Mol. Cells *30*, 173-183, September 30, 2010
DOI/10.1007/s10059-010-0146-4

Minireview

Molecules and Cells

Innate Immune Responses of the Airway Epithelium

Ji-Hwan Ryu[1,2], Chang-Hoon Kim[3,4], and Joo-Heon Yoon[1,2,3,4,*]

Barrier epithelia, especially airway epithelial cells, are persistently exposed to micro-organisms and environmental factors. To protect the host from these microbial challenges, many immune strategies have evolved. The airway epithelium participates in the critical innate immune response through the secretion of immune effectors such as mucin, antimicrobial peptides (AMP), and reactive oxygen species (ROS) to entrap or kill invading microbes. In addition, airway epithelial cells can act as mediators connecting innate and adaptive immunity by producing various cytokines and chemokines. Here, we present an overview of the role of mucosal immunity in airway epithelium, emphasizing the framework of bacterial and viral infections along with regulatory mechanisms of immune effectors in human cells and selected animal models. We also describe pathophysiological roles for immune effectors in human airway disease.

INTRODUCTION

The airway epithelium is positioned to confront microbes entering the multi-cellular bodies of eukaryotes. In addition to acting as physical barriers to block microbial infection, epithelial cells must sense pathogens with pattern recognition receptors and directly kill pathogens using immune effectors. Bacteria or virus recognition by airway epithelial cells induces the production of chemokines and cytokines that recruit and activate phagocytes to the site of infection, resulting in pathogen clearance by phagocytosis. The main bacteria or virus recognition receptors for airway epithelial cells are Toll like receptors (TLRs) and RIG-I-like receptors (RLRs). In the airway, several specialized cell types are present in the epithelial cells and submucosal glands. The nasopharynx, nasal sinuses, and conducting airways including trachea, bronchi, and bronchioles, are lined with a moderately tight pseudo-stratified surface epithelium (Willumsen and Boucher, 1989). The epithelium plays an essential role in regulating the host defense against pathogen infection by secreting osmotically coupled liquid in the airway lumen and controlling the composition of airway surface liquid (ASL). ASL contains many kinds of bacterial killing immune effectors including mucins, AMPs, and ROS. The characterization of the regulatory mechanisms and biological functions of these critical immune effectors against pathogens is important for understanding the innate immunity of the airway epithelium and causes of airway inflammatory diseases induced by chronic bacterial infection.

Microbial pattern recognition receptors (PRRs) in airway epithelium

Our general understanding of how the innate immune system recognizes various kinds of microbes has improved over the past several years (Akira et al., 2006; Fritz and Girardin, 2005; Medzhitov et al., 1997). The discrimination between "self" and "non-self" by TLRs as PRRs that recognize pathogen-derived molecular patterns is a key mechanism of innate immunity. Respiratory epithelia express various kinds of TLRs at the cell surface and endosomes. TLRs have extracellular ligand-binding domains consisting of 19-25 contiguous copies of a motif known as leucine-rich repeats (LRRs). They also have a conserved stretch of ~200 amino acids in their cytoplasmic region, known as the Toll/interleukin-1 (IL-1) receptor homology domains, which mediate signals to the nucleus through activating intracellular signaling molecules. To date, 13 mammalian TLRs have been described, 10 of which can be identified in humans (designated TLR1-TLR10). Each TLR recognizes a unique kind of molecular pattern, including peptidoglycans, bacterial lipopolysaccharide (LPS), lipoteichoic acid, lipoproteins, lipopeptides, fungal zymosan, bacterial flagellin, single- or double-stranded RNA, and CpG DNA. So far, a variety of ligands have been linked to specific TLRs, including lipoteichoic acid and lipoproteins for TLR2 (Schwandner et al., 1999), LPS for TLR4 (Poltorak et al., 1998), double-stranded viral RNA for TLR3 (Alexopoulou et al., 2001), bacterial flagellin for TLR5 (Hayashi et al., 2001), single-stranded viral RNA for TLR7 and TLR8 (Diebold et al., 2004; Heil et al., 2004) and unmethylated CpG DNA for TLR9 (Hemmi et al., 2000).

TLR4 in airway epithelium

The functions of TLR4 and TLR2 in combating bacterial infections have been well studied in the airway epithelium. Binding of bacterial LPS to TLR4 on airway epithelial cells initiates the immune response by inducing the expression of inflammatory cytokine genes such as IL-8 and IL-6 that serve to recruit and

[1]Research Center for Human Natural Defense System, Yonsei University College of Medicine, Seoul 120-752, Korea, [2]The Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Korea, [3]Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul 120-752, Korea, [4]The Airway Mucus Institute, Yonsei University College of Medicine, Seoul 120-752, Korea
*Correspondence: jhyoon@yuhs.ac

Received September 2, 2010; accepted September 5, 2010; published online September 17, 2010

Keywords: airway epithelium, antimicrobial peptides, innate immune responses, Mucin, pattern recognition receptor, reactive oxygen species, respiratory viruses

activate phagocytes to the site of infection. Although recruited neutrophils kill bacteria, their presence and the toxicity of their products also damage airway epithelial cells through the host inflammatory response. Accordingly, the level of expression and activity of TLR4 is critical for determining the intensity of the immune response to bacterial infection. In airway epithelial cells, TLR4-mediated signaling to LPS requires the cooperated action of accessory proteins, including soluble CD14, LPS-binding protein, and the costimulatory molecule MD2. Primary human epithelial cells are extremely hyporesponsive to endotoxin, in spite of normal expression of TLR4. The low response to endotoxin appears to be mainly due to low expression of MD2 (Jia et al., 2004). These findings suggest that a limited level of MD2 in airway epithelial cells restricts TLR-induced inflammation under resting conditions, ensuring that airways are not chronically inflamed by exposure to airborne microorganisms.

TLR4 is expressed in the cytosol and not mobilized to the cell surface by infection with *Pseudomonas aeruginosa (P. aeruginosa)* or *Staphylococcus aureus (S. aureus)*, indicating that lower expression of TLR4 on the cell surface supports the mechanism by which inflammation is normally silenced in the airway (Guillot et al., 2004). With repeated bacterial stimulation, however, the TLR4-mediated inflammatory response is critical for clearing infectious pathogens. The airway from cystic fibrosis (CF) patients is chronically infected with *P. aeruginosa*, and TLR-4 expression in the bronchial epithelium from CF patients is significantly lower than in healthy subjects, suggesting that the TLR4-mediated inflammatory response is required for removing chronic bacterial infection from CF airways (John et al., 2010). Smoking-induced severe chronic obstructive pulmonary diseases (COPDs) are also caused by the reduction of TLR4 gene expression in the airway epithelium (MacRedmond et al., 2007).

TLR2 in airway epithelium

TLR2 is expressed on the apical surface of polarized cells and mobilized into specialized lipid raft microdomains after bacterial stimulation (Soong et al., 2004). TLR2 expression level is increased by infection with Gram-positive and Gram-negative bacteria (Kajikawa et al., 2005; Knapp et al., 2004). The function of TLR2 in initiating the inflammatory immune response has been described in airway epithelia and professional immune cells (Greene et al., 2005; Muir et al., 2004). Survival of TLR2 null mice is dramatically reduced during systemic infection with *S. aureus* compared to wild-type (WT) mice (Takeuchi et al., 2000). *S. aureus* clearance is not affected in the lungs of TLR2 null mice infected with aerosolized *S. aureus*, but TLR2 presence is required for the production of inflammatory cytokines and chemokines (Skerrett et al., 2004). In addition, the survival rate of TLR2 null mice challenged with the aerosolized Gram-negative pathogen *P. aeruginosa* is not less than that of WT mice (Skerrett et al., 2007), indicating that unknown signaling pathways, in addition to TLR2 signaling, play a role in the response to respiratory pathogens. TLR2 has been involved in recognition of intracellular pathogens such as *Legionella pneumophila* (*L. pneumophila*) (Akamine et al., 2005), and TLR2-mediated signaling is required for clearance of *L. pneumophila* in the lung using a Legionnaires' disease mouse model (Archer and Roy, 2006). Taken together, these findings demonstrate that the protective role of TLR2-mediated signaling in airway epithelium is dependent on the type of infectious pathogen.

Recognition molecules for respiratory viruses in airway epithelium

Virus infection in mammals is detected by receptors that sense viruses as foreign molecules and initiate a variety of defense responses. The airway epithelium mainly limits viral infection by means of recognizing incoming viruses and alerting nearby immune cells to produce antiviral proteins and neutralizing antibodies. These immune responses are induced by signaling molecules, including type I interferons (IFN-α and IFN-β) and type III IFN (IFN-λ). Research on virus-sensing mechanisms by PRRs is extremely important for understanding the induction of IFN gene transcription in response to viral infection. So far, several virus recognition receptors have been identified and characterized, including TLR3, retinoic acid inducible gene I (RIG-I), melanoma differentiation factor-5 (MDA5), and laboratory of genetics and physiology-2 (LGP-2) (Pichlmair and Reis e Sousa, 2007). Although TLR3 is a member of the endosomal TLR family that responds to double-stranded-RNA (dsRNA) nucleotide structures of the main respiratory viruses, the protective role of TLR3 against viral infection has not been demonstrated. This discrepancy may be due to the localization of TLR3 in endosomes, which prevents detection of replicating virus in the cytoplasm.

RLR family members

Cytosolic virus recognition receptors of the RLR family such as RIG-I and MDA5 have been recently described (Fig. 1). RIG-I was first characterized as a gene that can activate IRF-induced gene expression in response to transfection with synthetic polyinosinic-polycytidylic acid (polyI:C) used as dsRNA mimic molecules (Yoneyama et al., 2004). RIG-I has a central DExD/H-box RNA helicase domain and two N-terminal caspase recruitment domains (CARDs). Overexpression of the N-terminal CARD domain constitutively activates IFN production without viral infection. Although overexpression of full-length RIG-I does not constitutively activate IFN production without viral infection, a significant increase in IFN-producing signaling occurs in response to viral infection. In addition, a mutant deficient in the CARD domain completely suppresses IFN-producing signaling in response to viral infection, indicating that CARD is required for mediation of the virus-induced IFN signaling pathway. The helicase domain modulates RIG-I function through binding to specific virus genomes during viral replication, and the adenosine triphosphate (ATP) binding region within the helicase domain is also responsible for activating IFN production signaling (Cui et al., 2008; Takahasi et al., 2008).

