

Review article

Important research questions in allergy and related diseases:
3-chronic rhinosinusitis and nasal polyposis – a GA²LEN study

Chronic rhinosinusitis is one of the most common health care challenges, with significant direct medical costs and severe impact on lower airway disease and general health outcomes. The diagnosis of chronic rhinosinusitis (CRS) currently is based on clinical signs, nasal endoscopy and CT scanning, and therapeutic recommendations are focussing on 2 classes of drugs, corticosteroids and antibiotics. A better understanding of the pathogenesis and the factors amplifying mucosal inflammation therefore seems to be crucial for the development of new diagnostic and therapeutic tools. In an effort to extend knowledge in this area, the WP 2.7.2 of the GA²LEN network of excellence currently collects data and samples of 1000 CRS patients and 250 control subjects. The main objective of this project is to characterize patients with upper airway disease on the basis of clinical parameters, infectious agents, inflammatory mechanisms and remodeling processes. This collaborative research will result in better knowledge on patient phenotypes, pathomechanisms, and subtypes in chronic rhinosinusitis. This review summarizes the state of the art on chronic rhinosinusitis and nasal polyposis in different aspects of the disease. It defines potential gaps in the current research, and points to future research perspectives and targets.

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A series has been started in Allergy for determining the important research issues in allergy (1). This paper is the third of the Series (2, 3).

Sinusitis and rhinitis usually coexist and are concurrent in most individuals; thus, the correct terminology for sinusitis is now *rhinosinusitis*. Attempts have been made to define rhinosinusitis in terms of pathophysiology, microbiology, radiology, severity of symptoms and their duration (4–6).

Rhinosinusitis is a significant health problem, also impacting the lower airways and it results in a large financial burden on society (7–9). However, the disease entity is badly defined, the diagnosis of chronic rhinosinusitis (CRS) is difficult and cannot be made on the grounds of symptoms only and the mechanisms are poorly understood. Data on (chronic) rhinosinusitis are therefore limited and difficult to interpret.

The last decade has seen the development of a number of guidelines, consensus documents and position papers on the epidemiology, diagnosis and treatment of acute and CRS and nasal polyposis (NP) (10–16). Although of considerable assistance, the available consensus documents on CRS and nasal polyps do not answer a number of relevant questions that would unify the information and current concepts that exist in epidemiology, diagnosis, treatment and research. In addition, none of these documents were evidence-based. The EP³OS document attempts to answer these questions (17).

Until recently, rhinosinusitis was usually classified, based on duration, into acute, sub-acute and chronic (6). This definition does not incorporate the severity of the disease as well as the mechanisms. A universal definition of CRS should be made available; however, there is also need to investigate the mechanisms underlying heterogeneous conditions in a more rigorous manner.

Prevalence

Chronic rhinosinusitis without nasal polyps

According to the National Institute of Allergy and Infectious Diseases Fact Sheet, CRS is the most commonly reported chronic disease, affecting 16.3% of the total population (nearly 32 million) in the United States in 1997 (18). The above figures have been arrived at without taking into account allergic rhinitis. A similar

number is reported by CDC for 2003 (29.7 million) (19). However, the prevalence of doctor-diagnosed CRS is much lower; a prevalence of 2% was found using ICD-9 codes as an identifier (20). In Canada, the prevalence of CRS, defined as an affirmative answer to the question 'Has the patient had sinusitis diagnosed by a health professional lasting for more than 6 months?' ranged from 3.4% in male to 5.7% in female subjects (21). By screening a non-ENT population, which may be considered representative of the general population in Belgium, 6% of subjects suffered from chronic nasal discharge (22).

Chronic rhinosinusitis with nasal polyps

Nasal endoscopy appears to be a prerequisite for an accurate estimate of the prevalence of NP (23). Surveys based on questionnaires asking for the presence of nasal polyps, provide an overestimation of the self-reported prevalence. Recently, a French ENT expert panel elaborated a diagnostic questionnaire/algorithm of nasal polyps with 90% sensitivity and specificity (24).

In a population-based study in Sweden (23), a prevalence of 2.7% was reported in the population. Based on a postal questionnaire survey in Finland, the prevalence of nasal polyps was 4% (25). Using a disease-specific questionnaire, the prevalence of NP was reported in 2.1% of the French population (24).

In general, nasal polyps occur in all races in adults and become more common with age (24, 26–28). Nasal polyps are uncommon in children except in cystic fibrosis (CF) (29).

Assessment of prevalence and clinical phenotypes of CRS

A prerequisite for any mechanistic study is to phenotype the patients on clinical grounds. The GA²LEN epidemiological survey forms the background for a large European survey already described (3). This survey will make it possible to assess the true prevalence of CRS and will have a major pharmacoeconomic impact as many patients diagnosed with CRS may only have nonallergic rhinitis.

The first step is ongoing in over 20 centres and the questionnaire has been developed incorporating nasal symptoms for consolidating the details on all patients with CRS.

