Review

Innate Immunity

16(3) (2010) 201–210

ISSN 1753-4259 (print)
10.1177/1753425910366058

Toll-like receptors and NOD-like receptors in pulmonary antibacterial immunity

Theivanthiran Balamayooran[1], Gayathriy Balamayooran[1], Samithamby Jeyaseelan[1,2]

[1]*Laboratory of Lung Biology, Department of Pathobiological Sciences and Center for Experimental Infectious Disease Research, Louisiana State University, Baton Rouge, Louisiana, USA*
[2]*Section of Pulmonary and Critical Care Medicine, Department of Medicine, LSU Health Sciences Center, New Orleans, Louisiana, USA*

Lung diseases caused by bacteria are a leading cause of death in both immunocompromised and immunocompetent individuals as well as in children. Although neutrophil recruitment is critical to augment the host defence, excessive neutrophil accumulation results in life-threatening diseases, such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Therefore, it is important to modulate excessive neutrophil influx in ALI/ARDS to mitigate lung damage and mortality. A better understanding of the basic mechanisms underlying neutrophil influx is crucial to designing novel and innovative treatment strategies for ALI/ARDS. Recognition of bacteria in the lung is the critical first step leading to neutrophil influx. Pattern recognition receptors, such as Toll-like receptors and NOD-like receptors, play an important role in the recognition of bacterial pathogens. Understanding the molecular and cellular mechanisms associated with the recognition of bacterial pathogens by the host is critical for the development of effective therapeutic strategies to control parenchymal damage via modulating neutrophil accumulation in the lung.

Keywords: TLRs, NLRs, lung innate immunity, bacterial lung infection, acute lung injury, acute respiratory distress syndrome

INTRODUCTION

Bacterial infections in the lung represent an important cause of morbidity, mortality and healthcare expenditure. In fact, bacterial pneumonia is the third leading cause of mortality world-wide. Pneumonia is the result of overwhelming infection of micro-organisms such as viruses, bacteria, fungi and parasites. Neutrophil recruitment is the pathological hallmark of pneumonia caused by bacteria. Although neutrophils play an important role in the pulmonary host defence during infection, excessive neutrophil accumulation leads to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).[1,2] Therefore, the development of drugs that target the excessive neutrophil accumulation could slow the progression of lung damage, thereby decreasing mortality during bacterial pneumonia.

Infections at mucosal sites caused by a broad range of pathogens are primarily defended by the innate immune mechanisms. The key players of innate immunity are phagocytes, such as neutrophils, and macrophages. Recognition of the pathogen is the first step in a multistep paradigm leading to innate immunity in the lungs and this function is primarily performed by pattern recognition receptors, such as membrane bound Toll-like receptors (TLRs) and cytosolic receptors, such as nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), Rig I like receptors (RLR), C-lectin receptors (CLR) and Scavenger receptors. The TLRs were identified more than a decade ago, whereas cytosolic receptors have been only recently discovered.[3] As a result, TLRs are the most extensively studied. In this article, we will review our current understanding of the TLRs and NLRs in the context of bacterial recognition in the lungs.

Received 11 January 2010; Revised 15 February 2010; Accepted 16 February 2010

Correspondence to: Samithamby Jeyaseelan, Pathobiological Sciences and Center for Experimental Infectious Disease Research, Louisiana State University, Baton Rouge, LA 70803, USA. E-mail: jey@lsu.edu

