Endotypes of Chronic Rhinosinusitis; relationships to disease phenotypes, pathogenesis, clinical findings and treatment approaches.

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Abstract

Chronic rhinosinusitis (CRS) is a common clinical syndrome that produces significant morbidity and costs to our health system. The study of CRS has progressed from an era focused on phenotype to include endotype based information. Phenotypic classification has identified clinical heterogeneity in CRS based on endoscopically observed features such as presence of nasal polyps, presence of comorbid or systemic diseases and timing of disease onset. More recently, laboratory-based findings have established CRS endotype based upon specific mechanisms or molecular biomarkers. Understanding the basis of widespread heterogeneity in the manifestations of CRS is advanced by findings that the three main endotypes, Type 1, 2 and 3, orchestrate the expression of three distinct large sets of genes. The development and use of improved methods of endotyping disease in the clinic is ushering in an expansion of the use of biological therapies targeting Type 2 inflammation now and perhaps other inflammatory endotypes in the near future. The purpose of this review is to discuss the phenotypic and endotypic heterogeneity of CRS from the perspective of advancing the understanding of the pathogenesis and improvement of treatment approaches and outcomes.

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Short Title: Relating endotypes, phenotypes and treatments in CRS

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Abstract

Chronic rhinosinusitis (CRS) is a common clinical syndrome that produces significant morbidity and costs to our health system. The study of CRS has progressed from an era focused on phenotype to include endotype based information. Phenotypic classification has identified clinical heterogeneity in CRS based on endoscopically observed features such as presence of nasal polyps, presence of comorbid or systemic diseases and timing of disease onset. More recently, laboratory-based findings have established CRS endotype based upon specific mechanisms or molecular biomarkers. Understanding the basis of widespread heterogeneity in the manifestations of CRS is advanced by findings that the three main endotypes, Type 1, 2 and 3, orchestrate the expression of three distinct large sets of genes. The development and use of improved methods of endotyping disease in the clinic is ushering in an expansion of the use of biological therapies targeting Type 2 inflammation now and perhaps other inflammatory endotypes in the near future. The purpose of this review is to discuss the phenotypic and endotypic heterogeneity of CRS from the perspective of advancing the understanding of the pathogenesis and improvement of treatment approaches and outcomes.

Introduction

The study of disease has progressed from an era focused on phenotype, where all information was collected by the physician in collaboration with the patient, with or without the use of tools such as the stethoscope, endoscope, X-ray and Ct scanner. We have witnessed the incorporation of ever more sophisticated laboratory-based findings, collected via microscopy and instruments developed for biochemistry, molecular biology and immunochemistry. Information gleaned by these modern techniques can provide information on the underlying cellular and molecular mechanisms that define the endotype. The purpose of this review is to discuss the phenotypic and endotypic heterogeneity of chronic rhinosinusitis from the perspective of advancing the understanding of the pathogenesis and improvement of treatment approaches and outcomes.

Definition of phenotype and endotype

To understand clinically observed variability in presentation and outcomes, many chronic diseases have been classified by genotype, phenotype and/or endotype. *Genotypic* classification subdivides disease based upon genetic polymorphisms and has been of limited utility in CRS [1], aside from identifying related monogenic conditions like cystic fibrosis [2] or ciliary dysmotility [3]. *Phenotypic* classifications utilize clinically observable characteristics and have helped advance understanding of natural history and treatment outcomes. In CRS, phenotypic classification has utilized endoscopically observed features, presence of comorbid or systemic illness and timing of disease onset. Classification of CRS by the presence (CRSwNP) or absence (CRSsNP) of nasal polyps has been the most widely applied phenotyping of CRS. CRSwNP is viewed as a diffuse

inflammatory process, while CRSsNP is linked, at least in part, to sinus outflow obstruction with secondary inflammation and infection, suggesting presence of a mechanical process[4]. Subclassification by the presence or absence of common comorbidities such as asthma [5] or allergies[6,7] has been used. Other phenotypic subclassification has embraced recognized presentations such as Aspirin Triad (AERD, NERD), Allergic Fungal Rhinosinusitis (AFRS), Eosinophilic Granulomatosis with Polyangiitis (EGPA) [8], Granulomatosis with Polyangiitis (GPA), sinonasal sarcoidosis and CRS with immunodeficiency [9], although these phenotypes are relatively rare and variably defined.

There has been a growing appreciation that establishment of endotype, which initially involved classification by histologic features such as presence of neutrophilia, eosinophilia, fibrosis, glandular hypertrophy and epithelial dysmorphosis, can provide insight into treatment response or pathobiology. Classification based on the presence of fungi or bacteria, has stimulated debate but not led to emergence of widely used clinical protocols [10-14]. Recent efforts seek to define CRS *endotypes* based upon specific molecular biomarkers or mechanisms. It is gratifying that endotypes have strong associations with phenotypes and histologic findings. Endotypic disease classification is challenging because considerable tissue is required, pathologist interpretations are variable, endotype assays are not readily accessible and results may be influenced by treatment or unstable [15]. Nonetheless, there is an inexorable shift of interest towards the molecular pathways that underlie endotypes that drive inflammation, remodeling and clinical phenotype[16]. In conjunction with new, specific and powerful interventions targeting molecular pathways, study of endotype holds great promise.

