INTRODUCTION

Exogenous proteases derived from a variety of different species such as mites, fungi, and bacteria are constantly challenging the body-own homeostasis and increasing the complexity of the protease network. Allergens with protease activity can be a seasonal trigger for atopic diseases like allergic rhinitis or allergic asthma, and the constant exposure of perennial allergens can persistently aggravate...
the chronic inflammation. Recently, studies have demonstrated that microbiota plays an essential role in fine-tuning the immune response. S aureus became an important player in the type 2 biased immune responses in the airways. S aureus is a Gram-positive, commensal organism with pathogenic properties. Over 30 percent of the human population are carriers of S aureus, harboring the bacteria either transiently or persistently in their nasal cavities. In the majority of human carriers, S aureus causes no detectable adverse effects. However, in patients with chronic rhinosinusitis with nasal polyps (CRSwNP), nasal colonization with S aureus and sensitization to S aureus enterotoxins are linked to a type 2 inflammatory response and asthma comorbidity. S aureus enterotoxin-specific immunoglobulin E was shown to be functional independent of the atopic status of patients. Importantly, S aureus enterotoxin sensitization is associated with allergic polysensitization in adolescents. Immunoproteomic analyses of the nasal polyps have identified detectable amounts of more than 600 S aureus proteins including α-hemolysin, γ-hemolysin, panton-valentine leukocidin, S aureus toxins, and S aureus protease-like proteins (Spls). Furthermore, Spls were shown to induce a specific immunoglobulin E (IgE) response in asthmatic patients.

Moreover, S aureus contributes to the initiation of a type 2 immune response by fast induction of innate cytokines and chemokines from airway epithelial cells. In murine models of co-exposure to Spls and ovalbumin (OVA), SplD breaks immune tolerance to the inert OVA, inducing the production of OVA-specific IgE and strong airway inflammatory responses. Recently, it has been stressed that IL-33 is a critical cytokine in allergic polysensitization and plays an essential role in an altered immune tolerance via activation of dendritic cells and the induction of a type 2 immune response. S aureus induces an increase in IL-33 protein levels in S aureus-infected nasal polytissue. It has been shown that mice treated with SplD could initiate an IL-33 mediated airway inflammation, supporting the role of S aureus-secreted factors. This mechanism could substantially contribute to the development of a severe and difficult to control airway disease (Figure 1).

In the current review, we position Spls among other allergens with protease activity and provide a critical overview of emerging roles of Spls in a type 2-biased immune response. Finally, we delineate the role of proteases in the activation of the IL-33 signaling pathway, which is upstream of a type 2 inflammatory response. We

**FIGURE 1**  Induction of an allergic immune response by *Staphylococcus aureus* serine protease-like protein D (SplD) in the airways. Airborne exposure to SplD causes a synergistic effect in allergic sensitization. SplD activates airway epithelial cells to release IL-33, which acts via ST2 receptors on innate lymphoid cells type 2 (ILC2), T helper cells type 2 (Th2), dendritic cells. These events cause an amplification of adaptive immunity and production of type 2 cytokines and result in reduced tolerance to allergens and aggravation of airway inflammation. Repeated exposures to SplD are associated with increased production of SplD-specific and allergen-specific immunoglobulin E (IgE). SplD facilitates the production of mucus due to proliferation of goblet cells. Airway hyperreactivity is also increased after SplD exposure.
discuss how interventions with the IL-33/ST2 signaling axis could modify allergic responses induced by bacterial allergens.

2 | EXOGENOUS PROTEASES—SEASONAL AND PERENNIAL ALLERGENS

2.1 | Airborne house dust mite allergens activate the airway epithelium

There are 24 groups of house dust mite (HDM) allergens out of which one comprises cysteine proteases and three groups are serine proteases. These proteases present a high IgE binding frequency, up to 100% in HDM allergic patients, and are thus major allergens. Der p 1 alone can decrease the epithelial barrier by disrupting tight junctions and increase specific IgE production due to its proteolytic activity, cleaving the low-affinity IgE receptor CD23, which leads to IgE upregulation, and the IL-2 receptor CD25 on T cells resulting in a shift toward Th2 cell activation.

