



Th2 inflammatory responses in the development of nasal polyps and chronic rhinosinusitis

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Purpose of review

Pathogenesis of nasal polyp has been largely studied based on innate and adaptive immunity of sinonasal mucosa. So far, various factors have been identified that trigger an inflammatory response in the pathogenesis of nasal polyps. In this review, we summarized recently updated information in the understanding of mechanisms in the development of chronic rhinosinusitis with nasal polyp (CRSwNP) focusing on Th2 inflammation.

Recent findings

Endotype of CRSwNP presented mainly Th2-skewed inflammation, and it has been associated with refractoriness and comorbidities. *Staphylococcus aureus* can drive Th2 inflammation by producing enterotoxins and serine protease-like protein. Moreover, *S. aureus* directly affected mucosal barrier function and enhanced Th2 cytokine production by fast induction of epithelial-derived innate cytokines. Epithelial-derived innate cytokines, including TSLP, IL-25, and IL-33, promote Th2 responses via the development of innate lymphoid cells. Mast cell expresses IL-5, IL-13, and periostin, and it plays a role in the pathogenesis of nasal polyps through orchestrating eosinophil infiltration. Formation of eosinophil extracellular traps and Charcot–Leyden crystals is strongly associated with disease severity and viscous mucus plug production. Therefore, it needs to be investigated mechanistically. The role of neutrophils in Th2 inflammation has been poorly understood but appears to enhance Th2 inflammation and make it more resistant to steroid therapy.

Summary

There is growing evidence of the role of *S. aureus* in innate and acquired immunity, which contribute to Th2 inflammation in CRSwNP. Innate immunity, including epithelial-derived cytokines, plays a crucial role in the development of CRSwNP by inducing various pathways and need to be investigated more as Th2-targeted biomarkers. Recently, the role of neutrophilic inflammation in Th2 inflammation has started to be studied but still remains unclear.

Keywords

eosinophil, IL-33, innate immunity, nasal polyp, rhinosinusitis, *Staphylococcus aureus*

INTRODUCTION

Chronic rhinosinusitis with nasal polyp (CRSwNP) is a multifactorial and heterogeneous inflammatory disease of the nose and the paranasal sinuses [1]. Sustained inflammation of sinonasal mucosa resulting in nasal polyp formation causes typical symptoms, such as nasal obstruction/blockage and purulent discharges. Epithelial barrier dysfunction, exposure to allergens or pathogens, and dysregulation of immune function are associated with nasal polypogenesis [2]. CRSwNP is dichotomously divided into eosinophilic and noneosinophilic nasal polyp based on the presence of tissue eosinophilia [3]. Accumulation of eosinophils in nasal polyp and infiltration of other inflammatory cells are strongly induced by Th2 cytokines expressed from CD4⁺ Th2 cells and group 2 innate lymphoid cells (ILC2) [4].

To date, endotyping of CRSwNP has been elucidated that inflammatory profiles of nasal polyp vary by regions, ethnicity, age, and comorbidities [3,5–9].

Additionally, endotype-driven treatment approach and patient consult would help care for CRS patients

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KEY POINTS

- *Staphylococcus aureus* directly modulates innate immune responses via epithelial-derived cytokines, such as IL-33 by producing exoproducts, such as SplD, in addition to T-cell-mediated immunomodulation via staphylococcal enterotoxins, contributing to Th2 inflammation.
- Epithelial-derived innate cytokines, including TSLP, IL-25, and IL-33, has the function as an alarmin, and promote Th2 responses via group 2 innate lymphoid cells, mast cells, and myeloid cells.
- Formation of eosinophil extracellular traps and Charcot-Leyden crystals is strongly associated with disease severity and need to be investigated mechanistically.
- The role of neutrophils in Th2 inflammation has been poorly understood but appears to enhance Th2 inflammation and make it more resistant to steroid therapy.

[10,11]. Nonetheless, Th2 inflammation is still a vital player of the pathogenesis of CRSwNP [3], and is associated with disease refractoriness and asthma comorbidity [12]. Therefore, several biologics targeting Th2-inflammation have been developed and proved its effects on nasal polyp [13–15]. Moreover, many researchers are focusing their efforts on unraveling mechanism of refractory CRSwNP characterized by the mixed inflammation of Th2 and Th17 types, which are resistant to steroid therapy [16].

The purpose of this review is to summarize recent updates of Th2 inflammation in the development of nasal polyps, focusing on potential factors of disease refractoriness.

