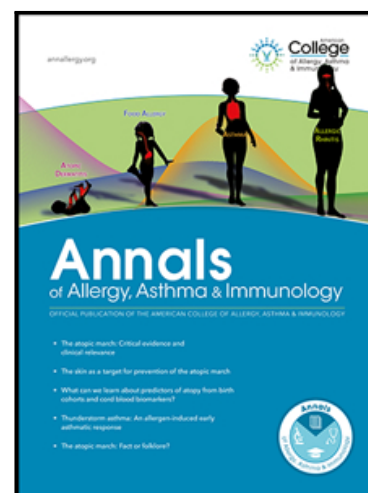


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**Pathophysiologic mechanisms of chronic rhinosinusitis and their roles in
emerging disease endotypes**

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Running title: Endotypes of CRS

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Key words: Chronic rhinosinusitis; endotype; immune response; nasal polyp; treatment

Abbreviations:

CRS, chronic rhinosinusitis; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; ILCs, innate lymphoid cells; Th, T helper; Tc, Cytotoxic T; Tfh, Follicular T helper; IL, interleukin; IFN- γ , interferon γ ; TGF- β , transform growth factor β ; TNF- α , tumor necrosis factor α ; MPO, myeloperoxidase; ECP, eosinophil cationic protein; TSLP, thymic stromal lymphopoietin; DC, dendritic cells; OX40L, OX40 ligand.

Key Messages

1. Heterogeneous groups of disorders with distinct pathogenic mechanisms are included under the terms of both CRSsNP and CRSwNP.
2. The heterogeneity of CRS influences the efficacy of medical and surgical treatments and contributes to the inadequate disease control.
3. Based on the emerging linkage between effector T cells in adaptive immunity and ILCs lineages, a convergence of innate and adaptive immune systems into type 1, type 2 and type 3 immune responses has been proposed.
4. An endotype of CRS with prominent type 2 inflammatory response is consistently identified. This endotype of CRS is characterized by inflammatory pathways involving IL-5, IL-13, and IgE, which highly associate with tissue eosinophilia, polyp formation, asthma comorbidity, severe disease, and recurrence after sinus surgery.
5. Epithelium derived cytokines TSLP and IL-33 may play a role in type 2-high CRS by regulating DCs and ILC2s, respectively. Local IgE production and IgE mediated mast cell activation may play an important role in tissue eosinophilia in type 2-high CRS.
6. Several biologic medications are available in type 2-high CRS.

ABSTRACT

Objective: Chronic rhinosinusitis (CRS) is a heterogeneous disorder with distinct pathophysiologic mechanisms. Based on the transcription factor expression and cytokine production patterns in different types of innate lymphoid cells (ILCs), in parallel with those of adaptive CD4⁺ T helper (Th) cells and CD8⁺ cytotoxic T (Tc) cells, new perspectives on endotypes of patients are emerging around the immune response deviation into type 1 (orchestrated by ILC1s, Tc1 and Th1 cells), type 2 (characterized by ILC2s, Tc2 and Th2 cells), and type 3 (mediated by ILC3s, Tc17 and Th17 cells) responses. Additionally, cluster analysis has been applied to the endotyping of CRS in recent years, which has provided additional novel insights into the CRS pathogenesis. This article aims to review pathological mechanisms of CRS on the basis of type 1, type 2, and type 3 immune responses and how they inform us to begin to understand CRS endotypes. This article also reviews the recent cluster analysis studies of CRS endotypes. Finally, the impact of endotype on therapeutic management of CRS is summarized.

Data Sources: Review of published literature.

Study Selections: Relevant literature concerning CRS endotypes and the possible underlying mechanisms were obtained from a PubMed search and summarized here.

Results and Conclusion: Both CRSwNP and CRSsNP are comprised of distinct endotypes with distinct deviated immune responses, pathogenic mechanisms, and different responses to medical and surgical treatment. An endotype of CRS with prominent type 2 immune responses is the most well studied endotype, and generally

can benefit from treatment with steroids and specific type 2 disrupting biologics.

Introduction

Chronic rhinosinusitis (CRS) is a complicated disorder characterized by persistent inflammation of sinonasal mucosa. It affects a considerable percentage of the population worldwide and is closely associated with the development and prognosis of lower airway diseases including asthma and chronic obstructive pulmonary disease.¹ Based on the presence or absence of nasal polyps, CRS is clinically classified into two phenotypes, CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSSNP), according to which current treatment regimens are established.¹ However, accumulating evidence suggests that such subclassification of CRS is unable to provide a full insight into the underlying pathophysiology in CRS, and heterogeneous groups of disorders with distinct pathogenic mechanisms may be included under the term of both CRSSNP and CRSwNP.^{2,3} This heterogeneity of CRS influences or even determines the efficacy of medical and surgical treatments and contributes to the inadequate disease control experienced when using “one-size-fits-all” treatment approaches based on traditional phenotype-based classification. For example, Wen et al. demonstrated that not all CRSwNP patients responded well to oral prednisone treatment.⁴ Oral prednisone was found to inhibit type 2 immune responses and shrink eosinophilic nasal polyps, whereas it failed to suppress the type 17 immune responses and neutrophilic inflammation in nasal polyps.⁴ Similarly, long-term low-dose macrolide treatment has been revealed to be effective only for CRSSNP patients without elevated IgE levels or with neutrophilic inflammation.^{5,6} In recent studies of biologics, variations in efficacy were found

