Chapter 8
Innate Immune Responses in Ventilator-Associated Pneumonia

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8.1 Introduction

Mechanical ventilation is a life-saving treatment of patients with acute and chronic respiratory failure. However, an adverse consequence of this intervention is the development of ventilator-associated pneumonia (VAP), which results in considerable morbidity and mortality in hospitalized patients (American Thoracic Society; Infectious Diseases Society of America 2005; Fujitani et al. 2011). VAP is defined as the development of pneumonia within 48–72 h after endotracheal intubation. Although the incidence of VAP is decreasing, still 9–27% of ventilated patients will develop this complication, with the highest incidence occurring in the first 10 days after intubation. Endotracheal intubation increases the risk of developing health care associated pneumonia by 6–20-fold. As compared to health care associated pneumonia (HAP) in non-intubated patients, both actual and attributable mortality is higher in VAP. Patients with certain underlying lung diseases, such as acute lung injury (ALI) and acute respiratory distress syndrome (Richardson et al. 1982), have a particularly high incidence of VAP (Richardson et al. 1982). Conversely, VAP represents a major risk factor for the development of ALI and ARDS.

8.2 Etiology of VAP

VAP can be caused by an array of Gram-negative and Gram-positive bacterial pathogens, and may be polymicrobial in up to a third of cases (American Thoracic Society; Infectious Diseases Society of America 2005; Fujitani et al. 2011).
The most common cause of VAP is by the Gram-positive bacteria *Staphylococcus aureus*, with methicillin resistant *S. aureus* (MRSA) representing over 60% of the *S. aureus* isolates in VAP. Other VAP-causing pathogens include aerobic Gram-negative bacilli such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* species, *Acinetobacter* species, and *Stenotrophomonas maltophilia*. *Legionella pneumophila* is an obligate intracellular bacterial pathogen that is an etiologic agent in both community acquired pneumonia (CAP), HAP and VAP. Viruses and fungi are unusual causes of VAP, although these organisms can modulate innate mucosal responses predisposing to the development of VAP. While the bacterial pathogens that cause VAP are similar to those that cause HAP in non-intubated patients, VAP is more frequently caused by pathogens with intrinsic resistance to multiple antimicrobial agents, including *P. aeruginosa*, *Acinetobacter* species, *S. maltophilia*, and MRSA. Mortality is considerably higher in patients with VAP due to *P. aeruginosa* strains that express the type III secretion system required for the secretion of pseudomonal exotoxins S, T, U, and Y (Roy-Burman et al. 2001; Sadikot et al. 2005). A recent and disturbing trend is the increasing prevalence of community acquired strains of MRSA (CA-MRSA) as a cause of nosocomial infections, including VAP(Kashuk et al. 2010). CA-MRSA, which is typically the USA300 strain, produce an array of exotoxins that promote extensive tissue necrosis and cavity formation. The intrinsic antibiotic resistance of these Gram-positive and Gram-negative bacterial strains contributes to increased mortality in patients with VAP (Fujitani et al. 2011). However, these pathogens are generally less virulent and invasive than pathogens that cause pneumonia in otherwise healthy individuals in the community, and tend to be invasive in hosts with anatomic defects in the respiratory tract or substantial impairment in lung mucosal innate immunity. Therefore, the presence of these bacterial species as pathogens identifies patients with profound anatomic defects or defects in lung innate immunity.

### 8.3 Pathogenesis of VAP

The vast majority of VAP cases develop as a result of microaspiration of bacteria colonizing the oropharynx (American Thoracic Society; Infectious Diseases Society of America 2005). Oropharyngeal colonization occurs very rapidly in critically ill patients. For example, nearly 75% of patients with underlying lung disease and/or undergoing oropharyngeal intubation were found to be colonized by pathogenic bacteria within 24 h of admission to the intensive care unit (Garroute-Orgeas et al. 1997). Reservoirs contributing to oropharyngeal colonization include the nasopharynx, sinuses, and stomach. Endotracheal tubes contribute to colonization by directly injuring mucosal surfaces of the upper respiratory tract, which facilitates bacterial adhesion. Organisms that cause VAP, including *P. aeruginosa* and *S. aureus*, promote biofilm formation with the endotracheal tube lumen, which can function as a nidus for direct inoculation of infected material into the distal airspaces. Less common sources of bacterial inoculation include colonization of the ventilator circuit or direct inoculation via infected aerosols or instrumentation, particularly suction catheters or
bronchoscopes. By comparison, hematogenous seeding of the lung as a cause of VAP is considerably less common, accounting for <15% of cases. Notable exceptions are hematogenous seeding from an intravascular *S. aureus* infection or gut bacterial translocation that can occur in immunocompromised patients with neutropenia.

Microaspiration is a common event in both healthy and critical ill patients. These events rarely result in infection in healthy subjects, primarily due to highly effective means to eradicate infectious or toxic insults of the respiratory tract, which include efficient mucocilliary clearance mechanisms and robust innate mucosal antimicrobial responses. In mechanically ventilated patients, impairments in both mucociliary transport and innate cellular responses results in the establishment of pulmonary infection. A summary of factors contributing to the pathogenesis of VAP is shown in Fig. 8.1.

### 8.4 Structural Changes in the Respiratory Tract in Mechanically Ventilated Patients

Ciliated, pseudostratified columnar epithelial cells line the tracheobronchial tree. These ciliated cells are critical to effective mucociliary transport and the cephalad movement of mucous, microbes, and acellular debris present within the conducting airways. Damage to ciliated cells can occur as a direct result of endotracheal intubation
or conditions that predispose the patient to respiratory failure (Nicholls et al. 2003; Piatti et al. 2005; Pittet et al. 2010). As discussed previously, denuding of columnar epithelial cells can result from the endotracheal tube or endotracheal tube cuff. Moreover, lung conditions that can result in mechanical ventilation, such as COPD, are associated with impaired mucocilliary transport (Piatti et al. 2005). Moreover, certain forms of infectious lung injury, including severe acute respiratory syndrome (SARS) is characterized by bronchial epithelial denudation and loss of cilia (Nicholls et al. 2003). Similarly, influenza infection predisposes to secondary bacterial infection, which is due not only to impairment in lung innate responses, but also disruption of mucocilliary transport mechanisms (Pittet et al. 2010).

8.5 Impairment in Innate Immunity

Many forms of critical illness result in a profound state of immune suppression affecting both the cellular and acquired arms of host immunity. This syndrome of immune suppression has been best characterized and is perhaps most severe in sepsis, but has also been described in trauma patients, burn injury patients, and patients during the peri-operative period. Sepsis is a complex clinical syndrome resulting from the interaction between microbe and host. Clinically, it is defined as the systemic inflammatory response syndrome (SIRS) with evidence of infection (Members of the American College of Chest Physicians/Society of Critical Care Medicine 2003). Changes in the population at risk for the development of sepsis, including an increase in the number of elderly and immunocompromised patients, has resulted in a steady rise in the incidence of severe sepsis (Martin et al. 2003). Despite improvements in supportive care and immunomodulatory therapies, the mortality rate from severe sepsis remains unacceptably high (Brun-Buisson 2000).

Host immune responses in critical illness, including sepsis can be conceptualized as occurring in distinct but overlapping phases. The initial response during critical illness, referred to as the systemic inflammatory response syndrome (SIRS), is characterized by the release of a number of pro-inflammatory mediators, including early responses cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin 12 (IL-12) leukocyte-active chemokines, adhesion molecules, and inflammatory leukotrienes (Dinarello 2000). SIRS is counter-regulated by the release of inhibitory molecules, including anti-inflammatory cytokines (e.g., interleukin 10 (IL-10), transforming growth factor-beta (TGF-β)), suppressors of pathogen recognition signaling cascades, immunomodulatory prostanoids and hormones. This counter-regulatory phase is referred to as the compensatory anti-inflammatory response syndrome (CARS) (Wesche et al. 1999; Bone 1996). Molecules released during CARS are believed to serve as a functional “brake” on systemic inflammation, and the expression of these mediators is induced by both microbial-derived and host-derived signals. SIRS and CARS overlap considerably, hence the overall immune status of the patient is dependent on which response predominates (Fig. 8.2) (van der Poll and van Deventer 1999). Recent evidence
suggests a third response to an inflammatory insult, referred to as the mixed antago-
nist response syndrome (MARS). This response is characterized by the secretion of
both pro- and anti-inflammatory mediators (specifically IL-6, IL-8, monocyte chemot-
actic protein (MCP)-1, macrophage inflammatory protein (MIP)-1β, IFN-γ, granulocyte-macrophage colony stimulating factor (GM-CSF), and IL-10) (Tamayo
et al. 2011). Consistent with this mixed systemic cytokine response, elevated levels of
IL-6 in circulation has been shown to predict the development of VAP (Ramirez et al.
2009). Whether the initial SIRS response drives the expression of molecules that
contribute to immune suppression or simply a marker of systemic inflammation
remains to be determined. A summary of innate immune events in critical illness is
shown in Fig. 8.2.

The compensatory release of anti-inflammatory molecules in sepsis is believed to
mediate immunosuppression during the peri-septic or post-injury period, during which
time immune cell function is substantially impaired (historically referred to as critical
illness-induced leukocyte “deactivation” or “immunoparalysis”). Recently, since the
altered leukocyte phenotype in critical illness involves selective regulation of some,
but not all innate genes, this phenomenon is now more appropriately referred to as
reprogramming. Leukocyte reprogramming appears to be of considerable clinical significance, as higher rates of nosocomial infection and increased mortality are observed in postoperative, burn injury or septic patients who display evidence of monocyte deactivation, either in the form of decreased monocyte HLA-DR expression, ex vivo cytokine production or impaired delayed-type hypersensitivity responses (Appel et al. 1989; Munoz et al. 1991). Septic patients are especially susceptible to nosocomial infections of the lung, particularly pneumonia from multidrug-resistant Gram-positive and Gram-negative organisms, including *S. aureus* and *P. aeruginosa* (Richardson et al. 1982; Mustard et al. 1991). Sepsis-induced immunosuppression is particularly prominent in patients with preexisting deficiencies in innate and acquired immunity, including the elderly and patients with chronic medical conditions (Hotchkiss and Karl 2003).

