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Toll-like receptor expression and function in airway epithelial cells

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Summary

Toll-like receptors (TLRs) belong to a family of transmembrane proteins that can recognize and discriminate a diverse array of microbial antigens. Following their activation by specific ligands, TLRs initiate intracellular signaling cascades that culminate in the activation of transcription factors and ultimately lead to changes in pro-inflammatory gene expression. The TLR family constitutes an important component of the innate immune system and, although most commonly considered to be associated with immune cell responses, TLRs are also known to be functionally expressed on a variety of other cell types. Epithelial cells represent a significant component of the cellular content of the airways. These cells provide both a barrier to infection and an active defense mechanism against invading microbes. The expression and function of TLRs on airway epithelial cells has been an area of increasing interest in the recent past. This review will summarize advances in our understanding of the role of TLRs in airway epithelial cells.

Key words:

Toll-like receptors • airway epithelial cells • inflammatory lung disease

Abbreviations:

CF – cystic fibrosis, **ds** – double-stranded, **HBD** – human β -defensin, **HSP** – heat shock protein, **ICAM-1** – intercellular adhesion molecule-1, **IFN** – interferon, **IKK** – I κ B kinase, **IL** – interleukin, **IL-1RI** – IL-1 type I receptor, **IP-10** – interferon inducible protein, **IRAK** – IL-1 receptor-associated kinase, **IRF** – interferon regulatory factor, **LPS** – lipopolysaccharide, **LTA** – lipoteichoic acid, **Mal** – MyD88 adaptor-like, **RANTES** – regulated on activation T cell expressed and secreted, **RSV** – respiratory syncytial virus, **SARM** – sterile α and HEAT-Armadillo motifs, **ss** – single-stranded, **TAB** – TAK1-binding protein, **TAK1** – transforming growth factor- β -activated kinase-1, **TB** – tuberculosis, **TBK1** – TANK-binding kinase 1, **TIR** – Toll/IL-1R, **TLR** – Toll-like receptor, **TRAF6** – tumor necrosis factor receptor-associated factor 6, **TRIF** – TIR domain-containing adaptor inducing interferon β , **TRAM** – TRIF-related adaptor molecule, **uCpG** – unmethylated CpG.

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INTRODUCTION

The lung is a unique organ. Although constantly exposed to inhaled contaminants and microbes present in the air breathed, up to 20,000 liters each day, it can effectively maintain a sterile environment. This is largely due to its innate immune defenses, a significant component of which is the Toll-like receptor (TLR) family. Both epithelial cells and dedicated immune cells within the lung express TLRs and together regulate lung homeostasis. Airway epithelial cells represent a significant portion of the cellular content of the airways and together constitute a vast surface area. The contribution of these so-called “non-immune” epithelial cells to the inflammatory response in the lung is an increasingly important area of research. Advances in our knowledge of the regulation, expression, function, and modification of TLRs in a variety of different tissues and cell types has led to the emerging concept that TLRs can behave in a cell-specific manner. This review will focus on our current understanding regarding the expression and function of TLRs in airway epithelial cells.

TOLL-LIKE RECEPTORS

The first TLR to be identified and characterized was in the fruit fly *Drosophila melanogaster*. This protein, called *Drosophila* or dToll, has an important role in embryogenesis, where it regulates dorsal-ventral axis formation in the developing fly embryo, but in the adult fly dToll acts as a key receptor regulating anti-fungal defense⁴⁸. In the early 1990s it was reported that dToll shared structural homology with the mammalian type I interleukin-1 receptor (IL-1RI)²⁴, an important receptor in innate immunity, and following that initial observation it has since been discovered that this homology also extends to functional responses. To date, ten functional human TLRs have been identified; all are germ-line encoded pattern-recognition receptors and each is postulated or proven to have a role in the innate immune response^{3, 93}. TLR expression is widespread, with tissues and cell types reported to express TLRs ranging from those of myeloid and lymphoid origin to endothelial and epithelial cells.