Early studies of CRS heterogeneity and indicators of underlying endotypes

Studies based on histology quantitated the numbers of eosinophils, mast cells and neutrophils [17-22]. In the West, CRSwNP patients had higher numbers of eosinophils and mast cells while CRSsNP expressed *relatively* higher levels of neutrophils. Heterogeneity of histology made it clear that there were multiple overlapping processes [23,24]. Specifically, many CRSwNP cases were associated with both eosinophilic and neutrophilic infiltrates, while CF polyps demonstrated a predominance of neutrophils [25]. Furthermore, a subset of CRSsNP cases exhibited elevated eosinophil counts [26]. Changes in remodeling including polypoid edema, glandular hypertrophy and fibrosis have been used to subdivide CRS [27-29]. The combination of tissue remodeling changes together with effector cell infiltrates has recently been proposed in a *structured histopathologic* classification system of CRS [30,31]. Histological phenotyping to distinguish endotypes is only as good as the specificity of inflammatory cell counts or tissue structural changes evaluated. For example, while current evidence indicates that polyposis reflects formation of a fibrin matrix, this feature can result from at least 3 distinct inflammatory pathways[32-34]. Although tissue eosinophilia and remodeling are probably of value, histopathologic features have not been defined in guidelines and remain experimental.

Early molecular studies indicated that IL-5 and IgE are important biomarkers in eosinophilic CRS while IL-8 is found in neutrophilic CRS[35-39]. Recognition of a T cell cytokine expression pattern was first made by Bachert and colleagues [40]. Interestingly, Asian CRSsNP expressed high levels of type 1 cytokines[41] similar to Caucasians, but Asian CRSwNP frequently expressed type 3 and type 1 cytokines, as in contrast to the type 2 skewing in Caucasian polyps (see below for discussions of the three types) [42]. A landmark first attempt to define the CRS endotypes was an international study that utilized a cluster analysis of the presence of pre-selected biomarkers to distinguish 10 endotypes[43]. The strength of the study was the differential association of these endotypes with the phenotypic presence of asthma or nasal polyposis. The 10 endotypes were further subdivided into 3 groups based on high IL-5, low IL-5 or absence of IL-5 [44]. Although the study did not associate endotype with outcome data, it was an important starting point, and this publication accelerated the search for biomarkers that could, at least in theory, define endotypes that would respond uniquely to endotype-specific therapeutics. 25%

Toward developing a current view of CRS molecular endotypes

Underlying immunological mechanisms

Pathology of CRSwNP has been well studied and tissue remodeling, epithelial dysfunction, activation of innate and adaptive inflammatory responses and fibrin deposition seem to be common features in

CRSwNP[45,46]. CRSwNP is frequently divided into two key endotypes, eosinophilic CRSwNP (ECRSwNP) and non-eosinophilic CRSwNP (NECRSwNP) based on the presence and absence of large numbers of eosinophils in nasal polyp (NP) tissue. Analysis of gene expression in CRS tissues ultimately shifted focus to molecules produced by T lymphocytes of types 1, 2 and 3 (also known as 17) that produce primary cytokines that drive the inflammatory patterns observed in tissues (referred to here as T1, T2 and T3 and Tun, signifying "untypeable").**Figure 1** shows microarray data from Chicago using samples from CRS patients illustrating the stark contrast between, and the strong similarity among, patients in the distinct endotypes, and **Figure 2** shows a summary/overview of the driving cytokine, source cells and effector cells in the tissues. ECRSwNP is characterized by presence of type 2 (T2) cytokines (IL-4, IL-5 and IL-13) and accumulation of type 2 immune cells including mast cells, basophils, CD4 T helper 2 (Th2) cells, group 2 innate lymphoid cells (ILC2), B cells, M2 macrophages and dendritic cells in addition to eosinophils. For this reason, ECRSwNP is also called T2 CRSwNP [45-55]. NECRSwNP can also be subdivided based on presence of inflammatory cytokines, including the type 1 (T1) endotype based on IFN-γ signaling and type 3 (T3) endotype based on IL-17A signaling; infiltrated cell types include neutrophils, lymphocytes and plasma cells [52,56-59]. NECRSwNP is highly heterogeneous and the frequency of each endotype varies geographically [56-58]. Transcriptomic approaches help to identify immunological mechanisms in NP [60-63]. Of note, two groups presented distinct gene expression profiles in NP between ECRSwNP and NECRSwNP in Asia and results also showed several similarities [56,63]. Both studies showed that eosinophil- and T2-associated genes including *CLC* , *CCL23* ,*CCL26* , *SIGLEC8* , *PRSS33* and *ALOX15* were upregulated in ECRSwNP, confirming that ECRSwNP is associated with the T2 endotype [56,63]. In contrast, NECRSwNP showed up-regulation of IFN-γ-induced genes (*CXCL9* , *CXCL10* and*CXCL11*), IL-17A-induced genes (serum amyloid A, CXCL6 and CHI3L1) and neutrophil chemokines (IL-8, CXCL1 and CXCL6), suggesting that NECRSwNP in Asia may display mainly a mixed T1 and T3 endotype with neutrophilia [56,63]. Indeed, Wang and colleagues reported that the T1 and T3 mixed (IFN-γ^+, IL-17A$^+$) endotype is the most common endotype in NECRSwNP in Beijing [56]. Although ECRSwNP and NECRSwNP showed distinct immunological mechanisms, both result in NP formation, suggesting that some phenotypic features, such as formation of polyps, are not reliable indicators of the transcriptomic pattern or molecular endotype. A summary of gene expression in the primary endotypes and mixed endotypes is found in **Figure 3** .

