THE UNIVERSITY *of* EDINBURGH

THE UNIVERSITY of EDINBURGH
Edinburgh Medical School

Biomedical Sciences

Heligmosomoides polygyrus-induced serum factors limit Respiratory Syncytial Virus titres; a potential role for CCL8

Lonalisa TKA Okwera

MSc by research

University of Edinburgh

2024

Declaration

I hereby declare that this thesis represents my own work which has been done throughout the year 2023-2024 as part of the infectious disease MScR programme. The work has been produced on my own as a result of working within the Schwarze group, whereby I contributed to the investigations presented below through various methods, research and analysis. This work is currently only being submitted as part of the MSCR degree and has not been submitted for any other purpose or elsewhere.

1 Contents

Acknowledgements

I would like to acknowledge the University of Edinburgh, specifically IRR institute and Ashworth, Kings building, for providing me with the necessary equipment and environment to complete this thesis.

Secondly, I would like to extend my gratitude to Dr. Henry J McSorley for his proteomic analysis which allowed this research to occur.

I would like to give thanks to my Principal investigator /supervisor Prof. Jurgen Schwarze and supervisor Dr. Matthew O Burgess for their continued support and encouragement. Prof. Schwarze continually assisted me in establishing the methodology and outline of my project and thesis, while Dr. Burgess offered expert knowledge, training, precedent, guidance and support throughout.

I would also like to thank Dr. Piotr Janas for his endless motivation, and kindness while providing me daily with expertise, advice and management for particular assays.

I also give thanks to Elaine Robertson for her time and support and the Amy Buck lab in allowing me access to their Helminth stock. In addition I extend my thanks to the Animal team at the Ashworth facility, Christopher Flockhart, operations manager, and David Mclver, animal technician. David for his incredible willingness to learn and endless support on animal experiments and animal training. Chris for helping me organise all training to enable experiments to run.

For analysis, Fiona Rossi for her tremendous amount of patience.

Finally, my loved ones, my Mother and God for encouraging me in what seemed like an exceptionally challenging year.

2 Abstract

Heligmosomoides polygyrus (*H. polygyrus*) is a naturally occurring intestinal nematode that employs immunomodulatory mechanisms towards the establishment of long term chronic infections within mice. The robust host Type 2 immune response to *H. polygyrus* infection can lead to a subdual of excess inflammation caused by a Type 1 immune response. Respiratory syncytial virus (RSV) is one of the most common viral respiratory infections among infants, young children, immunocompromised and elderly individuals. Infections with RSV lead to high rates of infant hospitalisation and mortality amongst low-income countries. Previously, there were no effective antiviral treatments, or vaccines for under 75's and those who were hospitalised received supportive care. Very recently RSV vaccination for those over 75 and passive immunisation against RSV in young infants (nirsevimab, maternal vaccination) have become available. Enhancing our understanding of the anti-viral response to RSV remains important to develop therapies that limit RSV burden and disease. *H. polygyrus* infection in mice has protective antiviral effects against RSV in the lung through induction of a microbiota-dependent Type I interferon response. *H. polygyrus* infection also leads to systemic monocytosis, contributing to elevated mononuclear phagocyte numbers in the lung, that reduces peak viral load of RSV. Despite this growing understanding of the anti-viral effects of *H. polygyrus*, a key question remains of how a strictly enteric infection induces these pulmonary responses.

Transfer of acellular serum from *H. polygyrus* infected mice (*H. polygyrus* serum) has been found to replicate the induction of interferons and reduction of RSV burden in recipient mice. A proteomic analysis of *H. polygyrus* serum revealed several elevated cytokines including Ccl8. This study aimed to investigate if *H. polygyrus* serum drives monocytosis and if Ccl8 chemokine is required for the antiviral effects of *H. polygyrus* serum to be sucsessful against RSV. Following transfer of *H. polygyrus* serum or control serum, flow cytometry of tail vein blood was undertaken to assess induction of monocytosis. To test the role of Ccl8 in *H. polygyrus* serum, antibody mediated depletion of Ccl8 was performed before transfer to naïve animals and subsequent infection with RSV 24 hours post serum transfer. Viral load within the lungs was assessed using RSV immunoplaque assays.

Mice that received *H. polygyrus* serum depleted of Ccl8 displayed a higher viral load 4 days post RSV infection, compared to mice that received control or non-depleted *H. polygyrus* serum. Furthermore, flow cytometry of blood samples taken 24 and 48 hours after serum transfer revealed that mice receiving *H. polygyrus* serum had lower levels of classical monocytes compared to mice that received control serum. Four days after the transfer, mice that received *H. polygyrus* serum also showed a

lower level of classical monocytes and higher levels of non-classical monocytes compared to mice that received control serum. However, it can be concluded that monocyte percentages tend to be lower with *H. polygyrus* serum transfer, but these differences were not statistically significant from day 1 to day 4 post serum transfer.

Based on the findings, it can be concluded that there is a recurring theme within mice receiving *H. polygyrus* serum displaying lower levels of classical monocytes in the blood throughout the 6 days. Additionally, the Ccl8 cytokine appears to play a crucial role in providing anti-viral protection as mice receiving *H. polygyrus* serum depleted of Ccl8 displayed a higher viral load compared to those receiving control or *H. polygyrus* serum without Ccl8 depletion. These results highlight the potential of *H. polygyrus* induced serum in modulating immune responses and reducing viral load, emphasising the importance of further understanding the mechanisms involved within the process of anti-viral protection.

3 Lay summary

Respiratory syncytial virus (RSV) is a common virus that can cause severe breathing difficulties, especially in infants, young children, and older adults with weakened immune systems, RSV often necessitates hospitalisation for many infants and can pose a significant burden in low-income nations. Presently, there are no approved medications to manage this infection; although in the UK those aged 75-79 and women who are 28-weeks pregnant and above are eligible to have an RSV vaccine which can reduce the severity of symptom upon infection. However, a treatment option is required, research has shown that in mice, RSV infection can be reduced when the animals are infected with the intestinal parasite *Heligmosomoides polygyrus* (*H. polygyrus*). The parasite stimulates a strong immune response that controls the excessive inflammation often seen with RSV infections. Despite a growing understanding of the anti-viral effects of *H. polygyrus* infection, a key question remains of how an intestinal infection induces certain pulmonary responses.

Experiments have shown serum taken from the blood of mice infected with *H. polygyrus* will reduce the viral load within the lungs of naïve mice who are infected with RSV. This research explores the protein(s) involved in this observation through analysis of the serum retrieved from *H. polygyrus* infected mice. A protein called Ccl8 was identified from the analysis, this cytokine may play a role in directing the immune response to sites of inflammation, with a particular influence of the recruitment of cells known as monocytes.

The removal of this protein from the serum of *H. polygyrus* mice was investigated, to look at the role of the Ccl8 and to assess if there were other protein components that were involved in this process. A test that calculated the amount of circulating virus – known as an immunoplaque assay – was used to measure any changes in the lungs following exposure to this serum in RSV infected mice.

Additionally, When comparing the impact of serum transfer from mice with (and without) *H. polygyrus* infections on white blood cell increase/decrease, there were subtle differences in the levels recorded, the observation was not significant.

The Ccl8 cytokine however, appears to play a crucial role in providing anti-viral protection, as mice receiving serum from animals exposed to *H. polygyrus* which was then depleted of Ccl8 displayed a higher viral load compared to those complete serum from mice infected with *H. polygyrus*. It is interesting to note that these viral levels were also far higher than mice receiving serum from uninfected mice, which would suggest the role of additional elements within this immune response.

In summary,the Ccl8 cytokine appears to play a crucial role in providing anti-viral protection, as mice receiving *H. polygyrus* serum depleted of Ccl8 displayed a higher viral load compared to those receiving uninfected serum or serum from mice infected

with *H. polygyrus*. These results highlight the potential of *H. polygyrus* infected serum in controlling immune responses and reducing RSV viral load and are suggestive of a complex immune response to RSV infection, where the absence of Ccl8 may be detrimental. Further research into this immunological process would be beneficial in advancing our understanding of the host response to RSV infections and may help identify targets for the development of anti-viral treatment.

4 Abbreviations

Full word	Abbreviation

Days post infection	**DPI**
Post serum transfer	**PST**
Alveolar macrophages	**AM**
Dendritic cells	**DC**
Natural killer cells	**NK**
Heligmosomoide polygyrus	***H. polygyrus***
H. polygyrus infected serum depleted of Ccl8	***H. polygyrus* – Ccl8**
Lower respiratory Tract infection	**LRTI**
Upper respiratory tract infection	**URTI**
Monocyte chemoattractant protein	**MCP**
Virtual memory CD8+ T cells	**TVM**
Helminth Excretory-Secretory	**HES**
Monocyte chemoattractant protein-2	**MCP-2**
West Nile virus	**WNV**
Antigen-presenting cells	**APCs**
Toll-like receptor	**TLR2**
Alternatively activated macrophages	**AAMs**

5 Introduction

Respiratory syncytial virus (RSV) can be considered one of the most common viral infections affecting people worldwide (Jain, 2023). RSV commonly presents in young children and infants, this can occur because of maternal immunoglobulins not adequately protecting infants from RSV (Kaler *et al.,* 2023). It is highly contagious, and most children will become infected with RSV in the first 2 years after birth (Langedijk & Bont, 2023). According to a study by Li *et al*., (2022), 97% of RSV-related deaths across all age groups occur in low-income and middle-income countries. This demonstrates that the burden of RSV disease remains high in low-income countries. Deaths from RSV in developed countries are rare. However, the hospitalisation of infants is still a significant concern. For example, in the United States, an estimated 80,000 RSV-associated hospitalisations occur each year for infants alone (McLaughlin *et al*., 2020).

Risk factors that increase the vulnerability to RSV include congenital heart disease, lung disease, Down syndrome and immunocompromised individuals, e.g. those suffering from chronic illness or undergoing chemotherapy. Patients with RSV can have an upper respiratory illness and may present with rhinorrhoea, nasal congestion, cough and sneezing. Infant infections can progress to lower respiratory tract infections resulting in bronchiolitis, wheezes, and in severe cases, it may also present with viral pneumonia, hypoxia, and acute respiratory failure. Children who have recovered from RSV bronchiolitis often experience subsequent chronic wheezing, and possibly asthma however, the relationship between RSV and subsequent wheezing is inconclusive (Midulla *et al.,* 2014; Bont *et al.,* 2000). RSV is considered a seasonal disease that peaks typically during the winter months and in tropical regions occurs during the rainy months. However, the annual epidemiology is impacted by the alternation of subtypes A and B and by genetic variations of strains (Rios Guzman & Hultquist, 2022). The peak incidence of RSV disease is between 6-12 months (Bont & Houben, 2011), and the associated hospitalization is 1.7% in the general population under 5 months of age in the United States and 1.8% in the first year after birth of healthy term infants in Europe (Hall *et al*., 2009).

Currently the treatments for RSV during hospitalisation rely heavily on supportive care, the use of aerosolised ribavirin is restricted to severe infections in immunocompromised patients and recently, the only option for immunoprophylaxis against severe lower respiratory tract illness in high-risk infants was palivizumab, a multiple-dose monoclonal antibody (mAb) (Griffiths *et al.,* 2017). Despite these management strategies, there is no cure for RSV infection however, for individuals over 75 and pregnant woman a vaccine has recently been made available in the UK, which reduces the symptoms associated with RSV e.g. pneumonia and bronchiolitis.

Heligmosomoides polygyrus (*H. polygyrus)* is a natural intestinal parasite occurring in wild mice which establishes chronic infections in their host, persisting for weeks. Despite this they can trigger a Type-2 (Th2) immune response within their host which can be considered protective (Valanparambil *et al.,* 2016), and the host response can be either adaptive or innate. An innate response would trigger macrophage, eosinophil, and lymphoid immune cells (Smith *et al.,* 2012). In an experimental setting, *H. polygyrus* is introduced via oral gavage. After being ingested, infective L3 larvae penetrate the submucosa of the small intestine within 24 hours and develop further before emerging back into the lumen as adult worms, which then feed on host intestinal tissue (Bansemir & Sukhdeo, 1994). The parasite can protect mice from RSV infection through the reduction of viral load that is dependent on type-I interferon signal.

This research paper will investigate the potential role of Ccl8 cytokine as a modulator for reducing RSV viral titre within the lungs of RSV infected mice.

5.1 RSV Biology

Respiratory syncytial virus (RSV) is categorised as a Pneumovirus, and its virions exhibit a diversity of sizes and shapes, consisting of a nucleocapsid encased within a lipid envelope. RSV is classified into two subgroups: A and B (Cane, P. 2006). The G glycoprotein, a type II transmembrane protein composed of 289-299 amino acids, facilitates RSV's attachment to host cells and is glycosylated. Additionally, the virus relies on the F protein, which is essential for both attaching and entering cells, representing a critical step in the viral lifecycle. Consequently, the majority of vaccine and therapeutic research has focused on these key surface glycoproteins (Mohapatra & Lockey, 2008), (Papi *et al.,* 2023).

RSV predominantly infects airway epithelial cells which line the nose, the epithelial cells are considered the first line of defence against the virus and can develop inflammation in association with the disease (Hacking & Hull, 2002). RSV infection involves initial viral entry into the host and binding of virions onto cell surfaces, followed by binding of the F protein to ensure fusion and engulfment into cell cytoplasm and transcription of the viral genome, creating cRNA from vRNA. (Cane, P. 2006).

5.2 RSV Epidemiology

RSV is responsible for approximately 30 million lower respiratory tract infections (LRTIs), and about 3 million hospitalisations per year as of 2015 (Hewitson *et al.,* 2009). However, most infants infected with RSV exhibit primarily upper respiratory tract symptoms, and 20% to 30% develop lower respiratory tract disease, e.g., bronchiolitis or pneumonia (Tan, T.Q. 2023). 60% to 80% of children are infected by RSV during their first year of life, with almost all infected by two years of age, RSV plays a significant role in child mortality worldwide, resulting in an estimated 66,000 to 199,000 deaths among children under the age of 5 annually. Nearly all these fatalities—about 99%—happen in low-income countries (Glezen, 1986 ; Stockman, 2012).

In the UK, a peak in RSV cases typically occurs between weeks 47 and 50, laboratory-confirmed cases of RSV in children younger than 5 years during the winter seasons from 2015–16 to 2019–20 resulted in an average/mean of around 8,697 cases between these 3 weeks. However, during the pandemic, starting from week 22 of 2021, there was a notable deviation in the trend of emergency department visits for acute bronchitis and bronchiolitis from the usual seasonal patterns (Bardsley *et al.,* 2023), this decrease could be owed to a majority of the population isolating and practising social distancing, lowering the potential for RSV incidence to increase, however, post-pandemic the previously high hospitalisation in younger children has risen and increased as expected.

There is currently no clear explanation for the repeated seasonal re-occurrence of RSV incidence, although meteorological factors are strongly associated with its prevalence. Other driving factors may involve behavioural factors such as increased exposure through overcrowding (Stensballe *et al.,* 2003). Despite there being lack of explanation on the occurrence of RSV it has been shown that those who fall sick with RSV can contract the infection from HIV or chronic obstructive pulmonary disease (COPD) patients. This further supports that RSV infection is contagious and patients who are immunocompromised can be reservoirs for RSV outside of RSV epidemics (Wedzicha & Seemungal, 2007).

5.3 Helminth infections

Helminths are large multicellular parasitic organisms, the majority of which are soil-transmitted worms that can cause gastrointestinal infections. Infection usually begins by ingestion of eggs within contaminated pasture or water (Mabbott, N.A. 2018). Some helminth larvae can also enter hosts through skin penetration e.g. hookworms, schistosomes and Strongyloides (Baron, S. 1996), as of 2009, helminths have been estimated to infect over 2 billion people worldwide (Brindley, P.J. *et al* ,. 2009).

When a helminth parasite infiltrates a host organism, it typically engages in the consumption of host tissues and blood. This interaction can have adverse effects on the host, leading to various pathological conditions, including chronic intestinal blood loss, anaemia, and compromised nutrient absorption. These disruptions can significantly impair the host's overall health and physiological functioning. Examples of common parasitic soil-transmitted infections are *Ascaris lumbricoides*, a roundworm which causes severe intestinal blockage, *Trichuris trichiura,* a whipworm which irritates the intestinal lining and *Necator americanus*, a hook worm which is associated with blood loss and anaemia (Lebu, S. *et al.,* 2023) .