Allergens with protease activity mediate their action through cleavage of a family of G protein–coupled receptors called proteinase-activated receptors (PARs). PAR2 is activated by cleavage of Der p 1. Toll-like receptor 4 (TLR4), expressed on structural lung cells, was shown to interact with PAR2, strongly supporting the inflammatory response toward HDM. PAR2 activation by HDM is important for IgE formation; however, airway inflammation occurs independently of PAR2. Furthermore, most allergens with protease activity (HDM, A. alternata, cockroach) induce release of damage-associated molecular patterns, such as IL-33, thymic stromal lymphopoietin (TSLP), adenosine triphosphate, and IL-1β damage-associated molecular patterns, such as IL-33, thymic epithelial cells in mice in vivo and airway epithelial damage. Der p 1 alone can decrease the epithelial barrier by disrupting tight junctions and increase specific IgE production due to its proteolytic activity, cleaving the low-affinity IgE receptor CD23, which leads to IgE upregulation, and the IL-2 receptor CD25 on T cells resulting in a shift toward Th2 cell activation.

2.2 | Fungal allergens as triggers for IL-33 production

Several fungal serine proteases from Aspergillus spp, Penicillium spp, Curvularia lunata, A. alternata, and others have been described as allergens (summarized in Table 1). Alternaria spp is a common outdoor mold but can also occur indoors in moldy dwellings. A alternata exposure in children is associated with elevated IgE levels and higher airway IL-33 levels. In mice, A alternata inhalation caused steroid-resistant airway hyperreactivity, dependent on IL-33. In humans, exposure to these fungal spores can lead to airway diseases such as allergic asthma, allergic rhinitis and sinusitis, allergic alveolitis, allergic pulmonary aspergillosis, or rhinosinusitis.

Alternaria alternata extract induces allergic asthma via IL-33 activation, dependent on the serine proteases in the extract. This further leads to activation of ILC2s and IL-5 production which causes the maturation of eosinophils in the bone marrow in response to A alternata. Next to the serine protease activity in the A alternata extract, an aspartic protease of A alternata activates PAR2 and triggers degranulation of eosinophils. Contradicting data can be found concerning the importance of protease activity in asthma induction. While heat-inactivated or protease inhibitor–treated A alternata extracts induce allergic asthma to the same extent as non-denatured extract in one study, another study shows that the protease activity is necessary for the development of lung inflammation and PAR2 activation.

Aspergillus fumigatus has four different proteases, the metallo-protease Asp f 5, an aspartic protease Asp f 10, and two serine proteases Asp f 13 and Asp f 18. Asp f 5 and Asp f 13 deletion mutants showed reduced inflammatory cell recruitment and IL-13 production compared to the wild type in a murine inhalation model; however, IgE levels and Th2 cytokine levels were comparable to the wild-type treated mice. Asp f 13 disrupts the epithelial barrier and the extracellular matrix of airway smooth muscle cells, leading to airway hyperreactivity in mice. Pen c 13, a serine protease from Penicillium citrinum, also increased epithelial permeability and allergic airway inflammation in mice.

2.3 | Bacteria as a source of allergens with protease activity

The fungal allergens Cur l 1 from Curvularia lunata and Cla h 9 from Cladosporium herbarum are subtilisin-like serine proteases. Subtilisin (also called Alcalase) is a serine protease from Bacillus spp acting as a bacterial allergen, as it can induce specific IgE and IgG4 formation, accumulation of eosinophils in the airway lumen and goblet cell hyperplasia in mice. The active subtilisin triggers an early release of amphiregulin, TSLP, IL-1β, and IL-33, while proteolytically inactive subtilisin cannot induce this response. IL-33 is processed to a more bioactive cytokine by subtilisin. In humans, subtilisin and savinase, another Bacillus spp protease, cause specific IgE formation. These findings are of special interest, because detergents contain protein engineered mutants of subtilisin and other bacterial
proteases that have an increased stability. Some of these bacterial proteases are also used by the food industry.46

