

DR. GEORGINA XANTHOU (Orcid ID : 0000-0002-9921-9859)

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Upper and lower airway remodelling mechanisms in asthma, allergic rhinitis and chronic rhinosinusitis: the one airway concept revisited

Konstantinos Samitas¹, Alison Carter², Harsha H. Kariyawasam^{2, 3*} and Georgina Xanthou^{1*}

¹Cellular Immunology Laboratory, Division of Cell Biology, Centre for Basic Research, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens 11527, Greece; ²Department of Allergy, Clinical Immunology and Medical Rhinology, Royal National Throat Nose Ear Hospital, London, UK and ³Department of Respiratory Medicine, University College London Hospital and University College London, UK

* These authors contributed equally

Corresponding Authors:

Georgina Xanthou, PhD

Cellular Immunology Laboratory, Biomedical Research Foundation of the Academy of Athens, Athens 11527, Greece

Tel/Fax: +30 210 65 97 336

Email: gxanthou@bioacademy.gr

Harsha Kariyawasam, PhD, MD FRCP

Department of Allergy, Clinical Immunology and Medical Rhinology, Royal National Throat Nose Ear Hospital and Department of Respiratory Medicine, University College London Hospital and University College London, UK

Email: harsha.kariyawasam@nhs.net

Phone/Fax: +44 7958 431 760

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Airway, asthma, remodelling, rhinitis, rhinosinusitis

Abbreviations

α -SMA	: alpha smooth muscle actin
ADAM33	: a disintegrin and metalloproteinase 33
AERD	: aspirin-exacerbated respiratory disease
AHR	: airway hyperresponsiveness
AR	: allergic rhinitis
ASM	: airway smooth muscle
BAL	: bronchoalveolar lavage
BM	: basement membrane
BMP	: bone morphogenetic protein
CRS	: chronic rhinosinusitis
CRSsNP	: chronic rhinosinusitis without nasal polyps
CRSwNP	: chronic rhinosinusitis with nasal polyps
ECM	: extracellular connective tissue matrix
EGFR	: epidermal growth factor receptor
EMTU	: epithelial mesenchymal trophic unit
FEV1	: forced expiratory volume in one second
HDM	: house dust mite
ICS	: inhaled corticosteroids
IFN- γ	: interferon- γ
IL	: interleukin
MMP	: matrix metalloproteinase
PAR	: perennial allergic rhinitis
RBM	: reticular basement membrane
SAR	: seasonal allergic rhinitis
TGF- β	: transforming growth factor-beta

TIMP : tissue inhibitor of metalloproteinase
VEGF : vascular endothelial growth factor

Abstract

Allergic rhinitis (AR), chronic rhinosinusitis (CRS) and asthma often co-exist. The one airway model proposes that disease mechanisms occurring in the upper airway may mirror lower airway events. Airway remodelling is the term used to describe tissue structural changes that occur in a disease setting and reflect the dynamic process of tissue restructuring during wound repair. Remodelling has been long identified in the lower airways in asthma and is characterized by epithelial shedding, goblet cell hyperplasia, basement membrane thickening, subepithelial fibrosis, airway smooth muscle hyperplasia and increased angiogenesis. The concept of upper airway remodelling has only recently been introduced, and data so far is limited and often conflicting, an indication that more detailed studies are needed. Whilst remodelling changes in AR are limited, CRS phenotypes demonstrate epithelial hyperplasia, increased matrix deposition and degradation along with accumulation of plasma proteins. Despite extensive research over the past years, the precise cellular and molecular mechanisms involved in airway remodelling remain incompletely defined. This review describes our current rather limited understanding of airway remodelling processes in AR, CRS and asthma, and presents mechanisms both shared and distinct between the upper and lower airways. Delineation of shared and disease-specific pathogenic mechanisms of remodelling between the sinonasal system and the lung may guide the rational design of more effective therapeutic strategies targeting upper and lower airways concomitantly and improving the health of individuals with inflammatory airway diseases.

Introduction

Tissue modelling can occur in any organ in relation to inflammation or mechanical injury with the aim of restoration to normal tissue architecture. Abnormal restitution or reconstruction of the damaged tissue is termed remodelling. Airway remodelling is well-recognised as a hallmark feature of the asthmatic lung that is often associated with more severe phenotypes of the disease [1]; still, its relevance in upper airway disease remains poorly defined. Moreover, the exact pathogenetic mechanisms that lead to airway remodelling are still mostly unexplored.

