Investigating the Role of Nucleotide-Binding Oligomerization Domain–Like Receptors in Bacterial Lung Infection

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Abstract

Lower respiratory tract infections (LRTIs) are a persistent and pervasive public health problem worldwide. Pneumonia and other LRTIs will be among the leading causes of death in adults, and pneumonia is the single largest cause of death in children. LRTIs are also an important cause of acute lung injury and acute exacerbations of chronic obstructive pulmonary disease. Because innate immunity is the first line of defense against pathogens, understanding the role of innate immunity in the pulmonary system is of paramount importance. Pattern recognition molecules (PRMs) that recognize microbial-associated molecular patterns are an integral component of the innate immune system and are located in both cell membranes and cytosol. Toll-like receptors and nucleotide-binding oligomerization domain–like receptors (NLRs) are the major sensors at the forefront of pathogen recognition. Although Toll-like

receptors have been extensively studied in host immunity, NLRs have diverse and important roles in immune and inflammatory responses, ranging from antimicrobial properties to adaptive immune responses. The lung contains NLR-expressing immune cells such as leukocytes and nonimmune cells such as epithelial cells that are in constant and close contact with invading microbes. This pulmonary perspective addresses our current understanding of the structure and function of NLR family members, highlighting advances and gaps in knowledge, with a specific focus on immune responses in the respiratory tract during bacterial infection. Further advances in exploring cellular and molecular responses to bacterial pathogens are critical to develop improved strategies to treat and prevent devastating infectious diseases of the lung.

Keywords: lung; bacterial infection; nucleotide-binding oligomerization domain–like receptors; inflammasome

Bacterial Lung Infections

Despite sophisticated advances in pulmonary medicine, lower respiratory tract infections remain a significant cause of morbidity, mortality, and health care costs in many countries regardless of socioeconomic status. The main bacterial pathogens causing bacterial lung infection in humans are *Streptococcus pneumoniae*, *Haemophilus influenzae*, group A *Streptococcus*, *Staphylococcus aureus*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas*

aeruginosa, and *Mycobacterium tuberculosis* (1). In the United States, bacterial pneumonia is common, with an incidence of 4 million adult cases per year, resulting in 1.1 million hospitalizations and 50,000 deaths annually despite the use of antibiotics and supportive care measures (1). Although antibiotics are the rational treatment for pneumonias, antibiotic-resistant *S. pneumoniae*, *H. influenzae*, *S. aureus*, and *M. tuberculosis* have been isolated from patients suffering from lower respiratory tract infections. Because of the emergence of multidrug-resistant bacteria, understanding innate immune response to

bacterial infection is a priority to ultimately formulate therapeutic strategies to augment host immune mechanisms to combat microorganisms.

The initial phase of a bacterial lower respiratory tract infection is characterized by neutrophil-mediated inflammation. Although neutrophil recruitment aids in the removal of bacteria, it also induces bystander injury to the lung parenchyma. When severe, this injury may lead to a clinical condition termed acute lung injury/acute respiratory distress syndrome. The relative contribution of hematopoietic cells, such as neutrophils and macrophages, versus nonhematopoietic

(Received in original form November 28, 2013; accepted in final form April 6, 2014)

Supported by a Flight Attendant Medical Research Institute (FAMRI) Young Clinical Scientist Award (FAMRI YCSA_092417) to R.K., and by Clinical Innovator Award CIA_113043 and National Institutes of Health Grant R01-HL 091958 to S.J.

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Am J Respir Crit Care Med Vol 189, Iss 12, pp 1461–1468, Jun 15, 2014

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Originally Published in Press as DOI: 10.1164/rccm.201311-2103PP on April 7, 2014

Internet address: www.atsjournals.org

cells (epithelial cells and endothelial cells) to neutrophil accumulation into the lung is dependent on the stimulus, because both are exposed to inflammatory components. In prior studies, it has been proposed that myeloid cells in the lung produce multiple neutrophil chemoattractants, such as keratinocyte-derived chemokine and macrophage inflammatory protein-2, to target lung resident cells, including epithelial cells and fibroblasts, to cause cytokine and chemokine expression. Neutrophils clear bacteria by phagocytosis followed by killing via proteases and reactive oxygen species. Both the activation of sentinel cells and the phagocytosis and killing by neutrophils are critically dependent on the recognition of pathogens by the innate immune system (2). As an additional mechanism, during inflammation, polymorphonuclear cells release neutrophil extracellular traps (NETs), which are composed of DNA and cytosolic antimicrobial agents. NETs have been shown to confine pathogens, including bacteria (3). However, the importance of NETs in host defense against respiratory bacterial pathogens has been largely unexplored.

