



Allergen-induced asthma, chronic rhinosinusitis and transforming growth factor- β superfamily signalling: mechanisms and functional consequences

Harsha H Kariyawasam & Simon B Gane

To cite this article: Harsha H Kariyawasam & Simon B Gane (2019): Allergen-induced asthma, chronic rhinosinusitis and transforming growth factor- β superfamily signalling: mechanisms and functional consequences, Expert Review of Clinical Immunology, DOI: [10.1080/1744666X.2020.1672538](https://doi.org/10.1080/1744666X.2020.1672538)

To link to this article: <https://doi.org/10.1080/1744666X.2020.1672538>



Accepted author version posted online: 24 Sep 2019.



Submit your article to this journal [↗](#)



View related articles [↗](#)



View Crossmark data [↗](#)

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Expert Review of Clinical Immunology*

DOI: 10.1080/1744666X.2020.1672538

Allergen-induced asthma, chronic rhinosinusitis and transforming growth factor- β superfamily signalling: mechanisms and functional consequences

Harsha H Kariyawasam^{1, 2, 3} and Simon B Gane^{2, 3}

¹Department of Adult Specialist Allergy and Clinical Immunology, Royal National ENT Hospital, University College London Hospitals NHS Foundation Trust, London, UK

²Department of Rhinology, Royal National ENT Hospital, University College London Hospitals NHS Foundation Trust, London, UK

³University College London, London, UK

Correspondence author and address

Harsha H Kariyawasam PhD FRCP

Royal National ENT Hospital and UCLH NHS Foundation Trust

London, England

harsha.kariyawasam@nhs.net

Abstract

Introduction: Often co-associated, asthma and chronic rhinosinusitis (CRS) are complex heterogeneous disease syndromes. Severity in both is related to tissue inflammation and abnormal repair (termed remodelling). Understanding signalling factors that can modulate, integrate the activation and regulation of such key processes together is increasingly important. The transforming growth factor (TGF)- β superfamily of ligands comprise a versatile system of immunomodulatory molecules that are gaining recognition as having an essential function in the immunopathogenesis of asthma. Early data suggests an important role in CRS as well. Abnormal or dysregulated signalling may contribute to disease pathogenesis and severity.

Areas covered: The essential biology of this complex family of growth factors in relation to the excess inflammation and remodelling that occurs in allergic asthma and CRS is reviewed. The need to understand the integration of signalling pathways together is highlighted. Studies in human airway tissue are evaluated and only selected key animal models relevant to human disease discussed given the highly context-dependent signalling and function of these ligands.

Expert opinion: Abnormal or dysregulated TGF- β superfamily signalling may be central to the excess inflammation and tissue remodelling in asthma, and possibly CRS. Therefore, the TGF- β superfamily signalling pathways represent an emerging and attractive therapeutic target.

Key words: asthma, chronic rhinosinusitis, immunopathogenesis, immune dysregulation, inflammation, remodelling, TGF- β superfamily

Article highlights

- TGF- β superfamily signalling is complex and current studies on airway disease are limited but suggest altered signalling pathways and therefore a dysregulated system is present. Such aberrant signalling likely contributes to the excess inflammation and remodelling seen in allergic asthma and CRS.
- Ligand signalling in asthma is rapid and not stand alone but appears to be integrated and modulated in a complex time-course manner.
- Further studies using better models of disease harnessing emerging tissue engineering methods and single cell technology is needed to further understand the integrated functional context and cell-specific multiple signalling pathways together in both health and disease. Only then can safe therapeutic strategies that can harness such powerful immune-modulatory growth factors to promote simultaneous inflammatory regulation and tissue repair be considered.

1. Introduction

1.1 Asthma

It is estimated that more than 339 million people worldwide have asthma [1]. From all aspects: immunopathogenesis, disease severity and therapeutic response, asthma is clearly a heterogeneous syndrome. Whilst most patients have mild to moderate disease, up to 5-10% of the asthma population are defined as having severe asthma where treatment response is often refractory to standard guideline-based treatment approaches [2]. Clinical symptoms of asthma are non-specific and broad, with shortness of breath, chest tightness and excess mucus production, sometimes with cough and audible wheeze, as common presentations. Such symptoms, whatever the exact immunopathogenesis, are interpreted as the result of airway inflammation and variable airflow obstruction arising from airway narrowing termed bronchoconstriction. This abnormal tendency of airway constriction is termed airway hyper-responsiveness (AHR). A significant proportion of asthmatics will also demonstrate early decline in lung function and can sometimes proceed to fixed airway obstruction. Overall it is the excessive airway narrowing that leads to severe asthma exacerbation and asthma-related death. Airway inflammation and structural change (termed remodelling) are both implicated in disease pathogenesis and severity.

1.2 Allergen driven asthma

Until recently asthma was broadly and too simplistically divided into just allergic and non-allergic disease based on the demonstration of sensitisation to a perennial allergen such as house dust mite (HDM) or cat and a history of asthma induction upon inhalational contact with that allergen. Allergic sensitisation is present in most asthma cohorts and represents approximately 70% of the disease group [3]. Whilst this broad

classification is still helpful, the emergence of more precisely targeted treatment strategies has required a greater diagnostic precision and therefore "endotyping" by inflammatory subtypes has led to a more detailed mechanism-specific classification [4].

1.3 High-T2 inflammatory asthma endotypes

The inflammatory airway process in allergic asthma is complex, involving predominantly CD4⁺ T cells, eosinophils, and mast cells. Macrophage [5] and neutrophil dysfunction [6], which have been so far ignored in this syndrome, are now also recognised as important contributors to asthma pathogenesis and exacerbations. T memory cells, on molecular presentation from DCs, can recognise specific antigens ranging from allergens and microbes to occupational agents. These activated T cells now migrate into the airway mucosa and 'help' (thus termed T-helper or Th2 cells) orchestrate the allergic cascade. Allergen-specific IgE production by B cells is driven by IL-4 whilst IL-5 recruits and maintains excess numbers of tissue eosinophils. Such chronic inflammation is maintained by cycles of acute inflammation where upon inhaled allergen airway impaction, IgE cross-linking on the surface of mast cells or basophils leads to degranulation release of immune mediators. Further T-cell activation occurs, with subsequent generation and release of key Th2 cytokines such as IL-4, IL-5, IL-9, and IL-13. Innate lymphoid cells (ILCs) also release excess IL-5 and IL-13 when activated from potent 'alarmin' type cytokines released from epithelium such as TSLP, IL-25 and IL-33 [7]. Given that such structural cells and other immune cells, not just T cells, produce such Th2 cytokines, the term Th2 inflammation has been replaced with just T2 inflammation. A complex inflammatory cascade leading to airway infiltration by further T cells, eosinophils, mast cells, macrophages and

neutrophils now occurs [8]. It is likely that airway vascular permeability rapidly increases leading to airway narrowing from oedema [9]. Prompt airway smooth muscle bronchoconstriction occurs leading to airflow obstruction. Induction of AHR is rapid and is sustained even 7 days after the acute allergen exposure event [8]. Recurrent allergen exposure leads to the establishment of chronic airway inflammation and daily fluctuating symptoms.

The prevalence of high-T2 inflammatory eosinophilic asthma endotypes are still probably under-estimated (as bio-marker profiling is still a new evolving entity) but considered to be as high as 50% of the asthma population [3] [10]. Whilst aero-allergen sensitisation can be demonstrated with up to 70 % prevalence [3] in such eosinophilic endotypes, suggesting allergic and non-allergic asthma are not mutually exclusive, most of this asthma subgroup is often termed 'non-allergic.' In this asthma subtype, aeroallergens often do not elicit asthma in terms of clinical history, symptoms started late-onset in life, patients often display more persistent airflow limitation, and a faster decline in lung function, particularly when co-existent smoking is or has been present [11]. This endotype is therefore more severe and presents a clinically challenging group of patients to treat [12]. The exact pathogenesis is still uncertain. Non-steroidal anti-inflammatory drug exacerbated respiratory disease represents up to 15% of this high-T2 group [13], often a severe asthma subtype frequently accompanied by by troublesome CRS with nasal polyps.

1.4 Airway remodelling

Inflammation can be viewed as the organ response to injury. Resolution of inflammation and tissue repair (termed modelling) must rapidly occur for restoration of health. In asthma there is a chronic inflammatory state and the failure to repair airway tissue to a normal state is termed remodelling. Here, the epithelium is often abraded and in an active state, the underlying reticular basement membrane thickened from excess collagen and other extracellular matrix (ECM) deposition. There is an excess of goblets cells increased in size (hyperplasia). The submucosa shows increased numbers of activated fibroblasts (myofibroblasts) and there is excess angiogenesis. Airway smooth muscle (ASM) is both hypertrophied and hyperplastic. The epithelium-fibroblast signalling pathway or system is often termed the epithelial-mesenchymal trophic unit (EMTU), given its resemblance to the organ embryogenesis unit that drives tissue modelling in human organogenesis [14]. In asthma, airway narrowing, and excessive reactivity will be the consequence of excess ECM deposition, mucus plugging, vascular oedema and ASM excess with enhanced contractility, all key components driving AHR. A detailed review of the mechanisms of airway remodelling in asthma and CRS was previously published [14].

1.5 Targeting airway inflammatory dysregulation and remodelling together in asthma

Given that full disease control is not possible in up to 86.5% of the asthma population, particularly in the more severe asthma cohorts, there is still an urgent unmet clinical need [15]. Addressing immune modulators that can address inflammation and tissue modelling together remains an unexploited strategy at present. Growth factors, particularly of the TGF- β super family of ligands, whilst key to organogenesis *in-utero* and maintenance of tissue homeostasis in health, can with aberrant signalling,

lead to both inflammatory dysregulation and abnormal tissue repair. Focus on potential ‘super’ modulators of such key processes as the TGF- β superfamily of ligands and the respective signalling pathways in the inflammatory airway may allow the development of effective immune-modulatory therapeutic interventions in the future.

2. Transforming growth factor (TGF) - β superfamily

2.1 TGF- β_1

In mammals, the transforming growth factor (TGF)- β superfamily is comprised of more than 35 members [16]. They include the TGF- β_{1-3} isoforms, activins, inhibins and bone morphogenetic proteins (BMPs) as well as the growth differentiation factors, müllerian inhibiting substance, nodal and leftys [16]. These factors demonstrate a remarkable diversity of biological function, from key roles in embryogenesis to regulation of cell growth, differentiation and apoptosis. The typical structural features of this family of proteins are two separate anti-parallel β sheets that are perpendicular to a four-turn α -helix. The structural hallmark of the TGF- β superfamily is the conserved six to nine cysteine residues in the mature domain, termed the cysteine knot [17]. The rigid cysteine knot scaffold serves to stabilise the entire structure and is the region required for dimerisation of the ligands via intermolecular disulphide bond formation. Such striking structural conservation of the family members leads to their characteristic physiochemical properties. However, such shared structural features do not necessarily predict shared biological functions within such superfamilies but indicate that these signalling factors will have evolved from a more restricted precursor in parallel to diversification of cellular function and may be a mechanism of improving target cell specificity.

An important characteristic of growth factors is that they act locally within the tissue compartment produced with both autocrine and paracrine effects. Given the complexity of cellular tissue, it is obvious that the cellular response to a specific growth factor has the potential to be modulated by the prior or concurrent action of other growth factors in the cellular microenvironment. Such a conceptual framework allows us to understand how the effect of a growth factor will be altered depending on the tissue and cellular setting present. The diverse functions of individual ligands are cell and tissue specific and must always be considered in the context of what other cellular and environmental signals are present.

