

Review

Staphylococcus aureus Orchestrates Type 2 Airway Diseases

Andrea R. Teufelberger,^{1,7} Barbara M. Bröker,² Dmitri V. Krysko,^{3,4,5} Claus Bachert,^{1,6,8} and Olga Krysko^{1,8,*}

Staphylococcus aureus persistently colonizes the nostrils of one-third of the population but colonizes the sinus mucosa in up to 90% of patients with nasal polyps, implying a possible role in airway disease. Recent findings give new mechanistic insights into the ability of *S. aureus* to trigger type 2 inflammatory responses in the upper and lower airways. This novel concept of a *S. aureus*-driven chronic airway inflammatory disease suggests a new understanding of disease triggers. This article reviews the role of *S. aureus* in chronic inflammatory airway diseases and discusses possible therapeutic approaches to target *S. aureus*.

Possible Connection of *S. aureus* Colonization and the Allergic Epidemic

Since the last century, allergic diseases have spread like an epidemic, currently affecting up to 30% of people in Europe and the USA [1]. Environmental factors could be a possible explanation for this increase because of the rapid spread, which is too fast to be explained by genetically predisposing effects. Modern lifestyle, housing conditions, food, and hygiene habits result in less exposure to bacterial contaminants and parasites and could increase the risk of allergies. The 'hygiene hypothesis' implies that exposure to a diverse range of microbes early in life is necessary to educate the immune system, which further induces the state of immune tolerance later in life via adequate induction of regulatory molecules and pattern recognition receptors [2,3]. The extensive use of antibiotics is an additional contributing factor for reduced microbial diversity, altering immune tolerance [4]. Importantly, some bacterial species start to flourish under these conditions. *S. aureus* is a Gram-positive bacterium, which can be a commensal organism as well as a pathogen due to facultative expression of virulence factors. These factors have different actions ranging from bacterial adherence and host cell invasion to evasion of the host's immune system. The *agr* system controls the expression of δ -toxin, enterotoxins, α -toxin, leukocidins, lipases and **serine protease-like proteins (Spl)** (see [Glossary](#)), which directly determine the degree of virulence [5]. Studies on the nasal microbiome have increased our understanding of the differences in *S. aureus* carriage between individuals and throughout life [6,7]. It is known that asthmatic, allergic rhinitis, and **chronic rhinosinusitis with nasal polyps (CRSwNP)** patients are significantly more likely to be colonized with *S. aureus* than controls [8,9], suggesting a causative role for this bacterium in these pathologies.

IgE formation against *S. aureus* enterotoxins occurs in most severe forms of CRSwNP and correlates with asthma severity [10,11]. Recently described actions of *S. aureus* serine protease-like proteins could further explain the observed disease association [12,13]. It could, therefore, be interesting to tackle *S. aureus* to prevent colonization of the nasal mucosa, which could potentially lead to disease resolution in patients with *S. aureus*-associated chronic **type 2 inflammatory response** in the airways. In this review, we first provide an update of *S. aureus* and host interactions during the development and exacerbation of type 2 inflammatory airway diseases. We further elaborate on possible treatment strategies to tackle *S. aureus* and discuss their benefits and consequences.

Highlights

S. aureus is a versatile bacterium, persistently colonizing the nasal mucosa of approximately one third of the general population.

A significant association of *S. aureus* colonization and sensitization with chronic airway inflammation has long been observed. However, recent mechanistic studies provide emerging evidence for *S. aureus* being an inducer of type 2 immunity.

This adds an infectious character to chronic inflammatory airway diseases such as asthma and chronic rhinosinusitis.

Novel strategies for effective *S. aureus* elimination are currently being developed to restore homeostasis in some patient subgroups of chronic inflammatory airway diseases.

¹Upper Airways Research Laboratory, Department of Otorhinolaryngology, Ghent University, Ghent, Belgium

²Department of Immunology, University Medicine Greifswald, Greifswald, Germany

³Institute of Biology and Biomedicine, National Research Lobachevsky State University of Nizhni Novgorod, Nizhny Novgorod, Russia

⁴Cell Death Investigation and Therapy Laboratory, Department of Human Structure and Repair, Ghent University, Ghent, Belgium

⁵Cancer Research Institute Ghent, Ghent, Belgium

⁶Division of ENT Diseases, CLINTEC, Karolinska Institute, Stockholm, Sweden

⁷Current address: Department of Medicine Solna, Immunology and Allergy Research Unit, Karolinska Institute, Stockholm, Sweden

⁸These authors share senior authorship

*Correspondence: olga.krysko@ugent.be (O. Krysko).