The second step consists of a full-length questionnaire with questions related to CRS and nonallergic rhinitis as well as a complete ENT examination including nasal endoscopy, measurement of the peak nasal inspiratory flow, and smell testing. The impact on lower airways is evaluated by spirometry with bronchodilatation. Impact of CRS on quality of life is studied using the EUROQOL instrument (EuroQol group, Europe). This epidemiological study has been essential to enrol patients who are representative of the European population and to avoid the selection bias (30).

Abbreviations: AERD, aspirin-exacerbated respiratory disease; AMP, antimicrobial peptide; CF-NP, cystic fibrosis nasal polyps; COX, cyclooxygenase; CRS, chronic rhinosinusitis; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; CSS, Churg-Strauss syndrome; DC, dendritic cell; ECP, eosinophilic cationic protein; IFN, interferon; Ig, immunoglobulin; IL, interleukin; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; SNP, single nucleotide polymorphisms; TGF, transforming growth factor; Th, T helper; TIMP, tissue inhibitor of metalloproteinases; TLR, toll-like receptor; Treg, T-regulatory; WP, Work Package.

A better understanding of the pathogenesis and the factors amplifying mucosal inflammation seems to be crucial for the development of new diagnostic and therapeutic tools. The GA²LEN network of excellence offers a unique opportunity to collaborate on a European scale to extend knowledge in this area. The GA²LEN Work Package (WP) 2.7.2 currently collects data and samples of 1000 CRS patients and 250 control subjects. The main objective of this project is to characterize patients with upper airway disease on the basis of clinical parameters, infectious agents, inflammatory mechanisms and remodelling processes. By using a cluster analysis approach on approximately 1000 patients and 250 controls, CRS patients will be categorized into clinically relevant subgroups based on clinical phenotyping and biomarker profiles (inflammatory and remodelling factors).

Samples from well-characterized patients will also be genetically evaluated using a candidate gene and a whole-genome-scan approach.

This collaborative research will result in better knowledge on patient phenotypes, pathomechanisms, and subtypes in CRS. This review summarizes the state-of-the-art details on CRS and NP in different aspects of the disease. It defines potential gaps in the current research, and points to future research perspectives and targets.

Assessment of phenotypes based on biomarkers

Diagnosis of CRS currently is based on clinical signs, nasal endoscopy and CT scanning, and therapy recommendations are focussing on two classes of drugs, corticosteroids and antibiotics. However, none of these drugs is able to change the fate of the patient, and to avoid surgery or at least maintain the postsurgical result. A better understanding of the pathogenesis and the factors amplifying mucosal inflammation is therefore crucial for the development of new diagnostic and therapeutic tools, which clearly are needed.

In an effort to further develop our knowledge in this area, the WP 2.7.2 of the GA²LEN network of excellence currently collects data and samples of 1000 CRS patients and control subjects, which will be studied in different scientific modules focussing on inflammation, remodelling, chemotaxis, dendritic cell (DC) and T-cell regulation, eicosanoids, the role of Staphylococcal enterotoxins, innate immunity and genetics. These biological data will be interrelated, and related to available clinical information. In fact, we hope to base our new interpretation and classification of chronic sinus disease on biomarkers instead of clinical signs, and may thus come to a meaningful understanding of disease subgroups, their clinical implications, and diagnostic markers as well as therapeutic targets. It is very likely that the current diagnostic tools will be insufficient, and need to be

amended by innovative 'omics' based approaches, which will cover several aspects of the disease such as type of inflammation, status of remodelling, amplification of disease by bacterial factors and others. However, with new therapies such as humanized anti-interleukin-5 (IL-5) antibodies or anti-metalloproteinase (MMP) approaches on the horizon, and the need to predict response to those treatments, also innovative diagnostic approaches are mandatory.

This new understanding will finally lead to the unravelling of the natural courses of disease subgroups, and the events progressing or inhibiting disease development as well as their co-morbidities.

Composition of inflammation in chronic rhinosinusitis, subgroup identification

Both European and US guidelines currently consider CRS as an umbrella for all sinus disease, although differentiation of sinus disease is granted for scientific and research purposes (10, 31). Clinical pattern of symptoms and signs are overlapping in patients with CRS with nasal polyps (CRSwNP) or without (CRSSNP). As a result, all chronic sinus disease is considered as one disease spectrum, 'CRS', which severely obstructs the development of pathophysiological knowledge and new therapeutic approaches. Innovative biological approaches to overcome this problem are urgently needed.

In a milestone investigation, inflammatory cells and mediators of the sinonasal mucosal tissue from CRSwNP, CF-NP, CRSSNP and control patients were investigated (32). This study clearly demonstrated that all three groups of CRS had characteristic differences in those markers, which allowed for a pathophysiologically meaningful differentiation with likely therapeutic consequences. Nasal polyps had significantly higher levels of eosinophil markers when compared with CRSSNP, controls and esp. Cystic fibrosis nasal polyp, which displayed a neutrophilic inflammation. Most importantly, CRSSNP was characterized by a Th1 polarization with high levels of interferon-gamma (IFN- γ) and transforming growth factor-beta (TGF- β), while CRSwNP showed a Th2 polarization with high IL-5 and immunoglobulin (Ig) E concentrations (Fig. 1) Thus, based on cellular and mediator profiles, CRSSNP, CRSwNP and CF-NP are distinct disease entities within the group of CRS. It is very likely that even within the CRSwNP group, subgroups may be differentiated in the near future, partially related to lower airway (asthma) or systemic (aspirin-exacerbated respiratory disease; AERD) co-morbidities.