### 8.6 Alterations of Leukocyte Function in Critical Illness and Mechanical Ventilation

Patients undergoing severe stress, including trauma, massive hemorrhage, burn injury, post-surgery, and sepsis exhibit significant defects in circulating and resident leukocyte populations. In addition, changes in the pulmonary microenvironment that occur as a result of mechanical ventilation substantially influence lung innate responses. Multiple leukocyte subtypes are affected and specific defects are shown in Fig. 8.3.

#### 8.6.1 Monocytes/Macrophages

While sepsis and similar stress-associated events have been shown to influence the effector activity of a variety of immune cells, the majority of studies have focused on peripheral blood monocytes (PBM), and to a lesser extent tissue macrophages. Changes in monocyte/macrophage function in sepsis resemble but are not identical to those observed in endotoxin-tolerized macrophages. Endotoxin tolerance describes the phenomena whereby upon initial exposure to LPS, cells become refractory to a secondary stimulus with LPS. Pathogen-associated molecular patterns (PAMPs) other than LPS can also induce a tolerance phenotype, and PAMPs of one type can induce cross tolerance to a different PAMP. Induction of tolerance results in suppression of multiple inflammatory genes, including both NF-κB and mitogen-activated protein kinase (MAPK)-dependent genes (e.g., TNF-α, IL-6, iNOS). Tolerance does not cause global suppression of all genes, as genes encoding certain antimicrobial and phagocytic proteins, including cathelicidin antimicrobial peptide, lipocalin, the scavenger receptor MARCO and the fMLP receptor, are indeed super-induced in response to sequential exposure to LPS (Foster et al. 2007). It is also noteworthy that the induction of this phenotype is not restricted to myeloid
cells, as structural cells, including alveolar epithelial cells, have been shown to develop a tolerance response upon repeated exposure to PAMPs. The LPS or PAMP tolerized phenotype is transient in nature and entirely reversible, and has been associated with remodeling of chromatin in the promoter region of several tolerizable genes (Foster et al. 2007; Chan et al. 2005).

Critical illness, like endotoxin tolerance, leads to inhibition of a broad range of NF-κB-dependent inflammatory genes in monocytes. Most notably, a significant reduction in the ex vivo production of inflammatory cytokines, including IL-1α, IL-1β, IL-6, and TNF-α has been observed in monocytes isolated from patients with sepsis (Munoz et al. 1991). This change in cytokine production may be a predictor of outcome, as peripheral monocytes isolated from those who survived sepsis regained their ability to produce cytokines in response to LPS stimulation, and...
monocytes isolated from the nonsurvivors did not (Munoz et al. 1991). Conversely, the production of certain anti-inflammatory proteins, including IL-10, IL-1 receptor antagonist, and the TNF soluble receptor I and II are enhanced in monocytes isolated from sepsis patients or patients with ventilator-induced lung injury (Frank et al. 2006). Patients with sepsis or early trauma have reduced monocyte HLA-DR expression (Appel et al. 1989; Adib-Conquy et al. 2006). This reduction in HLA-DR expression has been reported to directly correlate with the magnitude of sepsis (Volk et al. 2000) and may partially contribute to impaired cell-mediated immunity observed in patients with critical illness.

Similar critical illness-induced defects have been noted in macrophages residing in various tissues, which in some instances have been associated with evidence of enhanced macrophage apoptosis (Ayala et al. 1992; Gallinaro et al. 1994). In particular, alveolar macrophage function has been shown to be impaired in the setting of sepsis. For example, alveolar macrophages recovered from mice with abdominal sepsis (cecal ligation and puncture) display reduced production of inflammatory cytokines, chemokines, eicosanoids, nitric oxide, and respiratory burst (Reddy et al. 2001; Goya et al. 1992). Importantly, these phenotypic alterations in alveolar macrophage effector function are associated with a markedly enhanced susceptibility to intrapulmonary challenge with both Gram-positive and Gram-negative bacterial pathogens (Steinhauser et al. 1999; Deng et al. 2006). Little is known about alveolar macrophage phenotype in critically ill patients at risk for the development of VAP. However, we have performed Affymetrix microarray analysis on adherence purified alveolar macrophages recovered from patients with sepsis-induced ALI within 3 days of onset of sepsis. Relative to alveolar macrophages recovered from healthy subjects, lung macrophages from sepsis-induced ALI patients displayed a hybrid tolerized/alternatively activated phenotype, as characterized by minimal change or suppression of NF-κB-dependent genes (e.g., TNF-α, IL-1β, IL-6, iNOS), induction of antimicrobial genes (antimicrobial peptides, chemoattractant, and phagocytosis genes), and expression of makers of alternative (M2) rather than classical (M1) activation (high arginase, CCR2, IL-4Rα, MMP expression; low iNOS, interferon-γ, and IFN-inducible chemokine expression) (Gordon and Martinez 2010). Although this expression pattern may partially reflect the lung injury response, it is likely that the phenotype is shaped by systemic inflammation.

### 8.6.2 Neutrophils

Alterations in neutrophils (PMN), resembling those described in monocyte/macrophages, are present during the septic response and are predictive of adverse outcomes in these patients. Systemic inflammation promotes cytoskeletal changes in PMN cell membrane rigidity and reduced cellular deformability, resulting in impaired recruitment to sites of infection and deleterious accumulation and activation of PMN in vascular beds of distant organs. Directed migration is also impaired by nitric oxide-mediated inhibition of ICAM and VCAM-dependent adhesion and transmigration of PMN,
downregulation of the chemokine receptor CXCR2, and inhibition of G-protein
coupled receptor signaling (Benjamim et al. 2000; Cummings et al. 1999; Czermak
et al. 1999; Huber-Lang et al. 2001; Swartz et al. 2000). Microarray analysis of PMN
isolated from septic patients within 24 h of onset reveals a global suppression of immune
regulation and inflammatory response gene clusters, particularly genes regulated in an
NF-κB-dependent fashion (Tang et al. 2007). Conversely, the expression of selected
suppressive genes was enhanced, including the NF-κB inhibitor NFκBIA.

The discovery of neutrophil extracellular traps (NETs) has provided yet another
role for neutrophils in the containment of infection. NETs are complex structures
composed of nuclear chromatin, histones, a variety of granular antimicrobial pro-
teins and some cytoplasmic proteins (Urban et al. 2009). Formation occurs in
response to exposure of neutrophils to plasma from septic patients (Clark et al.
2007) as well as direct contact with microbial pathogens (Remijsen et al. 2011). Neutrophil elastase is released from azurophilic granules, assisting in the formation
of NETs via decondensation of nuclear chromatin, which along with other serine
proteases confer antimicrobial responses (Papayannopoulos et al. 2010). NET-
associated myeloperoxidase directly contributes to bacterial killing of
Staphylococcus aureus in the presence of H₂O₂ (Parker et al. 2012). NETs are capa-
ble of physically ensnaring bacteria and facilitating the interactions between bacte-
ria and antimicrobial effectors, ultimately leading to enhanced bacterial killing
(Mantovani et al. 2011). Despite their broad antimicrobial capacity, some bacteria
express nucleases to degrade NETs, thus avoiding capture and bacterial cell death
(Buchanan et al. 2006; Berends et al. 2010; Young et al. 2011). In some cases, NETs
may exert detrimental effects to the host. Increasing evidence links NET formation
to excessive inflammation and tissue damage in diseases such as sepsis (Clark et al.
2007). NET formation has recently been demonstrated in the alveoli of mice with
influenza H1N1 pneumonia, and these structures contribute to acute lung injury
responses in these animals (Narasaraju et al. 2011). While the presence of NETs has
not been clearly established in experimental bacterial pneumonia or in patients with
VAP, it is tempting to speculate that these structures may contribute to lung injury
that can occur in this setting.

8.6.3 Dendritic Cells

Dendritic cells (DC) are the most efficient professional antigen-presenting cells
(APC) in the lung and have the unique ability to induce primary immune responses in
 naïve T cells. DC are prevalent centrally within the spleen, lymphatics, and at mucosal
surfaces, most notably in gut and respiratory tract. Systemic endotoxin administration
in mice results in a brisk depletion in splenic DC by 24 h post-LPS. Similarly, there
is a prolonged loss of DC out to 15 days post-induction of abdominal sepsis in both
lung and spleen (Wen et al. 2008). In humans with lethal sepsis, follicular DC are
substantially diminished early in the course of disease (Hotchkiss et al. 2002).
Similarly, reductions in blood myeloid DC and plasmacytoid DC (27 and 53% of controls, respectively) have been observed in patients admitted to the hospital with pneumonia, and numbers of DC inversely correlated with procalcitonin levels, a marker of systemic inflammation (Dreschler et al. 2012). Endotoxin-tolerized DC or DC isolated from animals or humans with sepsis produce low levels of IL-12 and TNF-α, but high levels of IL-10 (Wen et al. 2008; Wysocka et al. 2001). This shift in cytokine profiles can persist for up to 6 weeks post-abdominal sepsis (CLP), and has been associated with posttranslational epigenetic modifications of histones binding to the IL-12 p35 and p40 promoters and increased susceptibility to pulmonary fungal challenge (Wen et al. 2008). Regulatory DC, or “tolerogenic” DC, are a newly described DC population that can be induced by incubation of bone marrow-derived DC with IL-10, resulting in DC that preferentially secrete IL-10 rather than IL-12, and induce T cell tolerance. A naturally occurring DC\textsubscript{reg} population has been identified in spleen (CD11c\textsuperscript{low}, CD45RB\textsuperscript{high}), and adoptive transfer of this cell population to septic mice diminished inflammatory cytokine production and sepsis-induced lethality (Fujita et al. 2006). Changes in the number, distribution, and function of these cells in lung, especially during critical illness, have not yet been explored.

8.6.4 Lymphocytes

Like other leukocyte populations, various lymphocyte populations are influenced by and likely contribute to the immunosuppressive effects of critical illness. This effect can be directly due to changes in lymphocytes numbers or effector functions, or indirectly due to changes in APC function, most notably DC. Studies consistently show that sepsis or other states of extreme stress (trauma, burn injury) generally result in anergy and a shift in T cell cytokine responses favoring a Th2-, rather than Th1-phenotype response.