2.2 TGF- β isoforms

There are three different isoforms in mammals termed TGF- β_1 , TGF- β_2 and TGF- β_3 which are the product of three separate genes located on chromosomes 19, 1 [18] and 14 [19, 20] respectively. It is TGF- β_1 that was first discovered as a 'factor' with ability to 'transform' function of cells [21] and much research has focused on this prototypic isoform. Valuable insight into isoform function has been gleaned from knock-out (KO) murine models. TGF- β_1 KO mice succumb to overwhelming widespread systemic inflammation in the perinatal period at 3-4 weeks [22] confirming the essential anti-inflammatory role of TGF- β_1 . The KO mouse for TGF- β_2 (perinatal mortality) and TGF- β_3 (death within 20 hours of birth) are not viable because of gross embryonic malformation [23] [24] and may therefore suggest that these isoforms maybe more functional during the process of epithelial-mesenchymal interaction and tissue repair. Whilst the exact roles (overlapping and or not) remain to

be defined all three isoforms are potent inhibitors of epithelial cell proliferation but can stimulate cells of mesenchymal origin [25].

2.3 Activin-A

Activin-A was isolated over three decades ago and functionally defined as an endocrine regulatory factor [26]. Whilst a pivotal role for activin-A has since been established in tissue developmental programmes, reproduction, cell growth and differentiation, it is only now that recognition of activin-A as playing a key role in the regulation of inflammation and the response to tissue injury and repair is beginning to occur [27]. Briefly, activin-A is now considered a key regulator of lower airway inflammation and abnormal signalling is implicated in immune dysregulation and excess tissue remodelling [28].

2.4 Bone morphogenetic proteins (BMPs)

The BMPs comprise the largest subgroup of proteins within the TGF- β superfamily [29]. Originally identified on the basis of their ability to induce endochondral bone formation [30], BMPs in fact regulate key developmental signalling pathways critical for embryogenesis and tissue generation such as in the kidney and the lung. BMP signalling pathways are also fundamental for the maintenance, regeneration and repair of tissue. At present very, little is known about the role of BMP ligands in such an organ-specific context as the lung, but it is likely that key roles in both regulation of inflammation and remodelling occur [31, 32], as discussed later.

2.5 Ligand synthesis and activation

There is very little sequence conservation between the promoter regions of the TGF- β isoforms indicating that isoform gene transcription is differentially regulated. The exact transcriptional programmes involved are probably context dependent and not fully identified. Additionally, all three TGF- β isoforms can up regulate their own expression [33]. Each TGF- β isoform is encoded as a 390-442 amino acid precursor protein that contains a signal sequence, essential for the proper folding and secretion of the isoform. The most critical post-translational modification is the proteolytic cleavage of this signal sequence by an endoprotease furin with the release of the 112-amino acid mature TGF- β ligand (C-terminal domain) [34]. The translational protein product common to the TGF- β superfamily is a 29 amino-acid signal sequence, a pro-peptide region termed the latency associated peptide (LAP) which now non-covalently associates with the C-terminal domain. The TGF-LAP product now dimerises by the formation of intermolecular disulphide bonds with the LAP region and another TGF- β mature region. For TGF- β and activin (presumably in other TGF- β superfamily members also) the LAP region is essential for this folding and dimerisation process. Without dimer formation TGF- β cannot be exported out of the cell. The dimeric molecule covalently bonds with a 135 kDa latent TGF- β binding protein (LTBP) which serves to anchor the latent TGF- β ligand to the ECM. The latency conferred to TGF- β by the LTBP prevents binding of secreted TGF- β to ubiquitously expressed receptors and assures a readily accessible extracellular reservoir of TGF- β family ligands that can be activated on demand. Thrombospondin (TSP-1) can activate latent TGF- β by binding to a specific site on LAP leading to a conformational change in the latent complex leading to an active state [35]. The epithelial integrins $\alpha_5\beta_6$ and $\alpha_4\beta_8$, up-regulated in response to lung injury, are

important activators of TGF- β ligands [36]. The $\alpha 5\beta 6$ knock out mouse fails to develop pulmonary fibrosis in response to bleomycin confirming the critical role of the epithelium and the TGF- β superfamily in initiating and regulating mesenchymal cell activation and function [37]. The activins have similar ligand activation steps [38] [39] and it is likely that other superfamily ligands are synthesised as precursors that too are activated 'on-demand' in response to 'real-time' needs rather than at random.

2.6 Receptors

Despite the diversity of functional responses undertaken, a remarkably simple principle underlies the major signalling pathway common to the TGF- β superfamily of ligands [40] (Figure 1.1). Upon activation, these ligands signal via a constitutively active serine-threonine kinase specific type II receptor that complexes with a type I receptor. The TGF- β_{1-3} isoforms signal through only the type II receptor T β RII (Figure 1.1). The activins can signal through either Activin RIIA (ActRIIA) or Activin RIIB (ActRIIB). BMP signalling is predominantly through BMPRII (BMP-2 and BMP-4) but can also occur via ActRIIA and ActRIIB (BMP-6 and BMP-7). The type I receptor for the TGF- β_{1-3} isoforms is predominantly activin-like kinase (ALK)-5 (T β RI). The activins signal through predominantly ALK-4 and BMPs via ALK-2, ALK-3 and ALK-6. ALK-1 is activated by TGF- β_1 . Variations seen are not discussed in detail for the sake of word constraints, but based on receptor signalling versatility, individual ligand families can modulate each other's function, such as activin-A inhibiting BMP-6 and BMP-9 via binding ALK-2 via ActRIIA or ActRIIB [41]. It becomes apparent that local tissue concentrations of an active ligand can thus determine signalling outcomes from other ligands. The specific type II receptor-type I receptor complex now propagates the downstream signalling by phosphorylating

receptor-regulated (R) SMADs that translocate to the nucleus to initiate gene transcription (Figure 1.2).

2.7 SMADs

The SMADs are divided into three distinct subfamilies, the receptor-activated SMADs (R-SMADs) 1,2,3,5 and 8, the common-partner SMADs (Co-SMADs) of which there is only SMAD4 in mammalian cells and finally the inhibitory SMAD6 and SMAD7 [40].

TGF- β ₁₋₃ and activin signalling is via SMAD2 and SMAD3 whilst SMAD1, SMAD5 and SMAD8 mediate BMP signals (Figure 1.1). SMAD2 and SMAD3 are activated by ALK-5 and ALK-4 with SMAD1, SMAD5 and SMAD8 being activated by ALK-1, ALK-2, ALK-3 and ALK-6 [40]. BMP-6 and BMP-7 activate only SMAD1 and SMAD5 but BMP-4 can activate all SMAD1-5-8, highlighting further versatility of this system and challenges faced in trying to select out cell specific functional pathways [42]. Whereas ALK-5 is widely expressed, ALK-1 is expressed predominantly in endothelial cells but also at sites of epithelial-mesenchymal interaction [43]. There may also be SMAD isoform specific signalling outcomes [44].

The C-terminal of R-SMADs display a characteristic -Ser-Ser-Xaa-Ser- motif of which the two latter serine residues are the target for phosphorylation (p) by activated type I receptors. The R-SMAD-Co-SMAD4 complex translocates into the nucleus.

The complex can participate in the transcription of specific genes by acting directly as a transcription factor on the regulatory sequences as well as by activating other transcription factors. As such, by interacting with multiple transcription factors or co-

activators and co-repressors, which in turn can interact with other multiple cellular signalling pathways, SMAD proteins provide a versatile system by which TGF- β superfamily ligands can achieve multiple context-dependent outcomes in a cell. Furthermore, the ligand activation of the type I receptor such as ALK-5 can also lead to activation of several alternative cell-signalling pathways. These pathways are SMAD independent and classified as non-canonical signalling that involves important pathways of the MAP kinase, phosphatidylinositol 3-kinase/AKT and Rho-like GTPase as important examples [45]. Such parallel modulatory pathway ‘cross-talk’ allows further signalling versatility but also add breath-taking complexity to understanding the functional outcomes from such ligands. These non-canonical systems are not discussed in this review due to word constraints.

Strict regulation of signalling activity is achieved through the induction of inhibitory SMAD7 [46], which by interacting with the type I receptor leads to receptor degradation and thus provides a negative feedback loop of inhibition on TGF- β superfamily signalling. SMAD7 expression is rapidly induced in cells in response to ligand signalling [47].

2.8 Regulation and diversity of signalling

Further strict control of ligand activation and signalling is achieved by incorporating several layers of active regulation by the presence of diffusible regulatory proteins that can bind ligand or accessory receptors that can target the key point of pathway activation between the type I receptor and transcription SMADs. An important example is of follistatin, a protein monomer that is a potent physiological inhibitor of the activins [48]. More recent work has demonstrated mechanisms involving ever so

more sophisticated pathways of control with short and long non-coding RNA molecules and epigenetic modifications of DNA and histone proteins, regulating ligand-SMAD and creating further 'cross-talk' pathways [49].

3. Human studies of TGF- β superfamily expression in asthma

3.1 Ligand expression (baseline asthma)

TGF- β_1

Whilst some studies demonstrate increased levels of TGF- β_1 in the asthmatic airway regardless of asthma severity, a similar number show expression levels similar to that of the normal airway [50]. Our own group evaluated both protein and mRNA expression of TGF- β_1 in mild asthmatics at baseline in relation to normal volunteers and found no significant difference. TGF- β_1 expression was ubiquitous throughout the airway with airway epithelium and submucosal inflammatory cells important sources of ligand [51].

Activin-A

Activin-A expression is markedly increased in both mild and severe asthma with almost absent expression in the normal airway [52, 53]. Interestingly, our work has shown that follistatin expression was less in the asthmatic airway (unpublished self observation). IL-13 is a potent inducer of activin-A expression in the murine airway [54] and it is likely that multiple inflammatory cytokines regulate activin-A expression in asthma. Epithelium and submucosal cells with mast cells, macrophages, CD4⁺ T cells and neutrophils were sources of activin-A in asthma [55], again supporting the concept that activin-A is induced in inflammatory tissue states. Increased activin-A levels have been identified in asthmatic serum [56].

Bone morphogenetic proteins (BMPs)

BMP 2, BMP-4 and BMP-7 expression in the baseline mild asthmatic airway was not altered compared to the normal airway tissue [57]. Again, epithelium and submucosal inflammatory cells were the main sites of ligand expression. Interestingly eosinophils were a significant inflammatory cell source of BMP-7 [57]. Like TGF- β_1 , given the role of BMPs in key homeostatic functions of tissue, one would expect the airway to store such ligands with rapid activation on-demand, as needed.

3. 2 Post-allergen provocation of mild allergic asthma

No modulation of ligand expression was seen for TGF- β_{1-3} , activin-A or BMP-2/4/7 at 24 hours, consistent with activation of local ‘on-demand’ ligand reservoirs [51, 55, 57]. However, submucosal cell TGF- β_1 along with epithelial and submucosal cell BMP-7 expression was significantly increased at the 7-day post-allergen time point (Figure 2.1 A-B and C-D respectively) [51, 57]. The clinical implications of such expression is discussed later.

3.3 Signalling pathway expression and modulation in mild allergic asthma

TGF- β superfamily signalling is rapid after allergen provocation in the asthmatic airway [58]. Airway remodelling is activated at 24 hours and sustained at 7 days post-allergen when inflammatory cell numbers return to baseline levels in mild allergic asthmatics [8]. The activation of TGF- β , activin and BMP signalling alongside specific receptor modulation in bronchial tissue obtained from the same cohort of volunteers is summarised in Figure 3. Detailed demography, baseline FEV₁ and details of allergen challenge on this volunteer cohort have already been published [8].