Role of *S. aureus*-Specific IgE in Asthma and CRS

Around 20–30% of the general population are persistently colonized with *S. aureus* on their nasal mucosa [14]. An association of *S. aureus* colonization with several chronic inflammatory diseases of the airways such as allergic rhinitis, CRSwNPs, and allergic asthma has been described. The nasal mucosa of 44% of perennial allergic rhinitis (AR) patients is colonized with *S. aureus* [15] and in inferior turbinate biopsies of AR patients, *S. aureus* is significantly more abundant in IgE^{high} than in IgE^{low} patients [16]. Also, 66.7% of CRSwNP patients with comorbid asthma, who are characterized as having high levels of IgE and IL-5, are colonized with *S. aureus* [8,10]. In contrast to CRSwNP, CRS without nasal polyps (CRSsNP), which is known to be mainly IL-5 negative and IgE^{low}, is not associated with an increased *S. aureus* colonization rate, even though around half of the patients suffer from allergies. Asthma comorbidity occurs in <20% of patients with CRSsNP, but up to 71% of CRSwNP patients, who produce measurable amounts of staphylococcal enterotoxin-specific IgE (SE-IgE), have asthma comorbidity [8,10]. Moreover, recent reports have demonstrated that SE-IgE levels in CRSwNP correlate with disease severity and asthma comorbidity, indicating their role as a major cofactor in chronic inflammatory airway diseases. A link between SE-IgE formation and asthma severity has been identified and it was found that 59.6% of severe asthma patients have SE-IgE, while only 13% of healthy control subjects are SE-IgE positive [17]. SE-IgE positively correlates with lower forced expiratory volume, higher oral steroid use, and more hospitalization [17]. Staphylococcal enterotoxin A (SEA)-specific IgE formation has been shown to be significantly associated with uncontrolled asthma compared with SEA-negative asthma patients [18]. These findings were confirmed in epidemiological studies in the USA between 2001 and 2013, which have indicated an increased prevalence of SE-IgE among both adults and children with asthma, compared with the general population [19]; and in a demographic study, where nasal *S. aureus* carriage was identified as a risk factor for asthma diagnosis in adolescents [20]. Importantly, no correlation between asthma severity and a specific IgE to common inhalant allergens, such as house dust mites (HDMs), has been observed [19]. In teenagers, SE-IgE correlates with atopy, asthma, and bronchial hyper-reactivity risk. However, also in nonatopic teenagers, SE-IgE positivity has been found to be a significant risk factor for bronchial hyper-reactivity [21]. In elderly patients with asthma, high levels of SE-IgE are associated with more severe asthma symptoms and sputum eosinophilia, than those with lower SE-IgE levels [22]. The IDENTIFY project investigated the sensitization of nonatopic asthmatics, to not commonly tested aeroallergens. These patients were classified as nonatopic due to their absence of IgE specific to commonly tested allergens, however, some showed IgE specificity for the tested alternative allergens [23]. Thus, *S. aureus* colonization in the upper airways is tightly linked to allergic sensitization and disease severity in patients with chronic inflammatory airway diseases – even in some asthmatics that are nowadays diagnosed to be nonatopic.

S. aureus Modulates the Type 2 Inflammatory Response in the Airways – Old and New Players

The clear association with type 2 inflammatory diseases suggests a possible causation of either *S. aureus* being attracted by already inflamed tissue or *S. aureus* creating its preferred milieu by triggering the host's type 2 response. It has been recently shown by single-cell RNA sequencing that CRSwNP has a strong induction of IL-4/IL-13 transcriptional programs [24]. When *ex vivo* nasal polyp tissue is infected with *S. aureus*, the bacterium induces upregulation of IL-33 and thymic stromal lymphopoietin (TSLP), suggesting an exacerbating effect of the type 2 response [25]. *S. aureus* also attracts eosinophils and triggers extracellular eosinophilic trap formation in CRSwNP, suggesting that type 2 inflammation is an antibacterial host defense mechanism [26]. The discussed *S. aureus* products promoting a type 2 response in the host's airway mucosa are summarized in Figure 1. *S. aureus* has, therefore, been shown to have exacerbating effects in type 2 inflamed tissue.

Glossary

Chronic rhinosinusitis with nasal polyps (CRSwNP):

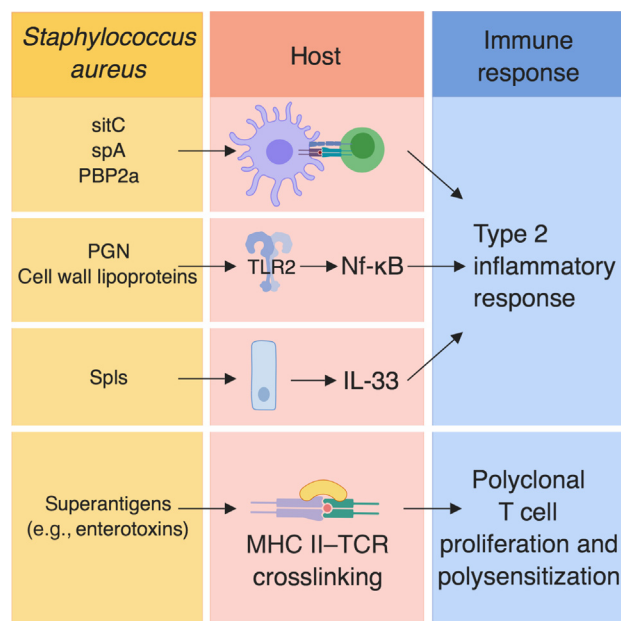
CRS is an inflammatory disease of the nose and paranasal sinuses with symptoms of nasal blockage and/or nasal discharge, persisting for more than 12 weeks. There are two clinical phenotypes of CRS: CRSwNP and CRS without nasal polyps (CRSsNP). These two phenotypes differ in their inflammatory patterns with CRSsNP being mostly Th1 and CRSwNP being mainly a Th2-mediated diseases.