Neutrophils contribute to initial host defence in the lung during bacterial infections.[4–6] Neutrophils are stored in bone marrow and are mobilised from the bone marrow into the circulation during infections. Neutrophil recruitment to the site of infection and inflammation is a multistep paradigm involving chemokine and cytokine production, up-regulation of cell adhesion molecules, margination of neutrophils, rolling and diapedesis.[7] In the inflammatory foci, activated neutrophils phagocytose microbes, degranulate, generate free radicals and degrade the microbes. Although neutrophils serve as the primary defence, their short life-span (<6 h) renders it difficult to investigate molecular mechanisms upon interaction with bacterial pathogens. A recently described long-term bone-marrow culture system will advance our knowledge of neutrophil biology in the near future.[8] Although neutrophils are considered as primary defenders of the innate immune system, a recent report has shown that neutrophil-derived interleukin (IL)-18, along with dendritic cell-derived IL-12, stimulate natural killer cells to release interferon (IFN)-γ,[9] illustrating their role in the induction of adaptive immunity. In addition to the intracellular killing mechanisms of neutrophils, recent studies have shown that neutrophils form extracellular traps that can bind, confine and destroy extracellular bacteria (neutrophil extracellular traps; NETs). The NETs can be formed by activated neutrophils. In this context, it has been shown that LPS, phorbol myristate acetate (PMA) and chemokines, such as IL-8, can activate neutrophils to form NETs.[10] These NETs are composed of chromatin meshwork containing several types of antimicrobial proteins from primary granules, including neutrophil elastase, cathepsin G and myeloperoxidase, secondary and tertiary granules, such as lacoferrins and gelatinase.[10] Although the formation of NETs is an exciting mechanism to destroy extracellular pathogens, forming NETs may be deleterious to the host because of extracellular noxious components released from neutrophils which can induce severe lung damage.

Toll-like receptors

The TLRs play a pivotal role against a variety of exogenous and endogenous pathogens. The TLRs are type 1 integral membrane glycoproteins which have an extracellular LRR domain and signalling cytoplasmic domain homologous to IL-1 receptor (IL-1R) and termed Toll–IL-1 receptor (TIR) domain.[11] There are 12 TLRs in mice and 10 TLRs in humans that have been reported so far.[3] The TLRs function as homodimers with the exception of TLR2 which dimerises with TLR1 and TLR6 with different ligand specificity. The TLRs activate similar, but not identical, molecules as in the IL-1R signalling cascade.[12] Stimulation of TLR ligands to their receptors recruit adaptor molecules to the cytoplasmic TIR domain and trigger the downstream signalling cascade, activation of NF-κB and production of pro-inflammatory cytokines and chemokines. TLR1, TLR2, TLR4, TLR5 and TLR6 are located on the cell surface and TLR3, TLR7 and TLR9 are located on the endosomal membrane. TLR1–TLR2 and TLR2–TLR6 heterodimers recognize lipoteichoic acid (LTA) and lipoproteins from Gram-positive bacteria and TLR4 recognizes lipopolysaccharide (LPS) of Gram-negative bacteria. TLR2 gene deficient mice showed high susceptibility to *Staphylococcus aureus* or *Streptococcus pneumoniae* upon challenge.[13] However, it protects only partially against *Legionella pneumophila*.[14–16] The TLR4 ligand LPS binds to TLR4 in association with LPS binding protein (LBP), CD14 and MD2.[3,17] TLR4 plays an important role in the pulmonary immunity against *Klebsiella pneumoniae*,[18] *Haemophillus influenzae*,[19] and *Strep. pneumoniae*.[20] During *Acinetobacter baumanii*[21] and *Pseudomonas aeruginosa*[22] infections, both TLR2 and TLR4 are shown to play an important role in the host defence. TLR5 is important for the recognition of flagellin, a constituent of flagella in motile bacteria. Mutations of TLR5 are associated with susceptibility to *L. pneumophila*[23] infections. TLR3, TLR7 and TLR8 recognize viral RNAs and their synthetic analogues. TLR9 is stimulated by bacterial unmethylated CpG DNA and protects the host against Gram-negative as well as Gram-positive bacterial infections.[24–26] Furthermore, TLR11 is important for the recognition of profilin and uropathogenic bacteria.[3]

Engagement of microbial components to the TLRs triggers the downstream signalling pathways and induction of genes involved in the host defence. There are five distinct adaptor molecules (MyD88, TRIF, TIRAP, TRAM and SARM) that are associated with TLR signalling which are recruited to the cytoplasmic TIR domain of the TLRs. Among the adaptor molecules, MyD88 is essential for almost all TLRs and it is recruited to the TIR domain by another adaptor molecule, TIRAP, in the TLR2 and TLR4 signalling cascade. TLR3 and TLR4 signalling involves the MyD88-independent pathway where the other adaptor, TRIF plays an essential role. TRAM is a key player in TRIF-mediated, MyD88-independent signalling. Recruitment of MyD88 facilitates the association of IL-1R associated kinases (IRAKs), IRAK4 and IRAK1, to the receptor complex; during this process, IRAK4 and IRAK1 become activated and promote the interaction of TRAF6 with the complex. This complex interacts again with another preformed complex comprising of TAK1, TAB1 and TAB2.[3] This clustering activates IKK and, subsequently, activates NF-κB. Activation of TAK1 also activates mitogen-associated protein kinase (MAPK)

and Janus kinase (JNK). This activation results in the expression of growth factors, chemokines and cytokines, and cell adhesion molecules. There are four different IRAKs (IRAK-1, IRAK-2, IRAK-M, and IRAK-4) that have been identified in mice and humans. Interestingly, recent studies have shown that IRAK-M serves as a negative regulator of TLR signalling and IRAK-M gene deficient mice show increased inflammatory response.[27]