Transcriptome analysis may have value in identifying genes that are associated with NP formation by extracting shared dysregulated genes in NPs from both ECRSwNP and NECRSwNP. Epithelial-to-mesenchymal transition (EMT) associated genes including HIF1A are elevated in both ECRSwNP and NECRSwNP, suggesting that EMT may be a key event in NP formation[46,56,64]. Although transcriptome analysis identifies similarities between ECRSwNP and NECRSwNP, the upstream pathway for the affected molecules may be different in each endotype. For example, reduced fibrinolysis associated with down-regulation of tissue plasminogen activator (tPA) is a common feature of NPs in T2wNP (ECRSwNP) and T1wNP (NECRSwNP) [32,34,46].

In contrast to CRSwNP, studies in CRSsNP have been complicated by the use of variable sinonasal biopsy sites which have inherent tissue-specific molecular differences that obscure the underlying heterogeneity of inflammation [57,58,62,65]. By the exclusive use of ethmoid sinus mucosa for microarray, we recently identified gene expression profiles in three inflammatory endotypes; T1sNP, T2sNP and T3sNP, and predicted molecular mechanisms and biomarkers for each endotype [55]. The gene signatures suggested that T1sNP is associated with T cells (Th1 cells and CD8$^+$ cytotoxic T cells), NK cells and antigen presenting cells (APC); T2sNP is associated with eosinophils, mast cells, basophils, Th2 cells, ILC2 and APC that are also found in T2wNP; and T3sNP is associated with neutrophils, Th17 cells, B cells and APC**(see Figure 2)** [55]. We further found that T1 (CXCL9 and CXCL10), T2 (eosinophilic proteins and CCL26) and T3 (CSF3) endotypic biomarkers can distinguish tissue endotypes in nasal lavage fluids from patients with CRSsNP [55].

Pathological mechanisms and their relationships to features and endotypes

As mentioned above, in Western countries, CRSwNP is primarily characterized by type 2 eosinophilic inflammation and mixed inflammatory histopathology, while both eosinophilic and non-eosinophilic patterns are

found in polyps from Asian patients [56,66]. Interestingly, there has been a shift in the endotype distribution over time with an increase in the degree of eosinophilia observed in NPs from Asian patients [67,68]. Furthermore, recent research suggests that neutrophilic inflammation may also play a role in the pathogenesis of Western NP [69]. Thus, it is clear that inflammatory patterns in CRSwNP show geographic variability across Europe, Asia, and Oceania [56]. While the neutrophilic inflammatory endotype has been demonstrated in parts of Asia and Europe, evidence is accumulating that, at least in the Western countries, CRSsNP, like CRSwNP, has a predominantly type 2 eosinophilic pattern[57,70].

Both innate and adaptive immune responses are important in the pathogenesis and endotypes of CRS. The NP tissue is characterized by dysregulated epithelium, elevated Th2 cells, innate lymphoid type 2 (ILC2) cells, B cells, mast cells, eosinophils, and basophils[48-51,54,71,72]. The sinonasal epithelium is the principal source of TSLP which is essential for type 2 inflammation and activates ILC2 cells and effector Th2 cells [45]. Investigators around the world have demonstrated elevated thymic stromal lymphopoietin (TSLP) in eosinophilic NP tissue [73-76]. As already mentioned, Th2 and ILC2 are important sources of type 2 cytokines, including IL-4, 5, and 13 [77]. IL-5 promotes eosinophilic inflammation, and IL-4 and IL-13 activate isotype switching, mucus production, M2 macrophage differentiation, and remodeling in CRSwNP [45]. Type 2 inflammation is believed to drive NP formation by promoting fibrin deposition and retention of plasma proteins and edema [33,78]. In addition to expansion of Th2 and ILC2, B cells and plasmablasts are also increased and produce IgE and other immunoglobulins in NP tissue[40,79]. While CRSwNP is mainly type 2 (T2) in the West, some patients manifest type 1 (T1), type 3 (T3), or mixed inflammatory patterns with a combination of T1, T2, and T3 inflammation. T1 and T3 inflammation are associated with elevated IFN γ and IL-17A, respectively. A subset of patients with CRS lacks an elevation of any T1, T2, or T3 markers and are classified as untypeable. This subgroup may represent another endotype of CRS whose inflammatory pattern is yet to be identified **(see Figure 2)** .