Serine protease-like proteins (Spl):

a group of six serine proteases, SplA to SplF, which are expressed by *S. aureus*. They are located on the *spl* operon and most *S. aureus* strains carry at least one Spl in their genome.

Superantigens: proteins that can crosslink the MHC II of APCs with the TCR of Th cells. This leads to a significant increase of polyclonal proliferation of T cells and APCs. Superantigens differ from conventional antigens as they do not require processing by APCs in order to activate T cells.

Type 2 inflammatory response: also called the type IVb hypersensitivity reaction; it is characterized by activated Th2 cells releasing the cytokines IL-4, IL-5, and IL-13. These cytokines recruit eosinophils and promote an immunoglobulin isotype class switching towards IgE, which leads to allergic sensitization.



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Figure 1. *Staphylococcus aureus* Products That Initiate a Type 2 Inflammatory Response and Exacerbate Allergic Sensitization in the Airway Mucosa. Dendritic cells presenting *S. aureus* antigens such as ABC transporter lipoprotein (sitC), surface protein A (SpA), and penicillin binding protein 2a (PBP2a) can activate T helper (Th)2 cytokine production in T cells *in vitro*. Peptidoglycan (PGN) and other cell wall compartments activate Toll-like receptor 2 (TLR2), which leads to a type 2 inflammatory response via NF-κB signaling. The serine protease-like proteins (Spls) initiate a type 2 response via activation of airway epithelial cells to produce interleukin (IL)-33. Superantigens such as enterotoxins can crosslink MHC II with T cell receptor (TCR) and thereby drive polyclonal T cell expansion. In a type 2 inflamed environment, this mechanism leads to polysensitization.

S. aureus Enterotoxins

S. aureus enterotoxins, which are one of the first identified *S. aureus* products, function as **superantigens** by crosslinking T cell receptor (TCR) variable regions and MHC II molecules on antigen-presenting cells (APCs) [27]. This action causes polyclonal proliferation and cytokine production in T cells and mucosa-associated T cells [28]. *S. aureus* enterotoxins also act as B cell superantigens via interaction with IgE molecules conventionally through complementarity-determining regions (CDRs) and unconventionally through the immunoglobulin heavy-chain variable region shared by immunoglobulins with different CDRs [29]. Enterotoxins also activate basophils by simultaneous binding as superantigens to the framework regions of IgE and IgE-FcεRI complexes and as antigens in the conventional manner to CDRs [29].

SEB, the enterotoxin most studied in airway allergy, is associated with polyclonal IgE production, which activates mast cells via crosslinking of the high affinity IgE receptor (FcεRI), causing their degranulation [30]. SE-IgE has also been shown to cause direct activation of mast cells and their degranulation, which facilitates type 2 inflammation [31]. Moreover, SEB stimulations of nasal tissue explants induces polyclonal T cell activation and increases the production of T helper (Th)1/Th2 cytokines, with prevalence of IL-4, IL-5, and IL-13 production in nasal polyp mucosa compared with healthy nasal tissue [30]. In mice, SEB increases airway hyper-reactivity, boosts IL-4, IL-5, IL-13, and IgE production, causes eosinophilia in the airways, and breaks tolerance to the inert protein ovalbumin [32]. A combination of SEB, peptidoglycan (PGN), and protein A has been shown to interact with CD48 on the surface of eosinophils to cause their degranulation [33]. Therefore, these data indicate that SEB is a potent direct activator of several cell types

including T and B cells, eosinophils, and mast cells. When SEB is repetitively given via endonasal applications to mice, it causes mucosal lesions with epithelial disruption and eosinophilic infiltration in the nasal mucosa, resembling nasal polyps [34]. Although these data provide extensive evidence on the role of SEB in driving type 2 immunity in human *ex vivo* tissue and murine models of allergic asthma, other *S. aureus* enterotoxins also play a role.

S. aureus Cell Wall Components

S. aureus cell wall components have immunomodulatory capacity and potentiate Th2 immune responses. Human dendritic cells loaded with the *S. aureus* antigens sitC, which is a lipoprotein and Toll-like receptor (TLR)2 agonist, staphylococcal surface protein A, and the penicillin binding protein 2a trigger the production of Th2 cytokines in T cells upon coculture *in vitro* [35]. In addition, PGN and several other lipoproteins of the staphylococcal cell wall trigger a Th2 response. They can activate TLR2 signaling in murine APCs and mast cells, resulting in production of type 2 cytokines [36], and thus actively shift the immune response towards Th2 by inhibition of the Th1/Th17 response [37,38]. In human peripheral blood mononuclear cells, *S. aureus* cell wall compounds other than PGN promote a Th2 response by inhibition of STAT1 signaling, causing down-regulation of IP-10 and Th1 cell recruiting chemokines such as CXCL9 and CXCL11 [39]. All these findings show that *S. aureus* cell wall compounds play an important role in modulating immune responses to inhibit the Th1 response and cause less efficient eradication of *S. aureus* infections. These mechanisms support the formation of long-term bacterial colonization. *S. aureus* cell wall components are skewing the antibacterial response of the host towards a type 2 response.

