Universität Hamburg
DER FORSCHUNG | DER LEHRE | DER BILDUNG

125 Years BNITM 1900-2025

Role of the C-type lectin receptor MINCLE in recognition of *Strongyloides ratti* and initiation of anti-helminth immune responses.

Dissertation

submitted in fulfillment of the requirements for the degree of
doctor rerum naturalium (Dr. rer. nat.)

Faculty of Mathematics, Informatics and Natural Sciences
Department of Biology
Universität Hamburg

&

Department of Immunology
Bernhard Nocht Institute for Tropical Medicine

Jennifer Antwi-Ekwuruke
Hamburg
2025

The present work was performed under the supervision of Prof. Dr. Minka Breloer at the Bernhard Nocht Institute for Tropical Medicine (BNITM) in Hamburg.

Evaluators

1st Reviewer:
Prof. Dr. Esther Schnettler
Bernhard-Nocht-Institut für Tropenmedizin
Bernhard-Nocht-Straße 74
20359 Hamburg

2nd Reviewer:
Prof. Dr. Minka Breloer
Bernhard-Nocht-Institut für Tropenmedizin
Bernhard-Nocht-Straße 74
20359 Hamburg

Day of oral defense: 08.10.2025

Eidesstattliche Versicherung:
Hiermit versichere ich an Eides statt, die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Hilfsmittel und Quellen benutzt zu haben.
Sofern im Zuge der Erstellung der vorliegenden Dissertationsschrift generative Künstliche Intelligenz (gKI) basierte elektronische Hilfsmittel verwendet wurden, versichere ich, dass meine eigene Leistung im Vordergrund stand und dass eine vollständige Dokumentation aller verwendeten Hilfsmittel gemäß der Guten wissenschaftlichen Praxis vorliegt. Ich trage die Verantwortung für eventuell durch die gKI generierte fehlerhafte oder verzerrte Inhalte, fehlerhafte Referenzen, Verstöße gegen das Datenschutz- und Urheberrecht oder Plagiate.

Affidavit:
I hereby declare and affirm that this doctoral dissertation is my own work and that I have not used any aids and sources other than those indicated. If electronic resources based on generative artificial intelligence (gAI) were used in the course of writing this dissertation, I confirm that my own work was the main and value-adding contribution and that complete documentation of all resources used is available in accordance with good scientific practice. I am responsible for any erroneous or distorted content, incorrect references, violations of data protection and copyright law or plagiarism that may have been generated by the gAI.

Hamburg, den 12.05.2025 Unterschrift/Signature

Acknowledgement

Looking back at my journey as a PhD student, I have come to appreciate that without the support of various people, I would not have been able to complete it. First of all, I would like to thank God for helping me through the challenging times and also allowing me to show kindness amidst difficult situations. A special thank you to Prof. Dr. Minka Breloer for the opportunity to do my PhD in her lab and her mentorship, Dr. Lara Linnemann for teaching me the techniques I needed for my project and the whole Breloer group. A big thank you to Prof. Tim Gilberger for being a mediator when I needed it the most. Thank you, Dr. Lidia Bosurgi, for the many advice that you gave me concerning my project. To Dr. Mine Altinli, thank you for being a great PhD confidant and a supporter. I would like to thank my parents Veronica and Samuel Antwi for the emotional support and also helping me take care of my children during this period. To my husband Christopher Ekwuruke, thank you for the respect, love and support. Thank you for putting your career on hold for 3 years so that I could go through this process and also for being there for our children when I couldn't be there for them. To all of you who helped me, I would like to announce to you that, your effort did not go to waste because I am now a postdoctoral fellow at Charité University Hospital Berlin.

"It is amateurs who have one big bright beautiful idea that they can never abandon. Professionals know that they have to produce theory after theory before they are likely to hit the jackpot."

Francis Crick, What Mad Pursuit: A Personal View of Scientific Discovery (1988)

Abstract

Strongyloides ratti is a parasitic nematode with tissue migrating and intestinal life stages. Recognition of pathogens and initiation of immune responses is promoted by interaction between pattern-recognition-receptors (PRRs) on immune cells and pathogen-associated-molecular-patterns (PAMPs) present on the parasite. Screening a crude *S. ratti*-derived protein lysate against a library of C-type lectin receptors (CLR), an ancient family of PRR, we found that Macrophage-inducible-C-type lectin receptor (MINCLE) binds immobilized *S. ratti* lysate and activates a MINCLE expressing reporter cell line. As this suggests presence of agonistic MINCLE-ligands in the S. ratti lysate, we analysed a putative function for MINCLE in vivo. Strikingly, MINCLE-deficient (KO) mice displayed reduced intestinal *S. ratti* parasite burden compared to their wildtype (WT) mice, suggesting an improved and not the expected impaired host defence in the absence of a stimulating PRR. To elucidate, which effector cell expressing MINCLE might be responsible for this protective immunity, we show that eosinophils and neutrophils represent the dominant MINCLE expressing cell population in vivo. Comparing their function in vitro, WT and MINCLE KO granulocytes inhibited motility of *S. ratti* third stage larvae (L3), indicating potential killing of L3. However, MINCLE-deficient eosinophils showed significantly increased capacity to impair *S. ratti* motility. These cells also produced more reactive oxygen species (ROS) compared to the WT. Therefore, indicating that, MINCLE-mediated signalling changes the function of eosinophils during *S. ratti* infection. Thus, expression of MINCLE on eosinophils protect *S. ratti* from immune response by host and as a result, contributing to high parasite adults in the intestine.

Summary

Helminths are large multicellular parasites that still infect approximately a quarter of the human population. The mammalian immune system protects us against helminth infection in the context of type 2 immune response. Infective third stage larvae (L3) live in the free world and actively penetrate the skin of their rodent host. L3 migrate within two days percutaneously partially via the lung to the nasofrontal region of the head and are swallowed subsequently to reach the small intestine. There, they moult via fourth larval stage to parasitic adults that live embedded in the mucosa of the small intestine and start to reproduce by day 5 post infection (dpi). Immune competent mice terminate the infection within a month and remain semi-resistant to subsequent infections. This process is initiated by the interaction between pattern-recognition-receptors (PRRs) on immune cells and pathogen-associated-molecular-patterns (PAMPs) present on the parasite. Macrophage-inducible C-type lectin receptor (MINCLE) is a PRR expressed by innate immune cells to recognise PAMPS. So far, the role of MINCLE in the immune response to helminth infections has only been addressed in the context of *Schistosomiasis*. Therefore, it became of interest to elucidate the role of MINCLE in anti-*Strongyloides* immune response.

Hence to begin this project, we first show that MINCLE binds immobilized *S. ratti* lysate and activates MINCLE expressing reporter cells. Therefore, indicating a presence of agonistic MINCLE-ligands in the *S. ratti* lysate. We then proceeded to find out the putative function for MINCLE in vivo. Strikingly, MINCLE-deficient (KO) mice displayed reduced intestinal *S. ratti* parasite burden compared to their wildtype (WT) mice, suggesting an improved and not the expected impaired host defense in the absence of a stimulating PRR. To elucidate which effector cell expressing MINCLE might be responsible for this protective immunity, we show that eosinophils and neutrophils are the dominant MINCLE expressing cell population in vivo. WT and MINCLE KO eosinophils expand significantly by 3dpi. However, by 6 dpi the frequency of WT eosinophils significantly decease in comparison to the MINCLE KO eosinophils. We also show that the frequency of neutrophils also increases significantly in both WT and MINCLE KO by 3 dpi. However, no difference is observed by 6 dpi. We then compared the function of these granulocytes in vitro. We show that WT and MINCLE KO granulocytes inhibit motility of

S. ratti L3, indicating potential killing of L3. Both WT and MINCLE KO neutrophils equally impair *S. ratti* L3. We also show that that in the presence of *S. ratti* L3 neutrophils do not release reactive oxygen species (ROS). However, MINCLE KO eosinophils significantly impair *S. ratti* motility compare to WT. Also, in the presence of *S. ratti* L3, MINCLE KO eosinophils produce more ROS compare to the WT.

Therefore, indicating that, MINCLE-mediated signalling changes the function of eosinophils during *S. ratti* infection. Thus, expression of MINCLE on eosinophils protect *S. ratti* from host immune response and as a result, contributing to high parasite adults in the intestine.

Table 1.
Abbreviations

AF	Alexa flour
APC	Antigen presenting cells
Bcl10	B-cell lymphoma/leukemia 10
BSA	Bovine serum albumin
BV	Brilliant violet
CARD9	Caspase recruitment domain-containing protein 9
CC	Chemokines
CLR	C type lectin receptor
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucliec acid
dNTP	Deoxynucleotide triphosphates
ECP	Eosinophil cationic proteins
EDN/EPX	Eosinophil-derived neurotoxin
ELISA	Enzyme linked immunosorbent assay
EPO	Eosinophil peroxidase
FACS	Flow cytometry
FEIA	Fluoro-enzyme-immunoassay
FLT3L	FMS-like tyrosine kinase 3 ligand
FSC	Forward scatter
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte-macrophage colony stimulating factor
HBSS	Hanks' Balanced Salt Solution
HEK	Human embryonic kidney cells
HEPES	Hydroxyethyle piperazineethanesulfonic acid
HRP	Horseradish peroxidase
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cell
ITAM	Immunoreceptor tyrosine based activation motifs
ITIM	Immunoreceptor tyrosine based inhibition motifs
IV	Intravenous
KO	Knockout
LPS	Lipopolysaccharide
MAC	Membrane attack complex
MACS	Magnetic-activated cell sorting
Malt1	Mucosa-associated lymphoid tissue lymphoma translocation protein 1
MBP	Major basic protein
MCL	Macrophage C-type lectin
MHC	Major histocompatibility complex
MINCLE	Macrophage inducible C-type lectin receptor
MMP9	Matrix metalloproteinase 9
MPO	Myeloperoxidase

NE	Neutrophil specific elastase
NET	Neutrophil extracellular traps
NF-κB	Nuclear factor kappa-light-chain enhancer of activated B cells
NLR	Nod-like receptor
PacBlue	Pacific blue
PAMP	Pathogen associated molecular pattern
PBS	Phosphate Buffered Saline
PD-L1	Programmed death ligand 1
PE-Cy	Phycoerythrin-Cyanine
PerCP	Peridinin-Chlorophyll
PMA	Phorbol-12-myristat-13-acetat
PRR	Pattern recognition receptor
RBC	Red blood cell
RIG	Retinoic acid-inducible gene I
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT	Room temperature
SAP130	Spliceosome-associated protein 130
SCF	Recombinant Murine Stem cell factor
SEAP	Secreted embryonic alkaline phosphatase
SHP1	Src homology region 2 domain-containing phosphatase-1
SIGNR-5 or RGS5	Regulator of G-protein signaling -5
siRNA	Small interfering RNA
SOD	Superoxide dimutase
SSC	Side scatter
Syk	Spleen Tyrosine Kinase
TDB	Trehalose-6,6 dibehenate
TDM	Trehalose -6,6-dimycolate
Th	T helper cells
TLR	Toll-like receptor
UV	Ultra-violet
WT	Wildtype

Table of content

1. Introduction

1.1. Helminths

Helminths are large multicellular parasites that still infect approximately a quarter of the human population (WHO key facts 2023; Hotez P.J, et. al., 2008; Viney 2017). They are worm like-parasites which are classified into two phyla called nematodes (roundworm) and platyhelminths. Platyhelminths are also categorized into two groups, where worms that are leaf-shaped flatworms also known as flukes are classified as Trematodes. A common example of trematodes is schistosomes (blood flukes), which infect humans (Castro GA. Helminths, 1996). Cestodes (Tapeworms) are also Platyhelminths and common examples are *Taenia saginata* (beef tapeworm) and *Taenia solium* (pork tapeworm) which also infect humans (Castro GA. Helminths, 1996; Sato MO, et. al., 2018).

1.1.1. Nematodes

Nematodes, also known as soil-transmitted helminths (STH), are one of the two major phyla of helminths, with the other being platyhelminths (which includes flatworms, such as cestodes and trematodes)(Hotez, P.J, et al., 2008). The transmission of these intestinal multicellular parasites is through ingestion of contaminated food, water or through contact with contaminated soil, where the parasite is able to penetrate the skin of the host (Stepek, et. al., 2006). *Ascaris lumbricoides* (roundworm), *Necator americanus* (hookworms), *Trichuris trichiura* (whipworm) and *Strongyloides stercoralis* (threadworm) are nematode species that infect humans, have great prevalence in tropical and sub-tropical countries (Stepek, et. al., 2006; Viney 2017). Although soil transmitted Helminths infections are not viewed as dangerous in comparison to parasite infections such as Malaria, it leads to serious illness of infected individuals especially children and pregnant women (Stepek, et. al., 2006). Therefore, it is still of interest to understand the immune mechanism by which infection is cleared, in order to develop better anti-helminth treatment.

1.1.2. *Ascaris lumbricoides*

Ascaris lumbricoides is the most prevalent soil transmitted helminth (Lagatie O, et. al., 2020). They also inhabit in the lumen and produce large numbers of eggs which are excreted into the environment. When the infective eggs are ingested through contaminated food or water, the eggs hatch into third stage (L3) Larvae and migrate via the portal blood vessel system to the liver, the larvae then migrate to lungs and penetrate the alveolar spaces and move to the pharynx, where they are coughed and swallowed into the small intestine where L3 larvae develop into adult worms. From the ingestions of infected eggs to adult worms takes about 66-76 days in human (TAKATA I 1951; Holland CV 2021). Symptoms experienced by patients infected *Ascaris lumbricoides* includes acute abdomen and upper gastrointestinal bleeding, (Wang, et. al., 2013)

1.1.3. *Necator americanus*

Necator americanus is a human gastrointestinal nematode that causes anaemia which in turn is linked to impaired development (Croese J, et. al., 2013). Humans are infected with these hookworms, when infected L3 larvae in contaminated soil penetrate the skin of the host. Through a host derived signal, L3 larvae migrate through the vasculature to the right side of the heart, then to the pulmonary vasculature. The larvae then rapture from the lung capillaries and enter the parenchyma, where they ascend the alveoli and trachea. They are coughed up and swallowed down into the gastrointestinal tract, where they moult twice in order to develop into adult worms (Hotez PJ, et. al., 2004).

1.1.4. *Trichuris trichiura*

Trichuris trichiura are large intestinal nematodes alslo known as whipworms. Similar to the other soil transmitted helminth, *Trichuris trichiura* infect host when contaminated soil or food

containing embryonated eggs is ingested. The eggs are hatched in the large intestine in response to a molecular signal triggered by bacteria. First stage lavae (L1) penetrates the gut epithelial cells and develop into second stage (L2) larvae. There L2 develop into L3, L4 and extend into the gut lumen, where they moult into adult worms (Hyes KS, et. al., 2010; Else KJ, et. al., 2020)

1.2. Strongyloides

Strongyloides are gastrointestinal parasites that infect terrestrial vertebrate (Viney 2017). The two species of the genus *Strongyloides* that infect humans are *S. fuelleborni* found in Africa and the *S. stercoralis* which is globally disseminated and clinically relevant (Grove D.I. 1996, Beknazarova M, et. al., 2016; Olsen, et. al., 2009; Schär, et. al., 2014). In addition to human host, *Stercoralis* can also infect primates, dogs or gerbils (Bonne-Année et. al., 2011; Breloer & Abraham, 2017). Infection of *S. stercoralis* leads to the disease strongyloidiasis, which is one of the most neglected tropical diseases (Olsen et. al., 2009; Schär et. al., 2014). Chronically infected individuals show symptoms such as urticarial rash, transient bronchitis and gastrointestinal complaints. (Arthur RP and Shelley WB 1958; Khieu V, et. al., 2013). However, due to limited and insufficient epidemiological surveys and control programmes for strongyloidiasis, is it still difficult to accurately diagnosis the disease. Thereby, infected individuals are treated with drugs such as albendazole and mebendazole which target other soil-transmitted helminths but have no or very low efficacy against strongyloidiasis (Bounfrate D, et. al., 2022). Human studies on *S. stercoralis* have shown the role of both innate and adaptive immune mechanism in the context of type 2 immune response (Anuradha R, et. al., 2016; Bock CN, et. al., 2017; Rajamanickam A, et. al., 2018). However, since the option of human studies is limited, mouse models have been used for further mechanistic studies to elucidate key areas that will help improve control and elimination of strongyloidiasis. In mice *S. stercoralis* larvae is incapable of developing beyond the third larval stage (Dawkins and Grove, 1982a; Bonne-Année et. al., 2011). However, infecting mice with the rodent specific *Strongyloides ratti* and *Strongyloides venezuelensis* has proven to be very important model to understand the interaction of strongyloides with its host, life cycle and migration route of the species. (Dawkins et. al., 1980; Breloer and Abraham, 2017; Viney and Kikuchi, 2017)

1.3. Life cycle and migration route of Strongyloides ratti

The life cycle of *S. ratti* was primarily described based on its life cycle in rats (Viney and Lok 2015). Free-living adults hatch eggs to form the first stage larvae (L1) which is either male (XO) or female (XX). Male larvae develop via L2-L4 stages into rhabditiform which is known as the indirect or heterogonic pattern of development. Female can also develop into rhabditiform females. However, most female develop via the direct, asexual or homogenic pathway, where first stage larvae female moult via L2 into infectious filariform L3 stage larvae (Figure 1). Infective L3 larvae dwell in moist soil and infect host via penetration into the skin of their rodent host. This process is imitated in laboratories whereby a defined number of L3 is deliberately injected subcutaneously (Tindall and Wilson 1988). Within 2 days of active penetration, the infective L3 migrate via the tissue to the nasofrontal region of the head. L3 is swallowed and moult via L4 stage larvae to parasitic adult by day 5 to day 6 post infection. The parasitic adults embed into the mucosa of the small intestine where they reproduce via parthenogenesis. (Tindall and Wilson 1988). Although the life cycle of *S. ratti and S venezuelensis are similar, S venezuelensis* differs in terms of its migration route where the infective L3 migrate exclusively via the lungs (Dawkins 1981 and Dawkins 1982). Also, between day 5 and 6 post infection, *S. venezuelensis* release eggs whereas *S. ratti* release hatched first stage larvae through faeces into the free world. Infected mice and rats that are immunocompetent terminate both *S. ratti and S venezuelensis* within a month and remain semi-resistant to subsequent infections. (Breloer M and Abraham 2017). To elucidate whether migration of *S. ratti* into the intestine follows a defined pattern, Ehrens and group compared the number of viable emigrating L3 after subcutaneous injection into the footpad. They found that 10 mins after infection, viable L3 were found under the skin and leg muscle of the rodent host. With 2 days viable L3 were found in the lung and in the head and by day 3 arrive in the intestine. All other tissues which are not mentioned as part of the migration route of *S. ratti* were all parasite-free. (Ehrens A et. al 2021).

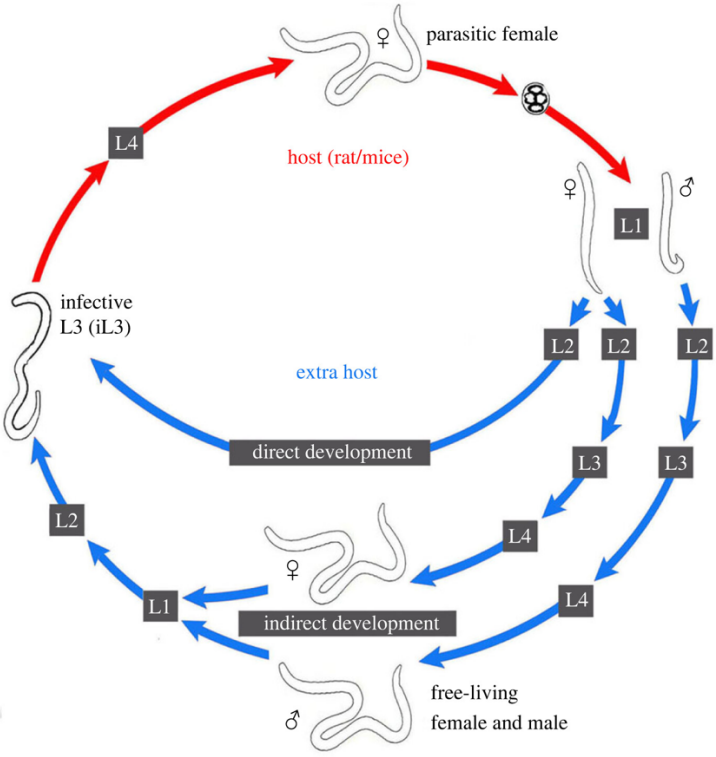

Figure 1. *Strongyloides ratti* life cycle. Parasitic adults lay eggs which hatch to L1 male or female. Male larvae develop via L2-L4 stages into rhabditiform which is known as the indirect or heterogonic pattern of development. Female can also develop into rhabditiform females or via the direct, asexual or homogenic pathway, where first stage larvae female moult via L2 into infectious filariform L3 stage larvae. Infective L3 larvae infect host via penetration into the skin of their rodent host, migrate to the head and are swallowed into the small intestine, where they molt via L4 to parasitic adult. Illustration from Minka Breloer and Lara linnemann 2024 review.

1.4. ***Pattern Recognition Receptors***

Pathogens are recognised by conversed pathogen associated molecular pattern (PAMP), which interacts with pattern recognition receptors (PRRs) found on innate immune cells (Janeway 1989). This interaction then leads to the appropriate immune defence needed by the infected host to eliminate pathogen (Medzhitov and Janeway 1997). There are four families of PRRs namely the Toll-like receptor (TLR), Nod-like receptor (NLR), Retinoic acid-

inducible gene I (RIG-I)-like receptors (RLRs) and C type lectin receptors (CLRs) families (Hardison and Brown 2012). Both TLR and RIG - I are PPRs that sense nucleic acids derived from viruses and initiate antiviral immune innate immune response (Yoneyama, et. al., 2004; Ishii, et. al., 2006). However, aside viruses, TLRs also recognize a variety of bacterial, fungal, and protozoal ligands (Takeda, et. al., 2001). NLRs sense fragments of bacterial peptidoglycans and triggers immune host response against bacteria (Chamaillard, et. al., 2003).

1.5. C type lectin receptors

Among the different types of PRRs C type lectin receptors (CLRs) are considered one of the largest family and known to recognise glycans in Ca^{2+} dependent manner (Furukawa, et. al., 2013). Dectin-1 was the first CLR that was identified and was found to recognize zymosan, a major yeast cell wall component, which in turn triggers immune response against fungal pathogens via signal transduction involving CARD9/Sky/Bcl10-Malt1 and NF-κB activation (Gross, et. al., 2006). Subsequent studies also showed that modulation of immune response by CLRs are via signalling through immunoreceptor tyrosine based activation motifs (ITAMs) or immunoreceptor tyrosine based inhibition motifs (ITIMs) independently or via associated adaptor proteins of ITAMs and ITIMs (Rogers, et. al., 2005; Turner, et. al., 2000).

1.6. Macrophage inducible C-type lectin receptor

Macrophage inducible C-type lectin receptor (MINCLE) is a CLR also known as Clec4e or Clecsf9, is predominately expressed by innate cells such as neutrophils, macrophages and dendritic cells (Linnemann, Antwi-Ekwuruke, et. al., 2024). In 2008, Yamasaki and group demonstrated that spliceosome-associated protein 130 (SAP130) which is released from dead cells activates MINCLE-expressing cells and triggers inflammatory response against pathogen. They also showed that MINCLE is type II transmembrane protein that is associated with ITAM and mediates Syk activation in a Fc receptor common γ-chain (FcRγ) and CARD9 dependent manner (Yamasaki, et. al., 2008). MINCLE has also been shown to recognise *Lactobacillus*

Kefiri and its S-layer glycoprotein (Malamud, et. al., 2020). Moreover, several studies have shown that MINCLE engages mycobacterial glycolipid called Trehalose 6,6-dimycolate (TDM) which subsequently leads to the recruitment of Syk-dependent adaptor protein CARD-9, BCL-10 and MALT1, which in turn induce NF-kB and promote proinflammatory immune responses (Ishikawa, et. al., 2009). In 2010, Schoenen and group showed that, both trehalose -6,6-dimycolate (TDM) and it's synthetic analog trehalose-6,6 dibehenate (TDB) induce macrophage activation via MINCLE and induce T cell immune responses against tuberculosis (Schoenen, et. al., 2010). Other studies have also shown that MINCLE deficiency impairs migration of neutrophils in bacterial infection (Lee, et. al., 2017). In 2009, Yamasaki reported that MINCLE is the first specific receptor for the fungal species *Malassezia*. MINCLE recognition of Malassezia induces cytokines and chemokines produced by macrophages (Yamasaki, et. al., 2009). So far, the role of MINCLE in the immune response to helminth infections has only been addressed in the context of *Schistosomiasis*. These reports show involvement of MINCLE with the CLRs Dectin 2 and SIGNR-5 in increasing production of dendritic cells to induce Th17 immune response, when schistosome egg ligands interacts with the CLRs. (Kalantari, et. al., 2018; 2019). However, the role of MINCLE in *anti-Strongyloides ratti* immune response has not yet been elucidated.

