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Chronic rhinosinusitis and asthma: novel understanding of the role of IgE 'above atopy'

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Chronic rhinosinusitis (CRS) affects more than 10% of the European population and is often associated with asthma. Phenotypes of CRS can be differentiated based on mucosal remodelling and inflammatory patterns. Understanding the role of central mediators, such as interleukin-5, in these different phenotypes may lead to the development of specific therapeutic approaches. The impact of staphylococcal superantigens has been shown to further modify the immune response, contributing to persistent severe disease via the activation of T and B cells and the formation of local IgE. It is clear that these mechanisms are involved in the systemic spread of upper airway disease with resulting asthma comorbidity, when IgE antibodies to staphylococcal enterotoxins are present at measurable levels in serum. Recent findings point to superantigens as possible causal agents in the intrinsic form of severe asthma, and an anti-IgE strategy has shown promising therapeutic potential in nonatopic patients with nasal polyps and asthma. These findings should lead to a clinically relevant endotyping of patients with upper and lower airway disease and to a new understanding of the role of IgE 'above atopy'.

Keywords: asthma, chronic rhinosinusitis, nasal polyps, *Staphylococcus aureus*, superantigens, T helper cells.

Introduction

Chronic rhinosinusitis (CRS) is defined by typical symptoms such as nasal obstruction, secretion, loss of smell and/or headache/facial pain persisting for longer than 12 weeks [1]. Recently, the GA2LEN study was conducted, using self-reported questionnaires, to determine the prevalence of CRS in 23 centres in Europe. Using these criteria, it was demonstrated that about 11% of the European population suffers from CRS, with a prevalence of 8.9% in Stockholm, Sweden, and 18.8% in Ghent, Belgium [2]. The questionnaire used for the GA2LEN study has been validated previously [3], and the prevalence of rhinosinusitis within the study centres was highly correlated with physicians' diagnosis, supporting the accuracy of these figures. CRS may be considered an underestimated disease in terms of prevalence, but also in terms of burden of disease especially amongst those patients with severe disease and comorbidity of the lower airways. In all study centres, there was a strong association between CRS and asthma, which was even stronger in those patients with CRS and allergic rhinitis [4]. CRS in the absence of nasal allergy was positively associated with late-onset asthma.

Although findings of the GA2LEN study provide important information about CRS in the European population, CRS was analysed as a spectrum of diseases rather than as defined phenotypes or endotypes. Clinical signs and symptoms may not be sufficient to allow for such differentiation, and biomarkers may need to be introduced, although they may be difficult to use in epidemiological approaches. CRS is a heterogeneous group of inflammatory diseases of the nasal and paranasal cavities either accompanied by polyp formation (CRSwNP) or without polyps (CRSsNP). Nasal polyps are an expression of a specific remodelling pattern of the nasal mucosa, which is also linked to a dominant inflammatory cell pattern at least in European patients [5]. A diverse spectrum of mucosal alterations involving histopathology, inflammatory cell and T-cell patterns, remodelling parameters, eicosanoid and IgE production, microorganisms and epithelial barrier malfunctions describe the heterogeneity of upper airway diseases. Novel evidence indicates that there is considerable heterogeneity within the CRSwNP subgroup, determining the risk of comorbid asthma. Thus, the characterization of specific disease subgroups is a challenging scientific and clinical task of

utmost importance for the development of diagnostic tools and the application of individualized treatments. In this review, we focus on recent evidence for the classification of distinct CRS phenotypes [6–9] based on clinical signs, and endotypes based on biomarkers, as well as their association with lower airway comorbidity.

Only limited information on CRS is available from studies on asthma, and specifically severe asthma. In a study of the National Institutes of Health-supported Severe Asthma Research Programme in the USA [10], 54% of patients with severe asthma reported sinus disease especially in late-onset asthma, and 27% reported previous surgery for sinusitis. When the group was enlarged and patients were differentiated using cluster analysis [11], one cluster was characterized by severe airflow obstruction and few patients with positive skin prick tests. Of these patients, 80% were severely asthmatic according to criteria of the American Thoracic Society (ATS); again, more than half of the patients suffered from sinus disease, and nearly half of those patients had undergone previous surgery for sinusitis. Unfortunately, upper airway disease findings were recorded by questionnaires only and lacked clinical confirmation by nasal endoscopy or computed tomography (CT) scan, making it impossible to differentiate CRS further into CRSsNP or CRSwNP in these patients. There is an urgent need for a large multicentre study to investigate both the lower and the upper airways, performed by the specialists in upper and lower airway disease, respectively, to extend current knowledge on airway comorbidity in general and particularly in severe airway disease.

In this review, we discuss the evidence that CRS can be differentiation into several subgroups based on specific remodelling and inflammatory cell and cytokine patterns. We also summarize current knowledge of factors that may predict asthma comorbidity in patients with CRS [12]. Finally, we confirm that the same factors are also associated with severe asthma independent of CRS comorbidity.

CRS with or without nasal polyps: the role of remodelling

Only with the use of a nasal endoscope can nasal polyps in the middle meatus be diagnosed or excluded before surgery in patients with symptomatic CRS; this clinical investigation is able to distinguish between CRSsNP and CRSwNP, although some patients may only have polyps within the sinus cavities not visible to nasal endoscopy, or may develop polyps after surgery for CRSsNP. However, in a postoperative observational study, it was found that <3% of patients switched between the CRSsNP and CRSwNP groups over a period of 4 years (unpublished observation). Thickening of the mucosa and polyp formation are typical signs of a disease-associated transformation of a functional and healthy mucosa. Histologically, CRSsNP is characterized by fibrotic mucosa, basement membrane thickening, goblet cell hyperplasia and mononuclear cell infiltration, whereas CRSwNP is characterized by an intense oedematous stroma with albumin deposition, formation of pseudocysts and subepithelial and perivascular inflammatory cell infiltration.