among different patient groups. The levels of interleukin (IL)-5 in the nose were found to predict response to reslizumab, an anti-IL-5 monoclonal antibody, in the treatment of nasal polyps.⁷ These are just a few examples making it clear that identification of endotypes of CRS with proper biomarkers can be valuable in selection of proper therapeutic approaches for particular patients. In recent years, this need has been significantly reinforced by the emerging clinical application of additional targeted biologics for the treatment of CRS. The purpose of this review is to summarize the current knowledge regarding the inflammatory endotypes of CRS, their probable underlying pathogenic mechanisms and how mechanisms form the basis of decision making in personalized therapeutic strategies. Allergic fungal rhinosinusitis, aspirin exacerbated respiratory disease (AERD), CRS with cystic fibrosis, CRS with cilia motility defect, and CRS with vasculitis are not included in this review because these are discrete disorders with unique pathophysiology. Our review mainly focuses on idiopathic inflammatory CRS that is characterized by distinct combinations of immune responses.

Immune polarization based upon T cell cytokine production

T cell polarization and activation play a critical role in inflammatory diseases; distinct T cell cytokines exert great control over the expression of chemokines and cytokines important for the recruitment and activation of distinct effector cells such as eosinophils, neutrophils, mast cells, and macrophages. Based on the production of signature cytokines and the expression of transcription factors controlling cell

differentiation, at least six subsets of CD4⁺ T helper (Th) cells have been identified.⁸ Type 1 Th (Th1) cells are distinguished by expression of the transcription factor T-box transcription factor (T-bet), and the production of interferon (IFN)- γ . Type 2 Th (Th2) cells are identified by the expression of the transcription factor GATA binding protein 3 (GATA-3) and cytokines IL-4, IL-5 and IL-13. Th17 cells are characterized by the expression of the transcription factor retinoic acid-related orphan receptor C (RORC) and cytokines IL-17A, IL-17F, and IL-22.⁹ Follicular Th (Tfh) cells are a novel subset of Th cells and now recognized as centrally important player in regulating B cells to support antibody responses.⁸ The master transcription factor B cell lymphoma 6 (BCL-6) controls the differentiation of Tfh cells and IL-21 is the signature cytokine of Tfh cells.⁸ In addition, type 9 Th cells and type 22 Th cells are identified on the basis of the production of signature cytokines IL-9 and IL-22, respectively.¹⁰

In parallel with CD4⁺ Th cells, CD8⁺ cytotoxic T (Tc) lymphocytes are classified into Tc1, Tc2 and Tc17 cells based on expression patterns of transcription factors and cytokines similar to that of their counterpart CD4⁺ Th cells.¹¹ In the last few years, a understanding of innate lymphoid cells (ILCs) has been gradually achieved. ILCs, which lack lineage markers and specific antigen receptors, have similarly been classified into three major subsets (ILC1, ILC2 and ILC3) based on their transcription factor and cytokine expression patterns, and these patterns also parallel those of Th cell subsets.¹² The polarization of naïve CD4⁺ and CD8⁺ T cells into different subsets is cytokine environment dependent. IL-12 and IFN- γ play an important role in

promoting Th1 and Tc1 cells development, whereas IL-4 induces Th2 and Tc2 cell differentiation.¹³ IL-6 or IL-21 in combination with transform growth factor β (TGF- β) promote Th17 or Tc17 cells differentiation.^{14, 15}

Based on the emerging linkage between effector T cells in adaptive immunity and ILC lineages, a convergence of the innate and adaptive immune systems into type 1, type 2, and type 3 immune responses has been proposed.¹³ For example, in this view, type 1 immune responses, characterized by type 1 cytokine IFN- γ production, are orchestrated by ILC1s, Tc1, and Th1 cells. The crucial function for type 1 immune responses is to protect the host against intracellular microbes, such as protozoa, intracellular bacteria and viruses.¹⁶ By producing the type 1 cytokines IFN- γ and tumor necrosis factor α (TNF- α), ILC1, Tc1, and Th1 cells activate mononuclear phagocytes, driving them to become robust effector cells.¹³ In addition, Tc1 cells display a potent specific cytolytic effect against infected cells by releasing cytotoxic molecules, such as granzyme B and perforin.¹⁷⁻¹⁹ Type 2 immune responses are characterized by the production of IL-4, IL-5 and IL-13 from ILC2s, Tc2 cells, and Th2 cells. Type 2 immune responses play an important role in parasite immunity and can induce allergic diseases characterized by eosinophilia, IgE production, and goblet cell hyperplasia.^{11,13,20,21} IL-4 and IL-13 drive IgE class switching in B cells.²² IL-5 promotes eosinophil activation and survival. Type 3 immune responses, featuring IL-17 and IL-22 production, are controlled by ILC3s, Tc17 cells, and Th17 cells. Type 3 cytokines, IL-17A, IL-17F and IL-22, can activate immune and nonimmune cells to produce inflammatory cytokines, antimicrobial peptides, matrix

metalloproteases, and neutrophil chemoattractant IL-8/CXCL8, thereby facilitating immune responses that oppose extracellular bacteria and fungi.^{13, 23}

Immune polarization in CRS

The selective expression of type 1, type 2, or type 3 immune responses promotes significant heterogeneity in CRSsNP and CRSwNP.^{24,25} Moreover, immune responses in CRS may vary across different geographic areas and populations with distinct racial backgrounds, further complicating the heterogeneity of CRS.^{26,27} Expression of biased immune responses either towards type 2 or type 1/type 3 direction defines certain endotypes and influences clinical manifestations.²⁸ Nevertheless, the coexistence or absence of type 1, type 2 and type 3 immune responses can also be found in some CRS patients.^{26,27,29} Currently, studies on how the intermingling of different types of inflammatory pathways impacts the downstream clinical behavior of CRS are limited. However, it seems that patients with high levels of type 2 inflammatory mediators combined with high levels of type 1, type 3 and/or neutrophilic inflammatory markers, have the worst prognosis or the most severe inflammation load.³⁰

