Respiratory Tract Mucin Genes and Mucin Glycoproteins in Health and Disease

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Rose, Mary Callaghan, and Judith A. Voynow. Respiratory Tract Mucin Genes and Mucin Glycoproteins in Health and Disease. Physiol Rev 86: 245–278, 2006; doi:10.1152/physrev.00010.2005.—This review focuses on the role and regulation of mucin glycoproteins (mucins) in airway health and disease. Mucins are highly glycosylated macromolecules (>50% carbohydrate, wt/wt). MUC protein backbones are characterized by numerous tandem repeats that contain proline and are high in serine and/or threonine residues, the sites of O-glycosylation. Secretory and membrane-tethered mucins contribute to mucociliary defense, an innate immune defense system that protects the airways against pathogens and environmental toxins. Inflammatory/immune response mediators and the overproduction of mucus characterize chronic airway diseases: asthma, chronic obstructive pulmonary diseases (COPD), or cystic fibrosis (CF). Specific inflammatory/immune response mediators can activate mucin gene regulation and airway remodeling, including goblet cell hyperplasia (GCH). These processes sustain airway mucin overproduction and contribute to airway obstruction by mucus and therefore to the high morbidity and mortality associated with these diseases. Importantly, mucin overproduction and GCH, although linked, are not synonymous and may follow from different signaling and gene regulatory pathways. In section 1, structure, expression, and localization of the 18 human MUC genes and MUC gene products having tandem repeat domains and the specificity and application of MUC-specific antibodies that identify mucin gene products in airway tissues, cells, and secretions are overviewed. Mucin overproduction in chronic airway diseases and secretory cell metaplasia in animal model systems are reviewed in section 2 and addressed in disease-specific subsections on asthma, COPD, and CF. Information on regulation of mucin genes by inflammatory/immune response mediators is summarized in section 3. In section 4, deficiencies in understanding the functional roles of mucins at the molecular level are identified as areas for further investigations that will impact on airway health and disease. The underlying premise is that understanding the pathways and processes that lead to mucus overproduction in specific airway diseases will allow circumvention or amelioration of these processes.
I. INTRODUCTION

A. Overview

The apical epithelial surfaces of mammalian respiratory, gastrointestinal, and reproductive tracts are coated by mucus, a mixture of water, ions, glycoproteins, proteins, and lipids. Mucosal components are secreted apically by goblet cells in polarized epithelium and by secretory cells in the submucosal glands (SMG). Mucus provides a protective barrier against pathogens and toxins and contributes to the innate defensive system in mucosal immunology (54).

Mucin glycoproteins (mucins) are the major macromolecular constituents of epithelial mucus and have long been implicated in health and disease. Mucins historically are large, highly glycosylated, viscoelastic macromolecules that are difficult to isolate and purify (218). The application of recombinant DNA technology to the mucin field resulted in the cloning of MUC genes that encode mucin protein backbones.1 This enlarged the definition of mucins to include membrane-tethered, as well as secreted, mucins. The development of molecular tools to identify mucins by their MUC protein backbone enabled studies on the expression and localization of MUC gene products in tissues, cells, and secretions. Currently, investigations on specific mucins and their roles in diseases, mucosal defense, and epithelial repair/remodeling are under way in many laboratories. Mucins implicated in cancer (110), lung diseases (153, 286), gastrointestinal diseases (53, 70), as well as mucins expressed in the eye (90) and ear (164), have recently been reviewed.

This review focuses on mucins in airway health and disease, which are expressed in the conducting airways (trachea, bronchi, bronchioles) of the lower respiratory tract. Airway mucins are major components of the soluble layer and/or viscoelastic gel that comprise lung mucus in healthy airways and contribute to the mucociliary defense system that protects the lungs against pathogens and environmental toxins (219). Mucin production entails several biological processes (Fig. 1), as the biosynthesis of even the simplest mature glycosylated mucin requires transcription of a MUC gene to encode a MUC mRNA, which is then translated into a MUC protein backbone that is posttranslationally modified by one or several glycosyltransferases (GT). Secretory mucins are stored in secretory granules and released at the apical surface in response to mucin secretagogues, while membrane-tethered mucins are integrated into the cell membrane.

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1 Abbreviations for human mucin genes and gene products follow the conventions in the Unigene database where human genes are capitalized and italicized, gene products are capitalized but not italicized, and numbers are not prefaced by a dash or space.
Mucins and mucins are overproduced in the airways of patients with chronic airway diseases, which greatly contributes to airway obstruction in patients with asthma, chronic obstructive pulmonary diseases (COPD), or cystic fibrosis (CF) (21, 123, 216, 224, 283). These diseases are characterized by inflammatory/immune response mediators, which are released into airway tissue and airway fluid/secretions. Some mediators can regulate MUC gene expression and/or stability, as well as mucin secretion (2, 47). However, the mechanisms that regulate mucin secretion are independent of the signal transduction mechanisms that lead to upregulation of MUC gene expression and thus increased mucin biosynthesis, and this has been well and succinctly overviewed (2). Mucin secretion in vivo is typically initiated by a secretagogue and is rapid, occurring within seconds to minutes (58, 282), in contrast to MUC gene regulation, which requires minutes to hours (155), and mucin biosynthesis, which requires 6–24 h (185). The term mucin hypersecretion is sometimes used to encompass all the processes that contribute to mucin overproduction in chronic airway diseases, rather than to describe a secretagogue-initiated secretory response. An increased understanding of the molecular mechanisms that regulate mucin secretion is evolving (159). However, secretion/hypersecretion is not covered herein; readers are referred to recent reviews on this topic (58, 129).

In section I, current information on the structure and properties of mucins, especially those expressed in the lower respiratory tract, as well as molecular tools used to identify MUC gene products in tissues, cells, and secretions, is presented. Information on mucin overproduction in chronic airway diseases is overviewed in section II and then addressed in disease-specific subsections on asthma, chronic bronchitis, and CF. Upregulation of MUC genes and goblet cell hyperplasia (GCH) are fundamental processes that contribute to and sustain mucin overproduction in the airways of patients with chronic lung diseases. Information on GCH and secretory cell metaplasia in animal models of airway diseases is integrated into each disease-specific subsection in section II, together with pertinent information on mucin gene regulation. However, the rapid increase in information on this latter topic warranted a separate section on “regulation of mucin genes” in section III. These studies have typically been carried out in airway epithelial cancer cell lines and/or primary normal human bronchial epithelial (NHBE) cells. When grown on an extracellular matrix and maintained at an air-liquid interface (ALI), NHBE cells differentiate to morphologically mimic conducting airway epithelium (reviewed in Refs. 2, 305), thus providing a useful model system for airway studies. A brief summary and overview of future directions for research in lung mucin biology are provided in section IV.

B. Mucin Glycoproteins

1. Mucin classification

Mucins are complex glycoproteins synthesized in epithelial cells and typically characterized by large molecular weight (2–20 × 10^5 Da), high carbohydrate content (50–90% by weight) reflecting a large number of O-glycans, and an extensive number of tandem repeats (TR) in the protein backbone (Fig. 2). The TR domains are the characteristic feature that distinguishes mucins from other glycoproteins, especially from membrane-bound glycoproteins/receptors that have extracellular regions high in serine, threonine, and proline and may be members of the immunoglobulin superfamily. Mucins are classified by their MUC protein backbone, which is encoded by a MUC gene. MUC genes are localized to chromosomes 1, 3, 4, 7, 11, 12, and 19 (Table I). MUC gene products vary in size. MUC transcripts range from 1.1 to >15 kb, while MUC proteins, which account for 10–50% (wt/wt) of mucin mass, have 377 to ≥11,000 amino acids in their protein backbones.

More than 20 human and murine mucin genes are deposited in GenBank. However, human MUC and rodent Muc genes are not always distinguished and the question of whether a macromolecule requires TR or only a significant amount of O-glycosylation sites for classification as a mucin needs to be resolved. For example, Muc10 is a murine gene and the ortholog of the human MUC7 gene (62), while Muc14 is a murine gene and the homolog of a gene that encodes human endomucin-1/2 (131).

Additionally, the definition of what determines a mucin gene is not always consistent. Perhaps serine/threonine/proline-rich TR domains (see sect. IB2) should define
a mucin, while a significant amount of serines, threonines, and a large number of O-glycosides would define a mucin-like glycoprotein. Three of the mucin genes in GenBank encode numerous serine and threonine residues, but do not encode TR domains in their protein backbones (Tables 2 and 3). These are MUC14, MUC15, and MUC18 (see above and Tables 2 and 3). If these three macromolecules are considered as serine/threonine-rich glycoproteins rather than mucins, then the present list of human mucins would have 18 members all of which had TR, e.g., MUC1, MUC2, MUC3A, MUC3B, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC9, MUC11, MUC12, MUC13, MUC16, MUC17, MUC18, and MUC20. Clarification of what constitutes a MUC gene product is evolving and should eventually be standardized. This process will be facilitated by the establishment of a mucin website (www.mucin.org), initiated by Dr. Yin Chen while in Dr. Reen Wu’s group at the University of California, Davis.

In addition to TR (see sect. IB2), additional modular motifs are present in the amino and carboxy termini of MUC protein backbones. These allowed classification as membrane-tethered or secretory mucins, with secretory mucins being further subdivided into those that are cysteine rich or cysteine poor (Table 2). MUC1, MUC4, MUC3A, MUC3B, MUC12, MUC13, MUC15, MUC16, MUC17, MUC18, and MUC20 mucins have transmembrane domains in their carboxy terminus and thus are type 1 membrane proteins. MUC2, MUC5AC, MUC5B, and MUC6 are large secretory mucins with cysteine-rich motifs. MUC19 is a secretory cysteine-rich mucin recently identified by in silico cloning and subsequently characterized (46). MUC7 is a small, secreted mucin that lacks cysteine-rich domains. MUC8 is a large, secreted mucin that lacks the von Willebrand factor (VWF) D4 or C1, C2 domains typically present in the carboxy end of large secretory mucins. Descriptions of modular motifs, as well as models of most of the identified MUC proteins, are detailed in two recent reviews (60, 110), to which readers are referred. Depictions of specific membrane-tethered mucins (MUC1 and MUC4), secretory cysteine-rich mucins (MUC2, MUC5AC, and MUC5B), and the secreted non-cysteine-rich mucin MUC7 are shown in Figure 3.

2. MUC protein backbones: TRs

Eighteen of the >20 mucin genes that are deposited in GenBank encode TRs, the exceptions being MUC14, MUC15, and MUC18 (see above and Tables 2 and 3). The tandem repeating nucleotide sequences are typically encoded by a single central exon in a MUC gene. Many MUC genes are polymorphic, with alleles having a variable number of TRs (VNTR) (81), as indicated in Table 3. Additional variations occur with minor changes in the length of the repeat unit (length polymorphisms) and repeating sequence (sequence polymorphisms). Thus different individuals can express different forms of the same MUC gene product. In addition, MUC genes may undergo alternative splicing (183). Changes in VNTR are reported in cancer (reviewed in Ref. 110), ulcerative colitis (143), and respiratory diseases (124).

### Table 1. Chromosomal localization of human mucin genes

<table>
<thead>
<tr>
<th>MUC Gene</th>
<th>Chromosome Locus</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC1</td>
<td>1q21–q24</td>
<td>264</td>
</tr>
<tr>
<td>MUC9</td>
<td>1q13</td>
<td>150</td>
</tr>
<tr>
<td>MUC13</td>
<td>3q13.3</td>
<td>302</td>
</tr>
<tr>
<td>MUC4</td>
<td>3q29</td>
<td>208</td>
</tr>
<tr>
<td>MUC20</td>
<td>3q29</td>
<td>108</td>
</tr>
<tr>
<td>MUC7</td>
<td>4q13.3</td>
<td>23</td>
</tr>
<tr>
<td>MUC3A</td>
<td>7q22</td>
<td>82,100</td>
</tr>
<tr>
<td>MUC3B</td>
<td>7q22</td>
<td>209</td>
</tr>
<tr>
<td>MUC11</td>
<td>7q22</td>
<td>301</td>
</tr>
<tr>
<td>MUC12</td>
<td>7q22</td>
<td>301</td>
</tr>
<tr>
<td>MUC17</td>
<td>7q22</td>
<td>101</td>
</tr>
<tr>
<td>MUC2</td>
<td>11p15.5</td>
<td>93,204</td>
</tr>
<tr>
<td>MUC5AC</td>
<td>11p15.5</td>
<td>179,187,204</td>
</tr>
<tr>
<td>MUC5B</td>
<td>11p15.5</td>
<td>69,204</td>
</tr>
<tr>
<td>MUC6</td>
<td>11p15.5</td>
<td>204,276</td>
</tr>
<tr>
<td>MUC15</td>
<td>11p14.3</td>
<td>197</td>
</tr>
<tr>
<td>MUC18</td>
<td>11q23.3</td>
<td>142</td>
</tr>
<tr>
<td>MUC19</td>
<td>12q12</td>
<td>46</td>
</tr>
<tr>
<td>MUC8</td>
<td>12q24.3</td>
<td>242</td>
</tr>
<tr>
<td>MUC16</td>
<td>19q13.22</td>
<td>307</td>
</tr>
<tr>
<td></td>
<td>19q13.22</td>
<td>192</td>
</tr>
</tbody>
</table>

The chromosomal localization of genes listed as mucin genes in GenBank but which lack tandem repeats, e.g., MUC15 and MUC18, are included.

