Modulation of Host Immune Response by *Staphylococcus aureus* Serine Protease-Like Proteins: Impact on Allergy and Infection

I N A U G U R A L D I S S E R T A T I O N

zur

Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Universität Greifswald

vorgelegt von

Maria Nordengrün
geboren am 21.09.1990
in Grevesmühlen

Greifswald, 22. März 2021

Dekan: Prof. Dr. Gerald Kerth

1. Gutachterin: Prof. Dr. Barbara M. Bröker

2. Gutachterin: Prof. Dr. Bettina Löffler

Tag der Promotion: 28. Juli 2021

"Science does not know its debt
to imagination."

- Ralph Waldo Emerson

TABLE OF CONTENT

OUTLINE OF THIS THESIS

Our modern understanding of the hygiene hypothesis is that bacteria are not only the cause of disease but also essential for a healthy immune response and regulation. Varied microbial exposure prenatally and in early childhood protects us from pathological immune reactions such as autoimmune diseases and allergies. Against this background, the hypothesis that bacteria can act as allergens appears paradoxical. Nevertheless, there is growing evidence that *Staphylococcus aureus* (*S. aureus)* is associated with allergic reactions and Spls produced by *S. aureus* have been identified as pacemakers of allergic reactions. The aim of this thesis was to elucidate the underlying mechanisms and the possible clinical relevance of this observation.

The objectives of the present thesis were

i) to shed light on the mechanisms underlying allergy induction by the Spls of *S. aureus*
ii) to analyze the Spl-specific immune response in relevant patient cohorts
iii) to correlate the findings with clinical parameters and
iv) to pave the way for future research on bacterial allergens

Chapter 1 provides an overview about the historical understanding of disease and pathological immune reactions such as allergy. It illustrates the current understanding of the hygiene hypothesis, which helps us to understand the clinical relevance of *S. aureus* as a commensal, pathogen and allergen.

Preliminary work identified *S. aureus* Spls as allergens, showing a Th2-biased immune response in healthy and asthmatic individuals as well as the Spls' capability to induce allergic inflammation *de novo* in a murine model of allergic asthma. To open prospects for treatment or causal therapy in patients at risk, the underlying mechanism of allergy induction was studied, which is presented in **Chapter 2**. Focusing on the IL-33 pathway of allergic airway inflammation, the study showed that Spls induce allergic airway inflammation via the IL-33/ST2 axis.

As part of this inflammatory cascade, dendritic cells (DCs) come into play. As producers of or responders to IL-33, these cells could initiate an allergic response against *S. aureus* and drive the immune reaction away from an anti-bacterial response toward allergic inflammation. The complex interplay of *S. aureus* and dendritic cells is summarized in **Chapter 3**.

While the causes of allergy induction by *S. aureus* Spls were addressed by investigating the underlying mechanisms, the consequences of this are also of interest: Does the pro-allergenic response to *S. aureus* affect patients exposed to *S. aureus* in their airways? **Chapter 4** therefore describes studies on the humoral and cellular immune response against Spls in cystic fibrosis patients who are more frequently colonized with *S. aureus* than the healthy population and suffer from frequent recurrent airway infections. Also, in this patient cohort a Th2-shift of the Spl-specific immune response became evident, which might impede antibacterial clearance and worsen the clinical picture. Larger clinical studies are needed to validate this notion by correlating the anti-*S. aureus* immune response with clinical parameters and testing new therapy options.

These results and findings shed light on a novel, possibly underestimated facet of the immune response against *S. aureus* and give impetus for further research on bacterial allergens in general, reaching beyond the species *S. aureus*. **Chapter 5** summarizes the current state of knowledge and the spectrum of methods available for this purpose.

1 INTRODUCTION

THE DISCOVERY OF BACTERIA AS A CAUSE OF INFECTION

" Everything was questioned, everything was unexplained, everything was doubtful, only the large number of dead was an undoubted reality." – Ignaz Semmelweis

Since the beginning of time, disease has threatened humankind. In order to reduce suffering and prolong life, medical research strived to understand the causes and development of diseases. Although detailed descriptions of infectious diseases can already be found in the literature of antiquity, precise knowledge about the origin of infectious diseases and bacteria as causative agents has only been gained in the last 150 years.

In ancient times, pathological bodily fluids were thought to be the cause of diseases. Hippocrates of Kos founded humoral pathology, the science of the body's juices, which considered a correct mixture or composition of the four bodily fluids – blood, yellow bile, black bile and mucus – a prerequisite for health.[1,2] Medical treatments aimed at restoring the balance and removing harmful substances from the body. Adherents of Hippocrates' doctrine believed that pathogenic substances in the air - so-called "miasmas" (Greek: míasma =

impurity) - were the actual triggers of disease. This theory was still taught until the 19th century.[3]

In 1677 Antoni van Leeuwenhoek was the first to observe bacteria under a light microscope and described three forms: Bacillus, coccus and spiral.[4] But it was not until about 200 years later that Robert Koch succeeded in proving the connection between microorganisms and disease. He described the life cycle of *Bacillus anthracis*, the anthrax pathogen, and showed that the transmission of bacteria to healthy individuals can cause disease.[5] Koch is thus the founder of bacteriology and his postulates – albeit modified – have survived to this day. From then on, Koch's school had many successes in the isolation and identification of pathogens and placed great emphasis on hygiene measures in public health: In 1861, Ignaz Semmelweis demonstrated the importance of hygiene and anti- and especially asepsis was increasingly introduced into medical practice in the following years by means of the prophylaxis of childbed fever.[6] Hygienic measures to reduce infectious germs and to prevent transmission from person to person drastically reduced infection rates and deaths.

Already in 1796 Edward Jenner had reached a milestone of a different strategy for infection control: prevention of infection by exposure. He inoculated a boy with cowpox, which protected him against symptoms from a subsequent smallpox inoculation, the current, high-risk preventive measure against smallpox. Jenner thus significantly improved the principle of preventing infections through immunization. He named his immunogenic agent vaccine (from Latin *vacca* "cow").[7] Many other vaccines were developed in the following decades.

Finally, at the beginning of the 20th century, the treatment of infectious diseases underwent another revolution: the discovery of the antibacterial effect of sulfonamides by Gerhard Domagk and of penicillin by Alexander Fleming made causal therapy of infections possible for the first time in human history.[8,9]

By the middle of the 20th century, medicine attributed a clear role to bacteria: bacteria make us sick. And people felt that they were prepared for this threat: hygiene, vaccinations and antibiotics promised prevention, therapy and cure.

FROM FOE TO FRIEND – BACTERIA PROTECT AGAINST ALLERGY

Both the frequency and intensity of epidemics have strongly decreased in recent decades. This is true even considering the current Covid-19 pandemic. The success in controlling infections is due to the unprecedented medical achievements of the 19th and 20th

centuries, which have given us vaccines, antibiotics, better hygiene and the modern medical infrastructure. A prime example is the success of the worldwide vaccination campaign against smallpox: Smallpox was considered the most dangerous human disease due to its high lethality and virulence. In 1967, 15 million people were still infected and 2 million died of the disease.[10] On May 8, 1980, the WHO declared the complete eradication of smallpox.[11] The victory was so comprehensive that the WHO has since discontinued smallpox vaccination.

Today, we vaccinate children at an early age against numerous potentially fatal bacterial and viral infections such as tetanus, diphtheria or poliomyelitis. Infant mortality has reached an all-time low: today less than 5 percent of children die before reaching adulthood.[12] Some former scourges of humankind can be considered defeated.

However, since the 1950s other diseases have become more common: allergies. In the past decades, the incidences of asthma, hay fever or food allergies have risen continuously, particularly in developed countries. At the end of the 1990s, scientists therefore assumed that there was a connection. In 1989, in his publication "Hay fever, hygiene, and household size", David P. Strachan published his observation that children with more siblings were less affected by hay fever or atopic eczema and attributed this to more infections in early childhood due to unhygienic contact with siblings.[13] This publication marks the birth of the hygiene hypothesis.

About 10 years later, it was shown that early contacts with many children in a crèche were inversely associated with the occurrence of hay fever in later life.[14,15] Within the framework of the original hygiene hypothesis these epidemiological findings were interpreted as a consequence of higher exposure to germs and parasites in early childhood, causing more frequent infections. In connection with the increase of hay fever and asthma during the last decades, the decrease in family size, the decrease of parasitic infestation as well as the structural change of agricultural enterprises and the related reduction of exposure to infectious germs were discussed.

Knowledge of immunological processes grew steadily, and so a mechanism underlying this phenomenon was soon hypothesized: Bacteria activate Th1 cells. Less contact to bacterial stimuli results in a lower stimulation of the Th1 response and thus a compensatory increased Th2 response, the hallmark of allergic reactions. The immune response was attributed the two extreme forms Th1 or Th2. The absence of one causes the predominance of the other.[16,17]

IT'S THE BALANCE THAT MATTERS – THE MODIFIED HYGIENE HYPOTHESIS

In the years after its publication, new findings questioned the hygiene hypothesis in its original form. In less-developed countries, parasite infestation is common, which is also associated with increased Th2 activity. Nevertheless, this is associated with fewer allergies and was even shown to be protective in the mouse model.[18] In addition, epidemiological studies on autoimmune diseases, such as type 1 diabetes, chronic inflammatory bowel disease or multiple sclerosis, also showed a dramatic increase in incidence in the last decades (Figure 1).[19] Autoimmune diseases can be characterized by an increased Th1 response rather than a decreased one.

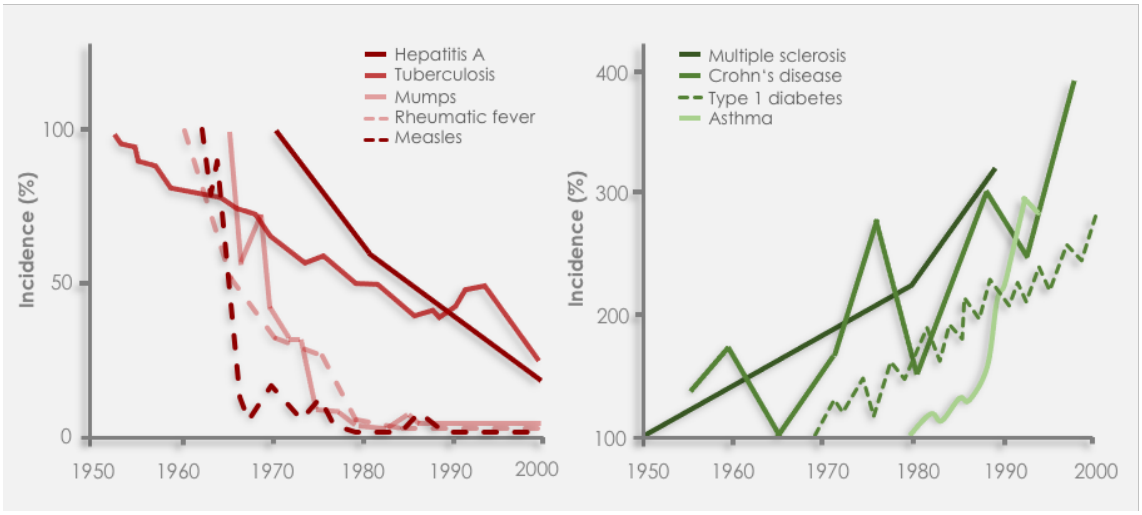


Figure 1: Inverse relation between the incidence of infectious diseases and the incidence of immune disorders from 1950 to 2000. Adapted from Bach *et al.*[19]

How can this apparent contradiction be explained? The immune system can tailor its effector functions to optimally respond to any immunogenic stimulus. As theoretical construct, three main types of immune effector modules can be distinguished, consisting of different effector T-cell and innate lymphoid cell (ILC) lineages: Type 1 immunity consists of T-bet$^+$ IFN-γ–producing group 1 ILCs (ILC1 and natural killer cells), CD8$^+$ cytotoxic T cells (T_C1), and CD4$^+$ Th1 cells.[20] It is biased towards cell-mediated cytotoxicity, protecting against intracellular pathogens. Type 2 immunity consists of GATA-3$^+$ ILC2s, T_C2 cells, and Th2 cells producing IL-4, IL-5, and IL-13.[20] Characteristic for type 2 inflammation are anti-parasite

activities involving the activation of mast cells, basophils and eosinophils, as well as IgE production by B cells.[21] However, Th2 polarization following release of the alarmin IL-33 can also cause considerable tissue damage and re-organization leading to fibrosis.[22] Type 3 immunity is mediated by RORα and/or RORγt$^+$ (retinoic acid–related orphan receptor γt) ILC3s, T_C17 cells, and Th17 cells producing IL-17, IL-22, or both, which recruit neutrophils and induce epithelial antimicrobial responses.[20,23] Type 3 immunity has an important function in protection against extracellular bacteria and some fungi.

But polarization of the immune system can also lead to pathological conditions: type 1 and type 3 immunity mediate autoimmune diseases, whereas type 2 responses can cause allergic diseases.[16,17] However, these effector functions are balanced by solid mechanisms of tolerance and anti-inflammation. Whether an endogenous or exogenous target cell is attacked or tolerated is decided in a complex control system in which regulatory T cells (Treg) play a key role. Tregs limit immunopathology by suppressing T cell effector responses and thus provide counter regulation. This physiological brake suppresses unwanted or excessive immune reactions against the body's own structures or harmless environmental antigens.

Thus, we are not dealing with a black-and-white situation, with a dead-end polarization towards autoimmune disease (Th1) on one side and allergy (Th2) on the other side, but rather with effector functions of types 1, 2 or 3 on one side that are balanced by regulatory functions on the other. The regulatory T cell response is able to suppress both forms of immune dysregulation, autoimmune and allergic reactions. This is essential for immune tolerance of one's own organism as well as foods, commensals and harmless environmental antigens and indispensable for a functioning immune system that can maintain a state of immune homeostasis.

Our current state of knowledge assumes that these tolerance mechanisms are established in early childhood and even prenatally and are not only based on the occurrence of infections. Instead, the role of commensal microflora in inflammatory homeostasis and immune regulation is coming into the focus: Babies born via Cesarean section experience lower initial colonization with "friendly" bacteria, like *Bifidobacterium*, and take longer time to establish a stable, healthy microbiome. The microbiota of bottle-fed babies also differ from those who were breast-fed and exposed to the bacteria of breast milk. Cesarian-section babies are five times more likely to develop allergies than babies who delivered normally. Breastfeeding was shown to protect against food allergy. In general, exposure to innocuous exogenous and endogenous microorganisms in early life can increase the number and diversity of bacteria in

the microbiome, which can protect against allergic diseases later in life.[24–28] Adding to this, a large number of epidemiological studies confirmed that growing up on a traditional farm with early-life contact with livestock and their fodder is a protective factor again the development of hay fever and asthma.[29–32]

"It's not about just learning what to attack but learning what to tolerate."

— Sally Bloomfield

Erika von Mutius and colleagues have gone on to compare urban and rural communities all over Europe and showed that children who grow up with traditional animal husbandry are significantly less likely to suffer from asthma and allergies than city children. The risk of getting asthma is only half as high on a farm. The probability of getting hay fever is only a third in farm children compared to other adolescents. This so called "farm effect" was observed in several studies. Children who grow up on a farm come into contact with a large number of microbial stimuli, which they inhale with the dust. This stimulates the child's immune system and prevents later pathological reactions.[33] The underlying mechanisms are likely to be numerous. Recently, Mutius and colleagues were able to elucidate one of these mechanisms: Using a mouse model of house dust mite (HDM)-induced asthma they were able to show that endotoxins contained in stable dust protect against allergic reactions by stimulating A20, a ubiquitin-modifying enzyme in the mucous membrane of the airway epithelium. A20 attenuates NF-kB activation by deubiquitinating key signaling intermediates down-stream of TLR, IL-1 receptor, and TNF–family receptors, thus stopping the inflammation cascade. Without A20, the protection failed: A20-deficient mice developed asthma symptoms when exposed to HDM despite daily endotoxin or stable dust intake and also showed increased sensitivity to inhaled HDM.[34] In humans, a variant of A20 correlates with increased susceptibility to asthma and allergy.[35]

Numerous factors seem of importance for inducing tolerance to allergens. Exposure to microbial stimuli very early in life, even *in utero*, is vital, as is the diversity of animal species with which children come into contact. These activate the immune system and are required to establish mature solid immune tolerance in the first place. The underlying mechanisms are

complex and require more research. But it is safe to conclude that bacteria are not only foes but also friends and that contact with them protects us from allergic disorders.

S. AUREUS IN THE CONTEXT OF ALLERGY

According to our current understanding of immune regulation, bacteria are essential for the development of solid tolerance mechanisms and a well-balanced immune response. They elicit primarily Th1-dominated immunity. Against this background it seems almost contradictory to talk about bacteria as triggers of allergies. Nevertheless, there is an increasing number of findings that associate allergic diseases with bacterial pathogens. One of them is *Staphylococcus aureus (S. aureus).*

S. aureus is well known as a commensal that colonizes about 20% of the healthy population without symptoms as well as a dangerous pathogen that can cause a wide range of local to systemic infections. These are an increasing threat to human health and health care systems due to the increased occurrence of antibiotic resistance.[36–38]

However, *S. aureus*, or the proteins it produces, also showed allergenic properties. The first indications resulted from analyzing patients with atopic dermatitis (AD). AD patients have a dramatically increased incidence of viral and bacterial infections.[39] *S. aureus* colonizes the skin of 80-100% of patients with AD and has been shown to play a crucial role in the pathophysiology of AD. The majority of AD patients have IgE antibodies against the *S. aureus* toxins ETA, TSST-1 and SEB and the toxin-specific serum IgE level correlates with the severity of the clinical picture.[40] IgE-mediated immune responses triggered by *S. aureus* toxins are thus assumed to play an important role in the pathophysiology of AD, which Laouini *et al.* confirmed in mouse models.[41]

In addition, there was an accumulation of evidence from the field of allergic respiratory diseases, such as allergic rhinosinusitis, nasal polyps and intrinsic asthma. These diseases are also characterized by an increased rate of colonization with *S. aureus* and are often associated with IgE binding to *S. aureus* superantigens (SAg).[42–44] Here too, the amount of IgE correlates with the severity of the disease. Bachert *et al.* therefore used an anti-IgE therapy (Omalizumab) in patients with nasal polyps and were able to demonstrate clinical efficacy. In the case of intrinsic asthma, they were also able to show that SAg-specific IgE rather than IgE against

common inhaled allergens was associated with a significantly increased risk of having asthma.[45–47]

These findings raised questions about a possible new quality of *S. aureus* as a pathogen: How is the microorganism able to modulate the human immune response and induce allergic reactions with Th2 dominance and production of IgE?

The work of Stentzel *et al.* gave a first answer to this question. They succeeded in identifying a group of allergens produced by *S. aureus*, the six serine protease-like proteins (Spls) A-F.[48] The Spls were named according to their high sequence homology to the staphylococcal serine protease V8, which is encoded by the same operon (ORF-2). In the ORF-2 operon, only one factor-independent termination sequence was detected below SplF, suggesting that the six Spls are co-transcribed. This was confirmed by Reed *et al.* in Northern Blot analyses.[49] The Spls appear to be common within the species *S. aureus*: when examining 167 different isolates, Zdzalik *et al.* were able to detect at least one Spl gene in 84% of *S. aureus* strains.[50]

However, the role of Spl proteases in staphylococcal physiology is not fully understood and the quest for physiological substrates cleaved by them is still ongoing. The substrate specificity of SplA, SplB, SplD and SlpE has been investigated focusing mostly on structural determinants[51–54]. These studies indicate that Spls have a restricted, non-overlapping range of substrate specificities. Using libraries of synthetic peptides and crystallographic models, Zdzalik and colleagues determined the consensus sequence cleaved by SplD. By subsequent BLAST analyses of the human genome, they detected the consensus sequence in a large number of human proteins. These include, in particular, proteins of the olfactory receptor family.[53] Building on *in silico* studies, Paharik and colleagues recently aimed to test cleavage of a putative human SplA substrate and to study the role of the spl operon in virulence. They demonstrated SplA-mediated cleavage of mucin 16, a human cell surface protein that is found on epithelial tissues, identifying the first human host protein cleaved by an Spl. In their rabbit model of pneumonia Spl-deficient mutants induced a more localized disease compared with the wild-type. The authors suggest that SplA-mediated cleavage of mucin 16 contributes to the spread of *S. aureus* in lung tissue.[55] However, infection experiments by Reed and colleagues showed no decisive influence of Spls on the virulence of *S. aureus*.[49]

Examining the human immune response against Spls, Stentzel and colleagues showed that Spls elicit a type 2 biased immune response in healthy individuals. In addition, they detected Spl-specific IgE in sera from asthmatic patients at higher levels than in healthy

individuals. Furthermore, intratracheal application of SplD induced allergic airway inflammation in mice, even without the addition of adjuvants.[48]

These findings identified Spls as pacemakers of allergic reactions and showed a new allergenic quality of the immune response against certain staphylococcal proteins. This is the starting point of the work in this thesis. The aim was to elucidate the underlying mechanisms of allergy induction by Spls and to investigate whether the Th2 shift also affects patients exposed to *S. aureus* and influences disease progression. If this was the case, the finding could help to develop new treatment options.

2

THE IL-33/ST2 AXIS IS CRUCIAL IN TYPE 2 AIRWAY RESPONSES INDUCED BY *STAPHYLOCOCCUS* AUREUS-DERIVED SERINE PROTEASE-LIKE PROTEIN D.

Teufelberger AR, **Nordengrün M**, Braun H, Maes T, De Grove K, Holtappels G, O'Brien C, Provoost S, Hammad H, Gonçalves A, Beyaert R, Declercq W, Vandenabeele P, Krysko DV, Bröker BM, Bachert C, Krysko O.

Published in the Journal of Allergy and Clinical Immunology. 2018 Feb;141(2):549-559.e7. doi: 10.1016/j.jaci.2017.05.004. Epub 2017 May 19.

AUTHOR CONTRIBUTIONS:

As a coauthor in this publication, **MN** had a significant contribution to conceiving and planning of experiments performed in this study as well as the execution of the experiment with wild type mice. **MN** actively participated in the discussion and analysis of the obtained results and in the scientific revision and editing of the final version of this manuscript.

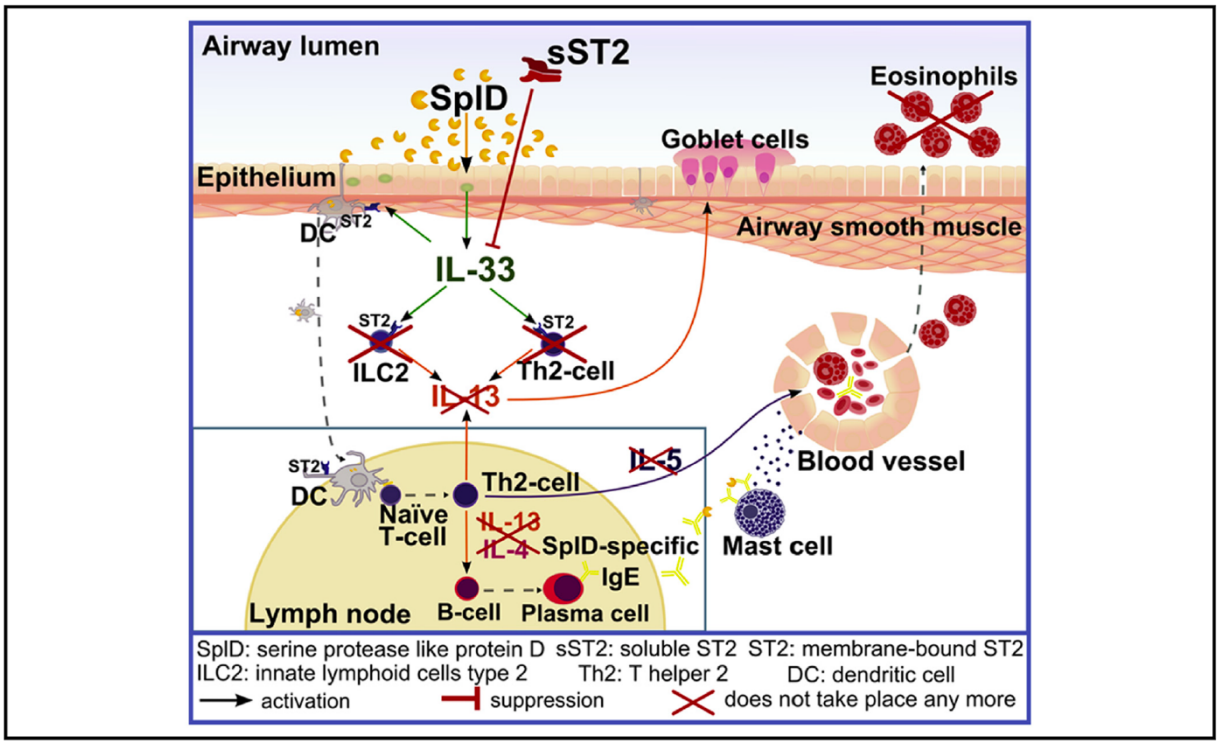
Study conception and design: ART, **MN**, CB and OK
Acquisition of data: ART, **MN**, HB, TM, KDG, GH, SP, HH, AG, RB, OK
Analysis and interpretation of data: ART, **MN**, DVK, BMB, CB and OK
Drafting of manuscript: ART, **MN**, CB and OK
Critical revision and editing: ART, **MN**, DVK, BMB, CB and OK

Maria Nordengrün | Prof. Barbara M. Bröker

The IL-33/ST2 axis is crucial in type 2 airway responses induced by *Staphylococcus aureus*–derived serine protease–like protein D

Andrea R. Teufelberger, MSc,[a,b,c] Maria Nordengrün, MSc,[d] Harald Braun, PhD,[c,e] Tania Maes, PhD,[f] Katrien De Grove, MSc,[f] Gabriele Holtappels, BSc,[a] Clara O'Brien, BSc,[a] Sharen Provoost, PhD,[f] Hamida Hammad, PhD,[c,g] Amanda Gonçalves, MSc,[c,h] Rudi Beyaert, PhD,[c,e] Wim Declercq, PhD,[b,c] Peter Vandenabeele, PhD,[b,c] Dmitri V. Krysko, PhD,[i] Barbara M. Bröker, PhD,[d] Claus Bachert, PhD,[a]* and Olga Krysko, PhD[a]* *Ghent, Belgium, and Greifswald, Germany*

GRAPHICAL ABSTRACT



From [a]the Upper Airways Research Laboratory, Department of Otorhinolaryngology, [c]the Department of Biomedical Molecular Biology, [f]the Laboratory for Translational Research in Obstructive Pulmonary Diseases, and [i]the Department of Basic Medical Sciences, Ghent University; [b]the Molecular Signalling and Cell Death Unit, [e]the Molecular Signal Transduction in Inflammation Unit, [g]Immunoregulation and Mucosal Immunology, and [h]VIB Bio Imaging Core Ghent, VIB Center for Inflammation Research, Ghent; and [d]the Department of Immunology, University Medicine Greifswald.

*These authors contributed equally to this work.

Supported by interuniversity attraction poles P7/30, BOF 14-GOA-019 and by the German Research Council (DFG: GRK 1870, TRR34). A.R.T. is a doctoral fellow paid by FWO-Vlaanderen. R.B. and H.B. are supported by an IOF UGent Advanced project grant (Ghent University) and the Fund for Scientific Research Flanders (FWO-Vlaanderen). S.P. is a postdoctoral researcher paid by FWO-Vlaanderen. M.N. is a doctoral fellow supported by the German Research Council (GRK 1870) and the Exzellenzförderprogramm Mecklenburg-Vorpommern, Germany (Card-ii-Omics). P.V.'s research group is supported by IAP 7/32, FWO G.0875.11, FWO G.0973.11, FWO G.0A45.12N, FWO G.0787.13N, FWO G.0E04.16N, FWO G.0C31.14N, BOF09/01M00709, and BOF16/MET_V/007.

Disclosure of potential conflict of interest: M. Nordengrün's institution received grant DFG RTG1870, which also provided support for travel to meetings for this study, and is employed by University Greifswald (DFG RTG1870). T. Maes personally received funding for travel from GlaxoSmithKline for other works and is a shareholder in Oryzon Genomics. B. Bröker's institution received grants from the German Research Council and Exzellenzföderprogramm Mecklenburg-Vorpommern and she personally received consulting fees from Novartis Pharma GmbH for this work; her institution received grants from grants from German Research Council, payment for lectures from the German Federal Ministry of Education and Research for other works, and payment for manuscript preparation from EU; she is personally employed by University Medicine Greifswald; and she has personally received royalties from Becton Dickinson for the mAb BNI3, which was not used in this study. The rest of the other authors declare that they have no relevant conflicts of interest.

Received for publication August 30, 2016; revised April 26, 2017; accepted for publication May 8, 2017.

Available online May 19, 2017.

Corresponding author: Olga Krysko, PhD, Upper Airways Research Laboratory, Department of Otorhinolaryngology, Ghent University, De Pintelaan 185, UZ Gent, MRB2, Ghent 9000, Belgium. E-mail: olga.krysko@ugent.be.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2017.05.004

Background: Chronic airway inflammatory diseases, such as chronic rhinosinusitis with nasal polyps and asthma, show increased nasal *Staphylococcus aureus* colonization. *Staphylococcus aureus*–derived serine protease–like protein (Spl) D and other closely related proteases secreted by *S aureus* have recently been identified as inducers of allergic asthma in human subjects and mice, but their mechanism of action is largely unknown.

Objective: We investigated the role of recombinant SplD in driving T_H2-biased responses and IgE formation in a murine model of allergic asthma.

Methods: Allergic asthma was induced in C57BL/6 J wild-type mice, Toll-like receptor (TLR) 4 knockout *(Tlr4$^{-/-}$)* mice, and recombination-activating gene (Rag2) knockout *(Rag2$^{-/-}$)* mice by means of repeated intratracheal applications of SplD. Inflammatory parameters in the airways were assessed by means of flow cytometry, ELISA, Luminex, and immunohistochemistry. Serum SplD-specific IgE levels were analyzed by using ELISA.

Results: We observed that repeated intratracheal exposure to SplD led to IL-33 and eotaxin production, eosinophilia, bronchial hyperreactivity, and goblet cell hyperplasia in the airways. Blocking IL-33 activity with a soluble ST2 receptor significantly decreased the numbers of eosinophils, IL-13$^+$ type 2 innate lymphoid cells and IL-13$^+$CD4$^+$ T cells and IL-5 and IL-13 production by lymph node cells but had no effect on IgE production. SplD-induced airway inflammation and IgE production were largely dependent on the presence of the functional adaptive immune system and independent of TLR4 signaling.

Conclusion: The *S aureus*–derived protein SplD is a potent allergen of *S aureus* and induces a T_H2-biased inflammatory response in the airways in an IL-33–dependent but TRL4-independent manner. The soluble ST2 receptor could be an efficient strategy to interfere with SplD-induced T_H2 inflammation but does not prevent the allergic sensitization. (J Allergy Clin Immunol 2018;141:549-59.)

Key words: *Allergy, asthma,* Staphylococcus aureus, *sensitization, serine protease*

Abbreviations used

AECII:	Airway epithelial cell type II
APC:	Allophycocyanin
BALF:	Bronchoalveolar lavage fluid
DC:	Dendritic cells
DMEM:	Dulbecco modified Eagle medium
FACS:	Fluorescence-activated cell sorting
FITC:	Fluorescein isothiocyanate
GFP:	Green fluorescent protein
HDM:	House dust mite
HRP:	Horseradish peroxidase
ILC2:	Type 2 innate lymphoid cell
MLKL:	Mixed lineage kinase domain–like protein
NF-κB:	Nuclear factor κB
OVA:	Ovalbumin
PAR:	Protease-activated receptor
PAS:	Periodic acid–Schiff
PE:	Phycoerythrin
PerCP:	Peridinin-chlorophyll-protein complex
pMLKL:	phosphorylated mixed lineage kinase domain–like protein
ProSPC:	Prosurfactant protein C
Rag2:	Recombination-activating gene
Spl:	*Staphylococcus aureus*–derived serine protease–like protein
sST2:	Soluble ST2 receptor
TLR:	Toll-like receptor
TSLP:	Thymic stromal lymphopoietin
TUNEL:	Terminal deoxynucleotidyl transferase dUTP nick end labeling

Staphylococcus aureus is a versatile germ frequently found colonizing patients with T_H2-biased diseases, such as atopic dermatitis and chronic rhinosinusitis with nasal polyps.[1-5] It actively manipulates the host immune response by releasing proteins that facilitate bacterial invasion and colonization.[6,7] These secreted proteins allow the bacterium to activate virulence and metabolic pathways required for bacterial survival and might exert immunosuppressive action on the mucosal environment.[8,9] Based on *in silico* analyses of the *S aureus* pangenome, it is estimated that the repertoire of secreted proteins comprises more than 1350 proteins, including enterotoxins, toxic shock syndrome toxin 1, and other virulence factors, and for many of these, the function is unknown.[10,11] It is important to understand the interplay between the immune proteome of *S aureus* and the immune response of the host and to elucidate its role in the initiation and persistence of chronic airway diseases. Asthmatic patients have increased specific IgE reactivity to various secreted *S aureus* proteins,[12] and several endotypes of chronic rhinosinusitis were proposed based on the presence of *S aureus*–specific IgE.[5,13]

Recently, we have observed increased levels of *Staphylococcus aureus* serine protease-like protein (Spl)–specific IgE in sera of asthmatic patients, indicating the clinical relevance of these proteases.[14] Spls are a group of 6 *S aureus* proteases (SplA-SplF) that belong to the small subfamily S1B (encompassing staphylococcal V8 protease, epidermolytic toxins, and Spl proteases). Eighty-four percent of *S aureus* strains contain at least 1 Spl protease–encoding gene.[15] Moreover, we could demonstrate that repeated exposure to pure SplD without the addition of any adjuvant results in a T_H2 response and SplD-specific IgE production in mice.[14] However, the exact mechanisms underlying this SplD-induced T_H2 bias are not yet unraveled and are the focus of the current study.

Allergens, such as house dust mite (HDM), cockroach, or *Alternaria alternata*, were shown to play an important role in allergy development in part through activation of cell surface protease-activated receptors (PARs)[16] in the airways, inducing cytokines and cleave intercellular epithelial tight junctions[17] and thereby amplifying the response to allergens. They can also cleave CXCR1 on the surfaces of neutrophils[18] and CD23 and CD25 receptors on immune cells,[19] thereby reinforcing allergy progression.

A key mediator of the type 2 inflammation of the airways is the cytokine IL-33. IL-33 binds to a heterodimeric cell-surface receptor consisting of IL-1 receptor accessory protein and ST2 on immune cells, such as T_H2 cells, type 2 innate lymphoid cells (ILC2s), invariant natural killer T cells, natural killer cells, basophils, eosinophils, mast cells, and dendritic cells (DCs), eventually activating intracellular signaling pathways and supporting allergic airway inflammation.[20-22] Among the 4 known isoforms of ST2, 2 are highly relevant for the regulation of allergic airway

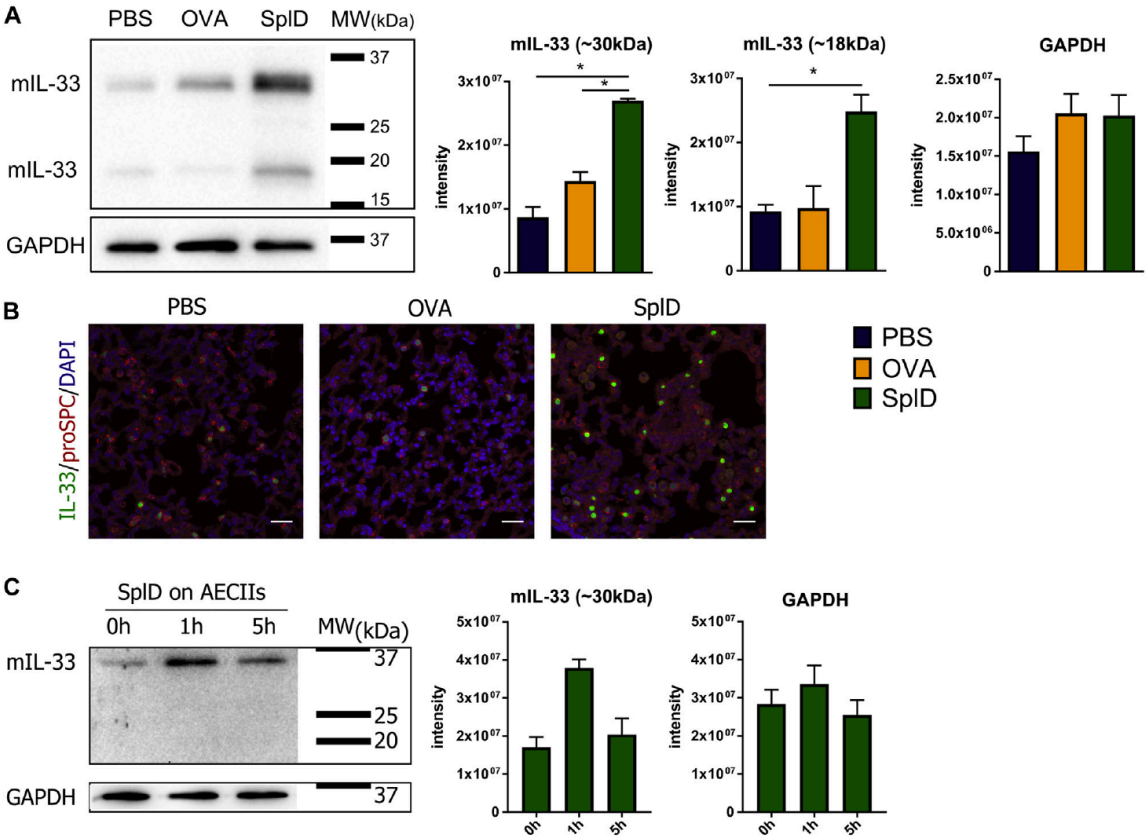


FIG 1. IL-33 upregulation on SpID exposure. **A,** Detection and semiquantitative analysis of full-length and short-form IL-33 in lung homogenates of PBS-, OVA-, or SpID-treated mice by means of Western blotting. Data are presented as means ± SEMs for 4 mice per group. **B,** Costaining of IL-33 (green), AECIIs (proSPC, red), and 4′-6-diamidino-2-phenylindole dihydrochloride (*DAPI*; blue). *Scale bars* = 25 μm. **C,** Western blot detection and semiquantitative analysis of IL-33 in isolated murine primary AECIIs stimulated with 25 μg/mL SpID for 0, 1, and 5 hours. N = 3-5. Results are presented as means ± SEMs. **P* < .05, Mann-Whitney *U* test. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase; *MW*, molecular weight.

inflammation in mice: the soluble ST2 receptor (sST2) and a transmembrane form (ST2).[23] It has been shown that sST2 antagonizes ST2, suppressing the IL-33–mediated activation of the nuclear factor κB (NF-κB) pathway, as well as downstream effects, such as T_H2 cytokine production, thereby attenuating allergic asthma symptoms in mice.[24,25] In this respect sST2 could be a promising therapeutic option for asthma.

In the current work we focused on the molecular mechanisms by which SpID drives allergic asthma in mice. We demonstrated that SpID-induced type 2 cytokine production and eosinophilia in the airways are largely IL-33 dependent, whereas production of SpID-specific IgE and goblet cell metaplasia occur independently of this pathway. The abovementioned effects are mediated by the adaptive immune system.

METHODS

Mice and sensitization protocol

Animals were maintained in a 12-hour/12-hour light/dark cycle and had access to water and food *ad libitum*. Animal experiments were approved by the local ethics committee of Ghent University. Female C57BL/6 J wild-type mice (Janvier, Heverlee, Belgium), Toll-like receptor (TLR) 4 knockout ($Tlr4^{-/-}$) mice and recombination-activating gene (Rag2) knockout ($Rag2^{-/-}$) mice (both kindly provided by VIB-IRC, Ghent, Belgium) were treated after achievement of light anesthesia with isoflurane/air (Ecuphar, Oostkamp, Belgium) 6 times every second day by means of intratracheal applications with either 45 μg of SpID, 45 μg of SpIF, or 100 μg of ovalbumin (OVA; Worthington Biochemical, Lakewood, NJ) in 50 μL of PBS (Gibco, Thermo Fisher Scientific, Erembodegem-Aalst, Belgium) and 1 additional intratracheal application of 50 μL of PBS after each treatment or 50 μL of PBS alone. In some experiments a combination of 45 μg of SpID and 100 μg of OVA was administered 6 times every second day by means of intratracheal application. sST2 was applied intratracheally at a dose of 25 μg/50 μL per mouse after each SpID application instead of the second PBS application. Forty-eight hours after the last intratracheal application, mice were killed with an intraperitoneal injection of 100 μL of Nembutal (Ceva Santé Animale, Libourne, France), and the lungs, serum, bronchoalveolar lavage fluid (BALF) and local draining lymph nodes were collected in each experiment.

Luminex analysis

Concentrations of murine IL-5, IL-13, IL-33, and eotaxin in lung homogenates and lymph node cell supernatants were analyzed by using Luminex Performance assays (R&D Systems, Oxon, United Kingdom). IL-25

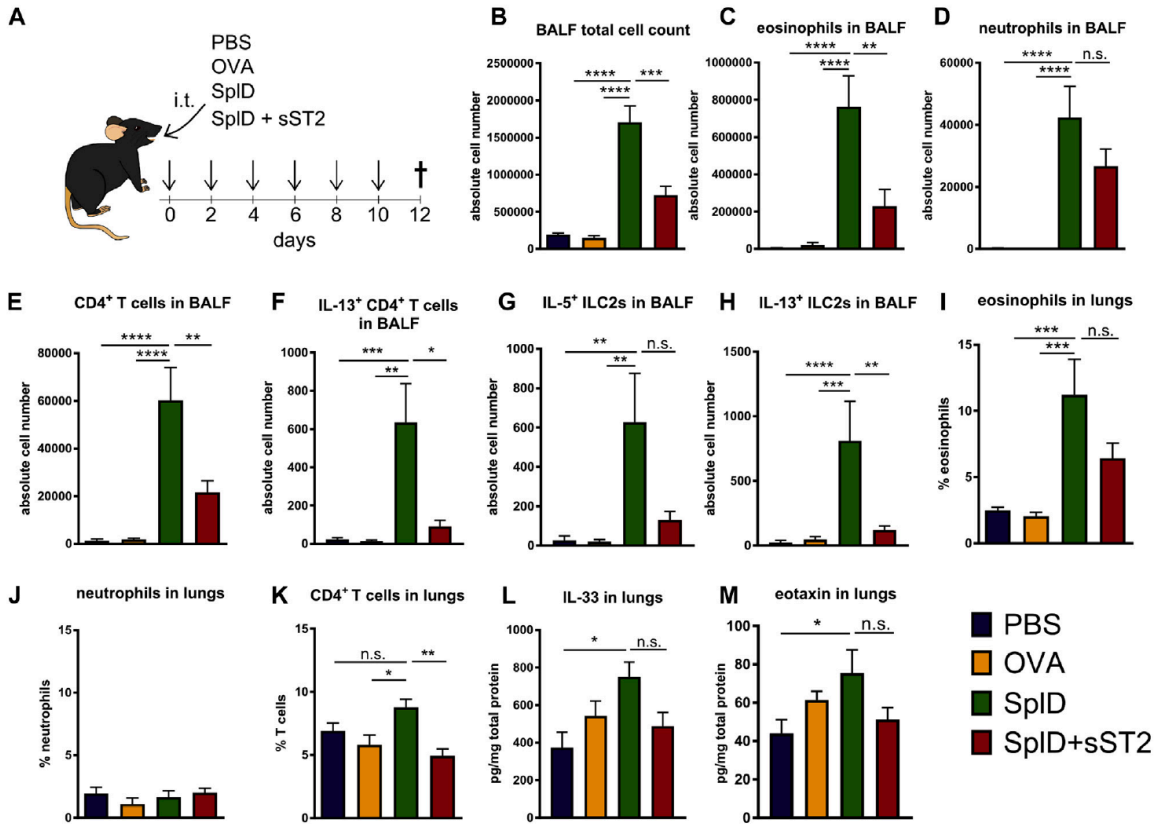


FIG 2. SpID-induced IL-33–dependent T_H2 inflammation. **A,** Application scheme. Different groups received 6 intratracheal *(i.t.)* applications every 48 hours of either PBS, OVA, SpID, or SpID in combination with sST2 and were killed 48 hours after the last application. **B-E,** Total cell count (Fig 2, *B*) and flow cytometric analysis of eosinophils ($CD11c^-Siglec\ F^+CD11b^+$; Fig 2, *C*), neutrophils ($GR1^+CD11b^+F4/80^-$; Fig 2, *D*), and $CD3^+CD4^+$ T cells (Fig 2, *E*) in BALF. **F-H,** IL-13–producing $CD4^+$ T cells (Fig 2, *F*) and IL-5–producing (Fig 2, *G*) and IL-13–producing (Fig 2, *H*) ILC2s in BALF. **I-K,** Eosinophils (Fig 2, *I*), neutrophils (Fig 2, *J*), and $CD4^+$ T cells (Fig 2, *K*) in lungs analyzed by using flow cytometry. **L** and **M,** IL-33 (Fig 2, *L*) and eotaxin (Fig 2, *M*) measurement of lung homogenates by using Luminex. Mean ± SEM values are presented. N = 7-13. $*P < .05$, $**P < .01$, $***P < .001$, and $****P < .0001$, Mann-Whitney *U* test. *n.s.*, Not significant.

and thymic stromal lymphopoietin (TSLP) levels were measured by means of ELISA (R&D Systems, Abington, United Kingdom).

Bronchial hyperreactivity test

Bronchial hyperreactivity in response to increasing doses of carbachol (0, 20, 40, 80, 160, 320, and 640 μg/kg) was measured 48 hours after the last intratracheal application by using the forced oscillation technique (flexiVent System, SCIREQ, Montreal, Quebec, Canada). Neuromuscular blockade was induced by injecting 1 mg/kg pancuronium bromide intravenously. A "snapshot perturbation" maneuver was imposed to measure the resistance of the whole respiratory system (airways, lung, and chest wall).

Flow cytometry

The list of antibodies used in the study and the gating strategy are provided in the Methods section in this article's Online repository at www.jacionline.org.

Statistics

Statistical analysis was performed by using GraphPad Prism version 6 software (GraphPad Software, La Jolla, Calif) and the unpaired nonparametric Mann-Whitney test.

A more detailed description of the materials and methods used in this study can be found in the Methods section in this article's Online Repository.

RESULTS

SpID induces key features of asthma and increased expression of IL-33 in mice

We established a sensitization protocol using C57BL/6 J wild-type mice that received 6 intratracheal applications of 45 μg of SpID. Mice treated with PBS or the inert antigen OVA served as negative control groups. With this model, we previously demonstrated that SpID could attract eosinophils and T cells to the airways, increase T_H2 cytokine production in local draining lymph nodes, and induce formation of SpID-specific IgE.[14] To study the mechanisms of the type 2–biased asthmatic response to SpID, we investigated IL-33 expression in this mouse model. IL-33 is a crucial cytokine in the setting of type 2 allergic inflammation.[20,26] After SpID exposure, mice expressed higher levels of IL-33 in lung homogenates than control mice. Full-length IL-33 and, even more distinctly, the cleaved form of IL-33 were

J ALLERGY CLIN IMMUNOL
VOLUME 141, NUMBER 2

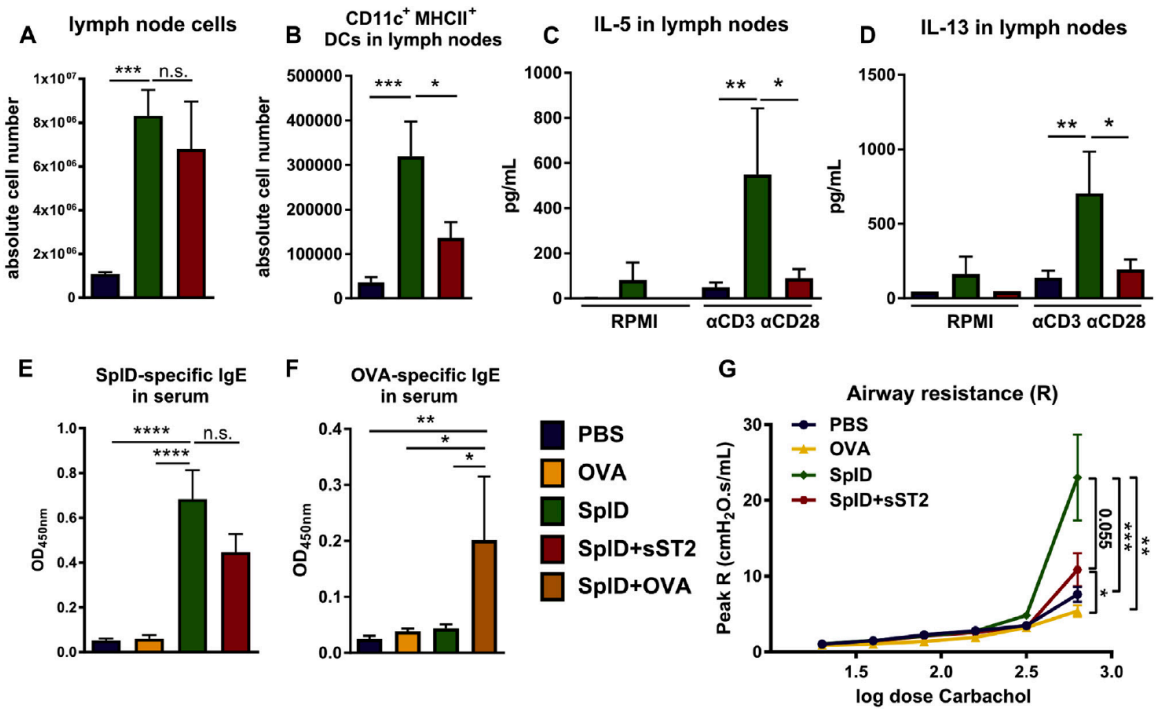


FIG 3. IL-33–dependent memory T-cell formation in local lymph nodes, specific IgE formation, and airway hyperreactivity assay. **A-D,** Total cell counts of lymph node cells (Fig 3, *A*), flow cytometric analysis of DCs in lymph nodes (Fig 3, *B*), and cytokine release by lymph node cells (Fig 3, *C* and *D*) after stimulation for 5 days with anti-CD3 and anti-CD28 antibodies or medium (RPMI, controls). **E** and **F,** SpID-specific IgE (Fig 3, *E*) and OVA-specific serum IgE (Fig 3, *F*) concentrations of mice treated with either PBS, OVA, SpID, or a combination of SpID and sST2 or SpID and OVA were measured by using ELISA. **G,** Airway resistance in response to increased doses of carbachol administered 48 hours after the last intratracheal challenge of PBS, OVA, SpID, or SpID and sST2 was measured by using a forced oscillation technique. N = 7-13. Results are presented as means ± SEMs. **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001, Mann-Whitney *U* test. *n.s.*, Not significant.

increased in SpID-treated mice (Fig 1, *A*). Immunofluorescent costaining of lung sections demonstrated that IL-33 had nuclear localization in airway epithelial cells type II (AECIIs; prosurfactant protein C [proSPC]$^+$) in all treatment groups (Fig 1, *B*), whereas more IL-33$^+$ cells were present in SpID-treated mice. Because IL-33 was shown to be an alarmin that can be passively released during necrosis,[27] we have analyzed cell death in the lungs of SpID-treated mice. Western blotting for phosphorylated mixed lineage kinase domain-like protein (pMLKL), which becomes phosphorylated on cell necrosis,[28] was not detectable in the experimental animals, irrespective of treatment (see Fig E1, *A*, in this article's Online repository at www.jacionline.org). In addition, no terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)–positive cells in the lung sections were detected either (see Fig E1, *B*), supporting that neither apoptotic nor necrotic cell death has a prominent role in the SpID-induced generation of IL-33 in murine airways. Isolated primary murine AECIIs responded to SpID with an increased expression of full-length IL-33, which peaked after 1 hour (Fig 1, *C*). We did not find an increase in TSLP, IL-25, and GM-CSF levels in the lungs of mice treated with SpID compared with those in control mice (data not shown).

