Peer-reviewed version available at J. Clin. Med. 2017, 6, 112; doi:10.3390/jcm6120112

Review

Airway Mucus and Asthma: The Role of MUC5AC and MUC5B

Luke R. Bonser [1], and David J. Erle [1, *]

[1] Lung Biology Center, University of California San Francisco, San Francisco, California, 94143-2922, USA; luke.bonser@ucsf.edu
[2] Lung Biology Center, University of California San Francisco, San Francisco, California, 94143-2922, USA; david.erle@ucsf.edu
* Correspondence: david.erle@ucsf.edu; Tel.: +1-415-514-4372

Abstract: Asthma is characterized by mucus abnormalities. Airway epithelial metaplasia results in changes in stored and secreted mucin and the production of a pathologic mucus gel. Mucus transport is impaired culminating in mucus plugging and airway obstruction, a major cause of morbidity in asthma. The polymeric mucins MUC5AC and MUC5B are integral components of airway mucus. *MUC5AC* and *MUC5B* gene expression is altered in asthma and recent work sheds light on their contribution to asthma pathogenesis. Herein we review our current understanding of the role of MUC5AC and MUC5B in mucus dysfunction in asthma.

Keywords: MUC5AC; MUC5B; asthma

1. Asthma

Asthma is a common, chronic, non-communicable disease that affects ~334 million people of all ages, races and ethnicities worldwide [1]. Dramatic increases in the prevalence of atopy and asthma have occurred in Westernized countries, and incidence is rising in less-developed countries [2]. Asthma causes approximately 250 000 deaths annually, is a major cause of lost school and work days, and imposes a substantial economic burden, particularly in low- to middle-income countries [3,4]. Asthma symptoms include wheezing, breathlessness (dyspnea), chest tightness and cough; all result from obstruction in airflow arising from a combination of inflammation induced airway smooth muscle constriction and impaired mucociliary clearance [5].

2. Mucociliary clearance

The conducting airways of the lung are lined by a pseudostratified columnar epithelium extending from the nasal cavity to the lower airway [6]. The epithelium is populated by several cell types. Ciliated cells are interspersed with secretory cells [6], which include club and goblet cells, and contribute secretions to the apical mucus gel [7]. In larger airways, the surface epithelium is contiguous with submucosal glands, which are situated between smooth muscle and cartilage plates [7]. Mucous cells within gland acini are a major mucus source [7] and mucous cells are also found within ducts which deliver gland secretions to the airway lumen. Basal cells anchor the epithelium to the underlying matrix and function as stem/progenitor cells for other airway cell types during natural turnover and in response to injury [8].

Together, the ciliated epithelium, periciliary layer and airway mucus gel form the mucociliary escalator [9]. Individual cilia atop ciliated cells beat in concert within the periciliary layer to propel airway mucus up and out of the lung [10]. In addition to providing a favorable environment for ciliary activity, the periciliary layer prevents compression from the overlying mucus gel layer, and provides a water reservoir to control water distribution [11]. Airway mucus is a hydrogel that functions as molecular flypaper, protecting the underlying epithelium by trapping potentially harmful inhaled particles, pathogens and dissolved chemicals within it [12].

Peer-reviewed version available at J. Clin. Med. 2017, 6, 112; doi:10.3390/jcm6120112

Effective mucociliary clearance is essential for maintaining an uninfected and unobstructed airway, and relies on ciliary activity and the physiochemical properties of the periciliary layer and mucus gel [9,12]. Failure of any component of the mucociliary apparatus can render clearance defective and lead to obstruction. For example, in primary ciliary dyskinesia (PCD), cilia absence and/or immotility impair mucociliary clearance, while in cystic fibrosis (CF) periciliary liquid depletion manifests as mucostasis [11]. In asthma, 'pathologically the outstanding feature of the asthmatic lung lies in the failure of clearance of the bronchial secretions' [13]. In fact, the principal cause of death in asthma is asphyxiation from intraluminal airway obstruction by mucus plugs [14–16]. Defective mucociliary clearance is observed even in mild stable asthma [17–19] and is also significantly impaired during acute exacerbation [20].

3. Polymeric mucins

Mucins are the products of secretory cells and the primary macromolecular components of mucus. Mucins are heterogeneous, densely glycosylated high molecular weight molecules [21]. To date ~20 mucin-like genes have been identified and fall into 2 broad classes: membrane-bound or cell surface mucins, and secreted mucins. Secreted mucins are further subdivided into polymeric and non-polymeric glycoconjugates [22]. 4 polymeric MUC genes, *MUC2, MUC5AC, MUC5B* and *MUC6*, are present in tandem on a conserved cluster of human chromosome 11p15 and likely arose by gene duplication [23]; the fifth, *MUC19*, is found on 12q12 [21,24].

Polymeric mucin gene products have complex, multidomain polypeptide structures important to their function [21]. They possess cysteine-rich von Willebrand factor (vWf) -like D-domains including 3 D-domains (D1,2 and 3) at the N terminus and a fourth at the C terminus (D4); a partial D domain (D') lies between the D2 and D3 domain [21,25]. Additional cysteine rich vWf-like domains (B, C, CK) are located at the C terminus. These domains are sites of mucin dimerization and polymerization, with mucin monomers forming disulfide-bonded polymers in both mass (2-50 mDa) and length (0.5-10 um) [9]. The capacity of polymeric mucins to polymerize is crucial to their gel-forming properties.

The hallmark of these proteins are the tandem repeat domains, encoded by a single large central exon and rich in proline, serine and threonine residues [9]. These regions are the site of *O*-glycosylation; the repetitive sequences create a dense array of glycan structures which contribute 50-90% weight by mass of the glycoprotein [21]. The extensive glycosylation extends and stiffens the mucin polypeptide chain. Terminal sulfation and sialyation of the *O*-glycans results in charge repulsion between neighboring oligosaccharide groups. Mucins therefore have a large hydrodynamic volume in solution, which is important for gel formation [21]. Charged polymers like mucins are also very effective lubricants in aqueous environments [26].

As aforementioned, mucins share sequence similarity with vWF, which also polymerizes through N and C-terminal disulfide linkages [25]. Studies on intact mucins, recombinant N and C terminal domains, as well as studies on porcine submaxillary mucin (PSM) have shown that polymeric mucins also share some basic pattern of polymer assembly with vWF [27–30]. The latter stages of assembly involving multimerization and packaging into secretory granules are less clearly resolved. Polymeric mucins are packaged highly condensed and dehydrated into secretory granules; calcium ions enable this through shielding charge on the polyanionic mucins [31,32]. A recent study characterized an additional association between MUC5B N-terminal D3 domains that enables secretory granule storage; uncoupling of the D3-mediated results in expansion during exocytosis [33]. As all polymeric mucins share sequence identity it is possible that the assembly mechanism is also shared.

Additionally, the mechanisms leading to mucus formation post-exocytosis are poorly understood. A 2-phase model has been proposed to explain the rapid and massive mucin expansion that occurs on secretion [34]. Following secretory granule fusion with the plasma membrane, calcium ions are exchanged by monovalent cations such as sodium and potassium and/or sequestered by bicarbonate [35,36]. This exposes the negatively charged terminal sugars on adjacent mucins, leading to their mutual repulsion and further expansion [36]. This process is followed by

Peer-reviewed version available at J. Clin. Med. 2017, 6, 112; doi:10.3390/jcm6120112

changes in mucin morphology, the molecules unfurling to attain a linear polymeric form in a process coined 'maturation' [34].

4. MUC5AC and MUC5B

In the airway, MUC5AC and MUC5B predominate, with little MUC2 detected and low level MUC19 expression reported; MUC6 is the only polymeric mucin not expressed [21,37]. MUC5B is produced by mucous cells in submucosal glands, and to a lesser extent secretory cells within the surface airway epithelium [38–40]. MUC5AC is produced by specialized secretory cells in the surface epithelium called goblet cells; MUC5AC staining can also be observed in the terminal secretory ducts of submucosal glands [38–40]. MUC5AC and MUC5B are secreted from different cells, or from different granules within the same cell, and remain largely segregated after secretion into the lumen [41,42]. They may also form distinct morphologic structures: lectins preferentially recognizing each mucin suggest MUC5B forms strands and MUC5AC threads and sheets in a porcine model.

