Mechanisms of Neutrophil Accumulation in the Lungs Against Bacteria

Gayathriy Balamayooran¹, Sanjay Batra¹, Michael B. Fessler², Kyle I. Happel³, and Samithamby Jeyaseelan^{1,3}

¹Laboratory of Lung Biology, Department of Pathobiological Sciences and Center for Experimental Infectious Disease Research, Louisiana State University (LSU), Baton Rouge, Louisiana; ²Laboratory of Respiratory Biology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina; and ³Section of Pulmonary and Critical Care Medicine, Department of Medicine, LSU Health Sciences Center, New Orleans, Louisiana

Bacterial lung diseases are a major cause of morbidity and mortality both in immunocompromised and in immunocompetent individuals. Neutrophil accumulation, a pathological hallmark of bacterial diseases, is critical to host defense, but may also cause acute lung injury/acute respiratory distress syndrome. Toll-like receptors, nucleotide-binding oligomerization domain (NOD)-like receptors, transcription factors, cytokines, and chemokines play essential roles in neutrophil sequestration in the lungs. This review highlights our current understanding of the role of these molecules in the lungs during bacterial infection and their therapeutic potential. We also discuss emerging data on cholesterol and ethanol as environmentally modifiable factors that may impact neutrophil-mediated pulmonary innate host defense. Understanding the precise molecular mechanisms leading to neutrophil influx in the lungs during bacterial infection is critical for the development of more effective therapeutic and prophylactic strategies to control the excessive host response to infection.

Keywords: bacterial pneumonia; lung inflammation; acute lung injury

The respiratory system continually encounters microorganisms. The pathogenicity of a microorganism not only depends on its virulence factors, but also on the host's immune defense and the environment. The myeloid and structural/resident cells of the respiratory system detect invading microorganisms by binding their pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs) to bacterial ligands. This recognition triggers a cascade of events leading to the activation of transcription factors, production of chemokines/cytokines, up-regulation of cell adhesion molecules, phagocytic cellular infiltration, and subsequent clearance of the microorganisms. The major players of the innate immune system are neutrophils, macrophages, and dendritic cells. Dendritic cells and macrophages not only produce proinflammatory mediators but also present antigens to induce eventual adaptive immune response. The innate immune system in vertebrates confers immediate defense against invading microbes via mech-

CLINICAL RELEVANCE

This review highlights the mechanisms underlying neutrophil influx in the lungs and discusses the potential avenues to attenuate neutrophil-mediated lung damage during bacterial pneumonia.

anisms such as phagocytosis, whereas the adaptive immune system armed with T and B lymphocytes plays an important role in chronic or recurrent infections.

Pneumonia is an important cause of mortality both in developed and in developing countries (1). When microbial infections overwhelm the innate immune defense, the result is pneumonia, which is associated with extensive lung pathology (1, 2). Although pneumonia can be caused by a variety of microbes, such as bacteria, viruses, and fungi, we have primarily focused upon bacterial pneumonia in this review. The initial phase of bacterial pneumonia is characterized by neutrophil-mediated inflammation (1, 2). While neutrophilic inflammation aids in removal of bacteria, it also induces bystander injury to the lung. When severe, this injury may lead to a clinical condition termed acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) (1, 2). It is therefore imperative to explore the molecular mechanisms that underlie pneumonia and ALI/ARDS to formulate therapeutic strategies to augment host immune mechanisms to combat microorganisms while attenuating excessive parenchymal damage.

This review addresses our current understanding of the innate immune responses of the host against different gram-positive and gram-negative bacteria during the acute phase of bacterial pneumonia, with special emphasis on the role of PRRs such as TLRs and NLRs, transcription factors, cytokines, and chemokines in neutrophil accumulation. Furthermore, we discuss emerging roles for cholesterol and alcohol in neutrophil recruitment to the lung during bacterial pneumonia.

NEUTROPHIL MOBILIZATION TO THE LUNGS

A large pool of neutrophils is stored in the bone marrow. Granulocyte-colony stimulating factor (G-CSF) stimulates the proliferation and differentiation of the granulocytic lineage in the bone marrow into mature neutrophils. These neutrophils are mobilized to the bloodstream during bacterial infections and are believed to be the first line defenders of the innate immune system against microbes. To reach sites of inflammation in various organs, including the lungs (3), neutrophils in the bloodstream adhere to the vascular endothelium and migrate across the endothelium. This process involves multiple adhesion

(Received in original form February 3, 2009 and in final form August 7, 2009)

This work was supported by a research grant from the American Lung Association (RG-22442-N to S.J.), a scientist award from the Flight Attendant Medical Research Institute (YCSA-062466 to S.J.), grants from the National Institutes of Health (R01 HL-091958 to S.J., K08 AA15163 to K.I.H.); and the Intramural Research Program of the NIH/NIEHS (to M.B.F.).

Correspondence and requests for reprints should be addressed to Samithamby Jeyaseelan, D.V.M., Ph.D., Pathobiological Sciences and Center for Experimental Infectious Disease Research, LSU, Baton Rouge, LA 70803. E-mail: jey@lsu.edu

Am J Respir Cell Mol Biol Vol 43, pp 5–16, 2010
Originally Published in Press as DOI: 10.1165/rncmb.2009-0047TR on September 8, 2009
Internet address: www.atsjournals.org

molecules from different families that are expressed on the surface of endothelial cells in response to different cytokines/chemokines. As these cells migrate to inflammatory foci, they get activated, generate free radicals, release granule contents, and phagocytose and degrade microbes. Neutrophils are the first phagocytic cells recruited to a site of bacterial infection, but they have a very short life span (<6 h) after release from the bone marrow.

Neutrophils are one of the critical contributors to host defense in the lungs, since (1) selective depletion of neutrophils results in substantial reduction in the clearance of *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Legionella pneumophila*; and (2) repletion of neutrophils in neutropenic mice improves host defense and survival in response to bacterial infection (4–6). Although the neutrophil is the critical cell type implicated in the pathogenesis of ALI/ARDS, its short life span renders it difficult to investigate the signaling cascades in response to stimuli using genetic manipulations. In this context, a novel method has recently been developed to generate mature neutrophils from bone marrow–derived progenitor cells via long-term bone marrow culture system (7). These neutrophils have been shown to be mature as determined by morphology, expression of surface markers (Gr1, CD11b, CD62L, and CXCR2), and functional capabilities, including superoxide generation, exocytosis of granular contents, chemotaxis, phagocytosis, and bacterial killing. Furthermore, these *in vitro* mature neutrophils are capable of migrating to inflammatory sites *in vivo*. We anticipate that this system will serve as an important tool to advance our knowledge in neutrophil biology in the future.

Despite the fact that various animal model systems are used to delineate molecular mechanisms underlying neutrophil accumulation and lung injury, murine models have been used extensively because (1) bacteria can induce substantial neutrophil influx and subsequent damage to the lungs of mice during pneumonia, (2) several mouse strains with a variety of gene deletions or disruptions are available, and (3) there is ample availability of reagents and pharmacologic agents to dissect out the signaling mechanisms in murine models.

RECOGNITION OF MICROBES

The innate immune system is responsible for recognizing invading pathogens and subsequent initiation of the inflammatory response and/or host defense. Distinguishing self from non-self is therefore an important hallmark of the immune system. The recognition of microbes relies on PRRs that can recognize molecular moieties common to microbes. The discoveries of both membrane-bound and cytoplasmic PRRs, including TLRs, NLRs, mannose receptors, and RNA helicases have stimulated investigations on the biology of the innate immune system. We have highlighted the roles of TLRs and NLRs in this review, since these receptors have recently been identified and have important roles in host defense against bacterial infections.

A single microbe generally has a variety of molecules called pathogen-associated molecular patterns (PAMPs) that can activate a single or multiple PRRs (8). The intracellular signaling cascades initiated by these receptors may lead to the activation of transcription factors, such as nuclear factor (NF)- κ B, activating protein (AP)-1, signal transduction and transcription (STAT) proteins, and interferon regulatory factor (IRF) families (8), thereby regulating the expression of proinflammatory mediators, such as cytokines/chemokines, and adhesion molecules. In turn, these proinflammatory mediators can induce infiltration of neutrophils via enhancing chemotaxis and activating neutrophils to release more cytokines/chemokines. While

neutrophils combat pathogens in a nonspecific manner as innate immune cells, antigen-presenting cells, such as dendritic cells and macrophages, present antigens to T lymphocytes to induce antigen-specific adaptive immune response. However, recent reports have suggested that neutrophil-derived IL-18 together with dendritic cell–derived IL-12 can induce IFN- γ synthesis in NK cells in response to intravenous *L. pneumophila* infection in mice (9). These recent findings reveal a new role for neutrophils in the control of bacterial infection, in addition to their classical phagocytosis/microbicidal functions.

TLRS

TLRs are type 1 transmembrane receptors, with an ectodomain composed of leucine-rich repeats (LRRs), and are members of a larger superfamily of interleukin-1 receptors (IL-1Rs). Toll was initially identified in *Drosophila* as a receptor essential for dorsoventral polarity during embryogenesis, and later was shown to also be important for antifungal host defense in insects. The members of this family share a conserved region of approximately 200 amino acids in the cytoplasmic region known as the Toll/IL-1R (TIR) domain (10), whereas the extracellular LRR region is diverse in nature and is directly involved in the recognition of microbes.

TLRs function as dimers, usually forming homodimers except for TLR2, which dimerizes with either TLR1 or TLR6, giving rise to different ligand specificity. To date, 12 TLRs in mice and 10 in humans have been identified (8). Several TLRs can recognize bacteria and/or their components. For example, TLR1, TLR2, and TLR6 recognize lipid and carbohydrate compounds, including lipoteichoic acid and lipoproteins, from gram-positive bacteria. TLR4 recognizes lipopolysaccharide (LPS), a cell-wall component of gram-negative bacteria and MD-2 is a key molecule important for the TLR4 recognition of LPS, whereas TLR5 recognizes bacterial flagellin. TLR3, -7, -8, and -9 are receptors for nucleic acid and its derivatives. Furthermore, TLR11 has shown to be involved in the recognition of profilin and uropathogenic bacteria (Figure 1).