The large respiratory epithelial surface encounters a multitude of inhaled pathogens with every breath, and the epithelium has evolved multiple mechanisms to prevent infection (4). First, the epithelium constitutes an impermeable barrier made of intercellular tight junctions (5). Second, mucociliary clearance achieves physical removal of pathogens (6). Third, epithelial cells secrete diverse antimicrobial peptides, including lactoferrin, defensins, and cathelicidins, as well as collectins, which exert direct antimicrobial activity and function as regulators of the innate and adaptive immune systems (7). Epithelial-derived oxidants also possess antimicrobial and proinflammatory effects (8). Fourth, epithelial cells (as well as other sentinel cells) express pattern recognition molecules (PRMs) that recognize microbial-associated molecular patterns (MAMPs) in their vicinity. Finally, epithelial-derived neutrophil chemoattractants, such as CXCL5 and lungkine, contribute to the immune response to bacterial pathogens (9, 10).

PRM signaling can result in the production of antibacterial molecules, stimulate autophagy, and/or regulate programmed cell death (11). The MAMP ligands for specific PRMs are highly conserved “non-self” molecular motifs of microbial origin; examples include LPS, peptidoglycan, flagellin, and CpG nucleotides

(11). PRMs can also interact with another set of molecular motifs known as damage (or danger)-associated molecular patterns (DAMPs), which are endogenous (“self”) molecules emanating from stressed (dying/infected/cancerous) cells. This pulmonary perspective focuses on the structure and function of nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and their importance in orchestrating the innate immune response to bacterial lung infections.

The NLR Family

After the discovery of membrane-bound Toll-like receptors (TLRs), it became clear that additional sensors were crucial for microbial surveillance in the cytoplasm. NLRs are cytosolic proteins that respond to diverse ligands ranging from bacterial and viral components to particulate matter and crystals. Intracellular pathogens or bacteria equipped with transmembrane secretion systems provide cytosolic MAMPs that may interact with NLRs (12, 13). In addition, extracellular gram-negative bacteria may shed membrane “blebs” that can be transported to the cytosol via lipid rafts to initiate NLR-dependent responses (14). Although it is well established that NLRs can sense

a wide array of ligands, the exact mechanisms for the transmembrane delivery of these ligands to the cytosol, and whether they directly bind NLRs, are in many instances not well understood.

All NLR family members are characterized by a tripartite domain structure with a C-terminal leucine-rich repeat (LRR) domain, a central NACHT (NAIP, CIIA, HET-E, TP1) NOD, and a variable N-terminal effector domain (15). NLRs are classified into four subfamilies based on the N-terminal effector domain they contain: NLRA members have transactivator activation domains (ADs); NLRBs have BIR (baculoviral inhibitor of apoptosis repeat) domains; NLRcs have CARD (caspase activation/recruitment domains), and NLRps have PYD (pyrin domains) (16). Each domain of the NLR molecule has a unique function. The C-terminal LRR sensing domain recognizes a variety of cytosolic ligands. This is followed by oligomerization of NACHT domains leading to the formation of an N-terminal platform where diverse adaptor molecules and downstream effectors may bind (16). The variable molecular makeup at the N terminus ascribes a degree of structural heterogeneity that in part dictates the recruitment of adaptor

Figure 1. A schematic comparing molecular structures of various NOD (nucleotide-associated oligomerization domain)-like receptor (NLR) family members relevant to bacterial lung infection. All NLRs have a tripartite domain organization comprising a C-terminal LRR, middle NACHT, and a variable N-terminal domain. The variability of the N-terminal domains is the basis for the division of NLRs into distinct subgroups. AD = activation domain; BIR = baculovirus inhibitor of apoptosis protein repeat; CARD = caspase recruitment domain; LRR = leucine-rich repeat; NACHT = NAIP, CIIA, HET-E, TP1; NAD = NACHT-associated domain; NAIP = NLR family apoptosis-inducing protein; PYD = pyrin domain; SH = superhelical domain; WH = winged helix domain.

molecules and the activation of downstream signaling pathways depending on the specificity of NLR and/or their ligands (Figure 1). The following sections highlight key structural differences among NLRs important in pulmonary inflammation and immunity.

