IL-33 Controls IL-22-Dependent Antibacterial Defense by Modulating the Microbiota

Inaugural-Dissertation
to obtain the academic degree
Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of
Biology, Chemistry, Pharmacy
of Freie Universität Berlin

by

Ivo Röwekamp

Berlin, 2024

This thesis was conducted under the supervision of

Prof. Dr. Bastian Opitz

at the

Department of Infectious Diseases
Respiratory Medicine and Critical Care
Charité – Universitätsmedizin Berlin

during the period

from 09/01/2019 to 01/23/2024.

1st reviewer:	Prof. Dr. Bastian Opitz
2nd reviewer:	Prof. Dr. Andreas Diefenbach
Date of defense:	10/07/2024

Acknowledgements

I would like to extend my heartfelt acknowledgments to the following individuals, whose support and contributions have been invaluable throughout my academic path:

First and foremost, I am deeply grateful to my supervisor, Prof. Dr. Bastian Opitz, for his unyielding guidance, mentorship, and expertise. Your steadfast commitment to my academic growth and your invaluable insights have been instrumental in shaping this research.

I would also like to show gratitude to my thesis advisory committee, including Prof. Dr. Andreas Diefenbach and Dr. Christoph Klose for their valuable input during our meetings.

I extend my sincere appreciation to Sandra, without whose dedicated support and commitment, this endeavor would not have been possible.

I want to express my heartfelt thanks to my own workgroup and all the other people including the groups of AG Sander and AG Witzenrath for their teamwork, comradeship, and the great vibes in the lab. Your support and enthusiasm made my time in the lab not only productive but also enjoyable.

I would also like to express my gratitude to Walter and Uschi for their role in nurturing and promoting my scientific thinking. Your encouragement and intellectual discussions have been very helpful.

Although she cannot witness this stage in my life, I know my mother would be proud. I never would have gotten to where I am now without your love and support.

Last but not least, I want to acknowledge my wife, Fine, for her enduring support and understanding throughout the demanding phases of my work. Her patience and love sustained me during these challenging times.

Each of you has played a crucial part in the success of my academic pursuits, and I am genuinely thankful for your contributions and encouragement.

Declaration of Independence

Herewith I certify that I have prepared and written my thesis independently and that I have not used any sources and aids other than those indicated by me.

Berlin, 01/23/2024

Ivo Röwekamp

Abstract

Infections of the lower respiratory tract represent the fifth most common cause of death worldwide. This study describes a mechanism that influences the course of pneumococcal pneumonia based on genetic determinants as well as environmental factors. Previous experiments have shown that animals deficient in IL-33 or its receptor ST2 exhibit an enhanced bacterial defense against *Streptococcus pneumoniae*.

Initially, associations between the susceptibility to human pneumococcal pneumonia and single nucleotide polymorphisms (SNPs) in the genes coding for IL-33 and ST2 were demonstrated. Furthermore, IL-33-deficient animals were shown to produce increased amounts of IL-22, and experiments using *Il33*$^{-/-}$ *Il22*$^{-/-}$ animals as well as recombinant IL-22 revealed that enhanced IL-22 production was responsible for the increased resistance of *Il33*$^{-/-}$ mice to *Streptococcus pneumoniae*. Classical type 2 immune responses driven by type 2 innate lymphoid cells (ILC2s) or IL-4/IL-13 signaling cascades were not involved in the IL-33-dependent regulation, while ST2-negative ILCs, most likely representing ILC3s, were identified as the main differential producers of IL-22.

Additionally, an association between bacterial defense and animal housing conditions was revealed. The IL-33-dependent negative regulation of pneumococcal pneumonia could only be reproduced in facilities with specific individual hygiene conditions. This effect was accompanied by differences in the composition of the intestinal microbiota of the animals, where increased abundance of *Lactobacillus spp.* correlated with a protective phenotype in *Il33*$^{-/-}$ mice. Finally, microbiota depletion and co-housing experiments resulted in equilibration of bacterial burden, while a fecal microbiota transplant was able to rescue the observed phenotype in microbiota-depleted animals. Taken together it was demonstrated, that the intestinal microbiota influences pneumococcal pneumonia.

The results presented in this study enhance our understanding of the bidirectional communication between microbiota and the immune system and demonstrate that genetic as well as environmental factors influence the defense against *Streptococcus pneumoniae*.

Zusammenfassung

Infektionen der unteren Atemwege stellen die fünfthäufigste Todesursache weltweit dar. In dieser Arbeit wird ein Mechanismus beschrieben, der den Verlauf einer Pneumokokken-Pneumonie in Abhängigkeit von genetischen Determinanten sowie Umwelteinflüssen beeinflusst. Vorangegangene Experimente haben gezeigt, dass Tiere mit Defizienz in IL-33 oder in seinem Rezeptor ST2 eine verbesserte bakterielle Abwehr gegen *Streptococcus pneumoniae* aufweisen.

In dieser Arbeit konnten zunächst Zusammenhänge zwischen *single nucleotide polymorphisms* (SNPs) in den Genen kodierend für IL-33 und ST2 und einer Anfälligkeit für humane Pneumokokkenpneumonie aufgezeigt werden. Darüber hinaus produzierten Tiere mit IL-33-Defizienz erhöhte Mengen an IL-22. Experimente unter Verwendung von *Il33*$^{-/-}$ *Il22*$^{-/-}$-Tieren sowie rekombinantem IL-22 zeigten eindeutig, dass die gesteigerte IL-22-Produktion für die erhöhte Resistenz von *Il33*$^{-/-}$-Mäusen gegenüber *Streptococcus pneumoniae* verantwortlich war. Klassische Typ-2-Immunantworten, getrieben von *type 2 innate lymphoid cells* (ILC2s) oder IL-4/IL-13 Signalkaskaden, waren nicht in die IL-33-abhängige Regulation involviert, während ST2-negative ILCs (sehr wahrscheinlich ILC3s) als differenzielle Hauptproduzenten von IL-22 identifiziert werden konnten.

Zusätzlich wurde eine Assoziation zwischen der bakteriellen Abwehr und den Haltungsbedingungen der Tiere nachgewiesen. Die IL-33-abhängige negative Regulation der Pneumokokkenpneumonie konnte nur in Einrichtungen mit spezifischen individuellen Hygienebedingungen reproduziert werden. Dieser Effekt ging mit Unterschieden in der Zusammensetzung der intestinalen Mikrobiota der Tiere einher, wobei eine erhöhte Frequenz von *Lactobacillus spp.* mit dem protektiven Phänotyp der *Il33*$^{-/-}$-Mäuse korrelierte. Weiterhin konnte durch Mikrobiotadepletion und co-housing ein Angleichen der bakteriellen Last zwischen WT- und *Il33*$^{-/-}$-Tieren nachgewiesen werden, während eine Transplantation fäkaler Mikrobiota zu einer Wiederherstellung des Phänotyps geführt hat.

Die in dieser Arbeit präsentierten Ergebnisse erweitern unser Verständnis der bidirektionalen Kommunikation zwischen Mikrobiota und Immunsystem und zeigen auf, dass sowohl genetische als auch Umweltfaktoren den Verlauf einer Pneumokokkenpneumonie beeinflussen.

Introduction

1.1 *Streptococcus pneumoniae* and pneumococcal pneumonia

1.1.1 Epidemiology, treatment and prevention of pneumococcal pneumonia

Streptococcus pneumoniae (*S. pneumoniae*) is an extracellular, facultative anaerobe, Gram-positive bacterium colonizing the nasopharynx. It is capable of infiltrating the lower respiratory tract in an opportunistic manner which can lead to pneumonia. *S. pneumoniae* can also cause sepsis, meningitis, or middle ear infections[1]. Lower respiratory tract infections (LRTIs) were responsible for more than 2.49 million deaths in 2019, according to the 2019 Global Burden of Death study[2]. These data show that LRTIs are the main cause of death due to infectious diseases globally, exceeding mortality from HIV (864,000 deaths) and tuberculosis (1.18 million deaths)[3]. The incidence of pneumonia-induced deaths correlates with sociodemographic status, poor healthcare and therefore strongly affects countries of the global south[4]. The disease pneumonia can be broadly categorized into community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP). *S. pneumoniae* is the most common cause of CAP, which is associated with a high morbidity and mortality rate[3]. Since the introduction of antibiotic therapy, pneumonia-associated deaths dropped significantly[5], but intensive care unit mortality still stays constantly high at around 10 %[6].

Pneumococci asymptomatically colonize the upper respiratory tract of 40 – 70% of healthy children below 5 years of age[7] and 5 – 20% of adults[8]. Individuals can transmit the pathogen via direct or indirect contact, droplets, and, under specific conditions, aerosols[1,9]. Hosts with well-functioning immune systems are usually capable of clearing *S. pneumoniae* infections before they cause serious disease. Therefore, the most susceptible populations are those with compromised immune functions, such as young children, the elderly or immunocompromised individuals[10,11]. The distribution of serotypes shows high variation depending on age and geographical location[7]. Serotype 3 is one of the most prevalent clinical isolates associated with a thicker capsule, greater virulence and higher mortality rate compared to other strains[12]. Although several antibiotics usually show high efficacy rates against severe pneumococcal pneumonia, the prevalence of antibiotic-resistant variants increases in many parts of the world[13]. Alternatively, vaccinations can prevent disease independently of antibiotic resistances. There are two types of vaccines available to date, the pneumococcal polysaccharide vaccine (PPSV) 23, covering 23 serotypes and pneumococcal conjugate vaccines (PCVs) which are authorized in several serotype combinations (PCV13, PCV15, PCV20)[14,15].

1.1.2 Biology and virulence factors

S. pneumoniae represents one of the most relevant human pathogens and 100 different serotypes have been described to date[16,17]. Serotypes are distinguished by their polysaccharide capsule, which represent one of their main virulence factors. Others include pneumolysin (PLY) and several pneumococcal surface proteins[4]. Depending on the capsule, *S. pneumoniae* is able to prevent phagocytosis by immune cells, to evade opsonization by complement factors and to resist immobilization by neutrophil extracellular traps (NETs)[18]. PLY is a toxin produced by *S. pneumoniae*, which assembles in multimers and forms transmembrane pores in host cells. It causes cell death by deregulating plasma membrane integrity and cellular homeostasis[19] which enables the pathogen to pass the epithelial barrier and to enter the bloodstream[20]. Moreover, PLY can activate the classical complement system and induce production of proinflammatory cytokines in immune cells[21]. Further virulence factors include PsaA, which confers resistance to oxidative stress and PspA, which prevents binding of C3 complement onto the pneumococcal surface[22].

1.2 Innate immunity in the respiratory tract

1.2.1 Pattern recognition receptors

For the efficient mounting of an immune response, reliable recognition of a pathogen is of importance. Nearly all cells utilize pattern recognition receptors (PRRs) to detect so-called pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). The group of PRRs contains Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), C-type lectin recptors (CLRs) and cytosolic DNA-sensors[23]. TLRs and CLRs are membrane bound, NLRs, RLRs and cytosolic DNA-sensors are located in the cytoplasm. PRRs are found on epithelial[24] and endothelial cells[25] as well as on alveolar macrophages (AMs) and dendritic cells (DCs)[26]. Upon activation, most PRRs induce inflammation by triggering the myeloid differentiation primary response 88 (MyD88)-dependent production of e.g., tumor necrosis factor (TNF), interleukin (IL)-1β precursor, IL-6, interferon (IFN)-β, C-X-C motif ligand (CXCL)1 and C-C motif ligand (CCL)2 via nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and interferon regulated factor (IRF)3/7[27].
TLRs can either be present on the cell surface or on endosomal membranes. TLR1 and TLR2 bind microbial lipoproteins, TLR3 double-stranded (ds) ribonucleic acid (RNA), TLR4 lipopolysaccharide (LPS), TLR5 flagellin, TLR9 CpG-rich desoxyribonucleic acid (DNA) and TLR13 ribosomal RNA[28–31]. Activation of TLRs lead to expression of pro-inflammatory cytokines, chemokines and antimicrobial peptides (AMPs)[32].
NLRs constitute a diverse family of cytosolic PRRs, but the functions of many remain elusive. Among this family, nucleotide-binding oligomerization (NOD)1[33–35] and NOD2[36] have been well-characterized[37]. Binding of γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP) and muramyl dipeptide (MDP), found in bacterial peptidoglycan activates NF-kB transcription and expression of effector cytokines[38,39]. Some NLRs including NLR family pyrin domain containing (NLRP)1[40], NLRP3[41] and NLRC4[42] are involved in the formation of inflammasomes which induce a specific form of programmed cell death called pyroptosis[43,44]. Once ac-

tivated, these NLRs bind to apoptosis-associated speck-like protein containing a CARD (ASC) and subsequently activate caspase-1, leading to cleavage and activation of IL-1β.
RLRs detect viral RNA which leads to activation of IRF3/7-dependent expression of type I IFNs as well as NF-kB-dependent production of inflammatory cytokines[45].
Another group consisting of cytosolic DNA sensors contains a multitude of different functional proteins. One of them, absent in melanoma 2 (AIM2), was described in 1997 as interferon-inducible gene[46]. It recognizes dsDNA and forms, together with the adaptor molecule ASC, an AIM2 inflammasome. Another well-described representative of cytosolic DNA sensors is cyclic GMP-AMP synthase (cGAS). After sensing dsDNA it binds to and activates its adaptor molecule stimulator of interferon genes (STING), subsequetly leading to type I interferon expression[47].
Immune cells can also be stimulated by the detection of DAMPs, which are endogenous molecules released upon necrotic cell death and tissue damage. Once released, they bind to either a range of PRRs including TLR2, TLR4, NLRP3, RIG-1 and Mincle or to specific receptors only detecting DAMPs[48,49]. Typical DAMPs include adenosine triphosphate (ATP) and uric acid derivates which are detected by P2X/Y receptors and IL-33 which binds to supression of tumorigenicity 2 (ST2)[50–53].

1.2.2 Innate antibacterial immune response in the respiratory tract

If a pathogen invades the lower parts of the respiratory tract, the first line of defense consists of mucociliary clearance, mediated by goblet cell derived mucus[54] and ciliated cells[55]. After overcoming this barrier and reaching the alveolar space, AMs are among the first immune cells to interact with the pathogen. AMs are mononuclear phagocytes that originate from the yolk sac or fetal liver during development and are continuously replenished by self-renewal[56] or by circulating bone marrow-derived macrophages (BMMs)[57]. Their primary role is to maintain homeostatic conditions within the alveoli[58]. Under steady state conditions, AMs clear microparticles or cell debris derived from apoptotic cells without inducing local inflammation[56,59].

In order to adapt to their environment, AMs are able to switch their activation status between classically activated (M1-like) and an alternatively activated (M2-like) phenotype[60]. The M2-like activation state is associated to steady state, while AMs acquire the M1-like activation state upon encountering PAMPs[61]. Once classically activated, AMs produce several proinflammatory mediators like TNF, IL-1β, IFN-α/β, CXCL1/2, CCL2 and IL-23 to stimulate alveolar epithelial cells and to recruit and stimulate other leucocytes to the site of infection[31,62–64]. However, the paradigm of two clear-cut activation states has been questioned[65] and the M1/M2 terminology rather describes a nuanced spectrum of plasticity and should be used cautiously. Beneath the previously described activation and recruitment of other cells, AMs are also capable of mounting direct effector mechanisms such as production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as well as proteases and peptides[66–68] to eliminate invading bacteria, fungi and viruses[58,69–71].

In addition to AMs, alveolar epithelial cells (AECs) play an important role in maintaining equilibrium and sensing danger[72]. The flat-shaped type I alveolar epithelial cells (AEC1s) make up 90% of the alveolar surface and are responsible for gas exchange between alveoli and capillaries. They differentiate from type II alveolar epithelial cells (AEC2s), which are cubic shaped and produce surfactant proteins, facilitating uptake and clearance of debris and pathogens by phagocytes[73–78]. AECs are also capable of PRR-dependent sensing of invading pathogens and of producing antimicrobial peptides and enzymes, including lysozyme, lactoferrin, α- and β-defensin, cathelicidins and complement factors, which all function to limit the spread of infection at early stages[24].

Residing in the interstitium of the lower respiratory tract, DCs constantly screen the alveolar environment for foreign molecules. DCs play an important role in activating the adaptive immune system by presenting antigens of encountered and phagocytized pathogens on major histocompatibility complex (MHC) molecules[79,80]. More relevant for the innate immune defense, DCs can also sense PAMPs via PRRs to produce inflammatory cytokines such as IL-1β, IL-6, IL-12 and IL-23, initiating subsequent activation and recruitment of other immune cells[31,80].

The next tier of an immune response is characterized by the influx of polymorphonuclear neutrophils (PMNs). Attracted by e.g., AM- and AEC- derived CXCL1, CXCL2 and CXCL5[81–83], PMNs infiltrate the site of infection shortly after detection of infection and initiate three main effector mechanisms: phagocytosis, degranulation of effector molecules and NET formation[84–86]. Degranulated vacuoles contain ROS,

RNS and AMPs such as elastase, cathepsin G and proteinase 3[86–88]. All these effector molecules also have the potential to harm the host[89]. Therefore, PMN-mediated inflammation needs to be terminated after pathogen removal by a process called efferocytosis, in which apoptotic PMNs are engulfed by surrounding macrophages[86]. Despite the danger of an excessive immune response and tissue damage, PMNs represent a crucial pillar in the defense of bacterial pathogens and dysfunction can lead to critical disease progression[90].

Shortly after the influx of PMNs, inflammatory monocytes (IMs) arrive at the site of infection attracted by CCL2 and CCL7[91–93]. Exiting the blood stream, IMs can differentiate either to phagocytic macrophages or to antigen-presenting DCs[94–96], thereby adapting to the individual situation when they arrive. IMs also produce inflammatory mediators including TNF, nitric oxide, IL-12 and IL-1β to directly or indirectly contribute to the elimination of invading pathogens[97–100].

Lastly, innate lymphoid cells (ILCs) are a type of lymphocyte able to react in an innate response, independently of adaptive activation[101–104]. The Family of ILCs includes cytotoxic natural killer cells (NK cells) and helper-like ILCs. Resembling the T cell nomenclature, helper-like ILCs are categorized in three groups: type 1, type 2 and type 3 ILCs (ILC1s, ILC2s, ILC3s).

The cytotoxic NK cells are characterized by the transcription factors EOMES and T-bet and are capable of producing high amounts of IFN-γ, TNF and in some cases IL-22 to further activate nearby AMs, PMNs and AECs, respectively[105,106]. One of their major products are granzymes, which are serine proteases capable of inducing apoptosis in targeted infected or cancer cells[107].

The helper-like ILC1s differ from NK cells by the lack of expression of the transcription factor EOMES and the ability to directly neutralize cells. Upon stimulation by IL-12 and IL-18, ILC1s produce the proinflammatory cytokine IFN-γ [108].

ILC2s are the major ILC population in the murine lung and depend on the transcription factor GATA-3[109]. Epithelial derived alarm signals like thymic stromal lymphoprotein (TSLP), IL-25 and IL-33 stimulate them to produce their signature cytokines IL-4, IL-5 and IL-13, mainly to repel parasitic and fungal pathogens. Another function of ILC2s is the production of amphiregulin (AREG) which induces repair processes after damage of the epithelial barrier[110–112].

ILC3s are characterized by presence of the transcription factor RAR-related orphan receptor gamma (RORγt) and become activated by IL-23 and IL-1β to produce IL-17A and IL-22 to defend against extracellular bacteria and fungi and help to

maintain epithelial integrity[113,114]. ILC3s are the predominant ILC population in the human lung, comprising about 60% of the total ILCs[115]. In contrast, they make up only 20-30% of the ILC population in murine lungs[116].
ILCs show high functional plasticity and have the capacity to differentiate into other subsets depending on the environmental stimuli[117]. Below many examples, Huang *et al.* showed that stimulation of ILC2s by IL-25 drove them to an inflammatory state, characterized by the ILC3-associated transcription factor RORγt and the expression of IL-17A[118].
Another tissue resident lymphoid population involved in innate immune responses are γδT cells, which can either act adaptively via their γδT cell receptor or in an innate manner via cytokine receptors and PRRs including TLR2 or Dectin-1[119,120]. When activated, they are capable of producing IL-17A and IL-22, which further increases PMN influx and stimulates production of AMPs by epithelial cells, respectively[121–124]. The T cell lineage comprises a multitude of distinct subsets, including cytotoxic T lymphocytes (CTLs), T helper cells (T_h cells), and regulatory T cells (T_{reg} cells), each contributing to the nuanced orchestration of immune responses. As lymphoid cells usually act in an adaptive manner, their detailed functions will not be extensively discussed in this work, which focuses on the early innate immune response in pneumococcal pneumonia. However, T_{regs} have been described to regulate the local inflammation in response to IL-33. Alvarez *et al.* showed in 2019 that IL-33 acting via its receptor ST2 increased forkhead box p3 (FOXP3) dependent suppressive characteristics of T_{regs} on the inflammatory environment, while lack of that signal drove the differentiation to a proinflammatory phenotype characterized by RORγt[125].

1.2.3 Recognition and elimination of *S. pneumoniae* by the pulmonary immune system

PRR signaling is fundamental in detecting *S. pneumoniae*. TLR2 recognizes lipoteichonic acid and lipoproteins[126,127]. *In vivo* studies showed that TLR2 deficiency in *S. pneumoniae*-infected mice led to an impairment of IL-1β, IL-6 and CXCL1 production and to decreased PMN numbers[128]. It is not certain whether TLR4 directly detects *S. pneumoniae*[129]. Some studies indicated PLY as ligand of TLR4, showing that high dosages of PLY led to an TLR4-dependent production of TNF, CXCL1 and CXCL2[130]. However, the question of whether PLY functions as a direct ligand or if PLY-induced cell damage triggers the release of DAMPs, subsequently activating TLR4, remains a subject of ongoing debate[131]. TLR9 detects pneumococcal unmethylated CpG motifs, and *Tlr9*$^{-/-}$ mice showed impaired pneumococcal defense manifesting in higher bacterial burden but without any differences in cytokine production[132].
While the absence of individual TLRs tends to result in relatively mild phenotypes, the deficiency in the adaptor molecule MyD88 led to severe impairment against pneumococcal infections in models of meningitis[133], sepsis[134] and pneumonia[135]. This discrepancy can be explained by the redundancy of TLR function compensating the lack of single TLRs, while MyD88 is activated downstream of all TLRs triggered by *S. pneumoniae* (Fig. 1.1). Another possible factor contributing to this effect, is that MyD88 is also a central adaptor molecule of e.g., IL-1R, IL-18R and ST2.

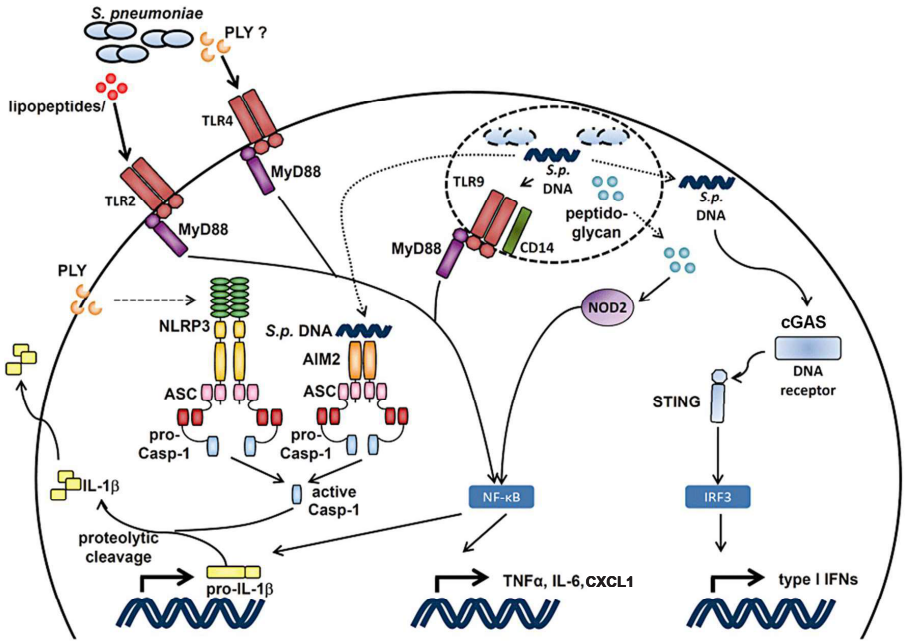

Figure 1.1: The detection of *S. pneumoniae* by functionally relevant PRRs. Pneumococci are recognized in the lung by a series of PRRs expressed in e.g., AMs, DCs, and AECs, which trigger intracellular signaling cascades leading to the production of e.g., proinflammatory cytokines and IFNs. For more details, see main text. Caspase (Casp), cluster of differentiation (CD). Figure edited from Koppe *et al.*[27].

The NLR NOD2 has been found to play a role in detecting *S. pneumoniae* by activating NF-kB pathways[136]. This activation led to increased production of CCL2, which, in turn, promoted greater monocyte influx and enhanced resistance against the infection[137]. Another member of the NLR family involved in immunity against *S. pneumoniae* is the inflammasome NLRP3. Murine *in vivo* models demonstrated that PLY triggers IL-1β production by macrophages and DCs[138] in a TLR4-independent but NLRP3-dependent manner. *Nlrp3*$^{-/-}$ mice were less resistant against *S. pneumoniae* infection and showed decreased lung barrier function[139]. AIM2, detects pneumococcal dsDNA, which leaked from phagosomes perforated by PLY. Subsequently, the inflammasome causes caspase-1 activation leading to IL-1β and IL-18 production[140,141]. As a member of the CLR family, mincle recognizes *S. pneumoniae* via the glycopeptide glucosyl-diacylglycerol but plays a limited role in pneumococcal infection[142,143]. Finally, cGAS and its adaptor molecule STING recognize

pneumococcal DNA fragments which led to IRF3/7-dependent production of type I IFNs by infected macrophages[27,144] (Fig. 1.1).

If *S. pneumoniae* overcomes the barrier of mucociliary clearance[145] and opsonization by complement[146] to reach distal airways and the alveolar space, a complex interplay between tissue and immune compartment is initiated. One of the first responders are AMs phagocytosing *S. pneumoniae*, via sensing by TLR2, mannose and scavenger receptors[147,148]. Another group of first line responders includes AECs, which sense invading *S. pneumoniae* by PRRs and mount direct or indirect immune responses. AECs are main producers of granulocyte-macrophage colony-stimulating factor (GM-CSF) and CXCL5 in *S. pneumoniae* infections, increasing the recruitment of PMNs to the alveolar space[149–153]. With the arrival of PMNs, degranulation of defensin- and lysozyme-containing ganules, phagocytosis and formation of NETs lead to an early control of bacterial burden[154,155]. However, ROS production by PMNs plays a minor role in neutralizing *S. pneumoniae*[86,88,156], and NET formation can be evaded by endonuclease A acitivity[157]. If these measures fail to bring the infection under control, the next line of defense arrives with IMs 24 to 48 hours post infection[158]. When entering the tissue, IMs have the capacity to either fuel the inflammatory immune response by differentiating into M1-like macrophages or initiate repair and suppress further inflammation by differentiating into M2-like macrophages. *In vivo* experiments demonstrated that overexpression of the macrophage attracting chemokine CCL2 in AEC2s increased bacterial resistance against *S. pneumoniae*[159].

Tissue resident lymphoid cells have been described to be involved in defense against *S. pneumoniae*. γδT cells promote PMN influx in an IL-17A dependent manner[160,161] and ILC3s are potent producers of IL-22, leading to improved antibacterial defense in murine pneumococcal infection[162,163]. Additionally, NK cells increase the phagocytic activity of macrophages by boosting IFN-γ secretion[164]. In 2017, Saluzzo *et al.* published a mechanism observed in newborn mice, where IL-33 was released from alveolar epithelial cells with the first breath after birth, activating ILC2s via ST2. Subsequently, IL-13 was released and alveolar macrophages shifted to an M2-like phenotype, thereby delaying the response to *S. pneumoniae* infection[165].

Augmenting the cellular response, humoral immunity also protects against *S. pneumoniae*, including the complement factor C3[146], surfactant proteins A and

D[166] and the B cell derived IgA[167]. All of these soluble factors opsonize the surface of the pathogen, flagging them for phagocytosis and immobilizing them for a better mucociliary clearance.

1.2.4 IL-22 and its role in antibacterial defense

IL-22 belongs to the large IL-10 family of cytokines, which is divided into three subfamilies. The first subfamily consists only of IL-10, which targets both innate and adaptive immune cells, playing a crucial role in mitigating excessive immune responses by exerting immunosuppressive effects. It is also crucial in the resolution phase of an infection and contributes to microbiota homeostasis[168,169]. The second group comprises the IL-20 subfamily of cytokines, including IL-19, IL-20, IL-22, IL-24 and IL-26. This group of cytokines mainly stimulates tissue epithelial and stromal cells to induce innate defense against pathogens as well as maintenance of tissue integrity and proliferation[170]. The IL-28 subfamily is composed of IL-28A, IL-28B and IL-29 also known as IFN-λ2, IFN-λ3 and IFN-λ1, respectively. These cytokines represent the type III IFNs and have partly overlapping functions with type I IFNs, stimulating tissue epithelial cells to defend against viral infections[171].
IL-22 was discovered simultaneously by two research groups, naming it IL-10-related T cell-derived inducible factor (IL-TIF) and IL-22[172,173]. Activation of intracellular signaling is initiated by binding first to the IL-22R1 subunit and subsequent recruitment of the IL-10R2 subunit[174]. Activation of the ligand-receptor complex leads to binding of just another kinase (JAK)1 and tyrosine kinase (TYK)2 and subsequent downstream phosphorylation of signal transducer and activator of transcription (STAT) proteins, especially STAT3[175,176]. The production of IL-22 is induced by IL-23[122,177], IL-1β [120,178], IL-7[179] and aryl hydrocarbon receptor (AhR)[180], while the soluble IL-22 binding protein (IL-22BP) is capable of neutralizing IL-22[181].
IL-22 is mainly secreted by αβT cells, γδT cells, natural killer T cells (NKT cells) and ILC3s[172,182–184]. Especially at mucosal sites, ILC3s comprise a major source of IL-22[104]. Numerous cytokines within the IL-1 family play a role in controlling the

production of IL-22 by ILC3s. In particular, IL-1β and IL-18 exert a direct influence on ILCs, promoting an increase in IL-22 production[185,186], while IL-36γ acts on $CD103^+CD11b^+$ cDCs to induce IL-23 release, thereby indirectly promoting IL-22 production. Mice deficient in IL-36γ were more susceptible to *C. rodentium* infection due to a lack of IL-22 expression[187]. Contrary to the described mechanisms, TSLP can suppress the expression of IL-22, which compromises host defense against *C. rodentium*[188].

The IL-22 receptor (IL-22R) is mainly expressed in epithelial cells and its activation executes functions in tissue regeneration after epithelial damage by inducing survival and inhibiting apopotosis[113]. Besides the described regenerative function, IL-22 can also directly induce production of AMPs including the Reg family of antimicrobial peptides and β-defensins[122,189]. Next to the direct antibacterial activity of AMPs, IL-22 can induce the production of other molecules contributing to host defense such as claudin-2 and fucosyltransferase 2[190,191]. Release of IL-22 triggers immune responses against pathogens including *Klebsiella pneumoniae*[192], *Citrobacter rhodentium*[170,189] and *Candida albicans*[193]. Recent investigations have also found an involvement of IL-22 signaling in defending against *S. pneumoniae*. In these studies, $Il22^{-/-}$ mice showed a higher bacterial burden upon pneumococcal infection due to decreased hepatic C3 production, leading to a reduced opsonic capacity. Primary IL-22 producers in this study were γδT cells, followed by ILC3s[163]. Van Maele *et al.* on the other hand, reported that ILC3s were main producers of IL-22 during pneumococcal pneumonia[162].

1.3 Regulation of IL-33/ST2 signaling

1.3.1 The alarmin IL-33 and its receptor ST2

First identified in 2005, IL-33 was described as a member of the IL-1 family[51]. It is located in the nucleus and plays a key role in innate and adaptive immunity, maintaining tissue homeostasis and contributing to environmental stress responses[194]. It is constitutively expressed by fibroblasts, endothelial and epithelial cells[195,196]. However, using a *Il33Citrin* reporter mouse, Hardman *et al.* found that AEC2s represent the main source of IL-33 in the lungs of naïve mice[197].

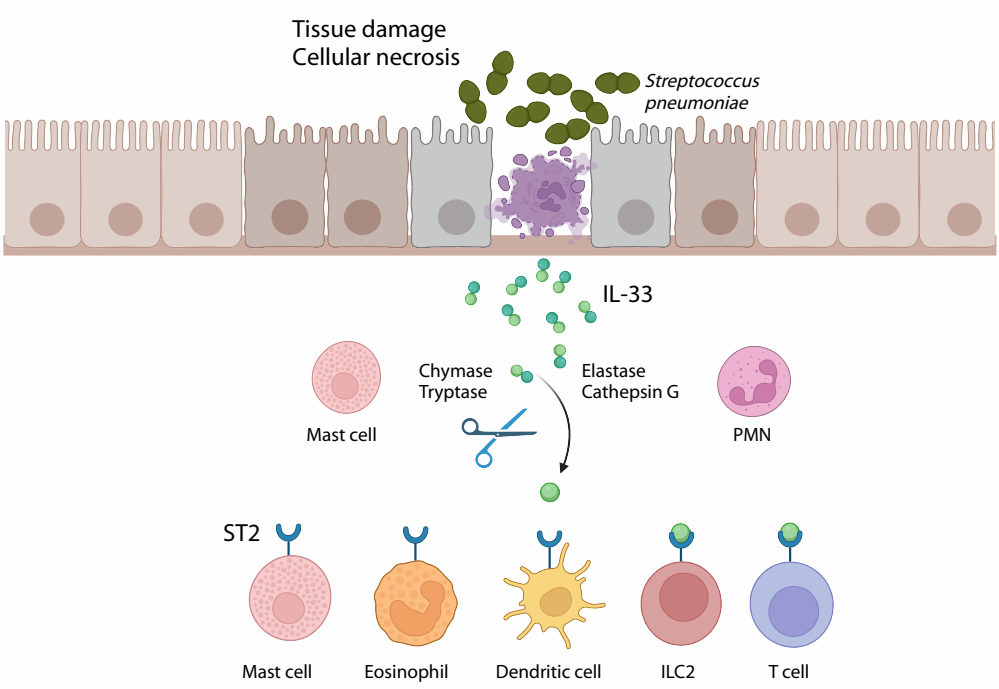

Figure 1.2: Extracellular release of IL-33 and activation by inflammatory proteases. Following tissue damage or necrotic cell death, full-length IL-33 is released from cells and cleaved by mast cell- and PMN-derived proteases. The mature form has a 10- to 30-fold increased bioactivity and activates cells harboring the IL-33 receptor ST2.

The murine *Il33* gene consists of 7 exons coding for a 31 kDa protein of 266 amino acids with two domains. The N-terminal domain contains a chromatin-binding motif[198] mediating the nuclear localization of IL-33. At the C-terminus, an IL-1-like cytokine domain is responsible for binding to the only known IL-33 receptor ST2[199]. Unlike other IL-1-family cytokines like IL-1β and IL-18, IL-33 does not need cleavage by caspase-1 to be released from the cell or to activate ST2[200]. However, extracellular PMN- or mast cell-derived proteases (e.g., cathepsin G, elastase, chymase and tryptase) are capable of cleaving full-length IL-33 to a length of 19 kDa, thereby increasing its bioactivity 10-30 fold[201,202] (Fig. 1.2).
The reason for the nuclear localization of IL-33 is not well studied. *In vitro* models overexpressing *Il33* demonstrated interaction with the N-terminal domain of NF-kB p65, delaying gene expression of downstream genes[203]. Other overexpression models showed that IL-33 can increase IL-13 expression by binding to regions of the *Il13* locus[204]. On the other hand, high-throughput proteomic analyses of endothelial cells completely lacking IL-33 or being exposed to IL-33 only lacking the N-terminus revealed that not nuclear, but extracellular IL-33 regulates protein expression[205]. The available evidence suggests that nuclear IL-33 may exert roles in regulating transcription, but these roles seem to be context-dependent and possibly specific to certain types of cells and conditions.
Depending on the environment and inflammatory state, a finely tuned regulation of IL-33 activity needs to be maintained. In an apoptotic environment, caspase-3 and caspase-7 cleave and inactivate IL-33 at Asp175 within the C-terminal domain[200,206]. Other mechanisms regulating the activity of IL-33 include oxidation of cysteins, formation of disulfide bridges to inactivate the protein[207], and neutralization of IL-33 by its decoy receptor soluble ST2 (sST2), which is an alternative splicing variant of ST2[208,209].

