Innate Immunity in the Respiratory Epithelium

Dane Parker[1] and Alice Prince[1]

[1]Department of Pediatrics, Columbia University, New York, New York

The airway epithelium represents the first point of contact for inhaled foreign organisms. The protective arsenal of the airway epithelium is provided in the form of physical barriers and a vast array of receptors and antimicrobial compounds that constitute the innate immune system. Many of the known innate immune receptors, including the Toll-like receptors and nucleotide oligomerization domain–like receptors, are expressed by the airway epithelium, which leads to the production of proinflammatory cytokines and chemokines that affect microorganisms directly and recruit immune cells, such as neutrophils and T cells, to the site of infection. The airway epithelium also produces a number of resident antimicrobial proteins, such as lysozyme, lactoferrin, and mucins, as well as a swathe of cationic proteins. Dysregulation of the airway epithelial innate immune system is associated with a number of medical conditions that can result in compromised immunity and chronic inflammation of the lung. This review focuses on the innate immune capabilities of the airway epithelium and its role in protecting the lung from infection as well as the outcomes when its function is compromised.

Keywords: innate immunity; respiratory; airway; signaling

CLINICAL RELEVANCE

The airway epithelium represents the first point of contact for inhaled foreign organisms. The airway epithelium uses a number of physical barriers and a vast array of receptors and antimicrobial compounds that constitute the innate immune system. This review focuses on the innate immune capabilities of the airway epithelium and its role in protecting the lung from infection as well as the outcomes when its function is compromised.

The airway epithelium represents the first line of defense of the lung. Airway epithelial cells provide a mechanical barrier to prevent infection but also produce chemokines and cytokines, such as IL-6, CXCL8, IL-1β, GM-CSF, and G-CSF, that recruit and activate phagocytic cells to eradicate organisms and infected cells. Because the lung is normally sterile, interactions with microorganisms typically cause an inflammatory response. This response can be due to direct cytopathic effects caused by the organism or can occur as a result of the host response to these organisms. The airway fluid contains a number of resident antimicrobial compounds, such as cationic defensins, or larger proteins such as lysozyme. In additional to resident antimicrobial proteins, the airway epithelium expresses an array of sensors to detect pathogens. Immune signaling can be activated by intact bacteria, viruses, fungi, or, more commonly, by the components of these organisms that are shed and gain access to surface or intracellular receptors. Even in the absence of direct epithelial contact, these shed components, such as LPS and flagella, referred to as pathogen-associated molecular patterns (PAMPs), can permeate the respiratory mucus layer to gain access to epithelial receptors stimulating inflammation. It is the recognition of PAMPs that constitutes what the innate immune system largely senses. The mucosal response, in particular the innate response, maintains the sterility of the lower airways by efficiently clearing sensed pathogens and rapidly controlling secondary effects associated with neutrophils and their products.

It is critical to regulate the intensity and duration of the proinflammatory signaling initiated in the airway. Perhaps more than at any other site, excessive inflammation (i.e., acute pneumonia) is associated with respiratory compromise and must be tightly controlled. Thus, a major component of mucosal immunity is the activation of the regulatory components of the innate immune system, which includes expression of NF-κB, activator protein 1, IFN regulatory factors (IRFs), and mitogen-activated protein kinases (MAPKs) (1, 2).

TLR SIGNALING

The Toll-like receptors (TLRs) are an important family of proteins involved in the recognition of microorganisms (Figure 1). The Toll protein was originally identified as being involved in dorsal-ventral patterning in *Drosophila* and later to be involved in fighting fungal infections (3, 4). Subsequent studies identified a number of homologs in humans that are involved in innate sensing of microbial products or PAMPs. The TLRs are integral membrane glycoproteins that, through homology, are part of a large family that includes IL-1 receptors (IL-1Rs). The cytoplasmic region contains a conserved TIR (Toll/IL-1R) domain (5), whereas the extracellular region differs between TLRs and IL-R by possessing leucine-rich repeats (LRRs), as opposed to an Ig-like domain. It is these LRRs that specify the target ligand for each TLR, also known as pattern recognition receptors.

There have been 11 TLRs identified in humans. TLRs recognize a diverse array of microbial components, such as lipoproteins (TLR1, -2, and -6) (6–9), LPS (TLR4) (10), flagellin (TLR5) (11), DNA (TLR9) (12), and RNA (TLR3, -7, and -8) (13–15). The nature of the TLR10 ligand is unknown, whereas TLR11 has been shown to recognize uropathogenic *E. coli* (16) and a profilin-like molecule from *Toxoplasma* (17). TLRs1, -2, -4, -5, and -6 are located at the plasma membrane, with TLR3, -7, -8, and -9 in the endoplasmic reticulum, and are then chaperoned to endolysosomes (18).