The IL-33/ST2 axis modulates type 2 inflammatory responses in a murine model of SpID-induced asthma

SpID-sensitized mice were treated with intratracheal applications of the recombinant soluble IL-33 receptor sST2 after each SpID treatment to block IL-33 signaling to investigate the role of IL-33 in SpID-induced asthma (Fig 2, *A*). sST2 treatment significantly reduced total cell numbers and eosinophil counts in BALF (Fig 2, *B* and *C*). The low number of neutrophils attracted to the BALF on SpID exposure was further reduced in sST2-treated mice (Fig 2, *D*). SpID treatment induced an infiltration of CD4$^+$ T cells into the BALF (Fig 2, *E*). Furthermore, activation of T_H2 cells and ILC2s was evident by the increased numbers of IL-13$^+$CD4$^+$ T cells and ILC2s and IL-5–producing ILC2s in the BALF, which were significantly reduced after sST2 treatment (Fig 2, *F-H*).

Eosinophil numbers were reduced in tendency in the lungs of SpID-exposed mice treated with sST2 (Fig 2, *I*). No neutrophil infiltration into the lungs of SpID-exposed mice was observed (Fig 2, *J*). sST2 caused a significant reduction in the number of CD4$^+$ T cells in the lungs (Fig 2, *K*). IL-33 and eotaxin levels

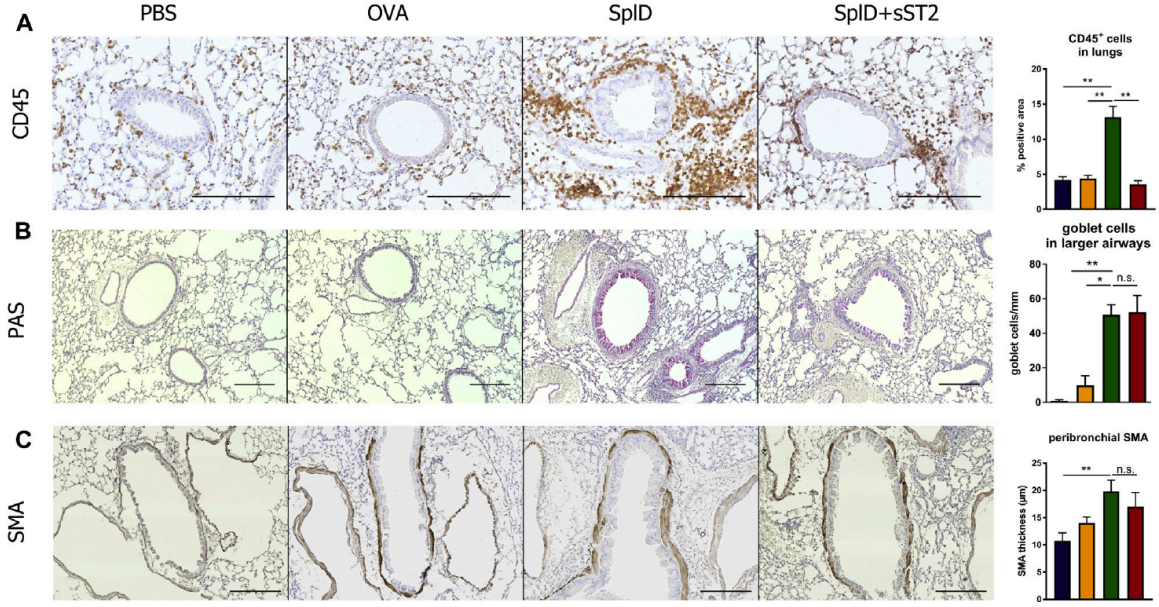


FIG 4. SplD-induced leukocyte infiltration and tissue remodeling in the lungs. Anti-CD45 staining in the lungs **(A)**, periodic acid–Schiff *(PAS)* staining of lung sections and goblet cell quantification in the airways (800-2000 μm perimeter; **B**), and anti–smooth muscle actin *(SMA)* staining **(C)** with measurement of the peribronchial airway smooth muscle thickness in the lungs of mice treated with PBS, OVA, SplD, and SplD with sST2 are shown. *Scale bars* = 200 μm. N = 5. Results are presented as means ± SEMs. $*P < .05$ and $**P < .01$, Mann-Whitney *U* test. *n.s.*, Not significant.

exhibited a tendency to decrease when sST2 was given in addition to SplD (Fig 2, *L* and *M*).

In the lung draining lymph nodes, the total cell count was not significantly altered by sST2 treatment (Fig 3, *A*); however, the increase in MHC class II^+CD11c^+ DCs that was observed after SplD treatment was significantly dampened when sST2 was given in addition (Fig 3, *B*). Local draining lymph node cells from mice receiving different experimental treatments were restimulated with αCD3 and αCD28 antibodies for 5 days to analyze the effect of the IL-33 signaling pathway on T-cell polarization. SplD-treated mice had a significantly higher T_H2 cytokine production (ie, IL-5 and IL-13 levels in the lymph node cell supernatants) compared with the PBS-treated groups, corroborating our earlier findings in the same model,[14] although T_H1 and T_H17 differentiation were not affected (data not shown). Remarkably, sST2 treatment resulted in a strong reduction of IL-5 and IL-13 levels in lymph node cell supernatants of SplD-treated mice (Fig 3, *C* and *D*), supporting the notion of an important role of IL-33 in SplD-induced T-cell activation. Although the inflammatory cell responses were dampened by sST2 treatment, serum levels of SplD-specific IgE were not significantly reduced by sST2 (Fig 3, *E*). Moreover, SplD exposure induced bronchial hyperreactivity, which was partially but not significantly decreased by sST2 treatment (Fig 3, *G*).

To further characterize our SplD sensitization model, we investigated lung histology. SplD-treated mice showed marked perivascular and peribronchial infiltration of $CD45^+$ leukocytes, which was largely prevented by sST2 (Fig 4, *A*). The key features of asthma, such as goblet cell hyperplasia in the airways (Fig 4, *B*) and thickening of the peribronchial airway smooth muscles, were present in the SplD-treated mice and indicate incipient airway remodeling (Fig 4, *C*). These were unchanged after addition of sST2.

In summary, our results underline the crucial role of IL-33 in SplD-induced type 2 inflammation, whereas airway remodeling features were less affected by intratracheal administration of sST2.

SplD acts as an adjuvant when coadministered with an inert allergen

As we have shown, SplD administration in the absence of adjuvants results in production of SplD-specific serum IgE in mice (Fig 3, *E*).[14] In contrast, OVA alone did not induce an OVA-specific IgE response under similar conditions because it is considered an inert antigen. Remarkably, intratracheal coadministration of SplD breaks the tolerance to OVA, as shown by significantly higher levels of OVA-specific serum IgE in the mice (Fig 3, *F*).

SplD induces attraction of $PAR2^+$ cells to the lungs

PAR2 is an important recognition receptor for protease allergens, such as HDM or *A alternata*, and its activation enhances a T_H2 response in the lungs.[29,30] After SplD treatment, numbers of $PAR2^+$ cells were significantly increased in the lungs, which could be efficiently counteracted by sST2 (Fig 5, *A*). This indicates that the increased PAR2 expression in the lungs is a consequence of the SplD-induced attraction of inflammatory cells rather than a direct activation of PAR2. Both an *in vitro* assay with NF-κB–dependent PAR2 reporter HEK-293 cells (Fig 5, *B*) and

J ALLERGY CLIN IMMUNOL
VOLUME 141, NUMBER 2

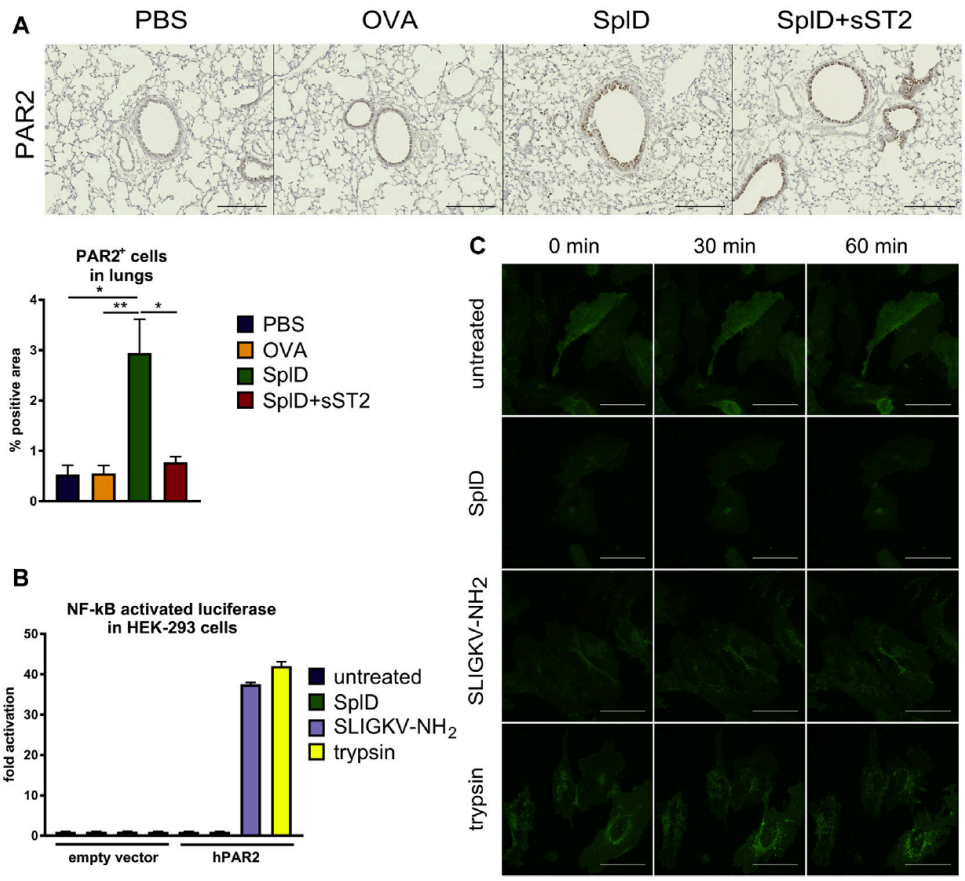


FIG 5. PAR2 is indirectly increased in SpID-treated mice. **A,** Anti-PAR2–stained lung sections and quantification of $PAR2^+$ area. N = 5. Results are presented as means ± SEMs. $*P < .05$ and $**P < .01$, Mann-Whitney *U* test. **B,** Luciferase activity–based activation assay of HEK-293 cells transfected with human PAR2 *(hPAR2)* or an empty vector control. Cells were either untreated or treated with SpID, the PAR2-activating peptide SLIGKV-NH_2, or trypsin for 10 hours. Luciferase values are presented as fold activation relative to untreated cells. Graphs represent mean values of quadruplicates ± SDs. **C,** Visualization of PAR2 translocation in HeLa cells transfected with a human PAR2–enhanced GFP fusion construct. Cells were either untreated or stimulated with SpID, SLIGKV-NH_2, or trypsin, and results were recorded for 60 minutes.

green fluorescent protein (GFP)–tagged PAR2-expressing HeLa cells (Fig 5, *C*) showed activation with the PAR2 activating peptide SLIGKV-NH_2 and trypsin by luciferase activity or internalization of PAR2 by endosome formation but not with SpID.

SpID does not activate TLR2 *in vitro*

TLR2 is a receptor that detects bacterial cell wall compartments, such as peptidoglycan, and is thus involved in recognizing *S aureus*.[31] SpID did not activate TLR2 reporter cells (HEK-Blue hTLR2) *in vitro* at any tested concentration, excluding a direct interaction of SpID with TLR2 (see Fig E2, *A*, in this article's Online repository at www.jacionline.org).

SpID induces asthma features independent from TLR4

Inhalation of allergens with proteolytic capacities, such as HDM, leads to asthma through initiation of the TLR4 signaling pathway.[32] However, the numbers of eosinophils, neutrophils, or T cells in BALF and lungs were comparable between wild-type and $Tlr4^{-/-}$ mice when treated with SpID (see Fig E2, *B-F*). In addition, TLR4 deficiency did not affect levels of SpID-induced IL-33 production (see Fig E2, *G*) and SpID-specific IgE (see Fig E2, *H*) production. This indicates that, in contrast to HDM, SpID-induced asthma is TLR4 independent.

The adaptive immune system is essential for SpID-induced asthma

To study the contribution of the adaptive immune system to SpID-induced allergic airway inflammation, we treated $Rag2^{-/-}$ mice, which lack mature T and B cells but not ILC2s,[33] with SpID or PBS. SpID-induced eosinophilia in the BALF was completely abolished in $Rag2^{-/-}$ mice (Fig 6, *A*), and neutrophil counts were significantly increased in BALF of SpID-treated $Rag2^{-/-}$ mice compared with those in control mice (Fig 6, *B*). IL-33 levels in

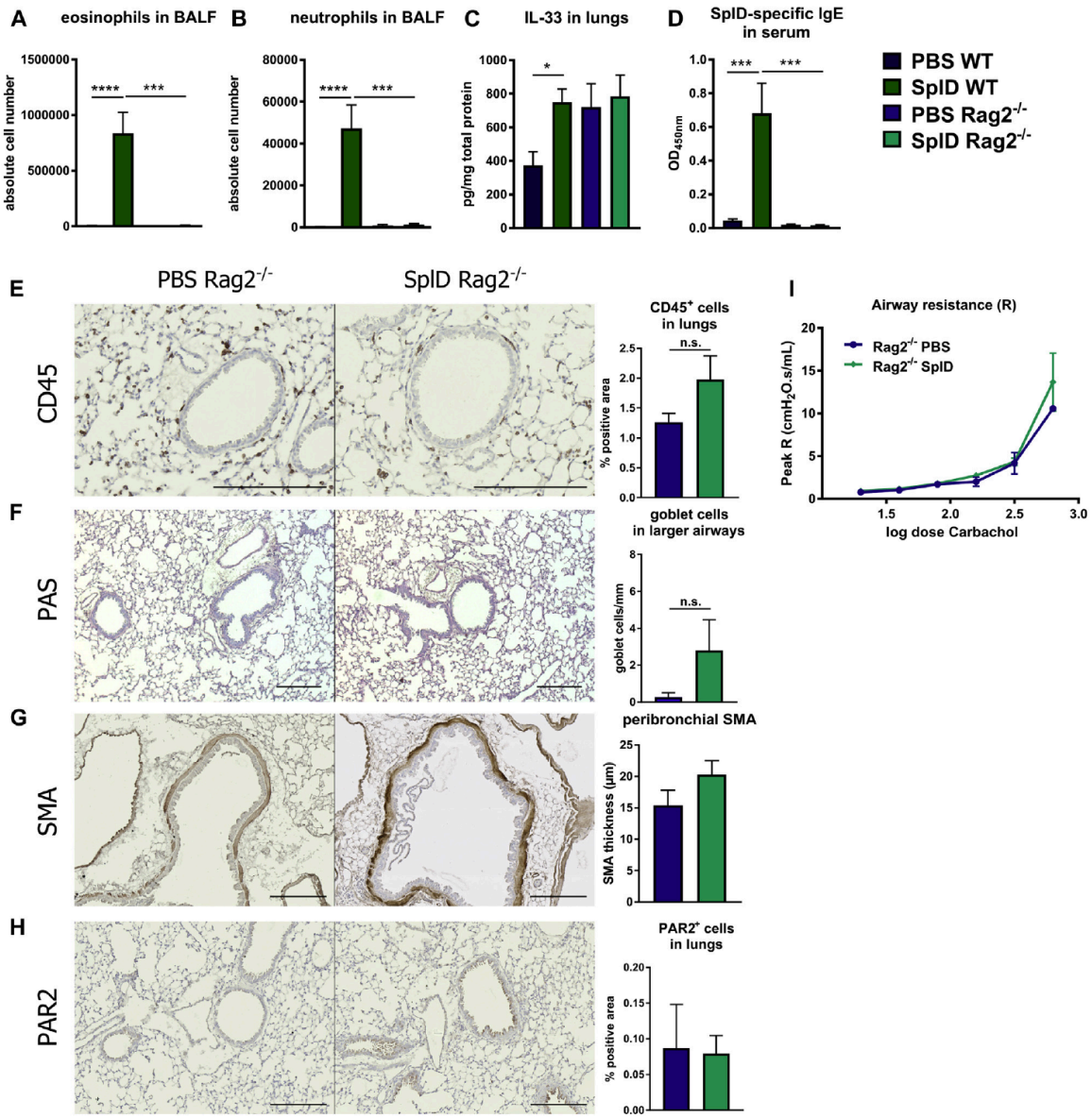


FIG 6. Role of the adaptive immune system in SpID-induced asthma by using *Rag2*$^{-/-}$ mice. **A-D,** Flow cytometric analysis of eosinophils (Fig 6, *A*) and neutrophils (Fig 6, *B*) in BALF, IL-33 measurement of lung homogenates by using Luminex (Fig 6, *C*), and SpID-specific IgE measurement in serum (Fig 6, *D*) by means of ELISA of PBS- or SpID-treated *Rag2*$^{-/-}$ mice in comparison with wild-type mice. **E-H,** Anti-CD45 staining and quantification (Fig 6, *E*), periodic acid–Schiff *(PAS)* staining and goblet cell count (Fig 6, *F*), anti–smooth muscle actin *(SMA)* with measurement of the peribronchial airway smooth muscle thickness (Fig 6, *G*), and anti-PAR2 staining and quantification on lung sections (Fig 6, *H*). **I,** Airway resistance in response to increased doses of carbachol administered 48 hours after the last intratracheal challenge of PBS- or SpID-treated *Rag2*$^{-/-}$ mice was determined by using the forced oscillation technique. *Scale bar* = 200 µm. N = 5-8. Means ± SEMs are presented. **P* < .05, ****P* < .001, and *****P* < .0001, Mann-Whitney *U* test. *n.s.*, Not significant.

the lungs of SpID-treated *Rag2*$^{-/-}$ mice were comparable with those of the control mice, indicating that IL-33 acts upstream of the adaptive immune system (Fig 6, *C*). No production of SpID-specific IgE could be measured in *Rag2*$^{-/-}$ mice (Fig 6, *D*). Histologic analysis of the lungs showed that *Rag2*$^{-/-}$ mice do not respond to SpID because they lack inflammatory cell infiltration, airway remodeling, or bronchial hyperreactivity (Fig 6, *E-I*), which were observed in the wild-type mice. The absence of a

J ALLERGY CLIN IMMUNOL
VOLUME 141, NUMBER 2

functional immune system in *Rag2*$^{-/-}$ mice does not allow analysis of the humoral immune response.

The serine protease–like protein SplF induces an inflammatory response similar to that of SplD

SplF is also encoded in the *Spl*-Operon and shares high sequence similarity with SplD. We tested the response to SplF *in vivo* by using the same protocol as with SplD. In the BALF of SplF-treated mice, total cell counts (see Fig E3, *A*, in this article's Online repository at www.jacionline.org), as well as numbers of eosinophils (see Fig E3, *B*), neutrophils (see Fig E3, *C*), and CD4$^+$ T cells (see Fig E3, *D*), were significantly higher than in the PBS-treated control mice. Eosinophil counts are also increased in the lungs (see Fig E3, *E*), whereas neutrophil counts remained low (see Fig E3, *F*). A trend toward increased expression of IL-33 and eotaxin was also observed (see Fig E3, *G* and *H*). After 6 applications of SplF, SplF-specific IgE was formed (see Fig E3, *I*). Representative images of CD45$^+$ leukocytes attracted to the lungs, goblet cells, peribronchial smooth muscles, and PAR2$^+$ cells show results comparable to those in SplD-treated mice (see Fig E3, *J*).

DISCUSSION

In this study we focus on the mechanisms of the T_H2-biased immune response induced by SplD. Our study reveals that sensitization with SplD causes allergic airway inflammation in mice accompanied by release of the innate cytokine IL-33, increased airway hyperreactivity, greater mucus production, and activation of the adaptive immune system with predominant release of T_H2 cytokines. Allergen recognition in the airways is characterized by release of IL-1α, IL-25, IL-33, TSLP, GM-CSF, and endogenous danger signals that activate the DC network and other innate immune cells, such as basophils and ILC2s.[34] In contrast to earlier observations in HDM sensitization models, we did not find an increase in TSLP, IL-25, and GM-CSF levels in the lungs of mice treated with SplD.

It became evident that IL-33 and its receptor ST2 are crucial in regulation of allergic inflammation.[24,35] IL-33 is expressed by airway smooth muscle cells and endothelial and epithelial cells, and it can also be produced by macrophages on activation in human subjects.[36] In murine lungs IL-33 is mostly localized in AECIIs.[37] IL-33 can be released as an alarmin from dying cells[27] or on allergic sensitization.[21,38-40] In this study we showed that IL-33 expression levels are increased in SplD-treated mice in comparison with the control mice. By stimulating isolated primary AECIIs with SplD *in vitro*, we demonstrated a fast and direct effect of SplD on IL-33 production that was independent of the presence of inflammatory immune cells. Importantly, IL-33 upregulation was not accompanied by cell death, as demonstrated by TUNEL staining of lung sections and Western blotting for pMLKL, suggesting that IL-33 is not passively released by dying cells but actively secreted by the airway epithelium. Bacterial and fungal cell wall components were shown to induce IL-33 production through TLR2 activation[41]; however, SplD did not directly activate TLR2 *in vitro*. Moreover, we demonstrated that the facilitating effects of SplD on allergy parameters, such as eosinophilia, T_H2 cytokine production, and SplD-specific IgE levels, appeared to be independent of TLR4. This observation is in sharp contrast to the mode of action described for HDM, where the presence of TLR4 on the structural lung cells is required for the development of allergic asthma.[32] This suggests the possibility that other receptors might be involved, such as PAR2, which is triggered, for example, by *A alternata*, to induce an asthmatic response independent of TLR4.[42] PAR2 activation in the lungs was shown to trigger and enhance a T_H2-biased inflammatory response to an inert protein antigen in mice.[43] We observed an increase in PAR2$^+$ cell counts in lung sections of SplD-treated mice. The higher amount of PAR2 in SplD-treated mice can be explained by PAR2 expression on immune cells, such as CD4$^+$ T cells[44] or macrophages infiltrating SplD-treated lungs, and by the effects of endogenous proteases that are released, such as by mast cells in response to the SplD-induced inflammation.[45] We have demonstrated that no direct protease-dependent activation of PAR2 by SplD occurs.

We have observed an increased production of IL-5 and IL-13 in lymph nodes of SplD-treated mice. Because IL-13 promotes continuous IL-33 production,[46] positive feedback loops could drive allergic airway inflammation in our model. The presence of both uncleaved and cleaved forms of IL-33 in the lungs of mice treated with SplD could be very important because the mature short form of IL-33 has a 30-fold increased capacity to activate ILC2s *ex vivo* through ST2.[47] We have ruled out the possibility that SplD could directly cleave IL-33 *in vitro* because no fragments were generated after coincubation with full-length IL-33 for up to 24 hours (data not shown). Alternatively, IL-33 activity can be regulated by endogenous proteases released during inflammation. Processing of IL-33 by neutrophil proteases, such as neutrophil elastase or cathepsin G, can also occur during bacterial, fungal, or viral infections.[48] In addition, mast cell tryptase and chymase were shown to generate the shorter mature forms of IL-33.[47] Although the numbers of attracted neutrophils and mast cells are negligibly small in our model (see Fig E5, *A*, in this article's Online repository at www.jacionline.org), they could be a potent source of IL-33–activating proteases, although SplD does not cause direct mast cell degranulation (see Fig E5, *B*). However, thus far, the mechanism of IL-33 cleavage in SplD-treated mice remains a matter of speculation.

To block the IL-33 pathway, we treated mice with intratracheal applications of sST2. In contrast to former studies involving sST2, we applied sST2 intratracheally to mimic the noninvasive administration route of therapeutic inhalers. sST2 treatment during SplD sensitization resulted in diminished eosinophilic attraction to the airways, lower IL-33 and eotaxin levels, lower numbers of IL-13$^+$ ILC2s and CD4$^+$ T cells in BALF, and less IL-5 and IL-13 production in local draining lymph nodes. In contrast, intratracheal application of sST2 did not reduce SplD-specific IgE levels. This is possibly due to the intratracheal administration route because the IL-33 pathway was shown to interfere with IgE production in ST2$^{-/-}$ mice by using HDM[49] or a mix of allergens from *A alternata*, *Aspergillus* species, or HDM.[50,51] In a study by Willart et al,[25] mice injected intraperitoneally with sST2 had reduced levels of HDM-specific IgE, whereas no effect on T_H2 cytokine levels in the lymph nodes could be seen. SplD-induced airway resistance was nonsignificantly decreased, and airway remodeling was unaffected by sST2 treatment.

DCs are known to be the primary sensors of antigens and infectious agents in the airways, and they initiate appropriate adaptive immune responses.[52] During allergic sensitization, IL-33 can activate DC maturation, resulting in expression of costimulatory molecules, priming of T cells, and ultimately production of T_H2 cytokines.[53,54] IL-33 treatment was shown to increase DC

numbers in the lungs, whereas local T- and B-cell populations were not affected.[54] We have demonstrated that SplD treatment attracts DCs to the lung, as well as the local lymph nodes, where they probably prime naive T cells for differentiation into T_H2 cells. We observe that SplD induced migration of DCs to the local lymph nodes. As expected, DC activation was IL-33 dependent because it was reduced in mice treated with sST2.

These results prompted us to analyze the role of the adaptive immune system in our experimental model of SpD-induced asthma by using *Rag2*$^{-/-}$ mice. Importantly, eosinophilia, SplD-specific IgE production and goblet cell hyperplasia were completely abolished in *Rag2*$^{-/-}$ mice that received SplD. This demonstrates an essential function of the adaptive immune system in SplD-induced allergic airway inflammation. Because IL-33 levels were unaltered in SplD-treated *Rag2*$^{-/-}$ mice, IL-33 must act upstream of T-cell sensitization, probably by tuning DCs. Moreover, data clearly show that the pronounced eosinophilia in the inflamed airways and lungs was not elicited by IL-33 directly but mediated by adaptive immune cells. In agreement with this, *Rag2*$^{-/-}$ mice treated with HDM had very few eosinophils in the airways and reduced goblet cell metaplasia.[33]

At first glance, our findings appear to contradict reports by Halim et al,[55,56] who showed that ILC2s rather than T cells are important at the initiation stage of papain-induced allergic inflammation (days 2-3), promoting type 2 cytokine production, eosinophilia, and mucus hyperproduction. However, at later stages (days 14-21), $CD4^+$ T cells were the main producers of T_H2 cytokines.[57] After 2 weeks of SplD treatment, we found, in addition to infiltration of $CD4^+$ T cells, a significant expansion of ILC2s in the airways of SplD-treated wild-type mice. It appears plausible that also in our model the initial response to SplD is carried out mainly by resident ILC2s. However, the lack of inflammation in *Rag2*$^{-/-}$ mice after 2 weeks of SplD exposure shows that an amplification of this initial ILC2-mediated response by T cells is required for development of the observed eosinophilic type 2 airway inflammation in wild-type mice.

We have previously shown that *Staphylococcus aureus* enterotoxin B, a potent T cell–activating agent, can break immune tolerance in the airways. In the presence of this toxin, intratracheal application of OVA, an otherwise inert protein, primes a T_H2 response characterized by bronchial hyperreactivity and the production of specific IgE.[58] This is regulated by activation of inflammatory DCs and polyclonal T cells independent of the IL-1 receptor pathway.[59] Similarly, SplD promoted production of OVA-specific IgE antibodies when given in combination with OVA. Importantly, in our experimental model intratracheal application of OVA alone did not elicit OVA-specific IgE production, which indicates that it functions as a model of airway tolerance. Prior priming by systemic application of OVA together with an adjuvant is usually required to render OVA allergenic.[60] Hence our data indicate that SplD is capable of breaking tolerance in the airways.

Next to SplD, we showed that SplF can also induce an eosinophilic inflammatory response and allergic sensitization in mice. SplF shares 94.6% sequence homology with SplD.[61] Considering its high prevalence of 71%[62] in *S aureus* strains, our findings make SplF another relevant *S aureus*–derived allergen.

Our observations support the hypothesis that *S aureus* is persistently colonizing the nasal mucosa of patients protected by a biofilm or inside of immune cells[4] and constantly producing a panel of factors that could initiate and/or aggravate a T_H2-biased immune response. Chronic exposure to *S aureus*–secreted proteins, such as Spls, would also hinder resolution of the inflammation, fostering chronification.

In summary, we have demonstrated that SplD causes a T_H2 response in an IL-33–dependent manner because T_H2 cytokine production and eosinophilic airway inflammation could be controlled by sST2 treatment. This identifies IL-33 as a potential therapeutic target in patients with chronic rhinosinusitis and asthma who are carriers of *S aureus*.

We thank T. Divert, I. De Borle, and K. De Saedeleer for their excellent technical assistance.

Key messages

- **Sensitization with SplD causes T_H2-biased allergic airway inflammation in mice accompanied by release of the innate cytokine IL-33.**
- **IL-33–targeted treatment could be an efficient therapeutic strategy to counteract the action of SplD on the immune system.**

REFERENCES

1. Reginald K, Westritschnig K, Werfel T, Heratizadeh A, Novak N, Focke-Tejkl M, et al. Immunoglobulin E antibody reactivity to bacterial antigens in atopic dermatitis patients. Clin Exp Allergy 2011;41:357-69.
2. Bachert C, Zhang N, Holtappels G, De Lobel L, van Cauwenberge P, Liu S, et al. Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma. J Allergy Clin Immunol 2010;126:962-8, e1-6.
3. Kobayashi T, Glatz M, Horiuchi K, Kawasaki H, Akiyama H, Kaplan DH, et al. Dysbiosis and *Staphylococcus aureus* colonization drives inflammation in atopic dermatitis. Immunity 2015;42:756-66.
4. Sachse F, Becker K, von Eiff C, Metze D, Rudack C. *Staphylococcus aureus* invades the epithelium in nasal polyposis and induces IL-6 in nasal epithelial cells in vitro. Allergy 2010;65:1430-7.
5. Tomassen P, Vandeplas G, Van Zele T, Cardell L-O, Arebro J, Olze H, et al. Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. J Allergy Clin Immunol 2016;137:1449-56.e4.
6. Thammavongsa V, Kim HK, Missiakas D, Schneewind O. Staphylococcal manipulation of host immune responses. Nat Rev Microbiol 2015;13:529-43.
7. Cho SH, Strickland I, Tomkinson A, Fehringer AP, Gelfand EW, Leung DY. Preferential binding of *Staphylococcus aureus* to skin sites of Th2-mediated inflammation in a murine model. J Invest Dermatol 2001;116:658-63.
8. Nakamura Y, Oscherwitz J, Cease KB, Chan SM, Muñoz-Planillo R, Hasegawa M, et al. Staphylococcus δ-toxin induces allergic skin disease by activating mast cells. Nature 2013;503:397-401.
9. Bröker B, Mrochen D, Péton V. The T cell response to *Staphylococcus aureus*. Pathogens 2016;5:31.
10. Kusch H, Engelmann S. Secrets of the secretome in *Staphylococcus aureus*. Int J Med Microbiol 2014;304:133-41.
11. Schlievert PM, Strandberg KL, Lin Y-C, Peterson ML, Leung DYM. Secreted virulence factor comparison between methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*, and its relevance to atopic dermatitis. J Allergy Clin Immunol 2010;125:39-49.
12. Bachert C, Van Steen K, Zhang N, Holtappels G, Cattaert T, Maus B, et al. Specific IgE against *Staphylococcus aureus* enterotoxins: an independent risk factor for asthma. J Allergy Clin Immunol 2012;130:376-81.e8.
13. Bachert C, Akdis CA. Phenotypes and emerging endotypes of chronic rhinosinusitis. J allergy Clin Immunol Pract 2016;4:621-8.
14. Stentzel S, Teufelberger A, Nordengrün M, Kolata J, Schmidt F, van Crombruggen K, et al. Staphylococcal serine protease-like proteins are pacemakers of allergic airway reactions to *Staphylococcus aureus*. J Allergy Clin Immunol 2017;139:492-500.e8.

J ALLERGY CLIN IMMUNOL
VOLUME 141, NUMBER 2

15. Zdzalik M, Karim AY, Wolski K, Buda P, Wojcik K, Brueggemann S, et al. Prevalence of genes encoding extracellular proteases in *Staphylococcus aureus*—important targets triggering immune response in vivo. FEMS Immunol Med Microbiol 2012;66:220-9.
16. de Boer JD, Van't Veer C, Stroo I, van der Meer AJ, de Vos AF, van der Zee JS, et al. Protease-activated receptor-2 deficient mice have reduced house dust mite-evoked allergic lung inflammation. Innate Immun 2014;20:618-25.
17. Steelant B, Farré R, Wawrzyniak P, Belmans J, Dekimpe E, Vanheel H, et al. Impaired barrier function in patients with house dust mite–induced allergic rhinitis is accompanied by decreased occludin and zonula occludens-1 expression. J Allergy Clin Immunol 2016;137:1043-53.e5.
18. Hartl D, Latzin P, Hordijk P, Marcos V, Rudolph C, Woischnik M, et al. Cleavage of CXCR1 on neutrophils disables bacterial killing in cystic fibrosis lung disease. Nat Med 2007;13:1423-30.
19. Takai T, Kato T, Ota M, Yasueda H, Kuhara T, Okumura K, et al. Recombinant Der p 1 and Der f 1 with in vitro enzymatic activity to cleave human CD23, CD25 and alpha1-antitrypsin, and in vivo IgE-eliciting activity in mice. Int Arch Allergy Immunol 2005;137:194-200.
20. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, 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 2005;23:479-90.
21. Kouzaki H, Iijima K, Kobayashi T, O'Grady SM, Kita H. The danger signal, extracellular ATP, is a sensor for an airborne allergen and triggers IL-33 release and innate Th2-type responses. J Immunol 2011;186:4375-87.
22. Oboki K, Nakae S, Matsumoto K, Saito H. IL-33 and airway inflammation. Allergy Asthma Immunol Res 2011;3:81-8.
23. Lloyd CM. IL-33 family members and asthma—bridging innate and adaptive immune responses. Curr Opin Immunol 2010;22:800-6.
24. Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. J Biol Chem 2007; 282:26369-80.
25. Willart MAM, Deswarte K, Pouliot P, Braun H, Beyaert R, Lambrecht BN, et al. Interleukin-1 controls allergic sensitization to inhaled house dust mite via the epithelial release of GM-CSF and IL-33. J Exp Med 2012;209:1505-17.
26. Fahy JV. Type 2 inflammation in asthma—present in most, absent in many. Nat Rev Immunol 2015;15:57-65.
27. Miller AM. Role of IL-33 in inflammation and disease. J Inflamm (Lond) 2011;8: 22.
28. Murphy JM, Czabotar PE, Hildebrand JM, Lucet IS, Zhang J-G, Alvarez-Diaz S, et al. The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. Immunity 2013;39:443-53.
29. Boitano S, Flynn AN, Sherwood CL, Schulz SM, Hoffman J, Gruzinova I, et al. *Alternaria alternata* serine proteases induce lung inflammation and airway epithelial cell activation via PAR2. Am J Physiol Lung Cell Mol Physiol 2011;300:L605-14.
30. Jacquet A. Innate immune responses in house dust mite allergy. ISRN Allergy 2013;2013:1-18.
31. Matsui K, Nishikawa A. Peptidoglycan from Staphylococcus aureus induces T(H) 2 immune response in mice. J Investig Allergol Clin Immunol 2012;22:80-6.
32. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via TLR4 triggering of airway structural cells. Nat Med 2009;15:410-6.
33. De Grove KC, Provoost S, Hendriks RW, McKenzie ANJ, Seys LJM, Kumar S, et al. Dysregulation of type 2 innate lymphoid cells and Th2 cells impairs pollutant-induced allergic airway responses. J Allergy Clin Immunol 2016;139:246-57.e4.
34. Lambrecht BN, Hammad H. Allergens and the airway epithelium response: gateway to allergic sensitization. J Allergy Clin Immunol 2015;134:499-507.
35. Bartemes KR, Iijima K, Kobayashi T, Kephart GM, McKenzie AN, Kita H. IL-33-responsive lineage- CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. J Immunol 2012;188:1503-13.
36. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. Nat Rev Immunol 2010;10:103-10.
37. Mohapatra A, Van Dyken SJ, Schneider C, Nussbaum JC, Liang H-E, Locksley RM. Group 2 innate lymphoid cells utilize the IRF4-IL-9 module to coordinate epithelial cell maintenance of lung homeostasis. Mucosal Immunol 2016;9:275-86.
38. Haenuki Y, Matsushita K, Futatsugi-Yumikura S, Ishii KJ, Kawagoe T, Imoto Y, et al. A critical role of IL-33 in experimental allergic rhinitis. J Allergy Clin Immunol 2012;130:184-94.e11.
39. Morita H, Arae K, Unno H, Miyauchi K, Toyama S, Nambu A, et al. An interleukin-33-mast cell-interleukin-2 axis suppresses papain-induced allergic inflammation by promoting regulatory T cell numbers. Immunity 2015;43:175-86.
40. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, et al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. J Allergy Clin Immunol 2013;131:187-200, e1-8.
41. Li C, Li H, Jiang Z, Zhang T, Wang Y, Li Z, et al. Interleukin-33 increases antibacterial defense by activation of inducible nitric oxide synthase in skin. PLoS Pathog 2014;10:e1003918.
42. Matsuwaki Y, Wada K, White TA, Benson LM, Charlesworth MC, Checkel JL, et al. Recognition of fungal protease activities induces cellular activation and eosinophil-derived neurotoxin release in human eosinophils. J Immunol 2009; 183:6708-16.
43. Ebeling C, Lam T, Gordon JR, Hollenberg MD, Vliagoftis H. Proteinase-activated receptor-2 promotes allergic sensitization to an inhaled antigen through a TNF-mediated pathway. J Immunol 2007;179:2910-7.
44. López ML, Soriano-Sarabia N, Bruges G, Marquez ME, Preissner KT, Schmitz ML, et al. Expression pattern of protease activated receptors in lymphoid cells. Cell Immunol 2014;288:47-52.
45. Rothmeier AS, Ruf W. Protease-activated receptor 2 signaling in inflammation. Semin Immunopathol 2012;34:133-49.
46. Christianson CA, Goplen NP, Zafar I, Irvin C, Good JT, Rollins DR, et al. Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33. J Allergy Clin Immunol 2015;136:59-68.e14.
47. Lefrançais E, Duval A, Mirey E, Roga S, Espinosa E, Cayrol C, et al. Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. Proc Natl Acad Sci U S A 2014;111:15502-7.
48. Lefrancais E, Roga S, Gautier V, Gonzalez-de-Peredo A, Monsarrat B, Girard J-P, et al. IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. Proc Natl Acad Sci U S A 2012;109:1673-8.
49. Zoltowska AM, Lei Y, Fuchs B, Rask C, Adner M, Nilsson GP. The interleukin-33 receptor ST2 is important for the development of peripheral airway hyperresponsiveness and inflammation in a house dust mite mouse model of asthma. Clin Exp Allergy 2016;46:479-90.
50. Iijima K, Kobayashi T, Hara K, Kephart GM, Ziegler SF, McKenzie AN, et al. IL-33 and thymic stromal lymphopoietin mediate immune pathology in response to chronic airborne allergen exposure. J Immunol 2014;193:1549-59.
51. Castanhinha S, Sherburn R, Walker S, Gupta A, Bossley CJ, Buckley J, et al. Pediatric severe asthma with fungal sensitization is mediated by steroid-resistant IL-33. J Allergy Clin Immunol 2015;136:312-22.e7.
52. van Helden MJ, Lambrecht BN. Dendritic cells in asthma. Curr Opin Immunol 2013;25:745-54.
53. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. J Allergy Clin Immunol 2009;123:1047-54.
54. Besnard A-G, Togbe D, Guillou N, Erard F, Quesniaux V, Ryffel B. IL-33-activated dendritic cells are critical for allergic airway inflammation. Eur J Immunol 2011;41:1675-86.
55. Halim TYF, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. Immunity 2012;36:451-63.
56. Halim TYF, MacLaren A, Romanish MT, Gold MJ, McNagny KM, Takei F. Retinoic-acid-receptor-related orphan nuclear receptor alpha is required for natural helper cell development and allergic inflammation. Immunity 2012;37:463-74.
57. Halim TYF, Steer CA, Math L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. Immunity 2014;40:425-35.
58. Huvenne W, Callebaut I, Plantinga M, Vanoirbeek JAJ, Krysko O, Bullens DMA, et al. *Staphylococcus aureus* enterotoxin B facilitates allergic sensitization in experimental asthma. Clin Exp Allergy 2010;40:1079-90.
59. Krysko O, Maes T, Plantinga M, Holtappels G, Imiru R, Vandenabeele P, et al. The adjuvant-like activity of staphylococcal enterotoxin B in a murine asthma model is independent of IL-1R signaling. Allergy 2013;68:446-53.
60. Maes T, Provoost S, Lanckacker E, Cataldo DD, Vanoirbeek JJ, Nemery B, et al. Mouse models to unravel the role of inhaled pollutants on allergic sensitization and airway inflammation. Respir Res 2010;11:7.
61. Reed SB, Wesson CA, Liou LE, Trumble WR, Schlievert PM, Bohach GA, et al. Molecular characterization of a novel *Staphylococcus aureus* serine protease operon. Infect Immun 2001;69:1521-7.
62. Zdzalik M, Kalinska M, Wysocka M, Stec-Niemczyk J, Cichon P, Stach N, et al. Biochemical and structural characterization of SplD protease from *Staphylococcus aureus*. PLoS One 2013;8:e76812.

METHODS

Recombinant protein production

Recombinant SplD and SplF were produced in the *Bacillus subtilis* strain 6051HGW LS8P-D, which lack the proprietary proteases WprA, Epr, Bpr, NprB, NprE, Vpr, Mpr, and AprE, as described in detail previously.[E1] Cell-free supernatants were collected and subjected to tangential flow filtration to exchange the buffer with 20 mmol/L Tris/HCl (pH 7.5). SplD was purified by means of ion-exchange chromatography on an SP Sepharose Fast Flow column (GE Healthcare, Fairfield, Conn), followed by a 2-step purification with centrifugal filter units (Amicon Ultra 30K/10K; Merck Millipore, Billerica, Mass). Thereby the buffer was exchanged with PBS. The quality of the native SplD preparation was verified by using SDS-PAGE. Recombinant murine sST2 was generated and purified with standard chromatographic procedures from sST2-His transfected HEK-293 cells at the VIB Protein Service Facility, VIB Center for Inflammation Research (Ghent, Belgium).

Organ processing

Blood was collected in EDTA Microvettes 200Z (Sarstedt, Nümbrecht, Germany) and centrifuged for 10 minutes at 3000 rpm, and serum was collected and stored at −20°C before further analysis. BALF was collected by rinsing the airways 3 times with 0.3 mL of 5% BSA (Sigma-Aldrich, Diegem, Belgium) in PBS with complete protease inhibitor cocktail (Roche Diagnostics, Anderlecht, Belgium) and 2 times with 1 mL of 0.2% ETDA (Sigma-Aldrich) in PBS and kept at 4°C. Red blood cells were lysed for 2 minutes with VersaLyse buffer (Beckman Coulter, Suarlée, Belgium). Cells were used for fluorescence-activated cell sorting (FACS) analysis. Lungs were perfused with 10 mL of 0.9% NaCl (Braun, Meslungen, Germany). The left lobe of the lung was fixed with 10% formalin (Sigma-Aldrich) for paraffin embedding, whereas the right lobes were either snap-frozen or minced and digested with 1% collagenase type II (Worthington Biochemical) for FACS analysis. Snap-frozen lungs were homogenized for 2 minutes at 50 oscillations per second with 10 times more wt/vol T-Per Tissue Protein Extraction reagent (Thermo Fisher Scientific, Waltham, Mass) with 1× HALT protease inhibitor cocktail kit (Thermo Fisher Scientific) by using the TissueLyser LT (Qiagen, Antwerp, Belgium). After 10 minutes of centrifugation at 3000 rpm, supernatants were collected and repeatedly centrifuged at 15,000 rpm for 3 minutes. Supernatants were collected as lung homogenates, and protein concentrations were measured with the Bio-Rad Protein assay (Bio-Rad, Temse, Belgium).

AECII sorting and *in vitro* stimulation with SplD

AECIIs were sorted from 3 naive C57BL/6J WT mice, as previously described. After sorting, cells were stimulated for 0, 1, and 5 hours with 25 μg/mL SplD in serum free IMDM medium (Gibco) and lysed with T-Per buffer (Thermo Fisher Scientific) containing 1× HALT protease inhibitor cocktail kit (Thermo Fisher Scientific). Protein concentrations were measured by using the Bio-Rad Protein assay (Bio-Rad).

Lymph node restimulation assay

Peribronchial lymph nodes were collected and put through a 70-μm mesh. Red blood cells were lysed with VersaLyse buffer (Beckman Coulter), and lymph node cells were seeded in triplicates per condition at 2×10^5 cells/well in RPMI medium (Gibco) with 10% FBS (Gibco) and Pen/Strep (Gibco) in a 96-well plate. The wells were either uncoated or coated with 2 μg/mL purified anti-mouse CD3 (eBioscience, San Diego, Calif) for 2 hours before cell incubation. Purified anti-mouse CD28 (eBioscience) was added to the CD3-stimulated cells to reach a final concentration of 2 μg/mL, and unstimulated controls were filled with RPMI medium to a final volume of 200 μL/well. Cells were incubated for 5 days at 37°C in a 5% CO_2 atmosphere, and supernatants were used for IL-5 and IL-13 analysis.

Western blotting

Twenty-five micrograms of protein of lung homogenates or lysed AECIIs per slot were loaded on a 15% Tris/HCl gel, separated by means of SDS-PAGE, and transferred to a nitrocellulose membrane (Bio-Rad). For Western blotting, mouse IL-33 antigen affinity-purified polyclonal goat IgG (dilution 1:300; R&D Systems) antibody and donkey anti-goat horseradish peroxidase (HRP; dilution 1:5,000; Santa Cruz Biotechnology, Heidelberg, Germany) and rabbit anti–glyceraldehyde-3-phosphate dehydrogenase antibody (dilution 1:10,000; Sigma-Aldrich) in combination with the donkey anti-rabbit HRP antibody (dilution 1:2,000; GE Healthcare) were used. For pMLKL and mixed lineage kinase domain–like protein (MLKL) Western blots, lungs were homogenized with the addition of PhosStop (Roche Diagnostics) in the above mentioned lysis buffer. A cell lysate of necrotic TNF-α–treated CT26 cells was used as a positive control. Anti-MLKL (phosphoS358, dilution 1:2,000; Abcam, Cambridge, United Kingdom) in combination with the donkey anti-rabbit HRP antibody (dilution 1:2,000; GE Healthcare) and anti-MLKL clone 3H1 (dilution 1:1,000; Merck Millipore) in combination with the anti-rat IgG HRP-linked antibody (dilution 1:2,000; Cell Signaling Technology, Leiden, The Netherlands) were used. Bands were visualized with the Immobilon Western Chemiluminescence HRP substrate (Merck Millipore). Four mice were tested per group for lung homogenates and 3 for AECIIs; one representative series is shown. Semiquantitative analysis of band intensities was performed by measuring the area under the peak of plotted lanes with ImageJ software (National Institutes of Health, Bethesda, Md).

Immunofluorescence staining

Formalin-fixed and paraffin-embedded lung tissues were cut into 5-μm sections. Sections were deparaffinized, and nonspecific binding sites were blocked with 3% normal horse serum (Vector Laboratories, Peterborough, United Kingdom) in PBS. Sections were incubated overnight at 4°C with the following primary antibodies: mouse IL-33 (dilution 1:50; R&D Systems) in combination with anti-proSPC antibody (1:1000; Merck Millipore). As secondary antibodies, donkey anti-goat Alexa Fluor 488 (1:750) antibody (Invitrogen, Thermo Fisher Scientific) was used in combination with donkey anti-rabbit Alexa Fluor 594 (1:750; Invitrogen). Slides were mounted with Vectashield Mounting medium for fluorescence with 4′-6-diamidino-2-phenylindole dihydrochloride (Vector Laboratories). Images were obtained with a Zeiss Axioplan 2 confocal microscope (Zeiss, Oberkochen, Germany).

Immunohistochemistry

Formalin-fixed and paraffin-embedded lung tissues were cut into 5-μm sections. Sections were deparaffinized and rehydrated, and antigen retrieval with citrate buffer (2 mmol/L citric acid and 10 mmol/L sodium citrate, Sigma-Aldrich), peroxidase blockage with 3% H_2O_2 (Sigma-Aldrich), and blockage of nonspecific binding sites with 3% goat serum (Sigma-Aldrich) in PBS with 0.4% Triton-X 100 (Sigma-Aldrich) were performed. Slides were stained with either anti-PAR2 antibody (dilution 1:100; Abcam) in combination with goat anti-rabbit HRP (1:100), with anti-smooth muscle actin (1:3000 dilution; Abcam) in combination with goat anti-rabbit HRP (1:200), or with purified rat anti-mouse CD45 (dilution 1:100; BD Biosciences, San Jose, Calif) in combination with biotin goat anti-rat immunoglobulin (dilution 1:500; BD Bioscience). The CD45 staining signal was amplified by using the Vectastain elite ABC kit (Vector Laboratories). All sections were developed with the DAB+ Substrate chromogen system (Dako, Glostrup, Denmark) and counterstained with hematoxylin (Sigma-Aldrich), dehydrated, and mounted with Pertex (Histolab, Gothenburg, Sweden). Images were taken with an AxioScanZ.1 (Zeiss, Germany) by using a 20× Plan-Apochromat 0.8 NA dry objective captured with a Hitachi HV-F202SCL camera. Percentages of $CD45^+$ and $PAR2^+$ area of lung sections were quantified by using the trainable WEKA segmentation tool of the Fiji software. Airway smooth muscle thickness was calculated as the radius of the positively smooth muscle actin–stained peribronchial smooth muscle area.

