MUC1 Mucin

A Peacemaker in the Lung

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MUC1 is a membrane-tethered mucin expressed on the surface of epithelial cells lining mucosal surfaces. Recent studies have begun to elucidate the physiologic function of MUC1 in the airways, pointing to an antiinflammatory role that is initiated late in the course of bacterial infection and is mediated through inhibition of TLR signaling. These new findings have great potential for clinical applications in controlling excessive and prolonged lung inflammation. This review briefly summarizes the protein structural features of MUC1 relevant to its function, the discovery of its antiinflammatory properties, and potential directions for future avenues of study.

Keywords: MUC1 mucin; antiinflammatory; airway infection

EXPRESSION OF MUC1 IN THE LUNG

Mucins are a family of high-molecular-weight glycoproteins responsible for the viscoelastic property of airway mucus and important for mucociliary clearance. Eighteen human *MUC* genes (*Muc* in nonhumans) have been cloned, 12 of which are expressed at the mRNA level in the airways (*MUC1*, 2, 4, 5A, 5B, 7, 8, 11, 13, 16, 19, and 20) (1–3). In 1987, we reported mucin-like glycoproteins present on the apical surface of hamster tracheal epithelial cells that were released by neutrophil elastase (NE), a potent mucin secretagogue (4). Subsequent gene cloning (5) and biochemical characterization of the plasma membrane proteins from primary airway epithelial cells (6) identified the hamster homolog of MUC1, originally cloned from human cancer cells (7, 8). In addition to tracheal epithelial cells, MUC1 expression has also been reported in alveolar epithelial cells (9).

STRUCTURE OF MUC1

MUC1 consists of a large ectodomain that is heavily glycosylated through GalNAc O-linkages, a single-pass transmembrane region, and an intracellular cytoplasmic tail (CT). The MUC1 ectodomain serves as a binding site for *Pseudomonas aeruginosa* flagella (10, 11), and the 72-amino acid CT contains seven evolutionally conserved tyrosines (12) (Figure 1). Our laboratory (13–18) and others (19–26) have shown that many of these tyrosines are phosphorylated, which may be necessary for binding to kinases and adapter proteins, including PI3K (Y²⁰HPM), Shc (Y²⁶PTY), PLC- γ (Y³⁵VPP),

CLINICAL RELEVANCE

This article reviews the discovery of a new antiinflammatory molecule in the lung and provides the future directions of the antiinflammatory research from both basic and clinical viewpoints.

c-Src (Y⁴⁶EKV), and Grb-2 (Y⁶⁰TNP). Binding of PI3K, c-Src, and Grb-2 to the CT have been demonstrated, while Shc and PLC- γ are only inferred based upon the sequence motifs (18, 21, 24). Other proteins bind to nontyrosine sites, including GSK3 β (D⁴²RSP), PKC- δ (T⁴¹DRS), and β -catenin (S⁵⁰AGNGGSSL) (20, 27, 28). Consensus sequences resembling an ITAM (immunoreceptor tyrosine-based activation motif) and ITIM (immunoreceptor tyrosine-based inhibitory motif) are present in the CT (12). Estrogen receptor α , p53, p120^{cas}, all ErbB members, APC, Hsp70, and Hsp90 also have been reported as binding partners of the CT, but specific amino acid residues have not been identified (29–34). Analysis of downstream signaling events indicated that the MUC1 CT activated a Ras $\rightarrow$ MEK1/2 $\rightarrow$ ERK1/2 pathway, but the mechanism is unclear (14, 16, 17, 33). For more details about MUC1 signaling, see the reviews by Hollingsworth and Swanson (1) and Hattrop and Gendler (3).

SUPPRESSION OF AIRWAY INFLAMMATION BY MUC1

A breakthrough in understanding the function of MUC1/Muc1 came with the development of gene knockout mice (35). Using an experimental model of bacterial lung infection, we reported that Muc1^{−/−} mice exhibited reduced lung colonization of *P. aeruginosa*, greater recruitment of leukocytes, and higher levels of TNF- α and keratinocyte chemoattractant (KC) (IL-8) in bronchoalveolar lavage fluid compared with their wild-type littermates (36). In addition, higher levels of TNF- α in culture medium of alveolar macrophages treated with flagellin, a TLR5 agonist, as well as greater levels of KC in medium of flagellin-treated primary tracheal epithelial cells from Muc1^{−/−} versus Muc1^{+/+} mice, suggested that Muc1 suppressed *P. aeruginosa*-induced airway inflammation. Knockdown of MUC1 expression by RNA interference in primary normal human bronchial epithelial cells enhanced flagellin-induced IL-8 production. This implied that the enhanced inflammatory responses from Muc1^{−/−} mice and cells to bacterial infection was not due to a compensatory mechanism resulting from the absence of Muc1 during embryonic development, but due to the absence of Muc1 function in the animals or cells. More importantly, overexpression of MUC1 attenuated NF- κ B activation and reduced TNF- α secretion in RAW264.7 cells in response to not only flagellin, but also to agonists for TLR2, 3, 4, 7, and 9, suggesting that MUC1 is a universal negative regulator of airway inflammation (37). The latter effects were lost when cells