The receptor ST2 was described before the discovery of its ligand IL-33 as an orphan receptor selectively expressed by T helper 2 cells (T_{h2} cells) and mast cells mediating allergic immunity[210,211]. Additional research has unveiled other cell types that respond to IL-33 by expressing ST2, including basophils[212], eosinophils[213], T_{regs}[214], DCs[215] and ILC2s[109]. Not only hematopoietic cells, but also endothelial cells, epithelial cells, fibroblasts, astrocytes and neurons have been reported to express ST2[216–219]. Interaction of IL-33 with the extracellular domain of ST2 initiates the binding of IL-1 receptor accessory protein (IL-1RAcP), leading to further

recruitment of the adaptor molecule MyD88 to the cytoplasmatic Toll-like/IL-1 receptor (TIR) domain. This induces a signaling cascade involving IRAK1, IRAK4 as well as TNF receptor associated factor (TRAF)6, leading to activation of NF-kB and mitogen-activated protein kinases (MAPKs)[51]. This signaling cascade results in the expression of genes inducing cell proliferation, survival signals and type 2 cytokines including IL-4, IL-5, IL-13 and the autocrine growth factor AREG, dependent on the cell type being activated by IL-33[220,221]. Mechanisms inhibiting IL-33/ST2 signaling include phosphorylation and ubiquitylation of the receptor, leading to internalization and degradation, or the inhibition of binding to IL-1RAcP by blockage of the TIR domain through single Ig IL-1 receptor-related molecule (SIGIRR)[222,223]. While the protein is called ST2, the gene name *IL1RL1* will be used throughout this work.

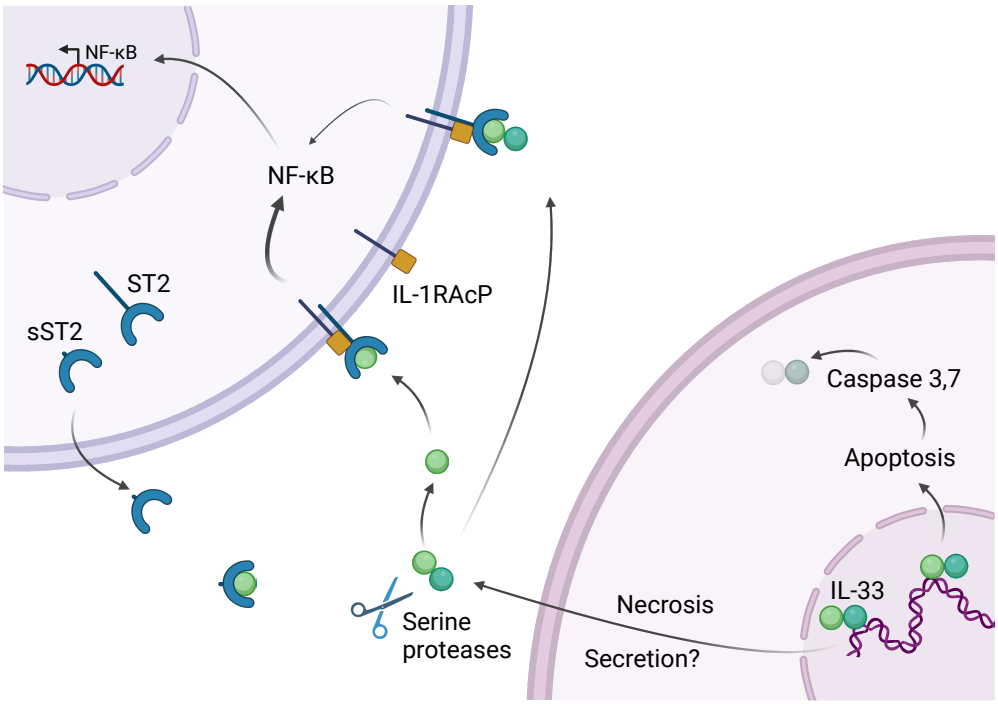

Figure 1.3: Molecular characteristics of IL-33/ST2 interaction. Located in the nucleus, full-length IL-33 is thought to be released to the extracellular space upon cellular damage and necroptotic cell death. In contrast, apoptotic death leads to deactivation of IL-33, facilitated by caspase-3- or caspase-7-driven cleavage. Upon release, full-length IL-33 is cleaved by serine proteases produced by PMNs and mast cells, generating forms with increased bioactivity. The receptor for IL-33, known as ST2, is generated in two variations: a short soluble variant sST2 and a longer membrane-bound variant ST2. While ST2 is consistently present on the cell surface, sST2 can be induced in response to tissue damage, where it binds IL-33 to limit its accessibility. Conversely, both full-length and processed versions of IL-33 bind ST2, associating with IL-1RAcP on target cells. This interaction triggers typical NF-kB and MAPK signaling pathways, culminating in cellular activation and proliferation.

1.3.2 The role of IL-33/ST2 signaling in regulating the immune response

Upon its release into the extracellular space, IL-33 functions as an alarm signal and communicates through its receptor ST2. Extracellular IL-33 primarily stimulates

type 2 immune reactions, playing a significant role in conditions such as allergies, rheumatic diseases, and the immune response against parasites[224,225]. For instance, the interaction between ST2 and IL-33 is protective in infections caused by *Trichuris muris*[226], *Toxoplasma gondii*[227], and *Nippostrongylus brasiliensis*[109]. It has also been demonstrated that IL-33 significantly amplifies innate immune responses at both mucosal and systemic levels, rather than adaptive immune responses[228]. This is a plausible observation, as ST2 is found in a broad spectrum of innate immune cells, while its presence is more restricted in certain subsets of adaptive immune cells[224]. Type 2 immune responses are characterized by the release of classical type 2 cytokines such as IL-4, IL-5, and IL-13, along with the promotion of tissue eosinophilia and an increase in goblet cell abundance. Dysregulation of these responses can contribute to the development of allergic diseases[229]. Type 2 immunity also plays a pivotal role in orchestrating and regulating the process of tissue repair and numerous studies strongly support the crucial role of ST2/IL-33 signaling in this process[230]. Activation of ST2 by IL-33 leads to the production of type 2 cytokines and other mediators from various immune cell types, including T_{h2} cells[51,210], mast cells[231,232], basophils[212], eosinophils[233,234], DCs[235] and ILC2s[109,111,236–238]. Furthermore, several studies indicate that IL-33 can activate downstream processes, to shift macrophage polarization towards an M2-like phenotype, triggering type 2 responses[165,239–242]. In recent years, a series of studies have compellingly demonstrated that IL-33 can also exert influence on type 1 immunity and is involved in the regulation of bacterial and viral infections[243–245]. In this context, IL-12-induced transient expression of ST2 on CTL, T helper 1 cells (T_{h1} cells) and NK cells enabled IL-33-dependent induction of IFN-γ production, contributing to the defense against pathogens[243,244,246–248]. Additionally, through the positive regulation of CXCR2 expression, which is crucial for PMN recruitment to the site of infection, IL-33 has been described to contribute to improved bacterial elimination in certain models[249]. Furthermore, it has been demonstrated that IL-33 enhances the iNOS-dependent antimicrobial capacity of macrophages in *Staphylococcus aureus*-infected skin[242]. However, there are also studies that provide evidence of a detrimental effect of the IL-33/ST2 axis on immune reactions. In 2010, Oboki *et al.* showed that the absence of IL-33 improved mouse survival during LPS-induced sepsis[228]. Furthermore, it has been documented that mice lacking ST2 exhibit greater resistance to subsequent *Pseudomonas aeruginosa* pneumonia following cecal ligation puncture-induced sepsis[250]. In two elegant studies, research groups around Sylvia Knapp *et al.* demonstrated that IL-33 in-

duced IL-13 release by ILC2s and basophils to imprint alveolar macrophages to an M2-like phenotype[165,251].

1.4 Microbiota and immunity

1.4.1 The microbiota of the gut and the respiratory tract

Mucosal surfaces, such as those found in the gastrointestinal and respiratory tracts, naturally harbor around 100 trillion bacteria, along with lesser amounts of fungi, archaea, and viruses. By far the largest abundance of microbiota resides in the gut.[252,253]. The microbiota plays a critical role in health and disease[254,255]. Most of the initial knowledge gained from the interplay between hosts and microbes and how it impacts the immune system, has primarily been generated by investigations involving germ-free and antibiotic-treated mice[256–258]. Recently, with the emergence of cost-effective high-throughput sequencing and a multitude of fecal microbiota transplant (FMT) studies in human and mice, a more in-depth understanding of the interplay between microbiota and immune system was achieved[259]. Microbial communities establish a symbiotic relationship with the host, wherein these microorganisms thrive in a stable, nutrient-rich environment to yield mutual benefits[260]. In turn, they carry out crucial functions for the host, including the fermentation of dietary components to produce nutrients, vitamins and metabolites[261], protection against infection through colonization resistance[167] and modulation of hormone secretion[262]. This symbiotic interaction also plays a fundamental role in the development and training of the immune system. In fact, emerging evidence highlights the importance of the constant recognition of microbes and their products in fine-tuning the immune system for optimal balance[263]. The healthy microbiota offers both local and systemic signals to regulate innate and adaptive immune responses, thereby facilitating the induction of protective responses against various pathogens[264,265].

Microbial abundance in the respiratory tract diminishes as proximity to the alveolar space increases. The composition in the upper respiratory tract is mainly composed of Proteobacteria, Firmicutes, Actinobacteria and Proteobacteria in the nasopharynx[266–269] and oropharynx[270–272]. Additionally, viruses, bacteriophages and fungi such as *Candida spp.* are present in the upper respiratory tract[272]. Going deeper down the respiratory tract, abundance decreases but the composition often mirrors

that of the oropharyngeal microbiota[273,274]. This observation can be explained by microaspiration of microbes[275], though similarity between upper and lower respiratory tract colonization varies in individuals[274,276]. This goes in line with reports that the rate of microaspiration of oropharyngeal fluids during sleep varies in healthy individuals[277]. Though it is likely that the microbes found in the lower airways represent a transient state of constantly aspirated microbes, it is still under debate if they may also represent a permanently colonizing community[278].

The microbiota of the upper respiratory tract is established at birth with a high abundance of microbes, maturating in diversity but decreasing over time, leading to a lower abundance of species[271,279]. Diversity and abundance of the airway microbiota are influenced by breastfeeding, type of birth delivery (natural vs. C-section) and duration of gestation[266–269,280,281]. In older individuals, microbial diversity continues to decrease, while oropharyngeal abundance increases, potentially resulting in an elevated abundance of pathogens[282,283]. The maturation of microbiota, which fluctuates between periods of increase and decrease as individuals age, demonstrates an inverse correlation with susceptibility to CAP. As a result, the risk of getting CAP is most pronounced in newborns and the elderly[278].

The gut microbiota of newborns shows lower diversity and undergoes a process of maturation characterized by the expansion of diversity and continuous improvement of function during the first few years of life[284–286]. Similar to the upper respiratory tract, the development of gut microbiota during early life is significantly influenced by factors such as the method of delivery and the choice between breastfeeding and formula feeding[284,287]. In the elderly, diversity of the intestinal microbiota decreases again, leading to instability in its composition. These alterations appear to be linked to concurrent health conditions, nutritional status, and immunosenescence, the decline in immune system function associated with aging[288,289].

Immune stimulation triggered by the microbiota is facilitated in several ways. One mechanism is the constant recognition of PAMPs by immune cells, modulating their activation and function[290]. Furthermore, microbiota-derived compounds, including substances such as bile acids and short-chain fatty acids (SCFAs), have the capacity to maintain health of the host organism by facilitating nutrition and energy, as well as by regulating the host's immune response. These mediators consist of products directly generated by commensal bacteria, or indirectly by metabolizing dietary compounds[291]. Primary bile acids, including chenodeoxycholic acids, exert bactericidal properties by inducing production of host AMPs[292]. Secondary bile

acids originated from the gut microbiota and symbiotic products like propionate also act againstv e.g., the colonization of *S. enterica*[293] or the bacterial pathobiont *C. difficile*[294] . SCFAs arising from the gut microbiota also influence conditions such as obesity, Parkinson's disease, and disorders that disrupt the integrity of the intestinal barrier[295–298]. Moreover, SCFAs promote maintenance of intestinal equilibrium through IL-10-associated immune modulation[299].

1.4.2 Influence of the respiratory microbiota on pneumococcal infections

The upper respiratory tract is colonized by a multitude of symbiotic and opportunistic microorganisms[272]. Colonization of *S. pneumoniae* has been described to be present in up to 50% of infants, but decreases in adulthood[300], while its hypo- or nasopharyngeal presence is associated with increased risk for pneumonia[301–303]. Pneumococcal colonization increases again in the elderly, which may be causative to the higher prevalence of pneumonia observed in the older population[304]. This effect was observed in humans and is supported by mouse studies showing that the control of pneumococcal colonization is impaired in aged mice[305]. A study by Pettigrew *et al.* demonstrated in 2012, that colonization by *Dolosigranulum spp.* and *Corynebacterium spp.* was associated to lower incidence of *S. pneumoniae* infections, while aberrant trajectories of microbiota development after birth led to higher abundance of respiratory tract infections[269,303,306]. Colonization of *S. epidermis* in children is also negatively correlated with carriage of *S. pneumoniae*. Proposed mechanisms behind these effects include the release of anti-pneumococcal metabolites such as free fatty acids[307] and bacteriocins inhibiting *S. pneumoniae* growth[308]. It is of high importance to recognize that not individual species, but the overall composition of a commensal microbial ecosystem is contributing to protection against infections[309].

1.4.3 Antimicrobial immunity regulated by the gut-lung axis

Disruption of a healthy microbiota leads to impairment of antimicrobial immunity[310,311]. Germ-free (GF) or microbiota-depleted mice show diminished pulmonary immunity against bacterial and viral pathogens[312–318]. Of special note, Schuijt *et al.* showed that the gut microbiota positively influences phagocytic capacity of alveolar macrophages and resistance of mice to *S. pneumoniae* infection. Moreover, their findings demonstrated that FMT in mice with depleted gut microbiota successfully restored pneumococcal defense. This restoration was marked by the normalization of TNF and IL-10 levels, comparable to those observed in untreated control mice[316]. A balanced microbiota influences immune cells by producing e.g., SCFAs and ligands for PRRs, including TLRs and NLRs. These compounds can reach circulation and thereby stimulate immune cells in distal body sites[312,319]. It was reported that microbiota depletion by antibiotics led to impaired expression of inflammasome components, leading to compromised immune reactions of $CD4^+$ T cells, $CD8^+$ T cells and B cells to influenza infection. Administration of TLR ligands either intrarectally or intranasally rescued immune responses[313]. In another study, pretreatment of GF mice with LPS before *K. pneumoniae* infection resulted in an increased PMN mobilization into the lungs and an ameliorated antibacterial defense compared to untreated controls. Peptidoglycans derived from the gut microbiota stimulated PMNs in the bone marrow of mice to increase NOD1-dependent antimicrobial capacity, thereby improving resistance to *S. pneumoniae*[312]. In another study, upper airway and gut derived peptidoglycans stimulated killing capacity of AMs in a NOD2, IL-17A and GM-CSF dependent manner[317]. Further studies contributed to the understanding of SCFAs modulating pulmonary immune functions by reporting of a G-protein-coupled receptor 43 (GPR43) dependent increase of antimicrobial activity by PMNs and AMs[320].

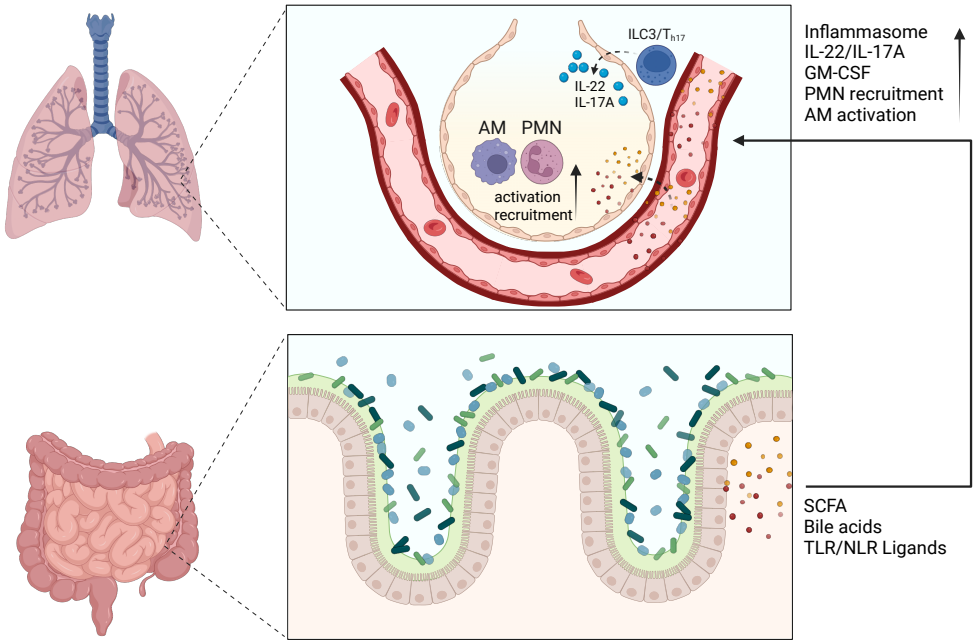

Figure 1.4: Soluble products derived from the gut microbiota influence defense mechanisms in the lung. Soluble molecules such as SCFAs, bile acids and TLR/NLR ligands produced by the intestinal microbiota enter circulation to reach the alveolar space. Once arrived, they can activate AMs and recruit PMNs, increase inflammasome activity and stimulate the production of IL-22, IL-17A and GM-CSF.

The intestinal microbiota also influences type 3 immune responses driven by IL-17A and IL-22. Ivanov *et al.* showed that intestinal segmented filamentous bacteria (SFB) are associated with IL-17A and IL-22 production by lung T helper 17 cells (T_{h17} cells)[321,322] and Gray *et al.* described a mechanism where the intestinal microbiota in newborn mice activates ILC3s to translocate to the lung and to produce IL-22, improving defense against *S. pneumoniae*[323]. SFB are also involved in the protection against *S. aureus* infections[315]. However, not only direct pathogen defense, but also disease tolerance is influenced by the microbiota. Schieber *et al.* demonstrated that intestinal colonization of an *E. coli* strain led to decreased muscle wasting upon lung infection by *B. thailandensis*[324]. This variety in different immune mechanisms regulated by the microbiota illustrates the importance of a healthy and diverse microbiota and demonstrates that dysregulation leads to impaired antimicrobial

resistance. However, it should be noted that variety in healthy microbiotas also contributes to differences in immune responses. Mice bred under special pathogen-free facility (SPF) conditions differ significantly in their microbiota from wild mice[325]. These alterations in the microbiota influence immune responses against pathogens by e.g., boosting plasma levels of IL-1β, TNF, IL-6 and IFN-γ [326,327]. Furthermore, baseline cytokine production in the lung of laboratory mice differs between various commercial vendors and is associated with alterations in the lung microbiome[328]. These observations may explain difficulties in applying animal studies to human clinical trials and highlight the importance of understanding the mechanistic interplay between microbiota and the immune system.

1.5 Preceding research findings leading to the current study

Former students in the Opitz laboratory initiated unpublished experiments to explore the potential involvement of DAMPs in pneumococcal pneumonia. These data show that ATP, uric acid and IL-33 were released from macrophages, lung epithelial cells and mouse lungs upon *S. pneumoniae* infection (Fig. 1.5 A – C).

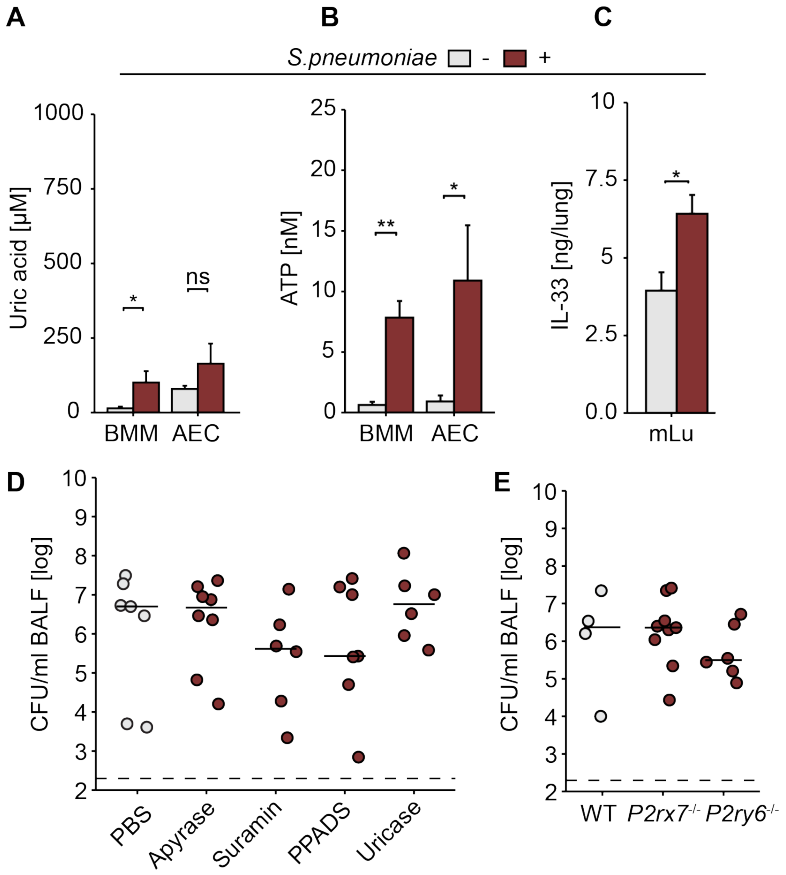

Figure 1.5: DAMP levels are increased upon *S. pneumoniae* infection and abrogation of ATP- and uric acid signaling does not influence antibacterial defense. Murine BMMs, AECs and murine lung tissue (mLu) were left untreated or infected with 10^6 CFU of *S. pneumoniae* for 16-24 h. Concentration of (A) Uric acid, (B) ATP and (C) IL-33 were measured in the supernatants. Data are shown as mean + SEM of 2-8 independent experiments carried out in duplicates or triplicates; Wilcoxon rank sum test; ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$. (D, E) WT mice were left untreated (PBS) or treated with different DAMP inhibitors (see main text) and WT, *P2rx7*$^{-/-}$ and *P2rx6*$^{-/-}$ mice were infected with *S. pneumoniae* and bacterial loads in BALF were measured. Lines represent median and dashed lines the lower detection limit. Data adapted from dissertation A. Rabes[329].

To understand the influence of these DAMPs on the course of infection, several *in vivo* experiments were conducted. Mice were either treated with different enzymes degrading ATP or uric acid (apyrase, uricase) or with nucleotide receptor antagonists (suramin, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS)). Additionally mice lacking receptors for ATP (*P2rx7*$^{-/-}$, *P2ry6*$^{-/-}$) or IL-33 (*Il33*$^{-/-}$) and its receptor ST2 (*Il1rl1*$^{-/-}$) were subsequently infected with *S.*

pneumoniae. Abrogation of ATP and uric acid signaling did not show any effect on the course of pneumococcal infection (Fig. 1.5 D, E), while lack of IL-33 or its receptor resulted in significantly decreased bacterial burden 48 hours post infection as compared to controls (Fig. 1.6 A, B, D, E). Also, *Il33*$^{-/-}$ and *Il1rl1*$^{-/-}$ mice exhibited less hypothermia (Fig. 1.6 C, F) and weight loss (data not shown).

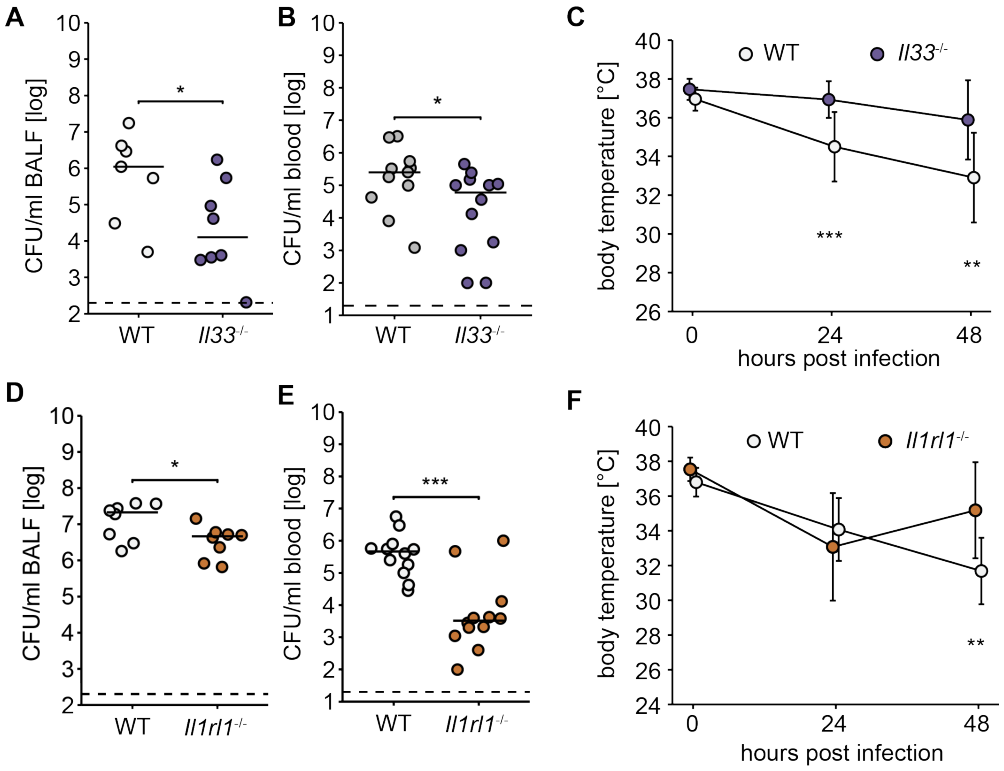

Figure 1.6: IL-33 and ST2 signaling exerts detrimental effects on pneumococcal pneumonia. WT and *Il33*$^{-/-}$ mice were infected intranasally with 5x10^6 CFU/mouse of *S. pneumoniae* and bacterial loads in (A) BALF and (B) blood were determined 48 h post infection and temperature was measured at the indicated time points (C). (D – F) WT and $^{-/-}$ were infected and bacterial loads in (D) BALF and (E) blood were determined 48 h post infection and temperature was measured (F). (A, B, D, E) Data are shown as individual data points. Lines represent median and dashed line the lower detection limit. (C, F) Data points represent mean values of n = 8-19. Wilcoxon rank sum test or Kruskal-Wallis followed by Dunn's posthoc test were used if more than two groups were compared. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Data adapted from dissertation A. Rabes[329].

To further elucidate the mechanism behind the detrimental effects of IL-33 and ST2 signaling on pneumococcal pneumonia, *Il33*$^{-/-}$ bone marrow chimeric mice were generated and infected. Interestingly, only if IL-33 was lacking in recipient animals, a positive effect on antibacterial defense compared to wild type (WT) controls was observable. In contrast, IL-33 deficiency in donor animals did not show any impact on the amount of bacteria measured in the bronchoalveolar lavage fluid (BALF) of infected animals, mirroring the WT phenotype. Furthermore, body temperature in *Il33*$^{-/-}$ recipient mice did not decrease as much as in mice lacking IL-33 in donor cells (Fig. 1.7 A, B). Interpreting this data, mechanistically relevant IL-33 rather derives from tissue cells than from hematopoietic cells, going in line with reports documented in the literature.

Fluorescence Activated Cell Sorting (FACS) measurements were conducted to examine infection associated innate immune cell populations (AMs, PMNs and IMs) in BALF of *S. pneumoniae*-infected WT and *Il33*$^{-/-}$ mice, but no differences in absolute numbers and frequency were observed (Fig. 1.7 C, D).

Although it was demonstrated that IL-33 negatively regulates the immunity against *S. pneumoniae*, the precise interplay of downstream mechanisms following the activation of ST2 and the specific cell types involved in this process remain elusive.

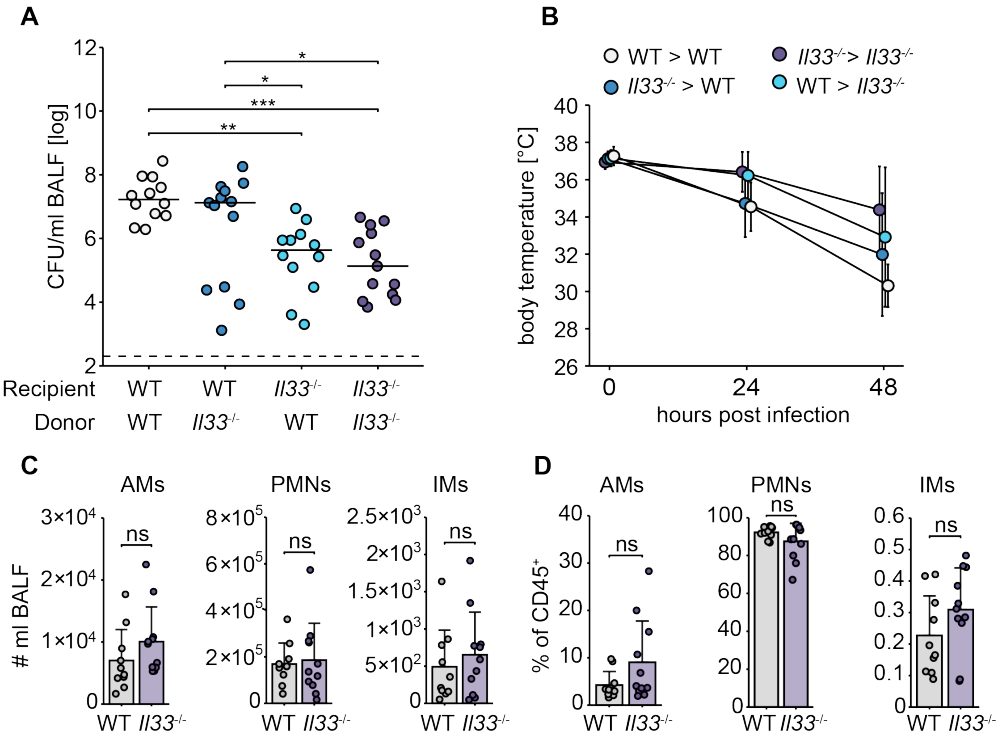

Figure 1.7: Lack of IL-33 in tissue cells results in decreased bacterial burden and abundance of AMs, PMNs and IMs is not influenced by IL-33. (A, B) Bone marrow chimeric mice were infected and bacterial loads in BALF were determined 48 h post infection, and temperature was measured. (C, D) Mice were infected and bronchoalveolar lavage was analyzed by flow cytometry 18 h post infection. Gating strategies: AMs ($CD45^+$ $CD11c^+$ Siglec-F^+); PMNs ($CD45^+$ $CD11b^+$ $Ly6G^+$); IMs ($CD45^+$ $CD11b^+$ $Ly6C^{++}$). (A) Lines represent median and dashed line the lower detection limit. (B) Data points represent mean values of n = 12-13. (C, D) Bars represent mean + SD. Wilcoxon rank-sum test or Kruskal-Wallis followed by Dunn's posthoc test were used if more than two groups were compared. Data adapted from dissertation L. Maschirow[330].

1.6 Objective of this work

Preliminary unpublished work of previous members of the Opitz laboratory show that IL-33 negatively regulates the defense against *S. pneumoniae*. The objective of this work is to elucidate the mechanism underlying the detrimental effects of IL-33 on the course pneumococcal pneumonia.

Material & Methods

2.1 Bacteria

For the murine *in vivo* model of pneumococcal pneumonia, serotype 3 of *S. pneumoniae* strain PN36 (NCTC7978) was used. Bacteria were plated on Columbia agar plates (5 % sheep blood), harvested after 9 hours of incubation at 37 °C + 5 % CO_2 and single colonies were picked and transferred into THY-medium to yield an OD_{600} = 0.03 – 0.04. Subsequently, the liquid culture was incubated at 37 °C for 2 – 3 hours to reach exponential growth with an OD_{600} = 0.3 – 0.4, when they were pelleted at 2700 g for 10 min at 4 °C and resuspended in phosphat buffered saline (PBS). The resuspension volume was determined by the assumption that an OD_{600} = 0.1 equals the amount of $1x10^8$ CFU/ml. As a control of infection dose the final bacterial dilutions were plated on blood agar plates and incubated over night at 37 °C and 5 % CO_2. The bacterial strain PN36 was kindly provided by Prof. Sven Hammerschimdt (Ernst-Moritz-Arndt-Universität Greifswald, Germany).

2.2 Mice

All animal experiments were approved by institutional (Charité – Universitätsmedizin Berlin) and governmental animal welfare committees (LAGeSo Berlin; approval IDs G0227/16, G0080/18 and T0014/12). *Il33*$^{-/-}$ mice[228] were kindly provided by Dr. Yasuhide Furuta (RIKEN Center for Developmental Biology, Kobe, Japan).

Il22$^{-/-}$ mice[331] were crossed with *Il33*$^{-/-}$ mice to generate a double knockout line *Il33*$^{-/-}$*Il22*$^{-/-}$. *Nmur1*$^{iCre\text{-}eGFP}$*Id2*$^{fl/fl}$ mice and *Id2*$^{fl/fl}$ littermate controls were described in Jarick *et al.* 2022[332] and *Il4ra*$^{-/-}$ mice were described in Barner *et al.* 1998[333]. All mice were bred in the animal facilities of the *"Forschungseinrichtungen für experimentelle Medizin (FEM), Charité – Universitätsmedizin Berlin"* and *Bundesinstitut für Risikoforschung (BfR)* and have a C57BL/6J background.

2.3 Murine pneumonia model

An established mouse model of transnasal infection with *S. pneumoniae*[334] was used to understand the role of the IL-33/ST2-axis in pneumococcal pneumonia

2.3.1 Transnasal infection of mice and preparation

Mice were anesthetized with an intraperitoneal (i.p.) injection of 80 mg/kg ketamine and 25 mg/kg xylazine and transnasally inoculated with 5x10^6 CFU *S. pneumoniae* in 20 µl PBS per mouse. Sham-infected mice received 20 µl PBS. Following infection, temperature and weight were measured every 12 hours to monitor the clinical course of disease. For analysis, mice received an i.p. injection of 160 mg/kg ketamine and 75 mg/kg xylazine and were sacrified 12, 18, 36 or 48 hours post infection (h.p.i.) by final blood withdrawal. Blood was centrifuged (1500 g, 10 min, 4 °C), serum was collected and lungs were perfused with 5 ml of PBS. All organs of interest were either snap frozen in liquid nitrogen or processed immediately.

2.3.2 Intranasal and intraperitoneal treatment of animals

The treatment of animals with recombinant IL-22 (rIL-22) (R&D Systems) was administered intranasally (1 μg in 10 µl PBS) at the time of infection (0 h.p.i.), and intraperitoneally at 24 h.p.i. (1 μg in 100 µl PBS). The same volumes of PBS were used for control animals.

2.3.3 Bronchoalveolar lavage of lungs

To analyze the influx of immune cells and the release of immunoregulatory mediators a bronchoalveolar lavage (BAL) was performed by flushing the lungs twice with 800 µl PBS containing protease inhibitors (one tablet per 10 ml). Both fractions were centrifuged (350 g, 10 min, 4 °C) and BALF was collected. The pelleted cells were combined and used for subsequent analyses (see 2.4.1).

2.3.4 Determination of bacterial load

The bacterial load was determined in BALF, blood and spleen of the sacrificed animals. Shortly after preparation, dilutions up to 10^6-fold were prepared from the first BALF, blood and spleen (passed through a 70 µm cell strainer into 10ml PBS), plated on blood agar plates and incubated at 37 °C + 5 % CO_2 over night. Colony forming units (CFU) were counted on the following day.

2.3.5 Digestion of murine lungs in preparation for RNA sequencing

After infection and preparation described in 2.3.1, lungs were perfused with 5 ml PBS followed by 2 ml dispase (5000U/ml, Corning) through the right heart ventricle. 700 µl dispase was applied intratracheally, followed by 500 µl lukewarm 1 % low melt agarose. After solidification of the agarose, lungs were removed and transferred into a petri dish containing 2 ml digestion medium (PBS + 5 % fetal calf serum (FCS) + 2 µg/ml Actinomycin D + 2 mg/ml collagenase + 0.5 mg/ml DNase) and trachea and other contaminating tissues were removed. To break down the tissue and increase its surface, lungs were mechanically shredded with tweezers and incubated for 30 min at 37 °C at 125 revolutions per minute (rpm) in a shaker. Afterwards, digested tissue was further dissolved by gently drawing up and down with an 18-gauge cannula. The homogenate was then discharged onto a 70 µm cell strainer and squeezed into a 50 ml Falcon tube with the plunger of a 5 ml syringe. The petri dish was washed with 10 ml of cold PBS (+ 2 % FCS) and filtered through the same cell strainer. Lung homogenates of each group were combined or not, centrifuged (300 g, 5 min, 4 °C) and resuspended in 2 ml red blood cell (RBC) lysis buffer (Gibco) for 2 min on ice. Reaction was stopped by adding 10 ml of PBS (+ 2 % FCS), centrifuged (300 g, 5 min, 4 °C), resuspended in 2 ml PBS (+ 2 % FCS) and passed through a 70 µm cell strainer with additional 8 ml of PBS (+ 2 % FCS). Cells were counted using a hemocytometer, adjusted to $1x10^8$ cells/ml and dead cells were removed with a magnetic bead based dead cell removal kit according to the manufacturer's instructions (EasySep STEMCELL Technologies).