MDA5 was originally cloned as a gene up-regulated by IFN treatment in a melanoma cell line (Kang et al., 2002). MDA5 shows 35% and 23% amino acid similarity to RIG-I in the helicase and N-terminal tandem CARD domains, respectively. MDA5 functions as a PRR for viral genomes, and a critical role for MDA5 in virus-induced IFN-producing signaling has been shown using KO mice (Gitlin et al., 2006; Kato et al., 2006). LGP2 is another RLR family member characterized as the helicase-like protein expressed in normal and neoplastic mammary tissue (Cui et al., 2001). The LGP2 protein has a high level of amino acid similarity to the helicase domain of RIG-1 and MDA5, but LGP2 is devoid of the CARD domain. LGP2 appears to play a suppressive role in RIG-I/MDA5-induced IFN signaling (Rothenfusser et al., 2005; Yoneyama et al., 2005), and yet LGP2 functions as a positive regulator in response to encephalomyocarditis virus (EMCV) infection, based on gene mutation analysis (Venkataraman et al., 2007).

Viral RNA recognition by RLRs

Given that both RIG-I and MDA5 recognize dsRNA such as poly I:C and induce IFN production via a common pathway, it is uncertain whether they have distinctive or redundant detection

systems for the recognition of viruses patterns. The analysis of RIG-I and MDA5 KO mice indicates a significant specificity for detecting various kinds of viruses (Gitlin et al., 2006; Kato et al., 2006). Mouse embryonic fibroblasts (MEFs) from RIG-I KO mice show diminished IFN production in response to infection with Newcastle disease virus (NDV), vesicular stomatitis virus (VSV), influenza A virus, Sendai virus (SeV), and Japanese encephalitis virus (JEV), whereas MDA5-deficient MEFs are unresponsive to picornaviruses, including Theiler's encephalomyelitis virus, encephalomyocarditis virus (EMCV), and mengovirus (Gitlin et al., 2006; Kato et al., 2006). Overall, a large portion of dsRNA viruses are detected by RIG-I. In contrast, murine norovirus and Picornaviridae are recognized by MDA5, even though they have a dsRNA genome. (Loo et al., 2008; McCartney et al., 2008). Several viruses are recognized in different ways. Reoviridae (a dsRNA virus) is recognized by either RIG-I or MDA5 in a length-dependent manner (Kato et al., 2008; Loo et al., 2008). Dengue virus and West Nile virus seem to be detected redundantly by both RIG-I and MDA5 (Fredericksen and Gale, 2006).

The difference in viral recognition by RIG-I and MDA5 is based on preferences for RNA structure from RNA virus genomes. RIG-I is selectively activated by RNA transcribed in vitro by phage polymerases (IVT-RNA). IVT-RNAs have an uncapped 5′-triphosphate (5′-PPP) that is responsible for promotion of binding of RIG-I and inducing IFN signaling. In contrast, MDA5 is specifically activated by poly I:C, which is made by annealing inosine and cytosine, which have 5′-diphosphate and 5′-monophosphate ends (Hornung et al., 2006; Kato et al., 2006; Pichlmair et al., 2006). This difference may explain why RIG-I does not respond to host cytoplasmic RNA; 5′-PPP moieties of most host mRNAs are either masked by a 7-methylguanosine capping and 5′-PPP moieties of most host rRNAs and tRNAs are removed by processing in the maturation process before they reach the cytoplasm. As mentioned above, poly I:C triggers MDA5-induced IFN production, and the optimal length of poly I:C is responsible for effective MDA5 activation. Shortening poly I:C to 1000 nucleotides or less converts it into a RIG-I agonist (Kato et al., 2008). These observations indicate that MDA5 detects long dsRNA generated during infection and replication of viruses. Although the initial research about viral recognition of RIG-I and MDA5 was carried out in hematopoietic cells, airway epithelial cells also express functional levels of these receptors. The expression of RIG-I and MDA5 is increased by respiratory virus infection, and increased MDA5 has a critical role in inducing IFN production (Wang et al., 2009). Future investigation into specific viral recognition will be required to determine which receptors respond to various kinds of respiratory viruses and mediate the IFN signaling pathway and pro-inflammatory cytokine production.

RLR-mediated signal transduction

The identification of RLRs was rapidly followed by the characterization of the downstream adaptor interferon-β promoter stimulator-1 (IPS-1), also called virus-induced signaling adaptor (VISA), mitochondrial antiviral signaling protein (MAVS), or CARD adaptor inducing IFN-β (CARDIF) (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005) (Fig. 1). IPS-1 contains an N-terminal CARD domain that forms homotypic interactions with the CARD domains of RIG-I and MDA5, which leads to activation of the C-terminal catalytic domain and triggering of a signaling transduction that culminates in IFN-β and pro-inflammatory cytokine gene expression. Interestingly, IPS-1 contains a transmembrane domain at its C-terminal end and is localized on the outer membrane of mitochondria (Seth et al., 2005). These results suggest that IPS-1 localization on mitochondria might be involved in RLR-induced signaling. In fact, truncation of its C-terminal transmembrane domain or enforced mislocalization of IPS-1 to the plasma membrane or endoplasmic reticulum leads to failure of IFN signal activation. The physiological role of mitochondrial localization of IPS-1 is not yet fully understood.

Viral evasion strategies from host anti-viral signaling

Viruses replicate expansively in infected host cells to guarantee successful transmission, and must, therefore, develop immune evasion strategies. Viruses can hinder IFN-α and -β synthesis by interfering with IFN receptor signaling or inhibiting the activation of antiviral effector proteins induced by IFNs (Weber et al., 2004). Here, we concentrate on the strategies used by viruses to inhibit RLR-mediated IFN signaling pathways. The simplest strategy for viruses to defend against antiviral signaling is to escape from the RLR recognition system. For example, RNA viruses such as CCHFV and BDV do not stimulate IFN signaling because they do not have a 5′-ppp structure in their own genome (Habjan et al., 2008). In addition, Picornaviridae and Calcioviridae express a VPg protein that modifies the 5′-terminal of the virus genome to escape RIG-I recognition. These two viruses are recognized by MDA5, however, suggesting that the host immune system has developed alternative detection machinery to hamper viral evasion strategies.

Viruses also escape detection through the production of viral proteins that directly inhibit RLRs or IPS-1 by interacting with them or breaking them down (Andrejeva et al., 2004; Childs et al., 2007; Komatsu et al., 2007). Non-structural protein 1 (NS1) of influenza A virus inhibits the function of RIG-I-mediated IFN signaling through interaction with RIG-I (Mibayashi et al., 2007; Opitz et al., 2007). In airway epithelial cells, NS1 of influenza A virus functions as an antagonist for the IFN signaling pathway by binding the RNA genome (Guo et al., 2007). Overall, although signaling molecules involved in RLR-induced IFN signaling have been identified, the exact interactions between viral proteins and antiviral signaling molecules remain largely unknown. In depth studies about these topics will support new insights into the host immune system against viral infection and may help develop new therapeutic approaches for viral infectious diseases.

ANTI-MICROBIAL EFFECTORS IN AIRWAY EPITHELIUM

Mucin

In eukaryotes, glycoproteins on the extracellular surface are known to play a critical role in mucosal defense (Fig. 2). Among them, mucins are the main glycoprotein components of mucus and have a peculiar structure of serine/threonine-rich tandem repeating amino acids which are the sites for O-linked glycosylation (Thornton et al., 2008). The presence of mucin at the airway epithelial surface is necessary to avoid desiccation of underlying cells, prevent endogenous and foreign particle accumulation, and defend against pathogen invasion. Here, we will focus on regulation of mucin gene expression and the function of mucin in normal and chronic inflammatory diseases.

Mucin genes in airway epithelium

Many MUC genes are expressed in airway epithelial cells, including MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8, MUC11, MUC13, MUC15, MUC19, and MUC20 (Rose and Voynow, 2006). The MUC proteins encoded by these genes are classified into three main families: membrane-associated mucins (MUC1, MUC4, MUC11, MUC13, MUC15,