S. aureus Exotoxins, Intracellular Persistence, and Phagocytosis

Several other toxins can contribute to the pathogenesis of allergic airway inflammation induced by *S. aureus*. For example, *S. aureus* δ -toxin, a pore-forming protein, which causes direct activation of mast cells *in vivo* and *in vitro* [40]. Moreover, δ -toxin (also called phenol soluble modulins (PSM)), PSM α 1, and PSM α 3 trigger tryptase production by the human mast cell line HMC1 *in vitro* [41]. It has been shown that patients with atopic skin disease have higher numbers of δ -toxin-producing *S. aureus* strains [40]. RNAIII promotes expression of virulence factors in the *agr* system and is the mRNA encoding δ -toxin [5]. Colonization of the murine skin with a δ -toxin-producing *S. aureus* strain promotes IgE and IL-4 secretion, supporting allergic skin disease in mice [40]. Therefore, these exotoxins might also contribute to the final outcome of allergic airway inflammation induced by *S. aureus*.

Furthermore, *S. aureus* colonization in chronic airway diseases is facilitated by the fact that *S. aureus* could persist in mast cells, macrophages, and airway epithelial cells in CRSwNP [42,43]. α -Hemolysin enables the intracellular persistence of *S. aureus* [44]. δ -Toxin and β -toxin facilitate the evasion of phagosomes by *S. aureus* [45]. Moreover, intramucosal persistence of *S. aureus* is also supported by deficient phagocytosis and bacterial killing in CRSwNP and severe asthma [46,47]. In addition, *S. aureus* promotes alternative macrophage polarization phenotype, M2, via Akt-1 signaling and subsequent regulation of the miR-155/SOCS1 axis [48]. Of note, M2 macrophages are predominantly present in the nasal polyp tissue [49], which could produce IL-10 and further inhibit host Th1 and Th17 responses, the recruitment of inflammatory cells, and prevent clearance of *S. aureus*. *S. aureus*-derived exotoxins are, therefore, supporting survival of the bacterium and, at the same time, promoting a type 2 response in the host.

S. aureus Serine Protease-Like Proteins

In search of the responsible inflammation inducing factor, it has been recently found that the Spls of *S. aureus* can act as inducers of a type 2 response (Box 1 and Figure 2). *S. aureus* can express up to six Spls: SplA–F. Asthmatics have higher titers of SplA-, SplB-, SplD-, and SplE-specific

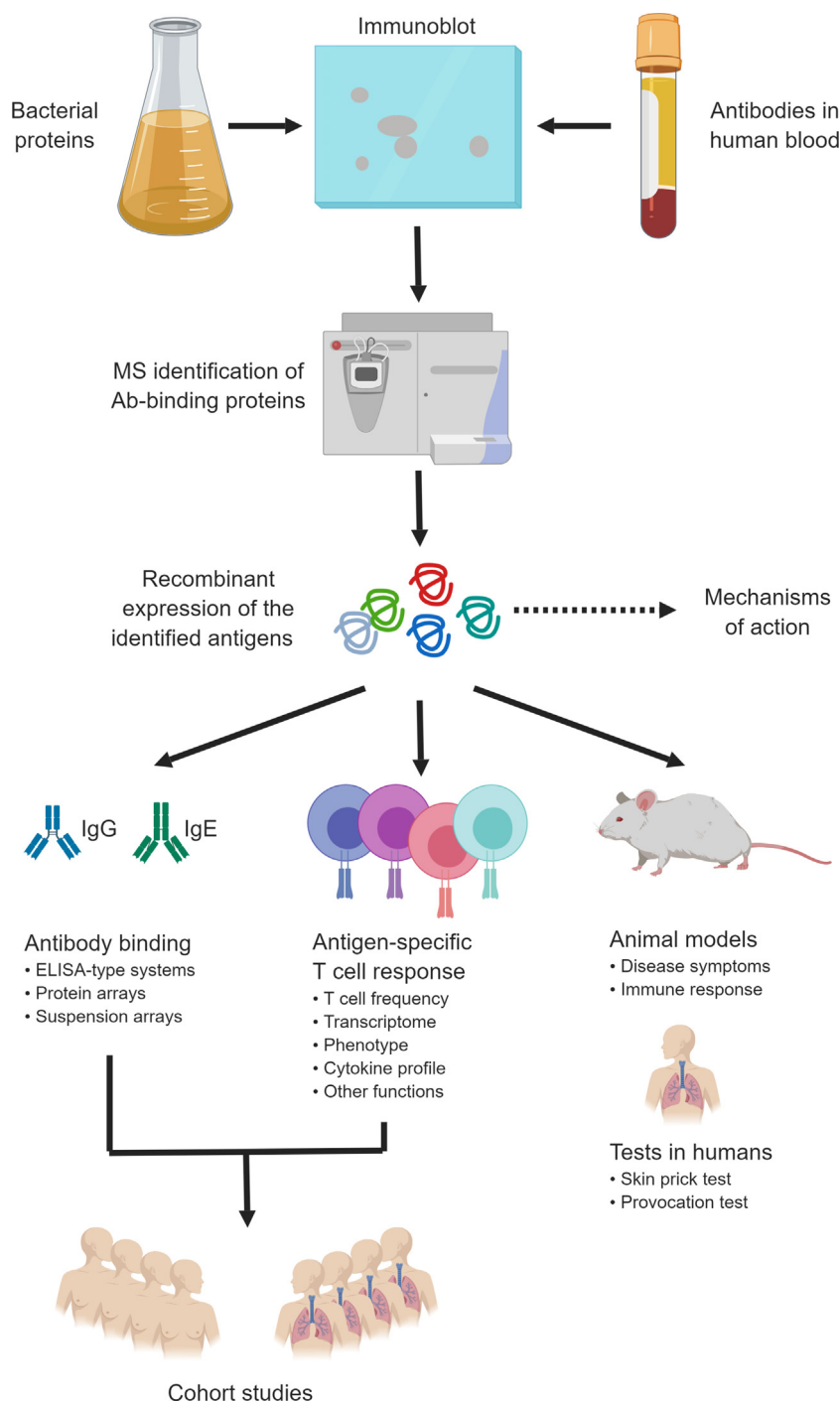
Box 1. Identification of *S. aureus*-Specific Immunoproteome