### Table 2. Classification of human mucins by MUC protein backbone structures

<table>
<thead>
<tr>
<th>Classification</th>
<th>Mucins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane-tethered with TR</td>
<td>MUC1, MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC13, MUC16, MUC17, MUC20</td>
</tr>
<tr>
<td>Secreted, cysteine-rich with TR</td>
<td>MUC6, MUC2, MUC5AC, MUC5B, MUC19</td>
</tr>
<tr>
<td>Secreted, cysteine-poor with TR</td>
<td>MUC7, MUC8, MUC9</td>
</tr>
<tr>
<td>Mucins without TR</td>
<td>MUC14, MUC15, MUC18</td>
</tr>
</tbody>
</table>

Classification of human mucins is as listed in GenBank. TR, tandem repeat.
TRs are unique in sequence and size (Table 3) and thus are the defining characteristic of each MUC protein. Among different mucins, TRs vary in length (5–375 amino acids) and in number (5–395 repeats). Some MUC proteins have two domains with different TR sequences, e.g., MUC2, MUC3A, MUC3B, and MUC5AC. The two major airway mucins, MUC5AC and MUC5B, have TR domains that are repeated four or five times in their protein backbones, respectively, and are separated by cysteine-rich domains (Fig. 3). Each TR contains proline and is rich in serine and/or threonine residues, the sites of O-glycosylation. Thus TRs largely determine a mucin’s degree of glycosylation, as longer or more highly repeated TR sequences can allow for hundreds of O-glycans per molecule (Figs. 2 and 3). MUC5B, for example, contains a repeat of 29 amino acids with a total of 16 serines and threonines. Seventy-two repetitions of the 29 amino acid sequence contribute 1,152 possible O-glycosylation sites to the MUC5B backbone. The number of TRs in a MUC protein also determines mucin size as they elongate the protein backbone and thus increase mucin molecular mass. They also likely contribute to the structural and functional diversity of mucins in ways that are not yet appreciated at the molecular or physiological level. For example, the TR domains of MUC2 are uninterrupted by cysteine-rich domains, in contrast to MUC5AC and MUC5B (Fig. 3). This perhaps reflects the need for MUC5AC/B to couple to the mucociliary escalator in the airways, whereas MUC2 may need to be more rigid to better protect the intestinal epithelium against proteolytic attack. Indeed, MUC2 is considered an insoluble mucin biochemically (10).

TRs are the primary domains to which O-glycans are attached, and O-glycans can alter mucin conformation. This has been shown for ovine submaxillary mucin, which undergoes a change from an extended filament to a globular form following enzymatic removal of the disaccharide O-glycans (228). Airway mucins typically present as flexible filamentous structures (227, 247), which on aggregation form a large interwoven network (227) quite different from the massive ropelike structures characteristic of sheep submaxillary mucin aggregates (50). Thus TR and their attached O-glycans influence the size, shape, and mass of mucins, thereby contributing to the biophysical and biological properties of mucus itself. For example, larger, bulkier, heavier mucins might result in more viscous mucus. Future studies should reveal the molecular details wherein MUC-specific TR contribute to the biochemical and physical properties of mucins.

TR domains are typically not conserved between human MUC and rodent Muc genes, whereas the non-TR motifs at the amino- and carboxy-terminal domains of many mucins are typically highly conserved between species, as first shown for MUC1/Muc1 (259).

3. Mucin glycosylation

Like mucins, many macromolecules, including cell adhesion markers and selectin ligands, have O-glycans attached to serine or threonine residues in their protein backbone (60, 219). However, mucins are 50–90% carbohydrate by mass and have numerous (dozens to several hundred) O-glycosylation sites per molecule due to the high number of TR in each MUC protein backbone (Table 3). Studies to determine and/or predict which serines or threonines in TRs or in mucins are O-glycosylated are challenging (88).

O-glycosylation is a major part of mucin biosynthesis and requires an N-acetylgalactosaminyl peptidyltransferase and one or more GTs depending on the final O-
glycan structure. O-glycosylation is initiated in the Golgi when an N-acetylgalactosaminyl peptidyltransferase transfers N-acetylgalactosamine (GalNAc) to a serine or threonine when a nascent MUC polypeptide chain traverses the Golgi, as depicted in Figure 1. Each O-glycan is then elongated by the stepwise addition of hexoses [galactose (Gal), N-acetylglucosamine (GlcNAc), fucose] or sialic acid by specific GT (33, 235). More than 30 GT have now been identified, of which at least a dozen participate in the synthesis of mucin O-glycans (34).

### Table 3. Tandem repeat (TR) sequences of MUC proteins

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC1</td>
<td>20</td>
<td>GSPAPPKGVTSAPDDRPAP</td>
<td>83, 84</td>
<td>21-125; 41 and 85 are most common</td>
</tr>
<tr>
<td>MUC2</td>
<td>23</td>
<td>PPITTTPSSPPTSTTTL</td>
<td>102, 200</td>
<td>20</td>
</tr>
<tr>
<td>MUC3A</td>
<td>17</td>
<td>TTTTETTSHSTPSFTSS</td>
<td>375</td>
<td>102, 200</td>
</tr>
<tr>
<td>MUC3B</td>
<td>17</td>
<td>ISETETTSHSTPSFTSS</td>
<td>375</td>
<td>102, 200</td>
</tr>
<tr>
<td>MUC4</td>
<td>16</td>
<td>ATPLPVIDTDSSASTGH</td>
<td>208</td>
<td>145-395</td>
</tr>
<tr>
<td>MUC5AC†</td>
<td>8</td>
<td>TSTITSSAP</td>
<td>73</td>
<td>(124, 17, 34, 66)†</td>
</tr>
<tr>
<td>MUC5B†</td>
<td>17</td>
<td>GTPPSGTMTATGQT</td>
<td>169</td>
<td>15-26</td>
</tr>
<tr>
<td>MUC6</td>
<td>16</td>
<td>ATPLPVIDTDSSASTGH</td>
<td>208</td>
<td>145-395</td>
</tr>
<tr>
<td>MUC7</td>
<td>13</td>
<td>TTPSPSPPPSPPTTTPPPPVT</td>
<td>276</td>
<td>15-26</td>
</tr>
<tr>
<td>MUC8</td>
<td>15</td>
<td>GSPPGTMTATGQT</td>
<td>169</td>
<td>15-26</td>
</tr>
<tr>
<td>MUC9</td>
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<td>15-26</td>
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<td>GSPPGTMTATGQT</td>
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</tr>
<tr>
<td>MUC11</td>
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<td>TTPSPSPPPSPPTTTPPPPVT</td>
<td>276</td>
<td>15-26</td>
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<tr>
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<td>GSPPGTMTATGQT</td>
<td>169</td>
<td>15-26</td>
</tr>
<tr>
<td>MUC13</td>
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<td>GSPPGTMTATGQT</td>
<td>169</td>
<td>15-26</td>
</tr>
<tr>
<td>MUC14</td>
<td>16</td>
<td>ATPLPVIDTDSSASTGH</td>
<td>208</td>
<td>145-395</td>
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<tr>
<td>MUC15</td>
<td>15</td>
<td>GSPPGTMTATGQT</td>
<td>169</td>
<td>15-26</td>
</tr>
<tr>
<td>MUC16</td>
<td>15</td>
<td>GSPPGTMTATGQT</td>
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<td>15-26</td>
</tr>
<tr>
<td>MUC17</td>
<td>15</td>
<td>GSPPGTMTATGQT</td>
<td>169</td>
<td>15-26</td>
</tr>
<tr>
<td>MUC18</td>
<td>15</td>
<td>GSPPGTMTATGQT</td>
<td>169</td>
<td>15-26</td>
</tr>
<tr>
<td>MUC19</td>
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<td>GSPPGTMTATGQT</td>
<td>169</td>
<td>15-26</td>
</tr>
<tr>
<td>MUC20</td>
<td>15</td>
<td>GSPPGTMTATGQT</td>
<td>169</td>
<td>15-26</td>
</tr>
</tbody>
</table>

*For MUC genes that exhibit VNTR, this number is reported as a range. † The number n of TR is different in specific regions of MUC5AC and MUC5B, as indicated in Fig. 3. ‡ The full DNA sequence for some mucins is not yet reported. The number of times a TR is present reflects data reported by mid 2005. § Repeats are degenerate. The sequence of the first repeat is listed here.
Four major core structures (Fig. 4) have been identified in O-glycans isolated from mucins (114). The transfer of Gal to C-3 of GalNAc yields a structure that will be core type 1 or 2. The next transferase can either add to Gal (elongation of core 1) or to GalNAc to form a branch (core 2). Similarly, GlcNAc can be added to GalNAc to form core 3, which can be branched to form core 4. Core structures can then be elongated by Gal and GlcNAc transferases to form Galβ1,3/4GlcNAc units, which can be terminated by blood group determinants, sialic acid, sulfate, or fucose. Sialic acid moieties and sulfates on Gal or GlcNAc moieties impart negative charges to mucins, which account for their low pI values, whereas fucose imparts hydrophobicity. Terminal sugars, because of their hydrophobicity or charge, are thought to contribute to or determine the physical and/or biological properties of mucins. Thus alterations in terminal glycosylation of mucins, which may occur in disease states (see sect. uD4), have the potential to alter the physical properties of mucins and the rheological properties of mucus.

Mucin glycosylation, like MUC gene expression, may have restricted tissue or cell expression, and the core structures of mucin O-glycans may be altered in disease (219). For example, O-glycans isolated from MUC1 mucin synthesized in vitro by breast cells from healthy women are predominantly core type 2, while those from breast cancer cell lines have core type 1 O-glycans (117). However, information even as to which cores are attached to mucins of known MUC backbones, let alone which specific serine or threonine residues, is still very limited. For example, O-glycans of mucins purified from normal colon tissue are predominantly core 3 structures with complex branching. In contrast, O-glycans from colon cancer cell lines have increases in short O-glycans (Tn antigen, TF antigen, and sialyl Tn), decreases in O-acetyl- and sialic acid and sulfation, and increases in sialyl LeX structures (40). However, several mucins are expressed in the colon, and information describing which O-glycans are attached to specific MUC protein backbone(s) has not been reported. Mucins of specific MUC backbones may be amenable to purification by affinity chromatography with MUC-specific antibodies.

A similar situation prevails for airway mucins. Over the last 30 years, several dozen O-glycans have been cleaved from mucins isolated from sputum from patients with airway disease and their structures determined by mass spectrometry or NMR (147). The four major core types in specific O-glycan structures (Fig. 4) have been identified in mucins from patients with airway diseases (278), whereas structural information from mucins isolated from healthy airways has not been reported. The identities of the MUC mucins to which specific O-glycans are attached are unknown, as O-glycans were cleaved from mucin-rich fractions. Thus it is not yet established whether the complexity of O-glycan structures reflects altered levels of specific mucins in airway secretions and/or alterations in glycosylation of specific MUC proteins in disease or inflammation (see sect. uD4). To add to the complexity, more than one core type O-glycan may be attached to a single MUC backbone, especially in mucins that have more than one TR type domain. A comprehensive analysis of O-glycan structures of specific MUC protein backbones will prove useful in determining whether specific mucins (defined both by MUC backbone and O-glycan core structures) are altered in airway diseases consequent to inflammation and/or in a recessive genetic disease, like CF (148).

While O-glycosylation is predominant in mucins, several MUC proteins contain one or more sites with the Asn-X-Ser/Thr sequence requisite for N-glycosylation. N-
glycosylation typically occurs cotranslationally in the ER; however, chemical identification of N-glycosides isolated from mucins secreted in vivo or in vitro has not been reported. Recently, a newly characterized linkage of C-mannose to tryptophan was identified in MUC5AC and MUC5B mucins synthesized in vitro (202). Low levels of mannose have occasionally been reported in secreted mucins, but they have generally been considered a contaminant. However, if mannose reflects a C-mannose linkage in airway mucins synthesized in vivo, these short glycans may contribute to the biological or physical properties of airway mucins.

C. Molecular Tools for MUC Identification

Mucin gene products are identified by nucleotide probes to specific MUC mRNA sequences or by antibodies that recognize specific MUC protein determinants. The sequence of the TR domains of each MUC gene product is unique (Table 3), which facilitated the use of MUC-specific oligonucleotide probes to evaluate MUC mRNA localization and expression in lower respiratory tract epithelial cells (8, 36, 212).

Antibodies raised against cognate sequences of MUC TR domains have been used in immunocytochemical studies to localize MUC protein expression, although antigen retrieval is generally required. However, these antibodies often do not detect mucins on Western blot analyses or ELISA, presumably because extensive O-glycosylation masks the TR epitopes and/or blocks accessibility of antibodies. In contrast, polyclonal antibodies raised against cognate sequences in the non-TR domains of secreted mucins identify mature, i.e., glycosylated, mucins in epithelial secretions by Western blot analyses (225, 271). MUC-specific polyclonal antibodies raised against cognate sequences in the cysteine-rich domains of MUC5AC (18, 225, 271), MUC2 (116), and MUC5B (273) recognize mature secreted airway mucins in epithelial secretions. Monoclonal antibodies raised against sequences in the non-TR domain of MUC5B and MUC7 and assayed in salivary secretions have recently been reported (230). Information on MUC-specific polyclonal or monoclonal antibodies used to investigate human MUC or rodent Muc mucins in respiratory tract cells or secretions is provided in Table 4. Detailed information on monoclonal antibodies used to investigate mucin expression in epithelial tissues and in cancer studies is available (304).