Of note, clinically comparable nasal polyp disease also seems to exist without abundantly activated tissue eosinophils (33). We demonstrated that nasal polyps from South China, which show a neutrophilic cellular pattern similar to other Asian regions (34), and polyps of European patients do not share the same T-effector cell polarization. South

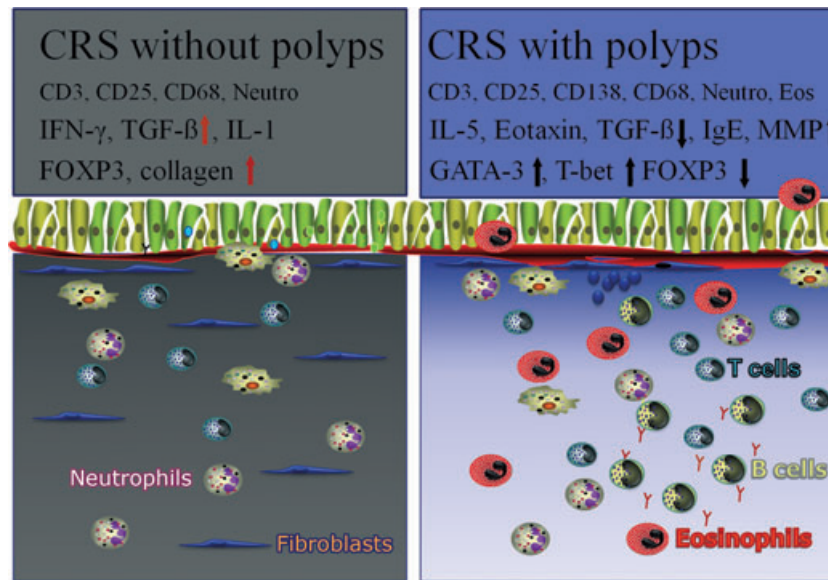


Figure 1. Overview of the differences between CRS with and without nasal polyps based on inflammatory mediators and remodelling factors.

Chinese CRSwNP did not show increased IL-5, eosinophilic cationic protein (ECP) or IgE concentrations within the tissue (33). Further studies revealed that instead of a Th2 effector cell pattern, Th17-related cytokines such as IL-6 and IL-17 were found in most nasal polyps of Chinese patients (35).

Eosinophils in Caucasian CRSwNP are activated, their survival is increased by IL-5-induced reduction of apoptosis (36, 37), and they are accumulated within the tissue by eosinophil-specific adhesion receptors and chemokines such as eotaxin (38, 39). Those cytokines, and consecutively the eosinophilic inflammation, are further increased in polyps with specific IgE to *Staphylococcus aureus* enterotoxins (SAEs), which serve as superantigens, inducing a polyclonal T-cell and B-cell activation with IgE formation, and amplifying the inflammation (40–42).

Consistent with the oedema formation in CRSwNP, upregulation of MMP-7 and MMP-9, but not of the tissue inhibitor of metalloproteinases (TIMP)-1 has been described, which is in line with a downregulation of TGF- β 1 (32). Very recently a significantly lower FOXP3 expression, but a significantly higher T-bet and GATA-3 expression in CRSwNP when compared with controls was observed, suggesting a deficit in T-regulatory (Treg) capacity, which leads to a strong increase in Th1 and Th2 effector cell signals (43).

The specific knowledge on the role of IL-5 in CRSwNP (36) and the possibility of its antagonism *in vitro* (37) has finally resulted in a study using humanized anti-IL-5 antibodies for the treatment of nasal polyps (44). Of interest, a subgroup of patients only responded to this treatment, again pointing to different subgroups within CRSwNP. A similar background encouraged studying the effect of a humanized anti-IgE-antibody in nasal

polyps; again, it is expected to find a subgroup of patients reacting to this approach, based on findings of high local IgE concentrations, particularly in relation to SAE effects (40, 45).

Departing from well-defined patient groups with CRS, the regulation of persistent mucosal inflammation and consecutive changes in the extracellular matrix need to be further unravelled. This will have to include Th1, Th2, Th17 and Treg cytokines, chemokines, metalloproteinases, ECM components, but also markers of innate immunity such as pathogen-associated molecular patterns, damage-associated molecular patterns (DAMPs), TLRs etc. As a confirmatory step, the analysis of selected markers in a random sample of several hundred CRS patients is currently planned. This analysis will also have to include the impact of environmental factors on the inflammation, such as cigarette smoke, allergens, bacteria and their products, viruses etc, as well as eventual deficits in appropriately responding to the environment e.g. based on genetic factors (gene–environment interaction). Today, multiplex techniques already allow the measurement and evaluation of a great number of factors simultaneously, and this progress will be enforced by the use of ‘-omics’ such as genomics, proteomics, etc. This should eventually lead to patient-specific ‘fingerprints’, which allow specifying the diagnosis and selecting the appropriate therapeutic target, and predicting its outcome.