The three major inflammatory endotypes are also present in CRSsNP[55,57,58]. It was initially proposed that type 1 inflammation associated with elevated IFN-γ was present in CRSsNP; however, this has not been confirmed in other studies[40,57,80]. Tan et al. investigated markers of inflammation in the ethmoid tissue from patients with CRS and controls and did not find a difference in IFN-γ among CRSsNP, CRSwNP, and controls. Type 2 markers of inflammation, including ECP, IL-5, and IL-13, were highest in NP and ethmoid tissue from patients with CRSwNP[57]. Interestingly, T2 markers of inflammation were also significantly elevated in the ethmoid tissue from patients with CRSsNP compared to the ethmoid tissue from controls. Furthermore, IL-17A, the primary marker of T3 inflammation, was elevated in the ethmoid tissue of a subset of patients with CRSsNP. More recently, Kato and colleagues have demonstrated that gene expression in CRSsNP is reminiscent of that in CRSwNP. As in CRSwNP, in CRSsNP, T1 is associated with T cells (Th1 and $CD8^+$), NK cells, and antigen-presenting cells (APC), whereas T2 is associated with eosinophils, mast cells, Th2 cells, ILC2, and APCs and T3 CRSsNP is associated with Th17 cells, B cells, neutrophils, and APCs[55]. Wang et al. have also demonstrated that type 1 inflammation is predominant in Chinese patients from Beijing with CRSsNP, whereas patients from Chendgu, China, lack elevation of T1, T2, or T3 markers [56]. **Figure 2** shows a hypothetical overview of the inflammatory patterns of cells and responses as related to the three primary endotypes, independent of the phenotype (i.e. the presence or absence of polyps). We have adopted a shorthand that encompasses both endotype and the major phenotype. Type 2 CRSwNP is *T2wNP* in this scheme, while mixed type 1 and 3 CRSsNP would be *T1,3sNP* , etc. **Figure 3** presents the pure and mixed endotypes and summarizes associated biomarkers. Occasionally, either CRSsNP or CRSwNP patients are identified that have all three endotypes elevated (T1,2,3sNP and T1,2,3wNP). The Tomassen paper identified 10 clusters/endotypes based on type 1 and 2 cytokines and inflammatory markers [43]. Clusters associated with low or no IL-5 resembled predominantly the CRSsNP phenotype and had a low likelihood of comorbid asthma. The highest IL-5 clusters were mostly CRSwNP patients expressing IgE to *Staphylococcus aureus*enterotoxins. One of their clusters expressed IL-17 and had a mixed CRSsNP/wNP phenotype.

Genomics, proteomics and metabolomics

Modern techniques including genomics (transcriptomics), proteomics and metabolomics provide comprehensive and un-biased approaches to study biological systems, identify previously un-recognized mechanistic pathways in health and disease, and establish endotypes within a disease[81-83]. Systems biology has provided a more holistic understanding of diseases and endotypes [81-83]. Proteomic analysis of nasal mucus and mucosa in CRS suggested a trend of increased presence of immunological, metabolic, tissue remodeling and apoptotic pathways in CRS [84,85]. Metabolomics analysis of low molecular weight compounds (up to 1,500 Da) has been performed in CRS [83]. Fazlollahi et al. found that fatty acids (palmitic, oleic, stearic, and lauric) were highly elevated in CRSwNP compared to CRSsNP and control tissues [86]. Miyata found impaired synthesis of cyclooxygenase- and lipoxygenase-derived mediators (including prostaglandin D_2[PGD_2], PGE_2, thromboxane B_2, 15-hydroxyeicosatetraenoic acid and lipoxin A_4) and selective upregulation of leukotriene D4 in nasal polyp-derived eosinophils compared to healthy peripheral blood eosinophils [87]. Future proteome and metabolome studies will require larger sample size and higher reproducibility to identify endotypes. In contrast, transcriptome analysis by microarray and RNA-Sequencing has been successfully used to characterize not only phenotype-specific (e.g. CRSsNP and CRSwNP) but also endotype-specific (e.g. eosinophilic and non-eosinophilic) gene expression profiles in CRS.

AERD as an informative phenotypic variant

As many as 15% of patients with CRSwNP have comorbid asthma and an intolerance to inhibitors of cyclooxygenase 1 (COX-1)[88,89]. This clinical triad is commonly referred to as Aspirin Exacerbated Respiratory Disease (AERD) in North America. The acronym NERD (NSAID Exacerbated Respiratory Disease) is often used in Europe but has not been adopted in North America as the word has negative connotations. While there is overlap between the phenotypes (and endotypes) of AERD and CRSwNP, important distinctions exist. AERD is the most severe sub-phenotype of CRSwNP. AERD patients typically have more severe sinonasal inflammation, their polyps grow quickly, and they undergo more sinus surgeries due to the recalcitrant nature of their disease [89,90]. Intolerance to COX-1 inhibitors has unique implications for clinical management of AERD. Aspirin desensitization followed by daily high-dose aspirin therapy can provide clinical benefit for patients with AERD but not for those with CRSwNP that tolerate COX-1 inhibitors [91,92]. AERD is predominantly characterized by type 2 inflammation. Studies are conflicting as to whether type 2 cytokine levels in AERD are similar or increased compared to CRSwNP, but levels are significantly elevated versus healthy controls [77,93]. In support of this, AERD patients clinically respond to type-2 biologics[94,95] and, in some studies, even more so than patients with CRSwNP [96]. As with observations in CRSwNP and CRSsNP, Type 1 and type 3 endotypes have recently been described in AERD[93,97]. A dysregulation of arachidonic acid metabolism uniquely distinguishes pathogenesis of AERD from CRSwNP. AERD patients have a characteristic over-production of cysteinyl leukotrienes and PGD_2 but reduced levels of PGE_2[98]. AERD patients also have marked activation of the 15 Lipoxygenase pathway, now thought to be important in CRS.[99,100] AERD patients with higher levels of urinary PGD_2 may fail to tolerate an aspirin desensitization compared to patients with lower PGD_2 levels, suggesting that sub-endotypes of AERD may also be present and clinically relevant[101].