2.3.6 Gut microbiota depletion

To study the effects of antibiotic treatment on the gut microbiota of mice, 8- to 9-week-old mice were housed in sterile cages and treated orally with a combination of imipenem (250 mg/L; Fresenius Kabi), metronidazole (1 g/L; Braun), vancomycin (500 mg/L; HIKMA Pharma), ciprofloxacin (200 mg/L; Fresenius Kabi) and ampi-

cillin (1 g/L; Ratiopharm) in the drinking water *ad libitum* for a period of 6 weeks (ABX mice). The antibiotic-containing water was refreshed every 3 days. Fecal samples were collected weekly for analysis of fungal outgrowth and depletion of gut microbiota. Any mice that showed fungal outgrowth were excluded from the study. Control mice were housed in the same animal facilities and rooms and were studied concurrently with the antibiotic-treated mice (ABX mice). Antibiotic treatment and monitoring was performed by our collaboration partners at AG Heimesaat.

2.3.7 Murine fecal microbiota transplantation

Microbiota depleted ABX mice (see 2.3.6) were transplanted with fecal samples derived from WT or *Il33*$^{-/-}$ mice originated from two different vivaria. Samples were collected, pooled by genotype and vivarium, resuspended in sterile PBS, and filtered using a 70 µM cell strainer. In sterile condition, 300 µl of fecal transplant sample was given once a day orally over the course of three days to WT and *Il33*$^{-/-}$ mice. Mice were infected with 5x10^{6} CFU/mouse *S. pneumoniae* five to eight days after transplantation (see 2.3.1). Antibiotic treatment, monitoring and FMT were executed by Alexandra Bittroff-Leben, Ulrike Fiebiger and Soraya Mousavi (AG Heimesaat).

2.4 Immunological methods

2.4.1 Flow cytometry

To analyze the recruitment of immune cells into the alveoli, pooled BALF cells (see 2.3.3) were centrifuged (300 g, 5 min, 4 °C) and resuspended in 50 µl surface master mix. After 20 min incubation at 4 °C, cells were centrifuged (300 g, 5 min, 4 °C) and incubated in 1 ml RBC lysis buffer for 2 min on ice. Reaction was stopped by adding

10 ml of PBS, cells were centrifuged (300 g, 5 min, 4 °C) and resuspended in 2 ml PBS (+ 2 % FCS). Cells were immediately used for analysis or fixed with FOXP3 Fix/Perm Kit (Biolegend).
For intracellular staining, lungs were prepared as described in 2.4.3, left unstimulated or stimulated with 50 ng/ml Phorbol-12-myristat-13-acetat (PMA), 1 µg/ml Ionomycin and 1 µg/ml Brefeldin A (all Sigma-Aldrich) for 5 h at 37 °C + 5 % CO_2. After incubation, cells were centrifuged (300 g, 5 min, 4 °C), resuspended in 50 µl surface master mix and incubated at 4 °C for 20 min and centrifuged (300 g, 5 min, 4 °C). Pellet was then resuspended in 100 µl FOXP3 Fix/Perm solution or 2% paraformaldehyde (PFA) and incubated for 30 min (FoxP3 Fix/Perm kit) or 1 h (2% PFA) in the dark. Following, cells were washed twice with 100 µl Perm Buffer and centrifuged (350 g, 8 min, 4 °C), resuspended in 50 µl intracellular master mix diluted in Perm Buffer and incubated for 60 min at room temperature in the dark. Then cells were washed with 150 µl Perm Buffer, centrifuged (350 g, 8 min, 4 °C) and resuspended in Perm Buffer. Before measurement at the flow cytometer, 50 µl of CountBright counting beads (Life Technologies) were added to each sample to determine absolute cell numbers.

2.4.2 Measurement of innate cells in the alveolar space

The cell pellets obtained from both lavages (see 2.3.3) were pooled in 1 ml PBS and analyzed with a FACSCanto II (BD Biosciences). The relative and absolute numbers of AMs ($CD45^+$ $CD11b^-$ $Siglec\text{-}F^+$), recruited PMNs ($CD45^+$ $CD11b^+$ $Ly6G^+$) and IMs ($CD45^+$ $Ly6G^-$ $CD11b^+$ $Ly6C^{++}$) were determined.

2.4.3 Measurement of lymphoid cells in the lung

To determine basal and infection-associated levels of lymphoid cells, lungs were perfused, isolated, cut into small pieces and incubated in 5 ml RPMI 1640 (+ 5 % FCS containing collagenase (2 mg/ml) and DNase (0.5 mg/ml) for 40 min at 37 °C while shaking. Digested lung tissue was passed through a 70 µm cell strainer and centrifuged (300 g, 5 min, 4 °C). Then cells were resuspended in 1 ml RBC lysis buffer, incubated for 2 min on ice, PBS-washed and centrifuged (300 g, 5 min, 4 °C). The pellet was solved in 0.5 ml FACS-buffer (PBS + 2 % FCS) and subsequently stained for flow cytometry as described in 2.4.1. Lymphoid populations were characterized as follows: αβT cells ($CD45^+$ $CD3^+$ $TCR\beta^+$), γδT cells ($CD45^+$ $CD3^+$ $TCR\gamma^+$), ILCs ($CD45^+$ $CD3^-$ $CD19^-$ $Ly6G^-$ $FceRIa^-$ $CD5^-$ $NK1.1^-$ $CD127^+$ $CD90^+$), ILC2s ($CD45^+$ L/D $CD3^-$ $CD19^-$ $Ly6G^-$ $FceRIa^-$ $CD5^-$ $NK1.1^-$ $CD127^+$ $CD90^+$ $GATA3^+$).

2.4.4 FACS sorting of ILCs

Following the digestion (see 2.3.5) during the Single-cell RNA sequencing (scRNAseq) experiment, half of the cell suspension was sorted for $CD45^+$ $CD90^+$ $CD127^+$ Lin^- ILCs and spiked back into the whole lung single cell solution. Sorting of the cell suspension was conducted by using a FACS AriaTM II sort flow cytometer cell sorter (BD Biosciences). After sorting, protocol was continued with scRNAseq library preparation (see 2.5.1). For the bulk RNA sequencing experiment, digested lung cells were sorted for ILC2s ($CD45^+$ L/D $CD3^-$ $CD19^-$ $Ly6G^-$ $Fc\epsilon RI\alpha^-$ $CD5^-$ $NK1.1^-$ $CD127^+$ $CD90^+$ $ST2^+$) and further processed as described in 2.5.2.

2.4.5 ELISA

The protein levels of various cytokines, chemokines and soluble receptors in BALF have been treated with commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, R&D Systems) or with a self-compiled multiplex assay (ProcartaPlex™, Thermo Fisher) (see 2.7). The protocols for ELISA kits and the multiplex assay were each performed according to the manufacturer's instructions. To determine the optical density a spectrophotometer (FilterMax™ F5 Multimode Microplate Reader, Molecular Devices) was used for the ELISA measurements and a Bio-Plex 200 (Bio-Rad) for the multiplex assay. Protein concentrations were calculated using a corresponding standard curve. BALF samples of WT and *Il33*$^{-/-}$ mice were measured with a 36-panel ProcartaPlex™ containing the analytes GM-CSF, IFN-α, IFN-γ, IL-1β, IL-12p70, IL-13, IL-18, IL-2, IL-5, IL-6, TNF-α, CXCL5, G-CSF, IL-1α, IL-28, LIF, M-CSF, IL-10, IL-15, IL-17A, IL-22, IL-23, IL-27, IL-31, IL-4, CCL2, CXCL1, CXCL10, CCL11, CCL7, CCL3, CCL4, CXCL2 and CCL5.

2.5 Molecular biology

2.5.1 Single cell RNA sequencing

After digestion to single cells (see 2.3.5) and enrichment of ILCs (see 2.4.4), cells were resuspended in an appropriate volume of PBS (Gibco), passed through a 40 µm cell strainer, counted utilizing a Neubauer counting chamber, adjusted to a concentration of 1x10^6 cells per ml and loaded into the Chromium Controller (10x). The following steps were conducted by Gitta Heinz (AG Mashreghi). Single Cell 5' reagent kit v2 was used for reverse transcription, cDNA amplification and library construction, followed by the detailed protocol provided by 10x Genomics. The generated libraries were sequenced on a NovaSeq 6000 S4 flowcell type with 200 cycles with 30.000 cells per lane and 4 lanes in total, targeting 2.500 million reads per lane. The raw sequencing data was subjected to de-multiplexing and quality-check

using the Cell Ranger pipeline. The process involved converting BCL files from each library to FASTQ reads with the 10x barcodes and TotalSeq antibodies specified in the respective sample sheet, using the bcl2fastq Conversion Software (Illumina). The reads were aligned to the reference genome provided by 10x Genomics (Mouse reference mm10), and a digital gene expression matrix was generated to record the number of UMIs for each gene in every cell. Quality metrics were applied to the count matrices, and thresholds were set for the number of genes (> 150 and < 4000) and the percentage of mitochondrial reads ($< 10\,\%$). All steps until the generation of the digital gene expression matrix were carried out by Frederik Heinrich (AG Mashreghi).

2.5.2 Bulk RNA sequencing

To prepare mouse lungs for RNA bulk sequencing, infected lungs were digested and FACS-sorted as described in 2.3.5 and 2.4.4. RNA was isolated using Direct-Zol Microprep Kit (Zymo Research, Cat#R2061). The following steps were conducted by the Max Delbrück Center For Molecular Medicine (MDC) next generation sequencing core facility. Library preparation were done with Takara SMARTer stranded Total RNA-Seq Kit v3 – Pico. Input and library was sequenced on a NovaSeq 6000 SP targeting 400 million reads. The following steps of preprocessing were done by Miha Milek from the Core Unit Bioinformatics (CUBI) of Berlin Institute of Health (BIH). Sequencing reads were mapped to the mm10 mouse genome sequences and further processed to obtain a normalized count-matrix which was used to analyze the data described in 2.6.

2.5.3 Fecal DNA extraction and shotgun sequencing

Mouse fecal samples were collected before mice were infected and stored at -80 °C until further use. For the DNA extraction a DNA Miniprep Kit from Zymo Research (Cat#D4300) was used, purity and concentration of the DNA was measured using a NanoDrop™ 2000 (Thermo-Fisher). Library prep and sequencing of the bacterial DNA was performed by Eurofins Genomics (Ebersberg, Germany) using a Illumina NovaSeq 6000, with 10 million reads and paired-end sequencing (2 x 150bp).

2.5.4 Shotgun Metagenome Data processing

After sequencing, preprocessing was conducted by Eurofins genomics. Poor quality bases, adapters and primers were removed before proceeding to the removal of host sequences. Taxonomic profiling was conducted by using the NCBI database of bacterial, archaeal, fungal, protozoan and viral genomes. FASTQ files were generated and a table containing normalized reads for each domain, phylum, class, order, family, genus and species was prodivded. This table was then loaded into R and used for subsequent frequency and correlation analysis.

2.5.5 Quantitative real time PCR to analyze feces samples

After DNA isolation described in 2.5.3, bacterial species in murine feces were quantified by quantitative real-time polymerase chain reaction (qPCR). For this purpose, a master mix was prepared for each reaction, consisting of 10 µl Power SYBR™ Green PCR Master Mix, 4 µl template DNA, and 0.5 µl of both, forward and reverse primers (10 µM each; SFBfwd 5'-GACGCTGAGGCATGAGAGCAT-3'; SFBrev 5'-GACGGCACGGATTGTTATTCA-3'; 16s rRNA primers for total bacterial DNA quan-

tification). 200 ng of DNA was then loaded into a 96-well plate, 15 µl of the appropriate master mix was added to each well. The qPCR was performed using the 7300 Real-Time PCR System (Applied Biosystems), with following settings: 2 min at 50°C, 10 min at 95°C, 40 cycles: 15 sec at 95°C + 1 min at 60°C.

2.5.6 SNP analysis

Genomic DNA from patients hospitalized with pneumococcal pneumonia and age and sex matched controls were either provided by the CAPNETZ competence network[335] or by the PolSenior program[336], respectively. DNA Genotyping of rs1420101, rs7044343, rs9500880 and rs1921622 was conducted by PCR utilizing fluorescent-labeled hybridization FRET probes followed by melting curve analysis in a LightCycler 480 (Roche Diagnostics). The following probes and primers were used: rs1420101: f-primer: TAgTTTggTgTCAgAgTTTCTgCAA, r-primer: TgAAgTgACTTACTCAAggCCA, anchor probe: LC640 CCAATgAgTATTACTAAAgATTAAgCTCTT PH, sensor probe: CAAAgCCTCTCATTAAACTTTgAA FL; rs7044343: f-primer: AggAATgAATATTgggTgACACTATg, r-primer: TACCCAAgTTCAAgAggCACTg, anchor probe: LC640 TCCTgTCTgCATgTAAAgCCACTC PH, sensor probe: ggTTACTTCTCAgggCATCA FL; rs9500880: f-primer: TTCAggAAATTAgAggTCTAATgTAA, r-primer: CTgCAgAgACATgCCAAAgACA, anchor probe: LC640 gACgAAAgCATTCTTAAATCTgATATTC PH, sensor probe: TgATTTCTAgTTCCACACTTATgA FL; rs1921622: f-primer: CACCAggATAACTCTgCCAC, r-primer: TAAATTTgCAAATgTTTCACCAAC, anchor probe: LC640 AgCCATAggCACTAgCTgAAATAC PH, sensor probe: TAAAAATTgATgAATTTTgTTCTgg FL.

2.6 Data analysis, visualization and statistics

Data analysis was performed using R, version 4.2.1. Wilcoxon rank-sum test was used for the comparison of two groups. If more than two groups were compared,

Kruskal-Wallis Test, followed by a Dunn's post-hoc test was utilized. SNP allele frequencies were analyzed using Fisher's exact test. To test for effect sizes in microbial communities obtained from fecal samples, Cliff's delta was calculated using the effsize package in R[337]. ILC2 bulkseq data was analyzed mainly with DeSeq2 package[338] in R. Differential genes were subjected to gene set enrichment analysis (GSEA) using the fgsea package[339] in R. The standard pipeline of the Seurat package (version 4.2.0)[340] was used for data integration, variable feature finding, data scaling, and PCA calculation based on highly variable genes. In case of batch effects, Harmony package[341] was utilized for correction. The Seurat functions RunUMAP(), FindNeighbours() and FindClusters() were employed to reduce dimensions and find shared nearest neighbours (SNN) in the dataset. To calculate differential gene expression, the Seurat function FindMarkers() was used. The flow cytometry results were analyzed using FlowJo™ v10.7.2 Software (BD Life Sciences). Figures 1.2, 1.3, 1.4 and 4.1 were created with in BioRender.com.

2.7 Materials

2.7.1 Reagents and media

Table 2.1: Reagents and Media

Reagents	Manufacturer
ACK Lysing Buffer	Gibco
Ampuwa®RNAse-free water	Fresenius Kabi
Bacto™ Todd Hewitt Broth	BD Biosciences
Bacto™ Yeast extract	BD Biosciences
BD FACS™ Lysing Solution	BD Biosciences
Brefeldin A	Sigma-Aldrich
BSA	Sigma-Aldrich
Collagenase II	Sigma-Aldrich
Columbia Agar + 5% sheep blood	BD Biosciences
cOmplete™, Mini Protease Inhibitor	Roche Diagnostics GmbH
CountBright™ Absolute Counting Beads	Molecular ProbesTM
Dispase	Corning
DMEM	Gibco
DMSO	Sigma-Aldrich
DNAse I	PanReac Applichem
Dynabeads® Biotin Binder	Thermo Fisher Scientific
EDTA	Roth
Ethanol	Merck
FCS	Capricorn Scientific
Fixable Viability Dye eFluor® 780	eBioscience
Ionomycin	Sigma-Aldrich
Isopropanol	Sigma-Aldrich
Ketamine (Ketavet)	Sigma-Aldrich
Low-melt Agarose	Bio-Rad

Continued on next page

Table 2.1 Reagents and Media

Reagents	**Manufacturer**
Methanol	Merck
NaCl (0.9%)	B. Braun
NaOH	Roth
PBS	Gibco
Phorbol-12-myristate-13-acetate	Sigma-Aldrich
Power SYBR Green® PCR Master Mix	Applied Biosystems
rIL-22	R&D Systems
RPMI 1640	Gibco
Sulfuric acid (H2SO4)	Roth
Thilo-Tears® Gel (Augengel)	Novartis Pharma GmbH
Tris-Base	Roth
Tris-HCl	Roth
Tween-20	Sigma-Aldrich
Xylazine (Rompun 2%)	Bayer
β-Mercaptoethanol	Sigma-Aldrich

2.7.2 Kits

Table 2.2: Kits

Kit	Manufacturer
BD Cytofix/Cytoperm™ Kit	BD Biosciences
Direct-zol RNA Microprep	Zymo Research
EasySep™ Dead Cell Removal Kit	STEMCELL Technologies
FOXP3 Fix/Perm Buffer Set	Biolegend
Next GEM Single Cell 5' Kit v2	10x Genomics
ZymoBIOMICS DNA Miniprep Kit	Zymo Research

2.7.3 Instruments and consumables

Table 2.3: Instruments and consumables

Instrument/consumable	Manufacturer
7300 Real-Time PCR System	Applied Biosystems
BD FACSAriaTM II	BD Biosciences
Bio-Plex®200 Systems	Bio-Rad
Chromium Controller	10x Genomics
DiscarditTM II syringe	BD Biosciences
EASYstrainerTM (40 µm, 70 µm)	Greiner Bio-One
ELISA-Multiplates (96-well)	Corning
FACSCanto II	BD Biosciences
FACS-Tubes	Sarstedt

Continued on next page

Table 2.3 Instruments and consumables

Instrument/consumable	**Manufacturer**
Falcon® Conical Tubes (15ml, 50 ml)	Falcon
FilterMax^{TM} F5 Microplate Reader	Molecular Devices
Heraeus Biofuge Fresco	Thermo Scientific
Heraeus Function Line Typ B20	Thermo Scientific
Heraeus Megafuge 1.0R	Thermo Scientific
Heraeus Typ BB 6220 CO2 incubator	Thermo Scientific
Heraeus Typ HS 18 safety bench	Thermo Scientific
Heraeus Typ HS 9 safety bench	Thermo Scientific
HerasafeTM KS safety bench	Thermo Scientific
Inoculation loops (1 µl and 10 µl)	Sarstedt
LightCycler 480	Roche Diagnostics
Mastercycler Gradient	Eppendorf
Microcentrifuge 5417R	Eppendorf
Mikrowaage CPA224S-OCE	Sartorius AG
Multipette® Plus	Eppendorf
NanoDrop 2000	Thermo Scientific
Neubauer-counting chamber	LO-Laboroptik GmbH
Omnifix®-F syringe (1 ml and 2 ml)	B. Braun
Petri dishes (100 x 15 mm)	Falcon
Pipet tips tips (Safe-Seal Tips®)	Biozym
Pipet tips, unsterile	Sarstedt
Pipetboy	Integra Biosciences AG
Pipets (10 µl, 100 µl, 200 µl, 1 ml)	Eppendorf
Precision scale EG300-3M	Kern
Rectal Probe for Mice RET-3	Bioseb
Safe-lock tubes (0.5 ml, 1.5 ml, 2 ml)	Eppendorf
Sterican® Injection canula	B. Braun
SW23 shaking waterbath	Julabo GmbH
Thermometer BAT-12	Physitemp Instruments
Tuttnauer Systec 2540 EL Autoclave	Systec GmbH

2.7.4 Antibodies

Table 2.4: Antibodies used for flow cytometry

Target	Fluorochrome	Clone	Manufacturer
B220	PE	RA3-6B2	BD Biosciencess
B220	APC-Cy7	RA3-6B2	Biolegend
B220	Biotin	RA3-6B2	Biolegend
CCR6	A647	140706	BD Biosciences
CCR6	BV786	140706	BD Biosciences
CD11b	BV421	M1/70	Biolegend
CD11c	APC-Cy7	N418	Biolegend
CD11c	APC	N418	eBioscience
CD127	PerCP-Cy5.5	A7R34	Biolegend
CD127	PE-eFluor610	A7R34	eBioscience
CD19	Biotin	6D5	Biolegend
CD25	A488	PC61	Biolegend
CD326	PE	G8.8	Biolegend
CD3e	PE-Cy7	145-2C11	eBioscience
CD3e	FITC	145-2C11	BD Biosciences
CD3e	BUV737	17A2	BD Biosciences
CD4	BV510	RM4-5	Biolegend
CD44	eFlour450	IM7	eBioscience
CD45	A488	30-F11	Biolegend
CD45	AF700	30-F11	Biolegend
CD5	Biotin	53-7.3	Biolegend
CD8a	PerCP	53-6.7	BD Bioscience
CD90.2	PE-Cy7	53-2.1	eBioscience
F4/80	APC-Cy7	BM8	Biolegend

Continued on next page

Table 2.4 Antibodies used for flow cytometry

Target	Fluorochrome	Clone	Manufacturer
FcεRIα	APC-Cy7	Mar-1	Biolegend
FcεRIα	Biotin	Mar-1	Biolegend
FoxP3	APC	FJK-16s	eBioscience
GATA-3	PE	TWAJ	eBioscience
GATA-3	AF647	L50-823	BD Biosciences
Gr-1	APC-Cy7	RB6-8C5	Biolegend
Gr-1	Biotin	RB6-8C5	Biolegend
IL-22	PerCP-Cy5.5	Poly5164	Biolegend
IL-23R	PE	12B2B64	Biolegend
Ly6C	PerCP	HK1.4	Biolegend
Ly6G	BV510	1A8	Biolegend
Nkp46	PE	29A1.4	Biolegend
NK1.1	PerCP-Cy5.5	PK136	eBioscience
NK1.1	PE-Cy7	PK136	eBioscience
NK1.1	SB702	PK136	eBioscience
RORγt	PE	B2D	eBioscience
RORγt	BV421	Q31-378	BD Biosciences
RORγt	BV650	Q31-378	BD Biosciences
Siglec-F	PE	E50-2440	BD Biosciences
Siglec-F	Biotin	S17007L	Biolegend
ST2	BUV395	U29-93	BD Biosciences
ST2	APC	RMST2-2	eBioscience
T-bet	A647	O4-46	BD Biosciences
T-bet	BV421	4B10	eBioscience
TCRβ	BV605	H57-597	BD Biosciences
TCRγδ	FITC	GL3	BD Biosciences
TCRγδ	PerCP-Cy5.5	GL3	Biolegend

Results

3.1 IL-33-dependent regulation of anti-pneumococcal immunity is independent of ILC2s, IL-4 and IL-13

3.1.1 Single-cell RNA sequencing reveals *Il1rl1* expression in ILC2s and no major differences in innate leukocyte populations between WT and *Il33*$^{-/-}$ animals

As described in the introduction, *Il33*$^{-/-}$ mice show a significantly increased bacterial defense upon *S. pneumoniae* infection, as indicated by lower bacterial loads in lungs and blood of *Il33*$^{-/-}$ compared to WT mice (Fig. 1.6). To characterize the underlying processes, scRNAseq was employed to analyze pulmonary cells from mice infected with *S. pneumoniae*, aiming to unravel the cellular dynamics involved. To enable the examination of rare ILCs, the whole lung was digested to single cells ($\frac{3}{4}$ of lung) and pooled with a fraction that was ILC-enriched by FACS-sorting CD45^{+} lineage^{-} CD90.2^{+} CD127^{+} cells ($\frac{1}{4}$ of lung) (Fig. 3.1 A). The distinct cellular populations were visualized by employing uniform manifold approximation and projection (UMAP) (Fig. 3.1 C).
Next, typical marker genes reported in literature were used to annotate clusters (Fig. 3.1 B). Subsequent analyses identified ILC2s as the primary source of *Il1rl1*, the gene coding for the IL-33 receptor (Fig. 3.1 D). The main source of *Il33* were AEC2s and mesenthelial cells.

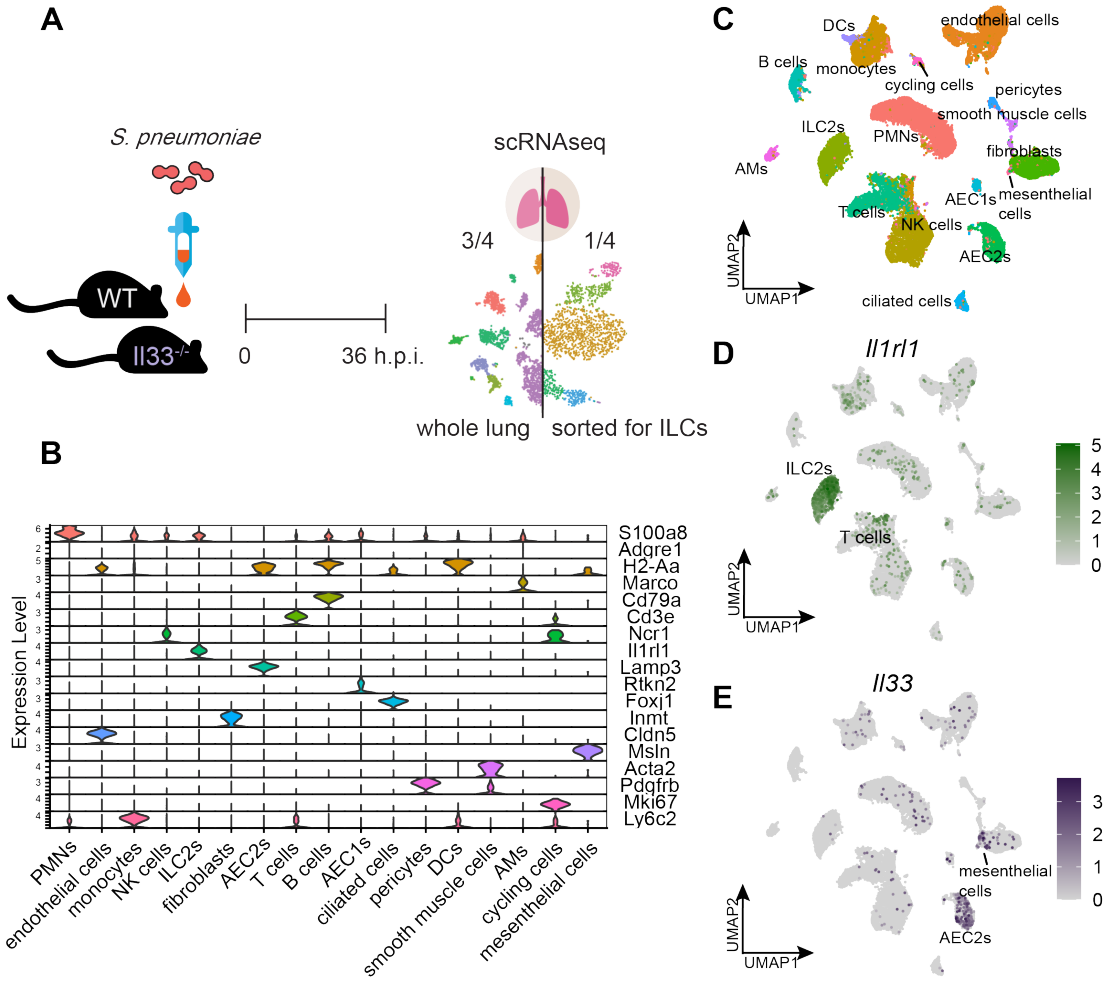

Figure 3.1: Single-cell sequencing of whole lung enriched with FACS-sorted ILCs. (A) Mice were infected with *S. pneumoniae*, sacrificed after 36 hours (n = 3-4 per group) and lungs were first digested into single cells and then pooled within the experimental group. $\frac{1}{4}$ of each single-cell solution was FACS-sorted for ILCs ($CD45^+$ $lineage^-$ $CD90.2^+$ $CD127^+$) and spiked back into the whole lung solution, before being subjected to scRNAseq. (B) Stacked violin plot displaying representative marker genes for each cell type. (C) Two-dimensional UMAP embedding on 24,612 computationally identified and cells. (D, E) Normalized expression levels of (D) *Il1rl1* and (E) *Il33* plotted in an UMAP embedding.

Additionally, unbiased subclustering of AMs, PMNs and monocytes revealed no notable differences in frequency (Fig. 3.2) or in infection-associated transcriptional pathways (not shown) between IL-33-deficient and WT mice. This is in line with flow cytometric measurements described in Maschirow, 2019[330], where no differences in frequency or absolute numbers of AMs, PMNs and inflammatory monocytes were observed (Fig. 1.7).

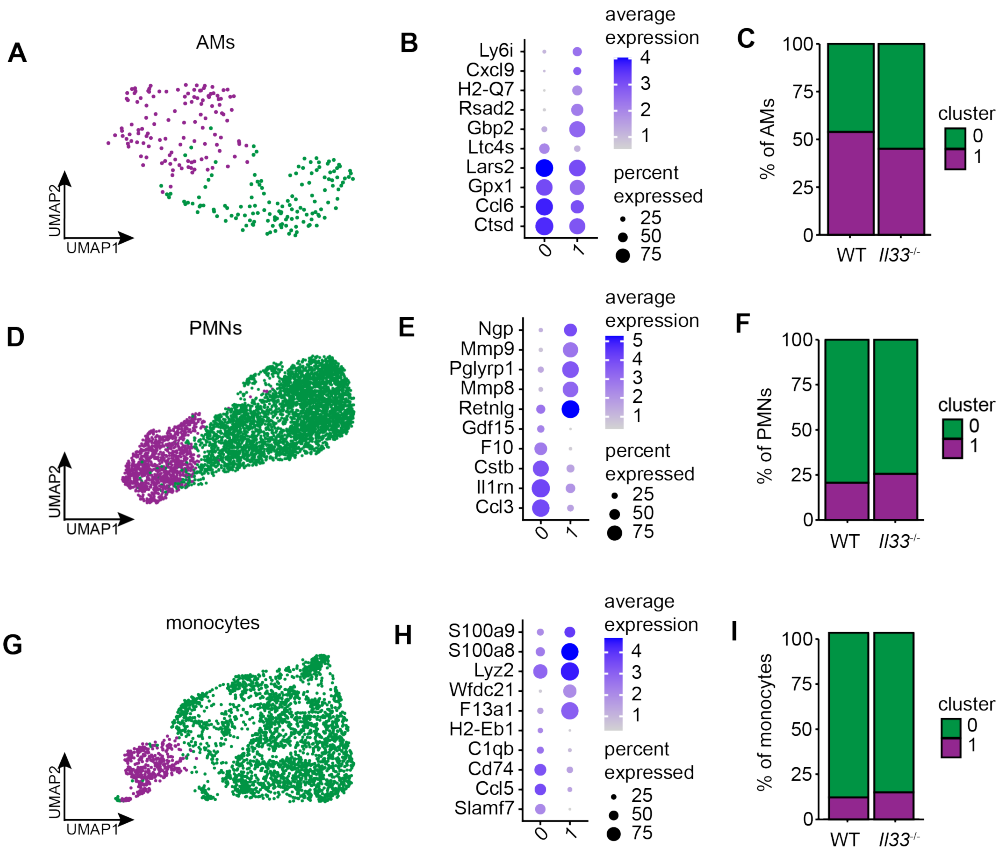

Figure 3.2: Subsets of AMs, PMNs and monocytes show no differences in frequency between WT and *Il33*$^{-/-}$ mice. Mice were infected, sacrificed after 36 hours (n = 3-4 per group) and lungs were subjected to scRNAseq. (A, D, G) Dataset was first subsetted on AMs, PMNs and monocytes and separated in two clusters by unbiased clustering. (B, E, H) Cluster specific marker genes of depicted cell types were visualized in a dotplot. Color strength correlates with average expression values, dot size increases with fraction of cells expressing a gene. (C, F, I) Frequencies of depicted cell types in WT and *Il33*$^{-/-}$ mice are represented in a barplot.

3.1.2 IL-33 induces expression of activation-associated gene sets in ILC2s

The prominent expression of the IL-33 receptor *Il1rl1* on ILC2s (Fig. 3.1 D) may implicate that IL-33 acts through ILC2s to negatively regulate anti-pneumococcal defense. In order to test this hypothesis, ILC2 numbers and proportions in the lungs of infected WT and *Il33*$^{-/-}$ mice were analyzed by flow cytometry. In detail, mice were infected with *S. pneumoniae* for 18 hours and the whole lung was enzymatically digested to single cells by collagenase and DNAse. Unexpectedly, no significant differences in absolute numbers or in frequencies were detected (Fig. 3.3 B, C).
To gain insights into the transcriptional patterns of ILC2s, a bulk RNA sequencing was performed on CD45^{+} Lin^{-} CD90^{+} CD127^{+} ST2^{+} FACS-sorted lung cells. A downregulation of several genes, including *Pi16*, *Ptx3*, *Prg4*, as well as gene sets associated with activation of MYC genes (HALLMARK_MYC_TARGETS) were observed in *Il33*$^{-/-}$ mice compared to WT mice during *S. pneumoniae* infection (Fig. 3.3 D, E). Upregulation of HALLMARK_OXIDATIVE_PHOSPHORYLATION in WT ILC2s implies an induction of oxidative phosphorylation pathways by IL-33 activation. Together, the present findings confirm an effect of IL-33 on ILC2s but most likely do not explain the detrimental effect of IL-33 signaling on pneumococcal pneumonia.

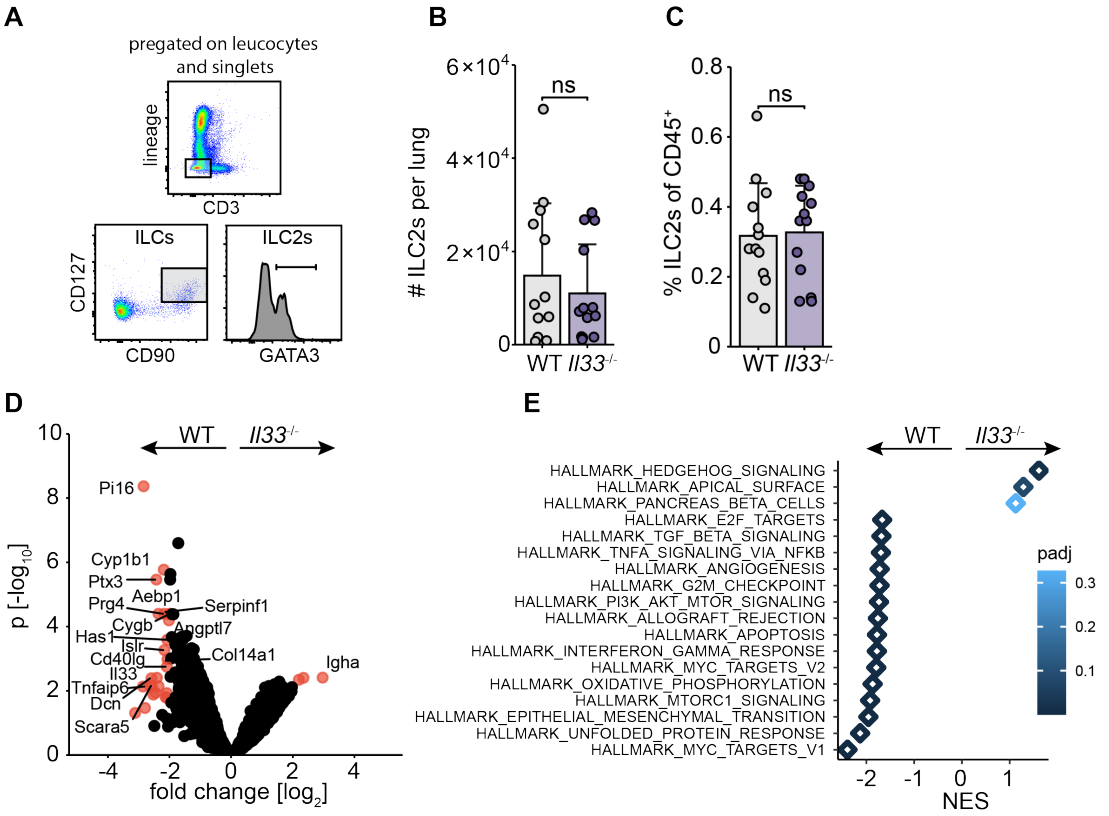

Figure 3.3: Lack of IL-33 influences gene expression but not abundance of ILC2s in lungs of *S. pneumoniae*-infected mice. (A) Gating strategy to quantify ILC2s by flow cytometry. (B) Numbers and (C) frequencies of pulmonary $CD45^+$ Lin^- $CD90^+$ $CD127^+$ $GATA3^+$ ILC2s from lungs of mice 18 h.p.i.. (D, E) Lungs were digested at 12 h.p.i. and FACS-sorted for ILC2s. RNA was isolated and quantified using bulk RNA-sequencing. (D) Volcano plot showing differentially expressed genes (DEGs) in ILC2s of WT and *Il33*$^{-/-}$ animals. Significant DEGs with a $\log_2$ fold change threshold of ± 2 and an adjusted p-value < 0.05 are indicated in red. (E) Gene set enrichment analysis (GSEA) analysis utilizing HALLMARK collection, normalized enrichment score (NES) and adjusted p-value (padj) are indicated. (B, C) Bars represent mean + SD. Wilcoxon rank-sum test, ns = $p > 0.05$

3.1.3 Depletion of ILC2s and abrogation of IL-4/IL-13 signaling does not affect antibacterial defense against *S. pneumoniae*

In order to investigate the functional role of ILC2s in the regulation of antibacterial defense against *S. pneumoniae* in the lungs, *Nmur1*$^{iCre\text{-}eGFP}$*Id2*$^{fl/fl}$ mice (ILC2^{cKO}) specifically lacking ILC2s were utilized[332,342]. *Nmur1* is a gene specifically expressed in ILC2s[343] and *Id2* is an essential transcriptional regulator for the development and maintenance of lymphoid cells[344]. This cell-specific depletion model utilizes a Cre/loxP system, specifically depleting ILC2s which is the only known cell type co-expressing *Nmur1* and *Id2*. ILC2^{cKO} mice and littermates were infected with *S. pneumoniae* and bacterial burden was assessed 48 hours later. Differences in bacterial loads were not detected, neither in BALF nor in blood (Fig. 3.4 A, B). This result demonstrates that ILC2s are not involved in the IL-33-dependent regulation of pneumococcal defense.