Toluidine blue staining

Lung sections were deparaffinized, rehydrated, and stained for 15 minutes with 0.1% Toluidine blue (Sigma-Aldrich) in 7% ethanol and 1% NaCl, pH 2.3, followed by dehydration with 100% ethanol and mounting with Pertex (Histolab). Mast cell numbers were quantified in 5 nonadjacent lung sections per mouse (n = 7). Images were acquired with an AxioScanZ.1 (Zeiss) with a

J ALLERGY CLIN IMMUNOL
VOLUME 141, NUMBER 2

20× Plan-Apochromat 0.8 NA dry objective captured with a Hitachi HV-F202SCL camera.

Periodic acid–Schiff staining

Deparaffinized and rehydrated lung sections were stained for mucus-producing goblet cells by using the periodic acid–Schiff kit (Sigma-Aldrich). The magenta stained goblet cells were counted in airways with a perimeter of 800 to 2000 μm by using Aviso software (Hillsboro, Ore).

TUNEL staining

Deparaffinized and rehydrated lung sections were stained with the TUNEL Apoptosis Detection Kit (Merck Millipore), according to the manufacturer's manual. As a positive control, 200 μg/mL DNAse I (Qiagen, Hilden, Germany) in 1 mg/mL BSA and Tris/HCl (pH 7.5) was applied for 10 minutes after the endogenous peroxidase-quenching step.

SplD-, SplF-, and OVA-specific IgE analysis

Sera of mice were taken 48 hours after the last SplD application, and titers of SplD-specific IgE were performed by using an ELISA, as described previously.[E1] For OVA- or SplF-specific IgE measurements, 5 μg/mL OVA or SplF was used to coat the plates; otherwise, the assay was performed in the same way as for SplD-specific IgE.

HEK-Blue hTLR2 cell stimulation assay

HEK-Blue hTLR2 (InvivoGen, San Diego, Calif) are TLR2 reporter cells that produce secreted embryonic alkaline phosphatase on TLR2 activation through NF-κB. HEK-Blue hTLR2 cells were stimulated with 50, 25, or 5 μg/mL SplD at 37°C in a 5% CO_2 atmosphere overnight. As a positive control, peptidoglycan was added at 1 μg/mL. Cell supernatants were diluted 1 in 10 with QUANTI-Blue (InvivoGen), according to the supplier's manual, and the secreted embryonic alkaline phosphatase activity was measured at 620 nm after 24 hours. Untreated cell supernatants were used as a negative control. All conditions were measured in duplicates, and the experiment was repeated 3 times.

Generation of PAR2 reporter HEK 293 T cells

Cells were seeded 1 day before transfection at a density of 4×10^4 cells/well in 1 mL of Dulbecco modified Eagle medium (DMEM) and 10% FCS medium in 24 well-plates. Cells were transfected by means of calcium-phosphate precipitation with an NF-κB–inducible luciferase reporter plasmid, a β-galactosidase reporter for normalization of the luciferase values, and a human PAR2 expression vector. For control transfections without PAR2, the total amount of DNA was equalized with empty vector DNA. After 4 hours of incubation at 37°C in a 5% CO_2 atmosphere, the medium was replaced with fresh DMEM with 10% FCS. Twenty-four hours after transfection, cells were used for the PAR2 activation assay.

PAR2 activation assay with HEK 293 PAR2 reporter cells

Medium was replaced with serum free DMEM, and cells were stimulated for 10 hours in quadruplicates with 25 μg/mL SplD, 50 μmol/L SLIGKV-NH_2 (Sigma-Aldrich), or 1 μmol/L trypsin (Gibco) or left untreated. After lysis of cells in 200 μL of luciferase lysis buffer (25 mmol/L Tris-phosphate [pH 7.8], 2 mmol/L dithiothreitol, 2 mmol/L 1,2-cyclohexaminediaminetetraacetic acid, 10% glycerol, and 1% Triton X-100), luciferase and β-galactosidase activities were measured. The obtained luciferase values were normalized with corresponding β-galactosidase values to correct for differences in transfection efficiencies.

Generation of PAR2–enhanced GFP–expressing HeLa cells and PAR2 receptor translocation assay

HeLa cells were transfected with a human PAR2–enhanced GFP fusion construct and stimulated with 25 μg/mL SplD, 100 μmol/L SLIGKV-NH_2 (Sigma-Aldrich), or 1 μmol/L trypsin or left untreated. The simulated cells were imaged every 2.5 minutes with the Zeiss Spinning Disk microscope for 1 hour. The experiment was repeated 3 times. Representative images at 0, 30, and 60 minutes of each experimental condition are shown.

Flow cytometry

Anti-mouse CD3–phycoerythrin (PE), CD4–fluorescein isothiocyanate (FITC), CD11b–peridinin-chlorophyll-protein complex (PerCP)–Cy5.5, purified CD16/32, CD25–allophycocyanin (APC), Gr-1–FITC, MHC class II–FITC, F4/80-APC, CD25-PECy7, CD90.2-APC, CD5-PerCP Cy5, NK1.1-PerCP-Cy5, T-cell receptor β–PerCP Cy5, CD11c-APCeFluo780, CD11b-APCeFluo780, CD45R-APCeFluo780, CD3-AF700, CD45-BV421, IL-13–PE, and IL-5–PE were purchased from eBioscience, and CD11c–PE-Cy7 (BD Biosciences) and Siglec F–PE (Miltenyi Biotec, Bergisch Gladbach, Germany) were used to stain mouse BALF and lungs. The secondary antibody HRP–APC-Cy7 was purchased from eBioscience. Cells were stained for 30 minutes at room temperature and washed with PBS, and LIVE/DEAD Fixable Aqua dead cell stain, LIVE/DEAD Fixable Near-IR dead cell stain (Invitrogen), or 7-AAD Viability Staining Solution (eBioscience) was used for 10 minutes to exclude dead cells. Cells were washed and analyzed by using flow cytometry with the FACSCanto II (BD Biosciences). For intracellular IL-5 and IL-13 staining, BALF cells were stimulated with the cell stimulation cocktail (plus protein transport inhibitors; eBioscience) for 4 hours at 37°C in a 5% CO_2 atmosphere, fixed, and permeabilized with the intracellular fixation and permeabilization buffer set (eBioscience). The ILC2 gating strategy in BALF is provided in Fig E4.

Mast cell degranulation assay

Bone marrow–derived murine mast cells were generated from C57Bl/6 J mice (n = 4). Mast cell growth medium was enhanced with 1 ng/mL rIL-3 (PeproTech, London, United Kingdom). Mast cells were grown for 4 weeks and analyzed for c-kit and FcεRI expression by using flow cytometry (c-kit–PE and FcεRI–PE-Cy7, both from eBioscience). Cells were used for the experiments at a purity of greater than 90% c-kit$^+$ and FcεRI$^+$ cells. Experiments were repeated 3 times. Mast cell degranulation was tested by using a β-hexosaminidase assay. Briefly, 2.4×10^5 cells/well were plated in 96-well plates and preincubated overnight with 1 μg/mL anti-DNP IgE (Sigma-Aldrich) and stimulated for 1 hour with 200 ng/mL DNP-HSA (Sigma-Aldrich). Cell pellets were lysed in 1% hexadecyltrimethylammonium bromide (Sigma-Aldrich) with 20 mmol/L EDTA for 10 minutes at room temperature and incubated with p-nitrophenyl-n-acetyl-β-d-glucosaminide (Sigma-Aldrich) for 1.5 hours at room temperature. The reaction was quenched with 100 μL of 2M NaOH, and absorbance was measured at 405 nm.

REFERENCE

E1. Stentzel S, Teufelberger A, Nordengrün M, Kolata J, Schmidt F, van Crombruggen K, et al. Staphylococcal serine protease-like proteins are pacemakers of allergic airway reactions to *Staphylococcus aureus*. J Allergy Clin Immunol 2017;139:492-500.e8.

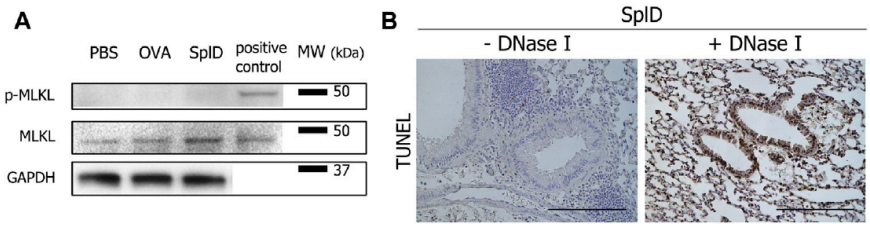


FIG E1. IL-33 upregulation on SpID exposure is cell death independent. **A,** Detection of pMLKL and unphosphorylated MLKL (MLKL) by means of Western blotting. Necrotic TNF-α–treated CT26 cell lysate served as a positive control. *GAPDH,* Glyceraldehyde-3-phosphate dehydrogenase; *MW,* molecular weight. **B,** TUNEL-stained lung sections of SpID-treated mice. The DNase I–treated lung section on the right served as a positive control. *Scale bar* = 200 μm.

J ALLERGY CLIN IMMUNOL
VOLUME 141, NUMBER 2

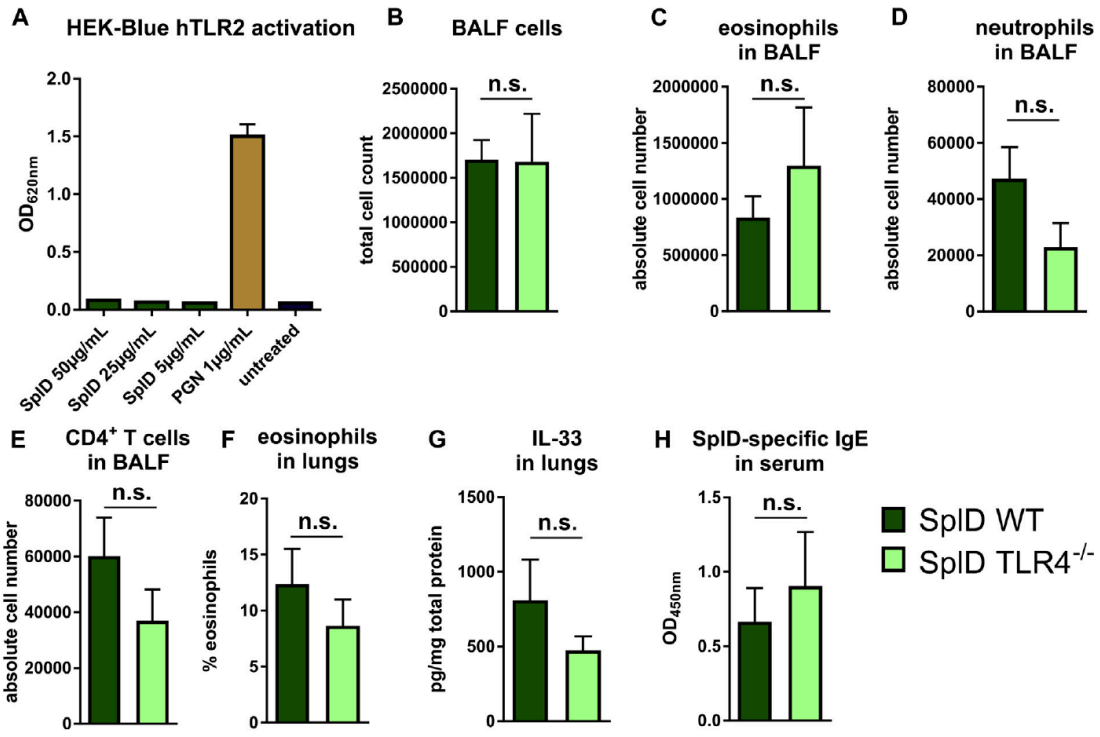


FIG E2. Possible interplay of SpID with the surface receptors TLR2 and TLR4. **A,** Secreted embryonic alkaline phosphatase activity of the TLR2 reporter cell line (HEK-Blue hTLR2) after overnight stimulation with different concentrations of SpID or peptidoglycan *(PGN).* Values were measured in duplicates, and the experiment was repeated 3 times. **B-F,** Total cell count (Fig E2, *B*) and flow cytometric analysis of eosinophils (Fig E2, *C*), neutrophils (Fig E2, *D*), and $CD4^+$ T cells (Fig E2, *E*) in BALF and eosinophils in lungs (Fig E2, *F*). **G,** IL-33 measurement in lung homogenates by using Luminex. **H,** SpID-specific IgE levels in serum measured by means of ELISA of SpID-treated *Tlr4*$^{-/-}$ mice in comparison with the results of SpID-treated wild-type mice. Results are presented as means ± SEMs. N = 4-11. Mann-Whitney *U* test. *n.s.*, Not significant.

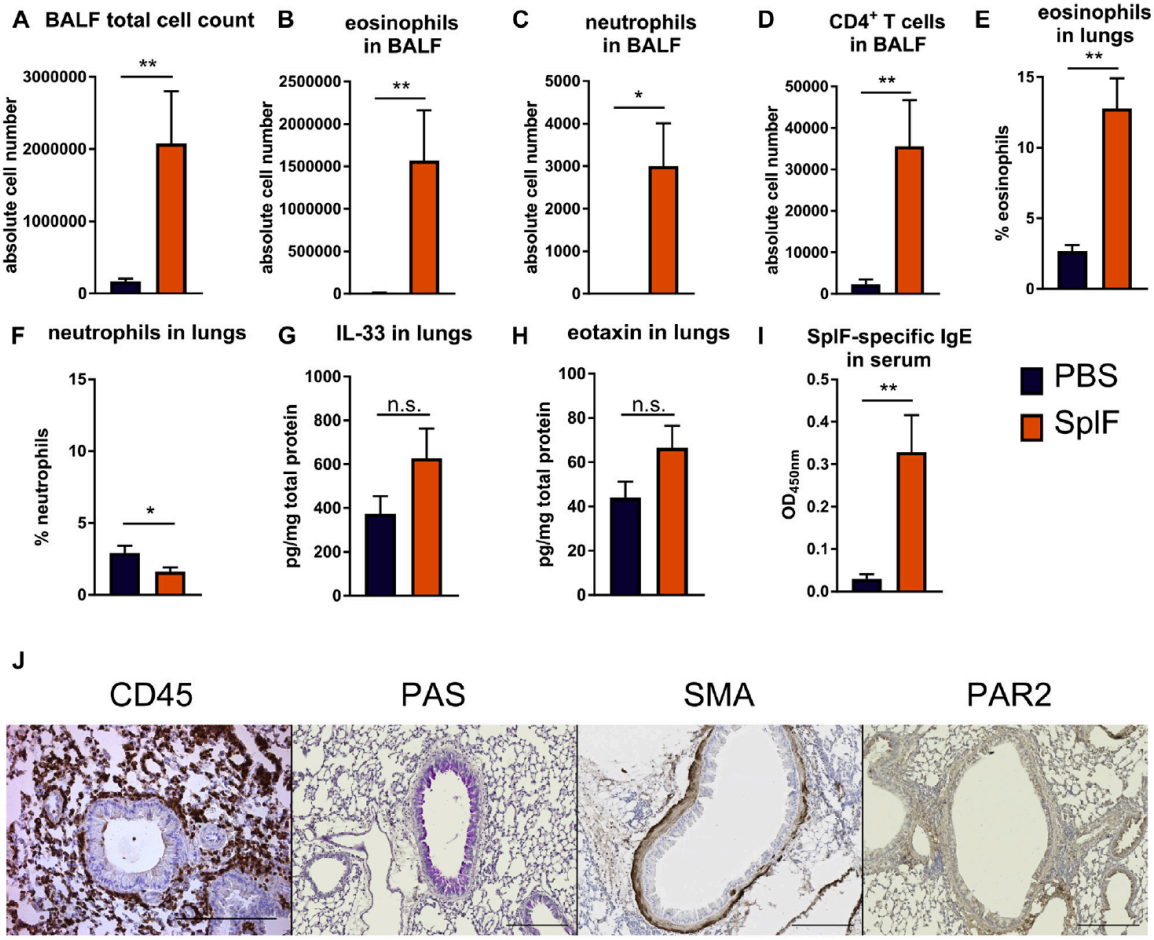


FIG E3. SpIF-induced airway inflammation. **A-D,** Total cell (Fig E3, *A*), eosinophil (Fig E3, *B*), neutrophil (Fig E3, *C*), and $CD4^+$ T-cell (Fig E3, *D*) counts in BALF. **E** and **F,** Eosinophil ($CD11c^-SiglecF^+CD11b^+$; Fig E3, *E*) and neutrophil ($GR1^+CD11b^+F4/80^-$; Fig E3, *F*) counts in the lungs, measured by means of flow cytometry. **G** and **H,** IL-33 (Fig E3, *G*) and eotaxin (Fig E3, *H*) levels measured in lung homogenates by using Luminex. **I,** SpIF-specific IgE levels in sera of PBS- or SpIF-treated mice. **J,** Representative images of anti-CD45, periodic acid–Schiff *(PAS)*, anti–smooth muscle actin *(SMA)*, and anti-PAR2 stainings of lung sections of SpIF-treated mice.

J ALLERGY CLIN IMMUNOL
VOLUME 141, NUMBER 2

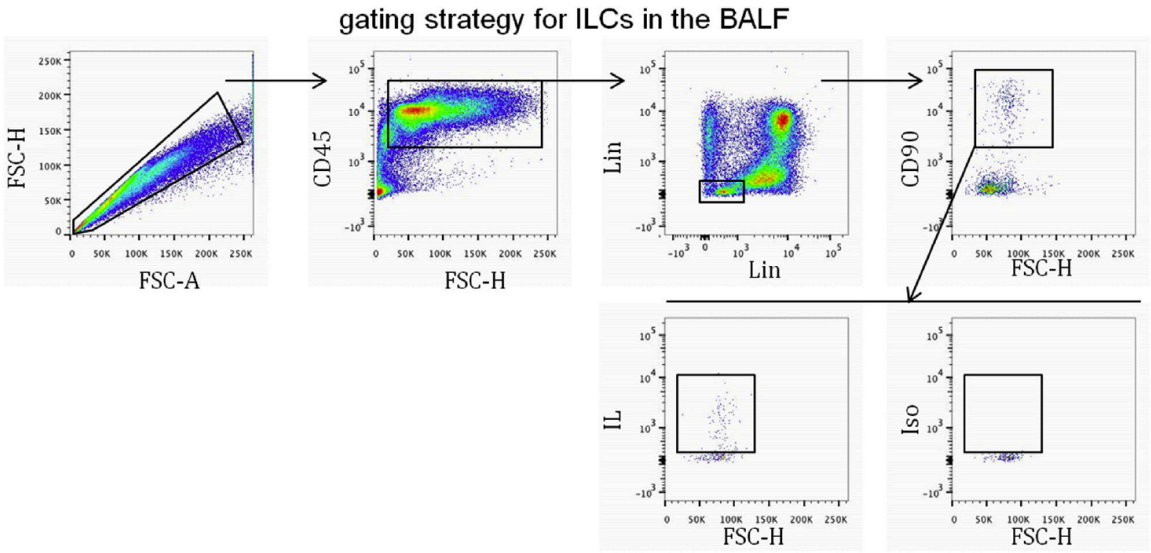


FIG E4. Representative dot plots and gating strategy of ILCs positive for intracellular IL-5 or IL-13 in BALF of wild-type C57BL/6 J mice repeatedly exposed to 45 μg of SpID. ILC2s were identified as Lin$^-$ (CD3$^-$, CD5$^-$, NK1.1$^-$, T-cell receptor β$^-$, CD11c$^-$, CD11b$^-$, and CD45R$^-$) and CD90$^+$ cells. *FSC*, Forward scatter; *Iso*, isotype control; *SSC*, side scatter.

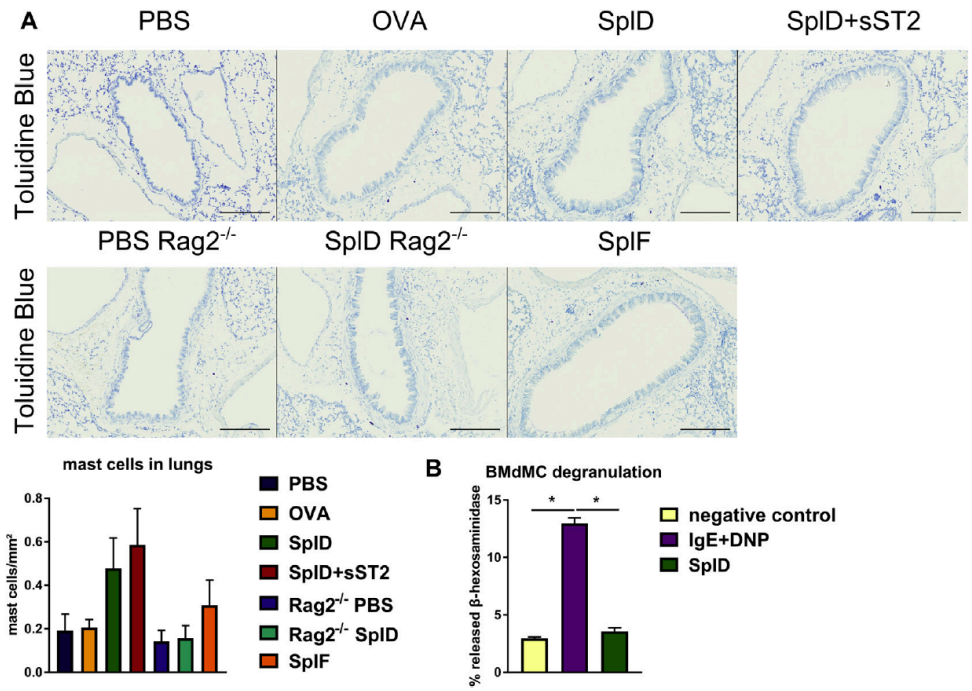


FIG E5. A, Mast cell quantification of toluidine blue–stained lungs. N = 5-13. Mast cell numbers are expressed per area of the section. **B,** β-Hexosaminidase assay of bone marrow–derived mast cells from C57BL/6 J mice to test mast cell degranulation. Results are presented as means ± SEMs. N = 4-11. *$P < .05$, Mann-Whitney U test.

3

MESSING WITH THE SENTINELS - THE INTERACTION OF *STAPHYLOCOCCUS* AUREUS WITH DENDRITIC CELLS

Darisipudi MN, **Nordengrün M**, Bröker BM, Péton V.

Published in Microorganisms. 2018 Aug 15;6(3). pii: E87. doi:10.3390/microorganisms6030087.

AUTHOR CONTRIBUTIONS:

As a coauthor in this publication, **MN** actively participated in the conception and design of this scientific review. **MN** prepared and summarized all the relevant information on *S. aureus*-induced respiratory diseases. **MN** designed the figure and made significant contributions to the scientific revision and editing of the final version of this manuscript.

Conception and design: MND, **MN**, BMB and VP
Collection and management of information: MND, **MN**, VP
Manuscript draft preparation: MND, **MN** and VP
Figure design: **MN**
Critical revision and editing: MND, **MN**, BMB and VP

Maria Nordengrün | Prof. Barbara M. Bröker

microorganisms

MDPI

Review

Messing with the Sentinels—The Interaction of *Staphylococcus aureus* with Dendritic Cells

Murthy N. Darisipudi, Maria Nordengrün, Barbara M. Bröker and Vincent Péton *

Department of Immunology, University Medicine Greifswald, Ferdinand-Sauerbruch-Straße DZ7, D-17475 Greifswald, Germany; venkata.darisipudi@uni-greifswald.de (M.N.D.); maria.nordengruen@uni-greifswald.de (M.N.); broeker@uni-greifswald.de (B.M.B.)

* Correspondence: vincent.peton@uni-greifswald.de; Tel.: +49-3834-865460

Received: 6 July 2018; Accepted: 14 August 2018; Published: 15 August 2018

check for updates

Abstract: *Staphylococcus aureus* (*S. aureus*) is a dangerous pathogen as well as a frequent colonizer, threatening human health worldwide. Protection against *S. aureus* infection is challenging, as the bacteria have sophisticated strategies to escape the host immune response. To maintain equilibrium with *S. aureus*, both innate and adaptive immune effector mechanisms are required. Dendritic cells (DCs) are critical players at the interface between the two arms of the immune system, indispensable for inducing specific T cell responses. In this review, we highlight the importance of DCs in mounting innate as well as adaptive immune responses against *S. aureus* with emphasis on their role in *S. aureus*-induced respiratory diseases. We also review what is known about mechanisms that *S. aureus* has adopted to evade DCs or manipulate these cells to its advantage.

Keywords: *S. aureus*; dendritic cells; innate immunity; adaptive immunity; immune evasion; infection

1. Introduction

Staphylococcus aureus (*S. aureus*) can act as a commensal bacterium in humans, where it frequently colonizes the airways, skin and gut. In most cases, the host can maintain equilibrium with the bacteria over long time periods. However, given the opportunity, *S. aureus* can cause a broad range of infections, ranging from mild, self-limiting skin and soft tissue infections to life-threatening diseases [1]. In the airways, *S. aureus* can cause pneumonia, and colonization with the bacteria is associated with allergic airway inflammation, especially with chronic rhinosinusitis with polyps and asthma [2]. Similarly, *S. aureus* is frequently found on inflamed skin of patients with atopic dermatitis (AD) [3].

Although *S. aureus* mainly has an extracellular lifestyle, the microorganism is also able to invade phagocytes as well as epithelial and endothelial cells and persist intracellularly [4]. To control the bacteria and the infected cells, the host immune system uses every level of its defense mechanisms [1]. Innate and adaptive immunity are involved, and both humoral and cellular effector mechanisms are required to keep the microorganism in check. Being at the interface between innate and adaptive immune responses, dendritic cells (DCs) must be central to the immune protection against *S. aureus* [5].

Recognition of *S. aureus* by professional phagocytes, such as monocytes (MOs), macrophages (Mφs) and DCs, induces the release of cytokines and chemokines, and the latter recruit neutrophils to the site of infection. Neutrophils are essential for killing the bacteria, either by phagocytosis or by NETosis. Phagocytosis is a process of engulfing and digestion of bacteria inside the cell, whereas NETosis involves trapping of bacteria in net-like structures, called neutrophil extracellular traps (NETs), which consist of DNA and histones as well as the content of neutrophil granules, such as anti-microbial peptides (AMPs) and elastase [1]. As a counter measure, *S. aureus* secretes many proteins that interfere with both the recognition by phagocytes and their chemotaxis to the infection site [6]. The microorganism is further capable of destroying NETs by nuclease production [7].

The humoral arm of the innate immune system, the complement cascade, is also indispensable in the defense against *S. aureus*. Complement factors or their fragments can promote opsonization to facilitate microbial clearance (C3b) and drive inflammation (C3a and C5a). These functions are mediated by specific complement receptors (CRs) on host cells. Moreover, complement can kill many bacterial species directly by forming pores in their membranes, the membrane attack complexes (MACs). In return, *S. aureus* interferes with complement function at many levels. A capsule and a thick peptidoglycan layer protect its membrane from MAC (reviewed in [1,8]). By inhibiting the central hub of the complement cascade, the C3 convertase, *S. aureus* reduces the production of C3b, C3a and C5a, interfering with both opsonization and inflammation [1,9]. These findings are in line with the previously observed role of C3 in controlling *S. aureus* bacteremia [10]. In addition, in a mouse model of *S. aureus*-septic arthritis, deficiency of C3 diminishes opsonization and phagocytosis of *S. aureus* and thereby impairs host defense [11].

Considering adaptive immunity, there is compelling evidence that antibodies contribute to clinical protection from *S. aureus* infection. Most human adults have a broad spectrum of *S. aureus*- specific antibodies in their body fluids with large inter-individual variation in terms of antibody titers and the spectrum of *S. aureus* antigens that are recognized [12,13]. High titers of specific antibodies are associated with a reduced risk of *S. aureus* infection and/or a less severe disease course [14]. Conversely, hyperimmunoglobulin E syndrome (HIES) patients are highly susceptible to recurrent *S. aureus* infection. In the majority of cases the disease is caused by heterozygous missense mutations and short deletions in signal transducer and activator of transcription 3 (*STAT3*) leading to an impairment of T cell development, in particular of Th17 cells. Recently it was shown that HIES patients also have very low anti-*S. aureus* antibody titers, although total serum IgG levels are in the normal range. Presumably, this is due to their impaired T cell response, which we discuss in detail below. Immunoglobulin (Ig)G replacement therapy significantly ameliorates *S. aureus* control, with concomitant antibiotic treatment, which makes a strong case of a protective role of antibodies [15,16].

Binding of IgG antibody to Fc receptor on phagocytes can opsonize the bacteria, whereas binding of IgG and IgM to bacteria triggers the complement cascade. Moreover, antibodies can neutralize *S. aureus* toxins and other virulence factors [12].

Recent studies have highlighted the importance of T cell-mediated immune response in *S. aureus* clearance. In a mouse model of persistent *S. aureus* infection, deficiency of T cells increased the susceptibility to *S. aureus* [17]. In addition, in murine models of nasal colonization and cutaneous infection, production of interleukin (IL)-17A by Th17 cells is required for bacterial clearance by promoting neutrophil influx to the site of pathogen invasion [18]. Furthermore, as discussed before, HIES patients with a defect in the STAT3 signaling pathway, display impaired Th17 differentiation and are highly susceptible to recurrent severe infections with *S. aureus* [19].

It is well known that most B cells require help by T cells to generate high affinity antibodies, such that the observation of a broad *S. aureus*-specific antibody repertoire indicates the existence of numerous *S. aureus*-specific T cells. Several recent studies have provided evidence for robust CD4+ and CD8+ T cell memory of staphylococcal antigens in humans [20–22].

There is limited knowledge about the mechanisms by which *S. aureus* activates the T cells and directs their differentiation into effector and memory T cell subpopulations, but DCs are bound to be critically involved. DCs have a central role as antigen-presenting cells (APCs) for T cells, and they have a decisive influence on the quality of the adaptive immune reaction.

In this article, we review different aspects of DC physiology and how these cells interact with *S. aureus* during colonization and infection. We also address the countermeasures *S. aureus* uses to divert and disturb the immune response triggered by recognition of the bacteria by DCs, including the induction of allergic inflammation (illustrated in Figure 1). We place emphasis on the airway environment, because multifaceted interactions between *S. aureus* and its host take place at this site: colonization, infection and allergy.

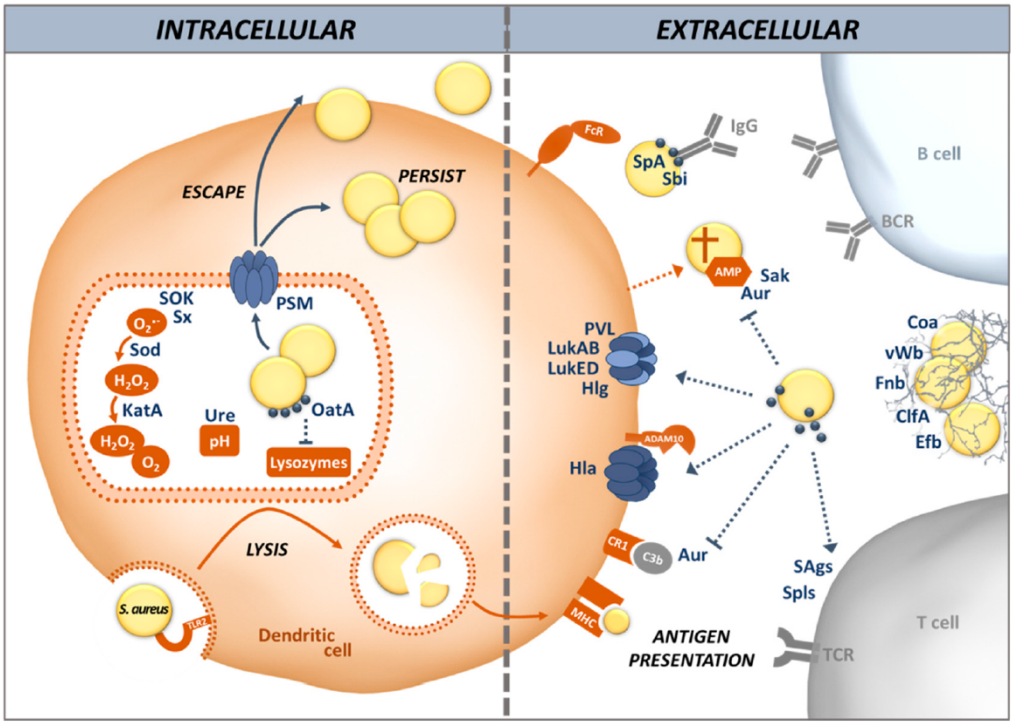


Figure 1. *S. aureus*' interaction with a dendritic cell during an infection. As antigen-presenting cells DCs are able to take up *S. aureus*, lyse the bacteria and present bacterial peptides on MHC class II to initiate a specific T cell response. However, *S. aureus* displays a broad range of mechanisms to avoid opsonization, phagocytosis and proteolytic degradation by DCs. In the extracellular space, *S. aureus* avoids opsonization by blocking antibody and complement function. SpA captures antibodies via their Fc region, thereby preventing recognition by Fc receptors. Aur cleaves complement factor C3 into non-functional C3b. ClfA, Efb and FnbAB bind to fibrinogen and fibronectin, respectively, facilitating the formation of a mesh that protects *S. aureus* from phagocytosis. The coagulases Coa and vWb potentiate this process by mediating the conversion of fibrinogen into fibrin. After engulfment by phagosomes, *S. aureus* can increase the local pH by producing Ure, preventing efficient lysis. OatA acetylates the peptidoglycan cell wall, rendering *S. aureus* resistant to lysozymes. The Sx and SOK have antioxidant properties, protecting *S. aureus* from membrane damage. In addition, *S. aureus* SodA, SodM and KatA can act in cascade to detoxify ROS. PSMs enable *S. aureus* to escape from the phagosomes, thus invading the cytoplasm and possibly killing the host cell, which releases *S. aureus* into the extracellular space. AMPs secreted by DCs can be degraded by Aur and Sak, protecting *S. aureus* from being killed. Furthermore, *S. aureus* produces several pore-forming toxins, among them Hla and the bi-component toxins LukAB, LukED, PVL and Hlg, that can directly kill DCs. Finally, *S. aureus* SAgs and Spls are able to modulate the balance of the initiated T cell response towards a more favorable Th2 profile. Brown: DC factors; blue: *S. aureus* factors. Abbreviations: ADAM10: A disintegrin and metalloproteinase domain-containing protein 10; AMP: Anti-microbial peptides; Aur: Aureolysin; BCR: B cell receptor; DCs: Dendritic cells; C3: Complement factor 3; Clf: Clumping factor; Coa: Coagulase; CR1: Complement receptor 1; Efb: Extracellular fibrinogen binding protein; Hla: Alpha-hemolysin; Hlg: Gamma-hemolysin; Fc: Fragment crystallizable; Fnb: Fibrinonectin-binding protein; Kat: Catalase; Luk: Leukocidin; MHC: Major histocompatibility complex; OatA: *O*-acetyltransferase A; PSM: Phenol-soluble modulin; PVL: Panton-Valentine leucocidin (PVL); ROS: Reactive oxygen species; SAgs: Superantigens; Sod: Superoxide dismutase; SOK: Surface factor promoting resistance to oxidative killing; SpA: *S. aureus* protein A; Spls: Serine protease-like proteins; Sx: Staphyloxanthin; TCR: T cell receptor; TLR2: Toll-like receptor 2; vWb: von Willebrand factor-binding protein; Ure: Urease.

2. Dendritic Cells

DCs were first described by Paul Langerhans in 1868 [23] as "branched skin cells resembling neurons", hence the term of Langerhans cells for skin DCs. Steinmann and Cohn proposed the term "dendritic cells" in 1973 and characterized their "tree-like" morphology (Greek, *dendron*) and tissue distribution in mice [24].

DCs are highly specialized phagocytic cells. Their main function is antigen presentation to T cells, and they have the unique ability to initiate and regulate both innate and adaptive immune responses against various antigens [25]. DCs originate in the bone marrow and travel through the blood into the tissues throughout the body, including the skin, mucosal tissues and lymphoid organs [26–28]. As sentinel cells in the tissues, DCs continuously take up antigen, sampling their microenvironment. Upon recognition of pathogen-associated and danger-associated signals, they initiate a response of the adaptive immune system. The DCs stop taking up additional antigen and migrate from the local tissue to secondary lymphoid organs, where they differentiate into mature DCs. The latter express high levels of major histocompatibility complex (MHC) class I and II, adhesins and costimulatory molecules, and can thus act as professional APCs for T cells [29]. They efficiently activate naïve antigen-specific T cells and strongly influence their differentiation into different subsets such as Th1, Th2, Th17 or Tregs.

In addition to their crucial role at the interface between innate and adaptive immune system as professional APCs, DCs contribute to the clearance of the opportunistic pathogen *S. aureus*. Although their ability to directly kill *S. aureus* is limited, they play a major role in the initiation and regulation of the immune response at the infection site [4,5,26,30]. By producing cytokines, DCs are essential for the recruitment of other effector cells specialized in killing bacteria, e.g., neutrophils [5,31]. In addition, IL-27 from APCs reduces the pH of phagolysosomes which boosts the ability of DCs to kill intracellular bacteria [32]. Moreover, activated platelets can stimulate DCs via CD40L production and improve their maturation, increasing cytokine secretion as well as antigen presentation in case of *S. aureus* infection [33].

DCs are generated during hematopoiesis from precursors of lymphoid or myeloid origin, which is an antigen-independent process. The progenitors in the bone marrow, called macrophage- and DC precursors (MDP), give rise to DCs and Mφs [34]. In recent years, awareness of the existence of several types of DCs has grown. Depending on their phenotype, function and tissue distribution [35,36], they are broadly classified into two major groups: conventional or classical DCs (cDCs) [24,37,38] and non-conventional DCs [39], with each group comprising more than one distinct subpopulation.

cDCs originate from pre-cDCs under the influence of granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-4. This type of bone marrow-derived DCs has been extensively studied in in vitro experiments, and they are the most valuable source of knowledge about human and murine DCs. In vivo, the precursors of DCs exit the bone marrow and migrate through the blood into lymphoid tissues, bone marrow, spleen and lymph nodes, where they differentiate into cDCs characterized by the integrin CD11c [40]. Since Mφs also express CD11c, additional markers are required. Tyrosine kinase receptor "fms-like tyrosine kinase 3" (Flt3 or CD135) is an excellent marker that distinguishes cDCs from Mφs. cDCs are further divided into subgroups based on their tissue localization and cell surface markers. In mice, lymphoid cDCs cells express CD8α and CD4 (referred to here as cDC1), which constitute about 20–40% of total spleen and LN cDCs, while human cDCs cells express CD370 (Clec9A) and the chemokine receptor XCR1 [41,42]. cDCs in non-lymphoid tissues are either CD103+ CD11b− or CD103− CD11b+ (referred to here as cDC2) and lack the marker CD8. However, both CD8+ cDCs and CD11b+ cDCs proliferate in response to Flt3 (reviewed in [42]). Human and murine cDC1 cells that reside in the barrier organs such as the skin, skin-draining lymph nodes and murine Peyer's patches, express the integrin CD103 [43].

cDCs are key players in the polarization of the T cell response. cDCs1 secrete Th1 polarizing cytokine IL-12p70, but are also capable of producing immune regulatory cytokines such as transforming growth factor beta (TGF-β) and IL-10, both important in immune tolerance [44]. cDC2 induce the Th2 immune response to helminth infections, whereas fungal pathogens induce the Th17 immune response.

In humans, cDC2 cells are characterized by expression of CD103, FcR1A and the alpha-chain of the high affinity receptor for IgE [45]. Compared to cDC1, cDC2 in mouse and human are less capable of presenting antigens via MHC class II [46].

Plasmacytoid DCs (pDCs) were first reported in 1958 by Lennert et al., and named by their appearance, which resemble plasma cells [39]. Precursors of pDCs express low levels of the GM-CSF but high amounts of the IL-3 receptor, and they differentiate into pDCs in response to IL-3 [47,48]. Human pDCs are characterized by their intermediate expression of CD11c, low levels of MHC class II and high density of CD123, but lack of CD11b [49,50]. In mice, pDCs exhibit surface markers that are shared with other cell types, e.g., B220 and Ly6C [51,52]. pDCs are found circulating in the blood and in peripheral organs such as bone marrow, spleen, thymus, lymph nodes, and the liver. They are known to play an important role in the production of type I interferons (IFN)-α/β by virtue of their capacity to sense viral nucleic acids [53,54].

3. Recognition and Uptake of *S. aureus* by DCs

DCs can efficiently recognize a wide range of invading microorganisms. The pattern recognition receptors (PRRs), which are expressed on their surface, can sense pathogen-associated molecular patterns (PAMPs) or damage associated molecular patterns (DAMPs). Among all PRRs, the Toll-like receptors (TLRs) are particularly well studied and characterized. They play a key role in inducing both direct and indirect DC maturation. To date, 10 human TLRs (TLR1–10) and 12 murine TLRs (TLR1–9, TLR11–13) have been identified. TLR1, 2, 4–6 and 11 are expressed at the cell surface while TLR3 and 7–9 are located intracellularly in the endosomal compartments [55].

TLR2 is the main receptor involved in *S. aureus* recognition, through the detection of lipoproteins, wall teichoic and lipoteichoic acid, as well as peptidoglycan [56,57]. In association with TLR1 or TLR6, TLR2 is able to sense diacyl or triacyl lipopeptides or lipoproteins [58]. Counteracting this, staphylococcal superantigen-like proteins (SSL) 3 and 4 inhibit TLR2 through interfering with both lipopeptide binding and TLR dimerization [59]. In a murine model of infection, *S. aureus* has been shown to evade TLR2 activation by secreting SSL3, indicating that TLR2 inhibition is important for staphylococcal pathogenesis [60]. The production of phenol soluble modulins (PSMs) increases the release of lipoproteins from the surface of *S. aureus*, whereas *S. aureus* strains producing low amounts of PSMs are less detected by TLR2 [56]. In AD, a chronic allergic inflammatory disease of the skin, DCs are less responsive to TLR2 stimulation, which could blunt their anti-*S. aureus* activity [61]. Colonization of lesional skin by *S. aureus* occurs in 90% of AD patients aggravating the inflammation and sometimes leading to severe invasive infections such as endocarditis or bacteremia [62]. TLR8 in MOs, Mφs and DCs senses *S. aureus* RNA, a vita-PAMP enabling the assessment of microbial viability [63,64]. TLR9 binds *S. aureus* CpG-DNA and induces type I IFN signaling [65]. In osteoblasts, TLR9 also improves the killing of *S. aureus* by increasing the production of reactive oxygen species (ROS) [66]. Persistent *S. aureus* carriers have a higher expression of TLR9 that is dependent on the carrier's TLR9 haplotype, sex and hormone status, which could explain why women are more susceptible to *S. aureus* septicemia than men [67,68].

To escape PRR-triggered phagocytosis and avoid the reaction of host cells to danger signals, *S. aureus* produces many proteins enabling it to invade different cell types, including non-phagocytic cells, via a zipper-type mechanism [69,70]. This is mediated by adhesins, known as microbial surface components, which recognize adhesive matrix molecules (MSCRAMMs) [71]. MSCRAMMs, including the fibronectin binding proteins (Fnb) A and B, are covalently linked to the peptidoglycan cell wall and involved in adhesion of *S. aureus* to the host cell matrix (Figure 1). *S. aureus* Fnb A and B bind to the host cell integrin $\alpha_5\beta_1$ via a fibronectin bridge. Other MSCRAMMs such as clumping factor (Clf) A and B are also involved in the attachment of *S. aureus* to host cells. Protein A (SpA), in contrast, interacts directly with host cell receptors [72]. These mechanisms stimulate actin rearrangement in the host cell and *S. aureus* is internalized without triggering TLRs [70,73,74].

Other adhesins, known as secretable expanded repertoire adhesive molecule (SERAMs), are released by *S. aureus* and re-attach to the bacterial surface non-covalently. The autolysin (Alt) family of proteins, AltA and Aaa, mediate adherence of *S. aureus* to host components such as fibronectin, gelatin and heparin, facilitating colonization and infection [75]. Among the SERAMs, the extracellular adherence protein (Eap) and extracellular matrix and plasma binding protein (Emp) have been shown to bind to host extracellular matrix components and plasma proteins, such as fibrinogen, fibronection, vitronectin, collagen, as well as to the endothelial cell adhesion receptor ICAM-1 [76,77]. Other SERAMs exploit the host's coagulation system. Staphylocoagulase (Coa) and von Willebrand factor-binding protein (vWbp) activate prothrombin by inducing a conformational change to form a complex that cleaves fibrinogen into fibrin. ClfA then binds to the resulting fibrin cables to form a mesh, thus protecting *S. aureus* from phagocytosis and inducing abscesses. In this way, *S. aureus* can aggregate and shield themselves from phagocytes in a tight network thanks to bacterial and host proteins [78].

Opsonization is an efficient way to help phagocytes, including DCs, to take up bacteria and target them for killing. Igs and complement components are the main opsonins in the body fluids. Phagocytes possess specific receptors for IgG as well as the main complement opsonin C3b. DCs express Fcγ receptors on their surface that bind to IgG, helping the DC to immobilize their target for phagocytosis [79]. However, *S. aureus* possesses a broad range of factors that can prevent opsonization, either by hiding the targets of the opsonins or by using decoys. Many *S. aureus* strains are encapsulated by sugar polymers that cover most of the immunogenic surface-exposed proteins [80]. The two main serotypes are capsular polysaccharide (CP) 5 and CP8, which represent about 75% of all clinical isolates. Although strains producing a capsule are more resistant to phagocytosis, this resistance is overcome when specific Igs bind to the capsule polymers [81]. Interestingly USA300, one of the main CA-MRSA clones, does not display any capsule, demonstrating that capsule targeting vaccines cannot cover the whole diversity of *S. aureus* clones. Moreover, all *S. aureus* clinical isolates produce SpA, a major virulence factor, which binds with high affinity to the Fc-portion of Igs, mostly IgG, rendering them unable to bind to the bacterial surface in the correct orientation and thus preventing opsonization [82]. SpA can also act as a B cell superantigen, since it binds to B cell receptors which use the V_HIII element, inducing apoptotic cell death. This can result in the complete deletion of the respective B cell clones [83].

DCs possess CR3 (CD11b/CD18) and CR4 (CD11c/CD18), such that coating of *S. aureus* with C3b facilitates phagocytosis [84]. However, *S. aureus* is adept in complement evasion. Many secreted proteins such as SpA, aureolysin (Aur), staphylokinase (Sak), extracellular fibrinogen-binding protein (Efb), and staphylococcal complement inhibitor (SCIN) interact with components of the complement pathways, preventing this system from fulfilling its purpose in opsonization and killing of pathogens (Reviewed in [1,85]). For instance, Aur cleaves C3 to produce a non-functional C3b fragment, hence preventing a normal activation of the complement and its opsonizing effects [86]. Furthermore, Sak and Aur can also bind and degrade secreted antimicrobial peptides such as α-defensins and LL-37, before these can cause pores in the bacterial membrane [87–89].

To kill before being killed is another strategy of *S. aureus*, which is effective even before the bacteria are taken up by the host cells. *S. aureus* produces bi-component pore-forming toxins called leukocidins (Luks) which play a pivotal role in killing host immune cells, including DCs. The target structures of these toxins on host cells have been identified over the past few years (reviewed in [90]). LukED targets the C-C chemokine receptor (CCR) 5 as well as the C-X-C motif chemokine receptor (CXCR) 1 and CXCR2, to kill DCs, T cells and Mφs [91,92]. LukAB specifically binds to the CD11b I domain in human but not murine polymorphonuclear cells (PMNs) and allows *S. aureus* to either kill its host cell or escape from phagosomes [93]. Since some subpopulations of DCs express CD11b, as part of the CR3, they can be targeted by LukAB [42,93–95]. LukMF′ is mostly found in *S. aureus* isolates from ruminants and is associated with bovine infections. It targets CCR2 and CCR5 on bovine and CCR1 on both human and bovine neutrophils and induces cell death [96]. These CCRs are also found on human DCs, which make them vulnerable targets [97]. The specificity of gamma-hemolysin

(Hlg) depends on the subunits forming the toxin. The heterodimer HlgAB has a hemolytic function by binding to the Duffy Antigen Receptor for Chemokine (DARC) on erythrocytes and can also target CXCR1, CXCR2 and CCR2 on DCs [97–99]. HlgCB binds to the same targets as Panton-Valentine leukocidin (LukSF-PV, also known as PVL), C5aR and C5L2 [98]. Moreover, some toxin subunits can cross-interact with others and form hybrid toxins, which might increase the number of targets on the surface of host cells. For instance, HlgB can compete with LukS-PV and LukD to interact with LukF-PV [100,101].

4. *S. aureus* Evades Killing by DCs as well as Antigen Processing and Presentation

After internalization by phagocytosis, *S. aureus* is exposed to bactericidal effector mechanisms in DCs. Phagosomes mature and fuse with lysosomes containing hydrolases. In the phagolysosomes, *S. aureus* is also subjected to oxidative conditions rich in ROS and reactive nitrogen species (RNS). NADPH oxidase (NOX2) consumes oxygen to produce superoxide radical anions ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). Oxidation modulates the activities of different groups of proteases and thus reduces proteolysis within phagosomes of DCs [102]. In addition to this oxidative stress, *S. aureus* is also submitted to acidic pH. Acidification of phagosomes in DCs is reduced compared to Mφs or neutrophils because the responsible enzyme, the V-ATPase, is incompletely assembled in immature DCs [30]. Proteases are therefore less active such that proteins are only partially degraded [103]. While reducing the ability of DC to truly clear the bacteria, incomplete protein degradation permits DCs to expose a higher diversity of peptides on MHC class II to efficiently prime T cell responses. Depending on the signals from their microenvironment, most importantly the nature of the infectious agent, DCs release various cytokines that direct the differentiation of naive CD4+ T cells into different effector and memory T cell subsets.

However, in many cases *S. aureus* manages to survive within eukaryotic host cells, including professional and non-professional phagocytes [104]. Acidification of the phagolysosome is counteracted by secretion of urease, which increases the pH by hydrolyzing urea into ammonia [105]. *S. aureus* is also highly resistant to oxidative stress, because staphyloxanthin (Sx), the main pigment of *S. aureus*, works as an antioxidant and prevents membrane peroxidation [106]. SOK (surface factor promoting resistance to oxidative killing) displays similar properties and is considered a virulence factor in endocarditis [107]. SodA, SodM and KatA act in cascade to detoxify O_2^- into H_2O_2 and then into $H_2O + O_2$ [108,109]. A recently discovered molecule, the staphylococcal peroxidase inhibitor (SPIN), inhibits myeloperoxidase (MPO) in neutrophils and protects *S. aureus* from oxidative stress during phagocytosis [110]. DCs lack MPO, but they are influenced by the neutrophil-derived enzyme, which inhibits antigen uptake and processing by DCs, as well as their migration to lymph nodes and, as a consequence, T cell priming [111,112]. MPO-inhibition by SPIN could, therefore, enhance *S. aureus* survival in neutrophils with a trade-off: promoting the induction of an adaptive immune response by DCs.

Modification of the bacterial cell wall is another way to avoid degradation. The *O*-acetyltransferase A (OatA) adds an acetyl group to *N*-acetylmuramic acid in the peptidoglycan, rendering *S. aureus* resistant to the lysozyme produced in the phagosome [113]. This may be one reason why lipoproteins that are embedded in the cell wall do not elicit a strong adaptive immune response [114].