3.3.1 TGF- β superfamily receptor modulation (**baseline asthmatic airway**)

The asthmatic airway demonstrates a markedly altered expression of key signalling receptors [51-53, 57]. The sole TGF- β_1 type II receptor T β RII was ubiquitously expressed throughout the airway in both asthmatic and normal airway tissue. However, the key type I receptors ALK-1 and ALK-5 (Figure 2.2 A-B) were significantly decreased. Activin related receptor expression was also altered in asthma with significantly less ActRIIA and ALK-4 expression. ActRIIB expression does not seem to be prominent in either the normal or asthmatic airway. The key BMP ligand receptors BMPRII and ALK-2/ALK-6 expression was attenuated in the asthmatic airway. The expression of pSMAD2 and pSMAD1/5 expression, however, was similar in both the asthmatic and normal airway. The expression of inhibitory SMAD7 was decreased in asthma (Figure 2.2 E-F), consistent with previous studies [59].

3.3.2 Post-allergen provocation of mild asthma

Figure 3 summarises TGF- β signalling changes seen in the asthmatic airway from baseline to 24 hours and 7 days post allergen challenge, in relation the human allergen-challenge model discussed earlier. Rapid activation of epithelial TGF- β , activin and BMP signalling was seen at 24 hours following allergen provocation of disease, as evidenced by the marked increase in pSMAD2 and pSMAD1/5 expression [55, 57]. There was also associated rapid modulation of selected ligand-specific receptor expression. Given that both TGF- β_1 and activin-A can activate pSMAD2, the up-regulation of epithelial and submucosal ALK-4 (activin-A) alongside further down-regulation of ALK-5 expression (i.e. TGF- β_1 signalling) (Figure 2.2 A-C) with

almost absent expression in the submucosal compartment, suggests a dominance of activin-A mediated signalling in response to allergen provocation of disease. At the 7-day post-allergen time point when remodelling was maximal [8] pSMAD2 and ALK-5 receptor expression had returned to baseline levels (Figure 2.2 D). Thus, initial activation of remodelling alongside pSMAD2 signalling is sustained at day 7 and suggests TGF- β and activin-A signalling precedes allergen-induced airway remodelling in asthma. Whilst rapid induction of pSMAD1/5 expression alongside ALK-2 and ALK-6 was evident 24 hours post-allergen, elevated expression was still evident at the 7-day time point [57]. Thus, BMP signalling is still active at one-week post-allergen when inflammatory resolution is occurring, but remodelling is active, suggesting BMPs are functionally implicated in both the regulation of inflammation and further propagation of the remodelling process. Further work is needed to confirm these possibilities.

Consistent with previous *in-vitro* studies, activation of ligand signalling was associated with significant induction of airway SMAD7 expression at both the 24 hours and 7-day time point (Figure 2.2 G-H). Eosinophils represented approximately 15 % of the submucosal cells staining for TGF- β_1 at the 7-day time point when remodelling was sustained but inflammatory cell numbers had returned to baseline levels (Figure 2.3 A-B) [51]. This is in keeping with previous findings suggesting that eosinophil derived TGF- β_1 plays a significant role in airway remodelling in asthma [60-63]. Whilst most eosinophil depletion studies demonstrate reduction of TGF- β_1 [64] there is at least one instance where TGF- β_1 levels have remained unchanged highlighting that vascular, structural and other inflammatory cell sources of TGF- β_1

contribute to airway repair processes [62, 65]. Only modulation of BMP-7 with increased expression at the 7-day time point was seen (Figure 2.1 C-D).

4.0 Functional studies

4.1 TGF- β_1 and inflammation

The inflammatory response to tissue injury is the initiation of a complex cascade of events leading to recruitment, migration and activation of inflammatory cells to the site of injury or antigen deposition. Resolution of the response is characterised by regulated apoptosis and matrix deposition [66]. The goal of this repair response is the elimination of the inciting agent, resolution of inflammation and the restoration of normal tissue architecture. TGF- β_1 has an essential role in all these processes.

TGF- β_1 was initially viewed as an anti-inflammatory cytokine because TGF- β_1 KO mice died of an overwhelming MHC Class II mediated autoimmune-type inflammatory response [22, 67] characterised by circulating autoantibodies, marked tissue leukocyte infiltration and increased IFN- γ expression [68]. Since then important roles for TGF- β_1 in both innate and adaptive immunity have been defined. TGF- β_1 is released from the α -granules of activated platelets, structural cells, inflammatory cells and ECM stores during tissue inflammation. Activated TGF- β_1 is involved early in the inflammatory response as it is a chemotactic factor for monocytes [69], lymphocytes [70], mast cells [71] and neutrophils [72] [73]. TGF- β_1 may also influence eosinophil chemotaxis as suggested by *in-vitro* experiments [74]. Cells can transmigrate out of the vessel wall at inter-endothelial cell junctions into tissues along a concentration dependent TGF- β_1 gradient [69]. By promoting integrin expression such as $\alpha_5\beta_1$ (the functional receptor for fibronectin) and $\alpha_3\beta_1$ (the ligand for laminin and Type IV collagen), TGF- β_1 also enables cell adhesion which is critical for regulated and

selective cell trafficking to inflammatory sites [75]. TGF- β_1 mediated induction of gelatinases (MMP-2 and MMP-9) which then degrade ECM in the path of the cell allows cell migration via tissues to occur [75]. TGF- β_1 initially potentiates the inflammatory cascade by the up-regulation of interleukin-1 β , TNF- α and IL-6 from activated monocytes and macrophages which act downstream on other inflammatory cells [76]. For example, IL-2 is an important lymphocyte proliferation cytokine. TGF- β_1 primes T lymphocytes such that the cells are more readily activated with an increased rate of proliferation and IL-2 production in response to subsequent stimulation [77]. TGF- β_1 may also contribute to T cell survival via delayed apoptosis [78]. It may have a critical role in shaping the T cell functional repertoire. TGF- β_1 modulates the differentiation and activation of DCs [79] that may subsequently determine the polarisation direction of naïve T cells into either the Th1 or Th2 type [80] [81]. In the thymus of the TGF- β_1 KO mouse, thymocytes are markedly sensitive to TCR-enforced lethality such that both positive and negative thymocyte selection is disrupted. This leads to escape of self-reactive T cells into the periphery that can initiate autoimmune responses.

Other cells are closely regulated by TGF- β_1 . Neutrophils undergo degranulation in response TGF- β_1 [82]. TGF- β_1 recruited macrophages have an essential role in phagocytosis of apoptotic cells, a key step towards the resolution of inflammation. By up-regulating CD16 (Fc γ RIII), the low affinity IgG Fc receptor with its important role in the capture and clearance of particles on monocytes and macrophages, TGF- β_1 can will clearly regulate early immune responses [83]. TGF- β_1 enhances macrophage recognition of phosphatidylserine, expressed on the outer membrane of apoptotic cells [84]. Enhanced phagocytic capacity is associated with increased respiratory burst activity and superoxide generation in response to TGF- β_1 by macrophages [85]. This

is then followed by inhibition of inflammatory cytokine and mediator production such as IL-1 β , IL-8, TNF- α , GM-CSF and LTC₄ [86].

Eosinophil clearance is crucial to the resolution of asthmatic inflammation [87] and is predominantly dependent on apoptosis. Apoptotic cell products are phagocytosed by macrophages [87]. TGF- β_1 has been demonstrated *in-vitro* to enhance the rate of eosinophil apoptosis. This may be through TGF- β_1 mediated inhibition of GM-CSF and IL-5 production [88]. Local tissue IL-3, GM-CSF and in-particular IL-5 effectively prolong tissue eosinophil survival and the absence of such factors leads to rapid apoptosis [89]. In addition, TGF- β_1 can inhibit eosinophil release of mediators [88]. Importantly, macrophage activation is followed by down-regulation of the macrophage TGF- β_1 receptor leading to decreased sensitivity to TGF- β_1 mediated signalling [76]. This is part of the resolution phase of inflammation. *In-vitro* studies indicate high concentrations of TGF- β_1 lead to deactivation of macrophage function such as suppression of the respiratory burst [90] and cytokine production with TNF- α (as one example) [91, 92]. TGF- β_1 mediated attenuation of MHC class II expression and costimulatory molecules on myeloid DCs [79] lead to loss of further T cell activation. TGF- β_1 inhibits proliferation and cytokine production by naïve T cells as well as Th1 and Th2 clones with post-activated T cells undergoing rapid apoptosis in response to TGF- β_1 [93]. Thus, initially TGF- β_1 initially propagates inflammation but later in the cascade becomes a potent anti-inflammatory agent. Thus, consideration of the context of TGF- β_1 signalling is important and is very much dependent on the state of cellular differentiation and the cytokine milieu in which the cell is present. It is therefore likely that the functional outcome of TGF- β signalling in asthma will be influenced by the disease microenvironment and suggests non-human asthma models of TGF- β signalling need to be interpreted with caution [94].

4.2 TGF- β_1 and remodelling

Rapid restoration of tissue structure following injury is essential for the maintenance of organ integrity and function. TGF- β_1 is one of the most fibrogenic factors known and therefore much research has focussed on its role in fibrosis. Airway epithelium is a very significant source of TGF- β ligands [95]. Autocrine effects of such ligands will have important disease implications. TGF- β_1 inhibits epithelial cell proliferation [96] although the exact mechanisms of such inhibition have not been fully elucidated. One mechanism by which TGF- β_1 can inhibit cell proliferation is by induction of the cyclin dependent kinase inhibitor p21waf that leads to arrest of the cell cycle in the G1 phase [97]. In the TGF- β_1 KO mice, expression of PCNA in inflamed tissues is elevated, indicative of uncontrolled cellular proliferation [68]. In asthma, the finding of markedly increased p21waf expression [98] in the absence of PCNA expression may then be a result of TGF- β_1 mediated epithelial effects which leads to inhibition of epithelial cell proliferation and therefore repair. TGF- β_1 mediated signalling thus contributes to aberrant epithelial repair. TGF- β_1 is an important fibroblast chemotactic factor [72] and fibroblast numbers have been shown to correlate with TGF- β_1 expression [99]. The TGF- β_{1-3} isoforms are all potent inducers of fibroblast proliferation with TGF- β_3 demonstrated the most potent [100]. The isoforms induce the differentiation of fibroblasts to α -SMA⁺ myofibroblasts [101] and promote the deposition of ECM components, particularly collagen [102]. All three isoforms can rapidly induce collagen I and III mRNA transcription [103], whilst at the same time limiting intracellular collagen degradation as demonstrated in foetal lung fibroblast *in-vitro* experiments [104]. TGF- β_1 leads to rapid synthesis of tenascin [60], biglycan [105], fibronectin [103] and hyaluronan [106]. By reducing the synthesis of MMPs, particularly collagenases and stromelysins and by increasing the expression of

molecules that inhibit them such as the TIMPs [107], as well as plasminogen activator inhibitor [108], ECM degradation is prohibited. TGF- β_1 is one of several mediators with effects on ASM including modulation of proliferation [109] and autocrine collagen synthesis [110].

From this background it becomes apparent that it is essential to strictly regulate TGF- β ligand activation and signalling. Any dysregulated function therefore has the potential to lead to impaired inflammatory homeostasis and tissue remodelling.

