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

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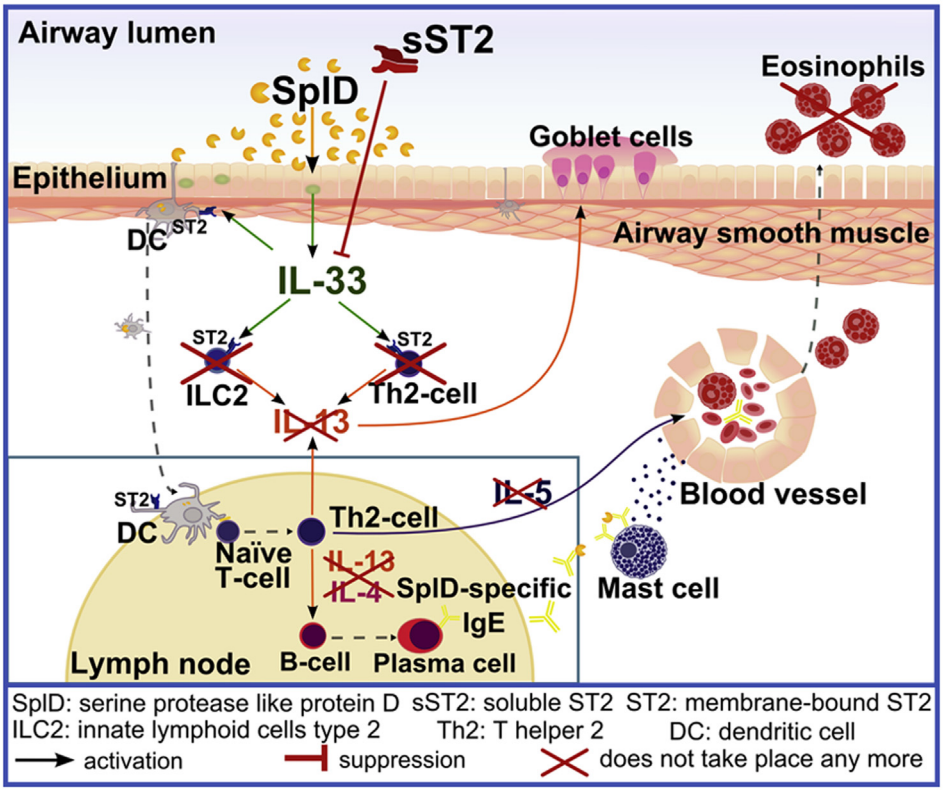
GRAPHICAL ABSTRACT



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

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

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

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

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

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

Abbreviations used

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

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

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

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

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