Several studies have elucidated the roles of adaptor molecules involved in the TLR pathways, such as MyD88-dependent cascade (MyD88 and TIRAP) and MyD88-independent cascade (TRIF and TRAM) in bacterial infections. For example, MyD88 is essential for the host defence against *Strep. pneumoniae*,[28] *S. aureus*,[29] *Escherichia coli*,[30] *K. pneumoniae*,[6] *H. influenzae*,[31] *P. aeruginosa*,[29,32] and *L. pneumophila*,[15,33] whereas TIRAP, an upstream molecule in the MyD88 cascade, is important for pulmonary host defence against *E. coli* and *K. pneumoniae*.[6,30] In general, MyD88 has been shown to be more critical for protection against bacterial infections than single or multiple TLRs (Table 1). Upon *E. coli*[30] and *P. aeruginosa*[34] challenge, TRIF plays an important role in the host defence. Regarding the relative importance of MyD88 and TRIF, MyD88 plays a much more important role than TRIF against *K. pneumoniae*.[35] These findings suggest that pathogens utilize both MyD88-dependent and MyD88-independent cascades in the host via different bacterial components.

Surfactant proteins (SPs) are a complex of lipoproteins that enhance pathogen clearance and primarily are expressed in the epithelial lining of the lung. The host-defence functions of surfactant are primarily mediated by SP-A and SP-D, which are members of the collectin family of proteins. These collectins opsonise bacterial pathogens in order to facilitate their phagocytic clearance. The surfactant proteins A and D potentially bind to several receptors and activate a number of signalling cascades, among which TLR2 and TLR4 are important. Activation of TLRs by surfactant proteins initiates a conserved series of responses that culminate in the production of inflammatory cytokines.[36]

NOD-like receptors

The NLRs comprise a large family of intracellular receptors that are characterized by the NOD family of proteins. They regulate both inflammation and apoptosis. There has been about 23 NLRs reported to date. The general structure of these proteins includes an amino-terminal caspase recruitment domain (CARD), pyrin, or baculovirus inhibitor repeat domains, a central NOD, and carboxyl-terminal leucine-rich repeats (LRRs) that detect specific PAMPs.[37,38] The family contains NALP (NACHT-, LRR-, and pyrin-domain containing proteins), NOD, CIITA (class II transactivator), IPAF (ICE-protease activating factor) and NAIP (neuronal apoptosis inhibitor protein). These receptors sense intracellular pathogens. The best characterized of the NODs are NOD1 and NOD2, where NOD1 has one CARD domain and NOD2 has two CARD domains in their amino terminus and they recognise different muropeptides (γ-D-glutamyl-mesodiaminopimelic acid and muramyl dipeptide) from the bacterial cell wall although how these peptides enter the cytosol is not clear.[37,38] The importance of NODs has been widely studied through their mutations associated with chronic inflammatory diseases, such as Crohn's disease. NOD1 is ubiquitously expressed whereas NOD2 is primarily found in antigen-presenting cells. Upon ligand binding to the NODs, the CARD-containing adaptor molecule serine/threonine kinase RIP2 (also known as RICK, CARDIAK, CCK, and Ripk2) transduces signals for antimicrobial inflammatory responses independent of TLR signalling.[39] RIP2 gene-deficient mice have shown increased susceptibility to intracellular *Listeria monocytogenes*.[40] Similar observations were reported in NOD1 and NOD2 gene-deficient mice against *List. monocytogenes* infection.[39] NOD1 is critical for the host immunity during *P. aeruginosa* infection[41] and NOD2 in *Mycobacterium tuberculosis*.[42] NOD1 and NOD2 activation results in nuclear factor (NF)-κB and MAPK activation and NOD-mediated NF-κB activation is essential for the protection of host during *Strep. pneumoniae* infection.[43]