IL-33 is known to induce the production of type 2 inflammation-associated cytokines such as IL-4 and IL-13 in a variety of immune cells[51]. To test if these cytokines exert regulatory effects on the early defense against *S. pneumoniae*, *Il4ra*$^{-/-}$ mice were analyzed. IL-4Rα is a crucial functional subunit of the receptor for IL-4 and IL-13[345]. Similar to ILC2^{cKO} mice, control of *S. pneumoniae* infection also remained unaffected in *Il4ra*$^{-/-}$ animals and bacterial burden did not differ compared to controls after 48 hours of infection (Fig. 3.4 D, E). In line with the lack of effect on bacterial burden, clinical parameters such as temperature (Fig 3.4 C, F) and relative weight loss (data not shown) were not affected by the absence of either ILC2s or IL-4Rα.

Hence, these findings demonstrate that, unlike IL-33 and ST2, regulatory effects on the early antibacterial defense against *S. pneumoniae* during pneumonia are not dependent on ILC2s or the type 2 inflammation-associated cytokines IL-4 and IL-13.

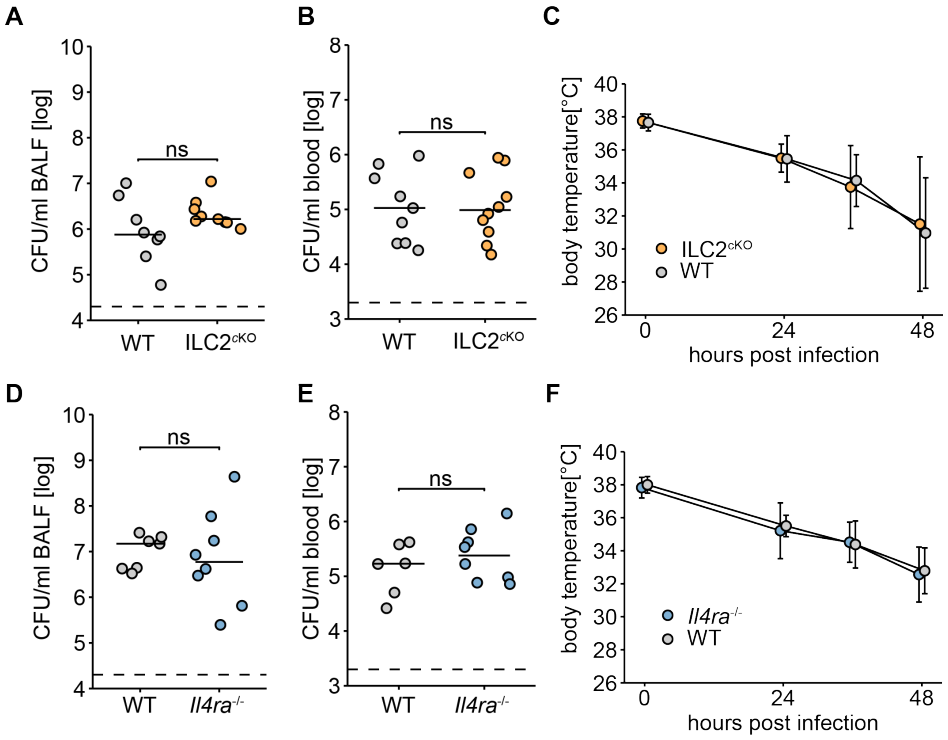

Figure 3.4: ILC2s as well as IL-4 and IL-13 signaling do not regulate early antibacterial defense during pneumococcal pneumonia. (A, B, C) *Nmur1*$^{\text{iCre-eGFP}}$*Id2*$^{\text{fl/fl}}$mice (ILC2$^{\text{cKO}}$) and controls were infected and bacterial loads in BALF and blood were assessed 48 hours post infection or temperature was measured every 12 h. (D, E, F) *Il4ra*$^{-/-}$ and control mice were infected and bacterial loads in BALF and blood were assessed at 48 hours post infection or temperature was measured every 12 h. (A, B, D, E) Lines represent median and dashed line the lower detection limit. Wilcoxon rank-sum test, ns = $p > 0.05$. (C, F) Data are shown as mean ± SD.

3.2 Protective effect of IL-33 deficiency in pneumococcal infection is mediated by enhanced IL-22 production

3.2.1 Inflammatory response of infected WT and *Il33*$^{-/-}$ animals

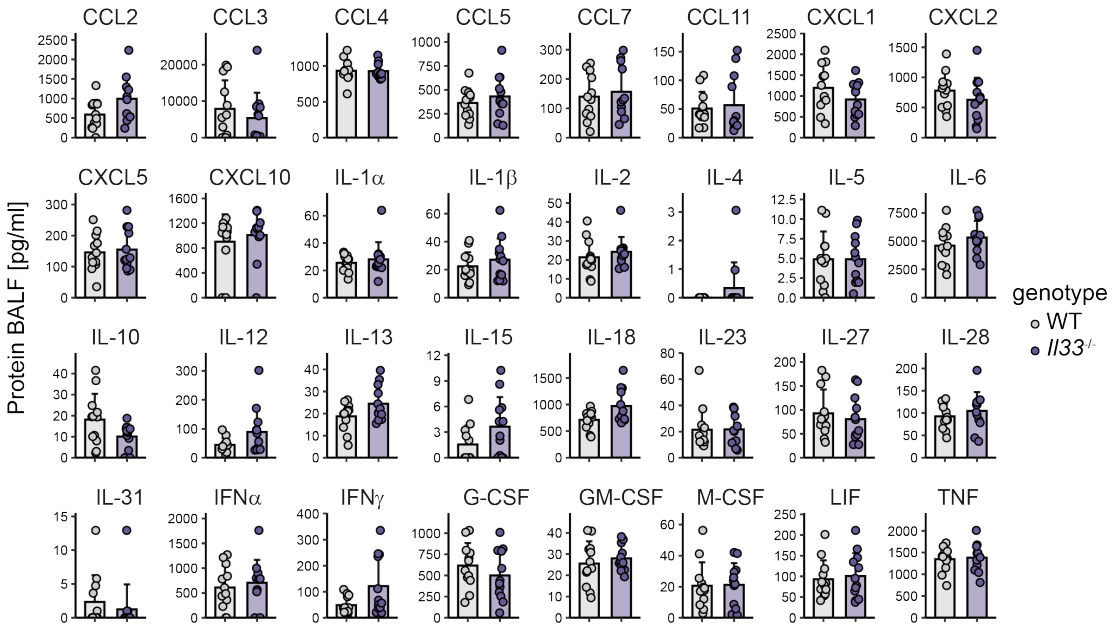

Figure 3.5: IL-33 deficiency does not influence levels of several inflammatory cytokines and chemokines in the lung. WT and *Il33*$^{-/-}$ mice were infected with *S. pneumoniae*. After 18 hours, cytokine and chemokine levels in BALF were quantified by multiplex ELISA. Data are shown as individual data points, bars represent mean + SD.

In the previous section, it was demonstrated that the impaired bacterial defense observed in mice capable of producing IL-33 is not linked to the typical type 2 immune response driven by IL-4 and IL-13 signaling. To obtain further insight into the mechanism underlying the different resistance of WT and IL-33-deficient mice, a multiplex ELISA with BALF samples from WT and *Il33*$^{-/-}$ mice infected for 18 hours was conducted. Notably, no apparent impact of IL-33 deficiency on the levels of chemokines and various cytokines in BALF was observable (Fig. 3.5). Typical cytokines downstream of IL-33 activation including IL-4, IL-5, IL-13, but

also less common mediators described in literature such as CXCL1, CXCL2, CCL20 and TNF[346] were not affected by abrogation of IL-33 signaling.

3.2.2 Lack of IL-33 leads to an increase in IL-22 production, resulting in enhanced protection against pneumococcal infection

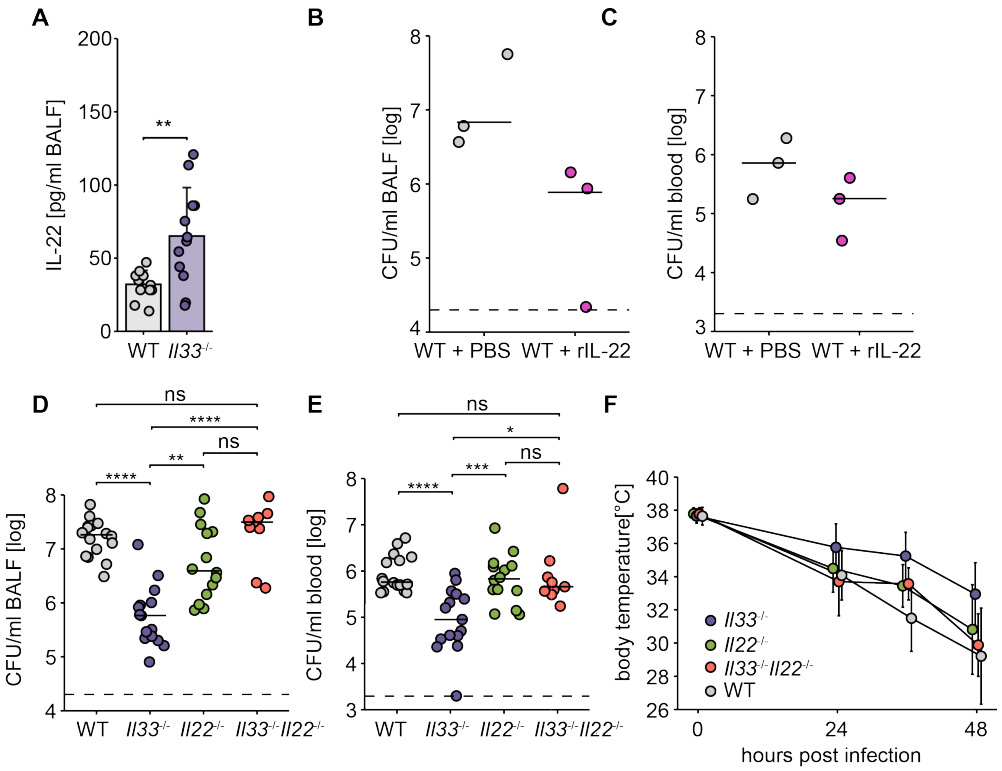

Figure 3.6: Protective effect of IL-33 deficiency in pneumococcal infection is driven by enhanced production of IL-22. (A) Mice were infected with *S. pneumoniae*, sacrificed after 18 hours and levels of IL-22 in BALF were measured by ELISA. Wilcoxon rank-sum test; ** = $p < 0.01$. (B, C) WT mice were treated with 1 µg rIL-22 or PBS as control and infected with *S. pneumoniae*. CFU were measured 48 h.p.i. in (B) BALF and (C) blood. (D, E, F) WT, *Il33*$^{-/-}$, *Il22*$^{-/-}$ and *Il33*$^{-/-}$*Il22*$^{-/-}$ mice were infected for 48 hours and bacterial loads were determined in (D) BALF and (E) blood. (F) Temperature was measured. data are shown as mean ± SD. (A) Bars represent mean + SD, Wilcoxon rank-sum test. (B, C, D, E) lines represent median and dashed line the lower detection limit, Kruskal-Wallis test followed by Dunn's posthoc test, ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

IL-22 plays a crucial role in the immune defense against bacterial infections at mucosal barriers, particularly in the lungs[170,347]. Protein measurements of BALF samples revealed that IL-22 production was significantly higher in *Il33*$^{-/-}$ mice compared to WT controls upon infection with *S. pneumoniae* (Fig. 3.6 A). To understand whether increased levels of IL-22, as seen in *Il33*$^{-/-}$ animals, enhance resistance of mice to pneumococcal pneumonia, WT mice were first treated with rIL-22. 1 µg was each administered intranasally and intraperitoneally to elicit local and potentially also systemic effects, respectively. Bacterial burden was decreased in BALF and blood of rIL-22-treated mice (Fig. 3.6 B, C) indicating that IL-22 confers a protective effect in context of the described model of pneumococcal pneumonia. To directly examine if IL-22 is involved in the protective effect of IL-33 deficiency, an *Il33*$^{-/-}$*Il22*$^{-/-}$ double knockout mouse was generated and bacterial loads after infection were compared to *Il33*$^{-/-}$, *Il22*$^{-/-}$, and WT animals. Interestingly, the bacterial loads in the lungs and blood of *Il33*$^{-/-}$*Il22*$^{-/-}$ mice were largely similar to those in WT mice and significantly higher than in *Il33*$^{-/-}$ mice (Fig. 3.6 D, E). Body temperature of *Il33*$^{-/-}$*Il22*$^{-/-}$, *Il22*$^{-/-}$ and WT mice were comparable, while *Il33*$^{-/-}$ show by trend less hypothermia during the course of infection (Fig. 3.6 F). These findings lead to the conclusion that increased production of IL-22 is responsible for the enhanced resistance of IL-33-deficient mice to pneumococcal lung infection.

3.2.3 IL-22 is differentially produced by ILCs

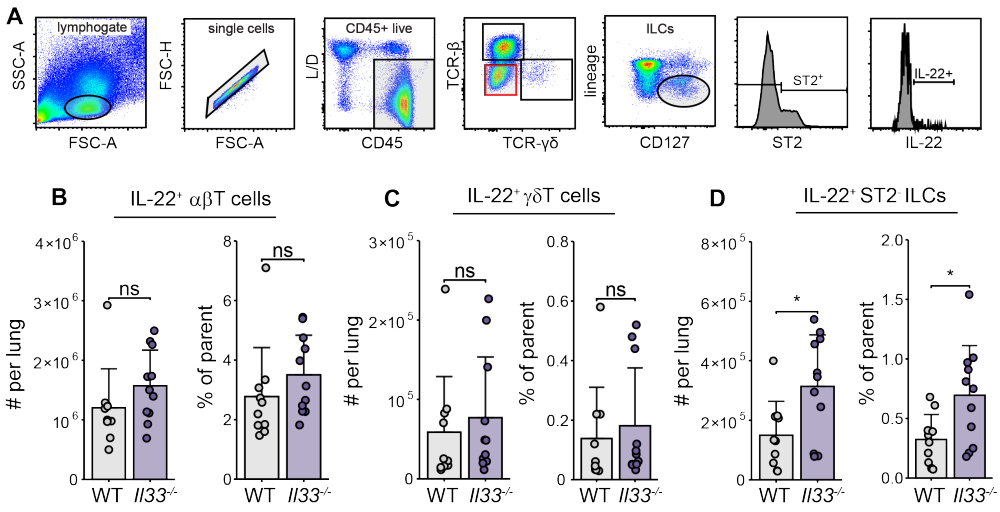

Figure 3.7: IL-22 is differentially produced by ILCs. (A) Representative gating strategy for analyzing IL-22$^+$ lymphoid cells by flow cytometry. (B, C, D) Frequencies and absolute numbers of IL-22-producing lymphoid cells derived from lungs of mice 18 hours after infection. Data are shown as individual data points, bars represent mean + SD. Wilcoxon rank-sum test, * = $p < 0.05$, ns = $p > 0.05$.

To reveal the source of IL-22 during infection with *S. pneumoniae* and how IL-22-producing cells were influenced by IL-33, pulmonary cells of infected WT and *Il33*$^{-/-}$ animals were analyzed by flow cytometry. After 18 hours of infection, lungs were digested to single cells and stained. Cells were then gated for lymphocytes, single cells, live CD45$^+$, TCRβ or TCRγδ. Double-negative cells were further gated on lineage$^-$ (CD3$^-$ CD19$^-$ Ly6G$^-$ FceRIa$^-$ CD5$^-$ NK1.1$^-$) and CD127$^+$ for ILCs. (Fig. 3.7 A). Significantly higher proportions and numbers of IL-22-producing ILCs were observed in the lungs of *Il33*$^{-/-}$ mice (Fig. 3.7 D). Further characterization of these cells revealed that they were ST2$^-$, thus likely representing ILC3s. However, no discernible differences in IL-22 production were observed in αβ- and γδT cells, which have also been described to be potential producers of IL-22 (Fig. 3.7 B, C). Thus, IL-22 production of pulmonary ILCs, most likely ILC3s, is affected by IL-33 during *S. pneumoniae* infection.

3.3 Protective effect of IL-33 deficiency in pneumococcal infection depends on microbiota alterations

3.3.1 The resistance of *Il33*$^{-/-}$ mice to *S. pneumoniae* varies between animals of different vivaria

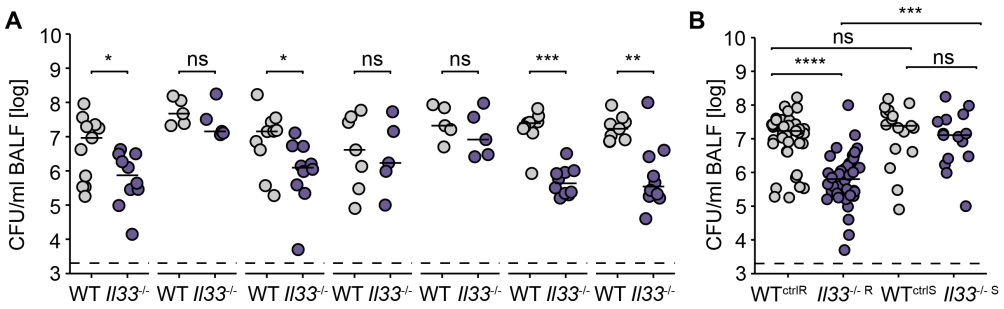

Figure 3.8: *Il33*$^{-/-}$ mice from different vivaria vary in their susceptibility to *S. pneumoniae* infection. (A) WT and *Il33*$^{-/-}$ mice from several animal vivaria were infected, sacrificed after 48 hours and bacterial loads in BALF were assessed. Data are shown as individual data points. Lines represent median and dashed line the lower detection limit. Wilcoxon rank-sum test. (B) Data from (A) were aggregated by phenotype (R = 'resistant', S = 'susceptible', ctrl = intra-vivaria control). Lines represent median and dashed line the lower detection limit. Kruskal-Wallis test followed by Dunn's posthoc test, ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

Due to organizational reasons, the process of breeding *Il33*$^{-/-}$ and WT control animals required multiple relocations from one vivarium to another within various institutional breeding facilities located in Berlin. Surprisingly, during the investigation of *S. pneumoniae* infection, the resistance of *Il33*$^{-/-}$ mice to *S. pneumoniae* varied depending on the facility the mice were bred. *Il33*$^{-/-}$ animals derived from vivaria with lower hygienic standards, characterized by e.g., conventional caging and unrestricted access for scientists rather than being limited only to responsible animal caretakers, tended to exhibit increased resistance to pneumococcal infection when compared to WT animals from the same vivaria. Conversely, *Il33*$^{-/-}$ mice originating from vivaria with higher hygienic restrictions tended to be more susceptible to the infection, behaving similarly to WT animals (Fig. 3.8 A). Of note, the susceptibility of WT mice to pneumococcal infection did not appear to be sig-

nificantly affected by the distinct housing environments, as demonstrated in Fig. 3.8 B. Based on these findings, it can be hypothesized that variations in microbial composition among different facilities lead to alterations in the gut microbiota of primarily *Il33*$^{-/-}$ mice, thereby influencing their defense against *S. pneumoniae* infection. WT and *Il33*$^{-/-}$ genotypes were pooled and referred to as 'resistant' and 'susceptible' phenotype (Categorization is based on aggregated data in Fig. 3.8 B). Although the hygienic status of the vivaria appeared to correlate with the phenotype of *Il33*$^{-/-}$ animals during *S. pneumoniae* infection, the underlying mechanism remains uncertain.

3.3.2 Microbiota changes modulate susceptibility to pneumococcal infection

In recent years, the role of microbiota in modulating immune responses and influencing susceptibility to infection has gained significant recognition in the scientific community[278,316,318,348–351]. To investigate whether differences in microbiota composition were responsible for the variable resistance observed in *Il33*$^{-/-}$ mice during *S. pneumoniae* infection, a comprehensive analysis of fecal samples obtained from WT and *Il33*$^{-/-}$ mice housed in different vivaria was conducted.
Initially, alpha diversities of microbiota samples from four groups (*Il33*$^{-/-R}$ = 'resistant', *Il33*$^{-/-S}$ = 'susceptible' and respective control groups WT^{ctrlR} and WT^{ctrlS}) were assessed and found to be comparable (Fig. 3.9 A).

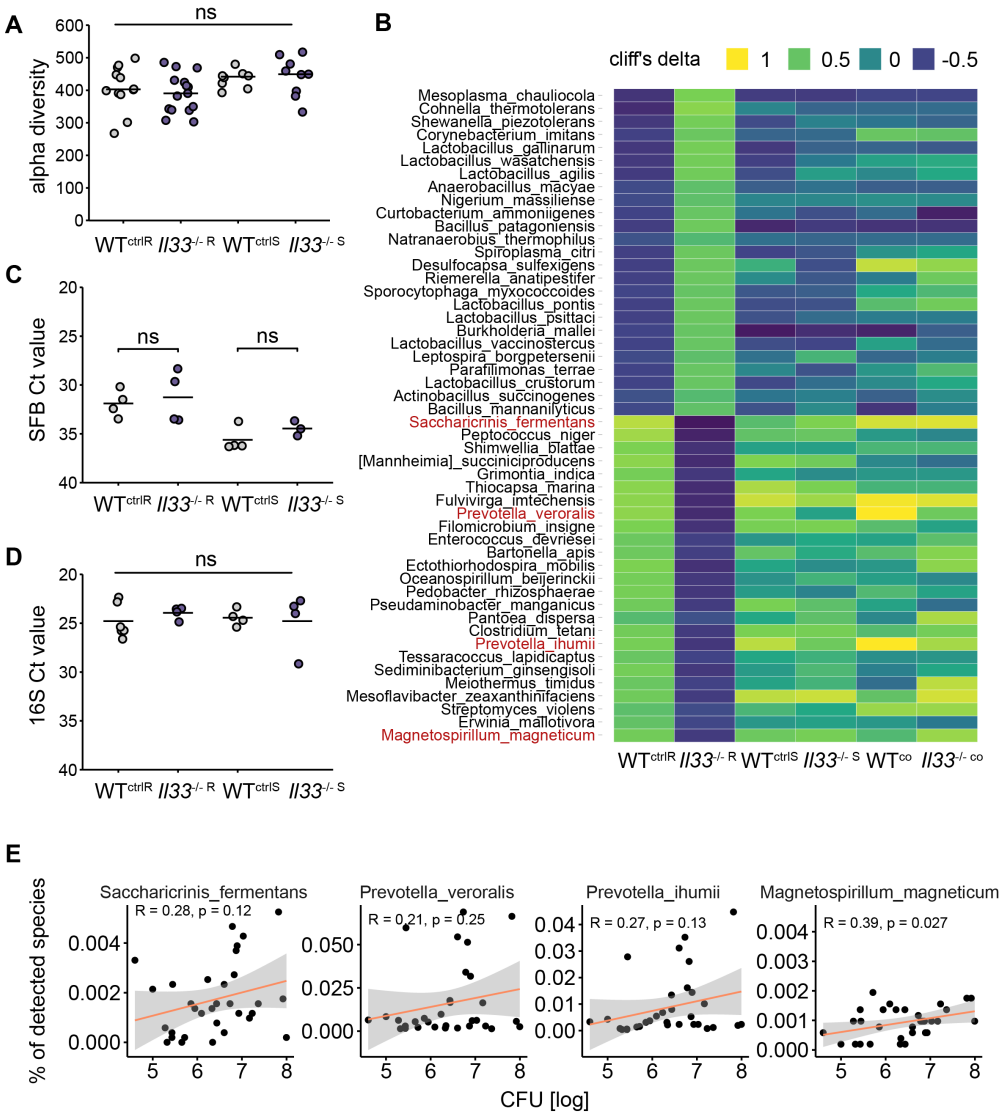

Figure 3.9: Bacterial microbiota of 'resistant' *Il33*$^{-/-}$ mice differs from 'susceptible' *Il33*$^{-/-}$, WT and co-housed animals. (A) Alpha diversity of microbiota samples from *Il33*$^{-/-}$ animals with 'resistant' (*Il33*$^{-/-R}$) and 'susceptible' (*Il33*$^{-/-S}$) phenotype and corresponding controls (WT^{ctrlR} and WT^{ctrlS}) is shown. (B) Heatmap depicting effect sizes of bacterial species derived from fecal samples of WT^{ctrlR}, *Il33*$^{-/-R}$, WT^{ctrlS}, *Il33*$^{-/-S}$ and co-housed animals (WT^{co}, *Il33*$^{-/-co}$). Cliff's delta was applied to visualize differences in effect sizes compared to *Il33*$^{-/-R}$. Species showing correlation with bacterial burden in lungs of mice infected with *S. pneumoniae* are depicted in red. (C, D) SFB and 16S rDNA was quantified in fecal samples from WT^{ctrlR}, *Il33*$^{-/-R}$, WT^{ctrlS} and *Il33*$^{-/-S}$ mice by qPCR. (A, C, D) Lines represent mean. Kruskal-Wallis test followed by Dunn's posthoc test, ns = $p > 0.05$ (E) Frequencies of depicted microbiota species and bacterial burden upon *S. pneumoniae* infection were plotted. Linear regression and confidence intervals were calculated and visualized in red and gray, respectively. Pearson correlation coefficient R and p-value was calculated.

However, further taxonomic profiling revealed differences in the composition of the microbiota of 'resistant' *Il33*$^{-/-}$ mice as compared to samples obtained from 'susceptible' *Il33*$^{-/-}$ mice and both WT controls. A statistical method calculating the effect size (Cliff's delta) of each species was applied to rank the most abundant species in each group. More than 60 bacterial species were differentially detected (Cliff's delta ± 0.5). Notably, the microbiota of 'resistant' *Il33*$^{-/-}$ mice harbored higher relative abundance of various *Lactobacillus spp.*, while showing lower abundance of *Saccharicrinis fermentans* and *Peptococcus niger*, in comparison to both WT groups and 'susceptible' *Il33*$^{-/-}$ mice (Fig. 3.9 B). Furthermore, bacterial composition of the microbiota was measured in feces of co-housed animals and effect sizes compared to *Il33*$^{-/-R}$ were calculated. As anticipated, the differences observed between WT^{ctrlR} and *Il33*$^{-/-R}$ were lost and the bacterial composition equalized between co-housed WT and *Il33*$^{-/-}$ animals (Fig. 3.9 B). Additionally, the 'resistant' *Il33*$^{-/-}$ mice displayed variations in their gut virome, archaeal, and eukaryotic communities in comparison to the other experimental groups (Fig. 3.10).

SFB were described to positively influence immune cells to produce IL-22 [321]. Therefore, relative abundance of this species was investigated by qPCR. However, no differences between WT and *Il33*$^{-/-}$ animals were observed, although the relative abundance of SFB was higher by trend in 'resistant' *Il33*$^{-/-}$ mice as compared to 'susceptible' *Il33*$^{-/-}$ mice (Fig. 3.9 C). To control for differences in the amount of input DNA used for the assay, total 16s DNA was measured and did not differ between groups (Fig. 3.9 D).

In order to identify candidate members of the gut microbiota responsible for influencing the animal's susceptibility to pneumococcal infection, bacterial loads after *S. pneumoniae* infection in individual animals were correlated with relative abundance of specific gut commensals. Correlations were then matched to species either differentially present or absent in 'resistant' *Il33*$^{-/-}$ mice visualized in Fig. 3.9 B. Species present in 'resistant' *Il33*$^{-/-}$ mice would be expected to be involved in boosting IL-22 production, while species absent in *Il33*$^{-/-}$ mice should suppress it. Interestingly, only species positively correlating with CFUs and absent in 'resistant' *Il33*$^{-/-}$mice were detected, namely *Saccharicrinis fermentans*, *Prevotella veroralis*, *Prevotella ihumii* and *Magnetospirillum magneticum* and marked in red showing correlations of 0.28, 0.21, 0.27 and 0.39, respectively (Fig. 3.9 E).

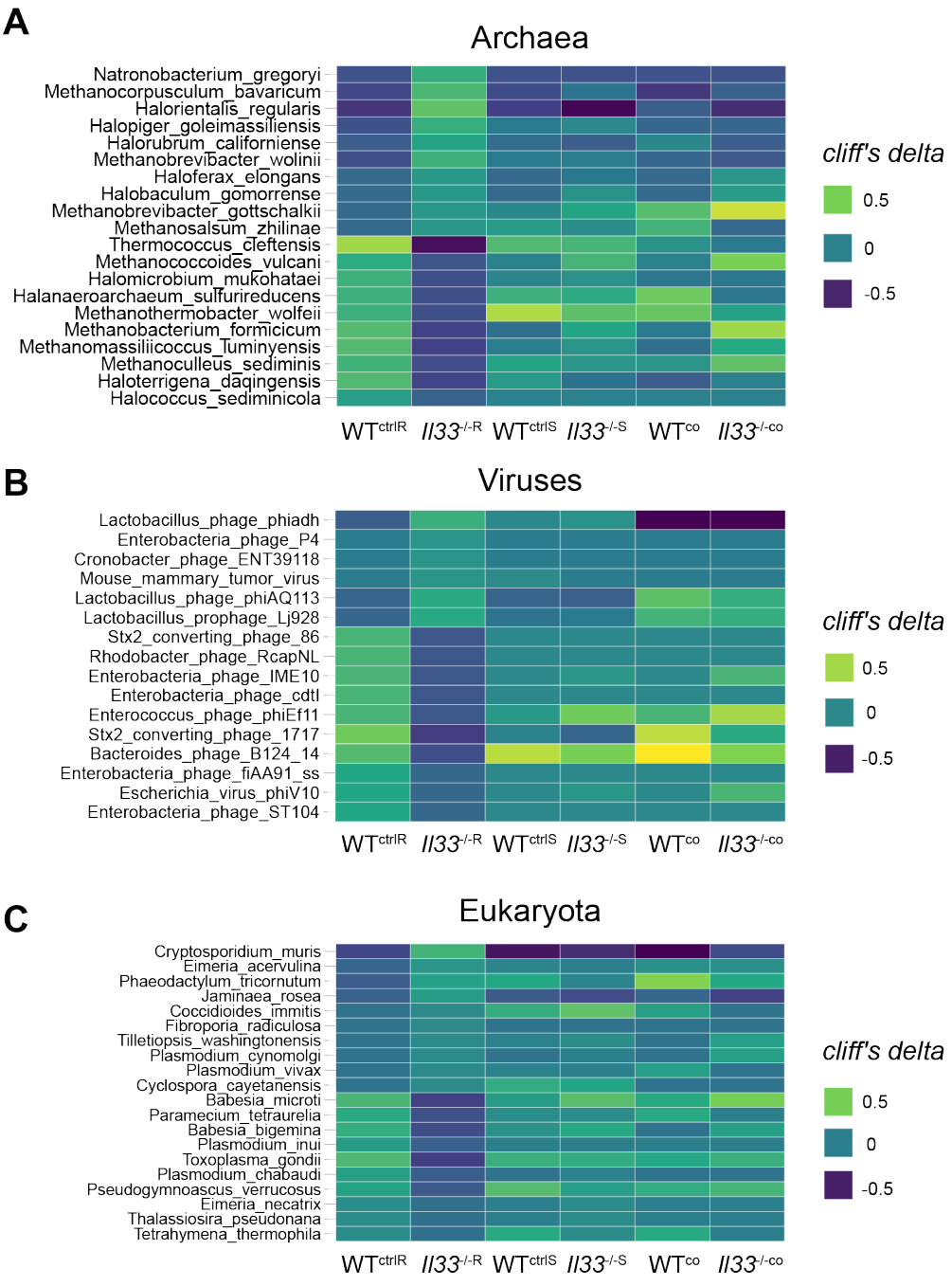

Figure 3.10: Non-bacterial microbiota of 'resistant' *Il33*$^{-/-}$ mice differs from 'susceptible' *Il33*$^{-/-}$, WT and co-housed animals. Fecal material was collected and shotgun sequenced. Effect sizes (Cliff's delta) were calculated to quantify differences of 'resistant', 'susceptible' and co-housed animals compared to *Il33*$^{-/-R}$. Species depicted represent the most abundant in *Il33*$^{-/-R}$ (upper half) and WT^{ctrlR} (lower half). Dataset was filtered for (A) Archaea, (B) Viruses and (C) Eukaryota.

To test if the microbiota differences were causally involved in the differential resistance of WT and *Il33*$^{-/-}$ animals, mice originating from a vivarium breeding *Il33*$^{-/-}$ mice with 'resistant' phenotype were treated with an antibiotic cocktail (imipenem, metronidazole, vancomycin, ciprofloxacin and ampicillin) for 8 weeks to systemically deplete their microbiota. As shown in Fig. 3.11 A and B, the difference in bacterial burden between WT and *Il33*$^{-/-}$ mice after infection with *S. pneumoniae* disappeared following microbiota depletion. Similarly, co-housing of WT and *Il33*$^{-/-}$ mice for four weeks equalized the resistance of WT and *Il33*$^{-/-}$ mice to *S. pneumoniae*, as indicated by similar bacterial loads in their lungs (Fig 3.11 D, E). These data suggest that the microbiota differences observed between WT and 'resistant' *Il33*$^{-/-}$ mice were responsible for their differential ability to control *S. pneumoniae* infection.

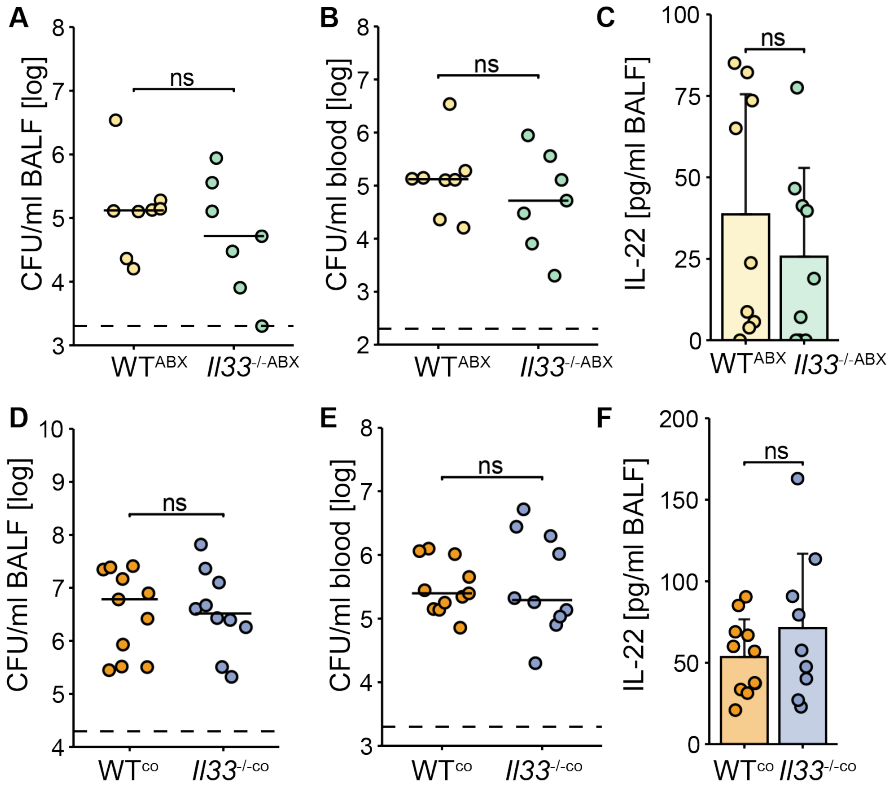

Figure 3.11: The influence of IL-33 on antibacterial defense depends on its modulatory effect on the microbiota. (A, B, C) WT and *Il33*$^{-/-}$ mice were microbiota-depleted by oral treatment with antibiotics (ABX), subsequently infected with 1x10^6 CFU/mouse and sacrificed after 48 h. Bacterial loads in (A) BALF and (B) blood were measured and IL-22 levels in (C) BALF were assessed. (D, E, F) WT and *Il33*$^{-/-}$ mice were co-housed for 4 weeks and infected for 48 h. Bacterial loads in (D) BALF and (E) blood were measured and IL-22 levels in (F) BALF were assessed. Data are shown as individual data points, lines represent median, dashed line the lower detection limit and bars represent mean + SD. Wilcoxon rank-sum test, ns = $p > 0.05$.

