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).

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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 maltiphila*. *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. maltiphila*, 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 stains 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 (Garrouste-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
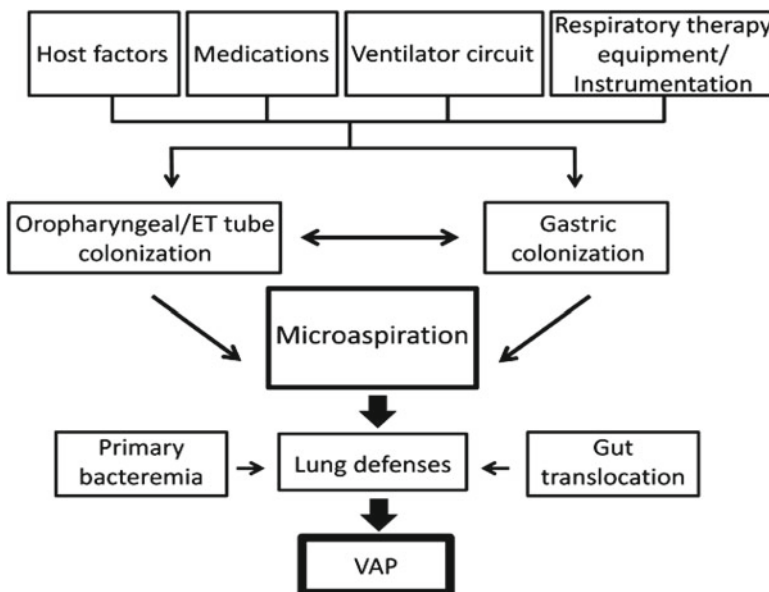
Fig. 8.1 Factors contributing to the pathogenesis of VAP. A variety of different contributing factors have been previously shown to contribute to the development of VAP. The end result from a combination of host factors, medication, and instrumentation is the introduction of infectious material into the sterile lung environment. These factors along with the immune state of host, contribute to the development of VAP

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 mucocilliary 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 mucocilliary 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
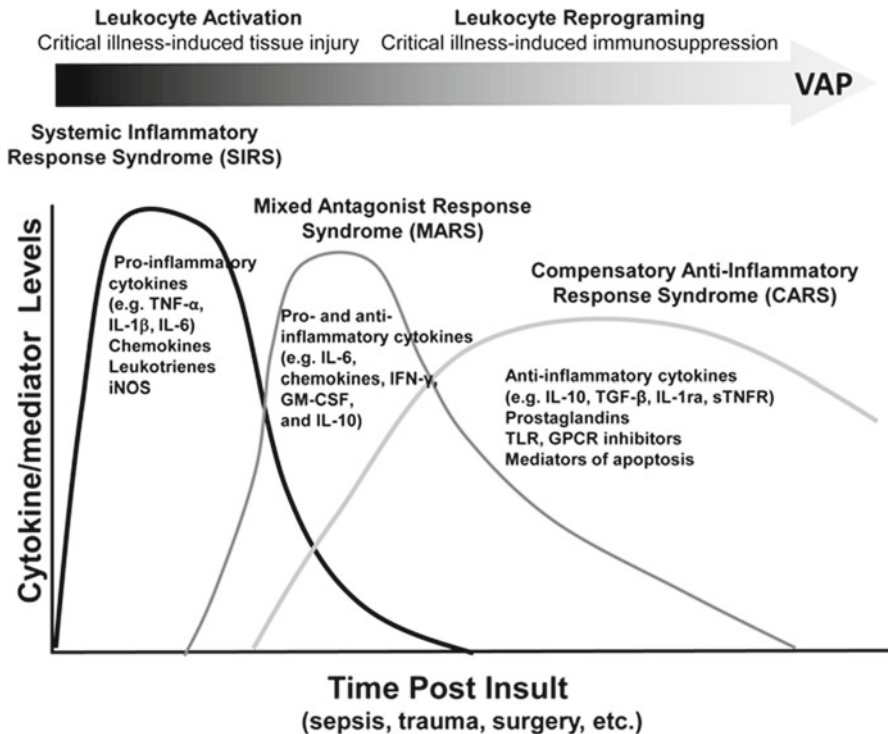
Leukocyte Activation
Critical illness-induced tissue injury

Leukocyte Reprograming
Critical illness-induced immunosuppression

VAP

**Systemic Inflammatory
Response Syndrome (SIRS)**

**Mixed Antagonist Response
Syndrome (MARS)**

Cytokine/mediator Levels

Pro-inflammatory
cytokines
(e.g. TNF-α,
IL-1β, IL-6)
Chemokines
Leukotrienes
iNOS

Pro- and anti-
inflammatory cytokines
(e.g. IL-6,
chemokines, IFN-γ,
GM-CSF,
and IL-10)

**Compensatory Anti-Inflammatory
Response Syndrome (CARS)**

Anti-inflammatory cytokines
(e.g. IL-10, TGF-β, IL-1ra, sTNFR)
Prostaglandins
TLR, GPCR inhibitors
Mediators of apoptosis

Time Post Insult
(sepsis, trauma, surgery, etc.)