STAPHYLOCOCCUS AUREUS AS A TH2 INDUCER IN NASAL POLYPS

S. aureus colonization has been observed more frequently in patients with CRSwNP compared with control participants [6,17]. CD4⁺ T cells, which express staphylococcal enterotoxins-targeting T-cell receptor V β genes are expanded in nasal polyps so that they can enhance polyclonal IgE production and contribute to Th2 inflammation in the airway mucosa [18,19]. In addition to its superantigenic properties, *S. aureus* actively modulates the host immune response by releasing proteins that facilitate bacterial invasion and colonization. Exoproducts from *S. aureus* induced lower repair rates and reduced lamellipodial protrusion length and velocity in primary nasal epithelial cells from CRSwNP than those from control participants, implying

S. aureus-induced physical epithelial dysfunction [20[¶]]. Additionally, aberrant immune responses of nasal polyp tissues facilitate entry of *S. aureus* into tissues. IFN- λ 1 stimulation induces MUC5AC and MUC5B in the epithelial layer in control tissue suggesting that in healthy nasal tissue, IFN- λ 1 can exert antibacterial function through upregulation of mucin synthesis and secretion, but in nasal polyp tissue, IFN- λ 1 is not protectively working, possibly because of defective mucin induction [21]. Defective expression of tight junction proteins and barrier leakiness were observed and were not upregulated with stimulation of *S. aureus* in nasal polyp tissues, whereas healthy tissues showed protective mechanism working [22]. Increased pro-inflammatory cytokines, such as IFN- γ and IL-4 within NPs induce a disruption in epithelial integrity, consequently forming surface deficits [23]. These defects in the epithelial layer may provide an opportunity for bacteria-related substance on the mucosal surface to disperse and penetrate the CRS tissue. Intracellular persistence of *S. aureus* can be identified in epithelial cells, inducing inflammatory cytokines, such as IL-6 [24] and also observed in mast cells in CRS tissues, which seems to be promoted by staphylococcal enterotoxins [25]. Internalization of *S. aureus* within cells help it survive with nutrition-rich cytosol and replicate by protecting it from antimicrobial activities, eventually allowing it to perpetuate chronicity of the disease.

S. aureus directly contributes to the initiation of Th2 inflammation by fast induction of epithelial-derived innate cytokines including thymic stromal lymphopoietin (TSLP) and IL-33 in nasal polyp tissues, not in healthy turbinate mucosa whereas *S. epidermidis* did not induce these cytokines. NF- κ B activation via toll-like receptor 2 pathway was involved in *S. aureus*-induced release of TSLP and IL-33 in BEAS-2B cell culture [26^{¶¶}]. Another evidence about the direct role of *S. aureus* in the immunoregulation of nasal polyps is that a significantly higher release of IL-5 was observed at the *S. aureus*-infected tissues. The killing of *S. aureus* by antibiotics or *S. aureus*-specific intravenous staphylococcal phages significantly reduced the IL-5 production from nasal polyp tissues [27]. Specifically, serine protease-like protein (Spl) D, one of the subtypes of six Spl (SplA-SplF) produced by *S. aureus*, causes Th2-biased inflammatory responses in an IL-33-dependent manner in allergic airway disease, such as asthma [28[¶],29^{¶¶}]. Repeated airway exposure to SplD led to IL-33 production, eosinophilic inflammation, and bronchial hyperactivity. Blocking IL-33 with a soluble ST2 (the ligand-binding chain of the IL-33 receptor) receptor significantly abrogated eosinophilic inflammation. SplD-induced eosinophilia in