D. Mucin Expression in Epithelial Tissues/Cells and Airway Cells

Mucin genes are expressed in most epithelial tissues (85, 287). MUC1 is a pan-epithelial membrane-tethered mucin expressed at the cell surface in numerous epithelial tissues, as well as in hematopoietic tissues (85). Expression of all other mucin genes is somewhat, but not exclusively, restricted in healthy tissues (reviewed in Ref. 222) and can be altered in diseases, especially in cancer (reviewed in Ref. 110). Secreted mucins are typically markers for goblet cells in the surface epithelium and glandular cells in the respiratory, salivary, gastrointestinal, and reproductive tracts. MUC genes are differentially expressed in goblet cells in diverse epithelial tissues. MUC5AC is the predominant mucin normally expressed in goblet cells in the lung, eyes, and stomach, while MUC2 is expressed in intestinal goblet cells. MUC5B and MUC19 mucins are typically restricted to glandular cells.

In the lower respiratory tract, expression of at least 12 human mucin genes (MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8, MUC11, MUC13, MUC15, MUC19, and MUC20) have been observed at the mRNA level in tissues from healthy individuals. This is reviewed or described in the following references: MUC1–MUC8 (212, 222), MUC11 (153), MUC13 (302), MUC15 (197), MUC19 (46), and MUC20 (108). In situ hybridization studies show that ciliated and basal cells in the surface epithelium of the lower (8, 212) and upper (9) respiratory tract, as well as goblet cells and SMG secretory cells, can express MUC genes.

In airway tissues from healthy individuals, goblet cells typically express MUC5AC mRNA or protein, while glandular mucosal cells express MUC5B and MUC8 (reviewed in Ref. 222) and MUC19 mRNA (46). MUC5B mRNA, which is typically expressed only in mucosal cells of SMG and not in goblet cells in tissues from normal adults, is also expressed in glandular neck cells and some goblet cells during midtrimester fetal development (212). Current thinking is that expression of MUC5B gene products in goblet cells in human adult lower respiratory tract epithelium is atypical and may be a marker of airway diseases (44). However, MUC5B mucin has also been reported in goblet cells in normal lung tissues from patients who died without pulmonary involvement (95, 96). These discrepancies may reflect regional variations within the airways and will require more attention to the locus from which tissues are obtained, as well as systematic morphometric analyses.

Interestingly, MUC7 mucin, which is well-expressed in mucosal and serosal cells in salivary glands (205), is typically localized to a subset of serous cells in SMG, but only in 15–20% of airway tissue from normal individuals (245). Studies on MUC gene expression in tissues from patients with airway diseases are limited in number and overviewed in section II.

Mucin expression in the in vitro NHBE and NHNE model cell systems has also been evaluated in cells derived from healthy airway tissues (19). Upregulation of MUC3, MUC4, MUC5AC, MUC5B, and MUC6 mRNA occurs during differentiation of NHBE cells. MUC1, MUC2, MUC7, and
MUC8 mRNAs are also expressed but do not appear to be strongly regulated as a function of differentiation. Expression of MUC5B mRNA is localized to goblet cells in these differentiated NHBE cell cultures that lack SMG (44). MUC5B expression in vitro thus differs from expression in vivo in adult airway tissue, but is similar to expression patterns observed during development where MUC5B is expressed in goblet, as well as glandular, cells (212).

### TABLE 4. MUC-specific antibodies and airway mucin expression analyses

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Type</th>
<th>Immunogen</th>
<th>Species</th>
<th>Reactivity</th>
<th>Applications</th>
<th>Reference Nos.</th>
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<td>CT1</td>
<td>Rabbit pAb</td>
<td>Last 17 AA of cytoplasmic domain: SSSLTYNPAVAATSANL</td>
<td>Human</td>
<td>W, IHC, IP</td>
<td>163, 201</td>
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<tr>
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<td>CT2</td>
<td>Hamster mAb</td>
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<td>Human</td>
<td>W, IHC, IP</td>
<td>191</td>
<td></td>
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<td></td>
<td>139H2</td>
<td>Mouse mAb</td>
<td>Core protein backbone</td>
<td>Human</td>
<td>IHC</td>
<td>186</td>
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<tr>
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<td>GP1.4*</td>
<td>Mouse mAb</td>
<td>Extracellular domain</td>
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<td>163</td>
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<tr>
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<td>C-20*</td>
<td>Goat pAb</td>
<td>COOH terminus</td>
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<td>92</td>
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<td>Mouse mAb</td>
<td>APDTR</td>
<td>Human</td>
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<tr>
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<td>MUC2</td>
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<td>NGLQPVREDPDGC</td>
<td>Human</td>
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<td>Tandem repeat: HSTPSFTSSITTETTSHSTPSFTSSITTETTS</td>
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<td>MUC4</td>
<td>ASGP-2</td>
<td>Rabbit pAb</td>
<td>ASGP-2 (rat MUC4β)</td>
<td>Rat</td>
<td>W, IHC</td>
<td>178</td>
<td></td>
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<td>LUM4</td>
<td>Rabbit pAb</td>
<td>Tandem repeat</td>
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<td>W</td>
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<td>Mouse mAb</td>
<td>MUC4β</td>
<td>Human, mouse, rat</td>
<td>W, IHC, ELISA</td>
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<td></td>
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<tr>
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<td>MUC4TR</td>
<td>Rabbit pAb</td>
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<td>Human</td>
<td>IHC</td>
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<td>4F12*</td>
<td>Mouse mAb</td>
<td>Rat Muc4</td>
<td>Rat &gt; mouse &gt; human</td>
<td>W, IP, IHC</td>
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<td>Rabbit pAb</td>
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<td>W, IHC, ELISA</td>
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<td>1–13M1*</td>
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<td>Gastric mucin M1 protein</td>
<td>Human</td>
<td>W</td>
<td>107,271</td>
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<tr>
<td></td>
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<td>300</td>
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<tr>
<td></td>
<td>MAN-5AC</td>
<td>Rabbit pAb</td>
<td>Non-tandem repeat, Cys-rich domain</td>
<td>Human</td>
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<td>300</td>
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<td>RGMH</td>
<td>Chicken pAb</td>
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<td>Mouse</td>
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<td>Rabbit pAb</td>
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<td>Human</td>
<td>W, IHC</td>
<td>107,300</td>
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<td>Mouse mAb</td>
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<td>Human</td>
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<td>PAN3</td>
<td>Mouse mAb</td>
<td>NH₂ terminus: CRPRLPSPNPKFPNPNHQP</td>
<td>Human</td>
<td>W, IHC</td>
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<tr>
<td></td>
<td>MUC8</td>
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<td>MUC13</td>
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<td>Anti- MUC8</td>
<td>Rabbit pAb</td>
<td>Cytoplasmic tail peptide: CMQNPSRHHSSMPDDY</td>
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<td>IHC, W</td>
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<td></td>
</tr>
</tbody>
</table>

IHC, Immunohistochemistry; W, Western blotting; ELISA, enzyme-linked immunosorbant assay; IP, immunoprecipitation. * Commercially available antibodies.
E. Mucins in Airway Secretions/Lung Mucus

Isolation and characterization of lung mucins have traditionally utilized the copious amounts of sputum from patients with airway diseases. However, comparative studies of mucins in healthy and diseased airways require procurement of airway secretions or mucus from individuals without airway disease. Healthy individuals produce much smaller amounts of airway secretions and do not expectorate mucus, but mucus samples from normal airways can be obtained following a standardized protocol of hypertonic saline exposure (270). Mucus samples can also be obtained by endotracheal suctioning of patients without airway diseases who are undergoing surgical procedures (231).

Identifying specific mucins in airway samples is now feasible because of the availability of MUC-specific antibodies. The large, cysteine-rich, gel-forming mucins MUC5AC (116, 194, 271), MUC5B (273), and MUC2 (116) mucins have been identified immunochemically in induced mucus or bronchial washings from normal airways, while MUC5AC (179, 194, 223, 271), MUC5B (57, 273, 300), MUC8 (241, 242), and MUC2 (57) mucins have been identified in sputum samples or bronchial washings from patients with chronic airway diseases.

Comparative analyses of mucin levels are more challenging than simply identifying specific mucins because of the inherent difficulties in solubilizing mucus or sputum samples (218) and in determining the best method (weight, volume, or protein) of normalizing mucin levels. The limited number of studies carried out are summarized below and presented in more detail in the disease-specific subsections in section II. Briefly, MUC5AC and MUC5B appear to be present at lower levels in mucus from non-diseased airways than in sputum from patients with asthma, chronic bronchitis, or CF (133). In contrast, in a recent study in which mucins are normalized on a weight basis, MUC5AC and MUC5B mucin levels are decreased in CF sputum compared with normal mucus (107). MUC2 mucin is detected only at very low levels in airway samples from control and diseased airways (116, 133), which is somewhat surprising as the MUC2 gene is upregulated in vitro by inflammatory mediators present in the airway secretions of patients with chronic lung disease (see sect. mB1). As indicated above, this may reflect the less soluble nature of MUC2 mucin.

Other mucins may be present in airway secretions, as 12 mucins have been identified at the mRNA level in the lower respiratory tract (see sect. ID). We have recently shown that MUC7 mucin is expressed in the airway secretions of most asthmatic pediatric patients but is absent in nonasthmatic pediatric patients (296). Neither levels of MUC19, a recently identified gel-forming mucin localized to mucosal cells in SMG (46), nor of MUC8, a mucin typically expressed in mucosal cells of SMG (242), have been evaluated in normal or diseased airway tissues or secretions. The question of whether extracellular glycopeptide fragments of membrane-tethered mucins are cleaved by proteases and thus present in the mucosal layer of inflamed or infected airways has also not yet been evaluated.

Levels of MUC5B and MUC5AC mucin have also been determined in secretions from differentiated NHBE cells. MUC5B mucins are present at greater than 10-fold higher levels than MUC5AC mucins (111), in agreement with the higher level of MUC5B mRNA expression in vitro (19).

F. Comparative Biology of Mucin Gene Expression in the Respiratory Tract

Respiratory tract expression of mucin genes in nonhuman species has been investigated mainly in the context of perturbations during disease, especially murine models. This is overviewed briefly below, and addressed in more detail in subsections in section II on asthma, COPD, and CF.

Normal, e.g., naive, murine lungs express membrane-tethered mucins. Muc1 protein (201) and Muc4 mRNA (63) have been identified. The glandular secretory mucins Muc5b (48) and Muc19 (46) have been localized to the mRNA level to the few SMG located in the proximal trachea of some murine strains (67). In contrast to human airways, only rare superficial epithelial cells express Muc5ac mRNA in naive airway epithelium (55). However, following allergen sensitization and exposure, Muc5ac mRNA is well-expressed (4) and Muc5ac mRNA/protein is localized to allergen-induced goblet cells in the surface airway epithelium (239). Additionally, mucin gene products typically localized to murine salivary glands, e.g., Muc5b or Muc10, are expressed in murine airway goblet cells induced following allergen sensitization by ovalbumin (OVA) (48) or interleukin (IL)-13 exposure (220), respectively.

Similar to human airways, rat lungs have been reported to express Muc1 protein (201), Muc2 mRNA (193), and Muc4 protein (178) in their conducting airway epithelium. Muc5ac protein/mRNA is expressed in rat goblet cells (103, 118). Expression of other Muc genes has not been evaluated in rat, as far as we are aware.

To date, there are little data on mucin expression in other species due to the limited number of Muc clones yet identified. The Muc1 gene is expressed in hamster lung (198), rhesus lung (GenBank accession no. AF176947), and across many other mammalian species (201). The Muc2 gene is expressed in guinea pig (160) and monkey (6) lungs. The Muc5ac gene is expressed in guinea pig (160) and horse (87) lungs. As more mammalian orthologs of human respiratory tract mucin genes are identified and characterized, non-murine animal models of chronic in-

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Inflammatory airway disease will be useful for evaluating mucin gene regulation and secretory cell remodeling.

II. MUCIN OVERPRODUCTION IN CHRONIC AIRWAY DISEASES

A. Overview

Mucin glycoproteins function as part of the normal mucociliary clearance and contribute to the innate immune system in the respiratory tract. Acute challenges to the respiratory tract, including environmental toxins, allergens, or infectious pathogens, activate lung inflammatory/immune response mediators. Some mediators initiate mucin hypersecretion by activating a secretory cascade that results in the rapid release (within minutes) of mucins from secretory granules of goblet cells in the surface epithelium and/or goblet cells in the SMG (Fig. 5). These hypersecreted mucins contribute to the innate mucosal defense system by protecting the airway epithelium in ways that are not yet well understood at the molecular level. They may entrap particles in the viscoelastic mucus gel, acting perhaps in conjunction with tethered mucins at the apical surface of epithelial cells (110), or bind receptors on inflammatory cells using motifs in specific MUC domains or in O-glycans. For example, the blood group Le⁴ oligosaccharides associated with MUC5AC gastric mucin are the primary receptor for *Helicobacter pylori* in the human stomach (277). Functional studies on bacterial interactions with specific O-glycan epitopes in airway mucins have not yet been carried out.

Sustained mucin secretion, e.g., hypersecretion, requires increased biosynthesis of mucins to replenish secretory granules, which in turn necessitates upregulation of MUC genes, as well as increased activity of GT or upregulation of GT genes (Fig. 5). The stimuli for upregulation of MUC genes during acute infection are presumably specific inflammatory/immune response mediators that are elicited in response to pathogens or environmental toxins, as such mediators upregulate expression of MUC genes in vitro (see sect. iii). The ability of inflammatory mediators to increase expression or activity of GT and sulfotransferases, enzymes required for mucin biosynthesis (Fig. 1), has been demonstrated (61, 306), but is far less well studied. Mucin hypersecretion and overproduction typically revert to baseline levels within days, presumably in response to anti-inflammatory mechanisms that abrogate proinflammatory mediators and restore homeostasis to the respiratory tract (80).