Moreover, *S. aureus* has means to destroy the phago(lyso)some membrane and escape into the cytoplasm. Upon internalization by professional phagocytes, the bacteria produce phenol-soluble modulins (PSMs), similar to the delta hemolysin, which can form membrane pores. In DCs, PSMs help *S. aureus* to escape from the phagosome, invade the cytoplasm and possibly kill the host cell [115–117]. PSMs are under the positive control of the *agr* system, a global regulator that is active in the phagolysosome environment [69]. This mechanism could allow *S. aureus* to interfere with antigen processing and presentation on the MHC class II of the DCs, reducing their T cell-priming ability. Another effect of PSMs is the modulation of cytokine production by the host cells [118–120]. In DCs, the activation of the p38-CREB pathway by the PSMs induces a tolerogenic phenotype with a reduction

of TLR2 signaling and production of inflammatory cytokines, leading to an increased priming of anti-inflammatory T_{regs} [118,119]. After escaping the phagosome, *S. aureus* is able to replicate in the cytosol and induce host cell death or survive in a dormant state for extended time periods in the shape of small colony variants (SCV) [69]. This ability is probably linked to the chronicity of infections. It is still unclear what determines the balance between killing the host cell and surviving inside. Chronic *S. aureus* infection are very difficult to treat, and novel approaches are required. Chloroquine improved clearance of *S. aureus* from lung epithelial cells in combination with antibiotic therapy [121,122].

Autophagy or, more precisely, xenophagy, is used by DCs to kill pathogens and present antigens, once the bacteria have escaped into the cytoplasm. The cytoplasmic invaders become surrounded by double-membrane vacuoles, named autophagosomes, which present microtubule-associated protein 1 light chain 3 (LC3) associated to phosphatidylethanolamine (LC3-II). These fuse with lysosomes, redirecting their cargo from the cytoplasm back into the lysosomal pathway, followed by digestion and presentation of antigenic proteins. Consequently, peptides derived from cytoplasmic antigens, which are usually presented on the MHC class I, are loaded onto the MHC class II molecules, a process known as cross-presentation of antigens.

The *agr* system conditions *S. aureus* survival during autophagy by preventing the fusion between autophagosomes and lysosomes. *S. aureus* strains with a highly active *agr* system are not killed in autophagosomes in vitro and display an extended intracellular survival within phagocytes in vivo [31]. *S. aureus* can prevent fusion of autophagosomes with lysosomes via a novel mechanism, involving activation of MAPK14 and ATG5 phosphorylation [123]. Autophagy can tolerize host cells to the effects of alpha-toxin (alpha-hemolysin, Hla), another pore-forming toxin produced by *S. aureus* under *agr* control [124]. Indeed, autophagy allows cells to recycle membranes affected by Hla and endure higher concentrations of pore-forming toxins. Remarkably, Hla has been identified as an important autophagy-inducing factor [31,125].

As previously mentioned, DCs produce cytokines to control the immune response, recruit immune cells on the infection site and activate T cells. *S. aureus* is able to modulate the cytokine production of immune cells with several mechanisms. The ESAT-6-like secretion system (Ess) is encoded in the conserved *ess* gene cluster [126]. The *ess*-encoded virulence factor EsxA dampens the pro-apoptotic response in infected DCs and may allow *S. aureus* to use these cells as a Trojan horse. EsxB dampens the production of regulatory and pro-inflammatory cytokines by the infected DCs, resulting in a reduced production of IFN-γ and IL-17 by activated CD4+ T cells [127].

S. aureus can also induce an overstimulation of the immune system by secreting superantigens [128]. The species harbors 25 genes encoding superantigens, comprising the toxic shock syndrome toxin-1 (TSST-1) and the staphylococcal enterotoxins, organized in the "enterotoxin gene cluster" *egc* or on mobile genetic elements [129,130]. As superantigens, they are able to interact with the MHC class II on the DCs and the Vβ region of the T cell receptor (TCR), bypassing the conventional antigen specific activation of T cells, to activate up to 20% of the T cell population [131].

5. Interaction of DCs with *S. aureus* in the Respiratory Tract

The airways are a site of extensive interaction between *S. aureus* and its host with extremely diverse outcome. Around 25 to 35% of adults persistently carry *S. aureus* in the nose, while the remainder is able to rapidly clear the bacteria from the upper airways [132]. However, *S. aureus* can also cause pneumonia, a life-threatening infection of the lungs. It may be community acquired, often in the context of an influenza infection, in which case the mortality rate can increase to 50% [133]. In hospitals, patients receiving mechanical ventilation are vulnerable to *S. aureus* infection and develop so-called ventilation-associated pneumonia. There is increasing evidence that, besides commensal and invasive behavior, *S. aureus* may also drive allergic airway inflammation [134,135]. Colonization with *S. aureus* is associated with childhood wheezing and asthma [136]. van Zele et al. have found that 66.7% of patients with nasal polys and co-morbid asthma are colonized with *S. aureus*, in contrast to 33.3% of healthy adults. In case of additional aspirin hypersensitivity, these were even 87.5% [137].

Moreover, IgE antibodies specific for *S. aureus* enterotoxins and serine protease-like proteins were found in asthmatic patients [138,139].

DCs play a critical role in shaping the adaptive immune response at mucosal sites. The modulation of the T helper cell response to *S. aureus* infection by lung DCs is of particular interest. Over the past years, many subsets of DCs have been described in lung immunity. Under steady state conditions, "immature" DCs in the lungs efficiently recognize and capture inhaled materials. DCs that have encountered antigens or allergens undergo maturation, leave the lung and migrate to draining regional lymphoid tissues, where they present the processed antigenic peptides to naïve T cells. This results in T cell activation and polarization, depending on the nature of the antigen [140]. It has been well documented that DCs induce protective immune responses against pathogens, but may also initiate inflammatory immune responses to harmless allergens, being thus involved in the pathophysiology of asthma and allergic rhinitis [141]. Both protection and allergy are relevant in the interaction of *S. aureus* with its host.

S. aureus-primed cDCs are highly responsive and induce T cell differentiation into IFN-γ-producing CD4+ (Th1) and CD8+ (Tc1) cells [142]. In fact, healthy donors and patients show a large pool of *S. aureus*-specific memory T cells that respond to *S. aureus* with the secretion of IFN-γ and/or IL-17 [21,22]. The existence of CD8+ T cell memory cells and their responses against staphylococcal antigens are important for minimizing inflammation and promoting T cell tolerance [20]. Moreover, long-term exposure of mice to *S. aureus* failed to produce IL-2 after an antigen-specific T cell response, suggesting that T cells undergo anergy during persistent infection [143]. The lungs are vulnerable to inflammation-induced organ damage interfering with gas exchange, which may rapidly become critical. A strong T cell response driven by DC recognition of *S. aureus* is therefore a double-edged sword, as has been demonstrated in murine pneumonia models (reviewed in [144]).

S. aureus colonization of the airways is associated with allergic airway disease, but the mechanisms of allergic sensitization or exacerbation by *S. aureus* are still poorly understood [136,145]. Asthma is defined by chronic airway inflammation with reversible airway obstruction, airway hyperresponsiveness, infiltration Th2 cells and eosinophils into the airway submucosa, mucus hypersecretion and airway remodeling. It has been more than two decades since Robinson et al. and other groups demonstrated that atopic asthma was associated with activation of Th2 type of T cell in the airways [146]. In addition, in murine model of asthma and allergy, adoptive transfer of Th2 cells, but not Th1 cells, induces airway hyper-responsiveness (AHR) [147]. The involvement of DCs in asthma was characterized in 1998 by Lambrecht et al. [148], who showed that cDCs were essential for triggering allergy in ovalbumin-sensitized mice (OVA). Conversely, pDCs were protective [141]. Recently, it has been demonstrated that a subtype of cDCs, CD117+ CD172α+, is a major mediator of inflammation in asthma by promoting the induction of Th2 immunity in spleens [149].

Some airborne allergens such as Der p1 from house dust mites disrupt the epithelial barrier by cleaving the tight junction proteins, thus gaining access to the DCs at the basolateral side of the epithelium [150]. It is tempting to speculate that *S. aureus* alpha-toxin (hemolysin alpha, Hla) may act similarly, because the pore-forming toxin activates the host metalloprotease ADAM10, which destroys epithelial adherens junctions [151,152]. Moreover, ADAM10 and its ligand Notch1 were shown to be essential for DCs to produce Th2 type cytokines in a murine model of IgE-mediated anaphylaxis, suggesting that ADAM10 activation by Hla could have pro-allergenic effects [153]. On the other hand, human Hla-specific T cells release mainly IFN-γ and IL-17, indicating that the toxin itself is not an allergen [21,154–156]. It is not known how Hla affects DCs, but in monocytes the toxin is able to induce IL-17, which is in line with the observed cytokine profiles of Hla-specific T cells [151]. While questions remain regarding the possible pro-allergenic effects of Hla, there can be no doubt that the toxin is a decisive virulence factor in *S. aureus* pneumonia [151,157]. Via ADAM10 the toxin induces the secretion of pro-inflammatory cytokines and cell death via the nucleotide-binding domain and leucine-rich repeat containing gene family, pyrin domain containing 3 (NLRP3) inflammasome [158]. In the lungs, epithelial tissue destruction could provide host-borne nutrients for bacterial growth, which may be

one reason for the disastrous effects of Hla in pneumonia [159]. Neutralization of *S. aureus* alpha toxin is under development as adjunct therapy with standard antibiotic treatment [151,160].

It was discovered that two classes of *S. aureus* proteins, SEs and serine protease-like proteins (Spls), readily cause allergic sensitization in the airways [161]. Affected humans and experimental animals elaborate antigen-specific IgE as well as Th2 cells [139,162]. This demonstrates that different *S. aureus* factors can elicit adaptive immune responses of different quality in the same individual. Similar observations have recently been reported for fungal antigens by Bacher et al. [163]. We conclude that many microbial proteins have adjuvant activity, determining the cytokine and antibody profiles of the specific T and B cells. As this is an emerging topic, there are many open questions: How are DCs involved in the process? Are the superantigenic or enzymatic activities of the staphylococcal factors important for their allergenic properties? Are there general features of bacterial allergens? How does intrinsic adjuvanticity of bacterial antigens affect the outcome of vaccination, especially, if non-adjuvanted bacterial factors are used as vaccines? Is *S. aureus* able to initiate the allergic march in susceptible individuals, or does it merely exacerbate pre-existing allergic inflammation? We hypothesize that *S. aureus* allergens may sensitize vulnerable persons whose allergic reaction is then potentiated by bacterial toxins and the PAMPs in the airways.

6. Conclusions

Coordination of the immune response at the interface between innate and adaptive defense mechanisms is an essential function of DCs. These phagocytic leukocytes sense microorganisms in tissues which border on the external environment. Not surprisingly, *S. aureus* has evolved means to prevent phagocytosis, to resist killing inside phagosomes, and manipulate DCs to its advantage. The multifaceted interactions between *S. aureus* and its host take place in the airways. The outcomes range from rapid clearance through symptom-free colonization to asthma or life-threatening pneumonia. Remarkably, single *S. aureus* proteins can elicit immune responses of distinctive cytokine and antibody profiles in the same individual, demonstrating that bacterial antigens have adjuvant properties. It will be worthwhile exploring more closely how DCs affect these processes and how their response is determined by various *S. aureus* virulence factors. A better understanding of the behavior of DCs, pivots of the immune system exhibiting great plasticity, will also benefit vaccine research.

Author Contributions: M.N.D., M.N., B.M.B. and V.P. wrote the manuscript.

Funding: This group's work was supported by grants from the German Research Foundation (TRR34, GRK840 and GRK1870), the German Ministry of Education and Research (Medical Infection Genomics, HICARE, InfectControl2020), Federal Excellence Initiative of Mecklenburg Western Pomerania and The European Social Fund (ESF) (Grants KoInfekt and Card-ii-Omics ESF_14-BM-A55-00xx_16).

Acknowledgments: We thank the members of our laboratory and our colleagues for inspiring discussions and their contributions to our understanding of the adaptive immune response to *S. aureus* and apologize to all those whose work could not be described due to space limitations.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Thammavongsa, V.; Kim, H.K.; Missiakas, D.; Schneewind, O. Staphylococcal manipulation of host immune responses. *Nat. Rev. Microbiol.* **2015**, *13*, 529–543. [CrossRef] [PubMed]
2. Feng, C.H.; Miller, M.D.; Simon, R.A. The united allergic airway: Connections between allergic rhinitis, asthma, and chronic sinusitis. *Am. J. Rhinol. Allergy* **2012**, *26*, 187–190. [CrossRef] [PubMed]
3. Abu-Humaidan, A.H.; Elvén, M.; Sonesson, A.; Garred, P.; Sørensen, O.E. Persistent Intracellular *Staphylococcus aureus* in Keratinocytes Lead to Activation of the Complement System with Subsequent Reduction in the Intracellular Bacterial Load. *Front. Immunol.* **2018**, *9*, 396. [CrossRef] [PubMed]
4. Nagl, M.; Kacani, L.; Müllauer, B.; Lemberger, E.-M.; Stoiber, H.; Sprinzl, G.M.; Schennach, H.; Dierich, M.P. Phagocytosis and Killing of Bacteria by Professional Phagocytes and Dendritic Cells. *Clin. Diagn. Lab. Immunol.* **2002**, *9*, 1165–1168. [CrossRef] [PubMed]

5. Schindler, D.; Gutierrez, M.G.; Beineke, A.; Rauter, Y.; Rohde, M.; Foster, S.; Goldmann, O.; Medina, E. Dendritic cells are central coordinators of the host immune response to *Staphylococcus aureus* bloodstream infection. *Am. J. Pathol.* **2012**, *181*, 1327–1337. [CrossRef] [PubMed]
6. Van Kessel, K.P.M.; Bestebroer, J.; van Strijp, J.A.G. Neutrophil-Mediated Phagocytosis of *Staphylococcus aureus*. *Front. Immunol.* **2014**, *5*, 467. [CrossRef] [PubMed]
7. Berends, E.T.M.; Horswill, A.R.; Haste, N.M.; Monestier, M.; Nizet, V.; Köckritz-Blickwede, M. von. Nuclease expression by *Staphylococcus aureus* facilitates escape from neutrophil extracellular traps. *J. Innate Immun.* **2010**, *2*, 576–586. [CrossRef] [PubMed]
8. Laarman, A.; Milder, F.; van Strijp, J.; Rooijakkers, S. Complement inhibition by gram-positive pathogens: Molecular mechanisms and therapeutic implications. *J. Mol. Med.* **2010**, *88*, 115–120. [CrossRef] [PubMed]
9. Rooijakkers, S.H.M.; van Kessel, K.P.M.; van Strijp, J.A.G. Staphylococcal innate immune evasion. *Trends Microbiol.* **2005**, *13*, 596–601. [CrossRef] [PubMed]
10. Von Köckritz-Blickwede, M.; Konrad, S.; Foster, S.; Gessner, J.E.; Medina, E. Protective role of complement C5a in an experimental model of *Staphylococcus aureus* bacteremia. *J. Innate Immun.* **2010**, *2*, 87–92. [CrossRef] [PubMed]
11. Na, M.; Jarneborn, A.; Ali, A.; Welin, A.; Magnusson, M.; Stokowska, A.; Pekna, M.; Jin, T. Deficiency of the Complement Component 3 but Not Factor B Aggravates *Staphylococcus aureus* Septic Arthritis in Mice. *Infect. Immun.* **2016**, *84*, 930–939. [CrossRef] [PubMed]
12. Bröker, B.M.; Holtfreter, S.; Bekeredjian-Ding, I. Immune control of *Staphylococcus aureus*—Regulation and counter-regulation of the adaptive immune response. *Int J. Med. Microbiol.* **2014**, *304*, 204–214. [CrossRef] [PubMed]
13. Holtfreter, S.; Kolata, J.; Bröker, B.M. Towards the immune proteome of *Staphylococcus aureus*—The anti-*S. aureus* antibody response. *Int. J. Med. Microbiol.* **2010**, *300*, 176–192. [CrossRef] [PubMed]
14. Stentzel, S.; Sundaramoorthy, N.; Michalik, S.; Nordengrün, M.; Schulz, S.; Kolata, J.; Kloppot, P.; Engelmann, S.; Steil, L.; Hecker, M.; et al. Specific serum IgG at diagnosis of *Staphylococcus aureus* bloodstream invasion is correlated with disease progression. *J. Proteom.* **2015**, *128*, 1–7. [CrossRef] [PubMed]
15. Stentzel, S.; Hagl, B.; Abel, F.; Kahl, B.C.; Rack-Hoch, A.; Bröker, B.M.; Renner, E.D. Reduced Immunoglobulin (Ig) G Response to *Staphylococcus aureus* in STAT3 Hyper-IgE Syndrome. *Clin. Infect. Dis.* **2017**, *64*, 1279–1282. [CrossRef] [PubMed]
16. Farmand, S.; Sundin, M. Hyper-IgE syndromes: Recent advances in pathogenesis, diagnostics and clinical care. *Curr. Opin. Hematol.* **2015**, *22*, 12–22. [CrossRef] [PubMed]
17. Spellberg, B.; Guidos, R.; Gilbert, D.; Bradley, J.; Boucher, H.W.; Scheld, W.M.; Bartlett, J.G.; Edwards, J. The epidemic of antibiotic-resistant infections: A call to action for the medical community from the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2008**, *46*, 155–164. [CrossRef] [PubMed]
18. Archer, N.K.; Harro, J.M.; Shirtliff, M.E. Clearance of *Staphylococcus aureus* nasal carriage is T cell dependent and mediated through interleukin-17A expression and neutrophil influx. *Infect. Immun.* **2013**, *81*, 2070–2075. [CrossRef] [PubMed]
19. Minegishi, Y.; Saito, M.; Nagasawa, M.; Takada, H.; Hara, T.; Tsuchiya, S.; Agematsu, K.; Yamada, M.; Kawamura, N.; Ariga, T.; et al. Molecular explanation for the contradiction between systemic Th17 defect and localized bacterial infection in hyper-IgE syndrome. *J. Exp. Med.* **2009**, *206*, 1291–1301. [CrossRef] [PubMed]
20. Uebele, J.; Stein, C.; Nguyen, M.-T.; Schneider, A.; Kleinert, F.; Tichá, O.; Bierbaum, G.; Götz, F.; Bekeredjian-Ding, I. Antigen delivery to dendritic cells shapes human CD4+ and CD8+ T cell memory responses to *Staphylococcus aureus*. *PLoS Pathog.* **2017**, *13*, e1006387. [CrossRef] [PubMed]
21. Kolata, J.B.; Kühbandner, I.; Link, C.; Normann, N.; Vu, C.H.; Steil, L.; Weidenmaier, C.; Bröker, B.M. The Fall of a Dogma? Unexpected High T-Cell Memory Response to *Staphylococcus aureus* in Humans. *J. Infect. Dis.* **2015**, *212*, 830–838. [CrossRef] [PubMed]
22. Brown, A.F.; Murphy, A.G.; Lalor, S.J.; Leech, J.M.; O'Keeffe, K.M.; Mac Aogáin, M.; O'Halloran, D.P.; Lacey, K.A.; Tavakol, M.; Hearnden, C.H.; et al. Memory Th1 Cells Are Protective in Invasive *Staphylococcus aureus* Infection. *PLoS Pathog.* **2015**, *11*, e1005226. [CrossRef] [PubMed]
23. Langerhans, P. Ueber die Nerven der menschlichen Haut. *Arch. Pathol. Anat.* **1868**, *44*, 325–337. [CrossRef]
24. Steinman, R.M. Identification of a novel cell type in peripheral lymphoid organs of mice: I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* **1973**, *137*, 1142–1162. [CrossRef] [PubMed]

25. Janeway, C.A.; Medzhitov, R. Innate immune recognition. *Annu. Rev. Immunol.* **2002**, *20*, 197–216. [CrossRef] [PubMed]
26. Banchereau, J.; Steinman, R.M. Dendritic cells and the control of immunity. *Nature* **1998**, *392*, 245–252. [CrossRef] [PubMed]
27. Steinman, R.M. The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* **1991**, *9*, 271–296. [CrossRef] [PubMed]
28. Steinman, R.M.; Hawiger, D.; Nussenzweig, M.C. Tolerogenic dendritic cells. *Annu. Rev. Immunol.* **2003**, *21*, 685–711. [CrossRef] [PubMed]
29. Kelly, B.; O'Neill, L.A.J. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res.* **2015**, *25*, 771–784. [CrossRef] [PubMed]
30. Savina, A.; Amigorena, S. Phagocytosis and antigen presentation in dendritic cells. *Immunol. Rev.* **2007**, *219*, 143–156. [CrossRef] [PubMed]
31. O'Keeffe, K.M.; Wilk, M.M.; Leech, J.M.; Murphy, A.G.; Laabei, M.; Monk, I.R.; Massey, R.C.; Lindsay, J.A.; Foster, T.J.; Geoghegan, J.A.; et al. Manipulation of Autophagy in Phagocytes Facilitates *Staphylococcus aureus* Bloodstream Infection. *Infect. Immun.* **2015**, *83*, 3445–3457. [CrossRef] [PubMed]
32. Jung, J.-Y.; Roberts, L.L.; Robinson, C.M. The presence of interleukin-27 during monocyte-derived dendritic cell differentiation promotes improved antigen processing and stimulation of T cells. *Immunology* **2015**, *144*, 649–660. [CrossRef] [PubMed]
33. Nishat, S.; Wuescher, L.M.; Worth, R.G. Platelets enhance dendritic cell responses against *S. aureus* through CD40-CD40L interactions. *Infect. Immun.* **2018**. [CrossRef] [PubMed]
34. Schraml, B.U.; Reis e Sousa, C. Defining dendritic cells. *Curr. Opin. Immunol.* **2015**, *32*, 13–20. [CrossRef] [PubMed]
35. Hart, D.N. Dendritic cells: Unique leukocyte populations which control the primary immune response. *Blood* **1997**, *90*, 3245–3287. [PubMed]
36. Ueno, H.; Klechevsky, E.; Morita, R.; Aspord, C.; Cao, T.; Matsui, T.; Di Pucchio, T.; Connolly, J.; Fay, J.W.; Pascual, V.; et al. Dendritic cell subsets in health and disease. *Immunol. Rev.* **2007**, *219*, 118–142. [CrossRef] [PubMed]
37. Steinman, R.M.; Cohn, Z.A. Identification of a novel cell type in peripheral lymphoid organs of mice: II. Functional properties in vitro. *J. Exp. Med.* **1974**, *139*, 380–397. [CrossRef] [PubMed]
38. Steinman, R.M.; Lustig, D.S.; Cohn, Z.A. Identification of a novel cell type in peripheral lymphoid organs of mice: III. Functional properties in vivo. *J. Exp. Med.* **1974**, *139*, 1431–1445. [CrossRef] [PubMed]
39. Lennert, K.; Remmele, W. Karyometrische Untersuchungen an Lymphknotenzellen des Menschen. *Acta Haematol.* **2004**, *19*, 99–113. [CrossRef] [PubMed]
40. Liu, K.; Victora, G.D.; Schwickert, T.A.; Guermonprez, P.; Meredith, M.M.; Yao, K.; Chu, F.-F.; Randolph, G.J.; Rudensky, A.Y.; Nussenzweig, M. In Vivo Analysis of Dendritic Cell Development and Homeostasis. *Science* **2009**, *324*, 392–397. [CrossRef] [PubMed]
41. Yamazaki, C.; Sugiyama, M.; Ohta, T.; Hemmi, H.; Hamada, E.; Sasaki, I.; Fukuda, Y.; Yano, T.; Nobuoka, M.; Hirashima, T.; et al. Critical roles of a dendritic cell subset expressing a chemokine receptor, XCR1. *J. Immunol.* **2013**, *190*, 6071–6082. [CrossRef] [PubMed]
42. Merad, M.; Sathe, P.; Helft, J.; Miller, J.; Mortha, A. The dendritic cell lineage: Ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu. Rev. Immunol.* **2013**, *31*, 563–604. [CrossRef] [PubMed]
43. Dress, R.J.; Wong, A.Y.W.; Ginhoux, F. Homeostatic control of dendritic cell numbers and differentiation. *Immunol. Cell Boil.* **2018**, *96*, 463–476. [CrossRef] [PubMed]
44. McGovern, N.; Schlitzer, A.; Gunawan, M.; Jardine, L.; Shin, A.; Poyner, E.; Green, K.; Dickinson, R.; Wang, X.-N.; Low, D.; et al. Human Dermal CD14 + Cells Are a Transient Population of Monocyte-Derived Macrophages. *Immunity* **2014**, *41*, 465–477. [CrossRef] [PubMed]
45. Bandola-Simon, J.; Roche, P.A. Dysfunction of antigen processing and presentation by dendritic cells in cancer. *Mol. Immunol.* **2018**. [CrossRef] [PubMed]
46. Collin, M.; Bigley, V. Human dendritic cell subsets: An update. *Immunology* **2018**, *154*, 3–20. [CrossRef] [PubMed]

47. Grouard, G.; Rissoan, M.-C.; Filgueira, L.; Durand, I.; Banchereau, J.; Liu, Y.-J. The Enigmatic Plasmacytoid T Cells Develop into Dendritic Cells with Interleukin (IL)-3 and CD40-Ligand. *J. Exp. Med.* **1997**, *185*, 1101–1112. [CrossRef] [PubMed]
48. Rissoan, M.C.; Soumelis, V.; Kadowaki, N.; Grouard, G.; Briere, F.; de Waal Malefyt, R.; Liu, Y.J. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* **1999**, *283*, 1183–1186. [CrossRef] [PubMed]
49. Wollenberg, A.; Günther, S.; Moderer, M.; Wetzel, S.; Wagner, M.; Towarowski, A.; Tuma, E.; Rothenfusser, S.; Endres, S.; Hartmann, G. Plasmacytoid Dendritic Cells: A New Cutaneous Dendritic Cell Subset with Distinct Role in Inflammatory Skin Diseases. *J. Investig. Dermatol.* **2002**, *119*, 1096–1102. [CrossRef] [PubMed]
50. MacDonald, K.P.A. Characterization of human blood dendritic cell subsets. *Blood* **2002**, *100*, 4512–4520. [CrossRef] [PubMed]
51. Onai, N.; Kurabayashi, K.; Hosoi-Amaike, M.; Toyama-Sorimachi, N.; Matsushima, K.; Inaba, K.; Ohteki, T. A Clonogenic Progenitor with Prominent Plasmacytoid Dendritic Cell Developmental Potential. *Immunity* **2013**, *38*, 943–957. [CrossRef] [PubMed]
52. Kamath, A.T.; Pooley, J.; O'Keeffe, M.A.; Vremec, D.; Zhan, Y.; Lew, A.M.; D'Amico, A.; Wu, L.; Tough, D.F.; Shortman, K. The development, maturation, and turnover rate of mouse spleen dendritic cell populations. *J. Immunol.* **2000**, *165*, 6762–6770. [CrossRef] [PubMed]
53. Fonteneau, J.-F. Activation of influenza virus-specific CD4+ and CD8+ T cells: A new role for plasmacytoid dendritic cells in adaptive immunity. *Blood* **2003**, *101*, 3520–3526. [CrossRef] [PubMed]
54. Kool, M.; van Nimwegen, M.; Willart, M.A.M.; Muskens, F.; Boon, L.; Smit, J.J.; Coyle, A.; Clausen, B.E.; Hoogsteden, H.C.; Lambrecht, B.N.; et al. An anti-inflammatory role for plasmacytoid dendritic cells in allergic airway inflammation. *J. Immunol.* **2009**, *183*, 1074–1082. [CrossRef] [PubMed]
55. Iwasaki, A.; Medzhitov, R. Control of adaptive immunity by the innate immune system. *Nat. Immunol.* **2015**, *16*, 343–353. [CrossRef] [PubMed]
56. Hanzelmann, D.; Joo, H.-S.; Franz-Wachtel, M.; Hertlein, T.; Stevanovic, S.; Macek, B.; Wolz, C.; Götz, F.; Otto, M.; Kretschmer, D.; et al. Toll-like receptor 2 activation depends on lipopeptide shedding by bacterial surfactants. *Nat. Commun.* **2016**, *7*, 12304. [CrossRef] [PubMed]
57. Fournier, B.; Philpott, D.J. Recognition of *Staphylococcus aureus* by the innate immune system. *Clin. Microbiol. Rev.* **2005**, *18*, 521–540. [CrossRef] [PubMed]
58. Fournier, B. The function of TLR2 during staphylococcal diseases. *Front. Cell. Infect. Microbiol.* **2012**, *2*, 167. [CrossRef] [PubMed]
59. Koymans, K.J.; Feitsma, L.J.; Brondijk, T.H.C.; Aerts, P.C.; Lukkien, E.; Lössl, P.; van Kessel, K.P.M.; de Haas, C.J.C.; van Strijp, J.A.G.; Huizinga, E.G. Structural basis for inhibition of TLR2 by staphylococcal superantigen-like protein 3 (SSL3). *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 11018–11023. [CrossRef] [PubMed]
60. Koymans, K.J.; Goldmann, O.; Karlsson, C.A.Q.; Sital, W.; Thänert, R.; Bisschop, A.; Vrieling, M.; Malmström, J.; van Kessel, K.P.M.; de Haas, C.J.C.; et al. The TLR2 Antagonist Staphylococcal Superantigen-Like Protein 3 Acts as a Virulence Factor to Promote Bacterial Pathogenicity in vivo. *J. Innate Immun.* **2017**, *9*, 561–573. [CrossRef] [PubMed]
61. Iwamoto, K.; Stroisch, T.J.; Koch, S.; Herrmann, N.; Leib, N.; Bieber, T. Langerhans and inflammatory dendritic epidermal cells in atopic dermatitis are tolerized towards TLR2 activation. *Allergy* **2018**. [CrossRef] [PubMed]
62. Aoyagi, S.; Oda, T.; Wada, K.; Nakamura, E.; Kosuga, T.; Yasunaga, H. Infective Endocarditis Associated with Atopic Dermatitis. *Int. Heart J.* **2018**, *59*, 420–423. [CrossRef] [PubMed]
63. Bergstrøm, B.; Aune, M.H.; Awuh, J.A.; Kojen, J.F.; Blix, K.J.; Ryan, L.; Flo, T.H.; Mollnes, T.E.; Espevik, T.; Stenvik, J. TLR8 Senses *Staphylococcus aureus* RNA in Human Primary Monocytes and Macrophages and Induces IFN-β Production via a TAK1-IKKβ-IRF5 Signaling Pathway. *J. Immunol.* **2015**, *195*, 1100–1111. [CrossRef] [PubMed]
64. Ugolini, M.; Gerhard, J.; Burkert, S.; Jensen, K.J.; Georg, P.; Ebner, F.; Volkers, S.M.; Thada, S.; Dietert, K.; Bauer, L.; et al. Recognition of microbial viability via TLR8 drives TFH cell differentiation and vaccine responses. *Nat. Immunol.* **2018**, *19*, 386–396. [CrossRef] [PubMed]
65. Parker, D.; Prince, A. *Staphylococcus aureus* induces type I IFN signaling in dendritic cells via TLR9. *J. Immunol.* **2012**, *189*, 4040–4046. [CrossRef] [PubMed]

66. Mohamed, W.; Domann, E.; Chakraborty, T.; Mannala, G.; Lips, K.S.; Heiss, C.; Schnettler, R.; Alt, V. TLR9 mediates *S. aureus* killing inside osteoblasts via induction of oxidative stress. *BMC Microbiol.* **2016**, *16*, 230. [CrossRef] [PubMed]
67. Mansur, N.; Hazzan, R.; Paul, M.; Bishara, J.; Leibovici, L. Does sex affect 30-day mortality in *Staphylococcus aureus* bacteremia? *Gend. Med.* **2012**, *9*, 463–470. [CrossRef] [PubMed]
68. Nurjadi, D.; Heeg, K.; Weber, A.N.R.; Zanger, P. Toll-like receptor 9 (TLR-9) promotor polymorphisms and gene expression are associated with persistent *Staphylococcus aureus* nasal carriage. *Clin. Microbiol. Infect.* **2018**. [CrossRef] [PubMed]
69. Fraunholz, M.; Sinha, B. Intracellular *Staphylococcus aureus*: Live-in and let die. *Front. Cell. Infect. Microbiol.* **2012**, *2*, 43. [CrossRef] [PubMed]
70. Foster, T.J.; Geoghegan, J.A.; Ganesh, V.K.; Höök, M. Adhesion, invasion and evasion: The many functions of the surface proteins of *Staphylococcus aureus*. *Nat. Rev. Microbiol.* **2014**, *12*, 49–62. [CrossRef] [PubMed]
71. Strobel, M.; Pförtner, H.; Tuchscherr, L.; Völker, U.; Schmidt, F.; Kramko, N.; Schnittler, H.-J.; Fraunholz, M.J.; Löffler, B.; Peters, G.; et al. Post-invasion events after infection with *Staphylococcus aureus* are strongly dependent on both the host cell type and the infecting *S. aureus* strain. *Clin. Microbiol. Infect.* **2016**, *22*, 799–809. [CrossRef] [PubMed]
72. Patti, J.M.; Allen, B.L.; McGavin, M.J.; Höök, M. MSCRAMM-mediated adherence of microorganisms to host tissues. *Annu. Rev. Microbiol.* **1994**, *48*, 585–617. [CrossRef] [PubMed]
73. Sinha, B.; François, P.P.; Nüsse, O.; Foti, M.; Hartford, O.M.; Vaudaux, P.; Foster, T.J.; Lew, D.P.; Herrmann, M.; Krause, K.H. Fibronectin-binding protein acts as *Staphylococcus aureus* invasin via fibronectin bridging to integrin alpha5beta1. *Cell. Microbiol.* **1999**, *1*, 101–117. [CrossRef] [PubMed]
74. Schwarz-Linek, U.; Werner, J.M.; Pickford, A.R.; Gurusiddappa, S.; Kim, J.H.; Pilka, E.S.; Briggs, J.A.G.; Gough, T.S.; Höök, M.; Campbell, I.D.; et al. Pathogenic bacteria attach to human fibronectin through a tandem beta-zipper. *Nature* **2003**, *423*, 177–181. [CrossRef] [PubMed]
75. Porayath, C.; Suresh, M.K.; Biswas, R.; Nair, B.G.; Mishra, N.; Pal, S. Autolysin mediated adherence of *Staphylococcus aureus* with Fibronectin, Gelatin and Heparin. *Int. J. Biol. Macromol.* **2018**, *110*, 179–184. [CrossRef] [PubMed]
76. Chavakis, E.; Aicher, A.; Heeschen, C.; Sasaki, K.-i.; Kaiser, R.; El Makhfi, N.; Urbich, C.; Peters, T.; Scharffetter-Kochanek, K.; Zeiher, A.M.; et al. Role of beta2-integrins for homing and neovascularization capacity of endothelial progenitor cells. *J. Exp. Med.* **2005**, *201*, 63–72. [CrossRef] [PubMed]
77. Hussain, M.; von Eiff, C.; Sinha, B.; Joost, I.; Herrmann, M.; Peters, G.; Becker, K. Eap Gene as novel target for specific identification of *Staphylococcus aureus*. *J. Clin. Microbiol.* **2008**, *46*, 470–476. [CrossRef] [PubMed]
78. McAdow, M.; Kim, H.K.; Dedent, A.C.; Hendrickx, A.P.A.; Schneewind, O.; Missiakas, D.M. Preventing sepsis through the inhibition of its agglutination in blood. *PLoS Pathog.* **2011**, *7*, e1002307. [CrossRef] [PubMed]
79. Guilliams, M.; Bruhns, P.; Saeys, Y.; Hammad, H.; Lambrecht, B.N. The function of Fcγ receptors in dendritic cells and macrophages. *Nat. Rev. Immunol.* **2014**, *14*, 94–108. [CrossRef] [PubMed]
80. Kuipers, A.; Stapels, D.A.C.; Weerwind, L.T.; Ko, Y.-P.; Ruyken, M.; Lee, J.C.; van Kessel, K.P.M.; Rooijakkers, S.H.M. The *Staphylococcus aureus* polysaccharide capsule and Efb-dependent fibrinogen shield act in concert to protect against phagocytosis. *Microbiology* **2016**, *162*, 1185–1194. [CrossRef] [PubMed]
81. O'Riordan, K.; Lee, J.C. *Staphylococcus aureus* Capsular Polysaccharides. *Clin. Microbiol. Rev.* **2004**, *17*, 218–234. [CrossRef] [PubMed]
82. Becker, S.; Frankel, M.B.; Schneewind, O.; Missiakas, D. Release of protein A from the cell wall of *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 1574–1579. [CrossRef] [PubMed]
83. Goodyear, C.S.; Silverman, G.J. Staphylococcal toxin induced preferential and prolonged in vivo deletion of innate-like B lymphocytes. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11392–11397. [CrossRef] [PubMed]
84. Lukácsi, S.; Nagy-Baló, Z.; Erdei, A.; Sándor, N.; Bajtay, Z. The role of CR3 (CD11b/CD18) and CR4 (CD11c/CD18) in complement-mediated phagocytosis and podosome formation by human phagocytes. *Immunol. Lett.* **2017**, *189*, 64–72. [CrossRef] [PubMed]
85. Pietrocola, G.; Nobile, G.; Rindi, S.; Speziale, P. *Staphylococcus aureus* Manipulates Innate Immunity through Own and Host-Expressed Proteases. *Front. Cell. Infect. Microbiol.* **2017**, *7*, 787. [CrossRef] [PubMed]

86. Laarman, A.J.; Ruyken, M.; Malone, C.L.; van Strijp, J.A.G.; Horswill, A.R.; Rooijakkers, S.H.M. *Staphylococcus aureus* metalloprotease aureolysin cleaves complement C3 to mediate immune evasion. *J. Immunol.* **2011**, *186*, 6445–6453. [CrossRef] [PubMed]
87. Jin, T.; Bokarewa, M.; Foster, T.; Mitchell, J.; Higgins, J.; Tarkowski, A. *Staphylococcus aureus* Resists Human Defensins by Production of Staphylokinase, a Novel Bacterial Evasion Mechanism. *J. Immunol.* **2004**, *172*, 1169–1176. [CrossRef] [PubMed]
88. Sieprawska-Lupa, M.; Mydel, P.; Krawczyk, K.; Wojcik, K.; Puklo, M.; Lupa, B.; Suder, P.; Silberring, J.; Reed, M.; Pohl, J.; et al. Degradation of Human Antimicrobial Peptide LL-37 by *Staphylococcus aureus*-Derived Proteinases. *Antimicrob. Agents Chemother.* **2004**, *48*, 4673–4679. [CrossRef] [PubMed]
89. Noore, J.; Noore, A.; Li, B. Cationic antimicrobial peptide LL-37 is effective against both extra- and intracellular *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2013**, *57*, 1283–1290. [CrossRef] [PubMed]
90. Spaan, A.N.; van Strijp, J.A.G.; Torres, V.J. Leukocidins: Staphylococcal bi-component pore-forming toxins find their receptors. *Nat. Rev. Microbiol.* **2017**, *15*, 435–447. [CrossRef] [PubMed]
91. Alonzo, F.; Kozhaya, L.; Rawlings, S.A.; Reyes-Robles, T.; DuMont, A.L.; Myszka, D.G.; Landau, N.R.; Unutmaz, D.; Torres, V.J. CCR5 is a receptor for *Staphylococcus aureus* leukotoxin ED. *Nature* **2013**, *493*, 51–55. [CrossRef] [PubMed]
92. Reyes-Robles, T.; Alonzo, F.; Kozhaya, L.; Lacy, D.B.; Unutmaz, D.; Torres, V.J. *Staphylococcus aureus* leukotoxin ED targets the chemokine receptors CXCR1 and CXCR2 to kill leukocytes and promote infection. *Cell Host Microbe* **2013**, *14*, 453–459. [CrossRef] [PubMed]
93. DuMont, A.L.; Yoong, P.; Day, C.J.; Alonzo, F.; McDonald, W.H.; Jennings, M.P.; Torres, V.J. *Staphylococcus aureus* LukAB cytotoxin kills human neutrophils by targeting the CD11b subunit of the integrin Mac-1. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 10794–10799. [CrossRef] [PubMed]
94. Ho, M.K.; Springer, T.A. Mac-1 antigen: Quantitative expression in macrophage populations and tissues, and immunofluorescent localization in spleen. *J. Immunol.* **1982**, *128*, 2281–2286. [PubMed]
95. DuMont, A.L.; Yoong, P.; Liu, X.; Day, C.J.; Chumbler, N.M.; James, D.B.A.; Alonzo, F.; Bode, N.J.; Lacy, D.B.; Jennings, M.P.; et al. Identification of a crucial residue required for *Staphylococcus aureus* LukAB cytotoxicity and receptor recognition. *Infect. Immun.* **2014**, *82*, 1268–1276. [CrossRef] [PubMed]
96. Vrieling, M.; Koymans, K.J.; Heesterbeek, D.A.C.; Aerts, P.C.; Rutten, V.P.M.G.; de Haas, C.J.C.; van Kessel, K.P.M.; Koets, A.P.; Nijland, R.; van Strijp, J.A.G. Bovine *Staphylococcus aureus* Secretes the Leukocidin LukMF′ To Kill Migrating Neutrophils through CCR1. *mBio* **2015**, *6*, e00335. [CrossRef] [PubMed]
97. Sozzani, S.; Luini, W.; Borsatti, A.; Polentarutti, N.; Zhou, D.; Piemonti, L.; D'Amico, G.; Power, C.A.; Wells, T.N.; Gobbi, M.; et al. Receptor expression and responsiveness of human dendritic cells to a defined set of CC and CXC chemokines. *J. Immunol.* **1997**, *159*, 1993–2000. [PubMed]
98. Spaan, A.N.; Reyes-Robles, T.; Badiou, C.; Cochet, S.; Boguslawski, K.M.; Yoong, P.; Day, C.J.; de Haas, C.J.C.; van Kessel, K.P.M.; Vandenesch, F.; et al. *Staphylococcus aureus* Targets the Duffy Antigen Receptor for Chemokines (DARC) to Lyse Erythrocytes. *Cell Host Microbe* **2015**, *18*, 363–370. [CrossRef] [PubMed]
99. Spaan, A.N.; Vrieling, M.; Wallet, P.; Badiou, C.; Reyes-Robles, T.; Ohneck, E.A.; Benito, Y.; de Haas, C.J.C.; Day, C.J.; Jennings, M.P.; et al. The staphylococcal toxins γ-haemolysin AB and CB differentially target phagocytes by employing specific chemokine receptors. *Nat. Commun.* **2014**, *5*, 5438. [CrossRef] [PubMed]
100. Meyer, F.; Girardot, R.; Piémont, Y.; Prévost, G.; Colin, D.A. Analysis of the specificity of Panton-Valentine leucocidin and gamma-hemolysin F component binding. *Infect. Immun.* **2009**, *77*, 266–273. [CrossRef] [PubMed]
101. Dalla Serra, M.; Coraiola, M.; Viero, G.; Comai, M.; Potrich, C.; Ferreras, M.; Baba-Moussa, L.; Colin, D.A.; Menestrina, G.; Bhakdi, S.; et al. *Staphylococcus aureus* bicomponent gamma-hemolysins, HlgA, HlgB, and HlgC, can form mixed pores containing all components. *J. Chem. Inf. Model.* **2005**, *45*, 1539–1545. [CrossRef] [PubMed]
102. Rybicka, J.M.; Balce, D.R.; Chaudhuri, S.; Allan, E.R.O.; Yates, R.M. Phagosomal proteolysis in dendritic cells is modulated by NADPH oxidase in a pH-independent manner. *EMBO J.* **2012**, *31*, 932–944. [CrossRef] [PubMed]
103. Ip, W.K.E.; Sokolovska, A.; Charriere, G.M.; Boyer, L.; Dejardin, S.; Cappillino, M.P.; Yantosca, L.M.; Takahashi, K.; Moore, K.J.; Lacy-Hulbert, A.; et al. Phagocytosis and phagosome acidification are required for pathogen processing and MyD88-dependent responses to *Staphylococcus aureus*. *J. Immunol.* **2010**, *184*, 7071–7081. [CrossRef] [PubMed]

104. Horn, J.; Stelzner, K.; Rudel, T.; Fraunholz, M. Inside job: *Staphylococcus aureus* host-pathogen interactions. *Int. J. Med. Microbiol.* **2017**. [CrossRef] [PubMed]
105. Bore, E.; Langsrud, S.; Langsrud, O.; Rode, T.M.; Holck, A. Acid-shock responses in *Staphylococcus aureus* investigated by global gene expression analysis. *Microbiology* **2007**, *153*, 2289–2303. [CrossRef] [PubMed]
106. Liu, G.Y.; Essex, A.; Buchanan, J.T.; Datta, V.; Hoffman, H.M.; Bastian, J.F.; Fierer, J.; Nizet, V. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J. Exp. Med.* **2005**, *202*, 209–215. [CrossRef] [PubMed]
107. Malachowa, N.; Kohler, P.L.; Schlievert, P.M.; Chuang, O.N.; Dunny, G.M.; Kobayashi, S.D.; Miedzobrodzki, J.; Bohach, G.A.; Seo, K.S. Characterization of a *Staphylococcus aureus* surface virulence factor that promotes resistance to oxidative killing and infectious endocarditis. *Infect. Immun.* **2011**, *79*, 342–352. [CrossRef] [PubMed]
108. Ballal, A.; Manna, A.C. Regulation of superoxide dismutase (sod) genes by SarA in *Staphylococcus aureus*. *J. Bacteriol.* **2009**, *191*, 3301–3310. [CrossRef] [PubMed]
109. Cosgrove, K.; Coutts, G.; Jonsson, I.-M.; Tarkowski, A.; Kokai-Kun, J.F.; Mond, J.J.; Foster, S.J. Catalase (KatA) and alkyl hydroperoxide reductase (AhpC) have compensatory roles in peroxide stress resistance and are required for survival, persistence, and nasal colonization in *Staphylococcus aureus*. *J. Bacteriol.* **2007**, *189*, 1025–1035. [CrossRef] [PubMed]
110. De Jong, N.W.M.; Ramyar, K.X.; Guerra, F.E.; Nijland, R.; Fevre, C.; Voyich, J.M.; McCarthy, A.J.; Garcia, B.L.; van Kessel, K.P.M.; van Strijp, J.A.G.; et al. Immune evasion by a staphylococcal inhibitor of myeloperoxidase. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 9439–9444. [CrossRef] [PubMed]
111. Odobasic, D.; Kitching, A.R.; Yang, Y.; O'Sullivan, K.M.; Muljadi, R.C.M.; Edgtton, K.L.; Tan, D.S.Y.; Summers, S.A.; Morand, E.F.; Holdsworth, S.R. Neutrophil myeloperoxidase regulates T-cell-driven tissue inflammation in mice by inhibiting dendritic cell function. *Blood* **2013**, *121*, 4195–4204. [CrossRef] [PubMed]
112. Scholz, W.; Platzer, B.; Schumich, A.; Höcher, B.; Fritsch, G.; Knapp, W.; Strobl, H. Initial human myeloid/dendritic cell progenitors identified by absence of myeloperoxidase protein expression. *Exp. Hematol.* **2004**, *32*, 270–276. [CrossRef] [PubMed]
113. Bera, A.; Herbert, S.; Jakob, A.; Vollmer, W.; Götz, F. Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan *O*-acetyltransferase OatA is the major determinant for lysozyme resistance of *Staphylococcus aureus*. *Mol. Microbiol.* **2005**, *55*, 778–787. [CrossRef] [PubMed]
114. Vu, C.H.; Kolata, J.; Stentzel, S.; Beyer, A.; Gesell Salazar, M.; Steil, L.; Pané-Farré, J.; Rühmling, V.; Engelmann, S.; Götz, F.; et al. Adaptive immune response to lipoproteins of *Staphylococcus aureus* in healthy subjects. *Proteomics* **2016**, *16*, 2667–2677. [CrossRef] [PubMed]
115. Giese, B.; Glowinski, F.; Paprotka, K.; Dittmann, S.; Steiner, T.; Sinha, B.; Fraunholz, M.J. Expression of δ-toxin by *Staphylococcus aureus* mediates escape from phago-endosomes of human epithelial and endothelial cells in the presence of β-toxin. *Cell. Microbiol.* **2011**, *13*, 316–329. [CrossRef] [PubMed]
116. Grosz, M.; Kolter, J.; Paprotka, K.; Winkler, A.-C.; Schäfer, D.; Chatterjee, S.S.; Geiger, T.; Wolz, C.; Ohlsen, K.; Otto, M.; et al. Cytoplasmic replication of *Staphylococcus aureus* upon phagosomal escape triggered by phenol-soluble modulin α. *Cell. Microbiol.* **2014**, *16*, 451–465. [CrossRef] [PubMed]
117. Surewaard, B.G.J.; de Haas, C.J.C.; Vervoort, F.; Rigby, K.M.; DeLeo, F.R.; Otto, M.; van Strijp, J.A.G.; Nijland, R. Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis. *Cell. Microbiol.* **2013**, *15*, 1427–1437. [CrossRef] [PubMed]
118. Armbruster, N.S.; Richardson, J.R.; Schreiner, J.; Klenk, J.; Günter, M.; Autenrieth, S.E. *Staphylococcus aureus* PSM peptides induce tolerogenic dendritic cells upon treatment with ligands of extracellular and intracellular TLRs. *Int. J. Med. Microbiol.* **2016**, *306*, 666–674. [CrossRef] [PubMed]
119. Armbruster, N.S.; Richardson, J.R.; Schreiner, J.; Klenk, J.; Günter, M.; Kretschmer, D.; Pöschel, S.; Schenke-Layland, K.; Kalbacher, H.; Clark, K.; et al. PSM Peptides of *Staphylococcus aureus* Activate the p38-CREB Pathway in Dendritic Cells, Thereby Modulating Cytokine Production and T Cell Priming. *J. Immunol.* **2016**, *196*, 1284–1292. [CrossRef] [PubMed]
120. Deplanche, M.; Alekseeva, L.; Semenovskaya, K.; Fu, C.-L.; Dessauge, F.; Finot, L.; Petzl, W.; Zerbe, H.; Le Loir, Y.; Rainard, P.; et al. *Staphylococcus aureus* Phenol-Soluble Modulins Impair Interleukin Expression in Bovine Mammary Epithelial Cells. *Infect. Immun.* **2016**, *84*, 1682–1692. [CrossRef] [PubMed]
121. Dey, S.; Bishayi, B. Killing of *Staphylococcus aureus* in murine macrophages by chloroquine used alone and in combination with ciprofloxacin or azithromycin. *J. Inflamm. Res.* **2015**, *8*, 29–47. [CrossRef] [PubMed]