Sepsis, trauma, and other critical states result in a substantial drop in the number of circulating lymphocytes. Lymphopenia develops early after the insult, and the persistence and magnitude of lymphopenia correlates with risk of nosocomial infection and death (Hotchkiss et al. 2001). Autopsy studies in septic patients revealed a profound loss of splenic B cells, CD4+ T cells, and follicular dendritic cells. No alterations in numbers of CD8+ T cells were observed. The loss of B and CD4+ T cells was mediated by caspase-9-dependent apoptosis. Similar changes, although not as uniform, could be observed in critically ill patients without sepsis (Hotchkiss et al. 2001).

In addition to changes in the absolute number of lymphocytes, the septic response can induce considerable alterations in lymphocyte effector function. For instance, the memory/effector CD8+/CD45RO+ T lymphocyte subset in nonsurviving septic patients demonstrate significantly decreased IFN-γ synthesis compared with survivors (Zedler et al. 1999). Similarly, T cell proliferative responses and cytokine production (IL-2, TNF-α) were significantly depressed in patients with abdominal sepsis, as compared to healthy controls, and the degree of IL-2 and TNF-α suppression directly correlated with patient survival (Heidecke et al. 1999). The proportion of Th2 T cells is increased in patients with sepsis, but not in non-septic critically ill
control patients and healthy subjects (Ferguson et al. 1999). Similar observations have been made in animal models of sepsis. Splenocytes isolated from mice undergoing CLP produced less IL-2, IL-12, and IFN-\(\gamma\), and more IL-4 and IL-10 than splenocytes isolated from healthy animals (Ayala et al. 1994; O’Sullivan et al. 1995). Given the importance of Th1 phenotype responses in host defense against both intracellular and extracellular microbial pathogens, this shift away from an appropriate Th1- and towards a dysregulated Th2-phenotype response has obvious implications for antimicrobial host immunity.

Regulatory T cells (Treg), are a limited but important population of CD4+, CD25+ T cells that universally express the transcription factor Forkhead box p3 (Foxp3). Treg inhibit CD4+ and CD8+ T cell effector functions, resulting in negative regulation of both innate and acquired immune responses.Suppressive effects of Treg are mediated by both direct cell–cell contact and through the release of soluble mediators, including but not limited to TGF-\(\beta\) and IL-10. An increase in the percentage (but not absolute number) of Treg has been found in blood, lymphatics, or spleen in septic mice and humans with sepsis or trauma (Venet et al. 2008; Scumpia et al. 2006; Wisnoski et al. 2007). Moreover, there is evidence of enhanced Foxp3 expression and suppressive function of Treg in mice with abdominal sepsis, and adoptive transfer of Treg into septic mice reduced overzealous TNF-\(\alpha\) production and improved mortality. However, the depletion of CD4+ CD25+ Treg in mice with polymicrobial sepsis had little impact on sepsis-induced mortality (Scumpia et al. 2006; Wisnoski et al. 2007). Thus, the role of Treg in controlling the systemic inflammatory response, or as a mediator of impaired innate and acquired immunity in critically ill patients at risk for VAP, is uncertain and requires further study.

A recently described B cell may play a critical role in innate responses during localized and systemic infection (Rauch et al. 2012). Innate response activator B (IRA-B) cells are a population of CD19+, B220+ cells that produce large quantities of GM-CSF during infection. This population expands in bone marrow and spleen in response to systemic LPS administration or abdominal sepsis, and the genetic deletion of these cells resulted in marked reduction of systemic cytokine responses, GM-CSF expression, and the ability to clear abdominal polymicrobial infection.

### 8.7 Alterations of Pathogen Recognition Receptors and/or Signaling Cascades in Critical Illness

Microbes and microbial components that initiate the septic response are recognized by both cell surface and intracellular pathogen recognition receptors (PRR), including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLR). Toll-like receptors are a family of evolutionarily conserved type I transmembrane receptors that respond to PAMPs expressed by a diverse group of infectious microorganisms, resulting in activation of the host’s immune system (Aderem and Ulevitch 2000; Akira and Hemmi 2003). There exist 13 distinct TLRs (10 in humans and 13 in mice) that have in common an extracellular domain with
leucine rich repeats and an intracytoplasmic domain shared with the IL-1 receptor (IL-1R). Binding of ligands to TLRs initiates a signaling cascade involving myeloid differentiation marker 88 (MyD88), IL-1R-associated kinases (IRAK 1 and 4), and TNFR-associated factor 6 (TRAF6), resulting in NF-κB translocation and MAPK activation, culminating in expression of genes involved in antimicrobial host defense (Aderem and Ulevitch 2000; Akira and Hemmi 2003). In addition, certain TLRs, such as TLR2, TLR3, and TLR4 can initiate a MyD88-independent signaling cascade that requires the adaptor proteins Toll-IL-1 receptor domain containing adaptor protein inducing interferon (TRIF) and TRIF-related adaptor molecule (TRAM), resulting in the expression of interferon responsive genes. The most relevant TLRs in lung antibacterial host defense include TLR2, which recognizes specific components of Gram-positive bacteria and fungi; TLR4, which is the major receptor for LPS; TLR5, which recognizes and is activated by bacterial flagellin; and TLR9, which is activated by unmethylated CpG motifs present in microbial but not mammalian DNA. In addition to PAMPs, TLRs can be activated by host-derived danger signals, referred to as damage-associated molecular patterns (DAMPs) or alarmins, and include heat shock proteins and matrix components (Ohashi et al. 2000). Also, high-mobility group box 1 protein (HMGB1) is a molecule released during the septic response that has recently been shown to activate TLR2 and TLR4 (Park et al. 2004). This is of particular relevance in the setting of sepsis and acute lung injury.

Multiple TLRs participate in lung host immunity against Gram-negative bacteria. For example, TLR4 recognizes the lipid A moiety of LPS, and is the major TLR mediating early innate responses and clearance of non-flagellated Gram-negative organisms that cause VAP, including *K. pneumoniae*, *H. influenza*, and *E. coli* (Schurr et al. 2005; Bhan et al. 2010; Wieland et al. 2005). In addition, mice deficient in TLR9 display impaired dendritic cell-mediated responses during experimental *Klebsiella or Legionella* pneumonia, culminating in reduced lung bacterial clearance and decreased survival (Bhan et al. 2007, 2008). Innate responses to the flagellated extracellular bacteria *P. aeruginosa* are mediated by several MyD88-dependent TLRs, predominantly TLR4 and TLR5 (Hajjar et al. 2005; Ramphal et al. 2008; Skerrett et al. 2004). Interestingly, both bone marrow-derived and stromal cells contribute to MyD88-dependent innate responses to *P. aeruginosa* in the lung (Hajjar et al. 2005).

Toll-like receptors appear to play a lesser role in host defense against *S. aureus*. For example, while TLR2 has been shown to mediate inflammatory responses to the staphylococcal toxin Panton-Valentine Leukocidin, neither TLR2, TLR4, nor MyD88 is required for effective anti-staphylococcal host immunity during respiratory infection (Skerrett et al. 2004; Zivkovic et al. 2011). The nucleotide-binding oligomerization domain (NOD)-like receptors (NLR) NOD1 and NOD2, which recognize the peptidoglycan component muramyl dipeptide (MDP), have been shown to be important in inflammatory cytokine release and bacterial eradication in a murine *S. aureus* skin infection model(Hruz et al. 2009; Inohara et al. 2005). More recently, mice deficient in RIP2, the shared NOD1/2 adaptor molecule, are considerably more susceptible to intrapulmonary challenge with *S. aureus* than wild-type mice, an effect which is dependent on downstream activation of inflammasome-caspase-1-dependent IL-1β release (unpublished observations, J. Deng). These later
observations suggest that NLRs, rather than TLRs, may be the predominant contributors to anti-staphylococcal immunity in the lung.

### 8.8 Suppression of PRR Expression, Binding or Downstream Signaling Cascades

#### 8.8.1 Alterations of Cell Surface Expression of TLRs and LPS Binding Partners

Some, but not all studies have identified changes in the cell surface expression of various TLRs during the septic response. In particular, either enhanced or reduced cell surface expression of TLR2 and TLR4 have been described in monocytes from sepsis patients and in tissue macrophages during experimental sepsis (Deng et al. 2006; Brunialti et al. 2006). Moreover, changes in monocyte cell surface expression of LPS binding partners MD2, CD14, and CD71 have also been observed in sepsis (Brunialti et al. 2006; Wolfs et al. 2008; Williams et al. 1998). Disparate findings are likely attributable to temporal differences in assessment of TLR expression and the heterogeneity of patient populations studied and animal models employed. The extracellular domains of certain TLRs can be shed from activated macrophages, and serve as sinks to bind extracellular PAMPs, and as a consequence dampen TLR-mediated signal transduction. For instance, soluble TLR2 (sTLR2) is released by human peripheral blood monocytes (PBM) and diminishes the cellular response to the TLR2 agonist Pam3Cys without affecting cellular responses to LPS (LeBouder et al. 2003). Both naturally occurring and recombinant soluble TLR4 have been shown to diminish responses to LPS (Iwami et al. 2000; Hyakushima et al. 2004). The contribution of soluble TLR2 and TLR4 to impaired innate responses during critical illness remains to be determined.

Illuminating the importance of TLRs in lung innate immunity during critical illness, combined loss of function polymorphisms in both TLR4 and the TLR4 adaptor TIRAP/Mal, or a homozygous TIRAP/Mal polymorphism have been causally linked to reduced circulating inflammatory cytokine levels, reduced ex vivo monocyte cytokine expression, and increased risk for serious postoperative infections, including VAP (Ferwerda et al. 2009).