NOD1 and NOD2

The cytosolic proteins NOD1 and NOD2 were the first NLRs discovered as pathogen sensors. Both NOD1 and NOD2 contain CARD domains at their N termini. Whereas NOD1 is expressed in a wide variety of cells and tissues, the expression of NOD2 is restricted to relatively few cell types including macrophages, dendritic cells, and lung epithelium (17). Principally described, bacterial ligands for NOD receptors are components of bacterial peptidoglycan. Specifically, *m*-DAP (L-Ala- γ -D-Glu-*m*-diaminopimelic acid) found in most gram-negative and some gram-positive bacteria binds NOD1 while the MDP (muramyl dipeptide) motif present in the peptidoglycans of both gram-positive and gram-negative bacteria binds NOD2 LRR (17). Peptidoglycan binding is followed by oligomerization of the central NACHT domains and recruitment of the cytosolic adaptor molecule receptor-interacting protein-2 (RIP2) at the N terminus by CARD–CARD interaction. RIP2 is then ubiquitinated, leading to the activation of downstream NF- κ B signaling and up-regulation of genes involved in host defense and apoptosis (17).

Inflammasomes

On ligand recognition, some NLR proteins form distinct hetero-oligomeric structures known as inflammasomes. Inflammasomes are platforms for the recruitment of pro-caspase-1 zymogen by CARD–CARD interaction followed by its activation to caspase-1 by proteolytic cleavage. Caspase-1 protease in turn activates pro-IL-1 β and pro-IL-18 to IL-1 β and IL-18, respectively, inducing inflammation. Under certain conditions, caspase-1 can also mediate pyroptosis, which is a caspase-1-dependent form of inflammatory programmed cell death. The CARD in an inflammasome

may belong to either a constituent NLR or to a CARD-containing adaptor protein, ASC (apoptosis-associated speck-like protein containing a C-terminal CARD) (16). NLRC4 and NLRP3 are the two most extensively studied inflammasome-forming NLRs that orchestrate immune responses to an array of important human pulmonary pathogens.

NLRC4 and NAIPs

NLRC4 and NAIP (NLR family apoptosis-inducing protein) are two structurally dissimilar NLR proteins that together form the NLRC4 inflammasome. NAIPs exhibit tripartite protein structure with a C-terminal LRR, a central NACHT, and N-terminal BIR domains akin to other NLR family members (Figure 1). After activation, the central NACHT of NLRC4 proteins oligomerizes with NAIP, resulting in the formation of an inflammasome (18). NAIPs and NLRC4 functionally complement one another as inflammasome constituents, with NAIP-LRR acting as an MAMP sensor while NLRC4-CARD recruits and activates pro-caspase-1. The role of the BIR domains in the organization of inflammasomes, their downstream signaling, and their relevance to immune defense against bacterial pathogens remains to be elucidated, although it is proposed that all three BIR domains are necessary for MAMP-induced oligomerization of NLRC4 (19). Because NLRC4 is equipped with its own CARD domain, whether the ASC adaptor is necessary for NLRC4 inflammasome function is not fully established (18). The majority of *in vitro* studies assessing cytokine production via NLRC4 have been performed primarily in macrophages, and although NLRC4 expression has been documented in epithelial cells in other anatomic locations, its role in pulmonary epithelium is undefined (20).

When comparing experiments using murine models and human cell lines it is important to note that there are four NAIP paralogs in mice, namely, NAIP1, NAIP2, NAIP5, and NAIP6, whereas only one functional protein, hNAIP, has been detected in humans (21). The murine NAIP paralogs are proposed to be involved in differential ligand recognition. For example, NAIP5 LRR and NAIP6 LRR selectively recognize flagellin, NAIP2 LRR recognizes

PrgJ (type III secretion system [T3SS] needle protein), and NAIP1 also recognizes T3SS needle protein (19, 22, 23). hNAIP and NAIP5 are both responsible for recognition of *L. pneumophila* flagellin and limiting growth of this bacterium within macrophages via assembly of the NLRC4 inflammasome (24). Interestingly, in human epithelial cells, hNAIP has been shown to inhibit *L. pneumophila* replication, despite the lack of NLRC4 expression in these cells, indicating the immune functions of NAIPs may extend beyond the NAIP/NLRC4 paradigm (24).

