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The quest for bacterial allergens

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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

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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.

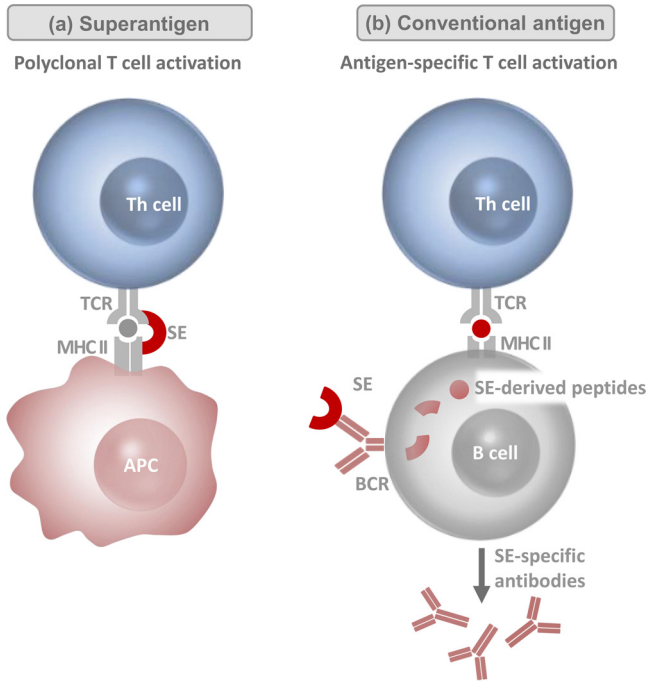

Fig. 1. Enterotoxins can function as superantigens (a) and as conventional antigens (b). For more explanations, please see Box 1. Abbreviations: TCR, T-cell receptor; MHC major histocompatibility complex; SEs, staphylococcal enterotoxins; APC, Antigen-presenting cell; BCR, B-cell receptor.

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 radio-immunoelectrophoresis (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

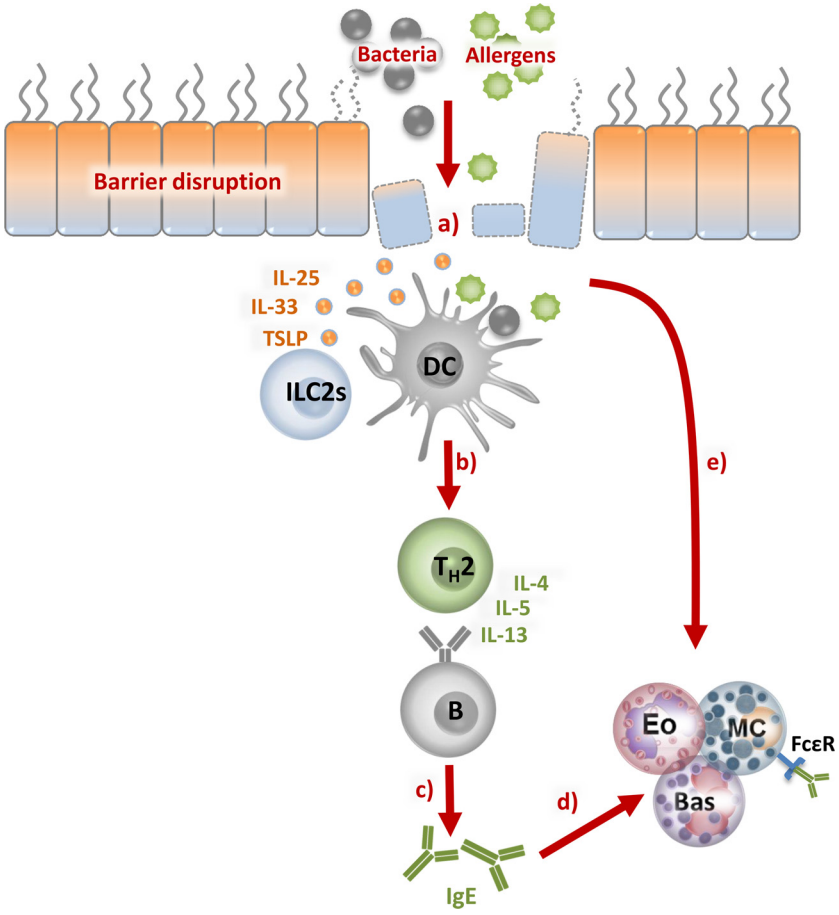

Fig. 2. Allergy-promoting mechanisms of bacteria.
Several pro-allergenic mechanisms have been described in bacteria. Bacterial proteases and toxins disrupt the epithelial barrier, facilitating microbial invasion and the influx of conventional allergens. This leads to local inflammation accompanied by 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

