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REVIEW

NF-κB and STAT3 signaling hubs for lung innate immunity

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Abstract Innate immune responses to lung pathogens involve the coordinated expression of myriad affector and effector molecules of innate immunity, which must be induced and appropriately regulated in response to diverse stimuli generated by microbes or the infected host. Many intercellular and intracellular signaling pathways are involved, but we propose NF-κB and STAT3 transcription factors to be especially important signaling hubs for integrating these pathways to orchestrate effective host defense without excessive inflammatory injury.

Keywords Pneumonia · Innate immunity · NF-κB · STAT3 · Transcriptional regulation

Introduction

Acute lower respiratory tract infection is a major public health concern. Amongst the poorest populations, it causes a greater burden of disease than cancer, heart attack, stroke, or any other type of infection (Mizgerd 2006). In wealthy countries such as the U.S., acute lower respiratory infection causes more deaths than any other infection, and the death rate has not substantively improved since antibiotics became of widespread use in the middle of the last century (Armstrong et al. 1999). New approaches for this persistent and pervasive disease are needed.

If microbes landing in the lungs are few and avirulent, then they can be eliminated by resident defenses such as the mucociliary escalator and alveolar macrophages. Larger numbers or more virulent microbes overwhelm or circumvent resident defenses and elicit an inflammatory response consisting of neutrophils and extravascular plasma (Mizgerd 2008). These neutrophils and plasma proteins are innate immune responses essential to host defense. However, neutrophils generate products that can kill cells and digest tissue, and the extravasation of plasma into alveoli leads to the pulmonary edema that is a defining feature of acute lung injury (Ware and Matthay 2000). Thus, these processes must be exquisitely controlled during lung infection in order to allow host defense while limiting inflammatory injury.

A tremendous number of molecules contribute to this control, including an ever-growing list of ligands, receptors, adaptor molecules, enzymes (including kinases, phosphatases, phospholipases, lipoxygenases, cyclooxygenases, oxidases, NO synthases, GTPases, proteases, isomerases, citrullinases, ubiquitin ligases, deubiquitinases, acetyltransferases, deacetylases, nucleotidyltransferases, ribonucleases), transcription factors, repressors, microRNAs (miRNAs), AU-rich element binding proteins (ARE-BPs; an ARE is a region with frequent A and U bases in a mRNA), and others. These myriad factors intersect in complex, intricate, and elegant networks. Signaling networks involve vast numbers of interactions with a substantially smaller number of hubs that are critical to determining the activity of the network (Ma'ayan 2009). We propose that two hubs critical to the signaling network of innate immune responses to microbes in the lung are nuclear factor kappa beta (NF-κB) and signal transducer and activator of transcription 3 (STAT3; Fig. 1). Diverse stimuli converge on these two transcription factors from myriad receptors through multiple signal transduction pathways, and these transcription factors then orchestrate responses by

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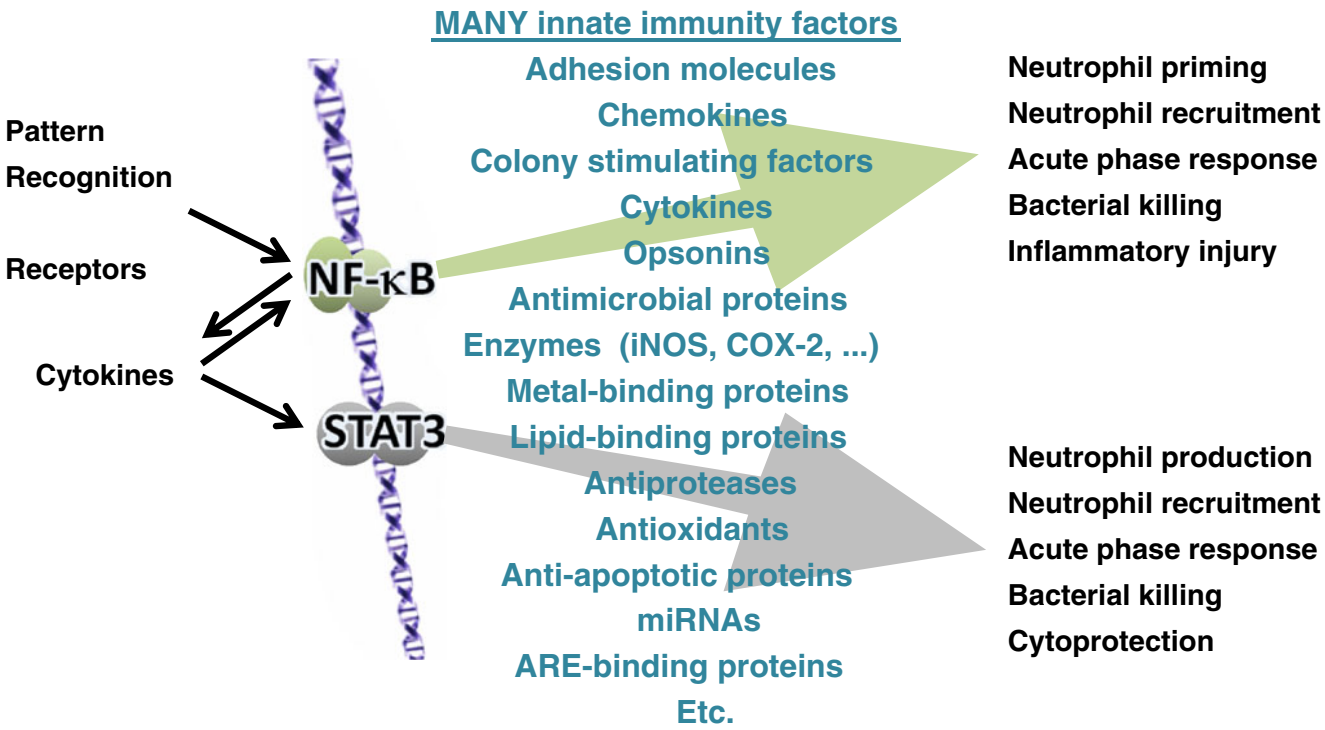