### 8.9 Inhibitors of TLR Signaling

#### 8.9.1 Interleukin-1 Receptor-Associated Kinase-M

Molecules have been identified that inhibit TLR signaling at multiple sites downstream of the receptor. Interleukin-1 receptor-associated kinase (IRA K)-1 and -4 are key kinases necessary for both MyD88-dependent and IL-1 receptor-mediated
signal transduction. Consequently, interruption of IRAK-1 and -4 phosphorylation or trafficking can have profound effects on the downstream expression of both NF-κB and MAPK-dependent inflammatory or antimicrobial genes. Interleukin-1 receptor-associated kinase-M (IRAK-M), also named IRAK-3, is a member of the IRAK family. However, IRAK-M differs from IRAK-1 and IRAK-4 in that this protein lacks kinase activity and IRAK-M has been shown to be a negative regulator of TLR signaling by blocking the disassociation of IRAK-1 from the Toll-IL-1 signaling domain. Bone marrow-derived or lung macrophages lacking IRAK-M display enhanced MAPK kinase activation and inflammatory cytokine production in response to TLR agonists and live bacteria (Wesche et al. 1999; Kobayashi et al. 2002). Importantly, IRAK-M is induced by endotoxin, the NOD-2 ligand muramyl dipeptide (MDP), and other PAMPs and is required for the development of tolerance to endotoxin and peptidoglycan (Kobayashi et al. 2002; Hedl et al. 2007; Nakayama et al. 2004). We have found that IRAK-M is upregulated in alveolar macrophages during experimental sepsis in a MyD88-dependent fashion, and mediates both the suppression of macrophage cytokine responses and impaired lung clearance of P. aeruginosa in septic mice (Deng et al. 2006; Lyn-Kew et al. 2010). IRAK-M has also been shown to suppress TLR-mediated responses in murine primary alveolar epithelial cells (Seki et al. 2010). Emerging data suggests that IRAK-M may be a major mediator and perhaps a biomarker for severity of disease in sepsis. IRAK-M is substantially induced in monocytes from healthy subjects administered LPS intravenously (van’t Veer et al. 2007). In patients with Gram-negative sepsis, blood monocytes demonstrate a more rapid and robust expression of IRAK-M when stimulated ex vivo with LPS (Escoll et al. 2003). Additionally, enhanced expression of IRAK-M mRNA has been noted in pediatric patients with sepsis, and high IRAK-M mRNA levels were associated with longer length of intensive care unit (ICU) stay, need for mechanical ventilation and death (Hall et al. 2007). We have also observed high constitutive expression of IRAK-M mRNA in alveolar macrophages and peripheral blooduffy coat cells isolated from patients with sepsis-induced ALI, as compared to similar cell populations from healthy subjects (T. Standiford, unpublished observations). In fact, IRAK-M was the only negative regulator of TLR signaling found to be significantly induced in this patient population.

8.9.2 Other Negative Regulators of TLR Signaling Cascades

Several other molecules have been causally linked with the development of endotoxin tolerance or hyperinflammatory responses to LPS in genetically deficient mice. Suppression of tumorigenicity 2 (ST2) is a transmembrane protein and soluble secreted protein that is expressed by a variety of cells, including T cells and macrophages. ST2 inhibits MyD88-dependent signaling by interfering with the ability of Mal/TIRAP and MyD88 to interact with downstream signaling molecules. This protein appears to contribute to sepsis-induced impairment in lung antibacterial
defense, at least in animal models (Holub et al. 2003). Specifically, CLP-induced impairment in anti-pseudomonal lung host defense is reversed in mice deficient in ST2. Interestingly, responsiveness of ST2−/− AM was not altered, whereas the expression of IFN-γ and TNF-α from CD4+ and CD8+ T cells was preserved in ST2−/− mice in the setting of abdominal sepsis, as compared to similarly treated wild-type animals.

Toll-like receptor signaling can also be modulated by both extracellular and intracellular decoys. Single immunoglobulin IL-1R-related protein (SIGIRR) is a member of the IL-1 receptor superfamily but is unable to signal. However, the extracellular domain of this molecule inhibits Toll-IL-1 signaling by interfering with binding of ligands to TLR4, TLR5, TLR9, and IL-1 receptor I, whereas the intracellular domain interferes with the complexing of IRAK-1 with TRAF-6 (Thomassen et al. 1999; Wald et al. 2003; Qin et al. 2005). SIGIRR is expressed predominantly by epithelial cells, including alveolar epithelial cells, but also to a lesser degree in monocytic populations. Mice deficient in SIGIRR have enhanced inflammatory responses to LPS challenge. Moreover, SIGIRR is upregulated in the PBM of septic patients, and is associated with the development of endotoxin tolerance in these cells (Adib-Conquy et al. 2006). MyD88 short (MyD88s) is an alternatively spliced variant of the parent molecule, MyD88. MyD88s functions as a dominant negative molecule by blocking recruitment of IRAK-4 to the toll-IL-1 signaling domain, resulting in reduced phosphorylation of IRAK-1 (Burns et al. 2003; Rao et al. 2005). The expression of MyD88s is induced in monocytes in response to LPS and is constitutively expressed in blood monocytes isolated from patients with sepsis (Adib-Conquy et al. 2006). Tollip disrupts IRAK-1 and IRAK-4 interactions, whereas microRNA 146 (miRNA 146) post-transcriptionally inhibits IRAK-1 and TRAF6 expression (Nahid et al. 2011). The suppressors of cytokine signaling (SOCS) are a family of molecules that predominately inhibit JAK-Stat signaling, but also disrupt TLR signaling cascades through a yet undefined mechanism. While these latter molecules could contribute to suppression of TLR-mediated responses during critical illness, there is no data to show enhanced expression and/or activity in blood monocytes or lung macrophages in patients at risk for the development of VAP.

8.10 Microenvironmental Factors that Regulate Innate Host Responses in VAP

8.10.1 Mechanical Ventilation

Initiation of mechanical ventilation (MV) is a vital therapeutic intervention in patients with respiratory failure. A consequence of mechanical ventilation is the inhomogeneous distribution of pressure and volumes to various regions of lung, resulting in excessive stretch in some alveolar units (referred to as volutrauma),
and repeated alveolar collapse in other regions (referred to as atelectotrauma) (Pugin et al. 1998). Excessive lung stretch results in activation of several transcriptional pathways, including the NF-κB and the MAPK kinase pathway (Fos, Jun), which contributes to the release of various inflammatory mediators such as TNF-α, IL-1β, IL-6, and IL-8 (Gharib et al. 2009; Halbertsma et al. 2005; Jaecklin et al. 2011). These cellular mediators not only trigger deleterious lung injury responses and possibly multiple organ dysfunction (An et al. 2011), but may also promote the reprogramming of leukocytes and structural cells that occurs in critical illness. Importantly, MV at moderate to high lung volumes can also prime the lung for enhanced lung injury or systemic organ failure in response to an infectious challenge (e.g., second hit). For instance, as compared to spontaneously breathing animals, the intrapulmonary administration of S. aureus or E. coli to mechanically ventilated mice results in enhanced lung inflammation and lung injury, without changes in lung bacterial clearance (Dhanireddy et al. 2006). Likewise, the i.p. administration of LPS to mice undergoing high tidal volume MV substantially increased lung and systemic cytokine expression and extrapulmonary organ injury, as compared to non-mechanically ventilated controls (O’Mahony et al. 2006). Mechanisms accounting for synergistic interactions between lung stretch and infectious challenge have not been clearly defined. However, previous work has shown that stretch of human alveolar epithelial cells increases the expression of TLR2 by sixfold (Charles et al. 2011). Moreover, mechanical ventilation increased the relative expression of TLR2 and TLR4 in lung tissue and increased the generation of endogenous ligands for TLR4 in bronchoalveolar lavage fluid (Vaneker et al. 2008). Recent work has shown that mechanical ventilation also generates other TLR4-independent and MyD88-dependent endogenous TLR ligands (Chun et al. 2010). Hyperinflation of the lung with high tidal volume not only promotes a significant increase in the expression of TLR4, but also paradoxically induces the expression of IRAK-M, an important negative regulator of TLR signaling (Villar et al. 2010).

A frequent consequence of mechanical ventilation and diseases that cause acute respiratory failure is alveolar collapse and atelectasis. Alveolar collapse is due, in part, to reductions in surfactant that occur in patients receiving mechanical ventilation and in patients with VAP (Nakos et al. 2003). Atelectasis has been shown to promote bacterial overgrowth, and use of open ventilation strategies and administration of exogenous surfactant can reduce bacterial numbers in an animal model of VAP (van Kaam et al. 2004). Moreover, administration of positive end-expiratory pressure (PEEP) at 5–8 cmH₂O to non-hypoxemic mechanically ventilated patients can reduce the incidence of VAP (Manzano et al. 2008). Surfactant proteins A and D can agglutinate P. aeruginosa, and SP-D can serve as an opsonin to enhance phagocytosis of P. aeruginosa (McCormack 2006). Pseudomonal elastase has been shown to degrade SP-A and SP-D, and these proteins are decreased in the lungs of patients with cystic fibrosis (Mariencheck et al. 2003). However, changes in SP-A and SP-D levels during mechanical ventilation and/or VAP have not been well characterized.
8.10.2 High Ambient Oxygen Concentrations

Administration of high concentrations of oxygen (FIO\textsubscript{2} >50\%) used during transient or prolonged mechanical ventilation is a common treatment for patients with respiratory failure (Gore et al. 2010). Although therapeutically necessary, hyperoxia results in the generation of reactive oxygen species (ROS), which promote the breakdown of critical barriers leading to systemic cellular and organ injury (Lee and Choi 2003). In the lung, ROS cause severe cellular damage and death, exposure of the basement membrane and disruption of the alveolar capillary membrane leading to increased pulmonary permeability, influx of inflammatory cells, and impaired gas exchange (Bhandari and Elias 2006). Hyperoxic exposure can also exacerbate alveolar epithelial injury and apoptosis in response to infectious challenge with \textit{P. aeruginosa} or \textit{L. pneumophila}, resulting in increased bacterial dissemination (Kikuchi et al. 2009). Moreover, high oxygen tensions inhibit the function of innate immune cells. For instance, macrophages exposed to elevated concentration of oxygen both in vitro and in vivo display reduced phagocytosis and killing of Gram-negative bacteria which correlated with changes in cell morphology and actin polymerization (O’Reilly et al. 2003). In addition, in vivo hyperoxia exposure increased the susceptibility to \textit{K. pneumoniae} lung infections, an effect that was partially attributed to reduced BAL GM-CSF levels and cell surface expression of TLR4 by AM (Baleeiro et al. 2003). Importantly, systemic treatment of these mice with GM-CSF during hyperoxia preserved macrophage functionality and decreased the severity of lung infection (Baleeiro et al. 2006). Taken together, hyperoxia is detrimental to the host by promoting greater alveolarcapillary injury, impairing local antibacterial responses, and increasing the risk of bacterial dissemination.