STRUCTURE

Structurally, TLRs are type I transmembrane proteins (Fig. 1). Similar to IL-1RI, each has an intracellular signaling domain with a conserved region 150–200 residues in length, termed the TIR or Toll/IL-1R domain, and a single transmembrane-spanning domain⁵⁹. Unlike IL-1RI, which has

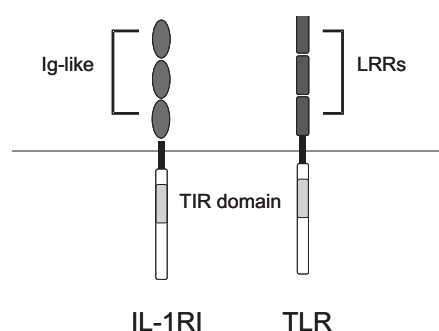


Figure 1. Structure of generic TLR and IL-1RI proteins. TLRs and IL-1RI are single transmembrane-spanning receptors. TLRs have leucine-rich repeats (LRRs) in their extracellular N-terminal domain, IL-1RI has immunoglobulin(Ig)-like domains. Both IL-1RI and TLRs have a conserved 150-200 amino-acid Toll/IL-1R (TIR) domain in their cytosolic carboxy-termini required for signaling.

immunoglobulin-like domains located extracellularly, TLRs have an extracellular domain composed of leucine-rich repeats. These are motifs commonly involved in protein-protein interactions and are likely to be the regions that confer specificity to TLRs with respect to their pattern-recognition properties and may also be involved in TLR dimerization¹¹.

The TIR domain is a key cytosolic region of all TLRs. Each contains three highly conserved regions, called Boxes 1, 2, and 3⁶¹. Box 1 is the signature sequence of the TIR domain. Box 2 forms an important loop in the TIR structure, which likely engages distal adapters⁷. The function of Box 3 remains to be elucidated, although it contains residues important in signaling⁷⁶. TIR domains are essential for the activation of a number of common signaling pathways, most notably those leading to the activation of nuclear factor (NF)- κ B and the three mitogen-activated protein kinase pathways p38, JNK, and ERK1/2. Although all TLR signaling events are dependent on the conserved TIR domain, individual TIR domains of these receptors are not functionally equivalent. For example, the TIR domain of TLR4 signals as a homodimer, whereas the TIR domain of TLR2 can only signal as a heterodimer cooperating with TLR1 or TLR6⁶⁴.

TLR LIGANDS

The generally accepted function of TLRs is to recognize and discriminate a diverse array of microbial antigens, derived from diverse species including bacteria, viruses, mycoplasma, yeasts, and protozoa (Fig. 2), and respond by activating intracellular signaling pathways culminating in gene expression changes³. The most widely studied and best characterized mammalian TLR to date is TLR4. This is the

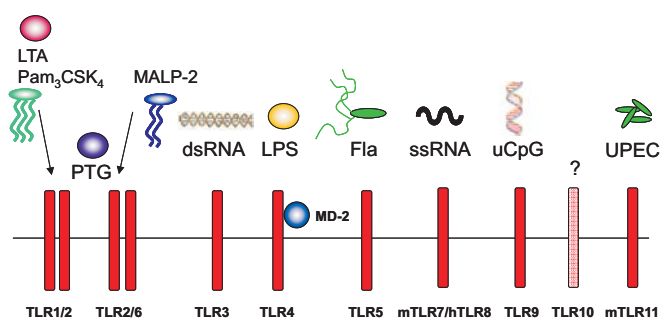


Figure 2. Summary of principal microbial TLR ligands. LTA – lipoteichoic acid, Pam₃CSK₄ – triacylated lipopeptide, PTG – peptidoglycan, MALP-2 – diacylated lipopeptide, dsRNA – double-stranded RNA, LPS – lipopolysaccharide, Fla – flagellin, ssRNA – single-stranded RNA, uCpG – unmethylated CpG dinucleotide motifs, UPEC – uropathogenic *Escherichia coli*, hTLR – human TLR, mTLR – murine TLR.