Fig. 1 Roles of nuclear factor kappa beta (*NF-κB*) and signal transducer and activator of transcription 3 (*STAT3*) as signaling hubs mediating innate immunity during acute lower respiratory tract infection (*iNOS* inducible NO synthase, *COX-2* cyclo-oxygenase-2, *miRNAs* microRNAs, *ARE* AU-rich element

regulating and coordinating the expression of many genes that determine the outcome of infection. These hubs function locally, in cells within the infected lungs. In addition, these hubs are important for integrating innate immune responses throughout the organ systems, even for localized infections such as a non-bacteremic pneumonia. Here, we present evidence for the crucial roles of these two transcription factors during pneumonia, highlighting their roles in intrapulmonary and in extrapulmonary cells.

Roles of NF-κB in lung innate immunity

NF-κB is a family of transcription factors including hetero- and homodimers of five different proteins (for a review, see Hayden and Ghosh 2008). At least some NF-κB proteins are expressed in all nucleated cells, being typically concentrated in the cytoplasm of resting cells by inhibitor kappa B (IκB) proteins. NF-κB becomes activated by a wide variety of stimuli relevant to infection and innate immunity, including pattern recognition receptors (PRRs) for microbial molecules, receptors for cytokines, and receptors for products released from damaged cells and tissues. Activation is mediated by the phosphorylation of IκB proteins, which leads to their degradation, after which NF-κB proteins become concentrated in the nucleus and bind κB sites in the DNA to regulate the transcription of nearby genes. To our knowledge, only two NF-κB proteins have been identified as translocating to the nucleus in response to microbial stimuli in the lungs, RelA (also known as p65) and p50 (Mizgerd et al. 2002); the other NF-κB proteins (c-Rel, RelB, and p52) have not been demonstrated to move into the nucleus during acute respiratory infection. Both IκB-α and IκB-β are degraded in mouse lungs after the instillation of lipopolysaccharide (LPS), and each of these IκB proteins associates with RelA in resting lungs (Mizgerd et al. 2002). These IκB proteins are probably regulatory steps that prevent spontaneous inflammation (Beg et al. 1995a; Cheng et al. 1998), with overcoming this inhibition being critical to innate immunity after microbial stimulation.

Targeted mutagenesis of the *Rela* gene causes embryonic lethality from which mice can be rescued by the additional interruption of tumor necrosis factor-α (TNF-α) or TNF receptor 1 (TNFR1; Alcamo et al. 2001). RelA-deficient mice on a TNFR1-deficient background are extremely susceptible to infection and die within weeks of birth unless they are kept under the most stringent barrier conditions and on an antibiotic regimen (Alcamo et al. 2001). In response to either LPS or *Streptococcus pneumoniae* in the lungs, RelA-deficient mice have extreme defects in the expression of multiple innate immunity genes,

including chemokines and adhesion molecules mediating neutrophil recruitment (Alcamo et al. 2001; Quinton et al. 2007). Accordingly, these mice have decreased neutrophil recruitment in the lungs and impaired bacterial clearance from the lungs (Alcamo et al. 2001; Quinton et al. 2007). Thus, the induction of innate immunity genes in the lungs requires NF-κB RelA.

In contrast, targeted mutation of the *Nfkb1* gene, which encodes NF-κB p50, neither results in infections nor compromises the health of mice in the absence of experimental challenges (Sha et al. 1995). In response to *Escherichia coli* or *E. coli* LPS in the lungs, p50 deficiency results in increased pulmonary expression of cytokines, including TNF-α, interleukin-1 (IL-1), IL-6, and chemokines (Mizgerd et al. 2003; Mizgerd et al. 2004a). The precise roles of p50 in limiting cytokine expression are complex and context-specific (Hayden and Ghosh 2008). The data available argue that p50 functions during acute bacterial pneumonia to repress (rather than promote) the expression of the multiple genes regulated by NF-κB. This can be disastrous for the health of the mice, resulting in increased lung injury and death despite no defect in intrapulmonary bacterial clearance during *E. coli* pneumonia (Mizgerd et al. 2003). Similarly, p50 deficiency results in increased cytokine expression, increased lung inflammation, and increased death after systemic administration of LPS (Gadjeva et al. 2004; Han et al. 2009). Thus, p50 is essential to braking the expression of innate immunity genes in the lungs in order to prevent or limit inflammatory injury during infection.