8.10.3 Microbial Flora Within the Lung Microenvironment

Emerging clinical and epidemiological data suggests a possible link between colonization with \textit{Candida} species and susceptibility to \textit{P. aeruginosa} pulmonary infection. \textit{Candida} species is among the most common organisms recovered from endotracheal tube biofilm and tracheal secretions in patients with VAP (Adair et al. 1999). Historically, \textit{Candida} has been considered a commensal organism rather than a true pathogen, and therefore believed to play no role in VAP disease pathogenesis. However, an observational study found a statistical association between airway colonization with \textit{Candida} species and the development of \textit{P. aeruginosa} VAP (Azoulay et al. 2006). In a rat model of \textit{P. aeruginosa} pneumonia, prior bronchial instillation of live but not heat-killed \textit{C. albicans} resulted in increased susceptibility to subsequent bacterial challenge (Roux et al. 2009). Mechanisms accounting for impaired in vivo clearance responses were not identified, but \textit{C. albicans} was found to inhibit AM respiratory burst ex vivo. While these intriguing findings require confirmation in other experimental model systems, they do raise the
possibility that Candida and perhaps other commensal organisms may contribute meaningful to VAP pathogenesis.

8.11 Novel Therapeutic Approaches to Reverse Critical Illness-Induced Immunosuppression

Antibiotics, prophylactic measures to reduce oropharyngeal colonization and microaspiration, and approaches to stimulate mucociliary transport are the mainstay of therapy to prevent and treat VAP. While these treatments are effective in some patients, adjuvant therapies are needed in others to bolster innate host responses, especially in the elderly and in patients with chronic immunosuppressive therapy. The recognition that critical illness can induce a profound state of immune dysregulation has prompted a reevaluation of potential immunologic approaches being used in the treatment of sepsis and other forms of critical illness (Pockros et al. 2007a). Effective immunoadjuvant therapy must necessarily promote antimicrobial effects without exacerbating deleterious lung inflammatory responses.

8.11.1 Immunostimulatory Therapy (Interferon-γ and GM-CSF)

Common features of both endotoxin tolerance and immune dysregulation of critical illness is impaired TLR signaling, NF-κB-dependent responses, reduced APC function, and a shift toward type 2 rather than type 1 immune responses. Two cytokines that have been shown to partially reverse these changes in vitro and in vivo are IFN-γ and GM-CSF. In endotoxin-tolerized monocytes, treatment with IFN-γ or GM-CSF can reverse the tolerance phenotype, in part by facilitating interactions between IRAK and MyD88, resulting in enhanced downstream activation of NF-κB (Adib-Conquy and Cavaillon 2002). Similarly, ex vivo treatment of blood monocytes from trauma patients with IFN-γ or GM-CSF, but not G-CSF, enhanced LPS-induced cytokine production, and HLA-DR expression (Lendemans et al. 2007).

These preclinical studies served as the foundation for several small clinical trials in patients with sepsis. Docke and colleagues administered IFN-γ to patients with sepsis in an attempt to reverse the cytokine imbalance and restore monocyte function (Docke et al. 1997). In this uncontrolled study, nine patients with evidence of sepsis-induced immunosuppression (decreased blood monocyte HLA-DR expression) were administered IFN-γ at a dose of 100 μg subcutaneously daily. Treatment with IFN-γ resulted in increased monocyte HLA-DR expression in all patients, along with a restoration of monocyte TNF-α production to levels observed in monocytes isolated from healthy subjects. Resolution of sepsis occurred in eight of the nine treated patients (Docke et al. 1997). In two small single center clinical trials, the i.v. administration of hrGM-CSF to patients with sepsis resulted in improvements
in ex vivo effector responses in PBMs or neutrophils (Nierhaus et al. 2003; Presneill et al. 2002). Moreover, one of the studies revealed improvements in \( \text{PaO}_2/\text{FiO}_2 \) ratios, as a measure of pulmonary gas exchange, suggesting reduced lung injury in the GM-CSF treated group (Presneill et al. 2002). Prevention of lung injury may be due, in part, to the fact that GM-CSF is an alveolar epithelial cell mitogen and can protect the alveolar epithelium against hyperoxic and bleomycin-induced injury (Baleeiro et al. 2006; Moore et al. 2000) and in a murine model of influenza pneumonia (Sever-Chroneos et al. 2011). These preclinical and clinical findings served as the basis for a multicenter randomized placebo controlled trial of subcutaneous GM-CSF administration in 38 patients with severe sepsis and evidence of monocyte deactivation (reduced HLA-DR expression). As compared to the placebo group, GM-CSF administration resulted in improved monocyte function (restored cell surface TLR2/4 expression, TNF production, and HLA-DR expression) and improved clinical outcomes, including reduced APACHE II scores, shorter time of mechanical ventilation, and a trend toward decreased length of ICU and hospital stay. These studies and others suggest that immunostimulatory therapy for treatment of critical illness-induced immune dysregulation or even end-organ injury appears to be a potentially viable therapeutic option that warrants larger controlled trials (Luedke and Cerami 1990). An obvious concern of immunostimulatory therapy in patients with severe sepsis and/or pneumonia is the potential of exacerbating the “cytokine storm” of SIRS. Fortunately, neither IFN-\( \gamma \) nor GM-CSF has precipitated worsening of hemodynamic compromise or multiorgan failure, even in patient with severe sepsis or septic shock (Docke et al. 1997; Nierhaus et al. 2003; Meisel et al. 2009). Additional consideration could be given to compartmentalized immunostimulatory therapy (e.g., aerosolized delivery) to prevent or treat VAP. However, this approach may be limited substantially by ventilation-perfusion mismatching that occurs in patients with lung disease, and the concern that the leukocyte reprogramming that occurs during critical illness is not limited to the lung microenvironment but almost certainly occurs more broadly in leukocyte populations systemically.

### 8.11.2 Inhibitors of Apoptosis

Activation of the PI3K/Akt pathway in certain leukocyte populations can lessen NF-\( \kappa \)B-mediated pro-inflammatory responses while stimulating pro-survival and antimicrobial responses (Williams et al. 2006; Wrann et al. 2007; Zhang et al. 2007). For example, the administration of selective activators of the PI3K/Akt signaling pathway (e.g., glucan, \( \alpha \)-lipoic acid) to LPS-challenged mice or mice undergoing CLP reduced apoptosis, inflammatory cytokine release, and improved mortality (Wrann et al. 2007; Zhang et al. 2007).

Interleukin 15 is a pleurapotential cytokine that regulates DC, T, and NK cell activation, proliferation, and survival. The administration of IL-15 to mice with abdominal sepsis (CLP) has been shown to block sepsis-induced apoptosis of NK cells, DC, and CD8 T cells, and to restore NK cell production of IFN-\( \gamma \) (Inoue et al.
Treatment with IL-15 also mitigated sepsis-induced apoptosis of gut epithelium. Importantly, IL-15 not only reduced mortality in CLP, but also in mice administered *P. aeruginosa* i.t.

Finally, caspase inhibitors have been shown to reduce lymphocyte apoptosis and increase survival in murine models of sepsis (Hotchkiss et al. 2000). A pan-caspase inhibitor (IDN-6556) have been employed in the treatment of liver disease in patients with Hepatitis C (Pockros et al. 2007b). However, trials targeting caspases or other pro-apoptotic molecules or administration of pro-survival factors (e.g., AKT activators, IL-15) in patients with sepsis or nosocomial pneumonia have not yet been reported.

8.12 Summary

In this review, we have defined the clinical features of VAP, and described the impact of critical illness and microenvironment factors introduced during mechanical ventilation on susceptibility to VAP, with special attention to specific molecules as potential mediators of immunosuppression and tissue injury. Increases in microbial resistance, combined with a burgeoning population of patients at risk, are trends that clearly make VAP a major clinical problem now and in the future. Preventative strategies and optimal ventilator management have been paramount in reducing the incidence of VAP. However, critical illness-induced reprogramming of leukocyte innate immune responses clearly contributes to susceptibility to VAP and VAP-induced tissue injury. Given our past failures, a paradigm shift in how we approach patients with evidence of immune dysregulation is required. In order for novel therapies to proceed, better clinical markers are needed to distinguish a deleterious innate response (e.g. SIRS) from a state of immunoparalysis (CARS) or mixed antagonist response syndrome (MARS) as the inflammatory response evolves (Wesche et al. 1999). Differentiating these quite disparate but overlapping responses in a patient-specific fashion will allow for better selection of patients in which immunoadjuvant therapy is more likely to be beneficial.

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References

Adair CG, Gorman SP, Feron BM, Byers LM, Jones DS, Goldsmith CE et al (1999) Implications of endotracheal tube biofilm for ventilator-associated pneumonia. Intensive Care Med 25(10):1072–1076

Aderem A, Ulevitch RJ (2000) Toll-like receptors in the induction of the innate immune response. Nature 406(6797):782–787
Adib-Conquy M, Cavaillon JM (2002) Gamma interferon and granulocyte/monocyte colony-stimulating factor prevent endotoxin tolerance in human monocytes by promoting interleukin-1 receptor-associated kinase expression and its association to MyD88 and not by modulating TLR4 expression. J Biol Chem 277(31):27927–27934

Adib-Conquy M, Adrie C, Fitting C, Gattolliat O, Beyaert R, Cavaillon JM (2006) Up-regulation of MyD88s and SIGIRR, molecules inhibiting Toll-like receptor signaling, in monocytes from septic patients. Crit Care Med 34(9):2377–2385

Akira S, Hemmi H (2003) Recognition of pathogen-associated molecular patterns by TLR family. Immunol Lett 85(2):85–95

American Thoracic Society; Infectious Diseases Society of America (2005) Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 171(4):388–416

An L, Liu CT, Yu MJ, Chen ZH, Guo XG, Peng W et al (2011) Heme oxygenase-1 system, inflammation and ventilator-induced lung injury. Eur J Pharmacol 677(1–3):1–4

Appel SH, Wellhausen SR, Montgomery R, DeWeese RC, Polk JHC (1989) Experimental and clinical significance of endotoxin-dependent HLA-DR expression on monocytes. J Surg Res 47(1):39–44

Ayala A, Perrin MM, Kisala JM, Ertel W, Chaudry IH (1992) Polymicrobial sepsis selectively activates peritoneal but not alveolar macrophages to release inflammatory mediators (interleukins-1 and -6 and tumor necrosis factor). Circ Shock 36(3):191–199