principal receptor for lipopolysaccharide (LPS), a toxic component present on the outer leaflet of the outer membrane of Gram-negative bacteria. The identity of TLR4 as the mammalian LPS receptor initially came from studies on the LPS hypo-responsive mouse strain C3H/HeJ⁶⁶. These mice can withstand challenges of lethal doses of LPS as a result of a point mutation in the TIR domain of their TLR4 gene. This mutation encodes a proline to histidine substitution at position 712 (Pro712His) and renders their TLR protein unresponsive to LPS. Other hyporesponsive mice exist (strains C57BL/ScCr and C57BL/ScN) that lack TLR4 mRNA expression due to chromosomal deletion of the gene. Amongst the TLRs, TLR4 is unique in that it requires the involvement of two accessory proteins for full responsiveness to its cognate ligand; MD-2, a soluble glycoprotein residing on the outer surface of the cell membrane in association with the N-terminal of TLR4, is unique to and necessary for full TLR4 responsiveness to LPS⁵⁵; CD14 is a glycosphosphatidyl inositol-anchored receptor which binds to LPS-LPS-binding protein complexes¹⁷. Together, these proteins enhance the responsiveness of TLR4 to LPS. In addition to LPS, TLR4 can also recognize other microbial ligands. These include envelope proteins from murine retroviruses and respiratory syncytial virus (RSV), flavobacterial flavolipins, and Hsp60 from *Chlamydia pneumoniae*^{26, 45, 68, 69}.

Of all the TLRs, TLR2 recognizes the broadest repertoire of ligands from such species as Gram-positive and Gram-negative bacteria, protozoa, mycobacteria, yeasts, and mycoplasma, and is interesting amongst the TLR family in that it can heterodimerize with other TLRs to confer responsiveness to these diverse ligands⁸⁹. For example, in conjunction with TLR1 it recognizes triacylated lipopeptides and

Gram-positive lipoteichoic acid (LTA), amongst others, whereas with TLR6 it can respond to diacylated lipopeptides such as MALP-2 from mycoplasma.

Other TLR ligands that have been identified include double-stranded (ds)RNA for TLR3⁴, which can be generated intracellularly during viral replication in infected cells, and flagellin for TLR5³³. Flagellin is the protein monomer of bacterial flagellae, the polymeric whip-like appendages extending from the outer membrane of Gram-negative bacteria that propel the microorganisms through aqueous environments. Interestingly, TLR2 may also have a role in the recognition of flagellin by TLR5¹. This is not altogether unexpected given TLR2's known ability to heterodimerize with other TLRs and respond to multiple ligands.

TLRs 7 and 8 were first shown to recognize imidazoquinoline anti-viral compounds such as imiquimod and also loxoribine and bropirimine^{35, 42}. More recently, however, it has emerged that the true ligands for these TLRs are guanosine- and uridine-rich single-stranded (ss)RNA found in many viruses, with TLR7 being the principal receptor in mice and TLR8 in humans^{20, 34}. Bacterial DNA activates TLR9³⁶. Unmethylated CpG (uCpG) dinucleotides are a motif that occur at a significantly higher frequency in bacterial versus mammalian DNA and, depending on the flanking sequence, e.g. GACGTT or GTCGTT, uCpG dinucleotides activate TLR9 signaling in either murine or human cells, respectively, with greater potency⁹.

The TLR10 gene is localized to chromosome 4p14. The specific ligands and functions of TLR10 are currently unknown; however, it has been postulated that TLR10 may be a potential asthma candidate gene⁴⁷. It is a highly polymorphic gene in which at least 78 single-nucleotide polymorphisms have been detected. The newest member of the TLR family to be identified is TLR11. In mice, TLR11 responds to a surface-exposed factor on uropathogenic bacteria⁹⁵. To date it is not clear whether humans express TLR11, as the murine Ser119 residue appears to be replaced by a stop codon in humans.

ENDOGENOUS TLR LIGANDS

In addition to microbial ligands, a number of endogenous TLR4 agonists have been reported. These include such factors as neutrophil elastase, heat shock proteins (Hsp60, Hsp70 Gp96), surfactant protein A, fibrinogen peptides, an alternatively spliced variant of fibronectin, hyaluronan oligosaccharides, and human β -defensin-2^{12, 29, 57, 58, 82–84}. The potential

of these agents to activate TLR4 have led to the “danger” or “altered self” hypothesis, which suggests that a mechanism exists whereby TLR4 can recognize molecular patterns of displaced factors or inflammatory mediators, become activated, and enhance the immune response. It remains to be shown whether the agonists interact directly with their cognate TLR or trigger TLR activation at the cell surface via binding intermediates.