Helminths can modulate the host's immune responses to benefit them and enhance their survival through adaptations and mechanisms which enable them to live within their hosts. These adaptations allow for the regulation and suppression of inflammatory responses used by the host to protect against parasitic infections (Lebu, S. *et al.,* 2023). Helminth parasites can interact with other pathogens when inside the host, a phenomenon termed co-infection, multiple pathogens reside within the host simultaneously (Dong & Xing, 2024). Helminth co-infection can involve bacteria or viruses and may result in detrimental effects to the host. Co-infection with helminths and bacteria can lead to different outcomes compared to viral co-infections. For instance, (Chen *et al.,* 2023) describes previous studies of co-infection in BALB/c mice with *H. Polygyrus* and *Listeria monocytogenes* in comparison to naïve mice infected with helminths only. Mice infected with *Listeria monocytogenes* and *H. polygyrus* experienced an expansion in virtual memory CD8+ T cells (TVM), cells which expand upon helminth infection and provide protective Immunity against *L. monocytogenes* Infection. The TVM expansion in helminth-infected mice was shown to be IL-4 signalling-dependent, indicating that when co-infected with certain bacterial strains helminths can aid in triggering a protective outcome for the hosts. Helminths have also been shown to reduce pulmonary inflammation caused by bacterial pneumonia by inducing a Type 2 (TH2) immune response, as exemplified by *T. spiralis* and *P. aeruginosa* co-infection (Njua-Yafi, C. *et al.,* 2016).

Furthermore, moving onto viral co-infections, a study was conducted in Cameroon with *P. falciparum* and malaria, in endemic zones where malaria and helminths frequently

co-infect the same individuals, this has been considered rather beneficial to children with malaria, as it leads to protection/lowered risk from anaemia, compared to individuals infected with *P. falciparum* alone. This suggests that helminth infection may be protective against anaemia when co-infected with a Plasmodium (Njua-Yafi, C. *et al.,* 2016).

Flaviviruses such as West Nile virus (WNV), which are transmitted into the skin by mosquitoes, and can disseminate to the brain and spinal cord. In a murine model of WNV , co-infection with *H. polygyrus* increased mortality and was observed through bowel blackening and tissue friability, indicating that WNV and helminth co-infection results in further exacerbation of WNV (Desai, P. *et al.,* 2021). Additionally, mice coinfected with *H. polygyrus* and WNV had higher mortality rates compared to WNV or *H. polygyrus* only infected mice. Therefore, coinfection of mice with *H. polygyrus* and WNV resulted in elevated viral burden in the gastrointestinal tract and central nervous system tissues, resulting in greater mortality. As a result, coinfection with enteric helminths can enhance host vulnerability to flavivirus infections via activation of a succinate-tuft cell-IL-25-IL-4R-intestinal circuit.

Another study involving mice coinfected with *N. brasiliensis* and influenza virus showed higher mortality (26% vs. 6%) and greater lung consolidation scores (41% vs. 26%) compared to mice infected with the influenza virus alone (Wescott, R.B. and Todd, A.C. 1966), suggesting that co-infections with helminths and viruses display detrimental effects to the host. Concerning RSV, studies from the Schwarze group indicate that infection with *H. polygyrus* is beneficial when RSV is present, through reduction of peak RSV viral burden and subsequent immunopathology, despite the tendency of helminths to act as immunomodulators and worsen bacterial and viral infections (Burgess, M. *et al.,* 2023 ; McFarlane *et al.,* 2017). Helminth parasites can migrate within the host and reside in different tissues within the body, not just in the gut, as a result, the host may experience long-term and unexpected effects of the infection, which could lead to a chronic condition. An example is when helminth infection and tissue infection affect host resistance to viral pathogens, the virus and helminth infect the same tissue which can lead to increased viral replication not only at the local site, but also in distant tissues which were not initially infected by the helminth, as can be seen in cases of HIV and helminth co-infection (Desai, P. *et al.,* 2021). Helminth infection can lead to an increased chance of physical damage to the tissue, as recently demonstrated by coinfection of mice with *N. brasiliensis* and Herpes simplex virus-2 (HSV-2). The study aimed to increase the recruitment of eosinophils to the female genital tract (FGT) in an attempt to combat intravaginal HSV-2, however, instead co-infection resulted in a local enhancement of eosinophils damaging the infected vaginal epithelium. This example demonstrates that certain viral/helminth co-infections can induce physical damage at the site of infection (Chetty, A. *et al,* 2021).

Furthermore, Zarek *et al*.,(2023) examined the effects of preexisting parasite infection with *H. polygyrus* on Murine Gammaherpesvirus 68 (MHV68), which is a virus that naturally infects rodents and can establish a lifelong and latent infection. In humans gammaherpesviruses are Epstein–Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) (Husain *et al*., 1999). Having previously infected murine models with gammaherpesvirus first then helminths, the study decided to reverse and infect with the parasite first, followed by MHV68. As a result, the study concluded that a previous helminth infection results in an elevated viral infection of tissue-resident macrophages during both acute and chronic herpesvirus infections. In the chronic stage of the infection, the co-infected mice exhibited a higher level of latent infection in tissue-resident macrophages and had increased frequent reactivation of herpesvirus, implying that infections with helminths worsen chronic herpesvirus infections

In addition, a study conducted by Osborne *et al*., (2014) investigates if helminth infection elicits immuno-modulation. The study conveys how co-infection with murine norovirus and the *Trichinella spiralis (Ts)* helminth led to a reduction in antiviral immunity and was related to alterations in the microbiota. However, further research indicated that helminth-induced immuno-modulation occurs independently of changes in the microbiota but is dependent on Ym1,a chitinase-like molecule related to activated macrophages, this was determined through Ym1 neutralisation which was able to partially restore antiviral immunity. These findings suggest that the immuno-modulation caused by helminths happens independently of microbiota changes but is reliant on Ym1. As a result, the data concluded that helminth co-infection can inhibit antiviral immunity via a pathway of innate immuno-modulation which is independent of changes in the microbiota, but was associated with induction of potent AAMac responses and Ym1-dependent inhibition of antiviral T cell responses

Despite the potential for helminths to induce a deteriorated immune response within its host, researchers have continued to research with helminth therapy, an experimental treatment involving the intentional introduction of helminth parasites to the host. For example,inflammatory bowel diseases (IBD) experimentations have suggested that the presence of helminths in the gut (pig whipworm *Trichuris suis*) does not prevent intestinal inflammation but rather activates several mechanisms suppressing gut inflammation (Weinstock, J.V. and Elliott, D.E. 2012). Therefore, it can be argued that helminth therapy is beneficial for gaining a further understanding for certain diseases.nevertheless, there is still little evidence supporting the efficacy of helminth infection as a treatment for IBD.

Overall, it is important to understand the immune responses to these infections to dissect any further potential protective mechanisms.

5.4 Host immunity in response to Helminth/RSV co- infection

Helminth infection elicits an immune response described as a Type 2 immune response (TH2) that is characterised by high levels of IL-4, IL-5 and IL-13. This immune response by cytokines triggers multiple downstream immune cells systemically by binding to cognate receptors on target cells while also activating macrophages. TH2 responses can result in an anti-inflammatory state and regulatory response characterised by the presence of Foxp3+ regulatory CD4+ T cells (Tregs), which acts as an immune response regulator, suppressing immune responses by producing cytokines (White *et al*., 2020).

When investigating helminth and RSV infection, it is essential to understand what mechanisms are necessary and contribute to protection against RSV, McFarlane, A.J*. et al*., (2017) reports on how enteric helminth infection induces Type 1 signalling that protects against pulmonary virus infection through interaction with microbiota. Gut microbiota are symbiotic microorganisms found within the digestive tracts, essential for maintaining homeostasis. In the specific context of infection with *H. polygyrus* and RSV simultaneously, McFarlane, A.J*. et al*., (2017) conveys how Infection with *H. polygyrus* is protective against RSV disease and inflammation, this was demonstrated through murine infection with *H. polygyrus*, 10 days later, when adult worms emerge into the lumen of the gut, mice were infected with RSV. It was concluded that *H. polygyrus* protected against RSV-induced weight loss, however, RSV infection led to inflammation in the lungs with an increase in immune cells like NK cells, CD8+ T cells, and conventional dendritic cells (cDCs). However, in mice that were also infected with *H. polygyrus,* RSV did not cause an increase in NK cell and B cell numbers, and the rise in cDCs was significantly lower. On day 2, the production of early inflammatory signals, IL-6 and TNF-α, were much lower in mice infected with *H. polygyrus* compared to those infected with RSV only. Although IFN-γ levels increased with RSV infection, they were not suppressed in mice with both infections, which suggests that the inhibition of immune response happens through a different pathway that does not involve IFN-γ. Furthermore, the report investigated whether *H. polygyrus* infection supressed the immune response or directly impacts how RSV infects the body, through RSV viral plaque assays it was determined that the viral load within the lungs reached its highest levels 4 days after RSV infection, however, when *H. polygyrus* was present, the viral levels decreased, but the speed at which the virus multiplied remained unchanged. To further consolidate findings and measure viral load in C57BL/6 mice , RSV L gene in the lungs was observed using Reverse transcriptase-polymerase chain reaction (RT-PCR)to estimate the viral load. Again, it was observed that the *H. polygyrus*-infected mice had significantly lower levels of the L gene. As a result, this suggest that *H. polygyrus* infection effectively reduces RSV-related

disease, lowers early inflammatory signals, and recruits various immune cells to the lungs, likely because it initially lowers the viral infection levels.

Moreover, the study determines that protection against RSV is dependent on the host microbiota and induction of type 1 interferon signalling. Intestinal helminths share their niche with the microbiome which is inclusive of viruses, fungi and bacteria. Helminth infections can significantly affect the microbiome in both humans and animals (Jenkins *et al.,* 2017). For instance, in the murine model of H. polygyrus, the infection promoted the growth of Lactobacillus in the small intestine (Walk *et al*., 2010). Therefore, it was hypothesised that gut microbiota may be a contributing factor in mice's protection against RSV due to helminths penetrating through the submucosa of the small intestine. Germ-free mice and fully colonised SPF mice were infected with RSV or *H. polygyrus* with RSV to determine if the presence of the gut microbiota is essential for *H. polygyrus* induced protection. *H. polygyrus* infection did not suppress viral loads in germ-free mice when compared to fully colonized SPF mice, making it evident that microbiota in the gut is essential for antiviral protection. Furthermore, irradiation of worms was conducted using 100gy to prevent their maturation into adults, however, this did not affect their ability to burrow into the intestinal wall and still proved to be protective against RSV, as a result, it was evident that maturation of the worms was not essential for anti-viral protection.

Furthermore, McFarlane, A.J. *et al*., (2017) discusses that *H. polygyrus*-induced protection against RSV infection requires type I IFN receptor signalling, therefore, IFNAR1-deficient mice which fail to signal in response to IFN-α and IFN-β was acquired for experimentation. This resulted in mice suppression of RSV load in *H. polygyrus* coinfection being lost, implying an essential role for this pathway in the protection against RSV.

An experiment was performed using IL-4ra deficient mice to determine whether a TH2 response was required for antiviral protection against RSV, these mice were incapable of responding to IL-4 or IL-13, the predominant type 2 signals and presented diminished response to TH2 immune responses. When the IL-4ra mice were infected with *H. polygyrus* and RSV, there were consistent reductions in RSV titres, indicating that the virus was suppressed independently of TH2 signalling. To determine if other adaptive immune responses were necessary for *H. polygyrus*-mediated protection against RSV infection, RAG1-deficient mice which lack T and B cells were acquired. Results concluded that RSV titres were significantly suppressed in both RAG1-/- mice and wild type controls following co-infection with *H. polygyrus*. Collectively, these findings indicate that both adaptive immune responses and TH2 responses are unnecessary for the protective influence of H. polygyrus against RSV infection.(McFarlane, A.J. *et al*., 2017).

Helminth Excretory-Secretory (HES) products refer to the cocktail of molecules released from helminths within the host (Lightowlers & Rickard, 1988). Proteins present in HES have been long considered as potential therapeutic agents for autoimmune disease or as immune regulators of the host immune responses (Hewitson *et al*., 2009). McFarlane, A.J. *et al*. (2017) investigated whether the HES released by *H. polygyrus* can provide protection against RSV and mimic a real helminth infection. No protective effects were observed, ruling out HES as a potential mediator of protection against RSV.

To conclude, the main findings of McFarlane , A.J. *et al*. (2017) have elucidated what the prerequisites for protective antiviral response in a co-infection model are, the active factor(s) driving the effects of *H. polygyrus* have not yet been identified.

5.5 Innate immune response to RSV infections

RSV infection begins in the nasopharyngeal epithelium and can occasionally rapidly spread to the lower airways. When the virus reaches the lungs, resident cells including dendritic cells (DC), epithelial cells and alveolar macrophages (AMS) initiate an innate immune response producing multiple cytokines and chemokines such as IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, IL-6, TNF-α, IL-1αβ, and IFN-αβ that enhance inflammation and attract innate immune cells into the lungs (Nuriev & Johansson, 2019). Leukocytes mediate the innate response, and the cytokines and chemokines produced assist in host protection. Recruited innate cells to RSV infection include neutrophils, eosinophils, basophils, NK-cells, monocytes/macrophages and mast cells (Resch *et al*., 2009). Neutrophils are the most prevalent cells in the airways, with 93% of neutrophils being present in the upper airways and 76% in the lower airways and are gathered by chemokines such as IL-8 which was previously found in large quantities of nasal discharge of infants suffering from RSV (Everard *et al*., 1994). Eosinophils also play a key role in inflammation from RSV and can be attracted to the lungs by chemokines such as CCL2 or CCL11 (Nuriev & Johansson, 2019). Natural killer cells (NK) and dendritic cells (DC) also play a part in the innate immune response. NK cells have the potential to limit or exacerbate immune response to RSV through promoting inflammatory response while DC are antigen-presenting which allows them to respond instantly to RSV infection, especially as they are resident within the lung (Banchereau *et al*., 2000).

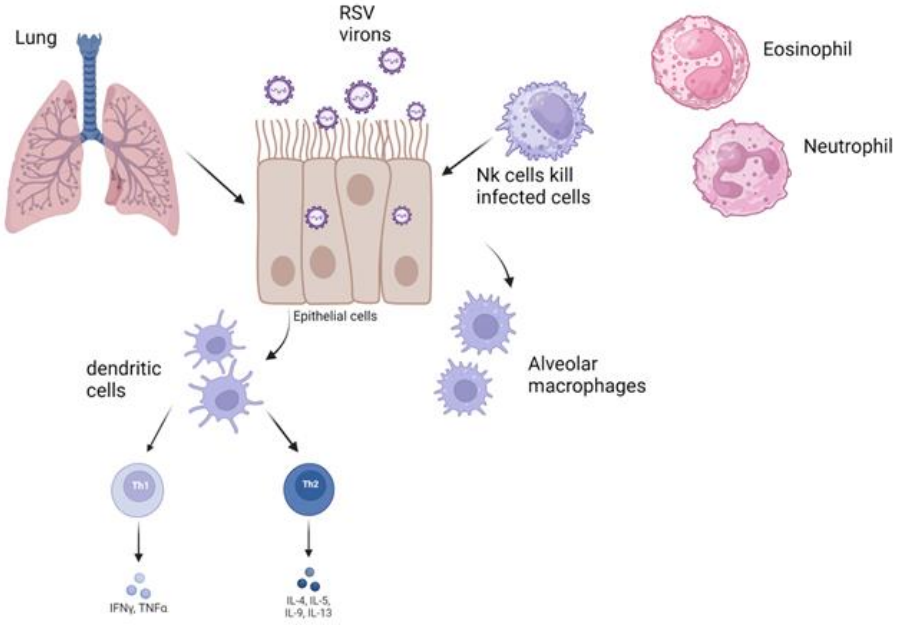

Figure 1: innate immune response during viral pathogenesis and the presence of cells which reside within the lung. The figure indicates the various cells present in early RSV lung infection and response, with RSV primarily infecting the lung epithelium. Lung epithelial cells secrete cytokines and chemokines which activate neutrophils and eosinophils, resulting in their recruitment into the lungs. NK cells and AM's are primed and migrate back to the infected epithelium to release further mediators and recruit additional inflammatory cells, including mononuclear cells to aid in anti-viral response.

Created in BioRender.com

5.6 Monocytes and their role in the reduction of disease

Monocytes are bone marrow derived leukocytes that circulate in the blood and spleen and are a component of the innate immune system. They play a crucial role within host defence, especially during infection and inflammation and can differentiate into macrophages and dendritic cells once recruited into tissue cells (Espinoza, 2023). Circulatory monocytes in a mouse can be distinguished into three phenotypic subtypes: classical, intermediate and non-classical monocytes. In mice classical monocytes are defined by membrane expression of Ly6CHi ,CCR2+ ,CD62L+ and CD43Lo, they are primed for phagocytosis but have a short circulation lifespan. While non-classical monocytes are characterised by Ly6Clow, CCR2Low, CD62L- CD43+) and exhibit lower proliferative activity while remaining in circulation for a longer duration and can be distinguished from classical monocytes by their association with

wound healing processes (Kapellos *et al*., 2019). Additionally, at steady states, rodent blood monocyte subsets often show the ability to develop, as previously observed, classical monocytes have been proven to develop into non-classical monocytes (Varol *et al*., 2006 ; Sunderkötter *et al*., 2004).