As the major matrix-forming macromolecules in airway mucus, the viscoelastic properties of airway mucus depend on MUC5AC and MUC5B [9]. Electron microscopy revealed that MUC5AC and MUC5B polymers are long, flexible linear threads [43,44]. However, MUC5AC and MUC5B differ in charge and shape [45]. MUC5B exists as 2 glycoforms, differing in charge due to glycosylation [46]. The high charge MUC5B variant has been identified in a subpopulation of submucosal gland cells suggesting a distinct cellular origin and glycosyltransferase repertoire [40]. MUC5AC has a lower sedimentation rate than MUC5B. As both form polymers of similar size, the difference in sedimentation is likely determined by the shape of the molecules: MUC5AC behaves more rod-like or extended in solution compared to MUC5B [44]. This characteristic of MUC5AC likely explains why MUC5AC polymers appear less polydisperse than MUC5B polymers as the extended structure gives poorer separation by sedimentation rate [40,44].

Targeting mouse mucin genes has provided insights into the roles of MUC5AC and MUC5B in the airway. MUC5B is critical for mucociliary clearance and airway defense [47]. MUC5B-deficient mice accumulate aspirated materials in the airway and develop chronic bacterial infections, severe inflammation, and airway obstruction [47]. Loss of MUC5B also inhibits innate inflammatory responses suppressing IL-23 that results in accumulation of alveolar macrophages whose phagocytosis and clearance of *Staphylococcus aureus* was impaired [47]. The role of MUC5B was also explored by crossing MUC5B-deficient mice with *Scnn1b*-Tg mice, which exhibit mucus hyperconcentration and airway surface adhesion due to overexpression of the epithelial sodium channel (ENAC) [48]. The magnitude of mucus obstruction in *Scnn1b*-Tg mice was significantly reduced in the absence of MUC5B, however mucus adhesion persisted and MUC5B deletion did not alleviate bacterial burden. Absence of MUC5B in *Scnn1b*-Tg mice was associated with increased airway inflammation suggesting that MUC5B is required to maintain immune homeostasis and is important in ant-bacterial defense.

MUC5AC-deficient mice have normal mucociliary transport and anti-bacterial defense and are protected from mucus plugging in an allergic asthma model [47,49]. Overexpression of MUC5AC confers resistance to viral infection but does not cause metaplasia or obstruction suggesting mucus hypersecretion alone is insufficient to trigger plugging [50]. However, MUC5AC appears to be detrimental in acute lung injury, enhancing neutrophil trafficking and inflammation [51].

Whether the polymeric mucins function similarly in humans has yet to be established. As aforementioned, the airways of normal mice more resemble human distal airways in respect to their diameter [52]. Additionally, the distribution of secretory cells differs between human and mice; submucosal glands are limited to the laryngeal region of trachea in mice [53]. Based on these cross-species anatomical differences one could hypothesize that MUC5B may perform baseline barrier and clearance functions in human distal airways, but its function may be augmented by MUC5AC in the proximal airways where MUC5AC production is greater. Notably, the proportion of

MUC5AC and MUC5B varies with the state of health and the effects of this in asthma are discussed below.

5. Regulation of *MUC5AC* and *MUC5B* expression in asthma

Variants in the 11p15 *MUC5B* and *MUC5AC* locus have been associated with AHR in asthma (6). Many individuals with asthma have increased *MUC5AC* mRNA levels but decreased *MUC5B* mRNA levels [54]. *MUC5AC* and *MUC5B* expression is sensitive to a wide variety of stimulants and developmental cues. Of particular pertinence to asthma are type 2 immune cells, including type 2 T helper (Th2) cells and innate lymphoid cells (iLC2s), which orchestrate allergic airway remodeling in asthma. IL-13 is produced by these cells during allergic inflammation, inducing characteristic changes in airway epithelial mRNA [55–57] and miRNA expression [58] in airway epithelial cells. The IL-13 transcriptional signature can be used to identify individuals with Th2 high and Th2 low asthma; approximately 50% of people with asthma are Th2 high [54,56]. Individuals with Th2 asthma have elevated levels of *MUC5AC* compared with healthy controls or individuals with Th2 low asthma; a substantial decrease in *MUC5B* expression is also observed in Th2 high asthma [54,59]. IL-13 significantly and consistently increases expression of *MUC5AC* in human airway epithelial cells in vitro and expression of *Muc5ac* in murine models [41,55,57,60,61]. The effect of IL-13 on MUC5B is more variable. IL-13 (and allergen challenge) induces *Muc5b* in mouse models but IL-13 frequently decreases *MUC5B* in cultured human airway epithelial cells [41,59].

The link between type 2 inflammation and airway structural cell dysfunction is incompletely understood. A recent study has suggested that Th2 inflammation is necessary but not sufficient for allergic asthma, and that the airway epithelium is more responsive to Th2 inflammation in people with asthma as measured by MUC5AC [62]. Whether this is because asthma cells are intrinsically more sensitive to type 2 inflammation or develop altered responses in a chronic inflammation environment remains undetermined [62].

Epidermal growth factor receptor (EGFR) signaling is also required for mucus production in vitro and in vivo [57,63,64]. EGFR levels are increased in individuals with asthma and expression correlates with disease severity [65]. Various stimuli (bacterial products, viruses, cigarette smoke and inflammatory cell products) and various ligands (EGF, TGF-α, amphiregulin) can trigger EGFR signaling in airway epithelial cells. EGFR signaling induces *MUC5AC* expression while EGFR tyrosine kinase inhibition blocked *MUC5AC* expression [63].

Recently, a murine inbred strain study revealed that a large fraction of the variation in secreted MUC5AC and MUC5B was attributable to strain specific genetic differences indicating heritability [66]. Although *MUC5AC* and *MUC5B* mRNA levels were strongly correlated, likely due to shared transcriptional regulation, neither mRNA correlated with protein production suggesting post-transcriptional events were important in mucin regulation [66]. Quantitative trait locus (QTL) mapping identified distinct, *trans* protein QTL for MUC5AC (chromosome 13) and MUC5B (chromosome 2) explaining 18% and 20% of phenotypic variance, respectively, indicating separate distal regulatory control [66]. Identifying additional QTL loci will inform mucin regulation further.

6. Goblet cell fate in asthma

A key feature of airway epithelial remodeling in asthma is increased goblet cell number, which accompanies the aforementioned increase in *MUC5AC* copy number. In fatal asthma a 30-fold increase in goblet cell number was reported [67]; increased goblet cell number is readily observed in mild to moderate disease too [68]. The mechanisms mediating goblet cell differentiation are incompletely understood with both hyperplasia and metaplasia proposed. In the human airways, understanding of secretory cell fate is limited and much of our knowledge derives from mouse models. During normal development, epithelial cells are thought to differentiate into ciliated and secretory lineages from basal and club cells that are considered to function as progenitor/stem cells. In the proximal airways, basal cells are the progenitors of ciliated and secretory cells [8]. The proportion of basal cells in the airway is highest in the large airways and progressively decreases

down the tracheobronchial tree, where club cells likely act as progenitors [69]. In murine models, where airways more resemble human distal airways, transition from both ciliated and club cells has been evidenced suggesting variable lineage [70,71].

Pathologic remodeling is caused by dysregulation of signaling cascades that govern normal differentiation. Notch signaling is an evolutionary conserved pathway that regulates cell fate decisions during development. Notch recently emerged as a pivotal regulator of basal cell differentiation in conducting airways with activation of secretory over ciliated lineages [72]. Notch2 is a common node downstream of IL-13 and is absolutely required for goblet cell metaplasia in vitro and in vivo [73]. Inhibition of Notch2 inhibits IL-13 and allergen driven goblet cell metaplasia in vivo [73]. In a mouse model of respiratory disease, inhibition of JAG, a ligand for the transmembrane Notch receptor, reduced goblet cell metaplasia when administered prior to an inflammatory stimulus, and reversed goblet cell metaplasia when administered post-stimulus (i.e. once metaplasia was established) [74].

At the transcriptional level, a number of transcription factors are thought to be involved in increased *MUC5AC* expression and mucous metaplasia. Sam pointed domain-containing ETS transcription factor (SPDEF) is sufficient and necessary for goblet cell metaplasia and for increasing *MUC5AC* and *MUC5B* expression [75]. *SPDEF* expression is increased in airway epithelial cells of patients with asthma compared to healthy controls [76], remains increased in spite of anti-inflammatory treatment, and is upregulated following IL-13 stimulation [77]. *SPDEF* induction following IL-13 stimulation was accompanied by DNA hypomethylation of several CpG sites within the SPDEF promoter [78]. Epigenetic editing of SPDEF suppressed *MUC5AC* expression in human airway epithelial cells [79]. In *SPDEF* deficient mice, goblet cells are absent, whilst overexpression of *SPDEF* causes goblet cell metaplasia [80].