INTRACELLULAR SIGNALING

An important and interesting feature of TLR signal transduction is that a highly conserved intracellular pathway is activated by the different TLRs^{13, 61}. Following their activation by specific factors, TLRs transduce intracellular signals to regulate proinflammatory gene expression. Classically, these signals are transduced via a number of kinases and adaptor proteins leading to activation of NF- κ B and induction of NF- κ B-regulated genes (Fig. 3)⁷⁹. TLR signaling can also lead to activation of AP1 and the MAP kinases JNK, p38, and ERK1/2⁷¹.

The signaling pathway leading to the activation of the transcription factor NF- κ B by TLR ligands has been

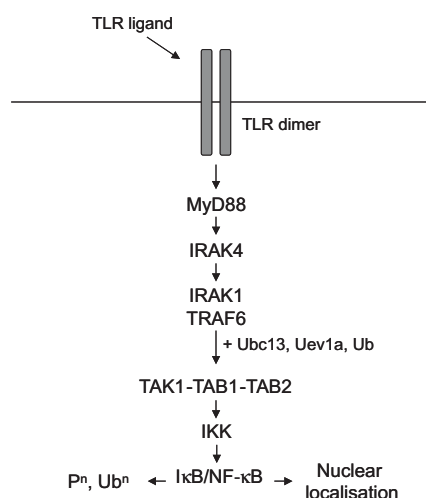


Figure 3. TLR signaling cascade leading to NF- κ B activation. Triggering of TLRs promotes interaction between the TIR domains of TLRs and MyD88. Next, IL-1 receptor-associated kinase-4 (IRAK-4) associates with MyD88 between death domains present in both proteins. Following the interaction of IRAK-1 and tumor necrosis factor receptor-associated factor 6 (TRAF6) with IRAK-4, the IRAK-1/TRAF-6 complex then dissociates from the receptor and associates with transforming growth factor β -activated kinase-1 (TAK1) and TAK1-binding proteins, TAB1 and TAB2. TRAF6 is ubiquitinated (Ub) on Lysine 63 by the E2 ligases Ubc13 and Uev1A. This activates TAK1 and leads to phosphorylation and activation of the I κ B kinase (IKK) complex, which in turn phosphorylates (Pⁿ) I κ B, targeting it for ubiquitination (Ubⁿ) and proteosomal degradation and releasing NF- κ B, which can then translocate to the nucleus to regulate gene expression.

well characterized. The current paradigm suggests that triggering of TLRs promotes the recruitment of the adaptor protein MyD88, which can associate with the cytosolic region of TLRs through its carboxyl-terminal TIR domain⁵⁰. Once recruited, MyD88 interacts with IL-1 receptor-associated kinase-4 (IRAK-4) via associations between death domains present in both MyD88 and IRAK-4⁷⁸. IRAK-1 then interacts with IRAK-4, followed by tumor necrosis factor receptor-associated factor 6 (TRAF6). The IRAK-1/TRAF-6 complex dissociates from the receptor and associates with transforming growth factor β -activated kinase-1 (TAK1) and TAK1-binding proteins, TAB1 and TAB2. Next TRAF6, TAK1, TAB1, and TAB2 form a larger complex with the E2 ligases Ubc13 and Uev1A, which catalyze the synthesis of a lysine 63-linked polyubiquitin chain on TRAF6¹⁹. This triggers the phosphorylation and activation of TAK1. Activated TAK1 phosphorylates and activates the I κ B kinase (IKK) complex, consisting of IKK α , IKK β , and NEMO/IKK γ ⁸⁵. I κ B proteins normally reside in the cytosol complexed to NF- κ B dimers, maintaining them in an inactive state. Phosphorylation of I κ B proteins by IKKs targets them for ubiquitination and proteosomal degradation and induces release and activation of NF- κ B, which can then translocate into the nucleus and transactivate expression of NF- κ B-regulated genes.