Type 2 immune responses and CRS

Independent of atopy, most Caucasian CRSwNP patients in Western countries demonstrate type 2-biased immune responses with high asthma comorbidity, which are featured by upregulated production of IL-4, IL-5, IL-13 and local IgE, and

profound tissue eosinophilia.^{1, 31, 32} In contrast, only about half of patients with CRSwNP in China and other East Asian countries show eosinophilic inflammation with typical type 2-biased immune response.³³ Noneosinophilic CRSwNP commonly demonstrates type1/type 3 predominant immune responses.³⁴ Thus, when taken as a whole, Asian patients with CRSwNP have lower frequency of type 2 cytokine expression, less eosinophilic inflammation, and lower asthma comorbidity as compared with their Caucasian counterparts.³³ Regarding CRSsNP, although most studies reported that the majority of patients presented noneosinophilic inflammation, a variable percentage of patients with a type 2-skewed inflammation could also be noted.^{27, 29, 35}

The type 2 immune responses in eosinophilic CRSwNP are likely orchestrated by Th2 cells, Tc2 cells and ILC2s. Increased numbers of Th2 and Tc2 cells were detected in CRSwNP and associated with mucosal eosinophilia in both Asian and Caucasian patients.^{36, 37} The number of ILC2s was also increased and associated with tissue eosinophilia, Th2 cells, and type 2 cytokines in CRSwNP (Fig 1).³⁸

IgE induction is an important feature of type 2 immune response. The elevated local production of IgE and its association with mucosal eosinophilia have been consistently reported in Caucasian and Asian patients with CRSwNP.^{32, 39} Increased mucosal infiltration of B cells, particularly germinal center B cells, and the presence of markers of class-switch recombination to IgE including epsilon germinal gene transcripts and epsilon circle transcripts in nasal polyps, strongly suggest local class-switch recombination to IgE in patients with eosinophilic CRSwNP.³⁹⁻⁴¹ Tfh

cells classically locate in germinal centers in secondary lymphoid tissues and play a crucial role in regulating B-cell responses. A recent study by Song et al. demonstrated that the formation of ectopic lymphoid tissues was more frequently observed in nasal polyps and Tfh cells resided in these structures in polyps (Fig 1).⁴² Zhang, Liu and collaborators found that nasal IL-4⁺ Tfh cells induced IgE production in B cells *in vitro* and correlated with local IgE production in nasal polyps *in vivo*.^{40,42} However, Feldman and collaborators did not detect an elevation of follicles or large clusters of lymphoid cells in their studies in Chicago.⁴³ In addition, large increases in extrafollicular plasmablasts expressing the marker Epstein-Barr virus-induced protein 2 have been reported by Hulse and her colleagues, suggesting that some B cells are activated outside of germinal centers in CRS tissues.⁴³ This could result in the emergence of autoreactive antibody producing cells, as reported by Tan et al.⁴⁴ Whether the causative antigens driving local immunoglobulin production in nasal polyps are exogenous or self antigens or both has not been completely clarified. In Caucasian patients with CRSwNP, it has been reported that *Staphylococcus aureus* enterotoxins, acting as both antigens and superantigens, may drive local IgE production.³² We found that local IgE against common aeroallergens, but not *Staphylococcus aureus* enterotoxins, was elevated in Chinese patients with eosinophilic CRSwNP.³⁹ Recently, by characterizing single cell-derived monoclonal antibodies, Takeda et al. discovered that about 20% of isolated IgE antibodies recognized nasal bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Haemophilus influenza*.⁴¹

Polyclonal IgE antibodies (IgE antibodies derived from different clones of plasma cells, i.e. distinct IgE antibodies to different epitopes of a particular antigen or multiple specific IgE antibodies to different antigens) are functional, and can activate mast cells in nasal polyps.⁴⁵ Mast cell hyperplasia has frequently been observed in CRSwNP.⁴⁶⁻⁴⁸ Cao et al. found that the local IgE levels positively correlated with the number of activated mast cells and eosinophilia in CRSwNP.³⁹ Recent studies by Wang et al. reported that IgE-mediated mast cell activation was promoted by kynurenine and aryl hydrocarbon receptor axis, which was upregulated in eosinophilic nasal polyps (Fig 1).⁴⁹ These studies highlight a contribution of local IgE mediated mast cell activation in eosinophilic inflammation in CRSwNP. In addition, increased infiltration of basophils was reported and associated with tissue eosinophilia in CRSwNP as well.⁵⁰ Basophil activation, as reflected by increased CD203cMFI positive basophil derived microparticles, was reported in both CRSwNP and AERD.⁵¹

A clustering analysis of CRS including CRSsNP and CRSwNP in Caucasians revealed that patients with increased type 2 inflammation were more likely to have nasal polyps, indicating a link of type 2 immune responses with the development of nasal polyps.⁵² Shi et al. consistently found that noneosinophilic CRSwNP was relatively less edematous and more fibrotic than eosinophilic CRSwNP.⁵³ Takabayashi et al found that in the type 2 environment in CRSwNP, type 2 macrophages enhanced the production of coagulation factor XIII-A, which associated with excessive production and cross-linking of fibrin.⁵⁴ In addition, type 2 immune responses downregulated the expression of tissue plasminogen activator, which would

be expected to diminish levels of plasmin and subsequent fibrinolytic degradation of cross-linked fibrin.⁵⁵ These events provide a potential mechanism for retention of water and formation of edema in polyps (Fig 1).