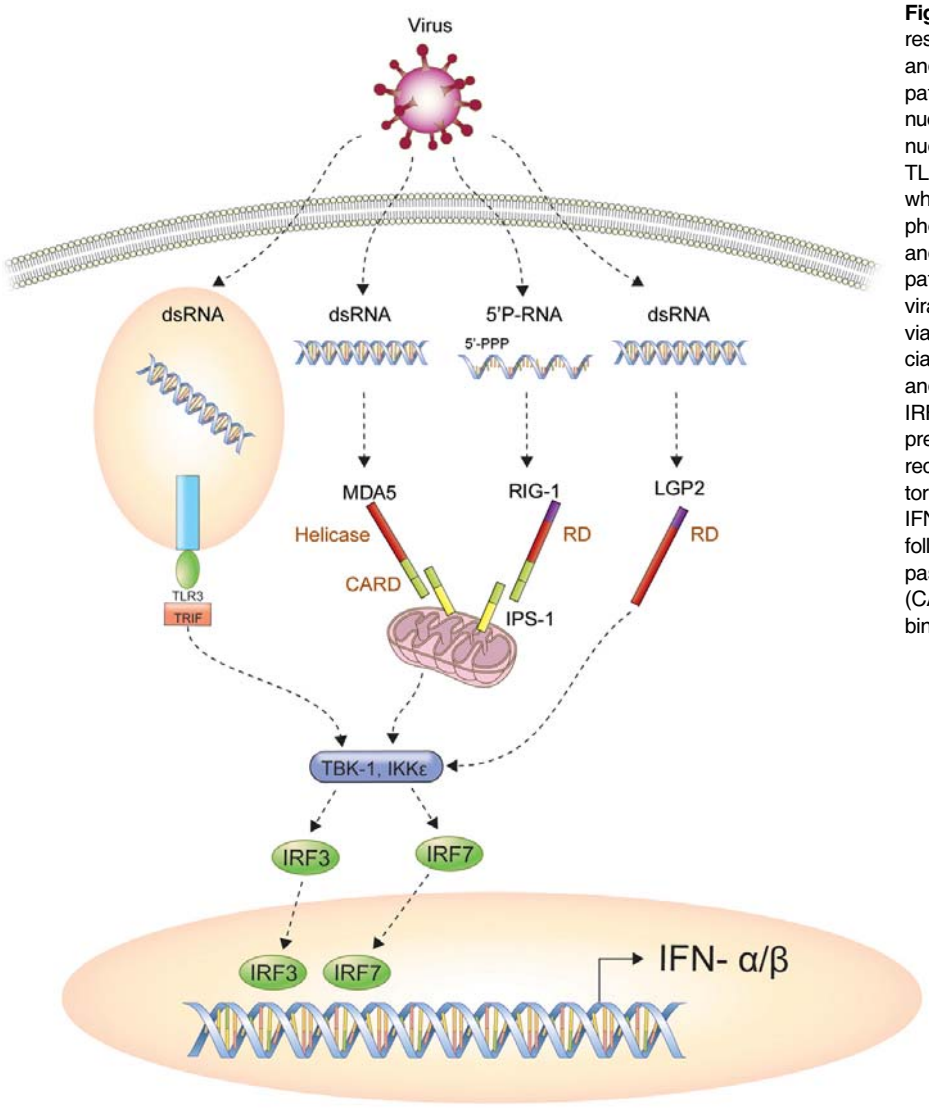

Fig. 1. General signaling pathways from respiratory virus recognition to IFN-α and -β gene expression. Endosomal pathway: respiratory viruses deliver their nucleic acids into endosomes, where nucleic acids are recognized by TLR3. TLR3 transfers the signal via TRIF, which activates TBK-1 and IKKε and phosphorylates IRF3 to induce IFN-α and -β gene expression. Cytosolic pathway: RIG-I and MDA5 recognize viral RNAs, which transfers the signal via the IPS-1, a mitochondrion-associated adaptor. IPS-1 activates TBK-1 and IKKε and phosphorylates IRF3 and IRF7 to induce IFN-α and -β gene expression. LGP2, a cytosolic dsRNA receptor, may use an alternative adaptor to activate TBK-1 and IKKε to induce IFN-α and -β gene expression. The following abbreviations are used: caspase activating recruitment domain (CARD), repressor domain (RD), RNA binding domain (Helicase).

MUC20), secreted gel-forming mucins (MUC2, MUC5AC, MUC5B, MUC8, and MUC19), and a secreted non-gel-forming mucin (MUC7). Membrane-associated mucins are bound to cells by a transmembrane domain and contain short cytoplasmic tails that associate with cytoskeletal elements and adaptor proteins involved in signal transduction. Thus, membrane-associated mucins might serve as cell-surface receptors or sensors in response to external stimuli (Hollingsworth and Swanson, 2004). Among the secreted mucins, MUC2 was the first MUC gene identified in the lung, although MUC5AC and MUC5B also seem to be major MUC genes expressed in human airway epithelium. MUC2 is up-regulated by infection with nontypable haemophilus influenzae (NTHi), an important human respiratory pathogen, through the TGF-β/NF-κB signaling pathway (Jono et al., 2002). In contrast, the MUC2 expression level in obstructive airway diseases makes up only 2.5% of the weight of whole gel-forming mucins, indicating that MUC2 is a minor mucin protein (Kirkham et al., 2002). Some reports indicate that MUC2 is aberrantly expressed in goblet-type mucinous carcinomas of the lung (Mesquita et al., 2004). Accordingly, MUC2 over-expression in the airway epithelium may be a marker of lung cancer.

MUC5AC is mainly expressed in goblet cells at the luminal surface in healthy airways, and is the most abundant gel-forming mucin in COPD, CF, and asthmatic airways (Davies et al., 1999; Groneberg et al., 2002; Kirkham et al., 2002). MUC5AC expression is up-regulated by *P. aeruginosa* in both bronchial explants and cultured airway epithelial cells (Dohrman et al., 1998). MUC5AC is also up-regulated in a mouse model of asthma and parainfluenza type 1 infection in mice (Walter et al., 2002; Zuhdi Alimam et al., 2000). Among gel-forming mucins, only MUC5AC is highly produced in ovalbumin-sensitized and -challenged mouse lungs (Young et al., 2007), indicating that MUC5AC over-expression is the central event in mucous metaplasia in the mouse airway. Apart from MUC5AC, MUC5B is constitutively expressed in the mucous cells of the submucosal gland (Schulz et al., 2005) and its production is not much greater in bronchial lavage fluid obtained from smokers and asthma patients compared to normal controls (Innes et al., 2006; Ordonez et al., 2001). Overall, compared to MUC5AC, MUC5B is not dramatically induced in the airway epithelium of patients with chronic inflammatory diseases. Investigating the

physiological differences between WT mice and mice with loss of function or gain of function in airway disease models will shed light on the significant role of MUC5B in airway epithelial cells.

Regulation of mucin gene expression

In innate and adaptive immune responses in the airway epithelium, mucin production is induced by stimulation from pathogens, inflammatory cytokines, and environmental toxins through various signaling pathways. It was first demonstrated that bacteria including *S. aureus*, *P. aeruginosa*, *Streptococcus pneu-moniae*, NTHi, and *Mycoplasma pnumoniae* induce MUC5AC and/or MUC2 gene expression through activation of the NF-κB signaling pathway (Chen et al., 2004; Ha et al., 2007; Lem-jabbar and Basbaum, 2002; Li et al., 1997; 1998; Wang et al., 2002). Respiratory viruses also induce mucin gene expression in airway epithelial cells. Rhinovirus, the main infectious virus of the upper airway, activates MUC5AC and MUC5B gene expression in airway epithelial cells (Inoue et al., 2006). Other respiratory viruses, including respiratory syncytial virus (RSV), influenza A virus, and paramyxoviruses, activate MUC5AC production involved in mucous metaplasia in mice (Buchweitz et al., 2007; Hashimoto et al., 2004; Tyner et al., 2006). Recently, a comprehensive analysis of the role of MUC5AC in a human infection model showed that rhinovirus infection induces asthma exacerbation by activating MUC5AC production (Hew-son et al., 2010).

Pro-inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α up-regulate MUC5AC gene expression (Lora et al., 2005; Song et al., 2003). In particular, TNF-α and IL-1β activate MUC5AC gene expression through a multi-signaling step, in which they activate CREB through p38 MAPK, ERK1/2, and MSK1. Activated CREB then binds to the MUC5AC promoter to trigger MUC5AC gene transcription in primary nasal epithelial cells (Song et al., 2003). Recently, transgenic mice over-expressing IL-1β in lung epithelial cells showed that MUC5AC production and mucous cell metaplasia lead to chronic inflammatory diseases in the airway (Bry et al., 2007; Lappalainen et al., 2005). IL-13, a critical Th-2 cytokine in airway remodeling, induces MUC5AC expression by upregulation of TGF-β2, Stat6 phosphorylation, and the secondary pathways of EGFR activation and suppression of the transcription factor FoxA2 (Chu et al., 2004; Wan et al., 2004; Wills-Karp et al., 1998; Zhen et al., 2007). Lastly, pollutants and oxidants including hydrogen peroxide, dual oxidase, tobacco smoke, residual oil ash, acrolein, and prostaglandin (PG) E_2 increase MUC5AC gene expression (Gensch et al., 2004; Gray et al., 2004; Kim et al., 2008; Longphre et al., 2000; Shao and Nadel, 2005; Takeyama et al., 2000).

In contrast, inhibitory mechanisms that suppress MUC5AC gene expression have been demonstrated in airway epithelial cells (Kim et al., 2009; Song et al., 2009a; 2009b). In many cases, MUC5AC-inducing stimulants activate EGFR-mediated or alternative signaling, resulting in the activation of NF-κB, specificity protein 1 (SP1), and activating protein 1 (AP1) (Thai et al., 2008; Yuan-Chen Wu et al., 2007). Another mechanism that regulates MUC gene production apart from transcriptional regulation has been reported (Rose and Voynow, 2006). Increased MUC5AC expression by neutrophil elastase treatment is mediated by enhanced post-transcriptional mRNA stabilization in human adenocarcinoma cells (Voynow et al., 1999). Transcriptional regulation of MUC genes likely cooperates with post-transcriptional regulation through mRNA stabilization to optimize MUC production in the airway epithelium.

Role of mucin in airway diseases

Mucus hypersecretion is a simple problem that is recognized as a short term irritant during seasonal allergies or colds caused by a respiratory infection. In contrast, mucus hypersecretion in diseased lungs is a fatal contributor to mortality (Bedrossian et al., 1976). The pathophysiology observed in obstructive pulmonary diseases is airway inflammation, mucus overexpression and hypersecretion, and impaired mucociliary clearance (Hogg et al., 2004; Mannino et al., 2002). Current therapies concentrate on reduction of inflammatory signals that eventually induce mucin synthesis. These approaches are not helpful in many cases, however, because mucus hypersecretion is a basic phenotype in CF without respiratory infection. In other words, the salt-fluid imbalance in the CF airway directly induces mucus hypersecretion. Further research is needed to identify the causes of mucus hypersecretion and methods for reducing inflammation or infection to develop effective therapies for chronic inflammatory airway diseases.

Antimicrobial peptides and proteins

In addition to mucin proteins, surface epithelial cells and submucosal glands secrete AMPs and proteins into the airway lining fluid (Fig. 2). These secretions function in a broad-spectrum mode, exerting antimicrobial effects against bacteria, viruses, and fungi. These antimicrobial effectors clear invading microbes primarily through disrupting cell walls, sequestrating nutrients and iron, and by providing attachment decoys for microbial survival and pathogenic processes (Brogden, 2005; Cole et al., 2002).