122. Leimer, N.; Rachmühl, C.; Palheiros Marques, M.; Bahlmann, A.S.; Furrer, A.; Eichenseher, F.; Seidl, K.; Matt, U.; Loessner, M.J.; Schuepbach, R.A.; et al. Nonstable *Staphylococcus aureus* Small-Colony Variants Are Induced by Low pH and Sensitized to Antimicrobial Therapy by Phagolysosomal Alkalinization. *J. Infect. Dis.* **2016**, *213*, 305–313. [CrossRef] [PubMed]
123. Neumann, Y.; Bruns, S.A.; Rohde, M.; Prajsnar, T.K.; Foster, S.J.; Schmitz, I. Intracellular *Staphylococcus aureus* eludes selective autophagy by activating a host cell kinase. *Autophagy* **2016**, *12*, 2069–2084. [CrossRef] [PubMed]
124. Maurer, K.; Reyes-Robles, T.; Alonzo, F.; Durbin, J.; Torres, V.J.; Cadwell, K. Autophagy mediates tolerance to *Staphylococcus aureus* alpha-toxin. *Cell Host Microbe* **2015**, *17*, 429–440. [CrossRef] [PubMed]
125. Mestre, M.B.; Fader, C.M.; Sola, C.; Colombo, M.I. α-hemolysin is required for the activation of the autophagic pathway in *Staphylococcus aureus* infected cells. *Autophagy* **2010**, *6*, 110–125. [CrossRef] [PubMed]
126. Korea, C.G.; Balsamo, G.; Pezzicoli, A.; Merakou, C.; Tavarini, S.; Bagnoli, F.; Serruto, D.; Unnikrishnan, M. Staphylococcal Esx proteins modulate apoptosis and release of intracellular *Staphylococcus aureus* during infection in epithelial cells. *Infect. Immun.* **2014**, *82*, 4144–4153. [CrossRef] [PubMed]
127. Cruciani, M.; Etna, M.P.; Camilli, R.; Giacomini, E.; Percario, Z.A.; Severa, M.; Sandini, S.; Rizzo, F.; Brandi, V.; Balsamo, G.; et al. *Staphylococcus aureus* Esx Factors Control Human Dendritic Cell Functions Conditioning Th1/Th17 Response. *Front. Cell. Infect. Microbiol.* **2017**, *7*, 330. [CrossRef] [PubMed]
128. Koymans, K.J.; Vrieling, M.; Gorham, R.D.; van Strijp, J.A.G. Staphylococcal Immune Evasion Proteins: Structure, Function, and Host Adaptation. *Curr. Top. Microbiol. Immunol.* **2017**, *409*, 441–489. [CrossRef] [PubMed]
129. Argudín, M.Á.; Mendoza, M.C.; Rodicio, M.R. Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins* **2010**, *2*, 1751–1773. [CrossRef] [PubMed]
130. Grumann, D.; Nübel, U.; Bröker, B.M. *Staphylococcus aureus* toxins—Their functions and genetics. *Infect. Genet. Evol.* **2014**, *21*, 583–592. [CrossRef] [PubMed]
131. Llewelyn, M.; Cohen, J. Superantigens: Microbial agents that corrupt immunity. *Lancet Infect. Dis.* **2002**, *2*, 156–162. [CrossRef]
132. Sollid, J.U.E.; Furberg, A.S.; Hanssen, A.M.; Johannessen, M. *Staphylococcus aureus*: Determinants of human carriage. *Infect. Genet. Evol.* **2014**, *21*, 531–541. [CrossRef] [PubMed]
133. Frazee, B.W. Severe methicillin-resistant *Staphylococcus aureus* community-acquired pneumonia associated with influenza—Louisiana and Georgia, December 2006-January 2007. *MMWR Morb. Mortal. Wkly. Rep.* **2007**, *56*, 325–329.
134. Barnes, P.J. Intrinsic asthma: Not so different from allergic asthma but driven by superantigens? *Clin. Exp. Allergy* **2009**, *39*, 1145–1151. [CrossRef] [PubMed]
135. Bachert, C.; Zhang, N. Chronic rhinosinusitis and asthma: Novel understanding of the role of IgE 'above atopy'. *J. Int. Med.* **2012**, *272*, 133–143. [CrossRef] [PubMed]
136. Davis, M.F.; Peng, R.D.; McCormack, M.C.; Matsui, E.C. *Staphylococcus aureus* colonization is associated with wheeze and asthma among US children and young adults. *J. Allergy Clin. Immunol.* **2015**, *135*, 811–813.e5. [CrossRef] [PubMed]
137. Van Zele, T.; Gevaert, P.; Watelet, J.-B.; Claeys, G.; Holtappels, G.; Claeys, C.; van Cauwenberge, P.; Bachert, C. *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. *J. Allergy Clin. Immunol.* **2004**, *114*, 981–983. [CrossRef] [PubMed]
138. Bachert, C.; van Steen, K.; Zhang, N.; Holtappels, G.; Cattaert, T.; Maus, B.; Buhl, R.; Taube, C.; Korn, S.; Kowalski, M.; et al. Specific IgE against *Staphylococcus aureus* enterotoxins: An independent risk factor for asthma. *J. Allergy Clin. Immunol.* **2012**, *130*, 376–381.e8. [CrossRef] [PubMed]
139. Stentzel, S.; Teufelberger, A.; Nordengrün, M.; Kolata, J.; Schmidt, F.; van Crombruggen, K.; Michalik, S.; Kumpfmüller, J.; Tischer, S.; Schweder, T.; et al. Staphylococcal serine protease–like proteins are pacemakers of allergic airway reactions to *Staphylococcus aureus*. *J. Allergy Clin. Immunol.* **2017**, *139*, 492–500.e8. [CrossRef] [PubMed]
140. Kapsenberg, M.L. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat. Rev. Immunol.* **2003**, *3*, 984–993. [CrossRef] [PubMed]
141. Condon, T.V.; Sawyer, R.T.; Fenton, M.J.; Riches, D.W.H. Lung dendritic cells at the innate-adaptive immune interface. *J. Leukoc. Biol.* **2011**, *90*, 883–895. [CrossRef] [PubMed]

142. Jin, J.-O.; Zhang, W.; Du, J.-Y.; Yu, Q. BDCA1-positive dendritic cells (DCs) represent a unique human myeloid DC subset that induces innate and adaptive immune responses to *Staphylococcus aureus* Infection. *Infect. Immun.* **2014**, *82*, 4466–4476. [CrossRef] [PubMed]
143. Ziegler, C.; Goldmann, O.; Hobeika, E.; Geffers, R.; Peters, G.; Medina, E. The dynamics of T cells during persistent *Staphylococcus aureus* infection: From antigen-reactivity to in vivo anergy. *EMBO Mol. Med.* **2011**, *3*, 652–666. [CrossRef] [PubMed]
144. Holtfreter, S.; Kolata, J.; Stentzel, S.; Bauerfeind, S.; Schmidt, F.; Sundaramoorthy, N.; Bröker, B.M. Omics Approaches for the Study of Adaptive Immunity to *Staphylococcus aureus* and the Selection of Vaccine Candidates. *Proteomes* **2016**, *4*, 11. [CrossRef] [PubMed]
145. Davis, M.F.; Ludwig, S.; Brigham, E.P.; McCormack, M.C.; Matsui, E.C. Effect of home exposure to *Staphylococcus aureus* on asthma in adolescents. *J. Allergy Clin. Immunol.* **2018**, *141*, 402–405.e10. [CrossRef] [PubMed]
146. Robinson, D.; Hamid, Q.; Bentley, A.; Ying, S.; Kay, A.B.; Durham, S.R. Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J. Allergy Clin. Immunol.* **1993**, *92*, 313–324. [CrossRef]
147. Romagnani, S. The role of lymphocytes in allergic disease. *J. Allergy Clin. Immunol.* **2000**, *105*, 399–408. [CrossRef] [PubMed]
148. Lambrecht, B.N.; Salomon, B.; Klatzmann, D.; Pauwels, R.A. Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. *J. Immunol.* **1998**, *160*, 4090–4097. [PubMed]
149. Andreas, N.; Riemann, M.; Castro, C.N.; Groth, M.; Koliesnik, I.; Engelmann, C.; Sparwasser, T.; Kamradt, T.; Haenold, R.; Weih, F. A new RelB-dependent CD117+ CD172a+ murine DC subset preferentially induces Th2 differentiation and supports airway hyperresponses in vivo. *Eur. J. Immunol.* **2018**, *48*, 923–936. [CrossRef] [PubMed]
150. Georas, S.N.; Rezaee, F. Epithelial barrier function: At the front line of asthma immunology and allergic airway inflammation. *J. Allergy Clin. Immunol.* **2014**, *134*, 509–520. [CrossRef] [PubMed]
151. Berube, B.J.; Bubeck Wardenburg, J. *Staphylococcus aureus* α-Toxin: Nearly a Century of Intrigue. *Toxins* **2013**, *5*, 1140–1166. [CrossRef] [PubMed]
152. Wilke, G.A.; Bubeck Wardenburg, J. Role of a disintegrin and metalloprotease 10 in *Staphylococcus aureus* alpha-hemolysin-mediated cellular injury. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13473–13478. [CrossRef] [PubMed]
153. Damle, S.R.; Martin, R.K.; Cockburn, C.L.; Lownik, J.C.; Carlyon, J.A.; Smith, A.D.; Conrad, D.H. ADAM10 and Notch1 on murine dendritic cells control the development of type 2 immunity and IgE production. *Allergy* **2018**, *73*, 125–136. [CrossRef] [PubMed]
154. Breuer, K.; Wittmann, M.; Kempe, K.; Kapp, A.; Mai, U.; Dittrich-Breiholz, O.; Kracht, M.; Mrabet-Dahbi, S.; Werfel, T. Alpha-toxin is produced by skin colonizing *Staphylococcus aureus* and induces a T helper type 1 response in atopic dermatitis. *Clin. Exp. Allergy* **2005**, *35*, 1088–1095. [CrossRef] [PubMed]
155. Niebuhr, M.; Gathmann, M.; Scharonow, H.; Mamerow, D.; Mommert, S.; Balaji, H.; Werfel, T. Staphylococcal alpha-toxin is a strong inducer of interleukin-17 in humans. *Infect. Immun.* **2011**, *79*, 1615–1622. [CrossRef] [PubMed]
156. Frank, K.M.; Zhou, T.; Moreno-Vinasco, L.; Hollett, B.; Garcia, J.G.N.; Bubeck Wardenburg, J. Host response signature to *Staphylococcus aureus* alpha-hemolysin implicates pulmonary Th17 response. *Infect. Immun.* **2012**, *80*, 3161–3169. [CrossRef] [PubMed]
157. Inoshima, I.; Inoshima, N.; Wilke, G.A.; Powers, M.E.; Frank, K.M.; Wang, Y.; Bubeck Wardenburg, J. A *Staphylococcus aureus* pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. *Nat. Med.* **2011**, *17*, 1310–1314. [CrossRef] [PubMed]
158. Ezekwe, E.A.D.; Weng, C.; Duncan, J.A. ADAM10 Cell Surface Expression but Not Activity Is Critical for *Staphylococcus aureus* α-Hemolysin-Mediated Activation of the NLRP3 Inflammasome in Human Monocytes. *Toxins* **2016**, *8*, 95. [CrossRef] [PubMed]
159. Hildebrandt, J.-P. Pore-forming virulence factors of *Staphylococcus aureus* destabilize epithelial barriers-effects of alpha-toxin in the early phases of airway infection. *AIMS Microbiol.* **2015**, *1*, 11–36. [CrossRef]
160. Giersing, B.K.; Dastgheyb, S.S.; Modjarrad, K.; Moorthy, V. Status of vaccine research and development of vaccines for *Staphylococcus aureus*. *Vaccine* **2016**, *34*, 2962–2966. [CrossRef] [PubMed]

161. Bernstein, J.M.; Allen, C.; Rich, G.; Dryja, D.; Bina, P.; Reiser, R.; Ballow, M.; Wilding, G.E. Further observations on the role of *Staphylococcus aureus* exotoxins and IgE in the pathogenesis of nasal polyposis. *Laryngoscope* **2011**, *121*, 647–655. [CrossRef] [PubMed]
162. Sørensen, M.; Klingenberg, C.; Wickman, M.; Sollid, J.U.E.; Furberg, A.-S.; Bachert, C.; Bousquet, J. *Staphylococcus aureus* enterotoxin sensitization is associated with allergic poly-sensitization and allergic multimorbidity in adolescents. *Allergy* **2017**, *72*, 1548–1555. [CrossRef] [PubMed]
163. Bacher, P.; Heinrich, F.; Stervbo, U.; Nienen, M.; Vahldieck, M.; Iwert, C.; Vogt, K.; Kollet, J.; Babel, N.; Sawitzki, B.; et al. Regulatory T Cell Specificity Directs Tolerance versus Allergy against Aeroantigens in Humans. *Cell* **2016**, *167*, 1067–1078. [CrossRef] [PubMed]

4

ALLERGIC REACTIONS TO SERINE PROTEASE-LIKE PROTEINS OF *STAPHYLOCOCCUS AUREUS*

Nordengrün M, Abdurrahman G, Treffon J, Wächter H, Kahl BC and Bröker BM

Accepted for publication in Frontiers Immunology. 08 Mar 2020.
doi: 10.3389/fimmu.2021.651060

AUTHOR CONTRIBUTIONS:

As a first author in this publication, **MN** conceived and planned experiments, carried them out, analyzed the data, drafted the manuscript and designed the figures. Additionally, **MN** contributed to the scientific revision and editing of the final version of this manuscript.

Study conception and design: **MN** and BMB
Acquisition of data: **MN**, JT
Analysis and interpretation of data: **MN**, GA, BMB, BK and HW
Drafting of manuscript: **MN** and GA
Critical revision and editing: GA, BMB, BK and **MN**

Maria Nordengrün | Prof. Barbara M. Bröker

frontiers in Immunology

ORIGINAL RESEARCH
published: 23 March 2021
doi: 10.3389/fimmu.2021.651060

Allergic Reactions to Serine Protease-Like Proteins of *Staphylococcus aureus*

Maria Nordengrün[1], *Goran Abdurrahman*[1*], *Janina Treffon*[2,3], *Hannah Wächter*[2], *Barbara C. Kahl*[2] *and Barbara M. Bröker*[1]

[1] Department of Immunology, Institute for Immunology and Transfusion Medicine, University Medicine Greifswald, Greifswald, Germany, [2] Institute of Medical Microbiology, University Hospital Münster, Münster, Germany, [3] Institute of Hygiene, University Hospital Münster, Münster, Germany

In cystic fibrosis (CF) infectious and allergic airway inflammation cause pulmonary exacerbations that destroy the lungs. *Staphylococcus aureus* is a common long-term colonizer and cause of recurrent airway infections in CF. The pathogen is also associated with respiratory allergy; especially the staphylococcal serine protease-like proteins (Spls) can induce type 2 immune responses in humans and mice. We measured the serum IgE levels specific to 7 proteases of *S. aureus* by ELISA, targeting 5 Spls (76 CF patients and 46 controls) and the staphopains A and B (16 CF patients and 46 controls). Then we compared cytokine release and phenotype of T cells that had been stimulated with Spls between 5 CF patients and 5 controls. CF patients had strongly increased serum IgE binding to all Spls but not to the staphopains. Compared to healthy controls, their Spl-stimulated T cells released more type 2 cytokines (IL-4, IL-5, IL-13) and more IL-6 with no difference in the secretion of type 1- or type 3 cytokines (IFNγ, IL-17A, IL-17F). IL-10 production was low in CF T cells. The phenotype of the Spl-exposed T cells shifted towards a Th2 or Th17 profile in CF but to a Th1 profile in controls. Sensitization to *S. aureus* Spls is common in CF. This discovery could explain episodes of allergic inflammation of hitherto unknown causation in CF and extend the diagnostic and therapeutic portfolio.

Keywords: cystic fibrosis, *Staphylococcus aureus*, allergy, type 2 immune response, IgE, Th2 cells

OPEN ACCESS

Edited by:
Rachel McLoughlin, Trinity College Dublin, Ireland

Reviewed by:
Alexander Horswill, University of Colorado Denver, United States
Marisa Mariel Fernandez, Institute of Studies on Humoral Immunity (IDEHU), Argentina

***Correspondence:**
Goran Abdurrahman
goran.abdurrahman@uni-greifswald.de

Specialty section:
This article was submitted to Microbial Immunology, a section of the journal Frontiers in Immunology

Received: *08 January 2021*
Accepted: *08 March 2021*
Published: *23 March 2021*

Citation:
Nordengrün M, Abdurrahman G, Treffon J, Wächter H, Kahl BC and Bröker BM (2021) Allergic Reactions to Serine Protease-Like Proteins of Staphylococcus aureus. Front. Immunol. 12:651060. doi: 10.3389/fimmu.2021.651060

INTRODUCTION

Cystic fibrosis (CF) is the most common life-shortening genetic disorder, afflicting around 7/100 000 of the general population in the US and the European Union (1). Recurrent bacterial lung infection and persistent airway inflammation gradually destroy the lung, ultimately resulting in respiratory failure (2). The causative agents are bacteria, prominently *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa*, but also fungi, especially the ubiquitous *Aspergillus fumigatus* (*A. fumigatus*) (3). Persistent colonization with *S. aureus* occurs early in the disease course in up to 70% of CF patients, a much higher percentage than in the general population (2, 4). In the long term, the pathogen adapts to the host, gradually reducing its virulence during airway infection (5). Nevertheless, recurrent pulmonary exacerbations gradually worsen the lung function and clinical condition of CF patients (6).

Prevention and therapy of chronic bacterial and fungal inflammation are therefore key in the treatment regimen of CF (6–8).

Besides infections, allergic immune responses play a crucial role in disease progression of CF. These are frequently associated with sensitization to *A. fumigatus* with 1-15% of patients suffering from allergic bronchopulmonary aspergillosis (ABPA), accelerating the decline of respiratory function (8, 9).

S. aureus is a frequent colonizer of nose and skin in the general population, but given appropriate circumstances, the microorganism can turn into a dangerous pathogen and cause a broad range of infections (10). *S. aureus* is also associated with allergic airway inflammation (11, 12). Recently we showed that the staphylococcal serine protease-like proteins (SplA – SplF) elicit a type 2-biased immune response in healthy individuals and especially in asthma patients. We observed serum IgE binding to these bacterial proteins in most asthmatics and a minority of healthy individuals. SplD was able to induce allergic airway inflammation *de novo* when applied intratracheally in a murine allergy model (13, 14).

To elucidate whether CF patients – many of whom are persistently exposed to *S. aureus* in their airways – react with type 2 inflammation to the Spls, we examined their specific IgE and T cell responses.

MATERIALS AND METHODS

Blood Donors

Serum samples from CF patients (n = 76) were obtained at the Institute of Medical Microbiology, University Hospital Münster, Germany. They comprise two cohorts, a multicenter study (n = 62) (5, 15) as well as a two center study that was conducted in Münster (n = 14). Samples from healthy individuals were obtained from in-house volunteers (n = 46). The median age of CF patients was 14.9, 52 patients (68.5%) were male, 24 (31.5%) were female. In 44 subjects from the multicenter study the *S. aureus* nasal colonization status was known; 28 were *S. aureus* nasal carriers and 16 were non-carriers. The median age of the healthy subjects was 23, 13 (28.2%) were male and 33 (71.8%) female; 16 (34.8%) were persistent *S. aureus* carriers. Five CF patients (from the two center study) and five healthy volunteers additionally donated peripheral EDTA blood samples. All blood donors gave informed consent (Approvals of the responsible Ethics Committees; Greifswald: IIIUV 23/06a, BB007/17; Münster: 2007-496-f-S, 2014-054-f-S).

Antigens

Recombinant Spls were generated as described (14). Lyophilized staphopain A and B were purchased from Sigma-Aldrich and reconstituted in PBS. When used in cell culture assays, the proteins were denatured at 95°C for 30 min.

Antibody Response

Enzyme-linked immunosorbent assays (ELISAs) were performed as previously described (14). Briefly, wells of 96-well microtiter plates (MaxiSorp, Nunc) were coated with 5 µg/mL recombinant Spls (50 µL/well). Serum samples were diluted 1:5 and added in duplicate wells. The bound IgE-antibodies were detected with biotinylated rabbit anti-human IgE antibody (10 µg/mL; antibodies online) followed by Streptavidin-HRPO (1:333; Dianova). TMB substrate reagent was added for 10 min, and the reaction was stopped with 20 µl 2N sulfuric acid. The optical densities (OD) were measured at 450 nm in Infinite M200 Pro (Tecan Austria GmbH). Negative controls were processed without the addition of serum. Each assay was repeated on two separate days.

Cellular Response to the Spls

PBMCs were isolated from 30 mL of whole blood using standard gradient methods and cryopreserved until analysis. After thawing, CD14+ monocytes were isolated from PBMCs by positive selection using CD14 MicroBeads (Miltenyi Biotec 30-050-201). Untouched T cells were isolated from the CD14-negative fraction using PAN T cell isolation kits (Miltenyi Biotec 130-096-535). The purity of the isolated T cells was assessed by flow cytometry and was > 95%.

The purified T cells were co-incubated with irradiated CD14+ feeder cells at a ratio of 10:1 in RPMI medium (PAN Biotech, P04-17500) supplemented with 5% human serum (PAN Biotech, P30-2401), 100 IU/mL penicillin, 200 µg/ml streptomycin, 4 mM glutamine, 50 µM β-mercaptoethanol, 1.0 mM sodium pyruvate, 0.1 mM non-essential amino acids (Sigma, M7145-100M). Cells were seeded in 24-well flat bottom plates and stimulated with a cocktail of recombinant SplA, SplB, SplD, SplE, and SplF (5 mg/mL each). On day 5, 750 µL of the medium was replaced by fresh medium supplemented with 20 IU/mL human recombinant IL-2 (Miltenyi Biotec).

On day 9, the supernatant was taken and stored at -80°C until analysis. The cytokine concentrations in the supernatant were measured using a 13-plex cytometric bead array (LEGENDplex Human Inflammation Panel, BioLegend 740721), and cytokine concentrations were determined with the corresponding LEGENDplex software.

The T cells were harvested in PBS and stained using fluorochrome-conjugated antibodies (**Supplementary Table 1A**). NIR (Biolegend, 423106) was used to exclude dead cells. Data were acquired on an LSR II (BD Bioscience, San Jose, CA, USA) and FlowJo (Treestar, Ashland, OR, USA) software was used for analysis. FSC-A vs. FSC-H blots identified singlets. After gating on live T cells (NIR-CD3+), CD4+ Th cell subsets were identified by their chemokine receptor expression patterns as shown in (**Supplementary Table 1B**).

RESULTS

Increased Spl-Specific Serum IgE Levels in CF Patients

We analyzed 76 CF patients from two cohorts, a multi-center study (n = 62) (5, 15) and a two-center study (n = 14), as well as 46 healthy adults. All patients were persistently colonized and

recurrently infected with *S. aureus* in their airways. We quantified Spl-specific IgE in the sera by ELISA. The antigens SplA, SplB, SplD, SplE, and SplF were tested, and we found IgE binding to all of them to be strongly elevated in CF patients compared with controls (**Figure 1A**). Only a minority of the healthy adults had measurable concentrations of Spl-specific serum IgE. The difference was robust and remained highly significant when the two patient cohorts were tested separately (see **Supplementary Figure 1**). There was pronounced variability in the patterns of each patient's IgE binding to the five Spls. This likely reflects the patients' history of exposure to these enzymes: Spl-specific IgE tended to be higher in CF patients that were persistently colonized with *S. aureus* not only in the lung but also in the nose; in the case of SplA this difference reached significance (**Table 1**). While all Spls are encoded in one operon that is present in around 80% of clinical *S. aureus* isolates, the composition of this operon is variable, indicating that in *S. aureus*-infected CF patients the immune system is confronted with different subsets of the Spl proteins (16).

Since protease activity is common in allergens, we next analyzed the IgE response against two other cysteine proteases of *S. aureus*, staphopain A (ScpA) and staphopain B (SspB). In contrast to the remarkably increased IgE response to the Spls in CF, the staphopain-specific IgE serum levels did not differ between CF patients (n = 14; subjects of the two-center study) and healthy controls (n = 46) (**Figure 1B**). This highlights that the ability to induce a strong IgE response is a specific property of the Spls rather than a general feature of *S. aureus* proteases or, in fact, staphylococcal antigens in general and corroborates earlier findings that *S. aureus* antigens can elicit immune responses of different quality in the same individual (14, 17). It is plausible to assume that the Spls' proteolytic activity has a role in causing the type 2 bias of the specific antibody response. The Spls of *S. aureus* are known to have distinctive and very selective preferences for cleavage motifs, indicating a narrow substrate range (18, 19). However, the knowledge about the Spls' pathophysiological substrates is very limited (20, 21). The extensive and long-term exposure of the CF patients' airways to *S. aureus* drives a strong antibody response to many *S. aureus* antigens, documented by high specific IgG titers (5, 15). However, this pronounced humoral immune reaction to the bacteria cannot be the only reason for the sensitization to the Spls in CF, which is very selective.

Th2 Bias in Spl-Reactive T Cells of CF Patients

Immunoglobulin class switch to IgE requires the help of antigen-specific Th2 cells. Therefore, we studied the Spl-specific T cell memory response in CF patients (n = 5) and healthy controls

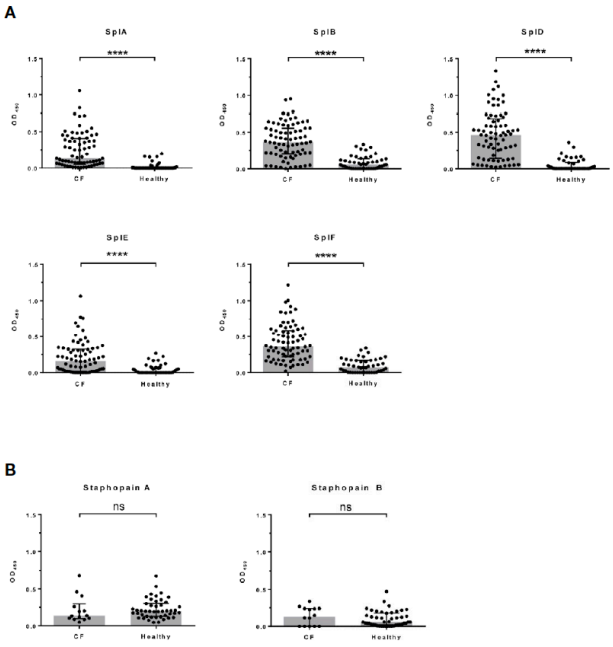


FIGURE 1 | *S. aureus* protease-specific IgE in sera of CF patients and healthy adults. Specific serum antibody binding was determined by ELISA. Each data point represents the mean of two technical replicates. Spl-specific serum IgE levels were significantly higher in CF patients (n = 76) than in healthy controls (n = 46) **(A)**, whereas no significant differences were seen in the staphopain A and staphopain B specific IgE levels (CF: n = 14; controls: n = 46) **(B)**. Medians (grey bars) with interquartile ranges are shown. ****$P < 0.0001$; Mann Whitney U test. CF, cystic fibrosis, ns, not significant, OD, optical density.

TABLE 1 | Correlation between nasal *S. aureus* carrier status and anti-SpI IgE.

SpI	Nasal *S. aureus* carriers[a] (n = 28)		*S. aureus* non-carriers (nose) (n = 16)		*P*-value[b]
	Mean ± SD	Median	Mean ± SD	Median	
SplA	0.29 ± 0.28	0.23	0.12 ± 0.18	0.05	0.035*
SplB	0.41 ± 0.26	0.36	0.26 ± 0.25	0.19	0.069
SplD	0.40 ± 0.29	0.38	0.52 ± 0.42	0.47	0.479
SplE	0.21 ± 0.25	0.13	0.11 ± 0.17	0.03	0.051
SplF	0.47 ± 0.29	0.43	0.31 ± 0.19	0.27	0.062

[a]) *Information about nasal S. aureus colonization was available for 44 CF patients, 28 carriers and 16 non-carriers.*
[b]) *Mann-Whitney U test; *) P < 0.05.*

(n= 5) and compared cytokine production and phenotype of the SpI-stimulated T cells. We isolated and co-cultured T cells and $CD14^+$ antigen-presenting cells from peripheral blood, stimulated them with a mixture of recombinant SplA, SplB, SplD, SplE, and SplF (each at 5 µg/mL) for nine days and then measured cytokines in the cell culture supernatants.

In healthy controls, type 2 cytokines (IL-4, IL-5, IL-13) were of low concentration or below the threshold of detection. In comparison, release of these cytokines was significantly increased in all cultures from CF patients. Similarly, IL-6 production was significantly higher in T cells isolated from CF patients than in those from controls, whereas IL-10 release tended to be lower in CF (**Figure 2**). We did not observe significant differences for IFNγ, IL-17A, IL-17F (**Figure 2**) nor for TNF, IL-9, IL-21 or IL-22 (not shown).

At the same time point, nine days after SpI stimulation, we assessed the phenotype of the T cells by flow cytometry and determined the proportions of $CD4^+$ T cell subtypes according to their chemokine receptor expression. Focusing on changes in the T cell subtype composition due to SpI exposure, we found a stronger Th2 and Th17 cell response in CF patients, whereas Th1 cells dominated the reaction in the control individuals (**Figure 3**). The slightly increased percentage of Th17 cells in CF patients was not reflected in the release of IL-17 in cell culture. This is not easily explained. It may reflect the known plasticity of Th17 cells, which developed differently in CF patients than in controls (22, 23).

DISCUSSION

The sensitization of CF patients to antigens of *S. aureus* that is colonizing and infecting their airways is reminiscent of ABPA, where a type 2 airway inflammation specific to the ubiquitous

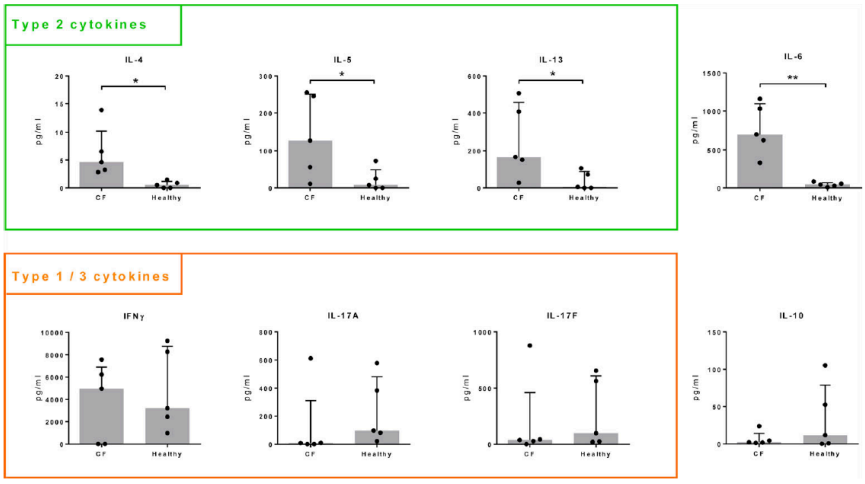


FIGURE 2 | Cytokine production by SpI-stimulated T cells. T cells were isolated from whole blood of CF patients (n = 5) and healthy volunteers (n = 5) and stimulated with a mixture of recombinant SplA, SplB, SplD, SplE and SplF (5 µg/mL each) in the presence of CD14+ antigen-presenting cells. Supernatants were taken at day 9 and cytokine concentrations measured by a cytometric bead array. Concentrations were normalized to 1 million T cells. Production of Th2 cytokines (IL 4, IL 5, IL 13) and IL 6 was significantly higher in CF patients compared to healthy controls, IL 10 release in tendency lower, while there were no significant differences in the concentrations of IFNγ, IL 17A, or IL 17F. Medians (grey bars) with interquartile ranges are indicated; *$P < 0.05$; **$P < 0.01$; Mann Whitney U test. CF, cystic fibrosis.

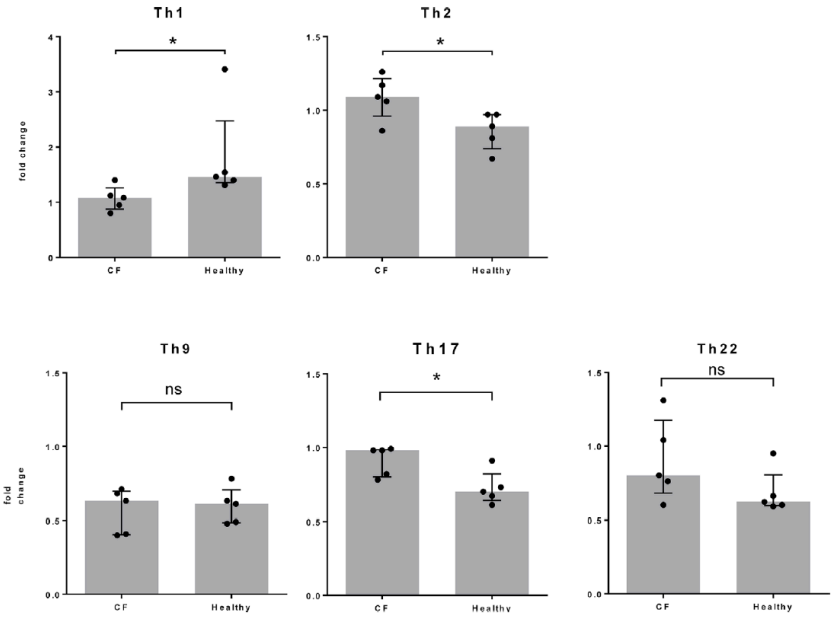


FIGURE 3 | T cell differentiation following Spl stimulation. From the same cell cultures described in **Figure 2**, T cells were harvested at day 9 and the proportion of each T cell subtype was determined by FACS. Fold changes between unstimulated and Spl-stimulated cells are shown. Compared to healthy controls, the changes in Th2 and Th17 cells were significantly higher and those in Th1 cells significantly lower in Spl-stimulated T cells from CF patients. *$P < 0.05$; Mann Whitney U test. CF, cystic fibrosis.

fungus *A. fumigatus* destroys lung function if left untreated (8, 24). In some patients the Spl-directed IgE response was very strong, and we propose that this could be an unrecognized cause of allergic lung exacerbation in CF patients harboring *S. aureus* in their airways. Type 2 immune responses may also favor bacterial colonization and infection because they counteract the immune clearance mechanisms, which are of a type1/type 3 profile. However, in our study, anti-Spl IgE levels did not differ significantly between CF patients who experienced lung exacerbations during the study period and those who did not, nor did they correlate with lung function (FEV_1% predicted). Probably our CF cohort was too small and too heterogeneous to show the influence of a single factor on the complex pathogenesis. Even in ABPA a bronchial provocation test was required to reveal the eosinophilic inflammation and reduction of FEV_1 in CF patients that were sensitized to *A. fumigatus* (25). Moreover, our analysis of the T cell response to Spls is limited by the small numbers of tested persons. The T cell analyses required substantial amounts of fresh blood, which only 5 patients in the second study cohort could safely provide. Nevertheless, the results clearly demonstrate skewing of the Spl-specific memory towards a type 2 profile in CF, possibly accompanied by a loss of tolerance that is indicated by the reduced IL-10 production. These findings corroborate the results of our IgE and cytokine measurements, underlining the specific type 2 quality of the adaptive immune response to the Spls of *S. aureus* in CF.

The discovery of allergic reactions to the Spls of *S. aureus* opens a new avenue for research and therapy. Further studies are now warranted to find out if CF patients develop allergic reactions to other colonizing or infecting bacteria as well. The quest for bacterial allergens is still in its beginning (21). However, sensitization to staphylococcal enterotoxins (SE) is well documented in chronic rhinosinusitis with nasal polyps where it is an independent risk factor for co-morbid asthma (26). Besides SE-specific IgE, many asthmatics have elevated serum IgE against *S. aureus* Spls (14). It is possible that sensitization to persistent colonizing and infecting bacteria significantly contributes to disease progression in some CF patients. In this case the therapeutic portfolio may be extended, because agents that selectively interfere with type 2 inflammation without hampering anti-microbial defense mechanisms are rapidly becoming available.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Approvals of the responsible Ethics Committees; Greifswald: IIIUV 23/06a, BB007/17; Münster: 2007 496-f S, 2014-054 f S. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Study concept and design: BB and BK. Designed and performed experiments: MN, GA, and JT. Wrote the manuscript: GA, BB, MN, BK, and JT. Analyzed the data: GA, BB, MN, BK, and HW. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by grants from: The German Research Foundation (DFG) [CRC TRR34 to BK and BB], The German Research Foundation [GRK1870 to GA and MN], European Social Fund [ESF/14 BMA55-0037/16 "Card-ii-Omics" to MN and BB], and Mukoviszidose e.V. [S05/07 to BK]. We acknowledge support for the Article Processing Charge from the DFG (German Research Foundation, 393148499) and the Open Access Publication Fund of the University of Greifswald.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.651060/full#supplementary-material

REFERENCES

1. Bell SC, Mall MA, Gutierrez H, Macek M, Madge S, Davies JC, et al. The future of cystic fibrosis care: a global perspective. *Lancet Respir Med* (2020) 8:65–124. doi: 10.1016/S2213-2600(19)30337-6
2. Cystic Fibrosis Foundation Patient Registry. *Annual Data Report*. Bethesda, Maryland: American Cystic Fibrosis Foundation (2018).
3. Hatziagorou E, Orenti A, Drevinek P, Kashirskaya N, Mei-Zahav M, de Boeck K. Changing epidemiology of the respiratory bacteriology of patients with cystic fibrosis-data from the European cystic fibrosis society patient registry. *J Cyst Fibros* (2020) 19:376–83. doi: 10.1016/j.jcf.2019.08.006
4. Goerke C, Kraning K, Stern M, Döring G, Botzenhart K, Wolz C. Molecular epidemiology of community-acquired *Staphylococcus aureus in* families with and without cystic fibrosis patients. *J Infect Dis* (2000) 181:984–9. doi: 10.1086/315331
5. Lange J, Heidenreich K, Higelin K, Dyck K, Marx V, Reichel C, et al. *Staphylococcus aureus* pathogenicity in cystic fibrosis patients-results from an observational prospective multicenter study concerning virulence genes, phylogeny, and gene plasticity. *Toxins (Basel)* (2020) 12:279. doi: 10.3390/toxins12050279
6. Stanford GE, Dave K, Simmonds NJ. Pulmonary exacerbations in adults with cystic fibrosis - a grown-up issue in a changing CF landscape. *Chest* (2021) 159:93–102. doi: 10.1016/j.chest.2020.09.084
7. Epps QJ, Epps KL, Young DC, Zobell JT. State of the art in cystic fibrosis pharmacology-Optimization of antimicrobials in the treatment of cystic fibrosis pulmonary exacerbations: I. Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) antibiotics. *Pediatr Pulmonol* (2020) 55:33–57. doi: 10.1002/ppul.24537
8. Epps QJ, Epps KL, Zobell JT, Young DC. Optimization of antimicrobials in the treatment of cystic fibrosis pulmonary exacerbations: II. Therapies for allergic bronchopulmonary aspergillosis. *Pediatr Pulmonol* (2020) 55:3541–72. doi: 10.1002/ppul.25080
9. Kaditis AG, Miligkos M, Bossi A, Colombo C, Hatziagorou E, Kashirskaya N, et al. Effect of allergic bronchopulmonary aspergillosis on FEV1 in children and adolescents with cystic fibrosis: a European cystic Ffbrosis society patient registry analysis. *Arch Dis Child* (2017) 102:742–7. doi: 10.1136/archdischild-2016-311132
10. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* (2015) 28:603–61. doi: 10.1128/CMR.00134-14
11. Davis MF, Ludwig S, Brigham EP, McCormack MC, Matsui EC. Effect of home exposure to *Staphylococcus aureus on* asthma in adolescents. *J Allergy Clin Immunol* (2018) 141:402–405.e10. doi: 10.1016/j.jaci.2017.06.031
12. Bachert C, van Steen K, Zhang N, Holtappels G, Cattaert T, Maus B, et al. Specific IgE against *Staphylococcus aureus* enterotoxins: an independent risk factor for asthma. *J Allergy Clin Immunol* (2012) 130:376–81.e8. doi: 10.1016/j.jaci.2012.05.012
13. Teufelberger AR, Nordengrün M, Braun H, Maes T, de Grove K, Holtappels G, et al. The IL-33/ST2 axis is crucial in type 2 airway responses induced by *Staphylococcus aureus*-derived serine protease-like protein D. *J Allergy Clin Immunol* (2018) 141:549–59.e7. doi: 10.1016/j.jaci.2017.05.004
14. Stentzel S, Teufelberger A, Nordengrün M, Kolata J, Schmidt F, van Crombruggen K, et al. Staphylococcal serine protease-like proteins are pacemakers of allergic airway reactions to *Staphylococcus aureus*. *J Allergy Clin Immunol* (2017) 139:492–500.e8. doi: 10.1016/j.jaci.2016.03.045
15. Junge S, Görlich D, den Reijer M, Wiedemann B, Tümmler B, Ellemunter H, et al. Factors associated with worse lung function in cystic fibrosis patients with persistent *Staphylococcus aureus*. *PloS One* (2016) 11:e0166220. doi: 10.1371/journal.pone.0166220
16. Zdzalik M, Karim AY, Wolski K, Buda P, Wojcik K, Brueggemann S, et al. Prevalence of genes encoding extracellular proteases in *Staphylococcus aureus* - important targets triggering immune response *in vivo*. *FEMS Immunol Med Microbiol* (2012) 66:220–9. doi: 10.1111/j.1574-695X.2012.01005.x
17. Kolata JB, Kühbandner I, Link C, Normann N, Vu CH, Steil L, et al. The fall of a dogma? unexpected high T-cell Memory response to *Staphylococcus aureus* in humans. *J Infect Dis* (2015) 212:830–8. doi: 10.1093/infdis/jiv128
18. Tam K, Torres VJ. *Staphylococcus aureus* secreted toxins and extracellular enzymes. *Microbiol Spectr* (2019) 7:GPP3–0039. doi: 10.1128/microbiolspec.GPP3-0039-2018
19. Stach N, Kalinska M, Zdzalik M, Kitel R, Karim A, Serwin K, et al. Unique Substrate Specificity of SplE Serine Protease from *Staphylococcus aureus*. *Structure* (2018) 26:572–79.e4. doi: 10.1016/j.str.2018.02.008
20. Paharik AE, Salgado-Pabon W, Meyerholz DK, White MJ, Schlievert PM, Horswill AR. The Spl serine proteases modulate *Staphylococcus aureus* protein production and virulence in a rabbit model of pneumonia. *mSphere* (2016) 1:e00208–16. doi: 10.1128/mSphere.00208-16
21. Nordengrün M, Michalik S, Völker U, Bröker BM, Gómez-Gascón L. The quest for bacterial allergens. *Int J Med Microbiol* (2018) 308:738–50. doi: 10.1016/j.ijmm.2018.04.003
22. Muranski P, Restifo NP. Essentials of Th17 cell commitment and plasticity. *Blood* (2013) 121:2402–14. doi: 10.1182/blood-2012-09-378653
23. Stadhouders R, Lubberts E, Hendriks RW. A cellular and molecular view of T helper 17 cell plasticity in autoimmunity. *J Autoimmun* (2018) 87:1–15. doi: 10.1016/j.jaut.2017.12.007
24. Elphick HE, Southern KW. Antifungal therapies for allergic bronchopulmonary aspergillosis in people with cystic fibrosis. *Cochrane Database Syst Rev* (2016) 11:CD002204. doi: 10.1002/14651858.CD002204.pub4
25. Eickmeier O, Zissler UM, Wittschorek J, Unger F, Schmitt-Grohé S, Schubert R, et al. Clinical relevance of *Aspergillus fumigatus* sensitization in cystic fibrosis. *Clin Exp Allergy* (2020) 50:325–33. doi: 10.1111/cea.13557

26. Bachert C, Humbert M, Hanania NA, Zhang N, Holgate S, Buhl R, et al. *Staphylococcus aureus* and its IgE-inducing enterotoxins in asthma: current knowledge. *Eur Respir J* (2020) 55:1901592. doi: 10.1183/13993003.01592-2019

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5

THE QUEST FOR BACTERIAL ALLERGENS

Nordengrün M, Michalik S, Völker U, Bröker BM, Gómez-Gascón L.

Published in the International Journal of Medical Microbiology. 2018 Aug;308(6):738-750. doi: 10.1016/j.ijmm.2018.04.003. Epub 2018 Apr 26. Review.

AUTHOR CONTRIBUTIONS:

As a first author in this publication, **MN** participated in the conception and design of this scientific review. **MN** researched, collected, filtered and analyzed all the relevant references needed in the preparation of the main manuscript. Furthermore, **MN** designed figures 1, 2 and 4. **MN** had a significant contribution to drafting of the manuscript as well as the scientific revision and editing of its final version.

Conception and design: **MN**, BMB, LGG
Collection and management of information: **MN** and LGG
Manuscript draft preparation: **MN**, SM, LGG
Figure design: **MN**, SM
Critical revision and editing: **MN**, SM, UV, BMB, LGG

Maria Nordengrün | Prof. Barbara M. Bröker

International Journal of Medical Microbiology 308 (2018) 738–750

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

The quest for bacterial allergens

Maria Nordengrün[a], Stephan Michalik[b], Uwe Völker[b], Barbara M. Bröker[a], Lidia Gómez-Gascón[a,*]

[a] Department of Immunology, University Medicine Greifswald, Ferdinand-Sauerbruch- Straße DZ7, D-17475, Greifswald, Germany
[b] Department of Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, University Medicine and Ernst-Moritz-Arndt University Greifswald, Felix-Hausdorff-Straße 8, D-17475, Greifswald, Germany

ARTICLE INFO

Keywords:
Allergy
Bacterial allergens
Serine-protease-like proteins
Staphylococcal enterotoxins
Superantigens

ABSTRACT

Allergies are complex diseases featuring local tissue inflammation, which is characterized by an exaggerated type 2 immune response to environmental compounds known as allergens. Pollens, environmental fungi, and house dust mites are examples of common allergens. Bacteria have a dual role in allergy. Usually, they are associated with protection, however, certain bacterial species promote the development and exacerbation of allergic inflammation. Notably, IgE antibodies specific for bacterial antigens are found in the sera of allergic individuals. This implies that some bacterial factors are allergens, eliciting a specific type 2 immune response. However, to date, only a few of these are molecularly defined. This review summarizes the current knowledge about known bacterial allergens, and it provides an overview of the available techniques for the discovery of new allergens as well as for measuring the immune responses directed against them.

1. Introduction

The prevalence of allergic diseases is very high and still increasing globally, particularly in low- and middle-income countries. Moreover, the complexity and severity of allergic diseases, including asthma, continue to increase, particularly in children and young adults (Masoli et al., 2004; Pawankar, 2014). To address these challenges and to fight these diseases, which place a huge burden on patients and health care systems worldwide, the molecular identification of allergens and their functional characterization is required. After briefly summarizing current knowledge about the role of bacteria in allergy, this review will focus on the nature and functions of bacterial allergens as well as on methods for their discovery and characterization.

1.1. The pathophysiology of allergy

Allergies are chronic inflammatory diseases caused by dysregulated immune responses to certain environmental substances, called allergens. Allergens are molecules that typically elicit IgE responses in the host. Besides, they have to meet additional criteria of the WHO/IUIS allergen nomenclature sub-committee, encompassing molecular and structural properties, that qualify them as allergens (Breiteneder and Chapman, 2014).

The most common allergens are found in pollens, environmental fungi, dust mites, and animal dander as well as in some foods and drugs (Ipci et al., 2016). A central feature of allergies is type 2 inflammation, characterized by increased numbers of Th2 cells, which release IL-4, IL-5, IL-9 and IL-13 upon allergen exposure, as well as by allergen-specific IgE, mast cell activation and tissue infiltration by eosinophils (Barnes, 2009; Wills-Karp et al., 2012). However, other types of helper T cells and their cytokines may also be involved (Farahani et al., 2014). Th17 cells, for example, can produce Th2-type cytokines (Cosmi et al., 2010; Raymond et al., 2011), and the Th9 subset releases large amounts of IL-9 (Koch et al., 2017). Moreover, Th22 cells, which secrete IL22 and IL-13, and Th25 cells, which secrete IL-25, are believed to be important in allergic reactions and airway inflammation (Angkasekwinai et al., 2007).

During airway inflammation, epithelial cells respond to allergens by producing potent mediators such as IL-25, IL-33 and thymic stromal lymphopoietin (TSLP). These mediators promote the recruitment and activation of specialized immune cells and affect their differentiation towards a type 2 immune response profile (Golebski et al., 2013). IL-33 enhances allergic inflammation through induction of other pro-allergenic cytokines and chemokines, such as IL-4, IL-5, and IL-13. Notably, ST2, an IL-33 receptor component, is primarily expressed by Th2 cells, mast cells, eosinophils and basophils (Borish and Steinke, 2011; Oboki et al., 2011).

Innate lymphoid cells (ILCs), which are related to natural killer cells, are emerging as important effectors in innate immunity because they are involved in protection against pathogens and associated with lymphoid tissue formation and tissue remodelling. There are three types of ILCs, which are differentiated based on their similarities to helper T

* Corresponding author.
E-mail address: lidia.gomezgascon@uni-greifswald.de (L. Gómez-Gascón).

https://doi.org/10.1016/j.ijmm.2018.04.003
Received 4 October 2017; Received in revised form 11 April 2018; Accepted 12 April 2018

cells. Among them, ILC2s have the ability to secrete type 2 cytokines such as IL-4, IL-5, IL-9 and IL-13. High levels of ILC2 cells have been observed in the tissues of patients with asthma or atopic dermatitis (AD). Thus, this subset of cells contributes to the immunopathology of chronic airway inflammation and to inflammation in other barrier organs (Bal et al., 2016; Mjosberg et al., 2012).

1.2. Bacteria counteract allergy development – the hygiene hypothesis

It is well documented that exposure to bacteria is associated with protection against allergy. Mycobacteria, for example, are potent inducers of Th1 responses including the release of IFN-γ, which counteract type 2 inflammation (Yoshida et al., 2002), and they elicit regulatory T cell (Treg) responses, which likely represent the main anti-allergic immune mechanism. Infection with *Mycobacterium tuberculosis* as well as vaccination with *Bacillus Calmette-Guérin* or other mycobacteria reduce the prevalence of allergy, both in humans and animals (Choi, 2014; Choi and Koh, 2002, 2003; Kim et al., 2014; Shirakawa et al., 1997; Umetsu et al., 2002). Moreover, there is a wealth of information available in the literature showing that bacterial products modulate the innate immune system. Innate pattern recognition receptors, e.g., the toll-like receptor (TLR) family including TLR4 mediate important anti-allergenic effects. Among these are antimicrobial responses such as phagocytosis, the induction of nitrogen oxide as well as the stimulation of the maturation of antigen-presenting cells (APCs). The latter increase the secretion of the type 1 cytokines, IL-6, TNF-α, IL-1, IFN-γ, and IL-12, and have a prominent role in B cell and T cell activation and differentiation (Chandler and Ernst, 2017; Freyne et al., 2018; Nagai et al., 2018; Shibata et al., 2018; Vandepapelière et al., 2008).