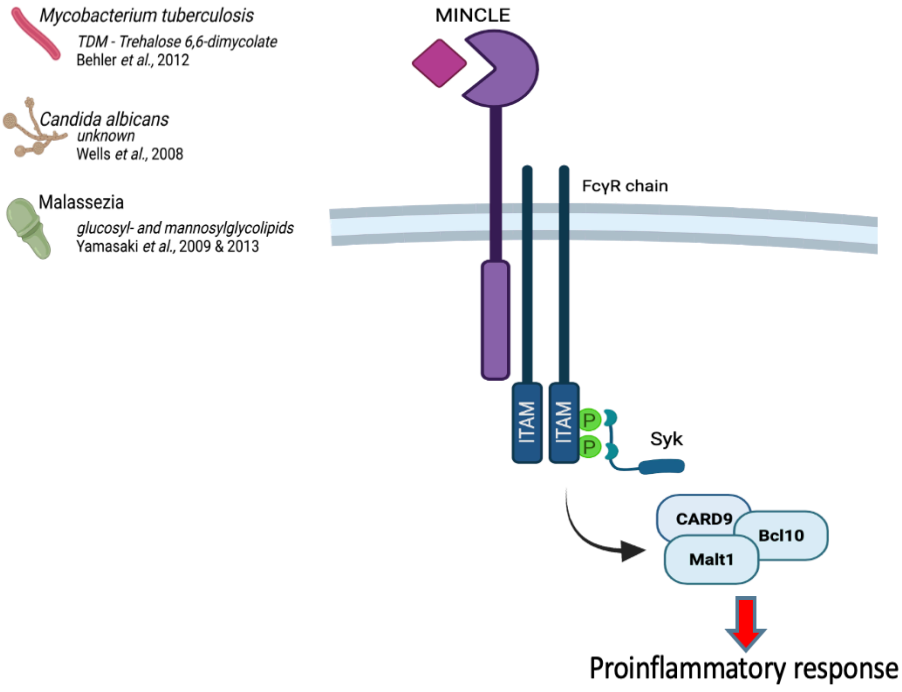

Figure 2. Activation of MINCLE. This illustration shows activation of MINCLE by some of the known ligands and its downstream effect. That is, binding of MINCLE agonist to the receptor leads to Syk-dependent recruitment of adaptor protein (CARD 9, BCL10 and MALT1), which subsequently promotes pro-inflammatory response.

1.7. Anti-*Strongyloides ratti* immune response

Although the recognition of *S. ratti* by pattern recognition receptors expressed on innate immune cells is still under investigation, there are evidence that migrating of *S.ratti* L3 in the skin tissue induce the release of the alarmin cytokine IL-33 via tissue disruption(Meiners, et. al. 2020; Imai, et. al., 2013; Osburn, et. al., 2017). IL-33 is nuclear cytokine which is expressed in and released by damaged or stressed epithelial barrier cells (Cayrol and Girard 2014). Further investigation show that once IL-33 is released, it acts on IL-9 producing group 2 innate lymphoid cells (ILC2) to expand IL-9 production, which in turn mediates the rapid activation of mucosal mast cells in the intestine and thereby, promoting parasite expulsion (Meiners, et. al. 2020). However in this study neutrophils and eosinophils which are known to intercept *S.ratti* during tissue migration were shown to have no involvement (Meiners, et. al. 2020; Ehrens, et. al. 2021). In 2021, Ehrens and group showed in a study that neutrophils and eosinophils play a vital role in the eradication of *S. ratti* L3 in tissue. Here they depleted neutrophils using anti-Gr-1 monoclonal antibody and as a result the number of *S.ratti* L3 in head and lung tissue elevated. Similarly using eosinophil deficient ΔdblGATA mice also resulted in the elevation *S. ratti* L3 in the head. It was further shown in the study that the efficient interception of *S.ratti* L3 at the site of entry by neutrophils and eosinophils was via extracellular DNA trap formation (Etosis) (Ehrens, et. al., 2021). Further investigations show that, migrating larvae are trapped with effector molecules such as myeloperoxidase (MPO) and Reactive oxygen species (ROS), which contribute to the formation of extracellular traps (Ehrens, et. al., 2021; Kirchner, et. al., 2012; Nishinaka, et.al., 2011).

1.8. Helminth infection and Neutrophils

For the longest time neutrophils were known to be the first line of defense against invading and eliminating bacterial, viral and fungal infections (Brinkmann, et. al., 2004; Toussaint, et. al., 2017; Sanches, et. al., 2021). However recent discoveries have shown that neutrophils also play a role in type 2 immunity, namely during helminth infection. Typically, a type 2 immunity is characterized by increased eosinophils, alternative activation of macrophages with upregulation of YMI, Arginase and RELMα proteins, expansion of T helper cells and ILC2, which produce cytokines such as IL-4, IL-5 and IL-9 and triggers induction of the anybody isotypes, immunoglobulin (Ig) IgG1 and IgE (Loke, et. al., 2002; Meiners, et. al. 2020; Ehrens, et. al. 2021). During infection by *Schistosoma (S) mansoni*, neutrophils are rapidly recruited to the skin within three hours in response to the release of excretory and secretory molecules from parasitic larvae (cercariae), followed by recruitment of other innate immune cells, leading to activation of acquired immunity (Paveley, et. al., 2009). Neutrophils have also been reported to play a role in *S. japonicum*, where Vγ2 γδ T cells produced IL-17A drives neutrophilia in blood and in spleen upon infection. When Vγ2 γδ T cells subsets were depleted, it led to significant decrease of neutrophils and as a consequence, induced liver fibrosis (Zheng, et. al., 2017). *Nippostrongylus brasiliensis*, a lung-migrating murine nematode, also triggers recruitment of neutrophils in the pulmonary by day two post infection in an IL-17A dependent manner. Subsequent depletion of neutrophils impaired parasite expulsion during secondary infection (Chen, et. al., 2014). While Larvae migration and tissue damage cause neutrophil activation during infection, it has also been demonstrated that bacteria associated with parasite also induce neutrophilia. In 2008, Pesce and collegues showed that, aside *Nippostrongylus brasiliensis,* bacteria from the faeces in which the parasite develop enters the host as well during infection and induce neutrophilia (Pesce, et. al., 2008). Interestingly, *Litomosoides sigmodontis*, also harbors *Wolbachia* bacteria in an endosymbiotic manner and during the skin stage infection of the parasite, *Wolbachia* triggers neutrophil recruitment. This subsequently leads to an increase killing of L3 larvae in the skin (Pionnier, et. al., 2016; Muhsin, et. al., 2018). *Heligmosomoides polygyrus* is also another nematode, described to cause neutrophil infiltration in to the mucosa and submucosa, where the larvae migrate to, to develop into adults before migrating back to the gut lumen. The rapid neutrophil accumulation around the larvae leads to type 2

immune response, where alternative macrophages (M2), $CD4^+$ T cells and eosinophils are activated (Morimoto, et. al., 2004; Anthony, et. al., 2006).

1.9. Eosinophils

Eosinophils are granulocytes that reside in mucosal tissues but are recruited from bone marrow and blood to the site of immune response. They are known to combat multicellular parasites or helminths and also participating in hypersensitivity or allergic response or allergic response (Steinbach, et. al., 1979; Gleich, et. al., 1986). The ability of eosinophils to kill helminth is through the generation of oxidants and cationic proteins. These toxins also damage airway epithelium by binding to negatively charged cell membrane and thereby distorting the arrangement of the lipid bilayer (Humbles, et. al., 2004). Aside toxins, eosinophils, also harbor cytokines (IL-4, IL-5, IL-6 IL-13, Il-25 and tumor necrosis factor α (TNF-α)), chemokines (CCL11 (eotaxin) and GM-CSF (granulocyte-macrophage colony stimulating factor) and growth factors (transforming growth factor β (TGF-β)), which are stored primarily within eosinophil granules or vesicles and are released in response to stimuli, which then affect immune microenvironment, leading to the relevant immune response (Broide, et. al., 1992; Luttmann, et. al., 1998; Gessner, et. al., 2005; Wang, et. al., 2007).

1.10. Eosinophils as non-professional Antigen Presenting Cells

Antigen presenting cells (APC) are categorized into two groups: professional and non-professional APCs. Professional APCs constitutively express major histocompatibility complex (MHC) class II molecules on their surface. They also express costimulatory molecules and capable of processing antigen and presenting to T cells. On the hand, non-professional APCs such as eosinophils are only able to express MHC class II, co stimulatory molecules, process antigens and present them to T cells, when activated by cytokines (Hansel, et. al., 1992; Weller, et. al. 1993; Ohkawara, et. al., 1996). A study was undertaken, where BALB/c mice

were infected with Microfilariae *Brugia malayi* via intra-peritoneal inoculation, resulted in the proliferation of $CD4^+$ T cells and its subsequent Type 2 immune response (Pearlman, et. al., 1993). *In vitro* and *In vivo* studies using *S. stercoralis* have demonstrated that antigens derived the parasite activate eosinophils and induce expression of MHC class II and T cell costimulatory molecules. These eosinophils were also capable of stimulating and priming $CD4^+$ T cells to induce production of antigen-specific type 2 cytokines (Padigel, et. al., 2006; 2007).

1.11. Eosinophil and Pattern Recognition Receptors

Eosinophils are also known to express PPRs such as TLRs which, when activated by their various ligands led to cellular activities such as expression of adhesion molecules, prolonged survival of eosinophils and induction of superoxide generation (Nagase, et. al.; 2003). In 2011, Kvarnhammar and colleagues, showed that eosinophils also express NLRs and RLRs. Moreover, they show that, alongside IL-5 and GM-CSF, NLRs induce activation of eosinophils via NF-kB signalling pathway, which in turn regulates Type 2 immunity (Kvarnhammar, et. al., 2011). Another study also shows that, eosinophils express a C-type lectin receptor called (1-3)-beta-D-glucan receptor or dectin-1. They then show that interaction between the gram-negative bacteria *Haemophilus influenzae* and eosinophil was found to be mediated via dectin-1 and thereby inducing innate inflammatory response (Ahrén, et. al., 2003).

Although, much is known about the ability of eosinophils and neutrophils and their potential to actively attack *S. ratti* during infection, there are no publications on how MINCLE expressing eosinophils and neutrophils contribute to immune response against *S. ratti* infection. Surprisingly too, no one has shown that eosinophils can also express MINCLE. Therefore, it became of interest to elucidate the role of MINCLE in anti-*Strongyloides* immune response.

1.12. Aims

1. Is there a MINCLE ligand in *S. ratti* L3 lysate, dead/viable L3 and supernatant of dead/viable L3? And can this ligand activate MINCLE reporter cells?

2. Which innate immune cells express MINCLE and at what frequency (in peripheral blood)? Does this frequency differ during infection?

3. What is the phenotype of MINCLE KO mice during *S. ratti* infection?

4. Which role do these innate immune cells play in anti-*Strongyloides* immune response?

2.1 Materials

Table 2- list of reagents

2.1.1 Reagents and Chemicals	**Company**	**Headquarter of company**
Amphotericin B (100x)	Capricorn Scientific	Ebsdorfergrung, Germany
Aqua bidest	BNITM	Hamburg, Germany
Bradford assay	Bio-Rad Laboratories, Inc	Hercules, CA, USA
BSA	PAA Laboratories, Inc., Thermo Fisher Scientific Inc.	Waltham, MA, USA
BSA standard	Thermo Fisher Scientific Inc.	Waltham, MA, USA
Cytofix/Cytoperm	BD Biosciences	New Jersey, USA
Deoxynucleotide triphosphates (dNTPs)	Invitrogen, Thermo Fisher Scientific Inc.	Waltham, MA, USA
Dimethyl sulfoxide (DMSO)	Roth	Karlsruhe, Germany
Ethanol	Merck Millipore	Burlington, USA
FC - Block	BNITM	Hamburg, Germany
Fetal Calf Serum (FCS)	PAA Laboratories	Pasching, Austria
FMS-like tyrosine kinase 3 ligand (FLT3L)	PeproTech	Rocky Hill, NJ, USA
Gentamycin Sulfate	Lonza	Verviers, Belgium
Granulocyte colony-stimulating factor (G-CSF)	Thermo Scientific	Wilmington, USA
Glutamax	Gibco	Waltham, MA, USA
Hanks' Balanced Salt Solution (HBSS)	BNITM	Hamburg, Germany
Hydroxyethyle piperazineethanesulfonic acid (HEPES)	Lonza	Verviers, Belgium
HotStartTaq Plus DNA Polymerase	Qiagen	Hilden, Germany
Hydrogen Peroxide (H_2O_2)	Sigma	Deisenhofen, Germany
Lipopolysaccharide (LPS)	Sigma	Deisenhofen, Germany
L929 supernatant	BNITM	Hamburg, Germany
Na2-EDTA	Merck Millipore	Burlington, USA
Na-Azide	Roth	Karlsruhe, Germany
Phosphate-buffered saline (PBS)	Roth	Karlsruhe, Germany
Penicillin/Streptamycin (Pen/Strep)	PAA Laboratories	Pasching, Austria
Phorbol-12-myristat-13-acetat (PMA)	Sigma	Deisenhofen, Germany
Recombinant murine IL-5	PeproTech	Rocky Hill, NJ, USA

Recombinant Murine Stem cell factor (SCF)	PeproTech	Rocky Hill, NJ, USA
Reverse Transcriptase	Thermo Scientific	Wilmington, USA
RPMI 1640 (with L-Glutamine)	Capricorn Scientific	Ebsdorfergrung, Germany
SybrGreen	Invitrogen	Darmstadt Germany
Sulfuric Acid (H_2SO_4)	Roth	Karlsruhe, Germany
Tryphan blue	Sigma	Deisenhofen, Germany
Trehalose-6,6-dibehenate (TDB)	InvivoGen	San Diego, USA
Tetramethylbenzidin (TMB)	Roth	Karlsruhe, Germany
Tween20	Sigma	Deisenhofen, Germany
Zymosan	Invivogen	San Diego, CA, USA

Table. 3 – list of consumables

2.1.2. Consumables	**Company**	**Headquater of company**
Cell culture microplate, 96 well, PS, F-Bottom, Chimney well, white, cellstar TC sterile.	Greiner bio-one	Frickenhausen, Germany
Cell culture plates (96-well, 24-well, F-bottom)	Greiner bio-one	Frickenhausen, Germany
Cell culture plates (96-well, U-bottom)	Greiner bio-one	Frickenhausen, Germany
Cell culture plates (96-well, V-bottom)	Greiner bio-one	Frickenhausen, Germany
Cell Strainer (50 µm and 70 µm)	Becton Dickinson	Heidelberg, Germany
Centrifuge tubes (15 ml, 50 ml)	Sarstedt	Nümbrecht, Germany
ELISA high binding F-bottom Miccolon plate	Greiner bio-one	Frickenhausen, Germany
FACS tube (5 ml, 75 x 12 mm)	Sarstedt	Nümbrecht, Germany
Gloves	Paul Hartmann AG.	Heidenheim, Germany
Microcentrifuge tube (1.5 ml, 2 ml)	Sarstedt	Nümbrecht, Germany
Needle 25G and 27G	Braun	Melsungen, Germany
Neubauer chamber (improved)	Brandt	Wertheim, Germany
Non-Tissue Culture-Treated Plate (6-well)	Corning	New York, USA
PCR-tube (8-strip, 100 µl)	Sarstedt	Nümbrecht, Germany
Petri dish (92 x 16 mm)	Sarstedt	Nümbrecht, Germany
Pipette (5 ml, 10 ml, 20 ml, 25 ml)	Brand	Wertheim, Germany

Pipette tips without filter (10 µl, 200 µl, 1000 µl)	Sarstedt	Nümbrecht, Germany
Pipette tips with filter (10 µl, 200 µl, 1000 µl)	Sarstedt	Nümbrecht, Germany
Serological pipette – plastic (5 ml, 10 ml, 20 ml, 25 ml)	Corning GmbH	Kaiserslautern, Germany
Serological pipette – glass (5 ml, 10 ml, 20 ml, 25 ml)		BNITM, Hamburg
Schott Bottles	Schütt Labortechnik	Göttingen, Germany
Syringe (10 ml)	Braun	Melsungen, Germany

Table 4 – list of Media and buffers

2.1.3. Culture Media, Buffer and Stock Solutions

ACK-Lysis buffer	80.24 g NH_4Cl 0.1 g $KHCO_3$ 37.2 mg Na2EDTA in 1L Aqua bidest.
Borate buffer	0,2M H_3B0_3 0,02M Na_2B4O_7 x 10 H_2O in Aqua. bidest
ELISA-Blocking buffer	1% BSA (w/v) in PBS (1x)
ELISA-Stop Solution	2 M H_2SO_4
ELISA-Substrate buffer	100 mM NaH_2PO_4
ELISA-Substrate solution	12 mL ELISA-Substrate buffer 200 µL TMB solution 1.2 µL H_2O_2
ELISA-Wash buffer	0.05 % Tween20 in PBS (1x)
Eosinophils base medium	Advanced RPMI 1640 500 mL 100 mL FCS 12.5 mL HEPES 5 ml Pen/Strep 5 mL GlutaMAX 0.5mL Gentamycine
Eosinophils growth medium	DAY 0 - 3 100 ng/mL SCF and FLT3L to base medium

	DAY 4 - 12 20ng/ mL IL-5 to base medium
FACS-buffer	10 mL Na-Azide (10 %) 10 mL FCS in 1L PBS (1x)
HBSS	NaCL: 4,0g KCL: 0,2g Na_2HPO_4 * 2H2O: 0,03g KH_2PO_4: 0,03g Glucose: 0,5g $NaHCO_3$: 0,12g in 500mL Aqua bidest
Luminol buffer	100 mM Luminol in DMSO 500 U/mL HRP in PBS 100 mg/mL Catalase in phosphate buffer 1mg/mL SOD in H_2O in Borate buffer
L3 washing buffer	RPMI1640 100 U/mL Pen/Strep 1µg/mL Gentamycin 10mM HEPES 2 µg/mL Amphotericin B
MACS buffer	2nM EDTA 0,5% BSA in 1x PBS
MINCLE HEK-blue reporter cell - initial medium	44.65 DMEM 5ml FBS 250 µl PEN/STREP 100 µL Normocin
MINCLE HEK-blue reporter cell - section medium	44.65 DMEM 5mL FCS 250µL PEN/STREP 100µL Normocin 150µL Blasticidin 5µL Puromycin 200µL HEK-Blue CLR selection
Normal cell culture medium	500 ml RPMI 1640 (with L-Glutamine) 25 mL FCS 5 mL PEN-STREP 2.5 mL Gentamycin
Neutrophils base medium	500 ml RPMI 1640 (with L-Glutamine) 50 mL FCS

	5 mL PEN-STREP 2.5 mL Gentamycin
PBS (10x)	80 g NaCl 2 g KCl 11.5 g Na_2HPO_4 2 g KH_2PO_4 in 1L Aqua bidest
Phosphate Buffer (50 mM)	0.0268 M K_2HPO_4 0.0232 M KH_2PO_4 in Aqua. bidest
TMD solution	6 mg Tetramethylbenzidin (TMB) in 1 mL DMSO
Trypan blue solution	2 % Trypan blue in PBS (1x)

Table 5 – List of hardware used

2.1.4. Hardware	**Version**	**Manufacturer**
BD LSRFortessa™ System		BD Biosciences, New Jersey, USA
Bio-Rad PowerPac 300	283BR Electrophoresis Power	Bio-Rad Laboratories, California, USA
CT 15RE centrifuge		Eppendorf, Hamburg, DE
Cytek Aurora (R0021)	R0021	Cytek Biosciences, Inc, Fremont, USA
ChemiDoc™ Touch Imaging System	Touch	Bio-Rad Laboratories, California, USA
Eppendorf Thermomixer	Comfort	Eppendorf Hamburg, Germany
Freezer & Refrigerator		Liebherr-Hausgeräte, Rostock, Germany
GFL Incubation/Inactivation Water Bath	1012	GFL GmbH, Burgwedel, Germany
Incubator 37 °C		New Brunswick Scientific, Nijmengen, Netherlands
Incubator 25 °C		Heraeus Instruments, Hanau, Germany
Laminar Air HBB	2448	Heraeus Instruments, Hanau, Germany
Laminar flow B-[MaxPro]-130	Max Pro 130	BERNER International, Elmshorn, Germany
Life Technologies EVOS FL Auto Imaging System	FL Auto	Thermo Fisher Scientific Inc., Waltham, USA

Megafuge 1.0 R centrifuge		Heraeus Instruments, Hanau, Germany
Multifuge 1 L-R centrifuge		Heraeus Instruments, Hanau, Germany
Optima Ultracentrifuge		Beckman Coulter, Brea, USA
Optical microscope		Helmut Hund, Wetzlar, Germany
Peqlab peqSTAR, Thermal Cycler	X cycler	Peqlab Biotechnology GmbH, Erlangen, Germany
Pipettes - single channel		Eppendorf, Hamburg, DE
Pipettes - multi channel		Thermo Scientific, Wilmington, USA
Pipette controller - pipetus		Cytek Bioscience, Fremont, USA
Plate Shaker		IKA Labortechnik, Staufen, Germany
Weighing Scale - Kern	Digital	Kern & Sohn, Balingen, Germany
Tecan Spark multimode microplate reader	Spark	Tecan Trading AG, Männedorf, Switzerland
Vortex Mixer		Heidolph Instruments, Schwabach, Germany

Table 6 – list of software used

2.1.5. Software	**Version**	**Manufacturer**
FlowJo	10.8.1	
GraphPad	Prism 9.5.1	GraphPad Software, San Diego, USA
LEGENDplex 7.1	7.1	BioLegend, San Diego, USA
Office	Office 365	Microsoft, Redmond, USA

Table 6 – list of antibodies

2.1.6. Antibodies	**Fluorochrome**	**clone**	**Dilution**	**Manufacturer**
Anti-mouse Zombie	UV, yellow		1: 1000	BioLegend, San Diego, USA
Anti-mouse CD11b	SparkUV	M1/70	1:500	BioLegend, San Diego,

				USA
Anti-mouse Ly6G	PacBlue	1A8	1:600	BioLegend, San Diego, USA
Anti-mouse F4/80	BV510	BM8	1:50	BioLegend, San Diego, USA
Anti-mouse Ly6C	BV785	HK1.4	1:1000	BioLegend, San Diego, USA
Anti-mouse CD45	SparkBlue550	30-F11	1:800	BioLegend, San Diego, USA
Anti-mouse CD11c	PE-Cy7	N418	1:250	BioLegend, San Diego, USA
Anti-mouse MINCLE	Biotin	1B6	1:50	MBL, Tokio, JPN
Anti-mouse Siglec F	AF647	E50-2440	1:200	BioLegend, San Diego, USA
Anti-mouse MHCII	AF700	M5/114.15.2	1:500	BioLegend, San Diego, USA
Anti-mouse CD19	BV711	6D5	1:400	BioLegend, San Diego, USA
Anti-mouse CD3	BV711	17A2	1:100	BioLegend, San Diego, USA
Anti-mouse CCR3	PerCP. 5.5	J073E5	1:100	BioLegend, San Diego, USA
Streptavidin	APC		1:100	BioLegend, San Diego, USA

2.2. Methods

2.2.1 Cultivation of MINCLE HEK-blue reporter cells

MINCLE HEK-blue reporter cells were obtained from Invivogen (#hkb-mmcl) and the persistent expression of MINCLE was persevered by using a selection medium which consist of RPMI1630, 10% fetal bovine serum (FBS), 50 U/mL Penicillin/Streptomycin (Pen/Strep) (10.000 U/mL), 100 µg/mL Normocin (#ant-nr-05), 30µg/mL Blasticidin (#ant-bl-05), 1 µg/mL Puromycin (ant-pr-1) and 1: 250 dilution of HEK-Blue CLR selection (#hb-csm). However, cell growth medium (RPMI1630, 10% fetal bovine serum (FBS), 50 U/mL Penicillin/Streptomycin (Pen/Strep) (10.000 U/mL) and 100 µg/mL Normocin (#ant-nr-05) was used for further subculturing.