Monocytes, when encountered by a viral infection, can protect through differentiating into DC's and play important roles in controlling the immune response in viral infections, not only by direct interaction with helper T cells and cytotoxic T cells but also by the production of cytokines. Monocytes can destroy some invading pathogens, and they have an important function as antigen-presenting cells (APCs), monocytes and macrophages internalise viral proteins and present these proteins on the cell membrane, along with dendritic cells to some degree. They seem to be involved in the acute inflammation associated with RSV bronchiolitis, RSV is generally able to induce monocyte activation (Kimpen, 2001; Meidaninikjeh *et al*., 2021). Research conducted by Barbalat *et al*., (2009) found that Ly6Chi inflammatory monocytes can effectively mediate responses to viral pathogens, this is achieved this by promoting type I interferon production through Toll-like receptor (TLR2) pathways, which helps differentiate between various pathogen types and activates specific antiviral responses.

When referring to respiratory viral infections there often seems to be a heightened amount of inflammation followed by monocyte infiltration within the lungs, The recruited monocytes ultimately occupy the space left by the lack of tissue-resident macrophages at the infection site (Li *et al*., 2022). These Ly6C+ monocytes can subsequently transform into macrophages, which play a key role in managing local inflammation (McQuattie-Pimentel *et al*., 2018).

Gotera *et al*., (2012) reported that patients suffering from asthma related to RSV infection had higher circulation levels of chemokines(MCP-1 and RANTES). Monocytes and chemokines play an important role in the inflammatory response to RSV infection, especially in asthma patients however, further study is required to understand the processes involved. Additionally, CCL5, a monocyte chemoattractant, is also important in the early stages of RSV infection. CCL5 binds to various receptors on different immune cells, including T cells, macrophages and dendritic cells. It has been shown that CCL5 has a significant anti-viral effect against RSV by blocking the fusion of RSV proteins (Nuriev & Johansson, 2019), (John, A.E., Berlin, A.A. and Lukacs, N.W. 2003). Additionally, Goritzka *et al*., (2015) reports that when lungs are infected with RSV, chemoattractant CCL2, CCL7, and CCL12 (all ligands for CCR2) are critical for monocyte recruitment to inflamed tissues, and all three chemokines are induced in lung tissue shortly after RSV infection.

Overall, monocytes are a crucial component of the innate immune system and play a multifaceted role in various physiological processes and conditions. They are involved

in host defence, inflammation, and tissue homeostasis and their subsets, including classical, intermediate, and non-classical monocytes, exhibit distinct functions and are differentially implicated in various diseases. However, further research is essential to further understand the complex role of monocytes within disease.

5.7 Helminth induced Monocytosis

Monocytosis is an increased concentration of circulating monocytes within the blood, and in a clinical setting monocytosis is a frequently encountered condition (Zachary, 2022). Some causes of monocytosis can be reactive, including bone marrow recovery, acute infections, stress induced, and exercise induced, to name a few (Mangaonkar et al., 2021). A host defence against helminth infection mainly involves alternatively activated macrophages (AAMs) (Rajamanickam & Babu, 2024) , AAMs can aid in expelling adult parasites and targeting helminth larvae through the production of growth factors and chemokines eventually repairing damaged tissue (Fabre *et al*., 2023).

Burgess *et al*., (2024) highlights how helminth induced monocytosis conveys protection from RSV infection in mice, some findings from infection of murine models found that *H. polygyrus* infection induces monocytosis within the blood and increases lung mononuclear phagocytes. This conclusion was reached following several experiments where early response to RSV infection alone in mice drove the recruitment of monocytes to the lung causing an accumulation of mononuclear phagocytes and in the initial hours of infection these cells were initially identified as Ly6Chi, later downregulating to Ly6C and becoming largely CD11c+. However, in comparison to infection with *H. polygyrus* alone where lungs were assessed for innate immune cell composition, It was concluded that there is an increase in the percentage of mononuclear phagocytes in the lungs between 7 and 10 DPI. This increase is characterised by the expansion of monocytes, which are predominantly identified as Ly6C+ Treml4− classical monocytes.

To further gain insight into whether protection or monocytosis persists after a prolonged helminth infection period, mice were infected with *H. polygyrus* up to 35 DPI and then with RSV. It is important to note that a long-term worm infection does not protect against RSV, additionally, the initial changes in immune cells observed after the infection faded after 35 days. Even when infected with an irradiated helminth, there was still a temporary increase in blood monocytes, this brief rise was enough to enhance similar cells in the lungs implying that the initial surge of blood immune cells caused by the helminth might be connected to some early protection against RSV.

Additional findings convey that *H. polygyrus* infection induces bone marrow monopoiesis, evidence was acquired 4 days post infection where there was an

increase in the percentage of monocyte-committed progenitors compared to uninfected controls therefore, suggesting that helminth infection is likely causing the bone marrow to produce increased amounts of monocytes. Furthermore, evidence suggests that helminth infection results in increased classical monocyte production although the observed elevation was short lived and lost by 10 dpi, and non-classical monocytes remained unchanged throughout *H. polygyrus* infection.

Moreover, Burgess *et al*., (2024) investigates how induced increases in blood monocytes and lung mononuclear phagocytes are IFN-I signalling dependent. Cell counts were assessed 10 dpi in *H. polygyrus* infected Ifnar1−/− mice, and an increase in blood and lung mononuclear phagocytes and circulatory monocytes were not visible in comparison to regular mice which. Therefore, monocyte increase is arguably reliant on IFN-I signalling.

RSV induces early monocyte influx, and to ascertain if this early influx was altered following *H. polygyrus* infection monocyte-mononuclear phagocyte compartment was assessed post RSV infection. RSV infection alone trended towards an increased percentage of mononuclear phagocytes in the lung , although after prior infection with *H. polygyrus* mononuclear phagocyte counts significantly increased compared to both *H. polygyrus* or RSV infection alone. Additionally, four days after RSV infection, numbers of Ly6C+ macrophages remained higher in mice with prior *H. polygyrus* infection, therefore, Burgess *et al*., (2024) reveals how *H. polygyrus* monocytosis results in higher numbers of lung mononuclear phagocytes early after RSV infection.

The study conducted by Burgess *et al*., (2024) illustrates the intricate relationship between helminth infections and the immune system, particularly concerning monocytosis and its role in defence against respiratory viral infections such as RSV. The findings reveal that infection with *H. polygyrus* leads to a notable increase in both circulating monocytes and lung mononuclear phagocytes, which seems to play a crucial role in enhancing the immune response during the early stages of RSV infection. Although prior helminth infection may result in a temporary rise in monocyte levels and bolster immediate immune protection against RSV, this benefit diminishes over time, highlighting the short-lived nature of this immune enhancement. Furthermore, the reliance on IFN-I signalling for monocyte proliferation underscores the significance of cytokine interactions in regulating the immune response. Overall, this research provides important insights into how helminth infections influence immune dynamics and their consequences for susceptibility to respiratory infections.

5.8 *H. polygyrus* and Viral titre

Lung RSV titres, assessed by immunoplaque assay, are reduced following *H. polygyrus* infection (McFarlane *et al*., 2017). However, despite the protective effects of *H. polygyrus* infection transmitting from the gut to the lungs, the specific factor(s) responsible for the antiviral effects remain unknown. To assess if a serum born factor was responsible for establishing the anti-viral state, acellular serum from the blood of mice infected with *H. polygyrus,* was transferred to naïve mice. Twenty-four hours later RSV was administered intranasally, and viral lung titres were assessed at the peak of infection. *H. polygyrus* infected serum was able to replicate the anti-viral effect, compared to the transfer of serum from naïve mice. To ascertain whether the protective serum factor was a protein, researchers within the Schwarze group performed a heat-inactivation of serum prior to transfer. The outcome indicated that the protective effect was evident in untreated serum, compared to the heat-treated group, where protection against RSV was lost. This finding confirmed that the serum factor is most likely to be a protein, as proteins denature at high temperatures. It was concluded that serum transfer of *H. polygyrus* infected serum alone could effectively reduce the viral load within the lung, and the physical presence of *H. polygyrus* within the gut was not necessary for this anti-viral effect. To identify proteins elevated in serum from *H. polygyrus* infected mice, in collaboration with Dr. Henry J McSorley, a proteomic analysis was conducted through abundant protein depletion (IgG and albumin) and mass spectrometry of naïve mouse serum and *H. polygyrus* infected mouse serum (Figure 2). The analysis revealed that Ccl8 cytokine was significantly elevated, prompting further investigation into its potential involvement in producing an anti-viral effect against RSV.

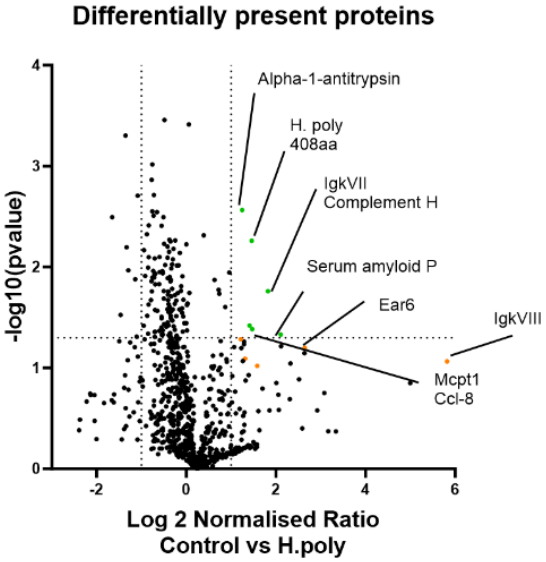

Figure 2: Proteomic analysis of serum retrieved from *H. polygyrus* and naïve mice.

The figure shows differentially elevated cytokines in both *H. polygyrus* infected serum and naïve serum samples after mass spectrometry analysis was conducted on serum .

5.9 The role of Ccl8 Chemokine (MCP-2) in immunity and Ccl8 rationale

The Ccl8 cytokine, previously known as monocyte chemoattractant protein-2 (MCP-2), is a chemoattractant for several immune cells including macrophages, monocytes, T cells, and NK cells, aiding in the development of inflammatory responses (Israr *et al*., 2022). Khalil *et al*., (2021) reports on the role of chemokines and chemokine receptors during COVID-19 infection and how they exert their anti-viral effects. Ccl8 is described as a trafficking mediator for neutrophils and is highly involved in inflammatory processes especially those associated with viral infections, previously Ccl8 levels within nasal fluid were discovered to correlate with the severity of acute respiratory tract infections (Henriquez *et al*., 2014).

CCR-2 is described as a chemotactic receptor and primary receptor on monocytes (Carter, 2002; She *et al*., 2022), Burgess *et al*., (2024) findings show that when assessing if *H. polygyrus* infection induced an increase of mononuclear phagocytes it led to an antiviral effect against RSV, show that CCR2-expressing cells are required for the expansion of circulatory monocytes (mononuclear phagocytes and classical monocytes) as prior anti-ccr2 treatment in the murine models prevented decrease in RSV load. Therefore, because CCR2 is a receptor for CcL8, Ccl8 can be considered an interesting target among the elevated cytokines which warrants further investigations.

Overall, Ccl8 is crucial in many immune responses and disease progression, highlighting its potential as a therapeutic target may be beneficial for those who are hospitalised against severe RSV.

5.10 Hypothesis and objectives

Current treatments for RSV are supportive and primarily focused on managing symptoms, vaccinations have been made available in the UK for over 75's and pregnant women however, there still remains a significant lack of anti-viral medications available for those infected and hospitalised. Identifying the factors in *H. polygyrus* serum which are responsible for driving anti-viral responses to RSV may contribute to the development of further therapeutic interventions.

This study hypothesises that Ccl8 is responsible for mediating the antiviral effects of *H. polygyrus* serum transfer against respiratory syncytial virus (RSV).

The overall objective of this study is to test if the presence of Ccl8 within serum is essential for the anti-viral effect that *H. polygyrus* serum confers against RSV infection and to assess if serum transfer replicates the monocytosis observed in a native *H. polygyrus* infection.

6 Methods

6.1 Hep2 cell culture

26

Cell culture media (RPMI Gibco cell culture media lot no: 31870-025, with 10% FBS, 1% penicillin streptomycin, 1% L-glutamine and 1% MEM non-essential amino acids lot no:2517555) was heated at 37ºC in water bath. HEp2 cells were retrieved from liquid nitrogen and transported in dry ice to cell culture hood. Vials containing HEp2 cells were rapidly thawed in a 37ºC water bath. Cells diluted to 5ml total in pre-warmed cell culture medium. The tube was centrifuged at 20ºC, 300 xg for 5 mins (Thermoscientific, Multifuge 3x FR Heratus). The supernatant was poured off and the pellet resuspended in fresh cell culture medium and transferred to a cell culture flask. Cells were left to grow in an incubator at 37ºC with 5% CO_2 and did not exceed 85% confluency where they were passaged by trypsin EDTA incubation. Cell culture medium was removed from flasks containing cells and a gentle wash of monolayer was performed with serum-free RPMI Gibco media (lot no:31870-025). Serum-free media was removed and 1ml of Trypsin EDTA was added to trypsinise cells, flasks were left in the incubator for 5-10 minutes. Cells were then observed to check adherence, once no longer adherent warm cell culture media was added to flask. All liquid from the flask was transferred into a falcon tube. When transferred, a small aliquot of cells were counted using tryphan blue. The remaining cells were centrifuged at 20 degrees, 300g's for 5 mins (Thermoscientific, Multifudge 3x FR Heratus). The supernatant was poured off and resuspended in cell culture media (media amount which was added was calculated based on percenntage of cells). Culture media was added into new flasks followed by the resuspended cells.

6.2 RSV viral growth with Hep2 cells

RSV A2, a lab adapted strain, was used and a multiplicity of infection (MOI) of 0.1 was desired with a viral stock of 5 x10^6 pfu/ml.

Hep2 cells were seeded in T175 flasks using cell culture media ,cells were left to incubate at 37ºC and 5% CO_2 until 90-95% confluency was reached. One flask of cells was dissociated for cell counting to calculate the required viral stock dilution for MOI 0.1. Next, RSV was thawed quickly, to maintain viral titre, in a water bath at 37ºC, diluted to MOI 0.1 and a total of 5ml was immediately added to each flask. Flasks were left to incubate at 37ºC for one hour and a half with flask rotations every 20mins to evenly distribute the virus between cells, and then 25mL of cell culture medium was added. Flasks were then incubated until 90% infection (dying cells and/or syncytia formation). After incubation ¾ of media was removed from each flask and transferred into a falcon tube stored on ice. Tubes were centrifuged at 4ºC ,900x*g* for 10mins, the supernatant was poured off and the pellet was resuspended and kept on ice. A cell scraper was used to remove the remaining cells from the base of the flask, the cells were then collected in the remaining media and transferred into falcon tubes. The tubes were then placed in a sonicator (Ultrawave sonicator 220-240 Voltz) with cold

water, water was pre-chilled with an ice bag prior to use. The pellets, on ice, were pooled together with the sonicated cells into one falcon tube and centrifuged. The supernatant was collected into a separate tube and aliquoted as 1ml in separate tubes on ice. The remaining pellet was vortexed for 3 mins until resuspended then centrifuged and aliquoted in separate tubes. All tubes were flash frozen in liquid nitrogen for transportation and then stored in liquid nitrogen for later use. The supernatant stock was used for murine infections.

6.3 *H. polygyrus* serum incubation with Ccl8 antibody

Serum derived from the blood of mice infected for 10 days with *H. polygyrus* was incubated with 100 µg/ml of anti-Ccl8 antibody purchased from Biotechne R&D systems (Lot no: JYC0323051) for 24 hours on a rotary shaker at 127rpm at 4ºC. Naïve/normal serum and remaining *H. polygyrus* serum which did not receive anti-Ccl8 was also kept at 4ºC, 127rpm for 24 hours to ensure both serums underwent similar conditions. (Not all *H.polygyrus* infected serum was incubated with anti-ccl8, some infected serum was kept aside creating 3 groups for experimentation: *H. polygyrus* infected serum depleted of Ccl8, *H. polygyrus* infected serum and naïve/normal serum)

6.4 Depletion of Ccl8 from *H. polygyrus* serum with Sepharose G column

Following incubation, the previously aliquoted *H. polygyrus* infected serum which had anti-Ccl8 was added to a separate pre-made Protein G (HP Spintrap^{TM}, Cytiva, lot no: 181790401) column. The columns were inverted 5-10 times by hand to resuspend the medium, the bottom cap of the column was removed and kept, and the column was placed in a 2ml Eppendorf tube. The top cap of the column was unscrewed slightly, and the column was centrifuged at 70-100 x*g* for 30 seconds to remove the storage solution using a microcentrifuge (accuSpin Micro17R, Fisher scientific). Immediately after centrifugation, the bottom cap was reinserted into the spin trap column to avoid leakage. 600 µl of binding buffer (0.33g Na_3PO_4, 10mls DH_2O, pH7) was added to the column to wash. The column was placed in a 2ml Eppendorf tube and was centrifuged to collect flow through. A maximum of 600 µl of sample was added to the column, both top and bottom caps were secured, and the column was incubated on a roller for 4 mins at room temperature. The column was placed in a new Eppendorf tube and the sample was eluted by centrifugation. The sample was aliquoted accordingly and placed in Eppendorf tubes for storage at –80ºC until further use. Only a select amount of *H. polygyrus* infected serum was incubated with anti-Ccl8 and depleted(as mentioned in the previous section), the remainder of the aliquoted *H. polygyrus* infected serum and naïve/normal serum also did not undergo Ccl8 depletion. However,

all serum samples did go through the Protein G tubes without prior anti-ccl8 incubation to maintain similar experimental conditions.