The observation of a sharp decline in infectious diseases accompanied by the steep rise in the incidence of allergy in recent decades has prompted the hygiene hypothesis: “The main factor in the increased prevalence of these allergic diseases in industrialized countries is the reduction in the incidence of infectious diseases in those countries over the past three decades” (Bach, 2002). This hypothesis was later modified, because the role of the commensal microflora in inflammatory homeostasis and immune regulation is being increasingly appreciated. Exposure to innocuous exogenous and endogenous microorganisms early in life protects against allergy. Generally, variations in the microbiome, both in terms of the number and diversity of bacteria, may significantly affect the incidence of allergic manifestations (Atkinson, 2013; Edwards et al., 2012; Hilty et al., 2010; Ipci et al., 2016; Medina et al., 2012; Ramsey and Celedon, 2005; Ribet and Cossart, 2015; Schaub et al., 2006). Because of these findings the capacity of certain species of the commensal gut microflora (probiotic strains), such as lactic acid bacteria including Lactobacillus or Bifidobacteria species, of enhancing immune tolerance is now being tested. Several excellent texts reporting the beneficial role of these strains in the primary prevention of allergic diseases are available (Chua et al., 2017; Chung, 2017; West et al., 2017).

1.3. Bacteria can promote allergy – epidemiological evidence

Conversely, there is increasing epidemiological evidence that colonization or infection with certain bacterial species can trigger or exacerbate allergies (Edwards et al., 2012; Emre et al., 1995; Seggev et al., 1996; Welliver and Duffy, 1993). In asthma, for example, bacteria may exacerbate disease symptoms alone or in conjunction with viruses such as human rhinovirus or respiratory syncytial virus (Barnes, 2009; Darveaux and Lemanske et al., 2014).

As early as the 1970s and 1980s, studies demonstrated a correlation between bacterial colonization and allergic diseases. Atypical bacteria such as *Chlamydia trachomatis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* are associated with an increased incidence of asthma, wheezing episodes and asthma exacerbations, as well as with lung remodelling. Similarly, these pathogens have been frequently identified in bronchoalveolar lavage fluid (BAL), nasal washes and sera from asthmatic patients (Emre et al., 1995; Hahn et al., 1991; Hahn and Peeling, 2008; Hahn et al., 2012; Huhti et al., 1974; Ikezawa, 2001; Johnston and Martin, 2005; Patel et al., 2012; Seggev et al., 1996; Tang et al., 2009; Wark et al., 2002; Webley et al., 2009; Yano et al., 1994; Ye et al., 2014). Regarding the common bacterial inhabitants of the human respiratory tract, colonization or infection with *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catharralis* and *Staphylococcus aureus* have been associated with the induction and exacerbation of asthma, chronic obstructive pulmonary diseases and recurrent wheezing early in life (Bachert et al., 2003; Barnes, 2009; Bisgaard et al., 2010; Bisgaard et al., 2007; Brarda et al., 1996; Darveaux and Lemanske et al., 2014; Davis et al., 2015; Hales et al., 2012; Hilty et al., 2010; Kjaergard et al., 1996; Pauwels et al., 1980). Moreover, in patients suffering from allergic disorders such as asthma, AD or nasal polyposis, *S. aureus* colonization appears to occur much more frequently (87%, 90%, 87%, respectively), in contrast to 20%–50% colonization of healthy adults (Holtfreter et al., 2016; Krismer et al., 2017; Mulcahy and McLoughlin, 2016; Ryu et al., 2014; Weidenmaier et al., 2012). In addition, asymptomatic colonization of neonates with *S. pneumoniae* or *M. catarrhalis* is associated with later development of recurrent wheezing and asthma (Bisgaard et al., 2007).

1.4. Bacterial mechanisms of allergy induction and exacerbation

Numerous pro-allergenic functions, both antigen-specific and non-antigen-specific, have been ascribed to bacteria. Bacteria have the ability to infect airway epithelial cells, thereby inducing inflammation, cell death and epithelial barrier failure. Moreover, pore-forming toxins, e.g., *S. aureus* α-toxin (Hla), and bacterial proteases contribute to epithelial barrier failure (Inoshima et al., 2011). Increased epithelial permeability facilitates microbial invasion and exposes the immune system to environmental pollutants and allergens.

On the other hand, antibacterial immune defense systems appear to be impaired in allergy. In response to bacterial invasion the innate immune system of human skin elaborates large amounts of antimicrobial peptides (AMPs) known as cathelicidins and beta-defensins. This response is defective in AD patients. Moreover, Th2 cytokines such as IL-4, IL-10, and IL-13 act synergistically to down-regulate AMP expression in the skin of AD patients. This results in a higher susceptibility to *S. aureus* colonization in AD patients, which in turn promotes the exacerbation of AD symptoms (Howell et al., 2006; Ong et al., 2002; Ryu et al., 2014; Takahashi and Gallo, 2017).

Respiratory pathogens can induce an excess of mediators of airway repair, resulting in airway remodelling accompanied by thickening of the airway walls and impairment of lung function. Fibroblast growth factors and vascular endothelial growth factors are involved in angiogenesis, airway smooth muscle proliferation and hypertrophy, collagen and fibronectin deposition as well as in the generation of new lymphatic vessels (Edwards et al., 2012; Smith-Norowitz et al., 2016). For example, in a murine asthma model, *M. pneumoniae* infection increases airway collagen deposition (Chu et al., 2003, 2005). In mice with chronic and recurrent *C. pneumoniae* infection, an increase in the thickness of the subepithelial basement membrane suggestive of airway remodelling was observed (Chen et al., 2009).

Some bacteria are able to elicit histamine release from human basophil leukocytes and mast cells via IgE-dependent or -independent mechanisms (Ahren et al., 2003; Clementsen et al., 1990; Emre et al., 1995; Kjaergard et al., 1996; Larsen et al., 1998; Nakamura et al., 2013; Pauwels et al., 1980; Seggev et al., 1996; Tee and Pepys, 1982; Welliver and Duffy, 1993; Ye et al., 2014). In asthmatic children infected with *M. pneumoniae*, elevated numbers of basophils are present in the peripheral blood and eosinophilia is observed in the BAL, suggestive of exacerbations of bronchial asthma (Tang et al., 2009). *H. influenzae* and *S. pneumoniae* activate eosinophils and potentiate the release of

inflammatory mediators by basophils and eosinophils when they are triggered by IgE-dependent or independent mechanisms (Ahren et al., 2003; Clementsen et al., 1990; Kjaergard et al., 1996). Nakamura and colleagues demonstrated that cell culture supernatants of *Staphylococcus epidermidis*, *S. aureus* and *Staphylococcus saprophyticus* elicited mast cell degranulation via δ-toxin independent of IgE and antigen (Nakamura et al., 2013).

Several studies indicate that bacteria can induce differentiation of naïve T cells into Th2 or Th17 cells and elicit Th2 cytokine release. Bacterial species such as *M. pneumonie* or *C. pneumoniae* induce the production of IL-4 in PBMCs and increase IL-4 levels as well as IL-4/IFN-γ ratios in the BAL from asthmatic patients (Koh et al., 2001; Smith-Norowitz et al., 2016; Ye et al., 2014; Yeh et al., 2016). Patients with bronchiectasis and *H. influenzae* infection develop a Th2 cytokine profile and increase serum concentrations of specific IgG1, IgG3 and IgG4 (King et al., 2003). Moreover, PBMCs derived from 6-month-old carriers of *S. pneumoniae*, *H. influenzae* or *M. catarrhalis* who developed asthma by 7 years of age produced more IL-5 and IL-13 upon exposure to these bacteria than PBMCs from those subjects who did not develop asthma (Larsen et al., 2014). *S. aureus* expresses virulence factors such as superantigens (SAgs, Box 1), fibronectin-binding protein A (FnBP) as well as δ-toxin, which can skew the cutaneous immune response towards a type 2 profile. This facilitates *S. aureus* attachment to the skin surface and survival of the microbes (Kim et al., 2006; Reginald et al., 2011; Taskapan and Kumar, 2000).

Induction of type 2 cytokines is expected to trigger an Ig class switch to IgE. Indeed, specific anti-bacterial serum IgE has been found in allergic individuals. For example, IgE antibodies directed against *C. trachomatis*, *C. pneumoniae, M. pneumoniae, H. influenzae*, *S. pneumoniae, M. catharralis* or *S. aureus* have been described (Bachert et al., 2003; Bisgaard et al., 2007; Brarda et al., 1996; Emre et al., 1995; Hahn et al., 2012; Hales et al., 2012; Ikezawa, 2001; Kjaergard et al., 1996; Patel et al., 2012; Pauwels et al., 1980; Yano et al., 1994; Ye et al., 2014). The clinical significance of these findings will be discussed in Section 3. Paradoxically, there is evidence indicating that exposure to *H. influenzae* or *S. pneumoniae* may confer protection from allergy, even if anti-bacterial IgE can be measured. In teenagers specific IgE against different proteins of these microbial species was inversely correlated with asthma risk. These observations underline that specific IgE per se does not equal symptomatic allergy. Moreover, they highlight the importance of epidemiological and mechanistic validation of allergen prediction.

Notably, it has recently been demonstrated that house dust mites (HDM) can act as carriers of antigens from bacteria colonizing the skin, the respiratory tract or the gut, such as *S. aureus* or *E. coli*. Thus HDM may trigger or facilitate sensitization to bacterial antigens. This could explain the frequent occurrence of IgE-reactivity to bacterial antigens in respiratory and skin manifestations of allergy (Dzoro et al., 2017).

S. aureus and *S. pyogenes* produce enterotoxins (staphylococcal enterotoxins: SEs; *S. pyogenes* enterotoxins: SPEs) (Foster, 2005; Thammavongsa et al., 2015). SEs and SPEs are very stable molecules and appear to have a dual role in allergy: on the one hand, they act as extremely potent SAgs, stimulating proliferation and effector functions in pre-existing effector and memory T cells, including Th2 cells. Some of them even skew the immune response towards a type 2 profile (Commons et al., 2014; Fraser and Proft, 2008; Grumann et al., 2014; Spaulding et al., 2013). On the other hand, SAgs are recognized by the immune system as conventional antigen targets, resulting in the development of specific antibodies directed against them (Box 1, Fig. 1).

Hence, bacteria command of general allergy-promoting mechanisms, and they can themselves become the target of type 2 immune responses characterized by specific Th2 cells and IgE antibodies. In the latter sense, bacteria and their compounds are discussed as allergens in this review. To date, only a few bacterial allergens have been defined at a molecular level, bacterial enterotoxins being the most prominent examples (Table 1). In Section 2, we will discuss state-of-the-art methodology for the discovery and validation of novel bacterial allergens, and in Section 3, we will review what has been achieved to date. An overview of the allergy-promoting mechanisms of bacteria is provided in Fig. 2.

2. Approaches for the identification of allergenic bacterial antigens

The identification of bacterial allergens adds a new dimension to our understanding as well as to diagnostic and treatment options. Allergens have been defined as antigens that induce the production of serum IgE and then bind to this IgE. Thus, the identification of allergens largely relies on the analysis of IgE binding (Chardin and Peltre, 2005; Zhuang and Dreskin, 2013). In addition, the criteria of the WHO/IUIS allergen nomenclature sub-committee, have to be met for a molecule to qualify as an allergen (Breiteneder and Chapman, 2014). Unbiased methods lend themselves to the discovery of new allergens, while targeted approaches are required to determine their pathophysiological importance.

As demonstration of IgE binding is key, researchers are facing the problem that free serum IgE is usually present at very low concentrations because circulating IgE antibodies rapidly attach to the high-affinity Fcε receptor 1 on mast cells and basophils. Thus, free serum IgE may not always reflect systemic total IgE levels (Amarasekera, 2011). Therefore, most serological assays that are used to examine the IgE antibody response, e.g., IgE immunoblotting, enzyme-linked immunosorbent assay (ELISA) or IgE inhibition assays, require substantially larger volumes of serum than tests for specific IgG, IgM or IgA. The depletion of IgG from the sera can increase the sensitivity of both ELISA and immunoblot analyses for allergen-specific IgE by reducing competition for antigen binding (Chardin and Peltre, 2005). Moreover, IgG4 holds promise as a surrogate marker for IgE. Since the production of both IgG4 and IgE antibodies depends on similar Th2 cytokine profiles, high antigen-specific IgG4 titres may indicate a Th2 bias (Aalberse et al., 2009; Stentzel et al., 2016b).

As described in this section, modern omics techniques provide promising tools for the discovery of allergen candidates.

2.1. Unbiased screening approaches for allergen discovery

In terms of the discovery of new allergens, bioinformatics tools for allergen prediction have evolved appreciably in recent years. Since the critical features constituting the allergenicity of a protein are not yet fully understood, all *in silico* prediction tools rely on similarities in primary or secondary structures with known allergens. According to the rules of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), a protein is classified as a putative allergen if it has at least six contiguous amino acids that are exact matches (rule 1) or a minimum of 35% sequence similarity over a window of 80 amino acids (rule 2) with known allergens (FAO/WHO, 2001). However, the FAO/WHO rules produce many false-positive results (Saha and Raghava, 2006; Wang et al., 2013b). Several computational prediction approaches have been developed, including sequence-, motif-, support vector machine (SVM)-, epitope- and allergen representative peptide (ARP)-based methods (McClain, 2017; Saha and Raghava, 2006; Wang et al., 2013a,b). As a matter of course, all predicted candidate allergens must be empirically validated. Table 2 summarizes the scopes and limitations of technologies that are suitable for this purpose.

Table 2 also lists unbiased methods for empirical allergen discovery. Well-established 1D or 2D immunoblot techniques have been used successfully to identify new allergenic proteins recognized by IgE in patient sera (Arcos et al., 2014; Chardin and Peltre, 2005; Ghosh et al., 2015; Reginald et al., 2011; Zhao et al., 2015; Zhuang and Dreskin, 2013).

The immunoblot technology relies on the separation of protein

Box 1
The Janus face of bacterial enterotoxins.

Enterotoxins, SEs and SPEs, are members of a large group of bacterial virulence factors known as superantigens (SAgs). In *S. aureus*, they comprise 23 proteins consisting of SEs and enterotoxin-like proteins (SEls) (Grumann et al., 2014). The streptococcal superantigens include SPEs (A, B, C, and G to M), streptococcal superantigen (SSA) and streptococcal mitogenic exotoxin Zn (SMEZn) (Spaulding et al., 2013).

(**a**) SAgs are notable for their capacity to stimulate a massive T cell response. These molecules, which are chemically extremely robust, bind to major histocompatibility complex (MHC) II molecules and to the T cell receptor (TCR) outside the typical antigen binding sites, thereby triggering a large fraction of T cells. Memory T cells respond with the release of effector cytokines reflecting their differentiation profile, e.g., Th1 or Th2. These processes can culminate in a life-threatening cytokine storm, notoriously known as toxic shock syndrome. By virtue of their enterotoxic properties, some SAgs cause food poisoning with vomiting and diarrhoea (Fraser et al., 2000; Fraser and Proft, 2008; Proft and Fraser, 2003; Spaulding et al., 2013).

(**b**) However, SAgs are also recognized by the immune system just like any other conventional antigen. They are taken up by antigen-presenting cells (APCs) and processed into peptides, which are then attached to the peptide-binding cleft of MHC II molecules and presented to T cells. Naïve T cells with the appropriate TCR – usually present at a very low frequency – react to these antigen complexes, divide and differentiate into effector and memory T cells. These cells may then help specific B cells to mount an antibody response. In this cognate interaction[1] of T cells and B cells, B cells act as APCs: they efficiently take up the (super)antigen with their specific B cell receptor (BCR), process it and present it to helper T cells. Indeed, SAgs are immunodominant bacterial proteins, and most adults have high titres of neutralizing antibodies in their body fluids, protecting them against toxic shock syndrome (Fraser et al., 2000; Grumann et al., 2011; Holtfreter et al., 2006). Many allergic patients harbour SE/SPE-specific IgE in their sera, which indicates that SAgs can act as allergens (see Section 3).

The superantigenic and antigenic properties of bacterial enterotoxins are depicted in Fig. 1.

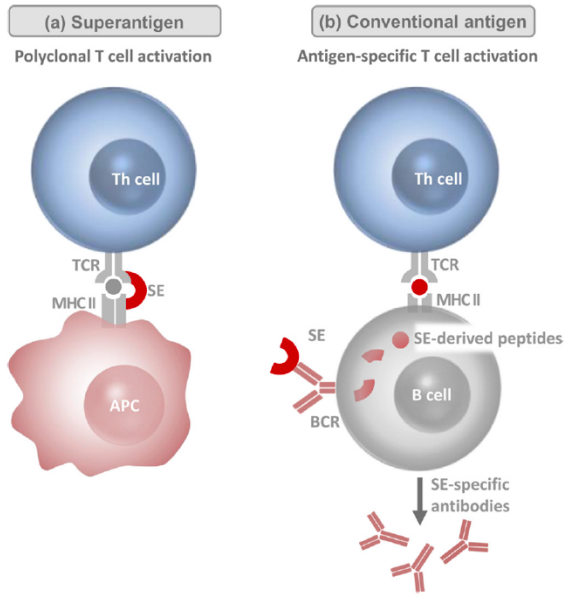


Fig. 1. Enterotoxins can function as superantigens (a) and as conventional antigens (b). For more explanations, please see Box 1. Abbreviations: TCR, T-cell receptor; MHC major histocompatibility complex; SEs, staphylococcal enterotoxins; APC, Antigen-presenting cell; BCR, B-cell receptor.

extracts according either to their molecular weight (MW) via 1D-SDS-PAGE or to their isoelectric point (pI) and MW via 2D-PAGE. Proteins are transferred to a membrane, probed with patient sera, and antibody binding is visualized using, e.g., anti-IgG4 or anti-IgE detection antibodies (Aalberse et al., 2009; Stentzel et al., 2016b). The Ig-binding protein bands or spots are then identified by mass spectrometry. This approach is powerful, albeit with some limitations. Since a single band on a 1D-gel/blot and even a single spot in a 2D-gel/blot may contain more than one protein or protein species (Lim et al., 2003), the results must be validated using other methods. Furthermore, only a specific "proteomic window" is accessed because 2D-PAGE covers only a certain pI and MW range and hydrophobic proteins are not resolved.

Conventional 1D immunoblotting has been further developed into a capillary-based automated 1D immunoblot system, Simple Western™, saving labour as well as antigen and patient material. (Rustandi et al., 2012; Stentzel et al., 2016a). The technique provides quantitative data about the antigen-antibody binding and information about the antigen size or isoelectric point. However, it does not permit the molecular identification of antigens in complex mixtures such as bacterial protein extracts. Thus, Simple Western™ technology is a good medium-throughput screening method.

Crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE) have also been used for analyses of individual IgE-binding proteins in complex mixtures. The common principle comprises two independent electrophoreses. Following separation in the first dimension, protein extracts are electrophoresed into an antibody-containing gel in the second dimension, which results in the formation of bell-shaped precipitates, each representing one antigen. The position of the precipitate reflects the nature and amount of the protein as well as the specific antibody concentration in the gel, such that relative quantification is possible. IgE-binding allergens are visualized by incubating CIE gels with IgE-containing patient sera followed by IgE-specific detection antibodies (Arlian et al., 2003; Hansen and Larsen, 2008).

In recent years, robust protein and peptide array technologies have emerged, enabling high-throughput screening for allergenic proteins as well as IgE and IgG4 epitope mapping of identified allergens (Lee et al., 2013). Array-based technologies can capture large numbers of proteins (up to 10.000) and also provide good quantification. Depending on the target molecules, forward protein arrays with specific proteins are distinguished from reverse phase proteins arrays, which contain complex protein mixtures as baits for antibody binding. On forward protein arrays selected proteins are coupled to a solid array surface and used to quantify specific antibodies in samples (Liotta et al., 2003). An interesting variant is the nucleic acid programmable protein array (NAPPA). Biotinylated target DNA (plasmid) containing the sequence of a protein of interest (GST-tagged) is spotted onto an array surface covered with both avidin and anti-GST-tag antibodies. Cell-free protein expression generates the recombinant proteins, which are immobilized at the position of the encoding DNA via their GST-tag. Thus, the NAPPA

[1] Cognate means that T cells and B cells recognize the same antigen.

Table 1
Bacterial proteins considered to be involved in the initiation and exacerbation of type 2 immune responses.

Bacteria	Putative allergens	Evidence for allergy	References
Chlamydia trachomatis	MOMP, CrpA, POMP, HSP60 Unidentified proteins (250 KDa, 64 KDa)	Specific-IgE in sera and BAL (patients)	Emre et al., 1995; Patel et al., 2012
Chlamydia pneumoniae	MOMP, CrpA, POMP, HSP60, LBP Unidentified proteins (98 KDa, 78 KDa, 58-60 KDa, 36 KDa)	Specific-IgE in sera and BAL(patients)	Emre et al., 1995; Larsen et al.,1998; Ikezawa, 2001; Hahn et al., 1991; Hahn and Peeling, 2008; Hahn et al., 2012
Mycoplasma pneumoniae	CARDS toxin	Induces allergic pulmonary inflammation with eosinophilia and Th2 cytokine secretion (murine model)	Medina et al., 2012
Staphylococcus aureus	SEs (A-U), TSST-1, FnBP, Spls (A-E)	SE-specific IgE in sera (asthma, chronic sinusitis/nasal polyposis, allergic rhinitis, chronic urticaria patients) Mast cell degranulation (in vitro and in vivo) High levels of FnBP-specific IgE, IgG4 and Th2 cytokines (atopic dermatitis patients, murine model) High levels of Spls-specific IgE, IgG4 and Th2 cytokines (human sera, PBMCs, murine model)	Bachert and Zhang, 2012; Tripathi et al., 2004; Kowalski et al., 2011; Liu et al., 2014; Ye et al. 2008; Nakamura et al., 2013; Reginald et al., 2011; Stentzel et al., 2016a
Haemophilus influenzae	P4 P6	Specific-IgE and IgG4 in sera (patients)	Hales et al., 2008; 2009; Hales et al., 2012; Hollams et al. 2010; Larsen et al., 2014
Streptococcus pneumoniae	PspC	Specific-IgE in sera (patients)	Hollams et al., 2010; Hales et al., 2012; Larsen et al., 2014
Streptococcus pyogenes	SPEs (A,C)	Specific-IgE in sera (chronic sinusitis/nasal polyposis patients)	Tripathi et al., 2004
Pseudomonas aeruginosa	OdDHL	IL-12 suppression and high levels of IgE (human immune cells) TNFα and IL-12 suppression and High levels of IgG1 (murine model)	Telford et al., 1998

Abbreviations: MOMP, major outer membrane protein; CrpA, cysteine-rich membrane protein; POMP, polymorphic outer membrane protein; HSP60, heat shock protein; LBP, lectin binding protein; CARDS toxin, community-acquired respiratory distress syndrome toxin; SEs, Staphylococcal enterotoxins; TSST, toxic shock syndrome toxin; FnBP, fibronectin-binding protein; Spls, staphylococcal serine protease-like proteins; PspC, pneumococcal surface protein C; SPEs, pyrogenic exotoxins of *S.pyogenes* ; OdDHL, *N*-(3-Oxododecanoyl)-L-homoserine lactone.

bypasses the need of overexpressing and purifying of the proteins of interest and reduces the costs. To date, the immunogenic potential of 1000 to 10,000 antigens can be simultaneously analyzed with NAPPA arrays (Katchman et al., 2017; Song et al., 2017). Further advantages of microarray-based immunoassays are the requirement of low serum volumes, robust statistical analysis, and the possibility of testing several immunoglobulin subclasses simultaneously (Kuhne et al., 2015; Lin et al., 2012; Martinez-Botas and de la Hoz, 2016).

2.2. Targeted approaches to quantify the allergic host response

Once (candidate) allergens are known, targeted strategies are required to explore their properties and determine their clinical relevance by measuring antigen-specific IgE in patients and controls (Breiteneder and Chapman, 2014). A quantitative, high-throughput, low-cost multiplex technique would be the ideal tool for the required large epidemiological studies.

Immunoblotting is well established as a robust targeted technique (Kumar et al., 2014). Selected recombinant antigens are separated by 1D SDS-PAGE, transferred to a membrane and probed with patient sera. The Simple Western™ technology described above is a gel-free variation of this technology.

Another well-known and highly versatile method is the enzyme-linked immunosorbent assay (ELISA). In the conventional single-plex setup, a defined antigen is immobilized on a microtitre plate surface and probed with patient serum. The readout for IgE binding is commonly accomplished using an enzyme-conjugated IgE-specific detection antibody. Provided that the conditions are optimized, ELISA results are precise, accurate and reproducible (Acker and Auld, 2014; Khan et al., 2014). In allergy diagnostics, the anti-IgE detection antibody is often conjugated to a radioactive label rather than an enzyme. In all other ways, this so-called radioallergosorbent test (RAST) works like an ELISA. The ELISA (or RAST) inhibition assay is a variant thereof, measuring IgE binding to soluble allergen in a fluid phase, which requires higher binding strength between the antibody and the antigen. Defined amounts of soluble allergen are added to the test sample, and the inhibition of serum IgE binding to the same allergen immobilized on a surface is determined (Pedersen et al., 2008; Schmitt et al., 2004).

Multiple ELISA-type tests can be performed in parallel. Alternatively, allergenic compounds may be immobilized on a solid support in a microarray format, optimizing multiplexing and saving sample volume. A prominent example of the multiplex ELISA technique is the ImmunoCAP® system, whereas its variant, the ImmunoCAP® ISAC system, is array-based. These tools are widely employed in allergy diagnostics (Arlian et al., 2003; Bonini et al., 2012; Dzoro et al., 2017; Liu et al., 2014). To date, ImmunoCAP® has integrated only five bacterial allergenic components, the *S. aureus* enterotoxins SEA, SEB, SEC, SED, and TSST1 (see Section 3).

Suspension arrays such as the Luminex's xMAP® technology utilize fluorescent beads as a solid matrix (Baker et al., 2012). This approach enables extensive multiplexing, and several hundred antigens can be tested simultaneously. Suspension protein arrays are easy to customize; antigen or DNA "printing" devices are not required. The workflow is depicted in Fig. 3. Antigens are selected based on prior knowledge, recombinantly expressed and covalently coupled to fluorescent beads such that each antigen can be identified by the specific and unique fluorescence code of the corresponding beads. If the recombinant antigens contain a sequence tag, protein coupling efficiency can be assessed using tag-specific fluorophore-conjugated antibodies. Subsequently, antigen-loaded beads are mixed to generate a multiplexed suspension array. After incubation with serum, antibody binding can be measured using specific detection antibodies coupled with a reporter fluorophore. The dual-laser flow-based classification of individual beads and quantification of the reporter signal has a very broad

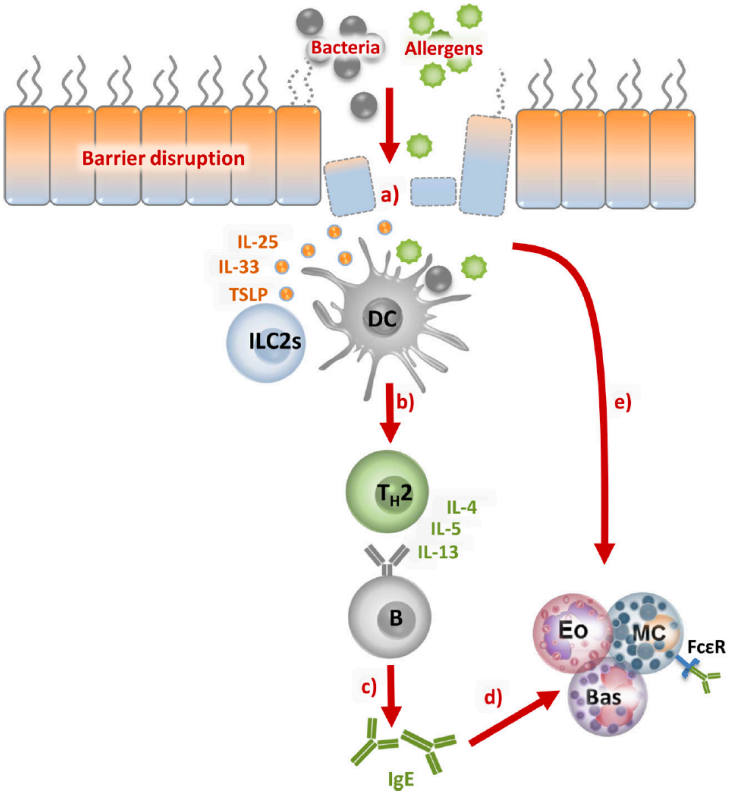


Fig. 2. Allergy-promoting mechanisms of bacteria.
Several pro-allergenic mechanisms have been described in bacteria. Bacterial proteases and toxins disrupt the epithelial barrier, facilitating microbial invasion and the influx of conventional allergens. This leads to local inflammation accompanied by the secretion of potent immune mediators (IL-25, IL-33 and TSLP) (**a**). This process promotes the recruitment of naïve T cells and their differentiation into effector T cells (Th2 or Th17), resulting in the release of pro-allergenic Th2 cytokines. Tissue resident ILC2s respond with type 2 cytokine secretion as well (**b**). An Ig class switch is induced in B cells, which differentiate into IgE-secreting plasma cells (**c**). IgE facilitates the recruitment and activation of mast cells, eosinophils and basophils (**d**). Bacterial components can also directly induce degranulation of these effector cells in an IgE-independent manner, exacerbating the allergic inflammation (**e**). Abbreviations: B, B cell; Bas, basophil; DC, dendritic cell; Eo, eosinophil; FCεR, high affinity immunoglobulin E receptor; MC, mast cell; Th, T helper cell; ILC2, Innate lymphoid cell type 2; TSLP, thymic stromal lymphopoietin.

Table 2
Advantages (blue bars) and disadvantages (red bars) of different techniques for measuring specific antibody responses.

	SimpleWestern™	2D immunoblot	ELISA	Protein array (NAPPA)	ImmunoCAP® (ISAC)	Suspension array (Luminex xMAP®)
Antigen discovery						
Specific analysis of known target						
Quantification of antibody binding						
Throughput						
Simplicity						
Availability of IgE assay						
Amount of sample material needed						
Cost						

dynamic range (Baker et al., 2012). Suspension array tests can be performed at high-throughput with comparably small amounts of antigen and patient material, making them a powerful and versatile tool for cohort studies (Table 2).

3. Bacterial allergens – state of the art

While IgE binding to protein extracts of *S. aureus*, *C. pneumoniae* and *C. trachomatis* has been demonstrated, only a handful of bacterial allergens have been molecularly defined (Emre et al., 1995; Patel et al., 2012; Reginald et al., 2011) (Table 1). *C. pneumoniae*-specific IgE was found in patients suffering from chronic respiratory disease and in asthma patients (Emre et al., 1995; Hahn et al., 2012; Patel et al., 2012). Cysteine-rich membrane protein A (CrpA), major outer membrane protein (MOMP), lectin binding proteins (LBPs), chlamydial heat shock protein 60 (HSP60) and lipopolysaccharide (LPS) were identified as the most prominent IgE-binding chlamydial compounds via Western blotting (Hahn et al., 2012). For the outer membrane proteins P4 and P6 of *H. influenzae* as well as for surface protein C (PspC) of *S. pneumoniae*, specific IgG4 and IgE antibodies were detected in allergic patients, and increased anti-bacterial IgE was observed during convalescence from asthma exacerbation, reaching titres similar to those

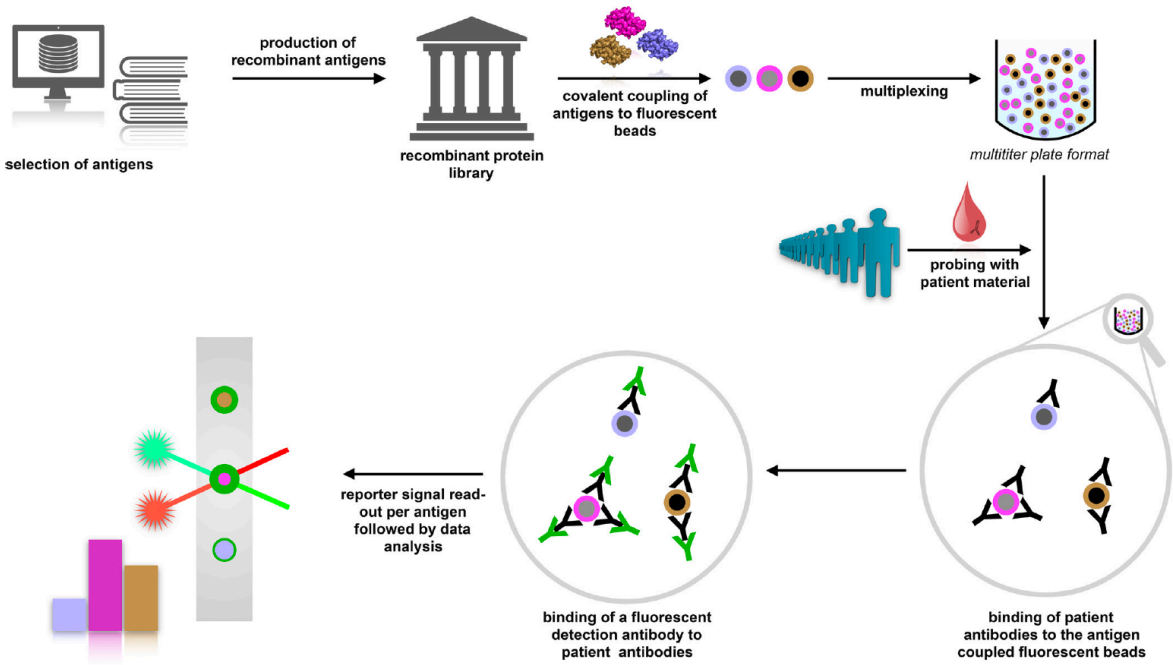


Fig. 3. Schematic representation of a suspension protein array workflow.
After selection and production, antigens are coupled to fluorescence-coded beads such that each fluorescence code corresponds to a single antigen. Following incubation with patient plasma/serum, antibody binding to the beads is visualized with a fluorophore-conjugated detection antibody (reporter signal). Based on the specific and unique fluorescence coding of each bead, the beads can be separated during readout, and each reporter signal can be attributed to the corresponding antigen.

induced by major aeroallergens (Hales et al., 2012; Hales et al., 2009; Hales et al., 2008; Hollams et al., 2010). In search of *M. pneumoniae* allergens, the recombinant CARDS (community-acquired respiratory distress syndrome) toxin, an ADP-ribosylating and vacuolating toxin, was tested as a candidate. In naïve mice, intranasal exposure to this toxin in the absence of adjuvant induced allergic pulmonary inflammation and production of Th2 type cytokines (Medina et al., 2012). Notably, CARDS toxin was detected in the airway secretions of therapy refractory asthma patients (Peters et al., 2011). Turning to *P. aeruginosa*, it was reported that the protein *N*-(3-Oxododecanoyl)-L-homoserine lactone (OdDHL), a molecule involved in quorum sensing of these bacteria, may influence the host's Th1-Th2 balance. In murine peritoneal macrophages, OdDH reduced the production of TNF-α and IL-12. Moreover, OdDHL, increased the relative amount of IgG1 in the antibody response elicited to keyhole limpet hemocyanin in spleen cells. IgG1 is an immunoglobulin isotype associated with type 2 immune responses in mice. In human immune cells, the IL-12 synthesis was reduced and the IgE antibody production was increased when PBMCs were incubated with OdDHL (Telford et al., 1998). However, conflicting data also exist. Other groups have described either a predominant Th1 immune response in the presence of OdDHL or that this protein inhibited the differentiation of both, Th1 and Th2 cells (Ritchie et al., 2007, 2003; Smith et al., 2002).

S. aureus SE-specific IgE has been detected in the sera and airways of patients with allergy. Anti-SE IgE is associated with increased asthma severity and with intrinsic asthma (Bachert et al., 2003; Huvenne et al., 2013; Kowalski et al., 2011; Liu et al., 2014; Tripathi et al., 2004). Likewise, *S. pyogenes* produces enterotoxins, and SPE-specific IgE has been observed in patients with chronic sinusitis/nasal polyposis (Proft and Fraser, 2003; Spaulding et al., 2013; Tripathi et al., 2004).

To date, *S. aureus* is considered the best-characterized bacterial species in terms of its pro-allergenic properties. Several secreted factors of *S. aureus* have been identified as IgE-reactive components, specifically SEs, fibronectin-binding protein (FnBP), serine protease-like proteins (Spls), extracellular vesicles (EVs) and δ-toxin. For this reason, studies conducted with *S. aureus* will be described in more detail in the following section to illustrate the research strategy spanning the identification of allergen candidates through in vitro and in vivo validation of their allergenic capacity to assessment of their immunodiagnostic potential.

3.1. Identification of IgG4/IgE-binding S. aureus proteins

Early in 1993, Leung et al. identified SEs as IgE-binding proteins in AD patients (Leung et al., 1993). Using enterotoxin-specific antibodies, these authors detected SEs in the secretome of *S. aureus* and screened a cohort of AD patients for SE-specific serum IgE by ELISA. In total, 57% of the tested patients showed IgE binding to SEA and SEB, identifying *S. aureus* SEs as IgE-inducing components. For a long time, SEs remained the only known IgE-binding proteins of *S. aureus*, and their role in allergy has been extensively investigated. Systematic screening experiments to characterize the *S. aureus* allergome were subsequently carried out by the groups of Reginald et al., 2011 and Stentzel (2016b). Reginald and co-workers screened a genomic *S. aureus* expression library using sera from AD patients who were positive for IgE antibodies against S. aureus extracts. They identified FnBP as an IgE-binding protein of *S. aureus* and also showed that FnBP-specific IgG4 levels were significantly higher in AD patients than in non-atopic individuals, indicating a Th2 bias (Reginald et al., 2011).

Stentzel and colleagues took a different approach and initially used IgG4 as a surrogate marker for IgE because immunoglobulin class switch recombinations to IgG4 and IgE are initiated by similar Th2-biased cytokine profiles. Employing 2D immunoblotting, they visualized protein spots with strong IgG4 binding. These spots were

identified by mass spectrometry to contain *S. aureus* Spls. The strong IgG4 bias of the Spl-specific antibody response was confirmed by analysis of specific IgG1- and IgG4-binding titres in the sera of healthy *S. aureus* carriers and non-carriers. The antibody response to Spls was strongly shifted towards IgG4 compared to the antibody response to *S. aureus* α-haemolysin (Hla) (Stentzel et al., 2016b).

S. aureus FnBP and Spls were thus proposed as major allergens of *S. aureus*.

This finding highlights modern omics techniques as powerful tools for the discovery of allergen candidates, marking the beginning of an experimental programme to validate their allergenic properties according to the WHO/IUIS criteria. This process is best exemplified by downstream analyses of the *S. aureus* allergen candidates FnBP and Spls.

3.2. Cell culture assays

Once an IgG4/IgE biased antibody response has been documented, the question arises as to whether this is the result of an underlying type 2 T cell response.

As all IgE-binding proteins of *S. aureus* characterized to date were found to be SAgs, Reginald and colleagues questioned whether FnBP exhibits superantigenic properties and compared the capacity of FnBP and SEs to induce a proliferative response in human T cells. T cells stimulated with SEs proliferated vigorously even when using fixed APCs, whereas FnBP induced a proliferative response only when viable APCs were present. This clarified that the induction of T cell proliferation by FnBP is dependent on antigen processing by viable APCs and clearly differs from polyclonal T cell activation by *S. aureus* SAgs. When PBMCs from patients with AD and from healthy donors were stimulated with FnBP, high levels of IFN-γ, IL-6 and TNF-α were detected. These reflect a pro-inflammatory cytokine milieu favouring the development of Th1 and Th17 cells, which can contribute to the inflammatory processes in AD. However, a Th2 bias of the FnBP-specific T cell response was not confirmed by these *ex vivo* experiments (Reginald et al., 2011).

Stentzel and colleagues analysed the Spl-specific memory T cell response in healthy adults. The cytokine profile elicited by Spls and *S. aureus* Hla clearly differed. The cytokine response upon Hla stimulation was dominated by Th1/Th17 cytokines (IFN-γ, IL-17, IL-6, TNF), as is typical of *S. aureus* antigens. Following Spl stimulation, these cytokines were released in very low amounts, whereas Th2 cytokines (IL-4, IL-5, IL-13) reached significantly higher amounts than following exposure to Hla (Stentzel et al., 2016b). These results revealed a Th2 bias of the Spl-specific T cell response, remarkably in non-allergic individuals.

3.3. Examining the local situation in mucosal tissue

In the context of allergic diseases, the local immune response against *S. aureus* provides strong hints regarding the allergenic potential of the bacteria and their secreted components.

Nasal polyp patients are a relevant cohort for these analyses because they are frequently colonized with *S. aureus* and often suffer from late-onset asthma, which is difficult to treat. *S. aureus* colonization is an independent risk factor for asthma development in these patients (Bachert et al., 2010). Bachert and co-workers analysed homogenates of mucosal tissue from nasal polyp patients for the presence of cytokines, eosinophilic cationic protein (ECP), SEs and SE-specific IgE. The ImmunoCAP™ system was used to measure IgE specific for SEs (SEA, SEC and TSST-1) directly in the tissue (Bachert et al., 2010). The researchers observed a Th2-biased local immune response reflected by the presence of IL-5, ECP and polyclonal as well as SE-specific IgE in polyp tissue. Significantly, ECP and IgE levels were positively correlated with the presence of SEs, suggesting that SEs are able to augment local mucosal inflammation. Moreover, patients whose tissue was positive for SE-specific IgE had a significantly higher prevalence of comorbid asthma (Bachert et al., 2010). Local polyclonal IgE was shown to induce mast cell degranulation upon exposure to various inhalant allergens, a mechanism that is expected to induce asthma symptoms (Bachert et al., 2003; Zhang et al., 2011). Taken together, these studies make a strong case for *S. aureus* SEs' ability to modify local mucosal inflammation in the airways, increasing type 2 inflammation as well as the incidence and severity of comorbid asthma.

To elucidate which *S. aureus* proteins are locally expressed, Schmidt and colleagues used high-resolution mass spectrometry to screen nasal polyp tissue and revealed the presence of SEs, FnBP and Spls. In addition, they detected IgG antibodies specific for SEs, FnBP and Spls in nasal polyp tissue extracts and quantified them using Luminex FLEXMAP 3D® technology (Schmidt et al., 2017). Hence, the question arises as to whether the presence of FnBP and Spls in the tissue is linked to allergic inflammation, as was observed for SEs, and whether these antigens are able to shift the local immune response towards type 2 inflammation.

3.4. Evidence from in vivo experiments

Whether a candidate allergen is able to cause allergy is a central issue that must be addressed in vivo. Murine models of allergic lung or skin inflammation lend themselves to this purpose. Mice are usually first sensitized with the proteins in question, either intra-tracheally or intraperitoneally, with or without adjuvants. The local and systemic immune responses elicited in the mice are then analysed upon antigen re-challenge, which takes place in vivo or *ex vivo* in cell culture.

The influence of SEB on ovalbumin (OVA)-induced airway inflammation was examined by Hellings et al. BALB/c mice were sensitized with the model antigen OVA intraperitoneally. Twenty days later, the mice were challenged by OVA inhalation. Prior intranasal or bronchial SEB exposure enhanced eosinophilic inflammation in the airway lumen and in the bronchial tissue. Higher mRNA expression of the Th2 cytokines IL-4, IL-5 and eotaxin-1 was observed in the bronchi of SEB-exposed mice, which was accompanied by increased IL-4 and IL-5 concentrations in the serum. Furthermore, SEB promoted sensitization to OVA because increased titres of OVA-specific IgE were measured in the sera of SEB-exposed mice (Hellings et al., 2006). Reginald et al. sensitized BALB/c mice subcutaneously with recombinant FnBP and alum as an adjuvant. The animals mounted an FnBP-specific IgE response, and FnBP-triggered basophil degranulation as well as FnBP-specific T cell proliferation were observed *ex vivo*. When splenocytes from FnBP-sensitized mice were restimulated with FnBP *ex vivo*, they showed a strong proliferative response, and, in contrast to the results obtained with human PBMCs, also released Th2 cytokines (Reginald et al., 2011). Recombinant SplD without adjuvant was used by Stentzel and colleagues to sensitize C57BL/6 mice. Repeated intra-tracheal application of SplD induced strong allergic lung inflammation accompanied by bronchial hyperresponsiveness, eosinophilic infiltration of the lungs and bronchoalveolar lavage fluid (BAL), as well as neutrophil and T cell infiltration into the BAL. Moreover, SplD-specific serum IgE was measurable after two weeks. SplD was also able to abrogate tolerance to OVA, which did not trigger lung inflammation or an IgE response in this model when administered alone. In contrast, the co-application of SplD and OVA led to the generation of OVA-specific IgE. These findings identified SplD as a driving allergen of *S. aureus,* triggering allergic lung inflammation *de novo*.

Regarding AD, murine models have helped to reveal the potential of *S. aureus* SEs to exacerbate allergic skin inflammation. Laouini's group used SEB for epicutaneous immunization of BALB/c mice. SEB treatment resulted in a local allergic skin inflammation accompanied by a systemic SEB-specific type 2 immune response, including elevated SEB-specific serum IgE titres (Laouini et al., 2003). In another model by Savinko and colleagues, epicutaneous immunization with SEB provoked the local accumulation of CD8 + T cells and a mixed Th1/Th2 type dermatitis. SEB treatment elicited specific serum IgE, and, similar to

SplD in the murine asthma model, the SAg was able to break the tolerance to OVA following co-administration.

Notably, all studies conducted with SEs to date have used the native toxins in their biologically active form. Therefore, it is impossible to determine whether the observed Th2 bias is the result of the pro-allergenic properties of the antigens in a strict sense or whether the toxins primarily amplify pre-existing type 2 inflammation by virtue of their superantigenic properties.

In addition to examining the influence of SEs, mouse models of allergic skin inflammation were used to identify novel *S. aureus* products that exacerbate skin inflammation. Nakamura et al. sensitized mice epicutaneously with either clinical *S. aureus* isolates from AD patients or mutant strains deficient in δ-toxin. Wild type S. aureus, but not the mutants, promoted IgE and IL-4 production as well as inflammatory skin disease (Nakamura et al., 2013). Hong et al. used a similar mouse model to elucidate the role of extracellular vesicles produced by *S. aureus* in the pathogenesis of AD. *In* vivo application of S. aureus EVs after tape stripping caused epidermal thickening in the mice accompanied by mast cell and eosinophil infiltration and enhanced cutaneous production of IL-4, IL-5, IFN-γ, and IL-17 (Hong et al., 2011). These results indicate that *S. aureus* SAgs, δ-toxin and EVs induce AD-like skin inflammation.

Once the allergenic potential of candidate proteins has been confirmed, it is possible to address the underlying pathogenetic mechanisms. δ-toxin facilitates mast cell degranulation, both in vitro and in vivo, in a phosphoinositide 3-kinase (PI3K)- and calcium-dependent manner. The δ-toxin-dependent degranulation is enhanced by IgE signalling in the absence of antigen (Nakamura et al., 2013). SplD, on the other hand, induces the local production of IL-33 and eotaxin. IL-33 is known to potently drive type 2 immune responses and is thus recognized as a key player in the pathophysiology of allergic airway inflammation. Co-administration of the soluble IL-33 receptor (sST2) with SplD blocks the downstream effects of IL-33 signalling, decreasing the numbers of inflammatory cells as well as IL-5 and IL-13 production by local lymph node cells. This finding identifies IL-33 as an essential factor in SplD-induced airway inflammation that is controlled by sST2 treatment (Teufelberger et al., 2017).

3.5. Cohort studies

To elucidate the clinical relevance of allergen candidates and assess individuals affected by allergic reactions to bacteria, epidemiological studies are key. The primary focus of such studies lies in the analysis of serum IgE in affected individuals. Elevated serum levels of total IgE and allergen-specific IgE are important immunodiagnostic criteria. Numerous clinical studies reported significant associations between levels of specific IgE to staphylococcal antigens and the severity of atopic disorders, namely urticaria, AD and allergic rhinitis, which can be accompanied by nasal polyposis and/or comorbid asthma.

Ye et al. could show that the prevalence of specific IgE to staphylococcal SAgs was significantly higher in patients with chronic urticaria than in healthy controls. 25.7% of urticaria patients but only 5% of controls had serum IgE specific for at least one of the SAgs SEA, SEB, and TSST-1 (Ye et al., 2008).

In the case of AD, many studies show an association of the frequency of allergic sensitization to *S. aureus* superantigens with the severity of disease symptoms (Breuer et al., 2000; Ide et al., 2004; Lin et al., 2000; Nomura et al., 1999; Sohn et al., 2003). Ong et al. observed a prevalence of allergic sensitization to staphylococcal SAgs of 38% in mild and 63% in moderate AD, sensitization to SEA and TSST-1 being most common (Ong et al., 2008). Nearly 80% of children with severe AD were sensitized to staphylococcal SAgs in a study by Nomura and colleagues (Nomura et al., 1999). However, these findings could not be confirmed in three other studies (Morishita et al., 1999; Rojo et al., 2014; Tada et al., 1996). Hence, a final conclusion about the association between *S. aureus*-specific IgE levels and the severity of AD is not yet possible, as was also the result of a recent meta-analysis conducted by Wit and colleagues (de Wit et al., 2017). However, SE-specific IgE is much more frequent in AD patients than in healthy controls: Pooled odds ratios were 8.37 (SEA), 9.34 (SEB) and 23.33 (TSST-1) (de Wit et al., 2017). Reginald and colleagues detected specific IgE reactivity to FnBP in one third of AD patients. FnBP-specific IgE was associated with more severe symptoms and with *S. aureus* skin superinfection (Reginald et al., 2011). SplB was found to be more frequent in *S. aureus* isolates from AD patients than in those from atopic controls (Rojo et al., 2014). Allergic sensitization to Spls in AD patients has not yet been shown.

Researchers have consistently reported that sensitization to SAg is significantly associated with asthma. The prevalence of SE-specific IgE in asthma patients is more frequent than in healthy controls. Song et al. conducted a meta-analysis of the available data and calculated a pooled odds ratio of 2.95 for asthma in SE sensitized individuals (Song et al., 2013). Rossi and Monasterolo analyzed SE-specific IgE (SEA, SEB, SEC, SED and TSST-1) in patients with allergic rhinitis and/or asthma with dust-mite allergy and found increased serum ECP levels in patients who were positive for SE-IgE, linking the presence of anti-SE-IgE to the severity of type 2 eosinophilic inflammation (Rossi and Monasterolo, 2004). Bachert and colleagues observed high-titre IgE binding to staphylococcal SEs and a positive correlation between anti-SE-IgE titres and the severity of disease symptoms (Bachert et al., 2003). This was supported by Kowalski et al., who found significantly higher serum levels of SE-specific IgE in patients with severe asthma compared to patients with non-severe asthma (Kowalski et al., 2011). Remarkably, the SE-specific IgE seems to be more closely related to asthma severity than sensitization to house dust mite or grass pollens (Bachert et al., 2003).

Stentzel and coworkers showed that Spl-specific IgE is significantly higher in the sera of asthma patients than in those of healthy *S. aureus* carriers and non-carriers (Stentzel et al., 2016a).

Together these results highlight the importance of *S. aureus* allergens in allergic diseases. Sensitization to staphylococcal allergens will aggravate the allergic inflammation in affected patients upon re-encounter with *S. aureus*. It is, therefore, of great clinical relevance to further study the pathogenetic mechanisms underlying the observed associations with the aim of minimizing the effects of potential microbial allergy triggers, including *S. aureus* and its superantigens.