To further characterize the mechanism leading to the loss of the 'resistant' phenotype, IL-22 levels were measured in BALF samples derived from antibiotic treated and co-housed mice (Fig. 3.11 C, F). In both groups the difference in IL-22 vanished to comparable levels. Considering that IL-22 is associated with increased bacterial defense (see 3.2.2), these results show a direct correlation between microbiota, IL-22 levels and the resistance to pneumococcal pneumonia.

3.3.3 Resistance of IL-33-deficient mice depends on the gut microbiota

The aforementioned results clearly associate microbiota composition and bacterial defense in *Il33*$^{-/-}$ mice. To definitely demonstrate that gut microbiota differences are responsible for the differential defense against *S. pneumoniae*, fecal material of WT and *Il33*$^{-/-}$ mice of two different vivaria (vivarium A and B) was first collected. Then, WT and *Il33*$^{-/-}$ mice were microbiota-depleted by antibiotic treatment and the collected fecal material was transplanted by oral gavage. After 5 days of reconstitution time, animals were infected with 5x10^6 CFU/mouse and bacterial load was assessed in BALF. While the phenotype of WT animals was not influenced by the transfer of microbiota of WT mice derived from two different vivaria, resistance to *S. pneumoniae* in *Il33*$^{-/-}$ animals was affected by type of *Il33*$^{-/-}$ gut microbiota (Fig. 3.12). These results further confirm that the resistance of *I33*$^{-/-}$ animals to pneumococcal infection depends on the gut microbiota.

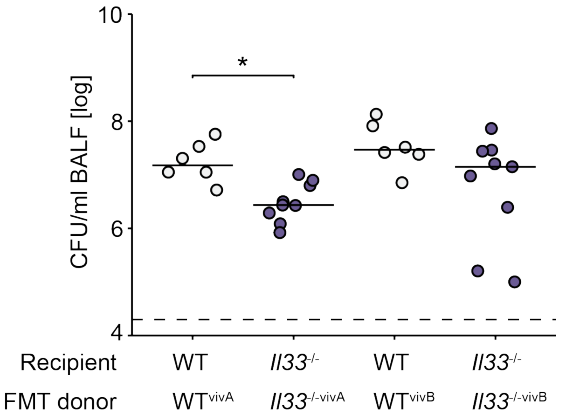

Figure 3.12: Fecal microbiota transplantation rescues resistant phenotype in *Il33*$^{-/-}$ mice. Fecal samples were collected from two different vivaria (A and B) and snap frozen at -80 °C. Then, microbiota in WT and *Il33*$^{-/-}$ mice was depleted by antibiotic treatment and collected fecal microbiota was transplanted. WT mice received WT microbiota and *Il33*$^{-/-}$ mice received *Il33*$^{-/-}$ microbiota. After a reconstitution time of 5 days, mice were infected with 5x10^6 CFU/mouse of *S. pneumoniae* and bacterial load was assessed 48 hours later. Data are shown as individual data points, lines represent median, dashed line the lower detection limit. Kruskal-Wallis test followed by Dunn's posthoc test, * = $p < 0.05$.

3.4 SNPs in *IL33* and *IL1RL1* are associated with pneumococcal pneumonia in humans

It is yet unknown if the detrimental effect of IL-33 on the capacity to defend against *S. pneumoniae* observed in mice is also existent in humans. To approach this question, an analysis of single nucleotide polymorphismss (SNPs) previously associated with type 2 inflammation and altered sST2 or IL-33 levels[352–357] was performed in 238 pneumococcal pneumonia patients and 238 age- and sex-matched controls. It was found that SNP alleles in the gene *IL1RL1* previously linked to lower levels of the decoy receptor sST2[352,353], and, by trend a SNP allele associated with higher IL-33 levels[354], were observed to be more frequent in pneumonia patients as compared to controls (Fig. 3.13 and Table 3.1). These findings are consistent with previous observations made in the murine model of IL-33 deficiency described in this work and demonstrate that potential changes in IL-33 and sST2 levels correlate with the capacity to defend against *S. pneumoniae*.

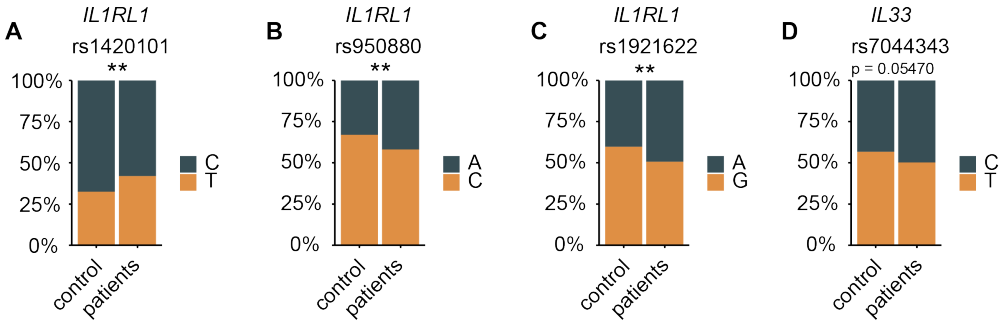

Figure 3.13: SNPs in *IL33* and *IL1RL1* are associated with pneumococcal pneumonia. (A – D) Frequencies of SNP alleles of the genes for *IL33* and *IL1RL1* were assessed in 238 patients with community-acquired pneumococcal pneumonia patients and 238 age- and sex- matched controls. Allele frequencies are visualized. Fisher's exact test; ** = $p < 0.01$, * = $p < 0.05$, ns = $p > 0.05$.

Table 3.1: List of screened SNPs correlating with pneumococcal pneumonia. Literature was screened for SNPs influencing IL-33 or ST2 levels. sST2 is a splice variant of ST2 acting as soluble decoy receptor for IL-33.

SNP	Gene	Effect on expression	SNP allele frequency	p value	Potential implication
rs1420101	*IL1RL1*	T-allele: decreased expression of sST2	CAPNETZ > control	0.0039 (**)	less sST2 in CAP patients
rs950880	*IL1RL1*	A-allele: decreased expression of sST2	CAPNETZ > control	0.006 (**)	less sST2 in CAP patients
rs1921622	*IL1RL1*	A-allele: decreased expression of sST2	CAPNETZ > control	0.0088 (**)	less sST2 in CAP patients
rs7044343	*IL33*	C-allele: increased expression of IL-33	CAPNETZ > control	0.055 (n.s.)	more IL-33 in CAP patients

Discussion

In this thesis, a reciprocal mechanism between the immune system and the microbiota that influences susceptibility to pulmonary bacterial infection is described. This mechanism is dependent on both genetic and environmental factors and has an impact on the efficiency of the immune system to defend against *S. pneumoniae*. Specifically, it was shown that IL-33 regulates the immune defense against *S. pneumoniae* through IL-22 production in pulmonary ILC3s. The regulation of defense relies on the ability of IL-33 to influence the composition of the gut microbiota, and this effect appears to be dependent on the environment, specifically on the housing conditions of the animals. A multitude of microbial species were observed to differ between WT and *Il33*$^{-/-}$ mice. One or several of these microbes only present in some vivaria may be suppressed by IL-33, but can thrive in *Il33*$^{-/-}$ animals. It is plausible to argue that metabolites produced by these microbes trigger IL-22 production in pulmonary ILC3s, leading to improved antibacterial defense in *Il33*$^{-/-}$ mice. Alternatively it may also be possible that a microbe inhibiting IL-22 production in ILC3s is more abundant in WT mice, leading to impaired antibacterial defense in WT as compared to *Il33*$^{-/-}$ animals. In contrast to *Il33*$^{-/-}$ mice, animals deficient in ILC2s or IL-4Rα did not show enhanced resistance to *S. pneumoniae* infection, indicating that the IL-33-dependent regulation of antibacterial defense is independent of these factors.

4.1 Microbiota and anti-pneumococcal defense

4.1.1 How IL-33 shapes the intestinal microbiota

To ascertain if the intestinal microbiota influences the capacity of *Il33*$^{-/-}$ mice to defend against *S. pneumoniae* infection, three experiments were conducted. Microbiota depletion and co-housing leveled out differences in antibacterial defense between WT and *Il33*$^{-/-}$ mice. Additionally, an FMT experiment in WT and *Il33*$^{-/-}$ mice was conducted to demonstrate that differences in the gut microbiota were responsible for the differential resistance of *Il33*$^{-/-}$ animals to *S. pneumoniae* infection. These results could be explained by a mechanism in which the presence of IL-33 suppresses proliferation of a still unknown gut commensal responsible for enhanced IL-22 production. In *Il33*$^{-/-}$ animals, this unknown microorganism might expand in the intestinal tract, thereby contributing to an increased IL-22-dependent antibacterial defense in the lung. This unknown microbe might only be present in some vivaria but not in all, thus explaining the variable phenotype of *Il33*$^{-/-}$ mice. However, more detailed conclusions about the composition of a protective microbiota and which particular microorganisms are responsible for the observed effect are still elusive. To investigate compositional differences between 'resistant' and 'susceptible' *Il33*$^{-/-}$ mice as well as of WT controls, and to identify potential candidate microbes responsible for the differences in infection susceptibility, fecal microbiota samples derived from mice of several vivaria were analyzed. More than 60 bacterial species were found to differ between *Il33*$^{-/-}$ and WT mice derived from 'resistant' vivaria, including *Lactobacillus spp.* and *Prevotella spp.* (Fig. 3.9 B). This high number of potential candidates, made it impossible to experimentally test which of these species were responsible for the differences in resistance against *S. pneumoniae* infection. To further narrow down the list of potential microbial candidates, a correlation analysis between relative abundance of such species and the burden of *S. pneumoniae* in the lung for each individual mouse was conducted. The rational for this approach was the assumption that if a microbiota species influences antibacterial defense, a correlation to abundance of *S. pneumoniae* in the lungs should be observable. Unexpectedly, no species was found correlate negatively

with pulmonary bacterial burden. However, a slightly positive correlation between CFU of *S. pneumoniae* and abundance of *Saccharicrinis fermentans*, *Prevotella veroralis*, *Prevotella ihumii* and *Magnetospirillum magneticum* was observed (Fig. 3.9 E marked in red). It appears unlikely that *S. fermentans* and *M. magneticum* play a relevant role in the modulation of immune responses in mice. They were isolated from marine mud[358] and sediment layers of low oxygen waters[359], respectively, and were never described in the context of murine microbiota. Nevertheless, *Prevotella spp.* are typical commensals in the respiratory and intestinal tract[360,361] and have been described to modulate immune functions, such as affecting susceptibility to arthritis[362] and gut dendritic cell activation[363]. However, it remains unlikely that these species are solely responsible for the observed phenotype, because their correlation with bacterial burden of $R = 0.21$ and $R = 0.27$ is relatively low. It is important to note that this correlation analysis specifically considers individual microorganisms as potentially responsible for the observed effect. Nonetheless, it is also plausible that collective influences of multiple gut commensals collaboratively influence defense against *S. pneumoniae*. More complex data analysis methods could shed light on multifactoral relationships between several parts of the microbiota and the susceptibility to *S. pneumoniae* infection. This effect may also be influenced by viral or fungal components of the microbiota.
The central hypothesis is that a species typically suppressed by IL-33, but present in *Il33*$^{-/-}$ mice, positively influences antibacterial defense in the lungs. Following this line of argumentation, it was unexpected to discover that there were no species enriched in 'resistant' *Il33*$^{-/-}$ mice exhibiting a negative correlation with the bacterial burden in the lung. The inability to detect such negatively correlating microbes may derive from an inadequate representation of the gut microbiota, achieved solely through the sampling of frozen feces samples rather than utilizing the entire intestine for microbial DNA extraction. In 2016, Malik *et al.* showed that IL-33 modulates microbiota composition, suppressing *Akkermansia muciniphila* and SFB[364]. Contrary to these findings, feces sequencing conducted in the course of this work did not show higher abundance of *Akkermansia muciniphila* and SFB in *Il33*$^{-/-}$ mice. One way to explain this discrepancy could be that in the study of Malik *et al.* the whole intestine of *Il33*$^{-/-}$ animals was digested and subsequently screened for microbiota, instead of just sampling feces.

As another approach to narrow down the list of potential candidate bacterial taxa

responsible for the augmented pulmonary defense, 'resistant' *Il33*$^{-/-}$ and WT mice could be infected after treatment with either polymyxin or vancomycin to approximate if gram-negative or gram-positive bacteria drive the phenotype, respectively. In subsequent experiments, single transplants with the most promising candidates of either gram-positive or -negative bacteria will be required to provide understanding of their modulatory effect on the microbiota. However, numerous questions pose challenges to the feasibility of transplanting individual species into a microbiota-depleted mouse. Is it possible to culture the desired microbe *in vitro* prior to transplantation? Does it survive the process of transplantation? Does it reach the gut and is it able to successfully colonize the intestinal tract? Is the observed effect dependent on synergistic effects of a combination of microorganisms only present in a complex microbiota? One can also not be sure, if antibiotic treatment completely eradicates the bacterial microbiota and if remaining microorganisms skew the outcome of infections. To avoid that, GF mice could be utilized in future experiments. This on the other hand, requires transfer of *Il33*$^{-/-}$ embryos into commercially avaliable GF recipient females.

Another recent study by Ignacio *et al.* elegantly demonstrated that the release of IL-33 by intestinal epithelial cells requires microbial colonization of the gut. This microbiota-dependent release of IL-33 resulted in an elevated eosinophil turnover rate and prevented villi blunting[365]. Conversely, GF mice exhibited reduced IL-33 release and impaired eosinophil functions. While this study does not offer a direct explanation for the mechanism behind increased immune defense in the lungs of *Il33*$^{-/-}$ animals, it does present a plausible scenario in which microbiota and IL-33 are interdependent.

As depicted in Fig. 3.10, differences in the microbiota composition of archaea, viruses and eukaryota have also been detected between WT and *Il33*$^{-/-}$ mice. Effect sizes were, however, much lower compared to the effect sizes calculated based on the bacterial microbiota. It could be that a fungus is responsible for the effect, since IL-33 has been shown to drive immune responses against fungi, including *Canida albicans*[366] and *Pneumocystis murina*[367]. Consequently, lack of IL-33 signaling could provide a niche for specific fungi to bloom. The outgrowth of such a fungus could in turn be positively influencing IL-22-dependent antibacterial responses to render an *Il33*$^{-/-}$ mouse more resistant to pneumococcal infections. This would agree with

data published by Bacher *et al.*, where intestinal *C. albicans* induced pulmonary T_{h17} responses against other fungal species in a cross-reactive manner[368]. However, this proposed mechanism is unlikely since a loss of phenotype was demonstrated upon antibiotic treatment. Therefore, it seems much more likely that differences in bacterial commensals are responsible for the differential resistance of WT and *Il33*$^{-/-}$ animals from different vivaria to *S. pneumoniae*.

Another important question is whether the composition of microbiota is only dependent on genetic background or on the maternal microbiota passed along at birth. Ubeda *et al.* found that TLR-deficient mice show different microbiota colonization patterns compared to WT mice[369]. Furthermore, they observed that when born from the same mother, littermates deficient in TLR and WT controls lost the characteristic microbiota patterns observed in isolated colonies. In this study, the maternal microbiota was the main factor influencing the microbiota composition of newborn mice. A similar approach was recently used to investigate microbiota differences between WT and *Il33*$^{-/-}$ animals[364]. The authors found that despite being born by the same mother, WT and *Il33*$^{-/-}$ littermates showed differences in microbiota composition. Future experiments should use littermates derived from heterozygous parents to show that the same initial maternal microbiota diverges into WT- and *Il33*$^{-/-}$-specific colonization patterns influencing resistance to *S. pneumoniae* infection.

4.1.2 The link between gut and lung

As reported in this work, ILCs lacking the IL-33 receptor ST2 (most likely ILC3s) are the main producers of IL-22 in *Il33*$^{-/-}$ mice during pneumococcal pneumonia. However, the factors leading to variations in IL-22 production between WT and *Il33*$^{-/-}$ mice remain unclear. Typical upstream regulators including IL-23 and IL-1β have not been found to be differentially produced; therefore they are unlikely to be responsible for the increased IL-22 production in 'resistant' *Il33*$^{-/-}$ mice. Nevertheless, it cannot be excluded that differences in the abundance of respective receptors or in intracellular signaling lead to increased IL-22 levels.

Another receptor enhancing IL-22 production in ILC3s is AhR, which can be activated e.g., by L-tryptophan-derived ligands[370,371]. Several microorganisms such as *Peptostreptococcus russellii*[372] and *Lactobacillus spp.*[373,374] have been described to produce AhR ligands and to subsequently induce IL-22 production in immune cells. Intriguingly, microbiota composition of 'resistant' *Il33*$^{-/-}$ mice revealed higher proportions of several *Lactobacillus spp.* compared to WT controls. In the list of 25 most abundant species in 'resistant' *Il33*$^{-/-}$ mice compared to WT controls, six species of *Lactobacillus* were detected (Fig. 3.9 B). A potential mechanism could lie in the increased production of AhR ligands by *Lactobacillus spp.* in the gut of *Il33*$^{-/-}$ mice, which spread via circulation and activate ILC3s in the lung to produce IL-22, leading to improved bacterial defense upon *S. pneumoniae* infection. However, the extent to which this impacts the production of IL-22 and if it causes the observed phenotype remains unknown. To test this hypothesis, specific microbiota transplant experiments with *Lactobacillus* species and measurements of AhR ligand concentrations would need to be conducted.

SFB have been described to colonize the small intestine of mammals, stimulating T_{h17} cells in the lamina propria to produce IL-22[321,375]. By treating mice with vancomycin and thereby depleting SFB, McAleer *et al.* showed that intestinal frequencies of T_{h17} cells and IL-22 levels were significantly decreased. Furthermore, they showed that this effect was dependent on SFB colonization and IL-22-induced Reg3γ expression[322]. Roy *et al.* demonstrated in 2021 that intestinal IL-22 production by T_{h22} cells was fostered by SFB[376]. Considering the reported influence of IL-33 on the gut microbiota, and the resulting effect on antibacterial defense, SFB represented an interesting candidate explaining the observed phenotype. However, though higher Ct-values were detected in fecal samples derived from a vivarium harboring animals with a 'resistant' phenotype, increased levels of SFB in *Il33*$^{-/-}$ mice compared to WT mice from the same vivarium were not detected (Fig. 3.9 C). On the other hand, SFB are known to be tightly associated to the intestinal epithelium[377,378] and a complete representation of all gut colonizing microorganisms may require extracting microbiota directly from the intestine. Therefore, an increased abundance of SFB in the microbiota of 'resistant' *Il33*$^{-/-}$ mice as compared to 'susceptible' *Il33*$^{-/-}$ and WT mice cannot be completely ruled out.

Another study published by Gray *et al.* in 2017 reported that newborn mice need

commensal microbiota to allow trafficking of IL-22-producing ILC3s to the lung[323]. This effect protected neonatal mice against pneumococcal pneumonia and was mediated by intestinal microbiota-stimulated DCs. Upon stimulation by these intestinal DCs, IL-22-producing ILC3s upregulated the lung-homing receptor CCR4 and relocated to the lung. Overall, these findings are potentially in line with the results reported in this work, which demonstrated that ILC3-derived IL-22 is an important factor in the defense against *S. pneumoniae*. Nonetheless, If this mechanism is dependent on intestinal DCs and CCR4 is not known. Further experiments should focus on either the blockage of CCR4 signaling on ILC3s or specific depletion of intestinal DCs to reveal their involvement in the improved bacterial defense in *Il33*$^{-/-}$.

In 2017, Geva-Zatorsky *et al.* presented a systematic study in which they monocolonized GF mice with 53 bacterial species derived from the human gut to test their immunomodulatory effects[379]. Besides other parameters, they looked at the potential of each single species to induce intestinal IL-22 production by ILCs in steady state. Using these data, the shotgun sequencing dataset generated from feces samples derived from several vivaria (Fig. 3.9 B) was screened for these candidates. *Acinetobacter baumannii*, a Gram-negative opportunistic pathogen described by Geva-Zatorsky *et al.* to be strongly inducing intestinal ILC-derived IL-22, was found to be enriched in 'resistant' *Il33*$^{-/-}$ and to be lacking in 'susceptible' *Il33*$^{-/-}$ animals and respective WT controls. It is conceivable that proliferation of *A. baumannii* is suppressed by IL-33 signaling, leading to increased abundance in *Il33*$^{-/-}$ mice, subsequently inducing enhanced IL-22 production by intestinal ILCs. Speculatively, *A. baumannii* is not present in the environment of 'susceptible' *Il33*$^{-/-}$ mice. Notably, there are limitations extrapolating these observations on the model described in this work. IL-22 production has been identified in the lungs during pneumococcal pneumonia. The local homeostatic IL-22 production in the gut, as documented by Geva-Zatorsky et al., does not appear to account for this phenomenon. In contrast, alternative studies, such as that by Gray *et al.*, have proposed the translocation of ILC3s from the gut to the lungs, providing a potential explanation for the observed IL-22 production during pneumococcal pneumonia[323]. Further experiments in which *A. baumannii* is transplanted into 'susceptible' *Il33*$^{-/-}$ mice should help determine if this effect is causative to the observations described in this work.

4.2 Role of IL-22 and type 3 immunity in antibacterial defense

The results in this work clearly describe a causality between IL-33 deficiency, enhanced IL-22 production and increased resistance to *S. pneumoniae*. Both, gain of function experiments, in which WT mice were treated with rIL-22, and loss of function experiments, in which *Il33*$^{-/-}$ *Il22*$^{-/-}$ mice were compared to single knockouts and WT controls, demonstrate the protective role of enhanced IL-22 production in pneumococcal pneumonia. However, the exact mechanism of how enhanced IL-22 levels induce defense against *S. pneumoniae* is not fully understood. One study conducted by Trevejo-Nunez *et al.* in 2016, demonstrated that IL-22/IL-22R signaling plays a crucial role in regulating host defenses against *S. pneumoniae* lung infections, with rapid induction of IL-22 production in the lungs during infection. Administration of rIL-22 reduced bacterial burden in lung and liver by enhancing C3 opsonization[163]. When comparing these results to those generated in this work, it must be pointed out that C3 levels were not influenced by IL-33[330]. Other studies have shown that IL-22 induces the production of various AMPs to promote antibacterial defense[122], such as lipocalin, β-defensins, S100A8/A9 and RegIIIγ [189,192,322,380]. Contrary to the effect of IL-22 described in literature, differences in production of several AMPs in the lungs between WT and *Il33*$^{-/-}$ animals were not detectable on transcriptome level (unpublished scRNAseq data) and on protein level[330]. Though no differences of the AMPs described above were detected in the lung, it is possible that they exist in the gut. McAleer *et al.* found RegIIIγ regulated by IL-22 in the gut, which led to a local control of microbiota[322]. Subsequent investigations should focus on the influence of IL-22 on gut AMP production to further elucidate if it shapes the gut microbiota in *Il33*$^{-/-}$ mice. Furthermore, IL-22 influences antibacterial defense by supporting mucosal repair mechanisms and maintaining barrier integrity in epithelial tissues[192,381,382]. However, no influence of IL-33 on lung barrier integrity as assessed by an assay involving murine serum albumin (MSA) was detected in *S. pneumoniae*-infected mice[330].

Most important producers of IL-22 described in literature are ILC3s, γδT cells and T_{h17} cells[383], but NK cells and NKT cells have also been described as sources of IL-

22[106,384]. Furthermore, in a murine *S. pneumoniae* infection model, Trevejo-Nunez *et al.* found γδT cells as main producer of IL-22, with an additional contribution of ILCs. Contrary to these findings, Van Maele *et al.* identified ILC3s as main source of *Il22* transcripts in a murine pneumococcal pneumonia model by FACS-sorting infected lung cells and subjecting them to qPCR[162,163]. Intriguingly, these data are in accordance with the findings reported in this work, showing that increased IL-22 production in ILCs could be detected (Fig. 3.7). Technical difficulties prevented further characterization of the ILC population, which is consistent with Van Maele *et al.* and Trevejo-Nunez *et al.* being unable to co-visualize the signal of transcription factors RORγt together with IL-22 in flow cytometric measurements. Nevertheless, it is reasonable to propose that ILC3s are the key producers, given that ILC1s and ILC2s are usually not considered relevant sources of IL-22. To overcome the technical difficulties of further characterizing the main IL-22 producer in the lung that is influenced by IL-33 signaling, an IL-22^{BFP} reporter mouse[385] was crossed with an *Il1rl1*$^{-/-}$ mouse. However, the IL-22^{BFP} signal strength in the lungs was very low and could not be detected during pneumococcal infection, which prevented further characterization of the IL-22-producing cell type (data not shown). To confirm ILC3s as a main IL-33-dependently regulated source of IL-22 in this model, future experiments should focus on either visualizing RORγt together with intracellular IL-22, measuring *Il22* transcripts in lysates of different FACS-sorted lymphoid cell populations derived from infected WT and *Il33*$^{-/-}$ mice or use another, brighter reporter mouse model.

IL-22BP can influence IL-22 activity by sequestering and subsequently inactivating it[181]. Studies have shown that *Il22bp*$^{-/-}$ mice display altered IL-22-dependent metabolic programs, resulting in increased antibacterial defense against *S. pneumoniae*[386] and antiviral defense in an influenza coupled superinfection model[387]. These effects were dependent on the complement factor C3 and the glycoprotein hemopexin, which, however, both have not been found to be influenced by IL-33 in the model of pneumococcal pneumonia[330].

Often, IL-22 is co-expressed with IL-17A, because expression of both cytokines is regulated by the same transcription factor RORγt[122,388]. IL-17A has the potential to influence bacterial infections by stimulating e.g., epithelial cells to produce mediators responsible for recruiting PMNs to the site of infection[119,383,389]. Unpublished

data generated in this work showed increased IL-17A levels in the lungs of infected *Il33*$^{-/-}$ mice. Although IL-17A is often co-expressed with IL-22, the main source of IL-33-dependently regulated IL-17A in the lungs of pneumococci-infected mice appear to be γδT cells but not ILC3s (own data not shown). It remains unclear if the elevated IL-17A levels of *S. pneumoniae*-infected *Il33*$^{-/-}$ mice contribute to the improved bacterial defense in these animals. In order to test whether increased IL-17A levels protect against pneumococcal pneumonia, infections with double knockout animals (*Il33*$^{-/-}$*Il17*$^{-/-}$) and respective controls could be conducted in the future.

In conclusion, the elevated levels of IL-22 in *Il33*$^{-/-}$ mice can be attributed to ILCs, most likely ILC3s. Although IL-22 levels were directly causative for improved bacterial defense in *Il33*$^{-/-}$ mice, the mechanisms underlying this effect were beyond the scope of this thesis. If and how increased IL-17A levels in *Il33*$^{-/-}$ mice contribute to the observed increased antibacterial defense remains elusive.

4.3 IL-33 in human pneumococcal pneumonia

To investigate if the findings regarding the role of IL-33 in pneumococcal pneumonia are also relevant in humans, several SNPs in the genes *IL33* and *IL1RL1* were examined. The study included 238 patients hospitalized with pneumococcal pneumonia (provided by the CAPNETZ foundation[335]) and 238 sex- and age-matched controls. Interestingly, SNPs in *IL33* and *IL1RL1* were more frequently found in pneumonia patients as compared to the controls. These SNPs were previously linked to changes in plasma levels of sST2 and IL-33[352–357] (Fig. 3.13). SNPs linked to decreased levels of the decoy receptor sST2 or increased levels of IL-33 were observed more frequently in pneumonia patients. These data thus suggest that also in humans, IL-33 signaling potentially increases susceptibility to pneumococcal pneumonia, and are thereby in line with the murine data presented here. Although these SNPs were previously described to influence the respective plasma levels of their gene product, no direct correlation between IL-33 or sST2 levels and pneumococcal pneumonia susceptibility or severity can be demonstrated. Future studies should assess the

levels of circulating IL-33 and sST2 in patients with pneumococcal pneumonia and analyze if they are associated with e.g., disease severity.

The 'resistant' phenotype of *Il33*$^{-/-}$ animals tended to be associated with vivaria of lower hygienic standards, which likely better reflect the 'real world situation' outside of unphysiologically clean laboratories. This is potentially in agreement with the observation of an improved antibacterial defense in 'wildling' mice, which better resemble humans in their immune responses than SPF mice bred under highly hygienic conditions. These 'wildling' mice are generated by embryo transfer of genetically distinct laboratory mice into wild mice harboring a 'natural world' microbiota, preserving tractable genetics but mimicking microbiota of wild mice[326,327]. Human individuals also live in the 'natural world' and harbor a microbiota rather comparable to 'wildling' mice than to SPF mice[390] and future experiments may benefit from utilizing 'wildling' mice instead of SPF mice. This approach aims to capture a more authentic representation of the human immune response and may help provide deeper insights into relevant human pathomechanisms. This shift in experimental design could contribute to a more comprehensive understanding of the biological processes under investigation, ultimately improving the translatability of research findings to human contexts.

4.4 Other potential mechanisms of how IL-33 influences antibacterial defense

Other mechanisms may also contribute to the IL-33-dependent effect on antibacterial defense. Two studies have been published recently, describing effects of IL-33 on *S. pneumoniae* infection and providing alternative explanations. In a comprehensive scRNAseq study, as reported by Cohen *et al.*, it was found that IL-33 produced by AEC2s activated resident basophils, subsequently priming AMs to adopt an M2-like phenotype[251]. A similar mechanism was described in a study by Saluzzo *et al.*, where the initial alveolar expansion in newborn mice triggered the release of IL-33 from AEC2s. This, in turn, lead to the production of IL-13 by ILC2s, reprogramming macrophages to an M2-like phenotype. The effect went hand in hand with a delayed

response to infection with *S. pneumoniae*[165]. Both mechanisms could also have explained the detrimental effect of IL-33 on pneumococcal pneumonia discussed in this work. However, the reported mechanisms are not consistent with several observations made in this study. First, IL-13 levels did not differ in BALF samples of infected WT and *Il33*$^{-/-}$ mice (Fig. 3.5). Second, scRNAseq data generated from infected WT and *Il33*$^{-/-}$ mice did not show differences in M2-like imprinting of macrophages (data not shown). Third, mice lacking IL-4Rα, a critical subunit for the IL-4 and IL-13 receptors, did not show enhanced resistance to *S. pneumoniae* in comparison to WT animals (Fig. 3.4).
However, a growing body of evidence suggests that another receptor, namely IL-13Rα2, binds IL-13 independently of IL-4Rα [391]. Initially observed in monocytes and subsequently validated in mouse models, the signaling of IL-13 through IL-13Rα2, coupled with AP1-induced TGF-β production, has been documented[392,393]. According to these studies, IL-13 can trigger production of TGF-β by upregulating IL-13Rα2, often induced by TNF. Recent research has revealed that this receptor can activate a diverse array of signals (such as WNT/β-Catenin, MAPK/ERK, AKT/PKB, Src/FAK, PIP3K) within normal or pathological contexts[394,395]. Since IL-13 levels did not differ between WT and *Il33*$^{-/-}$ mice, one could hypothesize that IL-13Rα2 is more abundantly expressed in WT mice, leading to yet unknown mechanistical effects. Further experiments characterizing the influence of an alternative IL-13 signaling pathway should shed light on the mechanism underlying the improved bacterial defense in *Il33*$^{-/-}$ animals.

ILC2s harbor high abundances of ST2 on their surface and are a compartment of lymphoid cells acting in an innate manner, which made them an interesting candidate mediator regulating IL-33-dependent anti-pneumococcal defense. Intriguingly, no differences in numbers or frequencies of ILC2s in the lungs of infected WT and *Il33*$^{-/-}$ mice were detectable (Fig. 3.3). Furthermore, transcriptomic profiles were generated by bulk-sequencing of FACS-sorted ILC2s. Here, utilizing gene set enrichment analysis, clear evidence of transcriptional influence of IL-33 on ILC2s was found. Gene sets associated with MYC transcription factor-associated proliferation were increased in WT mice. The upregulation of oxidative phosphorylation in WT ILC2s suggests an increased demand for ATP upon IL-33 stimulation. When observing differences on single gene level, several genes were regulated in ILC2s by IL-33. The most differentially upregulated gene in WT mice was *Pi16*, which

may be a technical artifact, since it has been described to be a marker of shear stress[396]. Other genes upregulated in WT ILC2s were *Ptx3*, a soluble PRR involved in opsonization[397] and *Prg4*, an anti-inflammatory TLR2/4 antagonist[398]. However, no gene found to be regulated by IL-33 in ILC2s seemed to explain the increased bacterial defense against *S. pneumoniae* in *Il33*$^{-/-}$ mice.
To finally prove that ILC2s do not play a role in this infection model, *Nmur1*$^{\text{iCre-eGFP}}$ *Id2*$^{\text{fl/fl}}$ (ILC2$^{\text{cKO}}$) mice, as a model of ILC2 deficiency was utilized and compared to control mice. *Nmur1* is specifically expressed in ILC2s and *Id2* is a common transcription factor necessary for the development of all ILC types[344,399]. Only ILC2s which express both proteins at the same time are depleted in ILC2$^{\text{cKO}}$ mice. After infecting ILC2$^{\text{cKO}}$ and littermate controls with *S. pneumoniae*, no differences in antibacterial defense were detected. With these results, ILC2s can be confidently excluded as relevant cell type involved in the IL-33-dependent regulation of anti-pneumococcal defense.

In a study conducted by Alvarez *et al.* in 2019, a mechanism in *Cryptococcus neoformans* infection was proposed, where IL-33 and IL-1 have opposing effects on priming ST2^{+} T_{regs}, leading to either a regulatory or inflammatory phenotype, respectively[125]. Projecting this mechanism on the discussed model of pneumococcal pneumonia, *Il33*$^{-/-}$ mice could harbor a higher abundance of pulmonary inflammatory T_{regs} which lead to an earlier induction of antibacterial programs. Similarly, Faustino *et al.* reported a mechanism where ST2^{+} T_{regs} were responsible for suppressing IL-17A producing γδT cells, subsequently delaying PMN influx to the site of infection[400]. When comparing the results presented in this work to those described above, it must be pointed out that *Il33*$^{-/-}$ mice did indeed show increased IL-17A levels in the lungs upon infection (data not shown). However, the increased IL-17A production did not result in an increased PMN influx, as described by Faustino *et al.* (Fig. 1.7 C, D). Furthermore, no differences in T_{reg} abundance and in their ST2 levels were detected by flow cytometry and transcriptomic analyses (data not shown). Thus, no evidence for a role of T_{regs} in the IL-33-dependent defense against *S. pneumoniae* was obtained.

Detecting the source of IL-33 is crucial for a comprehensive understanding of its impact on pneumococcal pneumonia. Expression of IL-33 has been attributed to nonhemapoietic cells, such as fibroblasts, epithelial and endothelial cells[195,196].

However, some studies have described active secretion of IL-33 from CD45$^+$ immune cells, such as macrophages and mast cells[236,401–405]. These observations stand in contrast to own RNA-sequencing data and publicly avaliable data[406] mainly locating *Il33* transcripts in nonhematopoietic cells. Furthermore, Cayrol and Girard discuss unpublished data demonstrating that the commonly used antibody to detect IL-33 in CD45$^+$ immune cells binds unspecific epitopes, inducing fluorescence in cells derived from IL-33-deficient mice[408]. Together, the present findings and publicly available data suggest a nonhematopoietic origin of IL-33.

In the context of acute pneumococcal lung infection, IL-33 appears to have a detrimental effect. However, it is reasonable to assume that the role of IL-33 might be beneficial in the later phases of the infection. Notably, IL-33 has been shown to influence infections by influenza through its interaction with ILC2s and the subsequent release of AREG, which contributes to tissue repair, as demonstrated in a study by Monticelli *et al.*[111]. Although *Areg* expression in ILC2s did not differ between WT and *Il33*$^{-/-}$ mice 36 hours after *S. pneumoniae* infection (own scRNAseq data not shown), IL-33-dependently produced AREG could play a beneficial role in the later resolution phase of infection. Future experiments using e.g., an inducible ILC2-specific *Areg* knockout model would be required to test if this assumption is correct. In such an experiment, inactivation of AREG could be induced at later phases of infection to avoid potentially overlapping effects of AREG during the earlier and later phases of infection.

4.5 Synopsis and concluding remarks

In this work, a mechanism is proposed in which environmental in combination with genetic factors modulate the antibacterial defense against *S. pneumoniae*. Specific microbes that might only be present in some but not all vivaria, colonize and expand in *Il33*$^{-/-}$ (but not WT) animals and enhance, possibly through production of specific metabolites, IL-22-dependent defense against *S. pneumoniae* in the lung (Fig. 4.1).