Several studies have unequivocally demonstrated the role of TLRs in pulmonary host defense (Table 1 and Figure 1). For instance, TLR2 has been shown to be essential for host defense against *Streptococcus pneumoniae* (11) and *Porphyromonas gingivalis* (12); however, TLR2 only mediates partial resistance to *L. pneumophila* (13–15). TLR4 contributes to a protective immune response against both *S. pneumoniae* (16) and *K. pneumoniae* (17), although its role is much more pronounced against *K. pneumoniae*. TLR4 also contributes to pulmonary host defense against *Haemophilus influenzae* (18). Also, MD-2 plays an important role during *Escherichia coli*–induced pneumonia (19). Both TLR2 and TLR4 have been shown to be significant for host defense against *Acinetobacter baumannii* (20) and *Pseudomonas aeruginosa* (21). TLR5 is an important regulator of neutrophil infiltration into the lung at early time-points (6 h), but not at late time points (24 h or beyond) during *L. pneumophila* infection (22). TLR9 is required for effective host defense not only against gram-negative pathogens, such as *L. pneumophila* (23) and *K. pneumoniae* (24), but also gram-positive pathogens, such as *S. pneumoniae* (25). These observations reveal (1) the activation of multiple TLRs in response to bacterial infection and (2) the time-dependent activation of TLRs in response to bacterial interaction.

LRRs of TLR2, -4, -5, and -6 are located outside of the cell, while the Toll–interleukin (IL)-1 receptor homology (TIR) domain is located inside the cell in all TLRs (10). Distinct adaptor molecules, including MyD88, TIRAP, TRAM, TRIF, and SARM, physically associate with the TIR domain of TLRs to transduce

It is important to note that individual TLRs can activate overlapping as well as distinct signaling cascades, ultimately providing diverse biological responses via activation of mitogen-associated protein kinases (MAPKs), and transcription factors, in turn resulting in the expression of growth factors, cytokines/chemokines and cell adhesion molecules (Figure 1). For example, MyD88-dependent TLR signaling cascades lead to early NF- κ B activation, whereas MyD88-independent TLR signaling pathways result in delayed NF- κ B activation. Of the TLR signaling cascades, the role of interleukin-1 receptor-associated kinases (IRAKs) in host defense has been well established. Four different IRAKs (IRAK-1, IRAK-2, IRAK-M, and IRAK-4) have been identified in mice and humans. Recently, patients with inherited IRAK-4 deficiency were reported who failed to respond to IL-1, IL-18, or to stimulation with TLR2, TLR3, TLR4, TLR5, and TLR9 agonists (26). In addition, findings with IRAK-M gene-deficient mice have shown that

Investigations have shown that TLR adaptor proteins play an important role in host defense against bacterial pathogens in the lung (Table 1). For instance, MyD88 is critical for host defense against numerous pathogens, including *S. pneumoniae*

TABLE 1. ROLE OF IMMUNE MOLECULES IN ACUTE LOWER RESPIRATORY BACTERIAL INFECTION

TLR	Infection (References)	Phenotype*			
		Survival	Neutrophil Influx [†]	Bacterial Burden [‡]	Bacterial Dissemination [§]
TLR2	<i>Acinetobacter baumannii</i> (20)	↓	↓	ND	ND
	<i>Legionella pneumophila</i> (13–15)	↓	↓	↑	↑
	<i>Porphyromonas gingivalis</i> (12)	ND	NS	↑	ND
	<i>Pseudomonas aeruginosa</i> (21)	ND	NS	↑early	ND
	<i>Streptococcus pneumoniae</i> (11)	↓	↓	↑	↑
TLR4	<i>A. baumannii</i> (20)	↓	↓	↑	↑
	<i>Haemophilus influenzae</i> (18)	↓	↓	↑	ND
	<i>Klebsiella pneumoniae</i> (17)	↓	ND	↑	ND
	<i>P. aeruginosa</i> (21)	ND	↓late	NS	ND
	<i>S. pneumoniae</i> (16)	↓	↓	↑	ND
TLR5	<i>L. pneumophila</i> (22)	ND	↓early	NS	ND
TLR9	<i>K. pneumoniae</i> (24)	↓	NS	↑	↑
	<i>L. pneumophila</i> (23)	↑	NS	↑	ND
	<i>S. pneumoniae</i> (25)	↓	NS	↑	↑
TLR Adaptor					
MyD88	<i>Escherichia coli</i> (35)	↓	↓	ND	ND
	<i>H. influenzae</i> (31)	ND	ND	↓	ND
	<i>K. pneumoniae</i> (6)	ND	↓	ND	ND
	<i>L. pneumophila</i> (14, 33, 34)	↓	↓	↑	↑
	<i>P. aeruginosa</i> (32, 33)	↓	↓	↑	↑
	<i>Staphylococcus aureus</i> (32)	ND	↓	NS	NS
	<i>S. pneumoniae</i> (30)	↓	↓	↑	↑
	<i>K. pneumoniae</i> (6, 36)	↓	↓	↑	↑
TIRAP	<i>E. coli</i> (6, 36)	ND	↓	↑	ND
TRIF	<i>E. coli</i> (36)	↓	↓	↑	↑
	<i>P. aeruginosa</i> (37)	ND	↓	↑	ND
Transcription Factor					
NF-κB p50	<i>E. coli</i> (48)	↓	↓	NS	↑
NF-κB RelA	<i>S. pneumoniae</i> (49, 50)	ND	↓	↑	ND
STAT3	<i>E. coli</i> (53)	ND	↓	↑	ND
STAT4	<i>K. pneumoniae</i> (51)	↓	ND	↑	↑
	<i>P. aeruginosa</i> (52)	ND	NS	↑	ND
Cytokine					
IL-23	<i>P. aeruginosa</i> (57)	ND	↓	NS	NS
	<i>K. pneumoniae</i> (56)	↓	ND	ND	ND
IL-17*	<i>K. pneumoniae</i> (59)	↑	↑	↓	ND
TNFα	<i>S. pneumoniae</i> (50)	↓	↓	↑	ND
IL-1	<i>S. pneumoniae</i> (50)	↓	↓	↑	ND
CXC chemokine receptor					
CXCR2	<i>P. aeruginosa</i> (62)	↓	↓	ND	ND
	<i>L. pneumophila</i> (64)	↓	↓	NS	ND
	<i>Nocardia asteroides</i> (63)#	↓	↓	↑	ND
CXC chemokine					
KC	<i>K. pneumoniae</i> (65)	↓	↓	ND	ND
MIP-2**	<i>K. pneumoniae</i> (66)	↓	↓	↑	↑
	<i>N. asteroides</i> (63)	NS	NS	NS	NS
	<i>K. pneumoniae</i> (67)	↓	↓ (24 h) ^{††}	↑	ND
Lungkine			↑ (48 h) ^{††}		

Definition of abbreviations: KC, keratinocyte-derived chemokine; MIP, macrophage inflammatory protein; ND, not determined; NS, no significant difference; STAT, signal transduction and transcription; TLR, Toll-like receptor.

* Phenotype was determined mainly by using gene-deficient or mutant mice after intrapulmonary infection and phenotypes established by transgenic mice are indicated separately;

[†] Neutrophil influx was determined in BALF and/or Lung parenchyma;

[‡] Bacterial burden was measured as CFUs in the lungs;

[§] Bacterial dissemination was measured as CFUs in blood or spleen;

* Studied with overexpression system.

^{||} Studied with transgenic mice.

** Abs only.

^{††} Significant in airspaces but not in lung parenchyma.

(25), *H. influenzae* (30), *P. aeruginosa* (31–33), *Staphylococcus aureus* (32), *K. pneumoniae* (31), *L. pneumophila* (14, 33, 34), and *E. coli* (31). Furthermore, TIRAP is reported to be essential for host defense against *E. coli* and *K. pneumoniae* (6, 35). In addition, MyD88 gene-deficient mice show more pronounced phenotype as compared with single or double TLR gene-deficient mice (14, 21, 34). Moreover, MyD88-independent, TRIF signaling has also been shown to be

important host defense in the lungs against *E. coli* (35) and *P. aeruginosa* (36). These observations demonstrate (1) the importance of MyD88 as an adaptor molecule for several TLRs, (2) the importance of MyD88 and TRIF as adaptor molecules for TLRs, and/or (3) the sequential activation of several TLRs during bacterial infection in the lungs. Our studies using TRIF-blocking peptide to attenuate the expression of IL-8, IL-6, and TNF-α in response to *E. coli* demonstrate the importance of

TRIF in humans (35). These results reveal the potential for using cell-permeable compounds to attenuate cytokine/chemokine production and thereby possibly be useful for reducing excessive neutrophil recruitment to the lungs.

NLRs

NLRs are the other types of PRRs involved in the innate immune system and are responsible for detecting pathogens and/or PAMPs. NLRs regulate both inflammation and apoptosis. To date, 22 NLR family members in humans have been reported (37). They have been further classified into subfamilies, including NODs and NALPs, the MHC Class II transactivator (CIITA), IPAF, and BIRC1. NLRs are characterized by the presence of a central NOD domain, a C-terminal LRR, and an N-terminal domain, including CARD or the putative protein-protein interaction (PYRIN) domain (37). The LRRs of NLRs are important for pattern recognition of microbial ligands.