NLRP3

NLRP3 has been principally studied in human and murine macrophages; however, NLRP3 inflammasome constituents are also expressed in human and murine airway epithelial cells on bacterial challenge (25). The defining feature of NLRP3 is the N-terminal PYD that homotypically binds PYD of ASC. The NLRP3 inflammasome is also prototypical in its requirement for two distinct signals for activation. The preassembly “priming” signal comes from TLR activation that induces NLRP3 expression via NF- κ B activation. Once the cytosolic amount of NLRP3 reaches a threshold, inflammasome assembly is initiated in response to a second signal originating from one or more NLRP3 ligands (26). The two-signal process may act as a cellular safeguard against hyperactivation of the NLRP3 inflammasome. Although other inflammasomes may not depend on TLR signaling for synthesis of their constituent molecules, it should be noted that TLR signaling does contribute to increased cytosolic expression of pro-IL-1 β , and production of mature IL-1 β by other inflammasomes may be impacted by TLR activation (25).

NLRP3 ligands are a curiously heterogeneous group of compounds ranging from exogenous materials including bacterial MAMPs, ozone, asbestos, silicon, and particulate matter to endogenous alarmins such as uric acid from DNA damage, ATP, and mitochondrial contents (27–35). The ability of the NLRP3 inflammasome to respond to an array of structurally and chemically diverse signals points to convergence on a

common subcellular event upstream of inflammasome assembly. The drop in the intracellular concentration of K^+ (K^+ efflux) has been shown to be a necessary and sufficient event that acts upstream of the ASC adaptor, resulting in assembly of the NLRP3 inflammasome and activation of caspase-1 (36). K^+ efflux is a common feature to the many cellular events such as membrane permeability or pore formation, lysosomal and mitochondrial damage, and reactive oxygen species production that are proposed to activate NLRP3 (36).

NLRs in Inflammation

Most studies support a pivotal role for NLRs as PRMs that recognize bacterial pathogens and induce downstream molecular pathways to generate cytokines and chemokines that play important roles in the pathophysiology of bacterial infections (Figure 2). The NLRs and bacterial components or PRMs that are

recognized by NLRs are listed in Table 1 regarding respiratory bacterial infection. Notably, *in vivo* correlates to assess leukocyte recruitment, bacterial clearance, or survival are either lacking or provide apparently discrepant results. For example, NOD1, NOD2, and/or RIP2 gene-deficient mice infected with *C. pneumoniae*, *S. aureus*, and *L. pneumophila* demonstrated reduced levels of pulmonary cytokines and chemokines and impaired neutrophil recruitment to the lungs (12, 37–39). Interestingly, *C. pneumoniae*-infected NOD1/2 and RIP2 gene-deficient mice had decreased bacterial clearance (38), *S. aureus*-infected wild-type and NOD2 gene-deficient mice showed similar pulmonary CFUs (37), while the pulmonary bacterial burden in NOD1/2 and RIP2 gene-deficient mice infected with *L. pneumophila* were enhanced (12, 39). In the human embryonic kidney cell line HEK293, activation of NF- κ B after invasion by *S. pneumoniae* was dependent on NOD2

(40). Experiments using HEK293 and the A549 human respiratory epithelial cell line showed that peptidoglycan from the gram-negative pathogen *H. influenzae* stimulated NF- κ B and production of IL-8 in a NOD1-dependent manner when cells were coincubated with an *S. pneumoniae* strain expressing the pore-forming toxin pneumolysin (41). The synergy between *H. influenzae* and pneumolysin of *S. pneumoniae* is an interesting model of NOD1 activation that may be relevant in cases of polymicrobial colonization of the respiratory tract (41). In response to *Mycobacterium* species, macrophages from NOD2 gene-deficient mice produced significantly less tumor necrosis factor- α as compared with wild-type control macrophages. In addition, human mononuclear cells harvested from patients homozygous for a frameshift loss of function mutation in NOD2 (3020insC) synthesized 65–80% less tumor necrosis factor- α than did

Figure 2. A schematic representation of NOD (nucleotide-associated oligomerization domain)-like receptor (NLR) signaling pathways. Cytosolic NLRs recognize bacterial components (microbial-associated molecular patterns) and activate downstream proinflammatory signaling cascades in the respiratory tract, resulting in host defense and/or excessive inflammation. ASC = apoptosis-associated speck-like protein containing a C-terminal CARD; BIR = baculovirus inhibitor of apoptosis protein repeat; CARD = caspase recruitment domain; IKK = I- κ B kinase; LRR = leucine-rich repeat; MDP = muramyl dipeptide; NACHT = NAIP, CIIA, HET-E, TP1; NAIP = NLR family apoptosis-inducing protein; PFT = pore-forming toxin; PGN = peptidoglycan; PYD = pyrin domain; RIP2 = receptor-interacting protein-2; T3SS = type III secretion system.