Function of small AMPs

Among the inducible bacteriocidal products in the airway epithelium, small cationic AMPs are dominant. Although hundreds of AMPs have been identified, human airway epithelium mainly expresses two types of AMPs: defensins and cathelicidins (Bartlett et al., 2008; Lai and Gallo, 2009). Defensins are characterized by six unique cysteine amino acids, giving rise to three disulfide bonds. The defensins are divided into two groups, α- and β-defensins, according to the distinctive tertiary structure caused by differences in the cysteine residues. β-defensins are expressed in lung epithelial cells and neutrophils. Human β-defensin-1 (HBD-1) is constitutively secreted into airway lining fluid, but HBD-2, -3, -4 are induced by epithelial cells in response to pathogens, inflammatory cytokines, or TLR ligands (Harder et al., 2000; Singh et al., 1998). Numerous β-defensins are induced in mouse lung epithelium by bacterial and viral infection, and they protect the host through killing of influenza viruses and fungi (Jiang et al., 2009; Wang et al., 2010).

Cathelicidins are another cationic AMP expressed in airway epithelia. Humans have only one cathelicidin propeptide (CAMP), which must be cleaved to the active antimicrobial peptide LL-37 (Bals et al., 1998a). LL-37 production is induced by respiratory pathogens such as *P. aeruginosa* and *pneumonia*, and activated LL-37 kills both in the mice airway, indicating that this molecule is a critical element of airway defense (Bals et al., 1998a; Herr et al., 2007). The direct killing mechanism of AMPs is not fully understood, but they kill microbes by inducing microbial permeabilization (Brogden, 2005). The biological importance of LL-37 is demonstrated by increased protection against bacterial challenge in LL-37 overexpressing mice (Bals et al., 1999). Furthermore, the importance of pathogen killing by LL-37 is confirmed by the reduced pathogen clearance and increased mortality of cathelicidin- or defensin-deficient mice infected with a number of bacterial species (Liu et al., 2006;

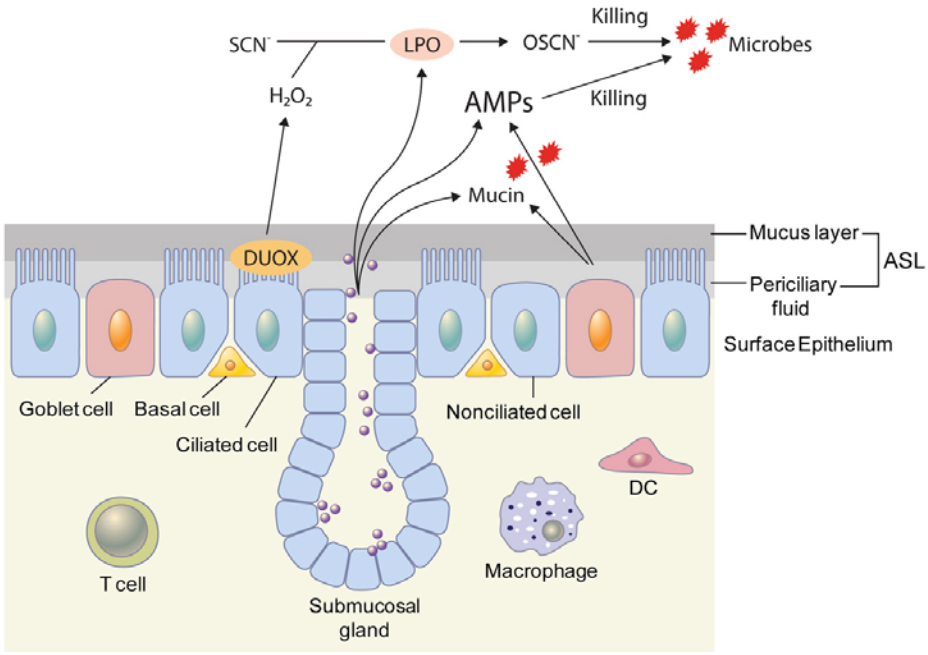

Fig. 2. Innate immunity components of airway epithelium. Bacterial killing immune effectors are secreted from airway epithelial cells and submucosal glands. Mucins are expressed and secreted in goblet cells and submucosal glands to trap microbes. AMPs and antimicrobial proteins are secreted in goblet cells and submucosal glands to kill microbes. LPO generates antibacterial $OSCN^-$ from DUOX-derived H_2O_2 and SCN^-. $OSCN^-$ is the main antibacterial ROS to kill microbes.

Moser et al., 2002). *In vivo* testing at the organism level indicates that AMPs in airway epithelium are important defenders for host protection. In addition to antimicrobial activity, AMPs have chemotactic activities for modulating the innate immune response in airway epithelium. For instance, HBD2 recruits mast cells, and HBD3 and -4 attract monocytes and macrophages. Similarly, LL37 is chemotactic for neutrophils, monocytes, mast cells, and T cells (De et al., 2000; Niyonsaba et al., 2002).

Function of large antimicrobial proteins

In addition to small AMPs, several large antimicrobial proteins have been defined (Kolls et al., 2008). Lysozyme was the first antimicrobial protein identified in ASL, and this polypeptide is expressed and secreted in epithelial cells and submucosal glands. Lysozyme induces lysis of Gram-positive bacteria by hydrolyzing the $\beta_{1\text{-}4}$ glycosidic bond in peptidoglycan, a bacterial cell wall component (Ganz, 2004). Lactoferrin, an 80 kDa protein, is activated by inflammatory and infectious stimuli. Lactoferrin not only inhibits bacterial growth through sequestration of iron from microbial pathogens, but also directly kills bacteria (Ganz, 2004). Antimicrobial proteins known as palate-lung-nasal-clone (PLUNC) are expressed in the upper airways, nose, and mouth and are divided into short (SPLUNC1 and -2) and long (LPLUNC) members. Though the direct antibacterial mechanism of PLUNC has not been determined, PLUNC may function as a host protector by inhibiting LPS-mediated cytokine secretion and LBP/LPS interactions (Bingle et al., 2009). In addition to its chemo-attractant role for leukocytes, CCL20 (also known as MIP-3α) has structural motifs similar to β-defensins. CCL20 gene expression in airway epithelium is induced by bacterial challenge, and elevated CCL20 levels have antibacterial activity against Gram-negative bacteria (Starner et al., 2003).

Collectins or C-type lectins are also inducible epithelial molecules with antimicrobial activity. A family of PRRs that contain an N-terminal cysteine-rich domain and a C-terminal carbohydrate recognition (lectin) domain, collectins have two members: Sp-A and Sp-D. The C-type lectins bind and recognize conserved carbohydrate patterns in respiratory pathogens, leading to phagocytosis by opsonization and neutralizing bacteria by modulation of interactions with dendritic cells and T cells (Pastva et al., 2007). Neutrophil gelatinase-associated lipocalin 2 (known as NGAL) is an antibacterial protein that binds siderophores, which are bacterial iron-binding molecules. Lipocalin 2 is critical for host defense against *K. pneumoniae* pneumonia through prohibiting bacterial growth by limiting the availability of iron (Flo et al., 2004). Protease inhibitors including secretory leukocyte proteinase inhibitor (SLPI) and elafin are known to be required for limiting host tissue damage caused by elastase and neutrophil proteases during airway inflammation (Williams et al., 2006). In addition to their protease inhibitor activity, both SLPI and elafin have antibacterial activity against various kinds of bacteria.

Many of these effector proteins cooperate to kill pathogenic microbes and more effectively protect the host. For example, the antibacterial activity of HBD-2 is improved by the addition of lysozyme and lactoferrin (Bals et al., 1998b). Similarly, treatment with the combination of lysozyme, lactoferrin, and SLPI causes much higher bacterial killing activity than the individual molecules alone (Cole et al., 2002). Thus, all antimicrobial effectors, including unidentified molecules in ASL, are likely to be important for full antimicrobial activity against various kinds of pathogens.

Function of ROS

The human airway produces significant amounts of ROS, and mucosal release of ROS is readily detected both in cell cultures and in the exhaled breath of humans in the normal uninflamed epithelium of the airways (Fischer, 2009). The main sources of released ROS are the dual oxidases DUOX1 and DUOX2 and the NADPH oxidase family found in epithelial cells. DUOX appears to be expressed by the apical part of ciliated cells, but not in nonciliated cells. Recent studies have focused on the antimicrobial function of ROS generated from DUOX in airway lining fluid. In bacterial infection in the airway epithelium, DUOX is

activated and generates H_2O_2 into the airway lumen where lactoperoxidase (LPO) converts DUOX-generated H_2O_2 to bactericidal $OSCN^-$, leading to killing of bacteria (Conner et al., 2002) (Fig. 2).

Regulation of DUOX expression in the airway epithelium

DUOX is mainly expressed from the apical side of ciliated cells in polarized airway epithelial cells (Schwarzer et al., 2004), and neither nonciliated cell nor basal cells express DUOX at a level detectable by immunocytochemistry (Fischer, 2009). LPO is expressed and released by goblet cells and submucosal glands, showing that DUOX-derived H_2O_2 production from ciliated cells and H_2O_2 utilization by LPO from goblet cells and glands are topologically separated, though the physiological reason is currently unknown. The observation that only ciliated cells, but not goblet cells, express DUOX may provide a clue for the cause of inflammatory airway diseases, because the airway epithelium in asthma, CF, and chronic bronchitis undergoes cellular changes to mucous cells or de-differentiated squamous cells, resulting in a large reduction of ciliated cells. These airway diseases are generally recognized by a reduced ability for bacterial killing in airway epithelium and following chronic infections. Given that DUOX expression and antimicrobial function have been robustly demonstrated in airway defense, the loss of ciliated cells in an abnormal de-differentiated cell condition is proposed to deprive the airway epithelium of a central defense mechanism.