6.5 Animals

Female BALB/c mice 10-12 weeks old were purchased from Charles River (Margate, Kent) and housed in the animal care facility of our institution. They were housed in filter cages with bedding, food, water, mouse loft and wood shavings at a room temperature of 27ºC degrees. Mice were allowed to acclimate for 1-2 weeks before experimentation. Mice were infected by oral gavage with 200 stage 3 *H. polygyrus* larvae, 10 days post administration mice were humanely culled and whole blood was collected in BD microtainer blood collection tubes for serum separation. Serum was similarly processed from mice sham infected with H_2O as a control. Serum was administered to naive mice intravenously, and either 24 hours or 48 hours later mice were intranasally infected with RSV. Guidelines provided by The University Of Edinburgh and The Home Office were followed and during experimentation, mice were monitored daily through visual inspection and weight checks.

6.6 Preparation of Third larval stage (L3) H. *polygyrus* stock

H. polygyrus larvae stocks were counted under a dissection microscope, stock was inverted and vortexed vigorously to resuspend larvae and the count was performed several times and averaged to account for sampling variation. Stocks were diluted to a concentration of 1 worm per µL such that 200 larvae were present in 200 µL of the *H. polygyrus* solution with dH_2O.

Stage 3 larvae were kindly provided by the Amy Buck lab from the University of Edinburgh.

6.7 Oral gavage administration

Prior to each gavage the larvae in the falcon tubes were vigorously resuspended by inversion and flicking to avoid clumping that can result in concentrated infection sites which can cause illness or death. Using a 1ml syringe, 200 µL of solution was drawn up; the BALAB/c mouse was scruffed to ensure a good straight alignment of the throat and oesophagus. The needle was passed via side of the mouth, along back of throat

and down the oesophagus, with minimal resistance. The 200 µL was dispensed into the stomach once the needle was correctly positioned.

6.8 Animal cull, blood withdrawal and serum retrieval

Mice were culled using the schedule 1 technique; cervical dislocation under injected anaesthesia of medetomidine (Domitor 1mg/ml, Orion pharma,pom-v: 06043) and Ketamine (Ketamidor, Chanelle, 100mg/ml). For confirmation, exsanguination of major blood vessel was performed, and blood was collected using a syringe into SSTD™ tubes (SST™ BD Microtainer Lot:1300563). Blood was stored at 4ºC to reduce the metabolic rate of cells and decrease the chance of degradation until use.

6.9 Preparation of serum for *in vivo* use

Blood was kept on ice and spun down with a microcentrifuge for 2 mins at 10,000 xg, 4ºC. Blood tubes were transferred into a class II microbiology safety cabinet (MBSC) and serum was taken from the different sample tubes and pooled together into a bijou tube. The pooled serum was filtered into a new bijou tube using a 1ml syringe and a 0.22 µm filter to remove any cellular debris. The filter was flushed with air, by drawing up the syringe after each injection of serum through the filter to remove excess residue from within the filter. Serum volume was measured and diluted 1:5 with sterile DPBS (GIBCO lot no: 140-091,–Mg –Ca). Diluted serum was either immediately processed for antibody depletion or aliquoted into Eppendorf tubes ready for serum transfer to naïve mice or frozen at -80ºC for later use.

6.10 Intravenous serum transfer

Naive mice were placed in a heating box for 10 minutes at 37ºC and were restrained using a mouse restrainer. 200 µL of naïve or infected serum was administered into the tail vein of naïve mice with a 25G needle. Mice were continuously monitored after injection to ensure no adverse effects and quick recovery.

6.11 RSV Intranasal

A MBS cabinet was used for this procedure, and an anaesthesia cage was connected inside to an isoflurane rig (vet tech solutions) filled with Isoflurane (Isofane, Henry Schein, REF: 988-3245). The rate of flow was set to 2L/minute with 4%-4.5% isoflurane in the presence of a connected Oxygenator (VTS vet tech) which was allowed to reach 95% before the Isoflurane rig was in use. An Aloabsober (VTS active scavenging unit, AN005) was also used. RSV was kept frozen on dry ice before use, the vial was then thawed rapidly immediately before use and each mouse was anaesthetised individually by placing them into the induction chamber. Their chest was monitored until their respiration rate slowed down. Then they were removed from the cage and 50 µL of RSV was pipetted into the nostrils of the mouse. Each mouse was laid in a holding cage and monitored for recovery.

6.12 Tail vein blood sample collection

Mice were weighed and following the University of Edinburgh blood sampling guidelines no more than 10% of blood according to each mouse's weight was withdrawn daily, and no more than 15% of blood according to the mouse's weight was drawn within 28 days of experimentation.

Blood sampling occurred from the tail vein, using a needle prick to induce bleeding, and no more than 50 µl was taken per mouse. The blood sample was stored on ice in an Eppendorf tube which contained 0.1x blood volume of EDTA 0.5mM, pH 8.0 purchased from Invitrogen (Lot: 2036899).

6.13 RSV Immunoplaque assay

A plaque assay was performed to quantify the viral titre in the lungs subsequent to Respiratory Syncytial Virus (RSV) infection and serum transfer.

24 hours prior to lung harvest, a concentration of 2x10^4 HEp2 cells at 80% confluency were transferred to a 96-well plate and left to incubate overnight. Cells were healthy, not overgrown or heavily passaged prior to use. Cell culture of Hep2 cells was performed from a T175 flask, after centrifugation and resuspension of cells with cell culture media 15 µL of cells were counted with 15 µL of Tryphan blue to determine the amount of cell culture media required to dilute cells to the desired concentration.

Lungs were homogenised using a Tissue Lyser (Qiagen) and then centrifuged using a microcentrifuge. In a biohood, the supernatant was pipetted into Eppendorf tubes and then re-centrifuged to get a smaller volume of lung homogenate and separate homogenate from debris. This was carried out on low temperatures, on ice.

Assay

In a MBSC 100 µL of serum-free RPMI media was added to a new 96 well-round bottomed plate, the plate was split according to the samples and left to incubate for 30mins. The plate was then placed on ice, and from the recentrifuged Eppendorf tubes 175-200 µL of lung homogenate was pipetted into the corresponding triplet sample wells of that plate. Next, a 10-fold dilution was performed down the plate by taking 100 µL.

Media from the plate which was incubated overnight containing the seeded cells was discarded, then replaced with 100 µL of warm serum-free RPMI media to wash over the monolayer, the plate was then left to incubate. Pipetting was done slowly and at the edge of the well to not disrupt the monolayer.

Media was discarded from the 96 well plate which contained Hep2 cells, and 50 µL from each well of the plate containing the viral lung homogenate was slowly pipetted into the corresponding row/well of the plate containing the HEp2 cells. The plate was

left to incubate for 1-2hours at 37ºC. After,150 µL of cell culture media (10%FBS) was added to each well and the plate was left to incubate for 24 hours for viral infection of cells.

24 hours later, media from the plate containing HEp2 cells was discarded, and cells were washed once with 180- 200 µL DPBS (+Ca, +Mg) . DPBS was thrown off and cells were fixed and incubated at 37ºC for 20 mins with 100 µL/well of methanol containing 2% hydrogen peroxide (SIGMA, Lot: STBH5484). Later 100 µL of BSA (1%PBS, no sodium azide,1g Bovine serum albumin Lot: SLCC3848,500ml PBS) was added to wash each well and discarded. Anti-RSV biotinylated antibody (Invitrogen, Lot no: XE3594374) was made in BSA with PBS (12ml PBS, 60 µL Anti-RSV) to create a solution and 100 µL was added to each well, the plate was left to incubate for 1 hour at RT.

The plate was then washed twice with DPBS(-Mg,-Ca), and 100 µL Extravidin peroxidase solution with PBS was added to each well(12ml PBS, 24 µL Extravidin peroxidase). The plate was incubated at room temperature for 30mins and washed twice. A solution using the AEC substrate kit (BD Pharmigen^{TM}, BD biosciences Lot no:2101893) was made, and 50 µL was added into each well. The plate was left to incubate at RT until plaques fully formed. The plate was finally washed twice with PBS to stop the reaction and reduce red background on plate. Plaques were counted under a microscope

6.14 Imaging/Microscopy

Plate wells were imaged using an EVOS XL core Microscope with software Version 1.0.198.

6.15 ELISA Assay

A sandwich ELISA was performed using Bio legend ELISA MAX™ DELUXE set (lot no: B414790) to measure cytokine Ccl8 concentration serum.

Reagents
Coating Buffer B (5x)
Capture antibody (200x)
Assay Diluent A (5x)
Detection antibody (200x)
Avidin-HRP (1000x)
Wash buffer- (0.05% Tween 20 + PBS)
Stop solution -2N H2SO4
Deionized water

Table 1: reagents for ELISA assay

Plate preparation

The previous day an ELISA plate (high binding 96 well plate) was prepared by coating with capture antibody solution to assist in antigen immobilization, sealed and incubated overnight between 2ºCand 8ºCdegrees.

Sample and standard preparation

The provided lyophilized standard was reconstituted (0.37 mL of 1x Assay Diluent A). And a 1:100-1:800 dilution series was performed. The serum samples were prepared through a 1:20-1:1280 dilution series using 1x Diluent A.

Assay

All washing steps were performed by adding 300 µL of washing buffer to each well, then tapping the plate on absorbent tissue paper.

All reagents were brought to room temperature before use and made into a 1x solution as recommended by the manufacturer. The plate was then washed 4 times with washing and blocked by adding 200 µL of 1X Assay Diluent A to each well to prevent non-specific binding and incubated at room temperature for 1 hour with shaking (500 rpm with a 0.3 cm circular orbit). The plate was then washed 4 times, and the standards were added to desired wells in duplicates. Samples were added to the wells, according to the dilution and the plate was incubated at RT with shaking for 2 hours. The plate was washed 4 times and 100 µL of diluted Detection Antibody solution was added to each well. The plate was incubated at room temperature for 1 hour with

shaking to allow for blocking. The plate was washed 4 times and 100 µL of diluted Avidin-HRP solution required for signal amplification was added to each well. The plate was incubated at room temperature for 30 minutes with shaking. Finally, the plate was washed 5 times with soaking for 30 seconds to 1 minute per wash. 100 µL/well of Substrate Solution C was added, and the plate was incubated in the dark for 10 minutes. Once colour change has occurred, 100 µL of stop Solution was added to each well. Absorbance was read at 450 nm and 570 nm within 15 minutes using a plate reader (BIOTEK synergy HT, Gen 5.3.11 program).

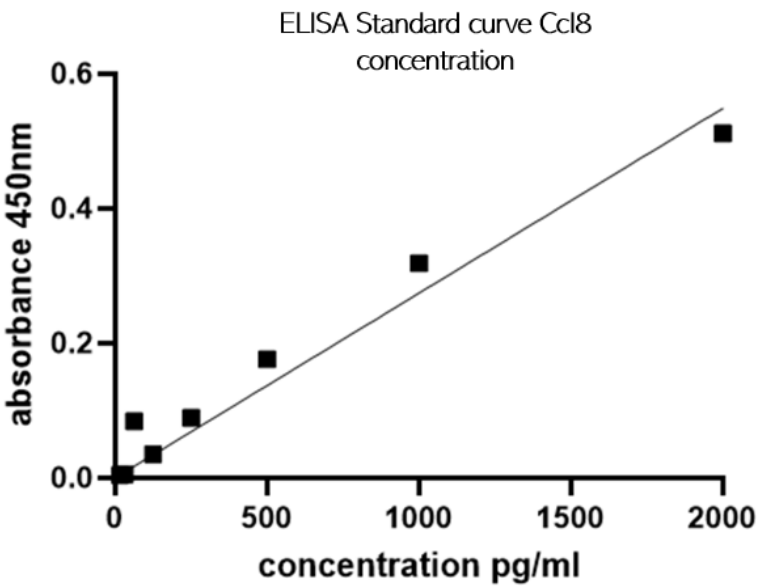

Figure 3: Standard curve of ELISA. Image conveying absorbance at varying concentrations of Ccl8 cytokine.

6.16 Flow cytometry of blood sample

Reagents:	Lot number:
Brilliant violent 421™ CD3	B359945
Brilliant violent 421™ CD19	B399079
Brilliant violent 510™ Ly-6G	B266675
Brilliant violent 605™ CD45	B375997
Brilliant violent 650™ CD11b	B291486
APC anti-mouse CD115	B277119
PE Treml4	143303 (cat no)
Brilliant violet ™ Alexa Flour 700 Ly6C	B388651
Near IR (780)	2486537

Table 2: reagents and lot number for flow cytometry

All steps were carried out at 4ºC, and Eppendorf tubes were prepared with 0.1x blood volume of 0.5mM EDTA for blood collection.

10x blood volume of 1x RBC lysis buffer (Biolegend, Lot no: 420301) was added to the Eppendorf tube of each sample, an incubation of 5mins was set and a long vortex step for each sample was performed (20 seconds).

100x blood volume of FACS buffer was added to each sample and centrifuged at 4ºC , 300 xg for 5 mins using a micro centrifuge. (accuSpin Micro17R, Fisher scientific). The supernatant was discarded and 10x blood volume of 1x RBC lysis buffer was added to each sample and an incubation of 5 minutes was set. A long vortex step for each sample was performed (20 seconds). Each Sample was centrifuged using a microcentrifuge. The supernatant was discarded and the pellet was resuspended. Control samples were created by taking an aliquot from each sample, creating pooled control samples. Samples were centrifuged at 300 xg, 4ºC for 5 mins. The supernatant was discarded and the pellet was resuspended with 100 µL of FACS buffer and 1 µL of FC block per sample, including controls. A 1:1000 Live dead fixable buffer solution was made and 100 µL of live dead stain was added to each sample (live dead fixable nearIR 780) and left to incubate in dark for 20 mins. Samples were washed 2x with 100 µL PBS and resuspended in 100 µL FACS buffer. A full stain solution/mix with FACS buffer and brilliant stain buffer (BD Horizon ™ Lot no: 3199146) was created and 100 µL of the solution was added to each sample

including a chosen control sample. Samples were left to incubate for 30mins at 37ºC in the dark and washed 2x in 100 µL of FACS buffer twice and resuspended in 200 µL of A 1:1 fixation buffer until required, and stored at 4ºC . When required samples were 2x washed and resuspended in 200 µL FACS buffer for Flow cytometry analysis.

Single stain controls

One drop of Ultracomp ebeads ™ (Invitrogen Lot no: 28502464) was added to empty Eppendorf tubes.1 µL of each single stain was added and stains were left to incubate in dark at 4ºC for 30 minutes.

100 µL of FACS buffer was added and stains were washed 2x and resuspended in 200 µL of FACS buffer.

6.17 Flow cytometry analysis

Analysis of blood was carried out on a NovoCyte Penteon by Aligent and analysed using the NovoExpress 1.6.2 software. Data was analysed using Flowjo software 10.10.

6.18 Gating strategies

Figure 4 shows the various gating steps, with the initial step showing the total amount of cells which were gated, excluding debris. Next doublet exclusion was performed on the total amount of cells to show singlets, gating continued, and dead cells were excluded with the aid of Near IR(780) staining while CD45+ cells were gated on. Following the gating of live cells, CD11b+ cells were identified allowing for CD115+ cells to be gated and identified followed by gating of monocytes (Ly6C+ Treml4-, Ly6C+ Treml4 +, Ly6C- Treml4+).