Type 1/type 3 immune responses and CRS

CRSsNP has previously been thought to present predominantly type 1 immune responses on the basis of upregulation of IFN- γ expression.^{24,25} However, studies on Americans in Chicago failed to replicate the upregulation of IFN- γ in CRSsNP.^{29,56} Such discrepancy might be due to the presence of different endotypes of CRSsNP in different geographical areas. Recently, a multicenter study including subjects from different continents demonstrated that IFN- γ levels were significantly higher in patients with CRSsNP than those in patients with CRSwNP and control subjects in Beijing only, but not in 5 other regions.²⁷

IL-17A expression has been considered to be elevated in patients with CRSsNP only in Asian countries.²⁸ However, recent studies have found that although the main endotype of CRSsNP was type 2 predominant, IL-17A levels were significantly increased in a minor subset of patients with CRSsNP compared to healthy controls in Chicago.²⁹ In the above mentioned multicenter study, significant variations in IL-17A expression in patients with CRSsNP were found across different areas, and the underlying mechanisms need further study.²⁷

Th1 cells counts were demonstrated to be increased in CRSsNP versus control in several studies, whereas inconsistent findings were reported regarding Th17 cell

counts in CRSsNP.^{36,57} Increased numbers of total CD8⁺ T cells were demonstrated in CRSsNP, but no study stratifying CD8⁺ T cells into different cell subsets is currently available. To our best knowledge, there is also no published data on ILC1 or ILC3 in CRS. In contrast to the finding in patients with CRSwNP, the number of mast cells was not elevated in patients with CRSsNP as compared with healthy controls.^{46,58} Neutrophil counts, and the levels of myeloperoxidase (MPO), IL-8 and IL-36 were higher in patients with CRSsNP than those in control subjects.^{27,34}

Although IFN- γ and IL-17A and neutrophil infiltration have been found upregulated in Caucasian patients with cystic fibrosis related CRSwNP, several studies have reported no increase of IFN- γ and IL-17A in Caucasian CRSwNP patients without cystic fibrosis.^{25,59} In comparison with Caucasians, patients with CRSwNP in China were reported to have higher IFN- γ and IL-17A expression.²⁸ Nevertheless, in a recent multicenter study, Wang et al reported that the concentrations of IL-17A in the CRSwNP group were higher than those in CRSsNP and control groups in Beijing as well as in Adelaide, but not in Berlin, Tochigi, and Chengdu.²⁷ Wang et al. confirmed that both eosinophilic and noneosinophilic CRSwNP in Wuhan, China had elevated expression of IFN- γ and IL-17A compared with control, and noneosinophilic CRSwNP had even higher IFN- γ and IL-17A expression compared to eosinophilic CRSwNP.³⁴ Consistently, Shi et al. and Ma et al. found that Th1 and Th17 cell counts, as well as Tc1 and Tc17 cell counts, were increased in noneosinophilic CRSwNP versus eosinophilic CRSwNP and control in Chinese.^{37,57} The number of ILC2s in noneosinophilic CRSwNP was comparable to

that in healthy controls, but decreased as compared with that in eosinophilic CRSwNP.⁶⁰

Asian patients with CRSwNP demonstrate neutrophil biased inflammation as reflected by a lower eosinophil cationic protein (ECP)/MPO ratio as compared with their Caucasian counterparts.^{27,28,34} In Chinese, both eosinophilic and noneosinophilic CRSwNP had elevated number of neutrophils with higher number of neutrophils in noneosinophilic CRSwNP than in the eosinophilic type.^{35,37,53} A study on patients in Chicago discovered that nasal polyps, which are highly eosinophilic, also have significant levels of neutrophils.⁶¹ Interestingly when comparing subjects with IL-17⁺ and those with IL-17⁻ inflammation, or comparing IFN- γ ⁺ with IFN- γ ⁻ subjects in Caucasians, the IL-17⁺ and IFN- γ ⁺ groups consistently had significantly higher levels of MPO and IL-1 β , indicating a contribution of types 1 and 3 immune responses to the development of neutrophilic inflammation.²⁶ The recently published study by Wang et al. demonstrated that increased production and activation of epithelial cell-derived IL-36 γ may act on neutrophils and further amplify neutrophilic inflammation in CRS (Fig 2).³⁴ IL-17A was able to upregulate the expression of IL-36 γ in nasal epithelial cells *in vitro*, which may further promote neutrophilic inflammation in CRS.³⁴ Neutrophils were found to produce TGF- β 2 in sinonasal mucosa, which may be involved in fibrosis in CRSsNP and noneosinophilic CRSwNP.⁵³ Interestingly, ectopic lymphoid tissues were also observed in noneosinophilic CRSwNP, potentially supporting the IgG production.⁴²

Epithelial barrier defect and reduced innate immunity in CRS

Barrier defects and reduced local innate immunity may initiate the immune and inflammatory responses in CRS. Epithelial barrier defects may be induced by secreted pro-inflammatory cytokines including IFN- γ and IL-4, which disrupt epithelial cell tight junctions and reduce transepithelial resistance.^{62,63} Eosinophil-derived DNA traps have recently been implicated in epithelium disruption in CRSwNP.⁶⁴ Neutrophils have been suggested to impair epithelial barrier function as well, potentially through releasing oncostatin M, an epithelial barrier disrupting cytokine.⁶¹ IFN- γ was reported to induce activated but insufficient autophagy, leading to p62-dependent apoptosis of nasal epithelial cells in CRSwNP.⁶⁵ In addition, impaired autophagy in myeloid cells was reported to aggravate eosinophilia, epithelial hyperplasia, and mucosal thickening in mice with eosinophilic CRS.⁶⁶ Decreased expression of antimicrobial S100 proteins in the epithelium of patients with CRS might lead to diminished innate immune responses and barrier function.^{67,68}