Whereas no humans have, to our knowledge, been identified with deficiencies of RelA or p50, human patient studies support the notion that diminished NF-κB activity increases susceptibility to infection and exaggerated NF-κB activity increases lung injury. For example, patients with deficiencies of MyD88 or interleukin-1 receptor-associated kinase 4 (IRAK-4) cannot activate NF-κB in response to IL-1 or diverse Toll-like receptor (TLR) ligands and are extremely susceptible to bacterial infections including pneumococcus in particular (Ku et al. 2007; von Bernuth et al. 2008). These pathways of NF-κB activation might be especially important for host defense against pyogenic bacteria (Bousfiha et al. 2010), but they probably also contribute to host defense against viruses and fungi in the lungs. Supporting this, TLR4 polymorphisms that decrease NF-κB activation are associated with increased occurrences of severe lung infections with respiratory syncytial virus or *Aspergillus* (Awomoyi et al. 2007; Tal et al. 2004; Carvalho et al. 2008). Conversely, for patients with sepsis, a TLR1 polymorphism that increases NF-κB activation is associated with increased acute lung injury and increased death (Wurfel et al. 2008). Thus, NF-κB is a signaling hub critical to respiratory infection and lung innate immunity in many species including humans.

Cell-specific roles of NF-κB in lung innate immunity

Bone-marrow transplant studies suggest that innate immune responses to microbes in the lungs are dictated by NF-κB in both hematopoietic and non-hematopoietic cells. As with humans (von Bernuth et al. 2008), MyD88 deficiency predisposes mice to bacterial infections, including *Pseudomonas aeruginosa* pneumonia (Skerrett et al. 2004a). Remarkably, reciprocal bone-marrow transplants demonstrate that, whereas MyD88 deficiency in all cells results in 5-log more bacteria in the lungs, MyD88 deficiency in hematopoietic cells alone has no significant effect on bacterial burdens, and MyD88 deficiency in non-hematopoietic cells alone has a significant but modest effect, increasing lung *P. aeruginosa* burdens by less than 1 order of magnitude (Hajjar et al. 2005). Although integrated host defenses are most markedly compromised by widespread MyD88 deficiency, phenotypes are apparent in the compartment-specific mutations. MyD88 deficiency in either the non-hematopoietic cells alone or the hematopoietic cells alone decreases neutrophil recruitment (Hajjar et al. 2005). Expression of the the ELR+CXC chemokines CXCL1 and CXCL2 is decreased by MyD88 deficiency in the non-hematopoietic cells, whereas expression of the early response cytokines TNF-α and IL-1β is diminished by MyD88 deficiency in hematopoietic cells (Hajjar et al. 2005). Thus, the widespread deficiency of MyD88 and downstream signaling such as NF-κB is disastrous, but deficiencies restricted to hematopoietic or non-hematopoietic cell types also show modest but significant phenotypes.

In which non-hematopoietic cells is the NF-κB signaling pathway important? Many studies implicate epithelial cells in the lung. Overexpression of a dominant negative (dn) IκB-α protein, in which an alanine residue replaces a serine whose phosphorylation by IκB kinase-β (IKK-β) is essential for signaling its proteasomal degradation and release of associated NF-κB proteins, is one strategy for diminishing the canonical pathway of NF-κB activation. Overexpressing dnIκB-α in alveolar epithelial cells (driven by the surfactant protein C [SPC] promoter) or in conducting airway epithelial cells (driven by the Clara Cell 10 [CC10] promoter) is sufficient to decrease the expression of cytokines elicited by LPS in the lungs, resulting in decreased neutrophil recruitment (Poynter et al. 2003; Skerrett et al. 2004b). Inhibition of NF-κB by SPC-driven dnIκB-α overexpression in alveolar epithelial cells compromises host defense during pneumococcal pneumonia, as measured by lung bacterial burdens (Quinton et al. 2007). Similarly, inhibition of NF-κB by CC10-driven deletion of IKK-β in airway epithelial cells compromises host defense during Group B *Streptococcus* (GBS) pneumonia, as measured by lung bacterial burdens (Fong et al. 2008).

Thus, NF-κB activity is required in epithelial cells specifically for effective innate immunity in the lung. Conversely, the overexpression of a constitutively active IκB kinase in airway epithelial cells (driven by an inducible CC10 system) is sufficient to activate NF-κB in these cells, inducing pulmonary inflammation (including cytokine expression and neutrophil recruitment) and causing lung injury (including pulmonary edema, arterial hypoxemia, and death; Cheng et al. 2007). Thus, excessive NF-κB activity in airway epithelial cells alone results in acute lung injury. Together, these studies indicate epithelial cells as being relevant sites of the NF-κB signaling hub for lung innate immunity.

Several lines of evidence implicate NF-κB signaling in hematopietic cells. In mice, reciprocal bone-marrow chimera studies demonstrate that MyD88 deficiency in hematopoietic cells decreases cytokines and neutrophil recruitment during *P. aeruginosa* pneumonia (Hajjar et al. 2005), that TLR4 deficiency in hematopietic cells decreases cytokines and neutrophil recruitment after LPS aerosolization (Hollingsworth et al. 2005), and that deficiency of RIP2 (a NOD-like receptor that activates NF-κB) in hematopietic cells increases bacterial burdens during *Chlamydiae pneumoniae* pneumonia (Shimada et al. 2009). In human patients, stem cell transplantation resulting in hematopoietic cells with a haplotype conferring a hyporesponsive TLR4 (stimulating diminished NF-κB activation) increases the risk of invasive aspergillosis (Bochud et al. 2008). Conversely, if mice are reconstituted with hematopoietic cells deficient in some of the brakes on the NF-κB system (such as IκB-α or NF-κB p50), then LPS-induced pulmonary inflammation as measured by neutrophil recruitment is increased (Han et al. 2009). Thus, leukocyte NF-κB plays pivotal roles in lung innate immunity.

