/ TCRβ-), **C)** ILC3s (live, single, CD45+, IL-7Rα+, lineage-, CD3ε/ TCRβ-, RORγt+, T-bet-), T-bet+ ILC3s (live, single, CD45+, IL-7Rα+, lineage-, CD3ε/ TCRβ-, RORγt+, T-bet+), ILC1s (live, single, CD45+, IL-7Rα+, lineage-, CD3ε/ TCRβ-, RORγt-, T-bet+), **D)** ILC2s (live, single, CD45+, IL-7Rα+, lineage-, CD3ε/ TCRβ-, RORγt-, T-bet-, GATA3+) and **E)** NK cells (live, single, CD45+, CD3ε/ TCRβ-, NK1.1+).

Overall, the total number of all ILCs show no significant changes at day 2 of *H. polygyrus* infection and this remains unchanged with the blockade of IFN-γ (Figure 4-13A). Further examination into the specific ILC subsets show slight fluctuations in the cell proportions with increased frequency of ILC3s during early infection, yet not when anti-IFN-γ was also administered (Figure 4-13B). Additionally, we found that NK cells had increased cell number and frequency during *H. polygyrus* infection, irrespective of IFN-γ blockade (Figure 4-13C). This suggests that although NK cells may be a potential source of IFN-γ, their cell numbers do not appear to be altered by IFN-γ expression, at least at this early timepoint.

Since NK cells were increased during infection and are known to produce IFN-γ, we decided to further explore the production of this IFN-γ at day 2 of *H. polygyrus* infection. Using a cytokine stimulation kit as part our flow cytometry staining protocol, we use a cell stimulation cocktail which contains components which can stimulate cells to produce cytokines. The cocktail also contains protein transport inhibitors to prevent the proteins from leaving the cell, enabling us to stain them and identify cells positive for cytokines of interest. With this kit, we were able to identify IFN-γ+ NK cells using the same gating as Figure 4-12E with the additional gating for IFN-γ+ cells shown in Figure 4-14A. The total frequency of NK cells expressing IFN-γ was significantly higher in infection compared to naïve, confirming that NK cells are likely a contributing source of the IFN-γ we identify in infection. Taken together, my data here show that ILCs do not appear to show major differences in infection, yet NK cells are not only increased at day 2 but also appear to be a source of our IFN-γ.

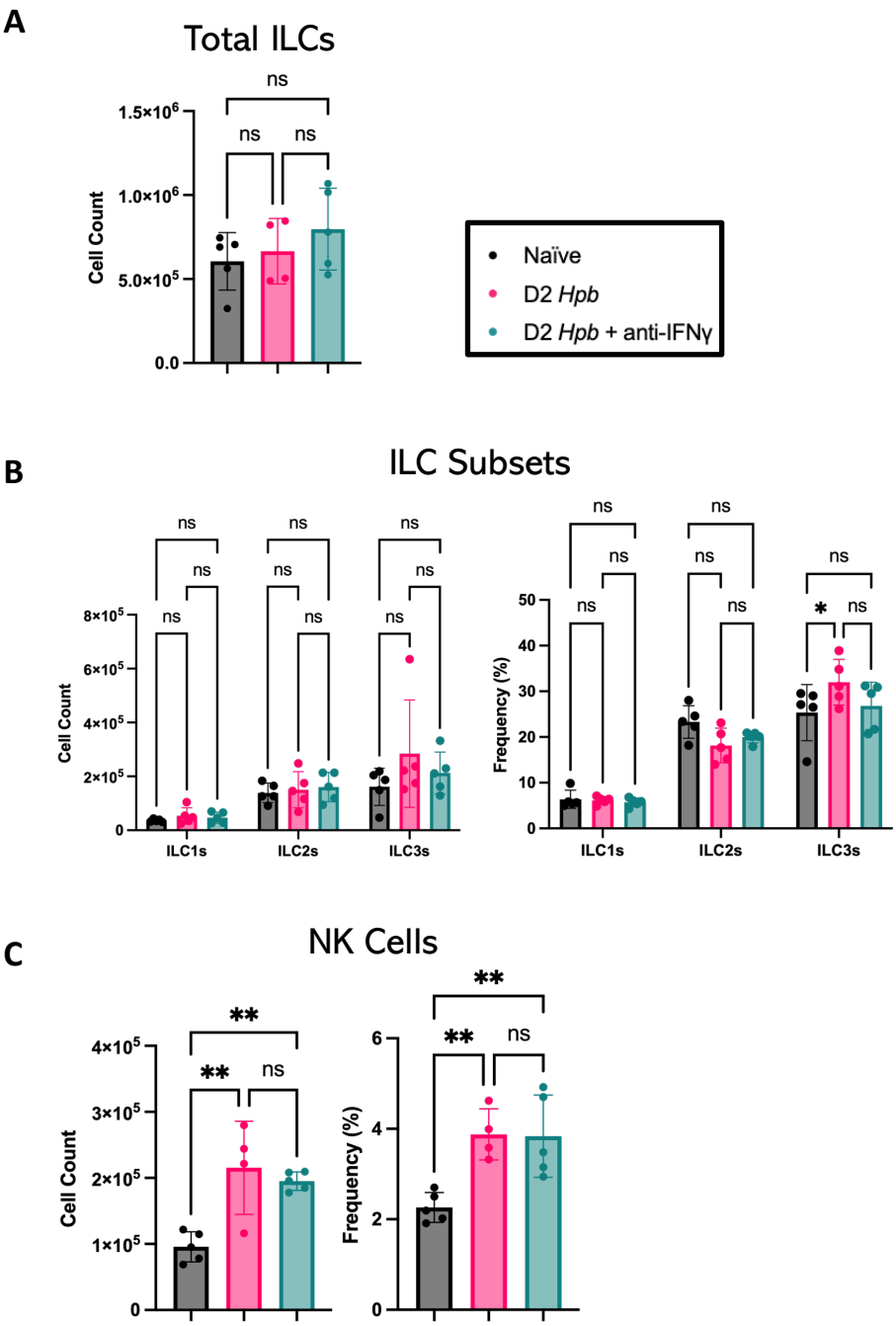

Figure 4-13 NK cells increased in early *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and a monoclonal antibody of IFN-γ was administered at day -1 of infection, samples were collected at day 2 post infection. Small intestinal lamina propria were digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 4-12). Frequency and absolute cell count of **A)** total ILCs, **B)** ILC subsets (1,2&3) and **C)** NK Cells plotted. Data shown with mean ±SD, with 4-5 mice per sample group. Statistical significance was calculated by a Two-Way ANOVA **(B)** or a One-Way ANOVA **(A&C)** followed by a Tukey's multiple comparisons test. (Significance *$p < 0.05$, **$p < 0.01$, NS, Non-significant).

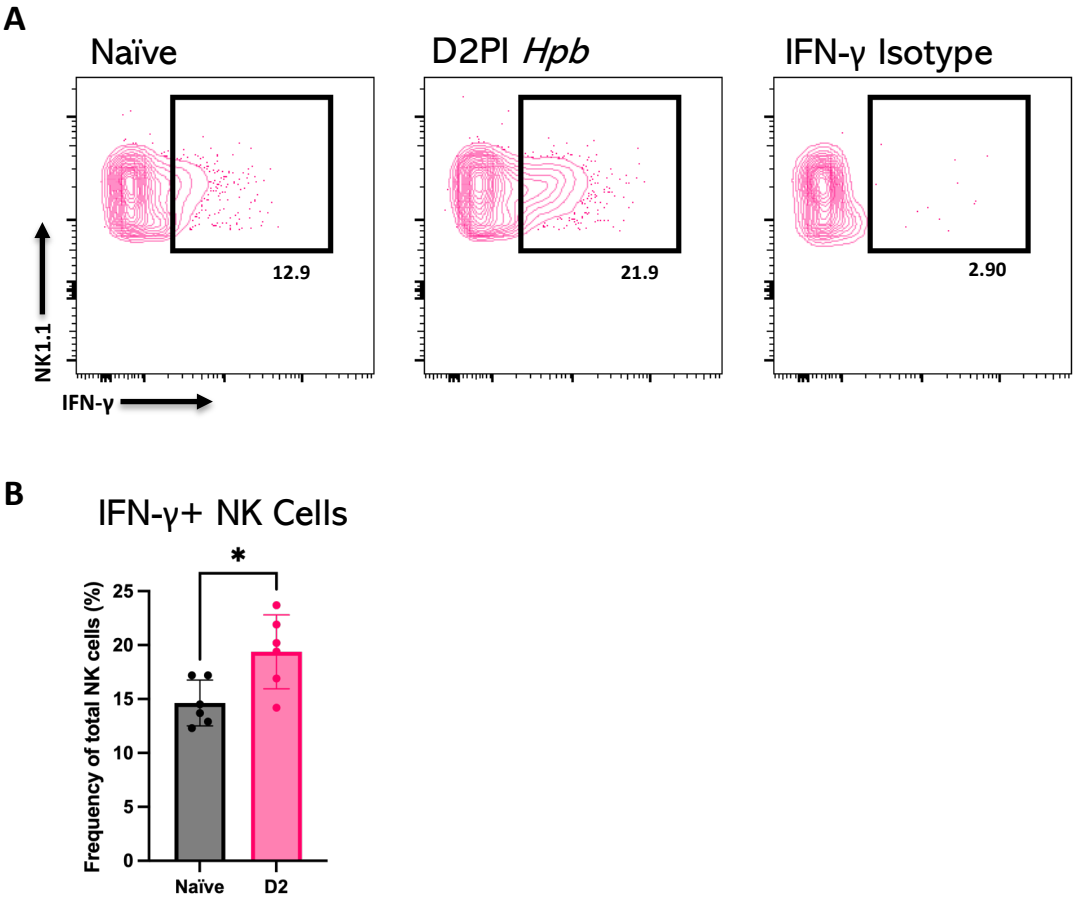

Figure 4-14 Proportion of IFN-γ expressing NK cells increases in early *Hpb* infection. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and samples were collected at day 2 post infection. Small intestinal lamina propria were digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 4-12). **A)** Gating of IFN-γ+ NK cells identified and labelled with frequency of 'parent' population. **B)** Frequency of total NK cells expressing IFN-γ plotted. Data shown with mean ±SD, with 6 mice per sample group. Statistical significance was calculated using a two-tailed unpaired t-test. (Significance $*p < 0.05$).

Overall, in this Chapter we sought to define a role for the early IFN-γ production in *H. polygyrus* infection, investigating whether it contributed to the alterations in antimicrobial responses and barrier disruption that we identified in Chapter 3. Our data illustrate that NK cell produced IFN-γ contributes to increased cell adhesion molecule expression and enhances antimicrobial responses through AMP expression and recruitment of myeloid cells.

4.4 Discussion

The aim of this chapter was to characterise and establish a role for the IFN-γ produced at day 2 of *H. polygyrus* infection. This question was particularly interesting in light of the disruption of barrier integrity and increases in antimicrobial defences that we had identified in early infection in Chapter 3. We were able to identify direct effects of IFN-γ on the intestinal epithelium in coordinating barrier integrity and inflammatory cell infiltrate. Using an *in vivo* blockade of IFN-γ in the early stages of *H. polygyrus* infection, we did not uncover a role for IFN-γ in the proliferation of the epithelial cells, but we did find that barrier integrity appeared either more disrupted or better repaired when IFN-γ was present during infection. IFN-γ was responsible for the enhancement of antimicrobial responses measured, and increased immune cell infiltrate during infection. The commensal bacteria of the host were still not found to translocate across the intestinal barrier upon helminth damage, even in the absence of IFN-γ and subsequent antimicrobial and barrier integrity. Finally, NK cells were confirmed producers of IFN-γ at day 2 of *H. polygyrus* infection and both total NK cell numbers and the frequency of IFN-γ producing NK cells were found to be higher, specifically at day 2 of infection. Together these data show that IFN-γ can signal directly to intestinal epithelial cells, promoting barrier integrity and antibacterial immunity; and can coordinate recruitment of innate immune cells into the tissue.

Overall, the data in this chapter show relatively modest differences between experimental groups, perhaps reflective that residual IFN-γ is sufficient to exert effects even with broad neutralisation by the monoclonal antibody. Our experimental model is limited by the fact that this residual IFN-γ may remain, however administration of the antibody to align with our infection timepoint enables us to assess effects of IFN-γ without introducing the complications associated with a systemic IFN-γ/ IFNGR KO mouse model.

4.4.1 IFN-γ and the intestinal epithelium

As described in this chapter's introduction, IFN-γ has a complex relationship with the intestinal epithelium, likely shaped by other surrounding stimuli. As we found in this chapter, IFN-γ can directly stimulate gene expression in intestinal

epithelial cells to both increase cell adhesion and barrier integrity and promote antimicrobial defence and protective responses. Although it has been reported that IFN-γ can cause epithelial cell death *in vivo* and in organoid cultures (Osaki et al., 2019), in this data, we did not find increased apoptotic signals upon IFN-γ stimulation, suggesting that IFN-γ is not directly damaging the epithelium. If IFN-γ is causing damage to epithelium this instead is likely to be through activation of neutrophils/ macrophages. The organoid data set that we used included sample groups that tested IFN-γ in combination with two other epithelial-relevant cytokines, IL-13 and IL-22 (Lindholm et al., 2022). Although the sole effects of IFN-γ are interesting, further analysis of all sample groups would be useful as *in vivo*, most cytokines act in combination.

In other nematode infections, IFN-γ has been shown to increase epithelial proliferation (Artis et al., 1999), but when we assessed the proliferation of the epithelium during early *H. polygyrus* infection we did not find any changes imposed by IFN-γ blockade at day 2 of infection. The study by Artis et al. used *T. muris* infection and measured the number of cells per crypt in an *in vivo* IFN-γ blockade model at day 21 PI (Artis et al., 1999), much later than our assay, perhaps explaining why our findings are inconsistent. So far, evidence of a more "foetal-like" epithelium during *H. polygyrus* infection and a proposed role for IFN-γ in this process and in tissue repair has been reported (Drurey et al., 2021b; Nusse et al., 2018b). Additionally, an axis through which IFN-γ signals via glial cells to contribute to reparative processes within intestinal tissue during *H. polygyrus* infection has been described (Progatzky et al., 2021). It is possible that the role of IFN-γ on the intestinal epithelium is not just to stimulate proliferation, but also to induce stem-like qualities and repair mechanisms, as suggested by these papers. In our data we did not find evidence for this stem-cell reversion however, we did see evidence of TJ regulation and altered permeability.

Both permeability assays used show high variability within sample groups. The FITC-dextran experiment showed decreased intestinal permeability in the anti-IFN-γ *H. polygyrus* infected mice although there were no significant differences shown between the naïve and infected groups. Repeating the experiment with an additional naïve with anti-IFN-γ treatment sample group would be an effective comparison. Also, the spread of data in the uninfected group was

large, and additional repeats might be useful to strengthen this data. The commensal antigen ELISA also had its own limitations. Whilst adapted from a published protocol (Hepworth et al., 2013), the readouts of Ig levels were not normalised against total protein concentration, since total protein content in serum could also be influenced by either infection or IFN-γ blockade. The lack of normalisation may emphasise technical variations (small pipetting errors, etc) and increase noise in the data. Recently, other studies have measured intestinal permeability later in *H. polygyrus* infection and found significant differences (Mules et al., 2024). Perhaps day 2 of measurement was too early for any significant changes to be detected and it is maybe at the later stage of maturation when the worm travels back into the lumen that is more likely to increase permeability. IFN-γ is still high on day 7 of *H. polygyrus* infection, as we show in Figure 3-13, but not to the same extent as during its early spike at day 2. Blockade at a later timepoint to assess its role in the latter breach would be interesting.

Our results show that IFN-γ blockade attenuated the increased TJ protein expression and reduced intestinal permeability. Perhaps together this indicates a role for IFN-γ in a reparative process in the gut epithelium. Further research is needed to determine whether IFN-γ drives a repair process following worm-induced damage or if it causes damage to facilitate the worm's breach, triggering the subsequent increased reparative response seen in the increased gene expression of TJ proteins. Research using *in vitro* intestinal epithelial cell cultures has identified a role for IFN-γ in inducing endocytosis of TJ proteins, resulting in a leakier and more permeable barrier (Bruewer et al., 2005; Utech et al., 2005). Additionally, these studies show that IFN-γ disrupts TJ localisation (Lee, 2015). In line with these findings, depletion of the IFN-γ receptor on organoid epithelial cells also disrupted the localisation of TJ proteins (Bardenbacher et al., 2019). Taken together these papers provide evidence for IFN-γ disrupting the intestinal barrier, potentially used to the advantage of the helminth as it's expression coincides with the first 'breach' point. IFN-γ has also been found to induce an increased expression of some TJs, resulting in barrier compromise (Capaldo et al., 2014; Garcia-Hernandez et al., 2017). As earlier mentioned, other helminth studies have suggested that worms are able to breach the intestinal barrier through facilitation of barrier breakdown via TJ

proteins (Schälter et al., 2022). Perhaps our research placed in the context of these findings suggest that IFN-γ is able to promote the internalisation of TJ proteins on the epithelial cells to enable a smooth entry for the helminth into the intestinal tissue. This hypothesis that IFN-γ could coordinate helminth 'breach' would also make the host more vulnerable to opportunistic pathogens at that moment and a dual role for IFN-γ in also promoting antimicrobial defences at this timepoint may be an essential protection against bacterial invasion.

4.4.2 IFN-γ and antimicrobial defence

In line with the above discussion, while stimulating epithelial cells to internalise their TJ proteins and allow entry of the worm, IFN-γ may also increase the epithelial AMP secretion and recruitment of other innate immune cells to defend against potential translocating bacteria. Our results show that IFN-γ is also able to enhance local antimicrobial responses by increasing AMP expression and the number of innate immune cells infiltrating the small intestine. Upon arrival of these myeloid cells, IFN-γ is likely to play a large role in the activation of neutrophils and polarisation of macrophages to a pro-inflammatory phenotype. Research has shown that KO or depletion of IFN-γ or its signalling mechanisms results in a compromised host that is prone to bacterial infections (Shtrichman and Samuel, 2001). IFN-γ has been shown to increase AMP expression in IECs and Paneth cells via STAT signalling (Yue et al., 2021b). Therefore, we hypothesise that IFN-γ is leading an antimicrobial response to prevent bacteria from translocating through the weakened barrier after a breach at day 2 of the *H. polygyrus* lifecycle.

Our data again found increases in neutrophils and other myeloid cells during early *H. polygyrus* infection, and these increases were lost or reduced upon IFN-γ blockade. Although neutrophils are often associated with being activated by IFN-γ and not recruited by this cytokine (Dallagi et al., 2015; Hoeksema et al., 2015; Nandi and Behar, 2011), IFN-γ has been shown to recruit neutrophils indirectly via epithelial stimulation (Andrews et al., 2018b). Together our data in the context of previously published research suggest to us that IFN-γ activates and enhances antimicrobial responses including neutrophil recruitment through an intestinal epithelial cell dependent interaction.

Despite IFN-γ's role in antimicrobial defence, we still found no evidence for bacterial translocation in *H. polygyrus* infection when IFN-γ was blocked. Perhaps this result would differ if the host was presented with a more damaging bacterial pathogen than commensal bacteria. Several studies have used co-infection models with bacteria and enteric helminths to show increased susceptibility to secondary infection during infection with a worm. Chen et al. found that co-infection with the bacteria *Citrobacter rodentium* alongside *H. polygyrus* resulted in bacterial translocation and increased mortality in mice (Chen et al., 2005). Other studies have explored co-infection using a model of the Schistosoma flatworms and *Salmonella* infection. Findings show that Schistosome eggs secrete glycoproteins to recruit basophils which subsequently release IL-4 and IL-13, suppressing the protective Th1 and Th17 responses against *Salmonella* infection (Schramm et al., 2018). Other researchers using a similar adult Schistosoma model found that *Salmonella* could bind to the outer cuticle of adult Schistosomes via their fimbrial proteins, providing them with a niche which prevented them from being killed by antibiotics (Barnhill et al., 2011). Likewise, these results have also since been reported in a *H. polygyrus* co-infection with *Salmonella*, where the helminth provides a niche for the bacterium (Brosschot et al., 2021). IFN-γ is only reported in two of these co-infection studies, at the latter timepoint of the *H. polygyrus* infection, the study by Chen et al. suggests that there is an increase in IFN-γ producing T cells compared to those of the control mice and Schamm et al., show an increase in IFN-γ expression at day 1 of schistosome infection, although this does not reach statistical significance (Chen et al., 2005; Schramm et al., 2018). Both studies find that IFN-γ is increased in co-infection when compared to naïve mice, although not as much as in sole infection with the bacterium in either scenario (Chen et al., 2005; Schramm et al., 2018). Hence, a co-infection experiment with helminth and bacterium in the context of IFN-γ blockade would deepen our insight into the antimicrobial role of IFN-γ in *H. polygyrus* infection.

4.4.3 IFN-γ production

Both NK cells and T cells have been implemented in the IFN-γ production during *H. polygyrus* infection. Previous work in our laboratory showed that in later *H. polygyrus* infection, IL-10 is able to suppress and regulate Th1 IFN-γ production (Webster et al., 2022). In relation to the earlier IFN-γ production, researchers

had suggested that IFN-γ was produced by NK cells and was key in tissue repair (Gentile et al., 2020). Our data confirms an influx of NK cells with increased IFN-γ production in early *H. polygyrus* infection. Since starting this project, the production of IFN-γ and its role in *H. polygyrus* infection has been explored by several other laboratories. In particular, a recent publication has explored similar questions and have shown that IFN-γ is produced by local tissue-resident CD8+ T cells and signals via stromal cells to recruit neutrophils to the infection site to aid in limiting tissue damage (Westfall et al., 2025). Some of these findings overlap with ours, giving confidence in our understanding. IFN-γ appears to be produced by multiple cells, and we hypothesise that the functionality and abundance of IFN-γ may differ depending on the source. The exact driver of the IFN-γ secretion remains unclear. Ordinarily, IFN-γ is known to be secreted in response to bacterial stimuli (Czarniecki and Sonnenfeld, 1993), whilst some research using schistosome larvae has suggested that these helminths are also able to modulate IFN-γ production (Paveley et al., 2011). The stimulus for production of this cytokine during early *H. polygyrus* infection remains a fundamental question to clearly understanding its role in intestinal helminth infection.

Overall, our data in this chapter characterise a role for IFN-γ in barrier integrity and in coordinating and enhancing local antimicrobial protection, including AMP expression and recruitment of innate immune cells such as neutrophils. Additionally, we reiterate NK cells as a key source of IFN-γ at day 2 of *H. polygyrus* infection. With our findings so far, the role of IFN-γ in relation to the breaches of the intestinal epithelium caused by *H. polygyrus* remains slightly unclear. The exact reason for IFN-γ production and whether it is a manipulation of a response coordinated by the helminth itself is yet to be defined. It is unclear whether IFN-γ and the antimicrobial defences are produced in response to bacterial stimuli or as a preventative protective measure upon helminth infection. Additionally, it is unclear whether a more dangerous bacterial pathogen than commensals would be able to translocate and colonise during *H. polygyrus* infection more easily in the absence of IFN-γ. Therefore, in our next chapter we decided to assess the production of IFN-γ and subsequent antimicrobial defences by eliminating bacteria and measuring these responses.

Chapter 5

5.1 Introduction

Our data so far illustrate that early *H. polygyrus* infection evokes a complicated immune response very different to the Th2 response found at the later stages of infection, including an IFN-γ co-ordinated antimicrobial response with increased barrier remodelling. Although these data are interesting, the order of events and in response to which stimuli remain unclear. We wondered whether the presence of IFN-γ in *H. polygyrus* infection is triggered in response to the helminth or as response to damage caused to the intestinal epithelium upon 'breaching' to prevent further infection or is actually produced in response to bacterial stimulation to coordinate antimicrobial responses. To address our questions, this chapter uses antibiotics to eliminate bacteria and determine whether IFN-γ and subsequent enhanced antimicrobial responses are measures in place to prevent bacterial invasion or are produced in response to bacterial stimulation.

As discussed in Chapter 4, IFN-γ has been associated with helminth infections for some time. IFN-γ is known to be involved in defence against invading bacterial pathogens, and its production is linked with bacterial stimulation (Czarniecki and Sonnenfeld, 1993; Mertowska et al., 2023). In helminth infection, a recent publication, argues that IFN-γ is produced by gut-resident CD8+ T cells in helminth infection, and is dependent on microbiota (Westfall et al., 2025). *H. polygyrus* and other helminths have been shown to manipulate the microbiota diversity within the infected host (Reynolds et al., 2014b; Walk et al., 2010b). Further research needs to be carried out to fully understand the effects that microbiota have on the helminth or resulting immune response. Germ free mice infected with intestinal helminths have enhanced Th2 immunity compared to similarly infected specific-pathogen-free (SPF) mice (Rausch et al., 2018), which suggests that commensal bacteria may suppress/ limit the Th2 response in early infection through stimulating a contrasting response such as type 1 immunity via IFN-γ production.

The intestine is a perfect niche for many bacteria due to the nutrient rich environment. Although many are essential for our survival and keeping us healthy, if not controlled by our effective immune responses there are several

bacterial species that can infect the host and cause severe health effects or even be fatal (Burd and Hinrichs, 2015). So far, our data and research from others have illustrated that *H. polygyrus* disrupts the intestinal barrier (Schälter et al., 2022c). We hypothesised that this breach could enable an opportunity for bacteria in the intestine to also translocate across the barrier, hence increasing the host's susceptibility to bacterial co-infection. Our data also record increases in antimicrobial responses during *H. polygyrus* infection, and we wondered whether these increases are provoked by, and indicative of, bacteria crossing the intestinal barrier and posing a threat to the immune system.

As mentioned in Chapter 3, papers suggest that antimicrobial responses associated with helminth infection are important in protecting the host from further infection (Horsnell and Oudhoff, 2022; Hu et al., 2021). Whether these are preventative measures or initiated by bacterial stimulation is unclear. There are several studies using co-infection models with enteric helminths and bacterial pathogens. These studies demonstrate the host's increased susceptibility to secondary bacterial infection when the host is already infected with a helminth (Elias et al., 2005; Potian et al., 2011; Reynolds et al., 2017b). Research has shown that in some cases helminths are able to provide an extra physical surface for bacterial species to reside on during infection, preventing effective clearance by the host of the respective pathogens (Barnhill et al., 2011). Indeed, the *H. polygyrus* larvae or mature worms may be able to carry surface bacteria and perhaps upon intestinal barrier entry, these hitchhikers induce bacterial-stimulated type 1 immunity.

We propose that the barrier breach and disruption caused by the worm is providing bacteria an entry point to the intestinal tissue. We therefore hypothesise that this additional bacterial contact with the host tissue and tissue-based immune system is provoking the IFN-γ -directed antimicrobial defences that we described in Chapters 3 and 4. To test this hypothesis, we eliminated bacteria through treatment of *H. polygyrus* infected mice with broad-spectrum antibiotics and investigated whether the IFN-γ and associated antimicrobial responses were still present. We also assessed the surface of the helminths and their interaction with bacteria to determine whether the worms could support bacterial survival by providing an environmental niche. Together, the data

contained in this chapter confirm a decisive impact of bacteria on the immune responses occurring early in helminth infection.

5.2 Aims

The objective for this final Chapter was to determine whether the antimicrobial and IFN-γ enhanced responses in *H. polygyrus* infection are a consequence of bacterial stimulation. To achieve this, we addressed the following aims:

- To investigate whether the antimicrobial responses that we have observed in early *H. polygyrus* infection are influenced by the presence of bacteria.

- To determine whether the increased IFN-γ in *H. polygyrus* infection is produced in response to bacteria.

- To explore the physical relationship between the *H. polygyrus* worm and intestinal bacteria, to understand whether they co-localise with each other.

5.3 Results

5.3.1 The effect of antibiotic treatment on *H. polygyrus* infection

To deepen our understanding of the relationship between *H. polygyrus* and potential bacterial threats, especially during the timepoints when the intestinal barrier is breached (D2 and D7), we decided to eliminate host microbiota and assess whether the infection-associated increases in antimicrobial peptide expression and immune infiltration that we previously observed were affected by the reduced bacterial load. Mice were treated in their drinking water with a broad-spectrum antibiotic (ABX) cocktail containing ampicillin, metronidazole, neomycin, gentamycin and vancomycin. This combination was used in the laboratory previously and in published research; it was found to be successful at reducing overall bacterial numbers in the gut and we used it with the intention of ablating the overall microbiome (Scott et al., 2018). Mice were given the ABX treatment starting 7 days prior to and throughout *H. polygyrus* infection. Mice were then culled on day 2 or 7 of infection (Figure 5-1A). Mice on the antibiotic treatment lost weight initially but regained it over time (Figure 5-1B), consistent with previously published work (Scott et al., 2018). These data gave confidence that the ABX treatment had been effective, but, as a further confirmation of successful microbiota depletion, the ceca of mice were also collected and weighed. Ceca were significantly larger in the antibiotic-treated mice (Figure 5-1C&D). Enlarged ceca have been previously reported to indicate bacterial depletion, due to the role that microbiota play in fermenting and digesting food (Ge et al., 2017; Puhl et al., 2012). The larger ceca we see in our results therefore suggest that the ABX treatment effectively reduced intestinal bacteria.