Dendritic cells and T lymphocytes

The role of the DCs or T lymphocytes in CRS or NP is not clear. Given the described co-morbidity between allergy/asthma and CRS/NP (17, 46), most research has

focused on local Th2 vs Th1 responses, whereas hardly any data is available on local Th17 or Treg responses. However, the Th2-skewed co-morbidities could mask an intrinsic CRS profile of immune-competent cells.

No comparison data on DC subsets is available for CRS. A recent manuscript (47) on a very few patients showed the expression DC-LAMP and DC-SIGN in nasal biopsies. A second study (48) showed the presence of IgE+ Langerhans' cells (CD1a). Other than that these cells types are present, no conclusion on their role in CRS/NP were drawn.

A well-controlled study showed that most tissue T lymphocytes are activated memory T cells (CD3+ CD45RO+) that display a mixed Th1/Th2 phenotype (49). Interestingly, these tissue cells are CD69^{high} CD25^{low}, which is opposite from the same cells tested in blood, but similar to cells observed in rheumatoid arthritis (50). They expressed IL-5 (Th2) and IFN- γ (Th1), but the absence of IL-4 (Th2) and missing data on IL-17 (Th17) or IL-10/TGF- β (Treg cells) does not allow firm conclusions. Expression data on CD4+ and CD8+ cells confirmed the IL-5 and IFN- γ expression, but in contrast also showed IL-2 and IL-4 expression (51). An independent approach showed a positive correlation for total IgE with CCR4+ cells and a negative correlation with CCR5+ cells, in line with a Th2-skew in CRS/NP (52).

Data on IL-17 (Th17) or IL-10 (Treg cells) was only available on whole tissue samples (53, 54) and not on a cellular level, which limits the scope of the conclusions. Similarly, TGF- β that is produced by a subset of Treg cells is often only considered in relationship to remodelling (55) and not to immune regulation (56). Recently, differences in key regulatory transcription factor expression were detected between healthy controls and CRS (with polyps or without polyps) (43). In CRS/NP, FOXP3 (Treg cells) and TGF- β 1 (Treg cells, remodelling) were expressed at a lower level than in controls, while T-bet (Th1) and GATA-3 (Th2) were expressed at a higher level. In CRS none of these proteins were affected, but showed a higher level of TGF- β 1 (Treg cells, remodelling) and IFN- γ (Th1) (43).

Our first goal should be to define the local and systemic cellular make-up in chronic sinusitis and NP. This should lead to a better understanding of the functional specializations of different sub-types of DCs and T lymphocytes (57, 58).

CC-chemokines and adhesion molecules

Although specific cytokines, chemokines and adhesion molecules that appear to play a central role in the pathogenesis of CRS have now been identified (10, 32, 59–63), their exact role and contribution to disease persistence remains unknown. There is a growing consensus that CRS is a heterogeneous group of sinus

diseases (31); thus, chemokines and adhesion molecules need to be considered for each subgroup separately.

Chemokines are more specific activators of target cells than cytokines. Moreover, chemokines play a key role in selective cell migration, and they have cell-selecting and activating properties. Even though there are no specific chemokines for the different cell types, one group of CC-chemokines, the eotaxins (eotaxin-1, 2, 3) have a preference in the activation of eosinophils via a single CC-chemokine receptor, the CCR-3 (64–66). Regulated on Activation, Normal T Expressed and Secreted (RANTES) acts via CCR-1, -3 and -5 on monocytes, T-lymphocytes, eosinophilic and basophilic granulocytes and is therefore unspecific for eosinophilic granulocytes expressing only CCR-3 (67). Recent studies have shown that ECP, IgE and IL-5 contribute to differentiate CRSwNP from CRS (59, 63). These findings confirm that eosinophils and related inflammatory products are the hallmark of NP-associated inflammation when compared with CRSsNP (32).

Dysregulation of epithelial CC-chemokine production, including eotaxin and RANTES in CRSwNP might be important in promoting the local chemotaxis of eosinophils (39, 68, 69). Furthermore, differences in the expression of eotaxin and RANTES between patient groups with NPs and different aetiological factors (allergy, aspirin-sensitivity) vs control groups have been described (62, 70). Several investigators have found increased expression of ICAM-1, VCAM-1, E-selectin and P-selectin on NP endothelium (60, 71) (8, 10).

However, there were only limited and contradictory data available concerning chemokines and adhesion molecules in CRSsNP, or comparisons of chemokine patterns in CRSwNP vs CRSsNP, often because of a lack of valuable classifications of sinus diseases until the EP³OS position paper (31).

Studies to distinguish CRSsNP and CRSwNP as disease entities need to include chemokines and adhesion molecules at the level of nasal tissue, blood vessels and circulating cells. With different comorbidities, subgroups within those poles may be found, relating to the underlying diseases.