Several forkhead box family members have also been implicated in airway polymeric mucin expression and mucous metaplasia. Forkhead box protein A2 (FOXA2) is negatively regulated by *SPDEF* and *MUC5AC* expression, and is a potent inhibitor of goblet cell differentiation [81]. FOXA2 is also regulated by DNA methylation [78]. Interestingly, both IL-13 and EGFR signaling cascades converge on FOXA2 inhibition, perhaps representing a common pathway for IL-13 and EGFR-induced mucous metaplasia [10]. Another family member, FOXA3, also functions as a goblet cell metaplasia regulator: it is highly expressed in patients with asthma, and is IL-13 and rhinovirus inducible [76]. HIF-1 is also downstream of IL-13 and EGF stimulation, plus a HIF-1 binding motif is conserved in mammalian *MUC5AC* promoters [82].

7. Altered MUC5AC and MUC5B properties

Abnormalities in goblet cell number are accompanied by changes in stored and secreted mucin. In asthma as in health, MUC5AC is produced in goblet cells from the surface epithelium, while MUC5B is largely produced in the submucosal glands. However, changes in the relative proportion of MUC5AC and MUC5B are observed in asthma (Figure 1). As at the gene expression level, elevated MUC5AC production is consistently reported, but there are conflicting reports regarding MUC5B. Increased MUC5AC and MUC5B protein have been reported in sputum from individuals with asthma [83]. Another study identified MUC5B as the predominant mucin in healthy secretions, while MUC5AC concentration increased and MUC5B decreased in individuals with asthma, including those in exacerbation [42]. Interestingly, within the asthma cohort, a higher ratio of MUC5AC to MUC5B correlated with type 2 inflammation (>2% eosinophils) [42]. in pediatric asthma, similar observations were made: increased MUC5AC was reported in children with both stable and acute asthma vs healthy controls. Median MUC5B concentration was non-significantly reduced, however, overrepresentation of a low-charge glycoform of MUC5B was observed [84]. A study on viscous mucus exudate from a patient who died in *status asthmaticus* demonstrated that the MUC5B low charge glycoform was the major constituent suggesting mucin glycosylation status may also be important in asthma [85]. Additionally, in mild asthmatics, large amounts of glandular MUC5B extracellular mucus was observed [86]. The contribution of glandular MUC5B to mucus dysfunction in asthma requires further exploration.

Peer-reviewed version available at J. Clin. Med. 2017, 6, 112; doi:10.3390/jcm6120112

It has been noted that asthmatic sputum is abnormally viscous [87]. Rheological measurements of sputum from patients with asthma during acute exacerbation demonstrated increased elastic and viscous moduli; the increased elastic response dominated suggesting increased crosslinking of mucin polymers as demonstrated recently in CF [88,89]. These differences are most visible in the form of mucus plugs, which occlude airways and prevent mucociliary clearance. Although mucins are primarily responsible for the biophysical properties of the gel, other constituents including DNA and albumin may also contribute to the increased viscoelasticity reported in asthma [87,88]. Several studies have compared the size distribution of MUC5AC and MUC5B in sputum from patients with asthma yet no discernible difference has been observed [84,88]. It has been suggested that mucin degradation is inhibited in asthma: protease dependent mucin degradation was inhibited at the height of exacerbation but restored during recovery [88]. Alterations in protease and antiprotease expression has been reported in asthma suggesting imbalance could affect mucus clearance and contribute to tethering and plugging.

Despite substantial plugging observed in the majority of patients with fatal asthma, the biochemical and biophysical mechanisms by which secreted mucus occludes airways is not fully understood. An autopsy study demonstrated a large increase in the frequency of goblet cells in continuity with intraluminal mucus in individuals with asthma [90]. We found that extracellular domains of MUC5AC-rich mucus were intimately associated or tethered to epithelial mucous cells, markedly impairing mucociliary transport [41]. Images from allergic mouse airways [49] are also consistent with tethering. MUC5AC-tethering probably leads to progressive luminal accumulation of mucus and airway plugging (Figure 1). The mechanism by which MUC5AC is tethered requires further investigation. It is possible that exocytosed MUC5AC is not fully released or expanded from goblet cells leading to tethering of mucus to the epithelium.

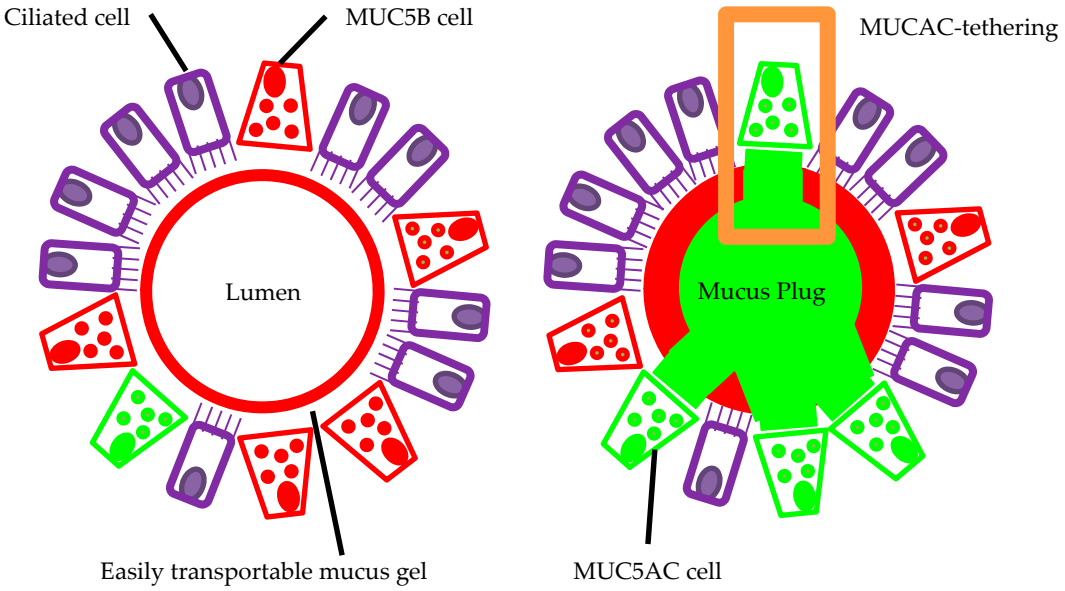

Figure 1. Alterations in MUC5AC and MUC5B contribute to mucus dysfunction in asthma. In health (left), MUC5B (red) is the predominant mucin expressed and the principal component of the airway mucus gel. The MUC5B-rich gel is readily transported by the ciliated epithelium (purple) maintaining an unobstructed and uninfected airway. In asthma (right), mucin expression is altered: MUC5AC (green) expression is upregulated, while MUC5B expression is reduced. This leads to the production of a heterogeneous airway mucus gel comprising distinct MUC5AC and MUC5B domains. Extracellular MUC5AC-domans remain tethered to MUC5AC-producing cells (orange box) compromising mucociliary clearance. Mucus accumulates forming mucus plugs which occlude the airway. Airway obstruction manifests clinically as breathlessness and wheeze; in some patients, intraluminal occlusion by mucus plugging can lead to asphyxiation.

Peer-reviewed version available at J. Clin. Med. 2017, 6, 112; doi:10.3390/jcm6120112

Interestingly, mucins from a viscous mucus plug appeared as 'tangled mases condensed around nodes from which many chains emanate', contrasting with the classic view of polymeric mucins as linear threadlike molecules [85]. These mucins resembled freshly secreted mucins implicating improper unpackaging of mucins post-secretion as a contributor to tethering [34]. Defective postsecretory maturation of MUC5B has been reported in CF [91]. Altered luminal pH could also contribute since sputum samples collected during asthma exacerbation have been shown to be more acidic compared with samples from people with stable asthma or with other respiratory diseases [90,92]. As aforementioned, a plethora of transcriptional alterations are observed in asthma which likely alter ciliary transport and ion transport and could impact airway surface pH, mucus adhesivity and mucociliary transport. The implications of altered pH on mucociliary transport is adequately demonstrated in cystic fibrosis [11].

We also demonstrated that MUC5AC and MUC5B form distinct extracellular domains and that IL-13 induces a heterogeneous gel, which could have implications for mucociliary clearance in asthma [41]. Differences in the biophysical properties of extracellular MUC5AC- and MUC5B-containing domains could be attributable to intrinsic properties of the MUC5AC and MUC5B core proteins. Other potentially important factors include posttranslational mucin modifications including glycosylation (low-charge MUC5B [84]) or disulfide cross-linking, hydration- and bicarbonate-mediated mucin expansion, non-mucin mucus constituents (e.g. intelectin-1 [93]), or other differences in the secretory cell or the luminal environment. Additionally, differences in airway mucus may be caused by differences in the clinical features of asthma, including exacerbation, severity, duration, smoking, and treatment.