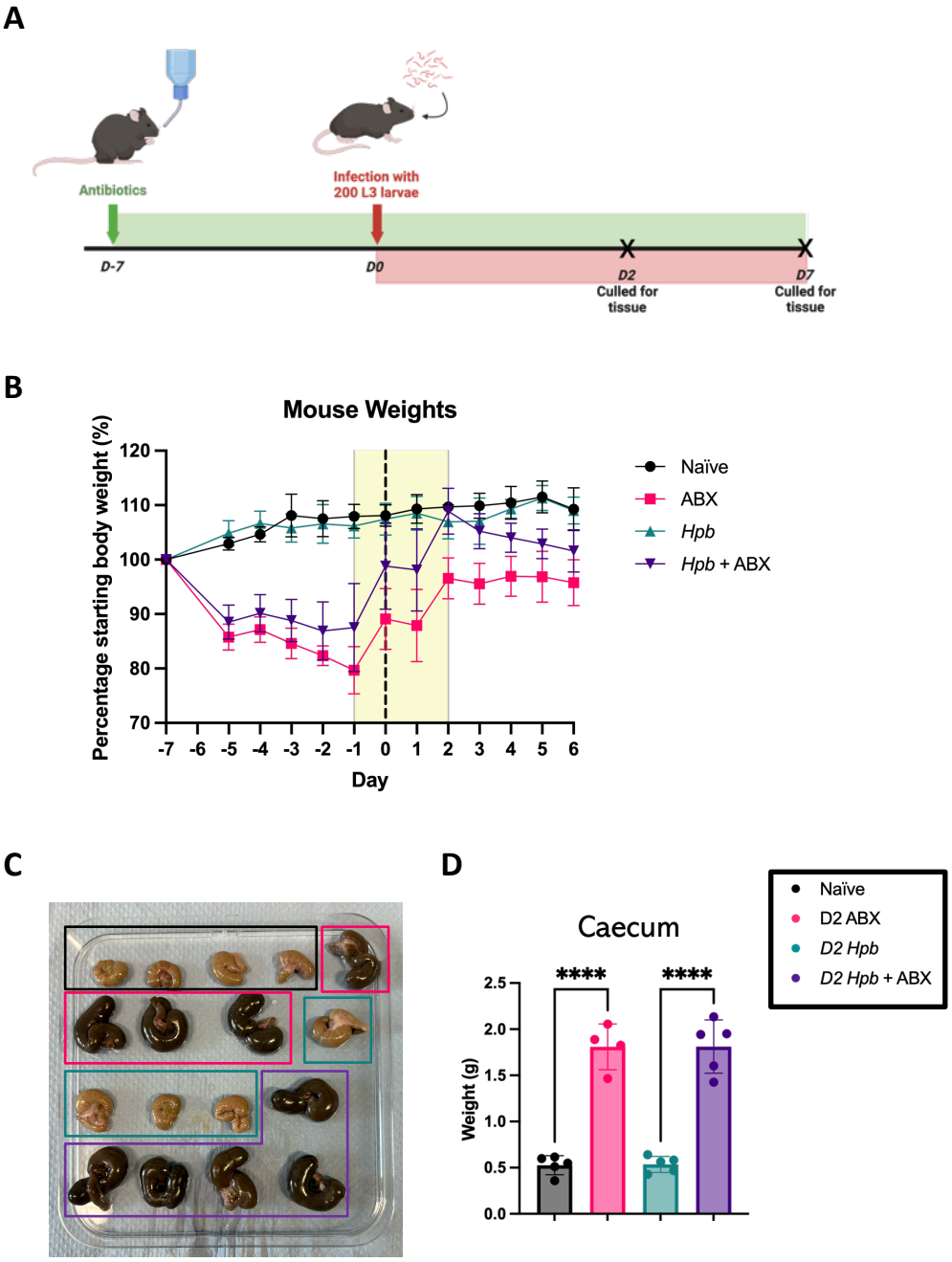

Figure 5-1 Successful *in vivo Hpb* infection with ABX treatment.
A) C57BL/6 mice were infected with 200 L3 *Hpb* larvae and given broad spectrum antibiotics in their drinking water for 7 days prior to and throughout infection, samples were collected at days 2 or 7 post infection. **B)** Mouse weights during infection and antibiotics treatment, plotted as percentage of starting body weight. Shaded area reflects time that mice were given supportive care. Dashed line reflects point of *Hpb* infection. **C)** Image of ceca collected at day 2 post infection. Outlines group ceca by colour, sample groups indicated by the key. **D)** Weight of c collected from mice in each sample group. Data shown with mean ±SD, 4-5 mice per sample group. Statistical significance was calculated by a One-Way ANOVA followed by a Tukey's multiple comparisons test. (Significance ****$p < 0.0001$).

To assess whether bacteria appear to influence *H. polygyrus* infection, we then looked at differences in pathology between sample groups. It should be noted

that tissue for the images in Figure 5-2 comes from standard sectioning of the closed duodenal lumen and not Swiss roll preparations as previously shown, hence overall histology looks different to previously shown sections. Examining the H&E-stained small intestinal samples found no obvious differences in intestinal structure or pathology with ABX treatment (Figure 5.2). Consistent with our earlier findings in Chapter 3, *H. polygyrus* infection shows a moderate inflammatory response surrounding the helminth in the intestinal tissue; the same is true for the ABX treated infected mice. Together our results so far have shown that ABX treatment effectively depletes bacteria, and there appears to be no major impact on tissue pathology.

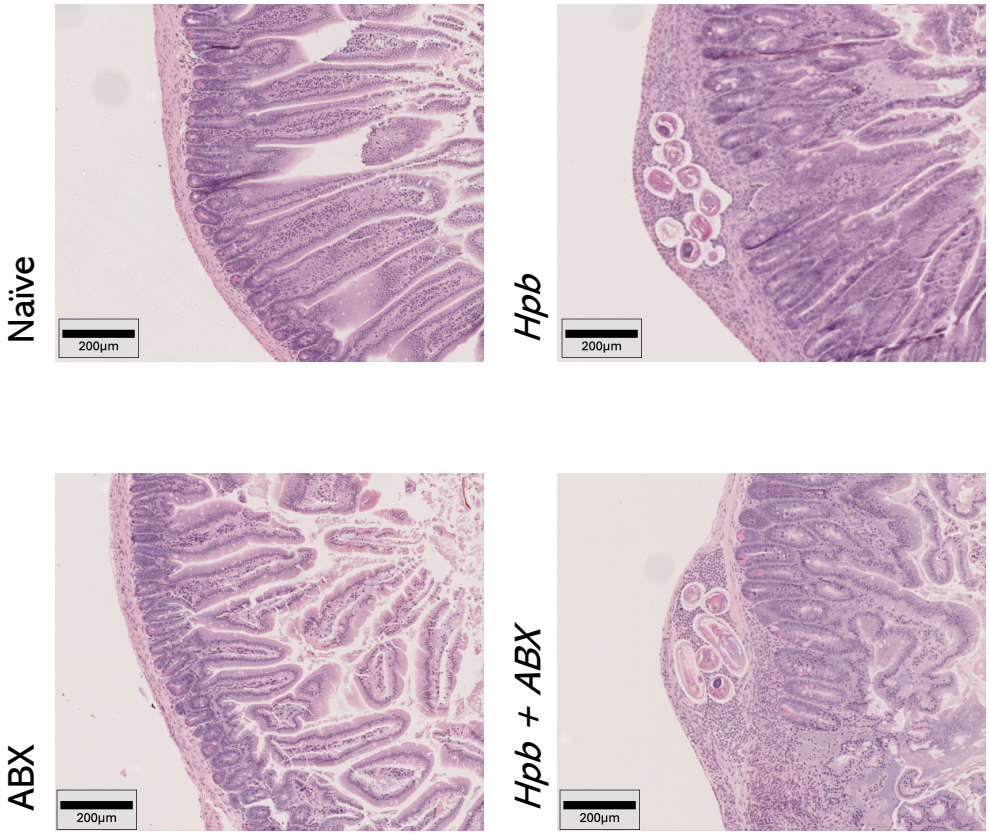

Figure 5-2 *Hpb* granuloma identified, representing successful and ordinary visual infection alongside ABX treatment.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and given broad spectrum antibiotics in their drinking water for 7 days prior to and throughout infection, samples were collected at day 7 post infection. H&E staining of small intestinal tissue at day 7 of infection with and without ABX treatment and with ABX or naïve control groups.

5.3.2 Antimicrobial changes in ABX treated *H. polygyrus* infected mice

Our data in previous chapters have shown that early *H. polygyrus* infection is associated with enhanced antimicrobial responses including increased expression of AMPs and immune cell influx. Therefore, we next aimed to evaluate these changes in a microbiota depleted setting to identify if the AMP expression and immune cell recruitment are enhanced in response to bacterial stimulation.

The previous increases in AMP expression we identified in early *H. polygyrus* infection using the markers Nos2 and Lcn2 was replicated in this experiment. Disappointingly, the expected increase in Ang4 expression we identified in early *H. polygyrus* infected mice when compared to uninfected controls, although higher, did not reach significance in this experiment (Figure 5.3A), contrasting to our earlier work, shown in Chapter 3 (Figure 3-4C). With additional ABX treatment, Ang4 and Nos2 expression was significantly reduced in infected mice, reducing the expression levels so much so that there were no significant differences between *H. polygyrus* infected ABX treated mice and naïve or just ABX treated mice. Lcn2 expression in mice infected and treated with ABX also showed a decreased trend when compared to *H. polygyrus* infected mice although this was not significant (Figure 5.3A-C). Together these data show that microbiota is required for the induction of AMPs in *H. polygyrus*, that epithelial damage caused by the worm is not enough alone, consistent with the hypothesis that these AMPs are a response to bacterial stimulation.

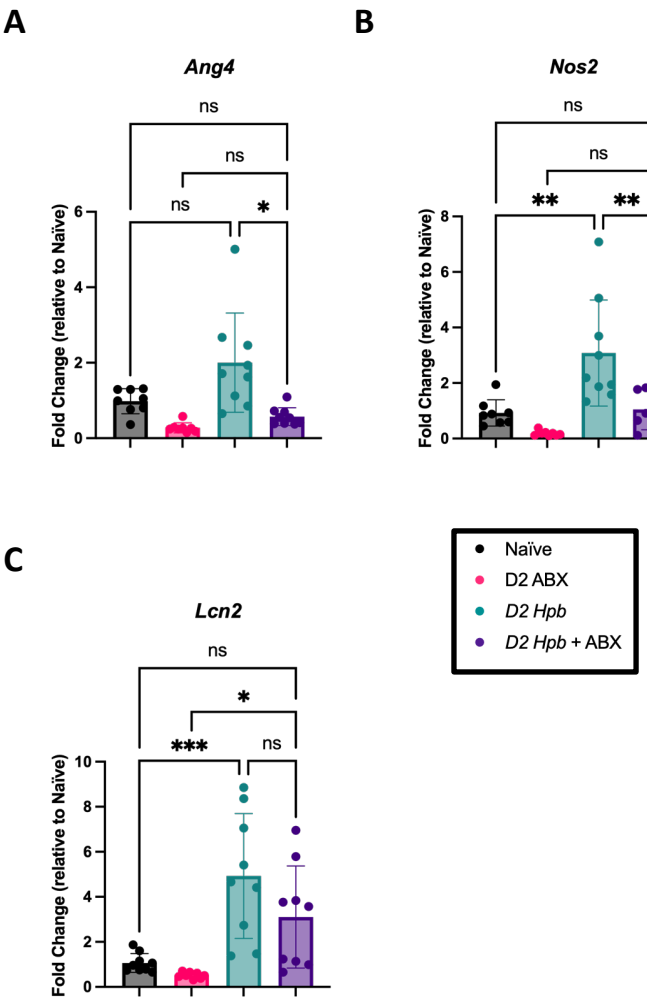

Figure 5-3 Increased AMP expression in early *Hpb* infection in response to microbiota. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and given broad spectrum antibiotics in their drinking water for 7 days prior to and throughout infection, samples were collected at day 2 post infection. Fold change of **A)** Ang4**, B)** Nos2, and **C)** Lcn2 in the duodenum compared to housekeeping gene (Rps29) and normalised to the mean of the naïve samples. Data shown with mean ±SD, 2 pooled experiments with 4-5 mice per experiment. Statistical significance was calculated by a One-Way Anova followed by a Tukey's multiple comparisons test or in cases where data was not normally distributed a Kruskal-Wallis test followed by Dunn's multiple comparisons test was used. (Significance *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS, Non-significant).

Next, we asked whether the immune cell infiltrate seen in early *H. polygyrus* infection was also affected by bacterial depletion. Using the same gating strategies as shown in Figure 3-5, we again measured myeloid cell populations in the small intestine at day 2 of infection. Consistent with our data in Chapter 3, both DCs and eosinophils did not show any significant changes in *H. polygyrus* infection as early as day 2 (Figure 5.4A&B). Additional ABX treatment did not impact these results in the DC data, although eosinophils had a small significant increase in proportion compared to naïve mice when both *H. polygyrus* and ABX were given. Since eosinophil cell numbers show no significant changes, this

suggests that ABX may be causing a decrease in other cell populations within the negative eosinophil gating. In contrast, Ly6G+ eosinophils show significant increases at day 2 of *H. polygyrus* infection compared to uninfected controls, which appear to be completely abrogated with ABX treatment and are even significantly lower than the levels in naïve mice (Figure 5-4C). In fact, ABX treatment alone appears to reduce this population, perhaps reflecting a role for the microbiota in maintaining the presence of these cells, even in the absence of infection.

Using the 'macrophage waterfall' shown in Chapter 3, we gated on CD11b and MHCII to identify the monocyte, intermediate and macrophage cell populations. Monocyte and intermediate cell numbers are not statistically different but show an increased trend during *H. polygyrus* infection when compared with the uninfected control. Both monocytes and intermediate myeloid cells were significantly higher in frequency at day 2 of infection vs non-infected animals (Figure 5-5). With ABX treatment given to infected animals, the higher frequency of the monocyte and intermediate monocyte cells is absent. Strikingly, macrophage populations are significantly higher in number and lower in proportion in *H. polygyrus* infected mice compared to naïve and these changes are completely nullified when mice are also treated with ABX and compared against the naïve.

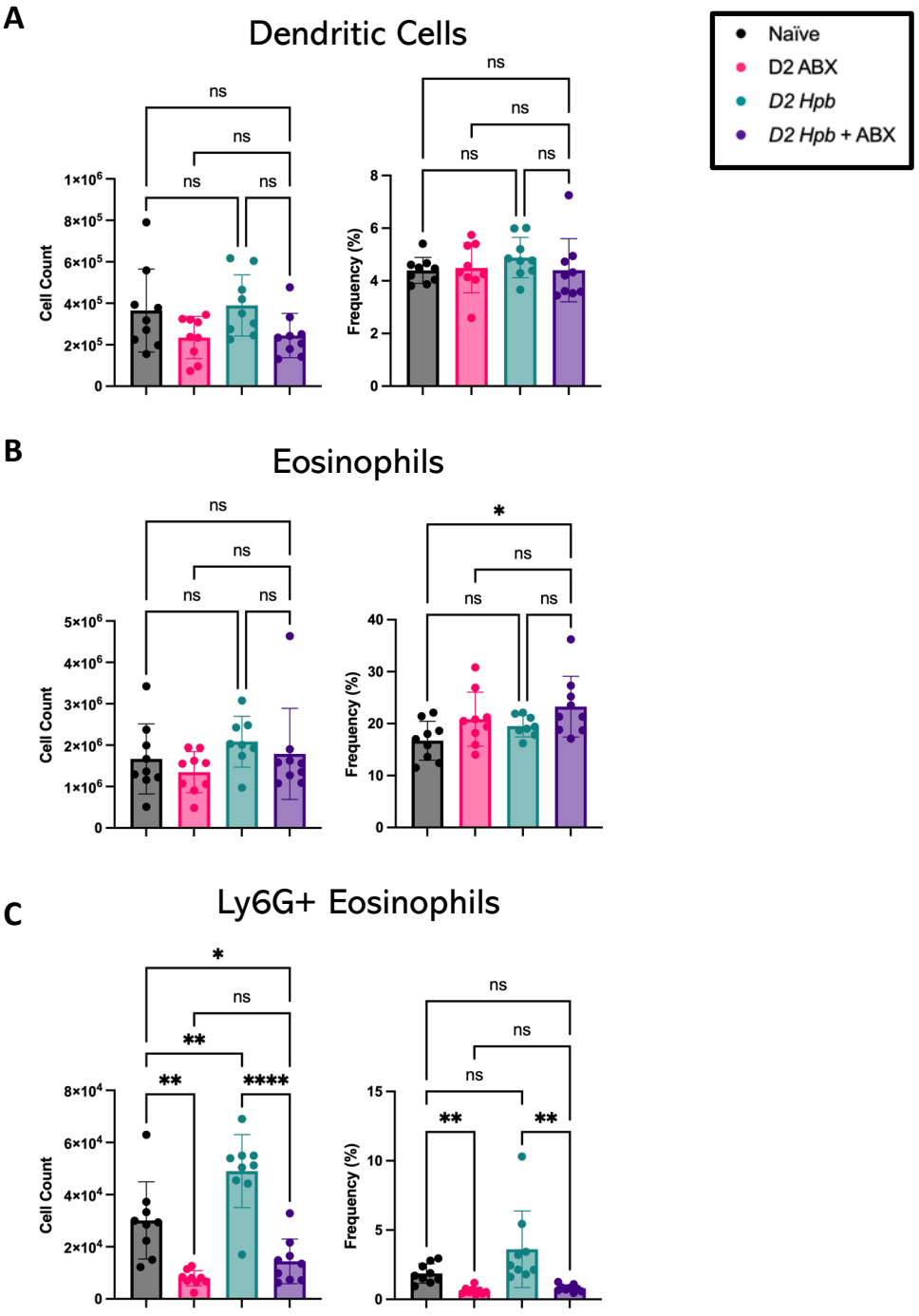

Figure 5-4 Myeloid population changes in early *Hpb* infection with and without ABX treatment.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and given broad spectrum antibiotics in their drinking water for 7 days prior to and throughout infection, samples were collected at day 2 post infection. Small intestinal lamina propria digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 3-5). Frequency and absolute cell count of **A)** DCs, **B)** eosinophils and **C)** Ly6G+ eosinophils plotted. Data shown with mean ±SD, 2 pooled experiments with 4-5 mice per experiment. Statistical significance was calculated by a One-Way Anova followed by a Tukey's multiple comparisons test or in cases where data was not normally distributed a Kruskal-Wallis test followed by Dunn's multiple comparisons test was used. (Significance *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, NS, Non-significant).

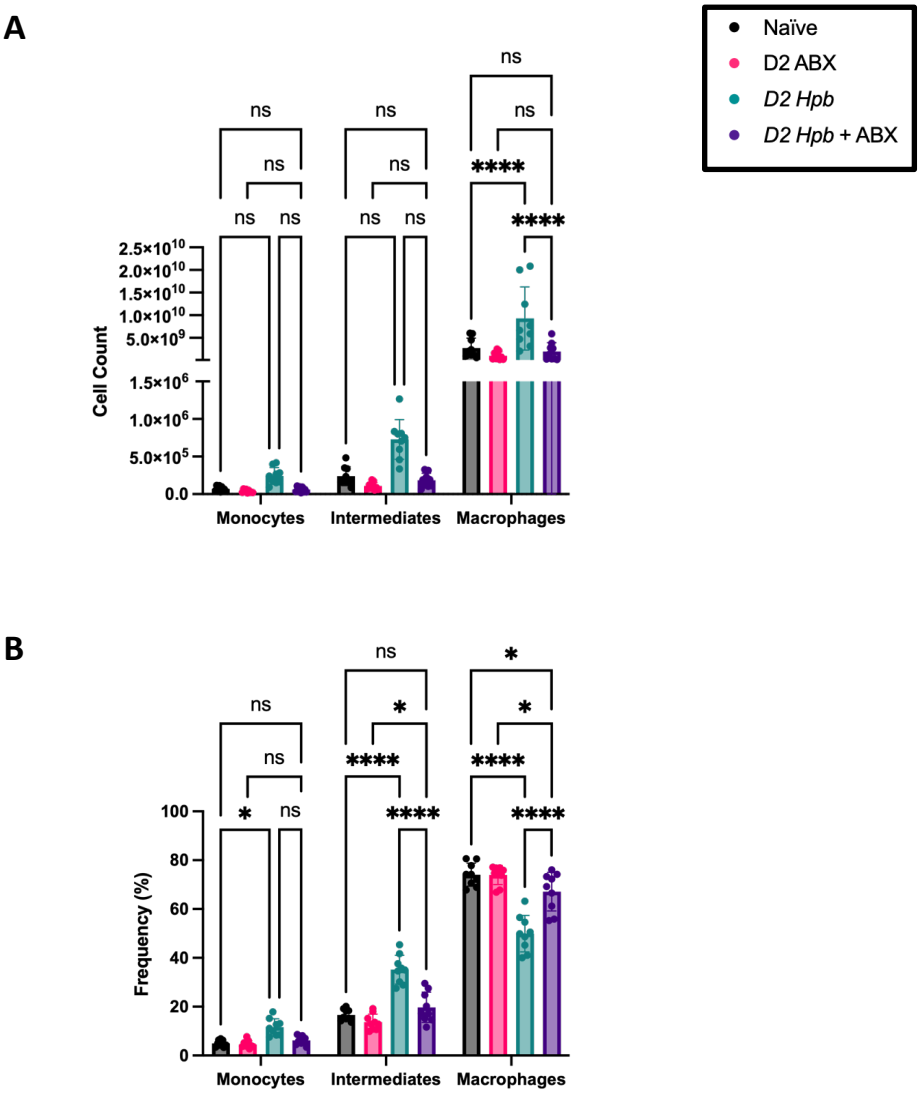

Figure 5-5 Changes to macrophage populations in early *Hpb* infection are microbiota dependent.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and given broad spectrum antibiotics in their drinking water for 7 days prior to and throughout infection, samples were collected at day 2 post infection. Small intestinal lamina propria digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 3-5). Frequency and absolute cell count of monocytes (MHCII-Ly6C+), intermediates (MHCII+Ly6C+) and macrophages (MHCII+Ly6C-) plotted. Data shown with mean ±SD, 2 pooled experiments with 4-5 mice per experiment. Statistical significance was calculated by a Two-Way ANOVA followed by a Tukey's multiple comparisons test. (Significance *$p < 0.05$, ****$p < 0.0001$, NS, Non-significant).

Finally, we looked at neutrophil populations. Gated in Figure 5-6A, neutrophils were significantly higher in frequency and cell number in mice 2 days PI when compared with naïve mice and similarly to the macrophages, these increases were completely ablated in infected mice whom also received ABX treatment (Figure 5-6B). In the experiments where *H. polygyrus* has successfully induced the expected myeloid infiltration into the small intestine, this cell influx does

not occur if the microbiota is ablated. This suggests that the immune infiltration is normally induced by the microbiome.

To assess whether the influx of neutrophils into the small intestine were only found during the early breach point of helminth infection or also later at the second 'breach' point, we also measured neutrophils at day 7 of *H. polygyrus* infection (Figure 5-6C). Neutrophil numbers and frequencies were again higher during infection at this later timepoint compared to uninfected mice. Although the infection-induced increase in the frequency of neutrophils was significantly reduced by ABX administration, any reduction in absolute cell number was not statistically significant. Taken together, our measurements of the myeloid cell influx and induction of AMPs that occur during *H. polygyrus* infection shows that ABX treatment removes multiple aspects of these immune responses. Our understanding is therefore that bacterial stimulation is essential to drive or enhance these antimicrobial responses.

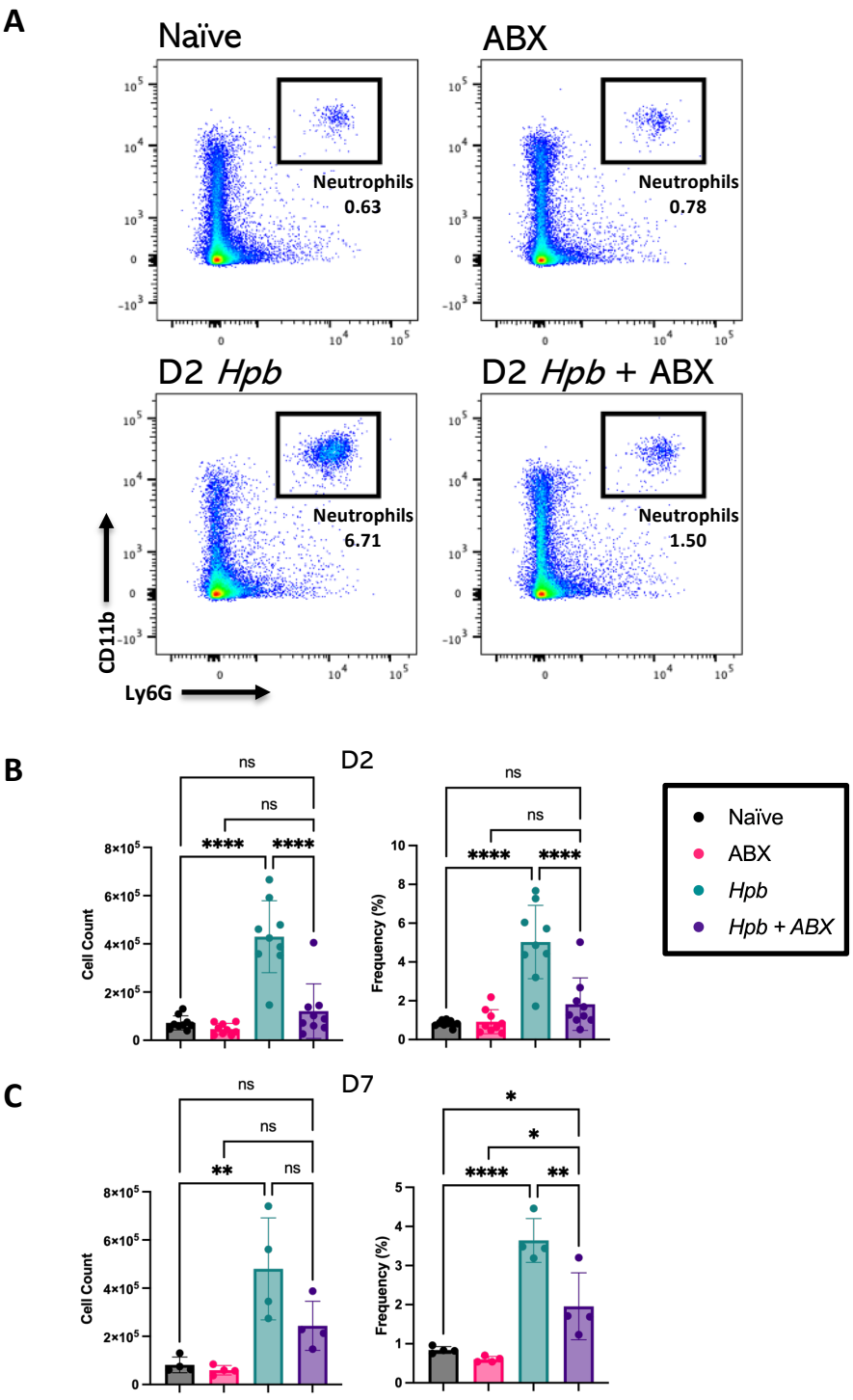

Figure 5-6 Neutrophil infiltration in early *Hpb* infection induced by microbiota. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and given broad spectrum antibiotics in their drinking water for 7 days prior to and throughout infection, samples were collected at day 2 post infection. Small intestinal lamina propria digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 3-5). **A)** Gating of neutrophils identified and labelled with frequency of 'parent' population. Frequency and absolute cell count of neutrophils at **B)** D2 and **C)** D7 plotted. Data shown with mean ±SD, 2 pooled experiments with 4-5 mice per experiment. Statistical significance was calculated by a One-Way Anova followed by a Tukey's multiple comparisons test was used. (Significance *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, NS, Non-significant).

5.3.3 IFN-γ expression in ABX-treated, *H. polygyrus*-infected mice

We next aimed to determine whether the local IFN-γ expression and production we identified during early *H. polygyrus* infection is also stimulated by bacteria. Consistent with our data shown so far, sole infection with *H. polygyrus* caused an increase in both duodenal gene expression and sera protein levels of IFN-γ (Figure 5-7). When ABX was administered alongside *H. polygyrus* infection, the increases in IFN-γ were no longer observed (Figure 5-7A&B).

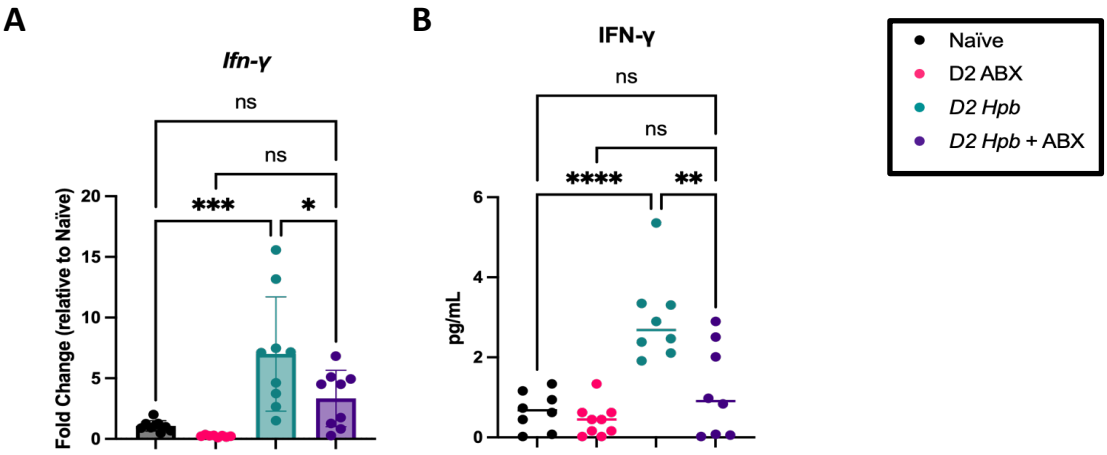

Figure 5-7 Increased IFN-γ expression in *Hpb* infection is dependent on microbiota. C57BL/6 mice were infected with 200 L3 *Hpb* larvae and given broad spectrum antibiotics in their drinking water for 7 days prior to and throughout infection, samples were collected at day 2 post infection. **A)** Fold change of IFN-γ in the duodenum compared to housekeeping gene (Rps29) and normalised to the mean of the naïve samples. **B)** Protein levels of IFN-γ measured in serum of mice using CBA assay. Data shown with mean ±SD, 2 pooled experiments with 4-5 mice per experiment. Statistical significance was calculated by a One-Way Anova followed by a Tukey's multiple comparisons test. (Significance *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, NS, Non-significant).

We then looked at NK cells, as we had confirmed in Chapter 4 that these cells were a source of the IFN-γ seen at day 2 of *H. polygyrus* infection. We used the same gating strategy as in Chapter 4 (Figure 4-12). Again, we found that sole infection increased both cell number and frequency of NK cells when compared to naïve mice. Interestingly, NK cell numbers did not seem to change between ABX treated infected mice and those with sole infection. Irrespective of infection, the frequency of NK cells was also significantly higher with ABX treatment alone when compared to naïve mice, indicating that other cells outside of this gating were lower in frequency since cell numbers did not significantly change. Additional markers and further analysis would be required

to assess the cells decreased in this gating with ABX treatment. Overall, these data demonstrate that IFN-γ is produced in response to bacteria and enhances local protective antimicrobial responses only in their presence.