There is now good evidence that, similar to other complex allergens, *S. aureus* confronts its host with a broad spectrum of antigens, which can elicit adaptive immune responses of different quality in the same individual [50,97,98]. To determine the bacterial allergome, those bacterial antigens that favor an IgE response in B cells and a Th2 response in T cells need to be identified by probing the human B and T cell memory in healthy individuals and allergic patients [96]. Immunomics is a powerful tool for obtaining a comprehensive, unbiased picture. Its foundation is modern mass spectrometry, which enables the highly sensitive analysis of bacterial subproteomes, for example, the entirety of extracellular, intracellular, or cell wall-associated proteins [99,100].

serum IgE than healthy controls, and mice that receive repeated endotracheal applications of SpID develop typical features of allergic asthma such as SpID-specific IgE formation, Th2 cytokine production in the local draining lymph nodes, and eosinophilic infiltration of the airways [50]. These mice also develop airway hyper-reactivity and goblet cell hyperplasia. The eosinophilic type 2 response is mainly mediated via IL-33, because antagonizing the IL-33/ST2 signaling axis with the soluble IL-33 receptor sST2 significantly dampens this inflammatory response towards SpID. Next to SpID, SpIF has comparable immunoregulatory functions [13]. IL-33 is an innate cytokine, activates group 2 innate lymphoid cells (ILC2s), which release IL-4, and as they are not antigen-specific, may orchestrate polyclonal IgE formation. In addition, activation of mast cells, T cells, dendritic cells, and eosinophils results in the production of Th2 cytokines such as IL-4, IL-5, and IL-13 and other proinflammatory mediators [51]. Mutations in the IL-33 gene were significantly associated with asthma and CRSwNP in genome-wide association studies [52,53]. Importantly, SpIs can be detected in nasal polyp tissue; however, their exact quantities have not yet been determined [54,55]. *S. aureus*, at least partially via production of SpID, causes a Th2-biased response in an IL-33-dependent manner. These data further identify IL-33 as a potential therapeutic target in patients with CRS and asthma who are carriers of *S. aureus*.

Polysensitization Induced by *S. aureus*

In humans, SE-specific IgE is associated with polysensitization to food allergens such as egg white, cow's milk, cod, wheat or peanuts, or inhalant allergens like birch, timothy or mugwort pollen, cat, horse, dog or HDM [56]. In a study of Korean school children with polysensitization to inhalant allergens and allergic multimorbidity (nonviral conjunctivitis, allergic rhinitis, asthma, and eczema) polysensitization correlated more strongly with disease severity than with allergic multimorbidity [57]. It could be of interest to measure SE and SpID-specific IgE levels in this cohort because it is also conceivable that SpID derived from *S. aureus* could play a role in this polysensitization, as it can act as an adjuvant in a mouse model in which SpID breaks the tolerance towards ovalbumin upon coadministration [13]. The upregulation of IL-33 upon SpID exposure could be responsible for the adjuvant effect observed for SpID, as IL-33 can act as an adjuvant on its own [58]. This cytokine also exacerbates the allergic airway inflammation when coadministered with ovalbumin in mice [59]. In this regard, *S. aureus* has recently been found in house dust samples of asthmatic patients, colonized with HDMs [60,61], which indicate a possible perennial exposure of the airways to *S. aureus* or *S. aureus* products via house dust, even without persistent nasal *S. aureus* carriage. As HDMs are carriers of *S. aureus*, *S. aureus* can potentially facilitate sensitization to HDM allergens due to its IL-33-activating function; sensitizations against both HDMs and *S. aureus* have been observed [60]. These findings indicate a possible perennial exposure of the airways to *S. aureus* or *S. aureus* products in the house even without persistent nasal *S. aureus* carriage. Therefore, it is conceivable that in combination with HDMs, *S. aureus* could potentially facilitate the sensitization to HDM allergens due to its IL-33-activating function. Under physiological conditions, the host is exposed to the whole bacterium and thereby confronted by a combination of the discussed *S. aureus* products. IL-33 release triggered by *S. aureus* seems to play a key role in this orchestrated manipulation of the host towards a type 2 response.



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Figure 2. Workflow for Identification of *Staphylococcus aureus* Allergens.