4. Why do bacteria induce type 2 inflammation?

The presented data provide strong evidence that colonization and infection by certain bacterial species increase the risk of allergy development and/or exacerbate allergic inflammation. Bacteria may benefit from an allergic deviation of the immune response towards a type 2 immune response profile because such a response counteracts anti-bacterial clearance mechanisms, which are primarily orchestrated by Th1 and Th17 cells (type 1). Hence, pro-allergenic mechanisms can be considered a means of immune evasion. This immune evasion may explain why certain species, such as *S. aureus*, have developed a whole arsenal of virulence factors favouring or even triggering allergic inflammation. The production of allergenic compounds by this species does not appear to be coincidental but rather the result of a concerted action favouring allergic deviation of the host immune response (Fig. 4). Conversely, it is plausible that type 2 immune deviation induced by bacteria renders the host more susceptible to bacterial colonization, thereby creating a vicious circle, which maintains and exacerbates allergic inflammation.

5. Conclusions

Allergic reactions to colonizing bacteria would indicate a poor prognosis for the affected individual because of the continuous presence of the allergens. Therefore, research efforts must be intensified to clarify the role of bacteria in allergy, especially in cases of hitherto

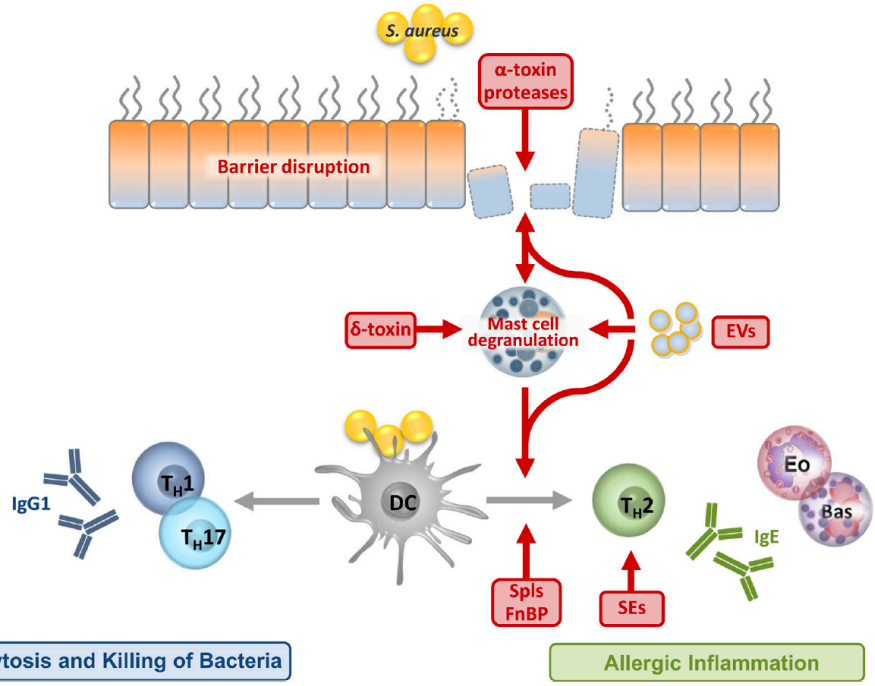


Fig. 4. Immune escape of *S. aureus* via the induction of type 2 inflammation.
Several *S. aureus* toxins facilitate allergen exposure by causing tissue damage. Cleavage of E-cadherin by Hla and bacterial proteases disrupt the epithelial barrier, facilitating the entry of other bacterial factors and aeroallergens. *S. aureus* δ-toxin and EVs exert potent mast cell degranulation activity, releasing endogenous proteases that will, in turn, exacerbate local barrier failure and create a pro-allergenic microenvironment. Moreover, secreted allergens of *S. aureus*, specifically Spls and FnBP as well as antigens contained in the EVs, trigger allergic inflammation *de novo*. Once atopic memory has been established in response to these bacterial allergens, they elicit allergic inflammation similar to other known allergens. SEs augment T cell-mediated inflammation but also function as allergens, eliciting a specific IgE response. As a result of this concerted action, *S. aureus* is able to shift the host immune response away from a Th1/Th17 profile, which would be required for the clearance of extracellular and intracellular bacteria, towards type 2 inflammation, which is less harmful to this microorganism. Abbreviations: Bas, basophil; Eo, eosinophil; Th, T helper cell; DC, dendritic cell; Evs, extracellular vesicles; SEs, staphylococcal enterotoxins; Spls, staphylococcal serine protease–like proteins; FnBP, fibronectin-binding protein.

unknown causation and in patients that are difficult to treat. The reasons why some bacterial species are more closely associated with allergy than others are of paramount interest for the development of prevention strategies. We must also increase our efforts to identify bacterial allergens and elucidate their functions to be able to interfere with them. High-throughput quantitative omics techniques will be crucial for adequately addressing this health problem of global dimensions.

6. Acknowledgement and funding statement

We are grateful to our colleagues in the departments of Immunology and Functional Genomics, University Medicine Greifswald, who provided insight and expertise. We wish to apologize to researchers whose work could not be cited in this review article due to space constraints. Our research groups are financially supported by the Deutsche Forschungsgemeinschaft (CRC-TRR 34, GRK 1870), the German Ministry of Education and Research (InfectControl 2020), and by the State of Mecklenburg Western-Pomerania (Card-ii-Omics, KoInfect).

References

Aalberse, R.C., Stapel, S.O., Schuurman, J., Rispens, T., 2009. Immunoglobulin G4: an odd antibody. Clin. Exp. Allergy 39 (4), 469–477. http://dx.doi.org/10.1111/j.1365-2222.2009.03207.x.

Acker, M.G., Auld, D.S., 2014. Considerations for the design and reporting of enzyme assays in high-throughput screening applications. Perspect. Sci. 1 (1), 56–73. http://dx.doi.org/10.1016/j.pisc.2013.12.001.

Ahren, I.L., Eriksson, E., Egesten, A., Riesbeck, K., 2003. Nontypeable Haemophilus influenzae activates human eosinophils through beta-glucan receptors. Am. J. Respir. Cell Mol. Biol. 29 (5), 598–605. http://dx.doi.org/10.1165/rcmb.2002-0138OC.

Amarasekera, M., 2011. Immunoglobulin E in health and disease. Asia Pac. Allergy 1 (1), 12–15. http://dx.doi.org/10.5415/apallergy.2011.1.1.12.

Angkasekwinai, P., Park, H., Wang, Y.H., Chang, S.H., Corry, D.B., Liu, Y.J., et al., 2007. Interleukin 25 promotes the initiation of proallergic type 2 responses. J. Exp. Med. 204 (7), 1509–1517. http://dx.doi.org/10.1084/jem.20061675.

Arcos, S.C., Ciordia, S., Roberston, L., Zapico, I., Jimenez-Ruiz, Y., Gonzalez-Munoz, M., et al., 2014. Proteomic profiling and characterization of differential allergens in the nematodes Anisakis simplex sensu stricto and A. pegreffii. Proteomics 14 (12), 1547–1568. http://dx.doi.org/10.1002/pmic.201300529.

Arlian, L.G., Morgan, M.S., Quirce, S., Maranon, F., Fernandez-Caldas, E., 2003. Characterization of allergens of Anisakis simplex. Allergy 58 (12), 1299–1303.

Atkinson, T.P., 2013. Is asthma an infectious disease? New evidence. Curr. Allergy Asthma Rep. 13 (6), 702–709. http://dx.doi.org/10.1007/s11882-013-0390-8.

Bach, J.F., 2002. The effect of infections on susceptibility to autoimmune and allergic diseases. N. Engl. J. Med. 347 (12), 911–920. http://dx.doi.org/10.1056/NEJMra020100.

Bachert, C., Zhang, N., 2012. Chronic rhinosinusitis and asthma: novel understanding of the role of IgE 'above atopy'. J. Intern. Med. 272 (2), 133–143. http://dx.doi.org/10.1111/j.1365-2796.2012.02559.x.

Bachert, C., Gevaert, P., Howarth, P., Holtappels, G., van Cauwenberge, P., Johansson, S.G., 2003. IgE to Staphylococcus aureus enterotoxins in serum is related to severity of asthma. J. Allergy Clin. Immunol. 111, 1131–1132 United States.

Bachert, C., Zhang, N., Holtappels, G., De Lobel, L., van Cauwenberge, P., Liu, S., et al., 2010. Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma. J. Allergy Clin. Immunol. 126 (5), 962–968. http://dx.doi.org/10.1016/j.jaci.2010.07.007. 968.e961-966.

Baker, H.N., Murphy, R., Lopez, E., Garcia, C., 2012. Conversion of a capture ELISA to a Luminex xMAP assay using a multiplex antibody screening method. J. Vis. Exp.(65). http://dx.doi.org/10.3791/4084.

Bal, S.M., Bernink, J.H., Nagasawa, M., Groot, J., Shikhagaie, M.M., Golebski, K., et al., 2016. IL-1beta, IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs. Nat. Immunol. 17 (6), 636–645. http://dx.

doi.org/10.1038/ni.3444.
Barnes, P.J., 2009. Intrinsic asthma: not so different from allergic asthma but driven by superantigens? Clin. Exp. Allergy 39 (8), 1145–1151. http://dx.doi.org/10.1111/j.1365-2222.2009.03298.x.
Bisgaard, H., Hermansen, M.N., Bonnelykke, K., Stokholm, J., Baty, F., Skytt, N.L., et al., 2010. Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. Bmj 341, c4978. http://dx.doi.org/10.1136/bmj.c4978.
Bisgaard, H., Hermansen, M.N., Buchvald, F., Loland, L., Halkjaer, L.B., Bonnelykke, K., et al., 2007. Childhood asthma after bacterial colonization of the airway in neonates. N. Engl. J. Med. 357 (15), 1487–1495. http://dx.doi.org/10.1056/NEJMoa052632.
Bonini, M., Marcomini, L., Gramiccioni, C., Tranquilli, C., Melioli, G., Canonica, G.W., Bonini, S., 2012. Microarray evaluation of specific IgE to allergen components in elite athletes. Allergy 67 (12), 1557–1564. http://dx.doi.org/10.1111/all.12029.
Borish, L., Steinke, J.W., 2011. Interleukin-33 in asthma: how big of a role does it play? Curr. Allergy Asthma Rep. 11 (1), 7–11. http://dx.doi.org/10.1007/s11882-010-0153-8.
Brarda, O.A., Vanella, L.M., Boudet, R.V., 1996. Anti-Staphylococcus aureus, anti-Streptococcus pneumoniae and anti-Moraxella catarrhalis specific IgE in asthmatic children. J. Investig. Allergol. Clin. Immunol. 6 (4), 266–269.
Breiteneder, H., Chapman, M.D., 2014. In: Lockey, Richard F., Ledford, Dennis K. (Eds.), Allergen Nomenclature. In Allergens and Allergen Immunotherapy: Subcutaneous, Sublingual and Oral, 5th edition. CRC Press, Taylor and Francis Group, Boca Raton, Florida, USA, pp. 37–49.
Breuer, K., Wittmann, M., Bosche, B., Kapp, A., Werfel, T., 2000. Severe atopic dermatitis is associated with sensitization to staphylococcal enterotoxin B (SEB). Allergy 55 (6), 551–555.
Chandler, C.E., Ernst, R.K., 2017. Bacterial lipids: powerful modifiers of the innate immune response. F1000Res 6. http://dx.doi.org/10.12688/f1000research.11388.1.
Chardin, H., Peltre, G., 2005. Allergome: the characterization of allergens based on a 2D gel electrophoresis approach. Expert Rev. Proteom. 2 (5), 757–765. http://dx.doi.org/10.1586/14789450.2.5.757.
Chen, C.Z., Yang, B.C., Lin, T.M., Lee, C.H., Hsiue, T.R., 2009. Chronic and repeated Chlamydophila pneumoniae lung infection can result in increasing IL-4 gene expression and thickness of airway subepithelial basement membrane in mice. J. Formos. Med. Assoc. 108 (1), 45–52. http://dx.doi.org/10.1016/s0929-6646(09)60031-0.
Choi, I.S., 2014. Immunomodulating approach to asthma using mycobacteria. Allergy Asthma Immunol. Res. 6 (3), 187–188. http://dx.doi.org/10.4168/aair.2014.6.3.187.
Choi, I.S., Koh, Y.I., 2002. Therapeutic effects of BCG vaccination in adult asthmatic patients: a randomized, controlled trial. Ann. Allergy Asthma Immunol. 88 (6), 584–591. http://dx.doi.org/10.1016/S1081-1206(10)61890-X.
Choi, I.S., Koh, Y.I., 2003. Effects of BCG revaccination on asthma. Allergy 58 (11), 1114–1116.
Chu, H.W., Honour, J.M., Rawlinson, C.A., Harbeck, R.J., Martin, R.J., 2003. Effects of respiratory Mycoplasma pneumoniae infection on allergen-induced bronchial hyperresponsiveness and lung inflammation in mice. Infect. Immun. 71 (3), 1520–1526.
Chu, H.W., Rino, J.G., Wexler, R.B., Campbell, K., Harbeck, R.J., Martin, R.J., 2005. Mycoplasma pneumoniae infection increases airway collagen deposition in a murine model of allergic airway inflammation. Am. J. Physiol. Lung Cell Mol. Physiol. 289 (1), L125–L133. http://dx.doi.org/10.1152/ajplung.00167.2004.
Chua, K.J., Kwok, W.C., Aggarwal, N., Sun, T., Chang, M.W., 2017. Designer probiotics for the prevention and treatment of human diseases. Curr. Opin. Chem. Biol 40, 8–16. http://dx.doi.org/10.1016/j.cbpa.2017.04.011.
Chung, K.F., 2017. Airway microbial dysbiosis in asthmatic patients: a target for prevention and treatment? J. Allergy Clin. Immunol. 139 (4), 1071–1081. http://dx.doi.org/10.1016/j.jaci.2017.02.004.
Clementsen, P., Milman, N., Kilian, M., Fomsgaard, A., Baek, L., Norn, S., 1990. Endotoxin from Haemophilus influenzae enhances IgE-mediated and non-immunological histamine release. Allergy 45 (1), 10–17.
Commons, R.J., Smeesters, P.R., Proft, T., Fraser, J.D., Robins-Browne, R., Curtis, N., 2014. Streptococcal superantigens: categorization and clinical associations. Trends Mol. Med. 20 (1), 48–62. http://dx.doi.org/10.1016/j.molmed.2013.10.004.
Cosmi, L., Maggi, L., Santarlasci, V., Capone, M., Cardilicchia, E., Frosali, F., et al., 2010. Identification of a novel subset of human circulating memory CD4(+) T cells that produce both IL-17A and IL-4. J. Allergy Clin. Immunol. 125 (1), 222–230. http://dx.doi.org/10.1016/j.jaci.2009.10.012. e221-224.
Darveaux, J.I., Lemanske Jr., R.F., 2014. Infection-related asthma. J. Allergy Clin. Immunol. Pract. 2 (6), 658–663. http://dx.doi.org/10.1016/j.jaip.2014.09.011.
Davis, M.F., Peng, R.D., McCormack, M.C., Matsui, E.C., 2015. *Staphylococcus aureus* colonization is associated with wheeze and asthma among US children and young adults. J. Allergy Clin. Immunol. 135 (3), 811–813. http://dx.doi.org/10.1016/j.jaci.2014.10.052. e815.
de Wit, J., Totte, J.E.E., van Buchem, F.J.M., Pasmans, S., 2017. The prevalence of antibody responses against Staphylococcus aureus antigens in patients with atopic dermatitis: a systematic review and meta-analysis. Br. J. Dermatol. http://dx.doi.org/10.1111/bjd.16251. [Epub ahead of print].
Dzoro, S., Mittermann, I., Resch, Y., Vrtala, S., Nehr, M., Hirschl, A.M., et al., 2017. House dust mites as potential carriers for IgE sensitisation to bacterial antigens. Allergy 73 (1), 115–124. http://dx.doi.org/10.1111/all.13260.
Edwards, M.R., Bartlett, N.W., Hussell, T., Openshaw, P., Johnston, S.L., 2012. The microbiology of asthma. Nat. Rev. Microbiol. 10 (7), 459–471. http://dx.doi.org/10.1038/nrmicro2801.
Emre, U., Sokolovskaya, N., Roblin, P.M., Schachter, J., Hammerschlag, M.R., 1995. Detection of anti-Chlamydia pneumoniae IgE in children with reactive airway disease. J. Infect. Dis. 172 (1), 265–267.
FAO/WHO, 2001. Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology.
Farahani, R., Sherkat, R., Hakemi, M.G., Eskandari, N., Yazdani, R., 2014. Cytokines (interleukin-9, IL-17, IL-22, IL-25 and IL-33) and asthma. Adv. Biomed. Res. 3, 127. http://dx.doi.org/10.4103/2277-9175.133249.
Foster, T.J., 2005. Immune evasion by staphylococci. Nat. Rev. Microbiol. 3 (12), 948–958. http://dx.doi.org/10.1038/nrmicro1289.
Fraser, J., Arcus, V., Kong, P., Baker, E., Proft, T., 2000. Superantigens - powerful modifiers of the immune system. Mol. Med. Today 6 (3), 125–132.
Fraser, J.D., Proft, T., 2008. The bacterial superantigen and superantigen-like proteins. Immunol. Rev. 225, 226–243. http://dx.doi.org/10.1111/j.1600-065X.2008.00681.x.
Freyne, B., Donath, S., Germano, S., Gardiner, K., Casalaz, D., Robins-Browne, R.M., et al., 2018. Neonatal BCG vaccination influences cytokine responses to Toll-like receptor ligands and heterologous antigens. J. Infect. Dis. http://dx.doi.org/10.1093/infdis/jiy069. [Epub ahead of print].
Ghosh, N., Sircar, G., Saha, B., Pandey, N., Gupta Bhattacharya, S., 2015. Search for allergens from the pollen proteome of sunflower (Helianthus annuus L.): a major sensitizer for respiratory allergy patients. PLoS One 10 (9), e0138992. http://dx.doi.org/10.1371/journal.pone.0138992.
Golebski, K., Roschmann, K.I., Toppila-Salmi, S., Hammad, H., Lambrecht, B.N., Renkonen, R., et al., 2013. The multi-faceted role of allergen exposure to the local airway mucosa. Allergy 68 (2), 152–160. http://dx.doi.org/10.1111/all.12080.
Grumann, D., Nubel, U., Broker, B.M., 2014. Staphylococcus aureus toxins–their functions and genetics. Infect. Genet. Evol. 21, 583–592. http://dx.doi.org/10.1016/j.meegid.2013.03.013.
Grumann, D., Ruotsalainen, E., Kolata, J., Kuusela, P., Jarvinen, A., Kontinen, V.P., et al., 2011. Characterization of infecting strains and superantigen-neutralizing antibodies in Staphylococcus aureus bacteremia. Clin. Vaccine Immunol. 18 (3), 487–493. http://dx.doi.org/10.1128/cvi.00329-10.
Hahn, D.L., Dodge, R.W., Golubjatnikov, R., 1991. Association of Chlamydia pneumoniae (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. Jama 266 (2), 225–230.
Hahn, D.L., Peeling, R.W., 2008. Airflow limitation, asthma, and Chlamydia pneumoniae-specific heat shock protein 60. Ann. Allergy Asthma Immunol. 101 (6), 614–618. http://dx.doi.org/10.1016/s1081-1206(10)60224-4.
Hahn, D.L., Schure, A., Patel, K., Childs, T., Drizik, E., Webley, W., 2012. Chlamydia pneumoniae-specific IgE is prevalent in asthma and is associated with disease severity. PLoS One 7 (4), e35945. http://dx.doi.org/10.1371/journal.pone.0035945.
Hales, B.J., Chai, L.Y., Elliot, C.E., Pearce, L.J., Zhang, G., Heinrich, T.K., et al., 2012. Antibacterial antibody responses associated with the development of asthma in house dust mite-sensitised and non-sensitised children. Thorax 67 (4), 321–327. http://dx.doi.org/10.1136/thoraxjnl-2011-200650.
Hales, B.J., Martin, A.C., Pearce, L.J., Rueter, K., Zhang, G., Khoo, S.K., et al., 2009. Antibacterial IgE in the antibody responses of house dust mite allergic children convalescent from asthma exacerbation. Clin. Exp. Allergy 39 (8), 1170–1178. http://dx.doi.org/10.1111/j.1365-2222.2009.03252.x.
Hales, B.J., Pearce, L.J., Kusel, M.M., Holt, P.G., Sly, P.D., Thomas, W.R., 2008. Differences in the antibody response to a mucosal bacterial antigen between allergic and non-allergic subjects. Thorax 63 (3), 221–227. http://dx.doi.org/10.1136/thx.2006.069492.
Hansen, G.N., Larsen, J.N., 2008. Immunoelectrophoresis for the characterization of allergen extracts. Methods Mol. Med. 138, 147–165. http://dx.doi.org/10.1007/978-1-59745-366-0_13.
Hellings, P.W., Hens, G., Meyts, I., Bullens, D., Vanoirbeek, J., Gevaert, P., et al., 2006. Aggravation of bronchial eosinophilia in mice by nasal and bronchial exposure to Staphylococcus aureus enterotoxin B. Clin. Exp. Allergy 36 (8), 1063–1071. http://dx.doi.org/10.1111/j.1365-2222.2006.02527.x.
Hilty, M., Burke, C., Pedro, H., Cardenas, P., Bush, A., Bossley, C., et al., 2010. Disordered microbial communities in asthmatic airways. PLoS One 5 (1), e8578. http://dx.doi.org/10.1371/journal.pone.0008578.
Hollams, E.M., Hales, B.J., Bachert, C., Huvenne, W., Parsons, F., de Klerk, N.H., et al., 2010. Th2-associated immunity to bacteria in teenagers and susceptibility to asthma. Eur. Respir. J. 36 (3), 509–516. http://dx.doi.org/10.1183/09031936.00184109.
Holtfreter, S., Grumann, D., Balau, V., Barwich, A., Kolata, J., Goehler, A., et al., 2016. Molecular epidemiology of Staphylococcus aureus in the general population in Northeast Germany: results of the study of health in Pomerania (SHIP-TREND-0). J. Clin. Microbiol. 54 (11), 2774–2785. http://dx.doi.org/10.1128/jcm.00312-16.
Holtfreter, S., Roschack, K., Eichler, P., Eske, K., Holtfreter, B., Kohler, C., et al., 2006. Staphylococcus aureus carriers neutralize superantigens by antibodies specific for their colonizing strain: a potential explanation for their improved prognosis in severe sepsis. J. Infect. Dis. 193 (9), 1275–1278. http://dx.doi.org/10.1086/503048.
Hong, S.W., Kim, M.R., Lee, E.Y., Kim, J.H., Kim, Y.S., Jeon, S.G., et al., 2011. Extracellular vesicles derived from Staphylococcus aureus induce atopic dermatitis-like skin inflammation. Allergy 66 (3), 351–359. http://dx.doi.org/10.1111/j.1398-9995.2010.02483.x.
Howell, M.D., Boguniewicz, M., Pastore, S., Novak, N., Bieber, T., Girolomoni, G., Leung, D.Y., 2006. Mechanism of HBD-3 deficiency in atopic dermatitis. Clin. Immunol. 121 (3), 332–338. http://dx.doi.org/10.1016/j.clim.2006.08.008.
Huhti, E., Mokka, T., Nikoskelainen, J., Halonen, P., 1974. Association of viral and mycoplasma infections with exacerbations of asthma. Ann. Allergy 33 (3), 145–149.
Huvenne, W., Hellings, P.W., Bachert, C., 2013. Role of staphylococcal superantigens in airway disease. Int. Arch. Allergy Immunol. 161 (4), 304–314. http://dx.doi.org/10.1159/000350329.

Ide, F., Matsubara, T., Kaneko, M., Ichiyama, T., Mukouyama, T., Furukawa, S., 2004. Staphylococcal enterotoxin-specific IgE antibodies in atopic dermatitis. Pediatr. Int. 46 (3), 337–341. http://dx.doi.org/10.1111/j.1442-200x.2004.01880.x.

Ikezawa, S., 2001. Prevalence of Chlamydia pneumoniae in acute respiratory tract infection and detection of anti-Chlamydia pneumoniae-specific IgE in Japanese children with reactive airway disease. Kurume Med. J. 48 (2), 165–170.

Inoshima, I., Inoshima, N., Wilke, G.A., Powers, M.E., Frank, K.M., Wang, Y., Bubeck Wardenburg, J., 2011. A Staphylococcus aureus pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. Nat. Med. 17 (10), 1310–1314. http://dx.doi.org/10.1038/nm.2451.

Ipci, K., Altintoprak, N., Muluk, N.B., Senturk, M., Cingi, C., 2016. The possible mechanisms of the human microbiome in allergic diseases. Eur. Arch. Otorhinolaryngol. 274 (2), 617–626. http://dx.doi.org/10.1007/s00405-016-4058-6.

Johnston, S.L., Martin, R.J., 2005. Chlamydophila pneumoniae and Mycoplasma pneumoniae: a role in asthma pathogenesis? Am. J. Respir. Crit. Care Med. 172 (9), 1078–1089. http://dx.doi.org/10.1164/rccm.200412-1743PP.

Katchman, B.A., Chowell, D., Wallstrom, G., Vitonis, A.F., LaBaer, J., Cramer, D.W., Anderson, K.S., 2017. Autoantibody biomarkers for the detection of serous ovarian cancer. Gynecol. Oncol. 146 (1), 129–136. http://dx.doi.org/10.1016/j.ygyno.2017.04.005.

Khan, P., Idrees, D., Moxley, M.A., Corbett, J.A., Ahmad, F., von Figura, G., et al., 2014. Luminol-based chemiluminescent signals: clinical and non-clinical application and future uses. Appl. Biochem. Biotechnol. 173 (2), 333–355. http://dx.doi.org/10.1007/s12010-014-0850-1.

Kim, K.H., Han, J.H., Chung, J.H., Cho, K.H., Eun, H.C., 2006. Role of staphylococcal superantigen in atopic dermatitis: influence on keratinocytes. J. Korean Med. Sci. 21 (2), 315–323. http://dx.doi.org/10.3346/jkms.2006.21.2.315.

Kim, Y.J., Kim, H.J., Kang, M.J., Yu, H.S., Seo, J.H., Kim, H.Y., et al., 2014. Bacillus Calmette-Guérin suppresses asthmatic responses via CD4(+)CD25(+) regulatory T cells and dendritic cells. Allergy Asthma Immunol. Res. 6 (3), 201–207. http://dx.doi.org/10.4168/aair.2014.6.3.201.

King, P.T., Hutchinson, P.E., Johnson, P.D., Holmes, P.W., Freezer, N.J., Holdsworth, S.R., 2003. Adaptive immunity to nontypeable Haemophilus influenzae. Am. J. Respir. Crit. Care Med. 167 (4), 587–592. http://dx.doi.org/10.1164/rccm.200207-728OC.

Kjaergard, L.L., Larsen, F.O., Norn, S., Clementsen, P., Skov, P.S., Permin, H., 1996. Basophil-bound IgE and serum IgE directed against Haemophilus influenzae and Streptococcus pneumoniae in patients with chronic bronchitis during acute exacerbations. Apmis 104 (1), 61–67.

Koch, S., Sopel, N., Finotto, S., 2017. Th9 and other IL-9-producing cells in allergic asthma. Semin. Immunopathol. 39 (1), 55–68. http://dx.doi.org/10.1007/s00281-016-0601-1.

Koh, Y.Y., Park, Y., Lee, H.J., Kim, C.K., 2001. Levels of interleukin-2, interferon-gamma, and interleukin-4 in bronchoalveolar lavage fluid from patients with Mycoplasma pneumonia: implication of tendency toward increased immunoglobulin E production. Pediatrics 107 (3), E39.

Kowalski, M.L., Cieslak, M., Perez-Novo, C.A., Makowska, J.S., Bachert, C., 2011. Clinical and immunological determinants of severe/refractory asthma (SRA): association with Staphylococcal superantigen-specific IgE antibodies. Allergy 66 (1), 32–38. http://dx.doi.org/10.1111/j.1398-9995.2010.02379.x.

Krismer, B., Weidenmaier, C., Zipperer, A., Peschel, A., 2017. The commensal lifestyle of Staphylococcus aureus and its interactions with the nasal microbiota. Nat. Rev. Microbiol. 15 (11), 675–687. http://dx.doi.org/10.1038/nrmicro.2017.104.

Kuhne, Y., Reese, G., Ballmer-Weber, B.K., Niggemann, B., Hanschmann, K.M., Vieths, S., Holzhauser, T., 2015. A novel multipeptide microarray for the specific and sensitive mapping of linear IgE-Binding epitopes of food allergens. Int. Arch. Allergy Immunol. 166 (3), 213–224. http://dx.doi.org/10.1159/000381344.

Kumar, S., Zheng, H., Mahajan, B., Kozakai, Y., Morin, M., Locke, E., 2014. Western blot assay for quantitative and qualitative antigen detection in vaccine development. Curr. Protoc. Microbiol. 33, 18. http://dx.doi.org/10.1002/9780471729259.mc1804s33. 14.11-11.

Laouini, D., Kawamoto, S., Yalcindag, A., Bryce, P., Mizoguchi, E., Oettgen, H., Geha, R.S., 2003. Epicutaneous sensitization with superantigen induces allergic skin inflammation. J. Allergy Clin. Immunol. 112 (5), 981–987. http://dx.doi.org/10.1016/j.jaci.2003.07.007.

Larsen, F.O., Norn, S., Mordhorst, C.H., Skov, P.S., Milman, N., Clementsen, P., 1998. Chlamydia pneumoniae and possible relationship to asthma. Serum immunoglobulins and histamine release in patients and controls. Apmis 106 (10), 928–934.

Larsen, J.M., Brix, S., Thysen, A.H., Birch, S., Rasmussen, M.A., Bisgaard, H., 2014. Children with asthma by school age display aberrant immune responses to pathogenic airway bacteria as infants. J. Allergy Clin. Immunol. 133 (4), 1008–1013. http://dx.doi.org/10.1016/j.jaci.2014.01.010.

Lee, J.R., Magee, D.M., Gaster, R.S., LaBaer, J., Wang, S.X., 2013. Emerging protein array technologies for proteomics. Expert Rev. Proteom. 10 (1), 65–75. http://dx.doi.org/10.1586/epr.12.67.

Leung, D.Y., Harbeck, R., Bina, P., Reiser, R.F., Yang, E., Norris, D.A., et al., 1993. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. J. Clin. Invest. 92 (3), 1374–1380. http://dx.doi.org/10.1172/jci116711.

Lim, H., Eng, J., Yates 3rd, J.R., Tollaksen, S.L., Giometti, C.S., Holden, J.F., et al., 2003. Identification of 2D-gel proteins: a comparison of MALDI/TOF peptide mass mapping to mu LC-ESI tandem mass spectrometry. J. Am. Soc. Mass Spectrom. 14 (9), 957–970.

Lin, J., Bruni, F.M., Fu, Z., Maloney, J., Bardina, L., Boner, A.L., et al., 2012. A bioinformatics approach to identify patients with symptomatic peanut allergy using peptide microarray immunoassay. J. Allergy Clin. Immunol. 129 (5), 1321–1328. http://dx.doi.org/10.1016/j.jaci.2012.02.012. e1325.

Lin, Y.T., Shau, W.Y., Wang, L.F., Yang, Y.H., Hwang, Y.W., Tsai, M.J., et al., 2000. Comparison of serum specific IgE antibodies to staphylococcal enterotoxins between atopic children with and without atopic dermatitis. Allergy 55 (7), 641–646.

Liotta, L.A., Espina, V., Mehta, A.I., Calvert, V., Rosenblatt, K., Geho, D., et al., 2003. Protein microarrays: meeting analytical challenges for clinical applications. Cancer Cell 3 (4), 317–325.

Liu, J.N., Shin, Y.S., Yoo, H.S., Nam, Y.H., Jin, H.J., Ye, Y.M., et al., 2014. The prevalence of serum specific IgE to superantigens in asthma and allergic rhinitis patients. Allergy Asthma Immunol. Res. 6 (3), 263–266. http://dx.doi.org/10.4168/aair.2014.6.3.263.

Martinez-Botas, J., de la Hoz, B., 2016. IgE and IgG4 epitope mapping of food allergens with a peptide microarray immunoassay. Methods Mol. Biol. 1352, 235–249. http://dx.doi.org/10.1007/978-1-4939-3037-1_18.

Masoli, M., Fabian, D., Holt, S., Beasley, R., Program, G. I. f. A. G, 2004. The global burden of asthma: executive summary of the GINA dissemination committee report. Allergy 59 (5), 469–478. http://dx.doi.org/10.1111/j.1398-9995.2004.00526.x.

McClain, S., 2017. Bioinformatic screening and detection of allergen cross-reactive IgE-binding epitopes. Mol. Nutr. Food Res. 61 (8). http://dx.doi.org/10.1002/mnfr.201600676.

Medina, J.L., Coalson, J.J., Brooks, E.G., Winter, V.T., Chaparro, A., Principe, M.F., et al., 2012. Mycoplasma pneumoniae CARDS toxin induces pulmonary eosinophilic and lymphocytic inflammation. Am. J. Respir. Cell Mol. Biol. 46 (6), 815–822. http://dx.doi.org/10.1165/rcmb.2011-0135OC.

Mjosberg, J., Bernink, J., Golebski, K., Karrich, J.J., Peters, C.P., Blom, B., et al., 2012. The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. Immunity 37 (4), 649–659. http://dx.doi.org/10.1016/j.immuni.2012.08.015.

Morishita, Y., Tada, J., Sato, A., Toi, Y., Kanzaki, H., Akiyama, H., Arata, J., 1999. Possible influences of Staphylococcus aureus on atopic dermatitis– the colonizing features and the effects of staphylococcal enterotoxins. Clin. Exp. Allergy 29 (8), 1110–1117.

Mulcahy, M.E., McLoughlin, R.M., 2016. Host-Bacterial crosstalk determines Staphylococcus aureus nasal colonization. Trends Microbiol. 24 (11), 872–886. http://dx.doi.org/10.1016/j.tim.2016.06.012.

Nagai, K., Domon, H., Maekawa, T., Oda, M., Hiyoshi, T., Tamura, H., et al., 2018. Pneumococcal DNA-binding proteins released through autolysis induce the production of proinflammatory cytokines via toll-like receptor 4. Cell. Immunol. 325, 14–22. http://dx.doi.org/10.1016/j.cellimm.2018.01.006.

Nakamura, Y., Oscherwitz, J., Cease, K.B., Chan, S.M., Munoz-Planillo, R., Hasegawa, M., et al., 2013. Staphylococcus delta-toxin induces allergic skin disease by activating mast cells. Nature 503 (7476), 397–401. http://dx.doi.org/10.1038/nature12655.

Nomura, I., Tanaka, K., Tomita, H., Katsunuma, T., Ohya, Y., Ikeda, N., et al., 1999. Evaluation of the staphylococcal exotoxins and their specific IgE in childhood atopic dermatitis. J. Allergy Clin. Immunol. 104 (2 Pt. 1), 441–446.

Oboki, K., Nakae, S., Matsumoto, K., Saito, H., 2011. IL-33 and airway inflammation. Allergy Asthma Immunol. Res. 3 (2), 81–88. http://dx.doi.org/10.4168/aair.2011.3.2.81.

Ong, P.Y., Ohtake, T., Brandt, C., Strickland, I., Boguniewicz, M., Ganz, T., et al., 2002. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. N. Engl. J. Med. 347 (15), 1151–1160. http://dx.doi.org/10.1056/NEJMoa021481.

Ong, P.Y., Patel, M., Ferdman, R.M., Dunaway, T., Church, J.A., 2008. Association of staphylococcal superantigen-specific immunoglobulin e with mild and moderate atopic dermatitis. J. Pediatr. 153 (6), 803–806. http://dx.doi.org/10.1016/j.jpeds.2008.05.047.

Patel, K.K., Anderson, E., Salva, P.S., Webley, W.C., 2012. The prevalence and identity of Chlamydia-specific IgE in children with asthma and other chronic respiratory symptoms. Respir. Res. 13, 32. http://dx.doi.org/10.1186/1465-9921-13-32.

Pauwels, R., Verschraegen, G., van der Straeten, M., 1980. IgE antibodies to bacteria in patients with bronchial asthma. Allergy 35 (8), 665–669.

Pawankar, R., 2014. Allergic diseases and asthma: a global public health concern and a call to action. World Allergy Organ J. 7 (1), 12. http://dx.doi.org/10.1186/1939-4551-7-12.

Pedersen, M.H., Holzhauser, T., Bisson, C., Conti, A., Jensen, L.B., Skov, P.S., et al., 2008. Soybean allergen detection methods–a comparison study. Mol. Nutr. Food Res. 52 (12), 1486–1496. http://dx.doi.org/10.1002/mnfr.200700394.

Peters, J., Singh, H., Brooks, E.G., Diaz, J., Kannan, T.R., Coalson, J.J., et al., 2011. Persistence of community-acquired respiratory distress syndrome toxin-producing Mycoplasma pneumoniae in refractory asthma. Chest 140 (2), 401–407. http://dx.doi.org/10.1378/chest.11-0221.

Proft, T., Fraser, J.D., 2003. Bacterial superantigens. Clin. Exp. Immunol. 133 (3), 299–306.

Ramsey, C.D., Celedon, J.C., 2005. The hygiene hypothesis and asthma. Curr. Opin. Pulm. Med. 11 (1), 14–20.

Raymond, M., Van, V.Q., Wakahara, K., Rubio, M., Sarfati, M., 2011. Lung dendritic cells induce T(H)17 cells that produce T(H)2 cytokines, express GATA-3, and promote airway inflammation. J. Allergy Clin. Immunol. 128 (1), 192–201. http://dx.doi.org/10.1016/j.jaci.2011.04.029. e196.

Reginald, K., Westritschnig, K., Linhart, B., Focke-Tejkl, M., Jahn-Schmid, B., Eckl-Dorna, J., et al., 2011. Staphylococcus aureus fibronectin-binding protein specifically binds IgE from patients with atopic dermatitis and requires antigen presentation for cellular immune responses. J. Allergy Clin. Immunol. 128 (1), 82–91. http://dx.doi.org/10.1016/j.jaci.2011.02.034. e88.

Ribet, D., Cossart, P., 2015. How bacterial pathogens colonize their hosts and invade deeper tissues. Microbes Infect. 17 (3), 173–183. http://dx.doi.org/10.1016/j.micinf.2015.01.004.

Ritchie, A.J., Whittall, C., Lazenby, J.J., Chhabra, S.R., Pritchard, D.I., Cooley, M.A.,

2007. The immunomodulatory pseudomonas aeruginosa signalling molecule N-(3-oxododecanoyl)-L-homoserine lactone enters mammalian cells in an unregulated fashion. Immunol. Cell Biol. 85 (8), 596–602. http://dx.doi.org/10.1038/sj.icb.7100090.

Ritchie, A.J., Yam, A.O., Tanabe, K.M., Rice, S.A., Cooley, M.A., 2003. Modification of in vivo and in vitro T- and B-cell-mediated immune responses by the Pseudomonas aeruginosa quorum-sensing molecule N-(3-oxododecanoyl)-L-homoserine lactone. Infect. Immun. 71 (8), 4421–4431.

Rojo, A., Aguinaga, A., Monecke, S., Yuste, J.R., Gastaminza, G., Espana, A., 2014. Staphylococcus aureus genomic pattern and atopic dermatitis: may factors other than superantigens be involved? Eur. J. Clin. Microbiol. Infect. Dis. 33 (4), 651–658. http://dx.doi.org/10.1007/s10096-013-2000-z.

Rossi, R.E., Monasterolo, G., 2004. Prevalence of serum IgE antibodies to the Staphylococcus aureus enterotoxins (SAE, SEB, SEC, SED, TSST-1) in patients with persistent allergic rhinitis. Int. Arch. Allergy Immunol. 133 (3), 261–266. http://dx.doi.org/10.1159/000076833.

Rustandi, R.R., Loughney, J.W., Hamm, M., Hamm, C., Lancaster, C., Mach, A., Ha, S., 2012. Qualitative and quantitative evaluation of Simon, a new CE-based automated Western blot system as applied to vaccine development. Electrophoresis 33 (17), 2790–2797. http://dx.doi.org/10.1002/elps.201200095.

Ryu, S., Song, P.I., Seo, C.H., Cheong, H., Park, Y., 2014. Colonization and infection of the skin by S. aureus: immune system evasion and the response to cationic antimicrobial peptides. Int. J. Mol. Sci. 15 (5), 8753–8772. http://dx.doi.org/10.3390/ijms15058753.

Saha, S., Raghava, G.P., 2006. AlgPred: prediction of allergenic proteins and mapping of IgE epitopes. Nucleic Acids Res. 34 (Web Server issue), W202–W209. http://dx.doi.org/10.1093/nar/gkl343.

Schaub, B., Lauener, R., von Mutius, E., 2006. The many faces of the hygiene hypothesis. J. Allergy Clin. Immunol. 117 (5), 969–977. http://dx.doi.org/10.1016/j.jaci.2006.03.003. quiz 978.

Schmidt, F., Meyer, T., Sundaramoorthy, N., Michalik, S., Surmann, K., Depke, M., et al., 2017. Characterization of human and Staphylococcus aureus proteins in respiratory mucosa by in vivo- and immunoproteomics. J. Proteom. 155, 31–39. http://dx.doi.org/10.1016/j.jprot.2017.01.008.

Schmitt, D.A., Cheng, H., Maleki, S.J., Burks, A.W., 2004. Competitive inhibition ELISA for quantification of Ara h 1 and Ara h 2, the major allergens of peanuts. J. AOAC Int. 87 (6), 1492–1497.

Seggev, J.S., Sedmak, G.V., Kurup, V.P., 1996. Isotype-specific antibody responses to acute Mycoplasma pneumoniae infection. Ann. Allergy Asthma Immunol. 77 (1), 67–73. http://dx.doi.org/10.1016/s1081-1206(10)63482-5.

Shibata, N., Kunisawa, J., Hosomi, K., Fujimoto, Y., Mizote, K., Kitayama, N., et al., 2018. Lymphoid tissue-resident Alcaligenes LPS induces IgA production without excessive inflammatory responses via weak TLR4 agonist activity. Mucosal Immunol. http://dx.doi.org/10.1038/mi.2017.103. [Epub ahead of print].

Shirakawa, T., Enomoto, T., Shimazu, S., Hopkin, J.M., 1997. The inverse association between tuberculin responses and atopic disorder. Science 275 (5296), 77–79.

Smith, R.S., Harris, S.G., Phipps, R., Iglewski, B., 2002. The Pseudomonas aeruginosa quorum-sensing molecule N-(3-oxododecanoyl)homoserine lactone contributes to virulence and induces inflammation in vivo. J. Bacteriol. 184 (4), 1132–1139.

Smith-Norowitz, T.A., Chotikanatis, K., Erstein, D.P., Perlman, J., Norowitz, Y.M., Joks, R., et al., 2016. Chlamydia pneumoniae enhances the Th2 profile of stimulated peripheral blood mononuclear cells from asthmatic patients. Hum. Immunol. 77 (5), 382–388. http://dx.doi.org/10.1016/j.humimm.2016.02.010.

Sohn, M.H., Kim, C.H., Kim, W.K., Jang, G.C., Kim, K.E., 2003. Effect of staphylococcal enterotoxin B on specific antibody production in children with atopic dermatitis. Allergy Asthma Proc. 24 (1), 67–71.

Song, L., Wallstrom, G., Yu, X., Hopper, M., Van Duine, J., Steel, J., et al., 2017. Identification of antibody targets for tuberculosis serology using high-density nucleic acid programmable protein arrays. Mol. Cell. Proteom. 16 (4 suppl 1), S277–s289. http://dx.doi.org/10.1074/mcp.M116.065953.

Song, W.J., Jo, E.J., Lee, J.W., Kang, H.R., Cho, S.H., Min, K.U., Chang, Y.S., 2013. Staphylococcal enterotoxin specific IgE and asthma: a systematic review and meta-analysis. Asia Pac. Allergy 3 (2), 120–126. http://dx.doi.org/10.5415/apallergy.2013.3.2.120.

Spaulding, A.R., Salgado-Pabon, W., Kohler, P.L., Horswill, A.R., Leung, D.Y., Schlievert, P.M., 2013. Staphylococcal and streptococcal superantigen exotoxins. Clin. Microbiol. Rev. 26 (3), 422–447. http://dx.doi.org/10.1128/CMR.00104-12.

Stentzel, S., Glaser, R., Broker, B.M., 2016a. Elucidating the anti-Staphylococcus aureus antibody response by immunoproteomics. Proteom. Clin. Appl. 10 (9–10), 1011–1019. http://dx.doi.org/10.1002/prca.201600050.

Stentzel, S., Teufelberger, A., Nordengrun, M., Kolata, J., Schmidt, F., van Crombruggen, K., et al., 2016b. Staphylococcal serine protease-like proteins are pacemakers of allergic airway reactions to Staphylococcus aureus. J. Allergy Clin. Immunol. 139 (2). http://dx.doi.org/10.1016/j.jaci.2016.03.045. 492-500.e8.

Tada, J., Toi, Y., Akiyama, H., Arata, J., Kato, H., 1996. Presence of specific IgE antibodies to staphylococcal enterotoxins in patients with atopic dermatitis. Eur. J. Dermatol. 6 (8), 552–554.

Takahashi, T., Gallo, R.L., 2017. The critical and multifunctional roles of antimicrobial peptides in dermatology. Dermatol. Clin. 35 (1), 39–50. http://dx.doi.org/10.1016/j.det.2016.07.006.

Tang, L.F., Shi, Y.C., Xu, Y.C., Wang, C.F., Yu, Z.S., Chen, Z.M., 2009. The change of asthma-associated immunological parameters in children with Mycoplasma pneumoniae infection. J. Asthma 46 (3), 265–269. http://dx.doi.org/10.1080/02770900802647557.

Taskapan, M.O., Kumar, P., 2000. Role of staphylococcal superantigens in atopic dermatitis: from colonization to inflammation. Ann. Allergy Asthma Immunol. 84 (1), 3–10. http://dx.doi.org/10.1016/s1081-1206(10)62731-7. quiz 11-12.

Tee, R.D., Pepys, J., 1982. Specific serum IgE antibodies to bacterial antigens in allergic lung disease. Clin. Allergy 12 (5), 439–450.

Teufelberger, A.R., Nordengrün, M., Braun, H., Maes, T., De Grove, K., Holtappels, G., et al., 2017. The IL-33/ST2 axis is crucial in type 2 airway responses induced by the Staphylococcus aureus protease SplD. J. Allergy Clin. Immunol. 141 (2). http://dx.doi.org/10.1016/j.jaci.2017.05.004. 549-559.e7.

Telford, G., Wheeler, D., Williams, P., Tomkins, P.T., Appleby, P., Sewell, H., 1998. The Pseudomonas aeruginosa quorum-sensing signal molecule N-(3-oxododecanoyl)-L-homoserine lactone has immunomodulatory activity. Infect. Immun. 66 (1), 36–42.

Thammavongsa, V., Kim, H.K., Missiakas, D., Schneewind, O., 2015. Staphylococcal manipulation of host immune responses. Nat. Rev. Microbiol. 13 (9), 529–543. http://dx.doi.org/10.1038/nrmicro3521.

Tripathi, A., Conley, D.B., Grammer, L.C., Ditto, A.M., Lowery, M.M., Seiberling, K.A., et al., 2004. Immunoglobulin E to staphylococcal and streptococcal toxins in patients with chronic sinusitis/nasal polyposis. Laryngoscope 114 (10), 1822–1826. http://dx.doi.org/10.1097/00005537-200410000-00027.

Umetsu, D.T., McIntire, J.J., Akbari, O., Macaubas, C., DeKruyff, R.H., 2002. Asthma: an epidemic of dysregulated immunity. Nat. Immunol. 3 (8), 715–720. http://dx.doi.org/10.1038/ni0802-715.

Vandepapelière, P., Horsmans, Y., Moris, P., Van Mechelen, M., Janssens, M., Koutsoukos, M., et al., 2008. Vaccine adjuvant systems containing monophosphoryl lipid A and QS21 induce strong and persistent humoral and T cell responses against hepatitis B surface antigen in healthy adult volunteers. Vaccine 26 (10), 1375–1386. http://dx.doi.org/10.1016/j.vaccine.2007.12.038.

Wang, J., Yu, Y., Zhao, Y., Zhang, D., Li, J., 2013a. Evaluation and integration of existing methods for computational prediction of allergens. BMC Bioinform. 14 (Suppl. 4), S1. http://dx.doi.org/10.1186/1471-2105-14-S4-S1.

Wang, J., Zhang, D., Li, J., 2013b. PREAL: prediction of allergenic protein by maximum relevance minimum redundancy (mRMR) feature selection. BMC Syst. Biol 7 (Suppl. 5), S9. http://dx.doi.org/10.1186/1752-0509-7-s5-s9.

Wark, P.A., Johnston, S.L., Simpson, J.L., Hensley, M.J., Gibson, P.G., 2002. Chlamydia pneumoniae immunoglobulin A reactivation and airway inflammation in acute asthma. Eur. Respir. J. 20 (4), 834–840.

Webley, W.C., Tilahun, Y., Lay, K., Patel, K., Stuart, E.S., Andrzejewski, C., Salva, P.S., 2009. Occurrence of Chlamydia trachomatis and Chlamydia pneumoniae in paediatric respiratory infections. Eur. Respir. J. 33 (2), 360–367. http://dx.doi.org/10.1183/09031936.00019508.

Weidenmaier, C., Goerke, C., Wolz, C., 2012. Staphylococcus aureus determinants for nasal colonization. Trends Microbiol. 20 (5), 243–250. http://dx.doi.org/10.1016/j.tim.2012.03.004.

Welliver, R.C., Duffy, L., 1993. The relationship of RSV-specific immunoglobulin E antibody responses in infancy, recurrent wheezing, and pulmonary function at age 7-8 years. Pediatr. Pulmonol. 15 (1), 19–27.

West, C.E., Dzidic, M., Prescott, S.L., Jenmalm, M.C., 2017. Bugging allergy; role of pre-, pro- and synbiotics in allergy prevention. Allergol. Int. 66 (4), 529–538. http://dx.doi.org/10.1016/j.alit.2017.08.001.

Wills-Karp, M., Rani, R., Dienger, K., Lewkowich, I., Fox, J.G., Perkins, C., et al., 2012. Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. J. Exp. Med. 209 (3), 607–622. http://dx.doi.org/10.1084/jem.20110079.

Yano, T., Ichikawa, Y., Komatu, S., Arai, S., Oizumi, K., 1994. Association of Mycoplasma pneumoniae antigen with initial onset of bronchial asthma. Am. J. Respir. Crit. Care Med. 149 (5), 1348–1353. http://dx.doi.org/10.1164/ajrccm.149.5.8173777.

Ye, Q., Xu, X.J., Shao, W.X., Pan, Y.X., Chen, X.J., 2014. Mycoplasma pneumoniae infection in children is a risk factor for developing allergic diseases. Sci. World J. 2014, 986527. http://dx.doi.org/10.1155/2014/986527.

Ye, Y.M., Hur, G.Y., Park, H.J., Kim, S.H., Kim, H.M., Park, H.S., 2008. Association of specific IgE to staphylococcal superantigens with the phenotype of chronic urticaria. J. Korean Med. Sci. 23 (5), 845–851. http://dx.doi.org/10.3346/jkms.2008.23.5.845.