All of the CC-chemokines act via a common receptor, the CCR-3. Thus, a promising target for a differentiated therapeutic approach is the antagonism of the CCR-3 receptor, possibly in combination with other anti-eosinophilic approaches. The modified chemokine met-RANTES or the anti-eotaxin antibody mAB711 were first steps to block CCR-3 (72, 73).

Eicosanoids and aspirin-exacerbated respiratory disease

The association of CRSwNP with aspirin hypersensitivity and asthma is referred to as 'aspirin triad' (73) or aspirin-induced asthma (74) where nasal polyp prevalence is 80–90% (74, 75). Recently this syndrome was renamed

AERD. In aspirin-sensitive asthmatics, bronchospasm and nasal symptoms (severe rhinorrhoea and nasal congestion) are induced by ingestion of aspirin and other nonsteroidal anti-inflammatory drugs, through a mechanism attributed to cyclooxygenase (COX)-1 inhibition (76, 77) with the influx of inflammatory cells, such as mast cells and eosinophils, and release of inflammatory mediators, such as tryptase, histamine, ECP and leukotrienes (78–80). Accordingly, different mechanisms underlying the pathogenesis of CRS and nasal polyps in aspirin-sensitive and -tolerant patients may be present (17, 81).

Tissue eosinophilia is a prominent feature of CRSwNP in aspirin-sensitive patients with an increased release of ECP (82, 83) and an upregulation of cytokines related to eosinophil activation and survival (IL-5, GMC-SF, RANTES, eotaxin) (68, 84, 85). An overproduction of IL-5 might intensify eosinophilic inflammation in aspirin-sensitive patients (86) and be correlated with increased levels of IgE to SAEs present in nasal polyp tissue (40).

Arachidonic acid metabolism abnormalities have been considered a distinctive feature of nasal polyps in aspirin-sensitive patients with a lower generation of both prostaglandin E₂ (PGE₂) (87) and COX-2 (88, 89), together with a reduced nuclear factor- κ B activity (90). Thus, an intrinsic defect in local COX-2 and PGE₂ generation could contribute to the development of severe eosinophilic inflammation mainly in aspirin-sensitive patients, but, to a lower extent, also in patients without aspirin-sensitivity (91, 92).

Some studies have not found any differences in leukotriene C₄ (LTC₄) release from nasal polyp-cultured cells (83) and the generation of COX and lipoxygenase (LO) products from whole blood cells between aspirin-sensitive and -tolerant patients (93), while others have observed an increased generation of leukotriene-producing enzymes (5-LO and LTC₄ synthase), cysLTs in nasal polyp tissue in aspirin-sensitive patients (80, 92, 94, 95). Moreover, increased expression of cysLT₁ receptors in the nasal mucosa from aspirin-sensitive patients was found (96, 97). CysLTs production correlates with tissue ECP concentration both in aspirin-sensitive and -tolerant nasal polyps suggesting that these mediators may be linked to tissue eosinophilia rather than to aspirin-sensitivity. In addition, upregulation of 15-LO metabolites (87) and downregulation of lipoxin A₄ have also been associated with nasal polyps in aspirin-sensitive patients (92).

The necessary investigation for understanding the underlying mechanisms and difficult clinical courses and management issues of AERD patients are on:

- 1 Genetic background [polymorphisms within COX-1/COX-2 pathways, 5-LO/15-LO pathways, receptors related to AA metabolites (EP₁₋₄, LT₁, LT₂), PLA₂ and others].
- 2 Natural history and role of infectious factors (*S. aureus*, viruses).

- 3 Role of arachidonic acid metabolites and receptors involved in its pathogenesis.
- 4 Local and systemic function of DCs and Treg cells.

TGF- β isoforms and receptors in chronic rhinosinusitis with or without nasal polyps

In recent years, it became clear that TGF- β could play a crucial role in airway disease. Transforming growth factor- β is a multifunctional and pleiotropic growth factor involved in many processes, with important immunomodulatory and fibrogenic characteristics. Many chronic diseases profit from the immunosuppressive effect of TGF- β , however, TGF- β has also been implicated in fibrosis formation and is suspected to play a major role in airway remodelling. In general, the function of TGF- β could be understood as a counter-regulatory cytokine to resolve inflammation and to initiate repair process.

One striking difference between chronic sinusitis with or without nasal polyps is the local tissue concentration of TGF- β . CRSsNP is characterized by significant higher levels of TGF- β when compared with controls, whereas TGF- β 1 has been found significantly lower in CRSwNP when compared with controls (32, 43, 55, 56). Two aspects seem to be of major importance in chronic sinus inflammation. Transforming growth factor- β has an important effect on extracellular matrix remodelling. The effect on extracellular matrix production is manifested by enhanced expression of extracellular matrix proteins and suppression of expression of matrix-degrading proteins. Transforming growth factor- β induces fibroblast proliferation, collagen production and collagen gel contraction (Fig. 2). Transforming growth factor- β also regulates the function of immune cells, for which it is a strong suppressor of activation of T cells and of antibody secretion by B cells. Recent research has