2.4 | Staphylococcus aureus proteases in type 2 immune responses

Staphylococcus aureus possesses ten major secreted proteases including a serine protease (V8 protease), a metalloprotease (aureolysin), two cysteine proteases (staphopain A and staphopain B), and six serine protease-like proteins (SplA, SplB, SplC, SplD, SplE, and SplF). Spls are proteases encoded by the spl operon, located on the pathogenicity island vSab.47 The S. aureus V8 protease and staphopains were shown to alter airway epithelial barrier integrity, modulate cytokine synthesis, and activate nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) in the airway epithelial cells, therefore predisposing to allergic sensitization.48 Recently, it has been shown that the majority of nasal polyp tissue colonized with S. aureus contained IgG binding to SplA, SplB, and SplD/F.49 Importantly, serum of asthmatic patients contained higher titers of SplA, SplB, and SplE-specific IgE compared to controls suggesting their role in the pathogenesis of asthma.5 Moreover, SplD is produced by S. aureus in measurable amounts in the airways of patients with CRSwNP.11 Ex vivo nasal tissue explants produce IL-5 in response to SplD and intratracheal applications of SplD in mice result in induction of SplD-specific IgE.5 All these data demonstrate that, next to their detrimental role in the airway epithelial barrier integrity, S. aureus proteases act as allergens by inducing specific IgE response in humans and in mice.

SplD-induced inflammation in mouse lungs is not accompanied by the release of TSLP, IL-25, or GM-CSF,5 which is in contrast to experimental models using other allergens with protease activity such as HDM and A. alternata extracts.49,50 However, repeated intratracheal exposure of mice to SplD leads to a sustained production of IL-33 in the lungs and the development of key features of

TABLE 1 Overview of allergens with proteolytic functions from different taxonomic kingdoms

<table>
<thead>
<tr>
<th>Protease type</th>
<th>Allergen name</th>
<th>Origin species</th>
<th>Class (kingdom)</th>
<th>Reference</th>
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<td>Arachnida (animalia)</td>
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nd, not determined.
allergic asthma including eotaxin production, eosinophilia, bronchial hyperreactivity, goblet cell hyperplasia, and ILC2s infiltration of the airways.\textsuperscript{5}

It has been shown that the release of IL-33 upon exposure to SplD could exacerbate features of allergic asthma and contribute to an altered immune tolerance to other allergens. IL-33 is sufficient to aggragate airway hyperresponsiveness and type 2 airway inflammation in mice exposed to OVA\textsuperscript{12} or house dust mite.\textsuperscript{51} SplD mediates an IL-33-dependent sensitization to OVA.\textsuperscript{5} Likewise, increased levels of IL-33 positively correlate with specific IgE to inhalant allergens.

It is important to mention that SplF shows 94.6% sequence homology with SplD.\textsuperscript{54} In mice, SplF induces airway inflammation comparable to SplD.\textsuperscript{5} These data suggest a novel potent group of bacterial allergens present in S. aureus.

However, we have shown that SplD does not directly activate PAR2 and its downstream signaling in vitro, while in vivo PAR2 is increased in the SplD-exposed lungs. This indicates an indirect activation of PAR2.\textsuperscript{5} Moreover, IL-33 is an important regulator in the SplD-induced PAR2 activation, since in mice treated with SplD and sST2, the levels of PAR2 in the lungs were significantly decreased.\textsuperscript{5} These data support the notion that endogenous proteases facilitate innate immune response induced by allergens.

### 2.5 The role of proteases in the activation of IL-33

Interleukin-33 is one of the major cytokines in the regulation of the innate and adaptive immunity in the allergic airways.\textsuperscript{22,55,56} Repeated exposure to environmental factors and allergens with protease activity triggers the activation of the airway epithelium and the release of TSLP, IL-25 and IL-33, which in turn triggers a sustained immune response in dendritic cells and ILC2s and activation of type 2 immune response.\textsuperscript{27,57,58} Once released, IL-33 mediates its function upon binding to the ST2 receptor, a member of the Toll-like/IL-1 superfamily of receptors,\textsuperscript{59} widely expressed on ILC2s, Th2 cells, eosinophils, invariant natural killer T cells, natural killer cells, basophils, and mast cells.\textsuperscript{59-63} IL-33 binding to ST2 leads to the formation of a complex with the interleukin-1 receptor, and a receptor accessory protein,\textsuperscript{64} resulting in the recruitment of the adaptor protein MyD88.\textsuperscript{65} This recruitment activates the interleukin-1 receptor-associated kinase-1,-4, mitogen-activated protein kinase, and TNF receptor-associated factor-6, which results in the activation of NF-κB, p36, and the protein kinase c-Jun N-terminal kinases.\textsuperscript{59}