It is clearly established that the upper and lower airways share common disease associations and immunopathological mechanisms [2]. The demonstration that nasal provocation with allergen can induce bronchial inflammation along with the observation that the converse is also true, confirmed the bi-directional nature of the one airway system [3;4]. As a result, the term ‘one airway’ or ‘united airways’ has been established [5-7]. In fact, up to 40% of allergic rhinitis (AR) patients report asthma symptoms and up to 80% of asthmatics have symptomatic AR [8]. Chronic rhinosinusitis (CRS) and asthma severity also co-associate [9]. Sinus disease is evident in up to 88% of mild-moderate asthmatics and in up to 100% in severe steroid-dependent asthma on sinonasal imaging [10].

Hence, the pressing question is to what extent upper airway remodelling occurs in sinonasal disease such as AR and CRS, and to what extent, if any, is there an overlap with coexistent lower airway remodelling in these patients. Whilst it is likely that tissue-specific characteristics and airway location influence this process, any common remodelling features between the upper and lower airway occurring in the same patient would suggest that shared mechanistic pathways exist. In this context, given the easy accessibility of sinonasal tissue

and the ability to provoke nasal disease via allergen, viral and bacterial challenge, and obtain nasal samples in a time course manner, it may be tempting to develop upper airway disease models to investigate remodelling changes [11]. Such approaches have the potential not only to enlighten immunopathogenesis in upper airway disease, but may provide essential insight into remodelling events in the lower airway in asthma. The present review describes the similarities and differences in the mechanisms involved in remodelling between the upper and the lower airways, and discusses advances in our knowledge pertinent to the implications of remodelling in AR, CRS and asthma pathogenesis.

Airway structure in health

The respiratory mucosa is made up of pseudostratified ciliated columnar epithelium and the supporting lamina propria. The airway epithelium acts as a physical barrier to the external environment, is continuously exposed to pollutants, allergens and microbes, and responds directly by modulating innate and adaptive immune responses [12]. The lower airway mucosa is identical to that of the upper airway apart from the presence of airway smooth muscle (ASM) that extends from the trachea to the terminal bronchiole. The vascular network remains substantial. Ciliated cuboidal epithelium often lines the terminal bronchioles.

The epithelium sits on the basement membrane (BM), which is composed of an upper layer called the basal lamina (true basement membrane) mainly consisting of type IV collagen and type V laminin [13]. The epithelium is attached to the lamina densa. Extending internally from the lamina densa is the lamina reticularis or reticular basement membrane (RBM) derived from the attenuated fibroblast sheath located below [13]. The BM compartmentalises the epithelium from the underlying mesenchymal components and allows epithelial and inflammatory cell migration [14]. The lamina propria below the BM contains mucus/serous

glands that support mucus secretion and airway hydration. It is composed of polysaccharides, collagens and water that confer tensile strength accompanied with elasticity and compressibility. It is these extracellular matrix (ECM) relative proportions of these components that contribute to the physical properties of the airway. The ECM has a pivotal role in regulating cellular function, acting as a substrate for cellular adhesion, migration, differentiation, proliferation and survival and as a scaffold [15]. Airway fibroblasts are localised beneath the BM and, when activated, become myofibroblasts, characterised by the expression of alpha smooth muscle actin (α -SMA), with rapid ECM production [16]. ECM turnover is orchestrated by the balance between the expression of matrix metalloproteinases (MMP) and their inhibitors (TIMP) [17].

Restoration of normal airway structure (modelling)

The airway mucosa must be resilient to environmental insults and rapidly induce tissue repair after damage. Airway injury leads to inflammation and is associated with the release of cytotoxic mediators, free oxygen radicals and collagenases by inflammatory and structural cells. If the airway surface is vulnerable, either intrinsically or as a result of disease, epithelial and BM integrity becomes compromised [18]. As a result, epithelial cells express adhesion molecules, cytokines and growth factors to induce tissue repair and restoration [19]. The repair process begins with the deposition of ECM proteins over the denuded epithelium [20]. The ECM matrix acts as a substrate over which the basal epithelial cells at the 'wound edge' can attach to, proliferate, flatten out and migrate over, and is composed of the remnant components of the BM, such as, type IV collagen and laminin, and blood-derived factors including fibrin and fibronectin. Activation of the attenuated fibroblast sheath is evident, particularly in response to signals from the transforming growth factor-beta (TGF- β) superfamily of cytokines. Myofibroblast numbers are increased and associated with enhanced

synthesis of collagen and other ECM components, in response to chemotactic gradients into the submucosa along ECM fibrils [21].