2.2.2. Determination of Cell Numbers

To determine the viable cell numbers, cell suspensions were diluted in trypan blue to the desired concentrations. Using a Neubauer chamber, 10uL of cell suspension was pipetted on the slide and all 4 squares were counted under the microscope. The concentration of the cell suspension was calculated with the formula:

$$\text{Concentration of cell suspension} = \frac{\text{Counted cell number x } 10000}{\text{Number of squares counted x dilution used}}$$

2.2.3 HEK-blue MINCLE reporter cell assay

HEK-blue MINCLE reporter cells were cultivated as described in 2.2.1. The assays were carried out by seeding 5 x 10^4 cells per well in 180 µL in a 96 well flat-bottomed plate. The cells were then stimulated with 20 µL of the following stimuli; 5 µg/mL TDB, isopropanol, 50 µg/mL *S. ratti* Lysate, 200 heat-killed *S. ratti* L3, supernatant from heat killed L3, washed heat killed L3, 14 µg/mL of HSP70 and incubated at 37 °C, 5% CO_2 for 20 h. To detect the secreted embryonic

alkaline phosphatase (SEAP) activity, 20 µL of supernatant was added to 180 µL QUANTI-Blue solution (Invivogen) and the colorimetric change of the QUANTI-Blue reagent (OD620 nm) was measured at a time interval of 5 min at 37 °C for 2 h, using Tecan Spark plate reader (Tecan Trading AG, Männedorf, Switzerland).

2.2.4. Animals and ethical statement

Experiments were conducted according to the German Animal Welfare Act and the relevant German authority (Behörde für Gesundheit und Verbracuherschutz, Hamburg). The approved animal licence numbers were N018/2021; T009/2022; N083/2022 and A20/2020). All animals were kept and bred at Bernhard Nocht Institute for Tropical Medicine (BNITM) under specific-pathogen free conditions, where a maximum of five mice were kept in cages with individual ventilation. We obtained the MINCLE Knockout (KO) mice line (Clec4e$^{tm1.1Cfg}$/ Mmucd) from the National Institutes of Health-sponsored MMRRC national system. The phenotype was maintained by back-crossing the MINCLE KO with C57BL/6 mice.

2.2.5. Genotyping of MINCLE mice

Homozygous wildtype and MINCLE KO mice were analysed using polymerase chain reaction (PCR) for their phenotype confirmation. Homozygous wildtype shows a band of 593 bp and homozygous MINCLE KO shows a band of 488 bp.

2.2.6. Isolating of S. ratti L3 larvae

Wistar rats were used for *S. ratti* life cycle. The rats were infected with 2500 L3 and after six, seven and eight dpi, their feces were collected on respective days, cultured in activated charcoal and incubated at 25 °C for six days. L3 larvae were recovered using the Baermann method. Isolated L3 were washed twice with PBS/Pen/Strep and used for further analysis. To

store larvae for lysate production, the larvae were was washed twice with aqua bidest after the PBS/Pen/Strep wash and stored at - 80 °C.

2.2.7. S. ratti L3 Lysate

The stored *S. ratti* L3 were thawed and transferred to a glass homogenizer. The larvae were pottered to obtained homogenizing lysate. The lysate was then centrifuged at 100.000 g for an hour at 8 °C. The supernatant was collected and filtered under sterile conditions. The protein concentration was measured using Bradford protein assay. Samples were prepared according to the following dilutions: undiluted, 1:2, 1:5 and 1:10 in PBS and the standard was prepared using BSA diluted in PBS to obtained the concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62 µg/mL, 31 µg/mL and blank). Using an ELISA high binding F-bottom Microlon, 10 µL sample or standard were pipetted in duplicates and 200 µL Bradford reagent (1x) was added. After 5 min of incubation the plate was measured, using an ELISA plate reader (Tecan) at 570 nm. The protein concentration of the *S. ratti* was calculated using the standard. The samples were diluted to the desired concentration for assays and the rest was stored at - 80 °C.

2.2.8. Isolation of S. ratti parasitic adults in the small intestine

Mice were sacrificed at 6 dpi and the small intestine were removed and placed in tap water. To wash away residual faeces, the small intestine was sliced open longitudinally and washed with tap water. After that intestines were placed in 50 mL tubes filled with tap water and incubated at 37 ° C for 3h with vigorous shaking of the tubes after every hour. The female parasitic adults were counted using a microscope.

2.2.9. Isolation of neutrophils from bone marrow

Wildtype (WT) C57Bl/6 mice, and MINCLE KO mice were euthanized using an overdose of CO_2. The femora and tibiae were removed. Then flesh around the bones were also removed. Each bone was cut open at the far ends and flushed with 5 mL of sterile PBS, using a 27 gauge needle and a 10 mL syringe. The collected bone marrow was centrifuged at 300 g, 5 min at 4 °C. The cell pellet was re-suspended in 5 mL red blood cell (RBC) lysis buffer and incubated for 5 min at room temperature (RT). The reaction was stopped using 15 mL RPMI medium. Cells were counted using a hemocytometer. Thus, 10 µL of the cell suspension were added to 90 µL Trypan blue and viable cells were counted using 10x microscope objective. To perform positive selection, the cells were re-suspended in *magnetic-activated cell sorting* (MACS) buffer, followed by Ly6G-MicroBeads and incubated for 10 min at 4°C. Cells were then placed on MS column in a magnetic field. The cells that bound to the Ly6G-MicroBeads were isolated by flushing the magnetic field with MACS buffer. The isolated cells were counted using a hemocytometer. Thus, 10 µL of the cell suspension was added to 90 µL Trypan blue and counted as described in in 2.2.2. To check neutrophil purity, 1 x 10^6 cells were blocked with 50 µL of Fc-block (PBS/1 % BSA and 0.1 % rat IgG) for 30 min at 4 °C in the dark, followed by staining with anti-Gr-1 *allophycocyanin* (APC) (1:100 in Fc-block). Afterwards cells were washed with flow cytometry staining buffer (FACS Buffer) and flow cytometry was performed using Cytek Aurora. Subsequently, the data was analysed using the FlowJo 10.4.2 software. The gating procedure is displayed in Figure 6, 7. First lymphocytes were gated using the forward (FSC-A) and side scatter (SSC-A). Duplets were excluded using FSC-A and the height forward scatter (FSC-H). Neutrophils were gated as Gr-1 positive cells. Unstained controls were used for the gating. Purity of the neutrophils culture was always > 96 %.

2.2.10. Isolation of eosinophils from bone marrow

For the generation of bone marrow-derived eosinophils, C57Bl/6 mice, and MINCLE KO mice were euthanized using an overdose of CO_2 and femora and tibiae were collected, flesh was removed and the bone marrow was harvested by cutting open the bones at the far ends and flushing them with 10 mL of Advanced RPMI 1640 medium with 10 % heat-treated fetal cave

serum (FCS), 1 % penicillin/streptomycin and 2 mM L-glutamine using a 27 gauge needle and a 10 mL syringe. The collected bone marrow was filtered through a 70 µm cell strainer and cells were centrifuged at 300 g, 5 min at 4 °C. The cell pellet was re-suspended in 5 mL red blood cell (RBC) lysis buffer and incubated for 5 min at room temperature (RT). The reaction was stopped using 15 ml RPMI medium. Cells were counted using a hemocytometer; 10 µL of the cell suspension were added to 90 µl Trypan blue and counted as described in 2.2.2. Bone marrow cells were seeded at a density of 1 x 10^6 cells/mL in Advanced RPMI 1640 medium with 20 % fetal bovine serum (FBS), 0.1 % gentamycin, 1 % penicillin/streptomycin, 2.5 % hydroxyethyl piperazineethanesulfonic acid (HEPES), and 1 % Glutamax. First, cells were cultured with 100 ng/ml FMS-like tyrosine kinase 3 ligand (FLT3L) and stem cell factor (SCF) for 4 days and then the medium was supplemented with 20 ng/ml IL-5. Every other day, half of the medium was exchanged and on day 8 all cells were transferred into a new cell culture flask. Cell densities were determined using hemocytometer as described before. After 12 days of culture, cells were harvested and checked for purity using flow cytometry. To check eosinophil purity, 1 x 10^6 cells were blocked with 50 µl of Fc-block (PBS/1 % BSA and 0.1 % rat IgG) for 30 min at 4 °C in the dark, followed by staining with anti-SiglecF- Alexa Fluor 647 (AF647) (1:100 in Fc-block). Afterwards cells were washed flow cytometry staining buffer (FACS Buffer) and flow cytometry was performed using Cytek Aurora and data was subsequently analysed using the FlowJo 10.4.2 software. The gating procedure is displayed in Figure 10-11. First lymphocytes were gated using the forward (FSC-A) and side scatter (SSC-A). Duplets were excluded using FSC-A and the height forward scatter (FSC-H). Eosinophils were gated as SiglecF positive. Unstained controls were used for the gating. Purity of the eosinophils culture was always > 90 %.

2.2.11. Isolation of eosinophils from peripheral blood

Peripheral blood eosinophils were obtained from either naive or day 3 infected mice. The animals were sacrificed and blood was taken from the *vena cava* using 25 G syringe. Heparin-Natrium 25000 I.E- ratiopharm was added to prevent blood clotting. The peripheral blood was lysed twice with 10 mL Erylysis buffer *(*0.1 M Tris pH7 and NH_4Cl*)* for 10 min and 30 mL of PBS was used to stop the reaction. Eosinophils were purified using Siglec-F MicroBeads and the

MACS cell separation system according to manufacture procedure (Miltenyi, Germany). Thus, peripheral blood cells were incubated with Siglec-F MicroBeads for 10 min in the fridge and added to a pre-equilibrated MS column in a magnetic field. The column was washed three times with MACS buffer and the column was removed from the magnetic field and cells were flushed out of the column. The cell count was determined using Neubauer Improved Hemocytometer Counting Chamber as described above. The purity of the eosinophils was analyzed using Cytek Aurora flow cytometer, where anti-mouse Zombie UV was used for staining dead cells and the surface with anti-mouse Siglec F Alexa Fluor 647. The cells were then used for L3 motility assay.

2.2.12. Reactive oxygen species (ROS) production *in vitro*

Bone marrow derived eosinophils were stimulated with *S. ratti* L3 and PBS to determine the sum of intra- and extracellular ROS using the luminol-amplified chemiluminescence assay (Kirchner T, et. al. 2012). Here, 2.23 x 10^6/mL eosinophils in HBSS + 0.5% FBS (without phenol were seeded in a flat bottom white chimney 96 - microplate and were pre-warmed for 20 -30 min at 37 °C, 5% CO_2. Luminol buffer consisting of borate buffer (0,2M H_3B0_3 and 0,02M $Na_2B_4O_7$ x 10 H_2O), 50 mM Phosphate Buffer (0.0268 M K_2HPO_4 and 0.0232 M KH_2PO_4), catalase, horseradish peroxidase (HRP) and superoxide Dimutase (SOD) was used to prepare the stimuli. Subsequently, the *S. ratti* L3 and PBS in Luminol buffer were added to cells and chemiluminescence resulting from ROS production was measured at a time interval of 2 min at 37 °C for 2 h, using Tecan Spark plate reader.

2.2.13. S. ratti L3 motility inhibition

To assess larval motility in the presence of eosinophils, L3 were isolated as described above, but washed additionally with RPMI1640 consisting of 100 U/mL Penicillin/Streptomycin (Pen/Strep), 1µg/mL Gentamycin, 10mM HEPES Bufffer and 2 µg/mL Amphotericin B. The L3 were finally resuspended in RPMI1640 /1% L-Glutamin /10% FCS /1 % penicillin/ streptomycin/ 0,1% gentamycin/ 2,5% HEPES. For the motility inhibition assay, L3 were

individually picked under a microscope and 20-30 L3 were added to each experimental well of a 96-well flat bottom plate and incubated at 37°C at 5% CO_2 for 24h to regain full motility. Then, 1 x 10^5 peripheral blood derived eosinophils in RPMI1640/1% L-Glutamin/10% FCS/1% penicillin/streptomycin/0,1% gentamycin/2,5% HEPES were added to the larvae. Wells containing only L3 were used as controls. The motility of each L3 was assessed daily for up to 4 days using a scoring system of 0 to 4. L3 that were fully viable and showed fast and continuous movements were scored 4, larvae that showed continuous but reduced speed of movement were scored 3, larvae that showed discontinuous movement were scored 2 and larvae that showed only sporadic movement were scored 1. Dead L3 was identified by no movement at all and were scored 0.

2.2.14. Characterization of antibodies present in mice plasma

Both wildtype and MINCLE KO mice were infected subcutaneously with 1000 *S. ratti* L3 for 6 days. On day 6 the mice were reinfected and sacrificed 6 days dpi. Blood was taken from *vena cava* and plasma was obtained by placing whole blood into a tube containing an anticoagulant. After centrifugation, the supernatant (plasma) was carefully removed from the cell pellet. The antibodies present in plasma of both Wildtype and MINCLE KO were quantified by coating ELISA High Binding Microlon plates with 1 µg/mL of *S. ratti* lysate overnight. Subsequently the plates were washed and blocked for 2 h with 100 µL 1% BSA in PBS. After that, the plates were incubated for 2 h with serially diluted plasma in duplicates, then washed and incubated for 1 h with HRP-labeled anti-mouse IgG1, IgG2a, IgG2b, IgG2c, IgG3, IgM and IgA. A further washing step was performed and the plates were developed with 100 µL tetramethylbenzidine (0,6 mg/mL in DMSO), 0.003% H_2O_2 in 100 mM NaH_2PO_4 (pH 5.5) for 25 min. By adding 50 µL 2 M H_2SO_4 to each well, the reaction was stopped and measured with a plate reader at 450 nm.

2.2.15. Identification of cell surface markers

Plates from the stimulation assays were kept on ice and the cells were harvested by flushing the plates with ice cold PBS twice and cells were transferred into 5 ml tubes. All washing steps were performed by re-suspending cells in FACS-buffer and centrifuged at 300 g, 5 min, 4◦C. For each staining 1 x 10^6 cells were used, followed by life/dead stain using 1mL Zombie Aqua (1:2000). The cells were incubated for 20-25 min at room temperature in the dark and 50 µL of primary antibodies diluted in Fc-Block were added to each sample. Cells were incubated for 30 min at 4◦C in the dark and washed. Subsequently, 50 µL of secondary antibodies in FACS buffer was added and cells were incubated on ice for 15 min in the dark. After washing the cells, they were re-suspended in 200 µL of Cytofix/Cytoperm solution and incubated for 20 min at 4◦C in the dark. After fixing cells, they were re-suspended in 100 - 200 µlL FACS buffer and either measured immediately or stored in the dark at 4◦C. All samples were measured using the flow cytometer "Cytek Aurora" and the results were analysed using the program "FlowJo".

3. Results

3.1. S. ratti L3 activates MINCLE reporter cells

To investigate the role of MINCLE in anti-*Strongyloides* immune response, preliminary work was done to show that *S.ratti* L3 lysate engages with MINCLE receptor. A master student (Annette Schlosser) used an ELISA based assay to show that supernatants derived from *S. ratti* L3 bind MINCLE- hFc receptor fusion protein. In detail, she prepared two different kinds of lysates from *S. ratti* L3, which was had membrane fragment (13 000 xg) or was membrane-free (100 000 xg). Both supernatants were immobilized on ELISA plates and incubated with MINCLE- hFc receptor fusion protein. To validate these results, further analyses by our collaboration partners (Prof. Dr. Bernd Lepenies and group) also showed that *S. ratti* L3 supernatant binds MINCLE receptor in a dose dependent manner, which we published together in 2024 (Linnemann, Antwi- Ekwuruke et. al. 2024).

To investigate whether the engagement of MINCLE and *S.ratti* L3 leads to activation, I used the HEK-blue MINCLE reporter cells, which were also used in other studies to screen for agnostic CLR ligands (Prado Acosta, et al 2021; Ishikawa T et al. 2013). I observed that *S. ratti* L3 activates HEK-blue MINCLE reporter cells (Figure 3A-C). Interestingly, both supernatant from dead *S. ratti* L3 and the dead L3 significantly activated the reporter cells, just as the MINCLE agonist TDB (Schoenen, et. al., 2010). To elucidate if the activation was associated specifically to the dead *S. ratti* L3 or it was selectively an excretory product, present in the supernatant, the dead L3 were washed twice after heat killing and incubated with the reporter cells. I observed that dead L3 itself do not activate MINCLE but rather the supernatant from the dead L3 activated MINCLE. On the other hand, viable *S. ratti* L3 did not activate the reporter cells, as a result of vigorous movement, which caused the reporter cells to lose their adherent characteristic, therefore interfering with the experiment. Although these results were reproduceable, the untreated cells showed a high background (Figure 1A-B). Hence, I reduced the concentration of the cells from 1×10^5 to 5×10^4 cells/well and repeated the experiments (Figure 1C). With the new concentration, I observed that *S. ratti* L3 lysate also activates MINCLE. In summary, these results showed that, the potential MINCLE

ligand activating the MINCLE reporter cells could be a water soluble PAMP which is released, when *S. ratti* L3 is killed.

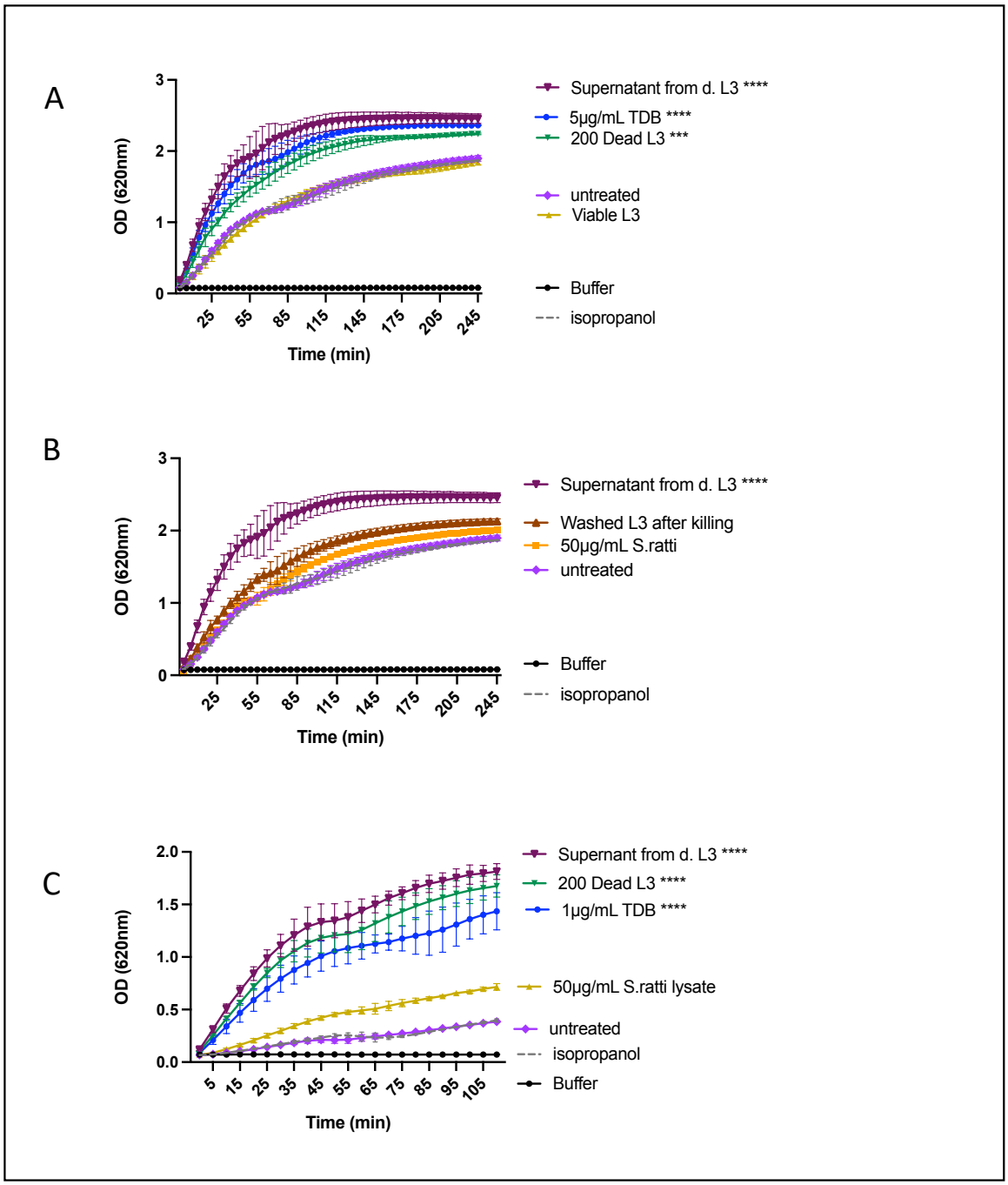

Figure 3. Activation of MINCLE reporter cells. (A-C) show SEAP activity over time using QUANTI-Blue™ to measure the activation of MINCLE HEK-blue reporter cells by supernatant derived from 200 heat killed *S. ratti* L3, 200 dead L3, 200 viable L3, 1 or 5 µg/mL Trehalose-6,6-dibehenate (TDB), washed L3 after heat killing, 50 µg/mL *S. ratti* lysate and controls (medium, buffer and isopropanol). Experiments were performed in triplicates and asterisks indicate significance compared to untreated control (Area under the curve (AUC) with one way ANOVA; p* <0,05; p** <0,01, p*** <0,001, p**** <0,0001).

3.2. Expression of MINCLE on innate cells

To investigate the biological relevance of MINCLE in initiating protective anti-*Strongyloides* immune response, I first analysed the presence of MINCLE on neutrophils and eosinophils in naïve peripheral blood leukocytes (PBL) using flow cytometry. These effector cells were of interest because, they had been described to play a vital role in anti-*Strongyloides* immune response (Ehrens, et. al. 2021). To identify the innate cells in peripheral blood, which express MINCLE, I had to preform initial gating to exclude unwanted cells and debris (Figure 4). I began by gating the single cells to exclude doublets. From that, I gated the live cells to exclude dead cells. I then gated $CD45^+$ cells from the live cells in order to exclude erythrocytes and debris. From the $CD45^+$ cells, I also excluded adaptive cells by gating out CD19/CD3 cells (B/T- cells). From the $CD19/CD3^-$, I then gated for the effector cells shown in Figure 5 and the presence of MINCLE on WT cells (Figure 4). In figure 5, I showed that Siglec F^+ cells (eosinophils) express MINCLE. $Ly6G/Ly6C^+$ cells (neutrophils) also express MINCLE. However, $CD11b^+$ (dendritic cells) and $F4/80^+$ (macrophages) showed no expression of MINCLE. Validation of the presence of MINCLE was based on the flow cytometry results obtained from the MINCLE KO cells.