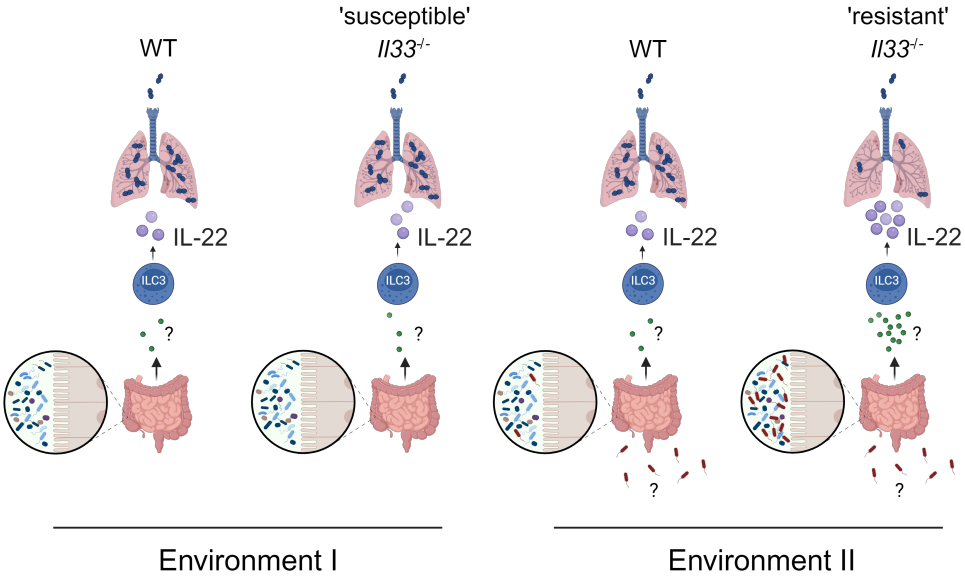

Figure 4.1: Proposed model of microbiota- and IL-33-dependent regulation of the immune defense against *S. pneumoniae*. Depending on the environment (i.e. the vivarium where mice are bred), *Il33*$^{-/-}$ mice differ in their susceptibility to *S. pneumoniae*. In 'resistant' *Il33*$^{-/-}$ mice, ILC3s produce enhanced levels of IL-22, leading to protection against *S. pneumoniae* infection of the lung. This increased IL-22 production in 'resistant' *Il33*$^{-/-}$ animals depends on microbiota alterations, which seem to be mediated by genetic factors (i.e., lack of IL-33) and the environment (most likely the hygenic status of the vivarium in the experimental setting). In the gut of *Il33*$^{-/-}$ mice, but not in WT mice, an unknown microbe—present only in certain vivaria—is able to expand and positively influence IL-22 production by ILC3s, perhaps by producing specific metabolites.

To improve our understanding, future investigations should aim to identify the specific gut colonizing microbe(s) influenced by IL-33 and their role in IL-22 production. Additionally, unraveling the mechanism by which IL-33 affects intestinal microbiota necessitates further research. Moreover, it is crucial to explore the potential effects of IL-22, particularly its influence on the airway epithelium, to better understand the underlying mechanism behind the protective effect in pneumococcal pneumonia. In conclusion, this study sheds light on the complex interplay between the microbiota and the immune system, impacting susceptibility to pulmonary infection by *S. pneumoniae*.

Bibliography

[1] Weiser, J. N., Ferreira, D. M., and Paton, J. C. Streptococcus pneumoniae: transmission, colonization and invasion. *Nature Reviews Microbiology*, 16:355–367, 6 2018. doi: 10.1038/s41579-018-0001-8.

[2] Vos, T., Lim, S. S., Abbafati, C., *et al.* Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, 396:1204–1222, 10 2020. doi: 10.1016/S0140-6736(20)30925-9.

[3] Torres, A., Cilloniz, C., Niederman, M. S., *et al.* Pneumonia. *Nature Reviews Disease Primers*, 7, 12 2021. doi: 10.1038/s41572-021-00259-0.

[4] Brooks, L. R. K. and Mias. Streptococcus pneumoniae's Virulence and Host Immunity: Aging, Diagnostics, and Prevention. *Frontiers in Immunology*, 2018. doi: 10.3389/fimmu.2018.01366.

[5] Ludwig, E., Bonanni, P., Rohde, G., *et al.* The remaining challenges of pneumococcal disease in adults. *European Respiratory Review*, 21:57–65, 2012. doi: 10.1183/09059180.00008911.

[6] Cillóniz, C., Liapikou, A., Martin-Loeches, I., *et al.* Twenty-year trend in mortality among hospitalized patients with pneumococcal community-acquired pneumonia. *PLoS ONE*, 13: 1–16, 2018. doi: 10.1371/journal.pone.0200504.

[7] Adegbola, R. A., DeAntonio, R., Hill, P. C., *et al.* Carriage of Streptococcus pneumoniae and other respiratory bacterial pathogens in low and lower-middle income countries: A systematic review and meta-analysis. *PLoS ONE*, 9, 2014. doi: 10.1371/journal.pone.0103293.

[8] Chao, Y., Marks, L. R., Pettigrew, M. M., and Hakansson, A. P. Streptococcus pneumoniae biofilm formation and dispersion during colonization and disease. *Frontiers in Cellular and Infection Microbiology*, 4:1–16, 2014. doi: 10.3389/fcimb.2014.00194.

[9] McCullers, J., McAuley, J., Browall, S., *et al.* Influenza Enhances Susceptibility to Natural Acquisition of and Disease due to Streptococcus pneumoniae in Ferrets. *The Journal of Infectious Diseases*, 202:1287–1295, 10 2010. doi: 10.1086/656333.

[10] Bogaert, D., Groot, R. D., and Hermans, P. W. Streptococcus pneumoniae colonisation: The key to pneumococcal disease. *Lancet Infectious Diseases*, 4:144–154, 2004. doi: 10.1016/S1473-3099(04)00938-7.

[11] Simon, A. K., Hollander, G. A., and McMichael, A. Evolution of the immune system in humans from infancy to old age. *Proceedings of the Royal Society B: Biological Sciences*, 282:20143085, 12 2015. doi: 10.1098/rspb.2014.3085.

[12] Sugimoto, N., Yamagishi, Y., Hirai, J., *et al.* Invasive pneumococcal disease caused by mucoid serotype 3 Streptococcus pneumoniae: a case report and literature review. *BMC Research Notes*, 10:1–6, 2017. doi: 10.1186/s13104-016-2353-3.

[13] Cherazard, R., Epstein, M., Doan, T.-L., *et al.* Antimicrobial Resistant Streptococcus pneumoniae: Prevalence, Mechanisms, and Clinical Implications. *American Journal of Therapeutics*, 24:e361–e369, 5 2017. doi: 10.1097/MJT.0000000000000551.

[14] Pletz, M. W. and Bahrs, C. [Pneumococcal vaccination]. *Der Internist*, 62:807–815, 8 2021. doi: 10.1007/s00108-021-01100-2.

[15] CDC. CDC - Pneumococcal Vaccination - https://www.cdc.gov/pneumococcal/vaccination.html, 2023.

[16] Geno, K. A., Gilbert, G. L., Song, J. Y., *et al.* Pneumococcal capsules and their types: Past, present, and future. *Clinical Microbiology Reviews*, 28:871–899, 2015. doi: 10.1128/CMR.00024-15.

[17] Ganaie, F., Saad, J. S., McGee, L., *et al.* A New Pneumococcal Capsule Type, 10D, is the 100th Serotype and Has a Large cps Fragment from an Oral Streptococcus. *mBio*, 11, 6 2020. doi: 10.1128/mBio.00937-20.

[18] Hyams, C., Camberlein, E., Cohen, J. M., *et al.* The Streptococcus pneumoniae capsule inhibits complement activity and neutrophil phagocytosis by multiple mechanisms. *Infection and Immunity*, 78:704–715, 2010. doi: 10.1128/IAI.00881-09.

[19] Marriott, H., Mitchell, T., and Dockrell, D. Pneumolysin: A Double-Edged Sword During the Host-Pathogen Interaction. *Current Molecular Medicine*, 8:497–509, 2008. doi: 10.2174/156652408785747924.

[20] Rubins, J. B., Duane, P. G., Clawson, D., *et al.* Toxicity of pneumolysin to pulmonary alveolar epithelial cells. *Infection and Immunity*, 61:1352–1358, 1993. doi: 10.1128/iai.61.4.1352-1358.1993.

[21] Pereira, J. M., Xu, S., Leong, J. M., and Sousa, S. The Yin and Yang of Pneumolysin During Pneumococcal Infection. *Frontiers in Immunology*, 13, 4 2022. doi: 10.3389/fimmu.2022.878244.

[22] Kadioglu, A., Weiser, J. N., Paton, J. C., and Andrew, P. W. The role of Streptococcus pneumoniae virulence factors in host respiratory colonization and disease. *Nature Reviews Microbiology*, 6: 288–301, 2008. doi: 10.1038/nrmicro1871.

[23] Takeuchi, O. and Akira, S. Pattern Recognition Receptors and Inflammation. *Cell*, 140:805–820, 2010. doi: 10.1016/j.cell.2010.01.022.

[24] Leiva-Juárez, M. M., Kolls, J. K., and Evans, S. E. Lung epithelial cells: Therapeutically inducible effectors of antimicrobial defense. *Mucosal Immunology*, 11:21–34, 2018. doi: 10.1038/mi.2017.71.

[25] Opitz, B., Eitel, J., Meixenberger, K., and Suttorp, N. Role of Toll-like receptors, NOD-like receptors and RIG-I-like receptors in endothelial cells and systemic infections. *Thrombosis and Haemostasis*, 102:1103–1109, 2009. doi: 10.1160/TH09-05-0323.

[26] Guilliams, M., Lambrecht, B. N., and Hammad, H. Division of labor between lung dendritic cells and macrophages in the defense against pulmonary infections. *Mucosal Immunology*, 6: 464–473, 2013. doi: 10.1038/mi.2013.14.

[27] Koppe, U., Suttorp, N., and Opitz, B. Recognition of Streptococcus pneumoniae by the innate immune system. *Cellular Microbiology*, 14:460–466, 2012. doi: 10.1111/j.1462-5822.2011.01746.x.

[28] Janeway, C. A. and Medzhitov, R. Innate immune recognition. *Annual review of immunology*, 20:197–216, 2002. doi: 10.1146/annurev.immunol.20.083001.084359.

[29] Kumar, H., Kawai, T., and Akira, S. Pathogen Recognition by the Innate Immune System. *International Reviews of Immunology*, 30:16–34, 1 2011. doi: 10.3109/08830185.2010.529976.

[30] Kawasaki, T. and Kawai, T. Toll-like receptor signaling pathways. *Frontiers in Immunology*, 5: 1–8, 2014. doi: 10.3389/fimmu.2014.00461.

[31] Iwasaki, A., Foxman, E. F., and Molony, R. D. Early local immune defences in the respiratory tract. *Nature Reviews Immunology*, 17:7–20, 1 2017. doi: 10.1038/nri.2016.117.

[32] Fitzgerald, K. A. and Kagan, J. C. Toll-like Receptors and the Control of Immunity. *Cell*, 180: 1044–1066, 2020. doi: 10.1016/j.cell.2020.02.041.

[33] Inohara, N., Koseki, T., del Peso, L., *et al.* Nod1, an Apaf-1-like Activator of Caspase-9 and Nuclear Factor-kB. *Journal of Biological Chemistry*, 274:14560–14567, 5 1999. doi: 10.1074/jbc.274.21.14560.

[34] Bertin, J., Nir, W.-J., Fischer, C. M., *et al.* Human CARD4 Protein Is a Novel CED-4/Apaf-1 Cell Death Family Member That Activates NF-kB. *Journal of Biological Chemistry*, 274:12955–12958, 5 1999. doi: 10.1074/jbc.274.19.12955.

[35] Viala, J., Chaput, C., Boneca, I. G., *et al.* Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. *Nature Immunology*, 5:1166–1174, 11 2004. doi: 10.1038/ni1131.

[36] Gilberts, E. C., Greenstein, A. J., Katsel, P., *et al.* Molecular evidence for two forms of Crohn disease. *Proceedings of the National Academy of Sciences of the United States of America*, 91: 12721–4, 12 1994. doi: 10.1073/pnas.91.26.12721.

[37] Chamaillard, M., Hashimoto, M., Horie, Y., *et al.* An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nature Immunology*, 4:702–707, 7 2003. doi: 10.1038/ni945.

[38] Magalhaes, J. G., Sorbara, M. T., Girardin, S. E., and Philpott, D. J. What is new with Nods? *Current opinion in immunology*, 23:29–34, 2 2011. doi: 10.1016/j.coi.2010.12.003.

[39] Mukherjee, T., Hovingh, E. S., Foerster, E. G., *et al.* NOD1 and NOD2 in inflammation, immunity and disease. *Archives of biochemistry and biophysics*, 670:69–81, 7 2019. doi: 10.1016/j.abb.2018.12.022.

[40] Thorpe, K. L., Abdulla, S., Kaufman, J., *et al.* Phylogeny and structure of the RING3 gene. *Immunogenetics*, 44:391–6, 1996. doi: 10.1007/BF02602785.

[41] Hoffman, H. M., Wright, F. A., Broide, D. H., *et al.* Identification of a locus on chromosome 1q44 for familial cold urticaria. *American journal of human genetics*, 66:1693–8, 5 2000. doi: 10.1086/302874.

[42] Geddes, B. J., Wang, L., Huang, W. J., *et al.* Human CARD12 is a novel CED4/Apaf-1 family member that induces apoptosis. *Biochemical and biophysical research communications*, 284: 77–82, 6 2001. doi: 10.1006/bbrc.2001.4928.

[43] Broz, P. and Dixit, V. M. Inflammasomes: mechanism of assembly, regulation and signalling. *Nature Reviews Immunology*, 16:407–420, 7 2016. doi: 10.1038/nri.2016.58.

[44] Xu, J. and Núñez, G. The NLRP3 inflammasome: activation and regulation. *Trends in biochemical sciences*, 48:331–344, 4 2023. doi: 10.1016/j.tibs.2022.10.002.

[45] Yoneyama, M., Kikuchi, M., Natsukawa, T., *et al.* The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nature Immunology*, 5: 730–737, 7 2004. doi: 10.1038/ni1087.

[46] DeYoung, K. L., Ray, M. E., Su, Y. A., *et al.* Cloning a novel member of the human interferon-inducible gene family associated with control of tumorigenicity in a model of human melanoma. *Oncogene*, 15:453–457, 7 1997. doi: 10.1038/sj.onc.1201206.

[47] Sun, L., Wu, J., Du, F., *et al.* Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science (New York, N.Y.)*, 339:786–91, 2 2013. doi: 10.1126/science.1232458.

[48] Rock, K. L., Latz, E., Ontiveros, F., and Kono, H. The sterile inflammatory response. *Annual review of immunology*, 28:321–42, 2010. doi: 10.1146/annurev-immunol-030409-101311.

[49] Kaczmarek, A., Vandenabeele, P., and Krysko, D. V. Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. *Immunity*, 38:209–23, 2 2013. doi: 10.1016/j.immuni.2013.02.003.

[50] Khakh, B. S., Burnstock, G., Kennedy, C., *et al.* International union of pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. *Pharmacological reviews*, 53:107–18, 3 2001.

[51] Schmitz, J., Owyang, A., Oldham, E., *et al.* IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*, 23:479–90, 11 2005. doi: 10.1016/j.immuni.2005.09.015.

[52] Abbracchio, M. P., Burnstock, G., Boeynaems, J.-M., *et al.* International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacological reviews*, 58:281–341, 9 2006. doi: 10.1124/pr.58.3.3.

[53] Marinho, Y., da Silva, C. M., Santana, P. T., *et al.* MSU Crystals induce sterile IL-1beta secretion via P2X7 receptor activation and HMGB1 release. *Biochimica et biophysica acta. General subjects*, 1864:129461, 1 2020. doi: 10.1016/j.bbagen.2019.129461.

[54] Whitsett, J. A. and Alenghat, T. Respiratory epithelial cells orchestrate pulmonary innate immunity. *Nature Immunology*, 16:27–35, 1 2015. doi: 10.1038/ni.3045.

[55] Bustamante-Marin, X. M. and Ostrowski, L. E. Cilia and Mucociliary Clearance. *Cold Spring Harbor Perspectives in Biology*, 9:a028241, 4 2017. doi: 10.1101/cshperspect.a028241.

[56] Perdiguero, E. G., Klapproth, K., Schulz, C., *et al.* Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature*, 518:547–551, 2 2015. doi: 10.1038/nature13989.

[57] van de Laar, L., Saelens, W., Prijck, S. D., *et al.* Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages. *Immunity*, 44:755–68, 4 2016. doi: 10.1016/j.immuni.2016.02.017.

[58] Kopf, M., Schneider, C., and Nobs, S. P. The development and function of lung-resident macrophages and dendritic cells. *Nature Immunology*, 16:36–44, 1 2015. doi: 10.1038/ni.3052.

[59] Guilliams, M., Kleer, I. D., Henri, S., *et al.* Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *Journal of Experimental Medicine*, 210:1977–1992, 9 2013. doi: 10.1084/jem.20131199.

[60] Hussell, T. and Bell, T. J. Alveolar macrophages: plasticity in a tissue-specific context. *Nature Reviews Immunology*, 14:81–93, 2 2014. doi: 10.1038/nri3600.

[61] Garbi, N. and Lambrecht, B. N. Location, function, and ontogeny of pulmonary macrophages during the steady state. *Pflügers Archiv - European Journal of Physiology*, 469:561–572, 4 2017. doi: 10.1007/s00424-017-1965-3.

[62] Happel, K. I., Dubin, P. J., Zheng, M., *et al.* Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. *The Journal of Experimental Medicine*, 202:761–769, 9 2005. doi: 10.1084/jem.20050193.

[63] Goritzka, M., Makris, S., Kausar, F., *et al.* Alveolar macrophage–derived type I interferons orchestrate innate immunity to RSV through recruitment of antiviral monocytes. *Journal of Experimental Medicine*, 212:699–714, 5 2015. doi: 10.1084/jem.20140825.

[64] Maier, B. B., Hladik, A., Lakovits, K., *et al.* Type I interferon promotes alveolar epithelial type II cell survival during pulmonary Streptococcus pneumoniae infection and sterile lung injury in mice. *European Journal of Immunology*, 46:2175–2186, 9 2016. doi: 10.1002/eji.201546201.

[65] Martinez, F. O. and Gordon, S. The M1 and M2 paradigm of macrophage activation: Time for reassessment. *F1000Prime Reports*, 6:1–13, 2014. doi: 10.12703/P6-13.

[66] Uribe-Querol, E. and Rosales, C. Control of Phagocytosis by Microbial Pathogens. *Frontiers in Immunology*, 8, 10 2017. doi: 10.3389/fimmu.2017.01368.

[67] Szulc-Dabrowska, L., Bossowska-Nowicka, M., Struzik, J., and Toka, F. N. Cathepsins in Bacteria-Macrophage Interaction: Defenders or Victims of Circumstance? *Frontiers in Cellular and Infection Microbiology*, 10, 12 2020. doi: 10.3389/fcimb.2020.601072.

[68] Herb, M. and Schramm, M. Functions of ROS in Macrophages and Antimicrobial Immunity. *Antioxidants*, 10:313, 2 2021. doi: 10.3390/antiox10020313.

[69] Ibrahim-Granet, O., Philippe, B., Boleti, H., *et al.* Phagocytosis and Intracellular Fate of Aspergillus fumigatus Conidia in Alveolar Macrophages. *Infection and Immunity*, 71:891–903, 2 2003. doi: 10.1128/IAI.71.2.891-903.2003.

[70] Arredouani, M., Yang, Z., Ning, Y., *et al.* The Scavenger Receptor MARCO Is Required for Lung Defense against Pneumococcal Pneumonia and Inhaled Particles. *The Journal of Experimental Medicine*, 200:267–272, 7 2004. doi: 10.1084/jem.20040731.

[71] Schneider, C., Nobs, S. P., Heer, A. K., *et al.* Alveolar Macrophages Are Essential for Protection from Respiratory Failure and Associated Morbidity following Influenza Virus Infection. *PLoS Pathogens*, 10:e1004053, 4 2014. doi: 10.1371/journal.ppat.1004053.

[72] Guillot, L., Nathan, N., Tabary, O., *et al.* Alveolar epithelial cells: Master regulators of lung homeostasis. *The International Journal of Biochemistry and Cell Biology*, 45:2568–2573, 11 2013. doi: 10.1016/j.biocel.2013.08.009.

[73] Crapo, J. Cell Number and Cell Characteristics of the Normal Human Lung. *American Review of Respiratory Disease*, 1982.

[74] Crapo, J. Morphometric characteristics of cells in the alveolar region of mammalian lungs. *American Review of Respiratory Disease*, 1983.

[75] Griese, M. Pulmonary surfactant in health and human lung diseases: state of the art. *European Respiratory Journal*, 13:1455–1476, 6 1999. doi: 10.1183/09031936.99.13614779.

[76] Wright, J. R. Immunoregulatory functions of surfactant proteins. *Nature Reviews Immunology*, 5:58–68, 1 2005. doi: 10.1038/nri1528.

[77] Whitsett, J. A., Wert, S. E., and Weaver, T. E. Alveolar Surfactant Homeostasis and the Pathogenesis of Pulmonary Disease. *Annual Review of Medicine*, 61:105–119, 2 2010. doi: 10.1146/annurev.med.60.041807.123500.

[78] Barkauskas, C. E., Cronce, M. J., Rackley, C. R., *et al.* Type 2 alveolar cells are stem cells in adult lung. *Journal of Clinical Investigation*, 123:3025–3036, 7 2013. doi: 10.1172/JCI68782.

[79] Thornton, E. E., Looney, M. R., Bose, O., *et al.* Spatiotemporally separated antigen uptake by alveolar dendritic cells and airway presentation to T cells in the lung. *Journal of Experimental Medicine*, 209:1183–1199, 6 2012. doi: 10.1084/jem.20112667.

[80] Persson, E. K., Uronen-Hansson, H., Semmrich, M., *et al.* IRF4 transcription-factor-dependent CD103(+)CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity*, 38:958–69, 5 2013. doi: 10.1016/j.immuni.2013.03.009.

[81] Lee, J., Cacalano, G., Camerato, T., *et al.* Chemokine binding and activities mediated by the mouse IL-8 receptor. *Journal of immunology (Baltimore, Md. : 1950)*, 155:2158–64, 8 1995.

[82] Craig, A., Mai, J., Cai, S., and Jeyaseelan, S. Neutrophil Recruitment to the Lungs during Bacterial Pneumonia. *Infection and Immunity*, 77:568–575, 2 2009. doi: 10.1128/IAI.00832-08.

[83] Kreisel, D., Nava, R. G., Li, W., *et al.* In vivo two-photon imaging reveals monocyte-dependent neutrophil extravasation during pulmonary inflammation. *Proceedings of the National Academy of Sciences*, 107:18073–18078, 10 2010. doi: 10.1073/pnas.1008737107.

[84] Brinkmann, V., Reichard, U., Goosmann, C., *et al.* Neutrophil Extracellular Traps Kill Bacteria. *Science*, 303:1532–1535, 3 2004. doi: 10.1126/science.1092385.

[85] Kaufmann, S. H. and Dorhoi, A. Molecular Determinants in Phagocyte-Bacteria Interactions. *Immunity*, 44:476–491, 3 2016. doi: 10.1016/j.immuni.2016.02.014.

[86] Pechous, R. D. With Friends Like These: The Complex Role of Neutrophils in the Progression of Severe Pneumonia. *Frontiers in Cellular and Infection Microbiology*, 7, 5 2017. doi: 10.3389/fcimb.2017.00160.

[87] Korkmaz, B., Moreau, T., and Gauthier, F. Neutrophil elastase, proteinase 3 and cathepsin G: Physicochemical properties, activity and physiopathological functions. *Biochimie*, 90:227–242, 2 2008. doi: 10.1016/j.biochi.2007.10.009.

[88] Standish, A. J. and Weiser, J. N. Human Neutrophils Kill Streptococcus pneumoniae via Serine Proteases. *The Journal of Immunology*, 183:2602–2609, 8 2009. doi: 10.4049/jimmunol.0900688.

[89] Grommes, J. and Soehnlein, O. Contribution of Neutrophils to Acute Lung Injury. *Molecular Medicine*, 17:293–307, 3 2011. doi: 10.2119/molmed.2010.00138.

[90] Grudzinska, F. S., Brodlie, M., Scholefield, B. R., *et al.* Neutrophils in community-acquired pneumonia: parallels in dysfunction at the extremes of age. *Thorax*, 75:164–171, 2 2020. doi: 10.1136/thoraxjnl-2018-212826.

[91] Sozzani, S., Zhou, D., Locati, M., *et al.* Receptors and transduction pathways for monocyte chemotactic protein-2 and monocyte chemotactic protein-3. Similarities and differences with MCP-1. *Journal of immunology (Baltimore, Md. : 1950)*, 152:3615–22, 4 1994.

[92] Si, Y., Tsou, C.-L., Croft, K., and Charo, I. F. CCR2 mediates hematopoietic stem and progenitor cell trafficking to sites of inflammation in mice. *Journal of Clinical Investigation*, 120:1192–1203, 4 2010. doi: 10.1172/JCI40310.

[93] Jia, T., Serbina, N. V., Brandl, K., *et al.* Additive Roles for MCP-1 and MCP-3 in CCR2-Mediated Recruitment of Inflammatory Monocytes during Listeria monocytogenes Infection. *The Journal of Immunology*, 180:6846–6853, 5 2008. doi: 10.4049/jimmunol.180.10.6846.

[94] Fogg, D. K., Sibon, C., Miled, C., *et al.* A Clonogenic Bone Marrow Progenitor Specific for Macrophages and Dendritic Cells. *Science*, 311:83–87, 1 2006. doi: 10.1126/science.1117729.

[95] Varol, C., Landsman, L., Fogg, D. K., *et al.* Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *The Journal of Experimental Medicine*, 204:171–180, 1 2007. doi: 10.1084/jem.20061011.

[96] Auffray, C., Sieweke, M. H., and Geissmann, F. Blood Monocytes: Development, Heterogeneity, and Relationship with Dendritic Cells. *Annual Review of Immunology*, 27:669–692, 4 2009. doi: 10.1146/annurev.immunol.021908.132557.

[97] Hsi, E. D. and Remick, D. G. Monocytes Are the Major Producers of Interleukin-1beta in an Ex Vivo Model of Local Cytokine Production. *Journal of Interferon and Cytokine Research*, 15:89–94, 1 1995. doi: 10.1089/jir.1995.15.89.

[98] Olliver, M., Hiew, J., Mellroth, P., *et al.* Human Monocytes Promote Th1 and Th17 Responses to Streptococcus pneumoniae. *Infection and Immunity*, 79:4210–4217, 10 2011. doi: 10.1128/IAI.05286-11.

[99] Soudja, S., Chandrabos, C., Yakob, E., *et al.* Memory-T-Cell-Derived Interferon-gamma Instructs Potent Innate Cell Activation for Protective Immunity. *Immunity*, 40:974–988, 6 2014. doi: 10.1016/j.immuni.2014.05.005.

[100] Seo, S.-U., Kamada, N., Muñoz-Planillo, R., *et al.* Distinct Commensals Induce Interleukin-1beta via NLRP3 Inflammasome in Inflammatory Monocytes to Promote Intestinal Inflammation in Response to Injury. *Immunity*, 42:744–755, 4 2015. doi: 10.1016/j.immuni.2015.03.004.

[101] Spits, H. and Santo, J. P. D. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nature immunology*, 12:21–7, 1 2011. doi: 10.1038/ni.1962.

[102] Spits, H., Artis, D., Colonna, M., *et al.* Innate lymphoid cells — a proposal for uniform nomenclature. *Nature Reviews Immunology*, 13:145–149, 2 2013. doi: 10.1038/nri3365.

[103] Marashian, S. M., Mortaz, E., Jamaati, H. R., *et al.* Role of Innate Lymphoid Cells in Lung Disease. *Iranian journal of allergy, asthma, and immunology*, 14:346–60, 8 2015.

[104] Vivier, E., Artis, D., Colonna, M., *et al.* Innate Lymphoid Cells: 10 Years On. *Cell*, 174:1054–1066, 8 2018. doi: 10.1016/j.cell.2018.07.017.

[105] Horowitz, A., Stegmann, K. A., and Riley, E. M. Activation of Natural Killer Cells during Microbial Infections. *Frontiers in Immunology*, 2, 2012. doi: 10.3389/fimmu.2011.00088.

[106] Xu, X., Weiss, I. D., Zhang, H. H., *et al.* Conventional NK Cells Can Produce IL-22 and Promote Host Defense in Klebsiella pneumoniae Pneumonia. *The Journal of Immunology*, 192:1778–1786, 2 2014. doi: 10.4049/jimmunol.1300039.

[107] Bots, M. and Medema, J. P. Granzymes at a glance. *Journal of Cell Science*, 119:5011–5014, 12 2006. doi: 10.1242/jcs.03239.

[108] Bernink, J. H., Peters, C. P., Munneke, M., *et al.* Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nature Immunology*, 14:221–229, 3 2013. doi: 10.1038/ni.2534.

[109] Neill, D. R., Wong, S. H., Bellosi, A., *et al.* Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature*, 464:1367–1370, 4 2010. doi: 10.1038/nature08900.

[110] Fallon, P. G., Ballantyne, S. J., Mangan, N. E., *et al.* Identification of an interleukin (IL)-25–dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *The Journal of Experimental Medicine*, 203:1105–1116, 4 2006. doi: 10.1084/jem.20051615.

[111] Monticelli, L. A., Sonnenberg, G. F., Abt, M. C., *et al.* Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nature immunology*, 12:1045–54, 11 2011. doi: 10.1031/ni.2131.

[112] Barlow, J. L., Bellosi, A., Hardman, C. S., *et al.* Innate IL-13–producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. *Journal of Allergy and Clinical Immunology*, 129:191–198.e4, 1 2012. doi: 10.1016/j.jaci.2011.09.041.

[113] Dudakov, J. A., Hanash, A. M., and van den Brink, M. R. Interleukin-22: Immunobiology and Pathology. *Annual Review of Immunology*, 33:747–785, 3 2015. doi: 10.1146/annurev-immunol-032414-112123.

[114] Melo-Gonzalez, F. and Hepworth, M. R. Functional and phenotypic heterogeneity of group 3 innate lymphoid cells. *Immunology*, 150:265–275, 3 2017. doi: 10.1111/imm.12697.

[115] Grove, K. C. D., Provoost, S., Verhamme, F. M., *et al.* Characterization and Quantification of Innate Lymphoid Cell Subsets in Human Lung. *PloS one*, 11:e0145961, 2016. doi: 10.1371/journal.pone.0145961.

[116] Cheng, H., Jin, C., Wu, J., *et al.* Guards at the gate: physiological and pathological roles of tissue-resident innate lymphoid cells in the lung. *Protein and Cell*, 8:878–895, 12 2017. doi: 10.1007/s13238-017-0379-5.

[117] Colonna, M. Innate Lymphoid Cells: Diversity, Plasticity, and Unique Functions in Immunity. *Immunity*, 48:1104–1117, 2018. doi: 10.1016/j.immuni.2018.05.013.

[118] Huang, Y., Guo, L., and Paul, W. E. IL-25-responsive, lineage-negative KLRG1hi cells are multipotential "inflammatory" type 2 innate lymphoid cells. *Nat Immunol.*, 56:481–495, 2015. doi: 10.1038/ni.3078.IL-25-responsive.

[119] Martin, B., Hirota, K., Cua, D. J., *et al.* Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. *Immunity*, 31:321–30, 8 2009. doi: 10.1016/j.immuni.2009.06.020.

[120] Sutton, C. E., Lalor, S. J., Sweeney, C. M., *et al.* Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity*, 31:331–41, 8 2009. doi: 10.1016/j.immuni.2009.08.001.

[121] Lockhart, E., Green, A. M., and Flynn, J. L. IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during Mycobacterium tuberculosis infection. *Journal of immunology (Baltimore, Md. : 1950)*, 177:4662–9, 10 2006. doi: 10.4049/jimmunol.177.7.4662.

[122] Liang, S. C., Tan, X.-Y., Luxenberg, D. P., *et al.* Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *The Journal of experimental medicine*, 203:2271–9, 10 2006. doi: 10.1084/jem.20061308.

[123] Umemura, M., Yahagi, A., Hamada, S., *et al.* IL-17-mediated regulation of innate and acquired immune response against pulmonary Mycobacterium bovis bacille Calmette-Guerin infection. *Journal of immunology (Baltimore, Md. : 1950)*, 178:3786–96, 3 2007. doi: 10.4049/jimmunol.178.6.3786.

[124] Shiromizu, C. M. and Jancic, C. C. gamma delta T Lymphocytes: An Effector Cell in Autoimmunity and Infection. *Frontiers in immunology*, 9:2389, 2018. doi: 10.3389/fimmu.2018.02389.

[125] Alvarez, F., Istomine, R., Shourian, M., *et al.* The alarmins IL-1 and IL-33 differentially regulate the functional specialisation of Foxp3+ regulatory T cells during mucosal inflammation. *Mucosal Immunology*, 12:746–760, 5 2019. doi: 10.1038/s41385-019-0153-5.

[126] Schröder, N. W. J., Morath, S., Alexander, C., *et al.* Lipoteichoic acid (LTA) of Streptococcus pneumoniae and Staphylococcus aureus activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. *The Journal of biological chemistry*, 278:15587–94, 5 2003. doi: 10.1074/jbc.M212829200.

[127] Schmeck, B., Huber, S., Moog, K., *et al.* Pneumococci induced TLR- and Rac1-dependent NF-kappaB-recruitment to the IL-8 promoter in lung epithelial cells. *American journal of physiology. Lung cellular and molecular physiology*, 290:L730–L737, 4 2006. doi: 10.1152/ajplung.00271.2005.

[128] Knapp, S., Wieland, C. W., van 't Veer, C., *et al.* Toll-Like Receptor 2 Plays a Role in the Early Inflammatory Response to Murine Pneumococcal Pneumonia but Does Not Contribute to Antibacterial Defense. *The Journal of Immunology*, 172:3132–3138, 3 2004. doi: 10.4049/jimmunol.172.5.3132.

[129] Wang, H., Lian, P., Niu, X., *et al.* TLR4 deficiency reduces pulmonary resistance to Streptococcus pneumoniae in gut microbiota-disrupted mice. *PLOS ONE*, 13:e0209183, 12 2018. doi: 10.1371/journal.pone.0209183.

[130] Dessing, M. C., Hirst, R. A., de Vos, A. F., and van der Poll, T. Role of Toll-like receptors 2 and 4 in pulmonary inflammation and injury induced by pneumolysin in mice. *PloS one*, 4:e7993, 11 2009. doi: 10.1371/journal.pone.0007993.

[131] Imai, Y., Kuba, K., Neely, G. G., *et al.* Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell*, 133:235–49, 4 2008. doi: 10.1016/j.cell.2008.02.043.

[132] Albiger, B., Dahlberg, S., Sandgren, A., *et al.* Toll-like receptor 9 acts at an early stage in host defence against pneumococcal infection. *Cellular microbiology*, 9:633–44, 3 2007. doi: 10.1111/j.1462-5822.2006.00814.x.

[133] Koedel, U., Rupprecht, T., Angele, B., *et al.* MyD88 is required for mounting a robust host immune response to Streptococcus pneumoniae in the CNS. *Brain : a journal of neurology*, 127:1437–45, 6 2004. doi: 10.1093/brain/awh171.

[134] Khan, A. Q., Chen, Q., Wu, Z.-Q., *et al.* Both innate immunity and type 1 humoral immunity to Streptococcus pneumoniae are mediated by MyD88 but differ in their relative levels of

dependence on toll-like receptor 2. *Infection and immunity*, 73:298–307, 1 2005. doi: 10.1128/IAI.73.1.298-307.2005.

[135] Albiger, B., Sandgren, A., Katsuragi, H., *et al.* Myeloid differentiation factor 88-dependent signalling controls bacterial growth during colonization and systemic pneumococcal disease in mice. *Cellular microbiology*, 7:1603–15, 11 2005. doi: 10.1111/j.1462-5822.2005.00578.x.

[136] Opitz, B., Püschel, A., Schmeck, B., *et al.* Nucleotide-binding Oligomerization Domain Proteins Are Innate Immune Receptors for Internalized Streptococcus pneumoniae. *Journal of Biological Chemistry*, 279:36426–36432, 8 2004. doi: 10.1074/jbc.M403861200.

[137] Davis, K. M., Nakamura, S., and Weiser, J. N. Nod2 sensing of lysozyme-digested peptidoglycan promotes macrophage recruitment and clearance of S. pneumoniae colonization in mice. *The Journal of clinical investigation*, 121:3666–76, 9 2011. doi: 10.1172/JCI57761.

[138] McNeela, E. A., Burke, A., Neill, D. R., *et al.* Pneumolysin activates the NLRP3 inflammasome and promotes proinflammatory cytokines independently of TLR4. *PLoS pathogens*, 6:e1001191, 11 2010. doi: 10.1371/journal.ppat.1001191.

[139] Witzenrath, M., Pache, F., Lorenz, D., *et al.* The NLRP3 Inflammasome Is Differentially Activated by Pneumolysin Variants and Contributes to Host Defense in Pneumococcal Pneumonia. *The Journal of Immunology*, 187:434–440, 2011. doi: 10.4049/jimmunol.1003143.

[140] Shoma, S., Tsuchiya, K., Kawamura, I., *et al.* Critical involvement of pneumolysin in production of interleukin-1alpha and caspase-1-dependent cytokines in infection with Streptococcus pneumoniae in vitro: a novel function of pneumolysin in caspase-1 activation. *Infection and immunity*, 76:1547–57, 4 2008. doi: 10.1128/IAI.01269-07.

