Regulation of mucin expression in respiratory diseases

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Abstract

Respiratory diseases such as asthma and COPD (chronic obstructive pulmonary disease) are characterized by increased numbers of goblet cells and excessive mucus production, which contribute to the underlying disease pathology. Mucins form a major component of the mucus contributing to its viscoelastic properties, and in the airways the mucins MUC5AC and MUC5B are found at increased levels in both asthmatic and COPD subjects. A diverse range of stimuli have been shown to regulate MUC5AC expression and cause increases in the number of mucus-producing goblet cells. Perhaps the best characterized of these mediators is the cytokine IL (interleukin)-13, which causes increases in MUC5AC-expressing goblet cells in the airways. Several transcription factors have been linked with goblet cell formation and mucus production and include STAT6 (signal transducer and activator of transcription 6), FOXA2 (forkhead box A2) and the SPDEF [SAM (sterile α motif) domain-containing prostate-derived Ets factor]. In mouse airways, goblet cells are normally rare or absent, but increase rapidly in number in response to certain stimuli. The origins of these goblet cells are not well understood, although Clara cells and ciliated cells have been implicated as goblet cell progenitors. An understanding of the origin and processes regulating goblet cell formation in human airway epithelial cells has important implications for the identification of therapeutic targets to treat respiratory diseases.

Mucus hypersecretion in respiratory diseases

Mucus overproduction has been linked to several of the pathological features of respiratory diseases such as asthma [1] and COPD (chronic obstructive pulmonary disease) [2]. Excessive mucus in the airways has been linked to an increase in the frequency and duration of infection, decline in lung function and increase in morbidity and mortality in respiratory diseases [2]. In the large airways, mucus is produced by goblet cells and submucosal glands, whereas in the small airways the only source of mucus is the goblet cell [3]. In healthy individuals there are a few mucus-producing cells distal to the trachea [4], whereas in asthma and COPD elevated numbers of goblet cells are coupled with excessive mucus production. The increase in the numbers of goblet cells is often termed goblet cell hyperplasia or metaplasia. Goblet cells can rapidly secrete mucus in response to certain stimuli by exocytosis to form a mucus layer that lines the airways (reviewed in [5]). This mucus layer normally plays a beneficial role that protects against inhaled pathogens, toxins and other foreign particles by a process termed mucociliary clearance. However, abnormal mucus production and clearance can contribute to respiratory disease pathologies [4,6]. In asthma and COPD, the major mucin components of airway mucus secretions are MUC5AC and MUC5B, which contribute to the viscoelastic properties of the mucus [3]. There have been several studies linking increases in mucin gene expression and mucus production with respiratory diseases. In asthmatic patients, mucus plugging of the airway lumen has been reported as a major contributing factor of fatal asthma [7]. Even in mild and moderate asthma, increases in airway goblet cell number and stored and secreted MUC5AC protein have been reported [8].

Increases in goblet cells and mucus production have also been reported in COPD subjects. Indeed, the progression of the disease has been reported to be strongly associated with accumulation of mucus in the lumen of the small airways and has been more recently associated with early death [9,10]. In COPD subjects, increased numbers of mucus-producing goblet cells have been described [11]. Studies analysing the mucin components of airway mucus in COPD patients have reported MUC5AC and MUC5B as the major mucins in sputum, with MUC5B being the predominant form [12,13]. As the contribution of excessive airway mucus to respiratory disease pathology is becoming clearer, factors regulating mucus production are also emerging.

Factors regulating mucin expression

COPD and asthma are inflammatory conditions of the airways associated with increases in inflammatory cells, and inflammatory mediators such as cytokines and chemokines. Indeed, many of the mediators that are associated with either asthma or COPD have been investigated *in vitro* and/or *in vivo* for their effects on goblet cell formation and mucin

Key words: epidermal growth factor (EGF), forkhead box A2 (FOXA2), goblet cell, interleukin 13 (IL 13), MUC5AC, signal transducer and activator of transcription 6 (STAT6).

Abbreviations used: COPD, chronic obstructive pulmonary disease; EGF, epidermal growth factor; EGFR, EGF receptor; FOXA2, forkhead box A2; HBEC, human bronchial epithelial cell; IL, interleukin; LPS, lipopolysaccharide; SPDEF, SAM (sterile α-motif) domain-containing prostate-derived Ets factor; STAT6, signal transducer and activator of transcription 6; TGF, transforming growth factor.