Airway mucins are overproduced by patients with chronic airway diseases like asthma, chronic bronchitis/CF, and CF that expectorate copious amounts of mucus. In these patients, increased mucin production in the absence of exacerbation likely reflects GCH in the airway epithelium, which increases the baseline level of mucin production (Fig. 5). Inflammatory mediators and by-products identified in the airway secretions or sputum of patients with chronic airway diseases are implicated in GCH, which is a characteristic feature and common out-

---

**FIG. 5.** Response of airway secretory cells to acute or chronic challenges. Inflammatory/immune response mediators are generated in the lung following exposure of normal human airway epithelium (*top*) to environmental irritants. During an acute attack (*bottom right*), some mediators can function as secretagogues to activate secretion of mucins from surface goblet cells and/or glandular secretory cells. Specific mediators can also upregulate MUC gene transcription, which is a process required to sustain mucin biosynthesis and thus hypersecretion. Mucin production returns to baseline levels (*top*) following reestablishment of airway homeostasis by anti-inflammatory mediators and mechanisms. Patients that develop chronic airway disease (*bottom left*) typically manifest goblet cell hyperplasia and glandular hyperplasia/hypertrophy due to airway remodeling by inflammatory/immune response mediators, thereby resulting in increased baseline levels of mucin production in patients with asthma, chronic obstructive pulmonary disease, or cystic fibrosis. Acute airway challenges or exacerbations contribute to mucus obstruction of the conducting airways of these patients.

**Environmental Toxins/Pathogens**

- **CHRONIC**
  - Mucin overproduction
  - Goblet Cell hyperplasia
  - Glandular hyperplasia

- **ACUTE**
  - Mucin hypersecretion
  - Mucin overproduction
  - MUC gene expression
  - GT expression and activity

**Mucus Layer**

- Goblet Cell
- Ciliated Cell
- Mucus Cell
- Serosal Cell
- Submucosal Gland

**Mucus obstruction in the airways:**
- Asthma, CF, Bronchitis
come in chronic airway diseases (Table 5). However, for each airway disease, specific and unique factors appear to be responsible for initiating pathways and processes that ultimately lead to airway obstruction. That GCH reflects airway remodeling is now an accepted paradigm (75); however, the mechanism(s) that lead to GCH in specific airway diseases are not well understood. Briefly, asthma is a multifactorial disease characterized by airway inflammation, hyperresponsiveness, and GCH/mucus obstruction that is likely initiated by an imbalance of TH cytokines (39, 75). CF airway pathophysiology, which results from mutations in the CFTR gene, is characterized by airway inflammation, bacterial infection, neutrophilia, and GCH/mucus obstruction, but the underlying mechanisms that result in CF airway disease are not yet elucidated (30, 206). Inflammation associated with cigarette smoke or pollutants likely mediate the development of GCH in chronic bronchitis. While the etiology and pathogenesis leading to GCH are specific to each airway disease, mechanisms that lead to GCH are likely to converge at a common pathway.

Patients with chronic airway diseases are susceptible to exacerbations induced by exposure to allergens or viral infections. These episodes typically result in massive mucin hypersecretion, presumably because a high number of goblet and/or mucus cells (due to GCH and/or SMG glandular hyperplasia/hypertrrophy) are poised to respond to the sudden increase in inflammatory mediators. Increased mucin biosynthesis is likewise easily maintained because of the high number of MUC templates accessible to inflammatory mediators (Fig. 5). Both processes result in mucin overproduction and can lead to airway obstruction by mucus plugs and airway occlusion; therefore, both likely contribute to the high morbidity and mortality associated with these diseases. Section II, B–D, addresses current information on mucin overproduction in asthma, chronic bronchitis, and CF, respectively. The approach has been to overview information on human pathophysiology with regard to altered mucus cells or mucin production, followed by a summary of studies using in vivo animal models or in vitro cellular models. In particular, we have attempted to distinguish whether mediator-induced increases in mucin gene expression reflect regulated MUC/Muc gene expression and/or secretory cell metaplasia. The latter term is often used generically when discussing GCH in humans or rats or goblet cell metaplasia (GCM) in murine models of airway diseases. At present there are no animal models of SMG hypertrophy/hyperplasia.

**B. Mucin Overproduction in Asthma**

1. Overview

Asthma, a major cause of chronic illness worldwide, has increased in prevalence by >80% in all age and ethnic groups over the past two decades (109, 196). Asthma is characterized in the airways by inflammation (TH2-driven eosinophilia), airway hyperresponsiveness, obstruction by mucus, and remodeling due to fibroblast and smooth muscle cell proliferation and GCH (38, 75). A hallmark feature of asthmatic patients who die after an acute exacerbation is the almost complete occlusion of airways by excessive mucus (3). Mucus plugging does not occur in all patients dying of asthma; however, incomplete plugs often encrust the airways of chronic asthmatics who have died from causes other than asthma (253). This indicates that mucus plug formation is a chronic process in asthmatic patients with varying disease severity, which progresses to airway occlusion and therefore may be a major contributor to disease mortality.

Excessive mucus in asthmatic patients reflects overproduction of mucins due to GCH and SMG hypertrophy, which are major pathological features of asthma. However, GCH is now thought to be a more consistent pathological feature compared with SMG hypertrophy (216). The latter feature is not common to all patients, even those with sputum production (120), whereas GCH is the most characteristic clinical finding even among newly diagnosed asthmatics (144) and in most (194), but not all (172), mild and moderate asthmatics. Airway GCH is especially marked in patients who die from status asthmaticus, with a 30-fold increase in the percentage of goblet cells compared with patients dying from nonasthmatic

**Table 5. Pathology of airway diseases**

<table>
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<th>Initiators</th>
<th>Asthma</th>
<th>CF</th>
<th>Bronchitis</th>
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<tbody>
<tr>
<td>Inflammatory cells</td>
<td>Allergens, TH2 cytokines</td>
<td>CFTR</td>
<td>Inhaled toxins, TH1 cytokines</td>
</tr>
<tr>
<td>Thickened basement membrane</td>
<td>+</td>
<td>+/−</td>
<td>−</td>
</tr>
<tr>
<td>Subepithelial fibrosis</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
</tr>
<tr>
<td>Smooth muscle hypertrophy</td>
<td>+</td>
<td>−</td>
<td>+/−</td>
</tr>
<tr>
<td>Bronchoconstriction/hyperresponsiveness</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Goblet cell hyperplasia</td>
<td>+</td>
<td>+</td>
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CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; +, yes; −, no. Asthma data are from Ref. 75. CF data are from Ref. 104. Bronchitis data are from Ref. 237.
respiratory diseases (3). Thus airway remodeling by GCH alters airway mucin composition, contributes to the obstruction of the conducting airways with mucus, and culminates in obstruction of the airways with mucus plugs, thereby greatly contributing to the severity of asthma (21, 109, 123, 215, 216, 224, 286).

2. MUC mRNA and protein in asthmatic cells

Expression and localization of some secretory mucins have been evaluated in airway tissues from asthmatic patients. Localization of MUC5AC mRNA or protein expression is not altered in airway cells of asthmatic patients, since MUC5AC gene products are restricted to goblet cells in normal (8, 212) and asthmatic (94) airway epithelium. However, a marked increase in MUC5AC expression has been observed in asthmatic epithelial samples obtained by fiberoptic bronchoscopy and bronchial biopsy (112), and a trend toward overexpression of MUC5AC mRNA has been observed in goblet cells of bronchial brushings of asthmatic patients (194).

On the other hand, altered localization of MUC5B gene products has been noted in airway tissues from asthmatic patients where MUC5B is expressed in goblet cells and glandular neck cells, as well as in mucosal SMG cells (94). Because dual localization of MUC5B in surface epithelium and mucosal glandular cells occurs during midtrimester fetal development (212), mediators in asthmatic airways may recapitulate events that occur during development. Neither MUC2 nor MUC19 mRNA/protein expression has yet been evaluated in asthmatic airway tissues, although MUC2 mucin has been evaluated in airway secretions, as discussed in the following subsection.

3. MUC mucins in asthmatic secretions and mucus plugs

Overproduction of airway mucins in asthmatic patients is a well-established paradigm clinically, and “mucus abnormalities” are considered a feature of asthma. However, as recently pointed out in a seminal review (216), it is not yet established whether these abnormalities result from overproduction of specific mucins, an intrinsic biochemical abnormality in asthmatic mucins, or interactions between mucins and other mucosal components.

Studies to date have focused on the secreted cysteine-rich mucins: MUC5AC, MUC5B, and MUC2. MUC5AC and MUC5B mucins have been identified in asthmatic airway secretions. MUC5AC mucin, initially isolated as tracheobronchial mucin from the lung mucus of a patient with bronchial asthma (179, 223), was subsequently shown to be present in airway secretions from both healthy individuals and asthmatic patients (116, 194, 271). MUC5B mucin has also been identified immunochemically and biochemically in asthmatic sputum, as well as in normal airway mucus (273, 300). Overall, MUC5AC and MUC5B mucin levels appear higher in secretions from asthmatic airways compared with nondiseased airways (133), likely reflecting GCH as well as SMG hypertrophy/hyperplasia in some asthmatic patients (216).

MUC2 mucin is present at very low levels in normal or asthmatic airway secretions (57, 116, 133), although it is well-detected in gastrointestinal tract secretions using the same antibody (271). Because MUC2 mRNA expression is increased ex vivo in human bronchial explants from nondiseased airway tissues (157) and in vitro in airway epithelial cell lines (13) by inflammatory mediators present in asthmatic airway secretions, it is surprising that MUC2 mucin is only a minor component of asthmatic airway secretions. This may reflect difficulties in clearing “insoluble” MUC2 mucin from the airways. MUC2 is considered to be an almost insoluble mucin, which protects the intestinal epithelium against damage (10). This likely reflects the very large TR domain of MUC2, which is not interspersed by cysteine-rich domains, in contrast to MUC5AC and MUC5B mucins (Fig. 3), which predominate in the airways. Interestingly, MUC2 mucin is detected in a higher percentage of secretions from asthmatic patients (3/10) than from induced sputum of healthy individuals (1/11) (133), suggesting that analysis of MUC2 mucin warrants further analysis in a larger cohort of patients.

Recent biochemical data indicate that MUC7 mucin is present in the airway secretions of asthmatic, but not nonasthmatic, pediatric patients (296). Other secretory mucins (MUC6, MUC8, and MUC19) may be present in asthmatic airways but have not yet been evaluated.

The molecular identity of mucins in asthmatic mucus obtained from inspissated airways following exacerbations has not been thoroughly investigated. A study carried out to biochemically investigate mucins from a patient who died in status asthmaticus (248) later identified MUC5B mucin in the mucus plug (246). MUC5AC and MUC5B mucins have been identified immunohistochemically in the lumen of patients with fatal asthma (94). A systematic evaluation of mucins that comprise the mucus plugs occluding the airways of patients who have died in status asthmaticus would be useful. The resultant data would provide fundamental information on mucin profiles following asthmatic exacerbations and thus direct future studies on regulation of MUC gene(s) activated by inflammatory/immune response genes during asthmatic exacerbations.

4. Genetic association of MUC genes and asthma

Genetic studies on mucin genes have been carried out to evaluate the potential involvement of mucins in airway obstruction in asthma and other airway diseases. Polymorphisms in the number and lengths of TR in
MUC1, MUC2, MUC4, MUC5AC, and MUC5B genes have been identified. Additionally, polymorphisms have been evaluated in atopic patients with or without asthma. No correlations of VNTR polymorphism in MUC1, MUC4, MUC5AC, or MUC5B genes were observed in patients with asthma, although a longer allele in the MUC2 gene is associated with a cohort of atopic, nonasthmatic patients (284). In contrast, an association of asthma with the MUC7 gene is reported, with a significantly lower frequency of the MUC7*5 allele in a small cohort of Northern European asthmatics (132).

5. Goblet cell metaplasia and mucin gene expression in murine models of asthma

Mice have proven useful models over the last decade for investigating the pathogenesis of allergic asthma. In vivo studies using OVA sensitization and challenge or direct cytokine exposure have linked Th2 cytokine-mediated inflammation and GCM. IL-13 is a central mediator of GCM in murine models of allergic asthma (97, 140, 303), and GCM induced by other Th2 cytokines is stimulated through a common, IL-13-mediated pathway (299). GCM is a characteristic phenotype used to monitor the pathophysiology of asthma, since goblet cells are very rarely seen in the airway epithelium of naive mice, while airway goblet cells predominate in mice exposed to inflammatory or immune-response mediators. Briefly, mice exposed to single or multiple doses of allergen or IL-13 develop GCM within 1–3 days after one or more allergenic challenges (4, 239, 303), although a few goblet cells are evident by 6 h after a single exposure to IL-13 (239) or OVA challenge (74).

Without continual exposure to allergens, GCM is transient in murine airways, although the temporal processes are impacted by allergen or mediator, dose, and murine strain. The epithelium reverts nearly to baseline levels 11 days after multiple challenges with OVA (22) and GCM, which is maximal at 3 days after a single OVA exposure, is minimal 8 days following OVA exposure (239). IL-13-induced GCM also reverts to baseline following multiple IL-13 exposures (97, 140, 303). Taken together, these results suggest that sustained GCM requires ongoing exposure of the airways to IL-13, either directly or indirectly following an allergenic exposure, and that a fairly rapid reversal of the processes leading to GCM occurs in the absence of IL-13 or genes activated by IL-13. Thus GCH manifested in patients with asthma or obstructive airway diseases may also be reversible if allergens or inflammatory mediators are removed or inhibited. If this were not so, the prevalence of GCH would be nearly 100% at any time in allergenic environments, whereas in the absence of environmental insults, GCH can revert to a normal morphology in conducting airways (238).