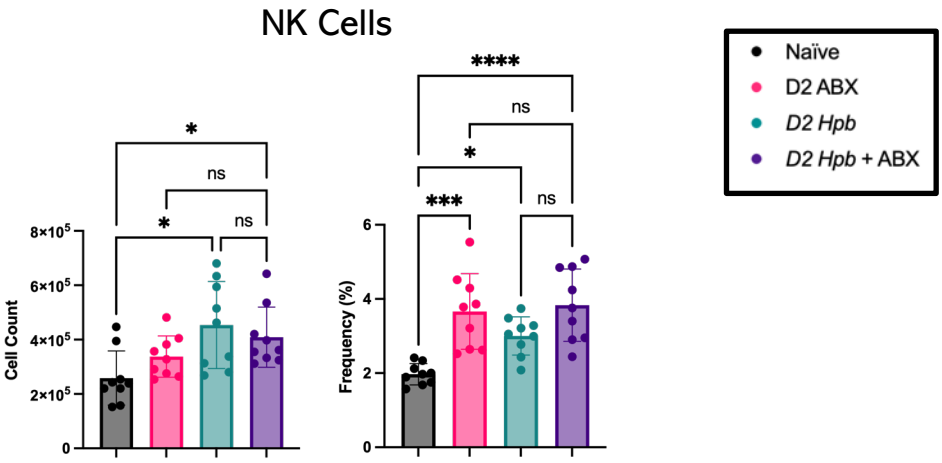

Figure 5-8 NK cell number is unaffected by additional ABX treatment during early *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and given broad spectrum antibiotics in their drinking water for 7 days prior to and throughout infection, samples were collected at day 2 post infection. Small intestinal lamina propria digested and analysed by flow cytometry populations gated and measured as previously identified (Figure 4-12). Frequency and absolute cell count of NK Cells plotted. Data shown with mean ±SD, 2 pooled experiments with 4-5 mice per experiment. Statistical significance was calculated by a One-Way Anova followed by a Tukey's multiple comparisons test or in cases where data was not normally distributed a Kruskal-Wallis test followed by Dunn's multiple comparisons test was used. (Significance *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$, NS, Non-significant).

5.3.4 Variability in bolus *H. polygyrus* infection

The data shown in this chapter so far are taken from two experiments in which there were clear differences between infected and uninfected animals, allowing us to assess the impact of ABX on those differences. However, several experiments in this chapter were afflicted by repeats in which there was no statistical differences between infected and uninfected in any of the parameters we measured. This outcome made it impossible to assess the impact of the microbiota and these repeats were excluded from our analysis, but an example is shown in Figure 5-9. In this figure, results of a third repeat of the antibiotics treated *H. polygyrus* day 2 infection groups are shown. The AMP expression in these mice was not altered in the infected mice relative to the naïve and neither was the IFN-γ expression (Figure 5-9A-D). Additionally, the neutrophil absolute number and frequency were not significantly higher in the *H. polygyrus* infection

as they usually are, although changes can be seen in some of the antibiotics treated *H. polygyrus* infected mice (Figure 5-9E). This experiment alongside one of a similar pattern in the IFN-γ blockade setting were excluded from our pooled data. Alongside these results, some mice kept until day 7 of *H. polygyrus* infection were found to have a lower than expected number of granulomas (data not shown), which are usually dominant along the duodenum of the small intestine. Whilst working on this project, this infection variability has been a discussion of interest with other researchers working with *H. polygyrus*. In conversations with Dr Constance Finney, University of Calgary, she informed us of similar issues within her own laboratory. Interestingly, a recent publication from another laboratory also working with *H. polygyrus* has defined a fungal commensal species in mice, *Kazachstania pintolopesii*, which they report to protect the host against parasitic infection, increasing resistance to helminths (Liao et al., 2024). It is possible that batches of wildtype mice, purchased for each of our experiments, may have been variable in their levels of colonisation by such commensal fungi, affecting our ability to achieve consistent *H. polygyrus* infections. To determine which experiments were excluded from the results, when day 7 mice were culled from an experiment at which day 2 mice were also culled, examination of the intestine for visual granulomas as shown in Figure 2-1A enabled assessment of successful infection. When no or extremely few granulomas were seen, infection was deemed unsuccessful, and results were excluded. Additionally, since our experiments were often repeated, data was examined and results from these mice did not align with repeated experiments under the same conditions and therefore these varied experiments were excluded upon careful examination and discussion with my supervisor.

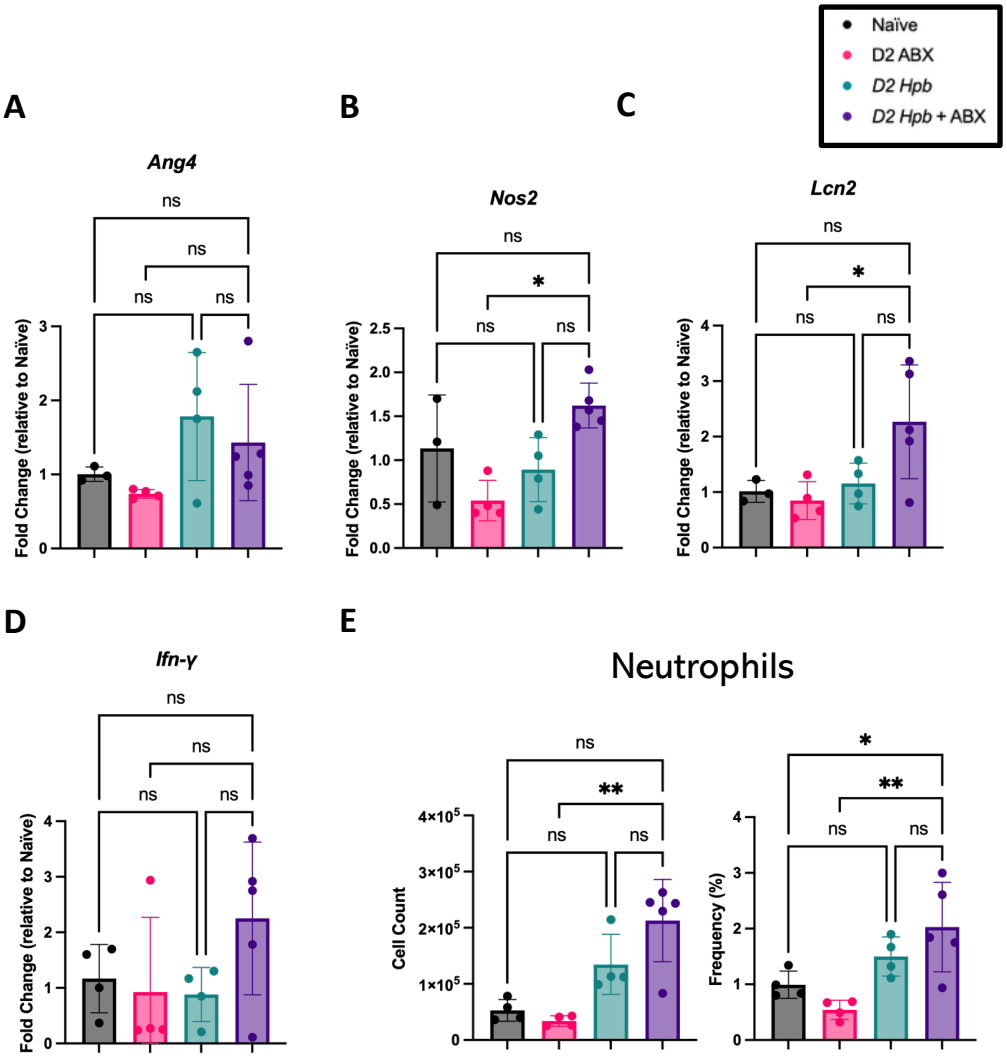

Figure 5-9 High levels of variability with bolus dose *Hpb* infection.
C57BL/6 mice were infected with 200 L3 *Hpb* larvae and given broad spectrum antibiotics in their drinking water for 7 days prior to and throughout infection, samples were collected at day 2 post infection. Fold change of **A)** Ang4**, B)** Nos2, **C)** Lcn2 and **D)** IFN-γ in the duodenum compared to housekeeping gene (Rps29) and normalised to the mean of the naïve samples. **E)** Frequency and absolute cell count of neutrophils plotted. Data shown with mean ±SD with 3-4 mice per sample group. Statistical significance was calculated by a One-Way Anova followed by a Tukey's multiple comparisons test or in cases where data was not normally distributed a Kruskal-Wallis test followed by Dunn's multiple comparisons test was used. (Significance *$p < 0.05$, **$p < 0.01$, NS, Non-significant).

5.3.5 *H. polygyrus* as a bacterial niche

Finally, with some of our data suggesting that the host response to *H. polygyrus* infection includes responses that happen upon bacterial stimulation, we went back to our original hypothesis, thinking that as *H. polygyrus* burrows into the intestinal wall it provides an entry point for bacteria, increasing the susceptibility to co-infection. We hypothesised that bacteria may not only use the epithelial breach as an entry point, but that they may also use the surface of

the worm as a physical niche to occupy, to avoid clearance and detection. A similar attachment of bacteria to a helminth surface has been shown in Schistosome infection (Barnhill et al., 2011). During a placement in the Reynolds laboratory at University of Victoria, I cultured *H. polygyrus* larvae with a fluorescent strain of *Salmonella* in a petri dish, *in vitro,* to assess whether the two pathogens co-localise. The image in Figure 5-10 shows both *H. polygyrus* L3 larvae and the fluorescent *Salmonella* in the same culture. The internal tissues have some autofluorescence, as seen in Figure 5-10. Control images of the worm alone and bacteria alone were sadly not taken so no formal conclusion can be made, but preliminary assessment of the cultures by eye did not suggest enhanced fluorescence in the co-culture wells, and the surrounding *Salmonella* bacteria did not appear to co-localise on the surface of the worm.

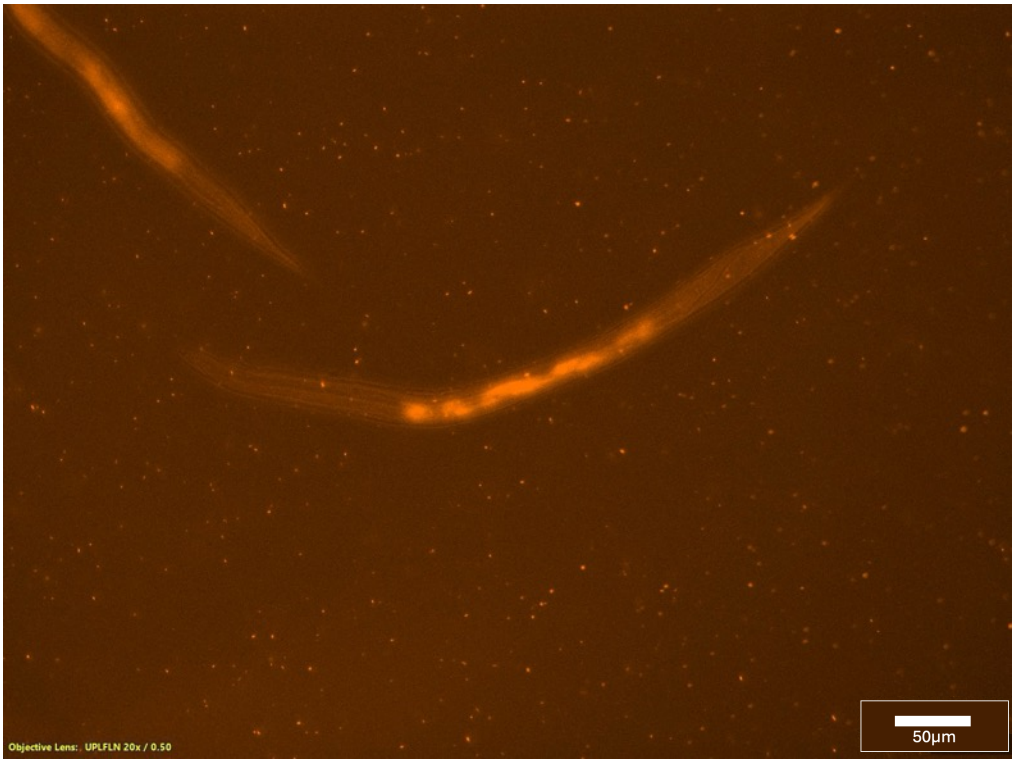

Figure 5-10 *Salmonella* does not co-localise with *Hpb* larvae.
Image of fluorescent *Salmonella* in co-culture with *Hpb* larvae. *H. polygyrus* larvae shows level of autofluorescence captured in image. Bacteria can be identified in image as fluorescent 'circles' in culture.

To further assess whether bacteria is able to bind to the surface of the helminth, at the later lifecycle stages of *H. polygyrus* infection, intestinal samples were taken from mice at days 8 and 21 of *H. polygyrus* infection and scanning electron

microscopy (SEM) was performed on the tissue. A recent publication shows the same assay used in *T. muris* infection with successful identification of both the helminth and local bacteria (de Oliveira et al., 2025). In our collected samples, the helminth is co-habiting the gut lumen with commensal bacteria, enabling us to assess the co-localisation of these organisms relative to each other. SEM allows detailed, high-resolution imaging of the surface of the worm (Figure 5-11). These samples were prepared by my supervisor, Georgia Perona-Wright, and processed and imaged by the head of our core imaging facility, Dr Leandro Lemgruber Soares. Figure 5-11A shows an image representative of the larger tissue sample where the spiral shape of the *H. polygyrus* worm at day 21, residing in the intestinal lumen, can be seen. Images of a higher magnification capture the worm in the lumen at day 8 and day 21 of infection (Figure 5-11B&C). The surface of the helminths shown in each image is clear and not obviously covered in adhered bacteria, perhaps giving a further indication that bacteria do not localise on the surface of the worm as we had hypothesised. However, it is also possible that these clean surfaces are a result of the washing and processing that the tissue was subjected to during sample preparation. Together, our current data perhaps does not support the idea of bacteria binding to and using the worm surface as a niche, although further work is needed to prove this conclusively. Overall, the data in this chapter suggest that microbiota are an influential presence, able to stimulate immune responses during early *H. polygyrus* infection.

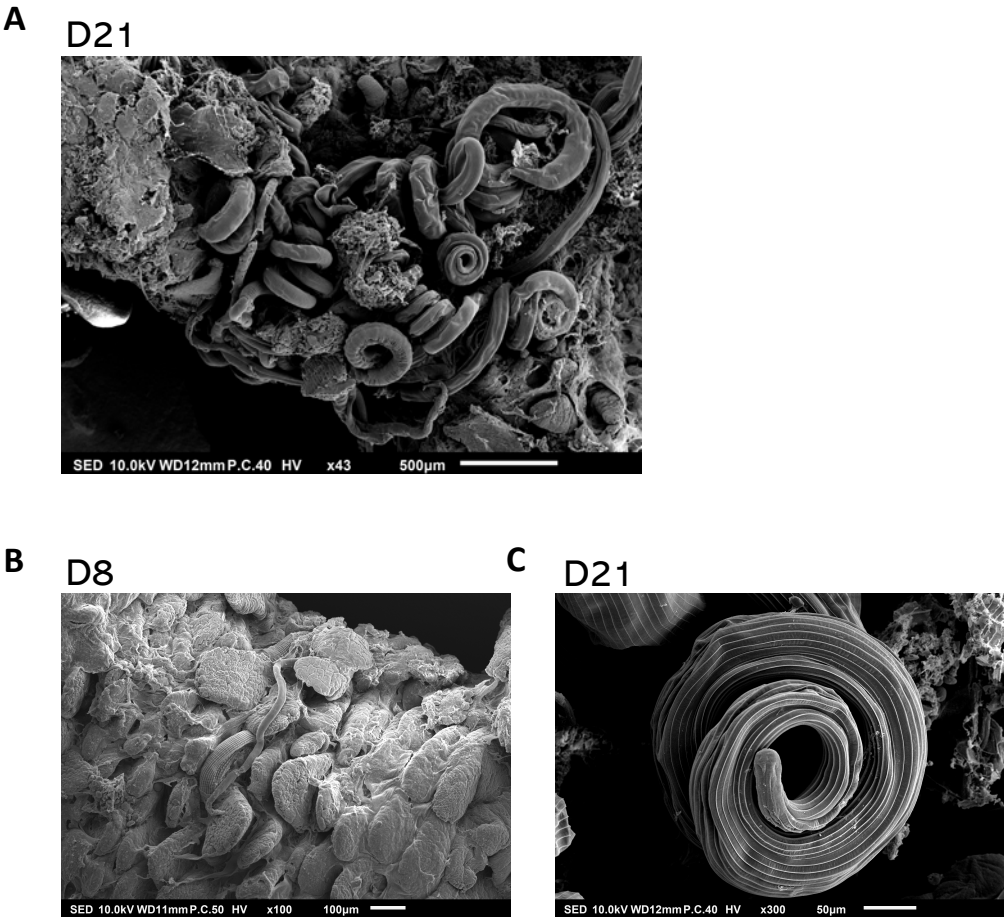

Figure 5-11 Bacteria undetected on surface of *H. polygyrus* helminths.
Scanning electron microscopy images of small intestine from C57BL/6 mice infected with 200 L3 *Hpb* larvae. **A &C)** D21 and **B)** D8 post-infection.

5.4 Discussion

Overall, the aim of Chapter 5 was to investigate whether the antimicrobial responses and IFN-γ production we demonstrated to be increased in *H. polygyrus* infection were related directly to bacterial stimulation. To test this hypothesis, our approach was to use antibiotic treatment to eliminate bacteria in mice infected with *H. polygyrus* to determine whether bacteria play a role in inducing our identified changes. We have defined a role for microbiota in stimulating and enhancing the IFN-γ-coordinated local immunity in the small intestine during *H. polygyrus* infection, suggesting that helminth entry allows bacteria to contact host tissue and the host immune system, acting as a stimulus for epithelial and immune responses. Additionally, to explore whether bacteria used the surface of the helminth as a niche, we took a small glance at the localisation and interactions of helminths and bacteria using co-cultures and ex vivo SEM imaging. Using these methods, no evidence was found to suggest that bacteria co-localise with the helminth. Collectively, the data in this chapter have furthered our understanding of the relationship between the host immune response and the helminth, defining that the type 1 immunity found in early infection is reflective of a local response to bacterial stimulation. We think that the epithelial disruption caused by early *H. polygyrus* infection allows commensals to interact with the host immune system, and stimulate an antimicrobial immune response, potentially protecting against opportunistic or concurrent bacterial infections.

5.4.1 Microbiota and antibiotics

Research using either antibiotic treatment or germ-free mice to eliminate bacteria are common experimental approaches. To deplete bacteria in our experiments, we decided to use a cocktail of broad-spectrum antibiotics to reduce overall bacterial load. Compared with germ-free mice, antibiotics are more accessible and offer precise timing of bacterial depletion (Kennedy et al., 2018). Whilst countless studies have used these experimental methods and they are known to be effective, they also have limitations. As well as depleting bacteria, antibiotics have been shown to have toxic effects on mammalian cells, causing mitochondrial damage (Morgun et al., 2015). Additionally, different antibiotics can specifically limit certain bacterial strains due to resistance or

specific targeting and the precise antibiotics cocktail used can make a difference to which bacteria remain. Similarly, however, germ-free mice also have caveats, causing several developmental impairments affecting both immune and neurological tissues (Delgado-Ocaña and Cuesta, 2024; Kennedy et al., 2018). In our experimental model, the antibiotics treatment we use has been used in previously published research and includes a cocktail designed to eliminate a broad range of bacterial strains. Each antibiotics added is most effective against certain bacterial groups; ampicillin is effective in eliminating many gram +ve bacteria and some gram -ve, metronidazole is effective against anaerobic bacteria, neomycin and gentamicin more specifically gram -ve anaerobes and vancomycin is effective at eliminating gram +ve bacterial strains. Therefore, the coverage of the treatment should be highly effective with any remaining bacterial strains likely to be more atypical or resistant to antibiotic treatment. Although we do not show efficacy of the antibiotics directly, we use weight loss and caecum size to confirm bacterial changes. Assessing bacterial elimination in the experimental mice, and specifically locally to the worms in the duodenum would have complemented the model nicely to confirm which bacterial species (if any) remain present.

Co-evolution of the host with microbiota has resulted in a complicated and often mutualistic relationship. It is well established that microbiota can influence immune responses in a variety of diseases, both infectious and autoimmune (Zheng et al., 2020). Dysbiosis of commensal bacteria has been linked to development of conditions such as IBD and Rheumatoid Arthritis and also exacerbated infection outcome with bacterial strains *Salmonella* and *Clostridioides difficile* (Hou et al., 2022; Pham and Lawley, 2014). Microbiota can protect against intestinal infections by preventing pathogens from colonising and secreting toxic antimicrobial peptides to inhibit growth of other bacteria (Tan et al., 2021). We show here that the microbiota are responsible for promoting an IFN-γ induced, antimicrobial response during helminth infection which we hypothesise acts to protect the host from future infection.

Research has demonstrated that the microbiota can alter immune cell subsets, inhibiting mucosal mast cell activation and promoting proinflammatory T cells and macrophage hyperactivation (Zheng et al., 2020). The commensal bacteria within a host intestine can also modulate epithelial responses including mucus

production, proliferation and secretion of AMPs (Tan et al., 2021). Our data show that the increased AMPs, IFN-γ and myeloid cell influx seen during *H. polygyrus* infection are influenced by the presence of microbiota. We did not directly look at the effects of antibiotic treatment on TJ protein expression, but a published study shows that microbial signals are able to upregulate epithelial TJ expression to strengthen the intestinal epithelial barrier (Bansal et al., 2010). These data perhaps suggest that the reparative, enhanced TJ protein expression we identified in early *H. polygyrus* infection could also be directed by intestinal microbes. In future, looking at the TJ proteins in this experimental setup would be beneficial to understand whether microbiota are contributing to cell adhesion in early *H. polygyrus* infection. Taken together, our data so far suggest that, since microbiota promote IFN-γ production, and we show that IFN-γ is responsible for enhancing TJ gene expression in *H. polygyrus*, it is likely that microbiota depletion would hinder TJ gene expression. As commensal bacteria and their relationship with diseases is a growing topic in research, several studies have explored microbiota effects on helminth infection.

5.4.2 *H. polygyrus* and microbiota

In 2010, it was first demonstrated that *H. polygyrus* helminths significantly alter and shape the commensal bacterial environment in the host (Walk et al., 2010b). This study and others have since shown that there is a significant increase in the abundance of certain bacterial species including *Lactobacillus* (Rausch et al., 2013; Reynolds et al., 2014b; Walk et al., 2010b). Part of the influence of the helminth on shaping the commensal bacteria is due to the worms exhibiting their own antimicrobial defences. These secreted proteins and peptides can kill certain bacterial species and perhaps enhance the growth of others to shape the host's microbiota (Rausch et al., 2018). The *H. polygyrus* moulded commensal environment is then able to provoke several immune responses, thought to be a direct consequence of helminth interaction. Thus, the relationship and interaction between the helminth and the host microbiota became a more active area of research to understand how bacterial reshaping in a similar pattern to the helminth may benefit the host (Blackwell et al., 2024; Su et al., 2018b; Zaiss et al., 2015). Methods were established to grow sterile *H. polygyrus* larvae to eliminate their own microbiota (Blackwell et al., 2024; Russell et al., 2021; Zaiss et al., 2015), as it had been hypothesised that the

microbiota carried by the *H. polygyrus* larvae themselves may contribute to already established populations in the host and favour the colonisation of certain species. However, a study identified and characterised the bacteria of the *H. polygyrus* larvae microbiome and found that the alterations in bacterial populations was not associated with the internal microbiome of the worm (Rapin et al., 2020). Together these data confirm that helminths can alter the microbiota, in turn influencing local immune responses. With our findings in this chapter illustrating that microbiota promote IFN-γ production and antimicrobial responses, perhaps the *H. polygyrus* helminth shapes the microbiota to promote these responses, protecting the host to benefit its own survival.

Whilst helminths and their infected hosts have co-evolved together for thousands of years, so have the microbiota and helminths. Hence, several studies have shown that microbiota are beneficial to helminths. In *T. muris* infection, Hayes et al. show that microbiota are essential for the helminth eggs hatching. Additionally, in germ-free mice infected with *H. polygyrus*, parasite growth and fecundity is hindered, illustrating the importance of the microbiota in parasite overall survival (Rausch et al., 2018). Other studies have shown that commensal bacteria provide a local increase in the short chain fatty acid, isovaleric acid, which enhances the fecundity and health of *H. polygyrus* during infection (Kennedy et al., 2021). In contrast, a recent paper found that a diverse microbiota increases host resistance to *H. polygyrus* (Moyat et al., 2022), suggesting that the helminth reshaping of microbiota we previously described may also help in preventing its clearance. Specifically, *Lactobacilli* has been shown to create a positive feedback with *H. polygyrus*, where the bacterial species enhance helminth infection and are then also promoted by the worms, working together in a mutualistic symbiotic environment (Reynolds et al., 2014). The mechanisms for how the helminths promote Lactobacilli abundance remain unclear, however this paper found that Lactobacilli can enhance Treg numbers, improving helminth outcome possibly by limiting the Th2 response usually responsible for helminth clearance (Reynolds et al., 2014). Although we did not specifically examine the outcome of infection with antibiotics treatment, our data show that IFN-γ production, AMP production and innate immune cell influx were each hindered. Since the above studies suggest that microbiota often provide a beneficial relationship to the host, it suggests that these impaired

responses would benefit the host, likely by hindering Th2 cells and perhaps by keeping the host from succumbing to further bacterial infection.

The effects of microbiota depletion during *H. polygyrus* infection have been primarily studied at later infection stages, with limited research on the early infection phase (Elizalde-Velázquez et al., 2023; Moyat et al., 2022). Similarly, studies on *H. polygyrus* in germ-free mice have also focussed on later stages. However, these studies have shown increased Th2 responses in germ-free mice, possibly complementing our data and suggesting that the absence of microbiota and the IFN-γ that the microbiota stimulates allows for an enhanced type 2 immune response (Rausch et al., 2018; Reynolds et al., 2014). Research has also illustrated that microbiota in helminth infection are important in driving protective type 1 interferon responses against secondary viral infection (McFarlane et al., 2017). Together, this complements our data nicely, suggesting that microbiota are important in driving protective immunity.

5.4.3 Antimicrobial defence in the intestine

Throughout this thesis we have shown that the AMPs are increased during helminth infection, and in this chapter we show that these AMPs are produced in response to bacteria. Some studies have suggested a role for AMPs in protecting the host from further infection during helminth infection (Horsnell and Oudhoff, 2022). In 2021, a study specifically investigating the expression of the AMP SPRR2A found that, after 2 weeks of infection with *H. polygyrus*, SPRR2A expression and production was increased in intestinal epithelium, and that these SPRR2A changes were promoted by the type 2 cytokines IL-4 and IL-13. The same study also showed that intestinal microbiota can induce SPRR2A through the TLR-MyD88 signalling pathway (Hu et al., 2021). Since our research focussed on earlier timepoints of helminth infection, prior to the dominant expression of type 2 cytokines, it is possible that the increased AMPs we see are a result of TLR signalling. This suggests that in *H. polygyrus* infection, there may be higher levels of bacterial stimulation, possibly due to the disruption of the barrier, responsible for the increases in AMP expression.

The data in this chapter show that the influx of myeloid cells, predominantly neutrophils, in early *H. polygyrus* infection is influenced and driven by the

presence of microbiota. In a healthy gut, microbiota contribute to the maintenance of gut homeostasis, at least in part by regulating and suppressing neutrophil recruitment via SCFA production (Belkaid and Harrison, 2017). When neutrophils are recruited, this is often due to pathogenic organisms being recognised and or upon intestinal bacterial translocation (Schwab et al., 2014). Neutrophils defend the host whilst in the intestinal tissue but can also migrate to the lumen to prevent translocation of invading bacterial species (Zhang and Frenette, 2019). Additionally, not only the recruitment of neutrophils but also their activation can be altered by microbiota. Studies have shown that neutrophil extracellular trap (NET) formation from neutrophils can be enhanced and driven by certain bacterial strains (Doolan and Bouchery, 2022). Interestingly NETs have also been shown to effectively trap and in some cases kill helminth larvae (Bonne-Année et al., 2014; Díaz-Godínez and Carrero, 2019). Together, these papers support our findings that microbiota can co-ordinate the influx of neutrophils in *H. polygyrus* infection and also demonstrate mechanisms by which neutrophils may be driven by microbiota to defend against further bacterial infection or even the helminth larvae.

We identified that the increased IFN-γ expression and secretion in *H. polygyrus* infection was ablated with antibiotics treatment and hence illustrated that bacterial stimulation provoked the production of this cytokine. Although we confirmed NK cells to be a source of IFN-γ in early *H. polygyrus* infection, their numbers and proportions did not significantly change in response to antibiotic treatment. These data perhaps suggest that *H. polygyrus* increases NK cell numbers irrespective of microbiota, but NK cell activation and increased cytokine production is stimulated by bacteria. This is complemented by other research which suggests that exposure of NK cells to commensal bacteria can activate them and induce NK cell production of IFN-γ (Aziz and Bonavida, 2016; Horowitz et al., 2012).

5.4.4 Co-infection

In a co-infection model with *H. polygyrus* and the respiratory bacterium, *Pseudomonas aeruginosa*, one study shows that bacteria induce the recruitment and activation of neutrophils in the lung and helminth infection enhances this, showing that helminth infection can potentiate neutrophil responses to other

infections (Long et al., 2019b). This shows that *H. polygyrus* can provide not just local but systemic enhanced immunity against invading pathogens. In contrast, neutrophil recruitment in *Salmonella* infection was shown to be reduced by co-infection with *H. polygyrus* (Su et al., 2014b). In scenarios of co-infection, the host must prioritise which pathogen to direct its immune response towards, while also avoiding immune pathology for the host. The impact of helminth co-infection on the host immune response and neutrophil recruitment may depend on the location and severity of the bacterial pathogen.

When combined with a secondary infection such as from food-borne bacteria, helminths can lead to severe disease outcomes. When immune responses tailored to the helminth disrupt effective immunity against concurrent pathogens, this is known as immune interference. This has been described in a number of instances in both helminth-virus and helminth-bacterial co-infections. Some research has demonstrated beneficial and protective influence of helminths on secondary infection (McFarlane et al., 2017). However, many co-infections result in higher pathogen burdens and more severe disease symptoms (Reese et al., 2014; Su et al., 2014b).

Reflecting on our data, the remaining questions are whether the immune changes driven by microbiota occur early, to prevent bacterial invasion; or are a consequence of bacteria actively translocating and posing a threat to the host. Therefore, our next steps would be to co-infect mice with *H. polygyrus* larvae and an additional more severe bacterial pathogen, known to infect and colonise in the small intestine. If we employed a fluorescent strain of bacteria, this could allow for visualisation of whether bacteria are indeed exploiting the worm's entry point in the intestinal epithelium. We could directly visualise whether fluorescent bacteria are colonising the intestinal tissue surrounding the helminth, and/or disseminating systemically. Recently, a paper has demonstrated a technique for more effectively visualising and monitoring bacterial translocation across the intestinal wall. The methods used allow for a real-time monitoring system by labelling gut microbiota and using two photon microscopy (Fan et al., 2025). In further research for this project, harnessing this technique would be an effective method of monitoring how helminth infection and consequent host responses can alter susceptibility to bacterial infection. Additionally, using co-infection models with a bacterial pathogen and

the *H. polygyrus* model alongside IFN-γ blockade methods could help us test the effectiveness of IFN-γ in preventing and reducing bacterial infections.