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gene expression. Studies have implicated multiple stimuli in the regulation of mucin gene expression and mediators regulating MUC5AC expression have been reviewed in detail [6]. These factors include the cytokines TNFα (tumour necrosis factor α), IL (interleukin)-1β, IL-9, IL-13, IL-17 [14–18], cigarette smoke [19], the growth factors EGF (epidermal growth factor), TGFα (transforming growth factor α), TGFβ2 [20,21] and proteases such as neutrophil elastase [22]. Additionally the bacterial pathogen *Pseudomonas aeruginosa* has been associated with increases in MUC5AC mRNA and protein expression in airway epithelial cell cultures [23]. The available data suggest that the EGFR (EGF receptor) pathway is involved in the regulation of mucin production by several stimuli, including the bacterial product LPS (lipopolysaccharide), cigarette smoke and neutrophil elastase (reviewed in [24]). Although many studies have examined regulation of MUC5AC expression, less is known about MUC5B, the predominant mucin expressed in COPD. *In vitro* studies using HBEC (human bronchial epithelial cell) cultures have indicated that retinoic acid can induce expression of MUC5B [25]. Additionally, cytokines IL-17 and IL-6 have been linked with increases in MUC5B, together with MUC5AC, expression in human airway epithelial cell cultures [18].

Several of the stimuli shown to induce MUC5AC or MUC5B expression *in vitro* have also been linked to mucin expression and goblet cell formation *in vivo*. Transgenic mice overexpressing IL-1β have increased numbers of MUC5AC-expressing goblet cells in the airways [26], and mice overexpressing IL-9 exhibit increased expression of MUC5AC, although this appears to be via an IL-13-dependent pathway [27]. The cytokine IL-4 has also been associated with goblet cell formation in mice, with direct delivery of IL-4 to the airways, resulting in increases in mucus-expressing goblet cells [28]. Neutrophil elastase, a protease found at elevated levels in airway secretions of COPD subjects, also induces expression of MUC5AC in goblet cells in mice [29]. Additionally, in a rat model a combination of cigarette smoke, a major contributor to the pathogenesis of COPD, and LPS resulted in an increase in mucus-expressing goblet cells [30].

Several studies have concentrated on the cytokine IL-13, which is an important mediator found at elevated levels in asthmatic airways (reviewed in [31]). In mice sensitized with the allergen ovalbumin, subsequent allergen challenge results in a marked increase in the numbers of goblet cells and expression of MUC5AC [32,33]. In ovalbumin-sensitized mice treated with a solubilized version of an IL-13 receptor, or in mice deficient in IL-13, goblet cell formation in response to allergen challenge is either absent or strongly attenuated [34,35]. In addition to these *in vivo* studies, the role of IL-13 in human respiratory epithelium has also been studied *in vitro* using primary HBECs collected *post mortem*. When cultured at the air/liquid interface in the presence of retinoic acid, HBECs differentiate to form a stratified epithelium containing mucus-containing cells and ciliated cells [36]. In untreated cultures a few goblet cells are present, but treatment with IL-13 results in up to a 10-fold increase in the density of goblet cells together with increased expression of MUC5AC compared with untreated cultures [17]. These studies indicate a central role for IL-13 in mediating goblet cell formation in both an *in vivo* model of allergic asthma and an *in vitro* model of human respiratory epithelium.

Signal transduction pathways leading to goblet cell formation

Evidence from knockout mice studies has identified a pivotal role for the transcription factor STAT6 (signal transducer and activator of transcription 6) in mediating IL-13-induced goblet cell formation. Mice deficient in STAT6 are completely protected from ovalbumin-induced goblet cell formation [37]. Overexpression of IL-13 in transgenic mice is also associated with mucus overproduction and mice lacking STAT6 are resistant to the effects of transgenic IL-13 expression [38]. Furthermore, in the above mentioned study, Kuperman et al. [38] found that reconstitution of STAT6 expression specifically in lung epithelial cells restored mucus-containing cells in transgenic mice, indicating that IL-13 acts directly on the epithelium. The authors of the present review have found that shRNA (small-hairpin RNA)-mediated knockdown of STAT6 expression in air/liquid interface cultures of HBECs also abrogates IL-13-induced goblet cell formation (J. Turner, S. Petit, J. Giddings, J. Roger and C.E. Jones), unpublished work), which indicates the importance of STAT6 in a human model.

Events downstream of STAT6 that effect the differentiation of goblet cells are less clear. As discussed earlier, IL-13 has been demonstrated to induce MUC5AC expression in a number of experimental systems; however, a direct role for STAT6 in regulating MUC5AC expression is unlikely since the MUC5AC gene promoter contains no consensus STAT6 binding motifs [39], suggesting the presence of additional signaling processes. Several candidates that regulate this process have recently come to light. Gene-targeting studies have revealed a key role for the FOXA2 (forkhead box A2) in regulating goblet cell hyperplasia. Deletion of FOXA2 from mouse lung epithelial cells resulted in goblet cell hyperplasia and, in addition, FOXA2 expression in lung tissue from patients with pulmonary diseases was inversely proportional to goblet cell hyperplasia [40]. It appears, therefore, that lack of FOXA2 expression is associated with goblet cell formation. Decreased expression of FOXA2 is also associated with increased numbers of MUC5AC-positive goblet cells in transgenic mice overexpressing IL-1β [26]. Additionally, FOXA2 has been implicated as a common mediator of both IL-13 and EGFR signalling in airway epithelial cells, and its expression is down-regulated by activation of either IL-13 or EGFR pathways, inversely correlating with MUC5AC expression [41]. FOXA2 may therefore represent a common signalling node for multiple goblet cell differentiation signalling pathways.