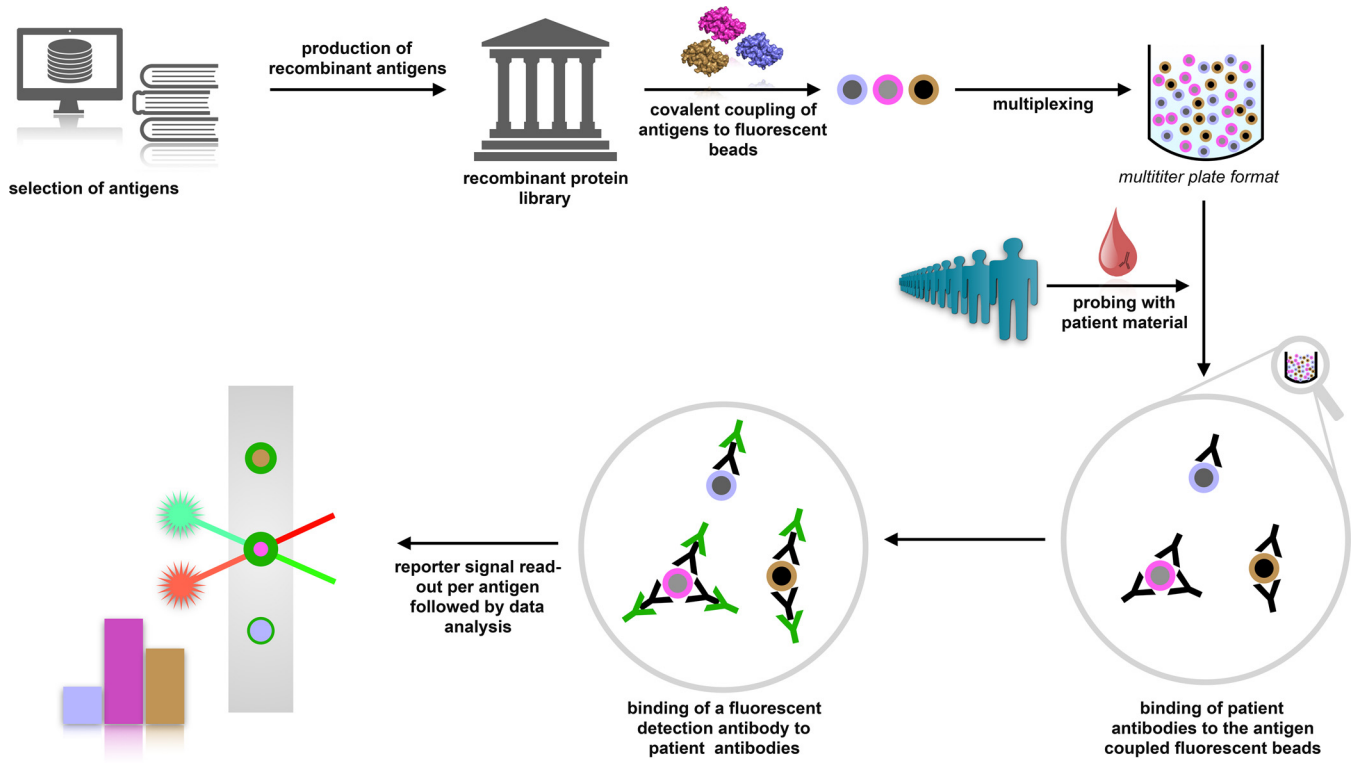

Fig. 3. Schematic representation of a suspension protein array workflow.
After selection and production, antigens are coupled to fluorescence-coded beads such that each fluorescence code corresponds to a single antigen. Following incubation with patient plasma/serum, antibody binding to the beads is 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-y, 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. *I*n 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

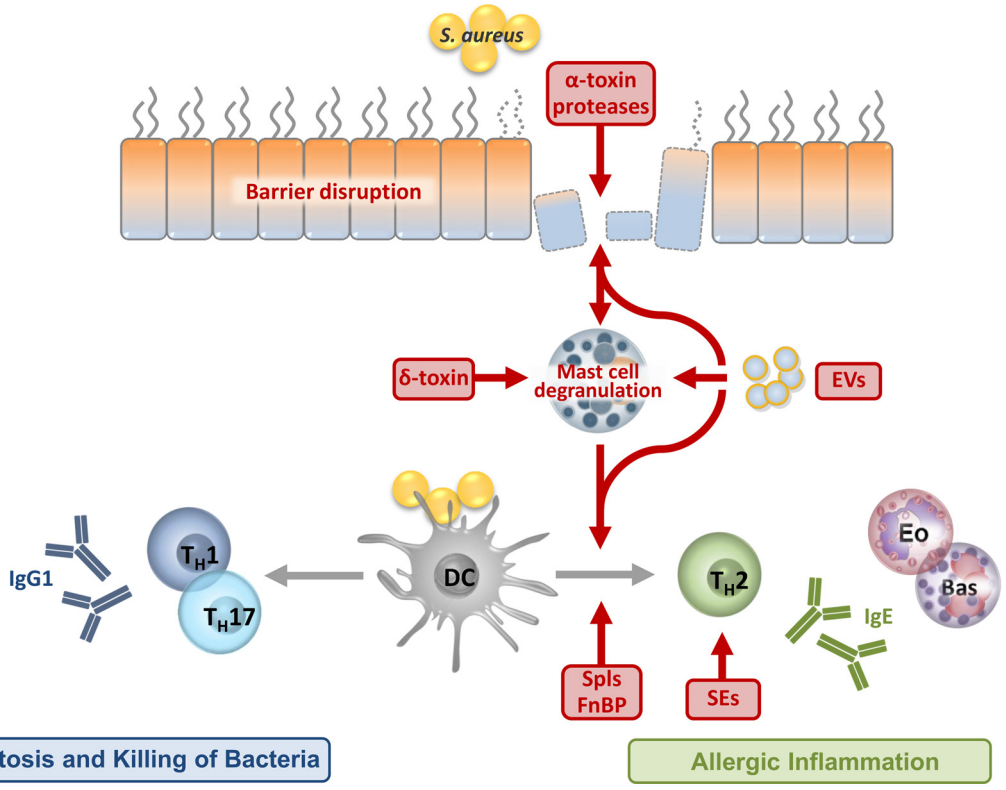

Fig. 4. Immune escape of *S. aureus* via the induction of type 2 inflammation.
Several *S. aureus* toxins facilitate allergen exposure by causing tissue damage. Cleavage of E-cadherin by Hla and bacterial proteases disrupt the epithelial barrier, facilitating the entry of other bacterial factors and 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.

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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.