In health, resolution of inflammation and tissue restoration is achieved. This also requires the clearance of activated immune cells through apoptosis and phagocytosis [22], as well as, the production of lipid pro-resolving mediators, including lipoxins, resolvins and protectins [23] that promote tissue repair. An emerging role for autophagy mechanisms in the resolution of inflammatory responses is also evident. Autophagy is a conserved process by which cytoplasmic materials are delivered to lysosomes for degradation when facing environmental and/or developmental changes [24]. Accumulating evidence proposes that autophagy plays critical protective roles in airway epithelial cell injury but may also contribute to pathologic airway remodeling and fibrosis [25]. Overall, the coordinated actions of all these tightly regulated processes guide tissue restoration and maintenance of airway homeostasis in healthy individuals.

Mechanisms involved in lower airway remodelling: the paradigm of allergic asthma

Currently much of our understanding of airway remodelling is obtained from studies on allergic asthma. Structural changes of the lower airways in asthmatics include epithelial shedding, goblet cell hyperplasia, RBM thickening, mucous gland and ASM hypertrophy, subepithelial fibrosis and angiogenesis. Despite extensive research over the last decade, the precise mechanisms underlying different aspects of lower airway remodelling and their clinical implications for allergic diseases remain incompletely explored.

Airway inflammation and remodelling: is there a link?

The prevailing model explaining the origins of airway remodelling was based on the hypothesis that chronic allergic inflammation is the initiating event of remodelling and the driving force behind most of its features. This hypothesis was supported by studies using transgenic mice that over-expressed interleukin (IL)-4, IL-5 and IL-9 in the lung and wherein marked mucus metaplasia, thickening of the RBM and induction of airway hyperresponsiveness (AHR) was observed [26;27]. IL-4 and IL-13 also induced fibroblast differentiation into myofibroblasts and enhancement of type III collagen release, while blockade of IL-13 reversed AHR [28] and IL-13 administration enhanced AHR, eosinophilic inflammation and mucus cell hyperplasia [29]. This may be related to the ability of IL-13 to induce the production of TGF- β 1, although IL-13 can also induce airway fibrosis in TGF- β 1-independent pathways [30].

Other cytokines with increased expression in the airways of asthmatics, such as osteopontin, are also closely associated with airway inflammation and remodelling [31-33]. Osteopontin is increased in the asthmatic airway and *in vitro* studies reveal a potent effect on fibroblast and ASM proliferation, while *osteopontin*^{-/-} mice demonstrate less airway remodelling following allergen challenge [34]. TGF- β 1, activin-A and bone morphogenetic proteins (BMPs) also play a role in epithelial cell proliferation, subepithelial fibrosis and ASM remodelling, as evidenced by a plethora of *in vitro* and *in vivo* studies in animal models and human subjects [35;36]. TGF- β 1 and activin-A signalling pathways are dysregulated in experimental asthma models and in the asthmatic airway, while rapid activation and modulation of signalling components occurs upon allergen-induced disease provocation in mild asthmatics [37-39]. Such dysregulated signalling seems to contribute to a sustained and excessive repair response in asthma. These cytokines can also restrain remodelling processes in the airways, through

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suppression of inflammation and/or directly targeting certain aspects of remodelling. Our recent studies have uncovered novel anti-angiogenic functions for activin-A, as evidenced by inhibition of vascular endothelial growth factor (VEGF)-induced proliferation and cytokine release by human pulmonary endothelial cells through IL-18 and VEGFR1-dependent pathways [40].

Apart from the cytokine milieu, it is important to consider other mechanisms by which inflammation may promote or modulate remodelling. Eosinophils are a rich source of mediators that can directly injure tissue and induce remodelling [41]. Eosinophil depletion attenuates airway remodelling, supporting also the view that eosinophil-derived TGF- β 1 is a key player [42]. In addition, eosinophil depleted mice are protected from peribronchial collagen deposition and ASM mass in response to chronic allergen exposure [43]. IL-5 deficient mice display markedly less total lung collagen, peribronchial collagen Type III and IV deposition, and α -SMA expression following chronic allergen challenge [44]. Interestingly, studies in humans using a monoclonal antibody against IL-5, demonstrated a reduction in airway eosinophils associated with decreased TGF- β 1 in bronchoalveolar lavage (BAL) and lower expression of the ECM components, tenascin, lumican and procollagen III [45].