Although the isoforms of DUOX1 and DUOX2 have high structural similarity, DUOX2 can produce more H_2O_2 than DUOX1 in cell-free systems (Ameziane-El-Hassani et al., 2005). Yet normal airway epithelium expresses higher levels of DUOX1 than DUOX2, according to semi-quantitative RT-PCR measurements (Schwarzer et al., 2004). Accordingly, the amount of H_2O_2 released from DUOX1 and DUOX2 may be almost the same in the normal airway lumen. The regulatory mechanisms of DUOX1 and DUOX2 expression are different under inflammatory conditions caused by cytokines or bacterial challenge, however. DUOX2 mRNA expression is upregulated by virus infection or the Th1 cytokine IFN-γ, while DUOX1 mRNA level is moderately but specifically induced by the Th2 cytokines IL-4 and IL-13 (Harper et al., 2005). These results suggest that inducible DUOX2 is required for response to infection and following inflammation, whereas steady inducible DUOX1 is involved in constitutive roles in the normal airway such as immune signaling and mucus production (Shao and Nadel, 2005; Wesley et al., 2007).

Function of DUOX

One mechanism of ROS-mediated bacterial killing at the airway epithelium is similar to the mechanism of NOX2-mediated killing of bacteria in phagocytes (Leto and Geiszt, 2006). There, NOX2 generates $O_2^{\bullet-}$ in the phagosome and it is dismutated to H_2O_2 by superoxide dismutase (SOD), and finally converted to bactericidal $HOCl^-$ by myeloperoxidase (MPO). In airway epithelium, lactoperoxidase (LPO) plays the same role as MPO in phagocytes (Fig. 2). LPO was initially identified in sheep airway secretions, where it constitutes approximately 1% of the soluble protein of the ASL (Conner et al., 2002). LPO is a heme-containing peroxidase expressed in submucosal glands and goblet cells in the airway epithelium. The initially proposed model of LPO-derived bacterial killing was incomplete, because of the absence of a verified H_2O_2 source. After DUOX was found to release H_2O_2 into ASL, the DUOX/LPO system was proposed as a main antibacterial model system in the respiratory tract (Forteza et al., 2005; Geiszt et al., 2003). In normal uninflamed airways, LPO generates antibacterial $OSCN^-$ from H_2O_2 and SCN^-, and $OSCN^-$ is the main antibacterial ROS in airway epithelium (Fig. 2). Recently, SCN^- concentration was reported as the rate-limiting factor for LPO-mediated $OSCN^-$ production. SCN^- is far more abundant than iodide in ASL, with a concentration of ~460 μM (Wijkstrom-Frei et al., 2003).

CONCLUSION

In airway epithelium, TLR2 and TLR4 are critical PRR for sensing infectious pathogens and signaling to leukocytes for host defense. MDA5 and RIG-I, the intracellular virus recognition receptors, are required to induce the production of type I IFNs, leading to virus clearance. Bacteria-killing immune effectors such as mucins, antimicrobial proteins, and ROS are significantly important for host defense against pathogen infection in ASL. The exact regulatory mechanisms that connect pathogen sensing to immune effectors for host defense have yet to be fully elucidated. In addition, it has not yet been determined whether stimulation of innate resistance in airway epithelial cells provides any clinical therapeutic value. A better understanding of innate resistance through modulation of immune effectors may supply insight into therapies for airway inflammatory diseases induced by chronic bacterial infection.

ACKNOWLEDGMENTS

This work was supported by a Korea Science and Engineering Foundation grant funded by the Korean Government (Ministry of Science and Technology) (R11-2007-040-02001-0) and a research grant from the Yonsei University College of Medicine, (6-2009-0115) to J.-H. Ryu.

REFERENCES

Akamine, M., Higa, F., Arakaki, N., Kawakami, K., Takeda, K., Akira, S., and Saito, A. (2005). Differential roles of Toll-like receptors 2 and 4 in *in vitro* responses of macrophages to *Legionella pneumophila*. Infect Immun. *73*, 352-361.

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Pathogen recognition and innate immunity. Cell *124*, 783-801.

Alexopoulou, L., Holt, A.C., Medzhitov, R., and Flavell, R.A. (2001). Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature *413*, 732-738.

Ameziane-El-Hassani, R., Morand, S., Boucher, J.L., Frapart, Y.M., Apostolou, D., Agnandji, D., Gnidehou, S., Ohayon, R., Noel-Hudson, M.S., Francon, J., et al. (2005). Dual oxidase-2 has an intrinsic Ca^{2+}-dependent H_2O_2-generating activity. J. Biol. Chem. *280*, 30046-30054.

Andrejeva, J., Childs, K.S., Young, D.F., Carlos, T.S., Stock, N., Goodbourn, S., and Randall, R.E. (2004). The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. Proc. Natl. Acad. Sci. USA *101*, 17264-17269.

Archer, K.A., and Roy, C.R. (2006). MyD88-dependent responses involving toll-like receptor 2 are important for protection and clearance of Legionella pneumophila in a mouse model of Legionnaires' disease. Infect. Immun. *74*, 3325-3333.

Bals, R., Wang, X., Zasloff, M., and Wilson, J.M. (1998a). The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. Proc. Natl. Acad. Sci. USA *95*, 9541-9546.

Bals, R., Wang, X., Wu, Z., Freeman, T., Bafna, V., Zasloff, M., and Wilson, J.M. (1998b). Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. J. Clin. Invest. *102*, 874-880.

Bals, R., Weiner, D.J., Meegalla, R.L., and Wilson, J.M. (1999). Transfer of a cathelicidin peptide antibiotic gene restores bacterial killing in a cystic fibrosis xenograft model. J. Clin. Invest. *103*, 1113-1117.

Bartlett, J.A., Fischer, A.J., and McCray, P.B., Jr. (2008). Innate immune functions of the airway epithelium. Contrib. Microbiol. *15*,