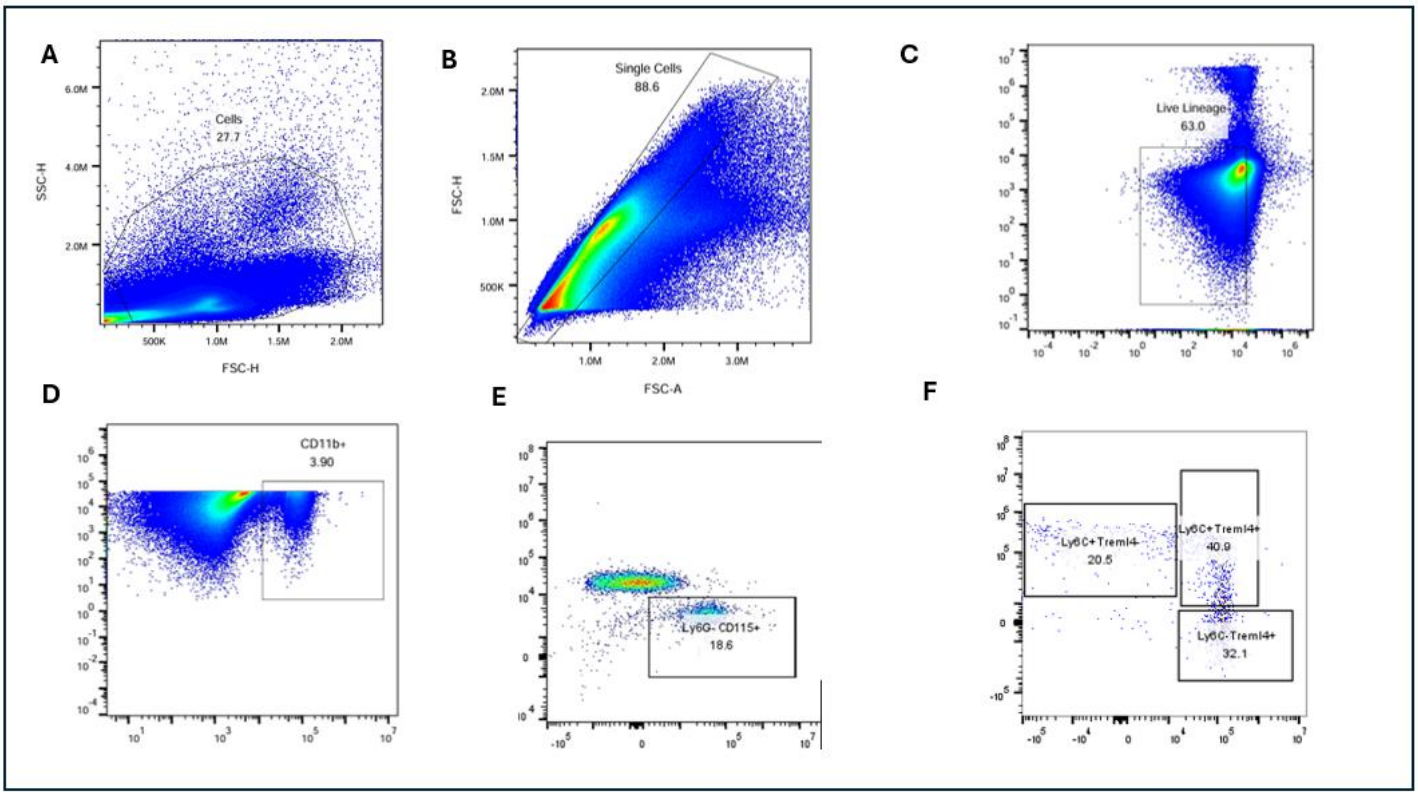

Figure 4: Cell gating strategy (**A**) Debris excluded from total cell count (**B**) doublets were excluded to show singlets (**C**) dead cells were excluded and CD45+ cells were gated (**D**) CD11b+ cells were gated (**E**) CD115+ cells were gated (**F**) Monocytes gated (Ly6C+ Treml4-, Ly6C+ Treml4 +, Ly6C- Treml4+).

6.19 Graphpad prism

10.2.3 software was used to generate statistical data and represent data in graphs. The Mann-Whitney U test was used to calculate statistical data.

7 Results

7.1 Blood monocyte levels 24, 48 and 144 hours post serum transfer.

It has previously been shown that ongoing *H. polygyrus* infection is protective against RSV infection, and induction of mononuclear cells in blood circulation and into the lungs is a key mechanism for the process of the anti-viral effect. As serum transfer of *H. polygyrus* serum is also able to induce an anti-viral response It was tested if serum transfer also enables the alteration of blood monocytes. Mice were administered 200 µL of serum, which was derived from either mice infected with *H. polygyrus* or naïve mice, the serum was also diluted 1:5 with PBS (-Ca,-Mg) and administered via intravenous injection. Blood samples were collected from the tail vein of recipients at 24- and 48-hours post-serum transfer, followed by flow cytometry analysis to evaluate monocyte levels. RSV was administered 48 hours after serum transfer, after blood withdrawal. The final blood sample was analysed 6 days post-serum transfer and 4 days post RSV intranasal.

Triplicate results of either mice receiving naïve or infected serum are displayed with error bars ± SEM. Results shown are a percentage (%) of gated CD45+ cells that are classical, intermediate or non-classical monocytes from each sample. On day one, (24 hours post-serum transfer) the percentage of CD115+ cells in the control group surpasses the number in the experimental group, with the intermediate subset possessing the increase in monocytes in both groups, closely followed by the classical subset (Figure 5). On day 2,(48 hours post serum transfer) the percentage of CD115+ cells in the control group (naïve serum) continued to surpass the experimental group and the intermediate subset conveyed the highest level of monocytes among both groups, with the control group surpassing the experimental group (Figure 6).

Overall, this suggests that serum transfer from *H. polygyrus* infected animals' results in reduced monocyte counts in compared to the increase in blood monocytes previously observed in animals with *H. polygyrus* infection. However, no significant statistical differences can be concluded between the two experimental groups. This highlights the need for further investigations and an increase in the sample size, which is currently limited to three per group.

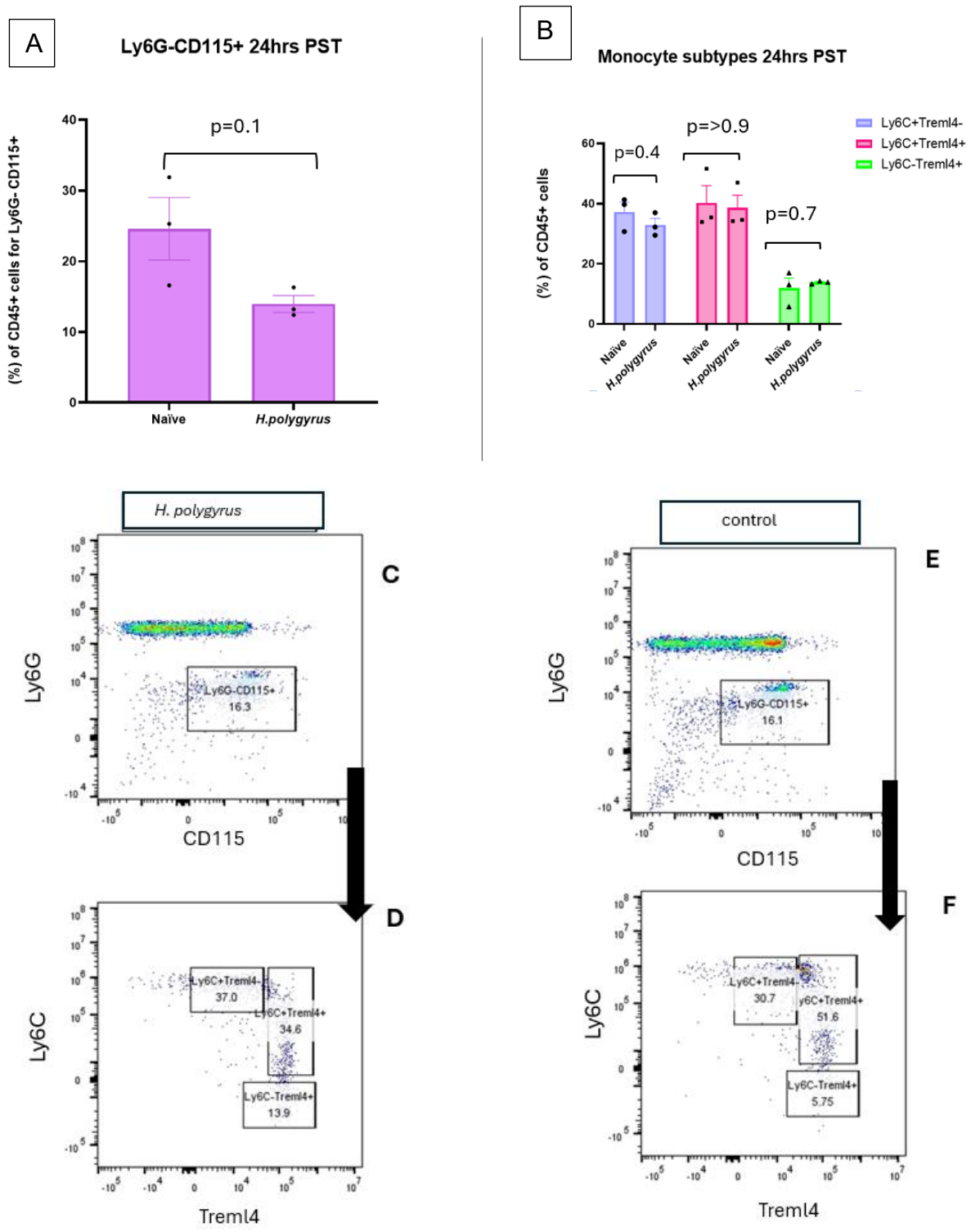

Figure 5: Monocyte percentages 24hrs post serum transfer: monocytes (CD115+ cells) are higher in mice receiving naïve serum at 24 hours and the intermediate

monocyte subset is the most expanded at 24 hours in both experimental groups. Mice were given 200 µL tail vein injection of either Naïve or *H. polygyrus* infected serum and 50 µL of blood was retrieved from tail vein for monocyte analysis n=3. Results show (**A**) Blood monocyte percentages 24hrs after serum transfer (**B**) Blood monocyte subtype percentages (**C-F**) Representative flow cytometry plots of gated monocytes in both experimental groups including classical monocytes (Ly6C+Treml4-), intermediate monocytes (Ly6C+ Treml4+), and non-classical monocyte (Ly6C-Treml4+). Statistical difference was determined using Mann-Whitney U test.

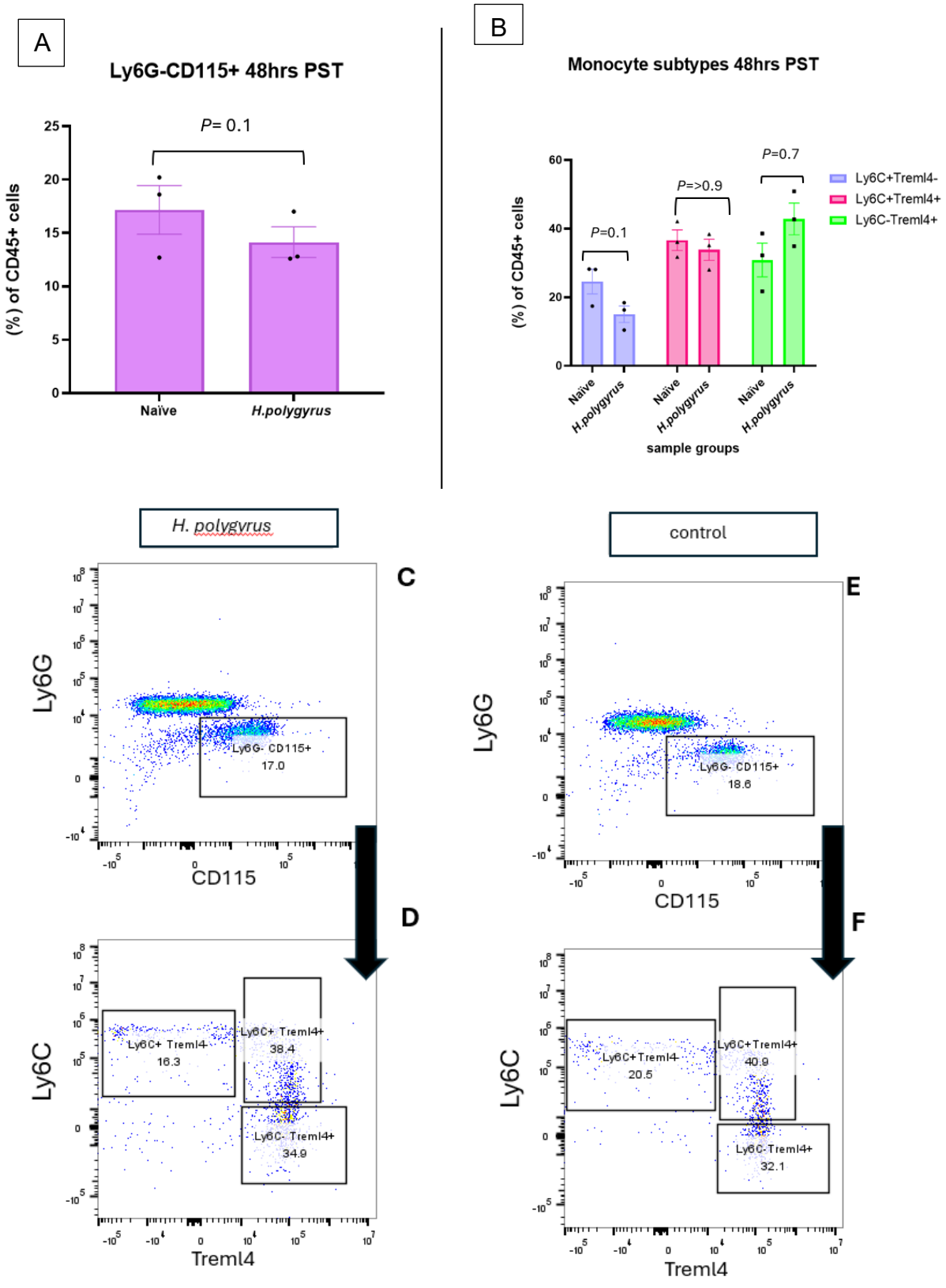
A
Ly6G-CD115+ 48hrs PST
P= 0.1
(%) of CD45+ cells
25
20
15
10
5
0
Naïve
H.polygyrus
B
Monocyte subtypes 48hrs PST
P=0.1
P=>0.9
P=0.7
Ly6C+Treml4-
Ly6C+Treml4+
Ly6C-Treml4+
(%) of CD45+ cells
60
40
20
0
Naïve
H.polygyrus
Naïve
H.polygyrus
Naïve
H.polygyrus
sample groups
H. polygyrus
control
C
Ly6G
Ly6G- CD115+
17.0
CD115
D
Ly6C
Ly6C+ Treml4-
16.3
Ly6C+ Treml4+
38.4
Ly6C- Treml4+
34.9
Treml4
E
Ly6G
Ly6G- CD115+
18.6
CD115
F
Ly6C
Ly6C+Treml4-
20.5
Ly6C+Treml4+
40.9
Ly6C-Treml4+
32.1
Treml4

Figure 6: Monocyte percentages 48hrs post serum transfer: monocytes (CD115+ cells) are higher in mice receiving naïve serum at 48hrs and the intermediate monocyte subset is the most expanded in both experimental groups. Mice were given 200 µL tail vein injection of either Naïve or *H. polygyrus* infected serum and 50 µL of blood was retrieved from tail vein for monocyte analysis n=3. Results show (**A**) Blood monocyte percentages 48hrs after serum transfer (**B**) Blood monocyte subtype percentages (**C-F**) Representative flow cytometry plots of gated monocytes in both experimental groups. including classical monocytes (Ly6C+Treml4-), intermediate monocytes (Ly6C+ Treml4+), and non-classical monocyte (Ly6C- Treml4+). Statistical difference was determined using the Mann-Whitney U test.