Of the NODs, NOD1, and NOD2 are known to recognize bacteria and/or their components. NOD1 and NOD2 recognize the bacterial peptidoglycan components γ -D-glutamyl-mesodiaminopimelic acid and muramyl dipeptide, respectively (37). The NODs interact with microbial molecules by means of a C-terminal LRR region and activate downstream gene transduction events through N-terminal CARD domains, which leads to apoptosis. These receptors transmit the signals by the serine threonine kinase CARDIAK/RICK/RIP2 and activate NF- κ B. Microbial molecules are detected by these proteins, resulting in their oligomerization and activation of caspases (37). Active caspases play an important role in inflammation via cleavage and maturation of proinflammatory cytokines. Recent investigations have shown that NOD1 is important for bacteria-induced host response. For example, NOD1 detects *P. aeruginosa* peptidoglycan, leading to NF- κ B activation. Cytokine secretion kinetics and bacterial killing are altered in NOD1-deficient cells infected with *P. aeruginosa* in the early stages of infection (38). When NOD1 and NOD2 gene-deficient mice were infected intravenously with *Listeria monocytogenes*, they showed reduced NF- κ B activation, and attenuated cytokines/chemokines and neutrophil influx in the liver and spleen (39). They also observed increased bacterial burden in the liver and spleen of NOD1 and NOD2 gene-deficient mice.

NALPs are the other members of the NLR family. NALPs have a PYRIN domain instead of a CARD domain at the N-terminus. This subfamily consists of 14 proteins, but most of their functions have not yet been determined. NALP1, NALP2, and NALP3 have been involved in the cleavage of pro-IL-1 β and pro-IL-18 to mature forms via caspase-1 (40). These NALPs form a complex of proteins called an “inflammasome,” which comprises adaptor proteins and caspases. NALPs have emerged as mediators of antibacterial host defense in recent years. NALP1 has been shown to be important for host defense against *Bacillus anthracis* (40). NALP3 is well studied and has been shown to be essential for host defense against *L. pneumophila* (41) and *Francisella tularensis* (42). IPAF is another NLR family member. It contains CARD domains and has been shown to recognize *Salmonella* flagellin and induce IL-1 β production (43). Flagellin of *L. pneumophila* is also responsible for IPAF-dependent caspase-1 activation (44). NALP5/NAIP5 (Birc1e) is an NLR protein that regulates host susceptibility to the intracellular pathogen *L. pneumophila*. NALP5 has been implicated in *L. pneumophila*-induced caspase-1 activation (44). Further understanding of the molecular pathways of NLRs in lung may lead to new strategies for controlling bacterial infectious diseases in the lungs.

TRANSCRIPTION FACTORS

Transcription factors are sequence-specific DNA-binding proteins that bind to the promoter or enhancer regions of specific genes and control the transfer of information from DNA to RNA. Although several transcription factors, including NF- κ B, AP-1, STAT, and IRFs, play important roles in inflammation, NF- κ B and STATs have been demonstrated to play important roles in bacterial pneumonia. NF- κ B, a protein complex found in almost all mammalian cell types, is the most studied transcription factor to date. NF- κ B exists in homodimeric and/or heterodimeric forms and consists of five family members, which include p50 (NF- κ B1), p52 (NF- κ B2), c-Rel, RelB, and RelA (p65) (45). All members of the family share the Rel homology domain. NF- κ B acts as a transcription factor in response to stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens. In normal cells, NF- κ B dimers are present in the cytoplasm and inhibited by I κ Bs (Inhibitor of κ B), proteins that contain multiple copies of a sequence called an ankyrin repeat. The I κ B proteins mask the nuclear localization signals of NF- κ B proteins and keep them in an inactive state in the cytoplasm. Degradation of I κ B leads to nuclear translocation of NF- κ B and its subsequent binding to the promoters of and “turn on” expression of specific genes.

NF- κ B can be activated by TLRs, NODs, and NALPs, and this triggers the expression of proinflammatory genes, including different cytokines and chemokines through MyD88-dependent and -independent cascades. Recently, it was found that NLRs also activate NF- κ B. Activation of NF- κ B contributes to neutrophil accumulation elicited in the lungs by LPS, which is largely in part through the up-regulation of several neutrophil chemokines (keratinocyte-derived chemokine [KC], macrophage inflammatory protein [MIP]-2, and CXCL5). Studies demonstrate that endogenous NF- κ B protects the mice during *E. coli* (46, 47) and pneumococcal pneumonia, and is essential for the survival during these infections (48). Neutrophil emigration to the alveoli during LPS-induced inflammation was severely reduced in TNF Receptor 1/RelA-double knockout mice, when compared with their control (TNFR1-deficient) mice (49). In a similar manner, it has been shown that deficiency of TNF- α and IL-1 receptor signaling reduces NF- κ B activation to *S. pneumoniae* (49). Furthermore, TRIF-deficient mice also showed reduced NF- κ B activation and chemokine expression in the lungs during *P. aeruginosa* infection (36).

Although the role of NF- κ B has been studied extensively in pneumonia, there are other transcription factors, such as STATs, that also play an important role in the innate immunity. STATs can be activated by cytokines, including IL-6. Cytokines bind to their homodimeric or heterodimeric receptors, which can bind to janus kinases (JAK). JAKs can be activated by transphosphorylation followed by phosphorylation of cytokine receptors, allowing STATs to bind cytokine receptors. The activated STATs translocate to the nucleus, where they bind DNA and induce transcription of genes. Studies have shown that the STAT4 is an integral component of the host innate immune response leading to cytokine production and bacterial clearance during *K. pneumoniae* infection in the lung (50). STAT4 also contributes to *P. aeruginosa*-induced inflammation, but is not essential for bacterial clearance (51). During *E. coli* pneumonia, IL-6 family members specifically activate alveolar epithelial STAT3 to promote neutrophil infiltration into the lungs (52).

CYTOKINES

Cytokines are polypeptides produced by variety of cell types. They have autocrine, paracrine, or endocrine functions to

regulate inflammation and immune defense. Cytokines bind to their cognate receptor and signal via second messengers, thereby increasing or decreasing the expression of other membrane proteins, and can be categorized as proinflammatory or anti-inflammatory. TNF- α , IL-1 β , IL-6, IL-8, and IFN- γ are major proinflammatory cytokines that participate in acute inflammation; IL-8 acts as a chemoattractant, whereas TNF- α and IL-1 β stimulate antigen presentation and increase the expression of cell adhesion molecules. On the other hand, IL-10, TGF- β , and IL-1Ra are major anti-inflammatory cytokines that down-regulate the inflammatory response in the lungs (53).

The importance of IL-12 in antibacterial host defense has been widely established. The IL-12-IFN- γ axis is known to be involved in host immune defense against bacterial pathogens and induces cell-mediated immune responses to clear infection. However, the role of the IL-23-IL-17 axis has only recently been recognized, as mice that lack both IL-12 and IL-23 are more susceptible to bacterial infections (54). IL-23 is a newly described cytokine synthesized by macrophages and dendritic cells. It shares the p40 subunit with IL-12, an important cytokine in the induction of the Th1 response (e.g., IFN- γ), but has a distinct p19 subunit. In general, IL-23 produces more IL-17 from CD4⁺ T cells than IL-12. Several recent studies suggest that bacterial infection can induce IL-23 expression by macrophages and/or dendritic cells. It has been documented that IL-23 is essential to mediate host defense in the lungs against *K. pneumoniae* and *P. aeruginosa* (55, 56).

IL-17, which is mainly produced by T cells via the Th17 immune cascade, has been proposed as a proinflammatory cytokine (57). Recently, five new IL-17 family members have been identified: IL-17B, IL-17C, IL-17D, IL-17E (also named IL-25), and IL-17F (57). Virtually all cells bear the IL-17 receptor, which has been shown to recruit neutrophils to the lung by stimulating the production of the ELR+CXC chemokines, IL-8 or KC (a mouse homolog of IL-8) (57), and G-CSF. In addition, IL-17A seems to be important for the early and the late phase of neutrophil accumulation in the lungs (52). Furthermore, IL-17 can induce the up-regulation of other cytokines, including IL-1 β and IL-6, and ICAM-1 by several types of cells (e.g., endothelial cells, epithelial cells) (57). Using gene-deficient mice, it has been shown that IL-17 is important for host defense in the lungs against respiratory infection in mice caused by *K. pneumoniae* (58).

CHEMOKINES

Cytokines that act as chemoattractants for other cells are known as chemokines. Chemokines are produced locally at sites of infection/inflammation and regulate the recruitment of specific subpopulations of leukocytes from the bloodstream into tissues. Chemokines enhance neutrophil adhesion and extravasation across the postcapillary venules and direct migration neutrophils to sites of infection/inflammation. According to the composition of a cysteine motif located near the N-terminus of these molecules, they are categorized into the C, CC, CXC, and CX3C subgroups. These chemokines have four cysteine molecules, the first two of which are separated by a nonconserved amino acid, and can be further categorized by the presence of the ELR (glutamic acid-leucine-arginine) motif immediately preceding the CXC sequence (59). All known ELR+CXC chemokines are neutrophil chemoattractants. Seven chemokines (IL-8; NAP-2; GRO α , β , and γ ; ENA-78; and GCP-2) have been identified in humans (59). Among these, IL-8 is the most potent neutrophil chemoattractant in bacterial pneumonia. Although a real homolog of human IL-8 has not been identified in rodents, KC, MIP-2, LPS-Induced CXC

Chemokine (LIX; CXCL5), and lungkine are important chemoattractants in mice in response to bacterial lung infection (60).