Remodelling is a dynamic process that balances extracellular matrix (ECM) production and degradation, which is regulated by diverse mediators amongst which transforming growth factor (TGF)-β has a central role. TGF-β is a critical factor implicated in the remodelling process in the airways via the attraction and induction of proliferation of fibroblasts and the upregulation of ECM synthesis. We previously reported that TGF-β1 and two protein concentrations, TGF-β receptor (R) I and RIII mRNA expression and the number of activated pSmad 2-positive cells (an indication of activation by TGF-β) were significantly higher in patients with CRSsNP compared with controls [8]. By contrast, in CRSwNP patients, TGF-β1 protein concentration, TGF-β RII and RIII mRNA expression and the number of activated pSmad 2-positive cells were significantly lower versus controls. These data confirm a major difference in the regulation of TGF-β and its signalling between these CRS subgroups, which is reflected in a lack of collagen formation in CRSwNP and excessive collagen deposition in the ECM in CRSsNP [8]. TGF-β signalling also contributes to the regulation of metalloproteinases (MMPs) and their natural tissue inhibitors (tissue inhibitors of MMPs; TIMPs); the overexpression of MMPs 7 and 9 and the lack of a counterbalance by TIMPs 1 and 4 is associated with ECM degradation, creation of vacuoles and albumin deposition in nasal polyp disease, whereas the upregulation of TIMPs in CRSsNP prevents oedema formation [9].

Of interest, the remodelling patterns described in European CRS patients were confirmed in Asian patients, indicating that the regulation of remodelling by TGF-β is a consistent phenomenon; as discussed later, this is different from inflammatory patterns, which may vary in different regions [5]. Thus, there seems to be a dissociation between remodelling and

inflammation, and various inflammatory patterns may be associated with a specific remodelling pattern and vice versa. Recent data also suggest that remodelling may precede inflammation at least in CRSsNP [13]. In a study measuring TGF-β and pro-inflammatory cytokines in the different sinuses in patients with early CRSsNP, TGF-β1 protein concentrations were significantly upregulated in the 'ostiomeatal complex' region, representing the central access to the sinuses, resulting in deposition of collagen and fibrosis formation. By contrast, T helper (Th)1-related and pro-inflammatory cytokines or myeloperoxidase protein as a marker of neutrophil activation were not upregulated. These findings underline the central role of TGF-β in remodelling in CRS and suggest that remodelling may be independent of and precede inflammation.

Inflammation in CRSwNP or CRSsNP: neutrophils and eosinophils

It is widely believed amongst otolaryngologists that eosinophils are involved in all forms of CRS, but are more abundant in patients with nasal polyps. This assumption is the main argument to support CRS as 'a spectrum of one diseases' and has been abused for the 'fungal hypothesis' claiming that all CRS was of fungal origin [14]. Recent data, however, demonstrate that there is a clear difference in the number and level of activation of eosinophils between CRSsNP and CRSwNP and that even within the group of patients with CRSwNP, there may be a lack of eosinophils. Recent findings demonstrate that inflammation in nasal polyps may be regulated by different T helper cell populations, including Th1, Th2 and Th17 cells, resulting in either predominantly eosinophilic or neutrophilic inflammation.

Whereas CRSsNP resembles a weak predominantly neutrophilic Th1-biased inflammatory profile, CRSwNP is mainly characterized by relatively strong eosinophilic Th2-biased inflammation at least in Caucasian patients [6]. As described previously, TGF-β expression is increased in CRSsNP, allowing for normal development of T regulatory cells; by contrast, TGF-β expression is low in nasal polyp disease and is associated with a deficit of T regulatory cells [7]. This deficit of T regulatory cells in nasal polyps has recently been confirmed by Kim *et al.* [15], who also suggested that attenuated migration of T regulatory cells in subjects with CRSwNP may explain the reduced number of regulatory cells. It may be speculated that the relative deficit of T regulatory cells accounts for the inability to suppress inflammation, resulting in a stronger inflammatory response in CRSwNP compared with CRSsNP patients. It is interesting that TGF-β downregulation and a T regulatory cell deficit have also been found in Asian CRSwNP patients, although the majority of nasal polyps in these subjects showed a neutrophilic rather than an eosinophilic pattern of inflammation. CRSwNP in Chinese patients clearly differs in terms of T-cell bias from CRSwNP in their European counterparts: in Caucasians, more than 80% of polyps express a Th2 profile with interleukin (IL)-5 protein and tissue eosinophilia; however, this profile is only found in <20% of Chinese CRSwNP patients, in whom inflammation is instead mediated by Th17 cells [5] (Fig. 1).

There is a major difference in asthma comorbidity between patients with CRS with and without nasal polyps, which might be related to the inflammatory profile found within the mucosal tissue. In a recent pan-European sinusitis cohort study within the GA2LEN research programme, we collected clinical data and nasal tissue from 825 patients with CRS. Asthma comorbidity was significantly higher in patients with nasal polyps (45%), but was not different from the control population or the CRSsNP group (13% and 18%, respectively). In parallel, the prevalence of aspirin-exacerbated respiratory disease (AERD) was 8.2% in the CRSwNP group, and significantly higher than in controls or in CRSsNP patients (1.4% and 1.5%, respectively) (unpublished observations). Of interest, asthma comorbidity in Caucasian CRSwNP patients was significantly more common than in their Asian counterparts. Considering the different inflammatory patterns described earlier, these findings suggest a link between mucosal inflammation in the upper airways and comorbid conditions of the lower airways.