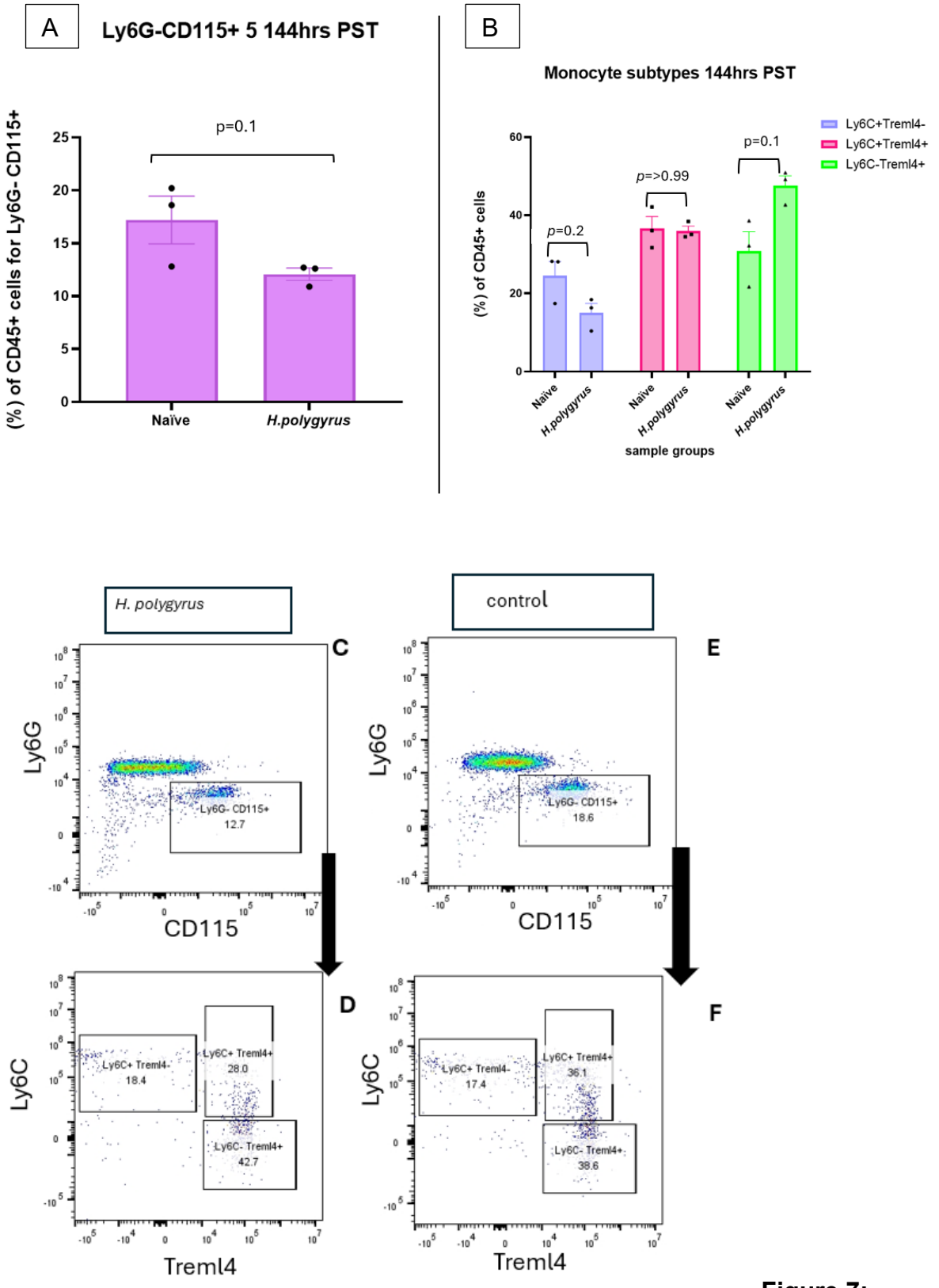

Figure 7:

Monocyte percentages 144hrs post serum transfer: monocytes (CD115+ cells) are higher in mice receiving naïve serum 144hrs post serum transfer and the non-

classical monocyte subset is the most expanded at 24 hours in the group receiving *H. polygyrus* serum experimental groups. Mice were given 200 µL tail vein injection of either Naïve or *H. polygyrus* infected serum, 24hrs later mice were given 50 µL of RSV intranasal and 50 µL of blood was retrieved from tail vein for monocyte analysis n=3. Results show (**A**) Blood monocyte percentages 144hrs after serum transfer (**B**) Blood monocyte subtype percentages (**C-F**) Representative flow cytometry plots of gated monocytes in both experimental groups including classical monocytes (Ly6C+Treml4-), intermediate monocytes (Ly6C+ Treml4+), and non-classical monocyte (Ly6C- Treml4+). Statistical difference was determined using the Mann-Whitney U.

7.2 Ccl8 concentrations in serum, ELISA assay

To find the active factor(s) of *H. polygyrus* infected serum, mass spectrometry proteomics had previously been performed, identifying candidates and differentially present proteins. The chemokine Ccl8 is higher in infected serum and known to play a role in monocyte dynamics so was selected for further investigation. Depletion of Ccl8 from serum from *H. polygyrus* infected mice was performed using Sepharose G columns and anti-Ccl8 antibody to test if its presence was required for the anti-viral effects. To confirm depletion, and validate the previous proteomics results, an ELISA assay was performed to determine Ccl8 concentration in each sample.

H. polygyrus infected serum, in comparison to naïve serum, contains an elevated concentration of Ccl8. Depletion of Ccl8 in *H .polygyrus* serum was successful, and its concentration was slightly lower than in naïve serum and substantially lower compared to *H. polygyrus* serum (Figure 7).

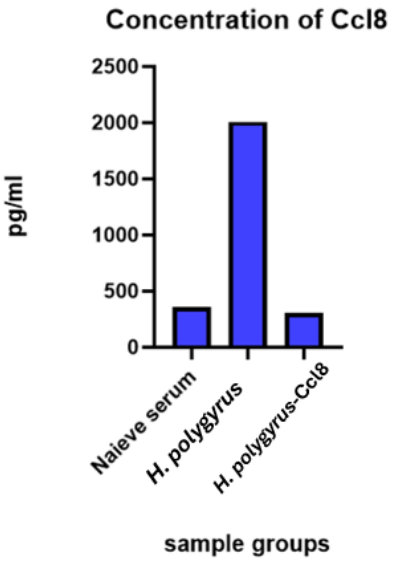

Figure 8: Comparative analysis of Ccl8 concentration across all three serum samples. *H. polygyrus* serum underwent Ccl8 depletion using Sepharose G columns, to assess the concentration of Ccl8 remaining and to determine the success of the depletion procedure, a sandwich ELISA assay was conducted. This assay enabled a comparative analysis of the levels of Ccl8 across all three serum samples. n=1

7.3

7.4 RSV plaque assay of viral load 5 days after Serum transfer and 4 days post RSV intranasal

It has previously been shown that helminth infection confers an anti-viral response against RSV infection. To confirm the antiviral activity of the sera batches, serum transfer was performed via IV. Naïve mice received 200 µL of *H.poygyrus* infected or naïve mice serum, followed by RSV intranasal infection 24 hours later and viral burden in the lung was assessed 4 days later via RSV plaque assay. Mice which received *H. polygyrus* serum transfer show a decrease in syncytia formation/ Viral load.

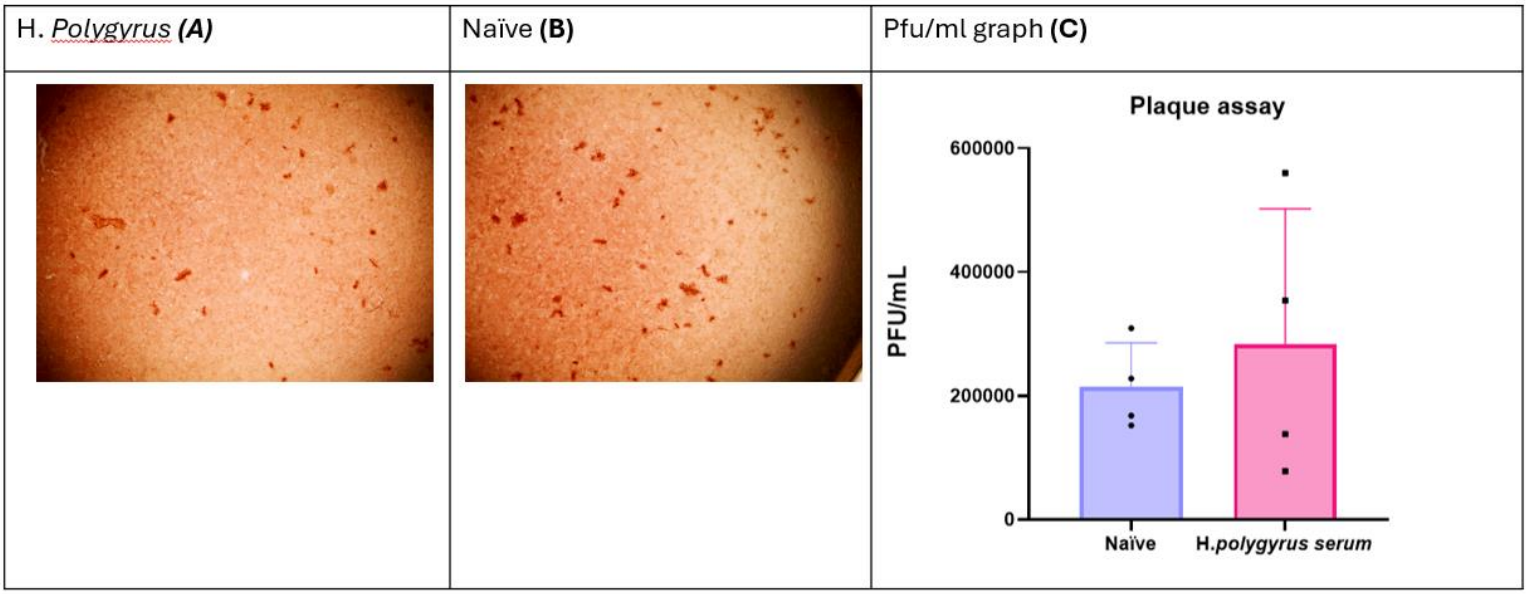

Figure 9: RSV immunoplaque assay: Representative images of RSV immunoplaque assay which highlight viral syncytia that stain positively for anti-RSV antibody binding n=4. (**A**) *H. polygyrus* serum recipients (**B**) Naïve serum recipients (**C**) mean of the total plaque forming units taken from the 10^{-3} fold dilution of mice which received infected or naive serum followed by RSV infection. Each group had 4 mice each and lung homogenates were kept separate for each mouse. A statistical difference was determined using the Mann-Whitney U test.

7.5 Effects of serum transfer of Ccl8 depleted *H. polygyrus* serum on RSV burden

Following confirmation that depleted *H. polygyrus* serum contained the lowest concentration of Ccl8, serum transfer was performed via IV to test if the depleted infected serum was still able to confer antiviral activity, despite lowered levels of Ccl8.

Naïve mice received 200 µL serum from *H. polygyrus* infected, naïve mice or Ccl8 depleted *H. polygyrus* serum, this was followed by RSV intranasal infection 24 hours later. Viral burden in lungs was assessed 4 days later via RSV immunoplaque assay (Figure 9). Mice which received *H. polygyrus* serum transfer show the lowest number of plaques, while mice recipients of naïve serum show an increased number of plaques. Mice which received Ccl8 depleted serum, however, display the highest amounts of plaques overall.

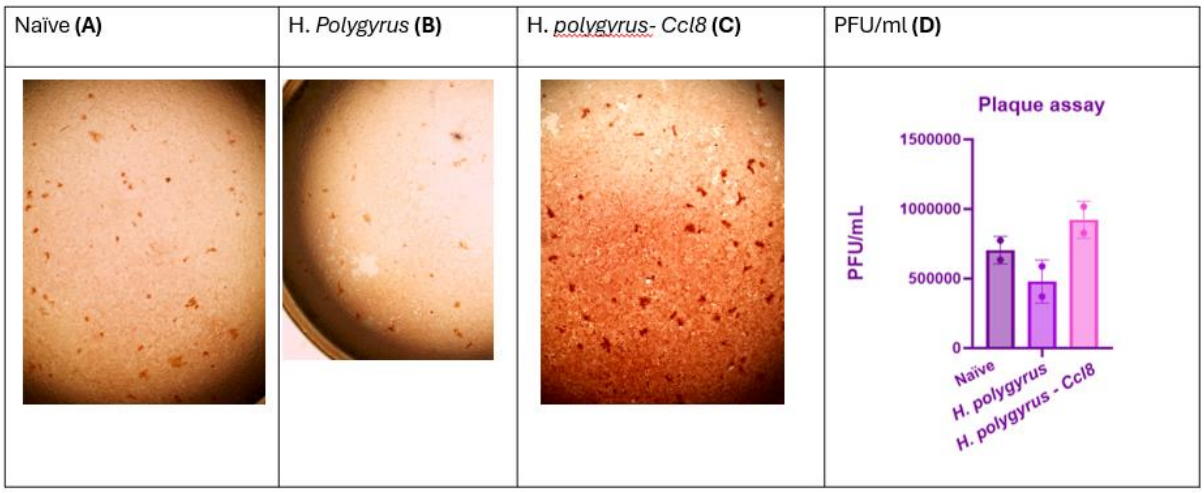

Figure 10: Table displays representative images of RSV immunoplaque assay which highlight viral syncytia that stain positively for anti-RSV antibody binding n=4. (**A**) Naïve (**B**) *H. polygyrus* serum recipients n=4 (**C**) depleted *H. polygyrus* serum n=4 (**D**)mean of the total plaque forming units taken from the 10^{-3} fold dilution of mice which received infected or naive serum. Each group had 4 mice each and lung homogenates were kept separate for each mouse. Statistical difference was determined using one-way Annova.

8 Discussion

These investigations aimed to show that the transfer of infected serum to naïve mice confers antiviral protection against RSV and to evaluate whether serum transfer affects monocytosis, investigations equally aimed to show that reduced levels of Ccl8 within *H. polygyrus* serum results in decreased anti-viral protection. Transfer of serum acquired from *H. polygyrus* infected mice to naïve mice reduced the percentage of monocytes circulating the blood at 48 hours post serum transfer, and 4 days post RSV infection. Serum transfer also increased the percentage of cells within the intermediate subset 48 hours post serum transfer in both experimental groups, followed by the non-classical subset which had a considerably lower percentage of cells. This monocyte results differ from the current literature surrounding helminth induced monocytosis in native infections, instead conveying a slightly higher percentage of intermediate monocytes in comparison to classical monocytes which are more likely to expand and continue to be elevated within the blood.

Importantly, the results for lung titre post serum transfer of Ccl8 depleted *H. polygyrus* infected serum show elevated viral titre in comparison to mice receiving *H. polygyrus* serum or naïve serum. Mice receiving naïve serum also show a high amount of viral titre.

Overall, it is important to note that although there are differences in monocyte numbers, no significant statistical differences can be concluded between both experimental groups, highlighting a need for further investigations and expansion within experimental size which is currently 4 per group. Despite this conclusion, the trends and potential differences in monocytes will still be discussed.

The use of Sepharose G protein column does not affect serum components

The abundant protein depletion that was performed for the mass spectrometry analysis removed IgG. Serum from both naïve mice and *H. polygyrus* infected mice was tested with and without IgG depletion and showed no effect upon providing adequate anti-viral protection, As a result, the use of Sepharose G beads for Ccl8 depletion would not be an issue, and the serums would not lack any other normally present antibodies.

Does monocytosis within the blood 24-48 hours post serum transfer result in changes in monocyte subset proportions?

CD115+ marker is expressed on monocytes, macrophages, and DCs (Kapanadze *et al*., 2023), it serves as a common marker for monocyte progenitors and is also important for mediating protection against monomicrobial systemic E. coli infections (Martin *et al*., 2023). Burgess *et al*., (2024) reported a significant rise in monocytes in peripheral blood from 4 to 14 days post-*H. polygyrus* infection, peaking at 7 days post-infection within mice which were recipients of infected *H. polygyrus* serum. However, in contrast, analysis of the blood of both experimental groups 24 and 48 hours after serum transfer (Figures 5A and 6A), indicates that mice receiving infected serum exhibited reduced levels of CD115+ cells, particularly at the 24-hour mark, suggesting that naïve serum facilitates greater recruitment of monocytes and macrophages.

Intermediate monocytes are specialised in antigen presentation and are commonly associated with later stages of inflammation (Kapellos *et al.,* ; 2019 Weber *et al*., 2000 ; Thieblemont *et al*., 1995). At 24 hours post-serum transfer (Figure 5B), the intermediate subset had a greater expansion compared to the non-classical subsets, with elevated levels of intermediate monocytes also observed at 48 hours post-transfer (Figure 6B) in both experimental groups compared to classical and non-classical monocytes. This observation indicates the possible presence of DC, especially in mice receiving naïve serum, Intermediate monocytes are characterised by their high expression of antigen presentation-related molecules, pro-inflammatory cytokines, and chemokines, however, it also indicates that a small proportion of intermediate subset monocytes may transition into monocytes associated with the classical subset (Patel *et al*., 2017). Existing literature suggests that *H. polygyrus* infection induces DC activation, with intestinal helminths playing a crucial role in modulating the host's response to enteric infections. Consequently, it would be reasonable to anticipate elevated intermediate monocyte levels following serum transfer from *H. polygyrus* infected mice (Chen *et al*., 2006).

Furthermore, the nature of intermediate monocytes means they possess a higher capacity within the blood to secrete cytokines, especially within an inflammatory environment (Ziegler-Heitbrock, 2015 ; Mossanen *et al*., 2020), they are also

recognised for secreting CCR5, a chemokine receptor and HIV coreceptor which also binds to Ccl8. Halvorsen *et al*., (2016) recently investigated its role in mitigating post-infarction inflammation by recruiting suppressive mononuclear cells (Dobaczewski *et al*., 2010). Therefore, the persistent elevation of intermediate subset at 24-48 hours post-serum transfer may be indicative of an adaptive response to counteract/resolve inflammation. Equally, during inflammation classical and intermediate monocytes are said to be tethered and invade tissue through interaction with MCPs (Wong *et al*., 2011). As a result, this may potentially explain the high percentage of the classical subset at 24hrs as there may have been some inflammation post serum transfer.

Additionally, the classical monocyte elevations displayed (Figure 5B) may be attributed to the tail vein injection procedure, as classical monocytes are known to rapidly migrate to sites if inflamed tissues. This inflammation could have been possibly elicited by local inflammation or bruising resulting from serum transfer, which required venepuncture (Turner *et al*., 2014; Sprangers et al., 2016). However, the expansion of the classical monocyte subset is short-lived, and cell numbers decrease notably as displayed at 48 hours post serum transfer (Figure 6B).