5.4.5 Infection variability

Our data also highlight some infection variability in our experimental models. As mentioned, this is not a problem limited to our laboratory and has been experienced by other research groups with a particular laboratory exploring this in a recent publication where they identify a commensal fungus responsible for increasing host resistance to helminths (Liao et al., 2024). To address the source of heterogeneity in the *H. polygyrus* model in our laboratory, several experiments could be used. In future experiments, screening could be carried out to measure the presence of such commensal fungi to assess whether these are altering infection efficacy. If presence of this fungus was confirmed, future experiments using this mouse model could include administering a preventative anti-fungal treatment to mice prior to *H. polygyrus* infection. However, using this treatment introduces further variables to the experiments which may introduce other issues and causes the experimental model to become further away from reflecting the true nature of infections in the wild. Finally, since our experiments are carried out early in *H. polygyrus* infection, egg and adult worm counts cannot be carried out. However, quantification of worm infection by qPCR could be a method to consider to enable determination of the infection level and be used to normalise experimental readouts.

5.4.6 Summary

Together, the data in this Chapter highlight that the IFN-γ found in early *H. polygyrus* infection is produced in response to bacteria and provides local antimicrobial protection only in their presence. Perhaps this data suggests that in the presence of a severe pathogen, the defence pathways we have identified as coordinated by local IFN-γ may be critical in preventing systemic, fatal infection. With our data, it appears that the mice prioritise avoiding additional infection by bacterial pathogens over clearing the helminths. In certain parasitic co-infection models, helminths have been found to provide a niche for bacteria, rendering antibiotic treatment less effective against infective pathogens (Barnhill et al., 2011). Therefore, understanding how helminth and host

responses interact with each other and influence other pathogen's ability to infect the host will enhance our understanding of how to better utilise helminths and effectively treat against them or bacteria during co-infections.

Chapter 6 Discussion

Much of the immune response to helminths, especially at later stages of infection is well characterised by a dominating Th2 driven immunity. A major event of many intestinal helminth lifecycles, however, involves barrier breach at earlier stages of infection, before adaptive immunity takes effect and where less research has focussed. The size of these multicellular worms is drastically larger than any individual mammalian cells and yet the mechanisms by which helminths can migrate across tissues is still unclear. These migrations often occur twice, to both enter and exit tissue. In the case of the murine *H. polygyrus* model, this migration is into and out of the duodenal tissue in the upper intestine (described in section 1.1.2). Although these helminths are large, little research has investigated the resulting state of the intestinal barrier or whether 'breach' points could create an opportunity for other pathogens to more easily infect the host. Previous studies have provided evidence for a granuloma surrounding the *H. polygyrus* helminth during its maturation in the intestinal tissue, and for anti-helminth responses at this stage. However, evidence for antimicrobial activity and investigation into responses contributing to host protection from further infection as a result of these migrations have not been explored. The cytokine, IFN-γ, is more typically associated with Th1 immunity, yet research has confirmed that it is increased at day 2 of *H. polygyrus* infection. However, the cause of its production and the role it plays in early helminth infection is unclear. The overall aim of this thesis was to investigate the breach in early helminth infection, to define the responses coordinated by IFN-γ and understand why these are provoked.

6.1 Characterising the response at the time of *H. polygyrus* breach across the intestinal barrier

Parasitic helminths can often cause tissue damage to the host either through feeding mechanisms, or as part of their lifecycle migration. In the case of *H. polygyrus*, a model representative of soil-transmitted helminths, two breach points can be identified upon entry and exit of the duodenal tissue at the beginning of infection. Our H&E images of the helminth in the intestine demonstrate just how large *H. polygyrus* is in comparison to the epithelium, even at just 2 days PI. Although it is well characterised that tissue damage

occurs during early infection as is evident by the release of alarmins and the initiated type 2 immune response (Inclan-Rico and Siracusa, 2018; Oyesola et al., 2020), how exactly the worm crosses the barrier and whether it enables an entry point for other pathogens has not been defined. Certain research has suggested that molecules secreted by the helminths can facilitate an opening of the barrier to enable the parasite to travel across. A proposed mechanism is that acetate is one of the components in *H. polygyrus* excretory-secretory (ES) molecules and can disrupt TJ proteins (Schälter et al., 2022). We demonstrate an increase in TJ gene expression at day 2 of *H. polygyrus* infection, coinciding with the time the worm migrates across the tissue, perhaps supporting the proposed mechanism in this study. The increased gene expression could be indicative of a reparative process by the epithelium to tightly reinforce cell adhesion after the worms passing across. A recently published study also explored the barrier disruption in *H. polygyrus* infection. Looking at the latter breach point, at day 7 of infection, the researchers also reported significant changes in the expression of tight junction proteins, complementing our data. Additionally, they further explore intestinal permeability using a FITC-dextran experimental assay and confirmed a significant increase in the permeability again at this latter breach point (Mules et al., 2024). Although the worm spends several days growing and maturing between these breaches, it is likely that this permeability may also be reflected in the initial breach at the day 2 timepoint. To further explore the barrier disruption, we also found other markers associated with intestinal permeability to be increased. Other studies have demonstrated that barrier disruption and intestinal permeability can lead to bacterial translocation, and subsequent infection (de Oliveira et al., 2025b; Di Tommaso et al., 2021; Hand et al., 2012b). Due to the bacteria-rich environment in the small intestine, the indication of permeability led us to question whether the barrier is disrupted enough to leave the host vulnerable to further infection by bacteria.

We therefore sought to investigate other immune responses local to the helminth which may indicate whether the barrier disruption enabled bacterial entry. Antimicrobial proteins (AMPs) are often expressed and secreted at low levels by epithelial cells, but this can be increased upon bacterial stimulation as a defence to kill invading bacteria (Gallo and Hooper, 2012; Hassan et al., 2022). Our results show increased AMP expression in the small intestine upon early *H.*

polygyrus infection. A previous study has shown that 2 weeks into infection, an increase in expression of the AMP, sprr2a, can prevent secondary infection in *H. polygyrus* infected mice (Hu et al., 2021). This suggestion supported our hypothesis that AMP expression is increased to prevent bacterial infection and we wanted to address this in the context of the helminth barrier disruption providing an opportunity for bacterial invasion. Through further exploration of local responses, we also confirmed an influx of myeloid cells, including those often associated with defending against bacterial pathogens; neutrophils and monocytes. Both cell types have previously been described in *H. polygyrus* infection but not reported quite as early as the day 2 timepoint at which the first breach takes place (Anthony et al., 2006; Hewitson et al., 2015; Webster et al., 2020). Together with the barrier disruption and evidence for responses characteristic of defence against bacteria, these findings suggested that bacteria could be present in the intestinal tissue. Other literature and this thesis do not provide any evidence of bacterial translocation; however more sensitive assays would be required to fully test this hypothesis. Use of a 16S probe to carry out fluorescent labelling of bacteria would be useful and an effective way to address our hypothesis. Part of our concern with this assay was that the host immune response to commensal bacterial is highly effective and therefore we wondered whether a more virulent strain of bacteria would be capable of colonising in the intestinal tissue where we imaged the *H. polygyrus* granuloma. To test this hypothesis, a co-infection model with *H. polygyrus* and a fluorescently labelled intestinal pathogenic bacterium would enable similar imaging techniques to assess the exact localisation of invading species and to determine whether the breach points created by the helminth can also be used as entry points by the bacteria. We also measured other responses known to be effective in bacterial clearance. Our data confirmed that *H. polygyrus* infection provokes IFN-γ secretion as other studies had identified (Gentile et al., 2020; Nusse et al., 2018), and we show that this is both local to the helminth as well as systemic.

6.2 IFN-γ coordinates a local antimicrobial and reparative response in early *H. polygyrus* infection

Even with anti-helminth responses often characterised by type 2 cytokines such as IL-4, IL-5 and IL-13, certain helminth infections including *T. muris* had identified a IFN-γ signature in much earlier research (Else et al., 1994). Research

found that in this infection, IFN-γ was important in promoting helminth survival and enabling chronic infection by limiting the Th2 response and thus preventing parasite clearance (Else et al., 1994b). In the context of *H. polygyrus*, several roles have been proposed for the IFN-γ identified in early infection, including aiding in the reparative process and also a similar responsibility as demonstrated in *T. muris* infection with limitation of Type 2 immunity to enable the helminth to persist (Gentile et al., 2020; Kapse et al., 2022; Progatzky et al., 2021). Our research reports a pivotal role for IFN-γ in strengthening the intestinal barrier through increasing TJ protein expression, co-ordinating antimicrobial responses including enhancing AMP expression and mediation of myeloid cell influx to the infection / breach site.

The enhanced expression of TJ protein expression can indicate repair upon damage (Namrata and Bai, 2021), which we suggest is what is happening in *H. polygyrus* infection. Further research is needed to determine whether IFN-γ drives a repair process following worm-induced damage or if it causes damage to facilitate the worm's breach, triggering the subsequent increased repair response identified by the TJ protein expression. Further analysing these proteins based on their surface expression and localisation during the breach point would be more effective than only measuring gene expression by qPCR. To test this, I propose using antibodies against specific TJ proteins to stain these and visualise them by immunofluorescence; this could be a next step used in our laboratory with the collection of Swiss roll samples from the experiments shown in this thesis. In other effects on the intestinal barrier, we explored permeability with a more effective assay during IFN-γ blockade. Whilst our FITC-dextran assay did not give readouts showing significant differences between *H. polygyrus* infected and naïve mice for intestinal permeability, IFN-γ blockade during infection reduced the levels of FITC-dextran found in the blood when compared to isotype treated, infected mice. A previous paper has reported that IFN-γ can internalise TJ proteins on intestinal epithelial cells (Bruewer et al., 2005; Utech et al., 2005), and our data placed in the context of this research suggests that IFN-γ may be facilitating barrier breach for the worms to enter the tissue.

Epithelial proliferation is also important in maintaining barrier integrity, and in contrast to other research, we found no evidence for altered proliferation of epithelial cells in *H. polygyrus*, irrespective of IFN-γ (Nusse et al., 2018b). This

study and others also provide interesting evidence of the ability of IFN-γ in altering the phenotypic state of the intestinal epithelium. Several papers show that IFN-γ can cause a foetal-like reversion of the epithelium during *H. polygyrus* infection (Drurey et al., 2021; Nusse et al., 2018). Although we did not explore this in our data, I wonder if this phenotypic change caused to the epithelium by IFN-γ could be responsible for the increases in AMP expression we identify. The AMP molecules are most often produced by cells of the epithelium, particularly Paneth cells, goblet cells and enterocytes so perhaps alterations to the numbers of these subsets may change the antimicrobial responses. Additionally, we wondered if the altered epithelium could also explain the myeloid cell recruitment which we found was enhanced by IFN-γ in *H. polygyrus* infection. Although IFN-γ is more often associated with activation of myeloid cells including neutrophils and monocytes, we found that the level of their influx during infection with *H. polygyrus* was very much dependent on IFN-γ. With the use of transcriptomics data generated by exposing intestinal organoids to recombinant cytokines, we found that stimulation with IFN-γ caused intestinal epithelial cells to upregulate expression of chemokines known to recruit neutrophils. We suggest that IFN-γ signals through epithelial cells to recruit innate immune cells to aid in bacterial defence.

NK cells, ILCs and T cells can each produce IFN-γ. We demonstrate production of IFN-γ by NK cells during infection, with both the number and the frequency of IFN-γ positive NK cells increasing during *H. polygyrus* infection. This is supported by previous research in which the authors found increased NK cells at the *H. polygyrus* breach sites, with IFN-γ production enhanced. These authors propose a reparative role for the NK cells and subsequent IFN-γ during infection (Gentile et al., 2020). In contrast, a more recent paper from the same team argues that IFN-γ is produced by gut-resident CD8+ T cells in helminth infection (Westfall et al., 2025). Here they explore a role for IFN-γ in signalling via a stromal cell subset to recruit neutrophils (Westfall et al., 2025). Whether the function of the IFN-γ is depending on its source of production remains unclear. Both of these papers are supported by our findings, that IFN-γ can promote both repair and neutrophil recruitment.

6.3 IFN-γ and subsequent antimicrobial responses stimulated by bacterial presence

Bacteria do not appear to translocate with the helminth during *H. polygyrus* infection, although their presence is essential for promoting the IFN-γ co-ordinated antimicrobial responses we define. Although we hypothesise that bacteria have easier access to the intestinal tissue due to the *H. polygyrus* 'breach' points and this triggers the immune changes, microbiota also may influence this in other ways. Instead of direct translocation of whole bacteria, PAMPs or bacterial metabolites may be able to translocate during *H. polygyrus* infection and initiate antimicrobial responses. At steady state, microbiota contribute to protecting the host from bacterial invasion by preventing colonisation by pathogenic bacteria, and by producing their own AMPs (Tan et al., 2021). Some research has shown that the microbiota can influence the production of IFN-γ in an infection setting. One particular study found that, during infection with the bacterium *Salmonella*, commensal bacteria enhanced IFN-γ production, contributing to better disease outcome with less *Salmonella* colonisation and prolonged survival of mice (Ost and Round, 2017; Thiemann et al., 2017). We suggest that our data implies a similar mechanism whereby microbiota are enhancing cytokine responses to protect from bacterial invasion. To assess whether the antimicrobial responses produced by IFN-γ are in place to prevent or protect against further bacterial infection, I would propose an experimental co-infection model should be used with both *H. polygyrus* and a pathogenic bacterium which invades the small intestine such as *Salmonella*. It has already been shown that *H. polygyrus* can attenuate infection to *Salmonella* (Brosschot et al., 2021b; Su et al., 2014b). Although these papers suggest this is due to more limited neutrophil recruitment in the co-infection than in sole bacterial infection, it may also be that the breach points in helminth migration are allowing the pathogenic bacteria easier access to the intestinal tissue. Administration of additional IFN-γ at early infection could provide indication of whether this further drives the responses we demonstrate; strengthening the barrier and driving antimicrobial responses by enhancing AMP expression and myeloid cell influx; and if these responses help to limit bacterial infection. In contrast, using a monoclonal antibody to neutralise IFN-γ in these co-infection models, as we have in this thesis, would enable determination of whether IFN-γ

is providing protection from other invading pathogens through these mechanisms.

The helminth faces a fine balance between protecting the host and weakening the host to facilitate infection. The helminth favours a weakened host to prevent worm clearance and to enable burrowing in the intestinal wall to allow successful maturation; although, it is in the interest of the helminth to protect its host as this provides a niche for survival and allows further spread of infection in passing of helminth eggs. Therefore, further research is needed to explore which immunomodulatory effects the helminth employs during early infection and whether it triggers the early antimicrobial IFN-γ response we have identified in order to protect the host from further infection. Research on hookworm infection has shown that a protein secreted by the hookworm can bind NK cells and induce them to produce cytokines, including IFN-γ, during infection, although the exact protein to achieve this has yet to be identified from the ES products (Hsieh et al., 2004). We show that microbiota are responsible for the promotion of IFN-γ production, which in turn can limit type 2 immunity, benefiting helminth survival. Not only have the host and microbiota evolved together, but the helminth has also evolved with host microbiota. Therefore, the helminth could use microbiota to shape the environment and elicit a type 1 response to dampen and prevent such a strong Th2 response to clear it. Additionally, whilst the parasite strives to establish its niche, the helminth disrupts the barrier and with it interrupts the mutualistic symbiosis between the host and microbiota. In disrupting barrier integrity, the helminth could provide an entry point for bacteria, some of which may be more harmful pathogens. I wonder whether the breach in barrier caused by the helminth serves a dual function, enabling the larvae to cross the barrier into an environment where it can grow and mature, and also causing damage to provoke other immune responses to evade being targeted during its early development.

6.4 Additional limitations

The context in which much of helminth research has been studied is in a laboratory setting with a very clean environment and using mice with no previous exposure to pathogens. Also, we infect by singular bolus infection to allow us to focus directly on the timepoints of the helminth breach as we aimed

to characterise the immune responses in this context. In a regular setting, a host is exposed to multiple pathogens continuously in everyday life and therefore the environment in the intestine is stimulated by numerous factors which are not present in the laboratory setting. Parasites can be ingested at multiple different times and infect the host across multiple days, therefore a host is often infected with parasites of different lifecycle stages continuously. A more representative way of mirroring helminth infections which the hosts are regularly exposed to is either by a trickle infection method whereby mice are administered larvae at multiple different timepoints in experimental setup (Ariyaratne et al., 2022), or by using wild mice as these are often naturally infected with the *H. polygyrus* parasite. With constant barrier breach occurring and adult helminths residing in the gut lumen, whether the antimicrobial responses and IFN-γ secretion are continuously produced remains unclear. Usually by day 14 of infection, an effective Th2 response is underway in helminth infection (Reynolds et al., 2012), working to eliminate the helminths, and the increased IFN-γ identified in early infection is no longer seen. Perhaps with trickle infection, each response continues and the IFN-γ can then limit the Th2 immunity, working in favour of the helminth. Otherwise, I suggest that the Th2 response may dominate, overpowering any IFN-γ responses and preventing antimicrobial defences, perhaps preventing a protective response which could prevent further infections by other pathogenic species.

Much of the published literature focusses on the later stages of *H. polygyrus* infection when the helminth resides in the intestinal lumen. I hypothesise that these earlier timepoints of infection when migration into and out of the intestinal tissue occurs is what can leave the host vulnerable to further infection. The data in this thesis contributes to current data by suggesting that bacteria are impacting the immune responses in helminth infection, potentially this could be by invading the intestinal tissue. Whilst we focus mainly at the earlier breach point at day 2 of infection, some of our data also explored the latter breach point at day 7 and found that changes such as some of our barrier disruption markers, IFN-γ production and myeloid cell influx were still significantly increased when compared to naïve mice. Other recent literature has indicated that the intestine is more permeable in *H. polygyrus* infected mice at day 7 PI (Mules et al., 2024), suggesting that this could provide an opportunity

for bacterial invasion. To extend the research in this project, a future step would be to further investigate the less-characterised immune responses at day 7 of *H. polygyrus* infection, potentially investigating again how IFN-γ and microbiota effect this through similar experimental setup to what we have used here. Whilst *H. polygyrus* has been an effective model for exploring the role of IFN-γ and the breach during infection, further investigating these in other helminth mouse models would enhance our overall understanding of co-infections.

As addressed in earlier data chapters, there were several other methodological limitations identified throughout this research project. From assay specific limitations with the commensal antigen ELISA and FITC-dextran assays having high variability within experimental groups, to more experimental design limitations such as the efficacy of both the antibiotics treatment and the monoclonal in vivo IFN-γ antibody.

6.5 *H. polygyrus* as a model

H. polygyrus as a model is generalisable to many helminth infection outcomes with similar patterns in Th2 immunity and immunomodulation. One of the unique points of this model is the lifecycle with the 'breach' points in the intestinal tissue. Whilst using the *H. polygyrus* model, the findings of this thesis are likely to be reflective of what could happen in human equivalent STH infections. Infection with the helminth *Ascaris lumbricoides* in humans also results in intestinal penetration into the mucosa, making the *H. polygyrus* model most similar to this infection type. As our research has aligned with the known 'breach' timepoints of *H. polygyrus*, perhaps these findings are reflective of similar immune patterns which may emerge during human infection. Co-infection with bacterial pathogens during enteric helminth infection is a known issue in human based infection. Therefore, our findings may contribute to the scientific field and aid in the development of treatments to provide enhanced antimicrobial defences during the early stages of infection, preventing such co-infections.

6.6 Concluding summary

With this project, we aimed to examine and characterise the immune responses at the early breach point of *H. polygyrus* helminth infection and specifically determine the role of IFN-γ at this timepoint, investigating its potential involvement in protecting the host from further infection. An overall summary diagram of the findings of this thesis is shown in Figure 6-1. Initially we found

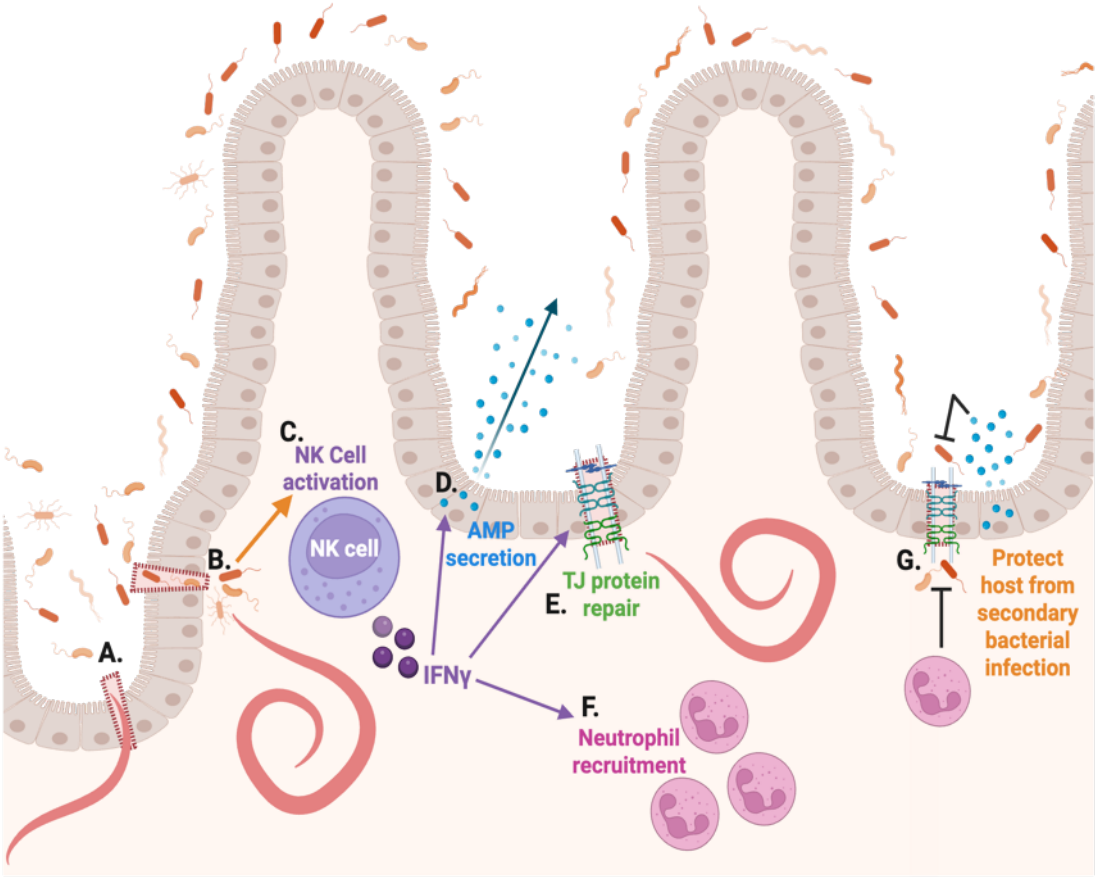

Figure 6-1 Graphical abstract.
Illustration summarising the data in this thesis. **A)** Worm breaches the duodenal barrier at day 2 post infection. **B)** As the breach occurs, microbiota gain access to host tissues and stimulate the intestinal immune response. **C)** This stimulation causes recruitment and/ or activation of innate lymphocytes such as NK cells, which then release IFN-γ. IFN-γ acts on intestinal epithelial cells to enhance their **D)** anti-microbial protein (AMP) secretion and **E)** tight junction (TJ) protein repair. **F)** IFN-γ also stimulates epithelial cells and macrophages to secrete chemoattractants that recruit and activate neutrophils and other inflammatory cells. **G)** We predict that the epithelial changes and immune influxes will result in enhanced host protection against concurrent bacterial pathogens. Illustration created using BioRender.com.

evidence for barrier disruption and enhanced antimicrobial responses at day 2 of *H. polygyrus* infection. The area surrounding the worm was further investigated and immune cell influx was confirmed to surround the helminth during

maturation, alongside a prominent spike in IFN-γ gene expression and secretion both locally and systemically. Using bulk RNA-seq datasets from intestinal organoids and *in vivo* blockade of IFN-γ, we demonstrated that NK cells can produce this early IFN-γ which in turn directly stimulates epithelial cells and contributes to increased cell adhesion expression and co-ordinates antimicrobial responses including AMP production and myeloid cell recruitment. Whilst no evidence for bacterial translocation was found in early *H. polygyrus* infection, irrespective of IFN-γ blockade, we hypothesised that our identified immune changes described above were due to bacterial stimulation accessing the small intestine at the helminth 'breach' points. To test this hypothesis, broad-spectrum antibiotics treatment was used alongside infection to eliminate bacteria and characterise the changes in immune responses. We reported that IFN-γ production, AMP expression and innate immune cell recruitment were each promoted by or dependent on microbiota. Further exploring the physical relationship between bacteria and *H. polygyrus* did not display co-localisation between these pathogens. Taken together, although we confirm bacterial presence is required for the IFN-γ co-ordinated responses we define, whether this is a protective host response promoted as a preventative measure by microbiota or due to bacteria accessing intestinal tissue through helminth-damaged areas remains unclear. We propose that bacteria use the helminth points of entry and exit to the intestinal tissue to gain access and invade host tissue. The data in this thesis contributes to our overall understanding of the ongoing immune response in early *H. polygyrus* infection. Whilst IFN-γ production in early *H. polygyrus* infection has recently been an area of interest, our data demonstrate a previously unreported role for IFN-γ in coordinating local antimicrobial protection dependent on bacterial presence. Experiments in future could assess these IFN-γ responses in the context of co-infection with a more pathogenic bacterial strain to determine whether this cytokine is providing protection from further infections. Sole infection with a helminth can have many devastating symptoms, some of which can have long-term impacts including malnutrition and stunted growth. However, due to the nature of infection and locations of high infection prevalence being associated with poorer living conditions, co-infections with bacteria are common and can often be fatal. Understanding how helminth and host responses interact with each other and

influence other pathogens' ability to infect will enhance our understanding of how to better treat co-infections.

References

Abo, H., Sultana, M.F., Kawashima, H., 2023. Dual function of angiogenin-4 inducing intestinal stem cells and apoptosis. Front Cell Dev Biol 11, 1181145. https://doi.org/10.3389/fcell.2023.1181145

Ajendra, J., 2021. Lessons in type 2 immunity: Neutrophils in Helminth infections. Seminars in Immunology, Resistance to parasites: lessons for type 2 immunity 53, 101531. https://doi.org/10.1016/j.smim.2021.101531

Allen, J.E., Wynn, T.A., 2011. Evolution of Th2 Immunity: A Rapid Repair Response to Tissue Destructive Pathogens. PLOS Pathogens 7, e1002003. https://doi.org/10.1371/journal.ppat.1002003

Andrews, C., McLean, M.H., Durum, S.K., 2018. Cytokine Tuning of Intestinal Epithelial Function. Front Immunol 9, 1270. https://doi.org/10.3389/fimmu.2018.01270

Andrusaite, A., Ridgewell, O., Ahlback, A., Webster, H., Yamaguchi, H., Peel, M., Frede, A., Al-Khalidi, S., Farthing, A., Heawood, A., Smith, A., Roberts, E., Mowat, A., Maizels, R., Perona-Wright, G., Milling, S., 2024. Intestinal helminth skews DC2 development towards regulatory phenotype to counter the anti-helminth immune response. https://doi.org/10.1101/2024.09.11.612410

Anthony, R.M., Rutitzky, L.I., Urban, J.F., Stadecker, M.J., Gause, W.C., 2007. Protective immune mechanisms in helminth infection. Nat Rev Immunol 7, 975–987. https://doi.org/10.1038/nri2199

Anthony, R.M., Urban, J.F., Alem, F., Hamed, H.A., Rozo, C.T., Boucher, J.-L., Van Rooijen, N., Gause, W.C., 2006. Memory TH2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. Nat Med 12, 955–960. https://doi.org/10.1038/nm1451

Antony, J.S., Ojurongbe, O., Meyer, C.G., Thangaraj, K., Mishra, A., Kremsner, P.G., Velavan, T.P., 2015. Correlation of Interleukin-6 levels and lectins during Schistosoma haematobium infection. Cytokine 76, 152–155. https://doi.org/10.1016/j.cyto.2015.04.019

Ariyaratne, A., Finney, C.A.M., 2019. Eosinophils and Macrophages within the Th2-Induced Granuloma: Balancing Killing and Healing in a Tight Space. Infection and Immunity 87, 10.1128/iai.00127-19. https://doi.org/10.1128/iai.00127-19

Ariyaratne, A., Kim, S.Y., Pollo, S.M.J., Perera, S., Liu, H., Nguyen, W.N.T., Coria, A.L., Luzzi, M. de C., Bowron, J., Szabo, E.K., Patel, K.D., Wasmuth, J.D., Nair, M.G., Finney, C.A.M., 2022. Trickle infection with Heligmosomoides polygyrus results in decreased worm burdens but increased intestinal inflammation and scarring. Front Immunol 13, 1020056. https://doi.org/10.3389/fimmu.2022.1020056

Artis, D., 2006. New weapons in the war on worms: Identification of putative mechanisms of immune-mediated expulsion of gastrointestinal nematodes. Int J Parasitol 36, 723–733. https://doi.org/10.1016/j.ijpara.2006.02.011

Artis, D., Grencis, R.K., 2008. The intestinal epithelium: sensors to effectors in nematode infection. Mucosal Immunology 1, 252–264. https://doi.org/10.1038/mi.2008.21

Artis, D., Potten, C.S., Else, K.J., Finkelman, F.D., Grencis, R.K., 1999. *Trichuris muris:* Host Intestinal Epithelial Cell Hyperproliferation during Chronic Infection Is Regulated by Interferon-γ. Experimental Parasitology 92, 144–153. https://doi.org/10.1006/expr.1999.4407

Atanga, R., Singh, V., In, J.G., 2023. Intestinal Enteroendocrine Cells: Present and Future Druggable Targets. Int J Mol Sci 24, 8836. https://doi.org/10.3390/ijms24108836

Atarashi, K., Tanoue, T., Ando, M., Kamada, N., Nagano, Y., Narushima, S., Suda, W., Imaoka, A., Setoyama, H., Nagamori, T., Ishikawa, E., Shima, T., Hara, T., Kado, S., Jinnohara, T., Ohno, H., Kondo, T., Toyooka, K., Watanabe, E., Yokoyama, S., Tokoro, S., Mori, H., Noguchi, Y., Morita, H., Ivanov, I.I., Sugiyama, T., Nuñez, G., Camp, J.G., Hattori, M., Umesaki, Y., Honda, K., 2015. Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. Cell 163, 367–380. https://doi.org/10.1016/j.cell.2015.08.058

Athie-Morales, V., Smits, H.H., Cantrell, D.A., Hilkens, C.M.U., 2004. Sustained IL-12 signaling is required for Th1 development. J Immunol 172, 61–69. https://doi.org/10.4049/jimmunol.172.1.61

Aziz, N., Bonavida, B., 2016. Activation of Natural Killer Cells by Probiotics. For Immunopathol Dis Therap 7, 41–55. https://doi.org/10.1615/ForumImmunDisTher.2016017095

Baldeón, M.E., Neece, D.J., Nandi, D., Monaco, J.J., Gaskins, H.R., 1997. Interferon-gamma independently activates the MHC class I antigen processing pathway and diminishes glucose responsiveness in pancreatic beta-cell lines. Diabetes 46, 770–778. https://doi.org/10.2337/diab.46.5.770