Anti-IL-5 as a marker of a polyp endotype and potential treatment approach

As discussed previously, IL-5 can be found in about 80% of nasal polyps in Caucasian subjects, associated with an abundant eosinophilia in the mucosal tissue, with a strong correlation between IL-5 and eosinophil cationic protein (ECP) or the number of eosinophils. This finding is independent of the atopic status of patients [16]. IL-5 has a major role in survival of eosinophils and may be produced by lymphocytes, mast cells and eosinophils themselves [17]. This critical role of IL-5 for eosinophil survival in nasal polyps has been demonstrated in *ex vivo* experiments in human nasal mucosa with neutralizing anti-IL-5 monoclonal antibodies, which induced

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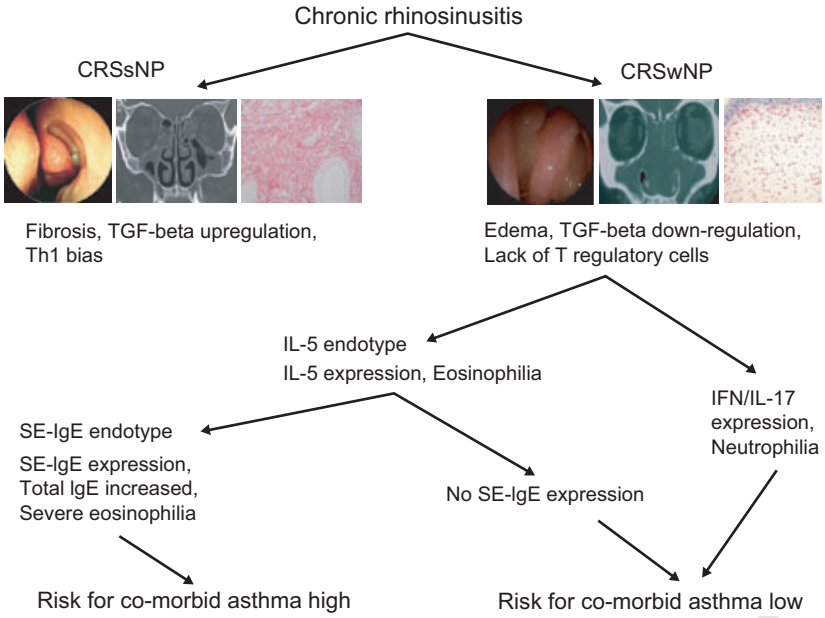

Fig. 1 *Phenotyping and endotyping of chronic rhinosinusitis (CRS) and the risk of comorbid asthma.*

eosinophil apoptosis and decreased tissue eosinophilia. Therefore, IL-5 is likely to represent an important cytokine responsible for delayed apoptosis in eosinophils in nasal polyps [17]. Consequently, we investigated the potential of inhibiting IL-5 as treatment of severe nasal polyposis using the humanized monoclonal antibody mepolizumab [18]. Thirty patients with severe nasal polyps (grades 3/4 or recurrent postsurgery) refractory to corticosteroid therapy were randomly assigned in a double-blind fashion to receive either two single intravenous injections of 750 mg mepolizumab (n = 20) or placebo (n = 10). A total of 60% of the patients receiving mepolizumab showed a significantly improved nasal polyp score and a decrease of polyp mass in the CT scan compared with 10% receiving placebo at weeks 8, versus baseline. Mepolizumab significantly reduced the size of nasal polyps for at least 2 months postdosing in most but not all patients. Therefore, IL-5 inhibition may be considered as a potential novel therapeutic approach in selected patients with severe eosinophilic nasal polyposis. This may serve as a proof-of-concept study for the role of IL-5 in CRSwNP; however, it also shows the limitations of this approach in terms of the dependence of the response on a specific IL-5-positive eosinophilic endotype. Thus, biomarkers are needed to select those patients likely to respond to this specific intervention; as discussed earlier, the proportion of suitable candidates for an anti-IL-5 approach may be very different in Europe and Asia.

Staphylococcus aureus enterotoxins modify mucosal inflammation

Staphylococcus aureus (SA) frequently colonizes the nostrils; up to a third of individuals in Europe are lifelong carriers of coagulase-positive SA. In some patients with CRS, particularly those with nasal polyps [19], SA also colonizes the middle nasal meatus, which is a key region at the entrance to the sinuses. Colonization rates were as high as 67% and 87% for CRSwNP patients with asthma and aspirin sensitivity, respectively. Furthermore, colonization with SA in Europe differs from that in China, with <5% positive swabs for SA in CRSwNP patients from Chengdu in Central China [20].

Staphylococcus aureus may not only colonize the mucosa, but particularly in patients with polyp may also form biofilms adherent to the mucosa. Recent studies have focused on the possible role of biofilms in the persistence of SA, serving as a reservoir for planktonic germs, which may repeatedly invade the mucosa [21]. Using peptide nucleic acid-fluorescence *in situ* hybridization, we recently demonstrated the presence of intramucosal SA especially in polyp tissue from patients with AERD; these bacteria were located intracellularly [22]. With the same technique, others recently added to these findings by showing that SA was present in the epithelium of polyps but not present in nasal epithelium in CRSsNP patients or control subjects [23]. It has been shown that SA not only

survives, but also replicates within nasal polyp epithelial cells. These findings underline the persistent character of SA under these conditions, making use of a deficit in the defence mechanism of the human polyp mucosa (i.e. the alternative activation of macrophages, see [24]). It is clear that the Th2-biased mucosal inflammation favours the programming of so-called M2 macrophages within the polyp tissue, which show a reduced capacity for phagocytosis and intracellular killing of SA.

Staphylococcus aureus recovered from the nose can synthesize and release a wide range of enterotoxins, amongst them the well-studied classical staphylococcal enterotoxins (SEs) SEA, SEC and SEE and toxic shock syndrome toxin-1 [25]. SEs, also known as superantigens, are able to activate T cells by binding to the variable β-chain of the T-cell receptors (TCR), which allows the polyclonal activation of a substantial number of T cells present in the tissue [26]. Furthermore, SEs may activate B cells, eosinophils, epithelial cells and others, resulting in a cytokine storm locally in the tissue and the generation of a strong inflammatory response. SEs are known for their ability to induce acute toxic shock syndrome, possibly leading to the sudden death of the patient caused by massive liberation of pro-inflammatory cytokines. We propose that SEs released in small amounts into the mucosal tissue by SA residing in mucosal cells leads to persistent stimulation of the local immune system, which creates changes in the innate [24] and adaptive immune response [27] to allow the long-term survival of the bacteria at the diseased airway. The creation of a severe Th2 bias within the tissue is part of this strategy (Fig. 2).