8. Therapeutics

Together with smooth muscle contraction, mucus obstruction is a major cause of airway obstruction in asthma [10]. A mucoactive drug is an agent capable of modifying mucus production, secretion, properties, or its interactions with the mucociliary epithelium [94]. Mucoactive agents facilitate mucus clearance (mucolytics) or inhibit mucus production or secretion (mucoregulators) [95,96].

No effective mucolytic treatments for asthma exist. Hypertonic saline is associated with mucus clearance but modest improvements in airflow [97]. The reducing agent N-acetylcysteine (NAC) is sometimes used as a mucolytic therapy but exhibits low efficacy (low mucolytic activity at high mucus concentrations and neutral pH) and tolerability: N-acetylcysteine can irritate the airways and cause bronchospasm in hyperreactive patients [98–103]. Thiol-modified carbohydrates have been proposed as novel mucolytics for CF and other lung diseases [89]. Pulmozyme, a human DNAse, improves pulmonary function and reduces pulmonary exacerbation in CF [104,105], but there is no evidence it is effective in other diseases, presumably since it does not target the mucins themselves. Recent evidence suggests mucin-specific approaches could be of benefit: complete MUC5B removal from the airway may be detrimental [48], whilst the identification of MUC5AC as an essential non-contractile mediator of AHR [49] and the role of MUC5AC in tethering [41] suggest MUC5AC-specific therapies could be of benefit in asthma.

There is rationale for development of cytokine specific antibodies as mucoregulators. For example, a recent trial with anti-IL-13 (lebrikizumab) demonstrated improved airflow (prebronchodilator FEV1) in patients with Th2 asthma. However anti-IL-13 only met its primary endpoint of significantly reducing exacerbation rates in one of two parallel Phase III studies, despite significantly improving FEV1 in both [106,107]. Although other mechanisms likely contribute, improved FEV1 could represent reduced mucus obstruction. An agent that inhibits a common subunit of IL-13 and IL-4 receptors also had beneficial effects in a clinical trial. Agents that reduce production of IL-13, including corticosteroids and antibodies against the epithelial-derived type 2-promoting cytokine TSLP likely act in part by inhibiting production of pathologic mucins [59,108].

Other approaches to regulating mucus obstruction might include specific targeting of mucin (especially *MUC5AC*) gene transcription or protein processing or blocking the differentiation of mucus-producing goblet cells. For example, targeting Notch through Jagged antagonism

reversed goblet cell metaplasia in a preclinical model [74]. Although controlling mucus hypersecretion is attractive therapeutically, a therapy which completely disrupts secretion and inhibits MUC5B may be detrimental. Overall, it is unclear which mucoactive drugs would offer optimal benefit [109] and further research is required.

9. Conclusions

MUC5AC and MUC5B are principal components of airway mucus. In the airway, their production is spatially separated, and they serve different functions (at least in mice). Altered MUC5AC and MUC5B gene expression is consistently observed in both asthma models and individuals with disease. Increases in goblet cell number accompany changes in mucin gene expression, which result in altered mucus composition and organization. These changes are associated with increased gel viscoelasticity, and are sufficient to impair mucus transport through MUC5AC-tethering, likely contributing to airway obstruction and mucus plugging. Continued research to understand the mechanisms underlying goblet cell fate and MUC5AC-tethering are crucial for the development of effective mucoactive agents.

Acknowledgments: This work was supported by NIH grants AI077439, HL138424 and HL099101, the Cystic Fibrosis Cell Models Core (funded by NIH grant DK072517 and Cystic Fibrosis Foundation grant DR613-CR11) and the UCSF Program for Breakthrough Biomedical Research Postdoctoral Independent Research Project Award.

Author Contributions: L.R.B and D.J.E wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Masoli, M.; Fabian, D.; Holt, S.; Beasley, R.; Global Initiative for Asthma (GINA) Program The global burden of asthma: executive summary of the GINA Dissemination Committee Report. *Allergy* **2004**, *59*, 469–478, doi:10.1111/j.1398-9995.2004.00526.x.
2. Pearce, N.; Aït-Khaled, N.; Beasley, R.; Mallol, J.; Keil, U.; Mitchell, E.; Robertson, C. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax* **2007**, *62*, 758–766, doi:10.1136/thx.2006.070169.
3. Fuhlbrigge, A. L.; Adams, R. J.; Guilbert, T. W.; Grant, E.; Lozano, P.; Janson, S. L.; Martinez, F.; Weiss, K. B.; Weiss, S. T. The Burden of Asthma in the United States. *Am. J. Respir. Crit. Care Med.* **2002**, *166*, 1044–1049, doi:10.1164/rccm.2107057.
4. Ferkol, T.; Schraufnagel, D. The Global Burden of Respiratory Disease. *Ann. Am. Thorac. Soc.* **2014**, *11*, 404–406, doi:10.1513/AnnalsATS.201311-405PS.
5. Fanta, C. H. Asthma. *N. Engl. J. Med.* **2009**, *360*, 1002–1014, doi:10.1056/NEJMra0804579.
6. Jeffery, P. K.; Li, D. Airway mucosa: secretory cells, mucus and mucin genes. *Eur. Respir. J.* **1997**, *10*, 1655–1662.
7. Fahy, J. V.; Dickey, B. F. Airway Mucus Function and Dysfunction. *N. Engl. J. Med.* **2010**, *363*, 2233–2247, doi:10.1056/NEJMra0910061.
8. Hackett, N. R.; Shaykhiev, R.; Walters, M. S.; Wang, R.; Zwick, R. K.; Ferris, B.; Witover, B.; Salit, J.; Crystal, R. G. The human airway epithelial basal cell transcriptome. *PloS One* **2011**, *6*, e18378, doi:10.1371/journal.pone.0018378.
9. Thornton, D. J.; Sheehan, J. K. From mucins to mucus: toward a more coherent understanding of this essential barrier. *Proc. Am. Thorac. Soc.* **2004**, *1*, 54–61, doi:10.1513/pats.2306016.
10. Erle, D. J.; Sheppard, D. The cell biology of asthma. *J Cell Biol* **2014**, *205*, 621–631, doi:10.1083/jcb.201401050.
11. Button, B.; Cai, L.-H.; Ehre, C.; Kesimer, M.; Hill, D. B.; Sheehan, J. K.; Boucher, R. C.; Rubinstein, M. A Periciliary Brush Promotes the Lung Health by Separating the Mucus Layer from Airway Epithelia. *Science* **2012**, *337*, 937–941, doi:10.1126/science.1223012.
12. Kim, W. D. Lung mucus: a clinician's view. *Eur. Respir. J.* **1997**, *10*, 1914–1917.
13. Dunnill, M. S. The pathology of asthma, with special reference to changes in the bronchial mucosa. *J. Clin. Pathol.* **1960**, *13*, 27–33.
14. Messer, J. W.; Peters, G. A.; Bennett, W. A. Causes of Death and Pathologic Findings in 304 Cases of Bronchial Asthma. *Dis. Chest* **1960**, *38*, 616–624, doi:10.1378/chest.38.6.616.