Bancroft, A.J., Else, K.J., Grencis, R.K., 1994. Low-level infection with Trichuris muris significantly affects the polarization of the CD4 response. Eur J Immunol 24, 3113–3118. https://doi.org/10.1002/eji.1830241230

Bankhead, P., Loughrey, M.B., Fernández, J.A., Dombrowski, Y., McArt, D.G., Dunne, P.D., McQuaid, S., Gray, R.T., Murray, L.J., Coleman, H.G., James, J.A., Salto-Tellez, M., Hamilton, P.W., 2017. QuPath: Open source software for digital pathology image analysis. Sci Rep 7, 16878. https://doi.org/10.1038/s41598-017-17204-5

Bansal, T., Alaniz, R.C., Wood, T.K., Jayaraman, A., 2010. The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. Proc Natl Acad Sci U S A 107, 228–233. https://doi.org/10.1073/pnas.0906112107

Bao, L., Shi, B., Shi, Y.-B., 2020. Intestinal homeostasis: a communication between life and death. Cell & Bioscience 10, 66. https://doi.org/10.1186/s13578-020-00429-9

Barbara, G., Barbaro, M.R., Fuschi, D., Palombo, M., Falangone, F., Cremon, C., Marasco, G., Stanghellini, V., 2021. Inflammatory and Microbiota-Related Regulation of the Intestinal Epithelial Barrier. Front. Nutr. 8. https://doi.org/10.3389/fnut.2021.718356

Bardenbacher, M., Ruder, B., Britzen-Laurent, N., Schmid, B., Waldner, M., Naschberger, E., Scharl, M., Müller, W., Günther, C., Becker, C., Stürzl, M., Tripal, P., 2019. Permeability analyses and three dimensional imaging of interferon gamma-induced barrier disintegration in intestinal organoids. Stem Cell Research 35, 101383. https://doi.org/10.1016/j.scr.2019.101383

Barnhill, A.E., Novozhilova, E., Day, T.A., Carlson, S.A., 2011. Schistosoma-associated Salmonella resist antibiotics via specific fimbrial attachments to the flatworm. Parasites & Vectors 4, 123. https://doi.org/10.1186/1756-3305-4-123

Barreau, F., Hugot, J., 2014. Intestinal barrier dysfunction triggered by invasive bacteria. Current Opinion in Microbiology, Host–microbe interactions: bacteria 17, 91–98. https://doi.org/10.1016/j.mib.2013.12.003

Bąska, P., Norbury, L.J., 2022. The Role of the Intestinal Epithelium in the "Weep and Sweep" Response during Gastro—Intestinal Helminth Infections. Animals (Basel) 12, 175. https://doi.org/10.3390/ani12020175

Beaurepaire, C., Smyth, D., McKay, D.M., 2009. Interferon-gamma regulation of intestinal epithelial permeability. J Interferon Cytokine Res 29, 133–144. https://doi.org/10.1089/jir.2008.0057

Becerra, S.C., Roy, D.C., Sanchez, C.J., Christy, R.J., Burmeister, D.M., 2016. An optimized staining technique for the detection of Gram positive and Gram negative bacteria within tissue. BMC Research Notes 9, 216. https://doi.org/10.1186/s13104-016-1902-0

Belkaid, Y., Harrison, O.J., 2017. Homeostatic immunity and the microbiota. Immunity 46, 562–576. https://doi.org/10.1016/j.immuni.2017.04.008

Berdnikovs, S., 2021. The twilight zone: plasticity and mixed ontogeny of neutrophil and eosinophil granulocyte subsets. Semin Immunopathol 43, 337–346. https://doi.org/10.1007/s00281-021-00862-z

Berg, R.D., 1999. Bacterial translocation from the gastrointestinal tract. Adv Exp Med Biol 473, 11–30. https://doi.org/10.1007/978-1-4615-4143-1_2

Berstad, A., Arslan, G., Folvik, G., 2000. Relationship between intestinal permeability and calprotectin concentration in gut lavage fluid. Scand J Gastroenterol 35, 64–69. https://doi.org/10.1080/003655200750024551

Bevins, C.L., Salzman, N.H., 2011. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nat Rev Microbiol 9, 356–368. https://doi.org/10.1038/nrmicro2546

Bick, F., Blanchetot, C., Lambrecht, B.N., Schuijs, M.J., 2025. A reappraisal of IL-9 in inflammation and cancer. Mucosal Immunology 18, 1–15. https://doi.org/10.1016/j.mucimm.2024.10.003

Birchenough, G.M.H., Schroeder, B.O., Sharba, S., Arike, L., Recktenwald, C.V., Puértolas-Balint, F., Subramani, M.V., Hansson, K.T., Yilmaz, B., Lindén, S.K., Bäckhed, F., Hansson, G.C., 2023. Muc2-dependent microbial colonization of the jejunal mucus layer is diet sensitive and confers local resistance to enteric pathogen infection. Cell Reports 42, 112084. https://doi.org/10.1016/j.celrep.2023.112084

Blackwell, K.H., Walk, H.M.G., Peters, L.R., Gunlikson, E.M., Bright, J.C., Kominsky, D.J., Walk, S.T., 2024. A simplified protocol for deriving sterile, infectious murine Heligmosomoides polygyrus bakeri larvae. STAR Protoc 5, 103144. https://doi.org/10.1016/j.xpro.2024.103144

Blonski, W., Buchner ,Anna M, Aberra ,Faten, and Lichtenstein, G., 2013. Teduglutide in Crohn's disease. Expert Opinion on Biological Therapy 13, 1207–1214. https://doi.org/10.1517/14712598.2013.815721

Bonne-Année, S., Kerepesi, L.A., Hess, J.A., Wesolowski, J., Paumet, F., Lok, J.B., Nolan, T.J., Abraham, D., 2014. Extracellular traps are associated with human and mouse neutrophil and macrophage mediated killing of larval Strongyloides stercoralis. Microbes Infect 16, 502–511. https://doi.org/10.1016/j.micinf.2014.02.012

Bouchery, T., Moyat, M., Sotillo, J., Silverstein, S., Volpe, B., Coakley, G., Tsourouktsoglou, T.-D., Becker, L., Shah, K., Kulagin, M., Guiet, R., Camberis, M., Schmidt, A., Seitz, A., Giacomin, P., Le Gros, G., Papayannopoulos, V., Loukas, A., Harris, N.L., 2020. Hookworms Evade Host Immunity by Secreting a Deoxyribonuclease to Degrade Neutrophil Extracellular Traps. Cell Host Microbe 27, 277-289.e6. https://doi.org/10.1016/j.chom.2020.01.011

Brandi, G., Calabrese, C., Tavolari, S., Bridonneau, C., Raibaud, P., Liguori, G., Thomas, M., Di Battista, M., Gaboriau-Routhiau, V., Langella, P., 2024. Intestinal Microbiota Increases Cell Proliferation of Colonic Mucosa in Human-Flora-

Associated (HFA) Mice. International Journal of Molecular Sciences 25, 6182. https://doi.org/10.3390/ijms25116182

Brosschot, T.P., Lawrence, K.M., Moeller, B.E., Kennedy, M.H.E., FitzPatrick, R.D., Gauthier, C.M., Shin, D., Gatti, D.M., Conway, K.M.E., Reynolds, L.A., 2021. Impaired host resistance to Salmonella during helminth co-infection is restored by anthelmintic treatment prior to bacterial challenge. PLOS Neglected Tropical Diseases 15, e0009052. https://doi.org/10.1371/journal.pntd.0009052

Brosschot, T.P., Reynolds, L.A., 2018. The impact of a helminth-modified microbiome on host immunity. Mucosal Immunology 11, 1039–1046. https://doi.org/10.1038/s41385-018-0008-5

Broz, P., Ohlson, M.B., Monack, D.M., 2012. Innate immune response to Salmonella typhimurium, a model enteric pathogen. Gut Microbes 3, 62–70. https://doi.org/10.4161/gmic.19141

Bruewer, M., Utech, M., Ivanov, A.I., Hopkins, A.M., Parkos, C.A., Nusrat, A., 2005. Interferon-gamma induces internalization of epithelial tight junction proteins via a macropinocytosis-like process. FASEB J 19, 923–933. https://doi.org/10.1096/fj.04-3260com

Burd, E.M., Hinrichs, B.H., 2015. Gastrointestinal Infections. Molecular Pathology in Clinical Practice 707–734. https://doi.org/10.1007/978-3-319-19674-9_50

Buys, J., Wever, R., van Stigt, R., Ruitenberg, E.J., 1981. The killing of newborn larvae of Trichinella spiralis by eosinophil peroxidase in vitro. European Journal of Immunology 11, 843–845. https://doi.org/10.1002/eji.1830111018

Campbell, L., Hepworth, M.R., Whittingham-Dowd, J., Thompson, S., Bancroft, A.J., Hayes, K.S., Shaw, T.N., Dickey, B.F., Flamar, A.-L., Artis, D., Schwartz, D.A., Evans, C.M., Roberts, I.S., Thornton, D.J., Grencis, R.K., 2019. ILC2s mediate systemic innate protection by priming mucus production at distal mucosal sites. Journal of Experimental Medicine 216, 2714–2723. https://doi.org/10.1084/jem.20180610

Capaldo, C.T., Farkas, A.E., Hilgarth, R.S., Krug, S.M., Wolf, M.F., Benedik, J.K., Fromm, M., Koval, M., Parkos, C., Nusrat, A., 2014. Proinflammatory cytokine-induced tight junction remodeling through dynamic self-assembly of claudins. Mol Biol Cell 25, 2710–2719. https://doi.org/10.1091/mbc.E14-02-0773

Capron, M., Torpier, G., Capron, A., 1979. In Vitro Killing of S. Mansoni Schistosomula by Eosinophils from Infected Rats: Role of Cytophilic Antibodies1. The Journal of Immunology 123, 2220–2230. https://doi.org/10.4049/jimmunol.123.5.2220

Castro, G.A., 1996. Helminths: Structure, Classification, Growth, and Development, in: Baron, S. (Ed.), Medical Microbiology. University of Texas Medical Branch at Galveston, Galveston (TX).

Chai, J.-Y., Jung, B.-K., Hong, S.-J., 2021. Albendazole and Mebendazole as Anti-Parasitic and Anti-Cancer Agents: an Update. Korean J Parasitol 59, 189–225. https://doi.org/10.3347/kjp.2021.59.3.189

Charles A Janeway, J., Travers, P., Walport, M., Shlomchik, M.J., 2001. The production of IgE, in: Immunobiology: The Immune System in Health and Disease. 5th Edition. Garland Science.

Chelakkot, C., Ghim, J., Ryu, S.H., 2018. Mechanisms regulating intestinal barrier integrity and its pathological implications. Exp Mol Med 50, 1–9. https://doi.org/10.1038/s12276-018-0126-x

Chen, C.-C., Louie, S., McCormick, B., Walker, W.A., Shi, H.N., 2005. Concurrent Infection with an Intestinal Helminth Parasite Impairs Host Resistance to Enteric Citrobacter rodentium and Enhances Citrobacter-Induced Colitis in Mice. Infect Immun 73, 5468–5481. https://doi.org/10.1128/IAI.73.9.5468-5481.2005

Chen, C.-C., Louie, S., McCormick, B.A., Walker, W.A., Shi, H.N., 2006. Helminth-Primed Dendritic Cells Alter the Host Response to Enteric Bacterial Infection. J Immunol 176, 472–483. https://doi.org/10.4049/jimmunol.176.1.472

Chen, F., El-Naccache, D.W., Ponessa, J.J., Lemenze, A., Espinosa, V., Wu, W., Lothstein, K., Jin, L., Antao, O., Weinstein, J.S., Damani-Yokota, P., Khanna, K., Murray, P.J., Rivera, A., Siracusa, M.C., Gause, W.C., 2022. Helminth resistance is mediated by differential activation of recruited monocyte-derived alveolar macrophages and arginine depletion. Cell Reports 38, 110215. https://doi.org/10.1016/j.celrep.2021.110215

Chen, F., Wu, W., Millman, A., Craft, J.F., Chen, E., Patel, N., Boucher, J.L., Urban, J.F., Kim, C.C., Gause, W.C., 2014. Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. Nat Immunol 15, 938–946. https://doi.org/10.1038/ni.2984

Chen, J., Gong, Y., Chen, Q., Li, S., Zhou, Y., 2024. Global burden of soil-transmitted helminth infections, 1990–2021. Infectious Diseases of Poverty 13, 77. https://doi.org/10.1186/s40249-024-01238-9

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., Zhao, L., 2017. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9, 7204–7218. https://doi.org/10.18632/oncotarget.23208

Choi, B., Lee, C., Yu, J.-W., 2023. Distinctive role of inflammation in tissue repair and regeneration. Arch Pharm Res 46, 78–89. https://doi.org/10.1007/s12272-023-01428-3

Classon, C.H., Li, M., Clavero, A.L., Ma, J., Feng, X., Tibbitt, C.A., Stark, J.M., Cardoso, R., Ringqvist, E., Boon, L., Villablanca, E.J., Rothfuchs, A.G., Eidsmo, L., Coquet, J.M., Nylén, S., 2022. Intestinal helminth infection transforms the CD4+ T cell composition of the skin. Mucosal Immunol 15, 257–267. https://doi.org/10.1038/s41385-021-00473-9

Cliffe, L.J., Grencis, R.K., 2004. The *Trichuris muris* System: a Paradigm of Resistance and Susceptibility to Intestinal Nematode Infection, in: Advances in Parasitology. Academic Press, pp. 255–307. https://doi.org/10.1016/S0065-308X(04)57004-5

Cliffe, L.J., Humphreys, N.E., Lane, T.E., Potten, C.S., Booth, C., Grencis, R.K., 2005. Accelerated Intestinal Epithelial Cell Turnover: A New Mechanism of Parasite Expulsion. Science 308, 1463–1465. https://doi.org/10.1126/science.1108661

Cole, J.J., Faydaci, B.A., McGuinness, D., Shaw, R., Maciewicz, R.A., Robertson, N.A., Goodyear, C.S., 2021. Searchlight: automated bulk RNA-seq exploration and visualisation using dynamically generated R scripts. BMC Bioinformatics 22, 411. https://doi.org/10.1186/s12859-021-04321-2

Collins, J.T., Nguyen, A., Omole, A.E., Badireddy, M., 2025. Anatomy, Abdomen and Pelvis, Small Intestine, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Cooke, J.P., 2019. Inflammation and its Role in Regeneration and Repair. Circ Res 124, 1166–1168. https://doi.org/10.1161/CIRCRESAHA.118.314669

Cooper, P.J., Ayre, G., Martin, C., Rizzo, J.A., Ponte, E.V., Cruz, A.A., 2008. Geohelminth infections: a review of the role of IgE and assessment of potential risks of anti-IgE treatment. Allergy 63, 409–417. https://doi.org/10.1111/j.1398-9995.2007.01601.x

Cote-Sierra, J., Foucras, G., Guo, L., Chiodetti, L., Young, H.A., Hu-Li, J., Zhu, J., Paul, W.E., 2004. Interleukin 2 plays a central role in Th2 differentiation. Proc Natl Acad Sci U S A 101, 3880–3885. https://doi.org/10.1073/pnas.0400339101

Croft, M., Salek-Ardakani, S., Song, J., So, T., Bansal-Pakala, P., 2013. Regulation of T Cell Immunity by OX40 and OX40L, in: Madame Curie Bioscience Database [Internet]. Landes Bioscience.

Czarniecki, C.W., Sonnenfeld, G., 1993. Interferon-gamma and resistance to bacterial infections. APMIS 101, 1–17. https://doi.org/10.1111/j.1699-0463.1993.tb00073.x

Dallagi, A., Girouard, J., Hamelin-Morrissette, J., Dadzie, R., Laurent, L., Vaillancourt, C., Lafond, J., Carrier, C., Reyes-Moreno, C., 2015. The activating effect of IFN-γ on monocytes/macrophages is regulated by the LIF–trophoblast-

IL-10 axis via Stat1 inhibition and Stat3 activation. Cell Mol Immunol 12, 326–341. https://doi.org/10.1038/cmi.2014.50

Damsker, J.M., Hansen, A.M., Caspi, R.R., 2010. Th1 and Th17 cells. Annals of the New York Academy of Sciences 1183, 211–221. https://doi.org/10.1111/j.1749-6632.2009.05133.x

Darwich, L., Coma, G., Peña, R., Bellido, R., Blanco, E.J.J., Este, J.A., Borras, F.E., Clotet, B., Ruiz, L., Rosell, A., Andreo, F., Parkhouse, R.M.E., Bofill, M., 2009. Secretion of interferon-γ by human macrophages demonstrated at the single-cell level after costimulation with interleukin (IL)-12 plus IL-18. Immunology 126, 386–393. https://doi.org/10.1111/j.1365-2567.2008.02905.x

De Benedetti, F., Prencipe, G., Bracaglia, C., Marasco, E., Grom, A.A., 2021. Targeting interferon-γ in hyperinflammation: opportunities and challenges. Nat Rev Rheumatol 17, 678–691. https://doi.org/10.1038/s41584-021-00694-z

de Oliveira, D.A., Oliveira, R., Braga, B.V., Straker, L.C., Rodrigues, L.S., Bueno, L.L., Fujiwara, R.T., Lopes-Torres, E.J., 2025. Experimental trichuriasis: Changes in the immune response and bacterial translocation during acute phase development illustrated with 3D model animation. PLoS Negl Trop Dis 19, e0012841. https://doi.org/10.1371/journal.pntd.0012841

de Weerd, N.A., Nguyen, T., 2012. The interferons and their receptors—distribution and regulation. Immunol Cell Biol 90, 483–491. https://doi.org/10.1038/icb.2012.9

Dea-Ayuela, M.A., Rama-Iñiguez, S., Bolás-Fernandez, F., 2008. Enhanced susceptibility to *Trichuris muris* infection of B10Br mice treated with the probiotic *Lactobacillus casei*. International Immunopharmacology 8, 28–35. https://doi.org/10.1016/j.intimp.2007.10.003

DeGruttola, A.K., Low, D., Mizoguchi, A., Mizoguchi, E., 2016. Current understanding of dysbiosis in disease in human and animal models. Inflamm Bowel Dis 22, 1137–1150. https://doi.org/10.1097/MIB.0000000000000750

Deitch, E.A., 2012. Gut-Origin sepsis; evolution of a concept. Surgeon 10, 350–356. https://doi.org/10.1016/j.surge.2012.03.003

Del, M., Sasiain, C., Barrera, S.D.L., Fink, S., Finiasz, M., Alemán, M., Fariña, M.H., Pizzariello, G., Valdez, R., 1998. Interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) are necessary in the early stages of induction of CD4 and CD8 cytotoxic T cells by Mycobacterium leprae heat shock protein (hsp) 65 kD. Clinical and Experimental Immunology 114, 196. https://doi.org/10.1046/j.1365-2249.1998.00702.x

Delgado-Ocaña, S., Cuesta, S., 2024. From microbes to mind: germ-free models in neuropsychiatric research. mBio 15, e02075-24. https://doi.org/10.1128/mbio.02075-24

Desai, P., Janova, H., White, J.P., Reynoso, G.V., Hickman, H.D., Baldridge, M.T., Urban, J.F., Stappenbeck, T.S., Thackray, L.B., Diamond, M.S., 2021. Enteric helminth coinfection enhances host susceptibility to neurotropic flaviviruses via a tuft cell-IL-4 receptor signaling axis. Cell 184, 1214-1231.e16. https://doi.org/10.1016/j.cell.2021.01.051

DeSesso, J.M., Jacobson, C.F., Williams, A.L., 2012. Anatomical and Physiological Parameters that Influence Gastrointestinal Absorption, in: Encyclopedia of Drug Metabolism and Interactions. John Wiley & Sons, Ltd, pp. 1–35. https://doi.org/10.1002/9780470921920.edm022

Deshmane, S.L., Kremlev, S., Amini, S., Sawaya, B.E., 2009. Monocyte Chemoattractant Protein-1 (MCP-1): An Overview. J Interferon Cytokine Res 29, 313–326. https://doi.org/10.1089/jir.2008.0027

Di Tommaso, N., Gasbarrini, A., Ponziani, F.R., 2021. Intestinal Barrier in Human Health and Disease. Int J Environ Res Public Health 18, 12836. https://doi.org/10.3390/ijerph182312836

Díaz-Godínez, C., Carrero, J.C., 2019. The state of art of neutrophil extracellular traps in protozoan and helminthic infections. Biosci Rep 39, BSR20180916. https://doi.org/10.1042/BSR20180916

Dong, L., Xing, L., 2024. Editorial: The biological mechanism and health effect of co-infection with multiple pathogens. Front. Cell. Infect. Microbiol. 14. https://doi.org/10.3389/fcimb.2024.1370067

Donohue, R.E., Cross, Z.K., Michael, E., 2019. The extent, nature, and pathogenic consequences of helminth polyparasitism in humans: A meta-analysis. PLOS Neglected Tropical Diseases 13, e0007455. https://doi.org/10.1371/journal.pntd.0007455

Doolan, R., Bouchery, T., 2022. Hookworm infections: Reappraising the evidence for a role of neutrophils in light of NETosis. Parasite Immunology 44, e12911. https://doi.org/10.1111/pim.12911

Doonan, J., Tarafdar, A., Pineda, M.A., Lumb, F.E., Crowe, J., Khan, A.M., Hoskisson, P.A., Harnett, M.M., Harnett, W., 2019. The parasitic worm product ES-62 normalises the gut microbiota bone marrow axis in inflammatory arthritis. Nat Commun 10, 1554. https://doi.org/10.1038/s41467-019-09361-0

Drurey, C., Lindholm, H.T., Coakley, G., Poveda, M.C., Löser, S., Doolan, R., Gerbe, F., Jay, P., Harris, N., Oudhoff, M.J., Maizels, R.M., 2021. Intestinal epithelial tuft cell induction is negated by a murine helminth and its secreted products.

Journal of Experimental Medicine 219, e20211140. https://doi.org/10.1084/jem.20211140

Duarte-Mata, D.I., Salinas-Carmona, M.C., 2023. Antimicrobial peptides´ immune modulation role in intracellular bacterial infection. Front Immunol 14, 1119574. https://doi.org/10.3389/fimmu.2023.1119574

Ekkens, M.J., Shedlock, D.J., Jung, E., Troy, A., Pearce, E.L., Shen, H., Pearce, E.J., 2007. Th1 and Th2 Cells Help CD8 T-Cell Responses. Infect Immun 75, 2291–2296. https://doi.org/10.1128/IAI.01328-06

Eley, B.S., Beatty, D.W., 2009. Chapter 8 - The basic immunology of tuberculosis, in: Schaaf, H.S., Zumla, A.I., Grange, J.M., Raviglione, M.C., Yew, W.W., Starke, J.R., Pai, M., Donald, P.R. (Eds.), Tuberculosis. W.B. Saunders, Edinburgh, pp. 75–86. https://doi.org/10.1016/B978-1-4160-3988-4.00008-1

Elias, D., Akuffo, H., Thors, C., Pawlowski, A., Britton, S., 2005. Low dose chronic Schistosoma mansoni infection increases susceptibility to Mycobacterium bovis BCG infection in mice. Clin Exp Immunol 139, 398–404. https://doi.org/10.1111/j.1365-2249.2004.02719.x

Elizalde-Velázquez, L.E., Yordanova, I.A., Liublin, W., Adjah, J., Leben, R., Rausch, S., Niesner, R., Hartmann, S., 2023. Th2 and metabolic responses to nematodes are independent of prolonged host microbiota abrogation. Parasite Immunology 45, e12957. https://doi.org/10.1111/pim.12957

Elliott, D.E., Metwali, A., Leung, J., Setiawan, T., Blum, A.M., Ince, M.N., Bazzone, L.E., Stadecker, M.J., Urban, J.F., Weinstock, J.V., 2008. COLONIZATION WITH HELIGMOSOMOIDES POLYGYRUS SUPPRESSES MUCOSAL IL17 PRODUCTION. J Immunol 181, 2414–2419.

Elliott, D.E., Setiawan, T., Metwali, A., Blum, A., Urban, J.F., Weinstock, J.V., 2004. Heligmosomoides polygyrus inhibits established colitis in IL-10-deficient mice. Eur J Immunol 34, 2690–2698. https://doi.org/10.1002/eji.200324833

Else, K.J., Finkelman, F.D., Maliszewski, C.R., Grencis, R.K., 1994. Cytokine-mediated regulation of chronic intestinal helminth infection. The Journal of experimental medicine 179, 347–351. https://doi.org/10.1084/jem.179.1.347

Eriguchi, Y., Nakamura, K., Yokoi, Y., Sugimoto, R., Takahashi, S., Hashimoto, D., Teshima, T., Ayabe, T., Selsted, M.E., Ouellette, A.J., 2018. Essential role of IFN-γ in T cell-associated intestinal inflammation. JCI Insight 3, e121886, 121886. https://doi.org/10.1172/jci.insight.121886

Esser-von Bieren, J., Mosconi, I., Guiet, R., Piersgilli, A., Volpe, B., Chen, F., Gause, W.C., Seitz, A., Verbeek, J.S., Harris, N.L., 2013. Antibodies Trap Tissue Migrating Helminth Larvae and Prevent Tissue Damage by Driving IL-4Rα-

Independent Alternative Differentiation of Macrophages. PLoS Pathog 9, e1003771. https://doi.org/10.1371/journal.ppat.1003771

Estes, J.D., Harris, L.D., Klatt, N.R., Tabb, B., Pittaluga, S., Paiardini, M., Barclay, G.R., Smedley, J., Pung, R., Oliveira, K.M., Hirsch, V.M., Silvestri, G., Douek, D.C., Miller, C.J., Haase, A.T., Lifson, J., Brenchley, J.M., 2010. Damaged Intestinal Epithelial Integrity Linked to Microbial Translocation in Pathogenic Simian Immunodeficiency Virus Infections. PLoS Pathog 6, e1001052. https://doi.org/10.1371/journal.ppat.1001052

Evaristo, C., Alegre, M.-L., 2013. IFN-γ: The Dr. Jekyll and Mr. Hyde of Immunology? American Journal of Transplantation 13, 3057–3058. https://doi.org/10.1111/ajt.12468

Everts, B., Smits, H.H., Hokke, C.H., Yazdanbakhsh, M., 2010. Helminths and dendritic cells: Sensing and regulating via pattern recognition receptors, Th2 and Treg responses. https://doi.org/10.1002/eji.200940109

Fan, X., Zhou, Y., Bai, W., Li, X., Lin, Liyuan, Lin, H., Yang, M., Yu, X., Wang, J., Lin, Liang, Wang, W., 2025. Intravital imaging of translocated bacteria via fluorogenic labeling of gut microbiota in situ. Proceedings of the National Academy of Sciences 122, e2415845122. https://doi.org/10.1073/pnas.2415845122

Faz-López, B., Morales-Montor, J., Terrazas, L.I., 2016. Role of Macrophages in the Repair Process during the Tissue Migrating and Resident Helminth Infections. BioMed Research International 2016, 1–11. https://doi.org/10.1155/2016/8634603

Fenton, T.M., Jørgensen, P.B., Niss, K., Rubin, S.J.S., Mörbe, U.M., Riis, L.B., da Silva, C., Plumb, A., Vandamme, J., Jakobsen, H.L., Brunak, S., Habtezion, A., Nielsen, O.H., Johansson-Lindbom, B., Agace, W.W., 2020. Immune profiling of human gut-associated lymphoid tissue identifies a role for isolated lymphoid follicles in the priming of region-specific immunity. Immunity 52, 557-570.e6. https://doi.org/10.1016/j.immuni.2020.02.001

Fernandez, M.-I., Heuzé, M.L., Martinez-Cingolani, C., Volpe, E., Donnadieu, M.-H., Piel, M., Homey, B., Lennon-Duménil, A.-M., Soumelis, V., 2011. The human cytokine TSLP triggers a cell-autonomous dendritic cell migration in confined environments. Blood 118, 3862–3869. https://doi.org/10.1182/blood-2010-12-323089

Fitzsimmons, C.M., Falcone, F.H., Dunne, D.W., 2014. Helminth Allergens, Parasite-Specific IgE, and Its Protective Role in Human Immunity. Front Immunol 5, 61. https://doi.org/10.3389/fimmu.2014.00061

Fournier, B.M., Parkos, C.A., 2012. The role of neutrophils during intestinal inflammation. Mucosal Immunol 5, 354–366. https://doi.org/10.1038/mi.2012.24

Fujita, T., Matsushita, M., Endo, Y., 2004. The lectin-complement pathway – its role in innate immunity and evolution. Immunological Reviews 198, 185–202. https://doi.org/10.1111/j.0105-2896.2004.0123.x

Furci, L., Secchi, M., 2018. Chapter 6 - AMPs and Mechanisms of Antimicrobial Action, in: Cho, C.H. (Ed.), Antimicrobial Peptides in Gastrointestinal Diseases. Academic Press, pp. 97–131. https://doi.org/10.1016/B978-0-12-814319-3.00006-4

Gallo, R.L., Hooper, L.V., 2012. Epithelial antimicrobial defence of the skin and intestine. Nat Rev Immunol 12, 503–516. https://doi.org/10.1038/nri3228

Garcia-Bonete, M.J., Rajan, A., Suriano, F., Layunta, E., 2023. The Underrated Gut Microbiota Helminths, Bacteriophages, Fungi, and Archaea. Life (Basel) 13, 1765. https://doi.org/10.3390/life13081765

Garcia-Hernandez, V., Quiros, M., Nusrat, A., 2017. Intestinal epithelial claudins: expression and regulation in homeostasis and inflammation. Ann N Y Acad Sci 1397, 66–79. https://doi.org/10.1111/nyas.13360

Gause, W.C., Maizels, R.M., 2016. Macrobiota — helminths as active participants and partners of the microbiota in host intestinal homeostasis. Current Opinion in Microbiology, Host-microbe interactions: parasites/fungi/viruses 32, 14–18. https://doi.org/10.1016/j.mib.2016.04.004

Ge, X., Ding, C., Zhao, W., Xu, L., Tian, H., Gong, J., Zhu, M., Li, J., Li, N., 2017. Antibiotics-induced depletion of mice microbiota induces changes in host serotonin biosynthesis and intestinal motility. Journal of Translational Medicine 15, 13. https://doi.org/10.1186/s12967-016-1105-4

Gentile, M.E., Li, Y., Robertson, A., Shah, K., Fontes, G., Kaufmann, E., Polese, B., Khan, N., Parisien, M., Munter, H.M., Mandl, J.N., Diatchenko, L., Divangahi, M., King, I.L., 2020. NK cell recruitment limits tissue damage during an enteric helminth infection. Mucosal Immunol 13, 357–370. https://doi.org/10.1038/s41385-019-0231-8