While most chemokines are secreted primarily by myeloid cells, lungkine and LIX are secreted by bronchial epithelial cells and type II alveolar epithelial cells, respectively. There are two receptors identified for CXC chemokines: CXCR1 and CXCR2, which are expressed in both humans and mice. CXCR2 binds to all ELR+CXC chemokines and is essential for neutrophil infiltration into the lungs during bacterial infection. In this context, *in vivo* studies have shown that neutrophil recruitment induced by *P. aeruginosa* (61), *Norcardia asteroides* (62) and *L. pneumophila* (63) involves the CXCR2 receptor (Table 1). Regarding the CXCR2 ligands, KC and MIP-2 are important for neutrophil influx during infection, as blocking these ligands impairs host defense (61, 64). Although lungkine is not important for neutrophil influx into the lung parenchyma, it is important for neutrophil accumulation in the airspaces (65) (Table 1). It has been reported that CXCL5 up-regulation increases neutrophil trafficking during infections with *P. aeruginosa*, *K. pneumoniae*, *L. pneumophila*, and *Bordetella bronchiseptica*. Subsequent studies have shown that LIX is an important molecule for neutrophil influx in the lungs during LPS-induced inflammation (66). However, the role of LIX in neutrophil infiltration in the lungs during bacterial infections remains to be determined.

EMERGING ROLES FOR CHOLESTEROL IN PMN RECRUITMENT TO THE LUNG

Cholesterol exerts complex effects upon the proinflammatory functions of macrophages, PMNs, and other cell types. Cholesterol overloading induces macrophages to produce cytokines, in part through endoplasmic reticulum stress (67–69), though it may also either enhance or attenuate macrophage responses to LPS (70, 71). Hypercholesterolemia primes PMNs for oxidant and granule protein release (72, 73), induces PMN adhesion to and emigration from postcapillary venules (74), and promotes mononuclear cell accumulation in vascular lesions by inducing endothelial chemokines (75, 76). The net effect of systemic dyslipidemia on organ-localized inflammation is likely, however, to be complex, as cholesterol loading also impairs leukocyte chemotaxis (77), and lipoproteins, the particles that carry cholesterol in the bloodstream, bind to and neutralize bacterial LPS (78) and inhibit leukocyte signaling responses to multiple TLR ligands (79). Further complicating expectations for the net effect of cholesterol upon inflammation in the lung in particular is the fact that the mechanism of, and requirements for, PMN transmigration from the vascular compartment into the airspace differ substantially from those at work in other organs (3).

While little is known about either the sensitivity of lung-resident cells to systemic dyslipidemia (i.e., circulating lipoprotein cholesterol) or the lung's local regulatory mechanisms for cholesterol, emerging reports interestingly do suggest that cholesterol trafficking and inflammation may be coupled uniquely in the lung. For example, genetic deletion of the cellular cholesterol efflux pump ATP-binding cassette transporter G1 (ABCG1) induces recruitment of multiple leukocyte subtypes, including PMNs, to the unexposed murine lung (67, 69), responses that reflect cooperative proinflammatory contributions from cholesterol-overloaded alveolar epithelium and alveolar macrophages. Conversely, the potential for therapies that reduce cellular and serum cholesterol to reduce PMN recruitment to the airspace has also been reported. Three different hydroxy-methylglutaryl coenzyme A reductase inhibitors ("statins") used to treat clinical hypercholesterolemia have now been shown to reduce PMN recruitment to and microvas-

cular injury within the rodent lung following LPS exposure (80–82), likely due to dual effects upon PMN chemotaxis and endothelium. Statins also enhance clearance of apoptotic PMNs by alveolar macrophages (83). While statins do exert some effects on leukocyte function by depleting cellular cholesterol (84), other effects stem from depletion of cellular isoprenoids and consequent inhibition of cellular protein prenylation (80). Systemic treatment of rodents with synthetic agonists of Liver X Receptor (LXR), a nuclear receptor that promotes cellular cholesterol efflux and reverse cholesterol transport, has also been shown to reduce PMN recruitment into the airspace induced by LPS and Gram-negative bacteria (85, 86). A potential untoward consequence of reduced PMN recruitment to the lung through cholesterol targeting is impaired antibacterial host defense, as both statins and LXR agonists also reduce clearance of bacteria deposited in the rodent lung (80, 86). In the case of statins, impaired bacterial clearance may also reflect drug-induced defects in PMN bactericidal function (80). This said, clinical expression of pneumonia and its complications reflect not only pathogen proliferation, but also bystander tissue injury from responding host cells.

In recent years, the relationship between statin use and risk of community-acquired pneumonia has been examined in observational studies. Several retrospective studies have reported that statins are independently associated with either reduced risk of pneumonia (87, 88) or reduced pneumonia-associated mortality (89–93). Prospective, randomized, controlled trials will be necessary to resolve a potential role for statins in pneumonia therapy, however, as a prospective cohort study of patients hospitalized for community-acquired pneumonia reported no significant relationship between statins and a composite outcome of in-hospital mortality or admission to an intensive care unit (94). Given these findings, it may be difficult to conclusively rule out persistent confounding or a “healthy user” effect (95). Moreover, publication bias cannot be excluded.

The effect of cholesterol upon PMN recruitment to the lung likely depends upon the cell types having dysregulated cholesterol, the specific molecular features of the dyslipidemic state, and perhaps the nature of the alveolar or hematogenous insult. Contrary to the effects of localized pulmonary cholesterol overload observed in ABCG1 deficiency, systemic dyslipidemia induced by either diet or genetic manipulation is associated with *reduced* recruitment of PMNs to the LPS-exposed airspace, reflecting both reduced airspace chemokine expression and impaired PMN chemotaxis; dyslipidemic rodents nevertheless have *increased* airspace fluid protein, suggesting aggravated injury to the alveolocapillary barrier (M. B. Fessler, unpublished observations). Collectively, these data indicate that cholesterol has the potential to either promote or inhibit PMN recruitment to the airspaces, and may also impact lung injury through independent, direct effects on the integrity of the air–blood barrier. Given the high prevalence of dyslipidemia and its pharmacologic treatment in modern-day society, further investigation of the complex effects of cholesterol upon PMN recruitment to the lung is clearly needed.

ALCOHOL

Alcohol abuse has been associated with increased risk for bacterial pneumonia. Observational data show that alcoholic men and women suffer increased mortality from bacterial pneumonia compared with control subjects (96). A key element to early host defenses against invading microbes is the robust recruitment of neutrophils into the infected lung. Extensive work has shown conclusively shown that alcohol intoxication interferes with both the recruitment and functional capacity of

neutrophils during bacterial infection of the lower respiratory tract (97–100).

The early expression of “alarm” cytokines, such as TNF- α , by alveolar macrophages is central to the development of the histologic hallmark of bacterial pneumonia: neutrophil infiltration (101, 102). Acute alcohol intoxication impairs lung TNF- α production in response to LPS at the post-transcriptional level (103, 104). By impairing the co-localization of TNF- α and the cell surface-bound TNF- α converting enzyme (TACE), acute ethanol exposure prevents cleavage of TNF- α from the producing cell’s surface (105). The effect of chronic ethanol exposure on TNF- α release is controversial. While some work suggests chronic intoxication increases TNF- α release via augmented TACE activity (106) and stabilization of TNF- α mRNA (107), other studies show suppression of TNF- α production resulting from chronic alcohol (108, 109).

Although TNF- α is not itself a chemoattractant for neutrophils, it stimulates lung expression of CXC chemokines, which in turn promote the recruitment of neutrophils from the vasculature. Animal models of infection have shown that acute alcohol intoxication suppresses the lung’s expression of the neutrophil chemokines MIP-2 and CINC, rodent orthologs of the human neutrophil chemokines IL-8 and Gro- α , which bind the CXCR2 receptor found on neutrophils (100, 110). This suppression is accompanied by decreased neutrophil recruitment, and exogenous chemokine administration has shown promise in restoring intrapulmonary neutrophil influx (111). The expression of neutrophil chemokines comes from many cell types in the lung, including the alveolar epithelium (112). Together, this suggests that alveolar parenchymal cells may be susceptible to alcohol. We recently investigated this hypothesis by examining the effect of acute intoxication on pulmonary expression of LIX, a neutrophil chemoattractant whose expression is limited to alveolar type II epithelial cells (113). Ethanol exposure decreased LIX expression in response to airway endotoxin challenge *in vivo*, and ethanol exposure down-regulated LIX in a dose-dependent manner in primary cultures of type II alveolar epithelium (114). These cells showed inhibition of NF- κ B and p38 MAP kinase pathways in response to relatively low (25 mM) ethanol concentrations, suggesting that they are exquisitely sensitive targets of ethanol. In addition to the effects of ethanol on the lung epithelium, Zhang and coworkers have shown that acute ethanol intoxication profoundly suppresses the expression of the neutrophil chemokines S100A8 and S100A9 by leukocytes (115).

In addition to decreased chemokine expression, alcohol exposure results in intrinsic defects in the neutrophil that impede migration. During pneumonia, circulating neutrophils up-regulate the β_2 -integrin adhesion molecule, CD11b/CD18. CD11b/CD18 mediates neutrophil firm attachment to the pulmonary capillary endothelium and their subsequent trans-endothelial migration. Alcohol inhibits the up-regulation of CD18 expression on neutrophils in response to inflammatory stimuli (116) and suppresses neutrophil adhesion to the endothelium during appropriate stimulation (117). Furthermore, studies of neutrophils taken from chronic alcohol abusers show that these cells are hyporesponsive to chemotactic stimuli (118, 119). In alcoholics with liver disease, it has been proposed that systemic LPS exposure (through increased intestinal permeability and portal blood LPS content) induces a chronic inflammatory state. Neutrophil chemokines (IL-8) and chemotactic complement fragments (such as C5a) are elevated in the peripheral circulation of patients with alcoholic liver diseases (120, 121). The chronic *in vivo* activation of neutrophils has been postulated to account for the blunted response of neutrophils to chemoattractants in these hosts. Compounding this

recruitment defect, alcohol-exposed neutrophils are less capable of killing bacteria owing to abnormalities in phagocytosis, degranulation, and superoxide generation (122, 123).