Relating endotypes to phenotypes and clinical findings of disease

Several groups have examined the association between endotypes and clinical phenotypes in CRS. In general, T2 inflammation is associated with NP (in the West) and asthma [43,102]. The type 2 eosinophilic inflammation is also associated with disease recurrence and severity in CRSsNP and CRSwNP [70,103,104]. A study of the association between endotypes and phenotypes was conducted by Stevens et al., who examined inflammatory endotypes using markers including IFN-γ (T1), eosinophilic cationic protein (T2), Charcot-Leyden crystal galectin (T2), and IL-17A (T3) in the ethmoid and NP tissue and related them to clinical parameters from medical and surgical records[58]. The T2 endotype was associated with the presence of NP, asthma comorbidity, smell loss, and allergic mucin in all CRS patients. The presence of pus was associated with the T3 endotype, and headache/migraine was negatively associated with the T1 endotype. When assessing patients with CRSsNP alone, smell loss and headache/migraine were associated with a T2 endotype, and the presence of pus was more common in T1 and T3 endotypes. Similarly, the T3 endotype was also associated

with pus in patients with CRSwNP, and the T2 endotype tended to be associated with smell loss in patients with mixed endotypes. In contrast to the CRSsNP subgroup, headache/migraine was lower in the presence of T2 endotype in patients with CRSwNP [58].**Figure 4** summarizes the relationship between phenotype and endotype based on these findings.

A study using cluster analysis showed that older adults with CRS were more likely to have neutrophilic inflammation in the sinus tissue and elevated proinflammatory cytokines, IL-1β, IL-9, TNF-α, and IL-6 in the mucus compared to younger individuals with CRS [105]. The neutrophilic inflammatory pattern observed in older individuals was clinically associated with purulent drainage and a higher likelihood of bacteria. Potentially, these patients with predominantly neutrophilic inflammation are less likely to respond to corticosteroids or biologics and may respond to macrolides. Finally, elevated local IgE in NP tissue compared to control sinonasal tissue has been observed in patients with CRSwNP. Bachert and others have reported elevated levels of specific IgE (sIgE) against *Staphylococcus aureus* enterotoxins (SAE) in the NP tissue and systemic circulation [106,107]. The presence of sIgE to SAE has been associated with comorbid asthma and more severe sinonasal disease [108,109].

Associations between endotypes and responses to surgical and medical treatments

Few studies have been performed evaluating the efficacy of specific treatments for CRS as a function of endotype. Outcome studies for CRS have generally not evaluated endotype prior to intervention, relying on phenotype to subdivide patients. Further, the definition of treatment success and the time point of evaluation post intervention have also been variable. Despite these limitations, some tentative statements can be made based on the presumed endotype of the population treated.

Short-Term Broad-Spectrum Antibiotics

Three and even six-week courses were recommended for CRSsNP prior to surgery for many years, based primarily on uncontrolled cohort studies[110]. Currently, no recommendation is made for or against the use of antibiotics for CRS, given the lack of placebo-controlled studies and avoidance of antibiotic overuse[111-113]. CRSsNP was historically presumed to be the result of infection or secondary to biofilms, anaerobes or intracellular pathogens [13]. Later work indicated alteration of the sinonasal microbiome rather than emergence of a specific pathogen[14]. Based on current dogma, the tissue endotype resulting from bacterial infection should presumably be T3, providing a theoretical rationale for the use of antibiotics in this setting**(see Figure 2)** [40]. More recent studies on CRSsNP in Chicago have indicated that slightly over 50% of CRSsNP patient tissues exhibit at least a partial T2 endotype[57]. It is reasonable to expect that a properly selected group of CRS patients with T3 endotype, including polyp patients, would be more likely to respond to broad-spectrum antibiotics. A small prospective trial using 4 weeks of Augmentin documented objective and subjective improvement in the non-T2 CRS subset only[114]. It has also been proposed that*Staphylococcus aureus* amplifies or causes T2 inflammation in a subset of CRS patients [115-117], but studies documenting efficacy of anti-staphylococcal antibiotics in association with a reduction in this bacterium in the tissue have not been performed to date.

Macrolides

Macrolides exhibit immunomodulatory as well as antibiotic properties and some mixed evidence exists to support long-term use in selected CRS patients [112,113]. The presumed mechanism of action is the suppression of pro-inflammatory cytokines [118-120]Two randomized, placebo-controlled trials have been performed in CRS.[121,122] *Post-hoc* analysis of treatment response indicated that patients with low serum IgE (and presumably non T2) responded best and these responders exhibited decreased IL-8 levels in the mucus post treatment [121]. Later cohort studies demonstrated a lack of efficacy for macrolides in eosinophilic CRSwNP patients [123,124]. It remains to be established whether responders exhibit T1, T3, null or mixed endotypes. Studies of whether macrolides might have an additive effect with other medications (e.g. corticosteroids or a biologic) dedicated to suppressing T2 inflammation are worth pursuing [125-127].