However, the linear model suggesting that environmental sensitisation leads to Th2 cell-driven allergic inflammation and subsequent airway remodelling has been challenged by studies in childhood asthma. Bronchial biopsies from asthmatic children show marked remodelling very early on in the disease [46] that may even predate the onset of symptoms by up to four years, suggesting that remodelling is an early feature of allergic asthma and not necessarily a result of chronic airway inflammation [47]. Other studies confirm that airway remodelling markers, such as, RBM thickening and abnormal structural cell activation are

consistently present in childhood asthma and may occur in the absence of eosinophilic inflammation [48]. Furthermore, inflammation and remodelling can be dissociated as shown by allergen provocation in adult asthma, wherein rapid and simultaneous activation of inflammation and airway remodelling occurs but dissociation of selected aspects of remodelling, such as, collagen deposition and AHR is evident at later time points [49]. In fact, whilst RBM thickening may reflect remodelling events deeper in the submucosa [50], there is little or no correlation of RBM thickness with the duration of asthma [46] or inflammation [51], suggesting that RBM thickening may be overall independent of inflammation. It is therefore conceivable that remodelling is initiated due to an intrinsic propensity of the lower airway of asthmatics for injury and abnormal repair and becomes later on exacerbated by aberrant inflammatory responses.

ADAM33 (a disintegrin and metalloproteinase)-33 is an asthma susceptibility gene that shows strong linkage to AHR, and supports the concept of airway tissue susceptibility and impaired repair [52]. ADAM33 is selectively expressed in fibroblasts and ASM and its activation induces the release of growth factors and modulates the expression of cell surface receptors. As such, ADAM33 may play an important role in the differentiation and proliferation of airway mesenchymal cells [53]. This is important in that fibroblast accumulation and ASM hypertrophy are the selective determinants of severe and persistent asthma symptoms [54]. Polymorphisms in ADAM33 are associated with a more rapid annual decline in post-bronchodilator forced expiratory volume in one second (FEV1) [55]. Such genetic associations may explain why severity and outcomes in adult asthma can to a certain extent be predicted in childhood, and do not necessarily relate to inflammatory biomarkers [56].

Increased ASM numbers seems to be the cause rather than the consequence of severe disease in asthma. ASM cells produce high levels of inflammatory cytokines and growth factors, such as GM-CSF, IL-1 β , IL-5, IL-6, eotaxin, bFGF, PDGF-B and VEGF in the airways [57]. Moreover, ASM cells of asthmatics release large amounts of IL-6 and IL-8 after stimulation with house dust mite (HDM) or following rhinovirus infection [58]. Interestingly, large-scale transcriptional profiling analyses of ASM cells from asthmatic versus non-asthmatic subjects, revealed a marked activation of pathways linked to cell proliferation and growth, vascularisation, inflammatory response genes and muscle-tissue specific responses, such as contractility in asthmatics [59]. Finally, ASM contraction can alter the mechanical properties of the airway wall, promoting mechanotransduction pathways that contribute to remodelling, and initiating cascades that synergise with other inflammatory mechanisms [60].

Enhanced vascularity correlates with asthma severity [61] and is a key feature in the airways of people who died from asthma [62]. Changes to the airway vasculature include increased numbers of vessels per unit area with enhanced vessel size and associated vasodilation and leakage leading to mucosal oedema. Increased vascularity is present even in mild asymptomatic asthma [63]. The model of airway wall remodelling proposed by Moreno *et al.* [64] suggests that even small increases in airway thickness significantly contribute to the airway obstruction observed after allergen challenge in asthma.

The role of the epithelial-mesenchymal trophic unit (EMTU) in lower airway remodelling

An important and possibly fundamental pathway involved in lower airway remodelling changes is the epithelium interacting with the underlying attenuated fibroblast sheath through which translation of environmental signals into the deeper submucosal compartment occurs.