Signal transduction from TLRs is typically referred to as MyD88-dependent or -independent. MyD88-dependent signaling (myeloid differentiation primary-response protein 88) (19) occurs through the adaptor protein MyD88 and its TLR binding

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Correspondence and requests for reprints should be addressed to Alice Prince, M.D., Department of Pediatrics, Columbia University, 650 West 168th St., Black Building BB4-416, New York, NY 10027. E-mail: asp7@columbia.edu

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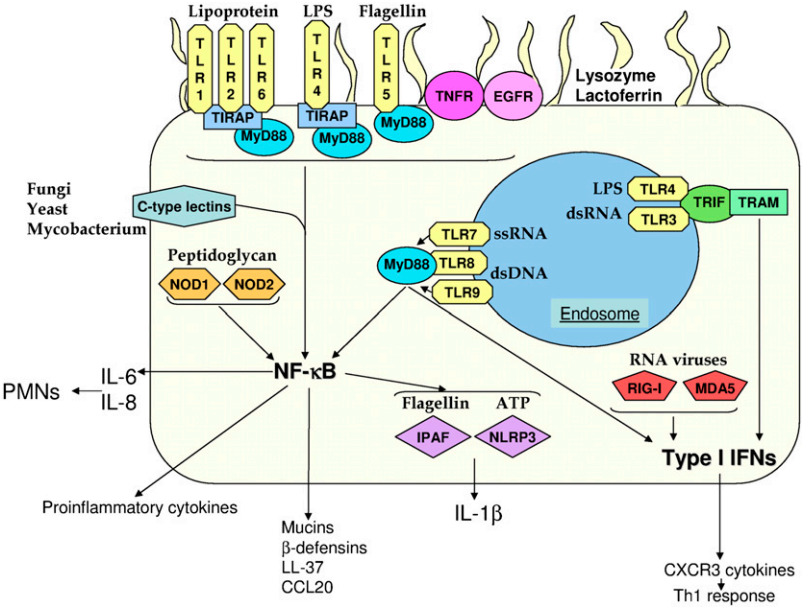

Figure 1. Innate immunity in the respiratory epithelium. Shown is an airway epithelial cell with the innate molecules discussed in this review and their ligands and surface receptors (Toll-like receptor (TLR)1, -2, -4, -5, -6; TNF receptor [TNFR]; and epidermal growth factor receptor (EGFR), endosomal receptors (TLR3, -4, -7, -8, and -9), cytosolic receptors (retinoic acid inducible gene [RIG]-I, melanoma differentiation–associated protein [MDA]5, nucleotide oligomerization domain [NOD]1, NOD2, IL1-β–converting enzyme protease activating factor [IPAF], and NOD-like receptor pyrin domain [NLRP3]) and antimicrobial proteins.

partner toll–IL-1 receptor domain containing adaptor protein (TIRAP) (20). All TLRs, with the exception of TLR3, use MyD88-dependent signaling. TIRAP is not used by TLR5, -7, -8, or -9 (20). The importance of MyD88 is highlighted by the observation that protective immunity is lost to a small group of pyogenic organisms in humans with MyD88 mutations (21). The MyD88-independent arm (discussed below) is initiated by TLR3 and TLR4 through the TRIF-related adaptor molecule (22, 23) that couples endocytosis of TLR4 to the TIR-domain–containing adapter-inducing IFN-β (TRIF) adaptor (13, 24, 25). Activation of a TLR and subsequent signaling through MyD88 initiates an extensive signal transduction cascade that proceeds through a number of kinases and transcription factors, leading to phosphorylation of IκBa, an NF-κB inhibitory protein, and allowing NF-κB to activate expression of proinflammatory genes such as TNF, IL-1β, IL-6, and CXCL8 (26, 27).

TLR SIGNALING IN THE AIRWAY EPITHELIUM

The airway epithelium expresses the full complement of TLRs, but their distribution and the availability of adaptor proteins is important in determining their participation in signaling the presence of PAMPs. The expression of each TLR has been investigated in a variety of primary and immortalized cell lines from the upper and lower airways, with the strongest gene expression present for TLRs 2 through 6; the expression of TLRs 7 through 10 is variable depending on the cell type studied (2, 28–32). TLRs 1 through 6 and 9 are present on the cell surface, identified through flow cytometry (33). However, other studies point to a more even distribution of the receptors throughout airway epithelial cells (28). Adaptors such as MyD88 and CD14 are not seen on the cell surface (28, 33). Reduced surface expression of CD14 and low levels of MD2 production provides a potential mechanism for the low endogenous responsiveness of airway epithelial cells to LPS (34).