15. Kuyper, L. M.; Paré, P. D.; Hogg, J. C.; Lambert, R. K.; Ionescu, D.; Woods, R.; Bai, T. R. Characterization of airway plugging in fatal asthma. *Am. J. Med.* **2003**, *115*, 6–11, doi:10.1016/S0002-9343(03)00241-9.
16. Evans, C. M.; Kim, K.; Tuvim, M. J.; Dickey, B. F. Mucus hypersecretion in asthma: causes and effects. *Curr. Opin. Pulm. Med.* **2009**, *15*, 4–11, doi:10.1097/MCP.0b013e32831da8d3.
17. Bateman, J. R.; Pavia, D.; Sheahan, N. F.; Agnew, J. E.; Clarke, S. W. Impaired tracheobronchial clearance in patients with mild stable asthma. *Thorax* **1983**, *38*, 463–467.
18. Agnew, J. E.; Bateman, J. R.; Pavia, D.; Clarke, S. W. Radionuclide demonstration of ventilatory abnormalities in mild asthma. *Clin. Sci. Lond. Engl. 1979* **1984**, *66*, 525–531.
19. O'Riordan, T. G.; Zwang, J.; Smaldone, G. C. Mucociliary Clearance in Adult Asthma. *Am. Rev. Respir. Dis.* **1992**, *146*, 598–603, doi:10.1164/ajrccm/146.3.598.
20. Messina, M. S.; O'riordan, T. G.; Smaldone, G. C. Changes in Mucociliary Clearance during Acute Exacerbations of Asthma. *Am. Rev. Respir. Dis.* **1991**, *143*, 993–997, doi:10.1164/ajrccm/143.5_Pt_1.993.
21. Thornton, D. J.; Rousseau, K.; McGuckin, M. A. Structure and function of the polymeric mucins in airways mucus. *Annu. Rev. Physiol.* **2008**, *70*, 459–486, doi:10.1146/annurev.physiol.70.113006.100702.
22. Evans, C. M.; Koo, J. S. Airway mucus: The good, the bad, the sticky. *Pharmacol. Ther.* **2009**, *121*, 332–348, doi:10.1016/j.pharmthera.2008.11.001.
23. Lang, T.; Hansson, G. C.; Samuelsson, T. Gel-forming mucins appeared early in metazoan evolution. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 16209–16214, doi:10.1073/pnas.0705984104.
24. Rose, M. C.; Voynow, J. A. Respiratory Tract Mucin Genes and Mucin Glycoproteins in Health and Disease. *Physiol. Rev.* **2006**, *86*, 245–278, doi:10.1152/physrev.00010.2005.
25. Perez-Vilar, J.; Hill, R. L. The Structure and Assembly of Secreted Mucins. *J. Biol. Chem.* **1999**, *274*, 31751–31754, doi:10.1074/jbc.274.45.31751.
26. Raviv, U.; Giasson, S.; Kampf, N.; Gohy, J.-F.; Jérôme, R.; Klein, J. Lubrication by charged polymers. *Nature* **2003**, *425*, 163–165, doi:10.1038/nature01970.
27. Asker, N.; Axelsson, M. A.; Olofsson, S. O.; Hansson, G. C. Human MUC5AC mucin dimerizes in the rough endoplasmic reticulum, similarly to the MUC2 mucin. *Biochem. J.* **1998**, *335 (Pt 2)*, 381–387.
28. Perez-Vilar, J.; Eckhardt, A. E.; DeLuca, A.; Hill, R. L. Porcine submaxillary mucin forms disulfide-linked multimers through its amino-terminal D-domains. *J. Biol. Chem.* **1998**, *273*, 14442–14449.
29. Perez-Vilar, J.; Eckhardt, A. E.; Hill, R. L. Porcine Submaxillary Mucin Forms Disulfide-bonded Dimers between Its Carboxyl-terminal Domains. *J. Biol. Chem.* **1996**, *271*, 9845–9850, doi:10.1074/jbc.271.16.9845.
30. Sheehan, J. K.; Kirkham, S.; Howard, M.; Woodman, P.; Kutay, S.; Brazeau, C.; Buckley, J.; Thornton, D. J. Identification of Molecular Intermediates in the Assembly Pathway of the MUC5AC Mucin. *J. Biol. Chem.* **2004**, *279*, 15698–15705, doi:10.1074/jbc.M313241200.
31. Verdugo, P.; Deyrup-Olsen, I.; Aitken, M.; Villalon, M.; Johnson, D. Molecular Mechanism of Mucin Secretion: I. The Role of Intragranular Charge Shielding. *J. Dent. Res.* **1987**, *66*, 506–508, doi:10.1177/00220345870660022001.
32. Forstner, G. Signal Transduction Packaging and Secretion of Mucins. *Annu. Rev. Physiol.* **1995**, *57*, 585–605, doi:10.1146/annurev.ph.57.030195.003101.
33. Ridley, C.; Kouvatsos, N.; Raynal, B. D.; Howard, M.; Collins, R. F.; Desseyn, J.-L.; Jowitt, T. A.; Baldock, C.; Davis, C. W.; Hardingham, T. E.; Thornton, D. J. Assembly of the Respiratory Mucin MUC5B A NEW MODEL FOR A GEL-FORMING MUCIN. *J. Biol. Chem.* **2014**, *289*, 16409–16420, doi:10.1074/jbc.M114.566679.
34. Kesimer, M.; Makhov, A. M.; Griffith, J. D.; Verdugo, P.; Sheehan, J. K. Unpacking a gel-forming mucin: a view of MUC5B organization after granular release. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* **2010**, *298*, L15–L22, doi:10.1152/ajplung.00194.2009.
35. Verdugo, P. Mucin exocytosis. *Am. Rev. Respir. Dis.* **1991**, *144*, S33-37, doi:10.1164/ajrccm/144.3_pt_2.S33.
36. Chen, E. Y. T.; Yang, N.; Quinton, P. M.; Chin, W.-C. A new role for bicarbonate in mucus formation. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* **2010**, *299*, L542–L549, doi:10.1152/ajplung.00180.2010.
37. Kim, K. C. Role of epithelial mucins during airway infection. *Pulm. Pharmacol. Ther.* **2012**, *25*, 415–419, doi:10.1016/j.pupt.2011.12.003.
38. Hovenberg, H. W.; Davies, J. R.; Carlstedt, I. Different mucins are produced by the surface epithelium and the submucosa in human trachea: identification of MUC5AC as a major mucin from the goblet cells. *Biochem. J.* **1996**, *318 (Pt 1)*, 319–324.
39. Hovenberg, H. W.; Davies, J. R.; Herrmann, A.; Lindén, C. J.; Carlstedt, I. MUC5AC, but not MUC2, is a prominent mucin in respiratory secretions. *Glycoconj. J.* **1996**, *13*, 839–847.
40. Wickström, C.; Davies, J. R.; Eriksen, G. V.; Veerman, E. C.; Carlstedt, I. MUC5B is a major gel-forming, oligomeric mucin from human salivary gland, respiratory tract and endocervix: identification of glycoforms and C-terminal cleavage. *Biochem. J.* **1998**, *334*, 685–693.