Upon cellular injury, activated epithelium secretes growth factors, particularly IL-13, TGF- β superfamily members [40;65], VEGF, MMPs and osteopontin [31;66;67], all of which activate the underlying mesenchymal cell unit and, ultimately lead to RBM thickening, subepithelial fibrosis and smooth muscle hyperplasia [68]. This epithelium-fibroblast signalling pathway is termed the epithelial mesenchymal trophic unit (EMTU) [69] and may, at least partly, explain the observed dissociation between inflammation and airway remodelling events.

In health, the epithelium is able to repair rapidly; still, in asthma there is increasing evidence that this process is defective with prolonged epithelial cell activation and signalling to the underlying mesenchymal cells, leading to myofibroblast transformation. Evidence for defective epithelial repair exists in that markers of proliferation, such as Ki67, are reduced at an early age [70]. Increased expression of the epidermal growth factor receptor (EGFR) with subsequent pro-fibrotic growth factor release also occurs in response to injury and is a marker of active epithelial repair [70]. In normal epithelium, elevated EGFR is only observed in areas of structural damage; still, in asthma, EGFR is induced in both damaged and normal epithelium, pointing to diffuse epithelial injury [71].

Tissue vulnerability to injury and/or inability to repair may have an origin in embryonic modelling programmes. The sinonasal lining is derived from ectoderm. The tracheal and bronchial epithelium originates from endoderm that undergoes a process of dichotomous branching onto the surrounding splanchnic mesenchyme with determined precision. Such epithelial-mesenchymal signalling is the same process as EMTU activation in remodelling states, and confirms the intrinsic ability of the airway to model itself. These ‘hard-wired’

repair programs seem to differ between the upper and lower airway in terms of remodelling capacity.

Overall, remodelling in the lower airways is a complex process in asthma and a definite unifying mechanistic pathway is uncertain. However, the observation that remodelling and asthma severity strongly correlate, suggests causality. Recently, the realisation that upper airway remodelling may have a similar impact on AR and CRS has initiated more focused research to dissect the relevance of structural changes in the immunopathology of these conditions.

Allergic rhinitis and nasal remodelling

AR is mainly driven by Th2 cell-associated inflammation. Up to 80% of asthmatics have AR and this has formed the basis of treatment guidelines reminding clinicians to treat AR in asthmatics [72]. Paired upper and lower airway bronchoscopic samples from patients with AR and allergic asthma confirm similar Th2 cell-driven inflammation in the nasal and bronchial mucosa [73]. If the linear model that inflammation leads to remodelling is followed, then persistent structural changes should be key findings in AR as is the case in asthma. However, the evidence for remodelling changes in AR is conflicting and suggests that remodelling does not occur or is not a prominent feature in AR. Moreover, animal models of AR are limited and not helpful as disease provocation is obtained via ovalbumin exposure, and thus does not relate to human disease. Hence, we will discuss current knowledge obtained predominantly through human studies.

Allergen exposure in individuals with perennial (PAR) and seasonal allergic rhinitis (SAR) leads to rapid activation and amplification of inflammatory pathways almost identical to those occurring in asthma. Both perennial (HDM related) and seasonal (pollen related) subtypes of disease increase eosinophilic mucosal inflammation post intranasal challenge. In SAR, nasal biopsies at baseline showed increased overall BM thickness compared to healthy tissue, but 24 hours after allergen challenge, despite eosinophil influx, further increase in BM thickening did not occur [74;75]. Moreover, no change in epithelial thickness or submucosal collagen deposition was observed. In individuals with PAR and HDM sensitisation BM thickness was also comparable to the normal nasal mucosa [74] and no further increase following HDM provocation was noted. In contrast, an early study reported loss of epithelial integrity in relation to the degree of eosinophilia in PAR [76]. Detailed analysis of nasal samples from PAR and PAR/SAR (in season) showed no differences in epithelial integrity, RBM thickening, angiogenesis and ECM collagen or other remodelling markers, such as, MMP/TIMP or α -SMA⁺ fibroblast numbers [77], even though high levels of IL-4, IL-5 and increased IL-13 was seen in the lavage from PAR/SAR in season subgroup. Given that airway remodelling probably has a genetic component, it would have been important to provide information regarding the asthma subgroup PC₂₀ and degree of FEV₁ reversibility and investigate whether the AR-asthma subgroup would have a greater tendency to develop nasal remodelling compared to the AR alone group. Still, other studies failed to detect nasal mucosal remodelling in AR compared to paired bronchial samples from the same individuals with allergic asthma [75], further supporting the concept that remodelling in the upper nasal airways does not represent a key feature of AR immunopathogenesis.