4. 3 Activin-A

Inflammation

Our group has previously published a detailed review of the immune functions of activin-A [28]. Several *in-vitro* approaches have suggested that activin-A is both a pro and anti-inflammatory cytokine. Function is context specific as with TGF- β_1 . Activin-A is highly expressed in inflammatory states with rapid induction in response to inflammatory signals such as IL-13, TNF- α , IL-1, IL-8, IFN- γ and interestingly TGF- β_1 itself. The pro-inflammatory effects range from potent induction of selected immune mediators such as TNF- α or IL-1 β , promoting cellular differentiation and activation of key cells such as mast cell progenitors [111] and mouse B cells *in-vitro* [112]. In contrast, activin-A blockade with follistatin enhances dendritic cell release of proinflammatory cytokines IL-6, IL-8, IL-12 and TNF- α and RANTES (CCL5) along with CD8⁺ T cell expansion [113]. Blood derived CD4⁺ T cells fail to proliferate in the presence of activin-A [56]. Activin-A can inhibit IL-6 in a dose dependent manner [114]. Critically, activin-A can convert peripheral mouse naïve T cells to CD4⁺CD25⁺ FoxP3⁺ Treg cells again in a dose dependent manner, and in synergy with TGF- β_1 [115]. We could not demonstrate a pro-inflammatory effect of activin-A on airway epithelium in either the basal or activated state [55]. TNF- α

induced the release of activin-A by normal human bronchial epithelial (NHBE) cells. IL-13-induced release of CXCL8/IL-8 by NHBE cells was significantly increased in the presence of follistatin. Furthermore, with follistatin, TNF- α -induced release of CXCL8/IL-8, CXCL10/IP-10 and CCL2/MCP-1, and IL-13 induced release of CXCL10/IP-10 from NHBE, suggesting that activin-A may act as a brake on cytokine-induced chemokine release by epithelial cells [55]. These findings, taken together with those discussed earlier suggests that activin-A may be anti-inflammatory rather than pro-inflammatory in its role in airway epithelium with the ability to induce epithelial inflammatory resolution and repair rather than injury. Using a murine model of repetitive ovalbumin induced acute airway inflammation, it has been shown that activin-A is a powerful suppressor of allergic airway inflammation and can protect against the development of AHR [52]. Antibody mediated neutralization of activin-A led to marked exacerbation of Th2-mediated allergic inflammation. ALK-4 blockade increased allergen-specific Th2 cell proliferation *in-vitro*. Thus, airway activin-A confers protection against allergic Th2-specific inflammation. Such activin-A-mediated immune suppression was associated with induction of a Treg cell population independent of FoxP3 expression [52] and recent work confirms activin-A in the inflammatory milieu of DCs promote a tolerogenic immunotype of DC [116].

4. 4 Remodelling

Activin-A induced primary human airway epithelial cell proliferation in a dose-dependent manner [55]. Activin-A can rapidly activate fibroblasts to ECM producing myofibroblasts [117] and induce ASM proliferation [118]. Endothelial cells secrete activin-A and an important role in the regulation of vascular remodelling has been shown [53]. Murine models of bleomycin-induced lung fibrosis demonstrate prominent immunoreactive activin-A [119]. The administration of follistatin in the same model led to attenuation in lung fibrosis. In a novel murine model engineered to over-express epithelial SMAD2, with HDM (not ova albumin-hence a more real-world like scenario) induced airway remodelling, activin-A with IL-25, not TGF- β_1 , was the dominant signalling pathway [120]. Our human studies confirm activin-A is strongly expressed in the asthmatic airway [52, 53] and signalling is rapidly activated alongside markers of airway remodelling following allergen challenge [8, 55].

4. 5 BMPs

Ur group has demonstrated BMP ligand and signalling pathway expression in the normal human airway along with markedly altered signalling expression in baseline asthmatic airway tissue [57]. Mechanistic studies are currently lacking and are difficult to fully undertake given the context-dependent signalling and functional outcomes of these complex ligands. The current *in-vitro* data would, however, predict an important regulatory role for BMPs in airway tissue injury, inflammation and repair events.

BMP-7 is rapidly gaining prominence as an anti-fibrotic factor by its ability to antagonise the effects of TGF- β_1 . BMP-7 can counteract the fibrotic effects of TGF- β_1 in the *in-vitro* setting [121, 122]. BMP-7 inhibits TGF- β_1 mediated collagen

expression, α -smooth muscle actin expression and TIMP-2 in lung-specific myofibroblasts [123]. In both the kidney and gut BMP-7 potently reduces inflammation and fibrosis [124, 125]. In an ovalbumin induced murine asthma model, BMP-7 was shown to potently antagonise the remodelling effects of TGF- β_1 whilst attenuating inflammation [126]. Other studies show the selective suppression in the expression of pro-inflammatory cytokines. BMPs have been shown to be potent suppressors of naïve and memory B cell function [127]. BMP-4 is a potent inhibitor of normal human lung fibroblasts proliferation and ECM production [128]. Both BMP-4 and BMP-7 prevented fibroblast to myofibroblast transformation by TGF- β_1 . However, BMP-7 fails to attenuate lung fibrosis in the bleomycin-induced model of lung injury [129] suggesting functional outcomes will be context specific as shown for both TGF- β_1 and activin-A. More detailed reviews of the potential role of the BMPs as regulators of inflammation and remodelling in asthma are available [32].

5.0 Altered TGF- β superfamily signalling in allergic asthma

5.1 TGF- β_1

Our data demonstrates that the TGF- β superfamily receptor distribution on the cell is very flexible and dynamic in response to activation of signalling by allergen. It remains uncertain as to why the key type I receptors ALK-2, ALK-6 (BMPs), ALK-1, ALK-5 (TGF- β_{1-3}) and ALK-4 (activin) have decreased expression in the basal asthmatic airway. One would speculate that the pro-inflammatory milieu of the asthmatic airway counter-regulates such expression. Decreased ALK-5 is demonstrated in more severe asthma and models of lung injury [130, 131]. Inflammatory cell expression of ALK-5 is determined to some extent by the level of differentiation and activation of the cell. For example, inactive monocytes express

high levels of ALK-5 but with cell activation there is down-regulation of ALK-5, leading to unresponsiveness to further TGF- β_1 signalling [132]. Thus, immune regulation is rapidly restored. In the human asthma model of allergen provocation, the demonstration of deficient ALK-5 expression at baseline and post-allergen suggests that the TGF- β_{1-3} anti-inflammatory and tissue repair signalling pathways may be impaired or dysregulated [55]. ALK-1 signalling may also contribute to disease. In the same model, at the 7-day time point after allergen challenge where inflammation returned to baseline levels, but remodelling and AHR was still markedly increased, we are confident that a significant proportion of ALK-1 staining submucosal cells were fibroblasts (fusiformic in shape with elongated nuclei) (2.3 C-D). The consequence for epithelial and fibroblast function of ALK-1 expression in the context of airway inflammation and remodelling in asthma remains to be determined. We would hypothesise that it may be related to regulation of ALK-5 mediated activation of epithelial-mesenchymal signalling [133]. However, it has been shown that BMP-9 can also utilise ALK-1 on fibroblasts to inhibit basic fibroblast growth factor induced proliferation [134]. Thus, it is possible that the modulation of ALK-1 expression seen in active asthma is co-related to activation of BMP ligand signalling [57].

5.2 Activin-A

ALK-4 expression was deficient in the asthmatic airway in both mild and severe asthma [52, 53]. There was an increase in numbers of submucosal cells expressing ALK-4 post allergen and a trend or increased expression in airway epithelium, reflecting either increased cellular recruitment or induction of ALK-4 expression in response to cell priming or activation [55]. Given that activin-A leads to epithelial cell proliferation *in-vitro* and inhibits cytokine production [55], collectively the

implication is that activin-A will induce epithelial repair and act to switch off aspects of epithelial activation and inflammation. Epithelium, however, fails to proliferate in the asthmatic airway leading to a denuded and highly activated state, suggesting a failure of activin-A function in asthma. In addition, activin-A regulation of submucosal inflammation may be impaired. Demonstration of high numbers of myofibroblast ALK-4 expression is consistent with the emerging role of activin-A in the induction of tissue remodelling, as has been demonstrated in other settings [117, 119]. Given the absence of significant ALK-5 expression in airway fibroblasts, it may suggest that activin-A rather than TGF- β_1 is the dominant pathway in allergen-induced airway remodelling in asthma. To support this, in animal models of lung fibrosis the activin antagonist follistatin abolishes fibrosis even in the presence of TGF- β_1 [135]. A murine model based study from the Lloyd group highlights airway remodelling in the absence of TGF- β_1 and confirms activin-A along with IL-25 as the dominant signalling factor implicated in house-dust mite induced airway remodelling [120, 136], consistent with our own findings specific to the human asthmatic airway using HDM, pollen and cat dander allergen provocation. Given that other murine studies implicating TGF- β_1 and the related isoforms in remodelling have so far used the artificial surrogate allergen ovalbumin [94], the suggestion is that activation of specific airway remodelling pathways will be related to the exact type of allergen or injury present.

5.3 BMPs

Given the essential anti-inflammatory role of TGF- β_1 and BMP-7, it is likely that the increased expression of these ligands at the 7-day time point of the human allergen asthma challenge model is related to regulation of inflammatory homeostasis.

Inflammatory cells return to baseline levels at the 7-day time point [8, 51, 57]. Furthermore, inflammatory cell derived active TGF- β_1 and BMP-7 may act to further interact with the EMTU leading to propagation of the remodelling process whilst latent ligands will contribute to replenishing ECM stores that were depleted because of the initial surge of TGF- β_1 -BMP-7 induced signalling. Again, decreased type II and type I expression may delay activation of BMP driven regulatory and tissue repair programmes in asthmatic airways. Default receptor combinatorial interactions may now occur with altered BMP functional immunological outcomes in the airway mucosa. Receptor processing is complex but it likely that inflammatory cells modulate the key type I BMP receptors, as has been shown for ALK-3 with eosinophil derived MBP and EDN in a primate asthma model of BMP signalling [137]. As discussed earlier, in human asthma eosinophils are a significant source of BMP-7 [57], suggesting these cells maybe contributing to regulation of inflammation and remodelling, and modulating receptor expression to allow BMP signalling to take place. Further work is urgently needed to take forward such concepts.

6.0 TGF- β superfamily expression in chronic rhinosinusitis (CRS)

CRS is subdivided into CRS with and without nasal polyps, and such CRS subgroups are composed of further distinct immunological endotypes [138].

Inflammation and tissue remodelling are extensive in both these subgroups. The one-airway concept, where shared upper and lower airway mucosal immune programmes lead to an interactive sinonasal-pulmonary system, is now an established concept [139]. As such, CRSwNP and at the least the more severe forms of asthma are strongly co-associated. Thus, it is tempting to hypothesise that TGF- β superfamily

ligand and the respective signalling pathway expression will reflect findings so far found in asthma.

An early study by the Bachert group demonstrated decreased TGF- β_1 expression in CRSwNP compared to normal nasal tissue [140]. There was associated down-regulation of T β RII, ALK-5 and pSmad2, suggesting overall suppressed TGF- β_1 signalling. This reflects the findings of decreased ALK-5 expression in the stable asthmatic airway, with further down-regulation of ALK-5 expression after allergen activation of asthma [8, 55]. T β RII expression in allergic asthma was not different to the normal airway and did not change with allergen induced activation. Given CRSwNP tissue is already severely remodelled [14], the lower levels of TGF- β_1 may reflect depleted tissue stores of the ligand. The study by Yamin *et al.* found the converse with increased TGF- β_1 and pSmad2/3 staining in CRSwNP [141].