Doxycycline

Doxycycline is an antibiotic that can also suppress cytokines, chemokines and remodeling factors [128-131]. Two small randomized controlled trials on Western CRSwNP patients (80-90% T2) indicated modest polyp shrinkage and symptom improvement with doxycycline, possibly by preventing enhancement of T2 responses by*Staphylococcus aureus* [71,132-134]. Based on these data, a role for doxycycline in the management of T2 CRSwNP has been proposed. Mechanistic studies on doxycycline are unclear, however, and a later clinical study indicated that low T2 biomarkers in CRSwNP patients were predictors of a clinical response[135-137].

Corticosteroids

For many years, corticosteroids have been considered a mainstay of treatment for CRS. Corticosteroids have potent anti-inflammatory properties and suppress T2 inflammation greater than T1 and T3, possibly explaining their better efficacy in CRSwNP than CRSsNP[138-141]. In the case of T2 inflammation, corticosteroids suppress ILC2 cells, Th2 cells, basophils and eosinophils [142-145]. Neutrophils are relatively resistant to corticosteroid effects, however [146,147]. Reduced activity against T3 inflammation may explain the decreased corticosteroid responsiveness observed in clinical trials of Asian CRSwNP as well as CRSsNP in general versus Western CRSwNP[66,147-154]. Topical corticosteroid sprays have limited access to inflamed sinus tissue but high-volume steroid irrigations, improved delivery devices and steroid-impregnated implants have improved efficacy [155-160]. Epithelial barrier remodeling defects and basal cell hyperplasia induced by T2 inflammation are partially reversed by corticosteroids [161,162]. Barrier defects may reflect expansions of basal epithelial cells due to epigenetically determined events in T2 CRS and may increase antigen access, heightening inflammation [46,112,163].

CRTH2 and Leukotriene Antagonists

A subset of AERD patients may express a discrete T2 subendotype with increased production of prostaglandin D2 (PGD_2) and cysteinyl leukotrienes compared to aspirin-tolerant T2 CRSwNP[101]. PGD_2 activates the chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2), which is important for eosinophil, basophil and lymphocyte recruitment and activation [164]. It is thus possible that CRTH2 and leukotriene antagonists could play a more significant role in managing patients with AERD compared to CRSwNP.

Surgery

Surgery is an option after failure of appropriate medical therapy[113,165]. Modern endoscopic sinus surgery (ESS) relieves sinus outflow obstruction, debrides inflamed tissue and provides improved access for topical agents [166]. Relief of obstruction is more relevant in mild to moderate CRSsNP[4] and balloon dilation may be sufficient in selected cases [167]. Mucus stasis from obstruction promotes microbial overgrowth and infectious inflammation predominantly in non-polypoid T1,3 inflammation. Relieving obstruction is of less value in CRS cases with diffuse inflammation as in CRSwNP and severe CRSsNP, in particular when associated with T2 inflammation[168,169]. Although high-level data is lacking, more extensive surgical procedures such as a 'full house ESS' are typically recommended for these cases [170-172]. Maximum surgical approaches are reserved for the most severe cases and involve removal of the floor of the frontal sinus and in some cases sinus mucosa[173-176]. Surgical recurrence rates are generally correlated with the intensity of T2 tissue inflammation[177-181]. Systemic markers of T2 inflammation such as blood eosinophilia are associated with surgical failure even in the absence of a T2 signature in the tissue [182]. In non-eosinophilic CRS, limited available data suggests that higher intensity of T1 and T3 inflammation also favors surgical failure[182,183].

Monoclonal Biologics

Biologic therapies targeting type 2 inflammation are increasingly used in patients with severe CRSwNP, which is associated with asthma comorbidity, worse disease severity, and recurrence after surgery (**Table 1**). Several monoclonal antibodies are either approved or under development for CRS. All inhibit aspects of T2 inflammation and have minimal side effects. Use of anti-T1 or T3 monoclonal antibodies for these respectively minor CRS endotypes (in the West) has not been attempted. Such treatment, if safe, might benefit patients with T1 or T3 endotypes, especially in Asia, where they are more prevalent.

Dupilumab is a monoclonal antibody that blocks IL-4 and IL-13 by binding to the α component of their shared receptors and inhibits T2 inflammation. In two phase 3 studies of only CRSwNP patients, SINUS-24 and SINUS-52, dupilumab reduced nasal polyp size, improved symptoms including nasal congestion and anosmia, and improved quality of life in patients with severe CRSwNP [94]. A pooled analysis of these studies showed that dupilumab reduced aggregate systemic corticosteroid use and nasal surgery by 76% compared to the placebo arm and substantially decreased type 2 inflammatory markers in serum and nasal secretions of patients with CRSwNP [184]. Based on these studies, dupilumab was approved for the treatment of inadequately controlled CRSwNP.