Ligand binding of IL-13 to the IL-4Ra/IL-13 receptor activates Stat6, which is a key regulator of IL-13-mediated GCM (140, 141). While the downstream genes and pathways that lead to GCM in murine models of allergic asthma remain to be identified, Foxa2, a winged helix transcription factor, is implicated in this pathway as mice that lack Foxa2 manifest GCM (294). Mechanisms that contribute to the reversal of GCM in rodents are also being investigated. Members of the Bcl-2 family, which inhibit apoptosis, play a role in restoring a metaplastic airway epithelium to a normal phenotype following allergenic challenges (250). A better understanding of the mechanisms that directly initiate or reverse GCM in murine airways may lead to pharmacological intervention that would ultimately decrease or circumvent GCH, and thus airway obstruction by mucus in human airways.

Cytokine-induced GCM correlates with increased expression of Muc5ac, an airway goblet cell marker. However, this correlation is not a demonstration that the cytokine of interest regulates mucin gene expression. For example, IL-4 (269) and IL-9 (171) are reported to increase Muc5ac gene expression and mucus production in vivo, but this likely reflects the IL-13-induced increase in goblet cells (299), which endogenously express Muc5ac mRNA/protein, rather than cytokine regulation of mucin gene expression. IL-4 does not upregulate MUC5AC/Muc5ac gene expression; the IL-9 story is less clear (Table 6, see sect. II-B). In addition to inducing GCM in murine airways, IL-13 may, however, directly or indirectly upregulate Muc5ac gene expression, as IL-13 increases the promoter activity of Muc5ac following transfection of a Muc5ac promoter-luciferase plasmid into murine transformed Clara cells (74). However, IL-13 does not upregulate MUC5AC mRNA expression in three human lung cancer cell lines (226) or in differentiated NHBE cells (45), suggesting differences in the sequences or regulation of Muc5ac and MUC5AC promoters.

Muc5b mucin is also induced in an OVA model of allergic asthma and mRNA expression is localized to goblet cells in the airway epithelium (48). Muc2 mRNA expression is induced in OVA and IL-13-induced models of allergic asthma (239). Muc10 mRNA and protein are also upregulated in murine airway epithelium and expressed in goblet cells induced by IL-13 exposure (220, 296). Thus several mucin genes are expressed in secretory cell metaplasia. Taken together, these data suggest that murine models of allergic asthma are pertinent to human asthma with regard to expression of secreted mucin gene products (see sect. II-B3). Thus mechanisms that direct GCM in murine models of allergic asthma are likely to provide insight into GCH in human models in vitro and warrant further investigation.

Two models of chronic asthma in which GCM is sustained and not transient have recently been established and may prove useful in investigating pathways...
that maintain GCH. In an OVA-induced model, airway remodeling manifested by GCM persists after resolution of acute allergen-induced airway inflammation (152). In a virally induced model, GCM develops following infection of murine airways with Sendai virus (SV) and subsequent viral clearance (293). Sendai virus, a murine RNA virus of the family Paramyxoviridae, is a natural pathogen of mice and causes reversible bronchiolitis and pneumonia in susceptible strains of mice (35, 199, 261), which parallels clinical features of childhood and adult asthma in the lower respiratory tract (149, 298). GCM is maintained for more than a year in C57BL/6 mice following SV infection, suggesting that genes and pathway(s) that lead to a chronic asthma phenotype are activated and maintained by the murine host in response to infection by a murine virus. Related work in rat strains has shown that Brown Norway rats are more likely than F344 rats to develop an asthma phenotype following SV inoculation (258).

C. Mucin Overproduction in COPD

1. Definition of COPD/chronic bronchitis: the inflammatory milieu

COPD is defined clinically as a complex of diseases characterized by airflow obstruction due to chronic bronchitis or emphysema. It is the fourth leading cause of patient deaths in adults in the United States. Approximately 14 million people in the United States have COPD; the majority of these patients have chronic bronchitis (237). The diagnostic criteria for chronic bronchitis are the presence of cough and sputum on most days for at least 3 mo per year in two consecutive years without another explanation (5). Epidemiologically, the major risk factor associated with the development of chronic bronchitis is exposure to environmental inhalant toxins, especially cigarette smoke. Exacerbations of airway disease may be provoked by viral or bacterial infections or by exposure to air pollutants.

The central airways of patients with chronic bronchitis are chronically inflamed with increased numbers of macrophages and T lymphocytes (237). Corresponding to the cellular inflammatory profile, there are increased levels of IL-6, IL-1β, IL-8, tumor necrosis factor-α (TNF-α), and monocyte chemotactic protein-1 in induced sputum or bronchoalveolar lavage from patients with stable COPD (52). During acute exacerbations, neutrophils and neutrophil chemokine and receptor expression increase in the airways of COPD patients (211). Neutrophils and macrophages release proteases that contribute to the inflammatory milieu and overwhelm antiprotease defenses. In addition, reactive oxygen species (ROS) generated by cigarette smoke or by inflammatory cells may produce airway and parenchymal injury (237). Together, these mediators injure the airway, activating programs for remodeling (including GCH) and/or MUC gene regulation.

2. Mucin changes in vivo

A major pathological feature of chronic bronchitis is an increase in secretory cells due to hypertrophy of SMG and to GCH, which can extend to the peripheral airways. Secretory cell hyperplasia is a prerequisite for sustained mucus hypersecretion/mucin overproduction. These phenotypes are a significant component of airway obstruction in chronic bronchitis and directly correlate with reduced pulmonary function (283).

Altered cellular localization and increased expression of mucins have been reported in patients with COPD. In airway tissues from patients with obstructive airway diseases, MUC5B mRNA is expressed in goblet, as well as glandular, cells, while MUC5B is not expressed in goblet cells of control patients. MUC5B mRNA is also expressed in the goblet cells of differentiated NHBE cells derived from these patient tissues, but expression levels are similar in cells from control patients and patients with obstructive airway diseases (44). In another study, no differences in alcian blue or periodic acid Schiff (PAS) staining are observed in peripheral lung sections from 9 smokers with COPD, 11 age-matched controls including smokers, and 6 lifelong nonsmokers with normal lung function, although intraluminal PAS staining is significantly more frequent among COPD subjects. MUC5AC protein expression is significantly higher in the bronchiolar epithelium of patients with COPD, and MUC5B expression is increased in the bronchiolar lumen MUC5B of COPD patients relative to controls (41).

Airway mucins from chronic bronchitis patients overall are similar in size and structure to mucus from healthy individuals, but appear to be less acidic (56). However, glycosylation patterns vary during acute exacerbations, as chronic bronchitis mucins are highly sialylated with increased sialylated and sulfated Lex structures (147). Despite biochemical characterization of the mucus gel, information about specific mucin glycoproteins in chronic bronchitis airway secretions is limited to a few studies. MUC5AC and MUC5B mucins are highly expressed constituents both in normal and chronic bronchitis mucus (133). The ratio of MUC5B: MUC5AC is increased, and MUC5B differs in charge in chronic bronchitis sputum compared with normal airway mucus (133). These observations suggest that both goblet and SMG cell secretions contribute to the mucus hypersecretion observed during exacerbations of chronic bronchitis.
3. Mucin regulation by inflammatory mediators in vitro and in vivo in animal models of chronic bronchitis

Because cigarette smoke is the single common factor related to development of chronic bronchitis, several in vivo models have been established to investigate the mechanism of smoke-induced airway inflammation and secretory cell metaplasia. Either inhaled tobacco smoke or cigarette smoke extract induce secretory cell metaplasia in rat main stem bronchi (146) or in BALB/c mice airways (181). In mice, exposure to smoke increases neutrophilic inflammation, mucin expression, and airway reactivity to methacholine. An aldehyde component of cigarette smoke, acrolein, induces expression of Muc5ac gene in vivo in rat (27) and mouse (28) lungs, concomitant with secretory cell metaplasia. In mice, these effects are dependent on macrophage, rather than neutrophil, inflammation (28). One mediator that regulates the secretory metaplastic response to cigarette smoke in guinea pig airways is platelet activating factor (137).

Air pollutants also induce secretory cell metaplasia in animal models in vivo. Sulfur dioxide exposure induces superficial secretory cell hyperplasia in rats (145) and mucus gland hypertrophy in dogs (236). Ozone induces mucus cell metaplasia in rat nasal airway epithelium (106). Neutrophil elastase (NE) proteolytic activity stimulates bronchial secretory cell metaplasia in hamsters (32) and in mice (288). It is noteworthy that inflammation associated with cigarette smoke or pollutants appears to mediate the development of secretory cell metaplasia. This concept is supported by evidence that glucocorticoid treatment mitigates the effect of cigarette smoke in guinea pig airways is platelet activating factor (137).

Bacteria, including Staphylococcus aureus (S. aureus), Streptococcus pneumoniae (S. pneumoniae), and Haemophilus influenzae (H. influenzae), are common pathogens that trigger exacerbations of bronchitis. Bacterial products regulate mucin gene expression in epithelial cell lines in vitro, as discussed in section III. In vivo, endotoxin or lipopolysaccharide (LPS), a component of Gram negative bacterial cell wall membranes, increase goblet cell number in rat nasal epithelium (251) and in murine respiratory epithila (306). Endotoxin also enhances ozone-induced secretory cell metaplasia in rat nasal epithelia (292), suggesting that infections and pollutants synergize to exacerbate secretory cell metaplasia. Paramyxoviruses also induce secretory cell metaplasia in vivo as a separate trait independent of intracellular adhesion molecule (ICAM)-1 expression and acute inflammation in a chronic model of murine asthma (293). These studies demonstrate the powerful effect of infectious agents on the induction of secretory cell metaplasia, thereby resulting in increased mucin production since goblet cells endogenously express mucin genes and secrete mucin glycoproteins.

The complement of inflammatory mediators expressed in the airways of patients with stable COPD and asthma are different, as TH1 cytokines predominate in COPD and TH2 cytokines predominate in asthma (Table 5). Interestingly, viral infections, cigarette smoke, and pollutants cause exacerbations in both patient populations, which are marked by neutrophilic inflammation. Mucus secretion is an important component of the innate immune system. Therefore, teleologically, it is possible that both TH1 and TH2 cytokines regulate mucin gene expression and secretory cell metaplasia in vivo and that individual cytokines have different functions in epithelial and immune cells. These possibilities are being investigated by numerous laboratories, and current data on mucin gene expression are presented in Table 6. It is likely that some mediators synergistically interact with pollutants, infectious agents, and neutrophilic mediators to significantly enhance remodeling that culminates in mucin overproduction and hypersecretion in airway disease states.

D. Mucin Overproduction in CF

1. The “mucus abnormality”

CF was initially called “mucoviscidosis” because of copious amounts of “mucoproteins” in the respiratory and gastrointestinal tracts of CF patients (76). Evidence for a fundamental mucus abnormality in CF is supported by ultrasound studies, wherein 90% of CF fetuses during the 17- to 19-wk period of gestation manifest inspissated meconium in the distal ileum, which is generally reabsorbed by birth (68). Gastrointestinal mucus obstruction is not life threatening except to the small number of CF infants born with meconium ileus.

In contrast, mucus obstruction in CF airways occurs postnatally (309), even though pulmonary complications resulting from airway mucus obstruction and predisposition to infection are the major cause of morbidity and mortality in CF patients (297). There are no marked morphological abnormalities in the airways of CF fetuses or neonates and the numbers and distributions of goblet cells in the epithelium, as well as the numbers and sizes of SMG, are within normal ranges at birth (263). Dilated acinar and duct lumens in SMG are observed in CF airways early in life; the earliest consistent pathological lesion and evidence of mucus obstruction presents in the bronchioles (262).

CF is a recessive genetic disease caused by mutations in the CFTR gene, which encodes the cystic fibrosis transmembrane regulator glycoprotein, CFTR (213). CFTR is expressed in mucus-rich epithelial tissues (respiratory, gastrointestinal, and reproductive tracts), non-mucus rich...
tissues (sweat glands and kidney), as well as nonepithelial tissues (heart and brain) (274). However, obstruction is typically observed only in mucus-rich tissues. The question of how CFTR initiates the primary pathogenetic event in CF airways is still unresolved and remains a question of paramount interest. There are several current hypotheses on the pathogenesis of CF lung disease. 1) Expression of mutant CFTR in CF respiratory cells produces defective chloride secretion and elevated sodium absorption (29), resulting in altered salt concentrations in airway secretions. However, the question of whether salt concentrations in CF airways are hypotonic or hypertonic is still unresolved (98, 268). 2) The concept that alterations in mucus volume impact on mucus hydration, and thus on the rheology of CF airway mucus to increase susceptibility to infection in CF airways, continues to gain credence (30). A marked decrease in the airway surface liquid volume in CF bronchial explants in vitro reflects the abnormal ion transport properties of CF airway epithelia in vivo (175). Similar behavior, as well as increased amounts of mucus and altered mucus transport and mucus adhesion to airway surfaces, is observed in vivo in mice that overexpress the β-subunit of the epithelial sodium channel, ENaC (174). 3) Lack of functional CFTR in lung cells could engender a hyperinflammatory state that alters homeostasis in CF airways. Such a situation is indicated by intrinsically high levels of inflammatory mediators in the airways of CF infants in the absence of evidence of previous infection (11, 127, 138, 184, 189). Many of these mediators increase expression of mucin genes in vitro (Table 6) and thus could prime CF airways for mucin overproduction in vivo, contributing to recurring cycles of infection followed by increased expression of mucins that culminates in airway obstruction with mucus. 4) If airway obstruction with mucus occurs before infection, then a primary or early result may be hypersecretion or accumulation of secretions with abnormal properties (297). This could include altered levels of CF mucin glycoproteins, as discussed in section uD3, or expression of CF mucins with alterations in terminal glycosylation, as discussed in section uD4. 5) Alternatively, mutant CFTR could lead to overexpression or altered regulation of specific glyconjugates that alter the ability of airway epithelium to protect against Pseudomonas and thus lead to obstruction with mucus. The glycolipid asialo GM1, a receptor for P. aeruginosa pilin, is expressed at higher levels in CF than non-CF primary airway epithelial cells (233). Interestingly, the extracellular domains of the MUC1 mucin protein backbone bind to P. aeruginosa flagella (161), resulting in phosphorylation of specific serine residues in the cytoplasmic tail of MUC1 and activation of the extracellular signal-related kinase (ERK) (162), suggesting a role for tethered mucins in CF airway defense. An explanation of how mutant CFTR leads to inflammation, chronic infection, and reduced airway clearance will require a more comprehensive understanding of the multifunctional roles of CFTR, as well as of the roles of mucins in healthy and CF airways (206).