Studies looking at activin-A expression in CRSwNP are also conflicting. The Bachert group show that activin-A levels are not significantly increased in CRSwNP [142], whilst the Yamin *et al.* study demonstrate increased levels of activin-A in CRSwNP. Another study shows activin-A expression at high levels in the normal nasal tissue [143]. Whilst staining in CRSwNP of activin-A was present in epithelium, it was less in the sub-epithelial mucosal compartment with also markedly less ALK-4 submucosal staining. In asthma, whilst activin-A expression is high, ALK-4 expression is decreased in both mild and severe asthma at baseline [52, 53], but ALK-4 expression increases with allergen challenge activation of disease. In the study by Tengroth *et al.* key type I receptor ALK-1 to ALK-7 expression on CRSwNP epithelial cells was high compared to normal tissue, again opposite to the findings of decreased ALK-5/ALK-4(submucosal)/ALK-2 and ALK-6 expression in the baseline

asthma airway [144]. However, CRSwNP tissue at surgery represents severe active disease so it maybe that such increased ALK expression is like the markedly increased ALK-4 and ALK-2/6 expression seen at the time point in the asthmatic airway when inflammatory resolution was present but AHR and airway remodelling still active [55, 57]. Thus, such high type I receptor expression could be hypothesised as reflection of the airway trying to promote TGF- β_1 , activin-A and BMP signalling to harness the presumed anti-inflammatory and tissue healing functions of these ligands. Early disease biopsies from CRS patients in a time course manner of disease, as has been undertaken in asthma, are needed to take forward this hypothesis.

Such conflicting data in CRSwNP is a clear indication that further work is still needed, and simple expression studies cannot be interpreted unless homogenous patient groups are studied. The limited TGF- β superfamily related studies in CRS must be interpreted with further caution for several reasons. Firstly, studies have been undertaken in nasal tissue obtained from patients undergoing routine sinus surgery, often an indication of a more severe disease endotype, and when inflammation and remodelling maybe at a 'burnt-out' phase. Thus TGF- β ligand and signalling pathway findings will reflect expression at a single severe disease time point, and will not indicate modulation of signalling as inflammatory and remodelling cascades set in. In addition, the nasal mucosa in more severe CRS is often colonised with staphylococcal aureus [138] which is a potent inducer of TGF- β_1 expression [145]. Activin-A is rapidly increased during sepsis [146]. Thus, upper airway expression studies of TGF- β ligand and signalling pathways are difficult to interpret in terms of the CRS disease process, and difficult to compare to findings in asthma and normal airways given the current limited data.

7.0 Proposed model of TGF- β superfamily signalling in asthma

It is possible to propose how allergen-induced airway injury and repair processes may proceed in allergic asthma in relation to TGF- β superfamily signalling (Figure 4).

Allergen impaction of the airway is associated with rapid inflammatory cell recruitment, associated with the induction and release of key cytokines but also TGF- β_{1-3} , activin-A, BMPs and other superfamily ligands along with 'injury' or 'alarmin' cytokines such as IL-25 and IL-33. Epithelium is an important source of these growth factors. Under normal circumstances injured epithelium can self-repair quickly, but this process is defective in asthma leading to marked epithelial cell activation and therefore maybe excessive TGF- β , activin-A and BMP ligand storage amounts, subsequent release and prolonged activation. The relative deficiency of key TGF- β signalling receptors in the asthmatic airway may lead to a delay in the activation of tissue mechanisms that regulate inflammation and activate tissue repair programmes as would otherwise occur in the normal healthy airway. In addition, such altered receptor expression along with a relative deficiency of regulatory SMAD7 may predispose to alternate receptor combination and further altered or dysregulated TGF- β superfamily signalling events that proceeds without the SMAD7 break. We would hypothesise that this would lead to an altered functional cellular and tissue outcome. Such aberrant signalling may thus contribute to excessive inflammation and induction and propagation of airway remodelling rather than tissue repair. With repetitive injury and such dysregulated repair, depending on the exact asthma phenotype and cytokine milieu present, progression to either excessive AHR or fixed airway obstruction may occur, thus contributing to disease symptoms and severity, particularly in the more severe asthma phenotypes. In more mild asthma, where the time course studies of TGF- β superfamily signalling were undertaken, despite repetitive allergen induced

exacerbation of disease, such patients usually respond to anti-inflammatory treatment and often do not demonstrate excessive AHR or airway remodelling. It is tempting to speculate that here, return of TGF- β signalling receptors to levels found in the normal airway allows ligands to eventually induce inflammatory resolution and some degree of tissue repair. Further work is urgently needed to take forward this proposed hypothesis.

8.0 Conclusion

The expression of the TGF- β superfamily signalling pathways are markedly altered in the airway of asthmatics and suggests dysregulated function. There is rapid activation and sustained modulation of TGF- β superfamily ligand signalling alongside inflammation and remodelling in response to allergen provocation. This is consistent with an important functional role for these ligands in asthma. Further work is still needed to dissect out the role of such ligands in the asthmatic airway, particularly in relation to severe asthma phenotypes. Expression studies in CRS are still at an early stage and findings conflicting and difficult to interpret in a disease context. Further work, particularly directed at understanding downstream signalling events in airway tissue, is now needed. Only by understanding the context-specific nature of TGF- β superfamily signalling can such pathways be manipulated with view to therapeutic intervention.

9.0 Expert opinion

Disappointingly, we are still at a very early stage of understanding the immune context and cell specific biology of this complex family of ligands in human airway disease. The main limitation to obtaining further insight into TGF- β superfamily

signalling in asthma and CRS is the lack of robust models of disease. Tissue from carefully pheno-endotyped patients, ideally with paired upper and lower airway tissue from the same individual, can be taken at baseline when in a stable disease state and then after provocation of disease with not just allergen but other known activators of disease such as LPS and live virus as examples, in a time course manner [147]. We appreciate such models are challenging to undertake given their likely costs, time requirements and difficulty in obtaining ethical approval. However, upper airway tissue is easy to safely access in the hands of skilled ENT surgeons. Bronchoscopy is an established clinical procedure and has been shown very safe even after repetitive lavage and bronchial biopsy in asthmatic individuals [148]. A collaborative multi-centre approach is needed when planning such ambitious studies. It is hoped that such ‘super-models’ of disease will be more helpful in understanding disease-specific biological pathways.

Inflammatory and structural cells differ both in cell numbers and amounts of stored TGF- β ligands in tissue compartments. Given that TGF- β ligand concentration may determine which signalling pathways dominate, researchers must begin to ask what individual cell populations rather than tissue blocks contribute to disease. Employment of new single cell technology will allow tissue heterogeneity to be dissected out together with better insight into the vectors of signalling identity that determine functional outcomes in a complex cellular setting of an organ system. Such approaches will allow better understanding of TGF- β superfamily biology.

Pressing questions include to what extent do current treatment strategies for asthma and CRS influence TGF- β superfamily ligand expression and signalling pathways, and can the mainstay anti-inflammatory approach undertaken restore receptor levels

to normal airway homeostatic function levels. It is always possible that failure to restore signalling pathways in an integrated manner may occur, enhancing further dysregulation and altered function, and thus active or progressive disease.

Given the critical homeostatic roles of these ligands in biological systems, therapeutic strategies that block or modulate entire ligand function are not the way forward. Work so far confirms that the TGF- β superfamily signalling pathways do not stand alone, but are heavily integrated with multiple immune cascades activated simultaneously in the same cell. Signalling is rapid and actively modulated. Understanding functional outcomes because of altered signalling and effects of blocking key points of the pathway such as the ALKs should thus provide more valuable functional insight. Future therapeutic strategies should aim to restore signalling pathways to that in normal tissue. Manipulating key receptors or regulatory proteins would be one strategy to consider.

It is hoped that more collaborative working between clinicians and researchers will have allowed the development of disease models more specific to humans. In particular, time-course models of actual human airway disease will be more established. Advances in tissue engineering technology and other 'biomimetic' systems will have allowed better understanding of context-specific cellular function and integration of the TGF- β superfamily signalling pathways. By understanding how any altered ligand signalling contributes to disease, the potential to modulate key pathway regulatory checkpoints with view to therapeutic potential will then, we hope, be a more realistic option.

Funding

This paper was not funded

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

References

Papers of interest (•) or significant interest (••) to readers have been highlighted.

1. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 2017; 390: 1211-1259.
2. Chung KF, Wenzel SE, Brozek JL et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; 43: 343-373.
3. Haldar P, Pavord ID, Shaw DE et al. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008; 178: 218-224.
4. Lotvall J, Akdis CA, Bacharier LB et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 2011; 127: 355-360.
5. Fricker M, Gibson PG. Macrophage dysfunction in the pathogenesis and treatment of asthma. *Eur Respir J* 2017; 50.
6. Toussaint M, Jackson DJ, Swieboda D et al. Host DNA released by NETosis promotes rhinovirus-induced type-2 allergic asthma exacerbation. *Nat Med* 2017; 23: 681-691.
7. Ray A, Oriss TB, Wenzel SE. Emerging molecular phenotypes of asthma. *Am J Physiol Lung Cell Mol Physiol* 2015; 308: L130-140.
8. Kariyawasam HH, Aizen M, Barkans J et al. Remodeling and airway hyperresponsiveness but not cellular inflammation persist after allergen challenge in asthma. *Am. J. Respir. Crit Care Med* 2007; 175: 896-904.

•• This study showed allergen provocation in allergic asthma activates inflammation and remodeling together, but later on inflammation returns to baseline levels whilst remodeling continues.

9. Kay AB, Ali FR, Heaney LG et al. Airway expression of calcitonin gene-related peptide in T-cell peptide-induced late asthmatic reactions in atopics. *Allergy* 2007; 62: 495-503.
10. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. *Lancet* 2018; 391: 783-800.
11. Coumou H, Westerhof GA, de Nijs SB et al. Predictors of accelerated decline in lung function in adult-onset asthma. *Eur Respir J* 2018; 51.
12. de Nijs SB, Venekamp LN, Bel EH. Adult-onset asthma: is it really different? *Eur Respir Rev* 2013; 22: 44-52.
13. White AA, Stevenson DD. Aspirin-Exacerbated Respiratory Disease. *N Engl J Med* 2018; 379: 1060-1070.
14. Samitas K, Carter A, Kariyawasam HH, Xanthou G. Upper and lower airway remodelling mechanisms in asthma, allergic rhinitis and chronic rhinosinusitis: The one airway concept revisited. *Allergy* 2018; 73: 993-1002.
15. Chang H, Brown CW, Matzuk MM. Genetic analysis of the mammalian transforming growth factor-beta superfamily. *Endocr. Rev* 2002; 23: 787-823.
16. Piek E, Heldin CH, Ten Dijke P. Specificity, diversity, and regulation in TGF-beta superfamily signaling. *Faseb j* 1999; 13: 2105-2124.
17. Sun PD, Davies DR. The cystine-knot growth-factor superfamily. *Annu Rev Biophys Biomol Struct* 1995; 24: 269-291.
18. de Martin R, Haendler B, Hofer-Warbinek R et al. Complementary DNA for human glioblastoma-derived T cell suppressor factor, a novel member of the transforming growth factor-beta gene family. *Embo j* 1987; 6: 3673-3677.
19. ten Dijke P, Geurts van Kessel AH, Foulkes JG, Le Beau MM. Transforming growth factor type beta 3 maps to human chromosome 14, region q23-q24. *Oncogene* 1988; 3: 721-724.
20. Lander ES, Linton LM, Birren B et al. Initial sequencing and analysis of the human genome. *Nature* 2001; 409: 860-921.
21. Assoian RK, Komoriya A, Meyers CA et al. Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization. *J Biol Chem* 1983; 258: 7155-7160.
22. Kulkarni AB, Ward JM, Yaswen L et al. Transforming growth factor-beta 1 null mice. An animal model for inflammatory disorders. *Am J Pathol* 1995; 146: 264-275.
23. Sanford LP, Ormsby I, Gittenberger-de Groot AC et al. TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. *Development* 1997; 124: 2659-2670.
24. Kaartinen V, Voncken JW, Shuler C et al. Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. *Nat Genet* 1995; 11: 415-421.
25. Fynan TM, Reiss M. Resistance to inhibition of cell growth by transforming growth factor-beta and its role in oncogenesis. *Crit Rev Oncog* 1993; 4: 493-540.
26. Vale W, Rivier J, Vaughan J et al. Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. *Nature* 1986; 321: 776-779.
27. Bloise E, Ciarmela P, Dela Cruz C et al. Activin A in Mammalian Physiology. *Physiol Rev* 2019; 99: 739-780.