Table 1. Role of Nucleotide-Binding Oligomerization Domain-like Receptors in Respiratory Bacterial Infection

Bacteria	MAMP	NLR	Phenotype*	Reference
<i>Bordetella pertussis</i>	CyaA	Unknown	<i>Snd Nnd</i> BB↑ BDnd (IL-1R ^{-/-} mice)	61
<i>Chlamydomphila (Chlamydia) pneumoniae</i>	Unknown	Unknown	S↓ <i>Nnd</i> BB↑ BDnd (caspase-1 ^{-/-} mice)	62
	PGN	NOD1/NOD2	S↓ N↑ BB↑ BDnd	38
<i>Klebsiella pneumoniae</i>	Unknown	NLRC4	S↓ N↓ BB↑ BD↑	49
	Unknown	NLRP3	S↓ N↓ BBnd BDnd	45
<i>Legionella pneumophila</i>	Flagellin	NLRC4	<i>Snd Nns</i> BB↑ BDnd	63
	PGN	NOD1/NOD2	S↓ N↓ BB↑ BDnd	12, 39
<i>Mycobacterium tuberculosis</i>	mAGP	NOD2	<i>Sns Nns</i> BBns BDnd	64
<i>Pseudomonas aeruginosa</i>	Flagellin/ExoUT3SS	NLRC4	<i>Sns Nnd</i> BB↑ BD↑	46, 65, 66
<i>Staphylococcus aureus</i>	MDP	NOD2	<i>Sns</i> N↓ BBns BDnd	37
<i>Streptococcus pneumoniae</i>	Pneumolysin	NLRP3	S↓ <i>Nns</i> BBns BDns	28

Definition of abbreviations: CyaA = adenylate cyclase toxin; ExoUT3SS = exoenzyme U type III secretion system; IL-1R = IL-1 receptor; mAGP = mycoarabinogalactan; MAMP = microbial-associated molecular pattern; MDP = muramyl dipeptide; NLR = NOD-like receptor; NOD = nucleotide-binding oligomerization domain; PGN = peptidoglycan.

*Phenotype represents outcomes in gene-deficient mice after infection. ↓ = decreased; ↑ = increased; BB = bacterial burden in the lungs; BD = bacterial dissemination; N = neutrophil influx; nd = not determined; ns = no significant difference in gene-deficient mice compared with wild-type mice; S = survival.

homozygous wild-type mononuclear cells when challenged with *M. tuberculosis* (42).

Pneumolysin and other bacterial pore-forming toxins, such as streptolysin O (*Streptococcus pyogenes*) and α-hemolysin (*S. aureus*), also induce NLRP3 inflammasomes (27–29, 43, 44). Pneumolysin-expressing strains of *S. pneumoniae* stimulate IL-1β production via NLRP3 in both human and murine macrophages. In addition, NLRP3 activation is protective for mice infected with pneumolysin-expressing *S. pneumoniae* (28). For *S. pneumoniae*, virulence factor polymorphism may be the means by which certain clinically relevant strains lacking pneumolysin expression evade this important detection system, and are able to establish invasive infections (28).

P. aeruginosa stimulates NLRC4 (also known as IPAF) activation via a T3SS used to inject various virulence factors into the host cytosol. The *P. aeruginosa* strain expressing the exoenzyme U (ExoU) phospholipase was able to suppress caspase-1-mediated cytokine production via NLRC4 (46). Although less than one-third of *P. aeruginosa* clinical isolates express ExoU, these strains are associated with more severe disease; however, this may relate to functions of the ExoU virulence factor that are independent of NLRC4 (46). In addition to its T3SS, *P. aeruginosa* flagellin provides another means of detection via NLRC4. Interestingly, it