In some cases (Hollingsworth et al. 2005; Shimada et al. 2009), the effects of hematopoietic deficiencies in NF-κB signaling pathways can be ameliorated by intratracheal instillation of wild-type macrophages, suggesting that the macrophage might be one hematopoietic cell in which NF-κB is important to lung innate immunity. Consistent with this, and providing the most direct evidence to date that NF-κB in myeloid cells is a determinant of the outcome of lung infection, the mutation of RelA driven by the M lysozyme (*LysM*) locus decreases neutrophil recruitment and increases susceptibility of mice to *P. aeruginosa* pneumonia (Hess et al. 2010). The RelA products from macrophages that are essential to integrated host defenses against bacteria in the lungs have not been identified in these studies. In what seems to be stark contrast, the myeloid deletion of IKK-β via LysM targeting results in increased neutrophils and fewer bacteria during GBS pneumonia (Fong et al. 2008). Further studies are needed to clarify the roles of NF-κB signaling in myeloid cells, and to identify specific roles of NF-κB in distinct myeloid cell subsets in the lung (such as alveolar macrophages, dendritic cells, recruited neutrophils, and exudate monocyte/macrophages). Activation of NF-κB in myeloid cells outside of the lung may also be critical to host defense in the lung, as discussed below.

Of course, neither alveolar macrophages nor epithelial cells function alone. Signaling in each cell type may be influenced by the other. An important NF-κB pathway during pneumonia is the activation of epithelial cells by the macrophage-derived cytokines TNF-α, IL-1α, and IL-1β. Some microbes (such as pneumococcus) do not directly activate alveolar epithelial cells (Quinton et al. 2007). During pneumococcal pneumonia, alveolar epithelial cell NF-κB activation requires TNF-α, IL-1α, or IL-1β (Quinton et al. 2007; Jones et al. 2005). Blocking TNF-α or IL-1 signaling pathways individually has little or no effect during pneumococcal pneumonia, but their simultaneous blocking results in pronounced defects in NF-κB signaling, cytokine expression, and neutrophil recruitment, rendering mice highly susceptible to pneumococcal pneumonia (Jones et al. 2005). A clinical trial involving rheumatoid arthritis patients has demonstrated that combining a TNF receptor fusion protein (etanercept) with an IL-1 receptor antagonist (anakinra) significantly increases the risk of serious infections, particularly pneumonia, compared with single agent treatment (Genovese et al. 2004). Thus, in both mice and humans, the cytokines TNF and IL-1 have overlapping roles that are essential to host defense in the lungs, probably attributable to the activation of NF-κB in alveolar epithelial cells.

Roles of STAT3 in lung innate immunity

STAT3 is a transcription factor that becomes activated by tyrosine phosphorylation, which stimulates STAT3 homodimerization and the dimerization of STAT3 with other proteins such as STAT1 (Schindler et al. 2007). Dimers of tyrosine-phosphorylated STAT3 concentrate in the nucleus and bind DNA, regulating transcription. Tyrosine-phosphorylated STAT3 is observed in the lungs of mice with bacterial or viral pneumonia or acute inflammation elicited by endotoxemia, immune complexes, ozone, or hyperoxia (Severgnini et al. 2004; Gao et al. 2004; Hokuto et al. 2004; Matsuzaki et al. 2006; Quinton et al. 2008; Kida et al. 2008; Ikegami et al. 2008; Lang et al. 2008). In addition to functioning as a transcription factor in the nucleus, metabolic roles of STAT3 in the mitochondria have recently been demonstrated (Gough et al. 2009; Wegrzyn et al. 2009), but any relevance to lung innate immunity for mitochondrial STAT3 is purely speculative at present.

The most compelling evidence that STAT3 is important for acute lower respiratory infections comes from human studies. Hyper-IgE syndrome (HIES, also known as Job's syndrome) is a rare genetic disease resulting from mutations in STAT3 rendering it transcriptionally inactive (Holland et al. 2007; Minegishi et al. 2007). Patients are heterozygous, and the dimerization of mutant allele products with themselves and with STAT3 from the functional allele results in ~25% normal STAT3 activity in patient cells (Minegishi et al. 2007). This defect in STAT3 signaling causes HIES patients to suffer from repeated bacterial pneumonias attributable to *S. pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, and other common causes of community-acquired pneumonia, implicating a defect in lung host defense (Freeman and Holland 2009). In childhood, these patients develop pneumatoceles, suggesting a defect in lung injury repair, and these pneumatoceles then become sites of opportunistic infections of the lung, especially by Gram-negative bacteria and fungi (Freeman and Holland 2009). Pneumonia is the direct or indirect cause of death for HIES patients (Freeman et al. 2007).