Using animal models, it has been demonstrated that SEs are able to aggravate airway inflammation in sensitized mice [28, 29]. SEB is able to break the normally developing tolerance and facilitate sensitization in mice when given intra-nasally together with an allergen [30]. Data suggest that SEB not only aggravates airway inflammation, but may also facilitate IgE formation in animals. There is some evidence to suggest that such effects can also been found in human nasal mucosa. We used an *ex vivo* human mucosal model to study the effects of SA-derived SEB and staphylococcal protein A [31]. Protein A stimulation of the mucosa resulted in significant degranulation of mast cells, whereas SEB stimulation over a period of 24 h induced considerable release of pro-inflammatory and Th2-associated cytokines including IL-4, IL-5 and IL-13 and also induced the release of IL-2, which further activates T effector cells. These cytokines skewed the T-cell responses even more towards the Th2 direction and at the same time against T regulatory cell activity, possibly contributing to the persistent severe predominantly Th2-biased inflammation in polyp disease.

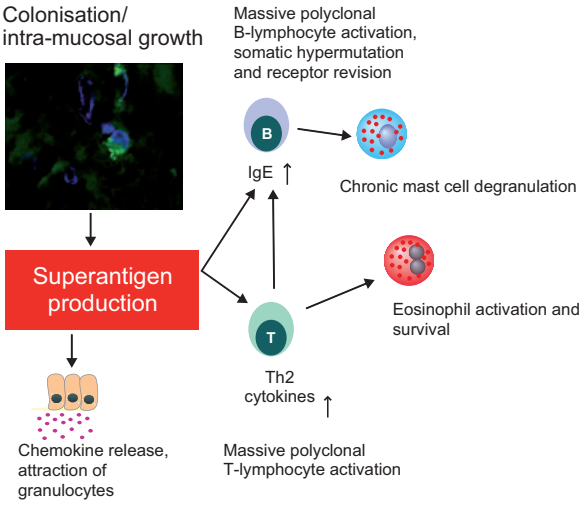

Fig. 2 *Principles of Staphylococcus aureus (SA) superantigen activity.*

SEB induces chemokine release from epithelial cells in a dose-dependent fashion and may also enhance the survival of granulocytes, exerting a direct pro-inflammatory effect on human nasal epithelial cells [32]. Amongst the most important activities of staphylococcal products such as SEA and protein A is the induction of IgE antibody formation [33, 34]. Such activation of B cells and their transformation into plasma cells can also be shown in nasal polyp tissue [35], resulting in mucosal IgE concentrations higher than 5000 kU L^{-1} IgE [36]. The formation of follicle-like structures in nasal polyps and the expression of activation-induced cytidine deaminase in polyp mucosa indicate that a truly local IgE class switch recombination and synthesis of IgE is possible within the diseased human airway mucosa [37, 38]. It is unclear whether this locally formed IgE – which is polyclonal owing to the impact of SEs – is functional and contributes to disease severity and persistence; specific IgE antibodies in polyp tissue only constitute a fraction of the total mucosal tissue IgE, which is also reflected in the serum of CRSwNP patients (forming about 3% in tissue and 0.6% of the mostly heavily increased serum total IgE) [39]. Hundreds of allergen-specific IgE antibodies may be formed as part of the total IgE concentration in tissue and serum, including IgE

antibodies against SEs (SE-IgE) (Table 1). When mucosal tissues were stimulated with anti-IgE, and inhalant allergens such as house dust mite, grass pollen or SEB, mast cell activation occurred dependent on the local IgE antibodies present, but independent from systemic IgE specificities [39]. Thus, house dust mite allergen was able to degranulate nasal polyp mast cells in the absence of specific IgE to mites in serum; this is an example of truly local mast cell activation. Such IgE reactivity was transferable to a basophil cell line (RBL SX38 cells), as would be expected from functional IgE antibodies. Similar mechanisms may also be relevant in the lower airways.

Asthma comorbidity can be predicted from analysis of nasal mucosal tissue

From clinical observations, it was clear that aspirin-sensitive subjects had a higher prevalence of asthma comorbidity and a higher degree of eosinophilic inflammation than patients without AERD [40]. However, it was it unclear whether asthma comorbidity in patients with nasal polyps was determined only by the intensity of inflammation, or whether it was possible to identify other responsible factors. We attempted to identify the mucosal factors associated with asthma comorbidity in European patients with nasal polyp disease with a high rate of asthma comorbidity (34%) and a second, independent group of Chinese CRSwNP patients with a low rate of comorbidity (9%) [12]. As discussed earlier, these populations showed marked differences in terms of the T helper cell profile within the polyp tissue, with a predominance of Th2 cells in European (Belgian) and a predominance of Th17 cells in Chinese patients [5]. To differentiate eosinophilic from neutrophilic polyps, we used the ECP/MPO ratio [5]; 54% of the Belgian, but only 8% of the Chinese samples showed predominantly eosinophilic inflammation, whereas the neutrophilic component was most common in all other polyps [12]. A cluster analysis approach identified IL-5 as the main positive determinant for the eosinophilic form of inflammation, which was expected from previous studies [16]. Of note, 83% of polyp samples from Belgian, but only 16% from Chinese patients were IL-5-positive, confirming the remarkable difference between the populations.

Within the Caucasian group, 37% of mucosal tissue samples contained IgE antibodies against SEs, compared with 17% in the Chinese group. However, SE-IgE in tissues was associated with significantly increased total IgE and ECP concentrations in both groups, indicating an amplification of the mucosal immune response in patients with SE-IgE. The vast majority of SE-IgE-positive samples also were IL-5-positive, suggesting that enterotoxins may need a Th2 background for optimal activity. A hypothesis-free modelling approach was performed in two ways, based on categorical (a marker is present/not present) or on continuous values (quantitative analysis). The categorical approach identified SE-IgE as positive and interferon (IFN)-γ as negative determinants for comorbid asthma in Belgian patients; the prevalence of comorbid asthma was significantly increased amongst those individuals who showed SE-IgE

Table 1 *Tissue IgE and specificities in nasal polyp mucosa: high total IgE concentrations and a polyclonal IgE characterizes those polyps with local SE-IgE*