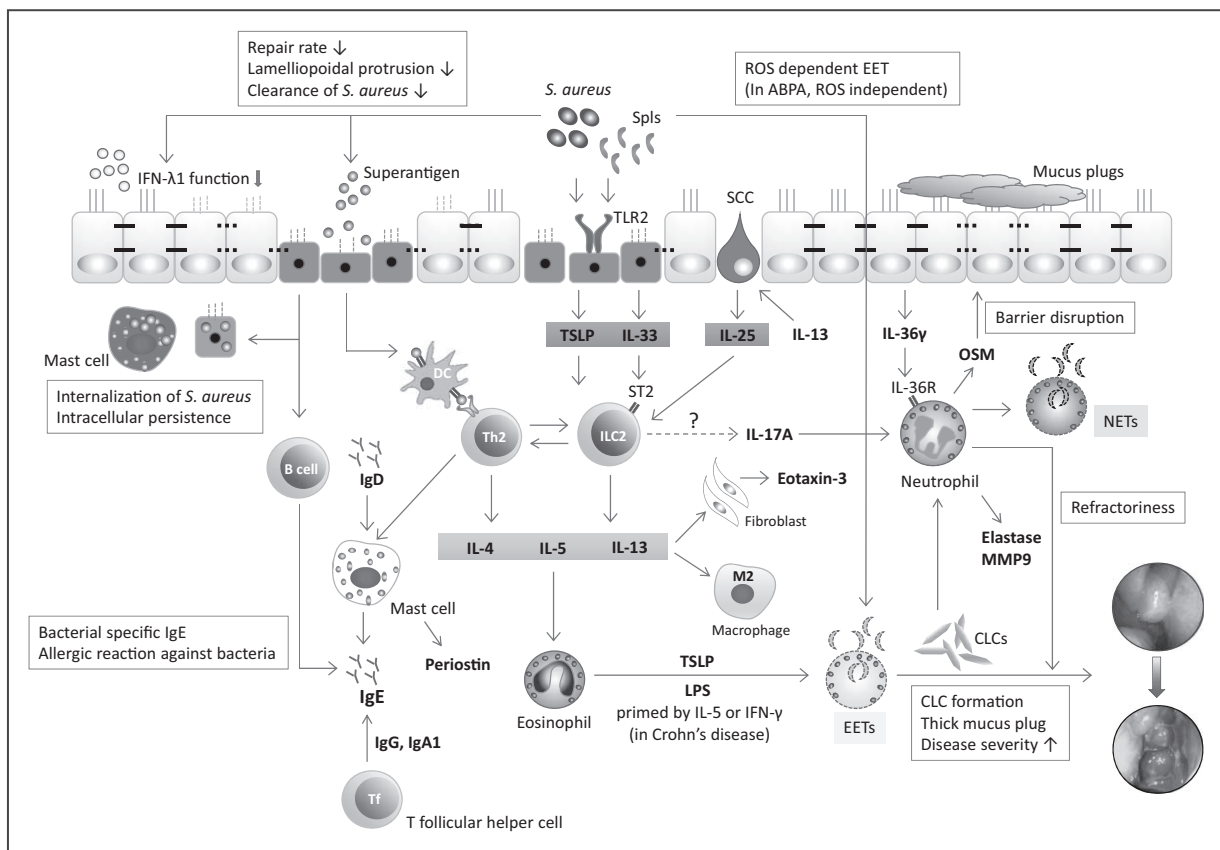


FIGURE 1. Overview of Th2 inflammatory pathways in the pathogenesis of nasal polyps. CLC, Charcot-Leyden crystal; DC, dendritic cell; EET, eosinophil extracellular trap; ILC2, group 2 innate lymphoid cell; LPS, Lipopolysaccharide; NET, neutrophil extracellular trap; OSM, oncostatin M; ROS, reactive oxygen species; SCC, solitary chemosensory cell; SpIs, serine protease-like protein; TLR2, toll-like receptor 2; TSLP, thymic stromal lymphopoietin.

the lung was completely abolished in Rag2^{-/-} mice, implying the adaptive immune systems is essential for SplD-induced airway inflammation [29[■]]. Interestingly, SpIs were detectable in nasal polyp tissues using proteomics analysis [27,30]. From previous data, it is postulated that *S. aureus* is involved in SplD/IL-33/ST2 signaling, contributing to Th2 inflammation in CRSwNP (Fig. 1). SplF were also found in nasal polyp tissues, which share 93% amino acid sequence with SplD, and IgG binding to SplA and SplG were also identified [28[■]]. The levels of Spl-specific IgE (SplA, SplB, SplD, and SplE) were increased in sera of asthma patients compared with healthy controls, and these were markedly associated with Th2 inflammation.