Downregulated production by epithelial cells of other host defense molecules, such as lactoferrin, has been observed in CRS.⁶⁹ Decreased expression of short, palate, lung, and nasal epithelial clone 1 and 2 (SPLUNC1 and 2) caused by decrease in the number of submucosal glands in CRSwNP may lead to a localized defect in innate defense as well.⁷⁰ Mucus hypersecretion is a hallmark of CRS. Th2-mediated pendrin expression may contribute to increased mucus production and decreased mucociliary clearance in CRSwNP.⁷¹ The bitter taste receptor, T2R38, was shown to be expressed in cilia of sinonasal epithelial cells, and activation of T2R38 regulates

calcium-dependent production of nitric oxide, resulting in stimulation of mucociliary clearance and direct antibacterial effects.⁷² Genetic variation in T2R38 has been shown to be associated with susceptibility to upper respiratory tract infections and CRS.^{72,73} In addition, substantial sinonasal microbiome dysbiosis has been demonstrated to be associated with the pathogenesis of CRS.⁷⁴

In addition to barrier function, epithelial cells play an important role in local immune regulation by interacting with immune cells. Cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), secreted by epithelial cells after exposure to environmental triggers (protease, allergen, virus, or fungi) may contribute to the development and activation of type 2 immunity in CRS.⁷⁵ TSLP is important for ILC2s survival, while ILC2s activation is more dependent on IL-33.⁷⁶ IL-33 has been demonstrated to drive IL-13 production from ILC2s in CRSwNP.⁷⁷ TSLP-activated dendritic cells (DCs) have been shown to preferentially drive Th2 cell differentiation in an OX40L-dependent manner.⁵⁷ The upregulation of TSLP have been consistently reported in eosinophilic CRSwNP, whereas there are controversies regarding the upregulation of IL-33 and IL-25 in CRSwNP.^{78,79} Therefore the importance of IL-33 and IL-25 in CRSwNP needs to be confirmed by further studies. Increased infiltration of OX40L⁺ myeloid DCs and OX40L⁺ plasmacytoid DCs were demonstrated in eosinophilic CRSwNP, which may promote Th2 cell differentiation.⁵⁷ In contrast, expression of osteopontin, which enhances Th1 and suppresses Th2 responses, was correlated with capacity of DCs to induce Th1 and Th17 cell differentiation in CRSwNP.⁵⁷

Identification of CRS endotypes by clustering analysis

With the awareness that a heterogeneous group of disorders with distinct pathophysiologic mechanisms is encompassed in the term of CRS, more powerful statistical efforts to endotype CRS on the basis of an array of cellular and molecular biomarkers have been made in recent years. Cluster analysis, a hypothesis-free method allowing an overview of a high number of variables without compromising information can lead to the discovery of associations and correlations without presumption and has been employed to analyze CRS endotypes. This unsupervised statistical method can result in novel insights with an unbiased technique.

By analyzing cellular markers only, Luo et al. identified 5 subject clusters in Chinese patients with CRSwNP.⁸⁰ The clusters were plasma cell-dominant, lymphocyte-dominant, mixed inflammatory pattern, neutrophil-dominant, and eosinophil-dominant, respectively.⁸⁰ It was found that patients in the eosinophil-dominant cluster had the highest rate of disease recurrence.⁸⁰ Patients who fell into plasma cell-dominant and lymphocyte-dominant endotype had relatively good prognosis.⁸⁰ Based on the protein levels of 41 cytokines and chemokines in tissue lysates, network mapping and unsupervised cluster analysis were conducted and 3 subject clusters were identified.⁵² Patients in subject cluster 1 were characterized by high expression of type 2 cytokines and related molecules, and the subjects in this cluster were all patients with CRSwNP and had the highest asthma prevalence. Patients in cluster 2 were characterized by the high expression of type

1/type 3 cytokines and relevant molecules and most of them were CRSsNP patients. Subjects in cluster 3 had high expression of growth factors and consisted of normal controls and patients with CRSsNP, with no patients with CRSwNP.

In an interesting multicenter study in Europe, inflammatory endotypes of CRS were analyzed based on a cluster analysis of 14 biomarkers and correlated to clinical characteristics.³⁰ It resulted in an optimal outcome of 10 clusters, in which 4 clusters with low or undetectable concentrations of IL-5, ECP, IgE, and albumin, and the other 6 clusters with high concentrations of those markers were identified. Three of the 4 of the IL-5-negative clusters resembled a predominant CRSsNP phenotype and had no increase of asthma comorbidity. In these 3 clusters, one cluster demonstrated no significant inflammation, one cluster was characterized by the expression of the type 3 cytokine IL-22, and the last cluster had a type 1 immune response and neutrophilic inflammation. The IL-5-positive clusters were further divided into a group of 3 clusters with moderate IL-5 concentrations, which were composed of a mixed group of patients with CRSsNP and patients with CRSwNP. The other 3 clusters with high IL-5 levels were comprised almost exclusively of patients with CRSwNP and increased asthma comorbidity. Among clusters with high IL-5 levels, two clusters had the highest concentrations of local IgE and prevalence of asthma, with all samples expressing local IgE against *Staphylococcus aureus* enterotoxins.³⁰ Clusters identified in this study showed a close relationship with the previously described clinical phenotypes and inflammatory features of CRSwNP and CRSsNP in Western countries. However, a limitation of this study is that it only investigated a limited number of

biomarkers, and some important inflammatory markers implied in the pathogenesis of CRS, such as epithelial cell-derived cytokines, chemokines, and tissue remodeling factors, were not included.