It is difficult to understand how eosinophilic inflammation and activated IL-4/IL-13 pathways fail to initiate some aspects of EMTU activation and remodelling in AR [78]. Whether this is because inflammation is not strong enough to injure the nasal tissue or whether it is because the nose is designed to withstand injury, remain important and interesting questions to answer. One should also take into consideration variables such as severity of disease, allergen type and dose and the methodology utilized for the examination of tissue remodelling. Time course studies of high-dose allergen challenge in severe AR would greatly assist in determining whether dose-dependent, allergen-induced activation of Th2 cell responses can initiate remodelling events. Failure in such a setting, would support the notion that inflammation and remodelling are separate events and dependent on local mucosal properties. In addition, comprehensive studies incorporating advanced imaging and molecular phenotyping techniques are needed before concrete conclusions on remodelling in AR can be reached.

Collectively, considering that the nasal mucosa is highly adapted to meet environmental insults one can speculate that it is more adept in responding to inflammation and can induce tissue restoration more effectively compared to the lower airway. In this context, patients defective in nasal tissue repair may have a yet unidentified, predisposing factor that renders them more susceptible to CRS and/or asthma [79].

Chronic rhinosinusitis and sinonasal remodelling

CRS is a broad term that incorporates a group of heterogeneous inflammatory disorders [80] and is subdivided into disease with and without nasal polyps (CRSwNP and CRSsNP, respectively), with even more inflammatory and remodelling subtypes [81]. CRS is very different to AR both in mucosal location of disease and the pattern of severity of

inflammation. Thus remodelling in CRS is expected to be different from any in AR, and maybe more relevant to studying remodelling in asthma. CRS and asthma are strongly associated and up to 14% CRSwNP and aspirin-exacerbated respiratory disease patients demonstrate severe asthma [82]. Such a strong relationship between two disease groups is intriguing and suggests common genetic predisposition and mechanistic pathways exist [83].

Unlike AR, tissue remodelling is a hallmark in CRS. CRSwNP is now emerging as CRS subgroup with different endotypes [83]. CRSwNP still represents a more defined immunological entity with a more Th2 driven inflammatory pathway. In contrast CRSsNP has more diverse immunological endotypes that remain poorly defined [84]. In CRSwNP, eosinophilic inflammation is present with amplification of IL-4, IL-5 and IL-13 driven pathways. Recent work has highlighted type II ILC infiltrated polyp tissue with Th2 skewed cells expressing IL-25R and IL-33R [85;86]. Inflammatory response amplification via bacterial super-antigens is also observed and leads to enhanced nasal IgE production [87]. In contrast, CRSsNP is characterised by a distinct inflammatory pattern, with a Th1 cell bias and excess interferon- γ (IFN- γ) and neutrophilic inflammation [88]. Whilst both CRS phenotypes have a high incidence of lower airway disease with asthma and bronchiectasis, the poor ability to subtype CRSsNP inflammatory profiles makes it difficult to compare remodelling changes with those occurring in the lower airway. Thus, we will compare remodelling in CRSwNP to asthma aiming to decipher upper and lower airway link in shared disease mechanisms.

In CRSwNP, epithelial cell disruption is extensive in whole nasal tissue samples [89] and excess mucus can be explained by goblet cell hyperplasia and mucin hypersecretion [90]. BM thickening relates to disease severity and duration, along with the presence of underlying

asthma [91] and seems to be independent of the degree of eosinophilic inflammation [92].

Immunohistochemical studies demonstrate that the sinonasal epithelium can transition to a mesenchymal phenotype, in both CRSwNP and CRSsNP, and markers, such as, E-cadherin, cytokeratin and vimentin expression correlate with fibrosis parameters, including BM thickening [93].