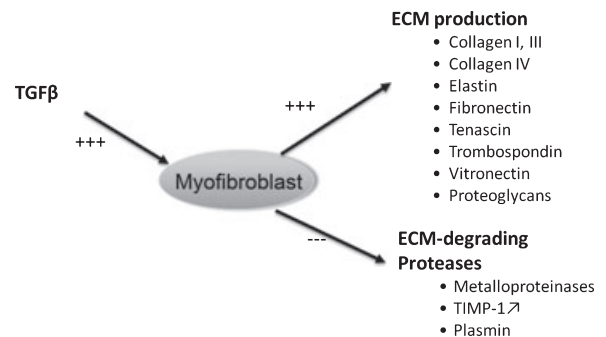


Figure 2. Regulation of extracellular matrix constitution by TGF- β . In myofibroblasts, TGF- β upregulates the expression of several extracellular matrix proteins, but it also downregulates the expression of matrix metalloproteinases and induces MMP-inhibitors such as TIMP-1.

suggested the role of TGF- β in Treg cells, where it participates in both suppression and differentiation. T-regulatory cells are able to suppress both Th1 and Th2 responses by producing TGF- β . It was hypothesized that decreased TGF- β expression in CRSwNP could be interpreted as a decreased Treg cell function (32), which has recently been confirmed (43). Low FOXP3 levels were found together with low levels of TGF- β 1 in CRSwNP while T-bet (Th1) and GATA-3 (Th2) were expressed at a higher level (43).

However, several aspects on TGF- β regulation need to be further unravelled. Until now, TGF- β 1 has been the most studied isoform. Little data are available regarding the specific distribution of the other isoforms in chronic sinus disease. Lower airway research revealed that in severe asthma TGF- β 2 is the predominant isoform expressed, associated with tissue eosinophilia (98). Further study of the distribution of TGF- β isoforms is needed in upper airway disease.

Secondly, little data are available regarding the expression of TGF- β receptors in CRS with or without NP. A better understanding of the expression of TGF- β receptors in different chronic sinus diseases would provide a better understanding of TGF- β signal transducing.

Thirdly, TGF- β is a profibrotic growth factor that stimulates the production of extracellular matrix components, such as tenascin-C, collagen, fibronectin and laminin. Analysis of extracellular matrix composition in relation to TGF- β tissue concentration will provide a better understanding of the remodelling phenomena observed in CRS. Finally, regulation and activation of TGF- β in chronic sinus disease is not fully understood. Until now, most techniques detect total TGF- β concentrations, including both active and latent forms. A better understanding of the balance between physiologically active and latent forms in diseased sinus mucosa is necessary.

MMP in chronic rhinosinusitis and nasal polyposis

Matrix metalloproteinase family comprise 23 structurally related endopeptidases (99). Their main function is in controlling tissue remodelling in embryonic development and tissue morphogenesis (100, 101). Matrix metalloproteinases are also important effectors molecules for immune cell recruitment and migration and they can modify immune responses (101, 102). In addition, certain MMPs exhibit anti-inflammatory properties (103–105). Tissue inhibitors of metalloproteinases are the major endogenous MMP inhibitors, maintaining the balance between extracellular matrix deposition and degradation in physiological processes (106, 107).

The current knowledge of MMP expression in CRS is limited. Matrix metalloproteinase-9 and TIMP-1 are found in CRSsNP, whereas in CRSwNP, MMP-9, but

not TIMP-1, is upregulated (108–111). In polyps, MMP-9 positive cells are found inside the pseudocysts, suggesting a direct degradative function, whereas upregulation of TIMP-1 over MMP-9 could explain the prominent fibrosis in CRSsNP (110). This could be secondary to the impaired wound repair, as elevated MMP-9 levels are found in the physiological repair process in the respiratory epithelium and could explain why high MMP-9 levels can predict poor healing after sinus surgery (112). Matrix metalloproteinase-9 together with MMP-2 controls also the transepithelial migration of inflammatory cells by controlling the transepithelial C-C-chemokine gradient, which may explain the accumulation of eosinophils in the respiratory epithelium in MMP-9 knockout mice after allergen exposure (103, 113). Furthermore, in CRSwNP, low MMP-9/TIMP-1 and MMP-8/TIMP-1 ratios are associated with enhanced eosinophilic inflammation (108), indicating synergistic functions of MMP-8 and MMP-9 in the control of Th2 inflammation in CRSwNP.

Mucous from CRSwNP patients contains high levels of MMP-8 (108, 114). In particular, the mesenchymal-type MMP-8 produced by the structural cells is converted to active forms (114). This activation is associated with elevated IL-8 levels, forming inductive cytokine-proteinase cascades in CRSwNP. Prominent MMP-1 expression is seen in nasal polyps especially in the areas with loose stroma, suggesting MMP-1-promoted extracellular matrix degradation (115).

Matrix metalloproteinase-7 is found in tissue homogenates from nasal polyps, but not in sinus mucous (108, 110). Matrix metalloproteinase-7 is constitutively expressed in the normal epithelium, and it has an important role in the repair of respiratory epithelium, and in innate immune responses (116–118). Enhanced MMP-7 expression in polyp tissue may reflect ongoing epithelial activation.