Besides NODs, there are other NLRs that play an important role in the antimicrobial host defence. The NALP subfamily has PYRIN instead of CARD in their N-terminus and has 14 members. NALP1, NALP2 and NALP3 form a multiprotein complex called 'inflammasome' comprising ASC (apoptosis-associated speck-like protein containing a CARD) and caspase. This complex activates caspase1 and leads to the cleavage of pro-IL-1β, pro-IL-18 and pro-IL-33 to their biologically active forms. NALP3 inflammasome has been shown to be essential for host defence against *L. pneumophila*,[44] *Francisella tulerensis*,[45] and *K. pneumoniae*,[46] whereas NALP1 is important for the host defence against *Bacillus anthracis*.[47] IPAF is another NLR that has a CARD domain and forms an inflammasome that recognises the bacterial flagellin of motile bacteria such as *Salmonella*, *Shigella*, *Legionella*, *Francisella* and *Pseudomonas*.[45,48–53]

Transcription factors

Several transcription factors such as NF-κB, AP-1, STAT and IRFs are involved in the regulation of host

Table 1. Role of innate immune molecules in acute respiratory bacterial infection

		Phenotype[a]			
	Infection	Survival	Neutrophil influx[b]	Bacterial burden[c]	Bacterial dissemination[d]
TLR					
TLR2	*Acinetobacter baumannii*[21]	↓	↓	ND	ND
	Legionella pneumophila[14–16]	↓	↓	↑	↑
	Pseudomonas aeruginosa[22]	ND	NS	↑ early	ND
	Streptococcus pneumoniae[13]	↓	↓	↑	↑
TLR4	*Acinetobacter baumannii*[21]	↓	↓	↑	↑
	Haemophillus influenzae[19]	↓	↓	↑	ND
	Klebsiella pneumoniae[18]	↓	ND	↑	ND
	Pseudomonas aeruginosa[22]	N	↓ late	NS	ND
	Streptococcus pneumoniae[20]	↓	↓	↑	ND
TLR5	*Legionella pneumophila*[23]	ND	↓ early	NS	ND
TLR9	*Klebsiella pneumoniae*[25]	↓	NS	↑	↑
	Legionella pneumophila[24]	↓	NS	↑	ND
	Streptococcus pneumoniae[26]	↓	NS	↑	↑
TLR adaptor					
MyD88	*Escherichia coli*[30]	↓	↓	ND	ND
	Haemophilus influenzae[31]	ND	ND	↓	ND
	Klebsiella pneumoniae[35]	↓	↓	↓	↓
	Legionella pneumophila[15,33]	↓	↓	↑	↑
	Pseudomonas aeruginosa[6,29,32]	↓	↓	↑	↑
	Staphylococcus aureus[29]	ND	↓	ND	ND
	Streptococcus pneumoniae[28]	↓	↓	↑	↑
TIRAP	*Klebsiella pneumoniae*[6,30]	↓	↓	↑	↑
	Escherichia coli[6,30]	ND	↓	↑	ND
TRIF	*Escherichia coli*[30]	↓	↓	↑	↑
	Pseudomonas aeruginosa[34]	ND	↓	↑	ND
	Klebsiella pneumoniae[35]	↓	↓	↑	↑
NLR					
NALP3	*Klebsiella pneumoniae*[46]	↓	↓	ND	ND
IPAF	*Pseudomonas aeruginosa*[52]	NS	ND	↑	↑
Transcription factor					
NF-κB p50	*Escherichia coli*[58,59]	↓	↓	NS	ND
NF-κB ReLa	*Streptococcus pneumoniae*[60]	ND	↓	↑	ND
STAT3	*Escherichia coli*[63]	ND	↓	↑	ND
STAT4	*Klebsiella pneumoniae*[64]	↓	ND	↑	↑
	Pseudomonas aeruginosa[65]	ND	NS	↓	ND
Cytokine					
IL-23	*Pseudomonas aeruginosa*[74]	ND	↓	NS	NS
	Klebsiella pneumoniae[72]	↓	ND	ND	ND
IL-17	*Klebsiella pneumoniae*[73]	↓	↓	↑	ND
TNF-α	*Streptococcus pneumoniae*[61]	↓	↓	↑	ND
IL-1	*Streptococcus pneumoniae*[61]	↓	↓	↑	ND
CXC chemokine receptor					
CXCR2	*Pseudomonas aeruginosa*[79]	↓	↓	ND	ND
	Legionella pneumophila[81]	↓	↓	NS	ND
	Norcadia asteroides[80]	↓	↓	↑	ND
CXC chemokine					
KC[$]	*Klebsiella pneumoniae*[83]	↓	↓	ND	ND
MIP2[#]	*Klebsiella pneumoniae*[84]	↓	↓	↑	↑
	Norcadia asteroides[80]	NS	NS	NS	NS
Lungkine	*Klebsiella pneumoniae*[85]	↓	↓ (24 h)* ↑ (48 h)*	↑	ND