ECM degradation maybe a key pathological step in CRSwNP as it explains how loosening of tissue architecture, tissue expansion and pseudocyst formation occurs. Data on MMP expression in CRS is conflicting. In CRSwNP, MMP-1 (collagenase) and MMP-2, MMP-9 (gelatinases which digest partially denatured collagen) and the elastase MMP-7 are increased [94;95]. ECM changes may explain the almost contradictory finding of decreased tissue remodelling in CRSwNP, compared to CRSsNP, which demonstrates markedly less collagen deposition [94;95]. The finding of lower TGF- β 1/2 levels along with diminished signalling pathway components in CRSwNP, compared to CRSsNP, suggests the latter is a more fibrotic subtype of rhinosinusitis [96]. The lack of TGF- β 1 could also explain the propensity to excess Th2 cell inflammation and the scarcity of regulatory T cells (Tregs) in polyp tissue and nasal mucosa [97]. Still, given that eosinophils produce TGF- β ₁ [42] and that IL-13 is powerful inducer of EMTU activity [98], both being present in CRSwNP tissue, one would expect to find increased collagen and other ECM production in CRSwNP. These contradictory findings may be due to the failure to report on location-specific expression and quantification of growth factors. Alternatively, and/or in conjunction, one should consider the structure of a nasal polyp. The findings of extensive pseudocyst formation filled with albumin, stromal oedema and less ECM, suggest that tissue in severe nasal polyps is almost at a stage of burn-out (as these studies have been conducted in discarded nasal tissue undertaken at surgery, indicating severe disease). As the key questions on how remodelling

begins and progresses, as well as, its relationship to inflammation and clinical features cannot be answered by studies reporting findings at the end stage of disease, more detailed analysis of tissue compartments accompanied by robust quantification strategies at earlier time points are needed.

A more recent study has attempted to capture remodelling using different markers and suggests that remodelling is actually excessive in CRSwNP [99]. Epithelial loss with decreased E-cadherin, ZO-1 and occludin expression was evident. Given that nasal polyps are histologically characterised by pseudocyst formation, the authors reported remodelling based on the turbinate and pedicle area of polyp. Whilst total collagen in polyps is less compared to normal tissue, the polyp mucosa towards the middle turbinate demonstrated increased collagen and fibronectin, alongside elevated numbers of α -SMA⁺, ECM-producing myofibroblasts [100]. ADAM33 expression is increased in nasal polyp tissue and it would be interesting to explore which polymorphisms relate to CRS remodelling and clinical phenotypes [100].

The findings that neutrophilic and eosinophilic subtypes of CRS demonstrate similar remodelling changes support the view that the type of the inflammatory milieu is not essential for the establishment of remodelling [101]. Moreover, the observation that eosinophils were found near the BM, away from the collagen-rich ECM areas does not mean they are independent events. It is conceivable, that inflammatory cells may contribute to remodelling by activating the epithelial aspects and attenuated fibroblast sheath rather than trafficking deeper into the ECM amongst myofibroblasts. TGF- β 1 and activin-A expression along with activated signalling via pSmad2/3 is evident [102]. Significantly higher concentrations of activin A and TGF- β 1 have been observed in CRSsNP compared to CRSwNP [103]. Excess

VEGF and angiopoietin is also seen in CRSwNP and is modulated by steroids. The finding then that there is no excess angiogenesis in this disease is somewhat conflicting [103]. Overall, descriptive studies at stand-alone time points of the disease do not provide detailed insight into potential remodelling mechanisms. Until definite time-course sinonasal remodelling studies are done, there is no evidence to support that inflammation either precedes or drives remodelling events in CRS. Moreover, information pertinent to growth factor expression and their signalling pathways is very limited in the upper airway. Further work incorporating studies at various sites in the nose and the polyp tissue and involving disease provocation with allergen, lipopolysaccharide (LPS), or aspirin should be conducted.

In Table 1, we summarise the gross histopathological changes seen in a remodelled airway in AR, CRS, and asthma. Regarding AR, no remodelling changes whatsoever are observed in the epithelium and submucosa, other than possibly some basement membrane thickening. In CRSwNP and allergic asthma, however, several changes do occur that have important similarities. In both, disruption and shedding of epithelial cells is excessive along with goblet cell hyperplasia. EMTU activation is extensive in both and maybe predisposed to because of epithelial vulnerability leading to active submucosal mesenchymal activation. Regarding the submucosa, similar remodelling processes in CRSwNP and allergic asthma include the hypertrophy of mucus glands and the excess production of ECM components, however significant differences also exist; pseudocysts exist only in CRSwNP while increased angiogenesis is observed only in allergic asthma. There is emerging overlap with common inflammatory and growth factor pathways in asthma and CRS pheno-endotypes, but more work is still needed to understand any over-lap pathways. Basic similarities and differences in remodelling events observed in AR, CRSwNP and allergic asthma are depicted in Figure 1.