Gerbe, F., Sidot, E., Smyth, D.J., Ohmoto, M., Matsumoto, I., Dardalhon, V., Cesses, P., Garnier, L., Bruschi, M., Harcus, Y., Taylor, N., Maizels, R.M., Jay, P., 2016. Intestinal epithelial tuft cells regulate type 2 mucosal responses required for expulsion of helminth parasites. Nature 529, 226–230. https://doi.org/10.1038/nature16527

Gibbons, D.L., Spencer, J., 2011. Mouse and human intestinal immunity: same ballpark, different players; different rules, same score. Mucosal Immunology 4, 148–157. https://doi.org/10.1038/mi.2010.85

Gilcrease, M.Z., 2007. Integrin signaling in epithelial cells. Cancer Letters 247, 1–25. https://doi.org/10.1016/j.canlet.2006.03.031

Gilmour, B.C., Corthay, A., Øynebråten, I., 2024. High production of IL-12 by human dendritic cells stimulated with combinations of pattern-recognition receptor agonists. npj Vaccines 9, 1–12. https://doi.org/10.1038/s41541-024-00869-1

Goldmann, O., Nwofor, O.V., Chen, Q., Medina, E., 2024. Mechanisms underlying immunosuppression by regulatory cells. Front Immunol 15, 1328193. https://doi.org/10.3389/fimmu.2024.1328193

Gomez, J.C., Yamada, M., Martin, J.R., Dang, H., Brickey, W.J., Bergmeier, W., Dinauer, M.C., Doerschuk, C.M., 2015. Mechanisms of Interferon-γ Production by Neutrophils and Its Function during Streptococcus pneumoniae Pneumonia. Am J Respir Cell Mol Biol 52, 349–364. https://doi.org/10.1165/rcmb.2013-0316OC

Grainger, J.R., Smith, K.A., Hewitson, J.P., McSorley, H.J., Harcus, Y., Filbey, K.J., Finney, C.A.M., Greenwood, E.J.D., Knox, D.P., Wilson, M.S., Belkaid, Y., Rudensky, A.Y., Maizels, R.M., 2010. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-β pathway. J Exp Med 207, 2331–2341. https://doi.org/10.1084/jem.20101074

Greer, R.L., Dong, X., Moraes, A.C.F., Zielke, R.A., Fernandes, G.R., Peremyslova, E., Vasquez-Perez, S., Schoenborn, A.A., Gomes, E.P., Pereira, A.C., Ferreira, S.R.G., Yao, M., Fuss, I.J., Strober, W., Sikora, A.E., Taylor, G.A., Gulati, A.S., Morgun, A., Shulzhenko, N., 2016. Akkermansia muciniphila mediates negative effects of IFNγ on glucose metabolism. Nat Commun 7, 13329. https://doi.org/10.1038/ncomms13329

Guan, Y., Watson, A.J.M., Marchiando, A.M., Bradford, E., Shen, L., Turner, J.R., Montrose, M.H., 2011. Redistribution of the tight junction protein ZO-1 during physiological shedding of mouse intestinal epithelial cells. Am J Physiol Cell Physiol 300, C1404–C1414. https://doi.org/10.1152/ajpcell.00270.2010

Gunawardene, A.R., Corfe, B.M., Staton, C.A., 2011. Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. Int J Exp Pathol 92, 219–231. https://doi.org/10.1111/j.1365-2613.2011.00767.x

Gurish, M.F., Bryce, P.J., Tao, H., Kisselgof, A.B., Thornton, E.M., Miller, H.R., Friend, D.S., Oettgen, H.C., 2004. IgE enhances parasite clearance and regulates mast cell responses in mice infected with Trichinella spiralis. J Immunol 172, 1139–1145. https://doi.org/10.4049/jimmunol.172.2.1139

Haber, A.L., Biton, M., Rogel, N., Herbst, R.H., Shekhar, K., Smillie, C., Burgin, G., Delorey, T.M., Howitt, M.R., Katz, Y., Tirosh, I., Beyaz, S., Dionne, D., Zhang, M., Raychowdhury, R., Garrett, W.S., Rozenblatt-Rosen, O., Shi, H.N., Yilmaz, O., Xavier, R.J., Regev, A., 2017. A single-cell survey of the small intestinal epithelium. Nature 551, 333–339. https://doi.org/10.1038/nature24489

Hamza, T., Barnett, J.B., Li, B., 2010. Interleukin 12 a Key Immunoregulatory Cytokine in Infection Applications. Int J Mol Sci 11, 789–806. https://doi.org/10.3390/ijms11030789

Hanada, T., Yoshimura, A., 2002. Regulation of cytokine signaling and inflammation. Cytokine & Growth Factor Reviews, Cytokines in Autoimmune Disease 13, 413–421. https://doi.org/10.1016/S1359-6101(02)00026-6

Hand, T.W., Dos Santos, L.M., Bouladoux, N., Molloy, M.J., Pagán, A.J., Pepper, M., Maynard, C.L., Elson, C.O., Belkaid, Y., 2012. Acute Gastrointestinal Infection Induces Long-Lived Microbiota-Specific T Cell Responses. Science 337, 1553–1556. https://doi.org/10.1126/science.1220961

Hang, L., Setiawan, T., Blum, A.M., Urban, J., Stoyanoff, K., Arihiro, S., Reinecker, H.-C., Weinstock, J.V., 2010. Heligmosomoides polygyrus infection can inhibit colitis through direct interaction with innate immunity. J Immunol 185, 3184–3189. https://doi.org/10.4049/jimmunol.1000941

Happel, K.I., Dubin, P.J., Zheng, M., Ghilardi, N., Lockhart, C., Quinton, L.J., Odden, A.R., Shellito, J.E., Bagby, G.J., Nelson, S., Kolls, J.K., 2005. Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. J Exp Med 202, 761–769. https://doi.org/10.1084/jem.20050193

Harnett, W., Harnett, M.M., 2009. Immunomodulatory Activity and Therapeutic Potential of the Filarial Nematode Secreted Product, ES-62, in: Fallon, P.G. (Ed.), Pathogen-Derived Immunomodulatory Molecules. Springer, New York, NY, pp. 88–94. https://doi.org/10.1007/978-1-4419-1601-3_7

Harris, J., Hope, Jayne.C., Keane, J., 2008. Tumor Necrosis Factor Blockers Influence Macrophage Responses to Mycobacterium tuberculosis. J INFECT DIS 198, 1842–1850. https://doi.org/10.1086/593174

Harris, N., Gause, W.C., 2011. B cell function in the immune response to helminths. Trends Immunol 32, 80–88. https://doi.org/10.1016/j.it.2010.11.005

Hase, K., Kawano, K., Nochi, T., Pontes, G.S., Fukuda, S., Ebisawa, M., Kadokura, K., Tobe, T., Fujimura, Y., Kawano, S., Yabashi, A., Waguri, S., Nakato, G., Kimura, S., Murakami, T., Iimura, M., Hamura, K., Fukuoka, S.-I., Lowe, A.W., Itoh, K., Kiyono, H., Ohno, H., 2009. Uptake through glycoprotein 2 of FimH+ bacteria by M cells initiates mucosal immune response. Nature 462, 226–230. https://doi.org/10.1038/nature08529

Hassan, M., Flanagan, T.W., Kharouf, N., Bertsch, C., Mancino, D., Haikel, Y., 2022. Antimicrobial Proteins: Structure, Molecular Action, and Therapeutic Potential. Pharmaceutics 15, 72. https://doi.org/10.3390/pharmaceutics15010072

Hedley, L., Wani, R.L.S., 2015. Helminth infections: diagnosis and treatment [WWW Document]. The Pharmaceutical Journal. URL https://pharmaceutical-

journal.com/article/ld/helminth-infections-diagnosis-and-treatment (accessed 4.4.25).

Helmby, H., 2015. Human helminth therapy to treat inflammatory disorders- where do we stand? BMC Immunol 16, 12. https://doi.org/10.1186/s12865-015-0074-3

Hepworth, M.R., Daniłowicz-Luebert, E., Rausch, S., Metz, M., Klotz, C., Maurer, M., Hartmann, S., 2012a. Mast cells orchestrate type 2 immunity to helminths through regulation of tissue-derived cytokines. Proceedings of the National Academy of Sciences 109, 6644–6649. https://doi.org/10.1073/pnas.1112268109

Hepworth, M.R., Maurer, M., Hartmann, S., 2012b. Regulation of type 2 immunity to helminths by mast cells. Gut Microbes 3, 476–481. https://doi.org/10.4161/gmic.21507

Hepworth, M.R., Monticelli, L.A., Fung, T.C., Ziegler, C.G.K., Grunberg, S., Sinha, R., Mantegazza, A.R., Ma, H.-L., Crawford, A., Angelosanto, J.M., Wherry, E.J., Koni, P.A., Bushman, F.D., Elson, C.O., Eberl, G., Artis, D., Sonnenberg, G.F., 2013. Innate lymphoid cells regulate CD4+ T cell responses to intestinal commensal bacteria. Nature 498, 113–117. https://doi.org/10.1038/nature12240

Hewitson, J.P., Filbey, K.J., Esser-von Bieren, J., Camberis, M., Schwartz, C., Murray, J., Reynolds, L.A., Blair, N., Robertson, E., Harcus, Y., Boon, L., Huang, S.C.-C., Yang, L., Tu, Y., Miller, M.J., Voehringer, D., Le Gros, G., Harris, N., Maizels, R.M., 2015. Concerted Activity of IgG1 Antibodies and IL-4/IL-25-Dependent Effector Cells Trap Helminth Larvae in the Tissues following Vaccination with Defined Secreted Antigens, Providing Sterile Immunity to Challenge Infection. PLoS Pathog 11, e1004676. https://doi.org/10.1371/journal.ppat.1004676

Hodge-Dufour, J., Marino, M.W., Horton, M.R., Jungbluth, A., Burdick, M.D., Strieter, R.M., Noble, P.W., Hunter, C.A., Puré, E., 1998. Inhibition of interferon γ induced interleukin 12 production: A potential mechanism for the anti-inflammatory activities of tumor necrosis factor. Proceedings of the National Academy of Sciences 95, 13806–13811. https://doi.org/10.1073/pnas.95.23.13806

Hoeksema, M.A., Scicluna, B.P., Boshuizen, M.C.S., van der Velden, S., Neele, A.E., Van den Bossche, J., Matlung, H.L., van den Berg, T.K., Goossens, P., de Winther, M.P.J., 2015. IFN-γ priming of macrophages represses a part of the inflammatory program and attenuates neutrophil recruitment. J Immunol 194, 3909–3916. https://doi.org/10.4049/jimmunol.1402077

Horowitz, A., Stegmann, K.A., Riley, E.M., 2012. Activation of Natural Killer Cells during Microbial Infections. Front Immunol 2, 88. https://doi.org/10.3389/fimmu.2011.00088

Horsnell, W.G.C., Dewals, B.G., 2016. RELMs in the Realm of Helminths. Trends in Parasitology 32, 512–514. https://doi.org/10.1016/j.pt.2016.04.011

Horsnell, W.G.C., Oudhoff, M.J., 2022. Helminths are positively AMPing up gut de-bugging. Cell Host & Microbe 30, 1–2. https://doi.org/10.1016/j.chom.2021.12.016

Hotez, P.J., Brindley, P.J., Bethony, J.M., King, C.H., Pearce, E.J., Jacobson, J., 2008. Helminth infections: the great neglected tropical diseases. J Clin Invest 118, 1311–1321. https://doi.org/10.1172/JCI34261

Hotez, P.J., Brooker, S., Bethony, J.M., Bottazzi, M.E., Loukas, A., Xiao, S., 2004. Hookworm Infection. New England Journal of Medicine 351, 799–807. https://doi.org/10.1056/NEJMra032492

Hou, K., Wu, Z.-X., Chen, X.-Y., Wang, J.-Q., Zhang, D., Xiao, C., Zhu, D., Koya, J.B., Wei, L., Li, J., Chen, Z.-S., 2022. Microbiota in health and diseases. Sig Transduct Target Ther 7, 1–28. https://doi.org/10.1038/s41392-022-00974-4

Howitt, M.R., Lavoie, S., Michaud, M., Blum, A.M., Tran, S.V., Weinstock, J.V., Gallini, C.A., Redding, K., Margolskee, R.F., Osborne, L.C., Artis, D., Garrett, W.S., 2016. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. Science 351, 1329–1333. https://doi.org/10.1126/science.aaf1648

Hsieh, G.C.-F., Loukas, A., Wahl, A.M., Bhatia, M., Wang, Y., Williamson, A.L., Kehn, K.W., Maruyama, H., Hotez, P.J., Leitenberg, D., Bethony, J., Constant, S.L., 2004. A secreted protein from the human hookworm necator americanus binds selectively to NK cells and induces IFN-gamma production. J Immunol 173, 2699–2704. https://doi.org/10.4049/jimmunol.173.4.2699

Hu, Z., Zhang, C., Sifuentes-Dominguez, L., Zarek, C.M., Propheter, D.C., Kuang, Z., Wang, Y., Pendse, M., Ruhn, K.A., Hassell, B., Behrendt, C.L., Zhang, B., Raj, P., Harris-Tryon, T.A., Reese, T.A., Hooper, L.V., 2021. Small proline-rich protein 2A is a gut bactericidal protein deployed during helminth infection. Science 374, eabe6723. https://doi.org/10.1126/science.abe6723

Huang, L., Appleton, J.A., 2016. Eosinophils in Helminth Infection: Defenders and Dupes. Trends in Parasitology 32, 798–807. https://doi.org/10.1016/j.pt.2016.05.004

Hurford, A., Day, T., 2013. Immune evasion and the evolution of molecular mimicry in parasites. Evolution 67, 2889–2904. https://doi.org/10.1111/evo.12171

Iizuka, M., Konno, S., 2011. Wound healing of intestinal epithelial cells. World Journal of Gastroenterology : WJG 17, 2161. https://doi.org/10.3748/wjg.v17.i17.2161

Inclan-Rico, J.M., Rossi, H.L., Herbert, D.R., 2022. “Every cell is an immune cell; contributions of non-hematopoietic cells to anti-helminth immunity.” Mucosal Immunol 15, 1199–1211. https://doi.org/10.1038/s41385-022-00518-7

Inclan-Rico, J.M., Siracusa, M.C., 2018. First responders: innate immunity to helminths. Trends Parasitol 34, 861–880. https://doi.org/10.1016/j.pt.2018.08.007

Ing, R., Su, Z., Scott, M.E., Koski, K.G., 2000. Suppressed T helper 2 immunity and prolonged survival of a nematode parasite in protein-malnourished mice. Proc Natl Acad Sci U S A 97, 7078–7083. https://doi.org/10.1073/pnas.97.13.7078

Inoue, T., Nakayama, J., Moriya, K., Kawaratani, H., Momoda, R., Ito, K., Iio, E., Nojiri, S., Fujiwara, K., Yoneda, M., Yoshiji, H., Tanaka, Y., 2018. Gut Dysbiosis Associated With Hepatitis C Virus Infection. Clin Infect Dis 67, 869–877. https://doi.org/10.1093/cid/ciy205

Ishida, Y., Kondo, T., Takayasu, T., Iwakura, Y., Mukaida, N., 2004. The Essential Involvement of Cross-Talk between IFN-γ and TGF-β in the Skin Wound-Healing Process1. The Journal of Immunology 172, 1848–1855. https://doi.org/10.4049/jimmunol.172.3.1848

Ishigame, H., Kakuta, S., Nagai, T., Kadoki, M., Nambu, A., Komiyama, Y., Fujikado, N., Tanahashi, Y., Akitsu, A., Kotaki, H., Sudo, K., Nakae, S., Sasakawa, C., Iwakura, Y., 2009. Differential Roles of Interleukin-17A and -17F in Host Defense against Mucoepithelial Bacterial Infection and Allergic Responses. Immunity 30, 108–119. https://doi.org/10.1016/j.immuni.2008.11.009

Ito, T., Wang, Y.-H., Duramad, O., Hori, T., Delespesse, G.J., Watanabe, N., Qin, F.X.-F., Yao, Z., Cao, W., Liu, Y.-J., 2005. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. J Exp Med 202, 1213–1223. https://doi.org/10.1084/jem.20051135

Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S.V., Tanoue, T., Imaoka, A., Itoh, K., Takeda, K., Umesaki, Y., Honda, K., Littman, D.R., 2009. Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. Cell 139, 485–498. https://doi.org/10.1016/j.cell.2009.09.033

Ivashkiv, L.B., 2018. IFNγ: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy. Nat Rev Immunol 18, 545–558. https://doi.org/10.1038/s41577-018-0029-z

Jepson, M.A., Collares-Buzato, C.B., Clark, M.A., Hirst, B.H., Simmons, N.L., 1995. Rapid disruption of epithelial barrier function by Salmonella typhimurium is associated with structural modification of intercellular junctions. Infect Immun 63, 356–359. https://doi.org/10.1128/iai.63.1.356-359.1995

Jessop, F., Buntyn, R., Schwarz, B., Wehrly, T., Scott, D., Bosio, C.M., 2020. Interferon Gamma Reprograms Host Mitochondrial Metabolism through Inhibition of Complex II To Control Intracellular Bacterial Replication. Infection and Immunity 88, 10.1128/iai.00744-19. https://doi.org/10.1128/iai.00744-19

Jin, S., Wetzel, D., Schirmer, M., 2022. Deciphering mechanisms and implications of bacterial translocation in human health and disease. Current Opinion in Microbiology 67, 102147. https://doi.org/10.1016/j.mib.2022.102147

Joardar, N., Sinha Babu, S.P., 2020. A review on the druggability of a thiol-based enzymatic antioxidant thioredoxin reductase for treating filariasis and other parasitic infections. International Journal of Biological Macromolecules 142, 125–141. https://doi.org/10.1016/j.ijbiomac.2019.09.083

Johansson, M.E.V., Hansson, G.C., 2016. Immunological aspects of intestinal mucus and mucins. Nat Rev Immunol 16, 639–649. https://doi.org/10.1038/nri.2016.88

Johnston, C.J.C., Robertson, E., Harcus, Y., Grainger, J.R., Coakley, G., Smyth, D.J., McSorley, H.J., Maizels, R., 2015. Cultivation of Heligmosomoides Polygyrus: An Immunomodulatory Nematode Parasite and its Secreted Products. J Vis Exp 52412. https://doi.org/10.3791/52412

Jorgovanovic, D., Song, M., Wang, L., Zhang, Y., 2020. Roles of IFN-γ in tumor progression and regression: a review. Biomarker Research 8, 49. https://doi.org/10.1186/s40364-020-00228-x

Kamal, M., Dehlawi, M.S., Brunet, L.R., Wakelin, D., 2002. Paneth and intermediate cell hyperplasia induced in mice by helminth infections. Parasitology 125, 275–281. https://doi.org/10.1017/s0031182002002068

Kanaya, T., Sakakibara, S., Jinnohara, T., Hachisuka, M., Tachibana, N., Hidano, S., Kobayashi, T., Kimura, S., Iwanaga, T., Nakagawa, T., Katsuno, T., Kato, N., Akiyama, T., Sato, T., Williams, I.R., Ohno, H., 2018. Development of intestinal M cells and follicle-associated epithelium is regulated by TRAF6-mediated NF-κB signaling. J Exp Med 215, 501–519. https://doi.org/10.1084/jem.20160659

Kanno, E., Tanno, H., Masaki, A., Sasaki, A., Sato, N., Goto, M., Shisai, M., Yamaguchi, K., Takagi, N., Shoji, M., Kitai, Y., Sato, K., Kasamatsu, J., Ishii, K., Miyasaka, T., Kawakami, Kaori, Imai, Y., Iwakura, Y., Maruyama, R., Tachi, M., Kawakami, Kazuyoshi, 2019. Defect of Interferon γ Leads to Impaired Wound Healing through Prolonged Neutrophilic Inflammatory Response and Enhanced MMP-2 Activation. Int J Mol Sci 20, 5657. https://doi.org/10.3390/ijms20225657

Kapse, B., Zhang, H., Affinass, N., Ebner, F., Hartmann, S., Rausch, S., 2022. Age-dependent rise in IFN-γ competence undermines effective type 2 responses to nematode infection. Mucosal Immunol 15, 1270–1282. https://doi.org/10.1038/s41385-022-00519-6

Karo-Atar, D., Ouladan, S., Javkar, T., Joumier, L., Matheson, M.K., Merritt, S., Westfall, S., Rochette, A., Gentile, M.E., Fontes, G., Fonseca, G.J., Parisien, M., Diatchenko, L., von Moltke, J., Malleshaiah, M., Gregorieff, A., King, I.L., 2022. Helminth-induced reprogramming of the stem cell compartment inhibits type 2 immunity. J Exp Med 219, e20212311. https://doi.org/10.1084/jem.20212311

Kennedy, E.A., King, K.Y., Baldridge, M.T., 2018. Mouse Microbiota Models: Comparing Germ-Free Mice and Antibiotics Treatment as Tools for Modifying Gut Bacteria. Front Physiol 9, 1534. https://doi.org/10.3389/fphys.2018.01534

Kennedy, M.H.E., Brosschot, T.P., Lawrence, K.M., FitzPatrick, R.D., Lane, J.M., Mariene, G.M., Wasmuth, J.D., Reynolds, L.A., 2021. Small Intestinal Levels of the Branched Short-Chain Fatty Acid Isovalerate Are Elevated during Infection with Heligmosomoides polygyrus and Can Promote Helminth Fecundity. Infection and Immunity 89. https://doi.org/10.1128/iai.00225-21

Khader, S.A., Gaffen, S.L., Kolls, J.K., 2009. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. Mucosal Immunology 2, 403–411. https://doi.org/10.1038/mi.2009.100

Khosravi, A., Mazmanian, S.K., 2013. Disruption of the gut microbiome as a risk factor for microbial infections. Curr Opin Microbiol 16, 221–227. https://doi.org/10.1016/j.mib.2013.03.009

Kim, J.-R., Hong, B.-K., Pham, T.H.N., Kim, W.-U., Kim, H.A., 2024. Interferon-gamma signaling promotes cartilage regeneration after injury. Sci Rep 14, 8046. https://doi.org/10.1038/s41598-024-58779-0

Kinnebrew, M.A., Pamer, E.G., 2012. Innate immune signaling in defense against intestinal microbes. Immunol Rev 245, 113–131. https://doi.org/10.1111/j.1600-065X.2011.01081.x

Klatt, N.R., Funderburg, N.T., Brenchley, J.M., 2013. Microbial translocation, immune activation and HIV disease. Trends Microbiol 21, 6–13. https://doi.org/10.1016/j.tim.2012.09.001

Kong, S., Zhang, Y.H., Zhang, W., 2018. Regulation of Intestinal Epithelial Cells Properties and Functions by Amino Acids. Biomed Res Int 2018, 2819154. https://doi.org/10.1155/2018/2819154

Kopf, M., Coyle, A.J., Schmitz, N., Barner, M., Oxenius, A., Gallimore, A., Gutierrez-Ramos, J.-C., Bachmann, M.F., 2000. Inducible Costimulator Protein (Icos) Controls T Helper Cell Subset Polarization after Virus and Parasite Infection. J Exp Med 192, 53–62. https://doi.org/10.1084/jem.192.1.53

Kretzschmar, K., Clevers, H., 2019. IFN-γ: The T cell's license to kill stem cells in the inflamed intestine. Science Immunology 4, eaaz6821. https://doi.org/10.1126/sciimmunol.aaz6821

Kühl, A.A., Kakirman, H., Janotta, M., Dreher, S., Cremer, P., Pawlowski, N.N., Loddenkemper, C., Heimesaat, M.M., Grollich, K., Zeitz, M., Farkas, S., Hoffmann, J.C., 2007. Aggravation of Different Types of Experimental Colitis by Depletion or Adhesion Blockade of Neutrophils. Gastroenterology 133, 1882–1892. https://doi.org/10.1053/j.gastro.2007.08.073

Kuo, W.-T., Odenwald, M.A., Turner, J.R., Zuo, L., 2022. Tight junction proteins occludin and ZO-1 as regulators of epithelial proliferation and survival. Annals of the New York Academy of Sciences 1514, 21–33. https://doi.org/10.1111/nyas.14798

Lacy, P., 2020. Gr1 makes an unexpected cameo appearance in eosinophils. Journal of Leukocyte Biology 107, 363–365. https://doi.org/10.1002/JLB.2CE1119-473R

Lai, Y., Gallo, R.L., 2009. AMPed Up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol 30, 131–141. https://doi.org/10.1016/j.it.2008.12.003

Lass, S., Hudson, P.J., Thakar, J., Saric, J., Harvill, E., Albert, R., Perkins, S.E., 2013. Generating super-shedders: co-infection increases bacterial load and egg production of a gastrointestinal helminth. Journal of The Royal Society Interface 10, 20120588. https://doi.org/10.1098/rsif.2012.0588

Laukoetter, M., Nava, P., Lee, W., Bruewer, M., Klapproth, J., Babbin, B., Parkos, C., Nusrat, A., 2008. O-014: IFN-Gamma induces apoptosis in inflammation by inhibition of the Wnt-pathway. Inflammatory Bowel Diseases 14, S4–S5. https://doi.org/10.1097/00054725-200801001-00014

Laukoetter, M.G., Nava, P., Lee, W.Y., Severson, E.A., Capaldo, C.T., Babbin, B.A., Williams, I.R., Koval, M., Peatman, E., Campbell, J.A., Dermody, T.S., Nusrat, A., Parkos, C.A., 2007. JAM-A regulates permeability and inflammation in the intestine in vivo. J Exp Med 204, 3067–3076. https://doi.org/10.1084/jem.20071416

Lebrusant-Fernandez, M., ap Rees, T., Jimeno, R., Angelis, N., Ng, J.C., Fraternali, F., Li, V.S.W., Barral, P., 2024. IFN-γ-dependent regulation of intestinal epithelial homeostasis by NKT cells. Cell Reports 43, 114948. https://doi.org/10.1016/j.celrep.2024.114948

Lee, S.C., Tang, M.S., Lim, Y.A.L., Choy, S.H., Kurtz, Z.D., Cox, L.M., Gundra, U.M., Cho, I., Bonneau, R., Blaser, M.J., Chua, K.H., Loke, P., 2014. Helminth Colonization Is Associated with Increased Diversity of the Gut Microbiota. PLoS Negl Trop Dis 8, e2880. https://doi.org/10.1371/journal.pntd.0002880

Lee, S.H., 2015. Intestinal Permeability Regulation by Tight Junction: Implication on Inflammatory Bowel Diseases. Intest Res 13, 11. https://doi.org/10.5217/ir.2015.13.1.11

Leta, G.T., Mekete, K., Wuletaw, Y., Gebretsadik, A., Sime, H., Mekasha, S., Woyessa, A., Shafi, O., Vercruysse, J., Grimes, J.E.T., Gardiner, I., French, M., Levecke, B., Drake, L., Harrison, W., Fenwick, A., 2020. National mapping of soil-transmitted helminth and schistosome infections in Ethiopia. Parasites & Vectors 13, 437. https://doi.org/10.1186/s13071-020-04317-6

Li, D., Wu, M., 2021. Pattern recognition receptors in health and diseases. Sig Transduct Target Ther 6, 1–24. https://doi.org/10.1038/s41392-021-00687-0

Liao, Y., Gao, I.H., Kusakabe, T., Lin, W.-Y., Grier, A., Pan, X., Morzhanaeva, O., Shea, T.P., Yano, H., Karo-Atar, D., Olsen, K.A., Oh, J.H., Vandegrift, K.J., King, I.L., Cuomo, C.A., Artis, D., Rehermann, B., Lipman, N., Iliev, I.D., 2024. Fungal symbiont transmitted by free-living mice promotes type 2 immunity. Nature 636, 697–704. https://doi.org/10.1038/s41586-024-08213-2

Lieberman, L.A., Hunter, C.A., 2002. Regulatory pathways involved in the infection-induced production of IFN-γ by NK cells. Microbes and Infection 4, 1531–1538. https://doi.org/10.1016/S1286-4579(02)00036-9

Liebing, E., Krug, S.M., Neurath, M.F., Siegmund, B., Becker, C., 2025. Wall of Resilience: How the Intestinal Epithelium Prevents Inflammatory Onslaught in the Gut. Cellular and Molecular Gastroenterology and Hepatology 19, 101423. https://doi.org/10.1016/j.jcmgh.2024.101423

Limkar, A.R., Mai, E., Sek, A.C., Percopo, C.M., Rosenberg, H.F., 2020. Frontline Science: Cytokine-mediated developmental phenotype of mouse eosinophils: IL-5-associated expression of the Ly6G/Gr1 surface Ag. Journal of Leukocyte Biology 107, 367–377. https://doi.org/10.1002/JLB.1HI1019-116RR

Lin, J., Mohrs, K., Szaba, F., Kummer, L., Leadbetter, E., Mohrs, M., 2019. Virtual memory CD8 T cells expanded by helminth infection confer broad protection against bacterial infection. Mucosal Immunol 12, 258–264. https://doi.org/10.1038/s41385-018-0100-x

Lin, L., Ibrahim, A.S., Xu, X., Farber, J.M., Avanesian, V., Baquir, B., Fu, Y., French, S.W., Jr, J.E.E., Spellberg, B., 2009. Th1-Th17 Cells Mediate Protective Adaptive Immunity against Staphylococcus aureus and Candida albicans Infection in Mice. PLOS Pathogens 5, e1000703. https://doi.org/10.1371/journal.ppat.1000703

Lin, Q., Rong, L., Jia, X., Li, R., Yu, B., Hu, J., Luo, X., Badea, S.R., Xu, C., Fu, Guofeng, Lai, K., Lee, M., Zhang, B., Gong, H., Zhou, N., Chen, X.L., Lin, S., Fu, Guo, Huang, J.-D., 2021. IFN-γ-dependent NK cell activation is essential to metastasis suppression by engineered Salmonella. Nat Commun 12, 2537. https://doi.org/10.1038/s41467-021-22755-3

Lindholm, H.T., Parmar, N., Drurey, C., Campillo Poveda, M., Vornewald, P.M., Ostrop, J., Díez-Sanchez, A., Maizels, R.M., Oudhoff, M.J., 2022. BMP signaling in the intestinal epithelium drives a critical feedback loop to restrain IL-13-

driven tuft cell hyperplasia. Sci Immunol 7, eabl6543. https://doi.org/10.1126/sciimmunol.abl6543

Liu, S.-K., Cypess, R.H., Van Zandt, P., 1974. Gastritis Caused by Multiple Nematospiroides dubius Infections. The Journal of Parasitology 60, 790–793. https://doi.org/10.2307/3278902

Livak, K.J., Schmittgen, T.D., 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262

Long, S.R., Lanter, B.B., Pazos, M.A., Mou, H., Barrios, J., Su, C.-W., Wang, Z.Q., Walker, W.A., Hurley, B.P., Shi, H.N., 2019. Intestinal helminth infection enhances bacteria-induced recruitment of neutrophils to the airspace. Sci Rep 9, 15703. https://doi.org/10.1038/s41598-019-51991-3