[a]Phenotype was determined mainly by using gene-deficient or mutant mice after infection.
[b]Neutrophil influx was determined in BALF and/or lung parenchyma.
[c]Bacterial burden was measured as CFUs in the lungs.
[d]Bacterial dissemination was measured as CFUs in blood or spleen.
*Significant in airspaces but not in lung parenchyma.
[$]Studied with transgenic mice.
[#]Abs only.
ND, not determined; NS, no significant difference.

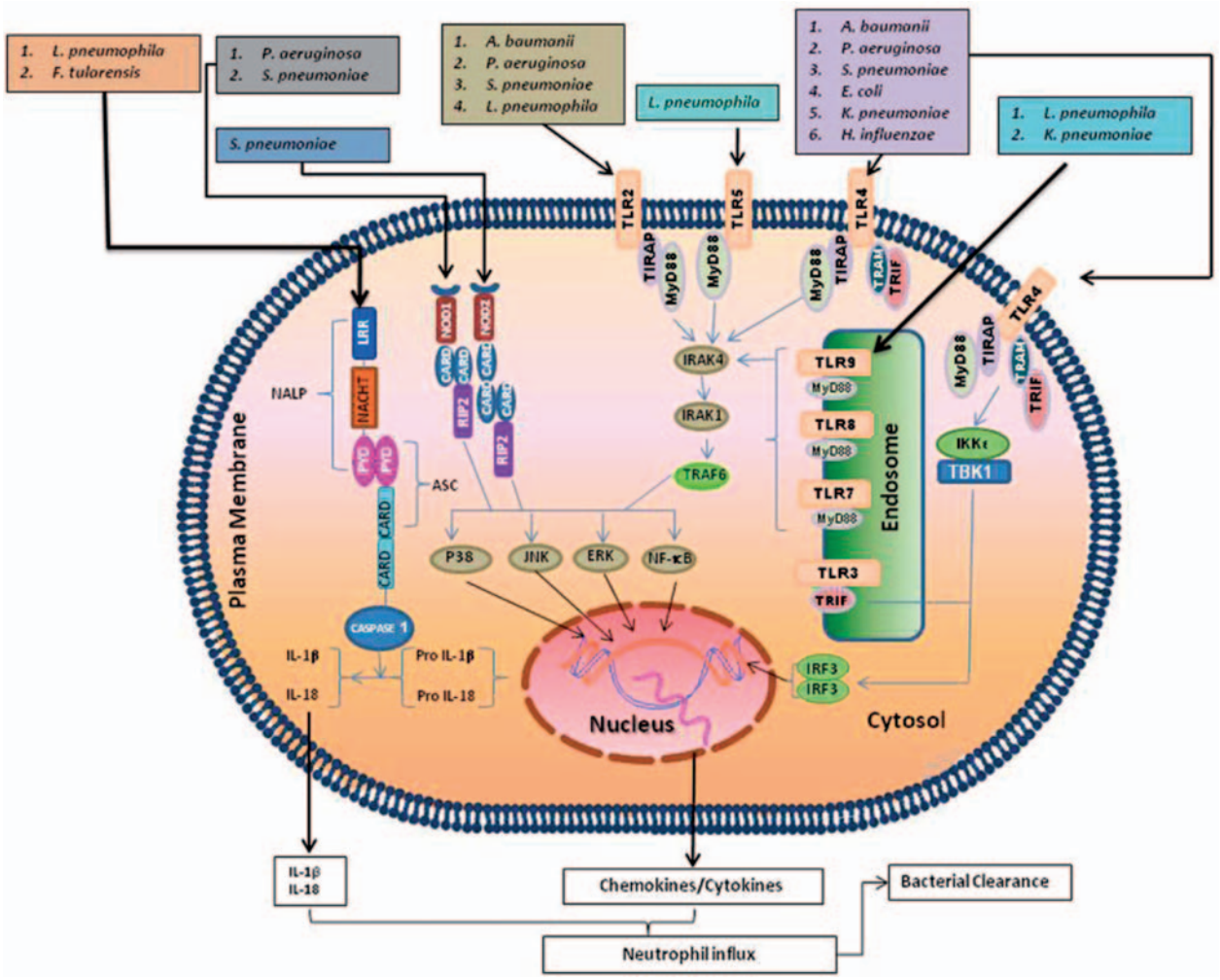

Fig. 1. Respiratory pathogens are recognised by membrane bound and cytoplasmic pattern recognition receptors. Plasma membrane-bound TLRs (TLR2, TLR4 and TLR5) and endosome membrane-bound TLRs (TLR3, TLR7, TLR8 and TLR9) recognise bacterial pathogens in the lungs. TLR2, TLR4, TLR5, TLR6, TLR7 and TLR9 recruit MyD88 whereas TLR2 and TLR4 recruit both TIRAP and MyD88. TLR3 and TLR4 recruit TRIF to induce downstream signalling cascades. Binding of pathogens and/or PAMPs to TLRs leads to complex signalling cascades, which result in transcription of pro-inflammatory mediators and activation of MAP kinases. Cytosolic NODs recognise bacterial pathogens in the lung and mediate signalling via RIP2, whereas NALPs use ASC to induce signalling cascades, which result in transcription of pro-inflammatory mediators and activation of MAP kinases. These pro-inflammatory mediators, including chemokines, recruit neutrophils to the lung in order to clear the causative organism during bacterial infection.

defence mechanism. NF-κB and STAT proteins have been studied in detail. However, NF-κB is considered as the central mediator of immune mechanisms. The TLR and NOD pathways converge to activate NF-κB-dependent gene transcription to mediate immune responses. TLR2 and TLR4 were both found to activate NF-κB, TLR2 in response to lipopeptides, and TLR4 in response to LPS stimulation, a response that is enhanced by the presence of CD14 and LBP.