IgE	SE-mix*	gx1*	hx2*	mx2*	tx9*	EA*	EB*	EC*	ED*	EE*	TSST*
4592.50	9.35	8.03	8.80	17.93	6.79	5.50	4.07	6.49	4.40	4.62	6.71
1073.75	BDL	4.69	4.14	6.43	4.25	BDL	BDL	BDL	BDL	BDL	BDL
2500.01	5.22	66.81	11.21	12.40	10.23	BDL	BDL	4.24	BDL	4.46	BDL
3281.62	16.16	13.67	8.03	68.56	7.81	BDL	4.99	6.83	5.21	29.29	8.14
2223.18	18.29	16.89	10.42	BDL	11.02	8.99	7.68	25.55	8.11	40.68	45.18
1577.67	6.88	7.65	15.75	4.89	BDL	4.77	BDL	BDL	BDL	8.43	6.65

gx1 (*Dactylis glomerata, Festuca elatior, Lolium perenne, Phleum pratense, Poa pratensis).*
tx9 (*Alnus incana, Betula verrucosa, Corylus avellana, Quercus alba, Salix caprea).*
hx2 (Hollister-Stier Labs., *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blatella germanica).*
mx2 (*Penicillium chrysogenum, Cladosporium herbarum, Aspergillus fumigatus, Candida albicans, Alternaria alternata, Setomelanomma rost).*
SE-IgEs: SEA, SEB, SEC, SED, SEE, TSST-1.
SE-mix: SEA, SEC and TSST-1.
TSST-1, toxic shock syndrome toxin-1.

positivity in nasal polyp tissue (57%) compared with SE-IgE-negative patients (20%, $P < 0.01$). Using the continuous approach, high total IgE and ECP concentrations within the polyp tissue were found to be the main positive predictors. As indicated earlier, both of these factors are upregulated by SEs. In line with these findings, eight of 93 of Chinese patients with CRSwNP had asthma, all of whom expressed IL-5 protein in the mucosal tissue and, again, IL-5 and increased IgE were highly predictive of asthma comorbidity, confirming the observations in European patients.

Thus, in a group of patients with nasal polyps, IL-5, a Th2 cytokine, and IgE, specifically IgE against SEs, were identified as indicators of asthma comorbidity. By contrast, asthma was highly unlikely in those CRSwNP patients who expressed the Th1 cytokine IFN-γ, in the airway mucosa, and patients lacking SE-IgE. These observations add support to the impact of staphylococcal superantigens on airway disease and furthermore extend their role to include severity of disease and lower airway involvement.

Is SE-IgE also a marker of asthma severity?

Asthma is a global health problem associated with high morbidity and socio-economic burden. In particular, patients with severe uncontrolled asthma are at risk of exacerbations and account for a significantly disproportionate amount of asthma-related healthcare costs. Although severe asthma may be associated with atopic disease in about 50% of patients, the impact of classical IgE-mediated pathomechanisms on asthma severity is unclear. Furthermore, it is well recognized that a substantial group of patients with severe asthma have raised total IgE levels, despite not being atopic [41]. On the basis of evidence from our own preliminary findings in asthma [42] and supported by a systematic review and meta-analysis [43], we proposed the hypothesis that IgE responses orchestrated by enterotoxins from SA may provide an explanation for disease persistence and severity in asthma, even in classically considered nonatopic asthma. There is indirect evidence for a possible impact of superantigens from studies on the TCR repertoire, demonstrating a Vbeta8 TCR bias and suggesting that superantigens may be involved in severe asthma [44]; staphylococcal superantigens have also been linked to corticosteroid insensitivity [45]. In mouse models, we and others have demonstrated that SA superantigens may amplify bronchial inflammation [28, 29].

In a collaborative study [46], we measured specific SE-IgE, total IgE and ECP concentrations in the serum of more than 100 patients with severe refractory asthma or nonsevere asthma according to ATS criteria. Total IgE levels were significantly higher in severely asthmatic compared with nonseverely asthmatic patients independent of levels of allergic sensitization. The presence of specific SE-IgE carried a significant risk of serum total IgE levels above 100 kU L^{-1}, and the mean serum total IgE levels were significantly higher in SE-IgE-positive than in SE-IgE-negative patients. About 80% of patients with severe asthma were SE-IgE-positive, and the mean level of enterotoxin-specific IgE was threefold higher in patients with severe refractory asthma compared to those with nonsevere asthma or controls. Of interest, concentrations of SE-IgE antibodies were significantly associated with respiratory function parameters and increased airway reversibility in response to albuterol. Aspirin hypersensitivity was also significantly more common in the severe asthma patients compared to those with mild-to-moderate asthma, and all AERD patients were SE-IgE-positive. It is also interesting that the number of SE-IgE-positive patients was not different between the CRSwNP and CRSsNP groups, and total serum IgE was even significantly higher in those without polyps. This argues for the possibility that the presence of SE-IgE is a phenomenon of severe asthma independent of the comorbidity of nasal polyps. The findings of this study suggest that clinical and immunological parameters of asthma severity are associated with specific immunological responses to SEs and support a role for staphyloccocal enterotoxins in the pathogenesis of severe asthma.

We recently extended these findings in a study involving more than 150 subjects with severe asthma and 150 subjects with nonsevere disease; severe asthma was defined as inadequately controlled disease despite high-dose inhaled corticosteroids plus at least two other controller therapies including oral steroids [47].

SE-IgE positivity was significantly more common in patients with severe asthma (59.6%) than in healthy controls (13.0%, $P < 0.001$). Logistic regression analyses demonstrated a significantly increased risk of any asthma in SE-IgE-positive subjects with an odds ratio (OR) of 7.2 [95% confidence interval (CI) 2.7–19.1], and of severe asthma with an OR of 11.1 (95% CI 4.1–29.6), compared with SE-IgE-negative subjects. By contrast, the presence of grass pollen or house dust mite IgE antibodies in serum was not

associated with either increased risk of asthma or severity in this study. In total, 21% of severely asthmatic patients with SE-IgE were considered nonatopic based on the absence of IgE to grass pollen and house dust mites and a negative skin prick test to the most frequent inhalant allergens; these patients typically had a total serum IgE level above normal values and reported a late-onset of disease (Table 2). Oral steroid use and hospitalizations were significantly increased in patients with SE-IgE and nonatopic asthma, confirming the impact of SE-IgE on asthma severity. Furthermore, grass pollen IgE was associated with a higher and enterotoxin IgE with a lower predicted FEV1%. In summary, staphylococcal enterotoxin IgE antibodies, but not IgE against inhalant allergens, were identified as a risk factor for asthma severity. We hypothesize that the presence of enterotoxin IgE in serum indicates the involvement of staphylococcal superantigens in the pathophysiology of severe asthma. This involvement is most likely to be mediated by both superantigen effects on T-cell activation, with a bias towards Th2 cells and against T regulatory cells and the production of IgE in mucosal plasma cells.