Ayala A, Deol ZK, Lehman DL, Herdon CD, Chaudry IH (1994) Polymicrobial sepsis but not low-dose endotoxin infusion causes decreased splenocyte IL-2/IFN-[gamma] release while increasing IL-4/IL-10 production. J Surg Res 56(6):579–585

Azoulay E, Timsit JF, Tafflet M, de Lassence A, Darmon M, Zahar JR et al (2006) Candida colonization of the respiratory tract and subsequent pseudomonas ventilator-associated pneumonia. Chest 129(1):110–117

Baleeiro CE, Wilcoxen SE, Morris SB, Standiford TJ, Paine R 3rd (2003) Sublethal hyperoxia impairs pulmonary innate immunity. J Immunol 171(2):955–963

Baleeiro CE, Christensen PJ, Morris SB, Mendez MP, Wilcoxen SE, Paine R III (2006) GM-CSF and the impaired pulmonary innate immune response following hypoxic stress. Am J Physiol Lung Cell Mol Physiol 291(6):L1246–L1255

Benjamim CF, Ferreira SH, Cunha FQ (2000) Role of nitric oxide in the failure of neutrophil migration in sepsis. J Infect Dis 182(1):214–223

Berends ET, Horswill AR, Haste NM, Monestier M, Nizet V, von Kockritz-Blickwede M (2010) Nuclease expression by Staphylococcus aureus facilitates escape from neutrophil extracellular traps. J Innate Immun 2(6):576–586

Bhan U, Lukacs NW, Osterholzer JJ, Newstead MW, Zeng X, Moore TA et al (2007) TLR9 is required for protective innate immunity in Gram-negative bacterial pneumonia: role of dendritic cells. J Immunol 179(6):3937–3946

Bhan U, Trujillo G, Lyn-Kew K, Newstead MW, Zeng X, Hogaboam CM et al (2008) Toll-like receptor 9 regulates the lung macrophage phenotype and host immunity in murine pneumonia caused by Legionella pneumophila. Infect Immun 76(7):2895–2904

Bhan U, Ballinger MN, Zeng X, Newstead MJ, Cornicelli MD, Standiford TJ (2010) Cooperative interactions between TLR4 and TLR9 regulate interleukin 23 and 17 production in a murine model of gram negative bacterial pneumonia. PLoS One 5(3):e9896

Bhandari V, Elias JA (2006) Cytokines in tolerance to hyperoxia-induced injury in the developing and adult lung. Free Radic Biol Med 41(1):4–18

Bone RC (1996) Sir Isaac Newton, sepsis, SIRS, and CARS. Crit Care Med 24:1125–1128

Brun-Buisson C (2000) The epidemiology of the systemic inflammatory response. Intensive Care Med 26(Suppl 1):S64–S74

Brunialti MK, Martins PS, Barbosa de Carvalho H, Machado FR, Barbosa LM, Salomao R (2006) TLR2, TLR4, CD14, CD11B, and CD11C expressions on monocytes surface and cytokine production in patients with sepsis, severe sepsis, and septic shock. Shock 25(4):351–357
Buchanan JT, Simpson AJ, Aziz RK, Liu GY, Kristian SA, Kotb M et al (2006) DNase expression allows the pathogen group A Streptococcus to escape killing in neutrophil extracellular traps. Curr Biol 16(4):396–400

Burns K, Janssens S, Brissone B, Olivos N, Beyaert R, Tschopp J (2003) Inhibition of interleukin 1 receptor/Toll-like receptor signaling through the alternatively spliced, short form of MyD88 is due to its failure to recruit IRAK-4. J Exp Med 197(2):263–268

Chan C, Li L, McCall CE, Yoza BK (2005) Endotoxin tolerance disrupts chromatin remodeling and NF-κB transactivation at the IL-1β promoter. J Immunol 175(1):461–468

Charles PE, Tissieres P, Barbar SD, Croisier D, Dufour J, Dunn-Siegrist I et al (2011) Mild-stretch mechanical ventilation upregulates toll-like receptor 2 and sensitizes the lung to bacterial lipopeptide. Crit Care 15(4):R181

Chun CD, Liles WC, Frevert CW, Glenny RW, Altemeier WA (2010) Mechanical ventilation modulates Toll-like receptor-3-induced lung inflammation via a MyD88-dependent, TLR4-independent pathway: a controlled animal study. BMC Pulm Med 10:57

Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM et al (2007) Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med 13(4):463–469

Cummings CJ, Martin TR, Frevert CW, Quan JM, Wong VA, Mongovin SM et al (1999) Expression and function of the chemokine receptors CXCR1 and CXCR2 in sepsis. J Immunol 162(4):2341–2346

Czermak BJ, Sarma V, Pierson CL, Warner RL, Huber-Lang M, Bless NM et al (1999) Protective effects of C5a blockade in sepsis. Nat Med 5(7):788–792

Deng JC, Cheng G, Newstead MW, Zeng X, Kobayashi K, Flavell RA et al (2006) Sepsis-induced suppression of lung innate immunity is mediated by IRAK-M. J Clin Invest 116(9):2532–2542

Dhanireddy S, Altemeier WA, Matute-Bello G, O’Mahony DS, Glenny RW, Martin TR et al (2006) Mechanical ventilation induces inflammation, lung injury, and extra-pulmonary organ dysfunction in experimental pneumonia. Lab Invest 86(8):790–799

Dinarello CA (2000) Proinflammatory cytokines. Chest 118:503–508

Docke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P et al (1997) Monocyte deactivation in septic patients: restoration by IFN-γ treatment. Nat Med 3(6):678–681

Dreschler K, Bratke K, Petermann S, Thamm P, Kuepper M, Virchow JC et al (2012) Altered phenotype of blood dendritic cells in patients with acute pneumonia. Respiration 83(3):209–217

Escoll P, del Fresno C, Garcia L, Valles G, Lendinez MJ, Arnalich F et al (2003) Rapid up-regulation of IRAK-M expression following a second endotoxin challenge in human monocytes and in monocytes isolated from septic patients. Biochem Biophys Res Commun 311(2):465–472

Ferguson NR, Galley HF, Webster NR (1999) T helper cell subset ratios in patients with severe sepsis. Intensive Care Med 25(6):106–109

Ferwerda B, Alonso S, Banahan K, McCall MB, Giamarellos-Bourboulis EJ, Ramakers BP et al (2009) Functional and genetic evidence that the Mal/TIRAP allele variant 180 L has been selected by providing protection against septic shock. Proc Natl Acad Sci U S A 106(25):10272–10277

Foster SL, Hargreaves DC, Medzhitov R (2007) Gene-specific control of inflammation by TLR-induced chromatin modifications. Nature 447(7147):972–978

Frank JA, Parsons PE, Matthay MA (2006) Pathogenetic significance of biological markers of ventilator-associated lung injury in experimental and clinical studies. Chest 130(6):1906–1914

Fujita S, Seino K, Sato K, Sato Y, Eizumi K, Yamashita N et al (2006) Regulatory dendritic cells act as regulators of acute lethal systemic inflammatory response. Blood 107(9):3656–3664

Fujitani S, Sun HY, Yu VL, Weingarten JA (2011) Pneumonia due to Pseudomonas aeruginosa: part I: epidemiology, clinical diagnosis, and source. Chest 139(4):909–919
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Gallinaro RN, Naziri W, McMasters KM, Peyton JC, Cheadle WG (1994) Alteration of mononuclear cell immune-associated antigen expression, interleukin-1 expression, and antigen presentation during intra-abdominal infection. Shock 1(2):130–134

Garrouste-Orgeas M, Chevret S, Arlet G, Marie O, Rouveau M, Popoff N et al (1997) Oropharyngeal or gastric colonization and nosocomial pneumonia in adult intensive care unit patients. A prospective study based on genomic DNA analysis. Am J Respir Crit Care Med 156(5):1647–1655

Gharib SA, Liles WC, Klaff LS, Altemeier WA (2009) Noninjurious mechanical ventilation activates a proinflammatory transcriptional program in the lung. Physiol Genomics 37(3):239–248

Gordon S, Martinez FO (2010) Alternative activation of macrophages: mechanism and functions. Immunity 32(5):593–604

Gore A, Muralidhar M, Espey MG, Degenhardt K, Mantell LL (2010) Hyperoxia sensing: from molecular mechanisms to significance in disease. J Immunototoxicol 7(4):239–54

Goya T, Abe M, Shimura H, Torisu M (1992) Characteristics of alveolar macrophages in experimental septic lung. J Leukoc Biol 52(2):236–243

Hajjar AM, Harowicz H, Liggitt HD, Fink PJ, Wilson CB, Skerrett SJ (2005) An essential role for non-bone marrow-derived cells in control of Pseudomonas aeruginosa pneumonia. Am J Respir Cell Mol Biol 33(5):470–475

Halbertsma FJ, Vaneker M, Scheffer GJ, van der Hoeven JG (2005) Cytokines and biotrauma in ventilator-induced lung injury: a critical review of the literature. Neth J Med 63(10):382–392

Hall MW, Gavrilin MA, Knatz NL, Duncan MD, Fernandez SA, Wewers MD (2007) Monocyte mRNA phenotype and adverse outcomes from pediatric multiple organ dysfunction syndrome. Pediatr Res 62(5):597–603

Hedl M, Li J, Cho JH, Abraham C (2007) Chronic stimulation of Nod2 mediates tolerance to bacterial products. Proc Natl Acad Sci 104(49):19440–19445

Heidecke C-D, Hensler T, Weighardt H, Zantl N, Wagner H, Siewert J-R et al (1999) Selective defects of T lymphocyte function in patients with lethal intraabdominal infection. Am J Surg 178(4):288–292

Holub M, Kluckova Z, Helcl M, Prihodov J, Rokyta R, Beran O (2003) Lymphocyte subset numbers depend on the bacterial origin of sepsis. Clin Microbiol Infect 9(3):202–211

Hotchkiss RS, Karl IE (2003) The pathophysiology and treatment of sepsis. N Engl J Med 348(2):138–150

Hotchkiss RS, Chang KC, Swanson PE, Tinsley KW, Hui JJ, Klender P et al (2000) Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. Nat Immunol 1(6):496–501