28. Kariyawasam HH, Semitekolou M, Robinson DS, Xanthou G. Activin-A: a novel critical regulator of allergic asthma. *Clin. Exp. Allergy* 2011.
29. Hogan BL. Bone morphogenetic proteins in development. *Curr. Opin. Genet. Dev* 1996; 6: 432-438.
30. Wang EA, Rosen V, Cordes P et al. Purification and characterization of other distinct bone-inducing factors. *Proc Natl Acad Sci U S A* 1988; 85: 9484-9488.
31. Wu DH, Hatzopoulos AK. Bone morphogenetic protein signaling in inflammation. *Exp Biol Med (Maywood)* 2019; 244: 147-156.
32. Kocwin M, Jonakowski M, Przemecka M et al. Selected bone morphogenetic proteins - the possibility of their use in the diagnostics and therapy of severe asthma. *Adv Respir Med* 2017; 85: 109-115.
33. Van Obberghen-Schilling E, Roche NS, Flanders KC et al. Transforming growth factor beta 1 positively regulates its own expression in normal and transformed cells. *J Biol Chem* 1988; 263: 7741-7746.
34. Jenkins G. The role of proteases in transforming growth factor-beta activation. *Int. J. Biochem. Cell Biol* 2008; 40: 1068-1078.
35. Schultz-Cherry S, Chen H, Mosher DF et al. Regulation of transforming growth factor-beta activation by discrete sequences of thrombospondin 1. *J Biol Chem* 1995; 270: 7304-7310.
36. Araya J, Cambier S, Morris A et al. Integrin-mediated transforming growth factor-beta activation regulates homeostasis of the pulmonary epithelial-mesenchymal trophic unit. *Am J Pathol* 2006; 169: 405-415.
37. Munger JS, Huang X, Kawakatsu H et al. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 1999; 96: 319-328.
38. Antenos M, Zhu J, Jetly NM, Woodruff TK. An activin/furin regulatory loop modulates the processing and secretion of inhibin alpha- and betaB-subunit dimers in pituitary gonadotrope cells. *J. Biol. Chem* 2008; 283: 33059-33068.
39. Altmann CR, Chang C, Munoz-Sanjuan I et al. The latent-TGFbeta-binding-protein-1 (LTBP-1) is expressed in the organizer and regulates nodal and activin signaling. *Dev Biol* 2002; 248: 118-127.
40. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 2003; 113: 685-700.
41. Olsen OE, Wader KF, Hella H et al. Activin A inhibits BMP-signaling by binding ACVR2A and ACVR2B. *Cell Commun Signal* 2015; 13: 27.
42. Aoki H, Fujii M, Imamura T et al. Synergistic effects of different bone morphogenetic protein type I receptors on alkaline phosphatase induction. *J Cell Sci* 2001; 114: 1483-1489.
43. Roelen BA, van Rooijen MA, Mummery CL. Expression of ALK-1, a type 1 serine/threonine kinase receptor, coincides with sites of vasculogenesis and angiogenesis in early mouse development. *Dev Dyn* 1997; 209: 418-430.
44. Matsuzaki K. Smad phospho-isoforms direct context-dependent TGF-beta signaling. *Cytokine Growth Factor Rev* 2013; 24: 385-399.
45. Zhang YE. Non-Smad pathways in TGF-beta signaling. *Cell Res* 2009; 19: 128-139.
46. Nakao A, Afrakhte M, Moren A et al. Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signalling. *Nature* 1997; 389: 631-635.
47. Afrakhte M, Moren A, Jossan S et al. Induction of inhibitory Smad6 and Smad7 mRNA by TGF-beta family members. *Biochem Biophys Res Commun* 1998; 249: 505-511.

48. Hashimoto O, Nakamura T, Shoji H et al. A novel role of follistatin, an activin-binding protein, in the inhibition of activin action in rat pituitary cells. Endocytotic degradation of activin and its acceleration by follistatin associated with cell-surface heparan sulfate. *J. Biol. Chem* 1997; 272: 13835-13842.
49. Suzuki HI. MicroRNA Control of TGF-beta Signaling. *Int J Mol Sci* 2018; 19.
50. Bosse Y, Rola-Pleszczynski M. Controversy surrounding the increased expression of TGF beta 1 in asthma. *Respir. Res* 2007; 8: 66.
51. Kariyawasam HH, Barkans J, Aizen M et al. Airway Submucosal Cells Expressing TGF- β 1 Are Significantly Increased at a Time Point When Airway Remodelling Is Maximal Post-Allergen. *Am. J. Respir. Crit Care Med* 179;2009:A1397
52. Semitekolou M, Alissafi T, Aggelakopoulou M et al. Activin-A induces regulatory T cells that suppress T helper cell immune responses and protect from allergic airway disease. *J. Exp. Med* 2009; 206: 1769-1785.
- **Key study to demonstrate a critical regulatory role for activin-A in the airway during Th2 inflammation.**
53. Samitas K, Poulos N, Semitekolou M et al. Activin-A is overexpressed in severe asthma and is implicated in angiogenic processes. *Eur. Respir. J* 2016; 47: 769-782.
54. Hardy CL, Lemasurier JS, Olsson F et al. Interleukin-13 regulates secretion of the tumor growth factor- β superfamily cytokine activin A in allergic airway inflammation. *Am. J. Respir. Cell Mol. Biol* 2010; 42: 667-675.
55. Kariyawasam HH, Pegorier S, Barkans J et al. Activin and transforming growth factor-beta signaling pathways are activated after allergen challenge in mild asthma. *J. Allergy Clin. Immunol* 2009; 124: 454-462.
- **Demonstrates the rapid modulation of the key type 1 receptors in allergic asthma after allergen provocation of disease.**
56. Karagiannidis C, Hense G, Martin C et al. Activin A is an acute allergen-responsive cytokine and provides a link to TGF-beta-mediated airway remodeling in asthma. *J. Allergy Clin. Immunol* 2006; 117: 111-118.
57. Kariyawasam HH, Xanthou G, Barkans J et al. Basal expression of bone morphogenetic protein receptor is reduced in mild asthma. *Am. J. Respir. Crit Care Med* 2008; 177: 1074-1081.
- **The first study to demonstrate altered BMP receptor expression in asthma and rapid modulation of signaling after allergen**
58. Phipps S, Benyahia F, Ou TT et al. Acute allergen-induced airway remodeling in atopic asthma. *Am J Respir Cell Mol Biol* 2004; 31: 626-632.
59. Nakao A, Sagara H, Setoguchi Y et al. Expression of Smad7 in bronchial epithelial cells is inversely correlated to basement membrane thickness and airway hyperresponsiveness in patients with asthma. *J. Allergy Clin. Immunol* 2002; 110: 873-878.
60. Phipps S, Ying S, Wangoo A et al. The relationship between allergen-induced tissue eosinophilia and markers of repair and remodeling in human atopic skin. *J. Immunol* 2002; 169: 4604-4612.
61. Flood-Page P, Menzies-Gow A, Phipps S et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J. Clin. Invest* 2003; 112: 1029-1036.
62. Kariyawasam HH, Robinson DS. The role of eosinophils in airway tissue remodelling in asthma. *Curr. Opin. Immunol* 2007; 19: 681-686.

63. Ohno I, Nitta Y, Yamauchi K et al. Transforming growth factor beta 1 (TGF beta 1) gene expression by eosinophils in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1996; 15: 404-409.
64. Cho JY, Miller M, Baek KJ et al. Inhibition of airway remodeling in IL-5-deficient mice. *J. Clin. Invest* 2004; 113: 551-560.
65. Humbles AA, Lloyd CM, McMillan SJ et al. A critical role for eosinophils in allergic airways remodeling. *Science* 2004; 305: 1776-1779.
66. Sugimoto MA, Vago JP, Perretti M, Teixeira MM. Mediators of the Resolution of the Inflammatory Response. *Trends Immunol* 2019; 40: 212-227.
67. Letterio JJ, Geiser AG, Kulkarni AB et al. Autoimmunity associated with TGF-beta1-deficiency in mice is dependent on MHC class II antigen expression. *J Clin Invest* 1996; 98: 2109-2119.
68. McCartney-Francis NL, Mizel DE, Redman RS et al. Autoimmune Sjogren's-like lesions in salivary glands of TGF-beta1-deficient mice are inhibited by adhesion-blocking peptides. *J Immunol* 1996; 157: 1306-1312.
69. Wahl SM, Hunt DA, Wakefield LM et al. Transforming growth factor type beta induces monocyte chemotaxis and growth factor production. *Proc. Natl. Acad. Sci. U. S. A* 1987; 84: 5788-5792.
70. Adams DH, Hathaway M, Shaw J et al. Transforming growth factor-beta induces human T lymphocyte migration in vitro. *J Immunol* 1991; 147: 609-612.
71. Gruber BL, Marchese MJ, Kew RR. Transforming growth factor-beta 1 mediates mast cell chemotaxis. *J Immunol* 1994; 152: 5860-5867.
72. Postlethwaite AE, Seyer JM. Identification of a chemotactic epitope in human transforming growth factor-beta 1 spanning amino acid residues 368-374. *J Cell Physiol* 1995; 164: 587-592.
73. Allen JB, Manthey CL, Hand AR et al. Rapid onset synovial inflammation and hyperplasia induced by transforming growth factor beta. *J Exp Med* 1990; 171: 231-247.
74. Luttmann W, Franz P, Matthys H, Virchow JC, Jr. Effects of TGF-beta on eosinophil chemotaxis. *Scand J Immunol* 1998; 47: 127-130.
75. Wahl SM, Allen JB, Weeks BS et al. Transforming growth factor beta enhances integrin expression and type IV collagenase secretion in human monocytes. *Proc Natl Acad Sci U S A* 1993; 90: 4577-4581.
76. McCartney-Francis NL, Wahl SM. Transforming growth factor beta: a matter of life and death. *J Leukoc Biol* 1994; 55: 401-409.
77. Cerwenka A, Bevec D, Majdic O et al. TGF-beta 1 is a potent inducer of human effector T cells. *J Immunol* 1994; 153: 4367-4377.
78. Cerwenka A, Kovar H, Majdic O, Holter W. Fas- and activation-induced apoptosis are reduced in human T cells preactivated in the presence of TGF-beta 1. *J Immunol* 1996; 156: 459-464.
79. Strobl H, Knapp W. TGF-beta1 regulation of dendritic cells. *Microbes Infect* 1999; 1: 1283-1290.
80. Foucras G, Coudert JD, Coureau C, Guery JC. Dendritic cells prime in vivo alloreactive CD4 T lymphocytes toward type 2 cytokine- and TGF-beta-producing cells in the absence of CD8 T cell activation. *J Immunol* 2000; 165: 4994-5003.
81. Van Weyenbergh J, MP PS, Bafica A et al. IFN-beta and TGF-beta differentially regulate IL-12 activity in human peripheral blood mononuclear cells. *Immunol Lett* 2001; 75: 117-122.