Furthermore, at 48 hours post-serum transfer, the findings differ from those reported by Burgess *et al*., (2024) concerning helminth-induced monocytosis, which identified an increase in classical monocytes following *H. polygyrus* infection. The notably lower levels of Classical monocytes may indicate reduced/controlled inflammation as the subset is known for reducing inflammation through tissue invasion. Therefore, it would be plausible to assume that the subset has aided in subsiding inflammation, and it may no longer be required to circulate with an expanded percentage of cells as before. However, Burgess *et al*., (2024) findings are based of native *H. polygyrus* infections and do not take into consideration the outcomes of serum transfer.

The amount of non-classical monocytes observed at 48 hours post serum transfer in the mice which received naïve serum continues to be lower, which may reflect the low proliferative capacity of the subset; therefore, it would be reasonable to anticipate an eventual increase in non-classical monocytes as the period after serum transfer increases. The eventual possible increase of the non-classical subset could be accompanied by a potential decrease in classical monocyte counts as the classical monocytes may transition into non-classical monocytes (Yona *et al*., 2013;Patel *et al*., 2017). This is due to their significant potential for further development before reaching a fully differentiated state (Villar *et al*., 2023), however, despite this, it is important to note that new classical monocytes are constantly emerging from the bone marrow.

Furthermore, it was reported that when monocytes were introduced into the bloodstream through adoptive transfer experiments, they were observed to rapidly lose their distinguishing features and transform into non-classical monocytes within 24 to 48 hours. Transformation could occur if classical monocytes are activated using

TLR7 activation (Mildner *et al.,* 2024). This mechanism could explain the sudden drop of classical monocytes compared to intermediate monocytes 24-48 hours post serum transfer (Mildner *et al*., 2017).

Despite the observed differences between the experimental groups, it can be hypothesised that 48 hours after transfer of either infected or naïve serum the intermediate subset is likely to expand. To strengthen this observation, an additional control cohort that receives no serum, should be included. This group would consist of blood from mice that did not receive any serum, however, the extracted serum would undergo the same handling and processing as experimental sera for accurate analysis and comparison.

Does the transfer of serum infected with *H. polygyrus* protect against RSV infection through monocytosis?

Monocytes are the second type of cells to infiltrate the lungs following RSV infection (Nuriev & Johansson, 2019), in the mice receiving *H. polygyrus* infected serum, there was a lower amount of CD115+ cells observed. This could reflect enhanced recruitment of monocytes into the lungs compared to the group receiving naïve serum.

Furthermore, when considering *H. polygyrus* infection and bone marrow monopoiesis, Burgess *et al*., (2024) reported similar trends in monocyte levels post *H. polygyrus* infection at specified time points, highlighting the absence of significant differences in CD115+ cells between groups receiving naive or infected serum and helminth infected groups at 4/10 days post infection. This may indicate that an extended infection period is necessary to observe changes in CD115+ levels, particularly in the context of infected serum transfer;, subset frequencies may be dependent on helminth exposure.

Figure 7B shows that at 144 hours (6 days) post serum transfer and 96 hours (4 days) post RSV intranasal, lowered levels of classical monocytes in the blood are observed for both experimental groups, compared to the levels of the intermediate subset which displays no significant difference between both experimental groups. This pattern mirrors observations in RSV-infected infants, who display increased intermediate monocytes alongside decreases in classical and non-classical subsets

in the peripheral blood, suggesting the intermediate-dominant profile may be a conserved feature of the host response to RSV Ahout *et al*., 2016). When comparing this observation to mice receiving naïve serum and RSV, we see high levels of the intermediate monocytes, a similar occurrence, as infants who were infected with RSV alone who also possessed high levels of intermediate monocytes, however, high intermediate subset levels are equally observed before RSV administration.

Moreover, the amount of the non-classical subset of mice receiving infected serum surpasses the levels of the mice which received naïve serum. Previously, it has been reported that patients infected with RSV show an increased frequency of non-classical monocytes in the blood. The heightened incidence of non-classical monocytes following RSV infection, particularly in the presence of *H. polygyrus* serum transfer, may suggest the subset's potential involvement of monocytes in cytokine production and migration to the airways, potentially aiding in the replacement of damaged alveolar cells through scavenging damaged cells and clearance of debris from the vascular endothelium (Epelman *et al*., 2014) as monocyte recruitment may aim to re-populate damaged cells within the lung. Furthermore, the sustained high levels of the intermediate subset may be associated with serum transfer, supported by the observation of prolonged circulation of intermediate monocytes in both experimental groups.

When observing figure 7B which depicts the levels of differing subsets classical monocytes show much lower levels in comparison to intermediate and non-classical subsets, however, when the 2 experimental groups are compared, the mice receiving naïve serum have a higher amount of classical cells. Classical cells are reported to initially mount an early inflammatory response but subsequently transition into a reparative state, where they help remove dead cells and restore normal tissue function. Therefore, it could be argued that in the mice receiving the naïve serum, there is an elevation of the classical monocytes to possibly aid in the removal of dead cells from RSV infection and restore lung tissue function.

Moreover, the correlation between the intermediate subset and DC is noteworthy, as DC play a critical role in initiating the adaptive immune response to viral infections. This is particularly significant in the context of RSV, given that there is a recruitment of DCs to sites of inflammation through various chemokines during RSV infection (Foti *et al*., 199911 ;Jensen & Gad, 2010;Shortman & Naik, 2006). The continued circulation of intermediate monocytes may indicate an ongoing adaptive immune response facilitated by the previous serum transfer. Additionally, non-classical monocytes exhibit a reduced tendency to migrate into tissues, and after adoptive transfer have been proposed to differentiate into lung resident macrophages, the marked rise in non-classical cells after intranasal RSV may support this observation and the sudden increase post-RSV administration may indicate the subsets willingness to eventually become resident lung macrophages, during respiratory infection as this increase was not visible with serum transfer alone (Mildner *et al*., 2017 ; Tacke *et al*., 2015).

Overall, it is evident that the transfer of different serums influences the circulatory immune response against RSV infection, the persistent presence of intermediate monocytes and the rise in non-classical monocytes post-RSV infection indicate each subset's potential contributions to producing an anti-viral response for lung recovery.

Additionally, the link between intermediate monocytes and DC indicates the importance of adaptive immunity in RSV pathogenesis. Overall, these findings suggest that targeted manipulation of monocyte functions could aid in reducing tissue damage and promoting recovery during respiratory viral infections.

Transfer of infected serum results in decreased viral titre/load.

Previous studies of RSV infection in the presence of *H. polygyrus* conclude that *H. polygyrus* coinfection substantially reduces RSV viral load in the lungs of mice without affecting the rate of viral replication (McFarlane, A.J. *et al.* 2017). This suggests that *H. polygyrus* may suppress the immune response to RSV rather than directly interfering with viral infectivity. Consistent with those findings, this data shows that mice receiving *H. polygyrus* serum still result in having a lower RSV viral load in comparison to mice receiving naïve serum. This result was expected and therefore, confirms that there is no need for physical helminth presence to achieve a reduced viral load in the lung and that a factor within *H. polygyrus* serum is responsible for driving antiviral protection. However, this does not dispute the previous research which indicates that to achieve an antiviral response against RSV *H. polygyrus* parasite must enter enterically into the host and microbiota must be present. These conditions must occur prior to the retrieval of infected serum.

It is possible to perform successful Ccl8 depletion on previously retrieved *H. polygyrus* serum.

The results indicate that the depletion of Ccl8 was successful, although Ccl8 was not completely eliminated from the serum, the data demonstrates a significant reduction in Ccl8 concentration was achieved when compared to undepleted *H. polygyrus* serum. This finding is critical, as previous research has identified *H. polygyrus* infection as providing anti-viral protection against RSV infection, as a result, lowered levels of Ccl8 may affect anti-viral activity. The notable reduction in Ccl8 levels facilitates for good experimental outcomes.

Ccl8 cytokine is required for anti-viral protection against RSV infection and to achieve a reduced viral load within the lungs.

No prior studies surrounding Ccl8 depletion in the context of RSV administration can be found, therefore, the results for serum transfer of *H. polygyrus* serum, naïve serum and Ccl8-depleted *H. polygyrus* serum indicate that mice which received depleted serum had a notably higher viral load than mice that received *H. polygyrus* infected serum alone. This shows evidence of a relationship between Ccl8 cytokine and the antiviral response of *H. polygyrus* serum against RSV. The results indicate that a lack of Ccl8 or reduced levels may result in reduced recruitment of Ccl8-responsive immune cells to the lungs, such as NK cells, monocytes, B cells and CD8T+ cells.

Additionally, Burgess, M.O. *et al.* (2024) previously reported that an increase in circulatory monocytes and lung mononuclear phagocytes is required for the *H. polygyrus*-induced protective effect against RSV infection. Depletion of Ccl8 may have had an adverse effect on circulatory monocyte and phagocyte recruitment to the lungs, resulting in an expected decreased protective effect.

9 Conclusion

This study investigated blood monocytosis after serum transfers and tested the ability of serum transfer to confer anti-viral protection against RSV. It was also investigated if Ccl8 was responsible for mediating the antiviral effects of *H. polygyrus* serum.

Monocyte populations showed trends indicative of altered subset distributions post-serum transfer, but no statistically significant differences were established, indicating a need for larger sample sizes in future studies. Overall, the findings highlight that the process of serum transfer itself is enough to modulate an immune response.

The findings from serum transfer indicate a significant role of the Ccl8 cytokine in the antiviral response against RSV, mice that received Ccl8-depleted serum displayed higher viral loads compared to those treated with *H. polygyrus* alone, highlighting the possible importance of Ccl8 in facilitating the recruitment of critical immune cells, including NK cells, monocytes, B cells, and CD8T+ cells, to the lungs. The depletion of Ccl8 may compromise the protective benefits usually conferred by *H. polygyrus* infection, suggesting that maintaining adequate Ccl8 levels is crucial for an adequate immune response to RSV. Further studies are warranted to explore the mechanisms by which Ccl8 influences immune cell dynamics and to establish potential therapeutic implications for RSV infections.

The findings also indicate that transfer of infected *H. polygyrus* serum decreases RSV viral load in the lungs of mice, indicating that *H. polygyrus* serum helps to modulate the immune response to RSV. Observations were made, highlighting that mice treated with *H. polygyrus* serum exhibit lower RSV load in the lung reinforces the idea that certain factors within the serum confer antiviral protection. However, it's important to note that prior studies emphasise the necessity of the physical presence of the *H. polygyrus* parasite and the involvement of the microbiota for an effective antiviral response against RSV. Thus, while serum factors may offer protection, the complete mechanistic understanding requires the parasite's entry into the host and the establishment of a conducive microbiota environment.

Finally, it can be acknowledged that monocytes associated with the intermediate subset are highly elevated 24-48 hours PST in both experimental groups, this can also be observed 4 days post RSV infection, however by this time point non-classical monocytes also become elevated, especially within the blood of *H. polygyrus* serum recipients. This highlights the potential role of monocytes in contributing to antiviral protection when serum transfer is administered.

10 Limitations

Throughout this project, numerous limitations were faced. The primary challenge was the lengthy process of obtaining a personal animal license. Although the course began in October, acquiring a personal animal license took until May. Furthermore, much of the work required the presence of a fully trained animal technician, which was difficult to arrange, and as a result, experiments were put on hold multiple times due to staffing issues. This significantly hindered progress, leaving less than four months to complete experimentations. Consequently, this limitation affected the quality of the work produced and the reliability of the conclusions, despite all protocols being followed correctly. Moreover, other elevated cytokines were present during proteomic analysis that also require further investigation and need to be compared to the previously transferred serums against RSV. Due to the difficulties surrounding sample quality lung flow cytometry was not able to be examined, this meant that there was a lack of results and discussion surrounding lung mononuclear phagocytes, this would be beneficial to understanding monocyte recruitment within the lungs after serum transfer. Additionally, there is a possibility that there was a remaining anti-Ccl8 antibody within the depleted serum, this may have had effects on cells recruitment post RSV infection.

11 Future work

Future work would involve further replication of the results acquired in order to have a solid conclusion on Ccl8 cytokine involvement in anti-viral response against RSV. Additionally, a lung harvest from the initial experiment which investigated monocytosis to further understand and consolidate how serum transfer affects monocyte recruitment within the lungs through analysis of mononuclear phagocytes would be beneficial. Further research on the mechanisms behind Ccl8 regulation within the blood in accordance to RSV infection would be paramount in order to understand if the cytokine affects innate immunity e.g recruitment of NK cells, potentially exacerbating RSV infection. Additionally, since it has already been established that between 7 and 10 days of *H. polygyrus* infection monocytes numbers increase, investigations into whether at this particular time point Ccl8 has any role in the recruitment of monocytes which kill RSV cells would also prove vital in understanding the overall role of the chemokine. Additionally, to further understand and consolidate the role of Ccl8 in antiviral protection it would be highly beneficial to use Ccl8 knockout mice (CCL8-/-) in further investigations surrounding serum transfer for dissecting the precise roles of the chemokine in RSV infection .

12 References

Agac, A. *et al.* (2023) 'Host responses to respiratory syncytial virus infection', *Viruses*, 15(10), p. 1999. doi:10.3390/v15101999.

Ahout, I.M. *et al.* (2016) 'Reduced expression of HLA-DR on monocytes during severe respiratory syncytial virus infections', *Pediatric Infectious Disease Journal*, 35(3). doi:10.1097/inf.0000000000001007.

Banchereau, J. *et al.* (2000) 'Immunobiology of dendritic cells', *Annual Review of I immunology*, 18(1), pp. 767–811. doi:10.1146/annurev.immunol.18.1.767.

Bansemir, A.D. and Sukhdeo, M.V. (1994) 'The food resource of Adult Heligmosomoides Polygyrus in the small intestine', *The Journal of Parasitology*, 80(1), p. 24. doi:10.2307/3283340.

Bardsley, M. *et al.* (2023) 'Epidemiology of respiratory syncytial virus in children younger than 5 years in England during the covid-19 pandemic, measured by laboratory, clinical, and syndromic surveillance: A retrospective observational study', *The Lancet Infectious Diseases*, 23(1), pp. 56–66. doi:10.1016/s1473-3099(22)00525-4.

Baron, S. (1996) *Medical microbiology*. 4th edn. Galveston, TX: University of Texas Medical Branch at Galveston.

Bont, L. and Houben, M.L. (2011) 'Commentary: Why are young healthy term infants protected against respiratory syncytial virus bronchiolitis?', *Pediatric Infectious Disease Journal*, 30(9), pp. 785–786. doi:10.1097/inf.0b013e31821fef9e.

Brindley, P.J. et al. (2009) 'Helminth genomics: The implications for human health', PLoS Neglected Tropical Diseases, 3(10). doi:10.1371/journal.pntd.0000538.

Burgess, M.O. *et al.* (2024) 'Helminth induced monocytosis conveys protection from respiratory syncytial virus infection in mice', *Allergy*, 79(8), pp. 2157–2172. doi:10.1111/all.16206.

Chen, C.-C. et al. (2006) 'Helminth-primed dendritic cells alter the host response to enteric bacterial infection', The Journal of Immunology, 176(1), pp. 472–483. doi:10.4049/jimmunol.176.1.472.

Chen, H. *et al.* (2023) 'The impact of helminth-induced immunity on infection with bacteria or viruses', *Veterinary Research*, 54(1). doi:10.1186/s13567-023-01216-3.

Chen, X.-J. *et al.* (2019) 'Hypoxia-induced ZEB1 promotes cervical cancer progression via CCL8-dependent tumour-associated macrophage recruitment', *Cell Death & Disease*, 10(7). doi:10.1038/s41419-019-1748-1.

Chetty, A. *et al.* (2021a) 'Il4ra-independent vaginal eosinophil accumulation following helminth infection exacerbates epithelial ulcerative pathology of HSV-2 infection', *Cell Host & Microbe*, 29(4). doi:10.1016/j.chom.2021.02.004.

Desai, P. et al. (2021) 'Enteric helminth coinfection enhances host susceptibility to neurotropic flaviviruses via a tuft cell-il-4 receptor signaling axis', Cell, 184(5). doi:10.1016/j.cell.2021.01.051.

Dobaczewski, M. *et al.* (2010) 'CCR5 signaling suppresses inflammation and reduces adverse remodeling of the infarcted heart, mediating recruitment of regulatory T cells', *The American Journal of Pathology*, 176(5), pp. 2177–2187. doi:10.2353/ajpath.2010.090759.

Dong, L. and Xing, L. (2024) 'Editorial: The biological mechanism and health effect of co-infection with multiple pathogens', Frontiers in Cellular and Infection Microbiology, 14. doi:10.3389/fcimb.2024.1370067.

Epelman, S., Lavine, K.J. and Randolph, G.J. (2014) 'Origin and functions of tissue macrophages', *Immunity*, 41(1), pp. 21–35. doi:10.1016/j.immuni.2014.06.013.