In addition to the direct role of bacterial substance in developing Th2 inflammation, nasal bacteria may be causative allergens in CRS, especially refractory CRSwNP, which had long disease duration and needed repeated surgeries [31[■]]. Takeda *et al.* characterized monoclonal antibody derived from nasal polyp-residing plasmablasts to investigate IgE reactivity of CRSwNP and demonstrated

that 10–37% of isolated IgE antibodies specifically recognized the surface structure of nasal bacteria including *S. aureus*, *Streptococcus pyogenes* and *Haemophilus influenzae* and contained relatively large numbers of mutations in the variable region of immunoglobulin heavy chain. Through high-throughput DNA sequencing immunoglobulin analysis, they proved that nasal polyp-derived IgE shared connectivity with IgG and IgA but not IgM, indicating class-switching to IgE within lineages after the accumulation of somatic mutations in nasal polyp. Nasal bacteria-specific IgE in nasal polyps originated from polyp-residing IgG⁺ and IgA⁺ germinal center B-like cells. Interestingly, previous studies reported that local IgE productions and local class-switch recombination from ectopic lymphoid tissues or tertiary lymphoid organs contribute to the development of eosinophilic nasal polyps and the refractoriness of CRSwNP [32,33]. In brief, a protective antibacterial response in healthy condition can be converted into Th2 inflammatory responses in CRSwNP.

EPITHELIAL-DERIVED CYTOKINES CONTRIBUTING TO TH2 INFLAMMATION IN THE PATHOGENESIS OF NASAL POLYPS

Epithelial-derived innate cytokines, including TSLP, IL-25, and IL-33, promote Th2 responses via the development of ILC2s in allergic disease [34,35]. Previous studies demonstrated that IL-25 and IL-33 were upregulated in human nasal polyp tissues and neutralizing antibodies to IL-25 and IL-33 reduced inflammation in murine nasal polyp model [36–38]. IL-25, also known as IL-17E, has a function as alarmin and is upregulated in patients with CRSwNP [39]. In asthma mice model, IL-25 can promote type 2 myeloid cells (T2M), which is classified type 2 cytokine-producing non-B, non-T cells, and T2M cells play a pivotal role in immediate response in asthma and prime type 2 responses before T-cell activation [40]. In nasal polyps, one of the major sources of IL-25 was reported to be specialized taster epithelial cells, such as solitary chemosensory cells (SCCs) in nasal polyps and exogenous IL-13 increased populations of SCCs by 40% and this effect was reversed by steroid treatment [41]. Although IL-25 is innate cytokines involving Th2 inflammation, serum and local IL-25 levels seem clinically recognized as a predictive biomarker of corticosteroid sensitivity in airway allergic diseases, such as asthma and nasal polyps [42,43]. IL-33, a member of the IL-1 cytokine family, is also an alarmin cytokine triggered by allergens or infection and shortly produce IL-5 and IL-13 by ILC2s [44]. ST2 was over-expressed in ILCs from CRSwNP tissues compared with chronic rhinosinusitis without nasal polyp (CRSsNP), and ST2-positive ILCs were a source of IL-13 in response to IL-33 [45]. However, there are still controversies about the expression levels of IL-33 in nasal polyp tissues. That may be associated with the fact that the cleaved IL-33 molecules strongly show more biological activity rather than full-length IL-33 concentration [46]. Therefore, we need to know information about the size of IL-33 in human samples from different diseases to apply IL-33 to a clinical setting as a biomarker. TSLP is upregulated in eosinophilic nasal polyps and degraded by proprotein convertase from nasal polyp extracts. Truncated TSLP strongly activates mast cells, myeloid dendritic cells, and group 2 innate lymphoid cells compared with mature TSLP, consequently contributing to Th2 polarization and eosinophilic inflammation [47,48]. Recently, in uncontrolled asthma, randomized, double-blind, placebo-controlled trials were performed using tezepelumab, a human monoclonal antibody specific for TSLP. An intriguing observation was that

patients with tezepelumab had lower rates of clinically significant asthma exacerbations than controls, independent of baseline blood eosinophil counts [49]. It implies that blocking TSLP may control non-Th2 inflammation as TSLP is an upstream pathway and influenced by nonallergic factors, such as virus, protease, and cigarette smoking as well as allergic factors.

Recently, the plasma level of eotaxin-3 showed correlations with IL-33 and TSLP and was suggested as a biomarker of eosinophilic CRS [50^{*}]. In this study, eotaxin-3 originated from nasal fibroblasts, which were activated by IL-13 stimulation. In another study, eotaxin-3 was induced by IL-13 stimulation from nasal epithelial cells, interestingly, IL-13-induced eotaxin-3 production is linked with nongastric H, K-ATPase, which is abrogated by proton pump inhibitors (PPI) [51]. *ATP12A* siRNA, used for knockdown of nongastric H, *K-ATPase* gene, significantly reduced the induction of eotaxin-3 by IL-13 in transfected epithelial cells. IL-13-induced phosphorylation of STAT6 (transcriptional signaling molecule of IL-13-induced eotaxin-3) was not affected by PPI, whereas PPIs enhanced decay of eotaxin-3 mRNA suggesting that posttranscriptional degradation of eotaxin-3 mRNA by PPI. However, prospective clinical trials have not been reported yet about the effectiveness of PPIs in CRSwNP.