82. Balazovich KJ, Fernandez R, Hinkovska-Galcheva V et al. Transforming growth factor-beta1 stimulates degranulation and oxidant release by adherent human neutrophils. *J Leukoc Biol* 1996; 60: 772-777.
83. Wahl SM, Allen JB, Welch GR, Wong HL. Transforming growth factor-beta in synovial fluids modulates Fc gamma RII (CD16) expression on mononuclear phagocytes. *J Immunol* 1992; 148: 485-490.
84. Rose DM, Fadok VA, Riches DW et al. Autocrine/paracrine involvement of platelet-activating factor and transforming growth factor-beta in the induction of phosphatidylserine recognition by murine macrophages. *J Immunol* 1995; 155: 5819-5825.
85. Welch GR, Wong HL, Wahl SM. Selective induction of Fc gamma RIII on human monocytes by transforming growth factor-beta. *J Immunol* 1990; 144: 3444-3448.
86. Fadok VA, Bratton DL, Konowal A et al. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest* 1998; 101: 890-898.
87. Woolley KL, Gibson PG, Carty K et al. Eosinophil apoptosis and the resolution of airway inflammation in asthma. *Am J Respir Crit Care Med* 1996; 154: 237-243.
88. Alam R, Forsythe P, Stafford S, Fukuda Y. Transforming growth factor beta abrogates the effects of hematopoietins on eosinophils and induces their apoptosis. *J Exp Med* 1994; 179: 1041-1045.
89. Yamaguchi Y, Suda T, Ohta S et al. Analysis of the survival of mature human eosinophils: interleukin-5 prevents apoptosis in mature human eosinophils. *Blood* 1991; 78: 2542-2547.
90. Oswald IP, Gazzinelli RT, Sher A, James SL. IL-10 synergizes with IL-4 and transforming growth factor-beta to inhibit macrophage cytotoxic activity. *J Immunol* 1992; 148: 3578-3582.
91. Tsunawaki S, Sporn M, Ding A, Nathan C. Deactivation of macrophages by transforming growth factor-beta. *Nature* 1988; 334: 260-262.
92. Nelson BJ, Ralph P, Green SJ, Nacy CA. Differential susceptibility of activated macrophage cytotoxic effector reactions to the suppressive effects of transforming growth factor-beta 1. *J Immunol* 1991; 146: 1849-1857.
93. Sillett HK, Cruickshank SM, Southgate J, Trejdosiewicz LK. Transforming growth factor-beta promotes 'death by neglect' in post-activated human T cells. *Immunology* 2001; 102: 310-316.
94. Kariyawasam HH, Robinson DS. Airway remodelling in asthma: models and supermodels? *Clin. Exp. Allergy* 2005; 35: 117-121.
95. Zhang S, Smartt H, Holgate ST, Roche WR. Growth factors secreted by bronchial epithelial cells control myofibroblast proliferation: an in vitro co-culture model of airway remodeling in asthma. *Lab Invest* 1999; 79: 395-405.
96. Fjellbirkeland L, Cambier S, Broaddus VC et al. Integrin alphavbeta8-mediated activation of transforming growth factor-beta inhibits human airway epithelial proliferation in intact bronchial tissue. *Am J Pathol* 2003; 163: 533-542.
97. Reddy KB, Hocevar BA, Howe PH. Inhibition of G1 phase cyclin dependent kinases by transforming growth factor beta 1. *J Cell Biochem* 1994; 56: 418-425.
98. Puddicombe SM, Torres-Lozano C, Richter A et al. Increased expression of p21(waf) cyclin-dependent kinase inhibitor in asthmatic bronchial epithelium. *Am. J. Respir. Cell Mol. Biol* 2003; 28: 61-68.

99. Vignola AM, Chanez P, Chiappara G et al. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1997; 156: 591-599.
100. McAnulty RJ, Hernandez-Rodriguez NA, Mutsaers SE et al. Indomethacin suppresses the anti-proliferative effects of transforming growth factor-beta isoforms on fibroblast cell cultures. *Biochem J* 1997; 321 (Pt 3): 639-643.
101. Thannickal VJ, Lee DY, White ES et al. Myofibroblast differentiation by transforming growth factor-beta1 is dependent on cell adhesion and integrin signaling via focal adhesion kinase. *J Biol Chem* 2003; 278: 12384-12389.
102. Coker RK, Laurent GJ, Shahzeidi S et al. Transforming growth factors-beta 1, -beta 2, and -beta 3 stimulate fibroblast procollagen production in vitro but are differentially expressed during bleomycin-induced lung fibrosis. *Am J Pathol* 1997; 150: 981-991.
103. Ignatz RA, Endo T, Massague J. Regulation of fibronectin and type I collagen mRNA levels by transforming growth factor-beta. *J Biol Chem* 1987; 262: 6443-6446.
104. McAnulty RJ, Chambers RC, Laurent GJ. Regulation of fibroblast procollagen production. Transforming growth factor-beta 1 induces prostaglandin E2 but not procollagen synthesis via a pertussis toxin-sensitive G-protein. *Biochem J* 1995; 307 (Pt 1): 63-68.
105. Romaris M, Heredia A, Molist A, Bassols A. Differential effect of transforming growth factor beta on proteoglycan synthesis in human embryonic lung fibroblasts. *Biochim Biophys Acta* 1991; 1093: 229-233.
106. Westergren-Thorsson G, Antonsson P, Malmstrom A et al. The synthesis of a family of structurally related proteoglycans in fibroblasts is differently regulated by TGF-beta. *Matrix* 1991; 11: 177-183.
107. Zeng G, McCue HM, Mastrangelo L, Millis AJ. Endogenous TGF-beta activity is modified during cellular aging: effects on metalloproteinase and TIMP-1 expression. *Exp Cell Res* 1996; 228: 271-276.
108. Lund LR, Riccio A, Andreasen PA et al. Transforming growth factor-beta is a strong and fast acting positive regulator of the level of type-1 plasminogen activator inhibitor mRNA in WI-38 human lung fibroblasts. *Embo j* 1987; 6: 1281-1286.
109. Cohen MD, Ciocca V, Panettieri RA, Jr. TGF-beta 1 modulates human airway smooth-muscle cell proliferation induced by mitogens. *Am J Respir Cell Mol Biol* 1997; 16: 85-90.
110. Coutts A, Chen G, Stephens N et al. Release of biologically active TGF-beta from airway smooth muscle cells induces autocrine synthesis of collagen. *Am J Physiol Lung Cell Mol Physiol* 2001; 280: L999-1008.
111. Funaba M, Ikeda T, Ogawa K et al. Role of activin A in murine mast cells: modulation of cell growth, differentiation, and migration. *J. Leukoc. Biol* 2003; 73: 793-801.
112. Ogawa K, Funaba M, Tsujimoto M. A dual role of activin A in regulating immunoglobulin production of B cells. *J. Leukoc. Biol* 2008; 83: 1451-1458.
113. Robson NC, Phillips DJ, McAlpine T et al. Activin-A: a novel dendritic cell-derived cytokine that potently attenuates CD40 ligand-specific cytokine and chemokine production. *Blood* 2008; 111: 2733-2743.
114. Brosh N, Sternberg D, Honigswachs-Sha'anani J et al. The plasmacytoma growth inhibitor restrictin-P is an antagonist of interleukin 6 and interleukin 11. Identification as a stroma-derived activin A. *J Biol Chem* 1995; 270: 29594-29600.

115. Huber S, Stahl FR, Schrader J et al. Activin A promotes the TGF-beta-induced conversion of CD4+. *J. Immunol* 2009; 182: 4633-4640.
116. Semitekolou M, Morianos I, Banos A et al. Dendritic cells conditioned by activin A-induced regulatory T cells exhibit enhanced tolerogenic properties and protect against experimental asthma. *J Allergy Clin Immunol* 2018; 141: 671-684.e677.
117. Ohga E, Matsuse T, Teramoto S et al. Effects of activin A on proliferation and differentiation of human lung fibroblasts. *Biochem Biophys Res Commun* 1996; 228: 391-396.
118. Cho SH, Yao Z, Wang SW et al. Regulation of activin A expression in mast cells and asthma: its effect on the proliferation of human airway smooth muscle cells. *J Immunol* 2003; 170: 4045-4052.
119. Matsuse T, Fukuchi Y, Eto Y et al. Expression of immunoreactive and bioactive activin A protein in adult murine lung after bleomycin treatment. *Am J Respir Cell Mol Biol* 1995; 13: 17-24.
120. Gregory LG, Mathie SA, Walker SA et al. Overexpression of Smad2 drives house dust mite-mediated airway remodeling and airway hyperresponsiveness via activin and IL-25. *Am. J. Respir. Crit Care Med* 2010; 182: 143-154.
121. Zeisberg M, Hanai J, Sugimoto H et al. BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat Med* 2003; 9: 964-968.
122. Zeisberg M, Bottiglio C, Kumar N et al. Bone morphogenic protein-7 inhibits progression of chronic renal fibrosis associated with two genetic mouse models. *Am J Physiol Renal Physiol* 2003; 285: F1060-1067.
123. Izumi N, Mizuguchi S, Inagaki Y et al. BMP-7 opposes TGF-beta1-mediated collagen induction in mouse pulmonary myofibroblasts through Id2. *Am J Physiol Lung Cell Mol Physiol* 2006; 290: L120-126.
124. Gould SE, Day M, Jones SS, Dorai H. BMP-7 regulates chemokine, cytokine, and hemodynamic gene expression in proximal tubule cells. *Kidney Int* 2002; 61: 51-60.
125. Maric I, Poljak L, Zoricic S et al. Bone morphogenetic protein-7 reduces the severity of colon tissue damage and accelerates the healing of inflammatory bowel disease in rats. *J. Cell Physiol* 2003; 196: 258-264.
126. Stumm CL, Halcsik E, Landgraf RG et al. Lung remodeling in a mouse model of asthma involves a balance between TGF-beta1 and BMP-7. *PLoS One* 2014; 9: e95959.
127. Huse K, Bakkebo M, Oksvold MP et al. Bone morphogenetic proteins inhibit CD40L/IL-21-induced Ig production in human B cells: differential effects of BMP-6 and BMP-7. *Eur. J. Immunol* 2011.
128. Pegorier S, Campbell GA, Kay AB, Lloyd CM. Bone morphogenetic protein (BMP)-4 and BMP-7 regulate differentially transforming growth factor (TGF)-beta1 in normal human lung fibroblasts (NHLF). *Respir. Res* 2010; 11: 85.
129. Murray LA, Hackett TL, Warner SM et al. BMP-7 does not protect against bleomycin-induced lung or skin fibrosis. *PLoS One* 2008; 3: e4039.
130. Balzar S, Chu HW, Silkoff P et al. Increased TGF-beta2 in severe asthma with eosinophilia. *J Allergy Clin Immunol* 2005; 115: 110-117.
131. Khalil N, Parekh TV, O'Connor RN, Gold LI. Differential expression of transforming growth factor-beta type I and II receptors by pulmonary cells in bleomycin-induced lung injury: correlation with repair and fibrosis. *Exp Lung Res* 2002; 28: 233-250.