Alcohol-abusing patients are frequently leukopenic, and they often do not mount an appropriate leukocytosis during pneumonia (124). Given the importance of neutrophils in pathogen clearance, it is not surprising that neutropenia in alcoholics increases mortality from pulmonary infection, particularly bacteremic pneumococcal pneumonia (125). During bacterial pneumonia, the lung expresses G-CSF (126, 127). In contrast to most cytokines, which are “compartmentalized” within the lung during infection, G-CSF readily exits the pulmonary tissue and enters the circulation during infection (126, 128). G-CSF thus serves as a means of communication between the infected lung and bone marrow (129). Studies of systemic and pulmonary infection have shown that acute alcohol inhibits the expression of G-CSF (130), and animal studies have shown that recombinant G-CSF treatment can partially restore lung neutrophil recruitment in response to a bacterial stimulus (131). Examination of bone marrow from chronic alcoholics often shows hypocellularity, maturation arrest, and vacuolization of myeloid progenitor cells (132, 133). Bone marrow cells treated with clinically relevant ethanol concentrations show impaired granulocyte colony formation, also suggesting a direct toxic effect on these tissues (134, 135). In the context of bacterial infection, we have recently shown that alcohol inhibits the increase in granulopoietic progenitor cell proliferation in mice challenged with *E. coli* (136). This effect is at least partially due to alcohol preventing the phenotypic reversion of hematopoietic precursors to a less differentiated state, a phenomenon described in hematopoietic precursors (129) which appears requisite for optimal granulocyte production.

The discovery of IL-17 has broadened our understanding of how the lung solicits neutrophil influx during infection. A product of T lymphocytes and related cell types, IL-17 induces CXC chemokine production, which promotes neutrophilic inflammation (137). In animal studies, inoculation with *K. pneumoniae* induces a TLR4-dependent pulmonary IL-17 expression within 12 hours, and animals deficient in the receptor for IL-17 show enhanced mortality from infection (138). Chronic alcohol intoxication inhibits IL-17 secretion in the lungs in response to *K. pneumoniae*, and *in vitro* stimulation of T cells confirms a dose-dependent inhibition of IL-17 by ethanol (139). IL-23 is induced in alveolar macrophages and myeloid dendritic cells during challenge and is the dominant trigger for IL-17 (140). The expression of IL-23 in the lung during bacterial challenge is strongly suppressed by acute ethanol intoxication (55). By pretreating animals with an adenoviral vector encoding IL-17 prior to *K. pneumoniae* inoculation, neutrophil recruitment is restored and survival improved in alcohol-treated animals, suggesting that transient pharmacologic gene expression therapies may offer promise in the treatment of immunosuppressed patients with bacterial pneumonia (55). A product of NK T cells, invariant NK T (iNKT) cells, γ delta T cells, and CD4+ T cells, IL-17 induces CXC chemokine production, which promotes neutrophilic inflammation (58, 137, 141, 142). While the precise source(s) of lung IL-17 expression during acute bacterial infection are not known, work has shown that depletion of CD4+ or CD8+ cells results in a decrease in lung IL-17 expression as expressed during *Klebsiella* infection. It is likely that several of these cell populations each contribute to the IL-17 response to infection.

CONCLUSIONS

Bacterial pneumonia is an important disease. Neutrophils are one of the first cells to reach the site of bacterial infection. An

ideal therapeutic strategy would be to attenuate the tissue-destructive potential of neutrophils without reducing their efficacy in antibacterial defense. Theoretically, one way to do this may be to identify therapeutic targets to attenuate excessive neutrophil accumulation during bacterial pneumonia. 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 cholesterol in neutrophil recruitment, and it has also been shown that alcohol affects neutrophil recruitment to the lungs during pneumonia. Although antibiotics are the rational treatment for pneumonias, antibiotic-resistant *S. 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 increasingly difficult. The future challenge will be to apply our current understanding of neutrophil function to design therapeutic methods to maintain the host defense potential of neutrophils while modulating their destructive potential. In this context, therapeutic potential of TLRs and NLRs against bacterial infection in the lungs remains to be explored.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgments: The authors are indebted to Scott Worthen for his support, guidance, and contribution to the work presented in this article. The authors thank Rachel Zemans, Ken Malcolm, Theivanthiran Balamayooran, and Kohila Mahadevan for critical reading of the manuscript.

References

- Mizgerd JP. Lung infection—a public health priority. *PLoS Med* 2006; 3:e76.
- Mizgerd JP. Acute lower respiratory tract infection. *N Engl J Med* 2008;358:716–727.
- Mizgerd JP. Molecular mechanisms of neutrophil recruitment elicited by bacteria in the lungs. *Semin Immunol* 2002;14:123–132.
- Garvy BA, Harmsen AG. The importance of neutrophils in resistance to pneumococcal pneumonia in adult and neonatal mice. *Inflammation* 1996;20:499–512.
- Tateda K, Moore TA, Deng JC, Newstead MW, Zeng X, Matsukawa A, Swanson MS, Yamaguchi K, Standiford TJ. Early recruitment of neutrophils determines subsequent T1/T2 host responses in a murine model of *Legionella pneumophila* pneumonia. *J Immunol* 2001;166: 3355–3361.
- Jeyaseelan S, Young SK, Yamamoto M, Arndt PG, Akira S, Kolls JK, Worthen GS. Toll/IL-1R domain-containing adaptor protein (TIRAP) is a critical mediator of antibacterial defense in the lung against *Klebsiella pneumoniae* but not *Pseudomonas aeruginosa*. *J Immunol* 2006;177:538–547.
- Zemans RL, Briones N, Young SK, Malcolm KC, Refaeli Y, Downey GP, Worthen GS. A novel method for long term bone marrow culture and genetic modification of murine neutrophils via retroviral transduction. *J Immunol Methods* 2009;340:102–115.
- Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;124:783–801.
- Sporri R, Joller N, Hilbi H, Oxenius A. A novel role for neutrophils as critical activators of NK cells. *J Immunol* 2008;181:7121–7130.
- O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat Rev Immunol* 2007;7: 353–364.
- Dessing MC, Florquin S, Paton JC, van der Poll T. Toll-like receptor 2 contributes to antibacterial defence against pneumolysin-deficient pneumococci. *Cell Microbiol* 2008;10:237–246.
- Hajishengallis G, Wang M, Bagby GJ, Nelson S. Importance of TLR2 in early innate immune response to acute pulmonary infection with *Porphyromonas gingivalis* in mice. *J Immunol* 2008;181:4141–4149.
- Fuse ET, Tateda K, Kikuchi Y, Matsumoto T, Gondaira F, Azuma A, Kudoh S, Standiford TJ, Yamaguchi K. Role of Toll-like receptor 2 in recognition of *Legionella pneumophila* in a murine pneumonia model. *J Med Microbiol* 2007;56:305–312.