Espinoza, V.E. (2023) *Histology, monocytes*, *StatPearls [Internet].* Available at: https://www.ncbi.nlm.nih.gov/books/NBK557618/ (Accessed: 14 October 2024).

Everard, M.L. *et al.* (1994) 'Analysis of cells obtained by bronchial lavage of infants with respiratory syncytial virus infection.', *Archives of Disease in Childhood*, 71(5), pp. 428–432. doi:10.1136/adc.71.5.428.

Fabre, T. *et al.* (2023) 'Identification of a broadly fibrogenic macrophage subset induced by type 3 inflammation', *Science Immunology*, 8(82). doi:10.1126/sciimmunol.add8945.

Foti, M. *et al.* (1999) 'Upon dendritic cell (DC) activation chemokines and chemokine receptor expression are rapidly regulated for recruitment and maintenance of DC at the inflammatory site', *International Immunology*, 11(6), pp. 979–986. doi:10.1093/intimm/11.6.979.

Glezen, W.P. (1986) 'Risk of primary infection and reinfection with respiratory syncytial virus', *Archives of Pediatrics & Adolescent Medicine*, 140(6), p. 543. doi:10.1001/archpedi.1986.02140200053026.

Goritzka, M. *et al.* (2015) 'Alveolar macrophage–derived type I interferons orchestrate innate immunity to RSV through recruitment of antiviral monocytes', *Journal of Experimental Medicine*, 212(5), pp. 699–714. doi:10.1084/jem.20140825.

Gotera, J. *et al.* (2012) 'Respiratory syncytial virus infection increases regulated on activation normal T cell expressed and secreted and monocyte chemotactic protein 1 levels in serum of patients with asthma and in human monocyte cultures', *Annals of Allergy, Asthma & Immunology*, 108(5), pp. 316–320. doi:10.1016/j.anai.2012.03.006.

Griffiths, C., Drews, S.J. and Marchant, D.J. (2017) 'Respiratory syncytial virus: Infection, detection, and new options for prevention and treatment', *Clinical Microbiology Reviews*, 30(1), pp. 277–319. doi:10.1128/cmr.00010-16.

Hacking, D. and Hull, J. (2002) 'Respiratory syncytial virus—viral biology and the host response', *Journal of Infection*, 45(1), pp. 18–24. doi:10.1053/jinf.2002.1015.

Hall, C.B. *et al.* (2009) 'The burden of respiratory syncytial virus infection in young children', *New England Journal of Medicine*, 360(6), pp. 588–598. doi:10.1056/nejmoa0804877.

Halvorsen, E.C. *et al.* (2016) 'Maraviroc decreases CCL8-mediated migration of CCR5+regulatory T cells and reduces metastatic tumor growth in the lungs', *OncoImmunology*, 5(6). doi:10.1080/2162402x.2016.1150398.

Han, Z. *et al.* (2023) 'The role of monocytes in thrombotic diseases: A Review', *Frontiers in Cardiovascular Medicine*, 10. doi:10.3389/fcvm.2023.1113827.

Henriquez, K.M. *et al.* (2014) 'Association of interleukin-8 and neutrophils with nasal symptom severity during acute respiratory infection', *Journal of Medical Virology*, 87(2), pp. 330–337. doi:10.1002/jmv.24042.

Hewitson, J.P., Grainger, J.R. and Maizels, R.M. (2009) 'Helminth immunoregulation: The role of parasite secreted proteins in modulating host immunity', *Molecular and Biochemical Parasitology*, 167(1), pp. 1–11. doi:10.1016/j.molbiopara.2009.04.008.

Husain, S.M. et al. (1999) 'Murine gammaherpesvirus M2 gene is latency-associated and its protein a target for CD8+T lymphocytes', Proceedings of the National Academy of Sciences, 96(13), pp. 7508–7513. doi:10.1073/pnas.96.13.7508.

Israr, M. *et al.* (2022) 'Role of chemokines in HPV-induced cancers', *Seminars in Cancer Biology*, 87, pp. 170–183. doi:10.1016/j.semcancer.2022.11.010.

Jain, H. (2023) *Respiratory syncytial virus infection in children*, *StatPearls [Internet].* Available at: https://www.ncbi.nlm.nih.gov/books/NBK459215/ (Accessed: 12 October 2024).

Jenkins, T.P. *et al.* (2017) 'Infections by human gastrointestinal helminths are associated with changes in faecal microbiota diversity and composition', *PLOS ONE*, 12(9). doi:10.1371/journal.pone.0184719.

Jensen, S.S. and Gad, M. (2010) 'Differential induction of inflammatory cytokines by dendritic cells treated with novel TLR-agonist and cytokine based cocktails: Targeting dendritic cells in autoimmunity', *Journal of Inflammation*, 7(1), p. 37. doi:10.1186/1476-9255-7-37.

John, A.E., Berlin, A.A. and Lukacs, N.W. (2003) 'Respiratory syncytial virus-induced CCL5/Rantes contributes to exacerbation of allergic airway inflammation', *European Journal of Immunology*, 33(6), pp. 1677–1685. doi:10.1002/eji.200323930.

Kaler, J. *et al.* (2023) 'Respiratory syncytial virus: A comprehensive review of transmission, pathophysiology, and manifestation', *Cureus* [Preprint]. doi:10.7759/cureus.36342.

Kapanadze, T. *et al.* (2023) 'Validation of CSF-1 receptor (CD115) staining for analysis of murine monocytes by flow cytometry', *Journliyal of Leukocyte Biology*, 115(3), pp. 573–582. doi:10.1093/jleuko/qiad147.

Kapellos, T.S. *et al.* (2019) 'Human monocyte subsets and phenotypes in major chronic inflammatory diseases', *Frontiers in Immunology*, 10. doi:10.3389/fimmu.2019.02035.

Karlmark, K., Tacke, F. and Dunay, I. (2012) 'Monocytes in health and disease — Minireview', *European Journal of Microbiology and Immunology*, 2(2), pp. 97–102. doi:10.1556/eujmi.2.2012.2.1.

Khalil, B.A., Elemam, N.M. and Maghazachi, A.A. (2021) 'Chemokines and chemokine receptors during COVID-19 infection', *Computational and Structural Biotechnology Journal*, 19, pp. 976–988. doi:10.1016/j.csbj.2021.01.034.

Langedijk, A.C. and Bont, L.J. (2023) *Respiratory syncytial virus infection and novel interventions*, *Nature News*. Available at: https://www.nature.com/articles/s41579-023-00919-w (Accessed: 12 October 2024).

Lebu, S. *et al.* (2023) 'Soil-transmitted helminths: A critical review of the impact of co-infections and implications for control and elimination', *PLOS Neglected Tropical Diseases*, 17(8). doi:10.1371/journal.pntd.0011496

Li, Y. *et al.* (2022) 'Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: A systematic analysis', *The Lancet*, 399(10340), pp. 2047–2064. doi:10.1016/s0140-6736(22)00478-0.

Lightowlers, M.W. and Rickard, M.D. (1988) 'Excretory–secretory products of Helminth parasites: Effects on host immune responses', *Parasitology*, 96(S1). doi:10.1017/s0031182000086017.

Liu, A.H. (2015) 'Revisiting the hygiene hypothesis for allergy and asthma', *Journal of Allergy and Clinical Immunology*, 136(4), pp. 860–865. doi:10.1016/j.jaci.2015.08.012.

Mabbott, N.A. (2018) 'The influence of parasite infections on host immunity to co-infection with other pathogens', Frontiers in Immunology, 9. doi:10.3389/fimmu.2018.02579.

Maizels, R.M. (2019) 'Regulation of immunity and allergy by Helminth parasites', Allergy, 75(3), pp. 524–534. doi:10.1111/all.13944.

Mangaonkar, A.A., Tande, A.J. and Bekele, D.I. (2021) 'Differential diagnosis and workup of monocytosis: A systematic approach to a common hematologic finding', Current Hematologic Malignancy Reports, 16(3), pp. 267–275. doi:10.1007/s11899-021-00618-4.

Martin, M.D. *et al.* (2023) 'CD115+ monocytes protect microbially experienced mice against E. coli-induced sepsis', *Cell Reports*, 42(11), p. 113345. doi:10.1016/j.celrep.2023.113345.

McFarlane, A.J. *et al.* (2017) 'Enteric helminth-induced type I interferon signaling protects against pulmonary virus infection through interaction with the microbiota', *Journal of Allergy and Clinical Immunology*, 140(4). doi:10.1016/j.jaci.2017.01.016.

McLaughlin, J.M. *et al.* (2020) 'Respiratory syncytial virus–associated hospitalization rates among US infants: A systematic review and meta-analysis', *The Journal of Infectious Diseases*, 225(6), pp. 1100–1111. doi:10.1093/infdis/jiaa752

Midulla, F. *et al.* (2014) 'Recurrent wheezing 36 months after bronchiolitis is associated with rhinovirus infections and blood eosinophilia', *Acta Paediatrica*, 103(10), pp. 1094–1099. doi:10.1111/apa.12720.

Mohapatra, S.S. and Lockey, R.F. (2008) 'Respiratory syncytial virus infection: From biology to therapy', *World Allergy Organization Journal*, 1(2), pp. 21–28. doi:10.1097/wox.0b013e31816549a2.

Mossanen, J.C. et al. (2020) 'Elevated circulating CD14++CD16+ intermediate monocytes are independently associated with extracardiac complications after cardiac surgery', Scientific Reports, 10(1). doi:10.1038/s41598-020-57700-9.

Mossanen, J.C. *et al.* (2020) 'Elevated circulating CD14++CD16+ intermediate monocytes are independently associated with extracardiac complications after cardiac surgery', *Scientific Reports*, 10(1). doi:10.1038/s41598-020-57700-9.

Njua-Yafi, C. *et al.* (2016) 'Malaria, helminths, co-infection and anaemia in a cohort of children from mutengene, south western cameroon', *Malaria Journal*, 15(1). doi:10.1186/s12936-016-1111-2.

Nuriev, R. and Johansson, C. (2019) *Chemokine regulation of inflammation during respiratory syncytial virus infection*, *F1000Research*. Available at: https://doi.org/10.12688/f1000research.20061.1 (Accessed: 24 October 2024).

Olingy, C.E., Dinh, H.Q. and Hedrick, C.C. (2019) 'Monocyte heterogeneity and functions in cancer', *Journal of Leukocyte Biology*, 106(2), pp. 309–322. doi:10.1002/jlb.4ri0818-311r.

Osborne, L.C. et al. (2014) 'Virus-helminth coinfection reveals a microbiota-independent mechanism of immunomodulation', Science, 345(6196), pp. 578–582. doi:10.1126/science.1256942.

Ożańska, A. (2020) Pattern of human monocyte subpopulations in health and disease - ożańska - 2020 - scandinavian journal of Immunology - Wiley Online Library, Pattern of human monocyte subpopulations in health and disease. Available at: https://onlinelibrary.wiley.com/doi/full/10.1111/sji.12883 (Accessed: 14 October 2024).

Papi, A. *et al.* (2023) 'Respiratory syncytial virus prefusion F protein vaccine in older adults', *New England Journal of Medicine*, 388(7), pp. 595–608. doi:10.1056/nejmoa2209604.

Patel, A.A. *et al.* (2017) 'The fate and lifespan of human monocyte subsets in steady state and systemic inflammation', *Journal of Experimental Medicine*, 214(7), pp. 1913–1923. doi:10.1084/jem.20170355.

Rajamanickam, A. and Babu, S. (2024) 'Monocytes/Macrophages in helminth infections: Key players in host defence, inflammation, and tissue repair', Results

and Problems in Cell Differentiation, pp. 315–340. doi:10.1007/978-3-031-65944-7_13.

Resch, B., Manzoni, P. and Lanari, M. (2009) 'Severe respiratory syncytial virus (RSV) infection in infants with neuromuscular diseases and immune deficiency syndromes', Paediatric Respiratory Reviews, 10(3), pp. 148–153. doi:10.1016/j.prrv.2009.06.003.

Rios Guzman, E. and Hultquist, J.F. (2022) 'Clinical and biological consequences of respiratory syncytial virus genetic diversity', *Therapeutic Advances in Infectious Disease*, 9. doi:10.1177/20499361221128091.

Sanford, D.E. *et al.* (2013) 'Inflammatory monocyte mobilization decreases patient survival in pancreatic cancer: A role for targeting the CCL2/ccr2 axis', *Clinical Cancer Research*, 19(13), pp. 3404–3415. doi:10.1158/1078-0432.ccr-13-0525.

Shortman, K. and Naik, S.H. (2006) 'Steady-state and inflammatory dendritic-cell development', *Nature Reviews Immunology*, 7(1), pp. 19–30. doi:10.1038/nri1996.

Smith, K.A. *et al.* (2012) 'Type 2 innate immunity in helminth infection is induced redundantly and acts autonomously following cd11c+cell depletion', *Infection and Immunity*, 80(10), pp. 3481–3489. doi:10.1128/iai.00436-12.

Sprangers, S., Vries, T.J. and Everts, V. (2016) 'Monocyte heterogeneity: Consequences for monocyte-derived immune cells', *Journal of Immunology Research*, 2016, pp. 1–10. doi:10.1155/2016/1475435.

Stensballe, L.G., Devasundraram, J.K, and Simoes, E.A,(2003) 'Respiratory syncytial virus epidemics: The ups and downs of a seasonal virus', *The Pediatric Infectious Disease Journal*, 22(Supplement). doi:10.1097/01.inf.0000053882.70365.c9.

Stockman, J.A. (2012) 'Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: A systematic review and meta-analysis', *Yearbook of Pediatrics*, 2012, pp. 530–531. doi:10.1016/j.yped.2010.12.025.

Sunderkötter, C. *et al.* (2004) 'Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response', *The Journal of Immunology*, 172(7), pp. 4410–4417. doi:10.4049/jimmunol.172.7.4410.

Tan, T.Q. (2023) *Red Book Atlas of Pediatric Infectious Diseases*. 5th edn. Itasca, IL: American Academy of Pediatrics.

Taylor, G. (2006) 'Immunology of RSV', *Perspectives in Medical Virology*, pp. 43–88. doi:10.1016/s0168-7069(06)14002-1.

Turner, J.D. *et al.* (2014) 'Circulating CD14BRIGHTCD16+ "intermediate" monocytes exhibit enhanced parasite pattern recognition in human helminth infection', *PLoS Neglected Tropical Diseases*, 8(4). doi:10.1371/journal.pntd.0002817.

Valanparambil, R.M. *et al.* (2016) 'Primary heligmosomoides polygyrus bakeri infection induces myeloid-derived suppressor cells that suppress CD4+ th2 responses and promote chronic infection', *Mucosal Immunology*, 10(1), pp. 238–249. doi:10.1038/mi.2016.36.

Varol, C. *et al.* (2006) 'Monocytes give rise to mucosal, but not splenic, conventional dendritic cells', *The Journal of Experimental Medicine*, 204(1), pp. 171–180. doi:10.1084/jem.20061011.

Villar, J. *et al.* (2023) 'Monocytes differentiate along two alternative pathways during sterile inflammation', *EMBO reports*, 24(7). doi:10.15252/embr.202256308.

Wedzicha, J.A. and Seemungal, T.A. (2007) 'COPD exacerbations: Defining their cause and prevention', *The Lancet*, 370(9589), pp. 786–796. doi:10.1016/s0140-6736(07)61382-8.

Weinstock, J.V. and Elliott, D.E. (2012) 'Translatability of helminth therapy in inflammatory bowel diseases', *International Journal for Parasitology*, 43(3–4), pp. 245–251. doi:10.1016/j.ijpara.2012.10.016.

Wescott, R.B. and Todd, A.C. (1966) 'Interaction of nippostrongylus brasiliensis and influenza virus in mice. I. Influence of the nematode on the virus', The Journal of Parasitology, 52(2), pp. 242–247. doi:10.2307/3276478

White, M.P., McManus, C.M. and Maizels, R.M. (2020) 'Regulatory t-cells in helminth infection: Induction, function and therapeutic potential', *Immunology*, 160(3), pp. 248–260. doi:10.1111/imm.13190.

Wu, C. *et al.* (2018) 'Spleen mediates a distinct hematopoietic progenitor response supporting tumor-promoting myelopoiesis', *Journal of Clinical Investigation*, 128(8), pp. 3425–3438. doi:10.1172/jci97973.

Yona, S. et al. (2013) 'Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis', Immunity, 38(1), pp. 79–91. doi:10.1016/j.immuni.2012.12.001.

Zachary, J.F. (2022) Pathologic basis of veterinary disease. St. Louis, MO: Elsevier.

Zarek, C.M. et al. (2023) 'Preexisting helminth challenge exacerbates infection and reactivation of gammaherpesvirus in tissue resident macrophages', PLOS Pathogens, 19(10). doi:10.1371/journal.ppat.1011691.

Ziegler-Heitbrock, L. (2015) ‘Blood monocytes and their subsets: Established features and open questions’, Frontiers in Immunology, 6. doi:10.3389/fimmu.2015.00423.