For a Figure360 author presentation of Figure 2, see the figure legend at <https://doi.org/10.1016/j.molmed.2019.05.003>. This strategy uses the antibody (Ab) response as an indicator for identifying allergen candidates to then test the corresponding T cell reaction in a second step. Spots of IgE binding are visualized on 2D immunoblots – using bacterial

(Figure legend continued at the bottom of the next page.)

Regulated Necrosis, *S. aureus*, and Type 2 Inflammation

It has been shown that *S. aureus* infection of nasal polyp tissue induces shedding of the epithelium and increases cell death [62]. In recent years it has become clear that *S. aureus* and its toxins induce damage in many cells including immune and epithelial cells. Toxins derived from those strains of *S. aureus* that cause a highly inflammatory necrotizing pneumonia, can induce necroptosis, a regulated form of necrosis, in human and murine immune cells [63–65]. Moreover, the damage caused by *S. aureus* in lung epithelial cells can be enhanced by tumor necrosis factor (TNF), which is associated with necroptosis mediated by receptor-interacting serine/threonine-protein kinase 3 (RIPK3) [66]. Of note, necroptosis is often associated with release of damage-associated molecular patterns including IL-33 [67,68], which outside the cells contribute to a pro-inflammatory response. Importantly, full-length IL-33 is released in response to necroptosis, which is bioactive, as it is able to activate basophils and eosinophils [67]. The authors showed that by blocking necroptosis, IL-33 is also reduced *in vitro*, leading to less eosinophilia in *Aspergillus fumigatus* extract-induced asthma *in vivo*; an allergic inflammation model that is highly dependent on IL-33. All these data suggest that necroptosis may be a direct mechanism for IL-33 release and it is implicated in type 2 immune responses induced by *S. aureus*. Recently, it has been shown that IL-33 is associated with ferroptosis [68], another type of regulated cell death which is morphologically, biochemically, and genetically distinct from other forms of necrotic cell death types [69,70]. In this regard it has recently been shown that ferroptosis might contribute to type 2 airway inflammation associated with asthma [71]. Both types of cell death (i.e., necroptosis and ferroptosis) are unlikely to occur in stable, controlled asthma but are highly likely to be present during disease-exacerbation-associated tissue damage [71]. Therefore, a molecular identification of the type of cell death implicated in type 2 inflammation may allow the development of therapeutic approaches based on targeting a specific cell death modality for disease intervention. Knowledge of the form of cell death in type 2 inflammation and its functional consequences is limited, and many more interesting and challenging findings are expected.

Novel Approaches in Targeting and Eliminating *S. aureus*

An ideal treatment option, which could effectively eradicate *S. aureus* from the nasal mucosa of patients suffering from type 2 inflammatory airway diseases, has not yet been found. Complete *S. aureus* elimination could be beneficial not only for chronic type 2 airway diseases but also for nosocomial infections, of which *S. aureus* is the most common cause [72]. Its fast adaptation and multidrug resistance, biofilm formation, intracellular growth, and intracellular persistence complicate the attempt to treat *S. aureus* with antibiotics. Also, the long survival of up to 1 month on surfaces, even after thorough cleaning, and its omnipresence in house dust can provoke recolonization in patients [60,61,73], which would demand constant antibiotics treatment, further increasing the risk of multidrug-resistant *S. aureus* carriage. The usage of antibiotics in health care but also in farm animals could also cause a dysbiosis in the gut microbiota, with systemic consequences. A decreased gut microbial diversity in the first month of life is associated

proteins as the antigens and human blood as the source of antibodies – and the corresponding proteins can be identified by mass spectrometry (MS) [91,92]. Since the allergen-specific IgE serum concentration is low, it may be helpful to increase the sensitivity of this initial survey by studying Ig-G4-binding as a proxy [50,93]. Alternatives to 2D immunoblots are bacterial protein expression libraries and arrays of recombinant proteins [94]. These strategies yield lists of candidate allergens, which need to be validated and tested for their antibody binding properties. This methodology is suitable for cohort studies which are conducted in order to determine associations of specific antibody responses with disease [95]. Recombinant proteins further enable the characterization of antigen-specific T cells by exposure of human T cells to the proteins in the presence of antigen-presenting cells, and their response can be analyzed on the transcriptomic, phenotypic, and functional levels. In humans, skin prick tests or provocation tests are currently the gold standard for confirming a decisive role for a bacterial candidate protein in allergic responses [96]. Animal models of allergy are instrumental to study the mechanism of immune recognition and immune response.

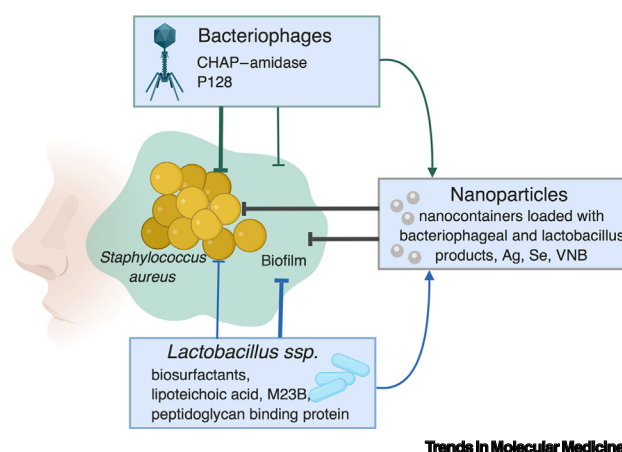
with an increased asthma risk at school age [74]. It has also been considered that antibiotic treatment of mice results in increased survival of *S. aureus* in neutrophils [75]. Therefore, treatment options alternative to antibiotics need to be developed. In this regard, several naturally occurring mechanisms of *S. aureus* eradication by other microbes could be adapted for therapeutic applications. These hypothesized treatment options of the future are summarized in Figure 3.