Unlike other members of the IL-1 family, such as IL-1β and IL-18, IL-33 does not require caspase-1 cleavage for its activation.\textsuperscript{66} IL-33 is inactivated by caspase-3- and caspase-7-mediated cleavage of its cytokine domain during apoptosis.\textsuperscript{67} It is known that the activity of IL-33 is regulated by endogenous proteases released during inflammation from epithelial cells, neutrophils, and mast cells. Endogenous proteases such as neutrophil elastase or cathepsin G were shown to activate IL-33 during bacterial, fungal, or viral infections.\textsuperscript{68} Cleavage of IL-33 by mast cell tryptase results in the generation of shorter mature forms of IL-33. Mature IL-33 is up to 30-fold more potent than the full-length IL-33 in its capacity to induce an expansion of ILC2s and Th2 cells, next to their IL-5 and IL-13 production.\textsuperscript{62} There are inconsistent data concerning the activation or degradation of IL-33 by human mast cell chymase.\textsuperscript{52,65} The murine mast cell chymase mMCP-4 has been shown to degrade IL-33.\textsuperscript{69,70} When IL-33 is incubated with allergens that have protease activity, it gets cleaved in its central cytokine domain, generating a shorter and more active form of IL-33, which efficiently activates ILC2s, basophils, mast cells, and eosinophils.\textsuperscript{27} Therefore, IL-33 acts as a sensor for aeroallergens with proteases activity to trigger the downstream inflammatory cascade.\textsuperscript{27}

It has been shown that the oxidation state of IL-33 affects the binding capacity of IL-33 to ST2.\textsuperscript{71} Oxidized IL-33 is most commonly found in the sputum from exacerbating asthmatics, which could be an intrinsic mechanism to limit an extensive inflammation. Both forms of IL-33, oxidized and reduced, are found during disease exacerbations in sputum of patients with moderate and severe asthma.\textsuperscript{71} Endogenous calpains, from damaged airway epithelial cells, can process full-length IL-33 and increase its alarmin activity up to ~60-fold.\textsuperscript{72}

It is important to point out that several alternatively spliced variants of IL-33 were detected in asthmatic patients.\textsuperscript{73} Among them, an IL-33 isoform lacking exons 3 and 4, that was shown to have a cytoplasmic localization in airway epithelial cells, could be actively secreted and activate mast cells and basophils.\textsuperscript{73} In asthmatic patients, this isoform is strongly associated with type 2 biased airway inflammation.\textsuperscript{73}

In CRSwNP mucosa, S. aureus induces epithelial cell-derived release of IL-33 and TSLP via cell wall compartment-induced TLR2 activation.\textsuperscript{15} However, it has been shown that SplD does not activate TLR2 directly\textsuperscript{5} suggesting that other recognition mechanisms are in place.
2.6 | Endogenous protease inhibitors in the airways

Natural protease inhibitors exist for serine, cysteine, metallo-, and aspartic proteases.74 Cystatin A is a cysteine protease inhibitor, and in combination with the serine protease inhibitor Kazal-type 5 (SPINKS5), it is involved in the regulation of the innate cytokine (IL-25, IL-33, and TSLP) response of *A alternata* extract or *S aureus* V8 protease, when exposed to human bronchial epithelial cells. In eosinophilic CRSwNP, the expression of cystatin A and SPINK5 is decreased compared to nasal tissue of noneosinophilic CRS.75

These findings underline the importance of antiprotease activity in the nasal mucosa for the prevention of detrimental effects of exposure to environmental allergens with protease activity and provide a possible explanation for the reduced capacity of epithelium to counteract the protease activity in CRSwNP, resulting in a sustained chronic type 2 inflammation. The role of this antiprotease network still needs to be discovered in the regulation of Spls' activity in chronic airway inflammation.