IGE-MEDIATED RESPONSES IN THE PATHOGENESIS OF NASAL POLYPS

The mast cell is one of the essential inflammatory cells in CRSwNP, and it expresses Th2 cytokines, including IL-5 and IL-13 [52,53]. Mast cells play a role in the pathogenesis of nasal polyps through orchestrating eosinophil infiltration and inducing tissue remodeling by extracellular matrix degradation. Previously, we demonstrated that mast cells in eosinophilic nasal polyps were a cellular source of periostin mediated by IgE stimulation, a potent inducer of Th2 inflammation, and mast cell interacts with epithelial cells via the periostin-integrin α V pathway, resulting TSLP secretion [54]. It has been reported that IgD is increased in CRSwNP and CRSsNP and IgD-activated mast cell-induced IgE production and contributed to eosinophilic nasal polyps [55–57]. In eosinophilic nasal polyps, kynurenine, an endogenous tryptophan metabolite, and aryl hydrocarbon receptor signaling promote IgE-induced mast cell activation through the upregulation of reactive oxygen species generation, and subsequently induce pathogenic progression of CRSwNP [58]. A recent study has shown that pro-inflammatory local IgE in CRSwNP was antagonized by the corresponding IgG indicating a regulatory

effect of IgG in IgE-mediated inflammation in CRSwNP [59].

EOSINOPHILIC EXTRACELLULAR TRAPS AND MUCUS FORMATION

It has been demonstrated that eosinophil extracellular trap (EET) is a part of the innate immune response and is observed in infectious, allergic, and autoimmune diseases [60,61]. Cytolysis of both live and lytic eosinophils releases nuclear materials, including histones and DNA, and granular proteins onto the extracellular matrix [62]. Extracellular DNA deposits were termed extracellular traps, which were first described in neutrophils [63]. Asthmatic airway specimen exhibited eosinophilic and/or neutrophilic extracellular DNA traps [64]. EET formations are morphologically characterized web-like chromatin structures through the breakdown of nuclear and plasma membranes, which are composed of mitochondrial DNA and toxic eosinophil granules. Lipopolysaccharide activated blood eosinophils primed by IL-5 or IFN- γ from patients with Crohn's disease, and many eosinophils released DNA *in vitro* [60]. TSLP, one of the epithelial-derived innate cytokine, can stimulate EET formation of eosinophils in atopic skin disease [65]. In CRSwNP, 8.8% of total eosinophils exhibited EETs, and it was associated with Th2 inflammation and *S. aureus* colonization [66]. These two prior researches suggested that *S. aureus* and its exotoxins induced EETs dependent on reactive oxygen species (ROS) production [65,66]. On the contrary, *Aspergillus fumigatus* in allergic bronchopulmonary aspergillosis (ABPA) patients produced EET independent of ROS [67].

During the extracellular trap, cell death (namely ETosis) in eosinophils, intracytoplasmic Charcot-Leyden crystals (CLCs) are formed, and plasma membrane rupture accelerates extracellular CLC formations [68]. Both EET and CLC associated with disease severity of CRS and corticosteroid response in CRSwNP [69,70]. CLCs are composed of galectin-10 which have a ability to self-aggregate and to stimulate innate and adaptive immunity. Neutrophils were prominently infiltrated after intratracheal injection of galectin-10 crystals in naïve and OVA-induced mice [71]. This pathway is probably one of the possible mechanisms that mixed inflammation occurs in disease with severe type 2 inflammation, such as asthma and CRSwNP. EETs add substantial effects on the viscosity of eosinophil-rich mucus in CRS, eosinophilic otitis media, and ABPA patients [61]. Eosinophil products, especially eosinophil peroxidase, mediates the formation of mucus plugs in patients with severe asthma [72]. In a murine asthma model, EET was increased in

bronchoalveolar lavage fluid and lung tissues, and treatment with recombinant DNase reduced airway resistance, goblet cell hyperplasia, and EET formation [73,74]. Thick mucus plugs sometimes containing CLCs in CRSwNP have been considered as a particular problem. Type 2 inflammation, promoting airway mucus plug formation might be applicable in new therapeutic target in CRSwNP.