147-163.
Bedrossian, C.W., Greenberg, S.D., Singer, D.B., Hansen, J.J., and Rosenberg, H.S. (1976). The lung in cystic fibrosis. A quantitative study including prevalence of pathologic findings among different age groups. Hum. Pathol. *7*, 195-204.
Bingle, L., Barnes, F.A., Lunn, H., Musa, M., Webster, S., Douglas, C.W., Cross, S.S., High, A.S., and Bingle, C.D. (2009). Characterisation and expression of SPLUNC2, the human orthologue of rodent parotid secretory protein. Histochem. Cell Biol. *132*, 339-349.
Brogden, K.A. (2005). Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat. Rev. Microbiol. *3*, 238-250.
Bry, K., Whitsett, J.A., and Lappalainen, U. (2007). IL-1beta disrupts postnatal lung morphogenesis in the mouse. Am. J. Respir. Cell Mol. Biol. *36*, 32-42.
Buchweitz, J.P., Karmaus, P.W., Harkema, J.R., Williams, K.J., and Kaminski, N.E. (2007). Modulation of airway responses to influenza A/PR/8/34 by Delta9-tetrahydrocannabinol in C57BL/6 mice. J. Pharmacol. Exp. Ther. *323*, 675-683.
Chen, R., Lim, J.H., Jono, H., Gu, X.X., Kim, Y.S., Basbaum, C.B., Murphy, T.F., and Li, J.D. (2004). Nontypeable Haemophilus influenzae lipoprotein P6 induces MUC5AC mucin transcription via TLR2-TAK1-dependent p38 MAPK-AP1 and IKKbeta-Ikappa Balpha-NF-kappaB signaling pathways. Biochem. Biophys. Res. Commun. *324*, 1087-1094.
Childs, K., Stock, N., Ross, C., Andrejeva, J., Hilton, L., Skinner, M., Randall, R., and Goodbourn, S. (2007). mda-5, but not RIG-I, is a common target for paramyxovirus V proteins. Virology *359*, 190-200.
Chu, H.W., Balzar, S., Seedorf, G.J., Westcott, J.Y., Trudeau, J.B., Silkoff, P., and Wenzel, S.E. (2004). Transforming growth factor-beta2 induces bronchial epithelial mucin expression in asthma. Am. J. Pathol. *165*, 1097-1106.
Cole, A.M., Liao, H.I., Stuchlik, O., Tilan, J., Pohl, J., and Ganz, T. (2002). Cationic polypeptides are required for antibacterial activity of human airway fluid. J. Immunol. *169*, 6985-6991.
Conner, G.E., Salathe, M., and Forteza, R. (2002). Lactoperoxidase and hydrogen peroxide metabolism in the airway. Am. J. Respir. Crit. Care Med. *166*, S57-61.
Cui, Y., Li, M., Walton, K.D., Sun, K., Hanover, J.A., Furth, P.A., and Hennighausen, L. (2001). The Stat3/5 locus encodes novel endoplasmic reticulum and helicase-like proteins that are preferentially expressed in normal and neoplastic mammary tissue. Genomics *78*, 129-134.
Cui, S., Eisenacher, K., Kirchhofer, A., Brzozka, K., Lammens, A., Lammens, K., Fujita, T., Conzelmann, K.K., Krug, A., and Hopfner, K.P. (2008). The C-terminal regulatory domain is the RNA 5′-triphosphate sensor of RIG-I. Mol. Cell *29*, 169-179.
Davies, J.R., Svitacheva, N., Lannefors, L., Kornfalt, R., and Carlstedt, I. (1999). Identification of MUC5B, MUC5AC and small amounts of MUC2 mucins in cystic fibrosis airway secretions. Biochem. J. *344 Pt 2*, 321-330.
De, Y., Chen, Q., Schmidt, A.P., Anderson, G.M., Wang, J.M., Wooters, J., Oppenheim, J.J., and Chertov, O. (2000). LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. J. Exp. Med. *192*, 1069-1074.
Diebold, S.S., Kaisho, T., Hemmi, H., Akira, S., and Reis e Sousa, C. (2004). Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science *303*, 1529-1531.
Dohrman, A., Miyata, S., Gallup, M., Li, J.D., Chapelin, C., Coste, A., Escudier, E., Nadel, J., and Basbaum, C. (1998). Mucin gene (MUC 2 and MUC 5AC) upregulation by Gram-positive and Gram-negative bacteria. Biochim. Biophys. Acta *1406*, 251-259.
Fischer, H. (2009). Mechanisms and function of DUOX in epithelia of the lung. Antioxid Redox Signal. *11*, 2453-2465.
Flo, T.H., Smith, K.D., Sato, S., Rodriguez, D.J., Holmes, M.A., Strong, R.K., Akira, S., and Aderem, A. (2004). Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature *432*, 917-921.
Forteza, R., Salathe, M., Miot, F., and Conner, G.E. (2005). Regulated hydrogen peroxide production by Duox in human airway epithelial cells. Am. J. Respir. Cell Mol. Biol. *32*, 462-469.
Fredericksen, B.L., and Gale, M., Jr. (2006). West Nile virus evades activation of interferon regulatory factor 3 through RIG-I-dependent and -independent pathways without antagonizing host defense signaling. J. Virol. *80*, 2913-2923.
Fritz, J.H., and Girardin, S.E. (2005). How Toll-like receptors and Nod-like receptors contribute to innate immunity in mammals. J. Endotoxin Res. *11*, 390-394.
Ganz, T. (2004). Antimicrobial polypeptides. J. Leukoc. Biol. *75*, 34-38.
Geiszt, M., Witta, J., Baffi, J., Lekstrom, K., and Leto, T.L. (2003). Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. FASEB J. *17*, 1502-1504.
Gensch, E., Gallup, M., Sucher, A., Li, D., Gebremichael, A., Lemjabbar, H., Mengistab, A., Dasari, V., Hotchkiss, J., Harkema, J., et al. (2004). Tobacco smoke control of mucin production in lung cells requires oxygen radicals AP-1 and JNK. J. Biol. Chem. *279*, 39085-39093.
Gitlin, L., Barchet, W., Gilfillan, S., Cella, M., Beutler, B., Flavell, R.A., Diamond, M.S., and Colonna, M. (2006). Essential role of mda-5 in type I IFN responses to polyriboinosinic:polyribocytidylic acid and encephalomyocarditis picornavirus. Proc. Natl. Acad. Sci. USA *103*, 8459-8464.
Gray, T., Nettesheim, P., Loftin, C., Koo, J.S., Bonner, J., Peddada, S., and Langenbach, R. (2004). Interleukin-1beta-induced mucin production in human airway epithelium is mediated by cyclooxygenase-2, prostaglandin E2 receptors, and cyclic AMP-protein kinase A signaling. Mol. Pharmacol. *66*, 337-346.
Greene, C.M., Carroll, T.P., Smith, S.G., Taggart, C.C., Devaney, J., Griffin, S., O'Neill S.J., and McElvaney, N.G. (2005). TLR-induced inflammation in cystic fibrosis and non-cystic fibrosis airway epithelial cells. J. Immunol. *174*, 1638-1646.
Groneberg, D.A., Eynott, P.R., Oates, T., Lim, S., Wu, R., Carlstedt, I., Nicholson, A.G., and Chung, K.F. (2002). Expression of MUC5AC and MUC5B mucins in normal and cystic fibrosis lung. Respir. Med. *96*, 81-86.
Guillot, L., Medjane, S., Le-Barillec, K., Balloy, V., Danel, C., Chignard, M., and Si-Tahar, M. (2004). Response of human pulmonary epithelial cells to lipopolysaccharide involves Toll-like receptor 4 (TLR4)-dependent signaling pathways: evidence for an intracellular compartmentalization of TLR4. J. Biol. Chem. *279*, 2712-2718.
Guo, Z., Chen, L.M., Zeng, H., Gomez, J.A., Plowden, J., Fujita, T., Katz, J.M., Donis, R.O., and Sambhara, S. (2007). NS1 protein of influenza A virus inhibits the function of intracytoplasmic pathogen sensor, RIG-I. Am. J. Respir. Cell Mol. Biol. *36*, 263-269.
Ha, U., Lim, J.H., Jono, H., Koga, T., Srivastava, A., Malley, R., Pages, G., Pouyssegur, J., and Li, J.D. (2007). A novel role for IkappaB kinase (IKK) alpha and IKKbeta in ERK-dependent up-regulation of MUC5AC mucin transcription by *Streptococcus pneumoniae*. J. Immunol. *178*, 1736-1747.
Habjan, M., Andersson, I., Klingstrom, J., Schumann, M., Martin, A., Zimmermann, P., Wagner, V., Pichlmair, A., Schneider, U., Muhlberger, E., et al. (2008). Processing of genome 5′ termini as a strategy of negative-strand RNA viruses to avoid RIG-I-dependent interferon induction. PLoS One *3*, e2032.
Harder, J., Meyer-Hoffert, U., Teran, L.M., Schwichtenberg, L., Bartels, J., Maune, S., and Schroder, J.M. (2000). Mucoid Pseudomonas aeruginosa, TNF-alpha, and IL-1beta, but not IL-6, induce human beta-defensin-2 in respiratory epithelia. Am. J. Respir. Cell Mol. Biol. *22*, 714-721.
Harper, R.W., Xu, C., Eiserich, J.P., Chen, Y., Kao, C.Y., Thai, P., Setiadi, H., and Wu, R. (2005). Differential regulation of dual NADPH oxidases/peroxidases, Duox1 and Duox2, by Th1 and Th2 cytokines in respiratory tract epithelium. FEBS Lett. *579*, 4911-4917.
Hashimoto, K., Graham, B.S., Ho, S.B., Adler, K.B., Collins, R.D., Olson, S.J., Zhou, W., Suzutani, T., Jones, P.W., Goleniewska, K., et al. (2004). Respiratory syncytial virus in allergic lung inflammation increases Muc5ac and gob-5. Am. J. Respir. Crit. Care Med. *170*, 306-312.
Hayashi, F., Smith, K.D., Ozinsky, A., Hawn, T.R., Yi, E.C., Goodlett, D.R., Eng, J.K., Akira, S., Underhill, D.M., and Aderem, A. (2001). The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature *410*, 1099-1103.
Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H., and Bauer, S. (2004). Species-specific recognition of single-stranded RNA via toll-like receptor