Anti-IgE in patients with nasal polyps and asthma: dependence on atopy

As mentioned earlier, SE-IgE in nasal polyps predicts asthma comorbidity. Consequently, one would expect a high level of SE-IgE positivity in a group of CRSwNP patients also suffering from asthma. Recently, amongst a group of asthmatic CRSwNP patients selected for a study with omalizumab, a monoclonal humanized anti-IgE antibody, we found that all patients except one were serum SE-IgE-positive. Omalizumab is an anti-IgE antibody with proven efficacy in severe asthma, only indicated for patients with an allergen-induced disease. However, it was unknown whether omalizumab would also be effective in SE-IgE-positive but otherwise nonatopic patients with severe airway disease [48].

In a pilot study, we investigated the clinical efficacy of omalizumab in patients with severe nasal polyps and comorbid asthma. Approximately, half of the patients were atopic (Gevaert P, Calus L, Van Zele T, *et al.*, in review).

Twenty-four patients with serum IgE concentrations between 30 and 700 kU L^{-1} were randomly assigned to receive subcutaneously administered omalizumab (n = 16) or placebo (n = 8) according to the regulatory dosing instructions for severe asthma treatment

Table 2 *Differentiation of patients with severe asthma based on atopy (IgE antibodies to grass and/or house dust mite allergens) and* Staphylococcus aureus *enterotoxin-specific IgE in serum. Atopic subjects report a significantly earlier onset of disease compared with nonatopic subjects; specifically SE-IgE-positive severe asthmatics show a late-onset of disease ('intrinsic asthma'). The combination of atopy and SE-IgE positivity significantly increases serum total IgE concentrations, whereas nonatopic SE-IgE-negative subjects have normal IgE values. Lung function and body mass index were not different between groups*

	Nonatopic, SE-IgE-negative (17.5%)	Atopic, SE-IgE-negative (24%)	Nonatopic, SE-IgE-positive (21%)	Atopic, SE-IgE-positive (37.5%)	Global *P*-value[a]	1 vs. 2[b]	1 vs. 3[b]	1 vs. 4[b]	2 vs. 3[b]	2 vs. 4[b]	3 vs. 4[b]
Age of onset, mean (SD)	32.8 (11.3)	20.0 (14.5)	36.7 (14.1)	24.9 (20.7)	**<0.001**	**<0.001**	0.123	**0.026**	**<0.001**	0.459	**0.009**
BMI, mean (SD)	28.6 (6.2)	27.7 (5.3)	29.0 (5.2)	30.2 (7.2)	n.s.						
tIgE (log), geom. mean (SD)	23.6 (4.6)	104.7 (3.5)	182.8 (2.7)	543.7 (3.1)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.066	**<0.001**	**<0.001**
FEV1, mean (SD)	61 (21.3)	68.7 (17.8)	63.3 (23.5)	60.5 (16.5)	n.s.						

[a]Kruskal–Wallis test. [b]Mann–Whitney *U*-test.

COLOR

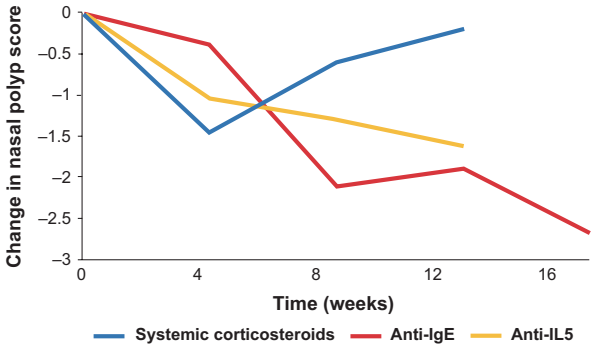

Fig. 3 *Effect of innovative treatment with the biological agents anti-IL-5 (mepolizumab) and anti-IgE (omalizumab) on nasal polyp score compared with standard oral glucocorticosteroid treatment (methylprednisolone 32-16-8 mg over 20 days).*

(Fig. 3). During the treatment period and follow-up, the clinical efficacy of therapy was assessed by nasal endoscopy, sinus CT scan, evaluation of nasal and asthma symptoms and sinus- and asthma-related quality-of-life questionnaires. We found a significant effect on the primary end-point, a substantial decrease in total polyp score after 16 weeks, in the omalizumab group compared with baseline, which was confirmed by CT scan. Omalizumab had a significant beneficial effect on upper and lower airway symptoms (nasal congestion, anterior rhinorrhoea, loss of sense of smell, wheezing and dyspnoea) and on the asthma-related quality-of-life score, irrespective of atopy. In summary, omalizumab demonstrated clinical efficacy for the treatment of nasal polyposis with comorbid asthma, supporting the importance and functionality of polyclonal IgE formation probably induced by SEs in atopic and nonatopic individuals. Larger studies in patients with severe asthma are needed to confirm these data, differentiating between classically atopic and nonatopic SE-IgE-positive patients, to provide new therapeutic options for nonatopic subjects with upper, lower and combined severe airway disease involving anti-IgE strategies.

Conclusions

Chronic rhinosinusitis affects more than 10% of the European population and increases the risk of asthma comorbidity. CRS, however, is not one disease; different disease phenotypes may be differentiated according to the remodelling pattern, the T helper cell profile and the impact of SEs on disease expression. Whereas CRS without polyps predominantly represents a Th1-biased fibrotic airway disease with the upregulation of TGF-β, CRS with nasal polyps is characterized by oedema formation owing to a relative lack of TGF-β production. This lack of TGF-β may also affect the number of T regulatory cells, which in turn may result in different types of persistent inflammation. Whereas a Th2 bias prevails in Europe, a Th17 bias is frequently found in Asia. This does not have a great influence on the remodelling pattern, but is critical for understanding new therapeutic approaches, including anti-IL-5, targeting specific immune patterns (endotypes) an individual patients. There is now evidence that anti-IL-5 is indeed effective in a subgroup of patients with polyp disease likely to be Th2 biased.