A number of TLRs are used by the airway to sense and initiate innate and adaptive immunity in response to pathogens. These organisms can induce the transcription of TLRs and their mobilization to the cell surface. Common airway pathogens, such as the viruses influenza, rhinovirus, and respiratory syncytial virus (RSV) and the bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*, are detected through the presence of PAMPs on the epithelial cell surface. In some cases, expression of the TLRs is induced. TLR3 is important in the detection of a number of viruses, and its transcription is induced when a cell is infected (35–37). As a potential by-product of continual viral insult, TLR3 ligands (e.g., poly(I:C)) give the strongest proinflammatory response (2, 38). Bacterial infection with *K. pneumoniae* causes induction of genes encoding TLR2 and -4, sensors of liporotein and LPS, two important receptors for gram-negative bacterial pathogens (39). Expression of TLR4 on airway epithelial cells is crucial in the allergic response LPS, as demonstrated using bone marrow chimeric mice (40). Interaction of the epithelium with *P. aeruginosa* involves TLR2, -4, and -5 (41, 42). The flagella of *P. aeruginosa*, recognized by TLR5, induce mobilization of the receptor to the surface of the cell (43). *P. aeruginosa* flagella also interact with TLR2 and asialoGM1. This signaling through asialoGM1 is facilitated by a TLR2 lipid raft complex (caveolin-1) (43). The importance of MyD88 signaling in epithelial cells in response to *P. aeruginosa* was shown using bone marrow chimeras (44). During the early phase of clearance, MyD88 null mice that received normal bone marrow still faired worse, indicating that MyD88-dependent signaling of non–bone marrow derived cells was important in initial *P. aeruginosa* clearance.

TLR REGULATION OF MUCIN PRODUCTION

Mucin gene expression is also regulated by proinflammatory/TLR signaling. Mucins are glycoproteins that constitute mucus, an important barrier component of the respiratory epithelium. Mucus not only partakes in the normal mucociliary clearance of the lung but also keeps the airway hydrated and traps particulate matter and potential pathogens. There are a large number of mucin genes, of which at least 12 are expressed in the airway (45). The most abundant mucins expressed are MUC1, MUC2, and MUC5AC; each is induced by a variety of gram-positive and gram-negative pathogens as well as viruses (46–51). Induction of mucin gene expression has been observed with TNF (52) and CXCL8 (53). Direct stimulation of TLR2 (54) and TLR3 (55) induces mucin expression as well as activating MAPK (56) and inducing epidermal growth factor receptor (EGFR) signaling (55, 57). It is also likely that mucins feedback

into the TLR signaling pathways. Ueno and colleagues (58) showed that MUC1 plays an antiinflammatory role by negatively regulating signaling as a result of TLR2, -3, -4, -5, -7, and -9 signaling. A comprehensive review of mucins in the airway has recently been published (59). There is a need to maintain balance of production and clearance of mucins and mucus in the airway, as can be seen in chronic diseases such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and asthma, which typically result in increased levels of mucus that reduce airway function (60–62). As an indication that excessive mucus is deleterious, mice lacking MUC1 were able to better clear *P. aeruginosa* with enhanced neutrophil recruitment and higher proinflammatory cytokine production (63).

MODIFICATION OF TLR SIGNALING IN DISEASE STATES

An altered ability to sense pathogens by TLRs can have a significant impact on health. Although viruses are not sensed by TLR4, infection with RSV induces expression of TLR4, resulting in a sensitized state to LPS and enhancing inflammatory signaling (64). Secondary bacterial pneumonia after influenza infection is associated with significant morbidity and mortality. Desensitization of TLRs by viral PAMPs may contribute to enhanced susceptibility to bacterial infection. The desensitization leads to reduced chemokine production and NF-κB activation (65). A tolerance state after repeated exposure is also the basis of the hygiene hypothesis in asthma, whereby exposure early in life to PAMPs reduces the likelihood of hyperinflammation later in life (66–68).