Lorenz, R.G., Newberry, R.D., 2004. Isolated lymphoid follicles can function as sites for induction of mucosal immune responses. Ann N Y Acad Sci 1029, 44–57. https://doi.org/10.1196/annals.1309.006

Löscher, T., Saathoff, E., 2008. Eosinophilia during intestinal infection. Best Practice & Research Clinical Gastroenterology, Eosinophils in Healthy Gut and Gastrointestinal Diseases 22, 511–536. https://doi.org/10.1016/j.bpg.2007.12.004

Loukas, A., Prociv, P., 2001. Immune Responses in Hookworm Infections. Clin Microbiol Rev 14, 689–703. https://doi.org/10.1128/CMR.14.4.689-703.2001

Luo, X.-C., Chen, Z.-H., Xue, J.-B., Zhao, D.-X., Lu, C., Li, Y.-H., Li, S.-M., Du, Y.-W., Liu, Q., Wang, P., Liu, M., Huang, L., 2019. Infection by the parasitic helminth Trichinella spiralis activates a Tas2r-mediated signaling pathway in intestinal tuft cells. Proc Natl Acad Sci U S A 116, 5564–5569. https://doi.org/10.1073/pnas.1812901116

Maciel-Fiuza, M.F., Muller, G.C., Campos, D.M.S., do Socorro Silva Costa, P., Peruzzo, J., Bonamigo, R.R., Veit, T., Vianna, F.S.L., 2023. Role of gut microbiota in infectious and inflammatory diseases. Front Microbiol 14, 1098386. https://doi.org/10.3389/fmicb.2023.1098386

Macierzanka, A., Mackie, A.R., Krupa, L., 2019. Permeability of the small intestinal mucus for physiologically relevant studies: Impact of mucus location and ex vivo treatment. Sci Rep 9, 17516. https://doi.org/10.1038/s41598-019-53933-5

Macpherson, A.J., Smith, K., 2006. Mesenteric lymph nodes at the center of immune anatomy. J Exp Med 203, 497–500. https://doi.org/10.1084/jem.20060227

Madara, J.L., 1990. Maintenance of the macromolecular barrier at cell extrusion sites in intestinal epithelium: Physiological rearrangement of tight junctions. J. Membrain Biol. 116, 177–184. https://doi.org/10.1007/BF01868675

Mah, A.T., Yan, K.S., Kuo, C.J., 2016. Wnt pathway regulation of intestinal stem cells. J Physiol 594, 4837–4847. https://doi.org/10.1113/JP271754

Mair, I., Wolfenden, A., Lowe, A.E., Bennett, A., Muir, A., Smith, H., Fenn, J., Bradley, J.E., Else, K.J., 2021. A lesson from the wild: The natural state of eosinophils is Ly6Ghi. Immunology 164, 766–776. https://doi.org/10.1111/imm.13413

Maizels, R.M., Gause, W.C., 2023. Targeting helminths: The expanding world of type 2 immune effector mechanisms. J Exp Med 220, e20221381. https://doi.org/10.1084/jem.20221381

Maizels, R.M., Newfeld, S.J., 2023. Convergent Evolution in a Murine Intestinal Parasite Rapidly Created the TGM Family of Molecular Mimics to Suppress the Host Immune Response. Genome Biol Evol 15, evad158. https://doi.org/10.1093/gbe/evad158

Maizels, R.M., Hewitson, J.P., Murray, J., Harcus, Y.M., Dayer, B., Filbey, K.J., Grainger, J.R., McSorley, H.J., Reynolds, L.A., Smith, K.A., 2012. Immune Modulation and Modulators in Heligmosomoides polygyrus infection. Exp Parasitol 132, 76–89. https://doi.org/10.1016/j.exppara.2011.08.011

Maizels, R.M., Smits, H.H., McSorley, H.J., 2018. Modulation of Host Immunity by Helminths: The Expanding Repertoire of Parasite Effector Molecules. Immunity 49, 801–818. https://doi.org/10.1016/j.immuni.2018.10.016

Marchi, L.F., Sesti-Costa, R., Ignacchiti, M.D.C., Chedraoui-Silva, S., Mantovani, B., 2014. In vitro activation of mouse neutrophils by recombinant human interferon-gamma: Increased phagocytosis and release of reactive oxygen species and pro-inflammatory cytokines. International Immunopharmacology 18, 228–235. https://doi.org/10.1016/j.intimp.2013.12.010

Marchiondo, A.A., Cruthers, L.R., Fourie, J.J. (Eds.), 2019. Chapter 2 - Nematoda, in: Parasiticide Screening, Volume 2. Academic Press, pp. 135–335. https://doi.org/10.1016/B978-0-12-816577-5.00007-7

Markey, K.A., Hill, G.R., 2017b. Chapter 13 - Cytokines in Hematopoietic Stem Cell Transplantation, in: Foti, M., Locati, M. (Eds.), Cytokine Effector Functions in Tissues. Academic Press, pp. 219–236. https://doi.org/10.1016/B978-0-12-804214-4.00012-9

Marsters, S.A., Pennica, D., Bach, E., Schreiber, R.D., Ashkenazi, A., 1995. Interferon gamma signals via a high-affinity multisubunit receptor complex that contains two types of polypeptide chain. Proc Natl Acad Sci U S A 92, 5401–5405.

Martinez-Guryn, K., Leone, V., Chang, E.B., 2019a. Regional Diversity of the Gastrointestinal Microbiome. Cell Host & Microbe 26, 314–324. https://doi.org/10.1016/j.chom.2019.08.011

Massacand, J.C., Stettler, R.C., Meier, R., Humphreys, N.E., Grencis, R.K., Marsland, B.J., Harris, N.L., 2009. Helminth products bypass the need for TSLP in Th2 immune responses by directly modulating dendritic cell function. Proc Natl Acad Sci U S A 106, 13968–13973. https://doi.org/10.1073/pnas.0906367106

Matta, B.M., Reichenbach, D.K., Blazar, B.R., Turnquist, H.R., 2017. Alarmins and their receptors as modulators and indicators of alloimmune responses. Am J Transplant 17, 320–327. https://doi.org/10.1111/ajt.13887

McCoy, K.D., Stoel, M., Stettler, R., Merky, P., Fink, K., Senn, B.M., Schaer, C., Massacand, J., Odermatt, B., Oettgen, H.C., Zinkernagel, R.M., Bos, N.A., Hengartner, H., Macpherson, A.J., Harris, N.L., 2008. Polyclonal and Specific Antibodies Mediate Protective Immunity against Enteric Helminth Infection. Cell Host & Microbe 4, 362–373. https://doi.org/10.1016/j.chom.2008.08.014

McFarlane, A.J., McSorley, H.J., Davidson, D.J., Fitch, P.M., Wilson, C., Mackenzie, K.J., Gollwitzer, E.S., Johnston, C.J.C., MacDonald, A.S., Edwards, M.R., Harris, N.L., Marsland, B.J., Maizels, R.M., Schwarze, J., 2017. Enteric helminth-induced type-I interferon signalling protects against pulmonary virus infection through interaction with microbiota. J Allergy Clin Immunol 140, 1068-1078.e6. https://doi.org/10.1016/j.jaci.2017.01.016

McGinty, J.W., Ting, H.-A., Billipp, T.E., Nadjsombati, M.S., Khan, D.M., Barrett, N.A., Liang, H.-E., Matsumoto, I., von Moltke, J., 2020. Tuft cell-derived leukotrienes drive rapid anti-helminth immunity in the small intestine but are dispensable for anti-protist immunity. Immunity 52, 528-541.e7. https://doi.org/10.1016/j.immuni.2020.02.005

McLoughlin, R.M., Witowski, J., Robson, R.L., Wilkinson, T.S., Hurst, S.M., Williams, A.S., Williams, J.D., Rose-John, S., Jones, S.A., Topley, N., 2003. Interplay between IFN-γ and IL-6 signaling governs neutrophil trafficking and apoptosis during acute inflammation. J Clin Invest 112, 598–607. https://doi.org/10.1172/JCI17129

McSORLEY, H.J., Loukas, A., 2010. The immunology of human hookworm infections. Parasite Immunology 32, 549–559. https://doi.org/10.1111/j.1365-3024.2010.01224.x

McSorley, H.J., Maizels, R.M., 2012. Helminth Infections and Host Immune Regulation. Clin Microbiol Rev 25, 585–608. https://doi.org/10.1128/CMR.05040-11

McSorley, H.J., O'Gorman, M.T., Blair, N., Sutherland, T.E., Filbey, K.J., Maizels, R.M., 2012. Suppression of type 2 immunity and allergic airway inflammation by secreted products of the helminth Heligmosomoides polygyrus. Eur J Immunol 42, 2667–2682. https://doi.org/10.1002/eji.201142161

McSorley, H.J., Smyth, D.J., 2021. IL-33: A central cytokine in helminth infections. Seminars in Immunology, Resistance to parasites: lessons for type 2 immunity 53, 101532. https://doi.org/10.1016/j.smim.2021.101532

Mertowska, P., Smolak, K., Mertowski, S., Grywalska, E., 2023. Immunomodulatory Role of Interferons in Viral and Bacterial Infections. Int J Mol Sci 24, 10115. https://doi.org/10.3390/ijms241210115

Michla, M., Wilhelm, C., 2022. Food for thought – ILC metabolism in the context of helminth infections. Mucosal Immunology 15, 1234–1242. https://doi.org/10.1038/s41385-022-00559-y

Monroy, F.G., Enriquez, F.J., 1992. *Heligmosomoides polygyrus*: A model for chronic gastrointestinal helminthiasis. Parasitology Today 8, 49–54. https://doi.org/10.1016/0169-4758(92)90084-F

Montenegro, S.M.L., Miranda, P., Mahanty, S., Abath, F.G.C., Teixeira, K.M., Coutinho, E.M., Brinkman, J., Gonçalves, I., Domingues, L.A.W., Domingues, A.L.C., Sher, A., Wynn, T.A., 1999. Cytokine Production in Acute versus Chronic Human Schistosomiasis Mansoni: The Cross-Regulatory Role of Interferon-γ and Interleukin-10 in the Responses of Peripheral Blood Mononuclear Cells and Splenocytes to Parasite Antigens. The Journal of Infectious Diseases 179, 1502–1514. https://doi.org/10.1086/314748

Moore, K.W., Malefyt, R. de W., Coffman, R.L., O'Garra, A., 2001. Interleukin-10 and the Interleukin-10 Receptor. Annual Review of Immunology 19, 683–765. https://doi.org/10.1146/annurev.immunol.19.1.683

Morgun, A., Dzutsev, A., Dong, X., Greer, R.L., Sexton, D.J., Ravel, J., Schuster, M., Hsiao, W., Matzinger, P., Shulzhenko, N., 2015. Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. Gut 64, 1732–1743. https://doi.org/10.1136/gutjnl-2014-308820

Morimoto, Motoko, Morimoto, Masahiro, Whitmire, J., Xiao, S., Anthony, R.M., Mirakami, H., Star, R.A., Urban, J.F., Jr, Gause, W.C., 2004. Peripheral CD4 T Cells Rapidly Accumulate at the Host:Parasite Interface during an Inflammatory Th2 Memory Response1. The Journal of Immunology 172, 2424–2430. https://doi.org/10.4049/jimmunol.172.4.2424

Moser, L.A., Carter, M., Schultz-Cherry, S., 2007. Astrovirus Increases Epithelial Barrier Permeability Independently of Viral Replication. J Virol 81, 11937–11945. https://doi.org/10.1128/JVI.00942-07

Mosmann, T.R., Coffman, R.L., 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 7, 145–173. https://doi.org/10.1146/annurev.iy.07.040189.001045

Motran, C.C., Silvane, L., Chiapello, L.S., Theumer, M.G., Ambrosio, L.F., Volpini, X., Celias, D.P., Cervi, L., 2018. Helminth Infections: Recognition and Modulation of the Immune Response by Innate Immune Cells. Front Immunol 9, 664. https://doi.org/10.3389/fimmu.2018.00664

Mouries, J., Brescia, P., Silvestri, A., Spadoni, I., Sorribas, M., Wiest, R., Mileti, E., Galbiati, M., Invernizzi, P., Adorini, L., Penna, G., Rescigno, M., 2019. Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development. J Hepatol 71, 1216–1228. https://doi.org/10.1016/j.jhep.2019.08.005

Mowat, A.M., 1989. Antibodies to IFN-gamma prevent immunologically mediated intestinal damage in murine graft-versus-host reaction. Immunology 68, 18–23.

Moyat, M., Lebon, L., Perdijk, O., Wickramasinghe, L.C., Zaiss, M.M., Mosconi, I., Volpe, B., Guenat, N., Shah, K., Coakley, G., Bouchery, T., Harris, N.L., 2022. Microbial regulation of intestinal motility provides resistance against helminth infection. Mucosal Immunol 15, 1283–1295. https://doi.org/10.1038/s41385-022-00498-8

Mühl, H., Bachmann, M., Pfeilschifter, J., 2011. Inducible NO synthase and antibacterial host defence in times of Th17/Th22/T22 immunity. Cellular Microbiology 13, 340–348. https://doi.org/10.1111/j.1462-5822.2010.01559.x

Mules, T.C., Tang, J.S., Vacca, F., Yumnam, B., Schmidt, A., Lavender, B., Maclean, K., Noble, S.-L., Waugh, C., van Ginkel, R., Camberis, M., Le Gros, G., Inns, S., 2024. Modulation of intestinal epithelial permeability by chronic small intestinal helminth infections. Immunol Cell Biol 102, 396–406. https://doi.org/10.1111/imcb.12749

Müller, A.J., Kaiser, P., Dittmar, K.E.J., Weber, T.C., Haueter, S., Endt, K., Songhet, P., Zellweger, C., Kremer, M., Fehling, H.-J., Hardt, W.-D., 2012. Salmonella Gut Invasion Involves TTSS-2-Dependent Epithelial Traversal, Basolateral Exit, and Uptake by Epithelium-Sampling Lamina Propria Phagocytes. Cell Host & Microbe 11, 19–32. https://doi.org/10.1016/j.chom.2011.11.013

Mumy, K.L., McCormick, B.A., 2009. The Role of Neutrophils in the Event of Intestinal Inflammation. Curr Opin Pharmacol 9, 697–701. https://doi.org/10.1016/j.coph.2009.10.004

Muniz, L.R., Knosp, C., Yeretssian, G., 2012. Intestinal antimicrobial peptides during homeostasis, infection, and disease. Front Immunol 3, 310. https://doi.org/10.3389/fimmu.2012.00310

Nacy, C.A., Fortier, A.H., Meltzer, M.S., Buchmeier, N.A., Schreiber, R.D., 1985. Macrophage activation to kill Leishmania major: activation of macrophages for intracellular destruction of amastigotes can be induced by both recombinant interferon-gamma and non-interferon lymphokines. J Immunol 135, 3505–3511.

Nakashige, T.G., Zhang, B., Krebs, C., Nolan, E.M., 2015. Human calprotectin is an iron-sequestering host-defense protein. Nat Chem Biol 11, 765–771. https://doi.org/10.1038/nchembio.1891

Namrata, K., Bai, B.-X., 2021. Role of Tight Junctions and Their Protein Expression in Atopic Dermatitis. International Journal of Dermatology and Venereology 04, 40–44. https://doi.org/10.1097/JD9.0000000000000114

Nandi, B., Behar, S.M., 2011. Regulation of neutrophils by interferon-γ limits lung inflammation during tuberculosis infection. J Exp Med 208, 2251–2262. https://doi.org/10.1084/jem.20110919

Naoshi Hikawa, H.F., Nagoya, M., Nagata, T., Minami, M., 1996. IFN-γ induces expression of MHC class I molecules in adult mouse dorsal root ganglion neurones. NeuroReport 7, 2951.

Nava, P., Koch, S., Laukoetter, M.G., Lee, W.Y., Kolegraff, K., Capaldo, C.T., Beeman, N., Addis, C., Gerner-Smidt, K., Neumaier, I., Skerra, A., Li, L., Parkos, C.A., Nusrat, A., 2010. Interferon-γ regulates intestinal epithelial homeostasis through converging β-catenin signaling pathways. Immunity 32, 392–402. https://doi.org/10.1016/j.immuni.2010.03.001

Nigg, J.C., Castelló-Sanjuán, M., Blanc, H., Frangeul, L., Mongelli, V., Godron, X., Bardin, A.J., Saleh, M.-C., 2024. Viral infection disrupts intestinal homeostasis via Sting-dependent NF-κB signaling in *Drosophila*. Current Biology 34, 2785-2800.e7. https://doi.org/10.1016/j.cub.2024.05.009

Nusse, Y.M., Savage, A.K., Marangoni, P., Rosendahl-Huber, A.K.M., Landman, T.A., de Sauvage, F.J., Locksley, R.M., Klein, O.D., 2018. Parasitic helminthes induce fetal-like reversion in the intestinal stem cell niche. Nature 559, 109–113. https://doi.org/10.1038/s41586-018-0257-1

Nutman, T.B., 2015. Looking beyond the induction of Th2 responses to explain immunomodulation by helminths. Parasite Immunol 37, 304–313. https://doi.org/10.1111/pim.12194

Oami, T., Abtahi, S., Shimazui, T., Chen, C.-W., Sweat, Y.Y., Liang, Z., Burd, E.M., Farris, A.B., Roland, J.T., Tsukita, S., Ford, M.L., Turner, J.R., Coopersmith, C.M., 2024. Claudin-2 upregulation enhances intestinal permeability, immune activation, dysbiosis, and mortality in sepsis. Proc Natl Acad Sci U S A 121, e2217877121. https://doi.org/10.1073/pnas.2217877121

Obata-Ninomiya, K., Ishiwata, K., Tsutsui, H., Nei, Y., Yoshikawa, S., Kawano, Y., Minegishi, Y., Ohta, N., Watanabe, N., Kanuka, H., Karasuyama, H., 2013. The skin is an important bulwark of acquired immunity against intestinal helminths. J Exp Med 210, 2583–2595. https://doi.org/10.1084/jem.20130761

Ohshima, Y., Yang, L.-P., Uchiyama, T., Tanaka, Y., Baum, P., Sergerie, M., Hermann, P., Delespesse, G., 1998. OX40 Costimulation Enhances Interleukin-4 (IL-4) Expression at Priming and Promotes the Differentiation of Naive Human CD4+ T Cells Into High IL-4–Producing Effectors. Blood 92, 3338–3345. https://doi.org/10.1182/blood.V92.9.3338

Okda, M., Spina, S., Safaee Fakhr, B., Carroll, R.W., 2025. The antimicrobial effects of nitric oxide: A narrative review. Nitric Oxide 155, 20–40. https://doi.org/10.1016/j.niox.2025.01.001

Omrani, O., Krepelova, A., Rasa, S.M.M., Sirvinskas, D., Lu, J., Annunziata, F., Garside, G., Bajwa, S., Reinhardt, S., Adam, L., Käppel, S., Ducano, N., Donna, D., Ori, A., Oliviero, S., Rudolph, K.L., Neri, F., 2023. IFNγ-Stat1 axis drives aging-associated loss of intestinal tissue homeostasis and regeneration. Nat Commun 14, 6109. https://doi.org/10.1038/s41467-023-41683-y

Osaki, L.H., Bockerstett, K.A., Wong, C.F., Ford, E.L., Madison, B.B., DiPaolo, R.J., Mills, J.C., 2019. Interferon-γ directly induces gastric epithelial cell death and is required for progression to metaplasia. J Pathol 247, 513–523. https://doi.org/10.1002/path.5214

Osborne, L.C., Monticelli, L.A., Nice, T.J., Sutherland, T.E., Siracusa, M.C., Hepworth, M.R., Tomov, V.T., Kobuley, D., Tran, S.V., Bittinger, K., Bailey, A.G., Laughlin, A.L., Boucher, J.-L., Wherry, E.J., Bushman, F.D., Allen, J.E., Virgin, H.W., Artis, D., 2014. Virus-helminth co-infection reveals a microbiota-independent mechanism of immuno-modulation. Science 345, 578–582. https://doi.org/10.1126/science.1256942

Ost, K.S., Round, J.L., 2017. A Few Good Commensals: Gut Microbes Use IFN-γ to Fight *Salmonella*. Immunity 46, 977–979. https://doi.org/10.1016/j.immuni.2017.06.010

Ouyang, W., Kolls, J.K., Zheng, Y., 2008. The Biological Functions of T Helper 17 Cell Effector Cytokines in Inflammation. Immunity 28, 454–467. https://doi.org/10.1016/j.immuni.2008.03.004

Oyesola, O.O., Früh, S.P., Webb, L.M., Tait Wojno, E.D., 2020. Cytokines and beyond: Regulation of innate immune responses during helminth infection. Cytokine 133, 154527. https://doi.org/10.1016/j.cyto.2018.08.021

Panneerselvam, D., Vaqar, S., 2025. Peyer Patches, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Paradis, T., Bègue, H., Basmaciyan, L., Dalle, F., Bon, F., 2021. Tight Junctions as a Key for Pathogens Invasion in Intestinal Epithelial Cells. Int J Mol Sci 22, 2506. https://doi.org/10.3390/ijms22052506

Patel, N., Kreider, T., Urban, J.F., Gause, W.C., 2009. Characterisation of effector mechanisms at the host:parasite interface during the immune response to tissue-dwelling intestinal nematode parasites. International Journal for Parasitology 39, 13–21. https://doi.org/10.1016/j.ijpara.2008.08.003

Paul, W.E., Zhu, J., 2010. How are TH2-type immune responses initiated and amplified? Nat Rev Immunol 10, 225–235. https://doi.org/10.1038/nri2735

Paveley, R.A., Aynsley, S.A., Turner, J.D., Bourke, C.D., Jenkins, S.J., Cook, P.C., Martinez-Pomares, L., Mountford, A.P., 2011. The Mannose Receptor (CD206) is an important pattern recognition receptor (PRR) in the detection of the infective stage of the helminth Schistosoma mansoni and modulates IFNγ production. Int J Parasitol 41, 1335–1345. https://doi.org/10.1016/j.ijpara.2011.08.005

Peck, A., Mellins, E.D., 2010. Precarious Balance: Th17 Cells in Host Defense. Infection and Immunity 78, 32–38. https://doi.org/10.1128/iai.00929-09

Peiseler, M., Kubes, P., 2019. More friend than foe: the emerging role of neutrophils in tissue repair. J Clin Invest 129, 2629–2639. https://doi.org/10.1172/JCI124616

Pelly, V.S., Kannan, Y., Coomes, S.M., Entwistle, L.J., Rückerl, D., Seddon, B., MacDonald, A.S., McKenzie, A., Wilson, M.S., 2016. IL-4-producing ILC2s are required for the differentiation of TH2 cells following *Heligmosomoides polygyrus* infection. Mucosal Immunology 9, 1407–1417. https://doi.org/10.1038/mi.2016.4

Peng, L., Li, Z.-R., Green, R.S., Holzmanr, I.R., Lin, J., 2009. Butyrate Enhances the Intestinal Barrier by Facilitating Tight Junction Assembly via Activation of AMP-Activated Protein Kinase in Caco-2 Cell Monolayers12. The Journal of Nutrition 139, 1619–1625. https://doi.org/10.3945/jn.109.104638

Perona-Wright, G., McSorley, H.J., 2022. Lessons from helminths: what worms have taught us about mucosal immunology. Mucosal Immunology 15, 1049–1051. https://doi.org/10.1038/s41385-022-00560-5

Perussia, B., 1991. Lymphokine-activated killer cells, natural killer cells and cytokines. Curr Opin Immunol 3, 49–55. https://doi.org/10.1016/0952-7915(91)90076-d

Peterson, J.W., 1996. Bacterial Pathogenesis, in: Baron, S. (Ed.), Medical Microbiology. University of Texas Medical Branch at Galveston, Galveston (TX).

Pham, T.A.N., Lawley, T.D., 2014. Emerging insights on intestinal dysbiosis during bacterial infections. Current Opinion in Microbiology, Host–microbe interactions: bacteria 17, 67–74. https://doi.org/10.1016/j.mib.2013.12.002

Phythian-Adams, A.T., Cook, P.C., Lundie, R.J., Jones, L.H., Smith, K.A., Barr, T.A., Hochweller, K., Anderton, S.M., Hämmerling, G.J., Maizels, R.M., MacDonald,

A.S., 2010. CD11c depletion severely disrupts Th2 induction and development in vivo. J Exp Med 207, 2089–2096. https://doi.org/10.1084/jem.20100734

Pinto, D., Gregorieff, A., Begthel, H., Clevers, H., 2003. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes Dev 17, 1709–1713. https://doi.org/10.1101/gad.267103

Platanias, L.C., 2005. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol 5, 375–386. https://doi.org/10.1038/nri1604

Pollo, S.M.J., Leon-Coria, A., Liu, H., Cruces-Gonzalez, D., Finney, C.A.M., Wasmuth, J.D., 2023. Transcriptional patterns of sexual dimorphism and in host developmental programs in the model parasitic nematode Heligmosomoides bakeri. Parasites & Vectors 16, 171. https://doi.org/10.1186/s13071-023-05785-2

Potian, J.A., Rafi, W., Bhatt, K., McBride, A., Gause, W.C., Salgame, P., 2011. Preexisting helminth infection induces inhibition of innate pulmonary anti-tuberculosis defense by engaging the IL-4 receptor pathway. J Exp Med 208, 1863–1874. https://doi.org/10.1084/jem.20091473

Poveda, M.C., Laidlaw, R.F., Hardy, O., Otto, T.D., Maizels, R.M., 2024. Spatial transcriptomics reveals focal induction of molecular responses and cellular interactions in the small intestine during Heligmosomoides polygyrus Infection. https://doi.org/10.1101/2024.02.09.579622

Progatzky, F., Shapiro, M., Chng, S., Garcia-Cassani, B., Classson, C.H., Sevgi, S., Laddach, A., Bon-Frauches, A.C., Lasrado, R., Rahim, M., Amaniti, E.-M., Boeing, S., Shah, K., Entwistle, L.J., Suárez-Bonnet, A., Wilson, M.S., Stockinger, B., Pachnis, V., 2021. Regulation of intestinal immunity and tissue repair by enteric glia. Nature 599, 125–130. https://doi.org/10.1038/s41586-021-04006-z

Puhl, N.J., Uwiera, R.R.E., Jay Yanke, L., Brent Selinger, L., Douglas Inglis, G., 2012. Antibiotics conspicuously affect community profiles and richness, but not the density of bacterial cells associated with mucosa in the large and small intestines of mice. Anaerobe 18, 67–75. https://doi.org/10.1016/j.anaerobe.2011.12.007

Pullan, R.L., Smith, J.L., Jasrasaria, R., Brooker, S.J., 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasites Vectors 7, 1–19. https://doi.org/10.1186/1756-3305-7-37

Rahman, M.T., Ghosh, C., Hossain, M., Linfield, D., Rezaee, F., Janigro, D., Marchi, N., van Boxel-Dezaire, A.H.H., 2018. IFN-γ, IL-17A, or zonulin rapidly increase the permeability of the blood-brain and small intestinal epithelial barriers: Relevance for neuro-inflammatory diseases. Biochemical and Biophysical

Research Communications 507, 274–279. https://doi.org/10.1016/j.bbrc.2018.11.021

Ramalho-Pinto, F.J., McLaren, D.J., Smithers, S.R., 1978. Complement-mediated killing of schistosomula of Schistosoma mansoni by rat eosinophils in vitro. Journal of Experimental Medicine 147, 147–156. https://doi.org/10.1084/jem.147.1.147

Rao, J.N., Wang, J.-Y., 2010. Intestinal Stem Cells, in: Regulation of Gastrointestinal Mucosal Growth. Morgan & Claypool Life Sciences.