Omalizumab is a humanized monoclonal that selectively binds to the Cε3 domain of IgE and prevents IgE from binding to the high-affinity IgE receptor on mast cells and basophils [185]. Elevated local IgE is found in NP and is associated with local eosinophilic inflammation, severe NP, and comorbid asthma[11,43,49,72,79,106]. The POLYP 1 and POLYP 2 phase 3 trials found that omalizumab reduced polyp size and improved sinonasal symptoms and quality of life in patients with CRSwNP[95].

Mepolizumab is a monoclonal antibody that inhibits IL-5, the cytokine that is key in promoting eosinophil recruitment, activation and survival. Phase 3 trials have demonstrated subjective and objective efficacy and FDA approval for CRSwNP is expected late in 2021. (ClinicalTrials.gov. NCT0308579). *Benralizumab* is a cytotoxic monoclonal antibody targeting the IL-5 receptor that eliminates eosinophils and has undergone Phase 3 trials for CRSwNP (ClinicalTrials.gov. NCT03401229). The completed phase 3 trials reportedly met their primary endpoints at the time of this review.

Overall, although the monoclonal antibodies above are effective drugs that target key elements of T2 inflammation, their efficacy relative to each-other is presently unclear. T2 sub-endotypes likely exist, based upon anecdotal reports of variable response to these monoclonal antibodies. Intuitively, patients with eosinophil-driven disease should respond best to mepolizumab or benralizumab, while patients with disease driven by mast cells and IgE should respond best to omalizumab and perhaps dupilumab; no head-to-head trials have been performed, and no strong recommendations can be made as to which biologic to use first in a patient with T2 disease. Nonetheless, expert panels have made some recommendations for clinicians [186]. There is the untested impression that dupilumab has the greatest objective efficacy and highest response rate of the currently available T2 targeting monoclonal antibodies.

The success of T2 biologics in CRSwNP established the importance of endotype targeted therapy in difficult to treat NP. Use of these drugs is only approved in T2 polyp patients with established endotype. The use of mucus samples to determine endotype is under development. Currently, the presence of asthma, AERD and serum eosinophilia (>300μl) are indicators of T2 CRSwNP in surgery naïve patients. In post-surgery patients, eosinophilic histology is definitive. T2 biologics are indicated for CRSwNP with severe symptoms despite INS and more than one oral prednisone burst per year. Questions remain about whether these drugs should be used in patients that have not undergone surgery as the surgical revision rate at 5 years is only approximately 20% [187-189]. While these drugs are effective, QOL does not return to normal, and polyposis does not completely resolve[94]. The most effective and practical treatment regimen for high risk, multi-recurrent patients may be surgery followed by a planned post-operative biologic agent to prevent recurrence and further reduce symptom burden.

Summary and conclusions

Understanding the basis of widespread heterogeneity in the manifestations of CRS is advanced by findings that the three main endotypes, T1, T2 and T3, orchestrate the expression of three distinct large sets of genes. It is clear that T2 inflammation can be found around the world and in both CRSwNP and CRSsNP phenotypes. Although the prevalence of T2 endotype in Asia was very low decades ago, it is increasing with industrialization. Another emerging view is that endotype, rather than the phenotype, can drive clinical features, such as the presence of comorbid asthma (T2sNP and T2wNP) and pus (T3sNP and T3wNP). Drugs blocking T2 inflammation can shrink nasal polyps in western countries; as trials are initiated in T2sNP patients, we expect that this very large group of patients will be found to benefit from blockade

of type 2 inflammation. Studies of the microbiome may discover that the higher prevalence of T3 forms of CRS in China reflect distinct microbiological exposures. The development and use of improved methods of endotyping disease in the clinic will likely usher in expansion of the use of biological therapies targeting T2 and introduction of treatments targeting other endotypes.

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Table 1

Biologic	**Dupilumab (anti IL-4R[?])[1]**	**Dupilumab (anti IL-4R[?])[1]**	**Omalizumab (anti IgE)[2]**	**Omalizumab (anti IgE)[2]**	**Mepolizumab (anti IL-5)[3]**	**Benralizumab (anti IL-5R[?])***
Study Name (Total numbers) Treatment Duration	**SINUS-24 (N=276) 24 weeks**	**SINUS-52 (N=448) 52 weeks**	**POLYP 1 (N=138) 24 weeks**	**POLYP 2 (N=127) 24 weeks**	**SYNAPSE (N=407) 52 weeks**	**OSTRO (N=413) 56 weeks**
Co-Primary Outcomes	LS mean difference in NPS vs placebo (scale 0-8) 95% CI (P value)	LS mean difference in NPS vs placebo (scale 0-8) 95% CI (P value)	Treatment arm difference in NPS vs placebo (scale 0-8) 95% Cl (P value)	Treatment arm difference in NPS vs placebo (scale 0-8) 95% Cl (P value)	Difference in median change from baseline for NPS (scale 0-8) 95% Cl (P value)	NPS
	-2.06 -2.43, -1.69 (P<0.0001)	-1.80 -2.10, -1.51 (P<0.0001)	-1.14 -1.59, -0.69 (P<0.0001)	-0.59 -1.05, -0.12 (P=0.0140)	-0.73 -1.11, -0.34 (P<0.001)	Met primary endpoint
	LS mean difference in NCS vs placebo (scale 0-3) 95% Cl (P value)	LS mean difference in NCS vs placebo (scale 0-3) 95% Cl (P value)	Treatment arm difference in NCS vs placebo (scale 0-3) 95% Cl (P value)	Treatment arm difference in NCS vs placebo (scale 0-3) 95% Cl (P value)	Difference in median change from baseline in nasal obstruction VAS score 95% Cl (P value)	Nasal blockage score
	-0.89 -1.07, -0.71 (P<0.0001)	-0.87 -1.03, -0.71 (P<0.0001)	-0.55 -0.84, -0.25 (P=0.0004)	-0.50 -0.80, -0.19 (P=0.0017)	-3.14 -4.09, -2.18 (P<0.001)	Met primary endpoint
Secondary Outcomes	Significant improvement with dupilumab vs placebo (week 24)	Significant improvement with dupilumab vs placebo (week 24)	Significant improvement with omalizumab vs placebo (week 24)	Significant improvement with omalizumab vs placebo (week 24)	Significant improvement with mepolizumab vs placebo (week 52)	NOT PUBLISHED