Conclusions

Airway remodelling is an active and highly complex process. The potential key pathways and factors involved in airway remodelling processes are summarised in Figure 2. Studies looking at upper airway remodelling are few and further research is needed to delineate the exact cellular and molecular mechanisms involved. The limited remodelling observed in AR, in the context of a highly inflammatory milieu, supports the view that inflammation by itself, does not induce remodelling and that other tissue susceptibility factors must be present for structural changes to develop. Findings from studies in CRS are also at an early stage but suggest progressive stages of remodelling seen at different sinonasal locations. In contrast, remodelling is extensive in the lower airways in allergic asthma. It is seen early-on in disease and a purely inflammatory model does not fully explain the extensive lower airway changes. The differences in aspects of remodelling between the upper and lower airways may reflect distinct intrinsic tissue vulnerability to environmental insults and tissue repair programs. Such disparities may be also linked to the different embryonic origins of the upper and lower airways. Further work focusing at paired tissue samples from the upper and lower airways in detailed time-course and disease provocation studies are needed to clarify the underlying mechanisms involved and to guide the design of more effective therapeutic strategies for allergic diseases. Only then can the clinical consequences of airway remodelling be understood.

Table 1. Summary of remodelling changes observed between the upper and lower airway in distinct inflammatory airway disease states

	Allergic Rhinitis	CRSwNP	Allergic Asthma
Epithelium	Normal	Disrupted/shedding Excess goblet cells Activated state EMTU active	Disrupted/shedding Excess goblet cells Activated state EMTU active
Basement membrane	Normal or thickened (based on allergen type)	Thickened	Thickened
Submucosa	Normal	Mucus gland hypertrophy Pseudocyst formation ECM excess and turnover depending on tissue site and disease state	Mucus gland hypertrophy ECM excess and turnover Increased angiogenesis ASM hypertrophy

ASM: airway smooth muscle

EMTU: epithelial mesenchymal transition unit

Figure Legends

Figure 1. Representative microphotographs of A) bronchial tissue from a healthy control individual and a patient with severe asthma depicting increased airway angiogenesis (CD34+ staining), reticular basement membrane thickening (H&E stain) and Goblet cell hyperplasia (PAS stain). B) Nasal mucosal tissue from a healthy control and a patient with AR with similar appearances on basic staining, and CRSwNP depicting typical remodelling changes with denuded epithelium and extensive pseudocyst formation. Nasal images courtesy of Miss Nara Orban, Imperial College London.

Figure 2. Potential key pathways and factors involved in airway remodelling processes in the upper and lower airways.

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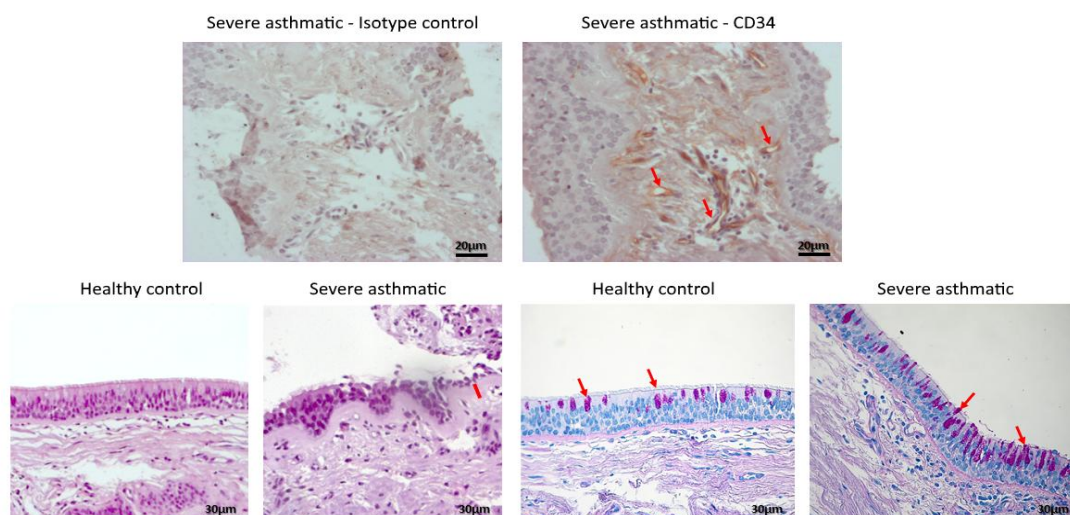
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