14. Hawn TR, Smith KD, Aderem A, Skerrett SJ. Myeloid differentiation primary response gene (88)- and Toll-like receptor 2-deficient mice are susceptible to infection with aerosolized *Legionella pneumophila*. *J Infect Dis* 2006;193:1693–1702.
15. Archer KA, Alexopoulou L, Flavell RA, Roy CR. Multiple myd88-dependent responses contribute to pulmonary clearance of *Legionella pneumophila*. *Cell Microbiol* 2009;11:21–36.
16. Dessing MC, Schouten M, Draing C, Levi M, von Aulock S, van der Poll T. Role played by Toll-like receptors 2 and 4 in lipoteichoic acid-induced lung inflammation and coagulation. *J Infect Dis* 2008;197:245–252.
17. Schurr JR, Young E, Byrne P, Steele C, Shellito JE, Kolls JK. Central role of Toll-like receptor 4 signaling and host defense in experimental pneumonia caused by gram-negative bacteria. *Infect Immun* 2005;73:532–545.
18. Wang X, Moser C, Louboutin JP, Lysenko ES, Weiner DJ, Weiser JN, Wilson JM. Toll-like receptor 4 mediates innate immune responses to *Haemophilus influenzae* infection in mouse lung. *J Immunol* 2002;168:810–815.
19. Cai S, Zemans RL, Young SK, Worthen GS, Jeyaseelan S. MD-2-dependent and -independent neutrophil accumulation during *Escherichia coli* pneumonia. *Am J Respir Cell Mol Biol* 2009;40:701–709.
20. Knapp S, Wieland CW, Florquin S, Pantophlet R, Dijkshoorn L, Tshimbalanga N, Akira S, van der Poll T. Differential roles of CD14 and Toll-like receptors 4 and 2 in murine acinetobacter pneumonia. *Am J Respir Crit Care Med* 2006;173:122–129.
21. Ramphal R, Balloy V, Huerre M, Si-Tahar M, Chignard M. TLRs 2 and 4 are not involved in hypersusceptibility to acute *Pseudomonas aeruginosa* lung infections. *J Immunol* 2005;175:3927–3934.
22. Hawn TR, Berrington WR, Smith IA, Uematsu S, Akira S, Aderem A, Smith KD, Skerrett SJ. Altered inflammatory responses in TLR5-deficient mice infected with *Legionella pneumophila*. *J Immunol* 2007;179:6981–6987.
23. Bhan U, Trujillo G, Lyn-Kew K, Newstead MW, Zeng X, Hogaboam CM, Krieg AM, Standiford TJ. Toll-like receptor 9 regulates the lung macrophage phenotype and host immunity in murine pneumonia caused by *Legionella pneumophila*. *Infect Immun* 2008;76:2895–2904.
24. Bhan U, Lukacs NW, Osterholzer JJ, Newstead MW, Zeng X, Moore TA, McMillan TR, Krieg AM, Akira S, Standiford TJ. TLR9 is required for protective innate immunity in gram-negative bacterial pneumonia: role of dendritic cells. *J Immunol* 2007;179:3937–3946.
25. Albiger B, Dahlberg S, Sandgren A, Wartha F, Beiter K, Katsuragi H, Akira S, Normark S, Henriques-Normark B. Toll-like receptor 9 acts at an early stage in host defence against pneumococcal infection. *Cell Microbiol* 2007;9:633–644.
26. Medvedev AE, Thomas K, Awomoyi A, Kuhns DB, Gallin JI, Li X, Vogel SN. Cutting edge: expression of IL-1 receptor-associated kinase-4 (IRAK-4) proteins with mutations identified in a patient with recurrent bacterial infections alters normal IRAK-4 interaction with components of the IL-1 receptor complex. *J Immunol* 2005;174:6587–6591.
27. Deng JC. Sepsis-induced suppression of lung innate immunity is mediated by IRAK-M. *J Clin Invest* 2006;116:2532–2542.
28. Krieg AM. Antiinfective applications of Toll-like receptor 9 agonists. *Proc Am Thorac Soc* 2007;4:289–294.
29. Barton GM. Viral recognition by Toll-like receptors. *Semin Immunol* 2007;19:33–40.
30. Wieland CW, Florquin S, Maris NA, Hoebe K, Beutler B, Takeda K, Akira S, van der Poll T. The Myd88-dependent, but not the Myd88-independent, pathway of TLR4 signaling is important in clearing nontypeable *Haemophilus influenzae* from the mouse lung. *J Immunol* 2005;175:6042–6049.
31. Jeyaseelan S, Manzer R, Young SK, Yamamoto M, Akira S, Mason RJ, Worthen GS. Toll-IL-1 receptor domain-containing adaptor protein is critical for early lung immune responses against *Escherichia coli* lipopolysaccharide and viable *Escherichia coli*. *J Immunol* 2005;175:7484–7495.
32. Skerrett SJ, Liggitt HD, Hajjar AM, Wilson CB. Cutting edge: myeloid differentiation factor 88 is essential for pulmonary host defense against *Pseudomonas aeruginosa* but not *Staphylococcus aureus*. *J Immunol* 2004;172:3377–3381.
33. Skerrett SJ, Wilson CB, Liggitt HD, Hajjar AM. Redundant Toll-like receptor signaling in the pulmonary host response to *Pseudomonas aeruginosa*. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L312–L322.
34. Archer KA, Roy CR. Myd88-dependent responses involving Toll-like receptor 2 are important for protection and clearance of *Legionella pneumophila* in a mouse model of legionnaires' disease. *Infect Immun* 2006;74:3325–3333.
35. Jeyaseelan S, Young SK, Fessler MB, Liu Y, Malcolm KC, Yamamoto M, Akira S, Worthen GS. Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF)-mediated signaling contributes to innate immune responses in the lung during *Escherichia coli* pneumonia. *J Immunol* 2007;178:3153–3160.
36. Power MR, Li B, Yamamoto M, Akira S, Lin TJ. A role of Toll-IL-1 receptor domain-containing adaptor-inducing IFM-beta in the host response to *Pseudomonas aeruginosa* lung infection in mice. *J Immunol* 2007;178:3170–3176.
37. Ting JP, Willingham SB, Bergstralh DT. NLRs at the intersection of cell death and immunity. *Nat Rev Immunol* 2008;8:372–379.
38. Travassos LH, Carneiro LA, Girardin SE, Boneca IG, Lemos R, Bozza MT, Domingues RC, Coyle AJ, Bertin J, Philpott DJ, et al. NOD1 participates in the innate immune response to *Pseudomonas aeruginosa*. *J Biol Chem* 2005;280:36714–36718.
39. Kim YG, Park JH, Shaw MH, Franchi L, Inohara N, Nunez G. The cytosolic sensors NOD1 and NOD2 are critical for bacterial recognition and host defense after exposure to Toll-like receptor ligands. *Immunity* 2008;28:246–257.
40. Sutterwala FS, Flavell RA. NLRc4/ipaf: a card carrying member of the NLR family. *Clin Immunol* 2009;130:2–6.
41. Case CL, Shin S, Roy CR. Asc and ipaf inflammasomes direct distinct pathways for caspase-1 activation in response to *Legionella pneumophila*. *Infect Immun* 2009;77:1981–1991.
42. Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M, Lee WP, Weinrauch Y, Monack DM, Dixit VM. Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 2006;440:228–232.
43. Franchi L, Amer A, Body-Malapel M, Kanneganti TD, Ozoren N, Jagirdar R, Inohara N, Vandenabeele P, Bertin J, Coyle A, et al. Cytosolic flagellin requires ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat Immunol* 2006;7:576–582.
44. Zamboni DS, Kobayashi KS, Kohlsdorf T, Ogura Y, Long EM, Vance RE, Kuida K, Mariathasan S, Dixit VM, Flavell RA, et al. The bircle cytosolic pattern-recognition receptor contributes to the detection and control of *Legionella pneumophila* infection. *Nat Immunol* 2006;7:318–325.
45. Hoffmann A, Baltimore D. Circuitry of nuclear factor kappaB signaling. *Immunol Rev* 2006;210:171–186.
46. Alcamo E, Mizgerd JP, Horwitz BH, Bronson R, Beg AA, Scott M, Doerschuk CM, Hynes RO, Baltimore D. Targeted mutation of TNF receptor I rescues the rela-deficient mouse and reveals a critical role for NF-kappa B in leukocyte recruitment. *J Immunol* 2001;167:1592–1600.
47. Mizgerd JP, Lupa MM, Kogan MS, Warren HB, Kobzik L, Topulos GP. Nuclear factor-kappaB p50 limits inflammation and prevents lung injury during *Escherichia coli* pneumonia. *Am J Respir Crit Care Med* 2003;168:810–817.
48. Quinton LJ, Jones MR, Simms BT, Kogan MS, Robson BE, Skerrett SJ, Mizgerd JP. Functions and regulation of NF-kappaB rela during pneumococcal pneumonia. *J Immunol* 2007;178:1896–1903.
49. Jones MR, Simms BT, Lupa MM, Kogan MS, Mizgerd JP. Lung NF-kappaB activation and neutrophil recruitment require IL-1 and TNF receptor signaling during pneumococcal pneumonia. *J Immunol* 2005;175:7530–7535.
50. Deng JC, Zeng X, Newstead M, Moore TA, Tsai WC, Thannickal VJ, Standiford TJ. Stat4 is a critical mediator of early innate immune responses against pulmonary *Klebsiella infection*. *J Immunol* 2004;173:4075–4083.
51. O'Sullivan R, Carrigan SO, Marshall JS, Lin TJ. Signal transducer and activator of transcription 4 (STAT4), but not IL-12 contributes to *Pseudomonas aeruginosa*-induced lung inflammation in mice. *Immunobiology* 2008;213:469–479.
52. Quinton LJ, Jones MR, Robson BE, Simms BT, Whitsett JA, Mizgerd JP. Alveolar epithelial STAT3, IL-6 family cytokines, and host defense during *Escherichia coli* pneumonia. *Am J Respir Cell Mol Biol* 2008;38:699–706.
53. Moldoveanu B, Otmishi P, Jani P, Walker J, Sarmiento X, Guardiola J, Saad M, Yu J. Inflammatory mechanisms in the lung. *J Inflamm Res* 2009;2:1–11.
54. Zhang Z, Hinrichs DJ, Lu H, Chen H, Zhong W, Kolls JK. After interleukin-12p40, are interleukin-23 and interleukin-17 the next therapeutic targets for inflammatory bowel disease? *Int Immunopharmacol* 2007;7:409–416.