[54,55] Furthermore, TLR5 regulates NF-κB activation during flagellin-induced sepsis.[56] Similarly, TLR3 activated by poly I:C mediates NF-κB activation.[57] The NF-κB family of structurally related transcription factors occur as homodimers or heterodimers in the cytoplasm of almost all mammalian cells.[58] The NF-κB commonly refers to p50–RelA heterodimer, which is one of the most avidly forming dimers present in cells. They control a large number of normal cellular processes, such as immune and inflammatory responses, developmental processes, cellular growth, and apoptosis. These NF-κB proteins are related through a highly conserved DNA-binding/dimerization domain called the Rel homology (RH) domain. The NF-κB protein activity is primarily regulated by interaction with inhibitory IκB proteins. In cells, NF-κB is present in a latent, inactive, IκB-bound complex form in the cytoplasm. The NF-κB–IκB interaction prevents NF-κB nuclear localisation as well as DNA binding ability. When a cell receives a signal, it can trigger

multiple signalling cascades that can activate the serine-specific IκB kinase (IKK) and eventually ubiquitinate the IκB and promote its degradation. This facilitates activation of NF-κB, nuclear localisation and DNA binding. NF-κB activation turns on several pro-inflammatory genes, more specifically the neutrophil chemo-attractants and result in neutrophil accumulation at the site of infection.[58] Studies of NF-κB have shown that endogenous NF-κB is essential for the host defence against *E. coli* and *Strep. pneumoniae* pneumonia.[59,60] It has been shown that NF-κB activity is affected by cytokine receptor signalling such as TNF-α and IL-1 receptors during pneumococcal infection.[61] During *P. aeruginosa*[34] and *K. pneumoniae*[35] infection TRIF, an adaptor molecule for TLR3 and TLR4, has been shown to reduce NF-κB activation and chemokine expression.