Biologic	Dupilumab (anti IL-4R[?])[1]	Dupilumab (anti IL-4R[?])[1]	Omalizumab (anti IgE)[2]	Omalizumab (anti IgE)[2]	Mepolizumab (anti IL-5)[3]	Benralizumab (anti IL-5R[?])*
	Quality of life (SNOT-22) Total symptom score Sense of smell (UPSIT) and loss of smell score Radio-graphic assessment (Lund-Mackay score) Increased time to oral corticos-teroids or surgery	Quality of life (SNOT-22) Total symptom score Sense of smell (UPSIT) and loss of smell score Radio-graphic assessment (Lund-Mackay score) Increased time to oral corticos-teroids or surgery	UPSIT and loss of smell score SNOT-22 score UPSIT score TNSS	UPSIT and loss of smell score SNOT-22 score UPSIT score TNSS	SNOT-22 VAS score Loss of smell Reduced need for surgery Reduced need for systemic corticos-teroid use	
Adverse reactions with active drug	Injection site reaction, transient eosinophilia, conjunctivitis (7 cases), EGPA (1 case), eosinophilia with arthralgia (1 case)	Injection site reaction, transient eosinophilia, conjunctivitis (7 cases), EGPA (1 case), eosinophilia with arthralgia (1 case)	Headache, injection site reaction, dizziness, upper abdominal pain	Headache, injection site reaction, dizziness, upper abdominal pain	Nasopharyngitis	

1. Bachert C, et al. Lancet 2019;394:1638-1650

2. Gevaert P, et al. J Allergy Clin Immunol 2020;146:595–605

3. Eur Respir J 2020;56:Suppl. 64, 4616

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LS: least squares; NPS: nasal polyp score; NCS: nasal congestion score; SNOT-22: Sino-nasal Outcome Test; UPSIT: University of Pennsylvania Smell Identification Test; VAS: visual analog scale; TNSS: Total nasal symptom score

EGPA: eosinophilic granulomatosis with polyangiitis

Figure Legends

Figure 1 . Gene expression patterns in sinonasal tissue from patients undergoing surgery in Chicago at Northwestern. Data are from samples from patients with no sinonasal disease (Control, n=11), CRSsNP (9

TunsNP, 5 T1sNP, 8 T2sNP and 5 T3sNP) and CRSwNP (8 ET and 9 NP). The heatmap shows genes with more than 3-fold elevated levels in T1sNP, T2sNP or T3sNP compared to controls. Inspection of the gene expression patterns shows that CRSwNP samples from ethmoid and from nasal polyps are closely aligned with the T2 patterns seen in ethmoid tissue from a subset of patients with CRSsNP. The figure was adapted from published studies by Klingler al [55]. Tun – untypeable, T1 – Type 1 endotype, T2 – Type 2 endotype and T3 – Type 3/17 endotype (see text). ET – ethmoid tissue, NP – nasal polyp tissue.

Figure 2 . Overview of the primary cytokines driving T1, T2 and T3 endotypes, the source cells producing the primary cytokines and the effector cells that are recruited to the tissue and activated. Classical endotype refers to earlier endotyping based on the presence or absence of eosinophils. The natural pathogenic targets of each immunological endotype are listed at the bottom of the figure.

Figure 3 . Summary of the major biomarker genes whose expression is elevated in the T1, T2 and T3 endotypes of CRS (whether CRSsNP or CRSwNP). Also shown are the "mixed" endotypes, as indicated by T1,3, T1,2 and T2,3. Not shown are T untypeable (Tun), which do not express the biomarker genes or T1,2,3, a rare group of patients that have elevated levels of all three sets of biomarker genes.

Figure 4 . Overview of recent studies linking disease phenotypic signs and symptoms with molecular endotype, showing the changes in prevalence of each phenotype in the indicated endotypes. It is anticipated that future studies will increasingly link phenotypic signs and symptoms with underlying endotype to better define pathogenic mechanisms and indicate appropriate treatment regimens.