Low levels of MMPs are needed in tissue homeostasis, whereas excessive MMP activity results in destructive processes. This makes MMPs an interesting therapeutic target also in CRSwNP. However, too strong an inhibition may prohibit its homeostatic functions, leading to side-effects and reduced efficacy (119). A weak MMP inhibitor, such as tetracycline derivatives and possibly macrolides, may more safely reduce tissue destruction without interfering with the physiological functions (119). There is a need for more detailed understanding of MMP functions and placebo-controlled studies of MMP inhibitors in different forms of CRS.

Innate immunity: from defensins to TLRs

Innate immunity mediates a first, early defense against invading infectious pathogens immediately after recognition of structural components of microorganisms by germ-line encoded receptors, independently of prior

exposure to the invading pathogen. By attacking essential structures of the microbe, which cannot easily be changed, resistance to these peptides is much lower than to chemical antibiotics. Human antimicrobial peptides (AMPs) such as defensins and cathelicidin are present in leucocytes and are also secreted by various epithelia in skin and mucosal surfaces (120–122).

Pathogen-recognizing receptors recognize in a selective way the conserved components on microbial surfaces with various degrees of affinity and contribute to the induction of an adequate innate and adaptive response. Among these, the toll-like receptors (TLRs) are transmembrane receptors with an extracellular leucine-rich repeat domain and an intracellular toll domain: IL-1 receptor domain that is responsible for initiating signalling pathways through protein–protein interactions (123). In their function as sensors of immunity, TLRs have been proposed as modifiers of the Th1/Th2 balance in allergic disorders. A genetic polymorphism encoding TLR2 (TLR2/-16934) was found to be associated with a reduced susceptibility to asthma and allergy in farmer's children (124).

Cellular innate responses depend on the activity of phagocytic cells (macrophages and neutrophils) and on the expression of proteins and peptides by epithelial cells and phagocytic cells. Also DCs, mast cells, natural killer cells, $\gamma\delta$ T cells and B-1 cells show direct innate immunity capacity (innate recognition of antigen without T-cell-mediated antigen presentation) (125).

Innate immunity mechanisms are an interesting target for therapeutic strategies. Triggering of the innate immune system (Lipopolysaccharides (LPS), CpG DNA and probiotics) may provide protection against and treatment of allergic disorders. As the problem of emergence of bacterial resistance to current antibiotic drugs continues to grow, there has been considerable interest in the development of AMPs as a novel therapeutic approach to treat infections. For AMPs, there are several different potential strategies for their general therapeutic application: (i) as single anti-infective agents; (ii) in combination with conventional antibiotics or antivirals to promote any additive or synergistic effects; (iii) as immunostimulatory agents that enhance natural innate immunity and (iv) as endotoxin-neutralizing agents to prevent the potentially fatal complications associated with bacterial virulence factors that cause septic shock. Of the potential pool of natural peptides, relatively few have actually proceeded into clinical trials based on promising data from *in vitro* and animal studies.

Superantigens modify inflammation

Staphylococcus aureus frequently colonizes the nasal mucosa in nasal polyp disease, and may release superantigens, which interfere with local T- and B cells (126). The finding of IgE-antibodies to SAEs in nasal polyp tissue homogenates (40) for the first time indicated that these

superantigens may be involved in the pathogenesis of eosinophilic CRSwNP (36–38).

An increased colonization rate of *S. aureus* and IgE to SAEs was reported in nasal polyps, specifically in subjects with asthma and AERD (127). Immunoglobulin E to SAEs also was co-incident with higher levels of IL-5, eotaxin and eosinophil cationic protein. Moreover, an increased number of T cells expressing the TCR β -chain variable region known to be induced by microbial superantigens was detected in CRSwNP and correlated with the presence of sIgE against SAEs (128).

In a recent *ex vivo* study, the modulatory effects of SAEB on nasal polyp tissue were determined (42). *Staphylococcus aureus* enterotoxin polarized the mucosal inflammation to a Th2-pattern, and may contribute to persistent inflammation by suppression of Treg lymphocytes (42).

A significant increase of local IgE antibodies can be observed in polyp patients, independent of serum IgE measurements, supporting the notion that SAEs locally activate B- and plasma cells (127). High concentrations of IgE, IgA and IgG were measured in nasal polyp tissue (45), and homogenates with detectable SAE-IgE had significantly higher concentrations of IgG4 and IgE abs.

Nasal polyps typically show upregulation of pro-inflammatory cys-LTs and down-regulation of PGE₂ (92). In polyp tissue of patients with an immune response to SAEs, the production of cys-LTs, LTB₄ and LXA₄ is further upregulated (129). *Staphylococcus aureus* enterotoxin B significantly downregulated PGE₂, COX-2 and prostanoid receptor EP₂ mRNA expression in fibroblasts, pointing to a direct role of bacterial superantigens in regulating eicosanoids (130).

There is a clear need to understand the factors determining the manifestation of immune effects of SAEs on a given tissue; these might be genetic host factors, local tissue or environmental influences. It is not clear yet, what pathways superantigens could use to impact the inflammation, apart from a direct interaction with the T cells via TCR, i.e. the functional role of local IgE in polyp disease needs to be investigated. Often disregarded as 'junk', multiclonal IgE could play an important role by facilitating a permanent stimulation of mast cell mediator release. Of great interest certainly are therapeutic approaches against *S. aureus* and SAE effects, such as antibiotic treatment or vaccination against this difficult-to-eradicate germ.