Increased TLR signaling is associated with several pulmonary diseases. Exposure to cigarette smoke has been shown to increase TLR4 expression, leading to heightened CXCL8 production and additional recruitment of polymorphonuclear cells to the airways (69). In patients with CF, there is typically a hyperinflammatory state in the lungs, which is also seen in CF cell lines with increased CXCL8 and NF-κB signaling (28, 70). This increased signaling is not a consequence of TLR4, which is reported to be reduced in CF (71). Although CF inflammation is enhanced by the sensing of flagellin by TLR5, *P. aeruginosa* typically becomes nonmotile in chronic infections of patients with CF over time (72), and loss of motility is not necessarily coupled with a loss of inflammatory activity (73). Changes in *P. aeruginosa* LPS also occur in the CF lung (74). Modification of the lipid A portion of *P. aeruginosa in vivo* was associated with resistance to antimicrobial peptides and increased proinflammatory signaling (75). A reduced ability to activate TLR signaling is also problematic. Mutations in TLR4 are associated with increased risk of infection after surgery and display reduced cytokine production in the context of ventilator-associated pneumonia (76).

TYPE I IFNS

Type I IFN signaling often involves the activation of an endosomally located sensor and, via the TRIF adaptor (TLR3 and -4), initiates the production of IFN-β via TANK binding kinase (TBK)1 and phosphorylated IRF3, -5, and -7 (Figure 1) (77–80). Interaction of IFN-β with its heterodimeric receptor (IFN-α/β receptor [IFNAR]) results in dimerization and phosphorylation of STAT1/2 via Jak1 and Tyk2, leading to the downstream transcription of many genes, including CXCL10 (81–85). It has been shown in the airway epithelium that IFNAR is located basolaterally in differentiated cells (86). Signaling through IFNAR also results in the activation of the MAPK and PI3K pathways (87, 88) and leads to NF-κB activation that can in turn activate type I IFN signaling (89).

Many bacterial pathogens, both intracellular and extracellular, induce the type I IFN response via recognition of PAMPs such as proteins, LPS, and DNA (90–92). TLRs3, -4, -7, -8, and -9 (93–96), nucleotide oligomerization domain (NOD) (97, 98), and RNA polymerase III, which was identified as a sensor for cytosolic DNA (99, 100), as well as DAI/Zbp1 (DNA-dependent activator of IFN genes) (101), can activate type I IFN signaling.

Viruses are potent activators of type I IFN signaling through endosomal TLRs as well as the retinoic acid inducible gene [RIG]-like receptors. The proteins that are able to recognize RNA viruses are RIG-I (102) and melanoma differentiation–associated protein 5 (MDA5) (103, 104), which converge to the mitochondrial-bound IPS-1 (also called mitochondrial antiviral signaling protein) (105, 106) before the signal goes to TBK1 and IRF3 and IRF7. RIG-I and MDA5 are produced in the airway epithelium and respond to a number of pathogens such as influenza, rhinovirus, and RSV (35–37, 107).

How nonphagocytic cells such as airway mucosal cells produce type I IFNs in response to extracellular pathogens is ill defined. Most of the pathogens studied to date that activate type I IFN signaling are intracellular in nature, and their signaling pathways have been studied in the context of DCs or macrophages. Recently, the importance of epithelial type I IFN signaling was shown (108) using a mouse lacking STAT1 in epithelial cells. In that study, STAT1 null mice were irradiated and reconstituted with healthy bone marrow. These epithelial-specific STAT1 null mice were still highly susceptible to viral infection, indicating that epithelial STAT1 signaling was important in mediating viral clearance. *S. aureus* induces type I IFN in the airway epithelium, a process dependent on the virulence factor, protein A (91).

The outcome of this type I IFN response is variable and dependent upon the organism and the nature of the infection. The ability to induce production of type I IFNs is a critical component of the host response to influenza infection (109) but has much more variable consequences in response to bacterial infection. Infection of *Ifnar*$^{-/-}$ mice by the intracellular organisms *Listeria* and *Legionella* have opposite consequences, with the *Ifnar*$^{-/-}$ mice being significantly protected from Listeriosis (110) but with enhanced susceptibility to *Legionella* (111). Many extracellular bacteria shed PAMPs in the airway that can be internalized by airway cells and gain access to receptors linked to type I IFN signaling, thereby functioning more like viruses in stimulating innate immune responses. The clinical outcome of these type I IFN signaling responses differs according to the specific organism. For example, type I IFN contributes to *S. aureus* virulence in the setting of pneumonia (91), possibly due to TNF-induced death (112, 113), but contributes to the clearance of *S. pneumoniae* (114). Consistent with type I IFN activation via LPS (115), mice lacking TRIF (116) or IRF3 (117) have reduced capacity to clear *P. aeruginosa* infection, indicating a role for type I IFNs in protection. A similar observation was observed with *E. coli* in a pneumonia model with TRIF-null mice (118). Type I IFN signaling also contributes to the development of secondary bacterial pneumonia after influenza infection (119). In inflammatory diseases such as COPD, higher levels of type I IFN production are observed (120), whereas nasal epithelial cells from smokers have reduced expression of type I IFN receptors, kinases, and reduced type I IFN–dependent cytokines after influenza infection (121).