55. Happel KI, Dubin PJ, Zheng M, Ghilardi N, Lockhart C, Quinton LJ, Odden AR, Shellito JE, Bagby GJ, Nelson S, *et al*. Divergent roles of IL-23 and IL-12 in host defense against *Klebsiella pneumoniae*. *J Exp Med* 2005;202:761–769.
56. Dubin PJ, Kolls JK. IL-23 mediates inflammatory responses to mucoid *Pseudomonas aeruginosa* lung infection in mice. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L519–L528.
57. Kolls JK, Linden A. Interleukin-17 family members and inflammation. *Immunity* 2004;21:467–476.
58. Ye P, Garvey PB, Zhang P, Nelson S, Bagby G, Summer WR, Schwarzenberger P, Shellito JE, Kolls JK. Interleukin-17 and lung host defense against *Klebsiella pneumoniae* infection. *Am J Respir Cell Mol Biol* 2001;25:335–340.
59. Lukacs NW, Hogaboam C, Campbell E, Kunkel SL. Chemokines: function, regulation and alteration of inflammatory responses. *Chem Immunol* 1999;72:102–120.
60. Baggiolini M, Dewaldm B, Moser B. Interleukin-8 and related chemotactic cytokines-cxc and cc chemokines. *Adv Immunol* 1994; 55:97–179.
61. Tsai WC, Strieter RM, Wilkowski JM, Bucknell KA, Burdick MD, Lira SA, Standiford TJ. Lung-specific transgenic expression of KC enhances resistance to *Klebsiella pneumoniae* in mice. *J Immunol* 1998;161:2435–2440.
62. Moore TA, Newstead MW, Strieter RM, Mehrad B, Beaman BL, Standiford TJ. Bacterial clearance and survival are dependent on cxc chemokine receptor-2 ligands in a murine model of pulmonary nocardia asteroides infection. *J Immunol* 2000;164:908–915.
63. Tateda K, Moore TA, Newstead MW, Tsai WC, Zeng X, Deng JC, Chen G, Reddy R, Yamaguchi K, Standiford TJ. Chemokine-dependent neutrophil recruitment in a murine model of *Legionella pneumoniae*: potential role of neutrophils as immunoregulatory cells. *Infect Immun* 2001;69:2017–2024.
64. Greenberger MJ, Strieter RM, Kunkel SL, Danforth JM, Laichalk LL, McGillicuddy DC, Standiford TJ. Neutralization of macrophage inflammatory protein-2 attenuates neutrophil recruitment and bacterial clearance in murine *Klebsiella pneumoniae*. *J Infect Dis* 1996;173:159–165.
65. Chen SC, Mehrad B, Deng JC, Vassileva G, Manfra DJ, Cook DN, Wiekowski MT, Zlotnik A, Standiford TJ, Lira SA. Impaired pulmonary host defense in mice lacking expression of the cxc chemokine lungkine. *J Immunol* 2001;166:3362–3368.
66. Jeyaseelan S, Chu HW, Young SK, Worthen GS. Transcriptional profiling of lipopolysaccharide-induced acute lung injury. *Infect Immun* 2004;72:7247–7256.
67. Baldan A, Gomes AV, Ping P, Edwards PA. Loss of abcg1 results in chronic pulmonary inflammation. *J Immunol* 2008;180:3560–3568.
68. Li Y, Schwabe RF, DeVries-Seimon T, Yao PM, Gerbod-Giannone MC, Tall AR, Davis RJ, Flavell R, Brenner DA, Tabas I. Free cholesterol-loaded macrophages are an abundant source of tumor necrosis factor- α and interleukin-6: model of NF- κ B- and MAP kinase-dependent inflammation in advanced atherosclerosis. *J Biol Chem* 2005;280:21763–21772.
69. Wojcik AJ, Skaflen MD, Srinivasan S, Hedrick CC. A critical role for abcg1 in macrophage inflammation and lung homeostasis. *J Immunol* 2008;180:4273–4282.
70. Haga Y, Takata K, Araki N, Sakamoto K, Akagi M, Morino Y, Horiuchi S. Intracellular accumulation of cholesteryl esters suppresses production of lipopolysaccharide-induced interleukin 1 by rat peritoneal macrophages. *Biochem Biophys Res Commun* 1989;160:874–880.
71. Yvan-Charvet L, Welch C, Pagler TA, Ranalletta M, Lamkanfi M, Han S, Ishibashi M, Li R, Wang N, Tall AR. Increased inflammatory gene expression in abc transporter-deficient macrophages: free cholesterol accumulation, increased signaling via toll-like receptors, and neutrophil infiltration of atherosclerotic lesions. *Circulation* 2008;118:1837–1847.
72. Maeda K, Yasunari K, Sato EF, Inoue M. Enhanced oxidative stress in neutrophils from hyperlipidemic guinea pig. *Atherosclerosis* 2005; 181:87–92.
73. Mazor R, Shurtz-Swirski R, Farah R, Kristal B, Shapiro G, Dorlechner F, Cohen-Mazor M, Meilin E, Tamara S, Sela S. Primed polymorphonuclear leukocytes constitute a possible link between inflammation and oxidative stress in hyperlipidemic patients. *Atherosclerosis* 2008;197:937–943.
74. Stokes KY, Clanton EC, Russell JM, Ross CR, Granger DN. Nad(p)h oxidase-derived superoxide mediates hypercholesterolemia-induced leukocyte-endothelial cell adhesion. *Circ Res* 2001;88:499–505.
75. Bjorkbacka H, Kunjathoor VV, Moore KJ, Koehn S, Ordija CM, Lee MA, Means T, Halmen K, Luster AD, Golenbock DT, *et al*. Reduced atherosclerosis in myd88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. *Nat Med* 2004;10:416–421.
76. Michelsen KS, Wong MH, Shah PK, Zhang W, Yano J, Doherty TM, Akira S, Rajavashisth TB, Arditi M. Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein e. *Proc Natl Acad Sci USA* 2004;101:10679–10684.
77. Qin C, Nagao T, Grosheva I, Maxfield FR, Pierini LM. Elevated plasma membrane cholesterol content alters macrophage signaling and function. *Arterioscler Thromb Vasc Biol* 2006;26:372–378.
78. Berbee JF, Havekes LM, Rensen PC. Apolipoproteins modulate the inflammatory response to lipopolysaccharide. *J Endotoxin Res* 2005; 11:97–103.
79. Shamshiev AT, Ampenberger F, Ernst B, Rohrer L, Marsland BJ, Kopf M. Dyslipidemia inhibits Toll-like receptor-induced activation of cd8 α -negative dendritic cells and protective th1 type immunity. *J Exp Med* 2007;204:441–452.
80. Fessler MB, Young SK, Jeyaseelan S, Lieber JG, Arndt PG, Nick JA, Worthen GS. A role for hydroxy-methylglutaryl coenzyme a reductase in pulmonary inflammation and host defense. *Am J Respir Crit Care Med* 2005;171:606–615.
81. Jacobson JR, Barnard JW, Grigoryev DN, Ma SF, Tudor RM, Garcia JG. Simvastatin attenuates vascular leak and inflammation in murine inflammatory lung injury. *Am J Physiol* 2005;288:L1026–L1032.
82. Yao HW, Mao LG, Zhu JP. Protective effects of pravastatin in murine lipopolysaccharide-induced acute lung injury. *Clin Exp Pharmacol Physiol* 2006;33:793–797.
83. Morimoto K, Janssen WJ, Fessler MB, McPhillips KA, Borges VM, Bowler RP, Xiao YQ, Kench JA, Henson PM, Vandivier RW. Lovastatin enhances clearance of apoptotic cells (efferocytosis) with implications for chronic obstructive pulmonary disease. *J Immunol* 2006;176:7657–7665.
84. Loike JD, Shabtai DY, Neuhut R, Malitzky S, Lu E, Husemann J, Goldberg IJ, Silverstein SC. Statin inhibition of fc receptor-mediated phagocytosis by macrophages is modulated by cell activation and cholesterol. *Arterioscler Thromb Vasc Biol* 2004;24:2051–2056.
85. Birrell MA, Catley MC, Hardaker E, Wong S, Willson TM, McCluskie K, Leonard T, Farrow SN, Collins JL, Haj-Yahia S, *et al*. Novel role for the liver x nuclear receptor in the suppression of lung inflammatory responses. *J Biol Chem* 2007;282:31882–31890.
86. Smoak K, Madenspacher J, Jeyaseelan S, Williams B, Dixon D, Poch KR, Nick JA, Worthen GS, Fessler MB. Effects of liver x receptor agonist treatment on pulmonary inflammation and host defense. *J Immunol* 2008;180:3305–3312.
87. Myles PR, Hubbard RB, McKeever TM, Pogson Z, Smith CJ, Gibson JE. Risk of community-acquired pneumonia and the use of statins, ace inhibitors and gastric acid suppressants: a population-based case-control study. *Pharmacoepidemiol Drug Saf* 2009; 18:269–275.
88. van de Garde EM, Hak E, Souverein PC, Hoes AW, van den Bosch JM, Leufkens HG. Statin treatment and reduced risk of pneumonia in patients with diabetes. *Thorax* 2006;61:957–961.
89. Chalmers JD, Singanayagam A, Murray MP, Hill AT. Prior statin use is associated with improved outcomes in community-acquired pneumonia. *Am J Med* 2008;121:1002–1007.
90. Mortensen EM, Pugh MJ, Copeland LA, Restrepo MI, Cornell JE, Anzueto A, Pugh JA. Impact of statins and angiotensin-converting enzyme inhibitors on mortality of subjects hospitalized with pneumonia. *Eur Respir J* 2008;31:611–617.
91. Mortensen EM, Restrepo MI, Anzueto A, Pugh J. The effect of prior statin use on 30-day mortality for patients hospitalized with community-acquired pneumonia. *Respir Res* 2005;6:82.
92. Schlienger RG, Fedson DS, Jick SS, Jick H, Meier CR. Statins and the risk of pneumonia: a population-based, nested case-control study. *Pharmacotherapy* 2007;27:325–332.
93. Thomsen RW, Riis A, Kornum JB, Christensen S, Johnsen SP, Sorensen HT. Preadmission use of statins and outcomes after hospitalization with pneumonia: population-based cohort study of 29,900 patients. *Arch Intern Med* 2008;168:2081–2087.
94. Majumdar SR, McAlister FA, Eurich DT, Padwal RS, Marrie TJ. Statins and outcomes in patients admitted to hospital with community acquired pneumonia: population based prospective cohort study. *BMJ* 2006;333:999.