7 and 8. Science *303*, 1526-1529.
Hemmi, H., Takeuchi, O., Kawai, T., Kaisho, T., Sato, S., Sanjo, H., Matsumoto, M., Hoshino, K., Wagner, H., Takeda, K., et al. (2000). A Toll-like receptor recognizes bacterial DNA. Nature *408*, 740-745.
Herr, C., Shaykhiev, R., and Bals, R. (2007). The role of cathelicidin and defensins in pulmonary inflammatory diseases. Exp. Opin. Biol. Ther. *7*, 1449-1461.
Hewson, C.A., Haas, J.J., Bartlett, N.W., Message, S.D., Laza-Stanca, V., Kebadze, T., Caramori, G., Zhu, J., Edbrooke, M.R., Stanciu, L.A., et al. (2010). Rhinovirus induces MUC5AC in a human infection model, & *in vitro* via NF-{kappa}B & EGFR pathways. Eur. Respir. J. (in press).
Hogg, J.C., Chu, F., Utokaparch, S., Woods, R., Elliott, W.M., Buzatu, L., Cherniack, R.M., Rogers, R.M., Sciurba, F.C., Coxson, H.O., et al. (2004). The nature of small-airway obstruction in chronic obstructive pulmonary disease. N. Engl. J. Med. *350*, 2645-2653.
Hollingsworth, M.A., and Swanson, B.J. (2004). Mucins in cancer: protection and control of the cell surface. Nat. Rev. Cancer *4*, 45-60.
Hornung, V., Ellegast, J., Kim, S., Brzozka, K., Jung, A., Kato, H., Poeck, H., Akira, S., Conzelmann, K.K., Schlee, M., et al. (2006). 5′-Triphosphate RNA is the ligand for RIG-I. Science *314*, 994-997.
Innes, A.L., Woodruff, P.G., Ferrando, R.E., Donnelly, S., Dolganov, G.M., Lazarus, S.C., and Fahy, J.V. (2006). Epithelial mucin stores are increased in the large airways of smokers with airflow obstruction. Chest *130*, 1102-1108.
Inoue, D., Yamaya, M., Kubo, H., Sasaki, T., Hosoda, M., Numasaki, M., Tomioka, Y., Yasuda, H., Sekizawa, K., Nishimura, H., et al. (2006). Mechanisms of mucin production by rhinovirus infection in cultured human airway epithelial cells. Respir. Physiol. Neurobiol. *154*, 484-499.
Jia, H.P., Kline, J.N., Penisten, A., Apicella, M.A., Gioannini, T.L., Weiss, J., and McCray, P.B., Jr. (2004). Endotoxin responsiveness of human airway epithelia is limited by low expression of MD-2. Am. J. Physiol. Lung Cell Mol. Physiol. *287*, L428-437.
Jiang, Y., Wang, Y., Kuang, Y., Wang, B., Li, W., Gong, T., Jiang, Z., Yang, D., and Li, M. (2009). Expression of mouse beta-defensin-3 in MDCK cells and its anti-influenza-virus activity. Arch. Virol. *154*, 639-647.
John, G., Yildirim, A.O., Rubin, B.K., Gruenert, D.C., and Henke, M.O. (2010). TLR-4-mediated innate immunity is reduced in cystic fibrosis airway cells. Am. J. Respir. Cell Mol. Biol. *42*, 424-431.
Jono, H., Shuto, T., Xu, H., Kai, H., Lim, D.J., Gum, J.R., Jr., Kim, Y.S., Yamaoka, S., Feng, X.H., and Li, J.D. (2002). Transforming growth factor-beta -Smad signaling pathway cooperates with NF-kappa B to mediate nontypeable Haemophilus influenzae-induced MUC2 mucin transcription. J. Biol. Chem. *277*, 45547-45557.
Kajikawa, O., Frevert, C.W., Lin, S.M., Goodman, R.B., Mongovin, S.M., Wong, V., Ballman, K., Daubeuf, B., Elson, G., and Martin, T.R. (2005). Gene expression of Toll-like receptor-2, Toll-like receptor-4, and MD2 is differentially regulated in rabbits with Escherichia coli pneumonia. Gene *344*, 193-202.
Kang, D.C., Gopalkrishnan, R.V., Wu, Q., Jankowsky, E., Pyle, A.M., and Fisher, P.B. (2002). mda-5: an interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties. Proc. Natl. Acad. Sci. USA *99*, 637-642.
Kato, H., Takeuchi, O., Sato, S., Yoneyama, M., Yamamoto, M., Matsui, K., Uematsu, S., Jung, A., Kawai, T., Ishii, K.J., et al. (2006). Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature *441*, 101-105.
Kato, H., Takeuchi, O., Mikamo-Satoh, E., Hirai, R., Kawai, T., Matsushita, K., Hiiragi, A., Dermody, T.S., Fujita, T., and Akira, S. (2008). Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. J. Exp. Med. *205*, 1601-1610.
Kawai, T., Takahashi, K., Sato, S., Coban, C., Kumar, H., Kato, H., Ishii, K.J., Takeuchi, O., and Akira, S. (2005). IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat. Immunol. *6*, 981-988.
Kim, H.J., Park, Y.D., Moon, U.Y., Kim, J.H., Jeon, J.H., Lee, J.G., Bae, Y.S., and Yoon, J.H. (2008). The role of Nox4 in oxidative stress-induced MUC5AC overexpression in human airway epithelial cells. Am. J. Respir. Cell Mol. Biol. *39*, 598-609.
Kim, H.J., Ryu, J.H., Kim, C.H., Lim, J.W., Moon, U.Y., Lee, G.H., Lee, J.G., Baek, S.J., and Yoon, J.H. (2009). ECG Suppresses Oxidative Stress-Induced MUC5AC Overexpression by Interaction with EGFR. Am. J. Respir. Cell Mol. Biol. *43*, 349-357.
Kirkham, S., Sheehan, J.K., Knight, D., Richardson, P.S., and Thornton, D.J. (2002). Heterogeneity of airways mucus: variations in the amounts and glycoforms of the major oligomeric mucins MUC5AC and MUC5B. Biochem. J. *361*, 537-546.
Knapp, S., Wieland, C.W., van 't Veer, C., Takeuchi, O., Akira, S., Florquin, S., and van der Poll, T. (2004). Toll-like receptor 2 plays a role in the early inflammatory response to murine pneumococcal pneumonia but does not contribute to antibacterial defense. J. Immunol. *172*, 3132-3138.
Kolls, J.K., McCray, P.B., Jr., and Chan, Y.R. (2008). Cytokine-mediated regulation of antimicrobial proteins. Nat. Rev. Immunol. *8*, 829-835.
Komatsu, T., Takeuchi, K., and Gotoh, B. (2007). Bovine parainfluenza virus type 3 accessory proteins that suppress beta interferon production. Microbes Infect. *9*, 954-962.
Lai, Y., and Gallo, R.L. (2009). AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol. *30*, 131-141.
Lappalainen, U., Whitsett, J.A., Wert, S.E., Tichelaar, J.W., and Bry, K. (2005). Interleukin-1beta causes pulmonary inflammation, emphysema, and airway remodeling in the adult murine lung. Am. J. Respir. Cell Mol. Biol. *32*, 311-318.
Lemjabbar, H., and Basbaum, C. (2002). Platelet-activating factor receptor and ADAM10 mediate responses to Staphylococcus aureus in epithelial cells. Nat. Med. *8*, 41-46.
Leto, T.L., and Geiszt, M. (2006). Role of Nox family NADPH oxidases in host defense. Antioxid Redox Signal. *8*, 1549-1561.
Li, J.D., Dohrman, A.F., Gallup, M., Miyata, S., Gum, J.R., Kim, Y.S., Nadel, J.A., Prince, A., and Basbaum, C.B. (1997). Transcriptional activation of mucin by Pseudomonas aeruginosa lipopolysaccharide in the pathogenesis of cystic fibrosis lung disease. Proc. Natl. Acad. Sci. USA *94*, 967-972.
Li, D., Gallup, M., Fan, N., Szymkowski, D.E., and Basbaum, C.B. (1998). Cloning of the amino-terminal and 5′-flanking region of the human MUC5AC mucin gene and transcriptional up-regulation by bacterial exoproducts. J. Biol. Chem. *273*, 6812-6820.
Liu, P.T., Stenger, S., Li, H., Wenzel, L., Tan, B.H., Krutzik, S.R., Ochoa, M.T., Schauber, J., Wu, K., Meinken, C., et al. (2006). Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science *311*, 1770-1773.
Longphre, M., Li, D., Li, J., Matovinovic, E., Gallup, M., Samet, J.M., and Basbaum, C.B. (2000). Lung mucin production is stimulated by the air pollutant residual oil fly ash. Toxicol. Appl. Pharmacol. *162*, 86-92.
Loo, Y.M., Fornek, J., Crochet, N., Bajwa, G., Perwitasari, O., Martinez-Sobrido, L., Akira, S., Gill, M.A., Garcia-Sastre, A., Katze, M.G., et al. (2008). Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. J. Virol. *82*, 335-345.
Lora, J.M., Zhang, D.M., Liao, S.M., Burwell, T., King, A.M., Barker, P.A., Singh, L., Keaveney, M., Morgenstern, J., Gutierrez-Ramos, J.C., et al. (2005). Tumor necrosis factor-alpha triggers mucus production in airway epithelium through an IkappaB kinase beta-dependent mechanism. J. Biol. Chem. *280*, 36510-36517.
MacRedmond, R.E., Greene, C.M., Dorscheid, D.R., McElvaney, N.G., and O'Neill, S.J. (2007). Epithelial expression of TLR4 is modulated in COPD and by steroids, salmeterol and cigarette smoke. Respir. Res. *8*, 84.
Mannino, D.M., Homa, D.M., Akinbami, L.J., Ford, E.S., and Redd, S.C. (2002). Chronic obstructive pulmonary disease surveillance-United States, 1971-2000. Respir. Care *47*, 1184-1199.
McCartney, S.A., Thackray, L.B., Gitlin, L., Gilfillan, S., Virgin, H.W., and Colonna, M. (2008). MDA-5 recognition of a murine norovirus. PLoS Pathog. *4*, e1000108.
Medzhitov, R., Preston-Hurlburt, P., and Janeway, C.A., Jr. (1997). A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature *388*, 394-397.
Mesquita, P., Almeida, R., Van Seuningen, I., and David, L. (2004). Coordinated expression of MUC2 and CDX-2 in mucinous carcinomas of the lung can be explained by the role of CDX-2 as