MYD88-INDEPENDENT SIGNALING

A common question posed regarding TLR signaling is how different TLR ligands can induce specific responses. One level of discrimination is at the level of ligand recognition, although it is now clear that a further degree of specificity is conferred due to the presence of a number of intracellular adaptor proteins which act as MyD88 homologues. Until recently, MyD88 was considered a unique member of the TLR/IL-1R family, being the only soluble protein; however, at least four additional MyD88 homologues are now known to also exist. These adaptor proteins include MyD88 adaptor-like (Mal, alternatively known as TIRAP)^{22, 40}, TIR domain-containing adaptor inducing interferon (IFN)- β (TRIF also known as TICAM-1)^{62, 92}, TRIF-related adaptor molecule (TRAM, also known as TICAM-2)^{23, 63, 91}, and sterile α and HEAT-Armadillo motifs (SARM)⁶⁰, and each is believed to transduce intracellular signals from different TLRs under different conditions. For example, all TLRs with the exception of TLR3 can signal via MyD88, TLRs 2 and 4 utilize both MyD88 and Mal, and other TLRs (TLR3 and TLR4) can engage TRIF and TRAM under certain circumstances. The role of SARM has yet to be characterized.

NF- κ B activation by MyD88 and Mal occurs via the

classical signaling cascade described. However engagement of TRIF and TRAM by TLR3 or TLR4 can also trigger an alternative signaling pathway involving the non-canonical IKKs, TANK-binding kinase 1 (TBK1) and IKK ϵ /IKK ι , culminating in the activation of the transcription factor interferon regulatory factors (IRF) 3 and 7 (Fig. 4)^{23, 43, 74, 90}. IRF3 and IRF7 regulate expression of the type I interferons, IFN- β and IFN- α , respectively. These, in turn, can then increase expression of other genes, such as IP-10 and RANTES, via activation of STAT1. This promotes activation of local dendritic cells, macrophages, and mast cells and, ultimately, T and B cell-mediated adaptive immunity. Although LPS fails to induce expression of RANTES from BEAS-2B airway epithelial cells³¹, TLR3 agonists have been shown to signal via TRIF to induce epithelial cell secretion of RANTES and IFN- β ^{30, 73}.

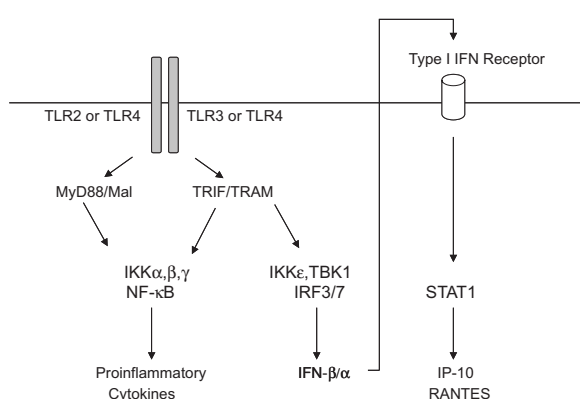


Figure 4. MyD88-dependent and MyD88-independent TLR signaling. TLR2 and TLR4, or TLR3 activate the IKK complex via MyD88/Mal, or TRIF, respectively, leading to classical NF- κ B activation. TLR3 and TLR4 also activate IKK ϵ and TBK1 via TRIF/TRAM, leading to IRF3 and IRF7 activation and production of IFN- β and - α , which are secreted and bind to the type I IFN receptor. This triggers STAT1 activation and induction of IFN-inducible protein (IP-10) and RANTES.

TLR EXPRESSION IN AIRWAY EPITHELIAL CELLS

To date, a number of studies have evaluated TLR expression in a variety of airway epithelial cell types. Cell lines that have been characterized include tracheal, bronchial, and alveolar type II cells with normal or cystic fibrosis (CF) phenotypes. Primary cultures of nasal polyp, tracheobronchial, airway, and type II alveolar cells have also been studied. Work from this laboratory has shown that CF and non-CF tracheal and bronchial epithelial cell lines express mRNA for TLRs 1-6 and TLR9²⁸. Muir et al.⁵⁴ have also shown that both normal and CF airway epithelial cells express mRNA for TLRs 1-10, and their confocal microscopy studies showed that the TLR2 protein

is localized to the apical surface of these cells, whereas TLR4 and TLR5 have a more basolateral distribution.