[141] Fang, R., Tsuchiya, K., Kawamura, I., *et al.* Critical roles of ASC inflammasomes in caspase-1 activation and host innate resistance to Streptococcus pneumoniae infection. *Journal of immunology (Baltimore, Md. : 1950)*, 187:4890–9, 11 2011. doi: 10.4049/jimmunol.1100381.

[142] Rabes, A., Zimmermann, S., Reppe, K., *et al.* The C-type lectin receptor Mincle binds to Streptococcus pneumoniae but plays a limited role in the anti-pneumococcal innate immune response. *PloS one*, 10:e0117022, 2015. doi: 10.1371/journal.pone.0117022.

[143] Behler-Janbeck, F., Takano, T., Maus, R., *et al.* C-type Lectin Mincle Recognizes Glucosyldiacylglycerol of Streptococcus pneumoniae and Plays a Protective Role in Pneumococcal Pneumonia. *PLoS pathogens*, 12:e1006038, 12 2016. doi: 10.1371/journal.ppat.1006038.

[144] Ruiz-Moreno, J. S., Hamann, L., Jin, L., *et al.* The cGAS/STING Pathway Detects Streptococcus pneumoniae but Appears Dispensable for Antipneumococcal Defense in Mice and Humans. *Infection and Immunity*, 86:1–13, 12 2017. doi: 10.1128/IAI.00849-17.

[145] Nelson, A. L., Roche, A. M., Gould, J. M., *et al.* Capsule Enhances Pneumococcal Colonization by Limiting Mucus-Mediated Clearance. *Infection and Immunity*, 75:83–90, 1 2007. doi: 10.1128/IAI.01475-06.

[146] Kerr, A. R., Paterson, G. K., Riboldi-Tunnicliffe, A., and Mitchell, T. J. Innate Immune Defense against Pneumococcal Pneumonia Requires Pulmonary Complement Component C3. *Infection and Immunity*, 73:4245–4252, 7 2005. doi: 10.1128/IAI.73.7.4245-4252.2005.

[147] Salina, A. C., Souza, T. P., Serezani, C. H., and Medeiros, A. I. Efferocytosis-induced prostaglandin E2 production impairs alveolar macrophage effector functions during Streptococcus pneumoniae infection. *Innate immunity*, 23:219–227, 4 2017. doi: 10.1177/1753425916684934.

[148] Li, H., Bradbury, J. A., Edin, M. L., *et al.* sEH promotes macrophage phagocytosis and lung clearance of Streptococcus pneumoniae. *The Journal of clinical investigation*, 131, 11 2021. doi: 10.1172/JCI129679.

[149] Yamamoto, K., Ferrari, J. D., Cao, Y., *et al.* Type I alveolar epithelial cells mount innate immune responses during pneumococcal pneumonia. *Journal of immunology (Baltimore, Md. : 1950)*, 189:2450–9, 9 2012. doi: 10.4049/jimmunol.1200634.

[150] Cleaver, J. O., You, D., Michaud, D. R., *et al.* Lung epithelial cells are essential effectors of inducible resistance to pneumonia. *Mucosal Immunology*, 7:78–88, 1 2014. doi: 10.1038/mi.2013.26.

[151] Yamamoto, K., Ahyi, A.-N. N., Pepper-Cunningham, Z. A., *et al.* Roles of lung epithelium in neutrophil recruitment during pneumococcal pneumonia. *American journal of respiratory cell and molecular biology*, 50:253–62, 2 2014. doi: 10.1165/rcmb.2013-0114OC.

[152] Dudek, M., Puttur, F., Arnold-Schrauf, C., *et al.* Lung epithelium and myeloid cells cooperate to clear acute pneumococcal infection. *Mucosal immunology*, 9:1288–302, 9 2016. doi: 10.1038/mi.2015.128.

[153] Nakatsuka, Y., Vandenbon, A., Mino, T., *et al.* Pulmonary Regnase-1 orchestrates the interplay of epithelium and adaptive immune systems to protect against pneumonia. *Mucosal immunology*, 11:1203–1218, 7 2018. doi: 10.1038/s41385-018-0024-5.

[154] Gardiner, E. E. and Andrews, R. K. Neutrophil extracellular traps (NETs) and infection-related vascular dysfunction. *Blood Reviews*, 26:255–259, 11 2012. doi: 10.1016/j.blre.2012.09.001.

[155] Kolaczkowska, E. and Kubes, P. Neutrophil recruitment and function in health and inflammation. *Nature reviews. Immunology*, 13:159–75, 3 2013. doi: 10.1038/nri3399.

[156] Hahn, I., Klaus, A., Janze, A.-K., *et al.* Cathepsin G and neutrophil elastase play critical and nonredundant roles in lung-protective immunity against Streptococcus pneumoniae in mice. *Infection and immunity*, 79:4893–901, 12 2011. doi: 10.1128/IAI.05593-11.

[157] Beiter, K., Wartha, F., Albiger, B., *et al.* An endonuclease allows Streptococcus pneumoniae to escape from neutrophil extracellular traps. *Current biology : CB*, 16:401–7, 2 2006. doi: 10.1016/j.cub.2006.01.056.

[158] Goto, Y., Hogg, J. C., Whalen, B., *et al.* Monocyte Recruitment into the Lungs in Pneumococcal Pneumonia. *American Journal of Respiratory Cell and Molecular Biology*, 30:620–626, 5 2004. doi: 10.1165/rcmb.2003-0312OC.

[159] Winter, C., Taut, K., Srivastava, M., *et al.* Lung-Specific Overexpression of CC Chemokine Ligand (CCL) 2 Enhances the Host Defense to Streptococcus pneumoniae Infection in Mice: Role of the CCL2-CCR2 Axis. *The Journal of Immunology*, 178:5828–5838, 2007. doi: 10.4049/jimmunol.178.9.5828.

[160] Nakasone, C., Yamamoto, N., Nakamatsu, M., *et al.* Accumulation of gamma/delta T cells in the lungs and their roles in neutrophil-mediated host defense against pneumococcal infection. *Microbes and infection*, 9:251–8, 3 2007. doi: 10.1016/j.micinf.2006.11.015.

[161] Ma, J., Wang, J., Wan, J., *et al.* Morphine disrupts interleukin-23 (IL-23)/IL-17-mediated pulmonary mucosal host defense against Streptococcus pneumoniae infection. *Infection and immunity*, 78:830–7, 2 2010. doi: 10.1128/IAI.00914-09.

[162] Maele, L. V., Carnoy, C., Cayet, D., *et al.* Activation of type 3 innate lymphoid cells and interleukin 22 secretion in the lungs during streptococcus pneumoniae infection. *Journal of Infectious Diseases*, 210:493–503, 2014. doi: 10.1093/infdis/jiu106.

[163] Trevejo-Nunez, G., Elsegeiny, W., Conboy, P., *et al.* Critical Role of IL-22/IL22-RA1 Signaling in Pneumococcal Pneumonia. *The Journal of Immunology*, 197:1877–1883, 2016. doi: 10.4049/jimmunol.1600528.

[164] Elhaik-Goldman, S., Kafka, D., Yossef, R., *et al.* The natural cytotoxicity receptor 1 contribution to early clearance of Streptococcus pneumoniae and to natural killer-macrophage cross talk. *PloS one*, 6:e23472, 2011. doi: 10.1371/journal.pone.0023472.

[165] Saluzzo, S., Gorki, A. D., Rana, B. M., *et al.* First-Breath-Induced Type 2 Pathways Shape the Lung Immune Environment. *Cell Reports*, 18:1893–1905, 2017. doi: 10.1016/j.celrep.2017.01.071.

[166] Hartshorn, K. L., Crouch, E., White, M. R., *et al.* Pulmonary surfactant proteins A and D enhance neutrophil uptake of bacteria. *The American journal of physiology*, 274:L958–69, 6 1998. doi: 10.1152/ajplung.1998.274.6.L958.

[167] Zhang, L., Li, Z., Wan, Z., *et al.* Humoral immune responses to Streptococcus pneumoniae in the setting of HIV-1 infection. *Vaccine*, 33:4430–6, 8 2015. doi: 10.1016/j.vaccine.2015.06.077.

[168] Engelhardt, K. R. and Grimbacher, B. *IL-10 in Humans: Lessons from the Gut, IL-10/IL-10 Receptor Deficiencies, and IL-10 Polymorphisms*, pages 1–18. 2014. doi: 10.1007/978-3-662-43492-5_1.

[169] Wang, X., Wong, K., Ouyang, W., and Rutz, S. Targeting IL-10 Family Cytokines for the Treatment of Human Diseases. *Cold Spring Harbor perspectives in biology*, 11, 2 2019. doi: 10.1101/cshperspect.a028548.

[170] Sonnenberg, G. F., Fouser, L. A., and Artis, D. Border patrol: Regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nature Immunology*, 12:383–390, 2011. doi: 10.1038/ni.2025.

[171] Lazear, H. M., Schoggins, J. W., and Diamond, M. S. Shared and Distinct Functions of Type I and Type III Interferons. *Immunity*, 50:907–923, 4 2019. doi: 10.1016/j.immuni.2019.03.025.

[172] Dumoutier, L., Louahed, J., and Renauld, J.-C. Cloning and Characterization of IL-10-Related T Cell-Derived Inducible Factor (IL-TIF), a Novel Cytokine Structurally Related to IL-10 and Inducible by IL-9. *The Journal of Immunology*, 164:1814–1819, 2 2000. doi: 10.4049/jimmunol.164.4.1814.

[173] Xie, M.-H., Aggarwal, S., Ho, W.-H., *et al.* Interleukin (IL)-22, a Novel Human Cytokine That Signals through the Interferon Receptor-related Proteins CRF2–4 and IL-22R. *Journal of Biological Chemistry*, 275:31335–31339, 10 2000. doi: 10.1074/jbc.M005304200.

[174] Bleicher, L., de Moura, P. R., Watanabe, L., *et al.* Crystal structure of the IL-22/IL-22R1 complex and its implications for the IL-22 signaling mechanism. *FEBS letters*, 582:2985–92, 9 2008. doi: 10.1016/j.febslet.2008.07.046.

[175] Kotenko, S. V., Krause, C. D., Izotova, L. S., *et al.* Identification and functional characterization of a second chain of the interleukin-10 receptor complex. *The EMBO journal*, 16:5894–903, 10 1997. doi: 10.1093/emboj/16.19.5894.

[176] Lejeune, D., Dumoutier, L., Constantinescu, S., *et al.* Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line. Pathways that are shared with and distinct from IL-10. *The Journal of biological chemistry*, 277:33676–82, 9 2002. doi: 10.1074/jbc.M204204200.

[177] Zheng, Y., Danilenko, D. M., Valdez, P., *et al.* Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature*, 445:648–51, 2 2007. doi: 10.1038/nature05505.

[178] Lee, Y., Kumagai, Y., Jang, M. S., *et al.* Intestinal Lin- c-Kit+ NKp46- CD4- population strongly produces IL-22 upon IL-1beta stimulation. *Journal of immunology (Baltimore, Md. : 1950)*, 190:5296–305, 5 2013. doi: 10.4049/jimmunol.1201452.

[179] Vonarbourg, C. and Diefenbach, A. Multifaceted roles of interleukin-7 signaling for the development and function of innate lymphoid cells. *Seminars in immunology*, 24:165–74, 6 2012. doi: 10.1016/j.smim.2012.03.002.

[180] Qiu, J., Heller, J. J., Guo, X., *et al.* The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. *Immunity*, 36:92–104, 1 2012. doi: 10.1016/j.immuni.2011.11.011.

[181] Kotenko, S. V., Izotova, L. S., Mirochnitchenko, O. V., *et al.* Identification, cloning, and characterization of a novel soluble receptor that binds IL-22 and neutralizes its activity. *Journal of immunology (Baltimore, Md. : 1950)*, 166:7096–103, 6 2001. doi: 10.4049/jimmunol.166.12.7096.

[182] Mabuchi, T., Takekoshi, T., and Hwang, S. T. Epidermal CCR6+ gammadelta T cells are major producers of IL-22 and IL-17 in a murine model of psoriasiform dermatitis. *Journal of immunology (Baltimore, Md. : 1950)*, 187:5026–31, 11 2011. doi: 10.4049/jimmunol.1101817.

[183] Goto, M., Murakawa, M., Kadoshima-Yamaoka, K., *et al.* Murine NKT cells produce Th17 cytokine interleukin-22. *Cellular immunology*, 254:81–4, 2009. doi: 10.1016/j.cellimm.2008.10.002.

[184] Spits, H. and Cupedo, T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. *Annual review of immunology*, 30:647–75, 2012. doi: 10.1146/annurev-immunol-020711-075053.

[185] Bergmann, H., Roth, S., Pechloff, K., *et al.* Card9-dependent IL-1beta regulates IL-22 production from group 3 innate lymphoid cells and promotes colitis-associated cancer. *European Journal of Immunology*, 47:1342–1353, 8 2017. doi: 10.1002/eji.201646765.

[186] Victor, A. R., Nalin, A. P., Dong, W., *et al.* IL-18 Drives ILC3 Proliferation and Promotes IL-22 Production via NF-kB. *The Journal of Immunology*, 199:2333–2342, 10 2017. doi: 10.4049/jimmunol.1601554.

[187] Ngo, V. L., Abo, H., Maxim, E., *et al.* A cytokine network involving IL-36gamma, IL-23, and IL-22 promotes antimicrobial defense and recovery from intestinal barrier damage. *Proceedings of the National Academy of Sciences*, 115, 5 2018. doi: 10.1073/pnas.1718902115.

[188] Giacomin, P. R., Moy, R. H., Noti, M., *et al.* Epithelial-intrinsic IKKalpha expression regulates group 3 innate lymphoid cell responses and antibacterial immunity. *Journal of Experimental Medicine*, 212:1513–1528, 9 2015. doi: 10.1084/jem.20141831.

[189] Zheng, Y., Valdez, P. A., Danilenko, D. M., *et al.* Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nature Medicine*, 14:282–289, 2008. doi: 10.1038/nm1720.

[190] Pham, T. A. N., Clare, S., Goulding, D., *et al.* Epithelial IL-22RA1-mediated fucosylation promotes intestinal colonization resistance to an opportunistic pathogen. *Cell host and microbe*, 16:504–16, 10 2014. doi: 10.1016/j.chom.2014.08.017.

[191] Tsai, P.-Y., Zhang, B., He, W.-Q., *et al.* IL-22 Upregulates Epithelial Claudin-2 to Drive Diarrhea and Enteric Pathogen Clearance. *Cell host and microbe*, 21:671–681.e4, 6 2017. doi: 10.1016/j.chom.2017.05.009.

[192] Aujla, S. J., Chan, Y. R., Zheng, M., *et al.* IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nature Medicine*, 14:275–281, 2008. doi: 10.1038/nm1710.

[193] Luca, A. D., Zelante, T., D'Angelo, C., *et al.* IL-22 defines a novel immune pathway of antifungal resistance. *Mucosal immunology*, 3:361–73, 7 2010. doi: 10.1038/mi.2010.22.

[194] Dwyer, G. K., D'Cruz, L. M., and Turnquist, H. R. Emerging Functions of IL-33 in Homeostasis and Immunity. *Annual Review of Immunology*, 40:15–43, 4 2022. doi: 10.1146/annurev-immunol-101320-124243.

[195] Moussion, C., Ortega, N., and Girard, J.-P. The IL-1-Like Cytokine IL-33 Is Constitutively Expressed in the Nucleus of Endothelial Cells and Epithelial Cells In Vivo: A Novel 'Alarmin'? *PLoS ONE*, 3:e3331, 10 2008. doi: 10.1371/journal.pone.0003331.

[196] Pichery, M., Mirey, E., Mercier, P., *et al.* Endogenous IL-33 is highly expressed in mouse epithelial barrier tissues, lymphoid organs, brain, embryos, and inflamed tissues: in situ analysis using a novel Il-33-LacZ gene trap reporter strain. *Journal of immunology (Baltimore, Md. : 1950)*, 188:3488–95, 4 2012. doi: 10.4049/jimmunol.1101977.

[197] Hardman, C. S., Panova, V., and Mckenzie, A. N. J. IL-33 citrine reporter mice reveal the temporal and spatial expression of IL-33 during allergic lung inflammation. *European Journal of Immunology*, 43:488–498, 2013. doi: 10.1002/eji.201242863.

[198] Roussel, L., Erard, M., Cayrol, C., and Girard, J.-P. Molecular mimicry between IL-33 and KSHV for attachment to chromatin through the H2A-H2B acidic pocket. *EMBO reports*, 9:1006–12, 10 2008. doi: 10.1038/embor.2008.145.

[199] Carriere, V., Roussel, L., Ortega, N., *et al.* IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, 104:282–7, 1 2007. doi: 10.1073/pnas.0606854104.

[200] Cayrol, C. and Girard, J.-P. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proceedings of the National Academy of Sciences of the United States of America*, 106:9021–6, 6 2009. doi: 10.1073/pnas.0812690106.

[201] Lefrançais, E., Roga, S., Gautier, V., *et al.* IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. *Proceedings of the National Academy of Sciences of the United States of America*, 109:1673–8, 1 2012. doi: 10.1073/pnas.1115884109.

[202] Lefrancais, E., Duval, A., Mirey, E., *et al.* Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. *Proceedings of the National Academy of Sciences*, 111:15502–15507, 2014. doi: 10.1073/pnas.1410700111.

[203] Ali, S., Mohs, A., Thomas, M., *et al.* The Dual Function Cytokine IL-33 Interacts with the Transcription Factor NF-kB To Dampen NF-kB–Stimulated Gene Transcription. *The Journal of Immunology*, 187:1609–1616, 8 2011. doi: 10.4049/jimmunol.1003080.

[204] Ni, Y., Tao, L., Chen, C., *et al.* The Deubiquitinase USP17 Regulates the Stability and Nuclear Function of IL-33. *International Journal of Molecular Sciences*, 16:27956–27966, 11 2015. doi: 10.3390/ijms161126063.

[205] Gautier, V., Cayrol, C., Farache, D., *et al.* Extracellular IL-33 cytokine, but not endogenous nuclear IL-33, regulates protein expression in endothelial cells. *Scientific Reports*, 6:34255, 10 2016. doi: 10.1038/srep34255.

[206] Lüthi, A. U., Cullen, S. P., McNeela, E. A., *et al.* Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity*, 31:84–98, 7 2009. doi: 10.1016/j.immuni.2009.05.007.

[207] Cohen, E. S., Scott, I. C., Majithiya, J. B., *et al.* Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation. *Nature communications*, 6:8327, 9 2015. doi: 10.1038/ncomms9327.

[208] Hayakawa, H., Hayakawa, M., Kume, A., and ichi Tominaga, S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *The Journal of biological chemistry*, 282:26369–80, 9 2007. doi: 10.1074/jbc.M704916200.

[209] Bandara, G., Beaven, M. A., Olivera, A., *et al.* Activated mast cells synthesize and release soluble ST2-a decoy receptor for IL-33. *European journal of immunology*, 45:3034–44, 11 2015. doi: 10.1002/eji.201545501.

[210] Löhning, M., Stroehmann, A., Coyle, A. J., *et al.* T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. *Proceedings of the National Academy of Sciences of the United States of America*, 95:6930–5, 6 1998. doi: 10.1073/pnas.95.12.6930.

[211] Moritz, D. R., Rodewald, H. R., Gheyselinck, J., and Klemenz, R. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. *Journal of immunology (Baltimore, Md. : 1950)*, 161:4866–74, 11 1998.

[212] Suzukawa, M., Iikura, M., Koketsu, R., *et al.* An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *Journal of immunology (Baltimore, Md. : 1950)*, 181:5981–9, 11 2008. doi: 10.4049/jimmunol.181.9.5981.

[213] Cherry, W. B., Yoon, J., Bartemes, K. R., *et al.* A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *Journal of Allergy and Clinical Immunology*, 121:1484–1490, 6 2008. doi: 10.1016/j.jaci.2008.04.005.

[214] Schiering, C., Krausgruber, T., Chomka, A., *et al.* The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature*, 513:564–568, 9 2014. doi: 10.1038/nature13577.

[215] Rank, M. A., Kobayashi, T., Kozaki, H., *et al.* IL-33–activated dendritic cells induce an atypical TH2-type response. *Journal of Allergy and Clinical Immunology*, 123:1047–1054, 5 2009. doi: 10.1016/j.jaci.2009.02.026.

[216] Yagami, A., Orihara, K., Morita, H., *et al.* IL-33 mediates inflammatory responses in human lung tissue cells. *Journal of immunology (Baltimore, Md. : 1950)*, 185:5743–50, 11 2010. doi: 10.4049/jimmunol.0903818.

[217] Maywald, R. L., Doerner, S. K., Pastorelli, L., *et al.* IL-33 activates tumor stroma to promote intestinal polyposis. *Proceedings of the National Academy of Sciences of the United States of America*, 112:E2487–96, 5 2015. doi: 10.1073/pnas.1422445112.

[218] Gadani, S. P., Walsh, J. T., Smirnov, I., *et al.* The glia-derived alarmin IL-33 orchestrates the immune response and promotes recovery following CNS injury. *Neuron*, 85:703–9, 2 2015. doi: 10.1016/j.neuron.2015.01.013.

[219] Liu, B., Tai, Y., Achanta, S., *et al.* IL-33/ST2 signaling excites sensory neurons and mediates itch response in a mouse model of poison ivy contact allergy. *Proceedings of the National Academy of Sciences of the United States of America*, 113:E7572–E7579, 11 2016. doi: 10.1073/pnas.1606608113.

[220] Molofsky, A. B., Savage, A. K., and Locksley, R. M. Interleukin-33 in Tissue Homeostasis, Injury, and Inflammation. *Immunity*, 42:1005–1019, 2015. doi: 10.1016/j.immuni.2015.06.006.

[221] Lott, J. M., Sumpter, T. L., and Turnquist, H. R. New dog and new tricks: evolving roles for IL-33 in type 2 immunity. *Journal of leukocyte biology*, 97:1037–48, 6 2015. doi: 10.1189/jlb.3RI1214-595R.

[222] Bulek, K., Swaidani, S., Qin, J., *et al.* The essential role of single Ig IL-1 receptor-related molecule/Toll IL-1R8 in regulation of Th2 immune response. *Journal of immunology (Baltimore, Md. : 1950)*, 182:2601–9, 3 2009. doi: 10.4049/jimmunol.0802729.

[223] Zhao, J., Wei, J., Mialki, R. K., *et al.* F-box protein FBXL19-mediated ubiquitination and degradation of the receptor for IL-33 limits pulmonary inflammation. *Nature immunology*, 13:651–8, 6 2012. doi: 10.1038/ni.2341.

[224] Sattler, S., Smits, H. H., Xu, D., and Huang, F.-P. The evolutionary role of the IL-33/ST2 system in host immune defence. *Archivum immunologiae et therapiae experimentalis*, 61:107–17, 4 2013. doi: 10.1007/s00005-012-0208-8.

[225] Duan, L., Chen, J., Gong, F., and Shi, G. The role of IL-33 in rheumatic diseases. *Clinical and developmental immunology*, 2013:924363, 2013. doi: 10.1155/2013/924363.

[226] Humphreys, N. E., Xu, D., Hepworth, M. R., *et al.* IL-33, a potent inducer of adaptive immunity to intestinal nematodes. *Journal of immunology (Baltimore, Md. : 1950)*, 180:2443–9, 2 2008. doi: 10.4049/jimmunol.180.4.2443.

[227] Jones, L. A., Roberts, F., Nickdel, M. B., *et al.* IL-33 receptor (T1/ST2) signalling is necessary to prevent the development of encephalitis in mice infected with Toxoplasma gondii. *European journal of immunology*, 40:426–36, 2 2010. doi: 10.1002/eji.200939705.

[228] Oboki, K., Ohno, T., Kajiwara, N., *et al.* IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proceedings of the National Academy of Sciences of the United States of America*, 107:18581–18586, 2010. doi: 10.1073/pnas.1003059107.

[229] Licona-Limón, P., Kim, L. K., Palm, N. W., and Flavell, R. A. TH2, allergy and group 2 innate lymphoid cells. *Nature Immunology*, 14:536–542, 2013. doi: 10.1038/ni.2617.

[230] Lopetuso, L. R., Scaldaferri, F., and Pizarro, T. T. Emerging role of the interleukin (IL)-33/ST2 axis in gut mucosal wound healing and fibrosis. *Fibrogenesis and tissue repair*, 5:18, 10 2012. doi: 10.1186/1755-1536-5-18.

[231] Enoksson, M., Lyberg, K., Möller-Westerberg, C., *et al.* Mast cells as sensors of cell injury through IL-33 recognition. *Journal of immunology (Baltimore, Md. : 1950)*, 186:2523–8, 2 2011. doi: 10.4049/jimmunol.1003383.

[232] Allakhverdi, Z., Smith, D. E., Comeau, M. R., and Delespesse, G. Cutting edge: The ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *Journal of immunology (Baltimore, Md. : 1950)*, 179:2051–4, 8 2007. doi: 10.4049/jimmunol.179.4.2051.

[233] Pecaric-Petkovic, T., Didichenko, S. A., Kaempfer, S., *et al.* Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood*, 113:1526–34, 2 2009. doi: 10.1182/blood-2008-05-157818.

[234] Stolarski, B., Kurowska-Stolarska, M., Kewin, P., *et al.* IL-33 exacerbates eosinophil-mediated airway inflammation. *Journal of immunology (Baltimore, Md. : 1950)*, 185:3472–80, 9 2010. doi: 10.4049/jimmunol.1000730.

[235] Besnard, A.-G., Togbe, D., Guillou, N., *et al.* IL-33-activated dendritic cells are critical for allergic airway inflammation. *European journal of immunology*, 41:1675–86, 6 2011. doi: 10.1002/eji.201041033.

[236] Chang, Y.-J., Kim, H. Y., Albacker, L. A., *et al.* Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity. *Nature immunology*, 12:631–8, 5 2011. doi: 10.1038/ni.2045.

[237] Yasuda, K., Muto, T., Kawagoe, T., *et al.* Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice. *Proceedings of the National Academy of Sciences*, 109:3451–3456, 2012. doi: 10.1073/pnas.1201042109.

[238] Price, A. E., Liang, H.-E., Sullivan, B. M., *et al.* Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proceedings of the National Academy of Sciences of the United States of America*, 107:11489–94, 6 2010. doi: 10.1073/pnas.1003988107.

[239] Kurowska-Stolarska, M., Stolarski, B., Kewin, P., *et al.* IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *Journal of immunology (Baltimore, Md. : 1950)*, 183:6469–77, 11 2009. doi: 10.4049/jimmunol.0901575.

[240] Hazlett, L. D., McClellan, S. A., Barrett, R. P., *et al.* IL-33 shifts macrophage polarization, promoting resistance against Pseudomonas aeruginosa keratitis. *Investigative ophthalmology and visual science*, 51:1524–32, 3 2010. doi: 10.1167/iovs.09-3983.

[241] Jiang, H.-R., Milovanović, M., Allan, D., *et al.* IL-33 attenuates EAE by suppressing IL-17 and IFN-gamma production and inducing alternatively activated macrophages. *European journal of immunology*, 42:1804–14, 7 2012. doi: 10.1002/eji.201141947.

[242] Li, C., Mu, R., Guo, J., *et al.* Genetic variant in IL33 is associated with susceptibility to rheumatoid arthritis. *Arthritis Research and Therapy*, 16:1–10, 2014. doi: 10.1186/ar4554.

[243] Smithgall, M. D., Comeau, M. R., Yoon, B. R. P., *et al.* IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK Cells. *International Immunology*, 20:1019–1030, 2008. doi: 10.1093/intimm/dxn060.

[244] Bonilla, W. V., Fröhlich, A., Senn, K., *et al.* The alarmin interleukin-33 drives protective antiviral CD8 T cell responses. *Science (New York, N.Y.)*, 335:984–9, 2 2012. doi: 10.1126/science.1215418.

[245] Rostan, O., Arshad, M. I., Piquet-Pellorce, C., *et al.* Crucial and diverse role of the interleukin-33/ST2 axis in infectious diseases. *Infection and immunity*, 83:1738–48, 5 2015. doi: 10.1128/IAI.02908-14.

[246] Yang, Q., Li, G., Zhu, Y., *et al.* IL-33 synergizes with TCR and IL-12 signaling to promote the effector function of CD8+ T cells. *European journal of immunology*, 41:3351–60, 11 2011. doi: 10.1002/eji.201141629.

[247] Kearley, J., Silver, J. S., Sanden, C., *et al.* Cigarette smoke silences innate lymphoid cell function and facilitates an exacerbated type I interleukin-33-dependent response to infection. *Immunity*, 42:566–79, 3 2015. doi: 10.1016/j.immuni.2015.02.011.

[248] Baumann, C., Bonilla, W. V., Fröhlich, A., *et al.* T-bet- and STAT4-dependent IL-33 receptor expression directly promotes antiviral Th1 cell responses. *Proceedings of the National Academy of Sciences of the United States of America*, 112:4056–61, 3 2015. doi: 10.1073/pnas.1418549112.

[249] Alves-Filho, J. C., Sônego, F., Souto, F. O., *et al.* Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection. *Nature medicine*, 16:708–12, 6 2010. doi: 10.1038/nm.2156.

[250] Hoogerwerf, J. J., Leendertse, M., Wieland, C. W., *et al.* Loss of suppression of tumorigenicity 2 (ST2) gene reverses sepsis-induced inhibition of lung host defense in mice. *American journal of respiratory and critical care medicine*, 183:932–40, 4 2011. doi: 10.1164/rccm.201006-0934OC.

[251] Cohen, M., Giladi, A., Gorki, A.-D., *et al.* Lung Single-Cell Signaling Interaction Map Reveals Basophil Role in Macrophage Imprinting. *Cell*, pages 1–14, 2018. doi: 10.1016/j.cell.2018.09.009.

[252] Lynch, S. V. and Pedersen, O. The Human Intestinal Microbiome in Health and Disease. *New England Journal of Medicine*, 375:2369–2379, 12 2016. doi: 10.1056/NEJMra1600266.

[253] Dickson, R. P., Erb-Downward, J. R., Martinez, F. J., and Huffnagle, G. B. The Microbiome and the Respiratory Tract. *Annual review of physiology*, 78:481–504, 2016. doi: 10.1146/annurev-physiol-021115-105238.

[254] Qin, J., Li, R., Raes, J., *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, 464:59–65, 3 2010. doi: 10.1038/nature08821.

[255] Consortium, H. M. P. Structure, function and diversity of the healthy human microbiome. *Nature*, 486:207–14, 6 2012. doi: 10.1038/nature11234.

[256] Pleasants, J. R. Rearing germfree cesarean-born rats, mice, and rabbits through weaning. *Annals of the New York Academy of Sciences*, 78:116–26, 5 1959. doi: 10.1111/j.1749-6632.1959.tb53099.x.

[257] Wostmann, B. S. The germfree animal in nutritional studies. *Annual review of nutrition*, 1:257–79, 1981. doi: 10.1146/annurev.nu.01.070181.001353.

[258] Donskey, C. J., Chowdhry, T. K., Hecker, M. T., *et al.* Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. *The New England journal of medicine*, 343:1925–32, 12 2000. doi: 10.1056/NEJM200012283432604.

[259] Hou, K., Wu, Z.-X., Chen, X.-Y., *et al.* Microbiota in health and diseases. *Signal Transduction and Targeted Therapy*, 7:135, 4 2022. doi: 10.1038/s41392-022-00974-4.

[260] Gensollen, T., Iyer, S. S., Kasper, D. L., and Blumberg, R. S. How colonization by microbiota in early life shapes the immune system. *Science (New York, N.Y.)*, 352:539–44, 4 2016. doi: 10.1126/science.aad9378.

[261] Krajmalnik-Brown, R., Ilhan, Z.-E., Kang, D.-W., and DiBaise, J. K. Effects of gut microbes on nutrient absorption and energy regulation. *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition*, 27:201–14, 4 2012. doi: 10.1177/0884533611436116.

[262] Neuman, H., Debelius, J. W., Knight, R., and Koren, O. Microbial endocrinology: the interplay between the microbiota and the endocrine system. *FEMS Microbiology Reviews*, 39:509–521, 7 2015. doi: 10.1093/femsre/fuu010.

[263] Budden, K. F., Gellatly, S. L., Wood, D. L. A., *et al.* Emerging pathogenic links between microbiota and the gut-lung axis. *Nature reviews. Microbiology*, 15:55–63, 1 2017. doi: 10.1038/nrmicro.2016.142.

[264] Rooks, M. G. and Garrett, W. S. Gut microbiota, metabolites and host immunity. *Nature reviews. Immunology*, 16:341–52, 5 2016. doi: 10.1038/nri.2016.42.

[265] Belkaid, Y. and Harrison, O. J. Homeostatic Immunity and the Microbiota. *Immunity*, 46: 562–576, 4 2017. doi: 10.1016/j.immuni.2017.04.008.

[266] Bogaert, D., Keijser, B., Huse, S., *et al.* Variability and diversity of nasopharyngeal microbiota in children: a metagenomic analysis. *PloS one*, 6:e17035, 2 2011. doi: 10.1371/journal.pone.0017035.

[267] Biesbroek, G., Bosch, A. A. T. M., Wang, X., *et al.* The Impact of Breastfeeding on Nasopharyngeal Microbial Communities in Infants. *American Journal of Respiratory and Critical Care Medicine*, 190:298–308, 8 2014. doi: 10.1164/rccm.201401-0073OC.

[268] Teo, S. M., Mok, D., Pham, K., *et al.* The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. *Cell host and microbe*, 17:704–15, 5 2015. doi: 10.1016/j.chom.2015.03.008.

[269] Bosch, A. A. T. M., de Steenhuijsen Piters, W. A. A., van Houten, M. A., *et al.* Maturation of the Infant Respiratory Microbiota, Environmental Drivers, and Health Consequences. A Prospective Cohort Study. *American journal of respiratory and critical care medicine*, 196: 1582–1590, 12 2017. doi: 10.1164/rccm.201703-0554OC.

[270] Lemon, K. P., Klepac-Ceraj, V., Schiffer, H. K., *et al.* Comparative Analyses of the Bacterial Microbiota of the Human Nostril and Oropharynx. *mBio*, 1, 8 2010. doi: 10.1128/mBio.00129-10.

[271] Stearns, J. C., Davidson, C. J., McKeon, S., *et al.* Culture and molecular-based profiles show shifts in bacterial communities of the upper respiratory tract that occur with age. *The ISME journal*, 9:1246–59, 5 2015. doi: 10.1038/ismej.2014.250.

[272] Man, W. H., de Steenhuijsen Piters, W. A., and Bogaert, D. The microbiota of the respiratory tract: gatekeeper to respiratory health. *Nature Reviews Microbiology*, 15:259–270, 5 2017. doi: 10.1038/nrmicro.2017.14.

[273] Charlson, E. S., Bittinger, K., Haas, A. R., *et al.* Topographical Continuity of Bacterial Populations in the Healthy Human Respiratory Tract. *American Journal of Respiratory and Critical Care Medicine*, 184:957–963, 10 2011. doi: 10.1164/rccm.201104-0655OC.

[274] Bassis, C. M., Erb-Downward, J. R., Dickson, R. P., *et al.* Analysis of the Upper Respiratory Tract Microbiotas as the Source of the Lung and Gastric Microbiotas in Healthy Individuals. *mBio*, 6, 5 2015. doi: 10.1128/mBio.00037-15.

[275] Dickson, R. P., Erb-Downward, J. R., Freeman, C. M., *et al.* Bacterial Topography of the Healthy Human Lower Respiratory Tract. *mBio*, 8, 3 2017. doi: 10.1128/mBio.02287-16.

[276] Segal, L. N., Alekseyenko, A. V., Clemente, J. C., *et al.* Enrichment of lung microbiome with supraglottic taxa is associated with increased pulmonary inflammation. *Microbiome*, 1:19, 12 2013. doi: 10.1186/2049-2618-1-19.

[277] Huxley, E. J., Viroslav, J., Gray, W. R., and Pierce, A. K. Pharyngeal aspiration in normal adults and patients with depressed consciousness. *The American Journal of Medicine*, 64:564–568, 4 1978. doi: 10.1016/0002-9343(78)90574-0.

[278] Thibeault, C., Suttorp, N., and Opitz, B. The microbiota in pneumonia: From protection to predisposition. *Science Translational Medicine*, 13:1–12, 2021. doi: 10.1126/SCITRANSLMED.ABA0501.

[279] Liu, Q., Liu, Q., Meng, H., *et al.* Staphylococcus epidermidis Contributes to Healthy Maturation of the Nasal Microbiome by Stimulating Antimicrobial Peptide Production. *Cell Host and Microbe*, 27:68–78.e5, 1 2020. doi: 10.1016/j.chom.2019.11.003.

[280] Biesbroek, G., Tsivtsivadze, E., Sanders, E. A. M., *et al.* Early respiratory microbiota composition determines bacterial succession patterns and respiratory health in children. *American journal of respiratory and critical care medicine*, 190:1283–92, 12 2014. doi: 10.1164/rccm.201407-1240OC.