α1-Antitrypsin (A1AT) inhibits trypsin, KLK7, KLK14, neutrophil elastase, proteinase 3, and matriptase. It is an acute-phase protein with a high abundance in serum. Apart from inhibiting neutrophil proteases, A1AT also inhibits the migration and activation of neutrophils,76 next to preventing the proteolytic maturation of matrix metalloproteinase-9 (MMP-9) by serine proteases from German cockroach extract.77 IL-6 and to a lesser extent LPS, tumor necrosis factor-α (TNF-α), and IL-33 stimulate A1AT secretion from monocytes.76 Proteome analysis of nasal secretion from allergic rhinitis (AR) patients and healthy controls revealed 2.5-fold higher levels of A1AT in AR patients.79 The mast cell chymase mMCP-1 can be inhibited by A1AT1, A1AT2, and A1AT4 in the serum.80

Serine protease inhibitor B1 is produced by neutrophils and inhibits the neutrophil proteases elastase, cathepsin G, and proteinase-3. In a lung-infection model of *Pseudomonas aeruginosa* with SERPINB1-deficient mice, SERPINB1 proved to be an important factor for the survival, bacterial clearance, and SP-D degradation.81 Furthermore, serum levels of SERPINB3 and SERPINB4 are increased in asthma, chronic obstructive pulmonary disease, and atopic dermatitis patients. SERPINB3 inhibits cathepsins S, K, L, and papain, while SERPINB4 inhibits mast cell chymase, cathepsin G, granzyme M, and Der p 1. IL-4 and IL-13-stimulated bronchial epithelial cells upregulate these two serpins. In mice, serpinb3a also inhibits papain, Der p 1 and the cathepsins S, K, L, and G, as well as V. The activation of serpinb3a is dependent on IL-13 as IL-13-treated serpinb3a-deficient mice have a significantly diminished inflammatory response. In an allergic asthma model, HDM-treated serpinb3a-deficient BALB/c mice developed a similar eosinophilic response as the wild-type mice, but have a decreased mucus production.82

Genetic variations within proteases and their inhibitors have also been associated with asthma, atopy, or chronic inflammatory lung diseases. For example, two polymorphisms of the MMP9 gene are associated with childhood atopic asthma risk, one of which upregulates the expression levels of MMP9.83 A polymorphism downstream of mast cell chymase gene (CMA1) is associated with bronchial asthma, atopic asthma, and IgE levels.84,85 Furthermore, one polymorphism in the promoter region of CMA1 is associated with atopic eczema.86 The variability in copy number of human α-tryptase can also have an impact on total and HDM-specific IgE levels in asthmatic patients. Patients carrying one copy of α-tryptase had lower IgE levels and worse lung function, but two copies correlated with higher total and HDM-specific IgE levels.87 Interestingly, α-tryptase is suggested to be enzymatically inactive.88 Hereditary loss of α1-antitrypsin/SERPINA1 results in early-onset chronic obstructive pulmonary disease and emphysema.89 A downstream mutation in serine protease inhibitor A3 (SERPINA3) has been identified as a possible susceptibility locus for childhood asthma.90 Interestingly, cystic fibrosis patients deficient of α1-antichymotrypsin/SERPINA3 have significantly milder symptoms of lung inflammation than non-α1-antichymotrypsin deficient patients.91

Whether there is a possible link of serine protease inhibitor production with the susceptibility of chronic inflammatory airway disease development upon *S aureus* colonization still needs to be investigated.