Becker et al.¹⁰ were the first to demonstrate that primary tracheobronchial cells express mRNA for TLRs 1-6. Later cell surface expression of TLR2 in primary airway epithelial cells was demonstrated³⁸; however, TLR4 appears to reside intracellularly in primary bronchial epithelial cells, with a mostly subapical localisation³¹. Adamo et al.¹ investigated TLR5 expression in polarized bronchial epithelial cells with tight junctions grown at an air-liquid interface and also reported a predominantly basolateral distribution for this TLR. However, following stimulation of these cells with flagella, TLR5 expression can up regulated and mobilized to the apical surface. This is in contrast to gut epithelial cells, which express TLR5 almost exclusively on the basolateral surface³⁷. In macrophages and dendritic cells, TLR9 resides in the endoplasmic reticulum (ER) and redistributes to uCpG-containing lysosomal compartments for ligand binding and signal transduction⁴⁶. Cell surface expression of TLR9 has been detected by fluorescence microscopy on a CF tracheal epithelial cell line and by flow cytometry on both immortalized and differentiated primary airway epithelial cells^{28, 65}. The role of the ER or other intracellular compartments in TLR-ligand interactions in non-phagocytic airway epithelial cells remains to be investigated.

The emerging consensus regarding TLR expression on bronchial and tracheal epithelial cells points to TLR2 as the predominant TLR expressed on the surface of these cells *in vivo*, with other TLRs (TLR3, TLR4, TLR5) residing mainly intracellularly or displaying only low-level surface expression. These TLRs, however, can be mobilized to the membrane following stimulation with microbial factors. For example, TLR4 cell surface localization is promoted by RSV infection⁵³ (Fig. 5).

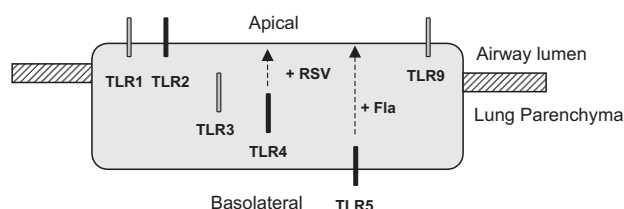


Figure 5. TLR protein expression in bronchial airway epithelial cells. TLR2 is the predominant TLR expressed on the apical surface. TLR3 and TLR4 reside intracellularly and TLR5 is located at the basolateral surface. TLR4 and TLR5 can be mobilized to the apical membrane following stimulation with RSV or flagellin (Fla), respectively. TLR1 and TLR9 have been detected on the apical surface. Black TLR – confocal data, gray TLR – flow cytometry or slide-based fluorescent cell counting data.

A549s are a type II alveolar cell line. TLR4 appears to be expressed at only low levels on this cell line³¹. However it has been demonstrated that both TLR2 and TLR4 are expressed on the surface of alveolar type II cells *in vivo*^{5, 21}. More extensive studies using alveolar cells should yield a clearer understanding of the localization of these and other TLRs in this cell type.

TLRS AND MULTIMERIC RECEPTOR COMPLEXES

In order for appropriate responses to inhaled microbes to be initiated, the relevant receptors must be present or mobilized to the exposed surfaces of the airway. Airway epithelial cells, in contrast to other mucosal surfaces such as the gut, are readily activated by superficial exposure to microbial factors and as such fulfil an important role in surveillance. It is not yet known whether microbial and/or endogenous TLR ligands interact directly with TLRs (although zymosan is believed to interact directly with TLR2⁷⁰) or whether the ligands are somehow displayed to TLRs or other membrane proteins that may co-exist in the multimeric protein complexes that assemble in lipid rafts. This concept has been given much credence by a recent, elegant study which showed that TLR2, asialo-GM1, caveolin-1, MyD88, IRAK-1, and TRAF6 can all be detected in a lipid raft receptor complex on the apical surface of airway epithelial cells after infection with *P. aeruginosa*⁷⁷. Furthermore, both TLR2 and TLR5 have also been detected in association with asialo-GM1 in flagellin-treated airway epithelial cells¹. These new findings add a further layer of complexity to our understanding of TLR activation, yet provide a more realistic model of the dynamic events that are likely to be taking place within a cell membrane exposed to an infective insult. The identity of other components of these complexes will no doubt follow soon.

TLR FUNCTION IN AIRWAY EPITHELIAL CELLS

Production of type I interferons, as discussed, is one way by which TLRs can signal to the adaptive immune response. However, activated TLRs more commonly enhance the pulmonary immune response by generating a number of other signals, including 1) production and secretion of diffusible chemotactic molecules and cytokines, 2) up-regulation of cell surface adhesion molecules, and 3) enhanced expression of antimicrobial peptides.