95. Thomsen RW. The lesser known effects of statins: benefits on infectious outcomes may be explained by "Healthy user" Effect. *BMJ* 2006;333:980-981.
96. Schmidt W, De Lint J. Causes of death of alcoholics. *Q J Stud Alcohol* 1972;33:171-185.
97. Pickrell KL. The effect of alcoholic intoxication and ether anesthesia on resistance to pneumococcal infection. *Bull Johns Hopkins Hosp* 1938;63:238-260.
98. Green GM, Kass EH. Factors influencing the clearance of bacteria by the lung. *J Clin Invest* 1964;43:769-776.
99. Astry CL, Warr GA, Jakab GJ. Impairment of polymorphonuclear leukocyte immigration as a mechanism of alcohol-induced suppression of pulmonary antibacterial defenses. *Am Rev Respir Dis* 1983;128:113-117.
100. Boe DM, Nelson S, Zhang P, Bagby GJ. Acute ethanol intoxication suppresses lung chemokine production following infection with *Streptococcus pneumoniae*. *J Infect Dis* 2001;184:1134-1142.
101. Kolls JK, Lei D, Nelson S, Summer WR, Greenberg S, Beutler B. Adenovirus-mediated blockade of tumor necrosis factor in mice protects against endotoxic shock yet impairs pulmonary host defense. *J Infect Dis* 1995;171:570-575.
102. Nelson S, Summer W, Bagby G, Nakamura C, Stewart L, Lipscomb G, Andresen J. Granulocyte colony-stimulating factor enhances pulmonary host defenses in normal and ethanol-treated rats. *J Infect Dis* 1991;164:901-906.
103. Nelson S, Bagby G, Summer WR. Alcohol suppresses lipopolysaccharide-induced tumor necrosis factor activity in serum and lung. *Life Sci* 1989;44:673-676.
104. Kolls JK, Xie J, Lei D, Greenberg S, Summer WR, Nelson S. Differential effects of in vivo ethanol on LPS-induced TNF and nitric oxide production in the lung. *Am J Physiol* 1995;268:L991-L998.
105. Zhao XJ, Marrero L, Song K, Oliver P, Chin SY, Simon H, Schurr JR, Zhang Z, Thoppil D, Lee S, et al. Acute alcohol inhibits TNF- α processing in human monocytes by inhibiting TNF/TNF- α -converting enzyme interactions in the cell membrane. *J Immunol* 2003;170:2923-2931.
106. Zhang Z, Bagby GJ, Stoltz D, Oliver P, Schwarzenberger PO, Kolls JK. Prolonged ethanol treatment enhances lipopolysaccharide/phorbol myristate acetate-induced tumor necrosis factor- α production in human monocyte cells. *Alcohol Clin Exp Res* 2001;25:444-449.
107. Kishore R, McMullen MR, Nagy LE. Stabilization of tumor necrosis factor α mRNA by chronic ethanol: role of a + u-rich elements and p38 mitogen-activated protein kinase signaling pathway. *J Biol Chem* 2001;276:41930-41937.
108. Omidvari K, Casey R, Nelson S, Olariu R, Shellito JE. Alveolar macrophage release of tumor necrosis factor- α in chronic alcoholics without liver disease. *Alcohol Clin Exp Res* 1998;22:567-572.
109. Standiford TJ, Danforth JM. Ethanol feeding inhibits proinflammatory cytokine expression from murine alveolar macrophages *ex vivo*. *Alcohol Clin Exp Res* 1997;21:1212-1217.
110. Zhang P, Bagby GJ, Stoltz DA, Summer WR, Nelson S. Granulocyte colony-stimulating factor modulates the pulmonary host response to endotoxin in the absence and presence of acute ethanol intoxication. *J Infect Dis* 1999;179:1441-1448.
111. Quinton LJ, Nelson S, Zhang P, Happel KI, Gamble L, Bagby GJ. Effects of systemic and local cxc chemokine administration on the ethanol-induced suppression of pulmonary neutrophil recruitment. *Alcohol Clin Exp Res* 2005;29:1198-1205.
112. Mason RJ. Biology of alveolar type II cells. *Respirology* 2006;11: S12-S15.
113. Jeyaseelan S, Manzer R, Young SK, Yamamoto M, Akira S, Mason RJ, Worthen GS. Induction of cxcl5 during inflammation in the rodent lung involves activation of alveolar epithelium. *Am J Respir Cell Mol Biol* 2005;32:531-539.
114. Walker JE Jr, Odden AR, Jeyaseelan S, Zhang P, Bagby GJ, Nelson S, Happel KI. Ethanol exposure impairs LPS-induced pulmonary LIX expression: alveolar epithelial cell dysfunction as a consequence of acute intoxication. *Alcohol Clin Exp Res* 2009;33:357-365.
115. Zhang P, Zhong Q, Bagby GJ, Nelson S. Alcohol intoxication inhibits pulmonary s100a8 and s100a9 expression in rats challenged with intratracheal lipopolysaccharide. *Alcohol Clin Exp Res* 2007;31: 113-121.
116. Zhang P, Bagby GJ, Xie M, Stoltz DA, Summer WR, Nelson S. Acute ethanol intoxication inhibits neutrophil β 2-integrin expression in rats during endotoxemia. *Alcohol Clin Exp Res* 1998;22:135-141.
117. MacGregor RR, Safford M, Shalit M. Effect of ethanol on functions required for the delivery of neutrophils to sites of inflammation. *J Infect Dis* 1988;157:682-689.
118. MacGregor RR, Gluckman SJ, Senior JR. Granulocyte function and levels of immunoglobulins and complement in patients admitted for withdrawal from alcohol. *J Infect Dis* 1978;138:747-755.
119. Gluckman SJ, Dvorak VC, MacGregor RR. Host defenses during prolonged alcohol consumption in a controlled environment. *Arch Intern Med* 1977;137:1539-1543.
120. Cook RT. Alcohol abuse, alcoholism, and damage to the immune system—a review. *Alcohol Clin Exp Res* 1998;22:1927-1942.
121. Sheron N, Bird G, Koskinas J, Portmann B, Ceska M, Lindley I, Williams R. Circulating and tissue levels of the neutrophil chemo-taxis interleukin-8 are elevated in severe acute alcoholic hepatitis, and tissue levels correlate with neutrophil infiltration. *Hepatology* 1993;18:41-46.
122. Tamura DY, Moore EE, Partrick DA, Johnson JL, Offner PJ, Harbeck RJ, Silliman CC. Clinically relevant concentrations of ethanol attenuate primed neutrophil bactericidal activity. *J Trauma* 1998; 44:320-324.
123. Sachs CW, Christensen RH, Pratt PC, Lynn WS. Neutrophil elastase activity and superoxide production are diminished in neutrophils of alcoholics. *Am Rev Respir Dis* 1990;141:1249-1255.
124. McFarland W, Libre EP. Abnormal leukocyte response in alcoholism. *Ann Intern Med* 1963;59:865-877.
125. Austrian R, Gold J. Pneumococcal bacteremia with especial reference to bacteremic pneumococcal pneumonia. *Ann Intern Med* 1964;60: 759-776.
126. Quinton LJ, Nelson S, Boe DM, Zhang P, Zhong Q, Kolls JK, Bagby GJ. The granulocyte colony-stimulating factor response after intra-pulmonary and systemic bacterial challenges. *J Infect Dis* 2002;185: 1476-1482.
127. Tazi A, Nioche S, Chastre J, Smiejan JM, Hance AJ. Spontaneous release of granulocyte colony-stimulating factor (G-CSF) by alveolar macrophages in the course of bacterial pneumonia and sarcoidosis: endotoxin-dependent and endotoxin-independent G-CSF release by cells recovered by bronchoalveolar lavage. *Am J Respir Cell Mol Biol* 1991;4:140-147.
128. Pauksen K, Elfman L, Ulfgren AK, Venge P. Serum levels of granulocyte-colony stimulating factor (G-CSF) in bacterial and viral infections, and in atypical pneumonia. *Br J Haematol* 1994;88: 256-260.
129. Kraggsbjerg P, Jones I, Vikerfors T, Holmberg H. Diagnostic value of blood cytokine concentrations in acute pneumonia. *Thorax* 1995;50: 1253-1257.
130. Shahbazian LM, Quinton LJ, Bagby GJ, Nelson S, Wang G, Zhang P. *Escherichia coli* pneumonia enhances granulopoiesis and the mobilization of myeloid progenitor cells into the systemic circulation. *Crit Care Med* 2004;32:1740-1746.
131. Bagby GJ, Zhang P, Stoltz DA, Nelson S. Suppression of the granulocyte colony-stimulating factor response to *Escherichia coli* challenge by alcohol intoxication. *Alcohol Clin Exp Res* 1998;22: 1740-1745.
132. Michot F, Gut J. Alcohol-induced bone marrow damage: a bone marrow study in alcohol-dependent individuals. *Acta Haematol* 1987;78:252-257.
133. Ballard HS. Hematological complications of alcoholism. *Alcohol Clin Exp Res* 1989;13:706-720.
134. Hernigou P, Beaujean F. Abnormalities in the bone marrow of the iliac crest in patients who have osteonecrosis secondary to corticosteroid therapy or alcohol abuse. *J Bone Joint Surg Am* 1997;79: 1047-1053.
135. Prakash O, Rodriguez VE, Tang ZY, Zhou P, Coleman R, Dhillon G, Shellito JE, Nelson S. Inhibition of hematopoietic progenitor cell proliferation by ethanol in human immunodeficiency virus type 1 tat-expressing transgenic mice. *Alcohol Clin Exp Res* 2001;25: 450-456.
136. Zhang P, Welsh DA, Siggins RW II, Bagby GJ, Raasch CE, Happel KI, Nelson S. Acute alcohol intoxication inhibits the lineage- c-kit+ sca-1+ cell response to *escherichia coli* bacteremia. *J Immunol* 2009;182: 1568-1576.
137. Colvin GA, Lambert JF, Moore BE, Carlson JE, Dooner MS, Abedi M, Cerny J, Quesenberry PJ. Intrinsic hematopoietic stem cell/progenitor plasticity: Inversions. *J Cell Physiol* 2004;199:20-31.
138. Aujla SJ, Dubin PJ, Kolls JK. Th17 cells and mucosal host defense. *Semin Immunol* 2007;19:377-382.

139. Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P, Oliver P, Huang W, Zhang P, Zhang J, *et al*. Requirement of interleukin 17 receptor signaling for lung cxc chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *J Exp Med* 2001;194:519–527.
140. Shellito JE, quan Zheng M, Ye P, Ruan S, Shean MK, Kolls J. Effect of alcohol consumption on host release of interleukin-17 during pulmonary infection with *Klebsiella pneumoniae*. *Alcohol Clin Exp Res* 2001;25:872–881.
141. Rachitskaya AV, Hansen AM, Horai R, Li Z, Villasmil R, Luger D, Nussenblatt RB, Caspi RR. Cutting edge: NKT cells constitutively express il-23 receptor and rorgammad and rapidly produce IL-17 upon receptor ligation in an IL-6-independent fashion. *J Immunol* 2008;180:5167–5171.
142. Braun RK, Ferrick C, Neubauer P, Sjoding M, Sterner-Kock A, Kock M, Putney L, Ferrick DA, Hyde DM, Love RB. IL-17 producing gammadelta t cells are required for a controlled inflammatory response after bleomycin-induced lung injury. *Inflammation* 2008; 31:167–179.