2. Mucin mRNA/protein expression in CF cells

There is limited information on quantitative comparisons of mucin gene and protein expression in CF and control airway cells and secretions. One study examining mucin gene expression in uninfected nasal cells from CF versus control subjects reports altered ratios of MUC5AC: MUC2 mRNA expression in CF cells (290). In situ hybridization studies show altered expression and localization of MUC2 (157) and MUC5AC (66) mRNA in CF versus non-CF bronchial explants and increased expression of mucin genes in response to P. aeruginosa. Briefly, MUC2 mRNA is sparsely detected in the surface epithelium and is not detected in the SMG of non-CF specimens, but is expressed both in the surface epithelium and SMG of CF specimens. Exposure of bronchial explants to P. aeruginosa supernatant results in a marked increase of MUC2 mRNA expression in the SMG of both patient groups (157). MUC5AC mRNA is expressed in cells of the surface bronchial epithelium of both CF and non-CF specimens, minimally expressed in the SMG of non-CF specimens, and, surprisingly, highly expressed in the SMG of CF specimens. Exposure of bronchial explants to P. aeruginosa supernatant results in a marked increase of MUC5AC mRNA expression in both the surface epithelium and the SMG of control patients (66). These results are intriguing, as MUC5AC gene products are not typically observed in SMG (8, 212). In contrast, no differences in the cellular localization of MUC5AC or MUC5B protein expression are observed between CF and non-CF lower respiratory tract epithelial biopsies (95). MUC5AC mucin is expressed in goblet cells and MUC5B in SMG mucus cells. However, MUC5B is also expressed in goblet cells in biopsies from both CF and healthy patients (95).

3. MUC mucins in CF airway secretions

MUC2 mucin glycoprotein is barely detectable in normal (115, 116) or CF airway secretions (57, 133), although MUC2 mRNA is well expressed in CF bronchial explants and its expression is upregulated by P. aeruginosa LPS (157) (see sect. uD2). MUC2 is considered an “insoluble” mucin (10), however, and may not be easily cleared by the airways.

On the other hand, MUC5AC and MUC5B mucins, which are well-expressed at the mRNA level in airway tissues, are major components of secretions and mucus from healthy airways as well as from sputum samples from patients with CF. The levels of these two mucins in CF sputum and non-CF mucus have been evaluated in two studies, with conflicting results. An initial study reported that MUC5AC and MUC5B are major mucins present at
variable concentrations in CF sputum and in airway mucus obtained from normal airways following exposure to hypertonic saline. These data indicate that both mucins are elevated in CF sputum and that the low-charge form of MUC5B is increased in CF sputum (133). However, a more recent study, in which mucus and sputum were processed similarly and normalized per volume, weight, or protein, indicate that MUC5AC and MUC5B mucins are present in lower concentrations in CF sputum (107). While this suggests reduced mucin secretion by CF cells, proteases present in CF sputum are able to digest lung mucins in vitro (221) and may fragment or remove an epitope in the cognate sequence, thereby reducing detection by antibodies. However, antibodies raised against the same cognate sequence were used in both studies (107, 133). Thus differences in procurement, processing, and normalization of mucus and sputum samples may account for the conflicting conclusions. Overall, comparative studies of mucus and CF sputum samples are confounded by the presence of chronic inflammatory cells and mediators in CF subjects that impact on mucin production and characterization, thereby rendering reliable quantitation of mucin gene and glycoprotein expression in the uninfected and uninfamed CF lower respiratory tract difficult.

Levels of MUC5B and MUC5AC mucins have also been compared in secretions from differentiated NHBE cells established from lung tissues of control and CF patients. MUC5B and MUC5AC levels are similar in NHBE cells from both patient populations, with MUC5B mucins present at >10-fold higher levels than MUC5AC mucins (111).

4. Alterations in terminal glycosylation of O-glycans

Several studies support altered glycosylation of CF mucins, although this remains to be conclusively demonstrated. Initially, studies focused on mucoprotein fractions from the duodenum, where increased levels of fucose and sulfate and decreased levels of sialic acid in CF samples relative to non-CF samples are observed (65). These data were reinforced in later analyses of CF mucosal samples (reviewed in Refs. 217, 234). Increased sulfation in CF glycoconjugates has also been reported (49, 308). The structures of several dozen neutral, sialylated and sulfated O-glycans from airway mucins of patients with CF or bronchitis have been determined (49, 114, 134, 166). A consistent finding has been increased expression of sialyl-Le$^x$ epitopes in mucin-rich fractions isolated from patients with CF. However, a similar finding has also been observed in mucins isolated from patients with chronic bronchitis, leading to the suggestion that increased sialyl-Le$^x$ expression in airway mucins correlates with severe infection or inflammation (59).

In support of a CF-specific alteration in terminal mucin glycosylation, sialylated and fucosylated structures in MUC5B mucins in salivary secretions from CF and non-CF individuals have been compared. Lectin staining demonstrated a shift from sialic acid α2,6 linkages in normal MUC5B mucins to sialic acid α2,3 linkages (Le$^x$ structures) in CF MUC5B (252). Because salivary glands are typically not infected in CF patients, the increased expression of sialylated Le$^x$ structures in MUC5B, if validated by structural studies of MUC5B O-glycans, would demonstrate a genetic consequence of mutated CFTR on glycosylation, rather than a consequence of infection/inflammation. However, MUC19, which has recently been shown to be expressed in salivary glands (46), may well be present in salivary secretions, which would confound analysis of large secreted mucins.

A recent study compared O-glycans isolated from mucins secreted in vitro by differentiated NHBE cells from CF and healthy individuals (111). Carbohydrate composition patterns are similar in CF and non-CF O-glycans. However, few structures are reported, preventing a comparison of terminal glycosylation. Interestingly, O-glycans have all four core types present, likely reflecting the mixture of MUC5B (both high- and low-charge glycoforms) and MUC5AC mucins. Thus the question of altered glycosylation between CF and non-CF mucins with the same MUC protein backbone is still not resolved, either for mucins secreted in vivo or in vitro. Elucidating the significance of alterations in terminal glycosylation in CF mucins will require molecular analyses of O-glycans released from specific MUC mucins isolated from CF and control individuals.

Biochemical and structural data on membrane glycoproteins from CF cells also continue to support differences, especially in altered fucosylation, in posttranslational modifications in CF glycoproteins expressed in epithelial cells, although the reported differences are on N-glycans (91, 234).

Differences in glycosylation of CF proteins could reflect alterations in GT activity, expression, or compartmentalization and could be a consequence of mutant CFTR, which is reported to alter the acidic microenvironment in the ER and Golgi (12), the sites of posttranslational glycosylation of secreted and membrane proteins. The concept of altered acidification in CF cells is not supported in later investigations, however (89). On the other hand, inflammatory mediators increase expression of several glycosyltransferases and sulfotransferases in vitro (61). Activation of an α1,3-fucosyltransferase, different from the Lewis enzyme α1,3-fucosyltransferase III, has been suggested to explain the increase in sialyl-Le$^x$ epitopes in severely infected patients phenotyped as Lewis negative (59). Additionally, the paucity of sialic acid α2,6 linkages in CF MUC5B salivary mucin (252) suggests a decreased expression, activity, or compartmentalization of one or more α2,6 sialyltransferases in CF secretory cells in vivo. Structural analyses of O-glycans
from MUC5B mucins secreted in vivo by CF patients and controls would clarify the significance of these findings.

Secretion of CF mucins with alterations in terminal glycosylation could alter the physical (rheological or viscoelastic) properties of airway mucus, as sialic acid and sulfate impart anionic properties to mucins, while fucosylation imparts hydrophobic properties. Additionally, alterations in the extent of O-glycosylation, e.g., number of chains per mucin protein backbone or the size or structure of O-glycans, could impact the biophysical or biological (binding to bacteria, viruses, cells) properties of specific mucins. Indeed, terminal α,1,4-linked N-acetylgalactosamine in O-glycans, which is expressed in the cells in gastric glands where MUC6 is synthesized, has a natural antibiotic function against Helicobacter pylori infection (125). Studies on biophysical and biological properties of specific airway MUC mucins with defined O-glycan chains will be facilitated when adequate amounts of purified or recombinant MUC mucins or specific MUC domains are available.

5. Models of airway obstruction in CF

In contrast to the murine models of allergic asthma discussed in section II B5, murine models of CF for investigating airway mucus obstruction have proven more difficult to generate. Mice typically lack airway SMG, and serosal cells in SMG glands are considered the major site of expression of CFTR in human airways (71). Mice that are deficient in CFTR, e.g., CF mice, do not mimic the physiology or pathology of CF airways, since the chloride channel abnormality observed in the airways of CF patients is not manifested in the airways of CF mice (255). P. aeruginosa (55) or S. aureus (254) do not colonize the airways of CF mice. Furthermore, CF and heterozygous mice, which exhibit GCM following OVA challenge, clear airways of CF mice. Furthermore, CF and heterozygous patients is not manifested in the airways of CF mice (255).

III. REGULATION OF MUCIN GENES

A. Overview

Respiratory tract infection and inflammation are characteristic features of patients with asthma, COPD, or CF. Bacterial or viral interactions with host cells trigger the activation of signal transduction pathways that modulate host responses and increase expression of inflammatory genes. Many inflammatory/immune response mediators, e.g., the pro-inflammatory mediators TNF-α, IL-6, IL-8, and by-products of bacterial or inflammatory cells, e.g., LPS or neutrophil elastase (NE), are elevated in airway secretions of patients with chronic airway disease (25, 52, 126, 184, 195). Investigations over the last decade have tested and validated the hypothesis that inflammatory/immune response mediators also regulate mucin gene expression in vitro (Fig. 6), thereby directly linking infection and inflammation to the copious amounts of mucus produced in chronic airway diseases (reviewed in Refs. 13, 226).

Current data on mucin gene regulation are depicted in Figure 6, summarized in Table 6, and presented in section III, B–D. Most studies have been carried out in airway epithelial cancer cell lines and/or primary differentiated NHBE cells. They support the paradigm that certain mediators 1) selectively regulate expression of specific MUC genes at steady-state equilibrium in airway epithelial cancer cell lines and/or primary differentiated NHBE cells and 2) regulate expression of individual MUC genes at the transcriptional and/or posttranscriptional level. Pathogen by-products, inflammatory/immune response mediators, and growth factors, such as the epidermal growth factor (EGF), upregulate mucin genes expressed in airway epithelial cells, as reviewed in section III, B and C. Mucin gene regulation studies initially focused on MUC2, since nucleotide sequences for MUC2 cDNA and promoter were available well before the MUC5AC and MUC5B genes were cloned and characterized.