Staphylococcus aureus frequently colonizes the human nose; it is significantly more common in European patients with nasal polyp disease than control subjects. SA has the potential to release various enterotoxins which may act as superantigens, polyclonally activating T and B cells within the human mucosa. It is clear that the Th2 bias favours SA survival either in biofilms or planktonic form, and intramucosal as well as intracellular survival of SA has recently been demonstrated. As SA is biologically active via superantigens, it may induce severe and persistent inflammation involving a variety of cells. Polyclonal activation of B cells can be demonstrated by the local production of IgE antibodies against a large number (probably several thousand) of allergens, amongst them the enterotoxins themselves. SE-IgE may therefore serve as a marker of this polyclonal activation either locally within the mucosa, or systemically in the serum. SE-IgE can be measured in serum in patients with CRSwNP and comorbid asthma, as well as

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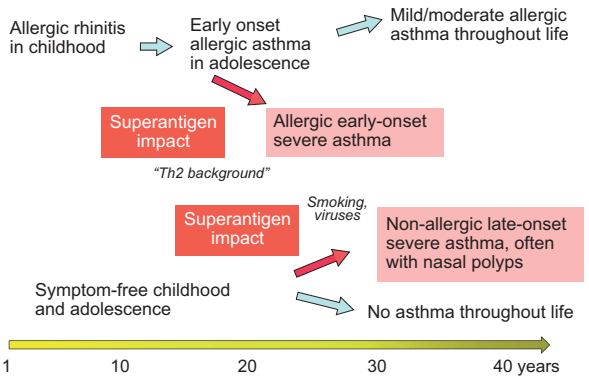

Fig. 4 *Diagram showing the hypothetical impact of Staphylococcus aureus (SA) superantigens on the development of IgE-related severe asthma.*

in patients with asthma alone, but seldom in those with isolated nasal polyps.

The presence of intramucosal SE-IgE in polyps predicts asthma comorbidity and, furthermore, serum SE-IgE levels predict asthma severity. About 60–80% of patients with severe asthma are SE-IgE-positive, and about one-third of these patients are nonatopic, suggesting that superantigens may be the cause of the so-called intrinsic form of asthma (Fig. 4). Serum SE-IgE positivity is associated with an increased number of hospitalizations each year, increased oral steroid intake and a decrease in lung function; in the same study, grass pollen and house dust mite IgE did not show any of these associations. SE-IgE is also associated with a stronger increase in total IgE than either grass pollen or house dust mite IgE antibodies, differentiating the impact of specific IgE antibodies on the disease. Finally, the use of anti-IgE antibodies is a valid treatment approach for nasal polyps and comorbid asthma in atopic and nonatopic patients. This demonstrates that SE-IgE-associated polyclonal IgE is functional. This will open new treatment approaches to selected patients with severe IgE-mediated, but nonatopic disease.

Conflict of interest statement

No conflict of interest to declare.

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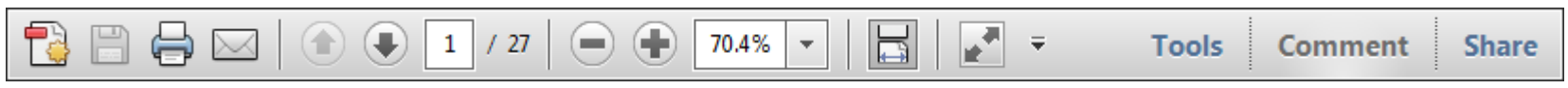

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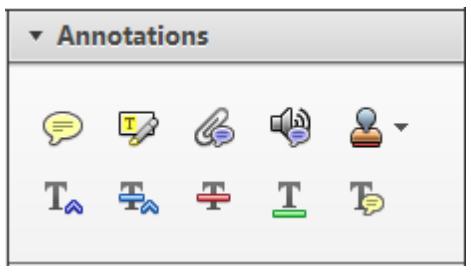

1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

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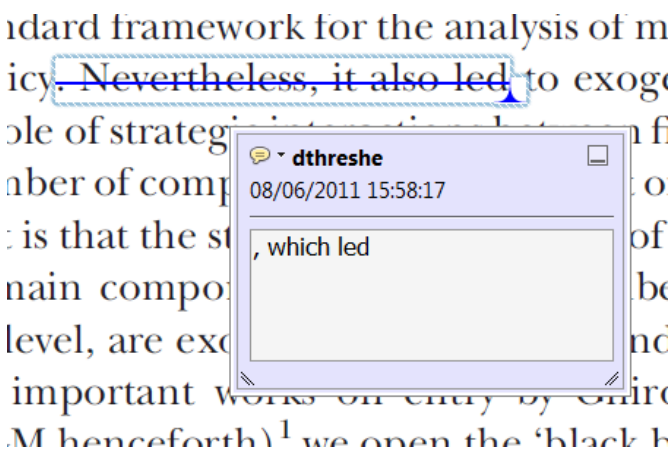

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- Highlight a word or sentence.
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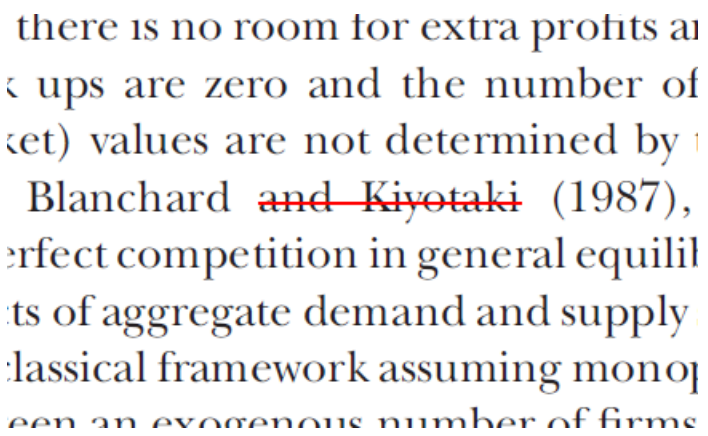