A recent study by Liao et al performed in Wuhan tried to identify multidimensional endotypes of CRS based on a cluster analysis of a larger number of parameters including 28 clinical parameters and 39 cellular and molecular parameters in Chinese patients.⁸¹ More importantly, the endotypes were correlated with treatment outcomes. In this study, seven CRS clusters with distinct biomarkers, clinical characteristics, and treatment outcomes were identified. Cluster 1 was comparable to the classic well-defined eosinophilic CRSwNP in Western countries, having the highest number of tissue eosinophils and prevalence of comorbid asthma, and the highest levels of IL-5, IL-13, eotaxin and IgE, and the highest proportion of difficult-to-treat cases. In clusters 2 and 4, the majority of patients had CRSsNP, demonstrating mild inflammation, but subjects in cluster 2 were highly atopic. Cluster 3 and cluster 6 were characterized by severe or moderate neutrophilic inflammation, respectively, and with high levels of IL-8. Subjects in these 2 clusters had high proportions of difficult-to-treat cases comparable to those in cluster 1, suggesting that either more severe eosinophilic or neutrophilic inflammation can lead to poorly controlled diseases. A previous study indicated that increased neutrophilia may reduce the response to glucocorticoid treatment in CRSwNP patients.⁴ In this cluster study, all patients were under a “standardized and glucocorticoid-centered” postsurgical treatment according to the current American and European position paper.¹ Therefore,

the high frequency of difficult-to-treat cases in the clusters characterized by neutrophilic inflammation again encourages a search for novel treatments targeting neutrophilic inflammation in CRS. Cluster 7 was characterized with both low eosinophilic and neutrophilic markers, which may resemble the “noneosinophilic and nonneutrophilic CRSwNP”. Cluster 5 was a unique group characterized by the highest levels of IL-10 and absence of difficult-to-treat cases, underscoring a potential critical role of IL-10, or the lack thereof, in the pathogenesis of CRS. The identification of the novel CRS endotype with high IL-10 expression highlights the power of cluster analysis since IL-10 expression was found upregulated in both CRSsNP and CRSwNP patients as a whole.⁸¹ In addition, when comparing difficult-to-treat and non-difficult-to-treat cases in the whole CRS group, an association of difficult-to-treat cases with IL-10, eosinophilia, and IgE, but not neutrophilia was identified.⁸¹ However, clustering analysis showed that difficult-to-treat CRS was associated with distinct clinical features and biomarkers in the different clusters. Association of difficult-to-treat CRS with increased neutrophilic inflammation and IL-8 levels was revealed in cluster 3, further supporting the advantage of cluster analysis for CRS endotype study. Immunodeficiencies such as common variable immune deficiency (CVID), X-linked agammaglobulinemia (XLA) and specific antibody deficiency (SAD) may also contribute to difficult control of CRS as well, which should be taken into consideration in future study design.^{82,83}

In a very recent study, cluster analysis was performed based on the expression of type 1/type 2/type 3-associated inflammatory mediators in nasal mucus from patients

with CRS in America.⁸⁴ Consistent results, particularly type 2-high CRS endotype, were revealed in this study. Importantly, this study underscored the feasibility to endotype CRS with samples more easily accessible.

There are several limitations to the study of CRS using cluster analysis. The clusters identified are highly dependent on the biomarkers and clinical parameters analyzed. It is therefore difficult to cover all the molecules implicated in the pathogenesis of CRS and inconsistent results reported by different studies are not uncommon. It is also difficult to offer a clear and clinical relevant definition for every endotype, although it may bring novel insights to CRS pathogenesis. Moreover, stability of clusters over time is uncertain and should be evaluated in future studies.

In the future, large cohort and multicenter studies with more parameters including clinical characteristics, molecular biomarkers, genetics, histopathology, epidemiology, and treatment response, may be helpful to distinguish and define different endotypes of CRS more precisely and comprehensively. Moreover, development of new assays to identify clinically accessible sensitive biomarkers to differentiate distinct endotypes to guide diagnosis and treatment selection hold significant translational promise.

Endotype-based therapy for CRS

In summary, both CRSwNP and CRSsNP are heterogeneous and composed of multiple different endotypes. Although current evidence is insufficient to give us a full characterization or clear definition of CRS endotypes, the improved insights into

the distinct pathophysiologic pathways of CRS and their associations with clinical characteristics provided by the endotyping studies performed around the world provide us with directions to better define pathophysiologic mechanisms and ultimately better targeted treatments.