Identifying genes involved in chronic rhinosinusitis and nasal polyposis

Allergic rhinitis and asthma are chronic inflammatory diseases of the airways linked by epidemiological, pathological and physiological characteristics and by a common therapeutic approach (131). The idea that a 'sensitization tendency' in these diseases might be genetically transmitted had originated nearly 100 years ago

(132) and their hereditary character has been substantiated in twin and association studies during the last decades (133, 134). However, the presence of a genetically transmitted component of CRS remains to be established.

Candidate gene approach

It is likely that many genes involved in the onset, course and severity of asthma, allergic rhinitis and CRS are common to these diseases. It is therefore a reasonable starting point to screen genes associated with asthma also in patients with CRS. A recent review by Ober and Hoffjan (134) summarizes the results of nearly 500 papers on asthma and atopy association studies. They identified a set of 25 highly replicated genes and an additional set of 54 genes replicated between two and five times. Markers covering these genes will be used to analyse a set of 380 cases and 190 controls. Using this strategy, we can detect a minimum odds ratio of 3.0 with 80% power at the 5% significance level when the minor allele frequency (MAF) is 5%. Single nucleotide polymorphisms (SNPs) that display allele frequency differences will be further analysed by analysis of the whole material.

Resequencing of genes found to be associated with CRS in the association study will then be performed to identify all common coding polymorphisms in 48 CRS cases. All genes will also be investigated for presence of putative deletions/duplications by multiplex ligation and probe amplification analysis.

Whole-genome scan approach

The use of the 500kSNP chip from Affymetrix (Affymetrix, Inc., Santa Clara, CA, USA) should provide information for the absolute majority of the 7–8 million SNPs with minor allele frequencies >0.05 that are present in the European population. A set of 380 individual cases and 190 controls will be analysed. Based on the results of this dense SNP scan, we will advance at least 500 of the most promising SNPs into the first replication study, where a set of 380 additional cases will be used to further investigate the candidate SNPs.

The aim is to get this analysis serve as a basis for selecting true positives for further characterization and replications in other populations.

Chronic rhinosinusitis – reflection of disease in blood and in lower airways

Chronic rhinosinusitis is not one disease but has many underlying contributory pathophysiologies, as yet incompletely understood (31). Some of these [e.g. aspirin hypersensitivity, Churg-Strauss syndrome (CSS), allergic fungal sinusitis] are usually associated with the formation

of nasal polyps, in others (e.g. hypogammaglobulinaemia) polyposis is usually absent. Rhinosinusitis is frequently the first manifestation of reduced immunity, either innate or acquired, and early diagnosis and treatment can reduce subsequent morbidity both within the respiratory tract and in other areas of the body (135). In evaluation and management of affected individuals, it is important to elucidate underlying causes as far as possible. Much of this is achieved by history taking and detailed upper respiratory tract examination; however, some diagnoses require blood tests e.g. immunoglobulin levels for immune deficiency, thyroxine levels for hypothyroidism; whilst others are rendered more secure by positive tests, e.g. aspergillus precipitins in allergic fungal sinusitis (136), anti-neutrophil cytoplasmic antibodies in Wegener's granulomatosis and in CSS (137).

Many patients with CRS have lower respiratory tract involvement. This again is heterogeneous with asthma frequently accompanying eosinophilic polypoid forms of rhinosinusitis, e.g. aspirin hypersensitivity, CSS. Predominantly infective forms of CRS, especially those with immune deficiency (CF, hypogammaglobulinaemia) are more likely to be accompanied by chronic lower respiratory tract (LRT) infection in adults and children (138, 139). Allergic fungal sinusitis may be associated with allergic broncho-pulmonary aspergillosis (140).

Wegener's granulomatosis presents in 95% of patients with upper respiratory tract (URT) symptoms; common manifestations are sinusitis, crusting of the nose and development of saddle nose deformity (137). Asthma is a cardinal symptom of CSS, often preceded by allergic rhinitis, frequently complicated by NP, sinusitis and sometimes otitis media with effusion. Sarcoidosis can involve any part of the body but lung pathology with bilateral hilar lymphadenopathy and interstitial fibrosis is relatively common. Presentation with nasal sarcoid should elicit investigation for disease elsewhere, particularly in the lungs (141).

Chest examination and a functional test such as spirometry or peak flow are mandatory (142). Exhaled nitric oxide levels are valuable in indicating inflammation or its absence in the LRT (143) as well as being useful in monitoring URT disease (144).

Challenges

- 1 Are these patients all immune-deficient in some way?
- 2 Does URT disease precede that in LRT – in adults? In children?
- 3 What are the most important U/LRT links?
- 4 Do they vary between different forms of CRS?
- 5 Does effective CRS therapy improve LRT function? And vice versa?
- 6 Can nNO levels aid in monitoring treatment and reduce the need for CT scans?

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