41. Bonser, L. R.; Zlock, L.; Finkbeiner, W.; Erle, D. J. Epithelial tethering of MUC5AC-rich mucus impairs mucociliary transport in asthma. *J. Clin. Invest.* **2016**, *126*, 2367–2371, doi:10.1172/JCI84910.
42. Lachowicz-Scroggins, M. E.; Yuan, S.; Kerr, S. C.; Dunican, E. M.; Yu, M.; Carrington, S. D.; Fahy, J. V. Abnormalities in MUC5AC and MUC5B Protein in Airway Mucus in Asthma. *Am. J. Respir. Crit. Care Med.* **2016**, *194*, 1296–1299, doi:10.1164/rccm.201603-0526LE.
43. Thornton, D. J.; Davies, J. R.; Kraayenbrink, M.; Richardson, P. S.; Sheehan, J. K.; Carlstedt, I. Mucus glycoproteins from 'normal' human tracheobronchial secretion. *Biochem. J.* **1990**, *265*, 179–186, doi:10.1042/bj2650179.
44. Sheehan, J. K.; Brazeau, C.; Kutay, S.; Pigeon, H.; Kirkham, S.; Howard, M.; Thornton, D. J. Physical characterization of the MUC5AC mucin: a highly oligomeric glycoprotein whether isolated from cell culture or in vivo from respiratory mucous secretions. *Biochem. J.* **2000**, *347*, 37–44, doi:10.1042/bj3470037.
45. Raclawska, D. S.; Ttofali, F.; Fletcher, A. A.; Harper, D. N.; Bochner, B. S.; Janssen, W. J.; Evans, C. M. Mucins and Their Sugars. Critical Mediators of Hyperreactivity and Inflammation. *Ann. Am. Thorac. Soc.* **2016**, *13 Suppl 1*, S98-99, doi:10.1513/AnnalsATS.201511-743MG.
46. Thornton, D. J.; Howard, M.; Khan, N.; Sheehan, J. K. Identification of Two Glycoforms of the MUC5B Mucin in Human Respiratory Mucus EVIDENCE FOR A CYSTEINE-RICH SEQUENCE REPEATED WITHIN THE MOLECULE. *J. Biol. Chem.* **1997**, *272*, 9561–9566, doi:10.1074/jbc.272.14.9561.
47. Roy, M. G.; Livraghi-Butrico, A.; Fletcher, A. A.; McElwee, M. M.; Evans, S. E.; Boerner, R. M.; Alexander, S. N.; Bellinghausen, L. K.; Song, A. S.; Petrova, Y. M.; Tuvim, M. J.; Adachi, R.; Romo, I.; Bordt, A. S.; Bowden, M. G.; Sisson, J. H.; Woodruff, P. G.; Thornton, D. J.; Rousseau, K.; De la Garza, M. M.; Moghaddam, S. J.; Karmouty-Quintana, H.; Blackburn, M. R.; Drouin, S. M.; Davis, C. W.; Terrell, K. A.; Grubb, B. R.; O'Neal, W. K.; Flores, S. C.; Cota-Gomez, A.; Lozupone, C. A.; Donnelly, J. M.; Watson, A. M.; Hennessy, C. E.; Keith, R. C.; Yang, I. V.; Barthel, L.; Henson, P. M.; Janssen, W. J.; Schwartz, D. A.; Boucher, R. C.; Dickey, B. F.; Evans, C. M. Muc5b is required for airway defence. *Nature* **2014**, *505*, 412–416, doi:10.1038/nature12807.
48. Livraghi-Butrico, A.; Grubb, B. R.; Wilkinson, K. J.; Volmer, A. S.; Burns, K. A.; Evans, C. M.; O'Neal, W. K.; Boucher, R. C. Contribution of mucus concentration and secreted mucins Muc5ac and Muc5b to the pathogenesis of muco-obstructive lung disease. *Mucosal Immunol.* **2017**, *10*, 395–407, doi:10.1038/mi.2016.63.
49. Evans, C. M.; Raclawska, D. S.; Ttofali, F.; Liptzin, D. R.; Fletcher, A. A.; Harper, D. N.; McGing, M. A.; McElwee, M. M.; Williams, O. W.; Sanchez, E.; Roy, M. G.; Kindrachuk, K. N.; Wynn, T. A.; Eltzschig, H. K.; Blackburn, M. R.; Tuvim, M. J.; Janssen, W. J.; Schwartz, D. A.; Dickey, B. F. The polymeric mucin Muc5ac is required for allergic airway hyperreactivity. *Nat. Commun.* **2015**, *6*, ncomms7281, doi:10.1038/ncomms7281.
50. Ehre, C.; Worthington, E. N.; Liesman, R. M.; Grubb, B. R.; Barbier, D.; O'Neal, W. K.; Sallenave, J.-M.; Pickles, R. J.; Boucher, R. C. Overexpressing mouse model demonstrates the protective role of Muc5ac in the lungs. *Proc. Natl. Acad. Sci.* **2012**, *109*, 16528–16533, doi:10.1073/pnas.1206552109.
51. Koeppen, M.; McNamee, E. N.; Brodsky, K. S.; Aherne, C. M.; Faigle, M.; Downey, G. P.; Colgan, S. P.; Evans, C. M.; Schwartz, D. A.; Eltzschig, H. K. Detrimental role of the airway mucin Muc5ac during ventilator-induced lung injury. *Mucosal Immunol.* **2013**, *6*, 762–775, doi:10.1038/mi.2012.114.
52. Rock, J. R.; Randell, S. H.; Hogan, B. L. M. Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis. Model. Mech.* **2010**, *3*, 545–556, doi:10.1242/dmm.006031.
53. Curran, D. R.; Cohn, L. Advances in Mucous Cell Metaplasia. *Am. J. Respir. Cell Mol. Biol.* **2010**, *42*, 268–275, doi:10.1165/rcmb.2009-0151TR.
54. Woodruff, P. G.; Modrek, B.; Choy, D. F.; Jia, G.; Abbas, A. R.; Ellwanger, A.; Arron, J. R.; Koth, L. L.; Fahy, J. V. T-helper Type 2–driven Inflammation Defines Major Subphenotypes of Asthma. *Am. J. Respir. Crit. Care Med.* **2009**, *180*, 388–395, doi:10.1164/rccm.200903-0392OC.
55. Kuperman, D. A.; Lewis, C. C.; Woodruff, P. G.; Rodriguez, M. W.; Yang, Y. H.; Dolganov, G. M.; Fahy, J. V.; Erle, D. J. Dissecting asthma using focused transgenic modeling and functional genomics. *J. Allergy Clin. Immunol.* **2005**, *116*, 305–311, doi:10.1016/j.jaci.2005.03.024.
56. Woodruff, P. G.; Boushey, H. A.; Dolganov, G. M.; Barker, C. S.; Yang, Y. H.; Donnelly, S.; Ellwanger, A.; Sidhu, S. S.; Dao-Pick, T. P.; Pantoja, C.; Erle, D. J.; Yamamoto, K. R.; Fahy, J. V. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 15858–15863, doi:10.1073/pnas.0707413104.
57. Zhen, G.; Park, S. W.; Nguyenvu, L. T.; Rodriguez, M. W.; Barbeau, R.; Paquet, A. C.; Erle, D. J. IL-13 and Epidermal Growth Factor Receptor Have Critical but Distinct Roles in Epithelial Cell Mucin Production. *Am. J. Respir. Cell Mol. Biol.* **2007**, *36*, 244–253, doi:10.1165/rcmb.2006-0180OC.