Hotchkiss RS, Tinsley KW, Swanson PE, Schmie RE Jr, Hui JJ, Chang KC et al (2001) Sepsis-induced apoptosis causes progressive profound depletion of B and CD4+ T lymphocytes in humans. J Immunol 166(11):6952–6963

Hotchkiss RS, Tinsley KW, Swanson PE, Grayson MH, Osborne DF, Wagner TH et al (2002) Depletion of dendritic cells, but not macrophages, in patients with sepsis. J Immunol 168(5):2493–2500

Hruz P, Zinkernagel AS, Jenikova G, Botwin GJ, Hugot JP, Karin M et al (2009) NOD2 contributes to cutaneous defense against Staphylococcus aureus through alpha-toxin-dependent innate immune activation. Proc Natl Acad Sci U S A 106(31):12873–12878

Huber-Lang M, Sarma VJ, Lu KT, McGuire SR, Padgaonkar VA, Guo RF et al (2001) Role of C5a in multiorgan failure during sepsis. J Immunol 166(2):1193–1199

Hyakushima N, Mitsuzawa H, Nishitani C, Sano H, Kuronuma K, Konishi M et al (2004) Interaction of soluble form of recombinant extracellular TLR4 domain with MD-2 enables lipopolysaccharide binding and attenuates TLR4-mediated signaling. J Immunol 173(11):6949–6954

Iinohara N, Chamaillard M, McDonald C, Nunez G (2005) NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. Annu Rev Biochem 74:355–383
Inoue S, Unsinger J, Davis CG, Muenzer JT, Ferguson TA, Chang K et al (2010) IL-15 prevents apoptosis, reverses innate and adaptive immune dysfunction, and improves survival in sepsis. J Immunol 184(3):1401–1409

Iwami K-I, Matsuguchi T, Masuda A, Kikuchi T, Musikcharoen T, Yoshikai Y (2000) Cutting edge: naturally occurring soluble form of mouse Toll-like receptor 4 inhibits lipopolysaccharide signaling. J Immunol 165(2):6682–6686

Jaeklin T, Engelberts D, Otulakowski G, O’Brodovich H, Post M, Kavanagh BP (2011) Lung-derived soluble mediators are pathogenic in ventilator-induced lung injury. Am J Physiol Lung Cell Mol Physiol 300(4):L648–L658

Kashuk JL, Moore EE, Price CS, Zaw-Mon C, Nino T, Haenel J et al (2010) Patterns of early and late ventilator-associated pneumonia due to methicillin-resistant Staphylococcus aureus in a trauma population. J Trauma 69(3):519–522

Kikuchi Y, Tateda K, Fuse ET, Matsumoto T, Gotoh N, Fukushima J et al (2009) Hyperoxia exaggerates bacterial dissemination and lethality in Pseudomonas aeruginosa pneumonia. Pulm Pharmacol Ther 22(4):333–339

Kobayashi K, Hernandez LD, Galan JE, Janeway CA, Medzhitov R, Flavell RA (2002) IRAK-M is a negative regulator of Toll-like receptor signaling. Cell 110(2):191–202

LeBouder E, Rey-Nores JE, Rushmere NK, Grigorov M, Lawn SD, Affolter M et al (2003) Soluble forms of Toll-like receptor (TLR)2 capable of modulating TLR2 signaling are present in human plasma and breast milk. J Immunol 171(12):6680–6689

Lee PJ, Choi AM (2003) Pathways of cell signaling in hyperoxia. Free Radic Biol Med 35(4):341–350

Lendemans S, Kreuzfelder E, Waydhas C, Schade FU, Flohe S (2007) Differential immunostimulating effect of granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and interferon gamma (IFN gamma) after severe trauma. Inflamm Res 56(1):38–44

Luedke CE, Cerami A (1990) Interferon-gamma overcomes glucocorticoid suppression of cachectin/tumor necrosis factor biosynthesis by murine macrophages. J Clin Invest 86(4):1234–1240

Lyn-Kew K, Rich E, Zeng X, Wen H, Kunkel SL, Newstead MW et al (2010) IRAK-M regulates chromatin remodeling in lung macrophages during experimental sepsis. PLoS One 5(6):e11145

Mantovani A, Cassatella MA, Costantini C, Jaillon S (2011) Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol 11(8):519–531

Manzano F, Fernandez-Mondejar E, Colmenero M, Poyatos ME, Rivera R, Machado J et al (2008) Positive-end expiratory pressure reduces incidence of ventilator-associated pneumonia in non-hypoxemic patients. Crit Care Med 36(8):2225–2231

Mariencheck WI, Alcorn JF, Palmer SM, Wright JR (2003) Pseudomonas aeruginosa elastase degrades surfactant proteins A and D. Am J Respir Cell Mol Biol 28(4):528–537

Martin GS, Mannino DM, Eaton S, Moss M (2003) The Epidemiology of Sepsis in the United States from 1979 through 2000. N Engl J Med 348(16):1546–1554

McCormack FX (2006) New concepts in collectin-mediated host defense at the air-liquid interface of the lung. Respirology 11(Suppl):S7–S10

Meisel C, Schebold JC, Pschowski R, Baumann T, Hetzger K, Gregor J et al (2009) Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. Am J Respir Crit Care Med 180(7):640–648

Members of the American College of Chest Physicians/Society of Critical Care Medicine (2003) American College of Chest Physicians/Society of Critical Care Medicine consensus conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 31:1250–1256

Moore BB, Coffey MJ, Christensen P, Sitterding S, Ngan R, Wilke CA et al (2000) GM-CSF regulates bleomycin-induced pulmonary fibrosis via a prostaglandin-dependent mechanism. J Immunol 165(7):4032–4039
Munoz C, Carlet J, Fitting C, Misset B, Blerlot J, Cavaillon J (1991) Dysregulation of in vitro cytokine production by monocytes during sepsis. J Clin Invest 88:1747–1754
Mustin RA, Bohnen JM, Rosati C, Schouten BD (1991) Pneumonia complicating abdominal sepsis. An independent risk factor for mortality. Arch Surg 126(2):170–175
Nahid MA, Satoh M, Chan EK (2011) Mechanistic role of microRNA-146a in endotoxin-induced differential regulation of TLR signaling. J Immunol 186(3):1723–1734
Nakayama K, Okugawa S, Yanagimoto S, Kitazawa T, Tsukada K, Kawada M et al (2004) Involvement of IRAK-M in peptidoglycan-induced tolerance in macrophages. J Biol Chem 279(8):6629–6634
Nakos G, Tsangaris H, Liokatis S, Kitsiouli E, Lekka ME (2003) Ventilator-associated pneumonia and atelectasis: evaluation through bronchoalveolar lavage fluid analysis. Intensive Care Med 29(4):555–563
Narasaraju T, Yang E, Samy RP, Ng HH, Poh WP, Liew AA et al (2011) Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonia. Am J Pathol 179(1):199–210
Nicholls JM, Poon LL, Lee KC, Ng WF, Lai ST, Leung CY et al (2003) Lung pathology of fatal severe acute respiratory syndrome. Lancet 361(9371):1773–1778
Nierhaus A, Montag B, Timmler N, Frings DP, Gutensohn K, Jung R et al (2003) Reversal of immunoparalysis by recombinant human granulocyte-macrophage colony-stimulating factor in patients with severe sepsis. Intensive Care Med 29(4):646–651
Ohashi K, Burkart V, Flohe S, Kolb H (2000) Cutting edge: heat shock protein 60 is a putative endogenous ligand of the Toll-like receptor-4 complex. J Immunol 164(2):558–561
O'Mahony DS, Liles WC, Altemeier WA, Dhanireddy S, Frevert CW, Liggitt D et al (2006) Mechanical ventilation interacts with endotoxemia to induce extrapulmonary organ dysfunction. Crit Care 10(5):R136
O'Reilly PJ, Hickman-Davis JM, Davis IC, Matalon S (2003) Hyperoxia impairs antibacterial function of macrophages through effects on actin. Am J Respir Cell Mol Biol 28(4):443–450
O'Sullivan ST, Lederer JA, Horgan AF, Chin DH, Mannick JA, Rodrick ML (1995) Major injury leads to predominance of the T helper-2 lymphocyte phenotype and diminished interleukin-12 production associated with decreased resistance to infection. Ann Surg 222(4):482–490, discussion 90–2
Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A (2010) Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol 191(3):677–691
Park JS, Svetkausaite D, He Q, Kim J-Y, Strassheim D, Ishizaka A et al (2004) Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem 279(9):7370–7377
Parker H, Albrett AM, Kettle AJ, Winterbourn CC (2012) Myeloperoxidase associated with neutrophil extracellular traps is active and mediates bacterial killing in the presence of hydrogen peroxide. J Leukoc Biol 91(3):369–376
Piatti G, Ambrosetti U, Santus P, Allegra L (2005) Effects of salmeterol on cilia and mucus in COPD and pneumonia patients. Pharmacol Res 51(2):165–168
Pittet LA, Hall-Stoodley L, Rutkowski MR, Harmsen AG (2010) Influenza virus infection decreases tracheal mucociliary velocity and clearance of Streptococcus pneumoniae. Am J Respir Cell Mol Biol 42(4):450–460
Pockros PJ, Schiff ER, Shiffman ML, McHutchison JG, Gish RG, Afdhal NH et al (2007a) Oral IDN-6556, an antiapoptotic caspase inhibitor, may lower aminotransferase activity in patients with chronic hepatitis C. Hepatology 46(2):324–329
Pockros PJ, Schiff ER, Shiffman ML, McHutchison JG, Gish RG, Afdhal NH, Makhviladze M, Huyghe M, Hecht D, Oltersdorf T, Shapiro DA (2007b) Oral IDN-6556, an antiapoptotic caspase inhibitor, may lower aminotransferase activity in patients with chronic hepatitis C. Hepatology 46(2):324–329
Presneill JJ, Harris T, Stewart AG, Cade JF, Wilson JW (2002) A randomized phase II trial of granulocyte-macrophage colony-stimulating factor therapy in severe sepsis with respiratory dysfunction. Am J Respir Crit Care Med 166(2):138–143

Pugin J, Dunn I, Jolliet P, Tassaux D, Magenat JL, Nicod LP et al (1998) Activation of human macrophages by mechanical ventilation in vitro. Am J Physiol 275(6 Pt 1):L1040–L1050