A number of studies have investigated the functional consequences of TLR activation in airway epithelial cells. To date these studies, using such diverse TLR ligands as Gram-positive or Gram-negative bacteria,

lipopeptides, LTA, peptidoglycan, zymosan, dsRNA, LPS, flagellin, or uCpG DNA, have shown that stimulation by these agonists can lead to a wide variety of immunological responses in respiratory epithelial cells^{1, 5, 6, 10, 27, 28, 30, 31, 38, 39, 41, 53, 54, 65, 73, 77, 86}. Of the proinflammatory cytokines examined, tumor necrosis factor α and IL-6 can be induced by TLR2, TLR4, and TLR9 agonists^{28, 39, 53}. The CXC chemokine IL-8, a potent neutrophil chemoattractant, is the most widely studied reporter gene in TLR studies of airway epithelium. The extensive repertoire of TLR agonists that have been shown to promote IL-8 mRNA and protein production include those that activate TLR2, TLR3, TLR4, TLR5, and TLR9 (Table 1). Such studies have been performed using both immortalized and primary respiratory epithelial cells. By activating inducible cell migration of neutrophils via increased epithelial expression of IL-8, surveillance, attack, containment, and clearance of invading microbes is enhanced. Another chemokine whose expression is increased by zymosan, dsRNA, LPS, and flagellin in airway epithelial cells, albeit much less potently than IL-8, is macrophage inflammatory protein-3 α of the CC chemokine family⁷³.

Table 1. Regulation of IL-8 expression in human airway epithelial cells by TLR agonists

Agonist	Cell type	Reference
Flagella	1HAEo-, 16HBE14o-	1
LPS, LTA	1° AECII	5
LPS, PTG	NCI-H292	27
Pam ₃ , LPS, uCpG	16HBE14o-, CFTE29o-	28
LPS	BEAS-2B	31
Pam ₃	1° airway	38
PTG	A549	39
LPS	A549	53
<i>S. aureus</i> , <i>P. aeruginosa</i>	9HTEo-pCep, pCepR (CF)	54
uCpG	1HAEo-, 1° airway cells	65
dsRNA	BEAS-2B	70
dsRNA, flagellin, LPS, uCpG, PTG, zymosan	BEAS-2B, 1° bronchial cells	73
<i>S. aureus</i> , <i>P. aeruginosa</i>	1HAEo-	77

Explanations: Pam₃ – triacylated lipopeptide, PTG – peptidoglycan.

Integrin ligands, such as the cell adhesion molecule intercellular adhesion molecule 1 (ICAM-1), facilitate the transepithelial passage of leukocytes to sites of infection. The microbial TLR ligands triacylated lipopeptide, LPS, and uCpG DNA are known to increase ICAM-1 expression on CF and non-CF airway epithelial cells²⁸. It is interesting that both IL-8 and ICAM-1 are positively regulated by TLRs, given their complementary roles in neutrophil-dominated airway diseases such as CF and pneumonia. dsRNA and influenza virus A are also potent inducers of ICAM-1 in BEAS-2B epithelial cells³⁰.

The mammalian innate immune system produces a variety of anti-microbial peptides as part of its host defense repertoire. Of these, human β -defensins (HBD) are produced directly by epithelial cells. HBD2 expression is induced in response to infective stimuli, including Gram-negative and, less potently, Gram-positive bacteria or their components. It has been demonstrated that activation of TLR2 by bacterial lipoprotein results in up-regulation of HBD2 in tracheobronchial epithelium³⁸. Other TLR2 agonists, such as LTA and peptidoglycan, are also known to increase HBD2 expression in both bronchial and alveolar airway epithelial cells^{39, 86}. LPS and Gram-negative bacteria such as mucoid *P. aeruginosa* are a more potent stimulus for HBD2 production. LPS can up-regulate HBD2 expression in immortalized and primary airway epithelial cells⁴¹.

To date, other gene products that have been shown to be increased in airway epithelial cells following TLR stimulation are granulocyte macrophage-colony stimulating factor, the kinin receptors B1 and B2, and serum amyloid A^{6, 73}.