HIES patients have a defect in Th17 cells (Milner et al. 2008; Ma et al. 2008), which may be one contributing factor in their susceptibility to lung infections. Th17 cells generate IL-17A, IL-17F, and IL-22, and signaling from these cytokines is important in lung host defense (Aujla et al. 2008; Ye et al. 2001). However, the sources of these cytokines in infected lungs have not been identified, and many cells other than Th17 cells can generate IL-17 and IL-22 (Mills 2008; Wolk et al. 2010). Whether Th17 cells specifically are important for lung host defense, or whether HIES patients have defects in IL-17 or IL-22 in their infected lungs is unclear. Mice deficient in STAT3 in all T cells or all CD4+ cells do not have a phenotype resembling HIES (Takeda et al. 1998; Harris et al. 2007), and bone-marrow transplantation in an HIES patient has failed to prevent immunodeficiency and infections (Gennery et al. 2000), results that, when taken together, suggest that STAT3 has roles beyond the development of Th17-like cells, which are essential to preventing lung infections and the resultant lung disease characteristic of HIES patients.

STAT3 becomes activated in the lungs of mice in response to microbial stimuli in the air spaces, including LPS, bacteria, or virus (Matsuzaki et al. 2006; Quinton et al. 2008; Ikegami et al. 2008), but STAT3 is not directly activated by signaling from PRRs for microbial molecules. IL-6 (which is induced by NF-κB during pneumonia; Quinton et al. 2007) is clearly one source of STAT3 activation during bacterial pneumonia, since the deficiency of IL-6 diminishes total levels of tyrosine-phosphorylated STAT3 in the lungs during pneumonia (Jones et al. 2006). IL-6 makes essential contributions to lung host defense, as mutation of IL-6 in mice leads to decreased neutrophil recruitment and bacterial clearance (Jones et al. 2006). These phenotypes do not correlate with the diminished expression of neutrophil chemokines or adhesion molecules. The molecular mechanisms responsible for decreased neutrophil recruitment and increased bacterial burdens in IL-6-deficient mice with pneumonia have yet to be identified.

All the IL-6 family cytokines can activate STAT3, and many IL-6 family cytokines are induced during bacterial pneumonia including oncostatin M (OsM), leukemia-inhibiting factor (LIF), and IL-11, in addition to IL-6 (Quinton et al. 2008). Using activation of an epithelial cell line as a reporter system for STAT3 activating stimuli in bronchoalveolar lavage fluid, Quinton et al. (2008) have demonstrated that the combination of IL-6 and LIF is responsible for virtually all STAT3 activation during the first 24 h of infection. Roles of LIF during lung infection are an important area for future research. By 48 h of infection with pneumonia, factors other than IL-6, LIF, and OsM are responsible for half of the STAT3-stimulating activity in the bronchoalveolar lavage fluid (Quinton et al. 2008), and the identity of these factors remains to be determined.

Several other cytokines that activate STAT3 are also essential determinants of lung innate immunity, although to our knowledge, no direct information is available about their roles in STAT3 activation during lung infection. IL-22 activates STAT3, and IL-22 blockade increases lung infection and lung injury during bacterial pneumonia (Aujla et al. 2008). The roles of IL-22 appear to involve epithelial cells particularly, with this cytokine enhancing barrier integrity and antimicrobial protein expression (Aujla et al. 2008). IL-23 activates STAT3, and IL-23 blockade increases lung infection during bacterial pneumonia (Happel et al. 2005). The role of IL-23 during pneumonia appears to be in driving the expression of IL-17 and IL-22 in the lungs, possibly from T cells (Aujla et al. 2008; Happel et al. 2005). IL-10 activates STAT3, and IL-10 receptor blockade increases lung injury elicited by influenza infection in the lungs (Sun et al. 2009a). The sites of IL-10 action may be the myeloid cells recruited to the infected lung, including monocyte/macrophages and neutrophils (Sun et al. 2009a). Increasing IL-10 expression can be protective against pneumonia-induced lung injury (Wang et al. 2005), but the anti-inflammatory effects of excess IL-10 compromise bacterial clearance (Sun et al. 2009b). STAT3 is essential to the anti-inflammatory actions of this cytokine in myeloid cells (Takeda et al. 1999), but the relevance to the lung of IL-10 signaling to macrophage STAT3 has not been specifically investigated. STAT3 can also be activated by other mediators implicated in lung host defense, such as leptin (Mancuso et al. 2002, 2006) or vascular endothelial growth factor (Yano et al. 2006), and others that may be

speculated to influence lung host defense (albeit not yet tested, to our knowledge), such as IL-21, IL-27, or IL-35. Thus, STAT3 is a probable signaling hub for IL-6, LIF, IL-22, IL-23, IL-10, and many other possible mediators contributing to lung innate immunity to determine the outcome of acute lower respiratory infection.

STAT3 has been mutated in epithelial cells in the lung by using Cre transgenes driven by SPC or CC10. Such mice demonstrate increased susceptibility to lung injury induced by diverse stimuli, including *E. coli* pneumonia, LPS-induced pulmonary inflammation, adenoviral infection, hyperoxia, and naphthalene cytotoxicity (Hokuto et al. 2004; Matsuzaki et al. 2006; Quinton et al. 2008; Kida et al. 2008; Ikegami et al. 2008). Protection against lung injury is probably attributable to the multiple roles of STAT3 including the prevention of apoptosis, the promotion of repair processes involving cell migration and proliferation, and the regulation of surfactant homeostasis. STAT3 in the alveolar epithelium also contributes to neutrophil recruitment and anti-bacterial host defense (Quinton et al. 2008). Although modest in comparison, these findings are consistent with such phenotypes resulting from IL-6 deficiency or HIES and suggest that STAT3 in the epithelium is one mechanism by which IL-6 contributes to host defense, and that the alveolar epithelial cell is one of the cells in which STAT3 functions to prevent lung infection.