2.7 | Interference with the ST2/IL-33 signaling axis for therapeutic purposes

Blocking of the IL-33 pathway by different pharmacological and genetic tools can be a promising and an efficient strategy to inhibit inflammatory changes induced by different groups of allergens. Indeed, the critical role of IL-33 in the initiation and modulation of allergic airway disease has been already proven (Table 2). Next to IL-33 blocking or IL-33 neutralizing antibodies, sST2 is an elegant way to inhibit the IL-33 signaling pathway. sST2 functions antagonistically to IL-33, acting as a decoy receptor and preventing IL-33 from binding to the membrane bound isoform ST2, thereby inhibiting its effector function.92,93 Anti-IL-33 antibodies as well as sST2 were shown to be efficient in reduction of the most prominent features of murine OVA-induced allergic asthma models such as eosinophil counts and IL-4, IL-5, and IL-13 in bronchoalveolar lavage fluid.94,95 Airway hyperreactivity to methacholine was also efficiently downregulated by this treatment in mice. However, the relatively short half-life of existing pharmacological compounds for binding active IL-33 does not allow the full prevention of airway remodeling and specific IgE production when using these compounds in the therapeutic regimen in a murine model of asthma, for example, induced by OVA or combination of HDM and diesel exhaust particles.96 A phase 1 clinical study to evaluate the safety and tolerability of a single dose of anti-IL-33R antibody (CNT1 7160) in healthy participants and multiple doses in participants with asthma and atopic dermatitis has been completed; however, no results have been reported yet. All these studies indicate that interference with the IL-33/ST2 signaling axis is a very promising strategy in preventing type 2 inflammation in diseases like CRSwNP and asthma induced by bacterial allergens.
TABLE 2 The role of different IL-33 targeting strategies on inflammatory changes in mouse models of allergic asthma

<table>
<thead>
<tr>
<th>Allergen</th>
<th>AHR</th>
<th>Inflammatory infiltration of lungs</th>
<th>Th2 cytokines/chemokines</th>
<th>IgE response</th>
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<td>OVA</td>
<td>↓ AHR</td>
<td>na</td>
<td>na</td>
<td>109</td>
</tr>
<tr>
<td>sST2 soluble receptor</td>
<td>OVA</td>
<td>na</td>
<td>↓ IL-4, IL-5, IL-13</td>
<td>na</td>
<td>95</td>
</tr>
<tr>
<td>OVA</td>
<td>na</td>
<td>↓ Eosinophils</td>
<td>↓ IL-4, IL-5, IL-13</td>
<td>na</td>
<td>110</td>
</tr>
<tr>
<td>OVA-DCs</td>
<td>na</td>
<td>↓ Eosinophils</td>
<td>na</td>
<td>na</td>
<td>111</td>
</tr>
<tr>
<td>HDM</td>
<td>na</td>
<td>↓ Eosinophils</td>
<td>ns</td>
<td>↓ Total IgE</td>
<td>49</td>
</tr>
<tr>
<td>S aureus SplD</td>
<td>↓ AHR</td>
<td>↓ Eosinophils</td>
<td>↓ IL-4, IL-5, IL-13</td>
<td>↓ Total IgE ns specific IgE</td>
<td>9, 5</td>
</tr>
</tbody>
</table>

DCs, dendritic cells; HDM, house dust mite; IgE, immunoglobulin E; na, not analyzed; ns, not significant; OVA, ovalbumin.

3 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In this review article, we underlined that the IL-33/ST2 signaling axis is activated in response to the major inhaled allergens from fungi, mites, and bacteria and highlighted the role of protease/anti-protease network in balancing the activity of exogenous proteolytic enzymes. Therefore, both signaling pathways represent potential targets for the future therapeutic interventions to prevent severe chronic type 2 biased airway inflammation. Deeper understanding of the interaction of S aureus-secreted compounds and subsequent immune responses in the host might help to understand how colonization of S aureus or exposure due to inhaled S aureus compounds can bias toward type 2 immune response in asthma and CRSwNP. Identification of these complex interactions between S aureus and the immune system may lead to the development of novel efficient therapeutics to prevent an exuberant activation of the immune system induced by pathogenic germs colonizing mucosal surfaces. It is important to state that many questions remain regarding the predisposition to a conversion of commensal S aureus lifestyle into pathologic colonization. It remains to be analyzed whether S aureus proteins contribute to initiation and acute exacerbations of human asthma and CRSwNP in the same way as shown in mice. Answers to these remaining questions will eventually provide us with the necessary insights on how to modulate either S aureus and its proteases or the host response in order to treat chronic type 2 diseases when other treatment strategies fail.

ACKNOWLEDGMENTS

This work was supported by grants from FWO-Vlaanderen G065319N, BOF 14-GOA-019 from Ghent University (CB, DVK). ART is a doctoral fellow paid by FWO-Vlaanderen. Research in the DVK group is supported by FWO-Vlaanderen (1506218N, 1507118N, and G051918N) and Ghent University (BOF 01/O3618).

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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