CXCR3

One group of cytokines that is regulated by type I IFNs provides a link between innate and adaptive immunity. The CXCR3

ligands CXCL9 (MIG), CXCL10 (IP-10), and CXCL11 (I-TAC) provide a mechanism for epithelial and other resident cells to recruit T cells (122–128). The production of CXCR3 chemokines such as CXCL10 preferentially attracts Th1 T cells (Figure 1) (127, 129) while antagonizing the recruitment of Th2 T cells (126).

The CXCR3 receptor and the CXCR3 cytokines are expressed in airway epithelial cells and are induced upon bacterial and viral stimulation in the airway (130–134). This results in $CD4^+$ T-cell chemotaxis (135) and contributes to inflammation (136, 137). The CXCR3 cytokines can exert direct antibacterial effects against gram-positive and gram-negative organisms (136, 138). CXCL9 has a bactericidal effect on *S. pneumonia*; however, CXCL9 knockout mice were not attenuated in pneumococcal clearance from the lung (130).

A correlation exists between respiratory infections and levels of CXCR3 cytokines, particularly CXCL10. Levels of CXCL10 correlated to disease severity, viral titer, and number of lymphocytes in patients infected with rhinovirus (134). Elevated levels of CXCR3-positive cells and cytokines have also been observed in smokers and patients with COPD and bronchitis (139–141).

NOD-LIKE RECEPTORS

The NOD-like receptor (NLR) family encompasses a family of proteins that sense PAMPs in the cytosol. The best characterized members of this family are the NOD proteins NOD1 and NOD2. The NODs contain a caspase recruitment domain (CARD), NOD, and LRR domains. The NOD proteins were initially observed for their role in NF-κB induction and Crohn's disease (NOD2) (142–146). NOD1 primarily senses gram-negative peptidoglycan, which contains γ-D-glutamyl-meso-diaminopimelic acid (147, 148), whereas NOD2 is considered a general sensor of peptidolgycan through recognition of muramyl dipeptide (Figure 1) (149). Signal transduction from either NOD converges on the RIP2 kinase that leads to NF-κB activation (150).

Because NLRs are relatively new, the knowledge of NLRs in the airway is still developing. Both NOD proteins are expressed in the airway epithelium and are induced with bacterial stimuli (30, 151–154). In the context of polymicrobial colonization in the airway, the pore-forming toxin pneumolysin from *S. pneumoniae* facilitates entry of peptidoglycan from *Haemophilus influenzae* to activate NOD1 (155). *In vivo* studies have shown that the NODs are involved in pulmonary clearance of a number of bacterial pathogens (156–159); in some cases they appear to have redundant roles, with attenuated clearance only observed in RIP2 knockout mice (157). Genetic polymorphisms in *nod1* have been linked to asthma (160).

BACTERIAL ACTIVATION OF THE INFLAMMASOME

The inflammasome is the term applied to the assembly of a number of proteins, including an NLR, pro–caspase-1, and the adapter apoptosis-associated speck-like protein (ASC) (161). An outcome of inflammasome activation is the production of caspase-1, which cleaves pro-proteins of IL-1β and IL-18 to their biologically active forms (162). Pro–IL-1β production is mediated by induction of the IL-1β gene through TLR and NOD stimulation, which is then processed by caspase-1 produced by recognition by the NLRs (163). The consequence of inflammasome activation is a form of cell death termed "pyroptosis." Pyroptosis results in membrane disruption and the release of IL-1β and other inflammatory cytokines (164). Two other NLR proteins are involved in inflammasome activation, an area that has not been studied in detail in the airway but is important in pulmonary defenses (165, 166).

IL1-β–converting enzyme protease activating factor (IPAF), also known as NLRC4 (NLR CARD domain), recognizes cytosolic flagellin (Figure 1) (167, 168), including that of *P. aeruginosa* (169, 170). Extracellular flagellin is not recognized by IPAF. Activation of IPAF via flagellin is complex because it involves the delivery of the ligand via a functional type III secretion system. In the case of *P. aeruginosa*, two different type III secreted toxins have been shown to inhibit caspase-1–dependent cytokine production (169–172). In human epithelial cells, it has been shown that IPAF controls replication of *Legionella pneumophila* (173).