132. Brandes ME, Wakefield LM, Wahl SM. Modulation of monocyte type I transforming growth factor-beta receptors by inflammatory stimuli. *J Biol Chem* 1991; 266: 19697-19703.
133. Goumans MJ, Lebrin F, Valdimarsdottir G. Controlling the angiogenic switch: a balance between two distinct TGF- β receptor signaling pathways. *Trends Cardiovasc Med* 2003; 13: 301-307.
134. Scharpfenecker M, van Dinther M, Liu Z et al. BMP-9 signals via ALK1 and inhibits bFGF-induced endothelial cell proliferation and VEGF-stimulated angiogenesis. *J Cell Sci* 2007; 120: 964-972.
135. Aoki F, Kurabayashi M, Hasegawa Y, Kojima I. Attenuation of bleomycin-induced pulmonary fibrosis by follistatin. *Am J Respir Crit Care Med* 2005; 172: 713-720.
136. Gregory LG, Jones CP, Walker SA et al. IL-25 drives remodelling in allergic airways disease induced by house dust mite. *Thorax* 2013; 68: 82-90.
- **Elegantly demonstrates how innate immunity is a potent inducer of remodelling and how activin-A is modulated.**
137. Lynn TM, Molloy EL, Masterson JC et al. SMAD Signaling in the Airways of Healthy Rhesus Macaques versus Rhesus Macaques with Asthma Highlights a Relationship Between Inflammation and Bone Morphogenetic Proteins. *Am J Respir Cell Mol Biol* 2016; 54: 562-573.
138. Fokkens WJ, Lund VJ, Mullol J et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. *Rhinol. Suppl* 2012; 3-298.
139. Kariyawasam HH, Rotiroti G. Allergic rhinitis, chronic rhinosinusitis and asthma: unravelling a complex relationship. *Curr. Opin. Otolaryngol. Head Neck Surg* 2013; 21: 79-86.
140. Van BN, Derycke L, Perez-Novoa CA et al. TGF-beta signaling and collagen deposition in chronic rhinosinusitis. *J. Allergy Clin. Immunol* 2009; 124: 253-259, 259.
141. Yamin M, Holbrook EH, Gray ST et al. Profibrotic transforming growth factor beta 1 and activin A are increased in nasal polyp tissue and induced in nasal polyp epithelium by cigarette smoke and Toll-like receptor 3 ligation. *Int Forum Allergy Rhinol* 2015; 5: 573-582.
142. Yang Y, Zhang N, Crombruggen KV et al. Differential Expression and Release of Activin A and Follistatin in Chronic Rhinosinusitis with and without Nasal Polyps. *PLoS One* 2015; 10: e0128564.
143. Chaker AM, Zissler UM, Poulos N et al. Activin-A Is a Pro-Inflammatory Regulator in Type-2-Driven Upper Airway Disease. *Int Arch Allergy Immunol* 2018; 176: 15-25.
144. Tengroth L, Arebro J, Larsson O et al. Activation of Activin receptor-like kinases curbs mucosal inflammation and proliferation in chronic rhinosinusitis with nasal polyps. *Sci Rep* 2018; 8: 1561.
- **Early data to support multiple TGF- β signalling pathways are active in several CRSwNP tissue compartments.**
145. Wu J, Ding Y, Wang J, Wang F. Staphylococcus aureus induces TGF-beta1 and bFGF expression through the activation of AP-1 and NF-kappaB transcription factors in bovine mammary epithelial cells. *Microb Pathog* 2018; 117: 276-284.
146. Jones KL, Mansell A, Patella S et al. Activin A is a critical component of the inflammatory response, and its binding protein, follistatin, reduces mortality in endotoxemia. *Proc Natl Acad Sci U S A* 2007; 104: 16239-16244.

147. Nicholson GC, Kariyawasam HH, Tan AJ et al. The effects of an anti-IL-13 mAb on cytokine levels and nasal symptoms following nasal allergen challenge. *J. Allergy Clin. Immunol* 2011; 128: 800-807.
148. Kariyawasam HH, Aizen M, Kay AB, Robinson DS. Safety and tolerability of three consecutive bronchoscopies after allergen challenge in volunteers with mild asthma. *Thorax* 2007; 62: 557-558.

Accepted Manuscript

Figure Legends

Figure 1.1 and 1.2: Summary of the TGF- β superfamily main ligand-receptor-SMAD signalling components and pathways

TGF- β superfamily of ligands signal by binding to a constitutively active serine-threonine kinase specific type II receptor that complexes with a type I receptor which subsequently propagates the signal downstream by phosphorylating receptor-regulated (R) SMADs that translocate to the nucleus to initiate gene transcription. TGF- β_{1-3} and activin signalling is via phosphorylated (p) SMAD2 and pSMAD3 whilst pSMAD1, pSMAD5 and pSMAD8 mediate BMP signals. TGF- β_1 via the type I receptor ALK-1 can lead to activation of BMP signalling pathways via pSMAD1 and pSMAD5. R-SMADs associate with the common (Co) SMAD4. SMAD7 expression is rapidly induced in cells in response to TGF- β_{1-3} , activin and BMP ligand signalling. SMAD7 inhibits further signalling by interacting with the type I receptor. Combinatorial interactions in the receptor complex allow differential ligand binding or differential signalling in response to the same ligand and this is cell specific. TGF- β ligands are regulated by synthesis but are also bound to ECM proteins as inactive forms which must be activated for example by thrombospondin or $\alpha_v\beta_6$ integrin. Thus, signalling analysis is required to detect activity of the factors.

Figure 2: Photomicrographs of selected pathway ligand and signalling expression

in paired volunteer bronchial biopsies pre and post-allergen provocation of asthma

TGF- β_1 expression was increased from baseline (2.1A) at the 7-day time point (2.1B).

There was also increased BMP-7 expression from baseline (2.1C) at 7 days (2.1D).

Figure 2.2A shows ALK-5 (type I receptor for TGF- β_1) expression in the normal airway to be high whilst expression the asthmatic baseline airway is less (2.2B). There is further decrease in ALK-5 expression 24 hours post allergen challenge of the asthmatic airway (2.2C) when inflammation is maximal, and remodelling is activated.

ALK-5 levels return to baseline levels (2.2D) with inflammatory resolution when remodelling is now maximal. High inhibitory SMAD7 expression is seen in the normal airway (2.2E) compared to asthma (2.2F), but allergen challenge increases SMAD7 expression (2.2G) when other TGF- β superfamily ligands are actively signalling, before returning to baseline levels of expression at Day 7 after allergen challenge (2.2H). The increased expression of TGF- β_1 , particularly in the submucosal cell population (2.3A arrowed) during the remodelling phase of disease is shown.

2.3A demonstrates submucosal cells stained for TGF- β_1 (green) co-localising to red stained MBP⁺ eosinophils and 2.3B (arrowed) shows an eosinophil releasing TGF- β_1 during the process of degranulation. ALK-1 expression was particularly increased from the baseline asthma at the 7 day time point and many cells displayed the morphology of fibroblasts (2.3C and D). All time-course slides are from the same individual asthmatic obtained in a time course manner.

Figure 3: Summary of TGF- β superfamily ligand and signalling expression in the

allergic asthmatic airway model before and after allergen challenge.

Bronchial biopsy-based expression studies of the relevant TGF- β superfamily ligands and the respective signalling component in the baseline asthmatic airway and modulation of expression 24 hours and 7 days after allergen challenge are summarised. Rapid activation of TGF- β , activin and BMP signalling was evident by the modulation of the type I receptors ALK-5, ALK-1, ALK-4, ALK-2 and ALK-6 along with pSMAD2/3 and pSMAD1/5. Activation is alongside inflammation and remodelling in asthma. At the 7-day time point, when inflammatory cell numbers had returned to baseline airway levels, remodelling was still very active. Here the main type I receptors for TGF- β 1 and activin-A (ALK-1, ALK-5 and ALK-4 respectively) had returned to baseline expression but ALK-2/ALK-6 expression (presumed mainly BMP signalling) were maximal and matched the normal airway expression levels. BMP-7 (considered predominantly to be an anti-inflammatory and pro-tissue repair protein) expression was high. Thus TGF- β superfamily ligand activation and signalling is rapid and follows a time course of expression in relation to inflammation and remodelling in asthma in an integrated and dynamic manner.

Figure 4: Proposed model of TGF- β superfamily signalling and dysregulation in allergic asthma

List of commonly used abbreviations

AHR- airway hyperresponsiveness
ALK-activin-like kinase
 α -SMA-alpha smooth muscle actin
ASM-airway smooth muscle
BMP-bone morphogenetic protein
CRS-chronic rhinosinusitis
DC-dendritic cell
ECM-extracellular matrix
EDN- eosinophil-derived neurotoxin
EMTU-epithelial mesenchymal trophic unit
ERK- extracellular signal-regulated kinase
GM-CSF-granulocyte macrophage colony stimulating factor
HDM-house dust mite
IgE –immunoglobulin E
IL-interleukin
ILCs-innate lymphoid cells
KO-knock-out
LAP-latency associated peptide
LPS-lipopolysaccharide
LTBP-latent TGF- β binding protein
MAPK-mitogen activated protein kinase
MBP-major basic protein
MC-mast cell
MMP-matrix metalloproteinases
NHBE-normal human bronchial epithelial
PCNA-proliferating cell nuclear antigen
PGs-proteoglycans
RANTES- regulated on activation, normal T cell expressed and secreted (CCL5)
RTK-receptor tyrosine kinase
TGF-transforming growth factor
TIMP-tissue inhibitor of matrix metalloproteinases
TNF-tumour necrosis factor
TSLP- Thymic stromal lymphopoietin
TSP-1-thrombospondin
VEGF-Vascular endothelial growth factor

Harsha H Kariyawasam PhD FRCP ^{1,2,3} and Simon B Gane MPhil FRCS ^{2,3}

¹Department of Adult Specialist Allergy and Clinical Immunology

²Department of Rhinology

Royal National ENT Hospital, London

University College London Hospitals NHS Foundation Trust, London

³University College London, London

Correspondence author and address

Harsha H Kariyawasam PhD FRCP

Royal National ENT Hospital and UCLH NHS Foundation Trust

330 Gray's Inn Road

London, WC1X 8DA

Email

harsha.kariyawasam@nhs.net

Telephone +44 20 3456 5242

Fax +44 203 456 5183

Figure 1.1

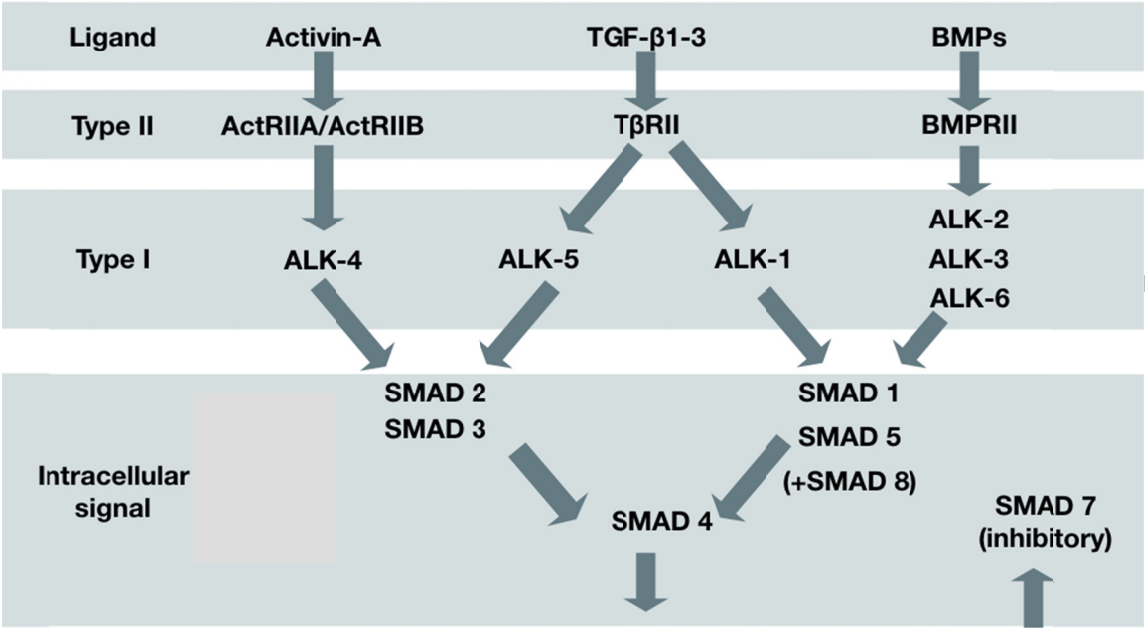


Figure 1.2