Many mediators that upregulate MUC2 gene expression also upregulate MUC5AC, as well as MUC8 or MUC4, genes (Table 6). However, many of these mediators [TNF-α, prostaglandin E2, 15-hydroxyeicosatetraenoic acid, or phorbol 12-myristate 13-acetate, a protein kinase C (PKC) activator] do not upregulate MUC5B gene expression (26), even though MUC5B mucin, unlike MUC2 mucin, is present at high levels in mucus from healthy and diseased airways (see sect. II). On the other hand, IL-6/IL-17 (45), UTP (47), and IL-8 (15) upregulate MUC5B, as well as MUC5AC, suggesting differential and specific regulation of mucin genes by inflammatory/immune response mediators. Considerable information is now known about some signal transduction pathways through which mucin gene expression can be mediated (Fig. 6). Minimal information is available on the specific cis-sequences in MUC promoters to which activated transcription factors bind to regulate MUC gene expression. Investigations on posttranscriptional regulation of mucin genes are also limited (see sect. III D). Further investigations into transcriptional and posttranscriptional regulation of airway mucin genes are warranted to better understand the pathways and
<table>
<thead>
<tr>
<th>Agonist</th>
<th>MUC Gene</th>
<th>Mechanism</th>
<th>Cell Line</th>
<th>RNA/Prt*</th>
<th>Reference Nos.</th>
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<td><strong>Bacteria, bacterial products, and viruses</strong></td>
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<td>H292</td>
<td>Both</td>
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<td>Transcriptional upregulation: bacterial LTA→PAFR→via G protein →ADAM10→release of EGF from HB-EGF→EGFR→Ras→MEK1/2→NFkB</td>
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<td>RNA</td>
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<td><strong>H. influenzae</strong></td>
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<td>Transcriptional upregulation: TLR2→MyD88→TAK1→NIK→IKK β/γ→InBos→NFkB</td>
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<td>A549</td>
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<td><strong>H. influenzae</strong> (and bacterial soluble cytoplasmic fraction)**</td>
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<td>Transcriptional upregulation: TLR2→MyD88→p38 MAPK</td>
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<td>RNA</td>
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<td><strong>Gram-negative bacteria flagellin</strong></td>
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<td>Transcriptional upregulation: flagellin binds to asialoGM1→ATP release→autocrine/paracrine binding to G protein-coupled P2Y2 receptor→PLC activation→inositol trisphosphate production→intracellular calcium mobilization→through calcium binding protein→MEK1/2→ERK1/2→transcription factor activation</td>
<td>HM3, H292</td>
<td>RNA</td>
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<td><strong>Bordetella pertussis</strong></td>
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<td>Transcriptional upregulation</td>
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<td><strong>DsRNA</strong></td>
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<td>Transcriptional upregulation: ATP release→P2Y receptor→PLC→PKC→p38 MAPK→NFkB</td>
<td>HM3 H292</td>
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<td><strong>Cytokines/chemokines/lipid mediators</strong></td>
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<td><strong>TNF-α</strong></td>
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<td>Expression increased by 30 min and maintained for 24 h. Regulation inhibited by PKC and tyrosine kinase inhibitors</td>
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<td>Posttranscriptional regulation</td>
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<td>RNA</td>
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<td>Regulation inhibited by RARα antagonist</td>
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<td>5AC</td>
<td>Transcriptional upregulation: ERK and p38 MAPK→MSK-1→CRE</td>
<td>H292, HNE</td>
<td>RNA</td>
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<td><strong>Cytokine-activated eosinophils</strong></td>
<td>5AC</td>
<td>TGF-α→EGFR activation</td>
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<tr>
<td><strong>Interleukin (IL)-1β</strong></td>
<td>2</td>
<td>Transcriptional upregulation: ERK and p38/MAPK→COX2→PGE2</td>
<td>H292</td>
<td>RNA</td>
<td>130</td>
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<td>Increased mRNA steady-state expression</td>
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<td>8</td>
<td>Transcriptional upregulation via ERK-RSK1-CREB-CRE pathway</td>
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### Table 6—Continued

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<td>IL-6/IL-17</td>
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<td>IL-6 → activates cell in autocrine/paracrine fashion → ERK; IL17→JAK2→IL-6 secreted</td>
<td>NHBE&lt;sub&gt;ALI&lt;/sub&gt;</td>
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<td>No effect</td>
<td>H292</td>
<td>RNA</td>
<td>26</td>
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</table>

**Proteases**

| Neutrophil elastase | 2         | Regulation inhibited by RARα antagonist | H292 | RNA | 139 |
|                    | 5AC       | Posttranscriptional regulation | NHBE | Both | 77 |
|                    | 5AC       | Proteolytic activity required, posttranscriptional regulation | A549, NHBE<sub>ALI</sub> | RNA | 291 |
|                    | 5AC       | ROS | NHBE<sub>ALI</sub> | Both | 78 |
| Human airway trypsin | 5AC       | TGF-α→EGFR→MEK1/2→ERK | H292 | Both | 135 |
| Tissue kallikrein   | 2         | Proteolytic activity and EGFR activation | HSG | Prt | 43 |

**Pollutants/reactive oxygen species**

| Hydrogen peroxide | 5AC       | Transactivation of EGFR→MEK1/2→ERK | H292 | Both | 266 |
| Cigarette/tobacco smoke | 5AC       | TACE-mediated TGF-α release→EGFR activation | H292 | Both | 244 |
| Cigarette/tobacco smoke | 5AC       | Transcriptional upregulation: ROS→Src-dependent JNK activation→JunD→AP-1 and/or EGFR→Ras/Raf-MEK→ERK→Fra-2→AP-1 | H292 | RNA | 86 |
| Acrolein            | 5AC       | Increased expression | H292 | RNA | 26 |
| Residual oil fly ash | 5AC       | No effect | H292 | RNA | 26 |

**Cell surface receptor agonists**

| Epidermal growth factor family of ligands | 5AC       | Augmented by TNF-α-mediated upregulation of EGFR surface expression→ERK/MAPK | H292 | Both | 136, 265, 266 |
|                                          | 2, 5AC    | Transcriptional upregulation: EGFR→Ras→Raf→ERK→Sp1 | H292 | RNA | 203 |
|                                          | 5B        | No effect | H292 | RNA | 203 |
| Uridine 5-triphosphate                  | 5AC, 5B   | Pertussin toxin-sensitive G protein and MEK1/2/MAPK dependent; PKC and PLC independent | NHBE<sub>ALI</sub> | RNA | 47 |

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Description of human cell lines: A549, a lung carcinoma cell line; HeLa, cervical adenocarcinoma cell line; HNE, human nasal epithelial cells; HSG, human SMG cells; H292, NCI-H292, a human pulmonary mucoepidermoid carcinoma cell line; NHBE, normal human bronchial or tracheal epithelial cells grown on Transwell membranes on a collagen layer; NHBE<sub>ALI</sub>, differentiated NHBE cells; HM3, colon carcinoma cell line; HMEEC-1, human middle ear epithelial cell line. See text for other definitions. * Gene product evaluated is mRNA and/or protein (Prt).
processes that lead to mucus overproduction in chronic airway diseases.

Few studies on the downregulation of mucin genes have been reported. The glucocorticoid dexamethasone decreases the abundance of MUC2 and MUC5AC mRNA in airway epithelial cell lines (122) and in rat primary airway epithelial cells grown submerged and at ALI (173). Interestingly, it has been shown that the decreased expression of Muc5ac mRNA in rat airway epithelial cells does not always produce a concomitant decrease in synthesis or expression of Muc5ac mucin (173). Identifying mechanisms that affect repression of mucin gene expression and/or mucin protein expression will be useful in ultimately reverting mucus overproduction in airway diseases.

B. Transcriptional Regulation by Infectious Mediators of Mucin Genes

Bacterial colonization and infection of the airways are major pathological features of CF and COPD. These infections are associated with increased mucin production and mucus hypersecretion, resulting in airway obstruction. Bacteria activate transcription of host defense genes via activation of specific signal transduction cascades. Bacteria-induced upregulation of mucin genes is now recognized as a primary innate defensive response in mammalian airways (13). This hypothesis, initially advanced by Professor Carol Basbaum, has significantly advanced our understanding of the signaling pathways that regulate mucin gene expression.

P. aeruginosa and S. aureus are the primary pathogenic bacteria found in the airways of CF patients, while S. aureus and H. influenzae are common pathogens that exacerbate bronchitis in patients with COPD. By-products of these bacterial infections activate mitogen-activated protein kinase (MAPK) pathways, which are important in transmitting extracellular signals from the cell surface to the nucleus to transcriptionally upregulate mucin genes (Fig. 6). This has been well-demonstrated for the MUC2 and MUC5AC genes (Table 6). The MAPK pathway is one example that culminates in activation of the transcription factor, nuclear factor kappa B (NFκB), which in turn regulates mucin gene expression (Fig. 6). However, NFκB is not the only transcription factor that mediates mucin gene expression, as SP1 and AP-1 also upregulate mucin gene expression following exposure to inflammatory/immune response mediators (86, 203).

1. MUC2 gene regulation

A seminal study on mucin gene expression showed that supernatant from P. aeruginosa increases MUC2 mRNA steady-state expression in bronchial explants and in airway and colon epithelial cell lines and that lipid A
transcriptionally upregulates MUC2 promoter activity by a MAPK pathway (157). The lipid A component of LPS is a potent activator of host defense responses following binding to receptors on cell surfaces. Subsequent studies showed that LPS increases MUC2 transcription by activation of c-Src-Ras-Raf-1, which leads to mitogen-activated protein kinase kinase (MEK)1/2 activation and subsequent ERK1/2 phosphorylation. ERK1/2 phosphorylation leads to activation of the 90-kDa ribosomal S6 kinase (pp90rsk), resulting in NFκB (p65/p50) binding to the NFκB cis-sequence at −1452/−1436 of the MUC2 promoter. A second NFκB/c-rel sequence at −234/−220 nt is not functional in LPS activation of MUC2 (158).

Gram-positive bacteria can also transcriptionally upregulate MUC2 gene expression as lipoteichoic acid (LTA) in the S. aureus cell wall, another potent activator of host defense responses, activates MUC2 gene expression (154). However, the signaling pathways activated by LTA interactions at epithelial cell surfaces are different from those activated by LPS, although both mediators can use the Ras-MEK1/2-ERK1/2 pathway that results in activation of NFκB. S. aureus-LTA binds to the platelet activating factor receptor (PAFR) resulting in G protein-mediated activation of ADAM10, a matrix metalloprotease. ADAM10 releases membrane-bound heparin binding-epidermal growth factor (HB-EGF), which binds to the EGF receptor (EGFR). HB-EGF activation of EGFR leads to downstream Ras and MEK1/2 activation, resulting in pp90rsk phosphorylation and activation of NFκB (154).

H. influenzae regulates MUC2 transcription by activating transforming growth factor (TGF)-β receptor type I/II, inducing Smad3/4 complex activation and downstream NFκB activation. Alternatively, H. influenzae can also bind to the toll-like receptor (TLR)2, and activate MyD88, TAK1, NIK, and IKKβ. This signal cascade results in release of IL-8 from the NFκB-IL-8 complex and therefore NFκB-mediated transcription of the MUC2 gene (121).

Bacterial flagella also activate MUC2 gene expression, but through a different cell surface binding and cellular signaling mechanism than LPS. P. aeruginosa flagellin, a major structural component of the bacterium flagella, binds to the epithelial cell surface ganglioside, ASGM1, causing release of ATP and subsequent activation of P2Y, a G protein-coupled receptor, with downstream activation of phospholipase C (PLC), formation of inositol trisphosphate, and calcium mobilization. This triggers MAPK signaling through phosphorylation of MEK1/2 and ERK1/2, which results in increased MUC2 transcription (177). As the MAPK pathway accounts for only half of the flagellin-induced signaling, the authors hypothesized that a postulated calcium binding protein activates MEK1/2 as well as an ERK independent pathway, thereby leading to increased MUC2 transcription. The cis-sequences on the MUC2 promoter and the transcription factors that regulate flagellin-induced ERK-dependent and --independent pathways have not yet been identified.

Respiratory viruses also induce mucin overproduction during infection and additionally trigger exacerbations in patients with asthma, bronchitis, or CF. Double-stranded (ds) RNA is a biologically active component of many respiratory viruses, and synthetic ds RNA can upregulate MUC2 transcription in stably transfected colon cancer cells. Like bacterial flagellin, ds RNA stimulates the extracellular release of ATP and activation of G protein-coupled ATP receptors on cell surfaces, resulting in stimulation of PLC. In the case of ds RNA, this induction activates PKC leading to activation of NFκB via the p38 MAPK, but not the c-Jun amino-terminal kinase JNK, pathway (167).

2. MUC5AC gene expression

Early studies on mucin gene regulation demonstrate that Gram-negative bacterial lysates, in addition to up-regulating MUC2 mRNA, also increase expression of MUC5AC mRNA in bronchial explants from normal or CF patients (66) and in epithelial cancer cell lines (156). This is not surprising as MUC5AC, like MUC2, has several putative NFκB cis-elements in its promoter sequence. P. aeruginosa upregulates MUC5AC expression following activation of the p42/44 MAPK pathway via an EGFR signaling cascade (136), analogous to how Gram-positive bacteria activate MUC2 expression (154). MUC5AC mRNA expression is increased following EGFR/EGFR ligand/receptor binding (see sect. mC) and subsequent activation of the MAPK cascade (265, 266).

Nontypeable H. influenzae also upregulates MUC5AC transcription via activation of NFκB, but does so via heat-stable cytoplasmic proteins rather than bacterial lipid oligosaccharides. Additionally, H. influenzae activates several signaling pathways through cytoplasmic proteins that may either positively or negatively regulate p38 MAPK. Although H. influenzae directly phosphorylates p38, it also activates negative regulators of p38, phosphoinositide-3-kinase and Akt, a serine-threonine kinase (295). The net result of these signaling cascades is upregulation of MUC5AC expression.

Bacterial and viral interactions with host cells can also trigger autocrine/paracrine nucleotide signaling, which mediates host defense responses at both the mucin secretory and gene expression levels. ATP up-regulates MUC2 gene expression in cancer cell lines (167, 177). UTP upregulates expression of MUC5AC and MUC5B genes in differentiated NIHBE cells, following activation of a MAPK pathway by a UTP-specific G protein-coupled receptor (47).

Ligand-dependent activation of EGFR increases transcription of the MUC2 (154) and MUC5AC (265, 266) genes. In addition to the above-mentioned studies demonstrating NFκB-mediated regulation, other mediators...
(TGF-α, EGF, and TNF-α) transcriptionally regulate MUC2 and MUC5AC and do so via the Sp1 transcription factor following activation of EGFR/Ras/RAP/ERK1/2 pathways. Functional investigations revealed that binding of Sp1 to Sp1 cis-elements within the MUC2 promoter (nucleotides −2627 to −2097) and MUC5AC promoter (nucleotides −202 to −1) contributes to the ability of the aforementioned mediators to regulate mucin gene expression. In contrast to an earlier study (265), TNF-α does not potentiate MUC5AC expression in the presence of EGFR ligands in H292 cells in this study (203).