A second inflammasome NLR is NLR pyrin domain (NLRP3). NLRP3 senses multiple PAMPS, such as peptidoglycan (174) and RNA (175), and results in an inflammasome if ATP is sensed or bacterial toxins facilitate entry of stimulating ligands (Figure 1) (176–179). NLRP3 has been shown to sense asbestos and uric acid as a result of lung injury (180–182). NLRP3 is present in the nasal epithelium, and *in vivo* NLRP3 null mice show reduced inflammation to bacterial and viral challenge but poor survival, showcasing the requirement for inflammation in clearing infections (151, 165, 166, 183).

NON-TLR SIGNALING

There are a number of receptors present on the cell surface that signal through a number of pathways that are not related to the TLRs or NLRs. These receptors, three of which are TNF receptor (TNFR)1, EGFR, and C-type lectins, respond to host components but are also used by pathogens and can be important in defense.

TNFR1

TNF is a major proinflammatory cytokine whose expression is briskly activated in response to many types of infection; thus, it is not surprising that many different cell types in the lung express receptors to TNF (TNFRs) (184). In the airway epithelium, TNFR1 is abundant on the cell surface (Figure 1) (185) and is linked to many signaling cascades involved in host defense. One of the most striking examples for the involvement of TNFR1 in host defense is its interaction with protein A from *S. aureus.* The IgG binding domain of protein A, which recognizes the Fc region of IgG and Fab of VH3 (185–187), activates the TNF cascade, inducing CXCL8 expression via TRAF2/p38 MAPK and NF-κB. This interaction is critical in the pathogenesis of *S. aureus* pneumonia because *spa* null mutants do not cause infection and TNFR1 null mice are highly resistant to infection (185, 187).TNFR1 signaling appears to be the primary sensing mechanism for *S. aureus* in the airway because MyD88 is not important in *S. aureus* pneumonia models *in vivo* (188). A similar requirement for TNFR1 in causing pneumonia was observed with *Stenotrophomonas maltophilia*, an opportunistic pathogen for patients with CF. TNFR1 mice faired significantly better for pneumonia and bacteremia when intranasally infected with *S. maltophilia* (189).

Elevated levels of TNFR1 expression have been observed in CF epithelial cells, and *Burkholderia cenocepacia*, also a CF pathogen, activates TNFR1 as well (190). TNFR1 is also important for the clearance of *P. aeruginosa* (191). TNFR1 also regulates expression of MUC1, which is an important anti-inflammatory component and binding site for *P. aeruginosa* on airway epithelial cells (47, 52, 63, 192).

EGFR

EGFR plays a number of roles in epithelial signaling in response to airway infection. EGFR is located apically on airway epithelial cells (Figure 1) and induces production of CXCL8 in response to a variety of stimuli (193, 194). *S. aureus* interacts

with EGFR through the IgG binding domain of protein A to activate TNF converting enzyme (TACE) (also called ADAM17). TACE participates in the regulation of inflammatory signaling by cleaving TNFR1 from the epithelial surface and inducing IL-6R shedding (195) and trans-signaling. The protein A-EGFR interaction induces TACE through a c-Src-erk1/2–mediated cascade (194). This signaling is not due to TGF-α because inhibition of protein A-EGFR binding prevented EGFR phosphorylation and TNFR1 cleavage.

EGFR signaling is central to the induction of mucin production in the airway. Activation of EGFR results in increased production of MUC5AC in the airway epithelium (196), and *P. aeruginosa* induces MUC5AC via activation of MAPK and EGFR (57, 197, 198). Increased mucin is a response to tobacco smoke (197), and EGFR serves as a gateway for cigarette smoke to mediate its damaging effects on adherens junctions and Wnt/β-catenin signaling (199). TACE is an integral component of this response because inhibiting TACE prevents the increased mucin expression as a result of reduce TGF-α shedding (198)

An interplay exists between EGFR signaling and the TLRs. TLR2, -3, -5, and -6 have been shown to activate EGFR. The mechanism leading to induction of CXCL8 occurs via a Duox1–TACE–TGF-α–EGFR pathway, with TGF-α acting as the ligand for EGFR signaling induced by the TLRs (200–202).