More recently, the transcription factor SPDEF [SAM (sterile α-motif) domain-containing prostate-derived Ets factor] has been implicated in regulating airway goblet cell hyperplasia. Increased expression of SPDEF was found in the

respiratory epithelium of mice challenged with either IL-13 or allergen and was associated with goblet cell hyperplasia [42]. Additionally, in the above mentioned report, expression of SPDEF in the respiratory epithelium of transgenic mice resulted in goblet cell hyperplasia. SPDEF appears to function downstream of STAT6, as the authors reported that IL-13-induced increases in SPDEF expression were absent in STAT6-knockout mice. Consequently, an understanding of the processes leading to goblet cell formation is important to elucidate how the epithelium responds functionally to insults such as allergens and how, in the event that these processes become perturbed, they may contribute to the pathogenesis of respiratory disease.

The cellular origins of goblet cells

The conducting airways comprising trachea, bronchi and terminal bronchioles leading to the alveolar regions of gas exchange are lined with a stratified epithelium. In addition to goblet cells, a few of which are present in healthy individuals, other prominent cell types are ciliated cells, non-mucus secretory Clara cells and underlying basal cells. In the murine ovalbumin model of allergic asthma, remodelling of the airway epithelium occurs rapidly with the first appearance of goblet cells 6 h after allergen challenge, and numbers reach a peak 7 days post-challenge [43]. In this acute *in vivo* model, the authors report that the numbers of goblet cells steadily decline from their peak during resolution of the mucus phenotype. This plasticity in phenotype has also been observed in air/liquid interface cultures of airway epithelial cells where IL-13-induced goblet cell formation is reversed by elimination of IL-13 from the cultures [44].

In mice, there is no significant change in epithelial proliferation at the time when goblet cells first appear in response to allergen, indicating that cell division does not play a role in this process [43]. Several studies have attempted to address the question of where the goblet cells arise from in response to signals that direct remodelling, in particular addressing the possibility that resident differentiated cell populations may actually act as progenitors of goblet cells. This is a process often termed transdifferentiation. In the respiratory epithelium of allergen-challenged mice, detailed ultrastructural analysis revealed the presence of cells sharing the characteristics of both goblet and Clara cells not present in the epithelium of control mice [45]. Similarly, in a second report, mucin expression was found in a subset of Clara cells located specifically in the proximal, but not distal, airways of allergen-challenged mice [43]. Further evidence in support of the hypothesis that goblet cells formed in response to allergen challenge may arise from Clara cells comes from a separate study where allergen-induced goblet cell formation in mice was accompanied by a decrease in the numbers of Clara cells [46]. Another candidate goblet cell progenitor that has been identified in the mouse is the ciliated cell. Using a mouse model of IL-13-dependent goblet cell formation inducible by Sendai virus infection, Tyner et al. [47] identified airway epithelial cells in which the ciliated cell marker β-tubulin is co-localized with the goblet cell mucin MUC5AC. A similar observation has been reported in IL-13-treated human bronchial airway epithelial cells cultured at the air/liquid interface and such observations may be accounted for by the presence of cells in transition from a ciliated to goblet cell phenotype [47,48]. These observations point to at least two sources of goblet cell progenitors in the airway epithelium. Which cell types act as progenitors of goblet cells may depend on their location within the lung and the specific stimulus received.

Studies such as those outlined above are suggestive that a process of transdifferentiation of cells already resident in the respiratory epithelium may underlie goblet cell formation in response to insult, although other sources of goblet cell progenitors cannot be ruled out. Conclusive evidence may be obtained from future studies using cell lineage tagging approaches such as the Cre/LoxP system (for a review see [49]), where individual cell types can be specifically and irreversibly labelled with reporter genes to facilitate the analysis of cell fate in response to differentiation stimuli. Finally, while murine models and the use of transgenic animals are undoubtedly powerful techniques, differences remain between human and mouse airway epithelial biology [50], indicating the importance of studying these processes in human model systems. For instance, Clara cells are restricted to the distal bronchioles in humans but are ubiquitous throughout the conducting airways of mice. Therefore adapting techniques such as cell lineage tagging to human *in vitro* models such as air/liquid interface cultures will be important to complement observations made in murine models.

Conclusions

In summary, a diverse range of mediators have been linked to airway mucus production, although the precise factors regulating goblet cell hyperplasia probably depend on the disease setting or model used. However, the detailed signalling pathways leading to mucin expression and mucus production still remain to be established. Several studies have linked IL-13 and EGFR pathways to mucin production and goblet cell hyperplasia, and evidence is now emerging for common elements of their signalling pathways. Identification of common regulators of mucin production and goblet cell hyperplasia promises to aid in the identification of new therapeutic approaches to target mucus hypersecretion in respiratory diseases such as asthma and COPD where excessive mucus production contributes to the underlying pathology.

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