Fig. 8.2 Innate immune events in critical illness. The dysregulation of the innate immune system is a main factor in the development of VAP. The progression of leukocyte activation, along with SIRS, followed by leukocyte reprograming, including MARS and CARS, contributes to the overall dysfunctions leading to the development of VAP

suggests a third response to an inflammatory insult, referred to as the mixed antagonist response syndrome (MARS). This response is characterized by the secretion of both pro- and anti-inflammatory mediators (specifically IL-6, IL-8, monocyte chemotactic 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 appropriated 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
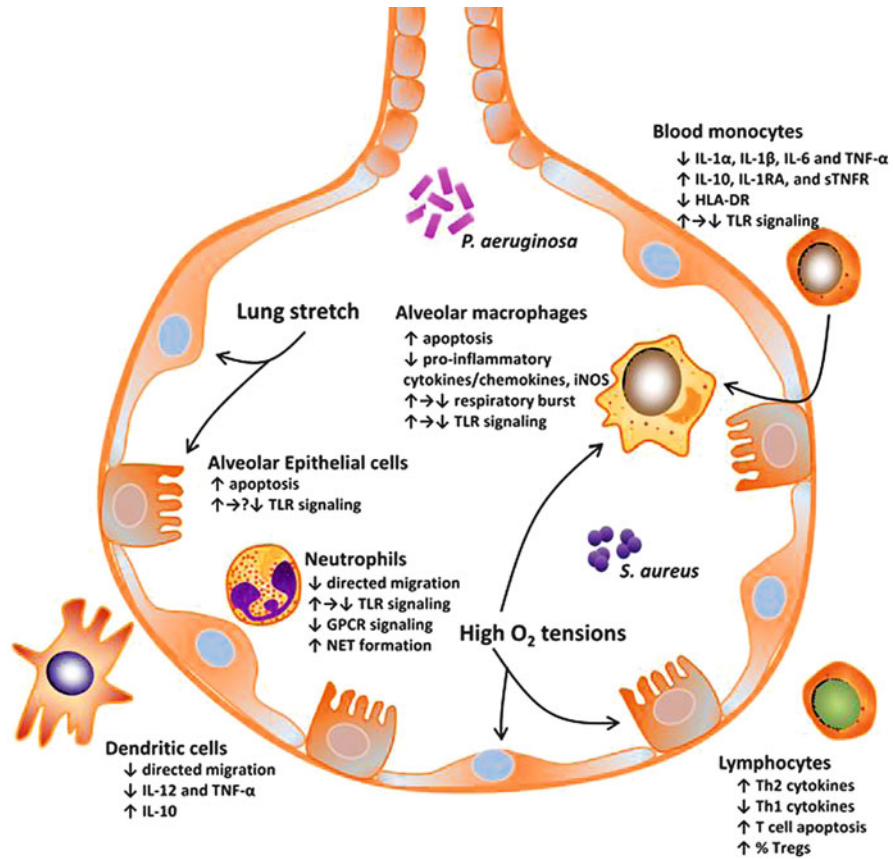
Fig. 8.3 Alterations and specific defects of leukocyte function in critical illness and mechanical ventilation. There are a variety of cellular, bacterial, and mechanical mediators which contribute to the impaired innate and acquired immune responses during critical illness. (*Upward arrow*) represent effects that enhance expression/function (*downward arrow*) represents effects that reduce expression/function

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 proteins 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 H_2O_2 (Parker et al. 2012). NETs are capable of physically ensnaring bacteria and facilitating the interactions between bacteria 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_{reg} population has been identified in spleen (CD11c^{low}, CD45RB^{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-γ, 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-β 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-α 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 initiated 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 anti-bacterial 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 oligodimerization 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 (IRAK)-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 blood buffy 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 treat 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-kB 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-dependant 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 cmH$_2$O 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_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 *P. aeruginosa* or *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 *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 *Candida* species and susceptibility to *P. aeruginosa* pulmonary infection. *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, *Candida* has been considered a commensal organisms 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 Candida species and the development of *P. aeruginosa* VAP (Azoulay et al. 2006). In a rat model of *P. aeruginosa* pneumonia, prior bronchial instillation of live but not heat-killed *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 *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 mucocilliary 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 PaO_2/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-γ 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-κB-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, α-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-γ (Inoue et al.

2010). 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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