C-TYPE LECTINS

The C-type lectin family of proteins has an important physical role in mediating cell–cell adhesion but also recognizes carbohydrates, an important mechanism to sense fungal, yeast, and mycobacterial infections (203). C-type lectins possess a distinct protein fold, termed the carbohydrate recognition domain, which is generated by two conserved disulfide bonds between cysteine residues at the base of a double loop structure (204). Members of this family include dectin-1, dectin-2, and mincle. The C-type lectins can recognize the β-glycans present on fungi, yeast, and mycobacterial cell walls (205, 206)

Dectin-1 has been shown to be important in *Pneumocystis carinii* respiratory infection (207), whereas its role in *Candida albicans* depends on the infection model (206, 207). Dectin-1 also plays a significant role in inflammatory signaling in response to *Aspergillus fumigatus* (208). Dectin-2 is another C-type lectin involved in sensing yeast that is expressed in the lung (209). Dectin-2 shows a preference for hyphae of *C. albicans* (210) and is important in host defense (211). A third C-type lectin is Mincle, which is been shown to be required for proinflammatory signaling in response to *C. albicans* (212). The CARD9 adaptor mediates dectin-1 and dectin-2 signaling in CARD9 (213, 214), and mice lacking CARD9 were unable to control respiratory infection of *Mycobacterium tuberculosis* (215).

The biology of the C-type lectins has been mainly characterized in myeloid cells. Their role, if any, in airway epithelial cells is not fully understood. One study has identified production of dectin-1 in airway epithelial cells (216), contrasting earlier work (217). Production of dectin-1 in A549 cells was induced upon stimulation with *M. tuberculosis*, and internalization of the organism was partially blocked by silencing dectin-1 (216).

ANTIMICROBIAL PRODUCTS

In response to the recognition of PAMPs via TLRs and NLRs, the airway itself participates in microbial killing. The airway secretes a number of antimicrobial products that act directly on invading pathogens (Figure 1). These products are resident in the airway fluid and inducible upon recognition of pathogen. The antimicrobial molecules produced by the airway can be small cationic molecules, such as the β-defensins, LL-37, and CCL20, or larger proteins, such lysozyme, lactoferrin, and mucin.

β-DEFENSINS

β-Defensins are small cationic peptides that play an important role in host defense against microbial pathogens in the airway epithelium. There are six β-defensins identified in humans (hBD1–6). Although hBD5 and hBD6 have shown antimicrobial activity, they are not expressed in the respiratory epithelium (218–220). hBD1 is constitutively expressed in the epithelium, whereas hBD2, -3, and -4 can be induced by a variety of bacterial, fungal, and viral pathogens (221–227).

Significant evidence exists for the regulation of β-defensin expression by TLRs. Initial evidence observed that proinflammatory cytokines such as TNF and IL-1β could induce expression of hBD2 (228, 229). Subsequently it was found that hBD2 could be induced through TLR2 signaling (230, 231). Interfering with NF-κB signaling abolishes this response (232), as does blocking MyD88 or Mal/TIRAP (233). The TLR4 signaling complex and its MyD88 portion of signaling are involved in β-defensin expression (229, 233, 234). Microbial DNA through TLR9 (31), bacterial flagellin through TLR5 (235), and viral dsRNA through TLR3 (235) induce β-defensin expression in the respiratory epithelium. β-Defensins can also induce signaling of T cells and dendritic cells by binding to the chemokine receptor CCR6 (236).

Levels of β-defensin expression correlate to lung disease. Elevated levels of hBD2 are associated with inflammation in patients with CF, inflammatory lung disease, and deterioration of lung function. β-Defensins are not usually detected in healthy bronchoalveolar lavage samples (221, 237, 238). Mucoid strains of *P. aeruginosa*, as selected in chronic infections in the CF lung, were capable of inducing hBD2, whereas nonmucoid strains were not (239). The ability to express β-defensins has also been shown to be reduced with long-term smoking (240) and may contribute to lung disease.

LL37

Another cationic host peptide peptide is LL-37, the only human member of the cathelicidin family of antimicrobial peptides (241). LL-37 is generated by the respiratory epithelium and possesses broad spectrum antimicrobial activity (242) that, when overexpressed in murine models, enhances bacterial clearance (243). Elevated levels of LL-37 have been observed in CF samples, correlating with severity of disease (237). This inflammatory correlate may be related to cell death because apoptosis of respiratory epithelial cells has been observed with physiologically relevant levels of LL-37 (244).