The JAK/STAT pathway is an important mechanism for a wide array of cytokines and growth factors. Intracellular activation of the JAK/STAT pathway occurs when ligand binding induces the multimerization of receptor subunits. Activated JAKs subsequently phosphorylate and activate STATs. Similar to NF-κB, STATs are also latent transcription factors that reside in the cytoplasm until activated. Phosphorylated and dimerised STATs enter the nucleus and bind specific regulatory sequences to activate or repress transcription of target genes.[62] During *E. coli* pneumonia, IL-6 has been shown to activate STAT3 to promote neutrophil recruitment into the lungs.[63] STAT4 is an essential component of the innate immune defence against *K. pneumoniae*[64] and *P. aeruginosa*[65] infections and bacterial clearance of *K. pneumoniae*.[64]

Cytokines

Cytokines are a diverse group of soluble proteins or peptides that bring biological responses at very low concentrations. These cytokines have autocrine, paracrine or endocrine activities to bring about immune functions. Specific binding of these cytokines to their cognate receptors results in signal transduction via secondary messengers, up-regulation or down-regulation of their receptors, cell proliferation and secretion. The major pro-inflammatory cytokines or the acute phase cytokines that are studied extensively are tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL-8 and IFN-γ. TNF-α, IL-1β and IL-6 play an important role in vasodilatation, increasing the vascular permeability and up-regulation of cellular adhesion molecules. IL-8 and IL-1β act as a potent chemokines and IFN-γ promotes the intracellular phagocytic killing of microbes. During Gram-negative bacterial infection, TLR4 has been shown to regulate the expression of TNF-α and IL-1β.[66,67] whereas, during *L. pneumophila* infection, TLR5 and TLR9 mediate the expression of TNF-α.[23,24] On the other hand, anti-inflammatory cytokines IL-10, TGF-β and IL-1Ra play a critical role to dampen the inflammatory response and resolve inflammation and aid in healing.[12] IL-12 is a pro-inflammatory cytokine that has been shown to have beneficial effects in host-acquired immunity. Effective delivery of IL-12 to the murine lung during *K. pneumoniae* infection protects the mice.[68] During *L. pneumophila* infection, TLR9 seems to regulate IL-12 production in lungs.[24] The IL-12/IFN-γ axis is important for intracellular bacterial host defence and phagocytic destruction of bacteria.

Recent studies have shown an emerging role of IL-23 in innate host defence during bacterial pneumonia. IL-23 is a heterodimer that has a p40 subunit identical to the IL-12 and a unique p19 subunit.[69] IL-23 is predominantly a cytokine that is produced by antigen-presenting cells and has been shown to stimulate the production IL-17 (IL-17A and IL-17F) by Th17 and γδ T-cells in a TLR-dependent manner. These cytokines promote the production of many other cytokines and chemokines.[70,71] IL-17 signalling is critical for the pulmonary host defence against, and survival during, *K. pneumoniae* infection.[72,73] Studies have also shown that IL-23 plays a critical role in the pulmonary immunity against *P. aeruginosa* infection.[74] The IL-23/IL-17 axis has been shown to regulate the expression of various other pro-inflammatory cytokines. In addition to IL-17A there are IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. IL-17 cytokine binds to type I cell surface receptor called IL-17R which has three different variants (IL-17RA, IL-17B and IL-17C). IL-17 signalling is critical for host defence against extracellular bacteria by regulating chemokine gradients for neutrophil emigration into infected tissue.[75] Recent reports demonstrate that the IL-17 family of cytokines can be regulated by IL-23, IL-15 and IL-12.[71]

Chemokines

Chemokines are cytokines that induce leukocyte infiltration into the site of infection/inflammation. Chemokines promote the expression of cellular adhesion molecules and extravasation of leukocytes. There are four types of chemokines depending on the presence of cysteine towards the N-terminus (C, CC, CXC and CX3C chemokines).[76] The members of ELR+CXC chemokines are neutrophil chemo-attractants. In humans there are seven chemokines (IL-8; NAP-2; GRO α, β and γ; ENA-78 and GCP-2) reported.[77] Among these, IL-8 is considered the most potent neutrophil chemo-attractant. In mice, keratinocyte-derived chemokine (KC), macrophage inflammatory protein (MIP)-2, LPS-induced CXC chemokine