Emerging extrapulmonary roles of NF-κB and STAT3 signaling in lung innate immunity

Effective immune responses within the confines of the intrapulmonary space are critical for both promoting lung host defenses and limiting the possibility of systemic disease. Integral components of the local milieu are, however, systemically derived blood constituents such as hematopoietic cells and extravasated plasma proteins. Much of our existing knowledge regarding innate immune responses within infected airspaces is born from research conducted to identify the functions and regulation of locally synthesized antimicrobial factors and/or factors enabling the movement of key extrapulmonary mediators into the lungs. Although the importance of these systemic elements is appreciated within the context of lung infections, our understanding of the way that extrapulmonary tissues respond to lung infections and of the extent of these responses is remarkably limited. The hepatic acute phase response (APR) and hematopoiesis represent two classical systemic reactions to infection and injury. As with local innate immunity, we propose that NF-κB and STAT3 serve as particularly important signaling hubs for these systemic innate immune responses during lung infection.

Roles of NF-κB and STAT3 in the hepatic APR

The APR was first recognized nearly 80 years ago in patients with pneumococcal pneumonia (Tillett and Francis 1930) and is now considered a hallmark of infection and injury. It is defined by significant changes (>25%) in circulating concentrations of acute phase proteins (APPs), of which there are at least 40 (Gabay and Kushner 1999). Select members of this family are routinely used as biomarkers of disease severity in patients, including those with pneumonia (Almirall et al. 2004; Smith et al. 1995; Yip et al. 2005). These proteins are typically liver-derived, based largely on studies revealing a sensitive capacity of hepatocytes to synthesize them (Andus et al. 1988; Ruminy et al. 2001) and seemingly disproportionate expression levels in the liver as compared with other tissues (Meek and Benditt 1986). The net influence of APPs on outcome during infections such as those in the lung is speculative, but known functions ascribed to individual APPs imply that their presence is generally beneficial to host defense and tissue protection. These functions are diverse, including but not limited to bacterial opsonization, bacteriostatic and bactericidal effects, cytokine induction, increased chemotaxis, anti-oxidant activity, metal transport, and protease inhibition (Gabay and Kushner 1999).

The quantity and diversity of APPs make the dissection of the regulatory mechanisms and functions of the APR, as an integrated biological response, a complex undertaking. Several transcription factors involved in the APR have been identified (Ruminy et al. 2001). Amongst these, NF-κB RelA and STAT3, the latter of which was originally known as the “acute phase response factor” (Akira et al. 1994; Zhong et al. 1994), might represent particularly important regulatory nodes for APP transcription in response to bacterial stimuli in the lungs (Quinton et al. 2009). Both transcription factors can directly mediate APP expression and are exquisitely responsive to cytokines that are present and essential during pneumonia, namely TNFα and IL-1 (activating RelA) and IL-6 (activating STAT3; Andus et al. 1988; Thorn et al. 2004). Requirements for RelA and STAT3 have been reported in vitro for APP gene expression (Quinton et al. 2009; Betts et al. 1993; Patel et al. 2007). RelA and STAT3 have also been shown to interact physically in a manner that is required for the transcriptional activity of at least some APPs (Hagihara et al. 2005; Uskokovic et al. 2007).

In vivo, the determination of the direct physiological roles of RelA and STAT3 during the APR or otherwise has historically been challenging, in large part because of the embryonic lethality of mice lacking functional genes for either factor (Beg et al. 1995b; Takeda et al. 1997). Conditional mutation, however, has revealed STAT3 as a requirement for maximal APP expression in mouse liver

during endotoxemia and polymicrobial sepsis (Alonzi et al. 2001; Sakamori et al. 2007). Site-specific targeting of NF-κB signaling has also been accomplished but has not yet been used to interrogate APP expression specifically. Interestingly, mutation of NF-κB RelA, IKKβ, or NEMO (NF-κB essential modulator), all of which are critical components of the canonical NF-κB signaling pathway (Hayden and Ghosh 2008), promotes liver injury in some contexts of severe inflammation (Geisler et al. 2007; Luedde et al. 2005), possibly influencing the extrapulmonary pathophysiology manifested during pneumonia or other conditions.

The early response cytokines TNFα and IL-1 together with IL-6 are well-recognized activators of APP synthesis in hepatocytes (Andus et al. 1988; Thorn et al. 2004; Kopf et al. 1994; Zahedi and Whitehead 1993). Moreover, as discussed above, these three cytokines are essential for host defense during pneumonia, albeit for reasons that are incompletely understood (Jones et al. 2005, 2006; Mizgerd et al. 2004b; van der Poll et al. 1997). It is plausible that hepatocytes are important targets of TNFα, IL-1, and IL-6 during an acute pulmonary inflammation. In response to bacteria or bacterial products administered into the lungs of mice, APPs are expressed in liver in association with the increased activation of NF-κB and STAT3 (Quinton et al. 2009; Mizgerd et al. 2001). In mice lacking a functional gene for IL-6, the induction of some APPs and STAT3 activation are nearly eliminated during bacterial pneumonia compared with the responses observed in their wild-type counterparts (Quinton et al. 2009). IL-6-dependent APP expression has also been demonstrated in response to intratracheal LPS (Vernooy et al. 2005; Gamble et al. 2008). Interestingly, the reliance upon IL-6 for STAT3 activation during a type of pneumonia elicited by Gram-positive or Gram-negative bacteria is considerably more pronounced in the liver compared with the lung (Jones et al. 2006; Quinton et al. 2009), reinforcing the possibility that hepatocytes are an important facilitator of IL-6-mediated immune responses. Mice devoid of all signaling receptors for TNFα and IL-1 also lack maximal liver APP expression (Quinton et al. 2009). Furthermore, signaling from TNFα and IL-1 receptors is required for NF-κB RelA activation in liver during pneumonia (Quinton et al. 2009; Mizgerd et al. 2001), suggesting that, like IL-6, immune properties conferred by early response cytokines during pneumonia may extend to hepatocytes.