LL-37 is induced by bacterial and mycobacterial infections, and this is dependent on MAPK (245–247). LL-37 is also capable of activating MAPK to induce CXCL8 secretion via activation of EGFR and IL-6 via NF-κB (248, 249). Although not investigated in epithelial cells, LL-37 can be induced by a variety of TLRs in macrophages (250).

CCL20

CCL20 (also known as LARC and MIP-3α) is another protein similar to the defensins. CCL20 is expressed in the respiratory epithelium and is stimulated by a variety of microorganisms, including bacteria and the dust mite (251, 252). CCL20 has also been shown to be regulated by TLR2, -3, and -5 as well as

TNF (253–256). By interacting with CCR6, CCL20 is able to attract immature DC and T cells. Clinically, elevated levels are observed in patients with CF (253), and cigarette smoke retards its induction (254).

LACTOFERRIN AND LYSOZYME

The large and abundant antimicrobial proteins in the airway are lysozyme and lactoferrin. Both proteins have proven antibacterial properties but act with differing mechanisms (257). Lysozyme targets the $\beta, 1 \rightarrow 4$ glycosidic bond between N-acetylglucosamine and N-acetylmuraminic acid in peptidoglycan (258) and subsequently is effective against gram–positive pathogens (257). Levels of lysozyme produced by epithelial cells correlate well to clearance of invading pathogens, and transgenic mice expressing elevated levels of lysozyme have significantly improved clearance of bacteria (259–261). Lactoferrin chelates iron away from bacteria but also has direct antimicrobial properties (257, 262, 263). Lactoferrin works with lysozyme to kill gram–negative pathogens by disrupting their membrane to expose susceptible peptidoglycan (264). A number of studies have investigated the correlation between elevated levels of lysozyme and lactoferrin in patients with CF (265) as well as individuals with chronic bronchitis and asymptomatic smokers, indicating a potential contribution to inflammation (266).

CONCLUSION

The airway epithelium is an important part of the innate immune system. Its collection of surface, endosomal, and cytosolic sensors that activate numerous proinflammatory signaling pathways and resident antimicrobial peptides offers significant mechanisms to deal with invading pathogens. It is a tremendously complex system, with many coregulated components. There is likely even greater complexity than we now appreciate; additional receptors are being identified continuously as is an appreciation for their role in epithelial cells. Despite the large amount of experimental data accrued, many questions remain. It remains unclear how the airway epithelium discriminates between commensal flora and pathogens that often colonize (*S. pneumoniae* or *S. aureus*) from the bacteria which initiate invasive infection. Not only does the host actively respond to the perceived threat of infection, but the organisms readily adapt to immune pressure, activating and repressing specific genes to facilitate proliferation despite the many effectors of immune clearance. A great deal has been learned by exploiting murine models of infection, which, despite their limitations, have facilitated a basic understanding of the major components of the innate immune system and their role in host defense of the respiratory tract. The importance of the innate immune system is highlighted by susceptibility to pathogens in specific transgenic mice studies and the correlations that exist with diseased states such as COPD, CF, and cigarette smoking. More complex models, such as the newly developed CF pig (267–269), as well as detailed genetic studies of polymorphisms in TLRs, NODs, and other receptors, should provide even more insights into the mechanisms through which the respiratory mucosa initiates host defenses against such a variety of pathogens.

FUTURE DIRECTION: THE ROLE OF EPITHELIAL SIGNALING IN MUCOSAL IMMUNITY

The participation of the airway epithelium in mucosal defenses has been well established; there is no question that airway epithelial cells provide much more than just a mechanical barrier to infection. However, many unanswered questions remain. The presence of the full complement of innate immune receptors, TLRs, NLRs, and the diverse intracellular receptors linked to the type I IFN cascade indicate that many airway epithelial cells have the potential to respond to a wide range of pathogens. What may be limiting is whether specific PAMPs can gain access to the corresponding receptors and whether they are superficially exposed or intracellular. Thus, the ability of the mucosal epithelium to distinguish commensal flora, which does not activate immune responses, from pathogens that do may lie in the ability of the pathogen to stimulate intracellular signaling. For many bacteria and viruses, this may include activating receptors linked to the type I IFN cascade, which are intracellular. The relative amounts and distribution of these receptors could account for major differences in the activation of epithelial cells at specific sites to respond to specific pathogens (e.g., the lack of TLR4 on the surface of polarized epithelial cells). A better understanding of how the mucosal epithelium responds to airway PAMPs and how signals from commensals are processed to prevent excessive damaging inflammatory responses and how the presence of a real pathogen is rapidly amplified to protect the lung are questions that are being actively investigated.

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