ROLE OF TH17 AND NEUTROPHILIC INFLAMMATION IN TH2 INFLAMMATION OF NASAL POLYPS

Most of CRSwNP have been reported highly eosinophilic in Western countries, unlike Asian countries. Refractory CRSwNP was characterized by severe eosinophilic inflammation mixed with neutrophils [6,16]. However, a few roles of neutrophils have been known in CRSwNP. Recently, neutrophils are reported to be a major source of Oncostatin M, which is involved in epithelial integrity during wound repair in CRSwNP and asthma [75]. Additionally, oncostatin M-producing neutrophils are supposed to express arginase 1 suggesting anti-inflammatory or tumorigenic N2 neutrophil phenotype, although it is not fully established. Wang *et al.* [76] proposed a novel mechanism by which neutrophils drive disease to persistence and chronic phase. IL-36 was the most abundant isoform and mainly expressed by epithelial cells in CRS. Tissue neutrophils mostly expressed the IL-36 receptor in nasal polyp tissues whereas IL-36R expression was almost absent in blood neutrophils. Cleaved IL-36 γ degraded by neutrophil elastase binds to IL-36R on neutrophils and activates it to produce matrix metalloproteinase 9, IL-17A, and chemokine (C-X-C motif) ligands 1, 2, and 8. IL-17A, in return, elicits IL-36 γ production in epithelial cells. Interestingly, the pro-inflammatory effect of IL-36 γ was not abrogated with dexamethasone treatment. The most intriguing investigation would be how neutrophils or Th17 inflammation regulates eosinophilic inflammation or Th2 responses. Unfortunately, there are few reports on this topic in CRSwNP. In a murine asthma model, blockade of Th2 cytokines, such as IL-4/IL-23 induced compensatory Th17 inflammation [77]. However, simultaneous blockade of IL-13 and IL-17 protected mice from eosinophilia, mucus hyperplasia, and airway hyperreactivity and abolished the neutrophilic inflammation suggesting the role of IL-17A in Th2 inflammation in asthma. The other studies showed that Th17-associated mediators, including IL-17A, IL-22, and IL-23 enhanced Th2-mediated eosinophilic inflammations in mice models or human research [78,79]. Dispersed nasal polyp cells (DNPC) from CRSwNP patients

constituting both Th2 and Th17 endotypes were stimulated by Th2 or Th17 cytokines to explore the interaction [80]. IL-4 and IL-13 inhibited the expression of Th17-related cytokines in DNPCs, whereas Th17 cytokines, IL-17, and TGF- β , increased levels of Th2 cytokines. This experiment using DNPCs could not explain the specific regulatory mechanism of Th2 and Th17 pathways, but it implicates that Th2 inflammation in CRSwNP is enhanced by Th17 stimulation. IL-33-induced neutrophil selectively produced Th2 cytokines, including IL-4, IL-5, IL-9, and IL-13 in allergic asthma model [81]. The previous investigation implies that Th2 and Th17 pathways regulate each other, favoring the development of a Th2 immune response in allergic airway diseases. Some subsets of neutrophils, CD16^{high} CD62L^{dim} neutrophils, were manifested in CRSwNP, and these neutrophils had abilities to phagocytose bacteria and induce reactive oxygen species [82].

CONCLUSION

A European study on endotypes of CRS showed that refractory CRS presented severe Th2 inflammation characterized by the presence of nasal polyp in nearly all cases and asthma comorbidity in up to 70%. Its endotype was characterized by higher concentrations of tissue staphylococcal enterotoxin-specific IgE and neutrophilic markers [6]. *S. aureus* contributes to Th2 inflammation by producing enterotoxins, which bypass interaction between T-cell receptor and MHC II eventually maximizing T-cell responses. Additionally, *S. aureus*-derived SpID is directly involved in IL-33/ST2 signaling contributing to Th2 inflammation. Antibacterial response in healthy condition can be aberrantly converted to allergic reaction against bacteria, contributing to Th2 inflammation in CRSwNP. Multiple biomarkers, such as epithelial-derived cytokines and eotaxin-3 have been suggested in clinical settings and end products of eosinophilic inflammation, such as ETT and CLC have an attraction in terms of diagnostic and therapeutic aspects (Fig. 1). Lastly, the role of neutrophils in Th2 inflammation has been poorly understood but appears to enhance Th2 inflammation and make it more resistant to steroid therapy.

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Conflicts of interest

There are no conflicts of interest.

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