58. Solberg, O. D.; Ostrin, E. J.; Love, M. I.; Peng, J. C.; Bhakta, N. R.; Hou, L.; Nguyen, C.; Solon, M.; Nguyen, C.; Barczak, A. J.; Zlock, L. T.; Blagev, D. P.; Finkbeiner, W. E.; Ansel, K. M.; Arron, J. R.; Erle, D. J.; Woodruff, P. G. Airway Epithelial miRNA Expression Is Altered in Asthma. *Am. J. Respir. Crit. Care Med.* **2012**, *186*, 965–974, doi:10.1164/rccm.201201-0027OC.
59. Lachowicz-Scroggins, M. E.; Finkbeiner, W. E.; Gordon, E. D.; Yuan, S.; Zlock, L.; Bhakta, N. R.; Woodruff, P. G.; Fahy, J. V.; Boushey, H. A. Corticosteroid and long-acting ß-agonist therapy reduces epithelial goblet cell metaplasia. *Clin. Exp. Allergy* n/a-n/a, doi:10.1111/cea.13015.
60. Kuperman, D. A.; Huang, X.; Koth, L. L.; Chang, G. H.; Dolganov, G. M.; Zhu, Z.; Elias, J. A.; Sheppard, D.; Erle, D. J. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat. Med.* **2002**, *8*, 885–889, doi:10.1038/nm734.
61. Laoukili, J.; Perret, E.; Willems, T.; Minty, A.; Parthoens, E.; Houcine, O.; Coste, A.; Jorissen, M.; Marano, F.; Caput, D.; Tournier, F. IL-13 alters mucociliary differentiation and ciliary beating of human respiratory epithelial cells. *J. Clin. Invest.* **2001**, *108*, 1817–1824.
62. Cho, J. L.; Ling, M. F.; Adams, D. C.; Faustino, L.; Islam, S. A.; Afshar, R.; Griffith, J. W.; Harris, R. S.; Ng, A.; Radicioni, G.; Ford, A. A.; Han, A. K.; Xavier, R.; Kwok, W. W.; Boucher, R.; Moon, J. J.; Hamilos, D. L.; Kesimer, M.; Suter, M. J.; Medoff, B. D.; Luster, A. D. Allergic asthma is distinguished by sensitivity of allergen-specific CD4$^+$ T cells and airway structural cells to type 2 inflammation. *Sci. Transl. Med.* **2016**, *8*, 359ra132-359ra132, doi:10.1126/scitranslmed.aag1370.
63. Takeyama, K.; Dabbagh, K.; Lee, H.-M.; Agustí, C.; Lausier, J. A.; Ueki, I. F.; Grattan, K. M.; Nadel, J. A. Epidermal growth factor system regulates mucin production in airways. *Proc. Natl. Acad. Sci.* **1999**, *96*, 3081–3086, doi:10.1073/pnas.96.6.3081.
64. Cras, T. D. L.; Acciani, T. H.; Mushaben, E. M.; Kramer, E. L.; Pastura, P. A.; Hardie, W. D.; Korfhagen, T. R.; Sivaprasad, U.; Ericksen, M.; Gibson, A. M.; Holtzman, M. J.; Whitsett, J. A.; Hershey, G. K. K. Epithelial EGF receptor signaling mediates airway hyperreactivity and remodeling in a mouse model of chronic asthma. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* **2011**, *300*, L414–L421, doi:10.1152/ajplung.00346.2010.
65. Takeyama, K.; Fahy, J. V.; Nadel, J. A. Relationship of epidermal growth factor receptors to goblet cell production in human bronchi. *Am. J. Respir. Crit. Care Med.* **2001**, *163*, 511–516, doi:10.1164/ajrccm.163.2.2001038.
66. Donoghue, L. J.; Livraghi-Butrico, A.; McFadden, K. M.; Thomas, J. M.; Chen, G.; Grubb, B. R.; O'Neal, W. K.; Boucher, R. C.; Kelada, S. N. P. Identification of trans Protein QTL for Secreted Airway Mucins in Mice and a Causal Role for Bpifb1. *Genetics* **2017**, genetics.300211.2017, doi:10.1534/genetics.117.300211.
67. Aikawa, T.; Shimura, S.; Sasaki, H.; Ebina, M.; Takishima, T. Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. *Chest* **1992**, *101*, 916–921.
68. Ordoñez, C. L.; Khashayar, R.; Wong, H. H.; Ferrando, R.; Wu, R.; Hyde, D. M.; Hotchkiss, J. A.; Zhang, Y.; Novikov, A.; Dolganov, G.; Fahy, J. V. Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am. J. Respir. Crit. Care Med.* **2001**, *163*, 517–523, doi:10.1164/ajrccm.163.2.2004039.
69. Crystal, R. G. Airway Basal Cells. The "Smoking Gun" of Chronic Obstructive Pulmonary Disease. *Am. J. Respir. Crit. Care Med.* **2014**, *190*, 1355–1362, doi:10.1164/rccm.201408-1492PP.
70. Park, K.-S.; Wells, J. M.; Zorn, A. M.; Wert, S. E.; Laubach, V. E.; Fernandez, L. G.; Whitsett, J. A. Transdifferentiation of Ciliated Cells during Repair of the Respiratory Epithelium. *Am. J. Respir. Cell Mol. Biol.* **2006**, *34*, 151–157, doi:10.1165/rcmb.2005-0332OC.
71. Rawlins, E. L.; Okubo, T.; Xue, Y.; Brass, D. M.; Auten, R. L.; Hasegawa, H.; Wang, F.; Hogan, B. L. M. The Role of Scgb1a1+ Clara Cells in the Long-Term Maintenance and Repair of Lung Airway, but Not Alveolar, Epithelium. *Cell Stem Cell* **2009**, *4*, 525–534, doi:10.1016/j.stem.2009.04.002.
72. Boucherat, O.; Morissette, M. C.; Provencher, S.; Bonnet, S.; Maltais, F. Bridging Lung Development with Chronic Obstructive Pulmonary Disease. Relevance of Developmental Pathways in Chronic Obstructive Pulmonary Disease Pathogenesis. *Am. J. Respir. Crit. Care Med.* **2015**, *193*, 362–375, doi:10.1164/rccm.201508-1518PP.
73. Danahay, H.; Pessotti, A. D.; Coote, J.; Montgomery, B. E.; Xia, D.; Wilson, A.; Yang, H.; Wang, Z.; Bevan, L.; Thomas, C.; Petit, S.; London, A.; LeMotte, P.; Doelemeyer, A.; Vélez-Reyes, G. L.; Bernasconi, P.; Fryer, C. J.; Edwards, M.; Capodieci, P.; Chen, A.; Hild, M.; Jaffe, A. B. Notch2 Is Required for Inflammatory Cytokine-Driven Goblet Cell Metaplasia in the Lung. *Cell Rep.* **2015**, *10*, 239–252, doi:10.1016/j.celrep.2014.12.017.
74. Lafkas, D.; Shelton, A.; Chiu, C.; de Leon Boenig, G.; Chen, Y.; Stawicki, S. S.; Siltanen, C.; Reichelt, M.; Zhou, M.; Wu, X.; Eastham-Anderson, J.; Moore, H.; Roose-Girma, M.; Chinn, Y.; Hang, J. Q.; Warming, S.; Egen, J.; Lee, W. P.; Austin, C.; Wu, Y.; Payandeh, J.; Lowe, J. B.; Siebel, C. W. Therapeutic antibodies

reveal Notch control of transdifferentiation in the adult lung. *Nature* **2015**, *528*, 127–131, doi:10.1038/nature15715.

75. Rajavelu, P.; Chen, G.; Xu, Y.; Kitzmiller, J. A.; Korfhagen, T. R.; Whitsett, J. A. Airway epithelial SPDEF integrates goblet cell differentiation and pulmonary Th2 inflammation. *J. Clin. Invest.* **2015**, *125*, 2021–2031, doi:10.1172/JCI79422.
76. Chen, G.; Korfhagen, T. R.; Karp, C. L.; Impey, S.; Xu, Y.; Randell, S. H.; Kitzmiller, J.; Maeda, Y.; Haitchi, H. M.; Sridharan, A.; Senft, A. P.; Whitsett, J. A. Foxa3 Induces Goblet Cell Metaplasia and Inhibits Innate Antiviral Immunity. *Am. J. Respir. Crit. Care Med.* **2014**, *189*, 301–313, doi:10.1164/rccm.201306-1181OC.
77. Park, K.-S.; Korfhagen, T. R.; Bruno, M. D.; Kitzmiller, J. A.; Wan, H.; Wert, S. E.; Khurana Hershey, G. K.; Chen, G.; Whitsett, J. A. SPDEF regulates goblet cell hyperplasia in the airway epithelium. *J. Clin. Invest.* **2007**, *117*, 978–988, doi:10.1172/JCI29176.
78. Song, J.; Heijink, I. H.; Kistemaker, L. E. M.; Reinders-Luinge, M.; Kooistra, W.; Noordhoek, J. A.; Gosens, R.; Brandsma, C. A.; Timens, W.; Hiemstra, P. S.; Rots, M. G.; Hylkema, M. N. Aberrant DNA methylation and expression of SPDEF and FOXA2 in airway epithelium of patients with COPD. *Clin. Epigenetics* **2017**, *9*, 42, doi:10.1186/s13148-017-0341-7.
79. Song, J.; Cano-Rodriquez, D.; Winkle, M.; Gjaltema, R. A. F.; Goubert, D.; Jurkowski, T. P.; Heijink, I. H.; Rots, M. G.; Hylkema, M. N. Targeted epigenetic editing of SPDEF reduces mucus production in lung epithelial cells. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* **2017**, *312*, L334–L347, doi:10.1152/ajplung.00059.2016.
80. Chen, G.; Korfhagen, T. R.; Xu, Y.; Kitzmiller, J.; Wert, S. E.; Maeda, Y.; Gregorieff, A.; Clevers, H.; Whitsett, J. A. SPDEF is required for mouse pulmonary goblet cell differentiation and regulates a network of genes associated with mucus production. *J. Clin. Invest.* **2009**, *119*, 2914–2924, doi:10.1172/JCI39731.
81. Wan, H.; Kaestner, K. H.; Ang, S.-L.; Ikegami, M.; Finkelman, F. D.; Stahlman, M. T.; Fulkerson, P. C.; Rothenberg, M. E.; Whitsett, J. A. Foxa2 regulates alveolarization and goblet cell hyperplasia. *Dev. Camb. Engl.* **2004**, *131*, 953–964, doi:10.1242/dev.00966.
82. Young, H. W. J.; Williams, O. W.; Chandra, D.; Bellinghausen, L. K.; Pérez, G.; Suárez, A.; Tuvim, M. J.; Roy, M. G.; Alexander, S. N.; Moghaddam, S. J.; Adachi, R.; Blackburn, M. R.; Dickey, B. F.; Evans, C. M. Central Role of Muc5ac Expression in Mucous Metaplasia and Its Regulation by Conserved 5′ Elements. *Am. J. Respir. Cell Mol. Biol.* **2007**, *37*, 273–290, doi:10.1165/rcmb.2005-0460OC.
83. Kirkham, S.; Sheehan, J. K.; Knight, D.; Richardson, P. S.; Thornton, D. J. Heterogeneity of airways mucus: variations in the amounts and glycoforms of the major oligomeric mucins MUC5AC and MUC5B. *Biochem. J.* **2002**, *361*, 537–546, doi:10.1042/bj3610537.
84. Welsh, K. G.; Rousseau, K.; Fisher, G.; Bonser, L. R.; Bradding, P.; Brightling, C. E.; Thornton, D. J.; Gaillard, E. A. MUC5AC and a glycosylated variant of MUC5B alter mucin composition in children with acute asthma. *Chest* **2017**, doi:10.1016/j.chest.2017.07.001.
85. Sheehan, J. K.; Richardson, P. S.; Fung, D. C.; Howard, M.; Thornton, D. J. Analysis of respiratory mucus glycoproteins in asthma: a detailed study from a patient who died in status asthmaticus. *Am. J. Respir. Cell Mol. Biol.* **1995**, *13*, 748–756, doi:10.1165/ajrcmb.13.6.7576713.
86. Groneberg, D. A.; Eynott, P. R.; Lim, S.; Oates, T.; Wu, R.; Carlstedt, I.; Roberts, P.; McCann, B.; Nicholson, A. G.; Harrison, B. D.; Chung, K. F. Expression of respiratory mucins in fatal status asthmaticus and mild asthma. *Histopathology* **2002**, *40*, 367–373.
87. Lopez-Vidriero, M. T.; Reid, L. Chemical markers of mucous and serum glycoproteins and their relation to viscosity in mucoid and purulent sputum from various hypersecretory diseases. *Am. Rev. Respir. Dis.* **1978**, *117*, 465–477, doi:10.1164/arrd.1978.117.3.465.
88. Innes, A. L.; Carrington, S. D.; Thornton, D. J.; Kirkham, S.; Rousseau, K.; Dougherty, R. H.; Raymond, W. W.; Caughey, G. H.; Muller, S. J.; Fahy, J. V. Ex Vivo Sputum Analysis Reveals Impairment of Protease-dependent Mucus Degradation by Plasma Proteins in Acute Asthma. *Am. J. Respir. Crit. Care Med.* **2009**, *180*, 203–210, doi:10.1164/rccm.200807-1056OC.
89. Yuan, S.; Hollinger, M.; Lachowicz-Scroggins, M. E.; Kerr, S. C.; Dunican, E. M.; Daniel, B. M.; Ghosh, S.; Erzurum, S. C.; Willard, B.; Hazen, S. L.; Huang, X.; Carrington, S. D.; Oscarson, S.; Fahy, J. V. Oxidation increases mucin polymer cross-links to stiffen airway mucus gels. *Sci. Transl. Med.* **2015**, *7*, 276ra27-276ra27, doi:10.1126/scitranslmed.3010525.
90. Shimura, S.; Andoh, Y.; Haraguchi, M.; Shirato, K. Continuity of airway goblet cells and intraluminal mucus in the airways of patients with bronchial asthma. *Eur. Respir. J.* **1996**, *9*, 1395–1401.