The net physiological consequence of the APR in the context of lung innate immunity or otherwise is unknown. Myriad APPs are involved, all which exhibit different and sometimes opposing effects on immune function and/or tissue protection. Studies have, however, implied important roles for individual APPs following an intrapulmonary challenge. Serum amyloid P (SAP), a prominent short pentraxin APP in mice similar in structure and function to human C-reactive protein (CRP), promotes the deposition of complement component C3 on the surface of pneumococcus and is required for bacterial clearance and survival in pneumonic mice (Yuste et al. 2007). In these studies, defects in SAP-deficient mice could be rescued by administration of human SAP (Yuste et al. 2007). Similarly, human CRP administration is protective in mice infected with *S. pneumoniae* (Mold et al. 1981). LPS-binding protein (LBP) facilitates the binding of LPS with CD14, thereby enhancing inflammatory responses during infections. LBP levels are elevated in mice in response to bacterial stimuli, and its presence is required for maximal innate immunity and host defense (Gamble et al. 2008; Branger et al. 2004; Brass et al. 2004; Fan et al. 2002; Knapp et al. 2003). As the field progresses, major challenges for investigators include: (1) determining the influence of all APP changes in response to a given physiological stress, rather than to single APPs, which are rarely if ever regulated as a lone entity; and (2) discriminating between the functions of baseline APP concentrations and those produced outside of homeostatic conditions. Most APPs are present in abundant concentrations in healthy subjects, and APP deletion may reveal distinct functions for APPs compared with eliminating acute phase changes in APP concentrations. Identifying and manipulating critical points of APP regulation will be necessary to gain true insight regarding the function and significance of the APR during lung innate immune responses. To date, research in this field suggests that NF-κB, STAT3, and the cytokines promoting their activity represent the foundation of a lung-liver axis and an important circuit through which the hepatocytes respond to infectious or other stimuli in the lung.

Roles of NF-κB and STAT3 in granulopoiesis

As potential pathogens subvert the resident defense mechanisms of the respiratory tract, recruitment of neutrophils becomes a critical innate immune component for preventing overwhelming infection. In animal models, the clearance of bacterial, viral, and fungal pathogens from the lung is markedly diminished in settings of reduced neutrophilic inflammation (Greenberger et al. 1996; Rehm et al. 1980; Tsai et al. 2000), and neutropenia or neutrophil dysfunction predisposes patients to opportunistic lung infections (Pennington 1986; Winkelstein et al. 2000). However, neutrophils are short-lived in the circulation (4–8 h), requiring a constant release of newly differentiated cells from the bone marrow to maintain normal baseline quantities and even greater demand in response to infections (Bicknell et al. 1994). So how does the supply meet the demand? During a pulmonary innate immune response, the bone marrow must be able to respond, directly or indirectly, to stimuli elaborated

within the airspaces. Further, cells within the bone marrow must be appropriately tuned to respond to pathogens at the site of infection. Evidence is now available supporting the idea that signaling from NF-κB and STAT3 is central amongst the processes required for eliciting the extrapulmonary neutrophil response.

Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic growth factor that strongly influences the differentiation, mobilization, and function of neutrophils (Demetri and Griffin 1991). The most well-recognized biological role of G-CSF is to control the maturation and release of granulocytes from the bone marrow under both normal and emergency conditions (Panopoulos and Watowich 2008; Lieschke et al. 1994; Liu et al. 1996). Indeed, the administration of recombinant G-CSF has long been used to re-establish blood neutrophil levels in neutropenic patients (Welte et al. 1996). Although signaling downstream of the G-CSF receptor involves numerous transcription factors, STAT3 activity has been shown to mediate its hematopoietic effects. Mutation of the STAT3-binding domain of the G-CSF receptor renders mice neutropenic, a phenotype that is ameliorated by over-expression of constitutively active STAT3 (McLemore et al. 2001). In addition, mice lacking functional STAT3 in myeloid progenitor cells have a reduced granulopoietic response following administration of G-CSF or *Listeria monocytogenes* (Panopoulos et al. 2006). STAT3-deficient neutrophils are also less chemotactic toward CXCR2 ligands, which are critical mediators of alveolar neutrophil recruitment during lung infections (Tsai et al. 2000), indicating that G-CSF is involved in both the quantity and quality of circulating neutrophils (Panopoulos et al. 2006).