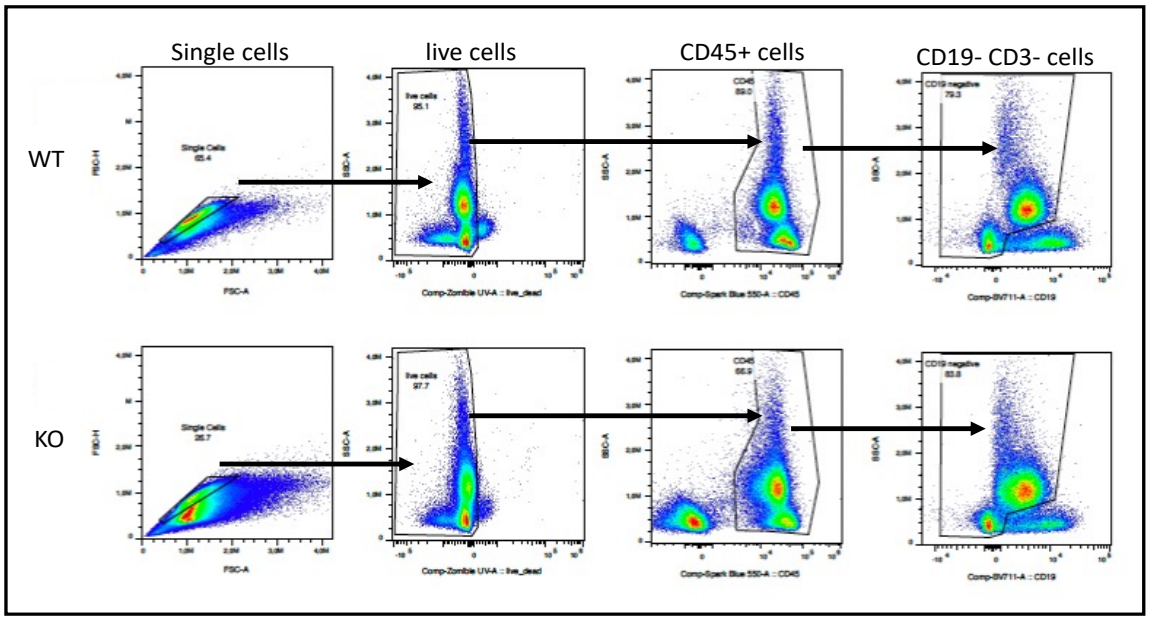

Figure 4: Gating strategy of peripheral blood cells. Shown is a respective staining and flow cytometry analysis of peripheral blood cells. FSC- height and FSC- area was used to gate for

singles cells , Zombie UV for live cells, CD45 spark blue for leukocyte population and CD19/CD3 to gate out adaptive immune cells.

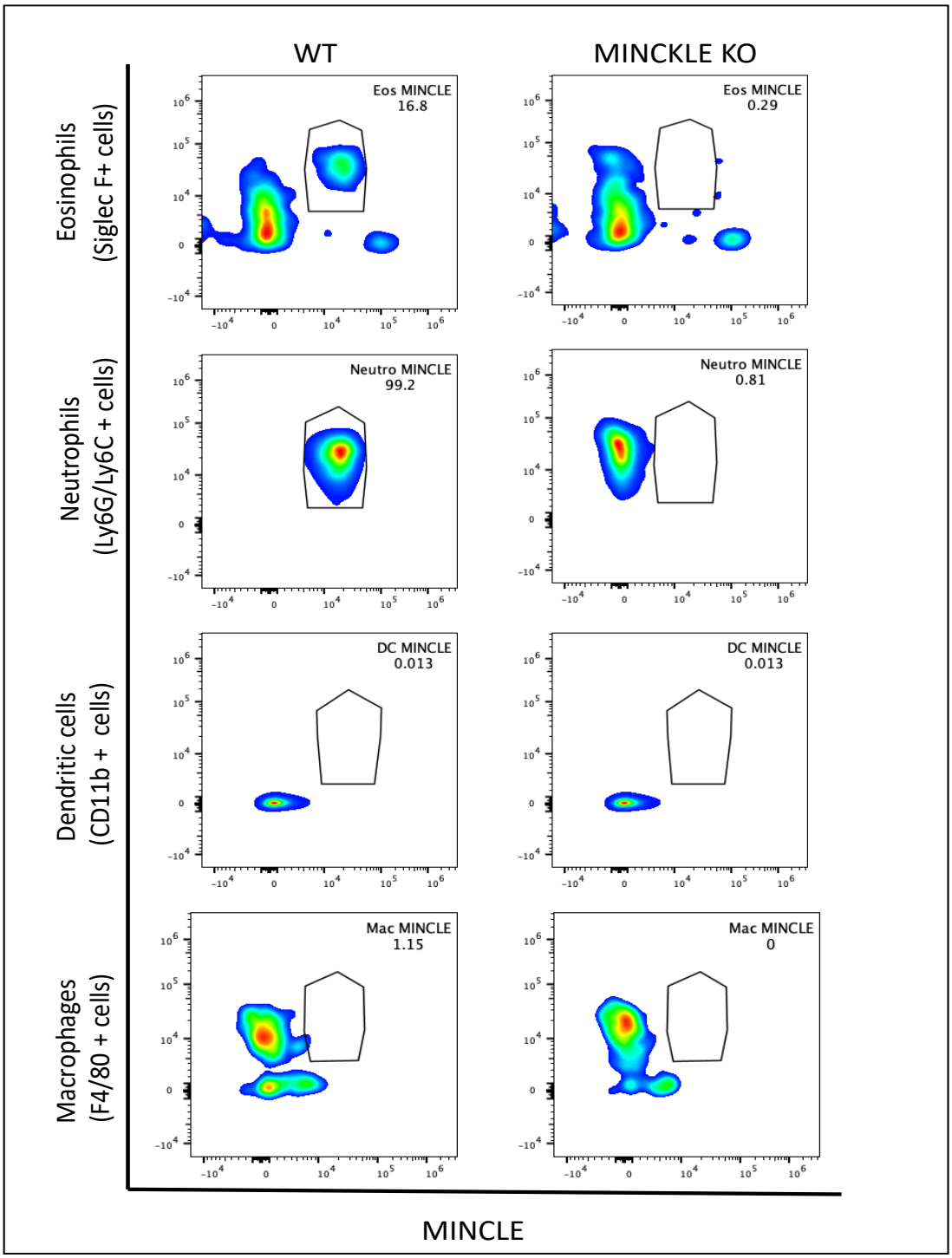

Figure 5. Eosinophils and neutrophils isolated from peripheral blood express MINCLE. Peripheral blood lymphocytes from naïve mice were stained and measured with flow cytometry to identify the expression of MINCLE. Eosinophils as Siglec F^+, neutrophils as Ly6G /Ly6C^+, dendritic cells as CD11b^+, macrophages as F4/80$^+$ cells. Shown are representative density blots of 8 individual naïve mice. Density blots found in a gate represent MINCLE expression (left) and MINCLE KO mice (right) were used for specificity control.

3.3. Expansion of eosinophils and neutrophils during S. ratti infection

Once it was established that both eosinophils and neutrophils express MINCLE. The question posed was, what is the frequency of these effector cells before and after infection in peripheral blood and could this help explain any phenotype observed during infection? The flow cytometry analyses showed that, the population of eosinophils expand by 3 dpi in both WT and MINCLE KO mice, when compared to naïve mice. However, by 6 dpi the population of WT eosinophils deceased whilst the MINCLE KO eosinophils increased (Figure 6A). The neutrophils population also increased significantly in both WT and MINCLE KO mice by 3dpi, as was observed for eosinophils. However, the significantly higher frequency of eosinophils in MINCLE KO mice by 6 dpi was not observed in the case of neutrophils. Instead, the population of MINCLE KO neutrophils remained constant from 3 dpi to 6 dpi. Macrophages, monocyte and dendritic cells were also analysed for any possible change in frequency; however, no specific differences were observed (Figure 6C-F).

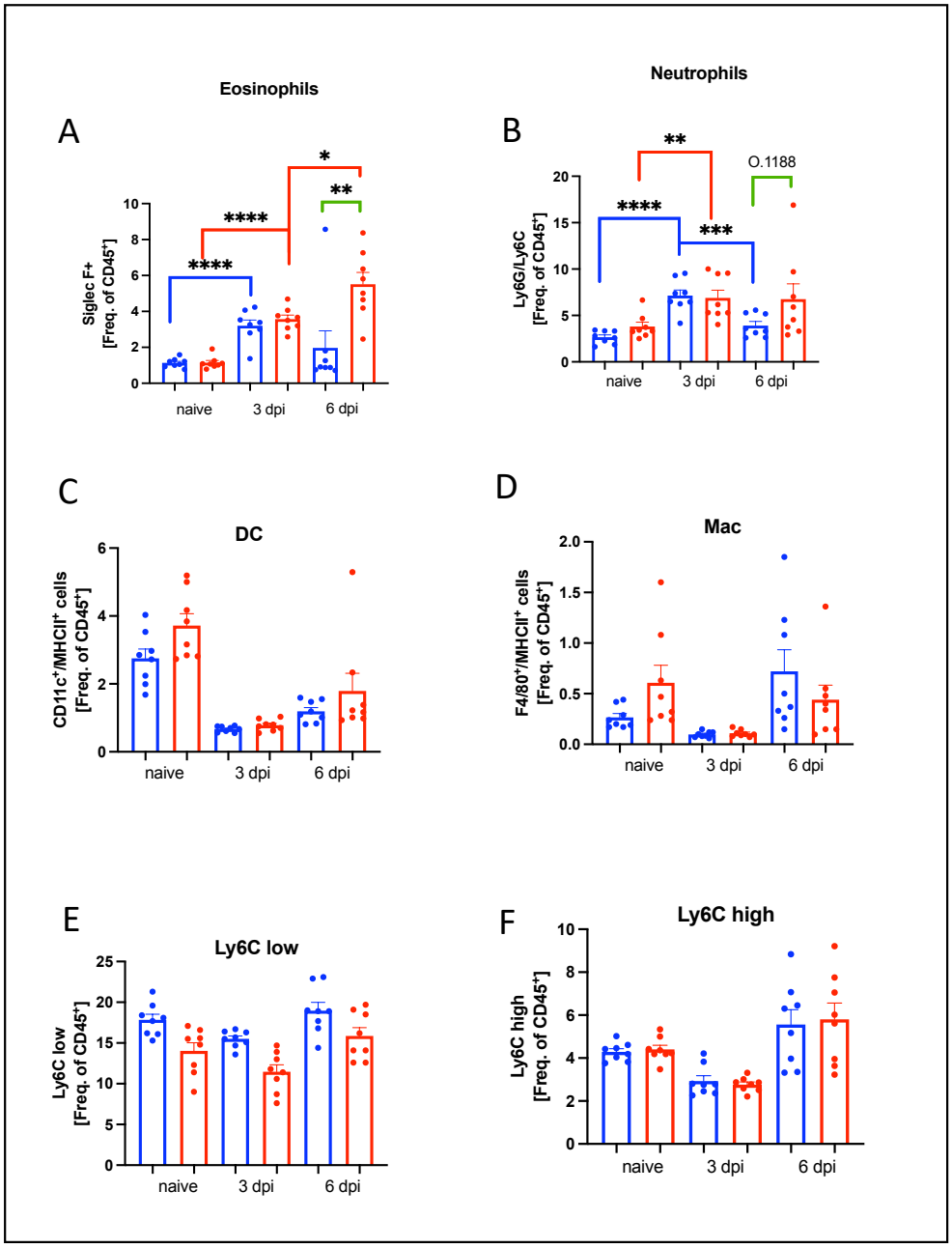

Figure 6. Frequency of eosinophils and neutrophils in peripheral blood of infect mice.
Wildtype (WT) and MINCLE Knockout (KO) mice were infected with 1000 *S. ratti* L3 in the footpad subcutaneously (s.c). Perheral blood samples were collected at day 3 and 6 post infection (dpi) via the facial vein. Naïve mice were used as control. (A)frequency of eosinophils were identified as Siglec F$^+$ within the CD45$^+$ gate (live cells), (B) neutrophils as Ly6G/ly6C (C) dendritic cells as CD11c$^+$/MHCII$^+$, (D) macrophages as F4/80$^+$/MHCII$^+$, (E-F) monocytes as Ly6C low and high. Comparison of WT vs WT is indicated in bule, MINCLE KO in red and between WT and MINCLE KO with green. Bars show the mean (SEM) and statistically significant differences are indicated as asterisks (p* <0,05; p** <0,01, p*** <0,001, p**** <0,0001)

3.4. S. ratti parasite burden in the presence and absence of MINCLE

The next question was, does the differences observed in Figure 6 have an implication for parasite burden in WT and MINCLE KO mice during *S. ratti* infection? Hence, I infected the mice with *S. ratti* L3 and on 6 dpi, I sacrificed them and counted the adult parasite in the small intestine as described in the method section 2.2.8. Surprisingly, I observed that MINCLE KO mice had a significantly decreased parasite burden in the small intestine compared to WT (Figure 7A). This result suggested an improved and not the expected impaired host defense in the absence of a stimulating PRR (Ishikawa, et. al., 2009). In summary the results showed that, absence of MINCLE and its mediated signalling in mice protects against *S. ratti* infection whilst the presence of MINCLE promotes increase parasite burden.

Since the parasite burden in WT mice was significantly high, I was curious to know which pro-inflammatory antibodies would be present in the peripheral blood of WT by 6 dpi. The ELISA analyses showed that there were significantly high levels of *S. ratti* specific IgG1, IgG2c, IgG2b in plasma from infected mice (pi) compared to plasma from naïve mice (Pn) (Figure 7B-E). Both pi and pn were used in the *in-vitro* experiments to have a better understand of the phenotype observed in Figure7A.

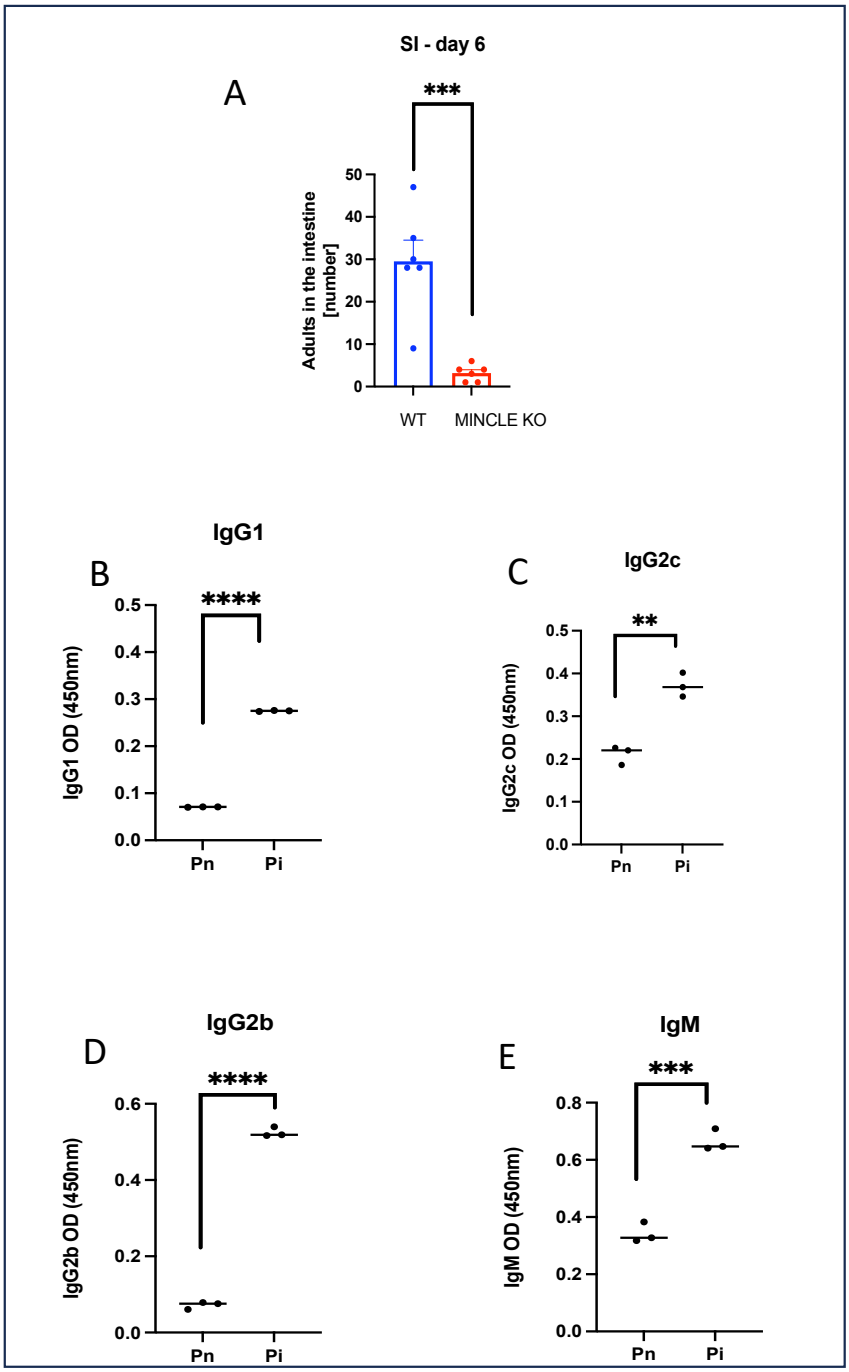

Figure 7. *S.ratti* parasite burden in the presence and absence of MINCLE.
C57BL/6 mice (WT) and MINCLE Knockout (KO) were s.c infected with 1000 S ratti L3 and sacrificed on day 6. Adults in the intestine were counted manually using a microscope. The circles represent the individual mouse. (A) Parasitic adults burden in the small intestine of WT is higher than in MINCLE KO. (B-C) Blood samples collected on day 6pi to identify antibodies present during infection in WT mice (IgG1, IgG2b, IgG2c and IgM) Bars show the mean (SEM) and statistically significant differences are indicated as asterisks (p* <0,05; p** <0,01, p*** <0,001, p**** <0,0001)

3.5. Bone-marrow derived Neutrophil

To further understand the function of the MINCLE expression cells. I performed series of *in-vitro* experiments based on the work of Ehrens, et. al. 2021, where they showed that bone-marrow derived neutrophils inhibit *S. ratti* L3 motility *in-vitro* in the context of ETosis. Based on the phenotype of MINCLE KO mice shown in Figure 6, we hypothesized that MINCLE KO neutrophils might inhibit *S. ratti* L3 motility better than the WT. To begin, I isolated neutrophils from bone-marrow cells using Ly6G beads and Gr-1 antibody. I then cultured the cells as described in 2.2.9 (method section). To exclude any differences between the WT and MINCLE KO neutrophil culture, I analysed the purity of the cells using Flow cytometry. The purity of both cultures was similar and > 96% (Figure 8). I also showed that only WT neutrophils express MINCLE (Figure 9).

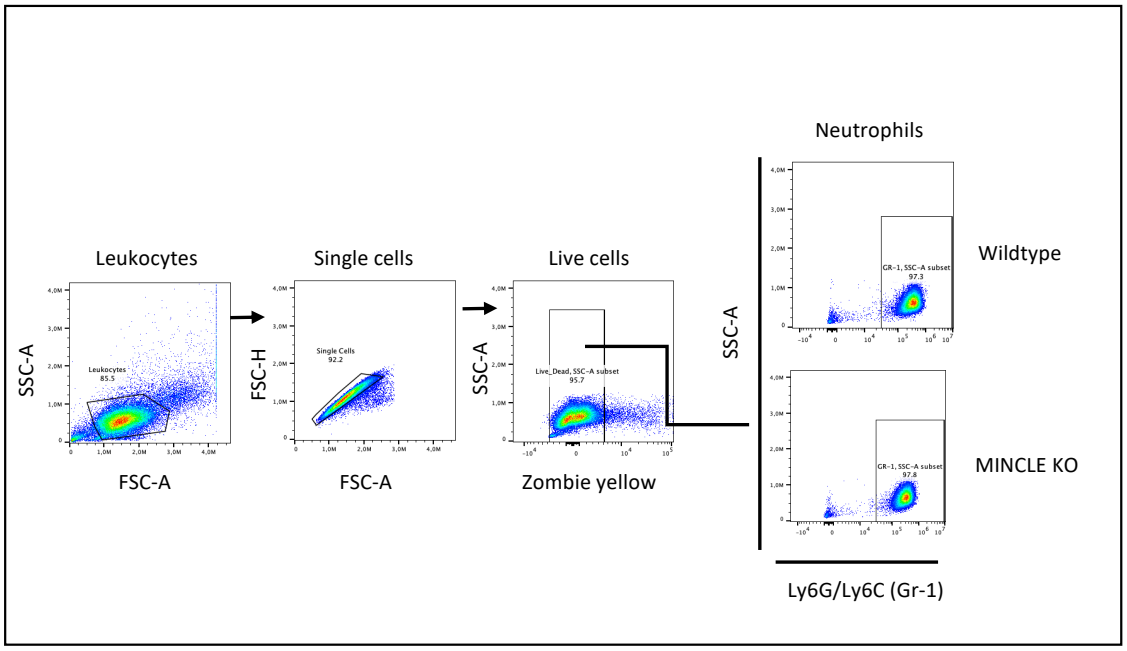

Figure 8. Purity of bone-marrow derived neutrophils Shown is a representative staining (4 biological replicates) and flow cytometry analysis of cultured neutrophils isolated from bone-marrow. FSC-area and SSC - area were used to gate for leukocytes, FSC- height and FSC- area for singles cells and Zombie yellow for live cells. Using Gr-1, neutrophils were identified from the live cells. Purity of cultured neutrophils were > 96.

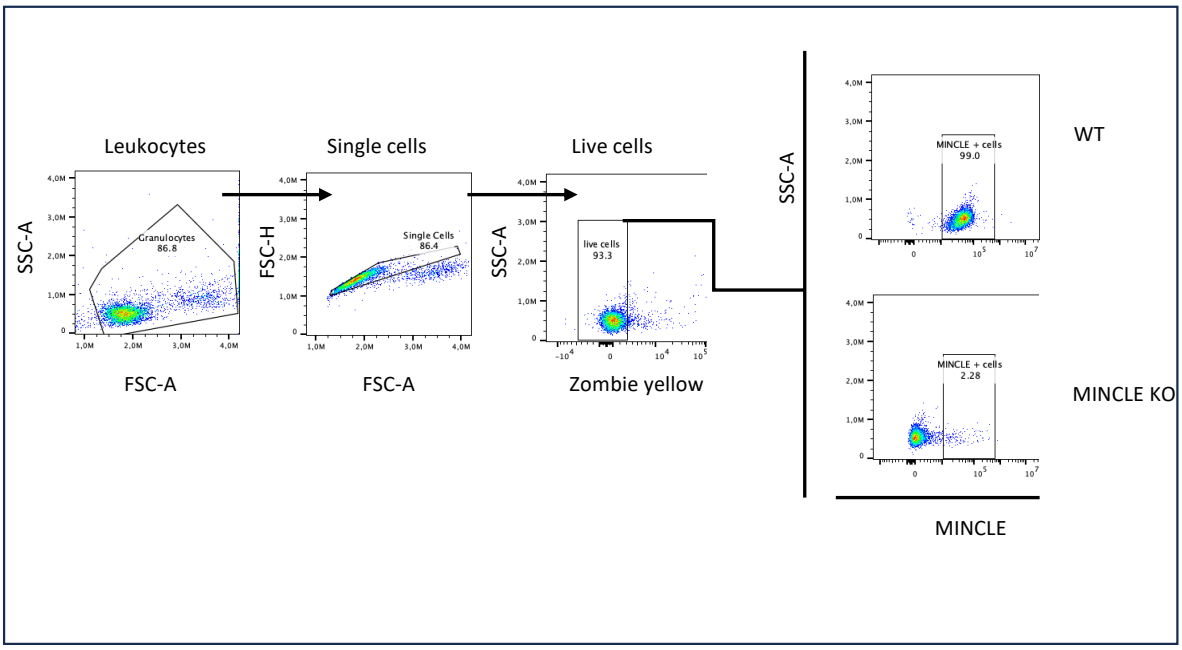

Figure 9: Neutrophils express MINCLE Shown is a representative staining (4 biological replicates) and flow cytometry analysis of cultured neutrophils isolated from bone-marrow. FSC-area and SSC - area were used to gate for leukocytes, FSC- height and FSC- area for singles cells and Zombie yellow for live cells. Using MINCLE antibody, neutrophils expressing MINCLE were identified. MINCLE KO neutrophils were used as specificity control.