An endotype of CRS with prominent type 2 immune responses is consistently identified in both Caucasians and Asians and it is the most well studied endotype (Fig 1). This type 2-high CRS is more likely to have polyp formation, asthma comorbidity, severe disease, and recurrence after sinus surgery. Biologics targeting type 2 immune responses, including anti-IL-5, anti-IL-4/IL-13 receptor, and anti-IgE, have been found to be effective in subgroups of Caucasian patients with CRSwNP. Anti-IgE monoclonal antibodies have been reported to reduce nasal polyp score in both allergic and nonallergic patients with CRSwNP and asthma, further supporting the potential significance of local IgE production.⁸⁵ Trials of IL-5 antagonists have shown reductions in polyp size and reduced the need for revision surgery.⁸⁶ Antibodies to IL-4R α antagonize the biological activity of both IL-4 and IL-13. Anti-IL-4R α therapy has been shown to reduce polyp size and restore sense of smell.⁸⁷ The biomarkers for the selection of proper patients for biologic treatments remain poorly explored for CRS, with one study demonstrating the need to identify patients with high levels of IL-5 to predict efficacy of anti-IL5 treatment.⁷ The use of biomarkers to predict responders to biologics targeting type 2 inflammation is even more important for Asian patients with reduced expression of type 2 immune responses compared to Caucasians. This applies equally for drugs targeting type 2 inflammation

by disrupting TSLP, IL-33, IL-25, or GATA binding protein 3 for the treatment of CRSwNP. Trials targeting these molecules have shown promising efficacy in treatment of asthma.

High type 2 immune responses with eosinophilia are generally responsive to glucocorticoids, which are routinely used as the drug of first choice and generally provide clinical benefit. These steroids are less effective against neutrophilic inflammation and previous studies showed that increased tissue neutrophilia reduced the response of nasal polyps to corticosteroid therapy.⁴ Low-dose and long-term macrolide treatment is sometimes utilized to target neutrophilic inflammation. However, it should be noted that the majority of relevant published studies of macrolides has limited study power and quality. Further studies with larger sample size, placebo controls, and proper stratification of subjects are obviously needed to determine the efficacy of glucocorticoids and macrolides for CRS with different endotypes. There are two FDA-approved agents that block IL-17A/IL-17R and are used in advanced management of psoriasis.^{88,89} The involvement of type 3 immune response in some of the subtypes of CRS suggests the potential applicability of specific use of these agents in specific subgroups with IL-17-mediated disease; this also deserves future study.

Summary and conclusions

Both CRSwNP and CRSsNP are comprised of distinct endotypes with different pathogenic mechanisms, different response to pharmacotherapy, and different risk of recurrence after surgery. An endotype of CRS with prominent type 2 immune

responses is the most well studied endotype, and generally can benefit from treatment with steroids and specific type 2 disrupting biologics. Although a complete definition of CRS endotypes and their clinical relevance have not fully been developed at our current stage, improving the understanding of endotypes of CRS will enable us to counsel our patients and better manage their disease in the near future, particularly with the rapid expansion in the availability of specific biologic therapies.

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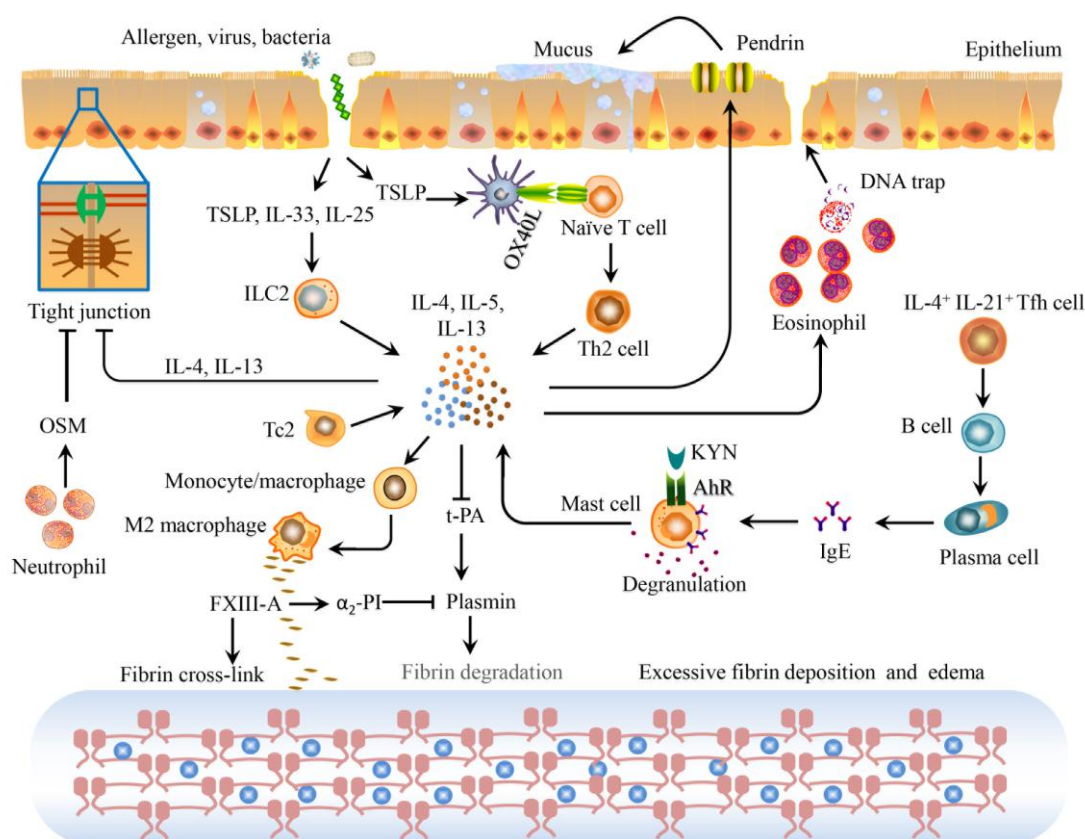