Qin J, Qian Y, Yao J, Grace C, Li X (2005) SIGIRIR inhibits interleukin-1 receptor- and Toll-like receptor 4-mediated signaling through different mechanisms. J Biol Chem 280(26):25233–25241

Ramirez P, Ferrer M, Gimeno R, Torno S, Valencia M, Piner R et al (2009) Systemic inflammatory response and increased risk for ventilator-associated pneumonia: a preliminary study. Crit Care Med 37(5):1691–1695

Ramphal R, Balloy V, Jyot J, Verma A, Si-Tahar M, Chignard M (2008) Control of Pseudomonas aeruginosa in the lung requires the recognition of either lipopolysaccharide or flagellin. J Immunol 181(1):586–592

Rao N, Nguyen S, Ngo K, Fung-Leung W-P (2005) A novel splice variant of interleukin-1 receptor (IL-1R)-associated kinase 1 plays a negative regulatory role in Toll/IL-1R-induced inflammatory signaling. Mol Cell Biol 25(15):6521–6532

Rauch PJ, Chudnovskiy A, Robbins CS, Weber GF, Etzrodt M, Hilgendorf I et al (2012) Innate response activator B cells protect against microbial sepsis. Science 335(6068):597–601

Reddy RC, Chen GH, Newstead MW, Moore T, Zeng X, Tateda K et al (2001) Alveolar macrophage deactivation in murine septic peritonitis: role of interleukin 10. Infect Immun 69(3):1394–1401

Remijesen Q, Kuipers TW, Wirawan E, Lippens S, Vandenameepe P, Vandenbega T (2011) Dying for a cause: NETosis, mechanisms behind an antimicrobial cell death modality. Cell Death Differ 18(4):581–588

Richardson JD, DeCamp MM, Garrison RN, Fry DE (1982) Pulmonary infection complicating intra-abdominal sepsis. Ann Surg 195(6):732–737

Roux D, Gaudry S, Dreyfuss D, El-Benna J, de Prost N, Denamur E et al (2009) Candida albicans impairs macrophage function and facilitates Pseudomonas aeruginosa pneumonia in rat. Crit Care Med 37(3):1062–1067

Roy-Burman A, Savel RH, Racine S, Swanson BL, Revadigar NS, Fujimoto J et al (2001) Type III protein secretion is associated with death in lower respiratory and systemic Pseudomonas aeruginosa infections. J Infect Dis 183(12):1767–1774

Sadikot RT, Blackwell TS, Christman JW, Prince AS (2005) Pathogen-host interactions in Pseudomonas aeruginosa pneumonia. Am J Respir Crit Care Med 171(11):1209–1223

Schurr JR, Young E, Byrne P, Steele C, Shellito JE, Kolls JK (2005) Central role of toll-like receptor 4 signaling and host defense in experimental pneumonia caused by Gram-negative bacteria. Infect Immun 73(1):532–545

Scumpia PO, Delano MJ, Kelly KM, O’Malley KA, Efron PA, McAuliffe PF et al (2006) Increased natural CD4+CD25+ regulatory T cells and their suppressor activity do not contribute to mortality in murine polymicrobial sepsis. J Immunol 177(11):7943–7949

Seki M, Kohno S, Newstead MW, Zeng X, Bhan U, Lukacs NW et al (2010) Critical role of IL-1 receptor-associated kinase-M in regulating chemokine-dependent deleterious inflammation in murine influenza pneumonia. J Immunol 184(3):1410–1418

Sever-Chrones Z, Murthy A, Davis J, Florence JM, Kurdowska A, Krupa A et al (2011) GM-CSF modulates pulmonary resistance to influenza A infection. Antiviral Res 92(2):319–328

Skerrett SJ, Liggitt HD, Hajjar AM, Wilson CB (2004) Cutting edge: myeloid differentiation factor 88 is essential for pulmonary host defense against Pseudomonas aeruginosa but not Staphylococcus aureus. J Immunol 172(6):3377–3381

Steinhauser ML, Hogaboam CM, Kunkel SL, Lukacs NW, Strieter RM, Standiford TJ (1999) IL-10 is a major mediator of sepsis-induced impairment in lung antibacterial host defense. J Immunol 162(1):392–399

Swartz DE, Seely AJ, Ferri L, Giannias B, Christou NV (2000) Decreased systemic polymorphonuclear neutrophil (PMN) rolling without increased PMN adhesion in peritonitis at remote sites. Arch Surg 135(8):959–966
Tamayo E, Fernandez A, Almansa R, Carrasco E, Heredia M, Lajo C et al (2011) Pro- and anti-inflammatory responses are regulated simultaneously from the first moments of septic shock. Eur Cytokine Netw 22(2):82–87

Tang BMP, McLean AS, Dawes IW, Huang SJ, Lin RCY (2007) The use of gene-expression profiling to identify candidate genes in human sepsis. Am J Respir Crit Care Med 176(7):676–684

Thomassen E, Renshaw BR, Sims JE (1999) Identification and characterization of SIGIRR, a molecule representing a novel subtype of the IL-1R superfamily. Cytokine 11(6):389–399

Urban CF, Ermert D, Schmid M, Abu-Abed U, Goossmann C, Nacken W et al (2009) Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS Pathog 5(10):e1000639

van der Poll T, van Deventer SJ (1999) Cytokines and anticytokines in the pathogenesis of sepsis. Infect Dis Clin North Am 13(2):413–426, ix

van Kaam AH, Lachmann RA, Herting E, De Jaegere A, van Iwaarden F, Noorduyn LA et al (2004) Reducing atelectasis attenuates bacterial growth and translocation in experimental pneumonia. Am J Respir Crit Care Med 169(9):1046–1053

Vaneker M, Joosten LA, Heunks LM, Snijder DG, Halbertsma FJ, van Egmond J et al (2008) Low-tidal-volume mechanical ventilation induces a toll-like receptor 4-dependent inflammatory response in healthy mice. Anesthesiology 109(3):465–472

van’t Veer C, van den Pangaart PS, van Zoelen MA, de Kruijf M, Birjimohun RS, Stroes ES, et al (2007) Induction of IRAK-M is associated with lipopolysaccharide tolerance in a human endotoxemia model. J Immunol 179(10):7110–20

Venet F, Chung C-S, Monneret G, Huang X, Horner B, Garber M et al (2008) Regulatory T cell populations in sepsis and trauma. J Leukoc Biol 83(3):523–535

Villar J, Cabrera NE, Casula M, Flores C, Valladares F, Díaz-Flores L et al (2010) Mechanical ventilation modulates TLR4 and IRAK-3 in a non-infectious, ventilator-induced lung injury model. Respir Res 11:27

Volk HD, Reinke P, Docke WD (2000) Clinical aspects: from systemic inflammation to ‘immunoparalysis’. Chem Immunol 74:162–177

Wald D, Qin J, Zhao Z, Qian Y, Naramura M, Tian L et al (2003) SIGIRR, a negative regulator of Toll-like receptor-interleukin 1 receptor signaling. Nat Immunol 4(9):920–927

Wen H, Dou Y, Hogaboam CM, Kunkel SL (2008) Epigenetic regulation of dendritic cell-derived interleukin-12 facilitates immunosuppression after a severe innate immune response. Blood 111(4):1797–1804

Wesche H, Hao X, Li X, Kirschning CJ, Stark GR, Cao Z (1999) IRAK-M is a novel member of the pelle/interleukin-1 receptor-associated kinase (IRAK) family. J Biol Chem 274(27):19403–19410

Wieland CW, Florquin S, Maris NA, Hoebe K, Beutler B, Takeda K et al (2005) The MyD88-dependent, but not the MyD88-independent, pathway of TLR4 signaling is important in clearing non-typeable haemophilus influenzae from the mouse lung. J Immunol 175(9):6042–6049

Williams MA, White SA, Miller JJ, Toner C, Wittington S, Newland AC et al (1998) Granulocyte-macrophage colony-stimulating factor induces activation and restores respiratory burst activity in monocytes from septic patients. J Infect Dis 177(1):107–115

Williams DL, Oztun-Skelton T, Li C (2006) Modulation of the phosphoinositide 3-kinase signaling pathway alters host response to sepsis, inflammation, and ischemia/reperfusion injury. Shock 25(5):432–439

Wisnosiński N, Chung CS, Chen Y, Huang X, Ayala A (2007) The contribution of CD4+ CD25+ T-regulatory-cells to immune suppression in sepsis. Shock 27(3):251–257

Wolfs TG, Dunn-Siegrist I, van’t Veer C, Hodin CM, Germeraad WT, van Zoelen MA et al (2008) Increased release of sMD-2 during human endotoxemia and sepsis: a role for endothelial cells. Mol Immunol. 45(11):3268–3277

Wrann CD, Tabriz NA, Barkhausen T, Kloas A, van Griensven M, Pape HC et al (2007) The phosphatidylinositol 3-kinase signaling pathway exerts protective effects during sepsis by controlling C5a-mediated activation of innate immune functions. J Immunol 178(9):5940–5948
Wysocka M, Robertson S, Riemann H, Caamano J, Hunter C, Mackiewicz A et al (2001) IL-12 suppression during experimental endotoxin tolerance: dendritic cell loss and macrophage hyporesponsiveness. J Immunol 166(12):7504–7513
Young RL, Malcolm KC, Kret JE, Caceres SM, Poch KR, Nichols DP et al (2011) Neutrophil extracellular trap (NET)-mediated killing of Pseudomonas aeruginosa: evidence of acquired resistance within the CF airway, independent of CFTR. PLoS One 6(9):e23637
Zedler S, Bone RC, Baue AE, von Donnersmarck GH, Faist E (1999) T-cell reactivity and its predictive role in immunosuppression after burns. Crit Care Med 27(1):66–72
Zhang WJ, Wei H, Hagen T, Frei B (2007) Alpha-lipoic acid attenuates LPS-induced inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway. Proc Natl Acad Sci U S A 104(10):4077–4082
Zivkovic A, Sharif O, Stich K, Doninger B, Biaggio M, Colinge J et al (2011) TLR 2 and CD14 mediate innate immunity and lung inflammation to staphylococcal Panton-Valentine leukocidin in vivo. J Immunol 186(3):1608–1617