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Figure 1. Structure of MUC1. (A) The extracellular region contains sites of O- and N-linked glycosylation. Serines and threonines are the sites of O-glycosylation, while asparagines in specific sequences (N-X-S/T) are potential sites of N-glycosylation. The 20-amino acid variable number of tandem repeats (VNTR) is also referred to as the PTS domain due to the preponderance of proline, threonine, and serine residues (colored ovals). Although only 6 VNTRs are shown, their actual number can vary from 25 to 125 in various tissues and/or individuals (3). Proteolysis at the indicated Gly-Ser site creates a heterodimer protein structure. (B) Tyrosine residues (purple) in the MUC1 CT are phosphorylated by the indicated kinases (arrows) and serve as binding sites (boxes) for kinases and adapter proteins. Additional binding sites for GSK3 β and β -catenin are indicated. The question mark indicates the action of an unknown kinase.

Figure 2. Proposed model for the roles of MUC1, TLRs, TNF- α , IL-8, and NF- κ B as major players in the response of airway epithelia to bacteria. (Step 1) During the early stage of infection by *Pseudomonas aeruginosa* (PA), bacterial PAMPs (e.g., flagellin) activate TLRs and NF- κ B on epithelial cells and macrophages (M) (2). Activation of NF- κ B leads to increased expression of TNF- α and of IL-8, which are subsequently secreted (3). IL-8 recruits neutrophils (N) across the epithelial barrier that release NE into the lumen of the airways (4). NE and TNF- α up-regulate MUC1 gene expression, resulting in increased expression of MUC1 mucin at the apical surface of lung epithelial cells (5). During the late stage of infection, tyrosine phosphorylation of MUC1 CT domain leads to inhibition of TLR signaling and down-regulation of inflammation.

were transfected with a MUC1 deletion mutant devoid of the CT, but not an ectodomain deletion, revealing the importance of the CT for its antiinflammatory effect. Although PI3K was initially proposed as a mediator of MUC1-TLR crosstalk, its involvement has recently been ruled out (18), and the exact mechanism of interaction between MUC1 and TLRs remains unknown.

REGULATION OF MUC1 LEVELS

Given the importance of host inflammatory responses to bacterial infection, it is important to understand how and when MUC1 levels are controlled in the context of airway inflammation. NE was demonstrated to up-regulate MUC1 expression *in vitro* by stimulating gene transcription through a PLC- γ $\rightarrow$ Duox1 $\rightarrow$ ROS $\rightarrow$ TACE $\rightarrow$ TNF- α $\rightarrow$ TNFR1 $\rightarrow$ ERK1/2 $\rightarrow$ Sp1 pathway (38, 39). These results suggested the involvement of inflammatory products in the regulation of airway MUC1 levels. Indeed, TNF- α (40) and IL-1 β (unpublished data) increased MUC1 levels in dose-dependent manners in cultured lung epithelial cells. In addition, while Muc1 levels in murine lungs were relatively low at early times after infection, they were drastically up-regulated after *P. aeruginosa*-induced increases in TNF- α levels. A similar increase in Muc1 was not observed in TNFR^{-/-} mice (unpublished data). Based on these studies, we proposed a working model to account for MUC1, TLRs, and TNF- α in the airway epithelial response to bacterial infection (Figure 2).

HOW DOES MUC1 FIT INTO THE CURRENT PARADIGM OF AIRWAY ANTIINFLAMMATION?

Given this background in concert with antiinflammatory processes that are recognized to operate in the airways (41, 42), we suggest two potential mechanisms associated with MUC1 that warrant further investigation, namely signaling through IL-10 and/or PPAR- γ . In the context of MUC1/Muc1, IL-10 plays a critical role during *P. aeruginosa* lung infection and inflammation. For example, IL-10^{-/-} mice have a phenotype similar to Muc1^{-/-} mice, suggesting that both proteins share a common mechanism of action (43). Furthermore, IL-10 production was up-regulated in response to flagellin, and treatment of dendritic cells with tumor-derived MUC1 increased IL-10 (44). On the other hand, PPARs are nuclear hormone transcription factors that play important regulatory roles in a variety of physiologic processes, including airway inflammation. Kim and coworkers (45) reported that the antiinflammatory role of PPAR- γ in the pathogenesis of asthma is partly mediated through its up-regulation of IL-10. Another study suggested that PPAR- γ induced mouse Muc1 expression through a PPAR- γ response element in its gene promoter (46). Thus, it is likely that the antiinflammatory effect of PPAR- γ involves both IL-10 and MUC1. In summary, *MUC1/Muc1* genes are transcriptionally activated during airway inflammation mainly as a consequence of increased TNF- α levels after bacterial infection. Increased MUC1/Muc1 protein levels suppress TLR signaling, resulting in attenuation of ongoing airway inflammation. While the detailed molecular mechanism of crosstalk between MUC1 and TLRs remain to be determined, signaling through IL-10 and/or PPAR- γ are likely mechanisms that deserve further study.

Conflict of Interest Statement: K.C.K. has applied to the U.S. PTA in May 2006 for a patent related to the anti-inflammatory role of MUC1. E.P.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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