Bacteriophages against *S. aureus*

Bacteriophages are natural predators for *S. aureus*, thus, making them a well-suited treatment option [76]. Eradication in an *ex vivo* model of *S. aureus*-infected nasal polyp tissue by bacteriophages showed an efficacy of 50% after 72 hours [54]. Nevertheless, treatment with the bacteriophage-derived murein peptidase LysK cysteine/histidine-dependent amidohydrolase/peptidase (CHAP) and amidase shows promising antimicrobial effects against *S. aureus*, multidrug-resistant *S. aureus*, *Staphylococcus epidermidis*, and *Enterococcus*, while it does not affect the growth of *Escherichia coli* and *Lactococcus lactis* [77]. Moreover, the enzyme P128, which is a combination of the tail-associated muralytic enzyme from phage K with a cell wall-binding domain derived from lysostaphin, demonstrated biofilm disrupting effects against sinus-derived *S. aureus* strains [78], suggesting that bacteriophages are a promising novel therapeutic strategy. Belgium is one of the first European countries that has implemented a magistral phage preparation framework to further explore phage applications and to overcome the regulatory challenges of phage medicine [79]. However it is not clear how the human immune system will respond to phages if applied several times, and purity and consistent quality are prerequisites for phage therapy. Therefore, more high-quality research is needed to clarify the safety and clinical efficiency of phage therapy.

Biofilm Prevention by *Lactobacillus* Treatment

S. aureus biofilm formation has been found in nasal polyp patients and associated with disease severity. It could be one of the factors that strengthens bacterial persistence [80]. In the search



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Figure 3. Novel Treatment Options against *Staphylococcus aureus* Colonization in Type 2 Inflammatory Diseases of the Airways. Bacteriophages and lactobacilli are natural sources of *S. aureus*-inhibiting compounds. Bacteriophage-derived proteins have antibacterial and, to a lesser extent, biofilm-disrupting activity. Fusion proteins such as cysteine/histidine-dependent amidohydrolase/peptidase (CHAP)-amidase, or P128, a fusion protein of the cell-wall-binding domain of lysostaphin and the peptidoglycan-degrading murein hydrolase derived from phage K, have been shown to be effective and specific to tackle *Staphylococcus* spp. Lactobacilli secrete molecules with a stronger biofilm-disrupting activity than antibacterial function. These *Lactobacillus* products comprise biosurfactants, lipoteichoic acid, M23B, or peptidoglycan-binding protein. Nanocontainers loaded with both, antibiotic proteins from bacteriophages, and biofilm-disrupting agents from *Lactobacillus* spp. could result in an efficient and specific local treatment to eradicate *S. aureus* in the airways of patients. Another local treatment option could be silver or selenium nanoparticles as well as vapor nanobubbles (VNBs).

Clinician's Corner

Clinicians, ENT (ear, nose, and throat) surgeons, and pulmonologists regularly face problems in the control of severe diseases such as CRSwNP (nasal polyps) and late-onset asthma, that often occur together. Typically, both diseases are characterized by increased blood eosinophils and total serum IgE concentrations, although approximately half of patients are not considered allergic, and allergens do not seem to play a role as classical symptom inducers. Nevertheless, anti-IgE and anti-IL5 strategies have been successfully used, pointing to the functional relevance of IgE and eosinophils.

S. aureus is a frequent colonizer in patients with atopic dermatitis, asthma, and nasal polyps, and has been shown to reside intramucosally and release a range of specific proteins known as superantigens and serine-like proteases into the airway mucosa, which in turn have been demonstrated to induce typical type 2 cytokines such as IL-4, IL-5, and IL-13. These cytokines are crucial for Th2 cell activation with subsequent formation of polyclonal IgE and recruitment and activation of eosinophils in the airways. In this process, SE-IgE is formed, which may serve as a biomarker of such a reaction, and eosinophils also have been demonstrated to be involved in the mucosal surface defense against *S. aureus*. SE-IgE is clearly associated with severe airway disease, including disease recurrence and comorbid asthma for nasal polyps and severe asthma, oral steroid use, decrease in lung function, and hospitalization for asthma.

Although antibiotics can suppress the growth of *S. aureus* temporarily, persistence of the bacteria in various forms is frequent, and colonization of type-2 immune diseases by *S. aureus* is frequent. New innovative approaches to tackle this problem are therefore needed and in progress.