3.6 Bone-marrow derived neutrophils inhibit S. ratti motility in-vitro

After culturing the neutrophils for 2 days, I incubated the cells with *S. ratti* L3 with and scored them for 3 to 4 days based on the following motility description: Score 4: Fast and continuous movement, Score 3: Still continuously moving but slower than 4, Score 2: Discontinuous movement, Score 1: Only moving at the end or clapping together like scissors and Score 0: No movement at all. I provided videos supporting these descriptions in a collaborative work with M. Cambra-Pelleja et. al. 2024, where she compared their larval migration inhibition assay with the motility inhibition assay I had established in our lab.

Similar to Ehrens and group, I observed that neutrophils can inhibit *S. ratti* L3 motility (Figure 10A). However, I did not observe any difference between WT and MINCLE KO. I, then added 5% of pi (Figure 7B-C) to the co-culture, to see if the presence of the plasma will improve the ability of the cells to impair *S. ratti* L3. As expected, I observed that the addition of the Pi, significantly accelerated the impairment of *S. ratti* L3 motility. However, this phenotype was observed for both WT and MINCLE KO cells. Again, no difference between WT and MINCLE KO was observed (Figure 10B). Moreover, I also observed that co-culturing *S. ratti* L3 with pi or pn without neutrophils significantly impaired larvae motility (Figure 10B-C).

To elucidate the effector molecules involved in inhibiting motility of *S.ratti* L3, I collected supernatant from the co-cultures after 6 hours and performed ELISA assays to detect myeloperoxidase (MPO), which contributes to the formation of extracellular traps (Ehrens, et.al. 2021; Kirchner, et. al. 2012). In Figure 11, I observed release of MPO by both WT and MINCLE KO. However, in the presence of *S. ratti* L3, MPO released by WT neutrophils was significantly higher than in MINCLE KO. Also, the addition of Pi to neutrophils and *S. ratti* L3, increased the level of MPO but again no significant difference between WT and MINCLE KO was observed (Figure 11). To test for functional difference, I incubated the neutrophils with TDB, which is a ligand of MINCLE (Schoenen, et. al. 2010) and as expected I observed a significant release of MPO by WT neutrophils compared to MINCLE KO. To test the specificity of TDB, L3 was also added to the co-culture. I observed release of MPO by MINCLE KO neutrophils was similar to level of MPO released, when only L3 was incubated with MINCLE KO neutrophils. For WT neutrophils, I observed that, addition of TDB to *S. ratti* L3 slightly increased the level of MPO released. Thereby, Indicating an additive effect. To rule out any intrinsic difference, I incubated the cells with zymosan and a combination of zymosan and *S. ratti* L3. Here, no difference between WT and MINCLE KO neutrophils was observed (Figure 11).

Aside MPO, I also checked for the release of oxygen species (ROS), which is also an effector molecule that is released when neutrophils release extracellular traps (Ehrens, et.al. 2021; Kirchner, et. al. 2012). To exclude any intrinsic differences, I used PMA and zymosan as positive controls. No difference was observed, when the cells were treated with PMA. However, for zymosan treatment, MINCLE KO released a significantly higher level of ROS

compared WT. The cells were also treated with *S. ratti* L3 only and no release of ROS in both WT and MINCLE KO was detected. In summary, both WT and MINCLE neutrophils impair *S. ratti* L3 motility. However, none of the results could explain the results seen in Figure 7A.

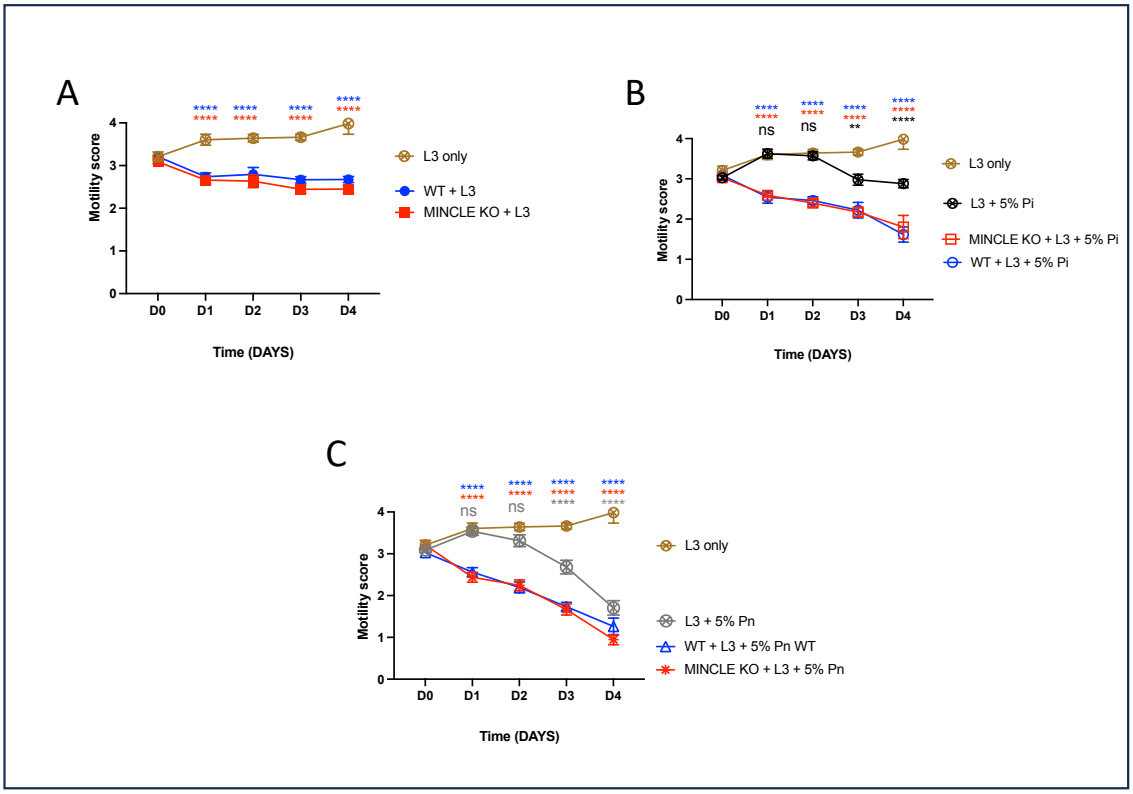

Figure 10. Motility inhibition of *S. ratti* L3 by neutrophils *in-vitro.* Using 96 well plates, 30 *S. ratti* L3 were placed in wells in triplicates and bone-marrow derived neutrophils were added or neutrophils and plasma from infected mice (Pi) or Plasma from naïve mice (Pn) and incubated for 4 days at 37 °C 5% CO_2. The motility of *S. ratti* was scored as described in the method section every day using light microscope. (A) show the incubation of L3 with WT and MINCLE KO neutrophils. (B)incubation of L3 with neutrophils and Pi (C) neutrophils with Pn. Shown is a mean of 4 biological replicates Bars show the mean (SEM) and statistically significant differences are indicated as asterisks (p* <0,05; p** <0,01, p*** <0,001, p**** <0,0001)

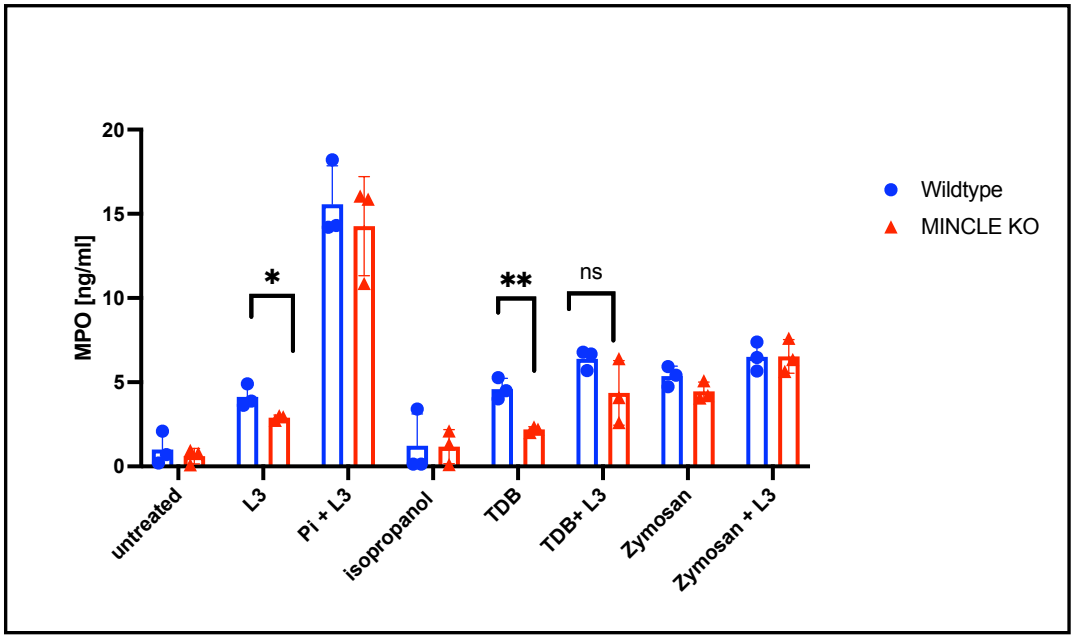

Figure 11: Neutrophils release myeloperoxidase in the presence of *S. ratti* L3.
Bone-marrow derived neutrophils were treated with 30 *S. ratti* L3, plasma from infected mice (Pi), 0.5 µg/mL TDB (functional control) and 50 µg/mL zymosan (intrinsic control). Using R&D ELISA kit, presence of myeloperoxidase (MPO) was measured. (B) 500 *S. ratti* L3, 50 nM PMA (intrinsic control) and 100 µg/mL zymosan (physiological intrinsic control). Shown is a representation of 2 biological replicates (6 technical replicates)

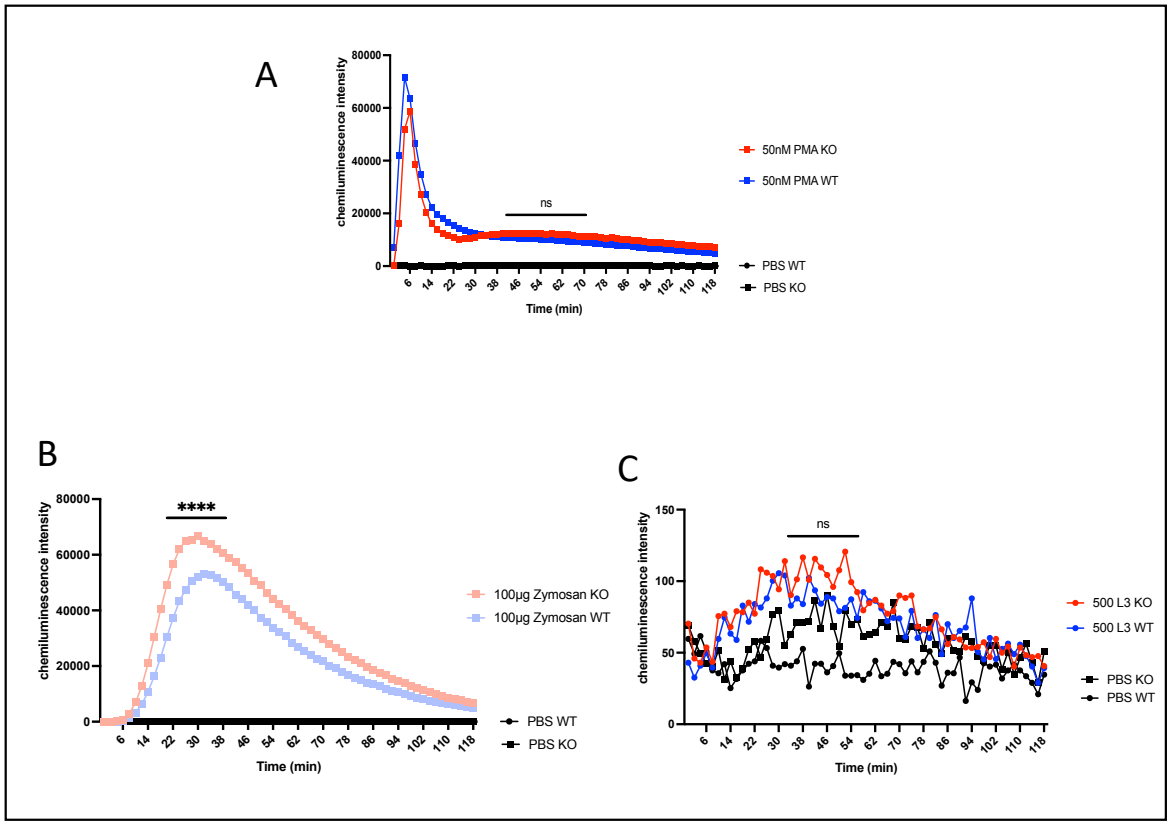

Figure 12: Release of effector molecules by neutrophils in the presence of *S. ratti* L3
Reactive oxygen species (ROS) was measured at an interval of 5 min, using Tecan reader and the Luminol chemiluminescence assay. Bars show the mean (SEM) and statistically significant differences are indicated as asterisks (p* <0,05; p** <0,01, p*** <0,001, p**** <0,0001). Shown is a representation of 3 biological replicates.

3.7. Eosinophils inhibit S. ratti motility in-vitro.

Considering the accumulated results by far, it became apparent that eosinophils might be the effector cells that *S. ratti* depends on to escape anti-*Strongyloides* immune response. Therefore, I began by analysing the expression of MINCLE by bone marrow derived eosinophils, using Flow cytometry and found out that they do not express MINCLE (Figure 13 and 14). Hence, I decided to use peripheral blood eosinophils, as these eosinophils express MINCLE (Figure 5). Also, to obtain similar cell count of eosinophils from both WT and MINCLE KO mice, naïve and 3 dpi mice were chosen (Figure 5A). In addition, I observed that the expression of CCR3, which is a chemokine receptor that is associated with eosinophil activation (Kampen GT, et. al. 2000) was similar at 0 dpi and 3 dpi (Figure 16D). Therefore, I isolated eosinophils from peripheral blood of 0 dpi and 3 dpi mice using Siglec F^+ antibody (detailed description in method section). I then gated the cells as shown in Figure 15. Briefly, I used FSC- height and FSC- area to gate for singles cells, Zombie UV for live cells, CD45 spark blue for the leukocyte population and I used CD19/CD3 BV711 to gate out adaptive immune cells. From those cells, I used Siglec F^+ to gate for eosinophils, MINCLE APC for $MINCLE^+$ cells and CCR3 PerCP-Cy5.5 for $CCR3^+$ positive cells. The results showed that the population of both WT and MINCLE eosinophils were similar and once again I observed that peripheral eosinophils do express MINCLE.

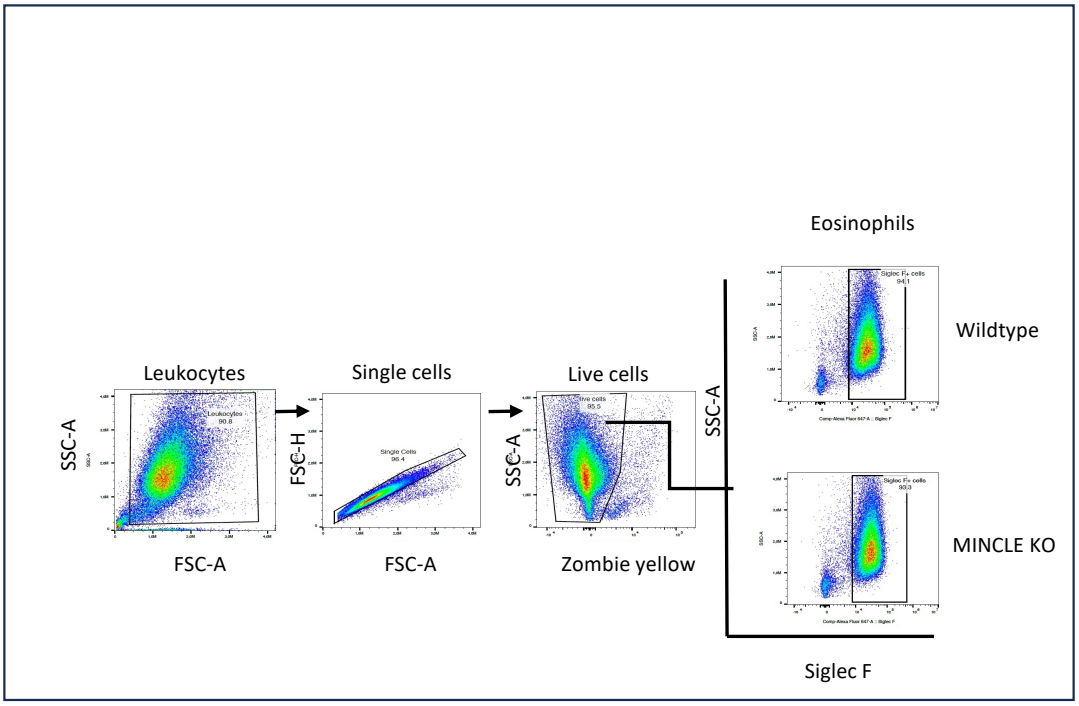

Figure 13: Purity of bone-marrow derived esoinophils
Shown is respective staining and flow cytometry analysis of cultured neutrophils isolated from bone-marrow. FSC-area and SSC - area were used to gate for leukocytes, FSC- height and FSC-area for singles cells and Zombie yellow for live cells. Using Siglec F antibody, eosinophils were identified from the live cells. Purity of cultured neutrophils were > 90.

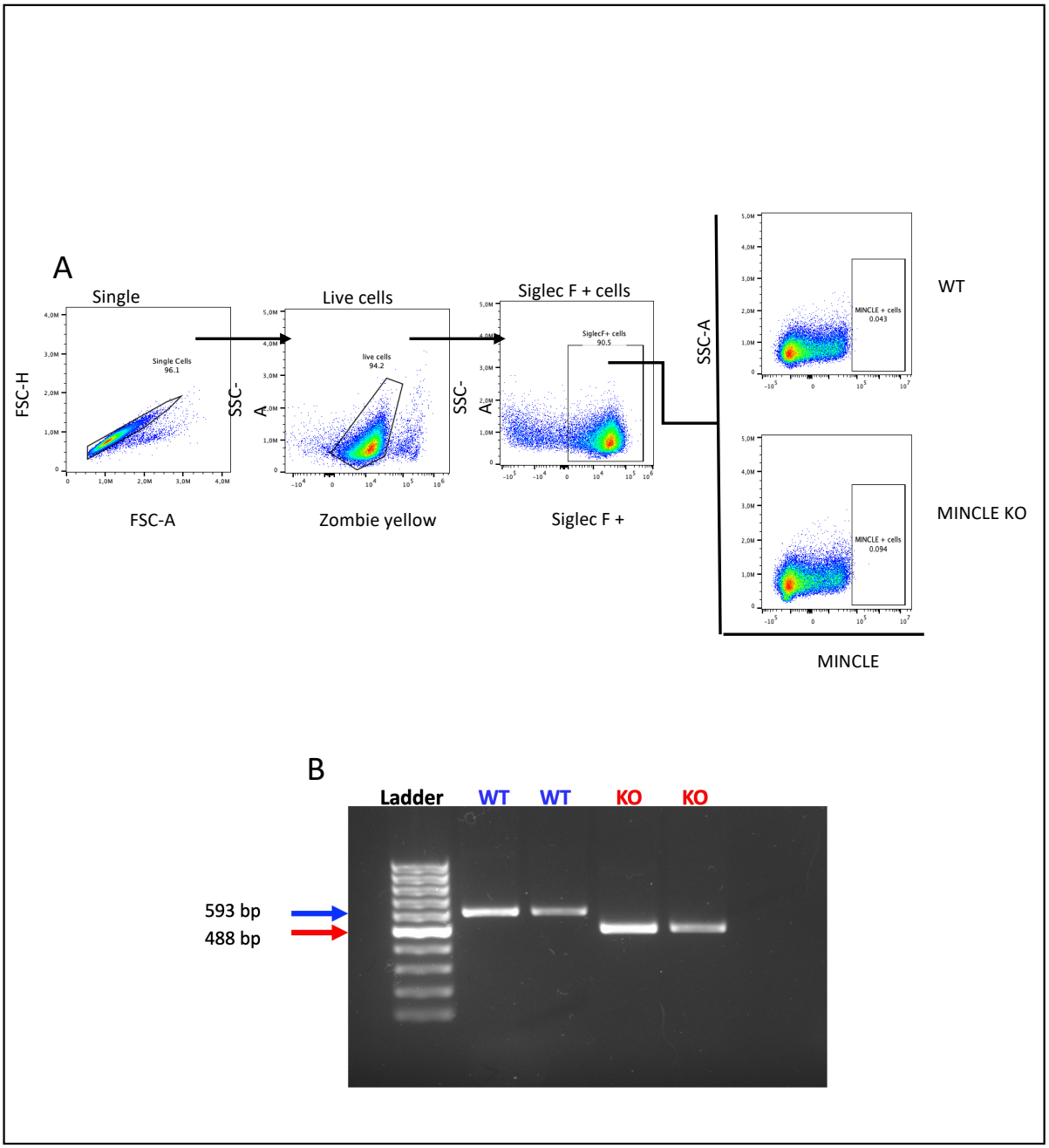

Figure 14: Bone-marrow derived eosinophils do not express MINCLE

(A) Shown is respective staining and flow cytometry analysis of cultured eosinophils isolated from bone-marrow. FSC- height and FSC- area were used to gate for singles cells and Zombie yellow for live cells. Using MINCLE antibody, eosinophils expressing MINCLE were identified. MINCLE KO eosinophils were used as specificity control. (B) shows genotyping polymerase chain reaction (PCR) analysis for WT and MINCLE KO phenotype confirmation. Wildtype shows a band of 593 bp and MINCLE KO shows a band of 488 bp.