(LIX/CXCL5), and lungkine are the four CXC chemokines associated with neutrophil recruitment to the lungs.[78] However, no rodent homologue of human IL-8 has been identified yet. KC and MIP-2 are myeloid cell derived chemokines, whereas lungkine and LIX are secreted by bronchial epithelial cells and type II alveolar epithelial cells, respectively. There are two receptors CXCR1 and CXCR2 which bind to CXC chemokines and present in both humans and mice. Between these receptors, CXCR2 binds to all ELR+CXC chemokines and is essential for neutrophil infiltration during bacterial infection, it is involved in the host defence and neutrophil recruitment upon challenge of *P. aeruginosa*,[79] *Nocardia asteroides*,[80] and *L. pneumophila*.[81] It has been shown that TLR2 down-regulates CXCR2 and impairs neutrophil migration during polymicrobial sepsis.[82] Studies have also shown that KC and MIP-2 play an important role in neutrophil accumulation during bacterial infection by using blocking peptides.[83,84] However, lungkine is not important for neutrophil recruitment into the lung parenchyma during pneumonia.[85] Unlike KC and MIP-2, CXCL5, also known as LIX, is a relatively newly reported CXC chemokine/neutrophil chemo-attractant. Up-regulation of CXCL5 increases neutrophil recruitment during infections with *P. aeruginosa*, *K. pneumoniae*, *L. pneumophila* and *Bordetella bronchiseptica*. Using TLR2 gene-deficient mice, it has been shown that TLR2 regulates the expression of KC and MIP-2 during LTA induced lung inflammation.[86] However, TLR4 is important for the regulation of KC and MIP-2 production during LPS-induced inflammation.[67,87] Furthermore, in flagellin challenged mice, TLR5 is critical for the production of KC and MIP-2.[88] Although LPS-induced inflammation has also shown that LIX is an important molecule for neutrophil influx in the lungs,[89] the role of LIX in neutrophil infiltration in the lungs during bacterial infections is unclear. Recently, a CC chemokine, monocyte chemo-attractant protein 1 (MCP1) which is primarily a monocyte chemokine, has been shown to play a role in neutrophil chemotaxis.[90]

Conclusions

Bacterial lung diseases are an essential public health concern. Neutrophils are amongst the first cells to reach the site of bacterial infection in order to clear bacteria. Our understanding of the molecular mechanisms that regulate neutrophil recruitment during infection/inflammation has improved substantially over recent years. Emerging studies indicate complex roles for TLRs and NLRs in neutrophil accumulation during pneumonia (Fig. 1). Although antibiotics are the rational treatment for pneumonias, antibiotic-resistant *Strep. pneumoniae*, *H. influenzae*, and *S. aureus* have been isolated from patients suffering from lower respiratory tract infections. The emergence of antibiotic-resistant pulmonary bacteria and the growing number of immunocompromised individuals have made the treatment of these infections difficult. As most of the TLR studies have been performed in murine models, the efficacy and safety of TLR therapies may not extrapolate to human responses. This is because: (i) of differences between the human and murine immune system; (ii) of differences in the activation profile of human and mouse, such as TLR8; and (iii) murine investigations are performed on in-bred strains that have minimal genetic variation. Though TLR9 agonists, such as CpG oligodeoxynucleotides have been shown to protect against numerous infectious agents in murine models, no human clinical studies have been reported, to our knowledge, using TLR9 agonists in bacterial infections. In addition, TLR3, TLR7, TLR8 and TLR9 can be activated upon intracellular bacterial infection, resulting in the production of IFN-α and, therefore, these receptors can be targeted to control bacterial infections. The studies using TLR adaptor-deficient mice in response to bacterial infection reveal the potential for using cell-permeable compounds to attenuate cytokine/chemokine production in order to reduce excessive neutrophil-mediated parenchymal damage. Unlike TLRs, NLRs have recently been identified and, therefore, their therapeutic potential to reduce bacteria-mediated neutrophil influx in the lungs remains to be evaluated. The future challenge will be to apply our current understanding of TLRs and NLRs to design therapeutic methods to attenuate uncontrolled neutrophil migration to the lungs in ALI/ARDS patients.

Acknowledgements

This work was supported by a research grant from the American Lung Association (RG-22442-N), a scientist award from the Flight Attendant Medical Research Institute (YCSA-062466), and grants from the NIH (R01 HL-091958 and R01 HL-091958S1 via ARRA) to SJ.

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