Rapin, A., Chuat, A., Lebon, L., Zaiss, M.M., Marsland, B.J., Harris, N.L., 2020. Infection with a small intestinal helminth, *Heligmosomoides polygyrus bakeri*, consistently alters microbial communities throughout the murine small and large intestine. International Journal for Parasitology 50, 35–46. https://doi.org/10.1016/j.ijpara.2019.09.005

Rausch, S., Held, J., Fischer, A., Heimesaat, M.M., Kühl, A.A., Bereswill, S., Hartmann, S., 2013. Small Intestinal Nematode Infection of Mice Is Associated with Increased Enterobacterial Loads alongside the Intestinal Tract. PLoS One 8, e74026. https://doi.org/10.1371/journal.pone.0074026

Rausch, S., Midha, A., Kuhring, M., Affinass, N., Radonic, A., Kühl, A.A., Bleich, A., Renard, B.Y., Hartmann, S., 2018. Parasitic Nematodes Exert Antimicrobial Activity and Benefit From Microbiota-Driven Support for Host Immune Regulation. Front Immunol 9, 2282. https://doi.org/10.3389/fimmu.2018.02282

Reese, T.A., Wakeman, B.S., Choi, H.S., Hufford, M.M., Huang, S.C., Zhang, X., Buck, M.D., Jezewski, A., Kambal, A., Liu, C.Y., Goel, G., Murray, P.J., Xavier, R.J., Kaplan, M.H., Renne, R., Speck, S.H., Artyomov, M.N., Pearce, E.J., Virgin, H.W., 2014. Helminth Infection Reactivates Latent γ-herpesvirus Via Cytokine Competition at a Viral Promoter. Science 345, 573–577. https://doi.org/10.1126/science.1254517

Reynolds, L.A., Filbey, K.J., Maizels, R.M., 2012. Immunity to the model intestinal helminth parasite Heligmosomoides polygyrus. Semin Immunopathol 34, 829–846. https://doi.org/10.1007/s00281-012-0347-3

Reynolds, L.A., Maizels, R.M., 2012. In the absence of TGF-β signaling in T cells, fewer CD103+ regulatory T cells develop, but exuberant IFN-γ production renders mice more susceptible to helminth infection. J Immunol 189, 1113–1117. https://doi.org/10.4049/jimmunol.1200991

Reynolds, L.A., Redpath, S.A., Yurist-Doutsch, S., Gill, N., Brown, E.M., van der Heijden, J., Brosschot, T.P., Han, J., Marshall, N.C., Woodward, S.E., Valdez, Y., Borchers, C.H., Perona-Wright, G., Finlay, B.B., 2017. Enteric Helminths Promote Salmonella Coinfection by Altering the Intestinal Metabolome. J Infect Dis 215, 1245–1254. https://doi.org/10.1093/infdis/jix141

Reynolds, L.A., Smith, K.A., Filbey, K.J., Harcus, Y., Hewitson, J.P., Redpath, S.A., Valdez, Y., Yebra, M.J., Finlay, B.B., Maizels, R.M., 2014. Commensal-pathogen interactions in the intestinal tract. Gut Microbes 5, 522–532. https://doi.org/10.4161/gmic.32155

Rook, G.A.W., 2023. The old friends hypothesis: evolution, immunoregulation and essential microbial inputs. Front Allergy 4, 1220481. https://doi.org/10.3389/falgy.2023.1220481

Rouaud, F., Sluysmans, S., Flinois, A., Shah, J., Vasileva, E., Citi, S., 2020. Scaffolding proteins of vertebrate apical junctions: structure, functions and biophysics. Biochimica et Biophysica Acta (BBA) - Biomembranes 1862, 183399. https://doi.org/10.1016/j.bbamem.2020.183399

Ruiz Silva, M., van der Ende-Metselaar, H., Mulder, H.L., Smit, J.M., Rodenhuis-Zybert, I.A., 2016. Mechanism and role of MCP-1 upregulation upon chikungunya virus infection in human peripheral blood mononuclear cells. Sci Rep 6, 32288. https://doi.org/10.1038/srep32288

Russell, G.A., Peng, G., Faubert, C., Verdu, E.F., Hapfelmeier, S., King, I.L., 2021. A protocol for generating germ-free Heligmosomoides polygyrus bakeri larvae for gnotobiotic helminth infection studies. STAR Protoc 2, 100946. https://doi.org/10.1016/j.xpro.2021.100946

Sad, S., Marcotte, R., Mosmann, T.R., 1995. Cytokine-induced differentiation of precursor mouse CD8+ T cells into cytotoxic CD8+ T cells secreting Th1 or Th2 cytokines. Immunity 2, 271–279. https://doi.org/10.1016/1074-7613(95)90051-9

Sahputra, R., Ruckerl, D., Couper, K.N., Muller, W., Else, K.J., 2019. The Essential Role Played by B Cells in Supporting Protective Immunity Against Trichuris muris Infection Is by Controlling the Th1/Th2 Balance in the Mesenteric Lymph Nodes and Depends on Host Genetic Background. Front Immunol 10, 2842. https://doi.org/10.3389/fimmu.2019.02842

Saito, A.C., Higashi, T., Fukazawa, Y., Otani, T., Tauchi, M., Higashi, A.Y., Furuse, M., Chiba, H., 2021. Occludin and tricellulin facilitate formation of anastomosing tight-junction strand network to improve barrier function. Mol Biol Cell 32, 722–738. https://doi.org/10.1091/mbc.E20-07-0464

Salgame, P., Yap, G.S., Gause, W.C., 2013. Effect of helminth-induced immunity on infections with microbial pathogens. Nat Immunol 14, 1118–1126. https://doi.org/10.1038/ni.2736

Salic, A., Mitchison, T.J., 2008. A chemical method for fast and sensitive detection of DNA synthesis *in vivo*. Proc. Natl. Acad. Sci. U.S.A. 105, 2415–2420. https://doi.org/10.1073/pnas.0712168105

Sanman, L.E., Chen, I.W., Bieber, J.M., Steri, V., Trentesaux, C., Hann, B., Klein, O.D., Wu, L.F., Altschuler, S.J., 2021. Transit-amplifying cells coordinate changes in intestinal epithelial cell type composition. Dev Cell 56, 356-365.e9. https://doi.org/10.1016/j.devcel.2020.12.020

Santaolalla, R., Abreu, M.T., 2012. Innate immunity in the small intestine. Curr Opin Gastroenterol 28, 124–129. https://doi.org/10.1097/MOG.0b013e3283506559

Savioli, L., Albonico, M., 2004. Focus: Soil-transmitted helminthiasis. Nat Rev Microbiol 2, 618–619. https://doi.org/10.1038/nrmicro962

Schälter, F., Frech, M., Dürholz, K., Lucas, S., Sarter, K., Lebon, L., Esser-von Bieren, J., Dubey, L.K., Voehringer, D., Schett, G., Harris, N.L., Zaiss, M.M., 2022. Acetate, a metabolic product of Heligmosomoides polygyrus, facilitates intestinal epithelial barrier breakdown in a FFAR2-dependent manner. Int J Parasitol 52, 591–601. https://doi.org/10.1016/j.ijpara.2022.04.004

Schneider, M.R., Dahlhoff, M., Horst, D., Hirschi, B., Trülzsch, K., Müller-Höcker, J., Vogelmann, R., Allgäuer, M., Gerhard, M., Steininger, S., Wolf, E., Kolligs, F.T., 2010. A Key Role for E-cadherin in Intestinal Homeostasis and Paneth Cell Maturation. PLoS One 5, e14325. https://doi.org/10.1371/journal.pone.0014325

Schnoor, M., 2015. E-cadherin Is Important for the Maintenance of Intestinal Epithelial Homeostasis Under Basal and Inflammatory Conditions. Dig Dis Sci 60, 816–818. https://doi.org/10.1007/s10620-015-3622-z

Schnupf, P., Gaboriau-Routhiau, V., Sansonetti, P.J., Cerf-Bensussan, N., 2017. Segmented filamentous bacteria, Th17 inducers and helpers in a hostile world. Current Opinion in Microbiology, Host-microbe interactions: bacteria 35, 100–109. https://doi.org/10.1016/j.mib.2017.03.004

Schorer, M., Kuchroo, V.K., Joller, N., 2019. Role of Co-stimulatory Molecules in T Helper Cell Differentiation, in: Azuma, M., Yagita, H. (Eds.), Co-Signal Molecules in T Cell Activation: Immune Regulation in Health and Disease. Springer, Singapore, pp. 153–177. https://doi.org/10.1007/978-981-32-9717-3_6

Schramm, G., Suwandi, A., Galeev, A., Sharma, S., Braun, J., Claes, A.-K., Braubach, P., Grassl, G.A., 2018. Schistosome Eggs Impair Protective Th1/Th17 Immune Responses Against Salmonella Infection. Front. Immunol. 9. https://doi.org/10.3389/fimmu.2018.02614

Schwab, L., Goroncy, L., Palaniyandi, S., Gautam, S., Triantafyllopoulou, A., Mocsai, A., Reichardt, W., Karlsson, F.J., Radhakrishnan, S.V., Hanke, K., Schmitt-Graeff, A., Freudenberg, M., von Loewenich, F.D., Wolf, P., Leonhardt, F., Baxan, N., Pfeifer, D., Schmah, O., Schönle, A., Martin, S.F., Mertelsmann, R., Duyster, J., Finke, J., Prinz, M., Henneke, P., Häcker, H., Hildebrandt, G.C., Häcker, G., Zeiser, R., 2014. Neutrophil granulocytes recruited upon

translocation of intestinal bacteria enhance graft-versus-host disease via tissue damage. Nat Med 20, 648–654. https://doi.org/10.1038/nm.3517

Scott, N.A., Andrusaite, A., Andersen, P., Lawson, M., Alcon-Giner, C., Leclaire, C., Caim, S., Le Gall, G., Shaw, T., Connolly, J.P.R., Roe, A.J., Wessel, H., Bravo-Blas, A., Thomson, C.A., Kästele, V., Wang, P., Peterson, D.A., Bancroft, A., Li, X., Grencis, R., Mowat, A.M., Hall, L.J., Travis, M.A., Milling, S.W.F., Mann, E.R., 2018. Antibiotics induce sustained dysregulation of intestinal T cell immunity by perturbing macrophage homeostasis. Sci Transl Med 10, eaao4755. https://doi.org/10.1126/scitranslmed.aao4755

Shao, S., Sun, X., Chen, Y., Zhan, B., Zhu, X., 2019. Complement Evasion: An Effective Strategy That Parasites Utilize to Survive in the Host. Front Microbiol 10, 532. https://doi.org/10.3389/fmicb.2019.00532

Shea-Donohue, T., Qin, B., Smith, A., 2017. Parasites, nutrition, immune responses, and biology of metabolic tissues. Parasite Immunol 39, 10.1111/pim.12422. https://doi.org/10.1111/pim.12422

Shiratori, H., Hattori, K.M., Nakata, K., Okawa, T., Komiyama, S., Kinashi, Y., Kabumoto, Y., Kaneko, Y., Nagai, M., Shindo, T., Moritoki, N., Kawamura, Y.I., Dohi, T., Takahashi, D., Kimura, S., Hase, K., 2024. A purified diet affects intestinal epithelial proliferation and barrier functions through gut microbial alterations. International Immunology 36, 223–240. https://doi.org/10.1093/intimm/dxae003

Shtrichman, R., Samuel, C.E., 2001. The role of gamma interferon in antimicrobial immunity. Current Opinion in Microbiology 4, 251–259. https://doi.org/10.1016/S1369-5274(00)00199-5

Shu, L.-Z., Ding, Y.-D., Xue, Q.-M., Cai, W., Deng, H., 2023. Direct and indirect effects of pathogenic bacteria on the integrity of intestinal barrier. Therap Adv Gastroenterol 16, 17562848231176427. https://doi.org/10.1177/17562848231176427

Smith, K.A., Harcus, Y., Garbi, N., Hämmerling, G.J., MacDonald, A.S., Maizels, R.M., 2012. Type 2 innate immunity in helminth infection is induced redundantly and acts autonomously following CD11c(+) cell depletion. Infect Immun 80, 3481–3489. https://doi.org/10.1128/IAI.00436-12

So, T., Song, J., Sugie, K., Altman, A., Croft, M., 2006. Signals from OX40 regulate nuclear factor of activated T cells c1 and T cell helper 2 lineage commitment. Proc Natl Acad Sci U S A 103, 3740–3745. https://doi.org/10.1073/pnas.0600205103

Sokol, C.L., Chu, N.-Q., Yu, S., Nish, S.A., Laufer, T.M., Medzhitov, R., 2009. Basophils Function as Antigen Presenting Cells for an Allergen-Induced TH2 Response. Nat Immunol 10, 713–720. https://doi.org/10.1038/ni.1738

Sorathia, S.J., Chippa, V., Rivas, J.M., 2025. Small Intestinal Bacterial Overgrowth, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Sorboni, S.G., Moghaddam, H.S., Jafarzadeh-Esfehani, R., Soleimanpour, S., 2022. A Comprehensive Review on the Role of the Gut Microbiome in Human Neurological Disorders. Clin Microbiol Rev 35, e00338-20. https://doi.org/10.1128/CMR.00338-20

Soumelis, V., Reche, P.A., Kanzler, H., Yuan, W., Edward, G., Homey, B., Gilliet, M., Ho, S., Antonenko, S., Lauerma, A., Smith, K., Gorman, D., Zurawski, S., Abrams, J., Menon, S., McClanahan, T., Waal-Malefyt, R. de, Bazan, F., Kastelein, R.A., Liu, Y.-J., 2002. Human epithelial cells trigger dendritic cell–mediated allergic inflammation by producing TSLP. Nat Immunol 3, 673–680. https://doi.org/10.1038/ni805

Souza, R.F., Caetano, M.A.F., Magalhães, H.I.R., Castelucci, P., 2023. Study of tumor necrosis factor receptor in the inflammatory bowel disease. World J Gastroenterol 29, 2733–2746. https://doi.org/10.3748/wjg.v29.i18.2733

Spencer, S., Wilhelm, C., Yang, Q., Hall, J., Bouladoux, N., Boyd, A., Nutman, T., Urban, J., Wang, J., Ramalingam, T., Bhandoola, A., Wynn, T., Belkaid, Y., 2014. Adaptation of Innate Lymphoid Cells to a Micronutrient Deficiency Promotes Type 2 Barrier Immunity. Science 343, 432–437. https://doi.org/10.1126/science.1247606

Stanbery, A.G., Smita, S., von Moltke, J., Tait Wojno, E.D., Ziegler, S.F., 2022. TSLP, IL-33, and IL-25: not just for allergy and helminth infection. J Allergy Clin Immunol 150, 1302–1313. https://doi.org/10.1016/j.jaci.2022.07.003

Stanford, A.H., Gong, H., Noonan, M., Lewis, A.N., Gong, Q., Lanik, W.E., Hsieh, J.J., Lueschow, S.R., Frey, M.R., Good, M., McElroy, S.J., 2020. A direct comparison of mouse and human intestinal development using epithelial gene expression patterns. Pediatr Res 88, 66–76. https://doi.org/10.1038/s41390-019-0472-y

Su, C., Su, L., Li, Y., Long, S.R., Chang, J., Zhang, W., Walker, W.A., Xavier, R.J., Cherayil, B.J., Shi, H.N., 2018. Helminth-induced alterations of the gut microbiota exacerbate bacterial colitis. Mucosal Immunol 11, 144–157. https://doi.org/10.1038/mi.2017.20

Su, L., Su, C., Qi, Y., Yang, G., Zhang, M., Cherayil, B.J., Zhang, X., Shi, H.N., 2014. Coinfection with an Intestinal Helminth Impairs Host Innate Immunity against Salmonella enterica Serovar Typhimurium and Exacerbates Intestinal Inflammation in Mice. Infection and Immunity 82, 3855–3866. https://doi.org/10.1128/iai.02023-14

Sultana, M.F., Suzuki, M., Yamasaki, F., Kubota, W., Takahashi, K., Abo, H., Kawashima, H., 2022. Identification of Crucial Amino Acid Residues for

Antimicrobial Activity of Angiogenin 4 and Its Modulation of Gut Microbiota in Mice. Front Microbiol 13, 900948. https://doi.org/10.3389/fmicb.2022.900948

Sun, J.C., Bevan, M.J., 2003. Defective CD8 T cell memory following acute infection without CD4 T cell help. Science 300, 339–342. https://doi.org/10.1126/science.1083317

Sun, K., Salmon, S.L., Lotz, S.A., Metzger, D.W., 2007. Interleukin-12 Promotes Gamma Interferon-Dependent Neutrophil Recruitment in the Lung and Improves Protection against Respiratory Streptococcus pneumoniae Infection. Infection and Immunity 75, 1196–1202. https://doi.org/10.1128/iai.01403-06

Sun, L., Su, Y., Jiao, A., Wang, X., Zhang, B., 2023. T cells in health and disease. Sig Transduct Target Ther 8, 235. https://doi.org/10.1038/s41392-023-01471-y

Suzuki, T., 2020. Regulation of the intestinal barrier by nutrients: The role of tight junctions. Anim Sci J 91, e13357. https://doi.org/10.1111/asj.13357

Takashima, S., Martin, M.L., Jansen, S.A., Fu, Y., Bos, J., Chandra, D., O'Connor, M.H., Mertelsmann, A.M., Vinci, P., Kuttiyara, J., Devlin, S.M., Middendorp, S., Calafiore, M., Egorova, A., Kleppe, M., Lo, Y., Shroyer, N.F., Cheng, E.H., Levine, R.L., Liu, C., Kolesnick, R., Lindemans, C.A., Hanash, A.M., 2019. T cell-derived interferon-γ programs stem cell death in immune-mediated intestinal damage. Science Immunology 4, eaay8556. https://doi.org/10.1126/sciimmunol.aay8556

Takiishi, T., Fenero, C.I.M., Câmara, N.O.S., 2017. Intestinal barrier and gut microbiota: Shaping our immune responses throughout life. Tissue Barriers 5, e1373208. https://doi.org/10.1080/21688370.2017.1373208

Tan, C.Y., Ramirez, Z.E., Surana, N.K., 2021. A modern world view of host–microbiota–pathogen interactions. J Immunol 207, 1710–1718. https://doi.org/10.4049/jimmunol.2100215

Tau, G., Rothman, P., 1999. Biologic functions of the IFN-γ receptors. Allergy 54, 1233–1251. https://doi.org/10.1034/j.1398-9995.1999.00099.x

Thiemann, S., Smit, N., Roy, U., Lesker, T.R., Gálvez, E.J.C., Helmecke, J., Basic, M., Bleich, A., Goodman, A.L., Kalinke, U., Flavell, R.A., Erhardt, M., Strowig, T., 2017. Enhancement of IFNγ Production by Distinct Commensals Ameliorates *Salmonella*-Induced Disease. Cell Host & Microbe 21, 682-694.e5. https://doi.org/10.1016/j.chom.2017.05.005

Turner, J.-E., Morrison, P.J., Wilhelm, C., Wilson, M., Ahlfors, H., Renauld, J.-C., Panzer, U., Helmby, H., Stockinger, B., 2013. IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. Journal of Experimental Medicine 210, 2951–2965. https://doi.org/10.1084/jem.20130071

Tyszka, M., Maciejewska-Markiewicz, D., Biliński, J., Lubas, A., Stachowska, E., Basak, G.W., 2022. Increased Intestinal Permeability and Stool Zonulin, Calprotectin and Beta-Defensin-2 Concentrations in Allogenic Hematopoietic Cell Transplantation Recipients. Int J Mol Sci 23, 15962. https://doi.org/10.3390/ijms232415962

Urban, J.F., Fayer, R., Sullivan, C., Goldhill, J., Shea-Donohue, T., Madden, K., Morris, S.C., Katona, I., Gause, W., Ruff, M., Mansfield, L.S., Finkelman, F.D., 1996. Local TH1 and TH2 responses to parasitic infection in the intestine: regulation by IFN-gamma and IL-4. Veterinary Immunology and Immunopathology, Proceedings of the International Veterinary Immunology Symposium 54, 337–344. https://doi.org/10.1016/S0165-2427(96)05708-X

Utech, M., Ivanov, A.I., Samarin, S.N., Bruewer, M., Turner, J.R., Mrsny, R.J., Parkos, C.A., Nusrat, A., 2005. Mechanism of IFN-γ-induced Endocytosis of Tight Junction Proteins: Myosin II-dependent Vacuolarization of the Apical Plasma Membrane. Mol Biol Cell 16, 5040–5052. https://doi.org/10.1091/mbc.E05-03-0193

Vacca, F., Le Gros, G., 2022. Tissue-specific immunity in helminth infections. Mucosal Immunol 15, 1212–1223. https://doi.org/10.1038/s41385-022-00531-w

Van der Sluis, M., De Koning, B.A.E., De Bruijn, A.C.J.M., Velcich, A., Meijerink, J.P.P., Van Goudoever, J.B., Büller, H.A., Dekker, J., Van Seuningen, I., Renes, I.B., Einerhand, A.W.C., 2006. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology 131, 117–129. https://doi.org/10.1053/j.gastro.2006.04.020

Vicanolo, T., Özcan, A., Li, J.L., Huerta-López, C., Ballesteros, I., Rubio-Ponce, A., Dumitru, A.C., Nicolás-Ávila, J.Á., Molina-Moreno, M., Reyes-Gutierrez, P., Johnston, A.D., Martone, C., Greto, E., Quílez-Alvarez, A., Calvo, E., Bonzon-Kulichenko, E., Álvarez-Velez, R., Chooi, M.Y., Kwok, I., González-Bermúdez, B., Malleret, B., Espinosa, F.M., Zhang, M., Wang, Y.-L., Sun, D., Zhen Chong, S., El-Armouche, A., Kim, K.K., Udalova, I.A., Greco, V., Garcia, R., Vázquez, J., Dopazo, A., Plaza, G.R., Alegre-Cebollada, J., Uderhardt, S., Ng, L.G., Hidalgo, A., 2025. Matrix-producing neutrophils populate and shield the skin. Nature 641, 740–748. https://doi.org/10.1038/s41586-025-08741-5

Viragova, S., Li, D., Klein, O.D., 2024. Activation of fetal-like molecular programs during regeneration in the intestine and beyond. Cell Stem Cell 31, 949–960. https://doi.org/10.1016/j.stem.2024.05.009

Vivier, E., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J.P., Eberl, G., Koyasu, S., Locksley, R.M., McKenzie, A.N.J., Mebius, R.E., Powrie, F., Spits, H., 2018. Innate Lymphoid Cells: 10 Years On. Cell 174, 1054–1066. https://doi.org/10.1016/j.cell.2018.07.017

Voehringer, D., 2009. The role of basophils in helminth infection. Trends in Parasitology 25, 551–556. https://doi.org/10.1016/j.pt.2009.09.004

Voehringer, D., Shinkai, K., Locksley, R.M., 2004. Type 2 Immunity Reflects Orchestrated Recruitment of Cells Committed to IL-4 Production. Immunity 20, 267–277. https://doi.org/10.1016/S1074-7613(04)00026-3

von Moltke, J., Ji, M., Liang, H.-E., Locksley, R.M., 2016. Tuft-cell-derived IL-25 regulates an intestinal ILC2–epithelial response circuit. Nature 529, 221–225. https://doi.org/10.1038/nature16161

Vremec, D., O'Keeffe, M., Hochrein, H., Fuchsberger, M., Caminschi, I., Lahoud, M., Shortman, K., 2007. Production of interferons by dendritic cells, plasmacytoid cells, natural killer cells, and interferon-producing killer dendritic cells. Blood 109, 1165–1173. https://doi.org/10.1182/blood-2006-05-015354

W.H.O, 2023. *Soil-transmitted helminth infections* [Online] Available: https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections

Walk, S.T., Blum, A.M., Ewing, S.A.-S., Weinstock, J.V., Young, V.B., 2010. Alteration of the murine gut microbiota during infection with the parasitic helminth Heligmosomoides polygyrus. Inflamm Bowel Dis 16, 1841–1849. https://doi.org/10.1002/ibd.21299

Walsh, R., Seth, R., Behnke, J., Potten, C.S., Mahida, Y.R., 2009. Epithelial stem cell-related alterations in Trichinella spiralis-infected small intestine. Cell Proliferation 42, 394–403. https://doi.org/10.1111/j.1365-2184.2009.00605.x

Walter, M.R., Windsor, W.T., Nagabhushan, T.L., Lundell, D.J., Lunn, C.A., Zauodny, P.J., Narula, S.K., 1995. Crystal structure of a complex between interferon-γ and its soluble high-affinity receptor. Nature 376, 230–235. https://doi.org/10.1038/376230a0

Webster, H.C., Andrusaite, A.T., Shergold, A.L., Milling, S.W.F., Perona-Wright, G., 2020. Isolation and functional characterisation of lamina propria leukocytes from helminth-infected, murine small intestine. J Immunol Methods 477, 112702. https://doi.org/10.1016/j.jim.2019.112702

Webster, H.C., Gamino, V., Andrusaite, A.T., Ridgewell, O.J., McCowan, J., Shergold, A.L., Heieis, G.A., Milling, S.W.F., Maizels, R.M., Perona-Wright, G., 2022. Tissue-based IL-10 signalling in helminth infection limits IFNγ expression and promotes the intestinal Th2 response. Mucosal Immunol 15, 1257–1269. https://doi.org/10.1038/s41385-022-00513-y

Weight, C.M., Jones, E.J., Horn, N., Wellner, N., Carding, S.R., 2015. Elucidating pathways of *Toxoplasma gondii* invasion in the gastrointestinal tract: involvement of the tight junction protein occludin. Microbes and Infection 17, 698–709. https://doi.org/10.1016/j.micinf.2015.07.001

Westfall, S., Gentile, M.E., Olsen, T.M., Karo-Atar, D., Bogza, A., Röstel, F., Pardy, R.D., Mandato, G., Fontes, G., Herbert, D., Melichar, H.J., Abadie, V., Richer, M.J., Vinh, D.C., Koenig, J.F.E., Harrison, O.J., Divangahi, M., Weis, S., Gregorieff, A., King, I.L., 2025. A type 1 immune-stromal cell network mediates disease tolerance against intestinal infection. Cell 188, 3135-3151.e22. https://doi.org/10.1016/j.cell.2025.03.043

White, M.P.J., Smyth, D.J., Cook, L., Ziegler, S.F., Levings, M.K., Maizels, R.M., 2021. The parasite cytokine mimic Hp-TGM potently replicates the regulatory effects of TGF-ß on murine CD4+ T cells. Immunology & Cell Biology 99, 848–864. https://doi.org/10.1111/imcb.12479

Williams, J.M., Duckworth, C.A., Burkitt, M.D., Watson, A.J.M., Campbell, B.J., Pritchard, D.M., 2015. Epithelial Cell Shedding and Barrier Function. Vet Pathol 52, 445–455. https://doi.org/10.1177/0300985814559404

Wojciechowski, W., Harris, D.P., Sprague, F., Mousseau, B., Makris, M., Kusser, K., Honjo, T., Mohrs, K., Mohrs, M., Randall, T., Lund, F.E., 2009. Regulation of type 2 immunity to H. polygyrus by effector B cells. Immunity 30, 421–433. https://doi.org/10.1016/j.immuni.2009.01.006

Wong, V., 1997. Phosphorylation of occludin correlates with occludin localization and function at the tight junction. American Journal of Physiology-Cell Physiology 273, C1859–C1867. https://doi.org/10.1152/ajpcell.1997.273.6.C1859

Woznicki, J.A., Saini, N., Flood, P., Rajaram, S., Lee, C.M., Stamou, P., Skowyra, A., Bustamante-Garrido, M., Regazzoni, K., Crawford, N., McDade, S.S., Longley, D.B., Aza-Blanc, P., Shanahan, F., Zulquernain, S.A., McCarthy, J., Melgar, S., McRae, B.L., Nally, K., 2021. TNF-α synergises with IFN-γ to induce caspase-8-JAK1/2-STAT1-dependent death of intestinal epithelial cells. Cell Death Dis 12, 864. https://doi.org/10.1038/s41419-021-04151-3

Wu, H.-J., Wu, E., 2012. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes 3, 4–14. https://doi.org/10.4161/gmic.19320

Wu, M., Zheng, W., Song, X., Bao, B., Wang, Y., Ramanan, D., Yang, D., Liu, R., Macbeth, J.C., Do, E.A., Andrade, W.A., Yang, T., Cho, H.-S., Gazzaniga, F.S., Ilves, M., Coronado, D., Thompson, C., Hang, S., Chiu, I.M., Moffitt, J.R., Hsiao, A., Mekalanos, J.J., Benoist, C., Kasper, D.L., 2024. Gut complement induced by the microbiota combats pathogens and spares commensals. Cell 187, 897-913.e18. https://doi.org/10.1016/j.cell.2023.12.036

Wu, S., Lim, K.-C., Huang, J., Saidi, R.F., Sears, C.L., 1998. Bacteroides fragilis enterotoxin cleaves the zonula adherens protein, E-cadherin. Proc Natl Acad Sci U S A 95, 14979–14984. https://doi.org/10.1073/pnas.95.25.14979

Wynn, T.A., Vannella, K.M., 2016. Macrophages in tissue repair, regeneration, and fibrosis. Immunity 44, 450–462. https://doi.org/10.1016/j.immuni.2016.02.015

Xie, S., Li, Y., Zhao, S., Lv, Y., Yu, Q., 2020. *Salmonella* infection induced intestinal crypt hyperplasia through Wnt/ß-catenin pathway in chicken. Research in Veterinary Science 130, 179–183. https://doi.org/10.1016/j.rvsc.2020.03.008

Xing, T., Benderman, L.J., Sabu, S., Parker, J., Yang, J., Lu, Q., Ding, L., Chen, Y.-H., 2020. Tight Junction Protein Claudin-7 Is Essential for Intestinal Epithelial Stem Cell Self-Renewal and Differentiation. Cellular and Molecular Gastroenterology and Hepatology 9, 641–659. https://doi.org/10.1016/j.jcmgh.2019.12.005

Xu, X., Foley, E., 2024. *Vibrio cholerae* arrests intestinal epithelial proliferation through T6SS-dependent activation of the bone morphogenetic protein pathway. Cell Reports 43, 113750. https://doi.org/10.1016/j.celrep.2024.113750

Ye, P., Rodriguez, F.H., Kanaly, S., Stocking, K.L., Schurr, J., Schwarzenberger, P., Oliver, P., Huang, W., Zhang, P., Zhang, J., Shellito, J.E., Bagby, G.J., Nelson, S., Charrier, K., Peschon, J.J., Kolls, J.K., 2001. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. J Exp Med 194, 519–527. https://doi.org/10.1084/jem.194.4.519

Yip, J.L.K., Balasuriya, G.K., Spencer, S.J., Hill-Yardin, E.L., 2021. The Role of Intestinal Macrophages in Gastrointestinal Homeostasis: Heterogeneity and Implications in Disease. Cell Mol Gastroenterol Hepatol 12, 1701–1718. https://doi.org/10.1016/j.jcmgh.2021.08.021

Yue, R., Wei, X., Zhao, J., Zhou, Z., Zhong, W., 2021. Essential Role of IFN-γ in Regulating Gut Antimicrobial Peptides and Microbiota to Protect Against Alcohol-Induced Bacterial Translocation and Hepatic Inflammation in Mice. Front. Physiol. 11. https://doi.org/10.3389/fphys.2020.629141

Zaini, A., Dalit, L., Sheikh, A.A., Zhang, Y., Thiele, D., Runting, J., Rodrigues, G., Ng, J., Bramhall, M., Scheer, S., Hailes, L., Groom, J.R., Good-Jacobson, K.L., Zaph, C., 2023. Heterogeneous Tfh cell populations that develop during enteric helminth infection predict the quality of type 2 protective response. Mucosal Immunol 16, 642–657. https://doi.org/10.1016/j.mucimm.2023.06.007

Zaiss, M.M., Rapin, A., Lebon, L., Dubey, L.K., Mosconi, I., Sarter, K., Piersigilli, A., Menin, L., Walker, A.W., Rougemont, J., Paerewijck, O., Geldhof, P., McCoy, K.D., Macpherson, A.J., Croese, J., Giacomin, P.R., Loukas, A., Junt, T., Marsland, B.J., Harris, N.L., 2015. The Intestinal Microbiota Contributes to the Ability of Helminths to Modulate Allergic Inflammation. Immunity 43, 998–1010. https://doi.org/10.1016/j.immuni.2015.09.012

Zhang, A., Sodhi, C.P., Wang, M., Shores, D.R., Fulton, W., Prindle, T., Brosten, S., O'Hare, E., Lau, A., Ding, H., Jia, H., Lu, P., White, J.R., Hui, J., Sears, C.L., Hackam, D.J., Alaish, S.M., 2020. A Central Role for Lipocalin-2 in the Adaptation to Short-Bowel Syndrome Through Down-Regulation of IL22 in Mice.

Cell Mol Gastroenterol Hepatol 10, 309–326. https://doi.org/10.1016/j.jcmgh.2020.04.006

Zhang, D., Frenette, P.S., 2019. Cross talk between neutrophils and the microbiota. Blood 133, 2168–2177. https://doi.org/10.1182/blood-2018-11-844555

Zhang, J., Yang, W., Roy, S., Liu, H., Roberts, R.M., Wang, L., Shi, L., Ma, W., 2023. Tight junction protein occludin is an internalization factor for SARS-CoV-2 infection and mediates virus cell-to-cell transmission. Proceedings of the National Academy of Sciences 120, e2218623120. https://doi.org/10.1073/pnas.2218623120

Zhang, M., Wu, C., 2020. The relationship between intestinal goblet cells and the immune response. Biosci Rep 40, BSR20201471. https://doi.org/10.1042/BSR20201471

Zheng, D., Liwinski, T., Elinav, E., 2020. Interaction between microbiota and immunity in health and disease. Cell Res 30, 492–506. https://doi.org/10.1038/s41422-020-0332-7

Zhou, F., 2009. Molecular Mechanisms of IFN-γ to Up-Regulate MHC Class I Antigen Processing and Presentation. International Reviews of Immunology 28, 239–260. https://doi.org/10.1080/08830180902978120