[281] Pattaroni, C., Watzenboeck, M. L., Schneidegger, S., *et al.* Early-Life Formation of the Microbial and Immunological Environment of the Human Airways. *Cell Host and Microbe*, 24:857–865.e4, 12 2018. doi: 10.1016/j.chom.2018.10.019.

[282] Whelan, F. J., Verschoor, C. P., Stearns, J. C., *et al.* The Loss of Topography in the Microbial Communities of the Upper Respiratory Tract in the Elderly. *Annals of the American Thoracic Society*, 11:513–521, 5 2014. doi: 10.1513/AnnalsATS.201310-351OC.

[283] de Steenhuijsen Piters, W. A. A., Huijskens, E. G. W., Wyllie, A. L., *et al.* Dysbiosis of upper respiratory tract microbiota in elderly pneumonia patients. *The ISME Journal*, 10:97–108, 1 2016. doi: 10.1038/ismej.2015.99.

[284] Bäckhed, F., Roswall, J., Peng, Y., *et al.* Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell host and microbe*, 17:852, 6 2015. doi: 10.1016/j.chom.2015.05.012.

[285] Bokulich, N. A., Chung, J., Battaglia, T., *et al.* Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Science translational medicine*, 8:343ra82, 6 2016. doi: 10.1126/scitranslmed.aad7121.

[286] Stewart, C. J., Ajami, N. J., O'Brien, J. L., *et al.* Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*, 562:583–588, 10 2018. doi: 10.1038/s41586-018-0617-x.

[287] Shao, Y., Forster, S. C., Tsaliki, E., *et al.* Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature*, 574:117–121, 10 2019. doi: 10.1038/s41586-019-1560-1.

[288] Claesson, M. J., Jeffery, I. B., Conde, S., *et al.* Gut microbiota composition correlates with diet and health in the elderly. *Nature*, 488:178–84, 8 2012. doi: 10.1038/nature11319.

[289] Odamaki, T., Kato, K., Sugahara, H., *et al.* Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC microbiology*, 16:90, 5 2016. doi: 10.1186/s12866-016-0708-5.

[290] Negi, S., Das, D. K., Pahari, S., *et al.* Potential Role of Gut Microbiota in Induction and Regulation of Innate Immune Memory. *Frontiers in immunology*, 10:2441, 2019. doi: 10.3389/fimmu.2019.02441.

[291] Lavelle, A. and Sokol, H. Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. *Nature Reviews Gastroenterology and Hepatology*, 17:223–237, 4 2020. doi: 10.1038/s41575-019-0258-z.

[292] Tremblay, S., Romain, G., Roux, M., *et al.* Bile Acid Administration Elicits an Intestinal Antimicrobial Program and Reduces the Bacterial Burden in Two Mouse Models of Enteric Infection. *Infection and Immunity*, 85, 6 2017. doi: 10.1128/IAI.00942-16.

[293] Jacobson, A., Lam, L., Rajendram, M., *et al.* A Gut Commensal-Produced Metabolite Mediates Colonization Resistance to Salmonella Infection. *Cell Host and Microbe*, 24:296–307.e7, 8 2018. doi: 10.1016/j.chom.2018.07.002.

[294] Winston, J. A. and Theriot, C. M. Impact of microbial derived secondary bile acids on colonization resistance against Clostridium difficile in the gastrointestinal tract. *Anaerobe*, 41:44–50, 10 2016. doi: 10.1016/j.anaerobe.2016.05.003.

[295] Peng, L., Li, Z.-R., Green, R. S., *et al.* Butyrate Enhances the Intestinal Barrier by Facilitating Tight Junction Assembly via Activation of AMP-Activated Protein Kinase in Caco-2 Cell Monolayers. *The Journal of Nutrition*, 139:1619–1625, 9 2009. doi: 10.3945/jn.109.104638.

[296] Qiao, Y., Qian, J., Lu, Q., *et al.* Protective effects of butyrate on intestinal ischemia–reperfusion injury in rats. *Journal of Surgical Research*, 197:324–330, 8 2015. doi: 10.1016/j.jss.2015.04.031.

[297] Feng, Y., Wang, Y., Wang, P., *et al.* Short-Chain Fatty Acids Manifest Stimulative and Protective Effects on Intestinal Barrier Function Through the Inhibition of NLRP3 Inflammasome and Autophagy. *Cellular Physiology and Biochemistry*, 49:190–205, 2018. doi: 10.1159/000492853.

[298] Descamps, H. C., Herrmann, B., Wiredu, D., and Thaiss, C. A. The path toward using microbial metabolites as therapies. *EBioMedicine*, 44:747–754, 6 2019. doi: 10.1016/j.ebiom.2019.05.063.

[299] Sun, M., Wu, W., Chen, L., *et al.* Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. *Nature Communications*, 9:3555, 9 2018. doi: 10.1038/s41467-018-05901-2.

[300] Hakansson, A. P., Orihuela, C. J., and Bogaert, D. Bacterial-Host Interactions: Physiology and Pathophysiology of Respiratory Infection. *Physiological Reviews*, 98:781–811, 4 2018. doi: 10.1152/physrev.00040.2016.

[301] Albrich, W. C., Madhi, S. A., Adrian, P. V., *et al.* Use of a Rapid Test of Pneumococcal Colonization Density to Diagnose Pneumococcal Pneumonia. *Clinical Infectious Diseases*, 54:601–609, 3 2012. doi: 10.1093/cid/cir859.

[302] Vissing, N. H., Chawes, B. L. K., and Bisgaard, H. Increased Risk of Pneumonia and Bronchiolitis after Bacterial Colonization of the Airways as Neonates. *American Journal of Respiratory and Critical Care Medicine*, 188:1246–1252, 11 2013. doi: 10.1164/rccm.201302-0215OC.

[303] Man, W. H., van Houten, M. A., Mérelle, M. E., *et al.* Bacterial and viral respiratory tract microbiota and host characteristics in children with lower respiratory tract infections: a matched case-control study. *The Lancet Respiratory Medicine*, 7:417–426, 5 2019. doi: 10.1016/S2213-2600(18)30449-1.

[304] van Deursen, A. M., van den Bergh, M. R., and Sanders, E. A. Carriage of Streptococcus pneumoniae in asymptomatic, community-dwelling elderly in the Netherlands. *Vaccine*, 34:4–6, 1 2016. doi: 10.1016/j.vaccine.2015.11.014.

[305] Thevaranjan, N., Whelan, F. J., Puchta, A., *et al.* Streptococcus pneumoniae Colonization Disrupts the Microbial Community within the Upper Respiratory Tract of Aging Mice. *Infection and Immunity*, 84:906–916, 4 2016. doi: 10.1128/IAI.01275-15.

[306] Pettigrew, M. M., Laufer, A. S., Gent, J. F., *et al.* Upper Respiratory Tract Microbial Communities, Acute Otitis Media Pathogens, and Antibiotic Use in Healthy and Sick Children. *Applied and Environmental Microbiology*, 78:6262–6270, 9 2012. doi: 10.1128/AEM.01051-12.

[307] Bomar, L., Brugger, S. D., Yost, B. H., *et al.* Corynebacterium accolens Releases Antipneumococcal Free Fatty Acids from Human Nostril and Skin Surface Triacylglycerols. *mBio*, 7, 3 2016. doi: 10.1128/mBio.01725-15.

[308] Santagati, M., Scillato, M., Patanè, F., *et al.* Bacteriocin-producing oral streptococci and inhibition of respiratory pathogens. *FEMS Immunology and Medical Microbiology*, 65:23–31, 6 2012. doi: 10.1111/j.1574-695X.2012.00928.x.

[309] de Steenhuijsen Piters, W. A. A., Jochems, S. P., Mitsi, E., *et al.* Interaction between the nasal microbiota and S. pneumoniae in the context of live-attenuated influenza vaccine. *Nature Communications*, 10:2981, 7 2019. doi: 10.1038/s41467-019-10814-9.

[310] Marsland, B. J. and Gollwitzer, E. S. Host–microorganism interactions in lung diseases. *Nature Reviews Immunology*, 14:827–835, 12 2014. doi: 10.1038/nri3769.

[311] Maschirow, L., Suttorp, N., and Opitz, B. Microbiota-Dependent Regulation of Antimicrobial Immunity in the Lung. *American Journal of Respiratory Cell and Molecular Biology*, 61:284–289, 9 2019. doi: 10.1165/rcmb.2019-0101TR.

[312] Clarke, T. B., Davis, K. M., Lysenko, E. S., *et al.* Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nature Medicine*, 16:228–231, 2010. doi: 10.1038/nm.2087.

[313] Ichinohe, T., Pang, I. K., Kumamoto, Y., *et al.* Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National Academy of Sciences*, 108:5354–5359, 3 2011. doi: 10.1073/pnas.1019378108.

[314] Fagundes, C. T., Amaral, F. A., Vieira, A. T., *et al.* Transient TLR Activation Restores Inflammatory Response and Ability To Control Pulmonary Bacterial Infection in Germfree Mice. *The Journal of Immunology*, 188:1411–1420, 2 2012. doi: 10.4049/jimmunol.1101682.

[315] Gauguet, S., D'Ortona, S., Ahnger-Pier, K., *et al.* Intestinal Microbiota of Mice Influences Resistance to Staphylococcus aureus Pneumonia. *Infection and Immunity*, 83:4003–4014, 10 2015. doi: 10.1128/IAI.00037-15.

[316] Schuijt, T. J., Lankelma, J. M., Scicluna, B. P., *et al.* The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. *Gut*, 65:575–583, 2016. doi: 10.1136/gutjnl-2015-309728.

[317] Brown, R. L., Sequeira, R. P., and Clarke, T. B. The microbiota protects against respiratory infection via GM-CSF signaling. *Nature Communications*, 8:1512, 11 2017. doi: 10.1038/s41467-017-01803-x.

[318] Robak, O. H., Heimesaat, M. M., Kruglov, A. A., *et al.* Antibiotic treatment-induced secondary IgA deficiency enhances susceptibility to Pseudomonas aeruginosa pneumonia. *Journal of Clinical Investigation*, 128:3535–3545, 2018. doi: 10.1172/JCI97065.

[319] Trompette, A., Gollwitzer, E. S., Yadava, K., *et al.* Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature Medicine*, 20:159–166, 2 2014. doi: 10.1038/nm.3444.

[320] Galvão, I., Tavares, L. P., Corrêa, R. O., *et al.* The Metabolic Sensor GPR43 Receptor Plays a Role in the Control of Klebsiella pneumoniae Infection in the Lung. *Frontiers in Immunology*, 9, 2 2018. doi: 10.3389/fimmu.2018.00142.

[321] Ivanov, I. I., Atarashi, K., Manel, N., *et al.* Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell*, 139:485–498, 10 2009. doi: 10.1016/j.cell.2009.09.033.

[322] McAleer, J. P., Nguyen, N. L. H., Chen, K., *et al.* Pulmonary Th17 Antifungal Immunity Is Regulated by the Gut Microbiome. *The Journal of Immunology*, 197:97–107, 7 2016. doi: 10.4049/jimmunol.1502566.

[323] Gray, J., Oehrle, K., Worthen, G., *et al.* Intestinal commensal bacteria mediate lung mucosal immunity and promote resistance of newborn mice to infection. *Science Translational Medicine*, 9:1–14, 2017. doi: 10.1126/scitranslmed.aaf9412.

[324] Schieber, A. M. P., Lee, Y. M., Chang, M. W., *et al.* Disease tolerance mediated by microbiome E. coli involves inflammasome and IGF-1 signaling. *Science*, 350:558–563, 10 2015. doi: 10.1126/science.aac6468.

[325] Beura, L. K., Hamilton, S. E., Bi, K., *et al.* Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature*, 532:512–516, 4 2016. doi: 10.1038/nature17655.

[326] Rosshart, S. P., Vassallo, B. G., Angeletti, D., *et al.* Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. *Cell*, 171:1015–1028.e13, 2017. doi: 10.1016/j.cell.2017.09.016.

[327] Rosshart, S. P., Herz, J., Vassallo, B. G., *et al.* Laboratory mice born to wild mice have natural microbiota and model human immune responses. *Science*, 365, 2019. doi: 10.1126/science.aaw4361.

[328] Dickson, R. P., Erb-Downward, J. R., Falkowski, N. R., *et al.* The Lung Microbiota of Healthy Mice Are Highly Variable, Cluster by Environment, and Reflect Variation in Baseline Lung Innate Immunity. *American Journal of Respiratory and Critical Care Medicine*, 198:497–508, 8 2018. doi: 10.1164/rccm.201711-2180OC.

[329] Rabes, A. ST2 and IL-33 differentially regulate the innate immune response to Streptococcus pneumoniae. 2014.

[330] Maschirow, L. Nicht-hämatopoetisches IL-33 beeinträchtigt die CCL2- und und IL-22-abhängige Immunantwort in der Pneumokokkenpneumonie. 2019.

[331] Kreymborg, K., Etzensperger, R., Dumoutier, L., *et al.* IL-22 Is Expressed by Th17 Cells in an IL-23-Dependent Fashion, but Not Required for the Development of Autoimmune Encephalomyelitis. *The Journal of Immunology*, 179:8098–8104, 12 2007. doi: 10.4049/jimmunol.179.12.8098.

[332] Jarick, K. J., Topczewska, P. M., Jakob, M. O., *et al.* Non-redundant functions of group 2 innate lymphoid cells. *Nature*, 11 2022. doi: 10.1038/s41586-022-05395-5.

[333] Barner, M., Mohrs, M., Brombacher, F., and Kopf, M. Differences between IL-4Ralpha-deficient and IL-4-deficient mice reveal a role for IL-13 in the regulation of Th2 responses. *Current Biology*, 8:669–672, 1998. doi: 10.1016/s0960-9822(98)70256-8.

[334] Saleh, M., Abdullah, M. R., Schulz, C., *et al.* Following in Real Time the Impact of Pneumococcal Virulence Factors in an Acute Mouse Pneumonia Model Using Bioluminescent Bacteria. *Journal of Visualized Experiments*, 2 2014. doi: 10.3791/51174.

[335] Welte, T., Suttorp, N., and Marre, R. CAPNETZ-community-acquired pneumonia competence network. *Infection*, 32:234–8, 8 2004. doi: 10.1007/s15010-004-3107-z.

[336] Neumann-Podczaska, A., Tobis, S., Antimisiaris, D., *et al.* Polypharmacy in Polish Older Adult Population-A Cross-Sectional Study: Results of the PolSenior Project. *International journal of environmental research and public health*, 19, 1 2022. doi: 10.3390/ijerph19031030.

[337] Torchiano, M. Effsize - a package for efficient effect size computation. *Zenodo*, 2016. doi: 10.5281/zenodo.1480624.

[338] Love, M. I., Huber, W., and Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15, 12 2014. doi: 10.1186/s13059-014-0550-8.

[339] Korotkevich, G., Sukhov, V., Budin, N., *et al.* Fast gene set enrichment analysis. *bioRxiv*, page 060012, 1 2021. doi: 10.1101/060012.

[340] Hao, Y., Hao, S., Andersen-Nissen, E., *et al.* Integrated analysis of multimodal single-cell data. *Cell*, 184:3573–3587.e29, 6 2021. doi: 10.1016/j.cell.2021.04.048.

[341] Korsunsky, I., Millard, N., Fan, J., *et al.* Fast, sensitive and accurate integration of single-cell data with Harmony. *Nature Methods*, 16:1289–1296, 12 2019. doi: 10.1038/s41592-019-0619-0.

[342] Tsou, A. M., Yano, H., Parkhurst, C. N., *et al.* Neuropeptide regulation of non-redundant ILC2 responses at barrier surfaces. *Nature*, 611:787–793, 11 2022. doi: 10.1038/s41586-022-05297-6. Nmur1 receptor for neuromedin is specific for ILC2s and not for other AREG producing type 2 lymphocytes.

[343] Klose, C. S. N., Mahlakõiv, T., Moeller, J. B., *et al.* The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature*, 549:282–286, 9 2017. doi: 10.1038/nature23676.

[344] Yokota, Y., Mansouri, A., Mori, S., *et al.* Development of peripheral lymphoid organs and natural killer cells depends on the helix–loop–helix inhibitor Id2. *Nature*, 397:702–706, 2 1999. doi: 10.1038/17812.

[345] Kotowicz, K., Callard, R. E., Friedrich, K., *et al.* Biological activity of IL-4 and IL-13 on human endothelial cells: functional evidence that both cytokines act through the same receptor. *International immunology*, 8:1915–25, 12 1996. doi: 10.1093/intimm/8.12.1915.

[346] Pinto, S. M., Subbannayya, Y., Rex, D. A. B., *et al.* A network map of IL-33 signaling pathway. *Journal of Cell Communication and Signaling*, 12:615–624, 9 2018. doi: 10.1007/s12079-018-0464-4.

[347] Ouyang, W. and O'Garra, A. IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation. *Immunity*, 50:871–891, 4 2019. doi: 10.1016/j.immuni.2019.03.020.

[348] Ansaldo, E., Farley, T. K., and Belkaid, Y. Control of Immunity by the Microbiota. *Annual Review of Immunology*, 39:449–479, 2021. doi: 10.1146/annurev-immunol-093019-112348.

[349] Wypych, T. P., Wickramasinghe, L. C., and Marsland, B. J. The influence of the microbiome on respiratory health. *Nature Immunology*, 20:1279–1290, 2019. doi: 10.1038/s41590-019-0451-9.

[350] Wolf, A. J. and Underhill, D. M. Peptidoglycan recognition by the innate immune system. *Nature Reviews Immunology*, 18:243–254, 4 2018. doi: 10.1038/nri.2017.136.

[351] Ganal, S. C., Sanos, S. L., Kallfass, C., *et al.* Priming of Natural Killer Cells by Nonmucosal Mononuclear Phagocytes Requires Instructive Signals from Commensal Microbiota. *Immunity*, 37:171–186, 2012. doi: 10.1016/j.immuni.2012.05.020.

[352] Gordon, E. D., Palandra, J., Wesolowska-Andersen, A., *et al.* IL1RL1 asthma risk variants regulate airway type 2 inflammation. *JCI Insight*, 1, 2016. doi: 10.1172/jci.insight.87871.

[353] Ho, J. E., Chen, W.-Y., huei Chen, M., *et al.* Common genetic variation at the IL1RL1 locus regulates IL-33/ST2 signaling. *Journal of Clinical Investigation*, 123:4208–4218, 10 2013. doi: 10.1172/JCI67119.

[354] Angeles-Martínez, J., Posadas-Sánchez, R., Llorente, L., *et al.* The rs7044343 Polymorphism of the Interleukin 33 Gene Is Associated with Decreased Risk of Developing Premature Coronary Artery Disease and Central Obesity, and Could Be Involved in Regulating the Production of IL-33. *PLOS ONE*, 12:e0168828, 1 2017. doi: 10.1371/journal.pone.0168828.

[355] Gudbjartsson, D. F., Bjornsdottir, U. S., Halapi, E., *et al.* Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nature Genetics*, 41:342–347, 2009. doi: 10.1038/ng.323.

[356] Reijmerink, N. E., Postma, D. S., Bruinenberg, M., *et al.* Association of IL1RL1, IL18R1, and IL18RAP gene cluster polymorphisms with asthma and atopy. *Journal of Allergy and Clinical Immunology*, 122:651–654.e8, 9 2008. doi: 10.1016/j.jaci.2008.06.030.

[357] Falahi, S., Mortazavi, S. H. R., Salari, F., *et al.* Association between IL-33 Gene Polymorphism (Rs7044343) and Risk of Allergic Rhinitis. *Immunological Investigations*, 51:29–39, 2022. doi: 10.1080/08820139.2020.1804399.

[358] Yang, S.-H., Seo, H.-S., Woo, J.-H., *et al.* Carboxylicivirga gen. nov. in the family Marinilabiliaceae with two novel species, Carboxylicivirga mesophila sp. nov. and Carboxylicivirga taeanensis sp. nov., and reclassification of Cytophaga fermentans as Saccharicrinis fermentans gen. nov., comb. nov. *International journal of systematic and evolutionary microbiology*, 64: 1351–1358, 4 2014. doi: 10.1099/ijs.0.053462-0.

[359] Maratea, D. and Blakemore, R. P. Aquaspirillum magnetotacticum sp. nov., a Magnetic Spirillum. *International Journal of Systematic Bacteriology*, 31:452–455, 10 1981. doi: 10.1099/00207713-31-4-452.

[360] Könönen, E. Pigmented Prevotella species in the periodontally healthy oral cavity. *FEMS immunology and medical microbiology*, 6:201–5, 3 1993. doi: 10.1111/j.1574-695X.1993.tb00327.x.

[361] Wu, G. D., Chen, J., Hoffmann, C., *et al.* Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. *Science*, 334:105–108, 10 2011. doi: 10.1126/science.1208344.

[362] Scher, J. U., Sczesnak, A., Longman, R. S., *et al.* Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. *eLife*, 2:e01202, 11 2013. doi: 10.7554/eLife.01202.

[363] Dillon, S. M., Lee, E. J., Kotter, C. V., *et al.* Gut dendritic cell activation links an altered colonic microbiome to mucosal and systemic T-cell activation in untreated HIV-1 infection. *Mucosal immunology*, 9:24–37, 1 2016. doi: 10.1038/mi.2015.33.

[364] Malik, A., Sharma, D., Zhu, Q., *et al.* IL-33 regulates the IgA-microbiota axis to restrain IL-1a-dependent colitis and tumorigenesis. *Journal of Clinical Investigation*, 126:4469–4481, 2016. doi: 10.1172/JCI88625.

[365] Ignacio, A., Shah, K., Bernier-Latmani, J., *et al.* Small intestinal resident eosinophils maintain gut homeostasis following microbial colonization. *Immunity*, 55:1250–1267.e12, 7 2022. doi: 10.1016/j.immuni.2022.05.014.

[366] Le, H. T., Tran, V. G., Kim, W., *et al.* IL-33 priming regulates multiple steps of the neutrophil-mediated anti-Candida albicans response by modulating TLR and dectin-1 signals. *Journal of immunology (Baltimore, Md. : 1950)*, 189:287–95, 7 2012. doi: 10.4049/jimmunol.1103564.

[367] Nelson, M. P., Christmann, B. S., Werner, J. L., *et al.* IL-33 and M2a alveolar macrophages promote lung defense against the atypical fungal pathogen Pneumocystis murina. *Journal of immunology (Baltimore, Md. : 1950)*, 186:2372–81, 2 2011. doi: 10.4049/jimmunol.1002558.

[368] Bacher, P., Hohnstein, T., Beerbaum, E., *et al.* Human Anti-fungal Th17 Immunity and Pathology Rely on Cross-Reactivity against Candida albicans. *Cell*, 176:1340–1355.e15, 3 2019. doi: 10.1016/j.cell.2019.01.041.

[369] Ubeda, C., Lipuma, L., Gobourne, A., *et al.* Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. *The Journal of experimental medicine*, 209:1445–56, 7 2012. doi: 10.1084/jem.20120504.

[370] Qiu, J., Heller, J. J., Guo, X., *et al.* The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. *Immunity*, 36:92–104, 1 2012. doi: 10.1016/j.immuni.2011.11.011.

[371] Pernomian, L., Duarte-Silva, M., and de Barros Cardoso, C. R. The Aryl Hydrocarbon Receptor (AHR) as a Potential Target for the Control of Intestinal Inflammation: Insights from an Immune and Bacteria Sensor Receptor. *Clinical reviews in allergy and immunology*, 59:382–390, 12 2020. doi: 10.1007/s12016-020-08789-3.

[372] Wlodarska, M., Luo, C., Kolde, R., *et al.* Indoleacrylic Acid Produced by Commensal Peptostreptococcus Species Suppresses Inflammation. *Cell Host and Microbe*, 22:25–37.e6, 7 2017. doi: 10.1016/j.chom.2017.06.007.

[373] Zelante, T., Iannitti, R., Cunha, C., *et al.* Tryptophan Catabolites from Microbiota Engage Aryl Hydrocarbon Receptor and Balance Mucosal Reactivity via Interleukin-22. *Immunity*, 39:372–385, 8 2013. doi: 10.1016/j.immuni.2013.08.003.

[374] Lamas, B., Richard, M. L., Leducq, V., *et al.* CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nature Medicine*, 22:598–605, 6 2016. doi: 10.1038/nm.4102.

[375] Klaasen, H. L., der Heijden, P. J. V., Stok, W., *et al.* Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. *Infection and Immunity*, 61:303–306, 1993. doi: 10.1128/iai.61.1.303-306.1993. initial SFB paper.

[376] Roy, U., de Oliveira, R. S., Galvez, E. J. C., *et al.* Induction of IL-22-Producing CD4+ T Cells by Segmented Filamentous Bacteria Independent of Classical Th17 Cells. *Frontiers in Immunology*, 12, 9 2021. doi: 10.3389/fimmu.2021.671331.

[377] Hampton, J. C. and Rosario, B. The attachment of microorganisms to epithelial cells in the distal ileum of the mouse. *Laboratory investigation; a journal of technical methods and pathology*, 14:1464–81, 8 1965.

[378] Klaasen, H., Koopman, J., Poelma, F., and Beynen, A. Intestinal, segmented, filamentous bacteria. *FEMS Microbiology Letters*, 88:165–180, 6 1992. doi: 10.1111/j.1574-6968.1992.tb04986.x.

[379] Geva-Zatorsky, N., Sefik, E., Kua, L., *et al.* Mining the Human Gut Microbiota for Immunomodulatory Organisms. *Cell*, 168:928–943.e11, 2 2017. doi: 10.1016/j.cell.2017.01.022.

[380] Dixon, B. R. E. A., Radin, J. N., Piazuelo, M. B., *et al.* IL-17a and IL-22 Induce Expression of Antimicrobials in Gastrointestinal Epithelial Cells and May Contribute to Epithelial Cell Defense against Helicobacter pylori. *PloS one*, 11:e0148514, 2016. doi: 10.1371/journal.pone.0148514.

[381] Broquet, A., Jacqueline, C., Davieau, M., *et al.* Interleukin-22 level is negatively correlated with neutrophil recruitment in the lungs in a Pseudomonas aeruginosa pneumonia model. *Scientific reports*, 7:11010, 9 2017. doi: 10.1038/s41598-017-11518-0.

[382] Barthelemy, A., Sencio, V., Soulard, D., *et al.* Interleukin-22 Immunotherapy during Severe Influenza Enhances Lung Tissue Integrity and Reduces Secondary Bacterial Systemic Invasion. *Infection and immunity*, 86, 7 2018. doi: 10.1128/IAI.00706-17.

[383] Valeri, M. and Raffatellu, M. Cytokines IL-17 and IL-22 in the host response to infection. *Pathogens and Disease*, 74:1–15, 2016. doi: 10.1093/femspd/ftw111.

[384] Witte, E., Witte, K., Warszawska, K., *et al.* Interleukin-22: A cytokine produced by T, NK and NKT cell subsets, with importance in the innate immune defense and tissue protection. *Cytokine and Growth Factor Reviews*, 21:365–379, 2010. doi: 10.1016/j.cytogfr.2010.08.002.

[385] Kleinschmidt, D., Giannou, A. D., McGee, H. M., *et al.* A Protective Function of IL-22BP in Ischemia Reperfusion and Acetaminophen-Induced Liver Injury. *Journal of immunology (Baltimore, Md. : 1950)*, 199:4078–4090, 12 2017. doi: 10.4049/jimmunol.1700587.

[386] Trevejo-Nunez, G., Elsegeiny, W., Aggor, F. E. Y., *et al.* Interleukin-22 (IL-22) Binding Protein Constrains IL-22 Activity, Host Defense, and Oxidative Phosphorylation Genes during Pneumococcal Pneumonia. *Infection and Immunity*, 87:1–11, 8 2019. doi: 10.1128/IAI.00550-19.

[387] Abood, R. N., Mchugh, K. J., Rich, H. E., *et al.* IL-22-binding protein exacerbates in fl uenza , bacterial super-infection. *Mucosal Immunology*, 2019. doi: 10.1038/s41385-019-0188-7.

[388] Ivanov, I. I., McKenzie, B. S., Zhou, L., *et al.* The Orphan Nuclear Receptor RORgammat Directs the Differentiation Program of Proinflammatory IL-17+ T Helper Cells. *Cell*, 126:1121–1133, 9 2006. doi: 10.1016/j.cell.2006.07.035.

[389] Archer, N. K., Adappa, N. D., Palmer, J. N., *et al.* Interleukin-17A (IL-17A) and IL-17F Are Critical for Antimicrobial Peptide Production and Clearance of Staphylococcus aureus Nasal Colonization. *Infection and Immunity*, 84:3575–3583, 12 2016. doi: 10.1128/IAI.00596-16.

[390] Gomaa, E. Z. Human gut microbiota/microbiome in health and diseases: a review. *Antonie van Leeuwenhoek*, 113:2019–2040, 12 2020. doi: 10.1007/s10482-020-01474-7.

[391] Donaldson, D. D., Whitters, M. J., Fitz, L. J., *et al.* The murine IL-13 receptor alpha 2: molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. *Journal of immunology (Baltimore, Md. : 1950)*, 161:2317–24, 9 1998.

[392] Fichtner-Feigl, S., Strober, W., Kawakami, K., *et al.* IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. *Nature medicine*, 12: 99–106, 1 2006. doi: 10.1038/nm1332.

[393] Brunner, S. M., Schiechl, G., Kesselring, R., *et al.* IL-13 signaling via IL-13Ralpha2 triggers TGF-beta1-dependent allograft fibrosis. *Transplantation research*, 2:16, 10 2013. doi: 10.1186/2047-1440-2-16.

[394] Fujisawa, T., Joshi, B., Nakajima, A., and Puri, R. K. A novel role of interleukin-13 receptor alpha2 in pancreatic cancer invasion and metastasis. *Cancer research*, 69:8678–85, 11 2009. doi: 10.1158/0008-5472.CAN-09-2100.

[395] He, C., Lee, C., Dela Cruz, C., *et al.* Chitinase 3-like 1 Regulates Cellular and Tissue Responses via IL-13 Receptor alpha2. *Cell Reports*, 4:830–841, 8 2013. doi: 10.1016/j.celrep.2013.07.032.

[396] Hazell, G. G. J., Peachey, A. M. G., Teasdale, J. E., *et al.* PI16 is a shear stress and inflammation-regulated inhibitor of MMP2. *Scientific Reports*, 6:39553, 12 2016. doi: 10.1038/srep39553.

[397] Garlanda, C., Bottazzi, B., Bastone, A., and Mantovani, A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annual Review of Immunology*, 23:337–366, 4 2005. doi: 10.1146/annurev.immunol.23.021704.115756.

[398] Richendrfer, H. and Jay, G. D. Lubricin as a Therapeutic and Potential Biomarker in Sepsis. *Critical care clinics*, 36:55–67, 1 2020. doi: 10.1016/j.ccc.2019.08.005.

[399] Yagi, R., Zhong, C., Northrup, D., *et al.* The Transcription Factor GATA3 Is Critical for the Development of All IL-7Ralpha-Expressing Innate Lymphoid Cells. *Immunity*, 40:378–388, 3 2014. doi: 10.1016/j.immuni.2014.01.012.

[400] Faustino, L. D., Griffith, J. W., Rahimi, R. A., *et al.* Interleukin-33 activates regulatory T cells to suppress innate gamma delta T cell responses in the lung. *Nature Immunology*, 21:1371–1383, 9 2020. doi: 10.1038/s41590-020-0785-3.

[401] Ohno, T., Oboki, K., Kajiwara, N., *et al.* Caspase-1, caspase-8, and calpain are dispensable for IL-33 release by macrophages. *Journal of immunology (Baltimore, Md. : 1950)*, 183:7890–7, 12 2009. doi: 10.4049/jimmunol.0802449.

[402] Hsu, C.-L., Neilsen, C. V., and Bryce, P. J. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. *PloS one*, 5:e11944, 8 2010. doi: 10.1371/journal.pone.0011944.

[403] Hsu, C.-L. and Bryce, P. J. Inducible IL-33 Expression by Mast Cells Is Regulated by a Calcium-Dependent Pathway. *The Journal of Immunology*, 189:3421–3429, 10 2012. doi: 10.4049/jimmunol.1201224.

[404] Fock, V., Mairhofer, M., Otti, G. R., *et al.* Macrophage-derived IL-33 is a critical factor for placental growth. *Journal of immunology (Baltimore, Md. : 1950)*, 191:3734–43, 10 2013. doi: 10.4049/jimmunol.1300490.

[405] Shimokawa, C., Kanaya, T., Hachisuka, M., *et al.* Mast Cells Are Crucial for Induction of Group 2 Innate Lymphoid Cells and Clearance of Helminth Infections. *Immunity*, 46:863–874.e4, 5 2017. doi: 10.1016/j.immuni.2017.04.017.

[406] BroadInstitute, B. Broad Institute single cell database - https://singlecell.broadinstitute.org/single_cell, 2023.

[407] Cayrol, C. and Girard, J. P. Interleukin-33 (IL-33): A critical review of its biology and the mechanisms involved in its release as a potent extracellular cytokine. *Cytokine*, 156, 8 2022. doi: 10.1016/j.cyto.2022.155891.

[408] Burton, O. IL-33 Antibody review - https://www.rndsystems.com/products/mouse-il-33-pe-conjugated-antibody-396118_ic3626p#product-reviews, 2016.

List of Abbreviations

AEC alveolar epithelial cell

AEC1 type I alveolar epithelial cell

AEC2 type II alveolar epithelial cell

AhR aryl hydrocarbon receptor

AIM2 absent in melanoma 2

AM alveolar macrophage

AMP antimicrobial peptide

AREG amphiregulin

ASC apoptosis-associated speck-like protein containing a CARD

ATP adenosine triphosphate

BALF bronchoalveolar lavage fluid

BMM bone marrow-derived macrophage

CAP community-acquired pneumonia

CCL C-C motif ligand

CD cluster of differentiation

CFU colony forming units

cGAS cyclic GMP-AMP synthase

CLR C-type lectin recptor

CTL cytotoxic T lymphocyte

CXCL C-X-C motif ligand

DAMP damage-associated molecular pattern

DC dendritic cell

DEG differentially expressed gene

DNA desoxyribonucleic acid

ds double-stranded

ELISA enzyme-linked immunosorbent assay

FACS fluorescence Activated Cell Sorting

FCS fetal calf serum

FMT fecal microbiota transplant

FOXP3 forkhead box p3

GF germ-free

GM-CSF granulocyte-macrophage colony-stimulating factor

GPR43 G-protein-coupled receptor 43

GSEA gene set enrichment analysis

h.p.i. hours post infection

HAP hospital-acquired pneumonia

iE-DAP γ-D-glutamyl-meso-diaminopimelic acid

IFN interferon

IL interleukin

IL-1RAcP IL-1 receptor accessory protein

IL-22BP IL-22 binding protein

IL-TIF IL-10-related T cell-derived inducible factor

ILC innate lymphoid cell

IM inflammatory monocyte

IRF interferon regulated factor

JAK just another kinase

LPS lipopolysaccharide

LRTI lower respiratory tract infection

M1-like classically activated

M2-like alternatively activated

MAPK mitogen-activated protein kinase

MDP muramyl dipeptide

MHC major histocompatibility complex

mLu murine lung tissue

MSA murine serum albumin

MyD88 myeloid differentiation primary response 88

NES normalized enrichment score

NET neutrophil extracellular trap

NF-kB nuclear factor kappa-light-chain-enhancer of activated B cells

NK cell natural killer cell

NKT cell natural killer T cell

NLR NOD-like receptor

NLRP NLR family pyrin domain containing

NOD nucleotide-binding oligomerization

padj adjusted p-value

PAMP pathogen-associated molecular pattern

PBS phosphat buffered saline

PCV pneumococcal conjugate vaccine

PFA paraformaldehyde

PLY pneumolysin

PMN polymorphonuclear neutrophil

PPADS pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid

PPSV pneumococcal polysaccharide vaccine

PRR pattern recognition receptor

qPCR quantitative real-time polymerase chain reaction

RBC red blood cell

rIL-22 recombinant IL-22

RLR RIG-I-like receptor

RNA ribonucleic acid

RNS reactive nitrogen species

RORγt RAR-related orphan receptor gamma

ROS reactive oxygen species

rpm revolutions per minute

S. pneumoniae *Streptococcus pneumoniae*

SCFA short-chain fatty acid

scRNAseq Single-cell RNA sequencing

SFB segmented filamentous bacteria

SIGIRR single Ig IL-1 receptor-related molecule

SNN shared nearest neighbours

SNP single nucleotide polymorphisms

SPF special pathogen-free facility

sST2 soluble ST2

ST2 supression of tumorigenicity 2

STAT signal transducer and activator of transcription

STING stimulator of interferon genes

T_{h17} cell T helper 17 cell

T_{h1} cell T helper 1 cell

T_{h2} cell T helper 2 cell

T_h cell T helper cell

T_{reg} cell regulatory T cell

TIR Toll-like/IL-1 receptor

TLR Toll-like receptor

TNF tumor necrosis factor

TRAF TNF receptor associated factor

TSLP thymic stromal lymphoprotein

TYK tyrosine kinase

UMAP uniform manifold approximation and projection

WT wild type

List of Figures

List of Tables