Recent research also shows that *S. aureus* is part of the microbiome of HDMs and releases proteins, including SEs and serine-like proteases, into house dust. These proteins may function as environmental allergens, triggering airway inflammation via inhalation, together with other inhalant allergens such as HDMs. More research is needed here to confirm the role of SEs as allergens.

for natural *S. aureus* biofilm-disrupting agents, *Lactobacillus*-derived products could be of therapeutic use. *Lactobacillus plantarum*-derived lipoteichoic acid has *S. aureus* biofilm-disrupting abilities, while it does not affect *S. aureus* growth [81]. Biosurfactants of *Lactobacillus casei* shows *S. aureus* biofilm-disrupting functions as well as antibacterial activity against *S. aureus* at a dose that has no negative effect on human epithelial cell growth [82]. Furthermore, the LysM-containing PGN-binding protein and the protease M23B secreted by *Lactobacillus salivarius* shows similar effects against *S. aureus* and could thus also be considered as novel therapeutic compounds [83]. Local application of these compounds with the help of nanomedicines could become the treatment of the future.

Nanomedicines with Antistaphylococcal Activity

Despite the plethora of preclinical data, clinical trials are still far from delivering a potential vaccine into clinical practice [84]. However, adaptation of *S. aureus* within the host rapidly alters bacterial phenotype, determining severity of the host immune response. The role of T cell immunity is being recognized in *S. aureus* immune defense mechanisms as a potential target for vaccination strategies [85]. Nanostructured materials offer a number of advantages over conventional antibacterial therapies for multidrug-resistant, intramucosal, biofilm-producing *S. aureus* infections, and allow reduction of the systemic dose needed to minimize adverse effects [86]. Different metalloid nanoparticles were shown to possess pronounced antimicrobial properties for drug-resistant strains; in particular, silver and selenium nanoparticles exert clear antimicrobial and antibiofilm activity against *S. aureus* strains [87,88]. The release of Ag⁺ ions from the surface of nanoparticles causes direct damage to bacterial membranes, reactive oxygen species production, and release of free radicals that damage proteins and lipids [87]. The recently developed technique of laser-induced vapor nanobubbles (VNBs) is efficient in destroying *S. aureus* biofilms and improving diffusion of antibiotics [89]. Loading bacteria with gold nanoparticles and subsequent laser illumination results in VNB formation inside the biofilms, disruption of biofilms, and increased tobramycin efficiency by several orders of magnitude [89].

Nanostructured materials offer several advantages for the delivery of antimicrobial drugs, as they are not only able to carry the drugs but also disrupt cellular membranes and act as bactericidal agents [90]. The nanoparticles facilitate targeted delivery by conjugation of vancomycin-loaded nanoparticles with the cyclic nine-amino-acid peptide CARGGLKSC, which specifically targets infected tissues [86]. It is conceivable that a combination of nanoparticles and advanced biomaterials will offer the unique sustainable delivery tools for chronic staphylococcal infections, and is a rapidly evolving field. Further stratification of patients is needed for choosing the most appropriate *S. aureus* targeting strategies.

Concluding Remarks

There are increasingly strong observations and associations that point to *S. aureus* and its proteins as inducers of persistent type 2 airway inflammation. Several pieces of evidence suggest that *S. aureus* could actively induce type 2 immune responses and profit from them, allowing for persistence. Interactions on several levels, for example, biofilm formation, intracellular persistence, and type 2 inflammation triggered by cell wall components as well as secreted proteins, are used by *S. aureus*. This complex interplay makes causality even more likely, but at the same time, increases the complexity to prove it (see Outstanding Questions). A critical element is the proof that *S. aureus* products, such as superantigens and Spls might act as allergens. Currently, studies with detoxified superantigens are planned to close this knowledge gap. This would at the same time allow causal studies in humans, which are currently impossible due to the inflammation-inducing properties of bacterial proteins. Causal human studies that indeed identify and solidify our hypothesis that *S. aureus* triggers severe airway diseases with epidemic dimensions,

Outstanding Questions

Why do some *S. aureus* carriers develop chronic type 2 immune responses while others do not? It is still unclear why there are different host responses towards *S. aureus*. It might either be due to a yet unknown genetic predisposition or the interaction of the host and *S. aureus* with other microbes in the airways.

What concentrations of Spls is the nasal mucosa exposed to under physiological conditions? Spls have been shown to have an asthma inducing function in mice *in vivo*; however, the concentration of Spls under physiological conditions could vary from those tested in the mouse model. Knowledge about the physiological concentrations might also explain different host responses towards *S. aureus*.

How effective is a noninvasive, local treatment against intracellular and intramucosal *S. aureus*? Nanoparticles loaded with anti-*S. aureus* compounds could be the treatment of the future. However, it is not clear how this treatment could access the intracellularly growing *S. aureus* populations in the airway mucosa.

What could be unwanted effects of *S. aureus* elimination in the airways? Since *S. aureus* does not colonize the majority of the population, it is expected that its elimination would have few to no adverse effects. However, the high complexity of the microbiome in the airways and the vaguely understood roles of each represented species make it difficult to predict the outcome. A shift in the microbial composition could lead to a dysbiosis, promoting the expansion of pathogens.

could pave the way for new treatment strategies. Avoidance and treatment options targeting *S. aureus* would then be needed. Novel approaches using naturally occurring anti-*S. aureus* compounds in combination with modern techniques of compound administration could be used to precisely eliminate this bacterium locally. This could eventually lead to the resolution of inflammation in a relevant subpopulation of patients.

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