Yeh, J.J., Wang, Y.C., Hsu, W.H., Kao, C.H., 2016. Incident asthma and Mycoplasma pneumoniae: a nationwide cohort study. J. Allergy Clin. Immunol. 137 (4), 1017–1023. http://dx.doi.org/10.1016/j.jaci.2015.09.032. e1011-1016.

Yoshida, M., Leigh, R., Matsumoto, K., Wattie, J., Ellis, R., O'Byrne, P.M., Inman, M.D., 2002. Effect of interferon-gamma on allergic airway responses in interferon-gamma-deficient mice. Am. J. Respir. Crit. Care Med. 166 (4), 451–456. http://dx.doi.org/10.1164/rccm.200202-095OC.

Zhang, N., Holtappels, G., Gevaert, P., Patou, J., Dhaliwal, B., Gould, H., Bachert, C., 2011. Mucosal tissue polyclonal IgE is functional in response to allergen and SEB. Allergy 66 (1), 141–148. http://dx.doi.org/10.1111/j.1398-9995.2010.02448.x.

Zhao, X., Li, L., Kuang, Z., Luo, G., Li, B., 2015. Proteomic and immunological identification of two new allergens from silkworm (Bombyx mori L.) pupae. Cent. Eur. J. Immunol. 40 (1), 30–34. http://dx.doi.org/10.5114/ceji.2015.50830.

Zhuang, Y., Dreskin, S.C., 2013. Redefining the major peanut allergens. Immunol. Res. 55 (1-3), 125–134. http://dx.doi.org/10.1007/s12026-012-8355-x.

6 SUMMARY AND DISCUSSION

Staphylococcus aureus is a versatile bacterium. It produces a variety of proteins that interact with the human immune system: they activate it, inhibit or modulate it potently, thus creating favorable conditions for bacterial invasion and colonization. The secretome of *S. aureus* comprises about 1350 proteins. For many of them, the exact function has not yet been elucidated.[56] However, a more detailed understanding of the interaction of *S. aureus* with our immune system is essential to tackle mechanisms of immune modulation.

Also, for Spls the physiological function is still unknown and physiological substrates have not yet been identified. *In silico* analyses identified the consensus sequences of the enzymatic cleavage sites, and Zdzalik and colleagues found these motifs in numerous human proteins, including proteins of the olfactory receptor family, which might be relevant in the colonization of *S. aureus*.[53] The wide distribution of the Spl genes in the species *S. aureus* suggests that they might play a relevant role in *S. aureus* physiology.[50]

Although the question of physiological Spl substrates is still open, an immune modulating role of the Spls has already been demonstrated: increased levels of Spl-specific IgE were found in asthma patients. SplD induced *de novo* Th2 responses in the mouse model after repeated intratracheal application, resulting in the production of Spl-specific IgE. This response was observed without the use of adjuvants.[48]

These findings shed light on the pathophysiological role of *S. aureus* in allergic reactions and raise questions about the causes of the type 2 bias of the anti-Spl immune response as well as about its possible clinical consequences: How is the type 2 reaction initiated? Which

molecular mechanisms underlie this reaction? Do Spls have an adjuvant function? Is their proteolytic activity crucial for this? And can they also shift the immune response towards Th2 in patients that are frequently colonized with *S. aureus* and suffer from recurrent infection? In these patients, polarization of the immune response by *S. aureus* could influence disease progression, prognosis and therapeutic options.

S. AUREUS INDUCES ALLERGIC AIRWAY INFLAMMATION VIA THE IL-33/ST2-AXIS

The pulmonary epithelium is the first line of defense of the human lung. It forms a barrier between the immune system and the external environment and plays a crucial role in promoting mucosal inflammatory responses. Damage of the epithelial cells causes the release of IL-33, IL-25 and thymic stromal lymphopoietin (TSLP).[57] A link of IL-33 and its receptor ST2 to asthma has been identified in genome-wide association studies.[58,59] In addition, IL-33 expression correlates with asthma severity. The release of IL-33 triggers a cascade of pro-allergic mechanisms: It is crucial for Th2 cell mobilization, attracting Th2 cells and enhancing the production of the Th2 cytokines IL-5 and IL-13.[60] In addition, IL-33 is able to activate basophils, mast cells, macrophages, ILC2s and eosinophils, the principal effector cells in allergic inflammation.[61] The main mechanisms involved in allergic airway inflammation that are induced or modulated by IL-33 are depicted in Figure 2.

In our murine asthma model C57BL/6 J wild-type mice were repeatedly exposed to SplD via intratracheal application. After two weeks a Th2-biased inflammatory response was observed in the airways: IL-33 and eotaxin production, eosinophilia, bronchial hyperreactivity, and goblet cell hyperplasia. Blocking IL-33 activity with a soluble ST2 receptor counteracted these effects: significantly decreased numbers of eosinophils, IL-13+ type 2 ILCs and IL-13+CD4+ T cells and IL-5 and IL-13 production by lymph node cells were observed.

These findings lead to the question of whether SplD directly triggers the release of IL-33 by degrading the pulmonary epithelial barrier. This ability has been described for numerous allergens: proteases from pollen diffusates contain proteases with serine and/or aminopeptidase activity that cleave the transmembrane adhesion proteins occludin, claudin-1 and E-cadherin. Der p1, the major allergen from house dust mites, disrupts the epithelial barrier by cleaving the tight junction proteins; and phospholipase A2, the major allergen in bee venom,

was shown to induce a type 2 response through the enzymatic cleavage of membrane phospholipids and release of IL-33.[62–64] However, TUNEL staining of lung sections in SplD-treated mice showed that IL-33 upregulation was not accompanied by cell death. This indicates that IL-33 may not be passively released by dying cells but actively secreted by the airway epithelium.

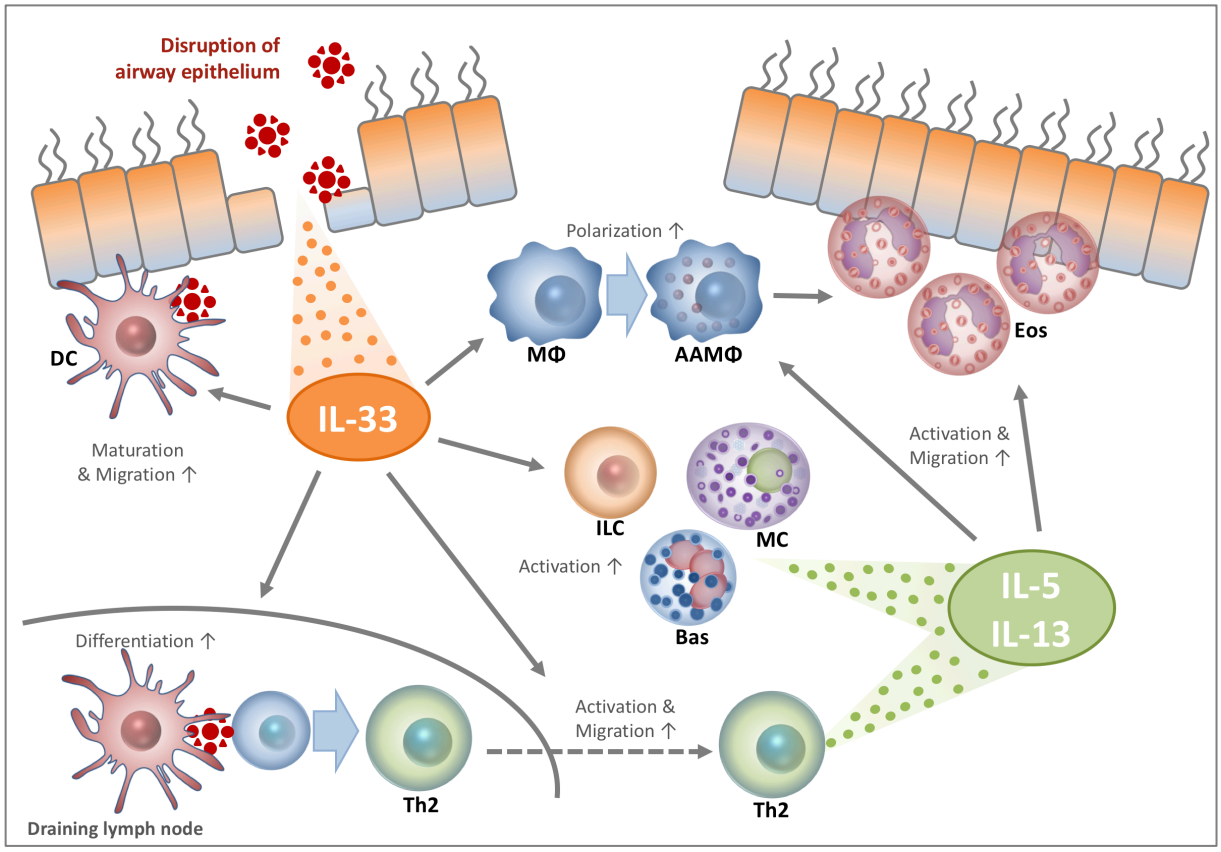


FIGURE 2: IL-33-INDUCED MECHANISMS OF ALLERGIC AIRWAY INFLAMMATION.

When IL-33 is released into the extracellular space of the lung, e.g. by proteolytic degradation of the epithelial barrier, it acts on numerous effector cells: dendritic cells are stimulated to migrate and mature and induce increased differentiation of Th2 cells in the draining lymphnodes. These cells migrate to the destroyed epithelial barrier where they produce IL-5- and IL-13, the key mediators of type 2 inflammation. In ILCs, mast cells and basophils, IL-33 also enhances the release of IL-5 and IL-13. Macrophages increasingly polarize to alternatively activated macrophages, which have reduced bactericidal functions. Finally, the released cytokines attract eosinophils to the site of action. The result is a self-amplifying non-protective Th2-type, which is partly responsible for the cardinal symptoms of allergic respiratory reactions. Abbreviations: **AAMΦ**: alternatively activated macrophage; **Bas**: basophil; **DC**: dendritic cell; **Eos**: eosinophil; **ILC**: innate lymphoid cell; **MΦ**: macrophage; **MC**: mast cell

Identification of the physiological substrates of the Spls could help to shed light on the molecular mechanism of allergy induction in the lungs. So far Spls are known to have distinctive and very selective preferences for cleavage motifs, indicating a narrow substrate range.

DENDRITIC CELLS COULD SHAPE THE MUCOSAL IMMUNE RESPONSE TOWARD TYPE 2

Dendritic cells are known to be the primary sensors of antigens and infectious agents. As professional antigen-presenting cells they are uniquely able to induce naïve T cell activation and effector differentiation and thus initiate appropriate adaptive immune responses. DCs are lining the epithelial layer where they "fish" antigens from the airway lumen and can thus recognize inhaled allergens. As soon as they have captured antigen the airway DCs migrate to the draining mediastinal lymph nodes, where they display allergen peptides to naïve CD4+ cells. Numerous factors like the type of antigen captured, the presence of microbial molecular patterns or alarmins or the genetic background of the host then determine the outcome of T cell polarization.[65–68] For the inhaled particles the usual outcome is tolerance, since they are immunologically inert. DCs will only initiate a T helper response if there is some sort of adjuvant activity present at the time of exposure to the allergen. This activity could be the presence of pathogen associated molecular patterns (PAMPs), damage associated molecular patterns (DAMPs), cytokines released upon cell activation, necrosis or oxidative stress. Proteolytic activity of an allergen itself can also provide an adjuvant signal: HDM, cockroach and many other allergens have proteolytic enzymes that can directly activate DCs or epithelial cells, and in this way promote Th2 sensitization.[69,70]

DCs perceive PAMPs with pattern-recognition receptors (PRRs), including the TLRs. TLR-activated DCs will be strongly activated, upregulate costimulatory molecules and produce pro-inflammatory cytokines (TNFα, IL-1, IL-6, and IL-12). In allergic setting the presence of TLR4 is required to induce allergic pulmonary Th2 responses to HDM.[71–73] Even antigens without any intrinsic activating properties, like the experimental allergen OVA, induce allergy via TLR4. This might sound surprising but is easily explained by the fact that most commercially available batches of OVA used in murine models of asthma were contaminated with LPS.[72] In contrast, the observed effects of SplD on allergy parameters appeared to be independent of TLR4. Other receptors such as PAR2 might play a role. PAR2 activation in the

lungs can trigger T_H2-biased inflammatory responses to an inert protein antigen in mice. In SplD-treated mice, an increase in $PAR2^+$ cell counts in lung sections was observed. However, this high amount of PAR2 in SplD-treated mice can be explained by an increase of infiltrating cells bearing the PAR2 receptor, rather than an upregulation of PAR2 expression. A direct protease-dependent activation of PAR2 by SplD did not occur.

Even in the absence of pathogens, DCs can react to molecules released by injured or necrotic cells that alert the body defense systems and are therefore also called 'alarmins', 'endogenous danger signals' or DAMPs. DAMPs contribute to inflammation by recruitment of innate inflammatory cells, and they interact with PRRs that are shared with the exogenous danger signals.[74] According to the current state of knowledge a tight collaboration between PAMPs and DAMPs is needed to initiate an immune response to allergens.[75,76]

We have demonstrated that SplD treatment attracts DCs to the lung, as well as to the local lymph nodes, where they initiated Th2 cell differentiation. In addition, SplD induced migration of DCs to the local lymph nodes and the DC activation was IL-33-dependent. It requires further investigation to determine if Spls have intrinsic adjuvanticity and activate DCs directly or if they stimulate epithelial cells and thereby indirectly act on DCs. This goes hand in hand with the question of whether the enzymatic activity of Spls is important for their allergenic properties.

CYSTIC FIBROSIS PATIENTS SHOW ALLERGIC REACTIONS TO SPLS

CF patients have an increased bacterial lung burden and susceptibility to infections, with *S. aureus* being one of the most frequent causes. Up to 80% of CF patients are persistently colonized with *S. aureus*. Recurrent bacterial infections worsen the lung function and clinical condition of CF patients. Prevention and therapy of chronic bacterial inflammation are therefore key in the treatment regimen of CF.[77–80]

Besides bacterial infections, allergic immune responses play a crucial role in disease progression of CF. Allergic bronchopulmonary aspergillosis (ABPA) frequently provokes allergic complications and is generally associated with a poor clinical condition.[81,82] A high percentage of CF patients shows immediate hypersensitivity to various inhalant allergens, and drug hypersensitivity reactions are common in CF, which further complicates adequate anti-bacterial therapy.[83,84]

S. aureus Spls were shown to elicit a type 2-biased immune response in healthy individuals and asthma patients. The question whether CF patients – many of whom are persistently exposed to *S. aureus* in their airways – also react with type 2 inflammation to the Spls was investigated in Chapter 4. The results demonstrate that CF patients show a Th2-driven immune response toward *S. aureus* Spls, including high Spl-specific serum IgE levels, strong induction of Th2 cell differentiation and production of type 2 cytokines following *ex vivo* stimulation with recombinant Spls. These effects were significantly more pronounced in CF than in control persons. The observed response seems to be specific for Spls rather than being a general feature of *S. aureus* proteases since other putative allergens of *S. aureus* (ScpA, SspB) did not show increased IgE binding in CF sera.

These findings raised the question of whether this allergic sensitization to bacterial antigens is of clinical relevance. A type 2-biased host immune response could impair the clearance of bacterial infection and facilitate bacterial survival, complicating anti-bacterial therapy. Allergic sensitization towards *S. aureus* antigens might aggravate the clearance of *S. aureus* in the lung of CF patients, leading to more frequent infections and exacerbations of disease symptoms. However, in our study, anti-Spl IgE levels did not correlate with lung exacerbations during the study period nor with lung function. Probably the CF cohort was too small and too heterogenous to show the influence of a single factor on these clinical parameters.

In conclusion, allergic sensitization towards *S. aureus* may limit bacterial clearance and increase the risk of lung infections in some CF patients. CF therapy using steroids that counteract inflammation is known to be beneficial, but also hinders anti-microbial clearance. However, there are therapeutic agents that selectively interfere with type 2 inflammation such as the anti-IgE monoclonal antibody Omalizumab. In ABPA, an allergic reaction to *Aspergillus fumigatus*, case reports showed efficacy of anti-IgE therapy (using Omalizumab) in CF patients. But randomized controlled studies have not been performed to date.[85] A similar approach should be tested in patients with strong type 2 response to *S. aureus* antigens as it could broaden the therapeutic portfolio. Additional studies are also warranted to find out if CF patients develop allergic reactions to other colonizing or infecting bacteria besides *S. aureus*. The quest for bacterial allergens has just begun.

MORE RESEARCH IS NEEDED TO CLARIFY THE ROLE OF BACTERIA IN ALLERGIES

Allergens of *S. aureus* can play an important role in both allergic and infectious diseases. Sensitization to staphylococcal allergens could aggravate the allergic inflammation in affected patients upon re-encounter with *S. aureus* and at the same time impede an effective anti-bacterial immune response. It is, therefore, of great clinical relevance to identify bacterial allergens and to study the underlying pathogenetic mechanisms. Chapter 5 explains the necessary steps to identify bacterial allergens:

1. Unbiased screening methods
   to discover new allergen candidates
2. Targeted approaches
   to investigate the immune response and to determine their pathophysiological importance
3. Cohort studies
   to elucidate the clinical relevance of allergen candidates and assess individuals affected by allergic reactions

The findings on Spls are an example for the successful execution of this three-step process. They were identified as allergen candidates in an unbiased screening approach: 2D immunoblots of antibody binding to the *S. aureus* exoproteome revealed the Spls as dominant IgG4-binding proteins. IgG4 can be used as a surrogate marker for IgE production to overcome the obstacle of very low serum concentrations of free IgE. Once identified, the Spls were recombinantly produced and used in targeted approaches: the Spl-specific immune response was assessed on a humoral and cellular level in in healthy individuals and revealed a shift towards type 2. A mouse model of allergic respiratory inflammation was well suited to demonstrate the Spls' ability to induce allergies *de novo*. Finally, cohort studies showed that asthmatics and CF patients are affected by the Th2-shift of the Spl-specific immune response.[48]

It appears likely that *S. aureus* Spls are not a unique case. However, to date, only a few bacterial allergens are molecularly defined, and few have been studied as thoroughly as the Spls. With the help of the methods and workflow described in Chapter 5 we are well prepared to discover more bacterial allergens in the future and to expand our knowledge in this rather new

field of infection immunology. In view of the numerous pathological conditions in which we are dealing with chronic exposure to bacteria, oscillating between colonization and infection, the modulating effect of bacterial allergens could be of pathogenic and therapeutic relevance.

BACTERIA CAN PROMOTE ALLERGY – BUT WHY?

We now know about a new quality in the immune response to *S. aureus*: The bacteria not only induce pro-inflammatory Th1 responses but are also able to modulate the human immune response and induce allergic reactions with Th2 dominance and production of IgE. But what effects does a Th2-dominated immune response have on *S. aureus* as a pathogen?

Numerous studies have demonstrated that Th2 dominance is not sufficient to protect against bacterial infection: it rather aggravates host symptoms and promotes the survival of the pathogen. One reason for this may be the different effector functions of Th1 and Th2 responses: Th2-dominated responses inhibit Th1-related microbicidal effector functions. Thus, for example, macrophages are inhibited, and the production of antimicrobial peptides is reduced.[86,87] For *S. aureus* it has also been shown that a Th2 cytokine profile enhances Hla-mediated cell death of keratinocytes.[88] A Th2 modulation of the immune response away from a protective Th1/Th17 response therefore creates more favorable conditions for bacteria during colonization or infection of the human host and represents an effective immune escape mechanism.

Starkl and colleagues however, made an opposite observation in a model of *S. aureus* skin infection: a type 2 response, particularly IgE antibodies and mast cells, increased antibacterial activity and protected against secondary lung and skin infection.[89] These results support the so-called "toxin hypothesis", which states that allergic reactions are an important, evolutionary conserved defense mechanism of the host against harmful substances such as toxins.[64,90] The authors refer to human studies showing that healthy individuals can have detectable levels of IgE specific for toxins, e.g., from Hymenoptera and *S. aureus*, in the absence of diagnosed atopic disease.[43,91–93] This may indicate that, at least in some individuals, toxin-specific IgE antibodies are not harmful but beneficial.

However, if we look at the evidence on *S. aureus* toxin-specific IgE in nasal polyposis and asthma, the correlation points to the opposite direction: the level of SAg-specific IgE correlates with the severity of the disease.[42,43] Even more convincingly, IgE does not confer protection in patients with hyperimmunoglobulin E syndrome (HIES): HIES patients show

elevated *S. aureus*-specific IgE serum levels but are highly susceptible to *S. aureus* infections.[94,95] The question of whether allergic immune reactions against bacterial antigens enhance or impair bacterial clearance, must therefore be considered and interpreted in the respective disease context.

These different disease patterns illustrate that immune polarization by bacterial factors can have pathophysiological consequences for the human host. Cleavage of substrates in airway cells, such as the shredding of mucin 16 by SplA, can facilitate the ability of other exogenous proteases and allergens to act on host cells, disrupt immune tolerance in the airways and predispose the organism to allergic sensitization. *S. aureus* enterotoxin sensitization has already been shown to be associated with allergic polysensitization in adolescents.[96] It is evident that allergies to bacteria that colonize the airways are refractory to established anti-inflammatory therapy, whether in asthma or CF. An intrinsic adjuvanticity of bacterial allergens could even affect the outcome of a future vaccination in these patients, especially, if non-adjuvanted bacterial factors are used. In active immunization against bacterial pathogens, the aim is to induce a Th1 response. Vaccines against Helicobacter pylori, which primarily generated Th2 responses, did not provide the desired protection.[97]

Future research will provide more insights into how to modulate either *S. aureus* and its proteases or the host immune response in order to develop novel therapeutics to treat chronic type 2 diseases and prevent pathologic colonization. Our knowledge of bacterial allergens is limited, and our understanding of the pathophysiological consequences and the potential impact on prevention and therapy of bacterial infections is still in its infancy. We paved the way for the quest for bacterial allergens, adding a new dimension to our understanding of host-pathogen interactions as well as to diagnostic and treatment options. Now it is up to us researchers to follow this path.

REFERENCES

1. Hippocrates (c. 460 – c. 370 BC). *Hippocratic Corpus, On The Sacred Disease.*

2. Gundolf Keil. Corpus hippocraticum. in *Enzyklopädie Medizingeschichte* vol. 1 (De Gruyter, 2011).

3. Last, J. M. L. M. miasma theory. in *A Dictionary of Public Health* (ed. Last, J. M.) (Oxford University Press, 2007).

4. Antony van Leeuwenhoek and his "Little Animals": being some Account of the Father of Protozoology and Bacteriology and his Multifarious Discoveries in these Disciplines. *Nature* **130**, 679–680 (1932).

5. Koch, R. Die Ätiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des Bacillus Anthracis (1876). in *Robert Koch : Zentrale Texte* (ed. Gradmann, C.) 19–43 (Springer, 2018).

6. Semmelweis, I. P. *Die Aetiologie, der Begriff und die Prophylaxis des Kindbettfiebers.* (Hartleben, 1861).

7. Riedel, S. Edward Jenner and the History of Smallpox and Vaccination. *Bayl. Univ. Med. Cent. Proc.* **18**, 21–25 (2005).

8. Domagk, G. Chemotherapie der bakteriellen Infektionen. *Angew. Chem.* **48**, 657–667 (1935).

9. Fleming, A. On a Remarkable Bacteriolytic Element Found in Tissues and Secretions. *Proc. R. Soc. Lond. Ser. B Contain. Pap. Biol. Character* **93**, 306–317 (1922).

10. Henderson, D. A. The eradication of smallpox – An overview of the past, present, and future. *Vaccine* **29**, D7–D9 (2011).

11. Fenner F, Henderson DA, Arita I, et al. (eds). Smallpox and its eradication. Geneva: World Health Organization, 1988.

12. United Nations, Department of Economic and Social Affairs, Population Division. Under-five mortality (both sexes combined) by region, subregion and country, 1950-2100 (deaths under age five per 1,000 live births). *World Popul. Prospects 2019* **Online Edition**, (2019).

13. Strachan, D. P. Hay fever, hygiene, and household size. *BMJ* **299**, 1259–1260 (1989).

14. Krämer, U., Heinrich, J., Wjst, M. & Wichmann, H. E. Age of entry to day nursery and allergy in later childhood. *Lancet Lond. Engl.* **353**, 450–454 (1999).

15. Ball, T. M. *et al.* Siblings, Day-Care Attendance, and the Risk of Asthma and Wheezing during Childhood. *N. Engl. J. Med.* **343**, 538–543 (2000).

16. Romagnani, S. Lymphokine production by human T cells in disease states. *Annu. Rev. Immunol.* **12**, 227–257 (1994).

17. Romagnani, S. The role of lymphocytes in allergic disease. *J. Allergy Clin. Immunol.* **105**, 399–408 (2000).

18. Yazdanbakhsh, M., Kremsner, P. G. & Ree, R. van. Allergy, Parasites, and the Hygiene Hypothesis. *Science* **296**, 490–494 (2002).

19. Bach, J.-F. The Effect of Infections on Susceptibility to Autoimmune and Allergic Diseases. *N. Engl. J. Med.* **347**, 911–920 (2002).

20. Spits, H. *et al.* Innate lymphoid cells — a proposal for uniform nomenclature. *Nat. Rev. Immunol.* **13**, 145–149 (2013).

21. Finkelman, F. D. *et al.* Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunol. Rev.* **201**, 139–155 (2004).

22. Kotsiou, O. S., Gourgoulianis, K. I. & Zarogiannis, S. G. IL-33/ST2 Axis in Organ Fibrosis. *Front. Immunol.* **9**, (2018).

23. Annunziato, F., Cosmi, L., Liotta, F., Maggi, E. & Romagnani, S. Defining the human T helper 17 cell phenotype. *Trends Immunol.* **33**, 505–512 (2012).

24. Schaub, B., Lauener, R. & Mutius, E. von. The many faces of the hygiene hypothesis. *J. Allergy Clin. Immunol.* **117**, 969–977 (2006).

25. Hilty, M. *et al.* Disordered Microbial Communities in Asthmatic Airways. *PLOS ONE* **5**, e8578 (2010).

26. Edwards, M. R., Bartlett, N. W., Hussell, T., Openshaw, P. & Johnston, S. L. The microbiology of asthma. *Nat. Rev. Microbiol.* **10**, 459–471 (2012).

27. Atkinson, T. P. Is Asthma an Infectious Disease? New Evidence. *Curr. Allergy Asthma Rep.* **13**, 702–709 (2013).

28. Ipci, K., Altıntoprak, N., Muluk, N. B., Senturk, M. & Cingi, C. The possible mechanisms of the human microbiome in allergic diseases. *Eur. Arch. Otorhinolaryngol.* **274**, 617–626 (2017).

29. Heinrich, J. *et al.* Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *Eur. Respir. J.* **20**, 617–623 (2002).

30. Douwes, J. *et al.* Does early indoor microbial exposure reduce the risk of asthma? The Prevention and Incidence of Asthma and Mite Allergy birth cohort study. *J. Allergy Clin. Immunol.* **117**, 1067–1073 (2006).

31. Gehring, U., Bischof, W., Fahlbusch, B., Wichmann, H.-E. & Heinrich, J. House Dust Endotoxin and Allergic Sensitization in Children. *Am. J. Respir. Crit. Care Med.* **166**, 939–944 (2002).

32. Braun-Fahrländer, C. & Lauener, R. Farming and protective agents against allergy and asthma. *Clin. Exp. Allergy* **33**, 409–411 (2003).

33. von Mutius, E. & Vercelli, D. Farm living: effects on childhood asthma and allergy. *Nat. Rev. Immunol.* **10**, 861–868 (2010).

34. Schuijs, M. J. *et al.* Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. *Science* **349**, 1106–1110 (2015).

35. Li, X. *et al.* Genome-wide association studies of asthma indicate opposite immunopathogenesis direction from autoimmune diseases. *J. Allergy Clin. Immunol.* **130**, 861-868.e7 (2012).

36. Kluytmans, J., Belkum, A. V. & Verbrugh, H. Nasal Carriage of Staphylococcus aureus: Epidemiology, Underlying Mechanisms, and Associated Risks. *CLIN MICROBIOL REV* **10**, 16 (1997).

37. Lowy, F. D. Staphylococcus aureus Infections. *N. Engl. J. Med.* **339**, 520–532 (1998).

38. Chambers, H. The Changing Epidemiology of Staphylococcus aureus? *Emerg. Infect. Dis.* **7**, 178–182 (2001).

39. Leung, D. Y. M. New Insights into Atopic Dermatitis: Role of Skin Barrier and Immune Dysregulation. *Allergol. Int.* **62**, 151–161 (2013).

40. Leung, D. Y. *et al.* Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *J. Clin. Invest.* **92**, 1374–1380 (1993).

41. Laouini, D. *et al.* Epicutaneous sensitization with superantigen induces allergic skin inflammation. *J. Allergy Clin. Immunol.* **112**, 981–987 (2003).

42. Bachert, C. *et al.* IgE to Staphylococcus aureus enterotoxins in serum is related to severity of asthma. *J. Allergy Clin. Immunol.* **111**, 1131–1132 (2003).

43. Van Zele, T. *et al.* Staphylococcus aureus colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. *J. Allergy Clin. Immunol.* **114**, 981–983 (2004).

44. Zhang, N. *et al.* An update on the impact of Staphylococcus aureus enterotoxins in chronic sinusitis with nasal polyposis. *Rhinology* **43**, 162–168 (2005).

45. Bachert, C., Gevaert, P., Zhang, N., van Zele, T. & Perez-Novo, C. Role of staphylococcal superantigens in airway disease. *Chem. Immunol. Allergy* **93**, 214–236 (2007).

46. Bachert, C. *et al.* Specific IgE against Staphylococcus aureus enterotoxins: An independent risk factor for asthma. *J. Allergy Clin. Immunol.* **130**, 376-381.e8 (2012).

47. Gevaert, P. *et al.* Omalizumab is effective in allergic and nonallergic patients with nasal polyps and asthma. *J. Allergy Clin. Immunol.* **131**, 110-116.e1 (2013).

48. Stentzel, S. *et al.* Staphylococcal serine protease–like proteins are pacemakers of allergic airway reactions to Staphylococcus aureus. *J. Allergy Clin. Immunol.* **139**, 492-500.e8 (2017).

49. Reed, S. B. *et al.* Molecular Characterization of a Novel Staphylococcus aureus Serine Protease Operon. *INFECT IMMUN* **69**, 7 (2001).

50. Zdzalik, M. *et al.* Prevalence of genes encoding extracellular proteases in Staphylococcus aureus - important targets triggering immune response in vivo. *FEMS Immunol. Med. Microbiol.* **66**, 220–229 (2012).

51. Stec-Niemczyk, J. *et al.* Structural and functional characterization of SplA, an exclusively specific protease of Staphylococcus aureus. *Biochem. J.* **419**, 555–564 (2009).

52. Popowicz, G. M. *et al.* Functional and structural characterization of Spl proteases from Staphylococcus aureus. *J. Mol. Biol.* **358**, 270–279 (2006).

53. Zdzalik, M. *et al.* Biochemical and structural characterization of SplD protease from Staphylococcus aureus. *PloS One* **8**, e76812 (2013).

54. Stach, N. *et al.* Unique Substrate Specificity of SplE Serine Protease from Staphylococcus aureus. *Structure* **26**, 572-579.e4 (2018).

55. Paharik, A. E. *et al.* The Spl Serine Proteases Modulate Staphylococcus aureus Protein Production and Virulence in a Rabbit Model of Pneumonia. *mSphere* **1**, (2016).

56. Kusch, H. & Engelmann, S. Secrets of the secretome in Staphylococcus aureus. *Int. J. Med. Microbiol. IJMM* **304**, 133–141 (2014).

57. Haraldsen, G., Balogh, J., Pollheimer, J., Sponheim, J. & Küchler, A. M. Interleukin-33 - cytokine of dual function or novel alarmin? *Trends Immunol.* **30**, 227–233 (2009).

58. Moussion, C., Ortega, N. & 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 (2008).

59. Moffatt, M. F. *et al.* A large-scale, consortium-based genomewide association study of asthma. *N. Engl. J. Med.* **363**, 1211–1221 (2010).

60. Smith, D. E. IL-33: a tissue derived cytokine pathway involved in allergic inflammation and asthma. *Clin. Exp. Allergy* **40**, 200–208 (2010).

61. Chan, B. C. L., Lam, C. W. K., Tam, L.-S. & Wong, C. K. IL33: Roles in Allergic Inflammation and Therapeutic Perspectives. *Front. Immunol.* **10**, (2019).

62. Gaspar, R. *et al.* Pollen Proteases Play Multiple Roles in Allergic Disorders. *Int. J. Mol. Sci.* **21**, (2020).

63. Wan, H. *et al.* Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J. Clin. Invest.* **104**, 123–133 (1999).

64. Palm, N. W. *et al.* Bee venom phospholipase A2 induces a primary type 2 response that is dependent on the receptor ST2 and confers protective immunity. *Immunity* **39**, (2013).

65. Kaiko, G. E., Horvat, J. C., Beagley, K. W. & Hansbro, P. M. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? *Immunology* **123**, 326–338 (2008).

66. Tzortzaki, E. G. & Siafakas, N. M. A hypothesis for the initiation of COPD. *Eur. Respir. J.* **34**, 310–315 (2009).

67. Hammad, H. & Lambrecht, B. N. Recent progress in the biology of airway dendritic cells and implications for understanding the regulation of asthmatic inflammation. *J. Allergy Clin. Immunol.* **118**, 331–336 (2006).

68. Shinagawa, K. & Kojima, M. Mouse model of airway remodeling: strain differences. *Am. J. Respir. Crit. Care Med.* **168**, 959–967 (2003).

69. Hammad, H. *et al.* Th2 polarization by Der p 1–pulsed monocyte-derived dendritic cells is due to the allergic status of the donors. *Blood* **98**, 1135–1141 (2001).

70. Jeong, S. K. *et al.* Mite and Cockroach Allergens Activate Protease-Activated Receptor 2 and Delay Epidermal Permeability Barrier Recovery. *J. Invest. Dermatol.* **128**, 1930–1939 (2008).

71. Kadowaki, N. *et al.* Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J. Exp. Med.* **194**, 863–869 (2001).

72. Eisenbarth, S. C. *et al.* Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J. Exp. Med.* **196**, 1645–1651 (2002).

73. Hammad, H. *et al.* House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat. Med.* **15**, 410–416 (2009).

74. Meylan, E., Tschopp, J. & Karin, M. Intracellular pattern recognition receptors in the host response. *Nature* **442**, 39–44 (2006).

75. Di Virgilio, F. Liaisons dangereuses: P2X(7) and the inflammasome. *Trends Pharmacol. Sci.* **28**, 465–472 (2007).

76. Yanai, H. *et al.* HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. *Nature* **462**, 99–103 (2009).

77. Cystic Fibrosis Foundation. Patient Registry 2018 Annual Data Report. 92 (2018).

78. Hatziagorou, E. *et al.* Changing epidemiology of the respiratory bacteriology of patients with cystic fibrosis–data from the European cystic fibrosis society patient registry. *J. Cyst. Fibros.* **19**, 376–383 (2020).

79. Stanford, G. E., Dave, K. & Simmonds, N. J. Pulmonary Exacerbations in Adults With Cystic Fibrosis: A Grown-up Issue in a Changing Cystic Fibrosis Landscape. *Chest* **159**, 93–102 (2021).

80. Epps, Q. J., Epps, K. L., Young, D. C. & Zobell, J. T. State of the art in cystic fibrosis pharmacology—Optimization of antimicrobials in the treatment of cystic fibrosis pulmonary exacerbations: I. Anti-methicillin-resistant Staphylococcus aureus (MRSA) antibiotics. *Pediatr. Pulmonol.* **55**, 33–57 (2020).

81. Mastella, G. *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. Epidemiologic Registry of Cystic Fibrosis. *Eur. Respir. J.* **16**, 464–471 (2000).

82. Kaditis, A. G. *et al.* Effect of allergic bronchopulmonary aspergillosis on FEV1 in children and adolescents with cystic fibrosis: a European Cystic Fibrosis Society Patient Registry analysis. *Arch. Dis. Child.* **102**, 742–747 (2017).

83. Taylor, B. W., Norman, A. P. & Soothill, J. F. Association of cystic fibrosis with allergy. 5.

84. Parmar, J. S. Antibiotic allergy in cystic fibrosis. *Thorax* **60**, 517–520 (2005).

85. Tanou, K., Zintzaras, E. & Kaditis, A. G. Omalizumab therapy for allergic bronchopulmonary aspergillosis in children with cystic fibrosis: A synthesis of published evidence. *Pediatr. Pulmonol.* **49**, 503–507 (2014).

86. Spellberg, B. & Edwards, J. E., Jr. Type 1/Type 2 Immunity in Infectious Diseases. *Clin. Infect. Dis.* **32**, 76–102 (2001).

87. Muraille, E., Leo, O. & Moser, M. Th1/Th2 Paradigm Extended: Macrophage Polarization as an Unappreciated Pathogen-Driven Escape Mechanism? *Front. Immunol.* **5**, (2014).

88. Brauweiler, A. M., Goleva, E. & Leung, D. Y. M. Th2 Cytokines Increase Staphylococcus aureus Alpha Toxin–Induced Keratinocyte Death through the Signal Transducer and Activator of Transcription 6 (STAT6). *J. Invest. Dermatol.* **134**, 2114–2121 (2014).

89. Starkl, P. *et al.* IgE Effector Mechanisms, in Concert with Mast Cells, Contribute to Acquired Host Defense against Staphylococcusaureus. *Immunity* **53**, 793-804.e9 (2020).

90. Profet, M. The function of allergy: immunological defense against toxins. *Q. Rev. Biol.* **66**, 23–62 (1991).

91. Langen, U., Schmitz, R. & Steppuhn, H. Häufigkeit allergischer Erkrankungen in Deutschland. *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz* **56**, 698–706 (2013).

92. Stemeseder, T. *et al.* Cross-sectional study on allergic sensitization of Austrian adolescents using molecule-based IgE profiling. *Allergy* **72**, 754–763 (2017).

93. Sintobin, I. *et al.* Sensitisation to staphylococcal enterotoxins and asthma severity: a longitudinal study in the EGEA cohort. *Eur. Respir. J.* **54**, (2019).

94. Berger, M., Kirkpatrick, C. H., Goldsmith, P. K. & Gallin, J. I. IgE antibodies to Staphylococcus aureus and Candida albicans in patients with the syndrome of hyperimmunoglobulin E and recurrent infections. *J. Immunol.* **125**, 2437–2443 (1980).

95. Park, B. & Liu, G. Y. Staphylococcus aureus and Hyper-IgE Syndrome. *Int. J. Mol. Sci.* **21**, (2020).

96. Sørensen, M. *et al.* Staphylococcus aureus enterotoxin sensitization is associated with allergic poly-sensitization and allergic multimorbidity in adolescents. *Allergy* **72**, 1548–1555 (2017).

97. Taylor, J. M., Ziman, M. E., Canfield, D. R., Vajdy, M. & Solnick, J. V. Effects of a Th1- versus a Th2-biased immune response in protection against Helicobacter pylori challenge in mice. *Microb. Pathog.* **44**, 20–27 (2008).

LIST OF ABBREVIATIONS

2D	Two-dimensional
ABPA	Allergic bronchopulmonary aspergillosis
AD	Atopic dermatitis
BLAST	Basic local alignment search tool
CD	Cluster of differentiation
CF	Cystic fibrosis
DAMP	Damage associated molecular patterns
DC	Dendritic cell
ETA	Exfoliative toxin A
HDM	House dust mite
HIES	Hyperimmunoglobulin E syndrome
Hla	Alpha-hemolysin
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cell
LPS	Lipopolysaccharide
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
ORF	Open reading frame
OVA	Ovalbumin
PAMP	Pathogen associated molecular patterns
PAR	Protease-activated receptor
PRR	pattern-recognition receptors
ROR	Retinoic acid–related orphan receptor
S. aureus	*Staphylococcus aureus*
SAg	Superantigen
ScpA	Staphopain A

SEB	Staphylococcal enterotoxin B
Spl	Serine protease-like protein
SspB	Staphopain B
ST2	Suppressor of tumorgenicity 2
Tc	Cytotoxic T cell
Th	Helper T cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	Regulatory T cell
TSLP	Thymic stromal lymphopoietin
TSST	Toxic shock syndrome toxin
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
WHO	World Health Organization

APPENDIX

EIGENSTÄNDIGKEITSERKLÄRUNG

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.
Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Unterschrift der Promovendin

LIST OF PUBLICATIONS AND OTHER SCIENTIFIC ACHIEVEMENTS

PUBLICATIONS

Stentzel S, Sundaramoorthy N, Michalik S, **Nordengrün M**, Schulz S, Kolata J, Kloppot P, Engelmann S, Steil L, Hecker M, Schmidt F, Völker U, Roghmann MC, Bröker BM. 2015. Specific serum IgG at diagnosis of *Staphylococcus* aureus bloodstream invasion is correlated with disease progression. J Proteomics. 2015 Oct 14;128:1-7. doi: 10.1016/j.jprot.2015.06.018. Epub 2015 Jul 5.

Stentzel S, Teufelberger A, **Nordengrün M**, Kolata J, Schmidt F, van Crombruggen K, Michalik S, Kumpfmüller J, Tischer S, Schweder T, Hecker M, Engelmann S, Völker U, Krysko O, Bachert C, Bröker BM. 2017. Staphylococcal serine protease-like proteins are pacemakers of allergic airway reactions to *Staphylococcus* aureus. J Allergy Clin Immunol. 2017 Feb;139(2):492-500.e8. doi: 10.1016/j.jaci.2016.03.045.

Teufelberger AR, **Nordengrün M**, Braun H, Maes T, De Grove K, Holtappels G, O'Brien C, Provoost S, Hammad H, Gonçalves A, Beyaert R, Declercq W, Vandenabeele P, Krysko DV, Bröker BM, Bachert C, Krysko O. 2018. The IL-33/ST2 axis is crucial in type 2 airway responses induced by *Staphylococcus* aureus-derived serine protease-like protein D. J Allergy Clin Immunol. 2018 Feb;141(2):549-559.e7. doi: 10.1016/j.jaci.2017.05.004. Epub 2017 May 19.

Nordengrün M, Michalik S, Völker U, Bröker BM, Gómez-Gascón L. 2018. The quest for bacterial allergens. Int J Med Microbiol. 2018 Aug;308(6):738-750. doi: 10.1016/j.ijmm.2018.04.003. Epub 2018 Apr 26.

Murthy N. Darisipudi, **Maria Nordengrün**, Barbara M. Bröker, Vincent Péton. 2018. Messing with the Sentinels—The Interaction of *Staphylococcus aureus* with Dendritic Cells. Microorganisms. 6(3): 87. Published online 2018 Aug 15. doi: 10.3390/microorganisms6030087

Agnes Bonifacius, Oliver Goldmann, Stefan Floess, Silva Holtfreter, Philippe A. Robert, **Maria Nordengrün**, Friederike Kruse, Matthias Lochner, Christine S. Falk, Ingo Schmitz, Barbara M. Bröker, Eva Medina, Jochen Huehn. 2020. *Staphylococcus aureus* Alpha-Toxin Limits Type 1 While Fostering Type 3 Immune Responses. Front Immunol. 2020; 11: 1579. Published online 2020 Aug 7. doi: 10.3389/fimmu.2020.01579

Andrea R. Teufelberger, Sharon Van Nevel, Paco Hulpiau, **Maria Nordengrün**, Savvas N. Savvides, Sarah De Graeve, Srinivas Akula, Gabriele Holtappels, Natalie De Ruyck, Wim Declercq, Peter Vandenabeele, Lars Hellman, Barbara M. Bröker, Dmitri V. Krysko, Claus Bachert and Olga Krysko. 2020. Mouse Strain-Dependent Difference Toward the *Staphylococcus* aureus Allergen Serine Protease-Like Protein D Reveals a Novel Regulator of IL-33. Front. Immonol. 11:582044. doi: 10.3389/fimmu.2020.582044

Maria Nordengrün, Goran Abdurrahman, Janina Treffon, Hannah Wächter, Barbara C. Kahl and Barbara M. Bröker. 2021. Allergic Reactions to Serine Protease-Like Proteins of Staphylococcus aureus. Front. Immunol. Accepted: 08 Mar 2021. doi: 10.3389/fimmu.2021.651060

CONFERENCES

Stentzel, S., Sundaramoorthy, N., Michalik, S., **Nordengrün, M.**, Hagl, B., Abel, F., Schmidt, F., Steil, L., Völker, U., Roghmann, M.-C., Kahl, B. C., Renner, E. D., Bröker, B. M. Are specific antibodies protective in *S. aureus* infection?. Annual Meeting of the German Society of Hygiene and Microbiology (DGHM). 2015. Münster, Germany

Stentzel, S., **Nordengrün, M.**, Kolata, J., Holtappels, G., Kumpfmüller, J., Michalik, S., Richter, E., Engelmann, S., Hecker, M., Holchgräfe, F., Schmidt, F., Völker, U., Schweder, T., Bachert, C., Bröker, B. M. Staphylococcal serine proteases – Inducers of airway allergies?. International Symposium on Immunoregulation in Allergy and Infection. 2015. Lübeck, Germany

Maria Nordengrün. Oral presentation: Allergic Reactions to *Staphylococcus aureus* In Airway Diseases. Joint Meeting of the RTG1870 and ILRS Jena. May 2015. Wittenberg, Germany

Maria Nordengrün, Andrea Teufelberger, Stephan Michalik, Sebastian Stentzel, Frank Schmidt, Olga Krysko, Uwe Völker, Claus Bachert & Barbara M. Bröker. Staphylococcal serine proteases are inducers of airway allergies. 3rd International Conference Pathophysiology of Staphylococci. September 2016. Tübingen, Germany

Maria Nordengrün, Andrea Teufelberger, Stephan Michalik, Sebastian Stentzel, Frank Schmidt, Olga Krysko, Uwe Völker, Claus Bachert & Barbara M. Bröker. Allergic reactions to Staphylococcal serine proteases in airway diseases. 47th Annual Meeting of the German Society of Immunology. September 2016. Hamburg, Germany

Maria Nordengrün, Andrea Teufelberger, Stephan Michalik, Sebastian Stentzel, Frank Schmidt, Olga Krysko, Uwe Völker, Claus Bachert & Barbara M. Bröker. Allergic reactions to Staphylococcal serine proteases in airway diseases. 1st Summer School Infection Biology. September 2019. Greifswald, Germany

Maria Nordengrün, Andrea Teufelberger, Stephan Michalik, Sebastian Stentzel, Frank Schmidt, Olga Krysko, Uwe Völke, Claus Bachert, Barbara M. Bröker. *S. aureus* serine proteases are inducers of airway allergies. 1st International Conference on Respiratory Pathogens (ICoRP). November 2017. Rostock, Germany

Maria Nordengrün, Andrea Teufelberger, Stephan Michalik, Narayana Murthy Darisipudi, Olga Krysko, Uwe Völker, Claus Bachert, Barbara M. Bröker. *Staphylococcus aureus* serine proteases are inducers of airway allergies. International Symposium - Allergy meets Infection. 2018. Lübeck, Germany

AWARDS

BEST TALK AWARD
For the oral presentation at the Joint Meeting of the RTG1870 and ILRS Jena 2015 in Wittenberg, Germany

POSTER AWARD
For the poster presentation at the International Symposium - Allergy meets Infection 2018 in Lübeck, Germany

FÖRDERUNG / FUNDING

Die vorliegende Dissertation wurde gefördert:

i) Im Graduiertenkolleg 1870 „Bacterial Respiratory Infections – Common and Specific Mechanisms of Pathogen Adaptation and Immune Defense“ durch die Deutsche Forschungsgemeinschaft (DFG).

ii) Im Rahmen des Forschungsprojekts „Card-ii-Omics – Kardiovaskuläre Implantat-entwicklung-Infektionen-Proteomics: Prävention, Diagnostik und Therapie von Implantatinfektionen“. Die Förderung des Projektes erfolgte aus Mitteln des Europäischen Sozialfonds (ESF) im Rahmen des Qualifikationsprogramms „Förderung von Nachwuchswissenschaftlern in exzellenten Forschungsverbünden – Exzellenzforschungsprogramm des Landes Mecklenburg-Vorpommern“ (ESF 14-BM-A55-0037/16).

DANKSAGUNG / ACKNOWLEDGEMENT

Barbara M. Bröker gilt mein herzlicher Dank für die exzellente wissenschaftliche Betreuung meiner Promotion, für Diskussionen, Anregungen und vor allem für das Vertrauen in meine Arbeit.

Dem Graduiertenkolleg 1870 „Bakterielle Atemwegsinfektionen – allgemeine und spezifische Mechanismen der Pathogenadaptation und Immunabwehr“ und dem Forschungsverbund Card-ii-Omics möchte ich für die Finanzierung meines Promotionsprojektes danken. Sylvia Kohler und Susann Räth danke ich für die hervorragende Koordination und die stete Hilfe bei allen Formalitäten. Besonders dankbar bin ich für Ermöglichung von Workshops, Seminaren sowie Konferenz- und Studienreisen, die meine Promotionszeit sehr lehrreich machten.

Claus Bachert, Olga Krysko und Andrea Teufelberger vom *Upper Airways Research Laboratory* in Gent danke ich für die überaus fruchtbare Zusammenarbeit bei den Studien zur murinen Antwort auf Spls. Mein Forschungsaufenthalt in der Arbeitsgruppe hat mich fachlich und persönlich sehr vorangebracht und ich denke gerne an diese Zeit zurück. Danke für die enorme Unterstützung.

Mein Dank gilt auch Uwe Völker und Stephan Michalik von der Arbeitsgruppe Funktionelle Genomforschung für die gute Zusammenarbeit, die stets prompte Unterstützung, spannende Diskussionen und die gemeinsamen Publikationen.

Barbara Kahl und Janina Treffon von der Medizinischen Mikrobiologie in Münster danke ich herzlich für die Zusammenarbeit bei der Untersuchung der Immunantwort von CF-Patienten, insbesondere für die Bereitstellung des wertvollen Patientenmaterials.

Allen Blutspendern danke ich für ihre Bereitschaft, Serum- und Vollblut für die Studien zur Immunantwort gesunder Probanden bereitzustellen.

Dem gesamten Team der Abteilung Immunologie danke ich für die freundliche und angenehme Arbeitsatmosphäre. Ihr habt ein Arbeitsumfeld geschaffen, in dem ich mich erneut

sehr wohl gefühlt habe. Für Motivation, Unterstützung und vor allem Denkpausen bedanke ich mich bei allen Aquariumsbewohnern.

Ganz herzlich danke ich auch allen Beteiligten am Mentoring-Programm für Doktorandinnen. Frau Hoppe und Frau Ehmler danke ich für ihre tolle Arbeit, die es mir möglich machte, meine persönlichen Kompetenzen auszubauen und über den Tellerrand hinauszuschauen.

Von ganzem Herzen danke ich meiner Mutter. Mit Rückhalt und Unterstützung stand sie mir während meiner Promotionszeit uneingeschränkt zur Seite und legte in jeder Hinsicht die Grundsteine für meinen bisherigen Weg.