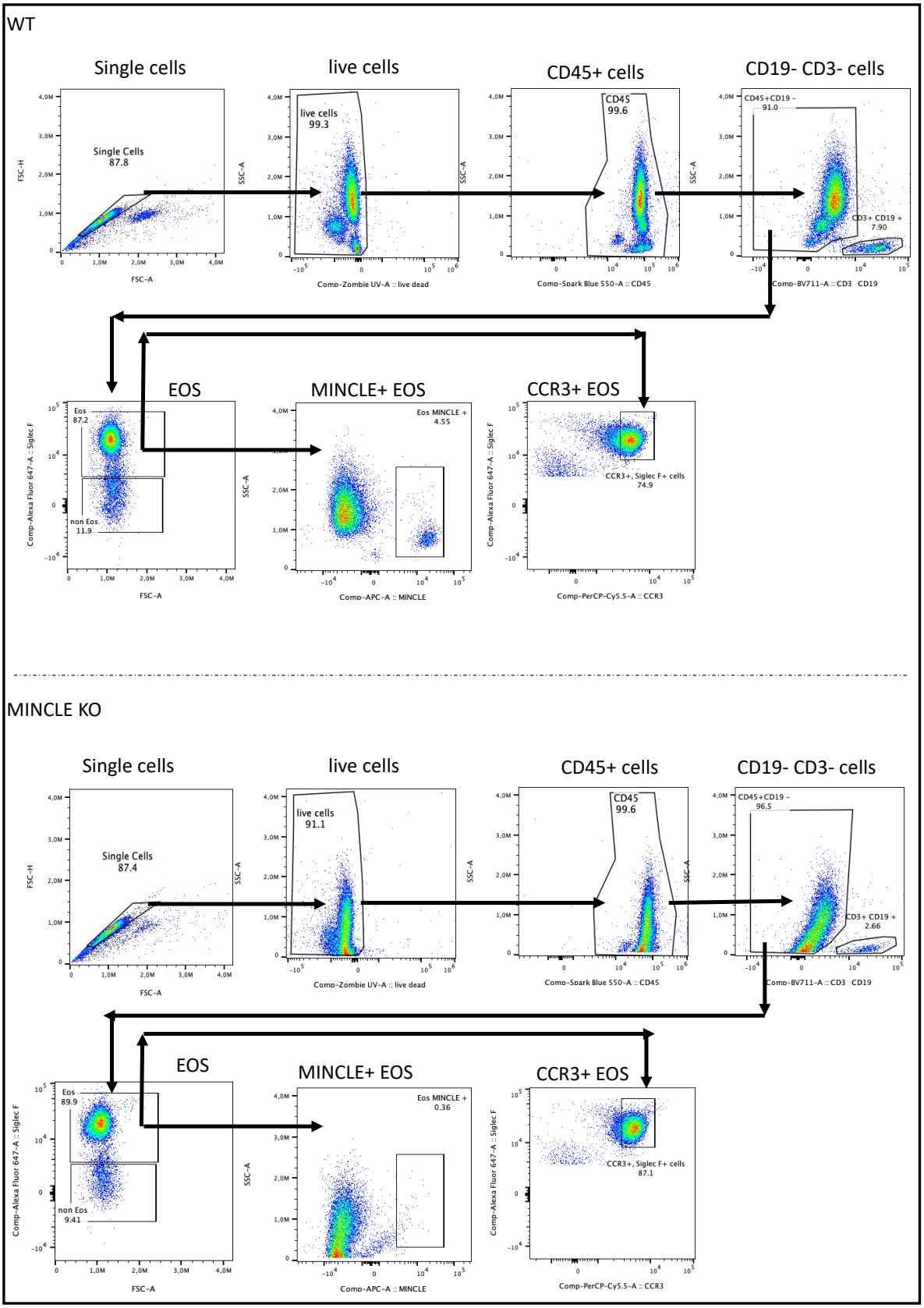

Figure 15: Gating strategy for positively selected peripheral blood eosinophils. Shown is a respresentative staining (4 WT and 4 MINCLE mice) and flow cytometry analysis of 3 dpi peripheral blood eosinophils. FSC- height and FSC- area was used to gate for singles cells ,

Zombie UV for live cells, CD45 spark blue for leukocyte population and CD19/CD3 BV711 to gate out adaptive immune cells, Siglec F^+ for eosinophils, MINCLE APC for $MINCLE^+$ cells and CCR3 PerCP-Cy5.5 for $CCR3^+$ positive cells.

3.8. MINCLE deficient eosinophils provide protective immunity against S. ratti infection.

The question now was, do MINCLE KO eosinophils protect *S. ratti* from anti-helminth immunity? And can I show this *in-vitro*? Hence, I performed both motility inhibition assay and ROS assay with these cells. I observed that, cells from both naïve WT and MINCLE KO were able to impair *S. ratti* L3 motility (Figure 16A). Furthermore, I observed that, MINCLE KO eosinophils significantly inhibited *S. ratti* L3 compared to WT eosinophils (Figure 16A). Eosinophils isolated from 3 dpi mice also showed similar results as the eosinophils from naïve mice. However, I saw a more pronounced and clear phenotypic difference between WT and MINCLE KO eosinophils in their inhibition of *S. ratti* L3 motility (Figure 16B). Also, I collected the supernatant from the motility assay after 6 h to check for presence of eosinophil peroxidase using ELISA. However, no eosinophil peroxidase was detected. Considering the results for the motility assay, I decided to use eosinophils isolated at 3 dpi to analyse ROS production. Analysis of ROS released by both WT and MINCLE KO eosinophils in the presence of *S. ratti* L3, also showed that MINCLE KO eosinophils significantly release more ROS between 14 and 30 min. WT eosinophils however did not produced ROS (Figure 16 C). Figure 15D, also showed that activation of MINCLE KO eosinophils significantly increases by 6 dpi, whereas WT CCR3 decreases.

In summary, the accumulated results depicts that the deficiency of eosinophils to express MINCLE, allows *S. ratti* to escape anti-helminth immunity.

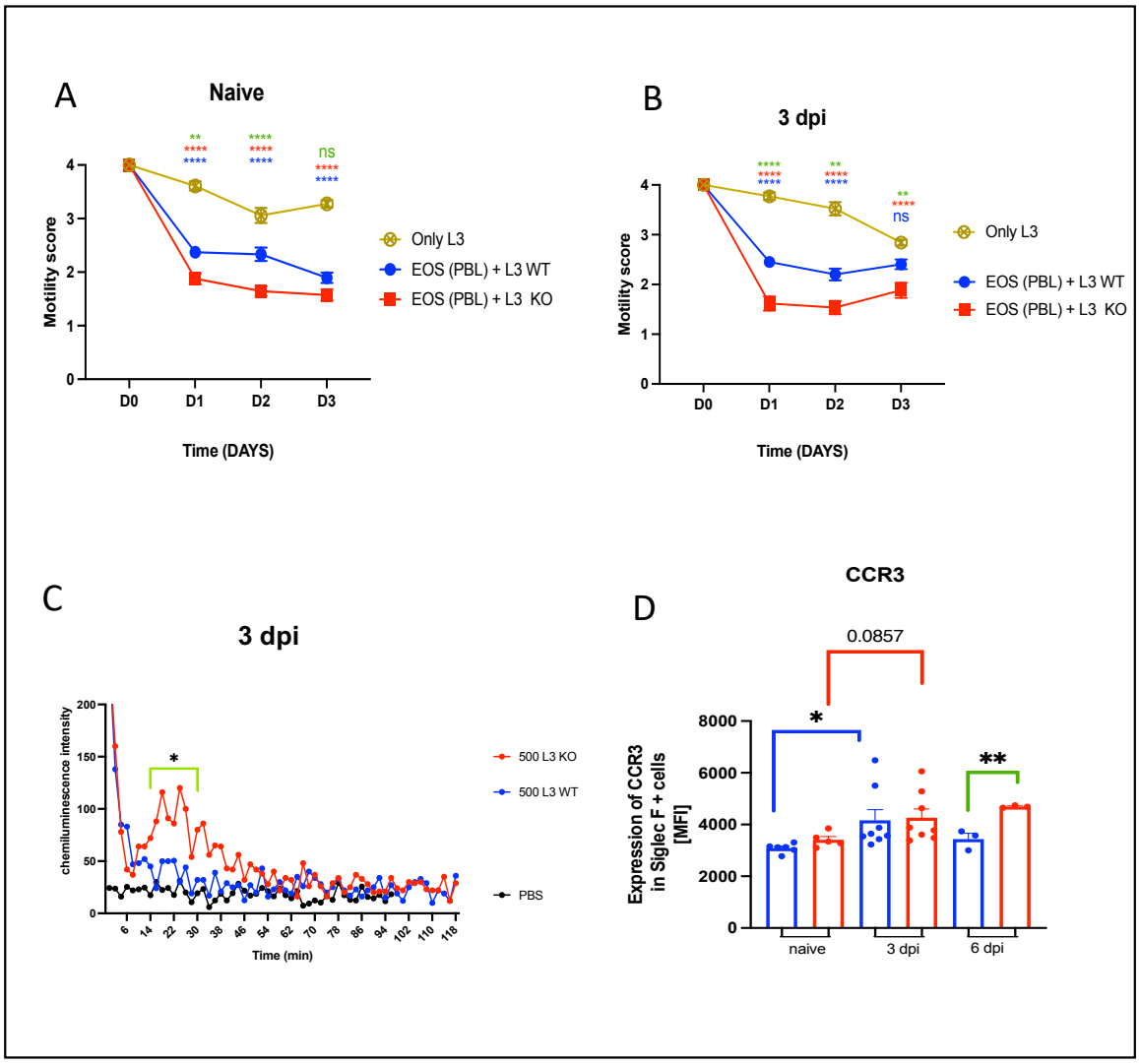

Figure 16: MINCLE deficient eosinophils protects against *S. ratti* infection.
(A-B) show motility inhibition of *S. ratti* L3, when treated with eosinophils isolated from peripheral blood before and after infection. (C)indicates release of reactive oxygen species (ROS), when *S. ratti* L3 were treated with 3 dpi eosinophils isolated from peripheral blood. (D) Flow cytometry analysis of peripheral blood to show activation of eosinophil via the expression of CCR3. Comparison of WT vs WT is indicated in bule, MINCLE KO in red and between WT and MINCLE KO with green. Bars show the mean (SEM) and statistically significant differences are indicated as asterisks (p* <0,05; p** <0,01, p*** <0,001, p**** <0,0001)

4.Discussion

4.1. Peripheral blood MINCLE-deficient eosinophils protect against S. ratti infection.

In this study, I found that MINCLE recognize *S. ratti* -derived ligands present in supernatant of dead *S. ratti* L3 lysate. Although in this study, I did not focus on identifying the *S. ratti* specific ligand activating the MINCLE reporter cells, a study by Younis and group identified *S. ratti* heat shock proteins (sHSP) in the excretory/secretory products (ESPs) of parasitic females. They also identified low levels of these sHSP genes in *S. ratti* L3 but the ELISA analysis of their ESPs showed undetectable levels of sHPS (Younis, et. al. 2011). Also, Dr. Vinayaga Gnanapragassam, a collaborative partner in charge of the molecular side of this project, also identified HSP70 in the lysate of *S. ratti* L3 and saw that HSP70 can also activate MINCLE reporter cells (unpublished). therefore, validating the observations made in this project.

Furthermore, I show that MINCLE KO mice display reduced parasite burden in the small intestine. For our publication in 2024, Dr. Lara Linnemann also shows that, the parasite burden in heterozygote MINCLE KO in the small intestine by 6 dpi was also reduced. Moreover, mice lacking CARD9, a central adaptor protein involved in MINCLE mediated signal transduction (Prado Acosta, et.al. 2021) also showed the same phenotype (Linnemann, Antwi-Ekwuruke, et. al. 2024). Thereby, enforcing the notion that the reduced parasite burden is associated with MINCLE.

I also show that eosinophils are the effector cells by which *S. ratti* is able to dampen host immune defense. Thus, *in-vivo* the population of eosinophils expands during infection (Figure 6A). Furthermore, MINCLE KO eosinophils show a more active phenotype with regards to CCR3 expression *in-vivo* (Figure 16D). Dr Lara Linnemann also show in our publication that, MINCLE KO eosinophils express more PD-L1 and CD80 *in-vivo* (Linnemann, Antwi-Ekwuruke, et. al. 2024), which is also indication for activated eosinophils (Gurtner, et. al. 2022).

In- vitro, MINCLE KO eosinophils also displayed increased ROS production (Figure 16C). ROS is also an indicator for eosinophil extracellular traps formation (Silveira, et. al. 2019). Silveira

and group showed how essential ROS are, for the formation of eosinophil extracellular traps. In the study, they inhibited ROS production with N-acetylcysteine (NAC) or diphenyleneiodonium (DPI), before treating the animals with ovalbumin and observed that ROS are needed for extracellular traps formation (Silveira et. al. 2019). In this study, I also observed the release of ROS upon *S. ratti* L3 exposure to peripheral blood eosinophils (Figure 16C). Consequently, I show that observed that, naïve and 3 dpi MINCLE KO eosinophils efficiently inhibit motility of *S. ratti* compared to WT (Figure 16A-B). To prove that, indeed eosinophils are the effector cells involve in the dampening of host immune response, Dr. Lara Linnemann, depleted eosinophils after tissue-migrating phase of *S. ratti* and this abrogated the advantage observed in the MINCLE KO mice (Linnemann, Antwi-Ekwuruke, et. al. 2024).

4.1. MINCLE mediates anti-inflammatory immune response via inhibitory ITAM (ITAMi)

Several studies have shown that inflammatory response leads to protective immune response (Chen, et.al. 2017) and the activation of CLRs is also associated with inflammatory response and consequently protective immune response for the host (Deng, et. al 2015). In response to glycolipids on the cell walls of bacteria such as trehalose-6,6-dimycolate (TDM) and its synthetic analogue trehalose-6,6 dibehenate (TDB), MINCLE has been described to trigger phosphorylation of immunoreceptor tyrosine-based activation motif (ITAM) tyrosine residues in the FcRγ chain by Src-Family kinases (Deng, et. al 2015). The phosphorylation of ITAM by phosphatase SHP2 scaffold then leads to recruitment and activation of the kinase Syk, which in turn generates an activating signal facilitated by CARD9 to cause inflammatory immune response and subsequently boost immunity to infection (Deng, et. al. 2015; Ishikawa, et. al. 2009; Schoenen, et. al. 2010, Shenderov, et. al. 2013, Sousa, et. al. 2011, Yamasaki, et. al. 2009). However, the observations made in Figure 7A, surprisingly suggest that the presence of MINCLE rather dampens immunity. Similar finding was reported by Wevers et. al 2014 in the study of *Fonsecaea monophora*, the fungal responsible for the chronic skin disease, Chromoblastomycosis. They observed that the presence of MINCLE interferes with IRF1, a transcription factor crucial for the recruitment of IL12A transcription, which is required for antifungal response. The induction of IRF1 degradation was found to be via E3 ubiquitin Ligase

Mdm2-dependent degradation pathway, which involves Syk/CARD9-mediated signaling (Wevers, et. al. 2014). Therefore, indicating that the activation of MINCLE by *Fonsecaea monophora* suppresses the antifungal immunity. Furthermore, in 2016 Iborra and group published their findings, where they showed the effect of MINCLE deficiency in correlation to *Leishmania major* parasitemia. They reported that *Leishmania* releases a soluble ligand that binds MINCLE on dendritic cells, which triggers an inhibitory effect mediated by the phosphatase SHP1 and FcRγ chain. This interaction then suppresses dendritic cell activation and as a result, dampens the priming of $CD4^+$ T cells. Thereby, allowing the parasite to evade the adaptive immune response of host (Iborra, et. al. 2016). They showed this by treating the dendritic cells with SHP1/2 inhibitor NSC-87877 and then exposing the cells to *Leishmania major*. Comparing the untreated cells to treated cells, they observed increased activation of dendritic cells. To further prove this notion, they intradermally inoculated *Leishmania major* parasite in the ears of wildtype, Clec4e -/- (MINCL KO) and CD11c∆*SHP1* (lacking SHP1) mice and observed lower parasitemia in Clec4e -/- and CD11c∆*SHP1* mice compared to wildtype (Iborra, et. al. 2016). The concept of inhibitory ITAM (ITAMi) refers to the inhibition of immune activation, which is the counterintuitive form of ITAM, a signaling that usually leads to immune activation. Thus, the MINCLE-FcRγ complex recruits SHP1, which dephosphorylates signaling intermediates and as a result leads to inhibition (Iborra, et. al. 2016). In 2012, Aloulou and group also reported, on how the engagement of monomeric IgG1 and intravenous immunoglobulin (IVlg) with FcRγRIII can covert traditionally activating immune receptor to an inhibitory one, through ITAMi mechanism (Aloulou, et. al. 2012). Therefore, based on these findings, *S. ratti* may exploit similar immune evasion strategy in modulating host defense mechanism and thereby able to dampen anti-*Strongyloides* response.

4.3. Role of neutrophils in MINCLE mediated immune response against S. ratti

Neutrophil mediated immune response against bacterial infection has been well studied (Balamayooran, et. al. 2012; Kovach, et. al. 2012)**.** The expression of MINCLE by neutrophils

and its role in immune response against pathogen has been described using Candida species, mycobacteria and *Klebsiella pneumoniae* (Lee, et. al. 2012; Vijayan, et. al. 2012; Sharma, et. al. 2014). The mechanism by which neutrophils are known to kill microbial, has also been described to be by the formation of extracellular traps (NETs), which are DNA fibrils with granular contents as myeloperoxidase (MPO), matrix metalloproteinase 9 (MMP9) and other proteases (Brinkmann, et. al. 2004; Ehrens et. al. 2021). In 2015, the first evidence of extracellular traps formation against the gastrointestinal nematode *Haemonchus contortus* was described. Using scanning electron microscopy, they showed that exposure of bovine neutrophils to third-stage larvae of *Haemonchus contortus* triggers extracellular traps formation (Muñoz-Caro, et. al. 2015). They went further to describe the different type of NETs structures which contributes to the immobilization of the Larval. Spread NETs consists of decondensed chromatin smooth and elongated web-like structures. They also observed antimicrobial proteins composed by thin fibers with a diameter of 15-17nm, which were detected in co-localization with DNA NET structures. Using Fluorescence-based analysis, specifically Sytox Orange staining, the antimicrobial proteins were confirmed to be MPO, neutrophil specific elastase (NE) and histones. Aggregated NETs on the other hand, were observed to have large cluster of NET structures, which significantly contributed to the entrapment of larvae and subsequently larval motility inhibition (Muñoz-Caro et. al 2015). In terms of *Strongyloides*, Bonne-Anné and group also showed that human neutrophils release NETs upon exposure to *S. stercoralis*. They also observed that, NETs trap the larvae but do not directly kill them (Bonne-Année, et. al. 2014). In 2021, similar experiment was performed, where treatment of *S. ratti* L3 led to release of NETs, histones and MPO and consequently impairment of Larvae motility (Ehrens et. al 2021). However, in this study I compared motility impairment of *S ratti* L3 *in-vitro,* with regards to both WT and MINCLE KO neutrophils. The results shown in Figure 9A, indicates that both WT and MINCLE KO neutrophils were able to significantly inhibit *S. ratti* motility at the same rate compared to untreated *S. ratti* L3. Although the results attained in Figure 11 showed that, treatment of *S. ratti* L3 with neutrophils leads to significant release of MPO in WT neutrophils compared to MINCLE KO neutrophils, similar results was not observed for ROS production (Figure 12D). Thus, exposure of *S. ratti* L3 to both WT and MINCLE KO neutrophils did not lead to ROS production. I expected the released of both MPO and ROS upon exposure to *S. ratti* L3 based on the work of both Bonne-Année, et. al.2014 and Kirchner, et. al. 2012. Bonne-Année and group showed

that MPO is a crucial component of NET formation. Kirchner and group on other hand showed the interdependence of MPO and ROS in the process of NETosis. Thus, for NETs to form, it requires ROS production via NADPH oxidase. At the same time, presence of MPO is also required for the antimicrobial function of NETs (Kirchner, et. al. 2012).

For instance, the study about the role of MINCLE in regulating neutrophil extracellular traps formation during *Klebsiella pneumoniae* infection, showed an impaired ability of neutrophil to form NETs in the absence of MINCLE (Sharma, et. al. 2014). They infected WT and MINCLE KO mice intranasally with *Klebsiella pneumoniae* and monitored disease progression. Their results showed that WT mice were able to clear infection and survive. However, compared to the MINCLE KO mice, diseased progressed daily and led to the death of all MINCLE KO mice by day 6 of infection. They, then examined the neutrophils isolated from bronchoalveolar lavage, by staining the neutrophil specific enzyme, elastase, which is known to cause chromatin decondensation through proteolysis of nuclear proteins (Kasperkiewicz, et. al. 2020). NETs observed in MINCLE KO neutrophils were impaired and lacked the web-like structure. Also, quantitative analysis showed that WT neutrophils produced significantly higher levels of NETs compared to MINCLE KO neutrophils (Sharma, et. al. 2014). However, the neutrophil specific enzyme elastase was not performed in this study to back this notion.

Examining the results obtained for ROS production, treatment of *S. ratti* L3 with WT and MINCLE KO neutrophils showed no significant release of ROS compared to untreated control (Figure 10D). The treatment of neutrophils with PMA, expectedly caused the production of ROS by both WT and MINCLE KO neutrophils at similar rate (Sharma, et. al. 2017). These results show that in the context of MINCLE, neutrophils do not participate in the dampen of immune response of host.

4.4. Possible role of complements in S. ratti infection.

In this study, I introduced plasma from infected and naïve mice to test for efficiency and differences in MPO release and also motility inhibition of *S. ratti* L3. I observed that more

MPO was released when plasma from infected mice was co-cultured with either WT or MINCLE KO cells and S. ratti L3. However, no differences were observed. Interestingly, addition of plasma from infected and naïve mice accelerated motility inhibition by both WT MINCLE KO neutrophils and eosinophils from infected mice. Also in these instances, no differences were observed (Figure 9 and 13). In addition, when *S. ratti* L3 were co-cultured with either plasma from infected or naïve mice without cells, I observed impairment of Larvae motility. To explain what could have been the cause of Larvae motility inhibition without the presence of effector cells, I show in Figure 6, the presence of IgG and IgM in both plasma from infected and naïve mice, where plasma from infected mice show significantly higher levels of antibodies. Even though I did not check for complements, it is known that plasma consist of complements (Haller L., et. al. 1978). Complements are humoral and cellular effector system in blood and other tissues which act in a cascade manner, resulting in inflammation and enhancement of adaptive immunity (Reid and Porter 1981). There are three pathways namely; classical, lectin and alternative pathway. The complements opsonize the surface of pathogens with opsonins C1q (Classical pathway), mannose binding lectin (Lectin pathway) and C3 (Alternative pathway) to initiate innate host defense. Thus phagocytes, which express complement surface receptors bind to these opsonins and activate the complement cascade (Janeway 5th edition). To achieve the ultimate goal of complement activation, the classical and alternative pathway produce anaphylatoxins C3a and lectin pathway produce C5, which in turn leads to the promotion of chemotaxis, myeloid cell activation and the membrane attack complex (MAC or C5b-9 complex), which disrupts and form pores in phospholipid bilayer to induce killing of pathogen (Janeway 5th edition).

Antibodies such as IgM and IgG activate the classical pathway of complement by binding to C1q, which is a complex of three protein (C1q, C1r and C1s). Due to the structure rearrangement of the IgM pentamer when bound to pathogens, they tend to have a higher infinity to bind C1q compared to IgG, which can only bind C1q in the presence of two or more IgG in close proximity. Moreover, the initiation of the complement cascade only takes place when the antibodies are bound to multiple sites on the surface of the pathogen (Janeway 5th edition). Therefore, this explain could explain why motility of *S. ratti* L3 is inhibited, when the larvae are co-cultured with plasma only.

4.5. Conclusion

In conclusion, I show that eosinophils express MINCLE during helminth infection, which was not known before and that these cells are negatively regulated by *S. ratti*-derived MINCLE ligands. Thus, in the presence of MINCLE, *S. ratti* is able to dampen immune response of host, thereby delaying parasite ejection from small intestine. These findings, suggest a novel helminth-induced immune evasive mechanism targeting innate intestinal immunity.

5. Outlook

5.1. Role of eosinophils in S. ratti infection

Similar to neutrophils, eosinophils are also known to release extracellular traps to exert their host defence against primarily multicellular helminths and also other pathogens (Mukherjee, et. al. 2018). Eosinophils has also been described to release extracellular traps co-localized with eosin granules (Muñoz-Caro et. al 2015). Specifically, eosinophils release four distinct granule which are cationic proteins (ECP), major basic protein (MBP), eosinophil peroxidase (EPO) and eosinophil-derived neurotoxin (EDN/EPX) to cause dysfunction and destruction of cells (Motegi and Kita 1998; Adamko, et.al.2004). Also, eosinophils release lipid mediators such as leukotriene C4, platelet-activating factor and liposins for host defence against pathogens (Tamura, et. al. 1988). However, an attempt to analyse eosinophil peroxidase in this study failed. Breifly, I co-cultured *S. ratti* L3 with both WT and MINCLE KO eosinophils, collected the supernatant after 6h and used ELISA to detect the presence of EPO but none was detected. In 2011, Dworski and group reported that release of eosinophil extracellular traps in human allergic asthmatic airways were associated with major basic protein (MBP). However, they did not observe any correlation with eosinophil specific cytokines (IL-5, IFN-γ) and chemokines (eotaxin) (Dworski, et. al. 2011). Therefore, it could be concluded that, the release of eosinophil extracellular traps and the specific inflammatory effector molecule may depend on the disease or pathogen.