AIRWAY EPITHELIAL CELLS, TLR POLYMORPHISMS, AND INFLAMMATORY LUNG DISEASE

Acute airway infections such as rhinitis, community acquired pneumonia, or exacerbations of chronic obstructive pulmonary disease are usually associated with bacterial or viral etiological agents and, as such, potentially involve the triggering and activation of many TLRs expressed by respiratory epithelial cells. Ideally, this results in a rapid and effective innate immune response being mounted, with quick recovery and eradication of the infective agent and resolution of any parenchymal damage. However, impaired TLR function can impact negatively on these events and may lead to more severe disease and, ultimately, sepsis. Over half of all incidents of sepsis are associated with Gram-negative bacteria⁸⁷, implicating TLR4 as an important target for new sepsis treatments. Indeed, a point mutation in the human TLR4 gene has been identified (Asp299Gly) that is associated with a decreased airway response to inhaled LPS and an increased risk of Gram-negative infection and sepsis^{2, 16, 72}.

In asthma, an increasingly common airways disease, LPS appears to have paradoxical roles depending on the timing and context of the LPS exposure. Many reports have demonstrated an increase in allergen-induced asthma severity following exposure to LPS^{51, 52}; however, early exposure to LPS (or other TLR ligands) can decrease the incidence of atopic asthma in later life^{15, 25}. With this in mind it is hardly surprising

that conflicting reports exist regarding the effect of the TLR4 Arg299Gly polymorphism on the overall incidence of asthma^{67, 88, 94}. The role of TLR4 in infective tuberculosis (TB) is also unclear at present, with conflicting reports suggesting that TLR4 either can or cannot enhance survival^{14, 75}. Two recent reviews discuss the role of TLR proteins in TB and/or asthma^{8, 18}.

CF is a genetic disease characterized by severe neutrophil-dominated airway inflammation. An important cause of inflammation in CF is *P. aeruginosa* infection. Other organisms commonly involved in the pathogenesis of pulmonary inflammation in CF are *Hemophilus influenza* and *Staphylococcus aureus*. The incidence of TLR4 or TLR2 polymorphisms in individuals with CF has not been studied. However, as the TLR2 Arg753Gln polymorphism has been implicated as a risk factor for staphylococcal infection, it may have implications in CF⁴⁹. A major goal in CF research is the development of improved therapies to treat pulmonary inflammation associated with this condition. Inhibitors based on MyD88 and Mal can abrogate IL-8 protein production by TLR agonists in CF tracheal epithelial cells, providing evidence of a potential role for these inhibitors as CF therapeutics²⁸. An important challenge for the future will be to develop suitable delivery methods for these inhibitors and determine their compatibility with current conventional CF therapies.

Other TLR polymorphisms that have been investigated to date in the context of inflammatory lung disease include the Arg753Gln polymorphism in TLR2 which is associated with an increased risk of developing TB⁵⁶, a TLR6 Ser249Pro mutation that may be linked with asthma⁸⁰, and a common stop codon polymorphism in the ligand binding domain of TLR5 (TLR5392STOP) that acts in a dominant fashion, is unable to mediate flagellin signaling, and is associated with susceptibility to *Legionella pneumophila*³².

FUTURE PERSPECTIVES

The lung represents the largest epithelial surface in the body and is a major portal of entry for microorganisms. It employs a number of efficient defense mechanisms to eliminate airborne pathogens encountered in breathing, with its epithelial surface providing the first line of defense against invading lung pathogens. Modulation of TLR function has important implications for inflammatory lung diseases. For example, suppression of TLR responses may reduce excessive inflammation in chronic diseases such as CF. This may be achieved by the use of TLR-neutralizing antibodies or molecules that inhibit ligand binding. Furthermore strategies designed to inhibit TLR

intracellular signaling have definite potential and the careful design of therapeutics that can selectively activate or inhibit specific TLRs in a reversibly controlled manner represents a major international goal.

Alternatively, it is possible that other airways diseases may be targeted by enhancing TLR responses. Stimulation of TLR3 activates the anti-viral response⁴, whilst uCpG can promote Th1 responses⁴⁴, suggesting that therapeutic administration of dsRNA or DNA could act as adjuvants and may also benefit patients likely to develop sepsis.

Finally, it will be important to evaluate the effects of current commonly used therapeutics on TLR responses in airway epithelial cells as it is becoming clear that agents such as inhaled corticosteroids can modulate TLR expression, and this may have a beneficial role in host defense mechanisms³⁸.

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