appears that recognition of *P. aeruginosa* via NLRC4 may depend not only on expression of a T3SS or flagellin, but also on motility, because caspase-1 activation and IL-1β production were reduced in peritoneal macrophages and bone marrow-derived dendritic cells exposed to nonmotile *P. aeruginosa* (47). This finding may have broad implications because loss of bacterial motility is described in a number of diseases and is thought to favor persistence in the host. As an example, the temporal loss of *P. aeruginosa* motility has been described during chronic infections in patients with cystic fibrosis (48). Interestingly, *K. pneumoniae*, which expresses neither flagellin nor T3SS, also activates NLRC4, indicating that NLRC4 must recognize other ligands that remain to be characterized. Furthermore, the exact molecular pathway leading to the activation of NLRC4 by extracellular pathogens such as *K. pneumoniae* has not been delineated (49). In addition, a slow-growing mycobacterial agent, *Mycobacterium kansasii*, activates NLRP3 within human macrophages, and generation of IL-1β by NLRP3 was shown to restrict intracellular growth of *M. kansasii* (50).

NLRs in Pyroptosis

Pyroptosis is a caspase-1-dependent programmed cell death pathway that is

downstream of inflammasome activation. Activated caspase-1 forms small cation-permeable pores in the cell membrane, allowing influx of calcium as well as cellular swelling and fluid imbalance, all of which ultimately contribute to cell death (51). However, inflammasome activation may not always result in pyroptosis. For example, human and murine macrophages incubated with *K. pneumoniae* generate IL-1β via NLRC4, although pyroptosis has not been observed in murine macrophages and neutrophils isolated from the lungs of *K. pneumoniae*-infected mice (49). In contrast, NLRC4-mediated pyroptosis occurs during infections with *L. pneumophila* and *P. aeruginosa* (46, 52). In experimental *L. pneumophila* infection pyroptosis appears protective, as caspase-1-mediated clearance of *L. pneumophila* *in vivo* was independent of IL-1β and IL-18 (52). In this model, cell lysis presumably liberates infectious agents into the extracellular space, where they may be more readily killed. However, as pyroptosis is inherently inflammatory, it may also contribute to morbidity in other models, as strong evidence for a protective role does not currently exist for all infectious inducers of pyroptosis.

How exactly cells modulate pyroptosis has largely remained elusive. In the case of NLRC4 differential expression of the adaptor protein ASC has some effect on pyroptosis, but plays a more pivotal role in cytokine production. NLRC4 inflammasomes can be formed with or

without ASC, and activation of both structures is required for maximal production of IL-1 β and IL-18 in macrophages infected with *L. pneumophila* (53). In contrast, NLRC4-NAIP-mediated pyroptosis is independent of ASC activity in this *L. pneumophila* model, and in ASC-deficient cells, pyroptosis is slightly enhanced (53). Similarly, ASC is required for maximal production of IL-1 β via NLRC4 but is dispensable for pyroptosis in a *P. aeruginosa* infection model (46).

Evidence suggests pyroptosis may be controlled in part by autophagy. Autophagy is a programmed cellular pathway by which cells engulf portions of their own cytoplasm, membrane, and organelles. This process may function in nutrient recycling, as well as degrading toxic metabolites such as reactive oxygen species during times of cellular stress (51). The various cascades that connect NLRs to autophagy during bacterial infection give rise to a complex network. In murine macrophages infected with *L. pneumophila* NAIP5 and NLRC4 stimulated autophagosome turnover. In addition, stimulation of autophagy appeared to inhibit pyroptosis, as caspase-1-dependent cell death occurred more frequently in cells when autophagy was blocked pharmacologically (54). These studies demonstrate that NLR activation increases autophagy, and that autophagy may be an important negative regulator of inflammasomes. Indeed, autophagy has been shown to deplete cytosolic IL-1 β and inflammasome constituents as well as suppress maturation of caspase-1 (55, 56). The prevailing paradigm from studies of autophagy and pyroptosis appears to be that autophagy decreases caspase-1 activation, but that increased levels of caspase-1 can also limit autophagy (51). Thus, if autophagy is effective in limiting caspase-1 activation, pyroptosis may be avoided; however, if caspase-1 prevails, inflammatory cell death occurs (51).

NLRs in Multifactorial Disease

Chronic obstructive pulmonary disease (COPD) is primarily a cigarette smoke (CS)-related multifactorial disease in which chronic exposure to an inhaled irritant leads to airway remodeling, reduced mucociliary

clearance, and destruction of the pulmonary parenchyma (57). Chronic ongoing inflammation contributes to disease progression and can be exacerbated by development of bacterial lung infections, which is favored because of compromised innate immune defenses.