For instance, an investigation of eosinophil dependent immunity against the nematode *Nippostrongylus brasiliensis*, showed eosinophil peroxidase (EPO) activity after treating mice with L3 *Nippostrongylus brasiliensis* (Giacomin, et. al. 2007). However, I did not observe this although *S. ratti* is also a nematode. Also, several studies have shown that, to reach a plausible conclusion on the function of eosinophils during infection, it is best to measure two or more eosinophil granules. In 1996, elevated serum level of eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN/EPX) were measured in patients with onchocerciasis, bancroftian filariasis and intestinal schistosomiasis as indicator of ongoing infection (Tischendor, et. al 1996). In 2006 Specht and colleagues also showed that infecting EPO and

MBP deficient mice with the rodent filaria *Litomosoides sigmodontis* led to significantly higher worm burden in these knockout mice compared to the wildtype (Specht, et. al. 2006). Therefore, in hindsight I should have investigated other effector molecules apart from EPO as this has not been elucidated in the study of *S. ratti* L3 infection.

Another strategy that could have helped show the toxicity of eosinophil granule proteins against *S. ratti*, would have been the strategy that Hamann and collogues implemented. They performed an *in-vitro* purification of four different types of eosinophils granule proteins, namely MBP, EPO, ECP and EDN. After that, *Brugia pahangi* and *Brugia malayi* which are microfilariae were treated with different doses of each eosinophil granule protein. ECP and MBP killed these worms in a dose-dependent manner. EDN could kill the microfilariae as well but compared ECP and MBP, a higher concentration was required. EPO alone was also shown to kill the worms and more interestingly, a combination of EPO, hydrogen peroxide (H_2O_2) and a halide (I-, Br- and Cl-) proved to be more toxic to the worms (Hamann KJ, et. al. 1990). This is because through the Haber- Weiss reaction, the enzyme EPO bind to the ionically charged surface of the helminths. This then catalyse H_2O_2 and Halides into H_2O and hypohalous acids. The hypohalous acids in turn form a far more stable and damaging products which leads to killing of the helminth (Callahan H.L., et. al. 1988).

Lastly in terms of *S. ratti* ligands, future experiments will show if the purified *S. ratti* ligands present in *S. ratti* L3 lysate, could also cause comparable mechanism mediated by MINCLE expressing eosinophils.

Publications/authorship

1. Linnemann L, **Antwi-Ekwuruke J**, Gnanapragassam V, Bang C, Rühlemann M, Ruland J, Hartmann W, Heepmann L, Dörken S, Yunus SM, Viebrock B, Schlosser A, Lepenies B, Breloer M. The C-type lectin receptor MINCLE interferes with eosinophil function and protective intestinal immunity in Strongyloides ratti-infected mice. Mucosal Immunol. 2025 Feb;18(1):220-231. doi: 10.1016/j.mucimm.2024.11.005. Epub 2024 Nov 22. PMID: 39581231.

2. Cambra-Pellejà M, Valderas-García E, Balaña-Fouce R, de la Vega J, Del Olmo E, **Antwi-Ekwuruke J**, Linnemann L, Heepmann L, Breloer M, Martínez-Valladares M. Evaluating alternative compounds for strongyloidiasis therapy: Novel insights from larval migration inhibition test. PLoS Negl Trop Dis. 2024 Oct 7;18(10):e0012532. doi: 10.1371/journal.pntd.0012532. PMID: 39374184; PMCID: PMC11458022.

6. References

Adamko DJ, Wu Y, Gleich GJ, Lacy P, Moqbel R. The induction of eosinophil peroxidase release: improved methods of measurement and stimulation. J Immunol Methods. 2004 Aug;291(1-2):101-8. doi: 10.1016/j.jim.2004.05.003. PMID: 15345309.

Ahrén IL, Eriksson E, Egesten A, Riesbeck K. Nontypeable Haemophilus influenzae activates human eosinophils through beta-glucan receptors. Am J Respir Cell Mol Biol. 2003 Nov;29(5):598-605. doi: 10.1165/rcmb.2002-0138OC. Epub 2003 Apr 14. PMID: 12689921.

Aloulou M, Ben Mkaddem S, Biarnes-Pelicot M, Boussetta T, Souchet H, Rossato E, Benhamou M, Crestani B, Zhu Z, Blank U, Launay P, Monteiro RC. IgG1 and IVIg induce inhibitory ITAM signaling through FcγRIII controlling inflammatory responses. Blood. 2012 Mar 29;119(13):3084-96. doi: 10.1182/blood-2011-08-376046. Epub 2012 Feb 14. PMID: 22337713.

Anthony RM, Urban JF Jr, Alem F, Hamed HA, Rozo CT, Boucher JL, Van Rooijen N, Gause WC. Memory T(H)2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. Nat Med. 2006 Aug;12(8):955-60. doi: 10.1038/nm1451. Epub 2006 Jul 30. PMID: 16892038; PMCID: PMC1955764.

Anuradha R, Munisankar S, Bhootra Y, Jagannathan J, Dolla C, Kumaran P, Shen K, Nutman TB, Babu S. Systemic Cytokine Profiles in Strongyloides stercoralis Infection and Alterations following Treatment. Infect Immun. 2015 Nov 23;84(2):425-31. doi: 10.1128/IAI.01354-15. PMID: 26597982; PMCID: PMC4730571.

ARTHUR RP, SHELLEY WB. Larva currens; a distinctive variant of cutaneous larva migrans due to Strongyloides stercoralis. AMA Arch Derm. 1958 Aug;78(2):186-90. doi: 10.1001/archderm.1958.01560080044007. PMID: 13558704.

Balamayooran G, Batra S, Theivanthiran B, Cai S, Pacher P, Jeyaseelan S. Intrapulmonary G-CSF rescues neutrophil recruitment to the lung and neutrophil release to blood in Gram-negative bacterial infection in MCP-1-/- mice. J Immunol. 2012 Dec 15;189(12):5849-59. doi: 10.4049/jimmunol.1200585. Epub 2012 Nov 5. PMID: 23129755; PMCID: PMC3518636.

Beknazarova M, Whiley H, Ross K. Strongyloidiasis: A Disease of Socioeconomic Disadvantage. Int J Environ Res Public Health. 2016 May 20;13(5):517. doi: 10.3390/ijerph13050517. PMID: 27213420; PMCID: PMC4881142.

Bock, C.N., S. Babu, M. Breloer, A. Rajamanickam, Y. Bhootra, M.-L. Brunn, A. A. Kühl, R. Merle, M. Löhning, S. Hartmann, S. Rausch. 2017. Th2/1 hybrid cells occurring in murine and human strongyloidiasis share effector functions of Th1 cells, Frontiers in Cellular and Infection Microbiology, doi: 10.3389/fcimb.2017.00261.

Bonne-Année S, Hess JA, Abraham D. Innate and adaptive immunity to the nematode Strongyloides stercoralis in a mouse model. Immunol Res. 2011 Dec;51(2-3):205-14. doi: 10.1007/s12026-011-8258-2. PMID: 22101674; PMCID: PMC6707741.

Breloer M, Abraham D. Strongyloides infection in rodents: immune response and immune regulation. Parasitology. 2017 Mar;144(3):295-315. doi: 10.1017/S0031182016000111. Epub 2016 Feb 24. PMID: 26905057.

Breloer M, Linnemann L. Strongyloides ratti infection in mice: immune response and immune modulation. Philos Trans R Soc Lond B Biol Sci. 2024 Jan 15;379(1894):20220440. doi: 10.1098/rstb.2022.0440. Epub 2023 Nov 27. PMID: 38008111; PMCID: PMC10676808.

Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. Science. 2004 Mar 5;303(5663):1532-5. doi: 10.1126/science.1092385. PMID: 15001782.

Broide DH, Paine MM, Firestein GS. Eosinophils express interleukin 5 and granulocyte macrophage-colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics. J Clin Invest. 1992 Oct;90(4):1414-24. doi: 10.1172/JCI116008. PMID: 1401075; PMCID: PMC443187.

Buonfrate D, Tamarozzi F, Paradies P, Watts MR, Bradbury RS, Bisoffi Z. The diagnosis of human and companion animal Strongyloides stercoralis infection: Challenges and solutions. A scoping review. Adv Parasitol. 2022;118:1-84. doi: 10.1016/bs.apar.2022.07.001. Epub 2022 Sep 2. PMID: 36088083.

Bustinduy AL, Sousa-Figueiredo JC, Adriko M, Betson M, Fenwick A, Kabatereine N, Stothard JR. Fecal occult blood and fecal calprotectin as point-of-care markers of intestinal morbidity in Ugandan children with Schistosoma mansoni infection. PLoS Negl Trop Dis. 2013 Nov 14;7(11):e2542. doi: 10.1371/journal.pntd.0002542. PMID: 24244777; PMCID: PMC3828154.

Callahan HL, Crouch RK, James ER. Helminth anti-oxidant enzymes: a protective mechanism against host oxidants? Parasitol Today. 1988 Aug;4(8):218-25. doi: 10.1016/0169-4758(88)90162-7. PMID: 15463102.

Castro GA. Helminths: Structure, Classification, Growth, and Development. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 86. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8282/

Cayrol C, Girard JP. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. Curr Opin Immunol. 2014 Dec;31:31-7. doi: 10.1016/j.coi.2014.09.004. Epub 2014 Sep 29. PMID: 25278425.

Chamaillard M, Hashimoto M, Horie Y, Masumoto J, Qiu S, Saab L, Ogura Y, Kawasaki A, Fukase K, Kusumoto S, Valvano MA, Foster SJ, Mak TW, Nuñez G, Inohara N. An essential role for

NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat Immunol. 2003 Jul;4(7):702-7. doi: 10.1038/ni945. Epub 2003 Jun 6. PMID: 12796777.

Chen F, Wu W, Millman A, Craft JF, Chen E, Patel N, Boucher JL, Urban JF Jr, Kim CC, Gause WC. Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. Nat Immunol. 2014 Oct;15(10):938-46. doi: 10.1038/ni.2984. Epub 2014 Aug 31. PMID: 25173346; PMCID: PMC4479254.

Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2017 Dec 14;9(6):7204-7218. doi: 10.18632/oncotarget.23208. PMID: 29467962; PMCID: PMC5805548.

Croese J., Gaze S. T., Loukas A. Changed gluten immunity in celiac disease by Necator americanus provides new insights into autoimmunity, International Journal for Parasitology,
Dawkins HJ, Grove DI. Kinetics of primary and secondary infections with Strongyloides ratti in mice. Int J Parasitol. 1981 Feb;11(1):89-96. doi: 10.1016/0020-7519(81)90031-x. PMID: 7228480.

Dawkins HJ, Thomason HJ, Grove DI. The occurrence of Strongyloides ratti in the tissues of mice after percutaneous infection. J Helminthol. 1982 Mar;56(1):45-50. doi: 10.1017/s0022149x00034994. PMID: 6461696.

Dawkins, H. J., Grove, D. I., Dunsmore, J. D. and Mitchell, G. F. (1980). Strongyloides ratti: susceptibility to infection and resistance to re-infection in inbred strains of mice as assessed by excretion of larvae. International Journal for Parasitology 10, 125–129.

Dawkins, H. J., Mitchell, G. F. and Grove, D. I. (1982a). Strongyloides ratti infections in congenitally hypothymic (nude) mice. The Australian Journal of Experimental Biology and Medical Science 60, 181–186.

Deng Z, Ma S, Zhou H, Zang A, Fang Y, Li T, Shi H, Liu M, Du M, Taylor PR, Zhu HH, Chen J, Meng G, Li F, Chen C, Zhang Y, Jia XM, Lin X, Zhang X, Pearlman E, Li X, Feng GS, Xiao H. Tyrosine phosphatase SHP-2 mediates C-type lectin receptor-induced activation of the kinase Syk and anti-fungal TH17 responses. Nat Immunol. 2015 Jun;16(6):642-52. doi: 10.1038/ni.3155. Epub 2015 Apr 27. PMID: 25915733; PMCID: PMC4439382.

Dillon S, Agrawal S, Banerjee K, Letterio J, Denning TL, Oswald-Richter K, Kasprowicz DJ, Kellar K, Pare J, van Dyke T, Ziegler S, Unutmaz D, Pulendran B. Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. J Clin Invest. 2006 Apr;116(4):916-28. doi: 10.1172/JCI27203. PMID: 16543948; PMCID: PMC1401484.

Dworski R, Simon HU, Hoskins A, Yousefi S. Eosinophil and neutrophil extracellular DNA traps in human allergic asthmatic airways. J Allergy Clin Immunol. 2011 May;127(5):1260-6. doi: 10.1016/j.jaci.2010.12.1103. Epub 2011 Feb 18. PMID: 21315435; PMCID: PMC3085562.

Ehrens A, Rüdiger N, Heepmann L, Linnemann L, Hartmann W, Hübner MP, Breloer M. Eosinophils and Neutrophils Eliminate Migrating Strongyloides ratti Larvae at the Site of Infection in the Context of Extracellular DNA Trap Formation. Front Immunol. 2021 Aug 12;12:715766. doi: 10.3389/fimmu.2021.715766. Erratum in: Front Immunol. 2022 Mar 21;13:878640. doi: 10.3389/fimmu.2022.878640. PMID: 34475874; PMCID: PMC8406770.

Else KJ, Keiser J, Holland CV, Grencis RK, Sattelle DB, Fujiwara RT, Bueno LL, Asaolu SO, Sowemimo OA, Cooper PJ. Whipworm and roundworm infections. Nat Rev Dis Primers. 2020 May 28;6(1):44. doi: 10.1038/s41572-020-0171-3. PMID: 32467581.

Foell D, Wittkowski H, Roth J. Monitoring disease activity by stool analyses: from occult blood to molecular markers of intestinal inflammation and damage. Gut. 2009 Jun;58(6):859-68. doi: 10.1136/gut.2008.170019. Epub 2009 Jan 9. PMID: 19136508.

Furukawa A, Kamishikiryo J, Mori D, Toyonaga K, Okabe Y, Toji A, Kanda R, Miyake Y, Ose T, Yamasaki S, Maenaka K. Structural analysis for glycolipid recognition by the C-type lectins Mincle and MCL. Proc Natl Acad Sci U S A. 2013 Oct 22;110(43):17438-43. doi: 10.1073/pnas.1312649110. Epub 2013 Oct 7. PMID: 24101491; PMCID: PMC3808641.

Gessner A, Mohrs K, Mohrs M. Mast cells, basophils, and eosinophils acquire constitutive IL-4 and IL-13 transcripts during lineage differentiation that are sufficient for rapid cytokine production. J Immunol. 2005 Jan 15;174(2):1063-72. doi: 10.4049/jimmunol.174.2.1063. PMID: 15634931.

Giacomin PR, Gordon DL, Botto M, Daha MR, Sanderson SD, Taylor SM, Dent LA. The role of complement in innate, adaptive and eosinophil-dependent immunity to the nematode Nippostrongylus brasiliensis. Mol Immunol. 2008 Jan;45(2):446-55. doi: 10.1016/j.molimm.2007.05.029. Epub 2007 Aug 1. PMID: 17675237.

Gleich GJ, Adolphson CR. The eosinophilic leukocyte: structure and function. Adv Immunol. 1986;39:177-253. doi: 10.1016/s0065-2776(08)60351-x. PMID: 3538819.

Grove D.I, Human Strongyloidiasis, Advances in Parasitology, Academic Press, Volume 38, 1996, Pages 251-309, ISSN 0065-308X, ISBN 9780120317387, https://doi.org/10.1016/S0065-308X(08)60036-6.

Gurtner A, Borrelli C, Gonzalez-Perez I, Bach K, Acar IE, Núñez NG, Crepaz D, Handler K, Vu VP, Lafzi A, Stirm K, Raju D, Gschwend J, Basler K, Schneider C, Slack E, Valenta T, Becher B, Krebs P, Moor AE, Arnold IC. Active eosinophils regulate host defence and immune responses in colitis. Nature. 2023 Mar;615(7950):151-157. doi: 10.1038/s41586-022-05628-7. Epub 2022 Dec 12. PMID: 36509106; PMCID: PMC9977678.

Haller L, Zubler RH, Lambert PH. Plasma levels of complement components and complement haemolytic activity in protein-energy malnutrition. Clin Exp Immunol. 1978 Nov;34(2):248-52. PMID: 216509; PMCID: PMC1537499.

Hamann KJ, Gleich GJ, Checkel JL, Loegering DA, McCall JW, Barker RL. In vitro killing of microfilariae of Brugia pahangi and Brugia malayi by eosinophil granule proteins. J Immunol. 1990 Apr 15;144(8):3166-73. PMID: 2324497.

Hansel TT, De Vries IJ, Carballido JM, Braun RK, Carballido-Perrig N, Rihs S, Blaser K, Walker C. Induction and function of eosinophil intercellular adhesion molecule-1 and HLA-DR. J Immunol. 1992 Sep 15;149(6):2130-6. PMID: 1355503.

Hardison SE, Brown GD. C-type lectin receptors orchestrate antifungal immunity. *Nat Immunol* (2012) **13**:817–22. doi: 10.1038/ni.2369

Hayes K. S., Bancroft A. J., Goldrick M., Portsmouth C., Roberts I.S, Grencis R.K. Exploitation of the intestinal microflora by the parasitic nematode Trichuris muris. Science 328, 1391–1394 (2010).

Holland CV. The long and winding road of Ascaris larval migration: the role of mouse models. Parasitology. 2021 Feb 22;148(14):1-9. doi: 10.1017/S0031182021000366. Epub ahead of print. PMID: 33612124; PMCID: PMC8660642.

Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. J Clin Invest. 2008 Apr;118(4):1311-21. doi: 10.1172/JCI34261. PMID: 18382743; PMCID: PMC2276811.

Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, Xiao S. Hookworm infection. N Engl J Med. 2004 Aug 19;351(8):799-807. doi: 10.1056/NEJMra032492. PMID: 15317893.

Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, Ghiran S, Gerard NP, Yu C, Orkin SH, Gerard C. A critical role for eosinophils in allergic airways remodeling. Science. 2004 Sep 17;305(5691):1776-9. doi: 10.1126/science.1100283. PMID: 15375268.

Iborra S, Martínez-López M, Cueto FJ, Conde-Garrosa R, Del Fresno C, Izquierdo HM, Abram CL, Mori D, Campos-Martín Y, Reguera RM, Kemp B, Yamasaki S, Robinson MJ, Soto M, Lowell CA, Sancho D. Leishmania Uses Mincle to Target an Inhibitory ITAM Signaling Pathway in Dendritic Cells that Dampens Adaptive Immunity to Infection. Immunity. 2016 Oct 18;45(4):788-801. doi: 10.1016/j.immuni.2016.09.012. Epub 2016 Oct 11. PMID: 27742545; PMCID: PMC5074365.

Imai Y, Yasuda K, Sakaguchi Y, Haneda T, Mizutani H, Yoshimoto T, Nakanishi K, Yamanishi K. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. Proc Natl Acad Sci U S A. 2013 Aug 20;110(34):13921-6. doi: 10.1073/pnas.1307321110. Epub 2013 Aug 5. PMID: 23918359; PMCID: PMC3752227.

Ishii KJ, Coban C, Kato H, Takahashi K, Torii Y, Takeshita F, Ludwig H, Sutter G, Suzuki K, Hemmi H, Sato S, Yamamoto M, Uematsu S, Kawai T, Takeuchi O, Akira S. A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. Nat Immunol. 2006 Jan;7(1):40-8. doi: 10.1038/ni1282. Epub 2005 Nov 13. Erratum in: Nat Immunol. 2006 Apr;7(4):427. PMID: 16286919.

Ishikawa E, Ishikawa T, Morita YS, Toyonaga K, Yamada H, Takeuchi O, Kinoshita T, Akira S, Yoshikai Y, Yamasaki S. Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J Exp Med. 2009 Dec 21;206(13):2879-88. doi: 10.1084/jem.20091750. Epub 2009 Dec 14. PMID: 20008526; PMCID: PMC2806462.

Ishikawa T, Itoh F, Yoshida S, Saijo S, Matsuzawa T, Gonoi T, Saito T, Okawa Y, Shibata N, Miyamoto T, Yamasaki S. Identification of distinct ligands for the C-type lectin receptors Mincle and Dectin-2 in the pathogenic fungus Malassezia. Cell Host Microbe. 2013 Apr 17;13(4):477-88. doi: 10.1016/j.chom.2013.03.008. PMID: 23601109.

Janeway CA Jr, Travers P, Walport M, et al., New York: Garland Science; 2001. Immunobiology: The Immune System in Health and Disease. 5th edition.

Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol. 1989;54 Pt 1:1-13. doi: 10.1101/sqb.1989.054.01.003. PMID: 2700931.

Kalantari P, Bunnell SC, Stadecker MJ. The C-type Lectin Receptor-Driven, Th17 Cell-Mediated Severe Pathology in Schistosomiasis: Not All Immune Responses to Helminth Parasites Are Th2 Dominated. Front Immunol. 2019 Jan 30;10:26. doi: 10.3389/fimmu.2019.00026. PMID: 30761125; PMCID: PMC6363701.

Kalantari P, Morales Y, Miller EA, Jaramillo LD, Ponichtera HE, Wuethrich MA, Cheong C, Seminario MC, Russo JM, Bunnell SC, Stadecker MJ. CD209a Synergizes with Dectin-2 and Mincle to Drive Severe Th17 Cell-Mediated Schistosome Egg-Induced Immunopathology. Cell Rep. 2018 Jan 30;22(5):1288-1300. doi: 10.1016/j.celrep.2018.01.001. PMID: 29386115; PMCID: PMC5815841.

Kampen GT, Stafford S, Adachi T, Jinquan T, Quan S, Grant JA, Skov PS, Poulsen LK, Alam R. Eotaxin induces degranulation and chemotaxis of eosinophils through the activation of ERK2 and p38 mitogen-activated protein kinases. Blood. 2000 Mar 15;95(6):1911-7. PMID: 10706854.

Kasperkiewicz P, Hempel A, Janiszewski T, Kołt S, Snipas SJ, Drag M, Salvesen GS. NETosis occurs independently of neutrophil serine proteases. J Biol Chem. 2020 Dec 18;295(51):17624-17631. doi: 10.1074/jbc.RA120.015682. PMID: 33454002; PMCID: PMC7762935.

Khieu V, Schär F, Marti H, Sayasone S, Duong S, Muth S, Odermatt P. Diagnosis, treatment and risk factors of Strongyloides stercoralis in schoolchildren in Cambodia. PLoS Negl Trop Dis. 2013;7(2):e2035. doi: 10.1371/journal.pntd.0002035. Epub 2013 Feb 7. PMID: 23409200; PMCID: PMC3566990.

Kirchner T, Möller S, Klinger M, Solbach W, Laskay T, Behnen M. The impact of various reactive oxygen species on the formation of neutrophil extracellular traps. Mediators Inflamm.

2012;2012:849136. doi: 10.1155/2012/849136. Epub 2012 Jan 26. PMID: 22481865; PMCID: PMC3317033.

Kovach MA, Standiford TJ. The function of neutrophils in sepsis. Curr Opin Infect Dis. 2012 Jun;25(3):321-7. doi: 10.1097/QCO.0b013e3283528c9b. PMID: 22421753.

Kvarnhammar AM, Petterson T, Cardell LO. NOD-like receptors and RIG-I-like receptors in human eosinophils: activation by NOD1 and NOD2 agonists. Immunology. 2011 Nov;134(3):314-25. doi: 10.1111/j.1365-2567.2011.03492.x. PMID: 21978001; PMCID: PMC3209571.

Lagatie O, Verheyen A, Van Hoof K, Lauwers D, Odiere MR, Vlaminck J, Levecke B, Stuyver LJ. Detection of Ascaris lumbricoides infection by ABA-1 coproantigen ELISA. PLoS Negl Trop Dis. 2020 Oct 15;14(10):e0008807. doi: 10.1371/journal.pntd.0008807. PMID: 33057357; PMCID: PMC7591086.

Lee WB, Kang JS, Yan JJ, Lee MS, Jeon BY, Cho SN, Kim YJ. Neutrophils Promote Mycobacterial Trehalose Dimycolate-Induced Lung Inflammation via the Mincle Pathway. PLoS Pathog. 2012;8(4):e1002614. doi: 10.1371/journal.ppat.1002614. Epub 2012 Apr 5. PMID: 22496642; PMCID: PMC3320589.