transcriptional regulator of MUC2. Am. J. Surg. Pathol. *28*, 1254-1255.
Meylan, E., Curran, J., Hofmann, K., Moradpour, D., Binder, M., Bartenschlager, R., and Tschopp, J. (2005). Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature *437*, 1167-1172.
Mibayashi, M., Martinez-Sobrido, L., Loo, Y.M., Cardenas, W.B., Gale, M., Jr., and Garcia-Sastre, A. (2007). Inhibition of retinoic acid-inducible gene I-mediated induction of beta interferon by the NS1 protein of influenza A virus. J. Virol. *81*, 514-524.
Moser, C., Weiner, D.J., Lysenko, E., Bals, R., Weiser, J.N., and Wilson, J.M. (2002). beta-Defensin 1 contributes to pulmonary innate immunity in mice. Infect. Immun. *70*, 3068-3072.
Muir, A., Soong, G., Sokol, S., Reddy, B., Gomez, M.I., Van Heeckeren, A., and Prince, A. (2004). Toll-like receptors in normal and cystic fibrosis airway epithelial cells. Am. J. Respir. Cell Mol. Biol. *30*, 777-783.
Niyonsaba, F., Iwabuchi, K., Matsuda, H., Ogawa, H., and Nagaoka, I. (2002). Epithelial cell-derived human beta-defensin-2 acts as a chemotaxin for mast cells through a pertussis toxin-sensitive and phospholipase C-dependent pathway. Int. Immunol. *14*, 421-426.
Opitz, B., Rejaibi, A., Dauber, B., Eckhard, J., Vinzing, M., Schmeck, B., Hippenstiel, S., Suttorp, N., and Wolff, T. (2007). IFNbeta induction by influenza A virus is mediated by RIG-I which is regulated by the viral NS1 protein. Cell Microbiol. *9*, 930-938.
Ordonez, C.L., Khashayar, R., Wong, H.H., Ferrando, R., Wu, R., Hyde, D.M., Hotchkiss, J.A., Zhang, Y., Novikov, A., Dolganov, G., et al. (2001). Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. Am. J. Respir. Crit. Care Med. *163*, 517-523.
Pastva, A.M., Wright, J.R., and Williams, K.L. (2007). Immunomodulatory roles of surfactant proteins A and D: implications in lung disease. Proc. Am. Thorac. Soc. *4*, 252-257.
Pichlmair, A., and Reis e Sousa, C. (2007). Innate recognition of viruses. Immunity *27*, 370-383.
Pichlmair, A., Schulz, O., Tan, C.P., Naslund, T.I., Liljestrom, P., Weber, F., and Reis e Sousa, C. (2006). RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. Science *314*, 997-1001.
Poltorak, A., He, X., Smirnova, I., Liu, M.Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., et al. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science *282*, 2085-2088.
Rose, M.C., and Voynow, J.A. (2006). Respiratory tract mucin genes and mucin glycoproteins in health and disease. Physiol. Rev. *86*, 245-278.
Rothenfusser, S., Goutagny, N., DiPerna, G., Gong, M., Monks, B.G., Schoenemeyer, A., Yamamoto, M., Akira, S., and Fitzgerald, K.A. (2005). The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible gene-I. J. Immunol. *175*, 5260-5268.
Schulz, B.L., Sloane, A.J., Robinson, L.J., Sebastian, L.T., Glanville, A.R., Song, Y., Verkman, A.S., Harry, J.L., Packer, N.H., and Karlsson, N.G. (2005). Mucin glycosylation changes in cystic fibrosis lung disease are not manifest in submucosal gland secretions. Biochem. J. *387*, 911-919.
Schwandner, R., Dziarski, R., Wesche, H., Rothe, M., and Kirschning, C.J. (1999). Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. J. Biol. Chem. *274*, 17406-17409.
Schwarzer, C., Machen, T.E., Illek, B., and Fischer, H. (2004). NADPH oxidase-dependent acid production in airway epithelial cells. J. Biol. Chem. *279*, 36454-36461.
Seth, R.B., Sun, L., Ea, C.K., and Chen, Z.J. (2005). Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell *122*, 669-682.
Shao, M.X., and Nadel, J.A. (2005). Dual oxidase 1-dependent MUC5AC mucin expression in cultured human airway epithelial cells. Proc. Natl. Acad. Sci. USA *102*, 767-772.
Singh, P.K., Jia, H.P., Wiles, K., Hesselberth, J., Liu, L., Conway, B.A., Greenberg, E.P., Valore, E.V., Welsh, M.J., Ganz, T., et al. (1998). Production of beta-defensins by human airway epithelia. Proc. Natl. Acad. Sci. USA *95*, 14961-14966.
Skerrett, S.J., Liggitt, H.D., Hajjar, A.M., and Wilson, C.B. (2004). Cutting edge: myeloid differentiation factor 88 is essential for pulmonary host defense against Pseudomonas aeruginosa but not Staphylococcus aureus. J. Immunol. *172*, 3377-3381.
Skerrett, S.J., Wilson, C.B., Liggitt, H.D., and Hajjar, A.M. (2007). Redundant Toll-like receptor signaling in the pulmonary host response to Pseudomonas aeruginosa. Am. J. Physiol. Lung Cell Mol. Physiol. *292*, L312-322.
Song, K.S., Lee, W.J., Chung, K.C., Koo, J.S., Yang, E.J., Choi, J.Y., and Yoon, J.H. (2003). Interleukin-1 beta and tumor necrosis factor-alpha induce MUC5AC overexpression through a mechanism involving ERK/p38 mitogen-activated protein kinases-MSK1-CREB activation in human airway epithelial cells. J. Biol. Chem. *278*, 23243-23250.
Song, K.S., Choi, Y.H., Kim, J.M., Lee, H., Lee, T.J., and Yoon, J.H. (2009a). Suppression of prostaglandin E2-induced MUC5AC overproduction by RGS4 in the airway. Am. J. Physiol. Lung Cell Mol. Physiol. *296*, L684-692.
Song, K.S., Kim, H.J., Kim, K., Lee, J.G., and Yoon, J.H. (2009b). Regulator of G-protein signaling 4 suppresses LPS-induced MUC5AC overproduction in the airway. Am. J. Respir. Cell Mol. Biol. *41*, 40-49.
Soong, G., Reddy, B., Sokol, S., Adamo, R., and Prince, A. (2004). TLR2 is mobilized into an apical lipid raft receptor complex to signal infection in airway epithelial cells. J. Clin. Invest. *113*, 1482-1489.
Starner, T.D., Barker, C.K., Jia, H.P., Kang, Y., and McCray, P.B., Jr. (2003). CCL20 is an inducible product of human airway epithelia with innate immune properties. Am. J. Respir. Cell Mol. Biol. *29*, 627-633.
Takahasi, K., Yoneyama, M., Nishihori, T., Hirai, R., Kumeta, H., Narita, R., Gale, M., Jr., Inagaki, F., and Fujita, T. (2008). Nonself RNA-sensing mechanism of RIG-I helicase and activation of antiviral immune responses. Mol. Cell *29*, 428-440.
Takeuchi, O., Hoshino, K., and Akira, S. (2000). Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. J. Immunol. *165*, 5392-5396.
Takeyama, K., Dabbagh, K., Jeong Shim, J., Dao-Pick, T., Ueki, I.F., and Nadel, J.A. (2000). Oxidative stress causes mucin synthesis via transactivation of epidermal growth factor receptor: role of neutrophils. J. Immunol. *164*, 1546-1552.
Thai, P., Loukoianov, A., Wachi, S., and Wu, R. (2008). Regulation of airway mucin gene expression. Annu. Rev. Physiol. *70*, 405-429.
Thornton, D.J., Rousseau, K., and McGuckin, M.A. (2008). Structure and function of the polymeric mucins in airways mucus. Annu. Rev. Physiol. *70*, 459-486.
Tyner, J.W., Kim, E.Y., Ide, K., Pelletier, M.R., Roswit, W.T., Morton, J.D., Battaile, J.T., Patel, A.C., Patterson, G.A., Castro, M., et al. (2006). Blocking airway mucous cell metaplasia by inhibiting EGFR antiapoptosis and IL-13 transdifferentiation signals. J. Clin. Invest. *116*, 309-321.
Venkataraman, T., Valdes, M., Elsby, R., Kakuta, S., Caceres, G., Saijo, S., Iwakura, Y., and Barber, G.N. (2007). Loss of DExD/H box RNA helicase LGP2 manifests disparate antiviral responses. J. Immunol. *178*, 6444-6455.
Voynow, J.A., Young, L.R., Wang, Y., Horger, T., Rose, M.C., and Fischer, B.M. (1999). Neutrophil elastase increases MUC5AC mRNA and protein expression in respiratory epithelial cells. Am. J. Physiol. *276*, L835-843.
Walter, M.J., Morton, J.D., Kajiwara, N., Agapov, E., and Holtzman, M.J. (2002). Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. J. Clin. Invest. *110*, 165-175.
Wan, H., Kaestner, K.H., Ang, S.L., Ikegami, M., Finkelman, F.D., Stahlman, M.T., Fulkerson, P.C., Rothenberg, M.E., and Whitsett, J.A. (2004). Foxa2 regulates alveolarization and goblet cell hyperplasia. Development *131*, 953-964.
Wang, B., Lim, D.J., Han, J., Kim, Y.S., Basbaum, C.B., and Li, J.D. (2002). Novel cytoplasmic proteins of nontypeable Haemophilus influenzae up-regulate human MUC5AC mucin transcription via a positive p38 mitogen-activated protein kinase pathway and a negative phosphoinositide 3-kinase-Akt pathway. J. Biol. Chem. *277*, 949-957.
Wang, Q., Nagarkar, D.R., Bowman, E.R., Schneider, D., Gosangi, B., Lei, J., Zhao, Y., McHenry, C.L., Burgens, R.V., Miller, D.J., et al. (2009). Role of double-stranded RNA pattern recognition receptors in rhinovirus-induced airway epithelial cell responses. J. Immunol. *183*, 6989-6997.
Wang, Y., Jiang, Y., Gong, T., Cui, X., Li, W., Feng, Y., Wang, B., Jiang, Z., and Li, M. (2010). High-level expression and novel

antifungal activity of mouse beta defensin-1 mature peptide in Escherichia coli. Appl. Biochem. Biotechnol. *160*, 213-221.
Weber, F., Kochs, G., and Haller, O. (2004). Inverse interference: how viruses fight the interferon system. Viral Immunol. *17*, 498-515.
Wesley, U.V., Bove, P.F., Hristova, M., McCarthy, S., and van der Vliet, A. (2007). Airway epithelial cell migration and wound repair by ATP-mediated activation of dual oxidase 1. J. Biol. Chem. *282*, 3213-3220.
Wijkstrom-Frei, C., El-Chemaly, S., Ali-Rachedi, R., Gerson, C., Cobas, M.A., Forteza, R., Salathe, M., and Conner, G.E. (2003). Lactoperoxidase and human airway host defense. Am. J. Respir. Cell Mol. Biol. *29*, 206-212.
Williams, S.E., Brown, T.I., Roghanian, A., and Sallenave, J.M. (2006). SLPI and elafin: one glove, many fingers. Clin. Sci. *110*, 21-35.
Wills-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T.Y., Karp, C.L., and Donaldson, D.D. (1998). Interleukin-13: central mediator of allergic asthma. Science *282*, 2258-2261.
Willumsen, N.J., and Boucher, R.C. (1989). Shunt resistance and ion permeabilities in normal and cystic fibrosis airway epithelia. Am. J. Physiol. *256*, C1054-1063.
Xu, L.G., Wang, Y.Y., Han, K.J., Li, L.Y., Zhai, Z., and Shu, H.B. (2005). VISA is an adapter protein required for virus-triggered IFN-beta signaling. Mol. Cell *19*, 727-740.
Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., Imaizumi, T., Miyagishi, M., Taira, K., Akira, S., and Fujita, T. (2004). The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat. Immunol. *5*, 730-737.
Yoneyama, M., Kikuchi, M., Matsumoto, K., Imaizumi, T., Miyagishi, M., Taira, K., Foy, E., Loo, Y.M., Gale, M., Jr., Akira, S., et al. (2005). Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J. Immunol. *175*, 2851-2858.
Young, H.W., Williams, O.W., Chandra, D., Bellinghausen, L.K., Perez, G., Suarez, A., Tuvim, M.J., Roy, M.G., Alexander, S.N., Moghaddam, S.J., et al. (2007). Central role of Muc5ac expression in mucous metaplasia and its regulation by conserved 5′ elements. Am. J. Respir. Cell Mol. Biol. *37*, 273-290.
Yuan-Chen Wu, D., Wu, R., Reddy, S.P., Lee, Y.C., and Chang, M.M. (2007). Distinctive epidermal growth factor receptor/e xtracellular regulated kinase-independent and -dependent signaling pathways in the induction of airway mucin 5B and mucin 5AC expression by phorbol 12-myristate 13-acetate. Am. J. Pathol. *170*, 20-32.
Zhen, G., Park, S.W., Nguyenvu, L.T., Rodriguez, M.W., Barbeau, R., Paquet, A.C., and Erle, D.J. (2007). IL-13 and epidermal growth factor receptor have critical but distinct roles in epithelial cell mucin production. Am. J. Respir. Cell Mol. Biol. *36*, 244-253.
Zuhdi Alimam, M., Piazza, F.M., Selby, D.M., Letwin, N., Huang, L., and Rose, M.C. (2000). Muc-5/5ac mucin messenger RNA and protein expression is a marker of goblet cell metaplasia in murine airways. Am. J. Respir. Cell Mol. Biol. *22*, 253-260.