CS-induced tissue damage can lead to formation of the NLRP3 ligands uric acid and calcium pyrophosphate, and in a model of acute CS inhalation, caspase-1 (now caspase-1/11) gene-deficient mice had reduced pulmonary inflammation, indicating inflammasome activation in response to CS challenge (57, 58). In a more recent publication, however, the degree of pulmonary inflammation after subacute CS exposure was independent of NLRP3 and caspase-1, but dependent on the IL-1 receptor type I. Both IL-1 α and IL-1 β were elevated in lung tissue and sputum samples from patients with COPD in this same study, indicating a potentially underappreciated role for IL-1 α in COPD-induced inflammation (57). The discrepancies between these studies may in part reflect differences in time course, as CS may induce inflammation by alternative mechanisms during the course of disease. In addition, these studies do not account for innate immune stimulation by bacterial agents known to exacerbate COPD.

Nontypeable *H. influenzae* (NTHi) is the most important bacterial species responsible for acute exacerbations of COPD (25). Stimulation with NTHi induced NLRP3 inflammasome up-regulation in human and murine macrophages and human bronchial epithelial cells (25). Moreover, stimulation with NTHi led to caspase-1 induction and production of IL-1 β and IL-18 in human lung tissue, indicating that NLRP3 may be an active player in acute inflammation during bacterial infections in patients with COPD (25).

Ventilator-associated pneumonia (VAP) is another multifactorial disease caused by environmental stressors and bacterial infection in severely injured patients. Although the role of NLRs in VAP has not been explored, studies have shown the role of inflammasomes in ventilator-induced lung injury (VILI). Although VILI is not infectious in nature, we reviewed some articles in this area because (1) VILI is an important pulmonary disease; (2) inflammation is associated with VILI; and

(3) the findings from VILI may help our understanding of the mechanisms in the pathogenesis of VAP. Mechanical ventilation can contribute to the injury of both healthy and previously injured lungs, that is, VILI. Even when lower tidal volumes are used to reduce barotrauma, in lungs with existing injury some areas may be collapsed, while others are intrinsically prone to hyperinflation and injury (59). VILI causes release from dying cells of DAMPs including uric acid, ATP, and hyaluronan, all known inducers of the NLRP3 inflammasome (59). In this regard, mechanical ventilation up-regulated NLRP3 and ASC mRNA, caspase-1, and IL-1 β in the lungs of mice and also increased NLRP3 expression in human and murine alveolar macrophages (59). VILI was reduced in the lungs of NLRP3 and ASC gene-deficient mice and in wild-type mice treated with an IL-1 receptor antagonist (59). Although these data strongly support a role for NLRP3 in VILI, the interplay between VILI, VAP, and NLRs is less clear. Although many of the pulmonary pathogens commonly isolated from VAP are known inducers of NLR activation, the combined impact of ventilator support and experimental infection needs to be explored in future studies.

Conclusions

Bacterial lung diseases are an important public health concern. Innate immune response to bacteria in the lung is a double-edged sword: an impaired response can result in life-threatening infection whereas an uncontrolled response can lead to life-threatening inflammatory disease. Although the importance of NLRs has emerged, much still remains to be learned, as new NLR family members, their ligands, and downstream signaling cascades are constantly being discovered. Molecular studies investigating cross-talk between TLRs and NLRs, and spatial association of NLR proteins with intracellular adaptors and autophagy machinery are needed for a more complete understanding of how antibacterial innate immune responses are integrated between immune sensors, and how these sensors bridge innate and adaptive responses. Understanding the connection between NLRs and autophagy is an exciting field in biology with many unanswered questions related to the

molecular mechanisms that warrant future studies. The translational relevance of NLR research is most evident in the novel therapeutic targets it has identified. Caspase-1, IL-1 β , and IL-1 receptor antagonists, and the uric acid inhibitors, uricase and allopurinol, have been tested in animal models with varied success in

combating deleterious NLR-mediated inflammation (57–60). The next logical step for NLR research may incorporate environmental stressors (i.e., CS, ventilator support), with bacterial challenge, thus enhancing our understanding of NLR-mediated antibacterial immunity within a framework relevant to human disease.

Moreover, the future challenge will be to apply our current understanding of NLRs to reducing excessive inflammation while augmenting host defense during respiratory bacterial infection. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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