Lee WB, Yan JJ, Kang JS, Zhang Q, Choi WY, Kim LK, Kim YJ. Mincle activation enhances neutrophil migration and resistance to polymicrobial septic peritonitis. Sci Rep. 2017 Jan 23;7:41106. doi: 10.1038/srep41106. PMID: 28112221; PMCID: PMC5253726.

Linnemann L, Antwi-Ekwuruke J, Gnanapragassam V, Bang C, Rühlemann M, Ruland J, Hartmann W, Heepmann L, Dörken S, Yunus SM, Viebrock B, Schlosser A, Lepenies B, Breloer M. The C-type lectin receptor MINCLE interferes with eosinophil function and protective intestinal immunity in Strongyloides ratti-infected mice. Mucosal Immunol. 2025 Feb;18(1):220-231. doi: 10.1016/j.mucimm.2024.11.005. Epub 2024 Nov 22. PMID: 39581231.

Lobato-Pascual A, Saether PC, Dahle MK, Gaustad P, Dissen E, Fossum S, Daws MR. Rat macrophage C-type lectin is an activating receptor expressed by phagocytic cells. PLoS One. 2013;8(2):e57406. doi: 10.1371/journal.pone.0057406. Epub 2013 Feb 28. PMID: 23468983; PMCID: PMC3585393.

Loke P, Nair MG, Parkinson J, Guiliano D, Blaxter M, Allen JE. IL-4 dependent alternatively-activated macrophages have a distinctive in vivo gene expression phenotype. BMC Immunol. 2002 Jul 4;3:7. doi: 10.1186/1471-2172-3-7. PMID: 12098359; PMCID: PMC117781.

Luttmann W, Franz P, Matthys H, Virchow JC Jr. Effects of TGF-beta on eosinophil chemotaxis. Scand J Immunol. 1998 Feb;47(2):127-30. doi: 10.1046/j.1365-3083.1998.00298.x. PMID: 9496687.

Malamud M, Cavallero GJ, Casabuono AC, Lepenies B, Serradell MLÁ, Couto AS. Immunostimulation by Lactobacillus kefiri S-layer proteins with distinct glycosylation patterns

requires different lectin partners. J Biol Chem. 2020 Oct 16;295(42):14430-14444. doi: 10.1074/jbc.RA120.013934. Epub 2020 Aug 13. PMID: 32817316; PMCID: PMC7573260.

Cambra-Pellejà M, Valderas-García E, Balaña-Fouce R, de la Vega J, Del Olmo E, Antwi-Ekwuruke J, Linnemann L, Heepmann L, Breloer M, Martínez-Valladares M. Evaluating alternative compounds for strongyloidiasis therapy: Novel insights from larval migration inhibition test. PLoS Negl Trop Dis. 2024 Oct 7;18(10):e0012532. doi: 10.1371/journal.pntd.0012532. PMID: 39374184; PMCID: PMC11458022.

Medzhitov R, Janeway CA Jr. Innate immunity: the virtues of a nonclonal system of recognition. Cell. 1997 Oct 31;91(3):295-8. doi: 10.1016/s0092-8674(00)80412-2. PMID: 9363937.

Meiners J, Reitz M, Rüdiger N, Turner JE, Heepmann L, Rudolf L, Hartmann W, McSorley HJ, Breloer M. IL-33 facilitates rapid expulsion of the parasitic nematode Strongyloides ratti from the intestine via ILC2- and IL-9-driven mast cell activation. PLoS Pathog. 2020 Dec 22;16(12):e1009121. doi: 10.1371/journal.ppat.1009121. PMID: 33351862; PMCID: PMC7787685.

Morimoto M, Morimoto M, Whitmire J, Xiao S, Anthony RM, Mirakami H, Star RA, Urban JF Jr, Gause WC. Peripheral CD4 T cells rapidly accumulate at the host: parasite interface during an inflammatory Th2 memory response. J Immunol. 2004 Feb 15;172(4):2424-30. doi: 10.4049/jimmunol.172.4.2424. PMID: 14764713.

Motegi Y, Kita H. Interaction with secretory component stimulates effector functions of human eosinophils but not of neutrophils. J Immunol. 1998 Oct 15;161(8):4340-6. PMID: 9780211.

Muhsin M, Ajendra J, Gentil K, Berbudi A, Neumann AL, Klaas L, Schmidt KE, Hoerauf A, Hübner MP. IL-6 is required for protective immune responses against early filarial infection. Int J Parasitol. 2018 Oct;48(12):925-935. doi: 10.1016/j.ijpara.2018.05.011. Epub 2018 Sep 1. PMID: 30176234.

Mukherjee M, Lacy P, Ueki S. Eosinophil Extracellular Traps and Inflammatory Pathologies-Untangling the Web! Front Immunol. 2018 Nov 26;9:2763. doi: 10.3389/fimmu.2018.02763. PMID: 30534130; PMCID: PMC6275237.

Muñoz-Caro T, Rubio R MC, Silva LM, Magdowski G, Gärtner U, McNeilly TN, Taubert A, Hermosilla C. Leucocyte-derived extracellular trap formation significantly contributes to Haemonchus contortus larval entrapment. Parasit Vectors. 2015 Nov 26;8:607. doi: 10.1186/s13071-015-1219-1. PMID: 26610335; PMCID: PMC4661960.

Mylemans M, Nevejan L, Van Den Bremt S, Stubbe M, Cruyssen BV, Moulakakis C, Berthold H, Konrad C, Bossuyt X, Van Hoovels L. Circulating calprotectin as biomarker in neutrophil-related inflammation: Pre-analytical recommendations and reference values according to sample type. Clin Chim Acta. 2021 Jun;517:149-155. doi: 10.1016/j.cca.2021.02.022. Epub 2021 Mar 6. PMID: 33689693.

Nagase H, Okugawa S, Ota Y, Yamaguchi M, Tomizawa H, Matsushima K, Ohta K, Yamamoto K, Hirai K. Expression and function of Toll-like receptors in eosinophils: activation by Toll-like receptor 7 ligand. J Immunol. 2003 Oct 15;171(8):3977-82. doi: 10.4049/jimmunol.171.8.3977. PMID: 14530316.

Nishinaka Y, Arai T, Adachi S, Takaori-Kondo A, Yamashita K. Singlet oxygen is essential for neutrophil extracellular trap formation. Biochem Biophys Res Commun. 2011 Sep 16;413(1):75-9. doi: 10.1016/j.bbrc.2011.08.052. Epub 2011 Aug 18. PMID: 21871447.

Ohkawara Y, Lim KG, Xing Z, Glibetic M, Nakano K, Dolovich J, Croitoru K, Weller PF, Jordana M. CD40 expression by human peripheral blood eosinophils. J Clin Invest. 1996 Apr 1;97(7):1761-6. doi: 10.1172/JCI118603. PMID: 8601642; PMCID: PMC507241.

Olsen A, van Lieshout L, Marti H, Polderman T, Polman K, Steinmann P, Stothard R, Thybo S, Verweij JJ, Magnussen P. Strongyloidiasis--the most neglected of the neglected tropical diseases? Trans R Soc Trop Med Hyg. 2009 Oct;103(10):967-72. doi: 10.1016/j.trstmh.2009.02.013. Epub 2009 Mar 27. PMID: 19328508.

Ometto F, Friso L, Astorri D, Botsios C, Raffeiner B, Punzi L, Doria A. Calprotectin in rheumatic diseases. Exp Biol Med (Maywood). 2017 Apr;242(8):859-873. doi: 10.1177/1535370216681551. Epub 2016 Jan 1. PMID: 27895095; PMCID: PMC5407536.

Osbourn M, Soares DC, Vacca F, Cohen ES, Scott IC, Gregory WF, Smyth DJ, Toivakka M, Kemter AM, le Bihan T, Wear M, Hoving D, Filbey KJ, Hewitson JP, Henderson H, Gonzàlez-Cìscar A, Errington C, Vermeren S, Astier AL, Wallace WA, Schwarze J, Ivens AC, Maizels RM, McSorley HJ. HpARI Protein Secreted by a Helminth Parasite Suppresses Interleukin-33. Immunity. 2017 Oct 17;47(4):739-751.e5. doi: 10.1016/j.immuni.2017.09.015. PMID: 29045903; PMCID: PMC5655542.

Padigel UM, Hess JA, Lee JJ, Lok JB, Nolan TJ, Schad GA, Abraham D. Eosinophils act as antigen-presenting cells to induce immunity to Strongyloides stercoralis in mice. J Infect Dis. 2007 Dec 15;196(12):1844-51. doi: 10.1086/522968. PMID: 18190266; PMCID: PMC3154724.

Padigel UM, Lee JJ, Nolan TJ, Schad GA, Abraham D. Eosinophils can function as antigen-presenting cells to induce primary and secondary immune responses to Strongyloides stercoralis. Infect Immun. 2006 Jun;74(6):3232-8. doi: 10.1128/IAI.02067-05. PMID: 16714550; PMCID: PMC1479274.

Paveley RA, Aynsley SA, Cook PC, Turner JD, Mountford AP. Fluorescent imaging of antigen released by a skin-invading helminth reveals differential uptake and activation profiles by antigen presenting cells. PLoS Negl Trop Dis. 2009 Oct 13;3(10):e528. doi: 10.1371/journal.pntd.0000528. PMID: 19829705; PMCID: PMC2759291.

Pearlman E, Hazlett FE Jr, Boom WH, Kazura JW. Induction of murine T-helper-cell responses to the filarial nematode Brugia malayi. Infect Immun. 1993 Mar;61(3):1105-12. doi: 10.1128/iai.61.3.1105-1112.1993. PMID: 8094378; PMCID: PMC302845.

Pesce JT, Liu Z, Hamed H, Alem F, Whitmire J, Lin H, Liu Q, Urban JF Jr, Gause WC. Neutrophils clear bacteria associated with parasitic nematodes augmenting the development of an effective Th2-type response. J Immunol. 2008 Jan 1;180(1):464-74. doi: 10.4049/jimmunol.180.1.464. PMID: 18097048; PMCID: PMC2288648.

Pionnier N, Brotin E, Karadjian G, Hemon P, Gaudin-Nomé F, Vallarino-Lhermitte N, Nieguitsila A, Fercoq F, Aknin ML, Marin-Esteban V, Chollet-Martin S, Schlecht-Louf G, Bachelerie F, Martin C. Neutropenic Mice Provide Insight into the Role of Skin-Infiltrating Neutrophils in the Host Protective Immunity against Filarial Infective Larvae. PLoS Negl Trop Dis. 2016 Apr 25;10(4):e0004605. doi: 10.1371/journal.pntd.0004605. PMID: 27111140; PMCID: PMC4844152.

Prado Acosta M, Goyette-Desjardins G, Scheffel J, Dudeck A, Ruland J, Lepenies B. S-Layer From *Lactobacillus brevis* Modulates Antigen-Presenting Cell Functions via the Mincle-Syk-Card9 Axis. Front Immunol. 2021 Mar 1;12:602067. doi: 10.3389/fimmu.2021.602067. PMID: 33732234; PMCID: PMC7957004.

Rajamanickam A, Munisankar S, Bhootra Y, Dolla CK, Thiruvengadam K, Nutman TB, Babu S. Altered levels of memory T cell subsets and common γc cytokines in Strongyloides stercoralis infection and partial reversal following anthelmintic treatment. PLoS Negl Trop Dis. 2018 May 24;12(5):e0006481. doi: 10.1371/journal.pntd.0006481. PMID: 29795573; PMCID: PMC5991401.

Reid KB, Porter RR. The proteolytic activation systems of complement. Annu Rev Biochem. 1981;50:433-64. doi: 10.1146/annurev.bi.50.070181.002245. PMID: 7023363.

Rogers NC, Slack EC, Edwards AD, Nolte MA, Schulz O, Schweighoffer E, Williams DL, Gordon S, Tybulewicz VL, Brown GD, Reis e Sousa C. Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. Immunity. 2005 Apr;22(4):507-17. doi: 10.1016/j.immuni.2005.03.004. Erratum in: Immunity. 2005 Jun;22(6):773-4. PMID: 15845454.

Sanches JM, Rossato L, Lice I, Alves de Piloto Fernandes AM, Bueno Duarte GH, Rosini Silva AA, de Melo Porcari A, de Oliveira Carvalho P, Gil CD. The role of annexin A1 in Candida albicans and Candida auris infections in murine neutrophils. Microb Pathog. 2021 Jan;150:104689. doi: 10.1016/j.micpath.2020.104689. Epub 2020 Dec 8. PMID: 33307121.

Sato MO, Sato M, Yanagida T, Waikagul J, Pongvongsa T, Sako Y, Sanguankiat S, Yoonuan T, Kounnavang S, Kawai S, Ito A, Okamoto M, Moji K. Taenia solium, Taenia saginata, Taenia asiatica, their hybrids and other helminthic infections occurring in a neglected tropical diseases' highly endemic area in Lao PDR. PLoS Negl Trop Dis. 2018 Feb 8;12(2):e0006260. doi: 10.1371/journal.pntd.0006260. PMID: 29420601; PMCID: PMC5821399.

Schär F, Guo L, Streit A, Khieu V, Muth S, Marti H, Odermatt P. Strongyloides stercoralis genotypes in humans in Cambodia. Parasitol Int. 2014 Jun;63(3):533-6. doi: 10.1016/j.parint.2014.01.010. Epub 2014 Feb 14. PMID: 24530857.

Schick J, Altunay M, Lacorcia M, Marschner N, Westermann S, Schluckebier J, Schubart C, Bodendorfer B, Christensen D, Alexander C, Wirtz S, Voehringer D, da Costa CP, Lang R. IL-4 and helminth infection downregulate MINCLE-dependent macrophage response to mycobacteria and Th17 adjuvanticity. Elife. 2023 Feb 8;12:e72923. doi: 10.7554/eLife.72923. PMID: 36753434; PMCID: PMC9908076.

Schoenen H, Bodendorfer B, Hitchens K, Manzanero S, Werninghaus K, Nimmerjahn F, Agger EM, Stenger S, Andersen P, Ruland J, Brown GD, Wells C, Lang R. Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. J Immunol. 2010 Mar 15;184(6):2756-60. doi: 10.4049/jimmunol.0904013. Epub 2010 Feb 17. PMID: 20164423; PMCID: PMC3442336.

Shenderov K, Barber DL, Mayer-Barber KD, Gurcha SS, Jankovic D, Feng CG, Oland S, Hieny S, Caspar P, Yamasaki S, Lin X, Ting JP, Trinchieri G, Besra GS, Cerundolo V, Sher A. Cord factor and peptidoglycan recapitulate the Th17-promoting adjuvant activity of mycobacteria through mincle/CARD9 signaling and the inflammasome. J Immunol. 2013 Jun 1;190(11):5722-30. doi: 10.4049/jimmunol.1203343. Epub 2013 Apr 29. PMID: 23630357; PMCID: PMC3719989.

Silveira JS, Antunes GL, Kaiber DB, da Costa MS, Marques EP, Ferreira FS, Gassen RB, Breda RV, Wyse ATS, Pitrez P, da Cunha AA. Reactive oxygen species are involved in eosinophil extracellular traps release and in airway inflammation in asthma. J Cell Physiol. 2019 Dec;234(12):23633-23646. doi: 10.1002/jcp.28931. Epub 2019 Jun 10. PMID: 31180592.

Sousa Mda G, Reid DM, Schweighoffer E, Tybulewicz V, Ruland J, Langhorne J, Yamasaki S, Taylor PR, Almeida SR, Brown GD. Restoration of pattern recognition receptor costimulation to treat chromoblastomycosis, a chronic fungal infection of the skin. Cell Host Microbe. 2011 May 19;9(5):436-43. doi: 10.1016/j.chom.2011.04.005. PMID: 21575914; PMCID: PMC3098964.

Specht S, Saeftel M, Arndt M, Endl E, Dubben B, Lee NA, Lee JJ, Hoerauf A. Lack of eosinophil peroxidase or major basic protein impairs defense against murine filarial infection. Infect Immun. 2006 Sep;74(9):5236-43. doi: 10.1128/IAI.00329-06. PMID: 16926417; PMCID: PMC1594830.

Steinbach KH, Schick P, Trepel F, Raffler H, Döhrmann J, Heilgeist G, Heltzel W, Li K, Past W, van der Woerd-de Lange JA, Theml H, Fliedner TM, Begemann H. Estimation of kinetic parameters of neutrophilic, eosinophilic, and basophilic granulocytes in human blood. Blut. 1979 Jul;39(1):27-38. doi: 10.1007/BF01008072. PMID: 223692.

Stepek G, Buttle DJ, Duce IR, Behnke JM. Human gastrointestinal nematode infections: are new control methods required? Int J Exp Pathol. 2006 Oct;87(5):325-41. doi: 10.1111/j.1365-2613.2006.00495.x. PMID: 16965561; PMCID: PMC2517378.

Sýkora J, Siala K, Huml M, Varvařovská J, Schwarz J, Pomahačová R. Evaluation of faecal calprotectin as a valuable non-invasive marker in distinguishing gut pathogens in young

children with acute gastroenteritis. Acta Paediatr. 2010 Sep;99(9):1389-95. doi: 10.1111/j.1651-2227.2010.01843.x. PMID: 20412103.

TAKATA I. Experimental infection of man with Ascaris of man and the pig. Kitasato Arch Exp Med. 1951 Mar;23(4):151-9; English transl, 49-59. PMID: 14874382.

Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol. 2003;21:335-76. doi: 10.1146/annurev.immunol.21.120601.141126. Epub 2001 Dec 19. PMID: 12524386.
Tamura N, Agrawal DK, Townley RG. Leukotriene C4 production from human eosinophils in vitro. Role of eosinophil chemotactic factors on eosinophil activation. J Immunol. 1988 Dec 15;141(12):4291-7. PMID: 2848892.

Tindall NR, Wilson PA. Criteria for a proof of migration routes of immature parasites inside hosts exemplified by studies of Strongyloides ratti in the rat. Parasitology. 1988 Jun;96 (Pt 3):551-63. doi: 10.1017/s0031182000080185. PMID: 3405640.

Tischendorf FW, Brattig NW, Büttner DW, Pieper A, Lintzel M. Serum levels of eosinophil cationic protein, eosinophil-derived neurotoxin and myeloperoxidase in infections with filariae and schistosomes. Acta Trop. 1996 Dec 16;62(3):171-82. doi: 10.1016/s0001-706x(96)00038-1. PMID: 9025985.

Toussaint M, Jackson DJ, Swieboda D, Guedán A, Tsourouktsoglou TD, Ching YM, Radermecker C, Makrinioti H, Aniscenko J, Bartlett NW, Edwards MR, Solari R, Farnir F, Papayannopoulos V, Bureau F, Marichal T, Johnston SL. Host DNA released by NETosis promotes rhinovirus-induced type-2 allergic asthma exacerbation. Nat Med. 2017 Jun;23(6):681-691. doi: 10.1038/nm.4332. Epub 2017 May 1. Erratum in: Nat Med. 2017 Nov 7;23(11):1384. doi: 10.1038/nm1117-1384a. PMID: 28459437; PMCID: PMC5821220.

Turner M, Schweighoffer E, Colucci F, Di Santo JP, Tybulewicz VL. Tyrosine kinase SYK: essential functions for immunoreceptor signalling. Immunol Today. 2000 Mar;21(3):148-54. doi: 10.1016/s0167-5699(99)01574-1. PMID: 10689303.

Vijayan D, Radford KJ, Beckhouse AG, Ashman RB, Wells CA. Mincle polarizes human monocyte and neutrophil responses to Candida albicans. Immunol Cell Biol. 2012 Oct;90(9):889-95. doi: 10.1038/icb.2012.24. Epub 2012 May 29. PMID: 22641025.

Viney M. Strongyloides. Parasitology. 2017 Mar;144(3):259-262. doi: 10.1017/S0031182016001773. Epub 2016 Oct 19. PMID: 27759560; PMCID: PMC5364833.
Viney ME, Lok JB. The biology of Strongyloides spp. WormBook. 2015 Jul 16:1-17. doi: 10.1895/wormbook.1.141.2. PMID: 26183912; PMCID: PMC5402216.

Viney M, Kikuchi T. Strongyloides ratti and S. venezuelensis - rodent models of Strongyloides infection. Parasitology. 2017 Mar;144(3):285-294. doi: 10.1017/S0031182016000020. Epub 2016 Mar 3. PMID: 26935155; PMCID: PMC5364835.

von Roon AC, Karamountzos L, Purkayastha S, Reese GE, Darzi AW, Teare JP, Paraskeva P, Tekkis PP. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and

colorectal malignancy. Am J Gastroenterol. 2007 Apr;102(4):803-13. doi: 10.1111/j.1572-0241.2007.01126.x. Epub 2007 Feb 23. PMID: 17324124.

Wang P., Li R.Z., Huang Z.Y., Tang C.W. Report on 16 cases of small intestine ascariasis diagnosed by capsule endoscopy, Chinese Journal of Parasitology & Parasitic Diseases, 31 (3) (2013), pp. 242-243

Wang YH, Angkasekwinai P, Lu N, Voo KS, Arima K, Hanabuchi S, Hippe A, Corrigan CJ, Dong C, Homey B, Yao Z, Ying S, Huston DP, Liu YJ. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. J Exp Med. 2007 Aug 6;204(8):1837-47. doi: 10.1084/jem.20070406. Epub 2007 Jul 16. PMID: 17635955; PMCID: PMC2118667.

Weller PF, Rand TH, Barrett T, Elovic A, Wong DT, Finberg RW. Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 alpha expression. J Immunol. 1993 Mar 15;150(6):2554-62. PMID: 8450230.

Wevers BA, Kaptein TM, Zijlstra-Willems EM, Theelen B, Boekhout T, Geijtenbeek TB, Gringhuis SI. Fungal engagement of the C-type lectin mincle suppresses dectin-1-induced antifungal immunity. Cell Host Microbe. 2014 Apr 9;15(4):494-505. doi: 10.1016/j.chom.2014.03.008. PMID: 24721577.

World health organization (WHO) key facts 2023, https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections.

Yamasaki S, Ishikawa E, Sakuma M, Hara H, Ogata K, Saito T. Mincle is an ITAM-coupled activating receptor that senses damaged cells. Nat Immunol. 2008 Oct;9(10):1179-88. doi: 10.1038/ni.1651. Epub 2008 Sep 7. PMID: 18776906.

Yamasaki S, Matsumoto M, Takeuchi O, Matsuzawa T, Ishikawa E, Sakuma M, Tateno H, Uno J, Hirabayashi J, Mikami Y, Takeda K, Akira S, Saito T. C-type lectin Mincle is an activating receptor for pathogenic fungus, Malassezia. Proc Natl Acad Sci U S A. 2009 Feb 10;106(6):1897-902. doi: 10.1073/pnas.0805177106. Epub 2009 Jan 26. PMID: 19171887; PMCID: PMC2644135.

Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol. 2004 Jul;5(7):730-7. doi: 10.1038/ni1087. Epub 2004 Jun 20. PMID: 15208624.

Younis AE, Geisinger F, Ajonina-Ekoti I, Soblik H, Steen H, Mitreva M, Erttmann KD, Perbandt M, Liebau E, Brattig NW. Stage-specific excretory-secretory small heat shock proteins from the parasitic nematode Strongyloides ratti--putative links to host's intestinal mucosal defense system. FEBS J. 2011 Sep;278(18):3319-36. doi: 10.1111/j.1742-4658.2011.08248.x. Epub 2011 Aug 24. PMID: 21762402; PMCID: PMC3718022.

Zheng L, Hu Y, Wang Y, Huang X, Xu Y, Shen Y, Cao J. Recruitment of Neutrophils Mediated by Vγ2 γδ T Cells Deteriorates Liver Fibrosis Induced by Schistosoma japonicum Infection in C57BL/6 Mice. Infect Immun. 2017 Jul 19;85(8):e01020-16. doi: 10.1128/IAI.01020-16. PMID: 28507072; PMCID: PMC5520426.