91. Abdullah, L. H.; Evans, J. R.; Wang, T. T.; Ford, A. A.; Makhov, A. M.; Nguyen, K.; Coakley, R. D.; Griffith, J. D.; Davis, C. W.; Ballard, S. T.; Kesimer, M. Defective postsecretory maturation of MUC5B mucin in cystic fibrosis airways. *JCI Insight* **2017**, *2*, e89752, doi:10.1172/jci.insight.89752.
92. Shimura, S.; Sasaki, T.; Sasaki, H.; Takishima, T. Chemical properties of bronchorrhea sputum in bronchial asthma. *Chest* **1988**, *94*, 1211–1215.
93. Kerr, S. C.; Carrington, S. D.; Oscarson, S.; Gallagher, M. E.; Solon, M.; Yuan, S.; Ahn, J. N.; Dougherty, R. H.; Finkbeiner, W. E.; Peters, M. C.; Fahy, J. V. Intelectin-1 Is a Prominent Protein Constituent of Pathologic Mucus Associated with Eosinophilic Airway Inflammation in Asthma. *Am. J. Respir. Crit. Care Med.* **2014**, *189*, 1005–1007, doi:10.1164/rccm.201312-2220LE.
94. Recommendations for guidelines on clinical trials of mucoactive drugs in chronic bronchitis and chronic obstructive pulmonary disease. Task Group on Mucoactive Drugs. *Chest* **1994**, *106*, 1532–1537.
95. Rogers, D. F.; Barnes, P. J. Treatment of airway mucus hypersecretion. *Ann. Med.* **2006**, *38*, 116–125, doi:10.1080/07853890600585795.
96. Sadowska, A. M. N-Acetylcysteine mucolysis in the management of chronic obstructive pulmonary disease. *Ther. Adv. Respir. Dis.* **2012**, *6*, 127–135, doi:10.1177/1753465812437563.
97. Daviskas, E.; Anderson, S. D.; Gonda, I.; Eberl, S.; Meikle, S.; Seale, J. P.; Bautovich, G. Inhalation of hypertonic saline aerosol enhances mucociliary clearance in asthmatic and healthy subjects. *Eur. Respir. J.* **1996**, *9*, 725–732.
98. Cotgreave, I. A.; Eklund, A.; Larsson, K.; Moldéus, P. W. No penetration of orally administered N-acetylcysteine into bronchoalveolar lavage fluid. *Eur. J. Respir. Dis.* **1987**, *70*, 73–77.
99. Shen, Y.; Cai, W.; Lei, S.; Zhang, Z. Effect of high/low dose N-acetylcysteine on chronic obstructive pulmonary disease: a systematic review and meta-analysis. *COPD* **2014**, *11*, 351–358, doi:10.3109/15412555.2013.858315.
100. Dano, G. Bronchospasm caused by acetylcysteine in children with bronchial asthma. *Acta Allergol.* **1971**, *26*, 181–190.
101. Gillissen, A.; Jaworska, M.; Orth, M.; Coffiner, M.; Maes, P.; App, E. M.; Cantin, A. M.; Schultze-Werninghaus, G. Nacystelyn, a novel lysine salt of N-acetylcysteine, to augment cellular antioxidant defence in vitro. *Respir. Med.* **1997**, *91*, 159–168.
102. Suk, J. S.; Boylan, N. J.; Trehan, K.; Tang, B. C.; Schneider, C. S.; Lin, J.-M. G.; Boyle, M. P.; Zeitlin, P. L.; Lai, S. K.; Cooper, M. J.; Hanes, J. N-acetylcysteine enhances cystic fibrosis sputum penetration and airway gene transfer by highly compacted DNA nanoparticles. *Mol. Ther. J. Am. Soc. Gene Ther.* **2011**, *19*, 1981–1989, doi:10.1038/mt.2011.160.
103. Rao, S.; Wilson, D. B.; Brooks, R. C.; Sproule, B. J. Acute effects of nebulization of N-acetylcysteine on pulmonary mechanics and gas exchange. *Am. Rev. Respir. Dis.* **1970**, *102*, 17–22, doi:10.1164/arrd.1970.102.1.17.
104. Fuchs, H. J.; Borowitz, D. S.; Christiansen, D. H.; Morris, E. M.; Nash, M. L.; Ramsey, B. W.; Rosenstein, B. J.; Smith, A. L.; Wohl, M. E. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. *N. Engl. J. Med.* **1994**, *331*, 637–642, doi:10.1056/NEJM199409083311003.
105. Laube, B. L.; Auci, R. M.; Shields, D. E.; Christiansen, D. H.; Lucas, M. K.; Fuchs, H. J.; Rosenstein, B. J. Effect of rhDNase on airflow obstruction and mucociliary clearance in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* **1996**, *153*, 752–760, doi:10.1164/ajrccm.153.2.8564129.
106. Corren, J.; Lemanske, R. F. J.; Hanania, N. A.; Korenblat, P. E.; Parsey, M. V.; Arron, J. R.; Harris, J. M.; Scheerens, H.; Wu, L. C.; Su, Z.; Mosesova, S.; Eisner, M. D.; Bohen, S. P.; Matthews, J. G. Lebrikizumab Treatment in Adults with Asthma. *N. Engl. J. Med.* **2011**, *365*, 1088–1098, doi:10.1056/NEJMoa1106469.
107. Hanania, N. A.; Korenblat, P.; Chapman, K. R.; Bateman, E. D.; Kopecky, P.; Paggiaro, P.; Yokoyama, A.; Olsson, J.; Gray, S.; Holweg, C. T. J.; Eisner, M.; Asare, C.; Fischer, S. K.; Peng, K.; Putnam, W. S.; Matthews, J. G. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir. Med.* **2016**, *4*, 781–796, doi:10.1016/S2213-2600(16)30265-X.
108. Corren, J.; Parnes, J. R.; Wang, L.; Mo, M.; Roseti, S. L.; Griffiths, J. M.; van der Merwe, R. Tezepelumab in Adults with Uncontrolled Asthma. *N. Engl. J. Med.* **2017**, *377*, 936–946, doi:10.1056/NEJMoa1704064.
109. Rogers, D. F. Mucoactive drugs for asthma and COPD: any place in therapy? *Expert Opin. Investig. Drugs* **2002**, *11*, 15–35, doi:10.1517/13543784.11.1.15.