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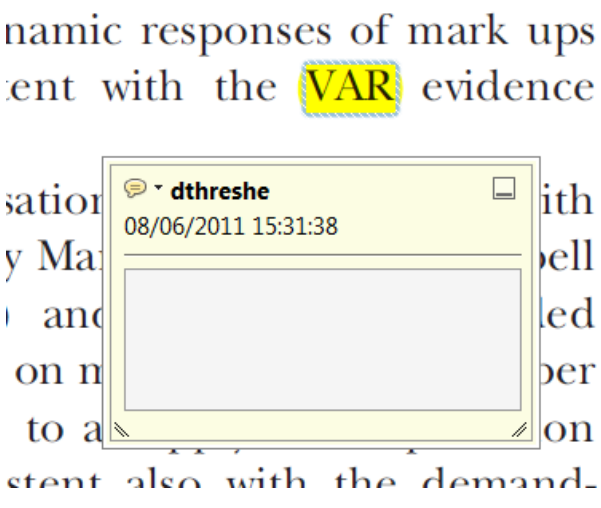

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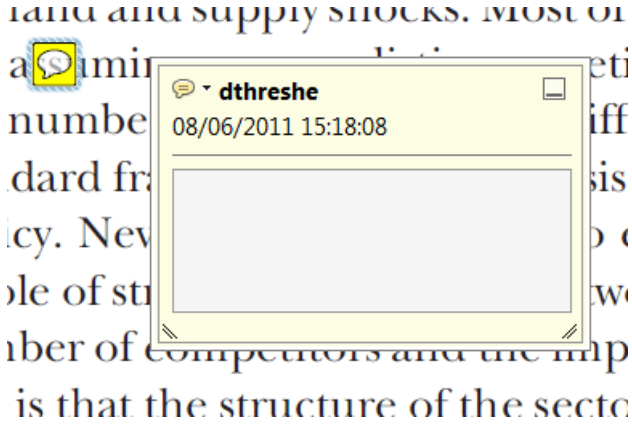

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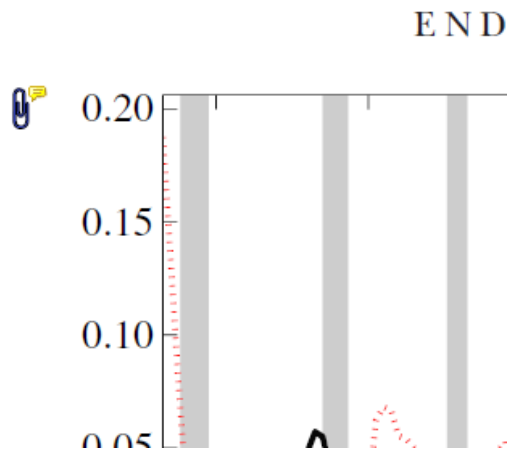

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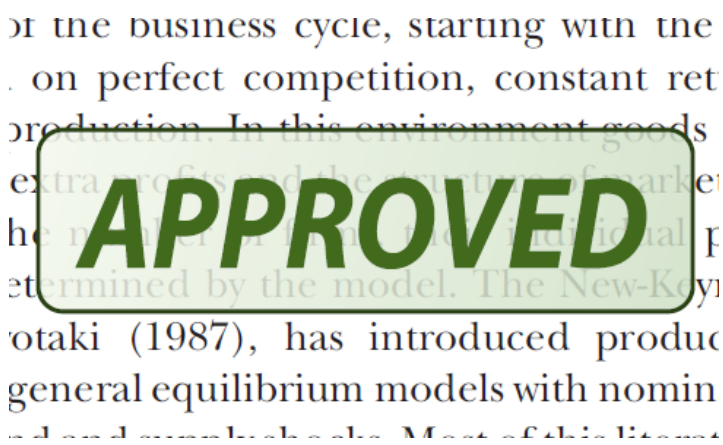

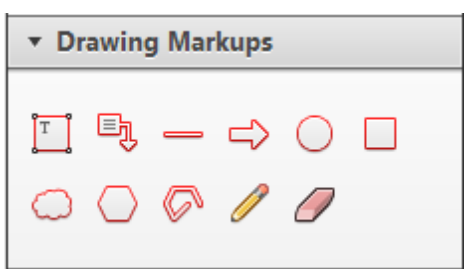

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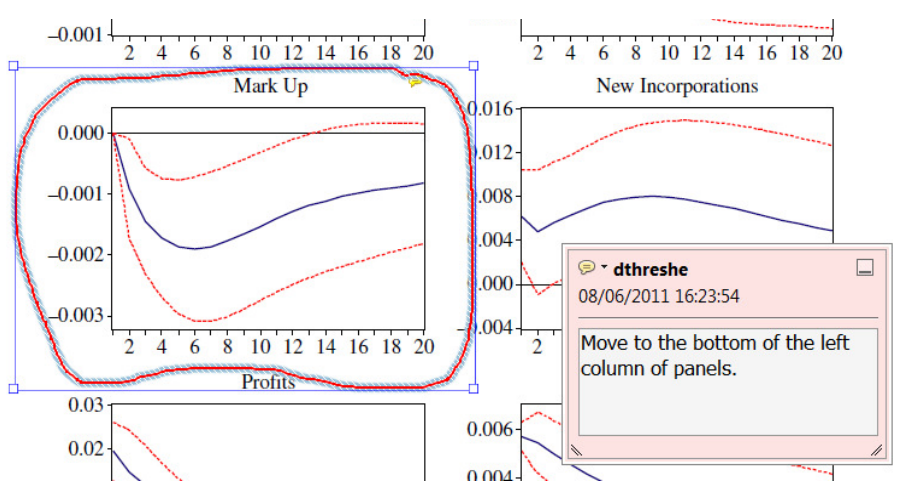

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- Click on one of the shapes in the Drawing Markups section.
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