G-CSF levels are highly elevated in the lungs and blood of both mice and humans in response to bacteria or bacterial products in the airspaces (O'Grady et al. 2001; Quinton et al. 2002). Indeed, the robust increase in circulating G-CSF content during an acute pulmonary inflammation stands somewhat in contrast to that of other cytokines, which are more compartmentalized within challenged airspaces (Boutten et al. 1996; Nelson et al. 1989). Following the administration of either recombinant G-CSF or *E. coli* into mouse lungs, circulating and bone-marrow neutrophil progenitors are increased in association with elevated bone-marrow STAT3 activity, suggesting that lung-derived G-CSF is sufficient to initiate STAT3-mediated granulopoiesis in the marrow (Shahbazian et al. 2004). During *P. aeruginosa* pneumonia, mice lacking the G-CSF receptor exhibit significant reductions in neutrophil numbers in the bone marrow, blood, and consequently air spaces of the lung (Gregory et al. 2007). This hematopoietic defect results in impaired bacterial clearance, worsened lung histopathology, and increased numbers of apoptotic neutrophils (which may reflect roles for STAT3 in cell survival), indicating that G-CSF signaling to STAT3 is critical for the innate immune response to lung infections (Gregory et al. 2007). G-CSF is a crucial means through which the lungs communicate with bone marrow and circulating neutrophils, allowing them to respond to localized infections in the lung.

NF-κB promotes the expression of numerous factors important for pulmonary innate immunity, including TNFα, IL-1, IL-6, and G-CSF (Quinton et al. 2007; Dunn et al. 1994; Pahl 1999), all of which, as described above, target extrapulmonary tissues in response to stimuli in the lung. In this capacity, NF-κB activity in the lung is a key initiator of extrapulmonary responses during an acute pulmonary inflammation. Recent evidence also identifies an additional extrapulmonary role for NF-κB tuning neutrophils in the bone marrow to be more effective at killing lung pathogens including *S. pneumoniae* and *S. aureus* (Clarke et al. 2010). The microbial flora of mice releases bacterial products that circulate through the blood and activate NF-κB in bone-marrow neutrophils. This NF-κB activation primes neutrophils and increases their ability to kill bacteria. In such a way, NF-κB signaling in the bone marrow might be essential to innate immune host defense in the lungs. The degree to which the NF-κB-mediated microbiome-to-bone-marrow circuit directly impacts pulmonary host defense and whether and when signals from the infected lung influence NF-κB activity and neutrophil priming in the marrow are compelling questions that remain to be addressed.

Roles of NF-κB and STAT3 in directing post-transcriptional regulation of lung innate immunity?

Whereas NF-κB and STAT3 are hubs of transcriptional regulation, innate immunity is also regulated at post-transcriptional levels (Anderson 2010; O'Connell et al. 2010). Roles of the major mediators of post-transcriptional regulation, miRNAs and ARE-BPs, in tuning innate immunity are complex and only beginning to be elucidated. Clearly, miRNAs and ARE-BPs regulate the expression of many extracellular and intracellular innate immunity mediators (Jing et al. 2005; Jones et al. 2009; Taylor et al. 1996), often to limit but also sometimes to enhance protein expression from targeted transcripts. The types of miRNA and ARE-BP change in response to receptor recognition of microbial products, and some of these changes alter innate immunity gene expression. Excitingly, many of these miRNAs and ARE-BPs are themselves under transcriptional regulation by NF-κB or STAT3. As but a few examples from the rapidly advancing field of miRNA regulation of innate immunity, TLR4 signaling can, in distinct settings, mediate NF-κB-dependent induction of mir-146 (which

targets TRAF6 ad IRAK-1 to tune TLR signaling; Taganov et al. 2006), of mir-9 (which targets *Nfkb1* to diminish NF-κB p50 and p105; Bazzoni et al. 2009), of mir-147 (which limits cytokine expression by as yet unknown means; Liu et al. 2009), of mir-21 (which targets PDCD4 to enhance pro-inflammatory cytokines; Sheedy et al. 2010), and of mir-155 (which targets SHIP1 to enhance pro-inflammatory kinase activity; McCoy et al. 2010). Similarly, the STAT3-mediated transcription of mir-21 can be essential to its anti-apoptotic function (Loffler et al. 2007), and the STAT3-mediated repression of mir-155 can be essential to the protective anti-inflammatory effects of IL-10 (McCoy et al. 2010). Although most of this review has focused on innate immune responses to pathogens in the lung, data about if, when, or how the regulation of miRNAs and/or ARE-BPs by NF-κB or STAT3 contributes to effective pulmonary host defense without excessive lung injury are virtually lacking. We suggest this is the crucial next frontier that needs to be crossed to further our understanding of innate immunity to pathogens in the lung.

Concluding remarks

NF-κB and STAT3 integrate inputs from diverse stimuli including microbial ligands and host-derived cytokines, and they mediate transcription levels for myriad genes including those for cytokines, chemokines, colony-stimulating factors, adhesion molecules, acute phase proteins, antimicrobial proteins, and anti-apoptotic proteins. As such, these signaling hubs coordinate and orchestrate innate immune responses to microbes in the lungs (Fig. 1). A current area of interest is the activity of these signaling hubs outside of the lung, as recent data suggest that extrapulmonary sites of transcriptional regulation contribute to innate immunity functions during acute lower respiratory infection. Finally, whereas NF-κB and STAT3 are the best recognized such hubs at present, other hubs are likely to emerge. Important future goals will to identify post-transcriptional hubs governing lung innate immunity and to determine how much these post-transcriptional hubs are themselves influenced by NF-κB and STAT3 activities during lung infection.

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