C. Transcriptional Regulation by Inflammatory/Immune Response Mediators

Many inflammatory/immune response mediators in the airway secretions or sputum of patients with asthma, COPD, or CF regulate mucin gene expression. These include cytokines, oxidants, and proteases, certain of which have been shown to increase MUC gene expression by a variety of signaling cascades in vitro in cancer cell lines or differentiated NHBE cells (Fig. 6, Table 6). In the first study of the effect of cytokines on mucin gene expression, TNF-α, the primary orchestrator of inflammatory responses, was shown to increase MUC2 mRNA steady-state expression in H292 cells (155). TNF-α also increases the abundance of MUC5AC, but not of MUC5B, mRNA and does so by increasing the stability of MUC5AC mRNA at the posttranscriptional level (26). TNF-α also regulates expression of MUC5AC at the transcriptional level (139, 256) by mechanisms similar to those observed by IL-1β (see below). NFκB cis-sequences in the promoter region of MUC5AC through which TNF-α upregulates MUC5AC expression have recently been identified (188).

IL-1β, an inflammatory mediator that is also expressed early in inflammation, induces MUC2 and MUC5AC expression in H292 or normal human nasal epithelial (NHNE) cells via MAPK activation of both ERK1/2 and p38 pathways. Three downstream mechanisms have been identified so far. These are 1) activation of cyclooxygenase-2 and prostaglandin E2 production (130), 2) presence of an active retinoic acid receptor α (139), and 3) signaling via the mitogen and stress-activated kinase 1 (MSK-1), with subsequent activation of the cAMP response element-binding protein (256).

The non-TH2 cytokines IL-6 and IL-17 also increase MUC5AC and MUC5B mRNA steady-state expression in differentiated NHBE cells. IL-17 upregulates MUC5B expression through an IL-6 paracrine/autocrine loop mediated by ERK signaling via JAK2-dependent signaling (45).

The TH2/Th2 cytokines, which are found in inflammatory airways and prominent mediators of asthmatic airway inflammation and central mediators of GCM in murine models of allergic asthma (see sect. iiB5), are reported to increase mucin gene expression in vivo. However, increased mucin gene expression may simply correlate with GCM as higher levels of steady-state mucin gene expression in models of allergic asthma reflect the increased number of goblet cells, which endogenously express mucin genes. The question of whether specific cytokines regulate mucin genes needs to be evaluated for each MUC/Muc gene of interest. Steady-state analyses of mucin mRNA expression following exposure to TH2 cytokines has shown that neither IL-4 nor IL-13 upregulates MUC5AC mRNA expression in human airway epithelial cancer cell lines (226) or in differentiated NHBE cells (45). Another study shows that IL-4 decreases MUC5AC expression in differentiated NHBE cells (119). However, the situation may be different for the murine Muc5ac gene, as IL-13 has recently been reported to increase the promoter activity of Muc5ac following transfection of a Muc5ac promoter-luciferase plasmid in murine Clara cells (74). The TH2 cytokines IL-5 and IL-9 do not alter MUC5AC or MUC5B mRNA abundance in differentiated NHBE cells (45). In contrast, IL-9 increases MUC5AC expression in NHBE cells cultured under submerged conditions and transcriptionally upregulates MUC5AC expression in H292 cells (168). IL-9 also increases MUC2 and MUC5AC mRNA steady-state expression in H292 cells and in submerged NHBE cells (171). These conflicting results may reflect differences in model systems or culture conditions. Pathways whereby IL-9 regulates mucin gene expression have not yet been reported.

Neutrophils are the predominant inflammatory cell in the airways of patients with CF, chronic bronchitis, and in acute, severe exacerbations of asthma. Neutrophil products such as proteases and oxidants have been evaluated for their role in regulating MUC gene expression. Neutrophil elastase, a serine protease, increases expression of at least two major respiratory tract mucin genes, MUC5AC (135, 291) and MUC4 (77). Neutrophil elastase can regulate MUC5AC by at least two different mechanisms: 1) NE induces oxidant stress in A549 and NHBE cells (79) and regulates MUC5AC, as well as MUC4 by a posttranscriptional mechanism, e.g., by prolonging mRNA half-life, as briefly discussed in section iiD; and 2) NE releases TGF-α resulting in EGFR activation and transcriptional regulation of MUC5AC in H292 cells (135). Other airway proteases such as kallikrein (43) and human airway trypsin (51) have recently been shown to also regulate MUC5AC expression following EGFR activation.

Extensive investigations of mucin gene expression have also focused on other intrinsic and extrinsic factors/compounds. Reactive oxygen species, which can be released by airway inflammatory cells, also regulate MUC5AC expression and do so by EGFR activation (266) and by TNF-α-converting enzyme (TACE) (243). Heavy metal particulates in fuel oil combustion products, particularly vanadium, also upregulate MUC5AC expression in

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airway epithelial cells (169). Acrolein, an aldehyde component of tobacco smoke, also increases MUC5AC expression (26). Tobacco smoke transcriptionally regulates MUC5AC by two different mechanisms: 1) ROS activation of EGFR via TACE-mediated release of amphiregulin or 2) EGFR-independent Src-Jnk activation of JunD/Fra-2 binding to AP-1 cis-elements in promoters (86).

An EGFR signaling cascade is a common pathway by which many stimuli, including oxidative stress, agarose plug instillation, cigarette smoke, and NE, induce MUC2 (14) or MUC5AC (136) gene expression in vitro (Table 6). The importance of this pathway in vivo is not fully established, as it requires expression of EGFR at the luminal surface of goblet cells to activate the signaling mechanisms that increase MUC5AC expression. There is not complete agreement on the expression and localization of EGFR in the conducting airways. In one study, EGFR immunoreactivity in healthy airways is rare and observed only in goblet cells. EGFR expression is increased in asthmatic airways and detected in goblet and basal cells (267). In other studies, EGFR expression in healthy airways is limited to the lateral junctions between columnar epithelial cells and their junctions with basal cells and is not observed on the luminal surfaces. EGFR expression is increased in asthmatic airways and is localized to the basolateral surface of pseudostratified columnar epithelium, except in areas of damaged epithelium, where EGFR is found on the apical surfaces (207). EGFR expression is also restricted to the basolateral aspect of pseudostratified columnar epithelium in normal and CF tissues (105, 289).

D. Posttranscriptional Regulation of Mucin Genes

Inflammatory-associated genes activated during lung inflammation, including TNF-α, endothelial and inducible nitric oxide synthase, and cyclooxygenase, can be regulated posttranscriptionally as well as transcriptionally (reviewed in Ref. 182). Several inflammatory mediators/products have now been shown to regulate mucin genes at the posttranscriptional level. TNF-α (26), NE (291), and IL-8 (16) increase MUC5AC expression in human epithelial cells by increasing mRNA stability. 12-O-tetradecanoylphorbol-13-acetate or forskolin increases MUC2 mRNA levels by a posttranscriptional mechanism in an intestinal epithelial cancer cell line, via PKC- or protein kinase A-dependent signal transduction pathways, respectively (280). MUC4 is posttranslationally regulated by TGF-β in rat mammary epithelial cells (210). While inflammatory/immune response mediators posttranscriptionally regulate MUC genes and significantly amplify gene expression, the mechanism(s) whereby they do so, and the relevance of these pathways in airway cells, remain to be determined.

IV. SUMMARY AND FUTURE DIRECTIONS

Mucins are glycoproteins characterized by TR domains that are high in serine or threonine and proline. A mucin protein backbone is encoded by a MUC gene, of which 18 human MUC genes have been identified by recombinant DNA technology over the last two decades. A majority of the TR serine/threonine residues are O-glycosylated, resulting in mucins being >50% carbohydrate by weight. Based on the primary structure of their MUC protein backbones, mucins are classified as either secretory or membrane-tethered. Standardization of nomenclature and molecular details on MUC genes and MUC gene products will be facilitated by the development of a website (www.mucin.org) wherein genomic and cDNA sequences, as well as protein sequences validated by peptide sequencing or by antibodies specific to MUC protein backbones, have been assessed.

MUC-specific antibodies have proven useful for identifying mucin localization in epithelial tissues, cells, and secretions and investigating mucin biosynthesis and secretion, as well as characterizing and semi-quantitating mucin levels in secretions from healthy individuals and those with diseases. They should likewise prove useful for isolating individual mucins for studies on mucin glycosylation and function. A comprehensive analysis of O-glycan structures isolated from mucins of known MUC protein backbones will establish whether specific mucins undergo altered glycosylation in airway diseases. This may occur in chronic airway diseases consequent to inflammation, while there may be an additional downstream effect of mutant CFTR on mucin glycosylation in a genetic disease like CF.

The concept that mucins have a role in mucosal immunity in the airways continues to gain validity. Both secretory and membrane-tethered mucins function as part of the innate immune defensive and mucociliary clearance systems that protect the airway epithelium against pathogens and environmental agents. The secretory mucins MUC5AC and MUC5B are major components of airway mucus from healthy airways and sputum from patients with chronic airway diseases. At least 10 other mucins are expressed at the mRNA level in lung epithelial cells; future studies will determine whether these gene products are also expressed at the protein level, and will lead to studies on the functional roles of specific membrane-tethered and secreted mucins. However, reliable quantitation and comparison of mucin levels in mucus samples from healthy airways and sputum samples from patients with chronic airway diseases remains a challenging problem encumbered by the difficulties in procuring and processing samples. Nevertheless, determining the identities of specific mucin gene products in airway epithelial cells and secretions is a crucially important step for investigations into the functional roles of specific mucins in airway ho-
meostasis, injury, and repair. Such studies will increase our understanding of airway health and diseases.

Several inflammatory airway diseases share common pathways of increased mucin gene expression and GCH; each process can contribute to mucin overproduction and mucus obstruction in the conducting airways of patients with asthma, COPD, or CF. There have been significant advances in our understanding of pathophysiological mechanisms leading to these findings over the last decade. Specific mediators differentially regulate mucin genes through various intracellular signaling pathways by activating transcriptional and/or posttranscriptional programs that regulate increased mucin production. Further investigations into transcriptional and posttranscriptional regulation of airway mucin genes are warranted to better understand the pathways and processes that lead to mucin overproduction in specific airway diseases by myriad mediators. Such studies will likely demonstrate that mediators, which trigger mucus obstruction of airways following exacerbations, also vary between diseases.

Increased mucin production in patients with airway diseases reflects in part an increased number of goblet cells in the conducting airway epithelium and/or glandular hyperplasia. The contributions of each are beginning to be elucidated. Importantly, mucin overproduction and GCH, although linked, are not synonymous and may follow from different signaling and gene regulatory pathways. Growth factors, inflammatory/immune response mediators, and pollutants are implicated in secretory cell metaplasia in models of airway diseases, analogous to the milieu of airway epithelia in hypersecretory diseases. Infections and pollutants may synergize to exacerbate mucin gene expression following establishment of secretory cell metaplasia in animal models and/or GCH in humans. Progress in evaluating pathways of GCM and mucin gene regulation will be facilitated by the development of genetically altered mice that lack the major airway mucin genes, Muc5ac and Muc5b. At present, knockout mice are available only for Muc1 and Muc2. Studies have shown that Muc1 plays a role in the progression of mammary carcinoma (260) while Muc2 has a protective role against colon cancer (281). The development of new animal models lacking respiratory tract mucin genes Muc5ac or Muc5b should yield basic information about mucin functions in response to infection and inflammation. Finally, further characterization of the signaling pathways leading to GCM in vivo may provide new biologic targets for therapies to prevent the development of GCH and airway obstruction by mucus in chronic airway diseases.

Future goals for research in the biology of airway mucins must not only focus on what goes wrong in chronic inflammatory diseases but also on what is essential about mucins in the normal innate immune defense of the lung. Such studies will require new fundamental observations about posttranslational processing of specific mucins. Studies on biophysical and biological properties of specific airway MUC mucins with defined O-glycan chains are also important and will be facilitated when adequate amounts of purified or recombinant MUC mucins are available. They will provide a basis for functional studies delineating how specific domains in MUC protein backbones, as well as in the attached O-glycans, contribute to and/or determine the biological and biophysical properties of mucin glycoproteins that impact on the role of mucins in mucosal immunity and thus on airway health and disease. The ultimate goal of research in this area of lung cell biology is to better understand airway health to diminish the morbidity and mortality so intimately associated with chronic airway diseases (whether inherited or acquired). Such information should facilitate the development of early detection methods for airway obstruction by mucus and of more efficacious pharmacological agents.

ACKNOWLEDGMENTS

We thank Dr. Bernard Fischer (Tables 4 and 6), Susan Nasr (Tables 2 and 3), Alan Watson (Figs. 1, 5, and 6; Table 4), Charles Kovach (Figs. 2 and 3), and Sami Mufarrij (Table 1) for their invaluable contributions to the tables and figures. We also thank Dr. Tracey Nickola, Susan Nasr, and Charles Kovach for critical editing of manuscript drafts and Dr. Inka Brockhausen for editing the mucin glycosylation section. The authors recognize that numerous investigators have contributed to the greatly expanded knowledge in this area of lung biology. We apologize for omissions or errors.

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REFERENCES

6. Atherton HC, Jones G, and Danahay H. IL-13-induced changes in the goblet cell density of human bronchial epithelial cell cul-
10. Aust MR, Madsen CS, Jennings A, Kasperebaru JL, and Gendler SJ. Mucin mRNA expression in normal and vasostronger inflam-
39. Carlson DM. Structures and immunomucchemical properties of oligo-
44. Chen Y, Zhao YH, and Wu R. Differential regulation of airway mucin gene expression and mucin secretion by extracellular nucleo-
45. Cheng PW, Boat TF, Cranfill K, Yankaskas JR, and Boucher RC. Increased sulfation of glycoconjugates by cultured nasal epi-

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282. Velich A and Augustin H. Stimulation of neuropeptide Y release from colonic mast cells by 10.220.33.1 on November 6, 2017 http://physrev.physiology.org/ Downloaded from