Figure 1. Type 2 immune responses and chronic rhinosinusitis

An overview of the type 2 immune responses in human chronic rhinosinusitis based on available evidence. After stimulation with innate immune activating stimuli, cytokines or injurious environmental agents such as proteases, epithelial cells produce thymic stromal lymphopoietin (TSLP), and perhaps in some cases interleukin (IL)-33 or IL-25, which activate group 2 innate lymphoid cells (ILC2s). Epithelial cell-derived TSLP up-regulates OX40L expression on dendritic cells (DCs), then DCs initiate the differentiation of naïve T cells into type 2 T helper (Th2) cells. Th2 cells, ILC2 and type 2 cytotoxic T (Tc2) cells orchestrate eosinophilic inflammation through production of type 2 cytokines. IL-4⁺IL-21⁺ T follicular helper (Tfh) cells initiate the differentiation of B cells into plasma cells, then mast cells are activated by IgE which is produced locally by plasma cells; the kynurenine (KYN)/aryl hydrocarbon (AhR) axis may be involved in this process. In addition, mast cells can also produce type 2 cytokines. Th2 inflammation can induce monocytes/macrophage differentiation into M2 macrophages. M2 macrophages produce coagulation factor XIII-A (FXIII-A) that induces excessive fibrin deposition by cross-linking of fibrin

and via the antifibrinolytic pathways through binding α_2 -Plasmin inhibitor (α_2 -PI, also known as α_2 antiplasmin) to fibrin. Meanwhile, tissue plasminogen activator (t-PA) levels are reduced in Th2 inflammation, causing impaired plasmin generation, which, in turn, decreases fibrinolysis. These events collectively result in retention of water and formation of edema in polyps. Th2 cytokine-mediated pendrin expression may increase mucus production. The cytokines IL-4 and IL-13 can diminish the expression of epithelial cell tight junction proteins. Neutrophil-derived oncostatin M (OSM) and eosinophil-derived DNA traps may also contribute to epithelium disruption.

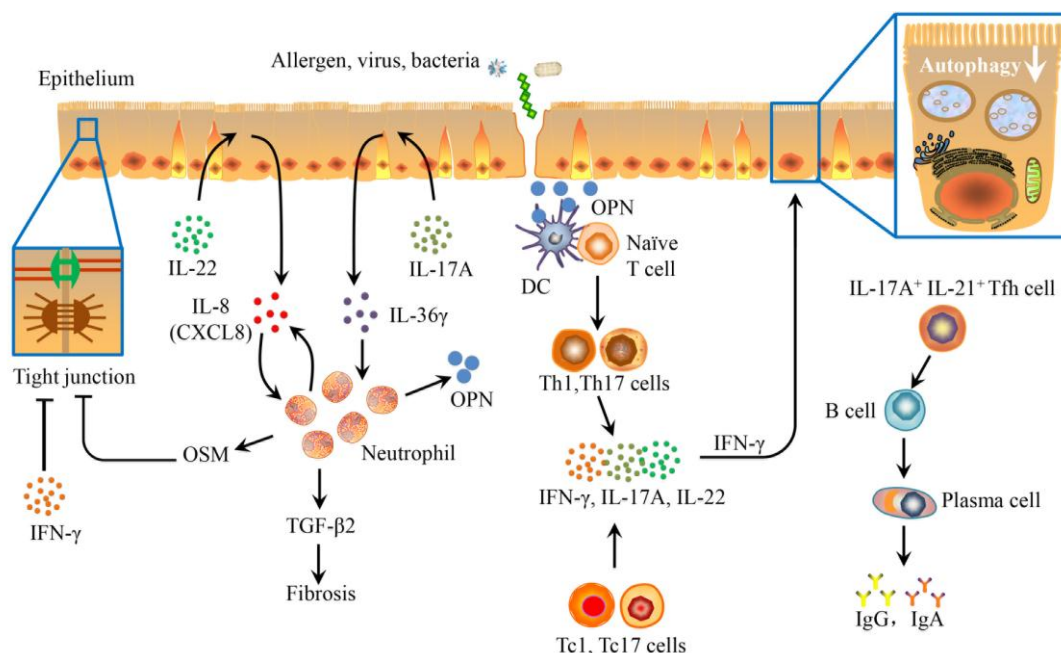


Figure 2. Type 1 and 3 immune responses and CRS

An overview of the type 1 and 3 immune responses in human chronic rhinosinusitis based on available evidence. In response to environmental stimuli, epithelial cells secrete osteopontin (OPN); OPN-stimulated dendritic cells (DCs) induce type 1 T helper (Th1) and type 17 T helper (Th17) cell differentiation. Th1 and Th17 cells as well as type 1 cytotoxic T (Tc1) and type 17 cytotoxic T (Tc17) cells orchestrate noneosinophilic inflammation through production of interferon γ (IFN- γ), interleukin (IL)-17A and IL-22. IL-17A upregulates the expression of IL-36 γ in epithelial cells, and IL-36 γ can act on neutrophils and further exaggerates neutrophilic inflammation by inducing IL-8/CXCL8 production from neutrophils. IL-22 induces epithelial cells to produce IL-8/CXCL8, then IL-8/CXCL8 act on neutrophils. Neutrophils can also produce oncostatin M (OSM), OPN and transform growth factor β 2 (TGF- β 2). TGF- β 2 may be involved in fibrosis. IFN- γ and OSM may disturb epithelial barrier function by diminishing the expression of epithelial cell tight junction proteins. IFN- γ can induce activated but insufficient autophagy, leading to apoptosis of nasal epithelial

cells. IL-17A⁺IL-21⁺ Tfh cells initiate B cell differentiation into plasma cells that produce IgG and IgA.